INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.
THE EFFECTS OF MODIFIED GLASSY CARBON SURFACES ON
ELECTRON TRANSFER KINETICS OF ORGANIC REDOX SYSTEMS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate School of
The Ohio State University

By

Hsueh-Hui Yang, B.S.

*****

The Ohio State University
2000

Dissertation Committee: Dr. Richard L. McCreery, Advisor
Dr. Larry B. Anderson
Dr. Susan V. Olesik

Approved by

P.J. McCreery
Advisor
Department of Chemistry
ABSTRACT

A systematic procedure was used to diagnose the kinetic effects of surface treatments on glassy carbon. First step is to classify redox systems into two different groups, “surface sensitive” and “surface insensitive” by comparing the electron transfer rate on polished GC and monolayer covered GC.

Ru(NH$_3)_6^{3+/2+}$, O$_2$, and six organic redox systems were examined in aqueous electrolytes on polished and modified glassy carbon electrodes. A plot of the natural log of the observed rate constant of Ru(NH$_3)_6^{3+/2+}$ vs. the monolayer thickness for a variety of chemisorbed monolayers was linear, with a slope of -0.21 Å$^{-1}$. The observations are consistent with a through-bond electron-tunneling mechanism. The tunneling constant of Ru(NH$_3)_6^{3+/2+}$ is between that reported for electron tunneling through conjugated polyene spacers (0.14 Å$^{-1}$) and that reported for phenyl-methylene spacers (0.57 Å$^{-1}$), on the basis of long-range electron transfer in rigid molecules.

Methyl viologen reduction to its cation radical exhibited a voltammetric peak potential difference which was insensitive to surface modification, with $k^0$ decreasing by only 50% when a chemisorbed monolayer was present. Methylene blue and three other phenothiazines adsorbed to polished GC, but the adsorption was suppressed by surface modification. However, the monolayers of electroinactive species had only minor effects
on $k^0$ for all phenothiazines. This minor change in $k^0$ was accompanied by a major
decrease in adsorption, apparently due to inhibition of dipole-dipole or $\pi-\pi$ interactions
between the phenothiazine and GC. A plot of the natural log of the observed rate
constant of chloropromazine (CPZ) vs. the monolayer thickness for a variety of
chemisorbed monolayers was linear, with a slope of -0.22 Å$^{-1}$. The observations are
consistent with a through-bond electron-tunneling mechanism for electron transfer to
methyl viologen and all four phenothiazines.

Oxygen and dihydronicotinamide adenine dinucleotide (NADH), are different
from the systems mentioned above. They are sensitive to the surface modifications. The
following chemical reaction of the reduction product of oxygen, the reaction of
superoxide radical with water, is catalyzed by the surface adsorption of superoxide
radical. On polished GC, the following chemical reaction is fast on the time scale of
cyclovoltammetry. Once the surface is modified with chemisorbed monolayers, the
adsorption is inhibited. Thus, the following reaction is slowed down. Reversible cyclovoltammograms are obtained on chemisorbed modified GC and irreversible cyclovoltammograms on polished GC at pH 14.

NADH undergoes a two electron and one proton oxidation on polished GC.
However, only the first electron transfer process happens on the chemisorbed monolayers.
The final product of the oxidation of NADH is nicotinamide adenine dinucleotide
($\text{NAD}^+$) on the polished GC and NAD dimer, ($\text{NAD}_2$) on the chemisorbed monolayer
modified GC.
Dedicated To My Grandparents

And

My Parents
ACKNOWLEDGMENTS

Instead of keeping quiet, as is my usual characteristic, I have to express my appreciation to those who made this dissertation possible and who made my graduate studies enjoyable. First, I would like to thank my advisor, Prof. Richard McCreery, whose guidance and encouragement have greatly helped me going through all the tough times in the graduate school, especially the final stage. Without those “deals”, I could not have finished this job on time.

I would also like to thank the McCreery group members for their friendship and help: Tzu-Chi Kuo, Kristin Frost, Jeremy Ramsey, Lin Xia, Stacy Duvall, Srikanth Ranganathan, Bill McGovern, Belinda Hurley, Bill Clark, Ilson Steidel, Jingya Wu, and Yangyan Hu. I wish all of you the best in achieving your goals.

I am grateful to my friends from Taiwan: Chien-Ni Chang, Ei-Wen Chang, Ling-Ling Yen, Yi-Wen Lee, and Hsiao-Ching Yang. With their companionship, the days without Fu-Chen and Michelle were not as lonely as I imagined.

I also wish to thank Fu-Chen’s labmates -- Aaron Rath, Heather Hall, and Christine Plecnik -- who spent their precious time proofreading my dissertation and making this work not look like “Chinese English”. Roman Kultyshev has been a very good friend of both Fu-Chen and I.
I would like to thank my grandparents and parents for their support and encouragement of my education and desire to learn. My sister, Wen-Hua, accompanied them for the days I spent in the USA. Without her, it would have been impossible for me to stay in the USA for five years.

Finally, the love, understanding, support, and patience from my husband, Fu-Chen, was essential for my success. He sacrificed his job opportunities to stay with me after his graduation. Without him taking care of our loving daughter, Michelle, alone in Taiwan during the last stage of my studies, I could not have finished the "impossible" so quickly. Fu-Chen, I really appreciate all of the things you have done to make this possible.
VITA

October 25, 1969 ................................................... Born; Taipei, Taiwan

1992 ....................................................................... B.S. in Chemistry
National Taiwan University
Taipei, Taiwan

1994-present ......................................................... Graduate Teaching and Research Associate
The Ohio State University
Columbus, Ohio

PUBLICATIONS


FIELDS OF STUDY

Major Field: Chemistry
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>ii</td>
</tr>
<tr>
<td>Dedication</td>
<td>iv</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>v</td>
</tr>
<tr>
<td>Vita</td>
<td>vii</td>
</tr>
<tr>
<td>List of Tables</td>
<td>xi</td>
</tr>
<tr>
<td>List of Figures</td>
<td>xii</td>
</tr>
<tr>
<td>Chapters:</td>
<td></td>
</tr>
<tr>
<td>1 Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Structure of Carbon Surfaces</td>
<td>2</td>
</tr>
<tr>
<td>Surface Oxides</td>
<td>7</td>
</tr>
<tr>
<td>Basics of Electroanalytical Chemistry</td>
<td>9</td>
</tr>
<tr>
<td>Relationships between Structure and Reactivity of Carbon Electrodes</td>
<td>12</td>
</tr>
<tr>
<td>Microstructure</td>
<td>12</td>
</tr>
<tr>
<td>Electron Transfer Mechanism</td>
<td>14</td>
</tr>
<tr>
<td>Surface Properties</td>
<td>19</td>
</tr>
<tr>
<td>Treatments of Carbon Electrodes</td>
<td>21</td>
</tr>
<tr>
<td>Polishing</td>
<td>21</td>
</tr>
<tr>
<td>Electrochemical Pretreatment</td>
<td>24</td>
</tr>
<tr>
<td>Laser Activation</td>
<td>26</td>
</tr>
<tr>
<td>Vacuum Heat Treatment</td>
<td>28</td>
</tr>
<tr>
<td>Other Surface Treatments</td>
<td>29</td>
</tr>
<tr>
<td>Objectives</td>
<td>30</td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Electrode Materials and Polished Procedure</td>
<td>159</td>
</tr>
<tr>
<td>Specific Adsorption</td>
<td>159</td>
</tr>
<tr>
<td>Electrolysis Procedures</td>
<td>159</td>
</tr>
<tr>
<td>Results</td>
<td>160</td>
</tr>
<tr>
<td>Conventionally Polished GC</td>
<td>160</td>
</tr>
<tr>
<td>Cyclohexane Polished GC</td>
<td>166</td>
</tr>
<tr>
<td>Nitrophenyl Derivatized GC</td>
<td>166</td>
</tr>
<tr>
<td>Discussions</td>
<td>173</td>
</tr>
<tr>
<td>Appendix A Spectral Data for Diazonium Salts</td>
<td>182</td>
</tr>
<tr>
<td>List of References</td>
<td>199</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table                                           Page
1.1    X-ray diffraction parameters for some common carbon materials       2
1.2    Comparison of HOPG and GC-20 properties                               6
2.1    Voltammetric peak potentials and derivatized potentials vs. Ag/Ag\(^+\)  43
2.2    Ru(NH\(_3\))\(_6\)\(^{3-\cdot2\cdot}\) kinetics results on modified surfaces  47
2.3    A\(_p\) and k\(^0\) of chlorpromazine for different surfaces            66
2.4    A\(_p\) and k\(^0\) of promazine for different surfaces                  67
2.5    A\(_p\) and k\(^0\) of triflupromazine for different surfaces           68
2.6    Chlorpromazine kinetics results on modified surfaces                 77
3.1    E\(_{1/2}\), A\(_p\) and k\(^0\) of oxygen at different pH and scan rate on methylphenyl derivatized GC 107
3.2    Parameters for the simulation of the oxygen reduction                 121
3.3    Peak potential and peak current at different pH on polished, AC/IPA cleaned and methylphenyl modified GC 129
3.4    Peak potential, peak current, and peak ratio at different pH on polished and AC/IPA cleaned GC 130
3.5    Calculated \(\alpha\) from the plot of ln \([i(t)/(I_1 - I(t))]\) vs. E 147
4.1    Chemically modified electrodes for electrocatalytic oxidation of NADH 157
### LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Crystallographic dimensions of sp(^2) carbon</td>
<td>3</td>
</tr>
<tr>
<td>1.2</td>
<td>Schematic representations of (A) highly ordered pyrolytic graphite and (B) glass carbon</td>
<td>5</td>
</tr>
<tr>
<td>1.3</td>
<td>Possible oxygen containing groups on carbon surfaces</td>
<td>8</td>
</tr>
<tr>
<td>1.4</td>
<td>Schematic illustrations of (A) outer-sphere and (B) inner-sphere electron transfer reactions</td>
<td>16</td>
</tr>
<tr>
<td>1.5</td>
<td>Schematic illustration of a redox mediated process</td>
<td>18</td>
</tr>
<tr>
<td>1.6</td>
<td>Classification of redox systems into kinetic subgroups according to their responses to changes in surface variables</td>
<td>22</td>
</tr>
<tr>
<td>1.7</td>
<td>Schematic illustration classification of redox systems</td>
<td>23</td>
</tr>
<tr>
<td>2.1</td>
<td>Illustration of chemical modification procedures</td>
<td>35</td>
</tr>
<tr>
<td>2.2</td>
<td>Illustration of long distance electron transfer procedures</td>
<td>37</td>
</tr>
<tr>
<td>2.3</td>
<td>Comparison of voltammograms of 1 mM Ru(NH(_3))(_6)(^{3+\cdot2-}) on polished and chemisorbed surfaces</td>
<td>44</td>
</tr>
<tr>
<td>2.4</td>
<td>Schematic representation of reduction of methyl viologen</td>
<td>48</td>
</tr>
<tr>
<td>2.5</td>
<td>Cyclic voltammograms of 1 mM MV(^{2-}) on polished and chemisorbed surfaces</td>
<td>49</td>
</tr>
<tr>
<td>2.6</td>
<td>Schematic illustration of reduction of methylene blue</td>
<td>51</td>
</tr>
<tr>
<td>2.7</td>
<td>Voltammograms of 10 (\mu)M methylene blue on polished, AC/IPA cleaned, and chemisorbed surfaces</td>
<td>52</td>
</tr>
</tbody>
</table>
2.8 Voltammograms of 1 mM methylene blue on polished and chemisorbed surfaces .................. 53
2.9 Structures of chlorpromazine, promazine, and triflupromazine ................................. 55
2.10 Schematic illustration of oxidation of chlorpromazine .................................................. 56
2.11 Voltammograms of 1 mM CPZ on polished GC in the absence and presence of 40 % MeOH ................................................................. 57
2.12 Semi-integral voltammograms for 1 mM CPZ ............................................................ 58
2.13 Adsorption isotherms for CPZ .................................................................................... 60
2.14 Log ip vs. log v for 1 mM CPZ ....................................................................................... 61
2.15 Comparison of voltammograms of 1 mM CPZ on polished, physisorbed, and chemisorbed surfaces, 0.2 V/sec ................................................................. 62
2.16 Structures of AQDS, BMB, and MB ............................................................................ 69
2.17 Comparison of voltammograms of 1 mM CPZ on polished and chemisorbed surfaces, 20V/sec ........................................................................................................ 70
2.18 Comparison of voltammograms of 1 mM CPZ on polished and chemisorbed surfaces, 0.2 V/sec ................................................................................................. 73
2.19 Plot of ln (k°) vs. monolayer thickness (d, Å) of Ru(NH3)6³⁻/²⁻ .................................... 78
2.20 Plot of ln (k°) vs. monolayer thickness (d, Å) of CPZ .................................................. 79
2.21 Illustration of tunneling parameters of different spacers ............................................. 82
2.22 Schematic illustration of classification of redox systems ............................................. 87
3.1 Comparison of voltammograms of 1 mM O₂ at different pH on polished, AC/IPA cleaned and methylphenyl derivatized GC ......................................................... 99
3.2 Voltammograms of 3 mM H₂O₂ in 1 M KOH on polished and methylphenyl derivatized GC ......................................................................................................................... 102
3.3 ip vs. v¹/² for 1 mM O₂ in 1 M KOH on polished, AC/IPA cleaned and methylphenyl derivatized GC ................................................................. 104
<table>
<thead>
<tr>
<th>Section</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.4</td>
<td>Voltammograms of 1 mM O₂ in 1 M KOH at different scan rate on methylphenyl derivatized GC</td>
</tr>
<tr>
<td>3.5</td>
<td>Voltammograms of 1 mM O₂ at different pH on methylphenyl derivatized GC</td>
</tr>
<tr>
<td>3.6</td>
<td>pH dependence of peak currents and peak potentials of oxygen reduction on methylphenyl derivatized GC</td>
</tr>
<tr>
<td>3.7</td>
<td>$i_p$ vs. $v^{1/2}$ for 1 mM O₂ at pH 14 and pH 7 on methylphenyl derivatized GC</td>
</tr>
<tr>
<td>3.8</td>
<td>Schematic illustration of the mechanism of oxygen reduction on methylphenyl derivatized GC</td>
</tr>
<tr>
<td>3.9</td>
<td>Comparison of voltammograms from experiment and simulation of 1 mM O₂ at different pH on methylphenyl derivatized GC</td>
</tr>
<tr>
<td>3.10</td>
<td>Comparison of $i_p$ vs. $v^{1/2}$ from experiment and simulation for 1 mM O₂ at pH 14 on methylphenyl derivatized GC</td>
</tr>
<tr>
<td>3.11</td>
<td>Comparison of voltammograms from experiment and simulation of 1 mM O₂ at pH 14 on methylphenyl derivatized GC</td>
</tr>
<tr>
<td>3.12</td>
<td>Comparison of voltammograms of 1 mM O₂ at different pH on polished and AC/IPA cleaned GC</td>
</tr>
<tr>
<td>3.13</td>
<td>Voltammograms of 1 mM O₂ at different pH on polished GC</td>
</tr>
<tr>
<td>3.14</td>
<td>Voltammograms of 1 mM O₂ at different pH on AC/IPA cleaned GC</td>
</tr>
<tr>
<td>3.15</td>
<td>pH dependence of peak potentials of oxygen reduction on polished, AC/IPA cleaned, and methylphenyl derivatized GC</td>
</tr>
<tr>
<td>3.16</td>
<td>pH dependence of peak currents of oxygen reduction on polished and AC/IPA cleaned GC</td>
</tr>
<tr>
<td>3.17</td>
<td>Comparison of voltammograms of 1 mM O₂ at pH = 10.5 on polished and AC/IPA cleaned GC at different scan rate</td>
</tr>
<tr>
<td>3.18</td>
<td>Schematic illustration of the mechanism of oxygen reduction on bare GC</td>
</tr>
<tr>
<td>3.19</td>
<td>Comparison of semi-integrals of 1 mM O₂ at different pH on polished, AC/IPA cleaned, and methylphenyl derivatized GC</td>
</tr>
</tbody>
</table>
3.20 Comparison of $\ln \left[ \frac{i(t)}{I(t) - I(t)} \right]$ vs. $E$ for Figure 3.19 at different pH .............. 145

3.21 Comparison of $i_p$ vs. $v^{1/2}$ for 1 mM $O_2$ at pH 10.5 on polished and AC/IPA cleaned GC ................................................................. 149

4.1 Structure of $\beta$-nicotinamide-adenine dinucleotide (NAD$^+$) and its reduced form, NADH ................................................................. 153

4.2 Voltammograms of 1 mM NADH at different scan rate on polished GC. .......... 161

4.3 $i_p$ vs. $v^{1/2}$ for 1 mM NADH on polished GC and nitrophenyl derivatized GC ... 162

4.4 $E_p$ vs. log $v$ for 1 mM NADH on polished GC and nitrophenyl derivatized GC 163

4.5 Voltammogram of 1 mM NADH on polished GC ............................................. 164

4.6 UV spectra of electrolysis of 1 mM NADH on polished and nitrophenyl derivatized GC ................................................................................ 165

4.7 Voltammograms of 1 mM NADH at different scan rate on cyclohexane polished GC .................................................................................. 167

4.8 Voltammograms of 1 mM NADH at different scan rate on nitrophenyl derivatized GC ........................................................................... 168

4.9 Comparison of voltammograms of 1 mM NADH on polished and nitrophenyl derivatized GC .................................................................. 170

4.10 Comparison of voltammograms of 1 mM NADH on polished and nitrophenyl derivatized GC, longer range .......................................... 172

4.11 Ultraviolet absorption spectra data of NADH and its oxidation products ....... 177

4.12 Calculated bond distance of NADH and the intermediates produced during electrochemical oxidation ................................................. 179

4.13 Schematic illustration of the mechanism of NADH oxidation on bare GC and nitrophenyl modified GC ...................................................... 181

A1 $^1H$ NMR spectrum of phenyl diazonium fluoborate .................................. 184

A2 Mass spectrum of phenyl diazonium fluoborate ........................................ 185
A3 $^1$H NMR spectrum of methyl phenyl diazonium fluoborate ......................... 186
A4 Mass spectrum of methyl phenyl diazonium fluoborate .............................. 187
A5 $^1$H NMR spectrum of trifluoromethyl phenyl diazonium fluoborate .......... 188
A6 Mass spectrum of trifluoromethyl phenyl diazonium fluoborate ................. 189
A7 $^1$H NMR spectrum of carboxyl phenyl diazonium fluoborate ..................... 190
A8 Mass spectrum of carboxyl phenyl diazonium fluoborate .......................... 191
A9 $^1$H NMR spectrum of ethyl phenyl diazonium fluoborate .......................... 192
A10 Mass spectrum of ethyl phenyl diazonium fluoborate ................................ 193
A11 $^1$H NMR spectrum of biphenyl diazonium fluoborate .............................. 194
A12 Mass spectrum of biphenyl diazonium fluoborate .................................... 195
A13 $^1$H NMR spectrum of acetoaminophenyl diazonium fluoborate ................. 196
A14 Mass spectrum of acetoaminophenyl diazonium fluoborate ....................... 197
A15 Mass spectrum of butyl phenyl diazonium fluoborate .............................. 198
CHAPTER I

INTRODUCTION

Carbon electrode materials have been applied widely in many industries. The applications include electrosynthesis, fuel cell manufacturing, and electrochemical sensors [1-3]. However, the understanding of the relationship between the surface structure and the electrochemical reactivity is one of the most important limitations, because it plays an essential role in the energy conversion efficiency. Even though carbon electrodes are widely used, it is hard to determine their surface chemistry and the nature of existing surface oxides. It has been a goal of electrochemists to find the relationship between the structure and the reactivity of carbon electrode surfaces. However, carbon surfaces are hard to prepare consistently and are prone to degradation via oxidation and impurity adsorption. A reliable method providing reproducible surface structure is desirable in order to study the relationship between the surface structure and the surface reactivity. This work provides a better method of producing the carbon surface structure and a better understanding of the relationship between the surface structure and the electrode reactivity at carbon electrodes.
Structure of Carbon Surfaces

Carbon materials have diverse structural and chemical characteristics [1]. Diamond and graphite, the two most common forms, have sp³ and sp² hybridized carbon structures, respectively. However, due to the limited electrical conductivity of diamonds, they are generally not considered as an electrode material. The basic building blocks of sp² carbon material are hexagonal rings composed of six bonded carbon atoms as shown in Figure 1.1. The C-C bond distance in all these sp² materials is essentially the same, which is 1.42 Å with predicted but undetermined variations near the edges of graphite planes. The structure parameters which distinguish one type of carbon material from another are the interplanar distance d₀₀₂ and the microcrystallite size both in the plane of the hexagonal lattice L₀ and in the direction perpendicular to the carbon hexagons Lₐ. These parameters are usually determined by X-ray diffraction (Table 1.1) [1,4,5].

<table>
<thead>
<tr>
<th></th>
<th>L₀ (Å)</th>
<th>Lₐ (Å)</th>
<th>d₀₀₂ (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon black</td>
<td>24</td>
<td>12</td>
<td>3.5-3.6</td>
</tr>
<tr>
<td>HOPG</td>
<td>10,000</td>
<td>100,000</td>
<td>3.35</td>
</tr>
<tr>
<td>Tokai GC-10</td>
<td>20</td>
<td>10</td>
<td>3.49</td>
</tr>
<tr>
<td>Tokai GC-20</td>
<td>25</td>
<td>12</td>
<td>3.48</td>
</tr>
<tr>
<td>Tokai GC-30</td>
<td>55</td>
<td>70</td>
<td>3.41</td>
</tr>
<tr>
<td>Carbon fiber</td>
<td>&gt;100</td>
<td>40</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Table 1.1. X-ray diffraction parameters for some common carbon materials.
Figure 1.1. Crystallographic dimensions of sp² carbon. $L_a$, $L_c$, and $d_{002}$ vary with carbon type.
Among all the carbon materials, highly ordered pyrolytic graphite (HOPG) and glassy carbon (GC) are of most interest to the present studies. HOPG is made by annealing pyrolytic graphite at high temperature under high pressure (e.g. 5700-7200 psi, 3000 °C) [6]. It has the most extended network of six member carbon rings and is the closest approximation to the ideal single crystal graphite. Figure 1.2A is a schematic representation of the structure of HOPG [7]. Sizes of graphite crystals vary with the grades of HOPG and typical values for the best grade are 2 μm for Lₐ and 10 μm for Lₖ. Its density is close to the single crystal density of 2.26 g/cm³, and its microcrystalline domains are up to 10,000 Å [1]. As shown in Figure 1.2, there are two distinct plane faces on HOPG, basal plane (the plane parallel to the a-axis) and edge plane (the plane perpendicular to the a-axis, also called the c-axis). Examples of the anisotropic properties of HOPG include electrical resistivity Ω_c/Ω_a = 3800 and thermal conductivity K_a/K_c = 250 at room temperature (Table 1.2) [1,8,9].

In contrast to HOPG, GC exhibits an average microcrystallite size of ca. 25 Å (Table 1.1). It is formed by heating polymeric resins. Figure 1.2B is a model of GC structure [10], which is a very complex structure with twisted graphite ribbons, structurally very different from that of HOPG. The higher temperature at which the GC precursor is treated, the more ordered the resulting material. GC is generally graded according to the temperature at which its precursor is treated: GC-30 (3000 °C), GC-20 (2000 °C), GC-10 (1000 °C). As can be seen in Table 1.1, the crystal dimensions change significantly with the temperature treatment. It is clear that these dimension are much smaller that those of HOPG. As a result of structural differences, HOPG and GC have
Figure 1.2. Schematic representations of (A) highly ordered pyrolytic graphite and (B) glass carbon [10].
very different physical properties. Structural and physical properties for both HOPG and GC are listed in Table 1.2.

<table>
<thead>
<tr>
<th>Properties</th>
<th>HOPG</th>
<th>GC-20</th>
</tr>
</thead>
<tbody>
<tr>
<td>L₂ (Å)</td>
<td>&gt;10,000</td>
<td>~25</td>
</tr>
<tr>
<td>Lₖ (Å)</td>
<td>&gt;100,000</td>
<td>12</td>
</tr>
<tr>
<td>d₀₀₂ (Å)</td>
<td>3.354</td>
<td>3.48</td>
</tr>
<tr>
<td>Resistivity (Ω-cm)</td>
<td>4 × 10⁻⁵ (a-axis)</td>
<td>4.2 × 10⁻³</td>
</tr>
<tr>
<td></td>
<td>0.17 (c-axis)</td>
<td></td>
</tr>
<tr>
<td>Apparent density (g/cc)</td>
<td>2.26</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Table 1.2. Comparison of HOPG and GC-20 properties [1,8,9].

As a consequence of its layer structure, HOPG exhibits intercalation characteristics for molecules and ions. In contrast to HOPG, GC is a rigid material and is not permeable to gases and liquids [1]. HOPG is an anisotropic material, which means its basal planes and edge planes have totally different properties such as electric conductivity, thermal expansion, thermal conductivity, etc. And the differences sometimes can be as large as a factor of thousands. On the other hand, GC is isotropic once the sample is larger than hundreds of angstroms.
Surface Oxides

Graphitic carbon materials are reactive to a variety of molecules whenever a graphitic sheet terminates because of unsatisfied valances. These unsatisfied valances of the edge sites are especially reactive toward oxygen, leading to a variety of functional groups on the surfaces. The glassy carbon surfaces are expected to have a high density of surface oxides because of the high edge to basal ratio. Figure 1.3 is a representation of the possible oxygen containing functional groups that may be on the carbon surfaces. These functional groups, including carbonyl, hydroxyl, carboxyl, quinone, lactone, and carboxylic acid, are similar to those found in many organic molecules. They vary in concentration on the glassy carbon but are believed to have similar chemical properties to the organic compounds containing the same functional groups. It is well accepted that surface oxides on carbon surfaces contribute to the diversity of carbon surface chemistry.

Acid-base titration [11] is one of the earliest methods for characterizing the surface oxide distribution using wet chemical techniques. However, due to the detection limits inherent to the old methods, a large surface area of carbon is required. Thus, materials like glassy carbon with low surface areas can not be characterized by the classical methods. Recently, x-ray photoelectron spectroscopy (XPS) [12-16], Fourier transform infrared spectroscopy (FT-IR) [17,18] and Raman spectroscopy [19] have been applied to the surface characterization of many different carbon surfaces. XPS is a powerful technique providing the information not only of elemental compositions of the surfaces but also the oxidation states of the elements. Sherwood et al. have conducted extensive studies on modifying carbon fibers using XPS techniques and established
Figure 1.3. Possible oxygen containing groups on carbon surfaces.
methods for analyzing different surface oxide contents [20,21]. Collier et al. used XPS to determine the amount of surface hydroxyl groups and carbonyl groups on glassy carbon surfaces after specific derivatizations of these functional groups [22,23].

Vibrational spectroscopy has the advantage of getting information from the substrate and surface adsorbed species at the same time. Thus, it is possible to get data in situ. Recently, Yang et al. and Lin et al. observed the formation of surface oxides during electrochemical oxidation using FT-IR in situ [24-26]. In addition to FT-IR, Raman has become a powerful tool in elucidating surface functionality. Fryling et al. developed a surface carbonyl probe, dinitrophenyl hydrazine (DNPH), and detected the adduct by resonance Raman spectroscopy using a 488 nm laser [19]. Liu et al. determined the orientation of DNPH when it reacts with HOPG at the edge plane using Raman [27].

**Basics of Electroanalytical Chemistry**

The fundamental principles of the electrodes and the properties of electrode/solution interfaces behind the electroanalytical techniques are the same even though there have been many advances in the techniques themselves. The following is a summary of the basic concepts and equations which are related to this work. Detailed understanding of electroanalytical chemistry can be found in Bard and Faulkner’s book [7].

In this work, the electrochemical reactions are discussed in terms of thermodynamic and kinetic considerations on glassy carbon electrodes. The observed heterogeneous electron transfer rate constant \( k_{\text{obs}} \) is different from, but related to the
homogeneous electron transfer rate constant where electron transfer happens between two reactants in solution. Heterogeneous electron transfer rate between the electrode and the redox active species is one of the fundamental variables which determine the usefulness of an electrode. Better results and higher sensitivity in analytical applications usually can be achieved by a higher electron transfer rate.

A heterogeneous electron transfer reaction is shown in Equation 1.1:

$$\text{Ox} + n\text{e}^- \xrightleftharpoons[k_f]{k_b} \text{Red}$$

where Ox is the oxidized form of the redox couple and Red is the reduced form, n is the number of the electrons involved in the redox reaction, $k_f$ and $k_b$ are the rate constants for the forward and backward reaction, respectively. By analogy to the reaction rates for a first order reaction, the rates for the forward and backward heterogeneous electrode reaction are expressed as:

$$v_f = k_f C_{\text{Red}(0,t)} = \frac{i_a}{nF}$$

$$v_b = k_b C_{\text{Ox}(0,t)} = \frac{i_c}{nF}$$

where $v_f$ and $v_b$ are the forward and backward rates of the reaction respectively, $C_{\text{Ox}(0,t)}$ and $C_{\text{Red}(0,t)}$ are the surface concentration of the oxidized and reduced form of the analyte, $i_c$ and $i_a$ are the cathodic (reduction) current and anodic (oxidation) current at the
time t, F is the Faraday constant, and A is the electrode area.

Assuming that the rate constant can be expressed in an Arrhenius form yields:

\[ k_f = A_j e^{-\Delta G_c^0 / RT} = A_j e^{-\Delta G_a^0 / RT} e^{-\alpha_f n_f E} \]  
\[ k_b = A_b e^{-\Delta G_c^0 / RT} = A_b e^{-\Delta G_a^0 / RT} e^{(1-\alpha)\eta_f E} \]

(1.4)
\( (1.5) \)

where \( \Delta G_c^0 \) and \( \Delta G_a^0 \) are the cathodic and anodic activation energies at the potential \( E \) (with respect to a reference electrode, for example, NHE scale defines the standard reduction potential of hydrogen reduction to be zero), \( \Delta G_{0c}^0 \) and \( \Delta G_{0a}^0 \) are the cathodic and anodic activation energies at the potential of zero V, \( \alpha \) is the transfer coefficient which depends on the symmetry of the potential energy curve, and \( f = F/RT \).

Consider a special case in which an electrode/solution interface is at equilibrium with a solution in which the bulk concentration of the oxidized form and the reduced form is the same (\( C_{Ox}^* = C_{Red}^* \)). According to the Nernst equation, \( E \) equals \( E^\circ \) (formal potential on NHE scale). Also, since it is at equilibrium, forward rate equals backward rate (\( k_f C_{Ox}^* = k_b C_{Red}^* \)). Thus, \( k_f = k_b \). Here a parameter called standard rate constant \( k^0 \) is defined in the following way:

\[ k^0 = A_j e^{-\Delta G_c^0 / RT} e^{-\alpha_f n_f E^\circ} = A_b e^{-\Delta G_a^0 / RT} e^{(1-\alpha)\eta_f E^\circ} \]

(1.6)
The physical meaning of the standard rate constant is the value of the forward and the
backward rate constants at formal potential ($E^0$) when the bulk concentrations of the
oxidized and the reduced forms are equal.

$k^0$ is an important electrochemical parameter in this study. It is used to determine
the reactivity of carbon electrodes. If Equations 1.2, 1.3, and 1.6 are combined, the
following equation is obtained for the total current flowing through the electrochemical
cell as a function of applied potential:

\[ i = i_c - i_a = nFA[k_f C_{ox}(o,t) - k_b C_{red}(o,t)] \]

\[ = nFAk^0 [C_{ox}(o,t)e^{-(\alpha\eta E - E^0)} - C_{red}(o,t)e^{(1 - \alpha)\eta (E - E^0)'}] \]  \hspace{1cm} (1.7)

where $i_c$ is the total current. This equation is called the Butler-Volmer equation.

**Relationships between Structure and Reactivity of Carbon Electrodes**

**Microstructure**

Bulk microstructure was discussed in the previous section, especially with respect
to differences in ordered (HOPG) versus disordered (GC) materials. Because HOPG has
the largest crystalline size and relatively well defined basal and edge graphite planes, it
has served as a model material for studying the relationship between the microstructure of
carbon materials and the reactivity of the electrode [28, 29]. In addition to having a well-
defined microstructure, HOPG samples possess low microscopic roughness and low
amounts of both chemisorbed and physisorbed impurities [30,31] which make control of the HOPG surface characteristics better than other types of carbon electrodes.

One interesting property of HOPG is its electrochemical anisotropy at basal planes and edge planes. Early work on HOPG indicated that electron transfer rates were lower on the basal plane than on the edge plane [32]. In later studies, the deliberate creation of defects at the basal plane surface by laser activation or electrochemical pretreatment (ECP) resulted in an increase in the $\text{Fe(CN)}_6^{3/-4}$ electron transfer rate constant [28]. The presence of edge plane regions for these treated surfaces was verified by Raman spectroscopy and scanning electron microscopy [28,33]. Besides the difference in the reactivity, basal planes also show lower capacitance than edge planes [28]. This has been attributed to a lower density of electronic states (the number of energy levels available to electrons in a unit energy level in a unit volume, DOS) near the Fermi level of HOPG basal planes. Microstructure studies on carbon electrodes using scanning tunneling microscopy (STM) [31] also concluded that edge defect regions have higher DOS at Fermi level than perfect basal plane regions and low electrode reactivity is believed to be attributed to low DOS.

Additional comparison of the adsorption of anthraquinone 2,6-disulfonate (AQDS) for edge and basal planes has been done [31,34]. AQDS adsorbs on and near graphite edge planes but not on basal planes, and after 1% of edge defects are created on a perfect HOPG basal plane, about 30% of a monolayer coverage of AQDS adsorption is produced. This result is attributed to the electronic disturbance caused by an edge step defect on a HOPG basal plane, which is a much larger area than the defect itself.
From Figure 1.2B, it is reasonable to suppose that GC has a higher electron transfer activity than HOPG because of a higher percentage of edge sites on a GC surface. So far the studies done by Kneten et al. agree with this statement [35]. However, the complexity of the GC micro structure in comparison with that of HOPG indicates that though the model works for HOPG, it may be too simplified. The models proposed for carbon electrodes depend on how electron transfer kinetics or thermodynamics of the redox systems respond to the changes of carbon surfaces. It is commonly believed that carbon surfaces may be involved in several different ways and the redox systems can be divided into classifications such as outer-sphere, inner-sphere, redox mediation, etc. according to the mechanisms involved in electron transfer processes [36].

**Electron Transfer Mechanisms**

Electrical double layer, the structure of a solid electrode and solution interface, is needed to be considered before the electron transfer mechanisms are studied. There are several different “layers” on the solution side of a double layer. The closest layer to the electrode contains solvent molecules and specifically adsorbed ions and molecules. The locus of the electric center of this layer is called the inner Helmholtz plane (IHP). The next layer consists of solvated ions which have charges opposite to the specifically adsorbed species on the IHP and the locus of its electrical center is called outer Helmholtz plane (OHP). The region in between the OHP and the bulk solution is called the diffusion layer.
An outer-sphere redox reaction involves the simplest electron transfer mechanism. There is no specific adsorption of electroactive species on the electrode surfaces and the closest place a redox particle can reach to the electrode is the OHP. The electrode acts simply like a sink of electrons. The interaction between the redox species and the electrode is long range, and primarily independent of their chemical properties. In other words, for outer-sphere systems, surface chemistry of electrodes are not important.

As for the case of an inner-sphere redox system, the redox species penetrate to the IHP and is in direct contact with the electrode surfaces. The penetration is caused by the specific interactions between the electroactive species and the active sites or functional groups on the electrode surfaces. Before electron transfer occurs, surface complexes may form and the redox products dissociate from the electrodes into the solution in the final step. Figure 1.4 illustrates the differences between outer-sphere and inner-sphere systems.

In addition to the difference in the location where the redox reactions take place for outer-sphere and inner-sphere redox systems, the rate constant is also different because of the change in the free energy. As indicated in Equation 1.6, the standard rate constant increases exponentially with a decrease in activation free energy. Most of the studies are done for homogeneous electron transfer reactions in order to understand the free energy barriers [37-39]. However, heterogeneous electron transfer processes are similar to the homogeneous electron transfer processes, thus many conclusions of homogeneous processes could be extended to heterogeneous processes. For an outer-sphere electron transfer process, part of the free energy barrier is called reorganization
Figure 1.4. Schematic illustrations of (A) outer-sphere and (B) inner-sphere electron transfer reactions.
energy, the free energy required to change the atomic position of the reactant (\(\Delta G_{\text{in}}^*\), inner-shell reorganization energy) and its solvation shell to the atomic position of the product (\(\Delta G_{\text{out}}^*\), outer-shell reorganization energy) and its solvation shell without allowing the electron to transfer. Marcus [39] and Hush [37] related \(\Delta G_{\text{out}}^*\) to the distance (d) between the centers of the two reactants and concluded that the larger the distance is, the larger \(\Delta G_{\text{out}}^*\) is. For heterogeneous electron transfer, this distance is between the center of the redox species and the electrode surface. Additionally, it is clear that this distance is smaller in the inner-sphere redox systems than it is in the outer-sphere redox systems. Thus, it is logical to say that the free energy barrier of a reaction is smaller for an inner-sphere electron transfer reaction compared to the same process reacting by an outer-sphere electron transfer reaction because of an interaction with the electrode, such as adsorption, lower the energy barrier. Therefore when the electron transfer rate is faster than the theoretically calculated values with the assumption of an outer-sphere reaction process, the existence of an inner-sphere electron transfer mechanism could be indicated.

Another common mechanism proposed for an electrode reaction is redox mediation. Figure 1.5 is a schematic illustration of a mediated electron transfer reaction. In this type of reaction, a second electroactive couple exchanges charge heterogeneously with the electrode, and this couple exchanges charge homogeneously with the redox couple of interest so that the redox couple of interest does not undergo direct electron exchange with an electrode. So the nature of the surface redox couple controls the potential at which this series of steps occur, not the one in solution. In order to have mediated reduction, the formal potential of the mediator should be more positive than that.
Figure 1.5. Schematic illustration of a redox mediated process.
of the analyte and in order to mediate oxidation, the formal potential of the mediator should be more negative than that of the analyte. Many electrochemical measurements of biological macromolecules and enzyme-based biosensors are based on the redox mediation mechanism [40,41]. Evans et al. proposed that surface quinoidal-like functional groups mediated the redox reaction of ascorbic acid and \(\beta\)-nicotinamide adenine dinucleotide (NADH) [42]. Mohan et al. also reported electrochemically generated quinone/hydroquinone mediating the electron transfer for ferrocene, ascorbic acid, and ferrocyanide [43].

**Surface Properties**

Because electrochemical reactions occur at the electrode surface, carbon surface variables contribute significantly to the electron transfer rate constant. It is well known that the different response to carbon surface conditions occurs for different redox systems. \(\text{Ru(NH}_3\text{)}^3\text{\textsuperscript{2-}}\) and \(\text{IrCl}_6\text{\textsuperscript{4-3-}}\) are relatively insensitive to the history of carbon electrodes and their electron transfer rate constants are not greatly affected by electrode preparation and pretreatments [44]. However, electron transfer rate constants of redox systems such as ascorbic acid, dopamine, ferrocyanide, etc. are largely decided by the way in which carbon electrodes are prepared and the environment experienced by these electrodes after preparation [45,46].

Specific chemical properties of carbon electrode surfaces also show a relationship with the electron transfer rate constant of redox systems. As mentioned before, Evans et al. suggested the involvement of quinoidal like functionalities in the electron transfer
reaction of NADH and that ascorbic acid behaved as redox mediators [42]. Also Cabaniss et al. proposed that phenolic like groups on carbon electrode surfaces are involved in proton coupled electron transfer thus increasing the electrode reaction rates for redox systems whose proton content changes upon oxidation [47]. McDermott et al. proposed a possible inner-sphere reaction mechanism for aquated metal ions such as Fe$^{3+}$-$2e^-$, V$^{3+}$-$2e^-$, and Eu$^{3+}$-$2e^-$ and related electron transfer kinetics of these systems to the amount of surface carbonyl groups on carbon electrode surfaces [48].

Recently, Chen et al. developed a systematic method to study the relationship between the surface structure and the electron transfer kinetics of redox systems [36]. Figures 1.6 and 1.7 are the illustrations of the method. First, redox systems are classified into two different groups, “surface sensitive” and “surface insensitive” by comparing the electron transfer rate on polished GC and monolayer covered GC. If, for a particular redox system, there are certain functional groups or certain sites on a polished electrode surface involved in the electron transfer process, the reaction rate for this redox system would be slower on a monolayer modified surface because the possible active sites on the surface are covered by the surface monolayers. In other words, with this type of modified surfaces, it is possible to distinguish an inner-sphere electrode reaction from an outer-sphere one. If a surface site is involved in the reaction, the second step is to find out whether oxide groups on carbon surfaces are involved in the surface sensitive reactions. If the electron transfer rate of a redox system is slower on an oxide-free surface, then the surface oxides is responsible for catalyzing this particular electron transfer reaction. If surface oxides are involved, the last step is to identify which surface oxide group is
responsible by modifying particular oxide groups. For example, surface carbonyl are
derivatized by a specific chemical reagent. After this reaction, carbonyl groups do not
exist on the surface and are not available. Thus, the reaction rate of those redox systems
catalyzed by carbonyl groups, the reaction rates should be slower on this surface.

Treatments of Carbon Electrodes

As mentioned before, it is hard to reproduce two carbon electrodes with the same
surface structure unless certain surface pretreatment procedures are applied. Carbon
electrodes generally require some surface pretreatment procedures before acceptable
voltammetric results may be obtained. These treatment procedures include polishing with
alumina slurries, electrochemical pretreatment, laser activation, and vacuum heat
treatment (VHT), etc.

Polishing

Polishing is a common preparation procedure for both metal and carbon
electrodes because this procedure is easy, inexpensive, and serves to eliminate any effects
of the electrode history. However, polished surfaces are dependent on the procedure and
the resulting surfaces yield large variations in electron transfer rate constants for some
redox systems [1]. Currently, GC surfaces are commonly prepared for electrochemical
experiments by what will be referred to as “conventional” polishing. This procedure
begins with a fine grit silicon carbide paper then utilizes successively finer sizes of
alumina on a polishing cloth. Polished GC is considered a structurally ill-defined
Figure 1.6. Classification of redox systems into kinetic subgroups according to their responses to changes in surface variables.
Figure 1.7. Schematic illustration classification of redox systems.
material and can contain a layer of polishing debris such as residual polishing material and carbon microparticles. Recently, Ranganathan et al. demonstrated that sonicating electrodes in an isopropanol solution in the presence of activated carbon changes the electron transfer rate constants for some redox systems [49]. A larger electron transfer rate constant for Fe(CN)$_{3-}^{+}$ and slower electron transfer rate constant for Fe$^{3+/2+}$ were obtained. A higher electron transfer rate constant is achieved because the surface itself is active and it is deactivated by physisorbed impurities. On the other hand, lower electron transfer rate is achieved because physisorbed impurities provide a more active electrode toward electron transfer. This result is in agreement with the model that physisorbed impurities exist on polished GC surfaces. Thus, polished surfaces does not serve well for fundamental research on finding electron transfer rate constants. It does, however, provide a common starting point for the effects of surface modification on electron transfer rates.

**Electrochemical Pretreatment**

Electrochemical pretreatment (ECP) is normally performed on an electrode immediately before an experiment. It involves applying a potential waveform to the carbon electrode in a solution. A variety of potential waveforms and solution conditions have been used to activate GC [47,50-53], HOPG [28,54,55] and carbon fiber electrodes [29]. Electron transfer rates for some redox systems are increased after ECP on all types of carbon electrodes studied. However, the degree of activation is largely dependent on what potential is applied and how long it is applied. It is generally believed that ECP
activates electrodes by removing contaminants from the surfaces, increasing the amount of oxygen containing functional groups, increasing electrode surface area by roughening and creating new graphite edge sites on the surfaces, etc [56].

Engstrom showed that GC electrodes can be activated or deactivated for different redox systems if a positive potential, sometimes followed by a negative potential is applied in a potassium nitrate solution containing electroactive species [50]. Hydroquinone was activated by applying a 1.5 V potential for 5 minutes and was deactivated by following with a negative potential of -1.5 V. Ferrocyanide was deactivated by the positive potential and activated by the successive negative potential. Bowling et al. reported that an ECP HOPG electrode at a potential larger than 1.8 V in 0.1 M KNO₃ increases electron transfer rate for both ferrocyanide and dopamine [28]. Wightman et al. found that the electron transfer rate for ascorbic acid and ferrocyanide are greatly improved as a result of ECP for carbon fibers [29].

Studies have been carried out to obtain information about the nature of changes which are caused by ECP and what is responsible for the changes of electrochemical properties of redox systems. A couple of conclusions have been drawn for GC. First, surface oxygen containing groups, including alcohols, ethers, carbonyls, and carboxylic acids are formed [47]. Second, Raman results show a change in microstructure after ECP [48]. Third, adsorbed impurities are removed during ECP [57]. Finally, the surface is more wettable by water after ECP [57].
Some of these conclusions can not be applied to HOPG. For example, ECP on HOPG probably does not involve removal of surface impurities, because HOPG basal planes are inert toward impurity adsorption. An anodized HOPG surface has been characterized by Raman spectroscopy \([28]\), STM \([53]\), and AFM \([54]\). These methods agree that ECP increases the fraction of edge sites on the HOPG basal plane. The formation of surface oxides during ECP procedures strains the graphite lattice which produces fracturing. Delimitation was also proposed and the few original defects may act as nuclei for the delimitation and fracturing of nearby graphite planes \([28]\). Although ECP also increases the amount of surface oxides on the HOPG basal plane, it is believed that the enhancement of the electron transfer rate is due to the increased percentage of edge sites.

In summary, ECP is a simple and powerful in situ electrode pretreatment technique which activates carbon electrodes for many redox systems. However, the activation mechanism for ECP is not completely understood and the choice of ECP potential waveforms and solution conditions remain largely empirical because of the very complex surface, poorly characterized regarding structure that ECP procedures introduce. Although ECP activates many redox systems, there are no general rules regarding what type of redox systems will benefit from ECP.

**Laser Activation**

Laser radiation has been used to both retard and initiate corrosion on metal surfaces \([58,59]\). Hershenhard *et al.* first used a pulsed second harmonic of a Nd:YAG
laser at 532 nm to treat GC electrodes [60]. They observed laser activation removes
passivating phenyl polymeric films on the surfaces. Later on, the fundamental beam of a
Nd:YAG laser at 1064 nm was used to activate carbon electrodes instead [30,61,62]. The
laser can cause surface heating, desorption, local melting and vaporization depending on
wavelength, power density, and number of pulses. At HOPG, using a laser power density
lower than 45 MW/cm², the electron transfer rate constants of ferrocyanide and dopamine
were not activated. When the laser power density was larger than 45 MW/cm²,
differential capacitance, AQDS adsorption and electron transfer rate constants for
ferrocyanide and dopamine have been reported to increase after laser activation [30,34].
After laser activation, the amount of edge sites on HOPG basal planes increases. Raman
spectroscopy [30], scanning electron spectroscopy (SEM) and STM [34] studies show
that laser power density larger than 45 MW/cm² cause significant and uniform damages to
graphite lattice.

In contrast, laser power density of 25 MW/cm² is large enough to activate GC
electrodes for ferrocyanide, dopamine, and ascorbic acid, etc [62]. STM [63] and SEM
[64,65] studies have demonstrated that laser activation of GC at 25 MW/cm² does not
alter the surface morphology. Adsorption coverage of phenanthrenequinone increases
about 50 % and the differential capacitance increases [62]. These increases are
considerably smaller than the corresponding increases in the electron transfer rate
constants, indicating that the laser activates the surface by some other means than through
roughening. Therefore, the activation mechanism of GC by laser pulses may be related to
surface cleaning.
Vacuum Heat Treatment

Vacuum heat treatment has been used on GC, carbon fibers, and graphite [21,66-69]. Temperatures utilized ranged from 550 - 750 °C and pressures range from 1 torr to $10^{-6}$ torr or less. The properties of resulting surfaces are dependent on the vacuum condition and the final heating temperature.

Characterization of heat treated surfaces with optical microscopy and SEM indicates that the layer of particles present after polishing is removed in the process of the heat treatment [70], however heat treated surfaces appear similar to polished surfaces in terms of roughness [29,69]. Electrode properties vary with changing heat treatment conditions. Heat treatment is speculated to desorb physisorbed impurities from the electrode surfaces [67], however the amount of chemisorbed surface oxides depends on the heat treatment conditions.

The species desorbed from carbon surfaces into gases during heat treatment under ultrahigh vacuum conditions have been investigated with thermal desorption mass analysis and they were related to functional groups on the surfaces prior to the heat treatments [66,68]. The desorbed gases are mainly composed of CO and CO$_2$, resulting from decomposition of surface oxygen-carbon complexes. The ratio of CO and CO$_2$ along the temperature axis was used to decide the nature of surface oxides from which they evolved.

Studies using different vacuum conditions indicate that pressures lower than $10^{-6}$ torr showed a decreased background double layer charge, while a pressure lower than 1 torr caused an increase in residual current [67,69,71]. The increases in residual current
may be indicative of electrode roughening or faradaic processes from surface oxides formed during the heat treatment under low vacuum conditions. XPS results also indicate that heat treatment under low vacuum conditions produces an increase in surface oxygen while heat treatment under high vacuum conditions yields in a decreased O/C ratio [67]. These results indicate that increased amount of surface oxides occur with treatment at low vacuum while decreased amounts of surface oxides occur under high vacuum conditions.

Heat treated GC electrodes under both low vacuum and high vacuum have been reported to have enhanced electrode reactivity for redox systems such as ferrocyanide and ascorbic acid. The activation mechanism of vacuum heat treatment is believed to be attributed to the changes in chemisorbed species and physisorbed species.

**Other Surface Treatments**

Many other surface treatments have been employed to optimized carbon electrodes. These surface treatments include radio frequency plasma [42,72,73], microwave plasma [74], hot filament [75], chemical and electrochemical induced surface bonding of various species [76-78]. Miller *et al.* applied radio frequency plasma of water, carbon dioxide, oxygen, ammonium, and ethylenediamine on both pyrolytic graphite and GC-20 surfaces. These treated surfaces show corrosion caused by plasma under SEM [72]. O/C ratios increased on the O₂ and H₂O plasma treated surfaces. Conversely, the O/C ratio decreased but N/C ratio increased on the NH₃ and ethylenediamine plasma treated surfaces. Xie *et al.* used an air microwave plasma to treat carbon fibers and found that the amount of the surface oxides increased after plasma treatment [74]. In addition to
the formation of oxides on the surface, nitrogen containing groups are also formed during air microwave plasma treatment. Recently, Kuo et al. used a hot filament technique to generate hydrogen radicals from a flow of hydrogen gas, and then the radicals attacked glassy carbon electrode surfaces. Gas-phase modification of glassy carbon (GC) was investigated in an attempt to make a C-H-terminated surface that is resistant to oxidation [75]. Fryling et al. developed DNPH as a surface carbonyl probe by chemical induced surface bonding [19]. There are many examples of using electrochemical methods to covalently bond chemical species to carbon surfaces. For instance, Saveant et al. electrochemically reduced various diazonium salts to the corresponding aryl radicals which covalently bond to GC in acetonitrile [77]. This reaction is discussed extensively in chapter 2.

Objectives

As described in the previous sections, there are many types of carbon electrode materials and a variety of electrode treatments available. Without the knowledge of the effects of carbon microstructure and surface chemistry on electrode reactivity, it is a difficult task for researchers to choose the right electrode for a particular application. Many studies have been done on the relationship between the microstructure and reactivity of carbon electrodes. However, the majority of the systems are inorganic redox systems.
The overall goal of this work is to gain further insight into structure-reactivity relations for carbon electrodes. This work examined a wide range of organic redox systems by using the systematic method Chen et al. developed [36]. Questions to be addressed include: (1) Is carbon surface chemistry important to all electron transfer processes and if not, which redox couples are sensitive to carbon surface chemistry? (2) Does surface modifications change the redox mechanism? (3) If the answer to question (2) is yes, then, how and why does the mechanism change?

To get these answers, various surface modifications have been used to control GC parameters. Several organic redox systems were examined on the modified electrodes and were classified into groups according to their response to the changes in the surface parameters. Chlorpromazine was studied extensively, and several other organic redox systems including methyl viologen, methylene blue, and derivatives of chlorpromazine were examined in less detail. The purpose is to test several organic redox systems and to draw some conclusions about the relationship between the structure of carbon surfaces and the reactivity toward different redox systems.

Chapter 2 deals with electron transfer kinetics and adsorption on GC. Several organic redox systems were explored to ascertain the effects of surface modifications on electron transfer kinetics and adsorption of phenothiazines and methyl viologen. Chapter 3 addresses the reduction of oxygen on nature and modified carbon electrodes in order to assess the importance of surface sites and oxygen chemisorption to the reduction mechanism. Finally, chapter 4 discusses the effects of surface modifications on the oxidation of dihydronicotinamide adenine dinucleotide (NADH).
CHAPTER 2

EFFECTS OF SURFACE MOMOLAYERS ON THE KINETICS OF VIOLOGEN AND PHENOTHIAZINE ELECTRON TRANSFER ON GLASSY CARBON ELECTRODES

Introduction

Through a variety of efforts from many laboratories, significant progress has been made toward understanding the electrochemical behavior of widely used carbon electrodes [1, 8, 79-83]. Since sp² carbon surfaces are difficult to prepare reproducibly and are prone to degradation via oxidation and impurity adsorption, our understanding of carbon electrode behavior has lagged that of metal electrodes, particularly mercury. This situation improved dramatically after more attention was paid to surface preparation, and the number of uncontrolled surface variables was reduced. In particular, several landmarks indicating reproducible performance of sp² carbon electrodes, mainly glassy carbon (GC), have been achieved:

1. Determination of the rapid heterogeneous electron transfer rate constant ($k^0$), for outer-sphere systems (e.g. Ru(NH₃)₆³⁺/²⁺, $k^0 > 0.2$ cm/sec) [36], comparable to those observed on Au and Pt [84].
2. Preparation of low oxide (O/C < 2 %) carbon surfaces which retain their low oxide levels for at least one month in air [67,75].

3. Structure characterization of organic monolayers and submonolayers on carbon with Raman spectroscopy [27,85,86].

4. Correlation of specific surface sites with electrocatalytic activity for various redox systems, including ascorbic acid, NADH, Fe^{3+/2+}, etc [36,87-89].

5. Systematic classification of redox systems according to their sensitivity to surface chemistry. Classes include outer-sphere systems (e.g. Ru(NH$_3$)$_6^{3+/2+}$, Co(en)$_3^{3+/2+}$, etc.), systems catalyzed by specific surface oxides (Fe$_{aq}^{3+/2+}$, V$_{aq}^{3+/2+}$, Eu$_{aq}^{3+/2+}$), and systems requiring a non-oxide surface site (Fe(CN)$_6^{3/-4+}$, ascorbate) [36,90].

With reproducible carbon electrodes in hand, it is possible to systematically examine the surface structure factors which control electron transfer reactivity, adsorption, capacitance, etc. In the experiments Chen and McCreery reported, several electrode modifications were used to classify a collection of mainly inorganic redox systems according to their sensitivity to surface structure [36]. Outer-sphere systems such as Ru(NH$_3$)$_6^{3+/2+}$ showed minor kinetic effects of surface modifications, even when a compact organic monolayer was chemisorbed to the surface before kinetic measurements. In contrast, rate constants for Fe$^{3+/2+}$ and related systems were dramatically affected by surface preparation, due to electrocatalysis by surface carbonyl groups [36]. For these systems, the outer-sphere rate in the absence of carbonyl groups was comparable to that
observed on metals, and carbonyl groups increased the observed inner-sphere rate by electrocatalysis as noted in Chapter 1.

While a framework relating surface modifications to kinetic effects on GC is quite useful for the about 15 redox systems classified to date, its generality is unknown. Ascorbic acid was the only organic redox system examined under the same procedure, and organic redox reactions are generally more complex, sometimes involving more than one electron, one or more protons, etc. The current work was undertaken to identify carbon surface structural effects on electron transfer kinetics for several organic redox systems, some with biological importance. Chlorpromazine, promazine, triflupromazine, methylene blue, and methyl viologen were subjected to the systematic analysis used previously for inorganic redox reactions.

This work extends the approach to the case of a covalently bonded monolayer to varies aryl group with different thickness. The surface modification is based on reductive formation of aryl radicals from diazonium derivatives, following by covalent binding to the carbon surface (Figure 2.1):

$$RC_6H_4N_2^- + e^- \rightarrow N_2 + RC_6H_4\cdot$$ (2-1)

$$RC_6H_4\cdot + GC \rightarrow GC-C_6H_4R$$ (2-2)

Saveant et al. have reported that the reduction of diazonium salts leads to a monolayer film of aryl groups on carbon, and that binding of aryl radical is nonselective for basal or
Figure 2.1. Illustration of chemical modification procedures.
edge plane carbon sites [78]. Furthermore, the functional groups on the surfaces have similar chemical properties to those found in organic molecules. Thus, once the aryl groups attached to the carbon surface, the functionalized aromatic groups could be modified by classical chemical reactions. For example, the 4-nitrophenyl group on the derivatized surfaces could be reduced electrochemically (in 10/90 EtOH/H₂O + 0.1 M KCl), by an irreversible 6 e⁻ process to a 4-aminophenyl group [78,86]. This derivatization method is an important tool in the design of carbon composite materials and in the field of sensors and aerospace industry [91].

When an electron transfer occurs between two species separated by a distance significantly exceeding the sum of their van der Waals radii, it is called long-range electron transfer. It was observed on thermal, photo-induced, and heterogeneous electron transfer [92-96]. The relationship between the observed rate constant \( k_{\text{app}}^0 \) and the distance \( d \) can be expressed as:

\[
k_{\text{app}}^0 = k^0 \exp (-\beta d)
\]  

(2-3)

where \( d \) is the distance in Å, and \( \beta \) is the tunneling constant. As illustrated in Figure 2.2, the plot of \( \ln (k^0) \) vs. \( d \) can be used to address the issue of long range electron transfer.

Structurally well-defined organic monolayers on solid surfaces allow researchers to simplify and model a larger variety of interfacial phenomena that are difficult to study at nature interfaces due to the poorly defined structure. Organic thios on gold have been explored extensively for the past years [92-95]. However, most of the organic
Figure 2.2. Illustration of long distance electron transfer procedures.
monolayers used were aliphatic and redox system terminated thios. Thus, the redox systems were bound to the electrode. Furthermore, little emphasis has been placed on the modification with different thickness of monolayers on carbon. In this chapter, carbon electrodes were modified with monolayers of various thickness and were used to study the effects of the modifications on the electron transfer rate of redox systems in solution. The results of this work reveal correlations between redox mechanism and surface effects. In addition, they also provide unexpected new information on the effects of surface structure on reactant adsorption.

**Experimental**

**Reagents**

Tetrabutylammonium tetrafluoroborate (NBu₄BF₄), 50 % fluoboric acid, disoldium 2,6-anthraquinonedisulfonate (AQDS), methylene blue, 1,4-bis(2-methylstyryl) benzene (BMB). aniline, p-toluidine, 4-ethylaniline, 4-(trifluoromethyl)aniline, 4-nitroaniline, 4-aminobiphenyl, and disperse orange 3 (4-nitro, 4'-amino azobenzene) were purchased from Aldrich Chemical Company and were used as received except AQDS was recrystallized from water. Chlorpromazine (CPZ), promazine (PMZ), triflupromazine (TPZ), methyl viologen, and sodium nitrite were purchased from Sigma Chemical Company and were used as received. Ether and cyclohexane were purchased from J T Baker and were used as received. Methylene blue and methyl viologen occur as chloride salts, and will be referred to herein as MB⁺ and MV²⁺ in solution.
Synthesis of Diazonium Tetrafluoroborate Salts

Diazonium tetrafluoroborate salts were synthesized according to the procedure described by Starkey et al. [97]. Primary aromatic amines on reaction with acids (e.g. fluoboric acid, or nitrous acid, etc.) at about 0 °C yield diazonium salts. The diazonium salts similarly derived from aliphatic primary amines decomposed readily even at this temperature to yield the corresponding alcohol and other products with the evolution of nitrogen.

\[
\text{ArNH}_2 + \text{NaNO}_2 + 2 \text{HBF}_4 \rightarrow \text{ArN}^+ \text{BF}_4^- + \text{NaBF}_4 + 2\text{H}_2\text{O}
\]

(2-4)

The acidified nitrite solution provides a source of nitrosonium ion (I) which electrophilically replaces the hydrogen in the primary amino group to form the N-nitroso derivative (II). This has a tautomeric structure, the hydroxydiazo form (III) yielding the diazonium ion (IV) under acidic condition.

\[
\begin{align*}
\text{H}^+ &\text{ArNH}_2 + \text{N}^=\text{O} &\rightarrow &\text{ArN}^+ \text{N}=\text{O} &\rightarrow &\text{H}^+ \text{ArN}^+\text{N}=\text{O} = \text{OH} \\
\text{(I)} & & &\text{(II)} & & &\text{(III)} \\
\text{H}^+ &\text{ArN}^+\text{N}=\text{O} &\rightarrow &\text{ArN}^+\text{N}=\text{N} &\rightarrow &\text{H}^+ \text{ArN}^+\text{N}=\text{N} + \text{H}_2\text{O} \\
\text{(IV)} & & & & &
\end{align*}
\]
The experimental conditions necessary for the preparation of diazonium salts are as follows. 0.1 mole of the corresponding amine precursor was dissolved in 44 ml of 50% fluoboric acid. The solution was placed in an ice bath and the solution was stirred with an efficient stirrer, then a cold solution of 0.1 mole of sodium nitrite in 14 ml of water was added dropwise. When the addition was complete, the mixture was stirred for several more minutes and then suction filtered on a sintered glass filter. The solid diazonium tetrafluoroborate was washed with cold fluoboric acid, ethanol, and ether. If necessary, the product was recrystallized in acetonitrile and ether. The products, with their NMR and mass spectroscopy data, are as follows:

Benzenediazonium fluoborate, NMR (d₆-DMSO, 250 MHz): δ=8.00, 8.27, 8.86 (m, 5H), MS (FAB) calcd for C₆H₅N₂ m/z 105.12, found m/z 104.98 (M-BF₄)⁻.

4-Methylbenzenediazonium fluoborate, NMR (d₆-DMSO, 250 MHz): δ=2.59 (s, 3H), 7.81, 8.56 (d, 4H, J = 8.45 Hz), MS (FAB) calcd for C₇H₇N₁ m/z 119.15, found m/z 119.07 (M-BF₄)⁻.

Trifluoromethylbenzenediazonium fluoborate, NMR (d₆-DMSO, 250 MHz): δ=8.30, 8.92 (d, 4H, J = 8.89 Hz), MS (FAB) calcd for C₇H₄N₂F₃ m/z 173.12, found m/z 173.05 (M-BF₄)⁻.

Ethylbenzenediazonium fluoborate, NMR (d₆-DMSO, 250 MHz): δ=1.25 (t, 3H, J = 7.5 Hz), 2.88 (q, 2H, J = 7.5 Hz), 7.85, 8.58 (d, 4H, J = 8.6 Hz), MS (FAB) calcd for C₈H₁₀N₂ m/z 133.17, found m/z 133.36 (M-BF₄)⁻.

Phenylbenzenediazonium fluoborate, NMR (d₆-DMSO, 250 MHz): δ=8.33, 8.74 (d, 4H, J = 8.81 Hz), δ=7.69 (m, 5H), MS (FAB) calcd for C₁₂H₉N₂ m/z 181.22, found
m/z 181.09 (M-BF₃)^+. Nitroazobenzene diazonium fluoborate, MS (FAB) calcd for C₁₂H₈N₃O m/z 254.3, found m/z 254.11 (M-BF₃)^+.

**Electrode Materials and Polished Procedure**

Commercial glassy carbon (GC 20) electrodes from Bioanalytical Systems Inc. (MF2070) were used in this work. Before any modification procedures, electrodes were polished successively in 1 μm, 0.3 μm, and 0.05μm alumina power (Buehler) slurries with Nanopure water (Barnstead) on microcloth polishing cloth (Buehler) and subsequently washed and sonicated in Nanopure water for about 10 minutes. A low oxide GC surface was prepared by polishing in cyclohexane/alumina power slurries instead of Nanopure water/alumina powers [36]. Cyclohexane was first saturated with argon for 15-20 minutes. 1, 0.3, 0.05 μm alumina powers were used successively. Electrodes were polished on bare glass plates and were sonicated in cyclohexane for 3 minutes and then in Nanopure water for another 10 minutes.

**Nonspecific Adsorption**

AQDS, methylene blue, and 1,4-bis(2-methylstyryl)benzene (BMB) were adsorbed onto glassy carbon according to the previously described procedures [36,85]. GC electrodes presorbed with 2,6-AQDS were prepared by placing a polished electrode in 10 mM 2,6-AQDS aqueous solutions for 10 minutes and rising with Nanopure water for three times. The electrodes were then transferred into electrochemical cells for
measuring of voltammetric data. GC electrodes pre-adsorbed with methylene blue were prepared by dipping a polished electrode in 0.1 mM methylene blue aqueous solutions for 10 minutes and rising with Nanopure water for three times. GC electrodes pre-adsorbed with BMB were prepared by placing a polished electrode in a 1 mM BMB/acetone solution for 10 minutes and rising with acetone for three times.

Specific Adsorption

Chemisorption of aryl radicals on GC surfaces was accomplished by the procedure developed by Saveant et al. [78,98]. Electrodes were polished successively in 1 μm, 0.3 μm, and 0.1 μm alumina power (Buehler) slurries with Nanopure water (Barnstead) on microcloth polishing cloth (Buehler) and subsequently washed and sonicated in Nanopure water for about 10 minutes and then rinsed with acetonitrile (ACN). These polished GC surfaces were used as cathodes in the electrolysis of the solution containing 1 mM of the appropriate diazonium salt and 0.1 M NBu₄BF₄ in acetonitrile at a potential 200 mV negative of Eₒ for diazonium reduction for 10 minutes. After the derivatization, the GC electrodes were rinsed with electrolyte and sonicated in acetonitrile for 3 minutes and then in Nanopure water for 10 minutes. The peak potentials for diazonium reduction are listed in Table 2.1, along with the amine precursor and deposition potentials.
Table 2.1. Voltammetric peak potentials and derivatized potentials vs. Ag/Ag⁺.

<table>
<thead>
<tr>
<th>Diazonium Salt</th>
<th>Amine Precursor</th>
<th>( E_p )</th>
<th>( E_{med} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>phenyl diazonium</td>
<td>aniline</td>
<td>-0.36</td>
<td>-0.56</td>
</tr>
<tr>
<td>4-methylphenyl diazonium</td>
<td>4-methyl aniline</td>
<td>-0.36</td>
<td>-0.57</td>
</tr>
<tr>
<td>4-trifluoromethylphenyl diazonium</td>
<td>4-trifluoromethyl aniline</td>
<td>-0.24</td>
<td>-0.44</td>
</tr>
<tr>
<td>4-nitrophenyl diazonium</td>
<td>4-nitro aniline</td>
<td>+0.04</td>
<td>-0.16</td>
</tr>
<tr>
<td>4-ethylphenyl diazonium</td>
<td>4-ethyl aniline</td>
<td>-0.42</td>
<td>-0.62</td>
</tr>
<tr>
<td>4-biphenyl diazonium</td>
<td>4-phenyl aniline</td>
<td>-0.15</td>
<td>-0.35</td>
</tr>
<tr>
<td>4-nitroazobenzene-4' diazonium</td>
<td>4-nitro-4'-amino azobenzene</td>
<td>+0.01</td>
<td>-0.19</td>
</tr>
</tbody>
</table>

Results

Ruthenium Hexaamine

Ruthenium hexaamine \((\text{Ru(NH}_3)_6^{3+/2-})\) is known as an example of outer-sphere inorganic redox system, electron transfer kinetics are relatively constant. Figure 2.3 shows the comparison of the cyclic voltammograms of the chemically modified surface and the polished surface. A chemically modified surface with a monolayer covalently bonded to GC surface has little effect on the voltammetry. The \( k^0 \) values for \( \text{Ru(NH}_3)_6^{3+/2-} \) determined from several repetitions of the experiment in Figure 2.3 is listed in Table 2.2.
Figure 2.3. Comparison of voltammograms of 1 mM Ru(NH$_3$)$_6$$^{3+/2+}$ in 1 M KCl, 20 V/sec on polished GC and (A) phenyl derivatized GC; (B) nitrophenyl derivatized GC.
Figure 2.3. (continued) Comparison of voltammograms of 1 mM Ru(NH$_3$)$_6$$^{3+/2+}$ in 1 M KCl, 20 V/sec on polished GC and (C) ethylphenyl derivatized GC; (D) biphenyl derivatized GC.
Figure 2.3. (continued) Comparison of voltammograms of 1 mM Ru(NH$_3$)$_6$$_{3+/2+}$ in 1 M KCl, 20 V/sec on polished GC and (E) nitroazobenzene derivatized GC.
Table 2.2. \( \text{Ru(NH}_3\text{)}_6^{3+/2+} \) kinetics results on modified surfaces.

Methyl Viologen

Methyl viologen is the simplest organic redox system studied in this work. It undergoes one electron reduction to its cation radical (Figure 2.4). This widely studied reaction is believed to be outer-sphere on carbon and diamond electrodes, forming a stable cation radical product [99]. Figure 2.5 shows a typical voltammogram for methyl viologen (MV\(^{2+}\)) on polished GC. Fairly rapid electron transfer is indicated by small \( \Delta E_p \) in this voltammogram. A chemically modified surface with a nitrophenyl monolayer covalently bonded to GC surface has little effect on the voltammetry. Only a slight decrease in background current is observed (Figure 2.5). The \( k^0 \) values for MV\(^{2+}/\text{MV}^-\) determined from several repetitions of the experiment in Figure 2.5 is 0.094 ± 0.025 cm/sec \( (N = 3) \) on polished GC, and 0.047 ± 0.013 cm/sec \( (N = 3) \) on the nitrophenyl modified GC surface. As was the case with \( \text{Ru(NH}_3\text{)}_6^{3+/2+} \), the nitrophenyl monolayer reduced \( k^0 \) by a factor of two, implying that both the MV\(^{2+/+} \) and \( \text{Ru(NH}_3\text{)}_6^{3+/2+} \) systems are

\[
\begin{array}{|l|c|c|c|}
\hline
\text{Monolayer} & d, (\text{Å}) & \Delta E_p \text{ (mV), 20 V/sec} & k^0 \text{ (cm/sec)} \\
\hline
\text{None} & 1.6 & 76.3 ± 2.3 & 0.24 ± 0.008 \\
\text{Phenyl (P)} & 5.9 & 80.7 ± 3.0 & 0.17 ± 0.009 \\
\text{Nitrophenyl (NP)} & 6.8 & 85.2 ± 5.6 & 0.11 ± 0.011 \\
\text{Ethylphenyl (EP)} & 8.1 & 97.6 ± 3.5 & 0.08 ± 0.009 \\
\text{Biphenyl (BP)} & 10.2 & 106.3 ± 4.1 & 0.06 ± 0.010 \\
\text{Nitroazobenzene (NAB)} & 14.0 & 183.7 ± 6.1 & 0.02 ± 0.012 \\
\hline
\end{array}
\]

\(^{a}\text{mean ± standard deviation for six determinations.}\)
Figure 2.4. Schematic representation of reduction of methyl viologen.
Figure 2.5. Cyclic voltammograms of 1 mM MV$^{2+}$ in 0.1 M NaCl on polished GC and nitrophenyl derivatized GC. 0.2 V/sec scan rate, no background subtraction.
acting as outer-sphere redox reactions.

**Methylene Blue**

Methylene blue (MB\(^*\)) is the second organic redox system studied in this work. It is generally considered to undergo a reversible 2 electron reduction (Figure 2.6). However, unlike, MV\(^{2-}/MV^-\), MB\(^-\) is prone to adsorption on to the GC surface [36]. Figure 2.7 shows voltammograms for MB\(^-\) at low concentration (10 \(\mu\)M), where the response is dominated by adsorbed MB\(^-\). Based on the geometric electrode area, the voltammetric peak area corresponds to 213 pmol/cm\(^2\) of MB\(^-\) adsorption. Pretreatment of the polished GC surface with isopropanol and activate carbon increases the coverage of MB\(^-\) on polished GC, to 265 pmol/cm\(^2\), presumably due to surface cleaning [49]. However, a nitrophenyl monolayer completely suppresses observable MB\(^-\) adsorption. Increasing the MB\(^-\) solution concentration to 1 mM permits observation of diffusing as well as adsorbed MB\(^-\). As shown in Figure 2.8, the diffusion wave centered at 0.2 V vs. Ag/AgCl is distinguishable from the adsorption wave at about 0.08 V. The nitrophenyl derivatization had little effect on the diffusion wave, but it suppressed adsorption. The voltammetry indicates that MB\(^-\) is also behaving as an outer-sphere system, with a minor decrease in electron transfer kinetics caused by a chemisorbed monolayer. Unlike, MV\(^{2-}\), however, MB\(^-\) exhibits adsorption which is strongly reduced by the presence of an otherwise inert monolayer.
Figure 2.6. Schematic illustration of reduction of methylene blue.
Figure 2.7. Voltammograms of 10 μM methylene blue in 0.1 M H₂SO₄, 2 V/sec on polished GC, nitrophenyl derivatized GC, and isopropanol/activation carbon cleaned GC.
Figure 2.8. Voltammograms of 1 mM methylene blue in 0.1 M H$_2$SO$_4$, 2 V/sec on polished GC and nitrophenyl derivatized GC.
Chlorpromazine, Promazine, and Triflupromazine

Chlorpromazine (CPZ) and two related phenothiazines, promazine (PMZ) and triflupromazine (TPZ) are more complex redox systems than MV$^{2-}$ and MB$^+$, and were examined in much more detail. Figure 2.9 shows the structures of these compounds and Figure 2.10 uses the oxidation of chlorpromazine as an example of the redox reaction of these compounds. One complication of this system has been described previously. CPZ, PMZ, TPZ are cation radicals which react with water or buffer components to yield a sulfoxide [100-102]. To avoid these complicating reactions, the pH was adjusted to 2.0 with HCl. The cation radicals are stable in this medium on the time scale of a voltammogram and the phenothiazines undergo a chemically reversible one electron oxidation. A second complication for the phenothiazines is their tendency to adsorb on to GC from aqueous solution. This property was identified when CPZ electrochemistry was first examined in detail [100], and has been exploited for electroanalytical applications [103,104]. The adsorption appears to arise largely from the hydrophobic nature of the phenothiazine ring system, and can be suppressed by adding 40 % methanol to the electrolyte. Figure 2.11 shows voltammograms of CPZ at polished GC with and without 40 % methanol. Methanol removes the obvious adsorption features but does not completely eliminate adsorption. Semi-integration of the CPZ voltammograms (Figure 2.12) shows the slight peak on the semi-integral plateau which indicates adsorption [105], even in the presence of 40 % methanol. This adsorption is increased by pretreatment with isopropanol and active carbon, as was observed for MB$^+$. Furthermore, the adsorption of CPZ on polished GC adheres to a Langmuir isotherm determined with chronocoulometry.
Figure 2.9. Structures of chlorpromazine, promazine, and trifluromazine.
Figure 2.10. Schematic illustration of oxidation of chlorpromazine.
Figure 2.11. Voltammograms of 1 mM CPZ in 0.2 M NaCl containing 0.01 M HCl, on polished GC in the absence and presence of 40 % MeOH. 2 V/sec.
Figure 2.12. Semi-integral voltammograms for 1 mM CPZ in 0.2 M NaCl, 0.01 M HCl, 20 V/sec. (A) polished GC, no MeOH; (B) polished GC, 40% MeOH; (C) nitrophenyl derivatized GC, no MeOH; (D) nitrophenyl derivatized GC, 40% MeOH.
(r^2 = 0.9980, Figure 2.13). Based on the geometric area, saturation coverage of 93 pmol/cm² is reached at about 1 mM solution concentration. When the GC was derivatized with a nitrophenyl monolayer, the adsorption in 40 % methanol became negligible as measured by chronocoulometry even for 10 mM CPZ in solution (shown as triangle in Figure 2.13).

Unlike MB⁻, CPZ adsorbed to the GC surface even when a nitrophenyl monolayer was present in the aqueous solution (without methanol). As shown in Figure 2.14, with methanol absent, the slope of a log (i_p) vs. log (ν) plot (for 0.02 to 20 V/sec) was 0.63 on polished GC, well above the value of 0.50 expected in the absence of adsorption. A nitrophenyl monolayer reduced the slope to 0.56. However, the combination of 40 % methanol and the nitrophenyl monolayer decreased the slope to 0.509, implying greatly reduced adsorption. These results indicate that neither 40 % methanol nor nitrophenyl derivatization can completely suppress CPZ adsorption by themselves, but the combination can. The chronocoulometric results (Figure 2.13), the semi-integrals (Figure 2.12), and the slopes of log (i_p) vs. log (ν) plots (Figure 2.14) plots all indicate that observable CPZ adsorption is suppressed for nitrophenyl derivatized surfaces in 40 % methanol.

The effects of various surface modifications on phenothiazine voltammetry were studied in the absence of methanol initially, since these modifications had been characterized previously without methanol present [36]. CPZ, PMZ, and TPZ voltammograms were acquired at four different scan rates (0.02, 0.2, 2, and 20 V/sec) on polished GC following several pretreatments. Figure 2.15 show the voltammograms of
Figure 2.13. Adsorption isotherms for CPZ in 0.2 M NaCl, 0.01 M HCl, 40 % MeOH on polished GC and nitrophenyl derivatized GC.
Figure 2.14. Log $i_p$ vs log $v$ for 1 mM CPZ in 0.2 M NaCl, 0.01 M HCl, (A) polished GC, no MeOH; (B) nitrophenyl derivatized GC, no MeOH; (C) nitrophenyl derivatized GC, 40% MeOH.
Figure 2.15. Comparison of voltammograms of 1 mM CPZ in 0.2 M NaCl, 0.01 M HCl, no MeOH, 0.2 V/sec on polished GC and (A) cyclohexane polished GC; (B) AQDS adsorbed GC.
Figure 2.15. (continued) Comparison of voltammograms of 1 mM CPZ in 0.2 M NaCl, 0.01 M HCl, no MeOH, 0.2 V/sec on polished GC and (C) BMB adsorbed GC; (D) MB adsorbed GC.
Figure 2.15. (continued) Comparison of voltammograms of 1 mM CPZ in 0.2 M NaCl, 0.01 M HCl, no MeOH, 0.2 V/sec on polished GC and (E) nitrophenyl derivatized GC.
CPZ on different surfaces at 0.2 V/sec as examples for these three systems. Anaerobic polishing with cyclohexane/alumina produced a GC surface with low oxide coverage [36]. AQDS (an anion), BMB (a neutral ion), and MB+ (a cation) were preadsorbed to polished GC before phenothiazine voltammetry. The structures of these three adsorbates are shown in Figure 2.16. Previous results demonstrated that these adsorbates remain on GC after transferred to aqueous electrolyte from the adsorption solutions [36,85]. The rate constants ($k^0$) and the peak separation voltage ($\Delta E_p$) from these experiments are tabulated in Table 2.3 to Table 2.5, along with those for the nitrophenyl modified surface. In all cases, $k^0$ did not show a significant trend with scan rate, and the standard deviations listed in the table are from the number of trials listed in the parentheses. Although some perturbation of these results is expected from adsorption, Table 2.3 to 2.5 do demonstrate that the effects of surface derivatization are minor for all three phenothiazines. Surface oxide coverage and the presence of anionic, neutral, or cationic adsorbates had little effect on the observed rates. Only a compact, chemisorbed monolayer of nitrophenyl groups has a significant kinetic effect, decreasing the rate constant by about 50%.

The covalently derivatized GC surfaces were examined in more detail, under conditions where adsorption was suppressed. Seven different aryl groups were used to modify GC surfaces. They formed different thickness of monolayer on GC surfaces. Voltammograms for CPZ on a series of derivatized surfaces in 40% methanol is shown in Figure 2.17 at the scan rate of 20 V/sec and Figure 2.18 at the scan rate of 0.2 V/sec. Recall that in these conditions, the nitrophenyl surface showed no CPZ adsorption and the CPZ electron transfer rate constant was invariant with scan rate between 0.02 and 20
<table>
<thead>
<tr>
<th>( \Delta E_p ) (mV)</th>
<th>20 mV/sec</th>
<th>200 mV/sec</th>
<th>2 V/sec</th>
<th>20 V/sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional Polished</td>
<td>62.54 ± 2.54 (13)</td>
<td>69.78 ± 2.49 (11)</td>
<td>91.10 ± 5.26 (10)</td>
<td>134.80 ± 6.46 (10)</td>
</tr>
<tr>
<td>Cyclohexane Polished</td>
<td>62.75 ± 1.26 (6)</td>
<td>68.00 ± 0.82 (6)</td>
<td>90.00 ± 1.73 (6)</td>
<td>132.75 ± 1.50 (6)</td>
</tr>
<tr>
<td>AQDS Adsorbed</td>
<td>62.29 ± 2.06 (6)</td>
<td>68.80 ± 1.79 (6)</td>
<td>90.60 ± 1.34 (6)</td>
<td>138.00 ± 10.79 (6)</td>
</tr>
<tr>
<td>BMB Adsorbed</td>
<td>62.50 ± 1.41 (6)</td>
<td>68.40 ± 3.29 (6)</td>
<td>87.75 ± 3.77 (6)</td>
<td>134.17 ± 8.68 (3)</td>
</tr>
<tr>
<td>Methylene Blue Adsorbed</td>
<td>62.17 ± 1.94 (6)</td>
<td>67.40 ± 2.88 (6)</td>
<td>87.00 ± 3.85 (6)</td>
<td>132.67 ± 7.92 (6)</td>
</tr>
<tr>
<td>NP Derivatized</td>
<td>65.72 ± 2.37 (18)</td>
<td>74.31 ± 6.34 (15)</td>
<td>105.43 ± 10.35 (14)</td>
<td>181.43 ± 12.40 (14)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>( k^0 ) (cm/sec)</th>
<th>20 mV/sec</th>
<th>200 mV/sec</th>
<th>2 V/sec</th>
<th>20 V/sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional Polished</td>
<td>0.032 ± 0.001 (13)</td>
<td>0.032 ± 0.002 (11)</td>
<td>0.030 ± 0.002 (10)</td>
<td>0.036 ± 0.002 (10)</td>
</tr>
<tr>
<td>Cyclohexane Polished</td>
<td>0.030 ± 0.001 (6)</td>
<td>0.036 ± 0.001 (6)</td>
<td>0.031 ± 0.001 (6)</td>
<td>0.038 ± 0.001 (6)</td>
</tr>
<tr>
<td>AQDS Adsorbed</td>
<td>0.035 ± 0.001 (6)</td>
<td>0.035 ± 0.001 (6)</td>
<td>0.031 ± 0.001 (6)</td>
<td>0.034 ± 0.003 (6)</td>
</tr>
<tr>
<td>BMB Adsorbed</td>
<td>0.033 ± 0.001 (6)</td>
<td>0.036 ± 0.002 (6)</td>
<td>0.034 ± 0.002 (6)</td>
<td>0.037 ± 0.002 (6)</td>
</tr>
<tr>
<td>Methylene Blue Adsorbed</td>
<td>0.036 ± 0.001 (6)</td>
<td>0.040 ± 0.002 (6)</td>
<td>0.038 ± 0.002 (6)</td>
<td>0.038 ± 0.003 (6)</td>
</tr>
<tr>
<td>NP Derivatized</td>
<td>0.016 ± 0.001 (18)</td>
<td>0.023 ± 0.002 (15)</td>
<td>0.019 ± 0.002 (14)</td>
<td>0.021 ± 0.001 (14)</td>
</tr>
</tbody>
</table>

Table 2.3. \( \Delta E_p \) and \( k^0 \) of chlorpromazine for different surfaces.
<table>
<thead>
<tr>
<th>$\Delta E_p$ (mV)</th>
<th>20 mV/sec</th>
<th>200 mV/sec</th>
<th>2 V/sec</th>
<th>20 V/sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional</td>
<td>63.67 ± 1.16 (3)</td>
<td>74.67 ± 1.53 (3)</td>
<td>94.33 ± 5.03 (3)</td>
<td>151.00 ± 10.00 (3)</td>
</tr>
<tr>
<td>Polished</td>
<td>65.67 ± 3.22 (3)</td>
<td>73.00 ± 5.00 (3)</td>
<td>96.67 ± 4.73 (3)</td>
<td>155.00 ± 7.00 (3)</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>64.00 ± 1.00 (3)</td>
<td>75.33 ± 5.51 (3)</td>
<td>94.00 ± 4.58 (3)</td>
<td>151.33 ± 12.10 (3)</td>
</tr>
<tr>
<td>Polished</td>
<td>64.00 ± 1.00 (3)</td>
<td>73.33 ± 4.93 (3)</td>
<td>93.67 ± 3.06 (3)</td>
<td>151.00 ± 7.00 (3)</td>
</tr>
<tr>
<td>AQDS Adsorbed</td>
<td>64.67 ± 2.52 (3)</td>
<td>72.67 ± 3.06 (3)</td>
<td>97.00 ± 9.16 (3)</td>
<td>147.67 ± 5.03 (3)</td>
</tr>
<tr>
<td>BMB Adsorbed</td>
<td>0.023 ± 0.004 (3)</td>
<td>0.023 ± 0.008 (3)</td>
<td>0.028 ± 0.004 (3)</td>
<td>0.028 ± 0.003 (3)</td>
</tr>
<tr>
<td>Methylene Blue</td>
<td>0.024 ± 0.004 (3)</td>
<td>0.028 ± 0.008 (3)</td>
<td>0.028 ± 0.003 (3)</td>
<td>0.029 ± 0.002 (3)</td>
</tr>
<tr>
<td>Adsorbed</td>
<td>0.024 ± 0.013 (3)</td>
<td>0.026 ± 0.005 (3)</td>
<td>0.026 ± 0.008 (3)</td>
<td>0.029 ± 0.001 (3)</td>
</tr>
<tr>
<td>NP Derivatized</td>
<td>0.012 ± 0.001 (3)</td>
<td>0.011 ± 0.002 (3)</td>
<td>0.013 ± 0.004 (3)</td>
<td>0.018 ± 0.006 (3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$k^\theta$ (cm/sec)</th>
<th>20 mV/sec</th>
<th>200 mV/sec</th>
<th>2 V/sec</th>
<th>20 V/sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional</td>
<td>0.024 ± 0.004 (3)</td>
<td>0.022 ± 0.002 (3)</td>
<td>0.028 ± 0.004 (3)</td>
<td>0.028 ± 0.003 (3)</td>
</tr>
<tr>
<td>Polished</td>
<td>0.021 ± 0.002 (3)</td>
<td>0.027 ± 0.009 (3)</td>
<td>0.026 ± 0.004 (3)</td>
<td>0.027 ± 0.002 (3)</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>0.023 ± 0.004 (3)</td>
<td>0.023 ± 0.008 (3)</td>
<td>0.028 ± 0.004 (3)</td>
<td>0.028 ± 0.003 (3)</td>
</tr>
<tr>
<td>Polished</td>
<td>0.023 ± 0.004 (3)</td>
<td>0.028 ± 0.008 (3)</td>
<td>0.028 ± 0.003 (3)</td>
<td>0.029 ± 0.002 (3)</td>
</tr>
<tr>
<td>AQDS Adsorbed</td>
<td>0.024 ± 0.013 (3)</td>
<td>0.026 ± 0.005 (3)</td>
<td>0.026 ± 0.008 (3)</td>
<td>0.029 ± 0.001 (3)</td>
</tr>
<tr>
<td>BMB Adsorbed</td>
<td>0.012 ± 0.001 (3)</td>
<td>0.011 ± 0.002 (3)</td>
<td>0.013 ± 0.004 (3)</td>
<td>0.018 ± 0.006 (3)</td>
</tr>
</tbody>
</table>

Table 2.4. $\Delta E_p$ and $k^\theta$ of promazine for different surfaces.
<table>
<thead>
<tr>
<th>$\Delta E_p$ (mV)</th>
<th>20 mV/sec</th>
<th>200 mV/sec</th>
<th>2 V/sec</th>
<th>20 V/sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional</td>
<td>63.80 ± 0.84</td>
<td>73.40 ± 4.83</td>
<td>112.4 ± 21.73</td>
<td>183.8 ± 10.99</td>
</tr>
<tr>
<td>Polished</td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>65.00 ± 2.00</td>
<td>73.33 ± 3.51</td>
<td>94.67 ± 2.52</td>
<td>159.33 ± 7.77</td>
</tr>
<tr>
<td>Polished</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>AQDS Adsorbed</td>
<td>64.67 ± 0.58</td>
<td>73.33 ± 3.51</td>
<td>97.00 ± 8.00</td>
<td>173.67 ± 17.90</td>
</tr>
<tr>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>BMB Adsorbed</td>
<td>63.67 ± 0.58</td>
<td>72.00 ± 1.73</td>
<td>100.67 ± 4.04</td>
<td>163.67 ± 5.86</td>
</tr>
<tr>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>Methylene Blue</td>
<td>65.00 ± 1.00</td>
<td>73.00 ± 2.00</td>
<td>93.67 ± 4.16</td>
<td>184.67 ± 7.51</td>
</tr>
<tr>
<td>Adsorbed</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>NP Derivatized</td>
<td>69.67 ± 3.06</td>
<td>84.67 ± 13.43</td>
<td>134.33 ± 21.94</td>
<td>218.67 ± 18.01</td>
</tr>
<tr>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$k^0$ (cm/sec)</th>
<th>20 mV/sec</th>
<th>200 mV/sec</th>
<th>2 V/sec</th>
<th>20 V/sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional</td>
<td>0.024 ± 0.003</td>
<td>0.026 ± 0.007</td>
<td>0.021 ± 0.007</td>
<td>0.020 ± 0.003</td>
</tr>
<tr>
<td>Polished</td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>0.020 ± 0.007</td>
<td>0.025 ± 0.006</td>
<td>0.027 ± 0.002</td>
<td>0.027 ± 0.002</td>
</tr>
<tr>
<td>Polished</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>AQDS Adsorbed</td>
<td>0.021 ± 0.002</td>
<td>0.025 ± 0.006</td>
<td>0.026 ± 0.007</td>
<td>0.021 ± 0.006</td>
</tr>
<tr>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>BMB Adsorbed</td>
<td>0.025 ± 0.002</td>
<td>0.026 ± 0.004</td>
<td>0.023 ± 0.003</td>
<td>0.025 ± 0.002</td>
</tr>
<tr>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>Methylene Blue</td>
<td>0.019 ± 0.004</td>
<td>0.025 ± 0.003</td>
<td>0.028 ± 0.004</td>
<td>0.020 ± 0.002</td>
</tr>
<tr>
<td>Adsorbed</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>NP Derivatized</td>
<td>0.011 ± 0.003</td>
<td>0.016 ± 0.008</td>
<td>0.012 ± 0.005</td>
<td>0.013 ± 0.003</td>
</tr>
<tr>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
</tbody>
</table>

Table 2.5. $\Delta E_p$ and $k^0$ of trifluromazine for different surfaces.
Figure 2.16. Structures of AQDS, BMB, and MB.
Figure 2.17. Comparison of voltammograms of 1 mM CPZ in 1 M NaCl, 0.01 M HCl, 40 % MeOH, 20 V/sec on polished GC and (A) phenyl derivatized GC; (B) nitrophenyl derivatized GC.
Figure 2.17. (continued) Comparison of voltammograms of 1 mM CPZ in 1 M NaCl, 0.01 M HCl, 40 % MeOH, 20 V/sec on polished GC and (C) trifluoromethylphenyl derivatized GC; (D) methylphenyl derivatized GC.
Figure 2.17. (continued) Comparison of voltammograms of 1 mM CPZ in 1 M NaCl, 0.01 M HCl, 40 % MeOH, 20 V/sec on polished GC and (E) ethylphenyl derivatized GC; (F) biphenyl derivatized GC.
Figure 2.18. Comparison of voltammograms of 1 mM CPZ in 1 M NaCl, 0.01 M HCl, 40 % MeOH, 0.2 V/sec on polished GC and (A) phenyl derivatized GC; (B) nitrophenyl derivatized GC.
Figure 2.18. (continued) Comparison of voltammograms of 1 mM CPZ in 1 M NaCl, 0.01 M HCl, 40 % MeOH, 0.2 V/sec on polished GC and (C) trifluoromethylphenyl derivatized GC; (D) methylphenyl derivatized GC.
Figure 2.18. (continued) Comparison of voltammograms of 1 mM CPZ in 1 M NaCl, 0.01 M HCl, 40 % MeOH, 0.2 V/sec on polished GC and (E) ethylphenyl derivatized GC; (F) biphenyl derivatized GC.
Figure 2.18. (continued) Comparison of voltammograms of 1 mM CPZ in 1 M NaCl, 0.01 M HCl, 40 % MeOH, 0.2 V/sec on polished GC and (G) nitroazobenzene derivatized GC.
V/sec. As shown in Figure 2.17, the polished, underivatized surface does show a larger \( i_p \) at 20 V/sec, due to a contribution from adsorbed CPZ, but this effect is greatly reduced for low scan rate (0.2 V/sec, shown in Figure 2.18.) The derivatization reagents were chosen to yield monolayers of increasing thickness (5.9 Å to 14.0Å), listed in Table 2.6.

<table>
<thead>
<tr>
<th>Monolayer</th>
<th>( d, (\text{Å}) )</th>
<th>( \Delta E_p (\text{mV}) ), 0.2 V/sec</th>
<th>( k_0^{\text{cpz}} (\text{cm/sec}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.6</td>
<td>63.3 ± 0.6</td>
<td>0.081 ± 0.007</td>
</tr>
<tr>
<td>Phenyl (P)</td>
<td>5.9</td>
<td>71.0 ± 1.0</td>
<td>0.029 ± 0.003</td>
</tr>
<tr>
<td>Methylphenyl (MP)</td>
<td>6.8</td>
<td>76.3 ± 1.2</td>
<td>0.020 ± 0.001</td>
</tr>
<tr>
<td>Trifluoromethylphenyl (TFMP)</td>
<td>6.8</td>
<td>77.0 ± 1.0</td>
<td>0.020 ± 0.001</td>
</tr>
<tr>
<td>Nitrophenyl (NP)</td>
<td>6.8</td>
<td>76.7 ± 0.6</td>
<td>0.020 ± 0.001</td>
</tr>
<tr>
<td>Ethylphenyl (EP)</td>
<td>8.1</td>
<td>81.3 ± 1.5</td>
<td>0.015 ± 0.001</td>
</tr>
<tr>
<td>Biphenyl (BP)</td>
<td>10.2</td>
<td>88.7 ± 1.1</td>
<td>0.010 ± 0.001</td>
</tr>
<tr>
<td>Nitroazobenzene (NAB)</td>
<td>14.0</td>
<td>98.7 ± 1.1</td>
<td>0.005 ± 0.001</td>
</tr>
</tbody>
</table>

\( ^b \) mean ± standard deviation for six determinations, all with a scan rate of 0.2 V/sec.

Table 2.6. Chlorpromazine kinetics results on modified surfaces.

The distance between the GC surfaces and the monolayer/solution interface \((d)\) was calculated by Spartan software with the structure optimized with molecular mechanics based on SYBYL force field. It presents the minimum distance between the surface and a redox system for a perfect monolayer. The observed rate constants and calculated \((d)\) values are listed in Table 2.6. Plots of \( \ln (k_0) \) vs. \( d \) are shown in Figure 2.19 for \( \text{Ru(NH}_3)_6^{3+/2+} \) and in Figure 2.20 for CPZ. Both plots show linear relationship between \( \ln \)
Figure 2.19. Plot of $\ln (k^0)$ vs monolayer thickness ($d$, Å) of $\text{Ru(NH}_3)_6^{3+/2-}$. 

$slope = -0.21 \, D^{-1}, \quad r^2 = 0.9479$
Figure 2.20. Plot of $\ln (k^0)$ vs monolayer thickness ($d$, Å) of CPZ.
Discussions

Approximately fifty years of investigation into organic electrochemistry has established that electron transfer between solid electrodes and organic redox systems can involved complex interactions with the electrode surface. The many examples of adsorption and electrocatalysis accompanying organic redox reactions, combined with multiple electron and proton transfers present formidable hurdles to understanding organic redox kinetics and mechanisms. For carbon electrodes, one has the added complications of variable surface condition and a propensity to adsorb organic molecules from solution. Much of the motivation from the current effort is the objective of examining organic redox reactions on reproducibly prepared GC electrodes. Using the approach developed for the inorganic redox systems [36], surface modification of the GC can provide insight into surface variables affecting kinetics and adsorption. The organic redox systems chosen for this initial study are relatively simple, involving only one electron and no protons for MV\textsuperscript{2+/1−}, CPZ, PMZ, TPZ, with MB\textsuperscript{−} following a more complex 2e\textsuperscript{−}/2H\textsuperscript{+} route.

Ru(NH\textsubscript{3})\textsubscript{6}\textsuperscript{3+/2−} is classified as an outer-sphere redox system and its electron transfer kinetics are relatively constant on different surfaces [36]. Chen \textit{et al.} concluded that Ru(NH\textsubscript{3})\textsubscript{6}\textsuperscript{3+/2−} transfer electron via tunneling. Ru(NH\textsubscript{3})\textsubscript{6}\textsuperscript{3+/2−} was studied first on several modified GC surfaces in order to observe the effects of modification on a well understood
redox system. The observed rate constants for Ru(NH₃)₆³⁺⁻²⁺ exhibit linear behavior in a
plot of ln (k⁰) vs. d (Figure 2.19). The slope of this plot is -0.21 which can be compared
to the “tunneling parameter” (β) discussed in studies of long-range electron transfer [92-
96]. Equation 2-3 has been invoked to explain the dependence of the observed rate
constant (k⁰_app) for metal electrodes modified with a self-assembled monolayer of
thickness d. β depends upon tunneling mechanism and the chemical structure of the
spacer, but values near 1.0 Å⁻¹ have been proposed for through-bond tunneling in aliphatic
chains and 1.3 - 1.8 Å⁻¹ for through space tunneling on modified gold electrodes [102]. β
values for through-bond tunneling in unsaturated spacers in rigid molecules are
substantially lower, ranging from 0.14 Å⁻¹ for a conjugated polyene spacer to 0.57 Å⁻¹ for
phenyl-methylene spacers (Figure 2.21). The closest electrochemical analog from the
literature examined the distance dependence of electron transfer for phenylene/ethynyl
spacers adsorbed to gold as thiolate monolayers [106]. For these conjugated spacer, the β
value was 0.57 ± 0.02 Å⁻¹. The absorbates used to construct Figure 2.19 all have aromatic
backbones, and are conjugated when more than one ring is present. The observed β value
of 0.21 Å⁻¹ is within the range of 0.14 to 0.57 Å expected for conjugated, unsaturated
spacers. However, the systems used in this chapter are different from self-assembled
monolayers. First, the redox systems are in the solution but not bound to the monolayers.
Second, it is C-C bond but not S-Au bond between electrode and monolayers. Thus,
under the assumption of through-bond tunneling, a β value of 0.21 Å⁻¹ is not unreasonable
for the series of absorbates considered here.
\[ k^0_{\text{app}} = k^0 \exp(-\beta d) \]

\[ \beta \quad (\text{Å}^{-1}) \]

0.14

1

0.57

0.57

0.21

\[ \text{Observed} \]

\[ \text{R} = \text{H, CH}_3, \text{CF}_3, \text{NO}_2, \text{C}_2\text{H}_5, \]

Figure 2.21. Illustration of tunneling parameters of different spacers [102,106].
Other than an apparently minor perturbation from MB' adsorption, MV^{2+/+} and MB' are remarkably insensitive to surface modification. A nitrophenyl monolayer decreases the apparent k^0's by about 50 %, and completely blocks MB' adsorption. Clearly, MB' adsorption is not required for fast kinetics, and diffusing MB' undergoes fast electron transfer even when the GC surface is occupied by nitrophenyl groups. Chen and McCreery concluded previously for inorganic redox systems that the electron tunnels through the nitrophenyl layer, causing a relatively small decrease in observed k^0 [36]. Further evidence for the tunneling mechanism for the case of CPZ is discussed below.

Table 2.3 to 2.5 indicate the CPZ, PMZ, and TPZ electron transfer kinetics are also insensitive to surface modifications, even when adsorption is partly responsible for the observed current. The rate constants in Table 2.3 to 2.5 are suspect due to adsorption, but the k^0 values and the \Delta E_p results from which they were determined show no major changes with surface modifications. Adsorption and electron transfer are uncorrelated and apparently independent, a conclusion which is inconsistent with an electron transfer mechanism involving electrocatalysis via chemisorption or redox mediation by surface groups. Like Ru(NH_3)_6^{3+/2+}, MB' and MV^{2+/+}, the phenothiazines behave as outer-sphere systems with respect to electron transfer.

The uncertainties about the effects of adsorption on kinetics observation are greatly reduced for the covalently modified surfaces in the presence of 40 % methanol. The adsorption of CPZ is presumably driven by dipole-dipole or \pi-\pi interactions between the graphitic surface and the phenothiazine ring system, as well as hydrophobic effects. The nitrophenyl modified surfaces show significantly weaker adsorption than a bare
polished surface, apparently because direct interactions between the carbon surface and the phenothiazine ring are blocked. There is still some residual adsorption to the nitrophenyl modified GC surface in the absence of methanol, presumably because hydrophobic effects are still present. As demonstrated in Figure 2.12, 2.13, and 2.14, the combination of nitrophenyl derivatization and 40 % methanol reduces CPZ adsorption below levels detectable by chronocoulometry or semi-integration.

The observed rate constants for CPZ obtained under conditions of negligible adsorption exhibit linear behavior in a plot of In (k⁰) vs. d (Figure 2.20). The point for polished GC at 1.6 Å is suspect due to adsorption, but the error was minimized by using a low scan rate. For the polished surface, the addition of methanol increases the apparent k⁰ from 0.0333 (Table 2.3) to 0.081 (Table 2.6). The slope of the ln (k⁰) vs. d plot of -0.22 Å⁻¹ (or -0.20 if polished GC is not included) is close to the slope of the ln (k⁰) vs. d plot, -0.21 for Ru(NH₃)₆³⁺⁻²⁺ which is an outer-sphere redox system. Thus, it is reasonable to conclude that the redox reaction of CPZ/CPZ⁺⁺ is controlled by tunneling. In other words, chemically modified surfaces inhibit adsorption but do not inhibit electron transfer kinetics of CPZ.

However, it is surprising that Figure 2.19 and 2.20 exhibits the linear ln (k⁰) vs. d dependence expected for the tunneling mechanism in the first place. The chemisorbed monolayers are not expected to self assemble on GC, since they are generated rapidly and their covalent bonds to the surface do not permit annealing. The initial GC surface is microscopically rough, so an ordered, regular monolayer is unlikely. However, the monolayers are likely to be low in pinholes, since any electroactive regions should have
been covered by additional diazonium reduction during derivatization. Furthermore, the coverage of the nitrophenyl film is close to that a compact monolayer, bonded to the GC at the para position, implying that the molecular axes are perpendicular to the local GC surface [86]. The linearity of Figure 2.19 and 2.20 is consistent with a compact monolayer which tracks the shape of the rough GC surface, and results in reasonable constant spacing between the GC substrates and the Ru(NH$_3$)$_6^{3-2+}$ or CPZ at the point of closest approach. Of course, some level of pinholes is inevitable as is some variation in Ru(NH$_3$)$_6^{3-2+}$ or CPZ approach to the surface near sharp corners, pits, etc. However, the results of Figure 2.19 and 2.20 imply that the average tunneling distance correlates with the size to the chemisorbed molecules.

There are several possible reasons why the phenothiazine electron transfer reactions are fast compared to many organic redox systems, but a strong candidate for the major factor involves inner-sphere reorganization energy. It has long been recognized that redox reactions requiring significant changes in bond distances are often slow, due to the high reorganization energy accompanying electron transfer. For example, Fe(H$_2$O)$_6^{3-2+}$ has a slow electron exchange rate [108] and a small $k^0$ on oxide free carbon [89] because the Fe-O bond distance must change by 0.13 Å between Fe$^{2+}$ and Fe$^{3+}$. Ferrocene (Fc) and anthracene are much faster, because delocalization of the positive charge upon oxidation leads to small changes in bond distances. For Fc/Fc$^+$, the bond lengths change by at most 0.04 Å upon oxidation [109-110]. The cation in CPZ$^{--}$ is quite delocalized, and one would not expect large bond length changes during the CPZ/CPZ$^{--}$ redox reaction, or those of the related phenothiazines, PMZ, TPZ and MB$^-$. Molecular
mechanics calculations based on ab initio geometry optimization, UHF-3TO-3G basis set were used to establish bond length changes for Fc/Fc" and CPZ/CPZ". The maximum change in bond length calculated for Fc/Fc" was 0.05 Å, while that for CPZ/CPZ" was 0.04 Å. The small reorganization energy associated with small changes in bond length is likely to contribute to the fast kinetics observed for the phenothiazines, compared to many other organic redox systems.

In contrast to Ru(NH₃)₆³⁺/²⁺, MB⁺, and CPZ, dopamine is an example that adsorption is critical for the electron transfer [111]. Duvall et al. reported that electron transfer for dopamine was profoundly inhibited by a monolayer of NP or (trifluoromethyl)phenyl (TFMP) groups on the GC surface, so that voltammetric waves were not observed. The ΔEₚ increased monotonically with surface coverage of TFMP groups. The results indicate that dopamine adsorption to GC is required for fast electron transfer.

Several conclusions (illustrated in Figure 2.22) about electron transfer of organic redox systems are available from the results presented in this chapter. First, the phenothiazines and MV²⁺/⁻ behave like Ru(NH₃)₆³⁺/²⁺ as outer sphere redox systems on GC and modified GC, with little kinetic sensitivity to surface modifications. Second, MB⁻ and CPZ are examples that chemisorbed monolayers greatly reduce their adsorption, but have minor effects (about 50 %) on their electron transfer rates. Third, dopamine is an example that chemisorbed monolayers reduce both adsorption and the electron transfer kinetics [111]. Now the next chapter will discuss the reduction of oxygen, which is another example of adsorption is critical in the electron transfer mechanism.
Figure 2.22. Schematic illustration of classification of redox systems.
CHAPTER 3

EFFECTS OF SURFACE MODIFICATIONS ON THE REDUCTION OF DIOXYGEN ON GLASSY CARBON ELECTRODES

Introduction

Redox reactions of dioxygen in both aqueous and nonaqueous solutions have received a great deal of attention due to their various applications in fuel cells and batteries, as well as in biological reactions [112,113]. Oxygen reduction occurs at the positive electrode in fuel cells and metal/air batteries to produce electricity while oxygen evolution takes place in rechargeable batteries during charge. Industrial H₂O₂ production uses O₂ as the reactant. In chlor-alkali cells, oxygen serves to lower the cell voltage in improving energy efficiency. But in metal corrosion reactions, oxygen promotes the reaction rate and is not desirable. Modern automobiles and industrial processes often use oxygen sensors as an integral part of emission control systems. In biological systems, many reactions also involve oxygen. A well known example is the photosynthesis reaction in green plants which produces oxygen to become the primary O₂ source in the atmosphere. Among these redox reactions of oxygen, the oxygen reduction reaction has received the most attention because of the interest in energy conversion devices such as
fuel cells.

The oxygen reduction reaction has been studied extensively on metal electrodes (Pt, Hg, Ag, and Au) and non-metal electrodes (carbon, diamond) [114-135]. Many different types of carbon have been used, such as highly ordered pyrolytic graphite (HOPG), pyrolytic graphite (PG), graphite powder, porous graphite, natural graphite, glassy carbon, pyrolytic carbon, active carbon, carbon black, and carbon single crystals. These materials exhibit a wide range of electrochemical activity for oxygen reduction because they each have different physicochemical properties. Since carbon has been widely employed in energy conversion applications where oxygen reduction reactions are involved, an understanding of the surface effects on the reduction of oxygen is invaluable. In particular, the reduction of dioxygen in alkaline media is critical to Zinc/air batteries, alkaline fuel cells, and air cathodes in general.

The redox chemistry of oxygen and its reduction products is heavily dependent upon mechanistic pathway, substrate, and solution acidity. The mechanism of oxygen ($O_2$) reduction to hydrogen peroxide anions ($HO_2^-$) on carbon materials in alkaline solutions have been examined extensively. Despite many efforts by electrochemists, there is still no clear agreement on the steps involved. The overall reactions in acidic and basic solutions are as follows:

In acid: $O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \quad E^0 = 1.229 \text{ V} \quad (3-1)$

In base: $O_2 + 2H_2O + 4e^- \rightarrow 4OH^- \quad E^0 = 0.401 \text{ V} \quad (3-2)$
On metal electrodes (Pt, Ag, Pd), four electron, four proton reduction of oxygen is possible, but the reaction does not occur in one single step. Instead, it usually involves a series of steps where intermediates may be adsorbed. On carbon, gold, and mercury, most workers agree that the reaction involves a peroxide pathway:

In acid: \[ \text{O}_2 + 2\text{H}^+ + 2e^- \rightarrow \text{H}_2\text{O}_2 \quad E^0 = 0.67 \text{ V} \quad (3-3) \]
followed by \[ \text{H}_2\text{O}_2 + 2\text{H}^+ + 2e^- \rightarrow 2\text{H}_2\text{O} \quad E^0 = 1.77 \text{ V} \quad (3-4) \]
or \[ 2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2 \quad (3-5) \]

In base: \[ \text{O}_2 + \text{H}_2\text{O} + 2e^- \rightarrow \text{HO}_2^- + \text{OH}^- \quad E^0 = -0.065 \text{ V} \quad (3-6) \]
followed by \[ \text{HO}_2^- + \text{H}_2\text{O} + 2e^- \rightarrow 3\text{OH}^- \quad E^0 = 0.867 \text{ V} \quad (3-7) \]
or \[ 2\text{HO}_2^- \rightarrow 2\text{OH}^- + \text{O}_2 \quad (3-8) \]

It is often observed that oxygen reduction proceeds only to the first two electron step (Equation 3-3 in acid and Equation 3-6 in base). This limitation is mainly caused by the high energy required to break the O-O bond in the reduction of the third and fourth electrons. To achieve four electron, four proton reduction, a very high overpotential can be used or a catalyst such as a metal porphyrin or phthalocyanine that can decompose peroxide or catalyze the peroxide reduction may also be used [113,119,130-135]. In the air cathode, a carbon surface reduces oxygen to \( \text{HO}_2^- \), then a catalyst produces \( \text{OH}^- \) and \( \text{O}_2 \).
Many detailed mechanistic studies have been conducted on oxygen reduction in basic solution on a carbon electrode because carbon is an effective electrocatalyst for oxygen reduction to peroxide. For graphite, Morcos et al. [126] proposed a three step reduction mechanism involving adsorption of oxygen on graphite electrodes followed by the reduction of adsorbed $O_2$ and the disproportionation of reduced $O_2^•$:

\[
\begin{align*}
O_2 & \rightarrow O_2(\text{ads}) \quad (3-9) \\
O_2(\text{ads}) + e^- & \rightarrow O_2^•(\text{ads}) \quad (3-10) \\
2O_2^•(\text{ads}) + H_2O & \rightarrow O_2 + HO_2^- + OH^- \quad (3-11)
\end{align*}
\]

With Equation 3-10 as the rate determining step, this mechanism is consistent with their experimental Tafel plot and does not involve O-O bond breaking.

Taylor et al. [127] concluded that the following mechanism on glassy carbon electrodes occurs:

\[
\begin{align*}
O_2 + e^- & \rightarrow O_2^•(\text{ads, before migration}) \quad (3-12) \\
O_2^•(\text{ads, before migration}) & \rightarrow O_2^•(\text{ads, after migration}) \quad (3-13) \\
2O_2^•(\text{ads, after migration}) + H_2O & \rightarrow O_2 + HO_2^- + OH^- \quad (3-14)
\end{align*}
\]

Equation 3-13, surface migration of adsorbed $O_2^•$ to the active sites was believed to be the rate determining step. Similarly, Zhang et al. [128] proposed two different forms of adsorbed $O_2$ on glassy carbon surfaces. The Equation 3-17 was suggested as the rate
Determining step:

\[ \text{O}_2 + e^- = [\text{O}_2(\text{ads})]^* \]  \hspace{1cm} (3-15)

\[ [\text{O}_2(\text{ads})]^* = \{\text{O}_2(\text{ads})\}^* \]  \hspace{1cm} (3-16)

\[ \{\text{O}_2(\text{ads})\}^* + \text{H}_2\text{O} = \text{HO}_2^*(\text{ads}) + \text{OH}^- \]  \hspace{1cm} (3-17)

\[ \text{HO}_2^*(\text{ads}) + e^- = \text{HO}_2^*(\text{ads}) \]  \hspace{1cm} (3-18)

\[ \text{HO}_2^*(\text{ads}) = \text{HO}_2^- \]  \hspace{1cm} (3-19)

where the \([\text{O}_2(\text{ads})]^*\) and \(\{\text{O}_2(\text{ads})\}^*\) represent two different forms of the superoxide on the surface with the structure like:

![Structure of superoxide forms](image)

Appleby et al. [116] proposed a mechanism involving a second electron transfer on carbon black electrodes in alkaline solution:

\[ \text{O}_2 + e^- = \text{O}_2^- \]  \hspace{1cm} (3-20)

\[ \text{O}_2^- + \text{H}_2\text{O} + e^- = \text{HO}_2^- + \text{OH}^- \]  \hspace{1cm} (3-21)

or

92
$$O_2 + H_2O + e^- = HO_2^{\cdot (ads)} + OH^-$$  \hspace{1cm} (3-22) \\
$$HO_2^{\cdot (ads)} + e^- = HO_2^{\cdot}$$  \hspace{1cm} (3-23)

where Equation 3-21 or Equation 3-23 is the rate determining step.

Recently, Xu et al. [123] explored several different carbon surfaces — electrochemically pretreated GC, laser treated GC, fractured GC, low-defect highly ordered pyrolyric graphite (HOPG) basal plane surfaces, and modified glassy carbon (GC). They proposed the following mechanism:

$$O_2 + e^- = O_2^{\cdot (ads)}$$  \hspace{1cm} (3-24) \\
$$O_2^{\cdot (ads)} + H_2O = HO_2^{\cdot} + OH^-$$  \hspace{1cm} (3-25) \\
$$HO_2^{\cdot} + O_2^{\cdot} = HO_2^{\cdot} + O_2$$  \hspace{1cm} (3-26) \\
or \\
$$HO_2^{\cdot} + e^- = HO_2^{\cdot}$$  \hspace{1cm} (3-27)

They concluded adsorption is critical to increasing the reduction rate by accelerating protonation of $O_2^{\cdot}$ (Equation 3-25). Without adsorption, $O_2^{\cdot}$ is a relatively weak base ($pK_{eq}^{25} = pK_b = pK_a - pK_a ~ 14 - 5 = 9$). They predicted that when $O_2^{\cdot}$ is adsorbed on the surface, it becomes a better base. The $pK_a$ for $O_2^{\cdot (ads)}$ shifts to around 10 ($pK_{eq}^{25} = pK_b ~ 14 - 10 = 4$). Thus, the reaction rates of protonation and subsequent disproportionation of $O_2^{\cdot}$ or reduction of $HO_2^{\cdot}$ are accelerated. Increases in adsorbed $O_2^{\cdot}$ from surface pretreatment can be caused either by formation of new adsorption sites or by increases in
microscopic surface area.

Besides the controversy in the rate determining step, the role of surface oxides is also poorly understood. Fagan et al. [67] and Zhang [128] concluded that o-quinone like structures are possible candidates to catalyze the reduction of oxygen. Evans et al. suggested that the oxygen-containing groups might serve as mediators between the electrode and the electroactive species [42]. Recently, Yeager et al. have observed oxygen reduction catalysis by adsorbed phenanthrenequinone in base, under conditions where the semiquinone anion (PQ\(^{-}\)) is formed [120]. They proposed that adsorbed PQ\(^{-}\) catalyzes oxygen reduction on a highly ordered pyrolytic graphite (HOPG) basal plane:

\[
PQ_{(ads)} + e^- \rightleftharpoons PQ^-_{(ads)} \tag{3-28}
\]

\[
PQ^-_{(ads)} + O_2 \rightleftharpoons PQ^-_{(ads)}...O_2^- \tag{3-29}
\]

\[
PQ^-_{(ads)}...O_2^- + H_2O + e^- \rightleftharpoons PQ_{(ads)} + HO_2^- + OH^- \tag{3-30}
\]

Nagaoka et al. [129] studied oxygen reduction at electrodes treated electrochemically and at ones on which quinone was adsorbed. They observed that potentials of the O\(_2\) reduction on anodized GC were relatively pH independent, whereas those for quinone adsorbed on GC shifted negative at a rate of -60 mV per pH, which is characteristic of a quinone/hydroquinone reaction. If the quinone mediates the O\(_2\) reduction, then the O\(_2\) wave should also be pH dependent on anodized GC. Thus, they argued that quinone catalysis is possible, but unlikely for oxidized carbon surfaces unmodified by quinone adsorption.
In addition to quinone, phthalocyanines or porphyrins of cobalt and iron are known as effective catalysts for oxygen reduction [130-133]. The reduction of the intermediate steps during the oxygen reduction was proposed since the rate of the overall reaction is often limited by one or more steps of the reduction. Thus, the other molecule, transition metal ion-Schiff-base complexes were of interested due to their capability to form adducts with oxygen [134]. Choi et al. reported the studies of oxygen reduction at a dinuclear Co(II)-Schiff-base complex, Co(II)$_2$-3,3',4,4'-tetra(salicylidene imino-1,1'-biphenyl tetrahydrate [Co(II)$_2$-disalophen; Co(II)$_2$-DSP·4H$_2$O], modified carbon electrodes [135]. The adsorbed complex was shown to have a large catalytic effect for oxygen reduction. The oxygen reduction was reasonably chemically reversible in alkaline solutions with an apparent number of electrons transferred of around one and an estimated exchanged rate constant of about 0.04 cm/sec at the Co(II)$_2$-DSP·4H$_2$O modified carbon electrodes.

This chapter is directed at establishing the nature of the interaction between carbon surfaces and oxygen which leads to electrocatalytic formation of HO$_2^-$ in the absence of transition metals. The approach exploits chemical modifications of carbon surfaces which have been shown to inhibit the adsorption of phenothiazines but the electron transfer kinetics of these redox systems are not significantly affected by the surface structure (chapter 2). In addition, a recently developed pretreatment of glassy carbon surfaces yielded unusually clean and reactive GC surfaces, and very high physisorption of catechols and methylene blue [49]. Since all of these mechanisms of oxygen reduction are believed to involve adsorption, the effects of blocking or enhancing
adsorption should be pronounced. Our objective is an understanding the effects of adsorption on carbon surfaces in oxygen reduction kinetics by studying carbon surfaces which were modified to suppress or enhance adsorption.

**Experimental**

**Reagents**

Tetrabutylammonium tetrafluoroborate (NBu4BF4), 50% fluoboric acid, and p-toluidine were purchased from Aldrich Chemical Company and were used as received. Sodium nitrite was purchased from Sigma Chemical Company and used as received. 1M KOH was made using pre-boiled Nanopure water and low carbonate KOH pellet (Millinckrodt). H2O2 solution was made from a 30% H2O2 stock (Fisher Scientific). All cyclic voltammograms were taken in aqueous buffers — pH 0, 1 M H2SO4 (reagent grade, J. T. Baker); pH 4, CH3COOH (reagent grade, J. T. Baker) + KOH; pH 7, K2HPO4·3H2O (reagent grade, J. T. Baker) + KH2PO4 (reagent grade, J. T. Baker) + KOH, (4/5 mole ratio of K2HPO4·3H2O to KH2PO4; 8 < pH < 12, H3BO3 (reagent grade, J. T. Baker) + KOH. Except H2SO4 solution, the total concentration of the buffer components was 0.1 M and 0.9 M of KCl was added to adjust the ionic concentration. All electrolytes were made fresh before use.

**Synthesis of Diazonium Tetrafluoroborate Salts**

Diazonium tetrafluoroborate salts were synthesized according to the procedure described in Chapter 2.
Electrode Materials and Polished Procedure

Commercial glassy carbon (GC 20) electrodes from Bioanalytical Systems Inc. (MF2070) were used in this work. Preparation procedures for the electrodes were stated in Chapter 2.

AC/IPA Cleaned GC

Electrodes were polished conventionally and then were solvent treated [49]. Activated carbon (AC) was mixed with 2-propanol (IPA) in a 1/3 (v/v) AC/IPA ratio to prepare the AC/IPA mixture. The mixture was covered and allowed to stand for 30 min before use. IPA was of reagent grade and was used without further purification. For solvent treatment, the electrode was sonicated in the AC/IPA mixture for 10 min, followed by an additional 10 min sonication in Nanopure water.

Specific Adsorption

Chemisorption of methylphenyl radicals on GC surfaces was accomplished by the procedure described in Chapter 2. The NMR and mass spectroscopy data of 4-methylbenzenediazonium fluoborate were listed in Chapter 2. 4-Methylbenzenediazonium fluoborate instead of 4-nitrobenzenediazonium fluoborate was chosen due to its electrochemical inertness during the potential where oxygen reduction occurred.
**Electrochemical Measurements**

Cyclic voltammetry was performed in a three-electrode cell. The reference electrode was Ag/AgCl/3 M KCl with a glass frit, the auxiliary electrode was a platinum wire. The electrolyte was saturated with argon (Linde Gas, pre-purified) or oxygen (Linde Gas, ultra-high purity) for 20 min prior to voltammetry. During data acquisition, the purging gas was passed above the solution without disturbing it. The saturated concentration and the diffusion coefficient of the oxygen used in the simulation were 1 mM [113] and $1.65 \times 10^{-4}$ cm$^2$/s [136], respectively.

**Results and Discussions**

A chemically modified surface with a methylphenyl monolayer covalently bonded to a GC surface was achieved by the reduction of methylphenyl diazonium salt at a potential 200 mV negative of $E_p$ for diazonium reduction for 10 minutes. Figure 3.1 shows the comparison of voltammograms for the reduction of oxygen over a pH range from 14 to 0 on conventionally polished, AC/IPA cleaned and methylphenyl derivatized GC. Unlike on the AC/IPA cleaned or polished GC, a chemically reversible peak appears on the methylphenyl modified GC at pH 14. The reverse oxidation peak decreases as the pH decreases. In addition, the AC/IPA pretreatment yields a large, more positive reduction wave compared to the polished surface. Figure 3.2 shows the comparison of the cyclic voltammograms of $\text{H}_2\text{O}_2$ oxidation on the conventionally polished GC and methylphenyl derivatized GC at pH 14. Neither surface reduces $\text{HO}_2^-$ in the potential
Figure 3.1. Comparison of voltammograms of 1 mM O$_2$ at (A) pH = 14, (B) pH = 10.5 on polished, AC/IPA cleaned and methylphenyl derivatized GC, 200 mV/sec, background subtracted.
Figure 3.1. (continued) Comparison of voltammograms of 1 mM \( \text{O}_2 \) at (C) \( \text{pH} = 9 \), (D) \( \text{pH} = 7 \) on polished, AC/IPA cleaned and methylphenyl derivatized GC, 200 mV/sec, background subtracted.
Figure 3.1. (continued) Comparison of voltammograms of 1 mM O$_2$ at (E) pH = 4, (F) pH = 0 on polished, AC/IPA cleaned and methylphenyl derivatized GC, 200 mV/sec, background subtracted.
Figure 3.2. Voltammograms of 3 mM H$_2$O$_2$ in 1 M KOH on polished and methylphenyl derivatized GC, 200 mV/sec, background subtracted.
range shown, and both exhibit comparable $\text{HO}_2^-$ oxidation waves. For the modified surface, the oxygen generated by $\text{HO}_2^-$ oxidation is reduced on the subsequent scan, again exhibiting chemical reversibility. On polished GC, the reduction of electrogenerated oxygen is similar to that observed for an oxygen solution at the same pH.

Figure 3.1 and 3.2 demonstrate a profound change in oxygen reduction chemistry on methylphenyl derivatized surface compared to either polished surface or AC/IPA cleaned GC. Although unexpected, the reverse wave on the modified surface implies formation of superoxide ion which is stable at pH 14 on the voltammetric time scale. As noted in a previous chapter (Chapter 2), the chemically modified surfaces inhibited the adsorption of phenothiazines, thus, methylphenyl modification should prevent adsorption of oxygen or its reduction products on the carbon surface, causing a dramatic effect on oxygen voltammetry. In order to understand the more complex voltammetry on the unmodified GC surface, methylphenyl derivatized GC was studied in some detail. After describing the results and conclusions for the modified surface, the unmodified GC will be revisited in greater detail.

**Methylphenyl Derivatized GC**

Figure 3.3 and 3.4 show the scan rate dependence of the oxygen reduction wave at pH 14. Both the reduction and oxidation peak heights show a linear relationship with $(v^{1/2})$ and $\Delta E_p$ increases with scan rate. This shows that the peak current is due to a diffusion controlled process. The slope for the reduction peak on the methylphenyl modified GC is about half of that on polished GC ($49.31 \mu A \cdot \text{sec}^{1/2} / V^{1/2}$ vs. 98.53...
Figure 3.3. $i_p$ vs $v^{1/2}$ for 1 mM O$_2$ in 1 M KOH on polished, AC/IPA cleaned and methylphenyl derivatized GC.
Figure 3.4. Voltammograms of 1 mM O₂ in 1 M KOH at different scan rate (100 mV/sec to 2 V/sec) on methylphenyl derivatized GC, background subtracted.
μA·sec$^{1/2}$/V$^{1/2}$) and corresponds approximately to that expected for a one electron reduction. Both ($i_p$) and ($i_p^*$) track ($ν^{1/2}$), implying chemical reversibility over the time scale of 0.5 to 10 seconds. These observations are completely consistent with a one electron reduction of O$_2$ to O$_2^·$, without adsorption.

For a chemically reversible reaction, the expected slope can be estimated from the following equation [7]:

$$\text{Slope} = \frac{i_p}{ν^{1/2}} = 2.69 \times 10^5 \times n^{3/2} \times D^{1/2} \times C_0^* \times A$$

(3-31)

where $n$ is the number of electrons involved in the reaction, $D$ is the diffusion coefficient of O$_2$(aq), $A$ is the electrode area, and $C_0^*$ is the concentration of the O$_2$ in the bulk solution. Given $n = 1$, $D = 1.65 \times 10^{-3}$ cm$^2$/s, $A = 0.071$ cm$^2$ and $C_0^* = 1$ mM, the calculated slope is 77.58 μA·sec$^{1/2}$/V$^{1/2}$, higher than the observed 49.31 μA·sec$^{1/2}$/V$^{1/2}$ on the methylphenyl derivatized GC. The deviation could be explained by the following chemical reaction which will be described later.

Several pH values were examined for their effects on the reduction of oxygen on methylphenyl derivatized GC. Since the reduction of oxygen on the methylphenyl derivatized GC at high pH is one electron reduction as stated in the previous section, the electron transfer rate constant can be calculated. Table 3.1 lists the voltammetric results for 0.1 to 2.0 V/sec, along with the electron transfer rate constants ($k^o$) determined from $ΔE_p$. Reverse voltammetric peaks were observed at pH 12-14, and the results for three pH values are shown. Both the reduction potential ($E_{1/2}$, determined from the average of
<table>
<thead>
<tr>
<th>Scan Rate (V/sec)</th>
<th>$E_{1/2}$ (mV)</th>
<th>$\Delta E_p$ (mV)</th>
<th>$^a k^0$ (cm/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>-382 ± 3</td>
<td>145 ± 5</td>
<td>0.0035 ± 0.0003</td>
</tr>
<tr>
<td>0.2</td>
<td>-381 ± 2</td>
<td>172 ± 3</td>
<td>0.0035 ± 0.0002</td>
</tr>
<tr>
<td>pH = 14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>-383 ± 2</td>
<td>230 ± 3</td>
<td>0.0032 ± 0.0003</td>
</tr>
<tr>
<td>1</td>
<td>-380 ± 4</td>
<td>264 ± 6</td>
<td>0.0032 ± 0.0005</td>
</tr>
<tr>
<td>2</td>
<td>-379 ± 5</td>
<td>314 ± 8</td>
<td>0.0028 ± 0.0008</td>
</tr>
<tr>
<td>pH = 13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>-381 ± 4</td>
<td>147 ± 6</td>
<td>0.0033 ± 0.0004</td>
</tr>
<tr>
<td>0.2</td>
<td>-382 ± 2</td>
<td>171 ± 3</td>
<td>0.0035 ± 0.0002</td>
</tr>
<tr>
<td>0.5</td>
<td>-378 ± 3</td>
<td>235 ± 5</td>
<td>0.0030 ± 0.0005</td>
</tr>
<tr>
<td>1</td>
<td>-382 ± 5</td>
<td>270 ± 8</td>
<td>0.0028 ± 0.0007</td>
</tr>
<tr>
<td>2</td>
<td>-383 ± 7</td>
<td>318 ± 9</td>
<td>0.0029 ± 0.0010</td>
</tr>
<tr>
<td>pH = 12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>-382 ± 4</td>
<td>146 ± 7</td>
<td>0.0032 ± 0.0005</td>
</tr>
<tr>
<td>0.2</td>
<td>-379 ± 5</td>
<td>172 ± 5</td>
<td>0.0035 ± 0.0004</td>
</tr>
<tr>
<td>0.5</td>
<td>-381 ± 2</td>
<td>237 ± 3</td>
<td>0.0029 ± 0.0003</td>
</tr>
<tr>
<td>1</td>
<td>-380 ± 5</td>
<td>271 ± 9</td>
<td>0.0028 ± 0.0008</td>
</tr>
<tr>
<td>2</td>
<td>-378 ± 6</td>
<td>320 ± 8</td>
<td>0.0029 ± 0.0008</td>
</tr>
</tbody>
</table>

$^a$mean ± standard deviation for three determinations.

Table 3.1. $E_{1/2}$, $\Delta E_p$ and $k^0$ of oxygen at different pH and scan rate on methylphenyl derivatized GC.
\( E_{p}^{\circ} \) and \( E_{p}^{\circ} \) and the rate constant \( (k^{0}) \) for the reduction of oxygen on the methylphenyl derivatized GC show minor changes at the pH range of 12-14. The means and standard deviations of 45 determinations for five scan rates and three pH values are:

\[
E_{1/2} = -380.7 \pm 3.9 \text{ mV vs. Ag/AgCl} \\
k^{0} = 0.0031 \pm 0.0005 \text{ cm/sec}
\]

As mentioned in Chapter 2, for several outer-sphere redox systems such as Ru(NH\(_3\))\(_6^{3+/2-}\), methyl viologen\(^{2+/+}\), and chlorpromazine, a thin monolayer on GC has minor effects on observed electron transfer rate constants. For a nitrophenyl monolayer, a decrease in \( k^{0} \) of a fact of two was observed for the Ru(NH\(_3\))\(_6^{3+/2-}\), methyl viologen\(^{2+/+}\), and chlorpromazine, consistent with electron tunneling through the monolayer. The \( k^{0} \) of O\(_2\)/O\(_2\)^• on bare GC was estimated as 0.005 cm/sec in a previous report, based on a small reverse wave observed in tetraethylammonium hydroxide electrolyte [123]. The methylphenyl modification used in the this work exhibits a \( k^{0} \) of 0.003 cm/sec, consistent with outer-sphere electron transfer to the O\(_2\)/O\(_2\)^• couple through a methylphenyl monolayer.

The pH dependence of O\(_2\) voltammetry on methylphenyl derivatized GC is shown in Figure 3.5 and the trends of \( E_{p} \) and \( i_{p} \) are shown in Figure 3.6. As pH is decreased, \( i_{p} \) increases by approximately a factor of two while \( E_{p} \) shows minor variation. The lack of a pH dependence of \( E_{p} \) indicates no proton transfers are involved in the initial reduction reaction, consistent with electrogeneration of O\(_2\)^• at about -0.5 V vs. Ag/AgCl.
Figure 3.5. Voltammograms of 1 mM O₂ at different pH on methylphenyl derivatized GC, 200 mV/sec, background subtracted.
Figure 3.6. pH dependence of peak currents and peak potentials of oxygen reduction on methylphenyl derivatized GC.
Figure 3.7. $i_p$ vs $v^{1/2}$ for 1 mM O$_2$ at pH 14 and pH 7 on methylphenyl derivatized GC.
Figure 3.7 shows the relationship between peak current \( (i_p^*) \) and \( (v^{1/2}) \) on methylphenyl derivatized GC at pH 14 and pH 7. At pH 7, the plot of \( (i_p^*) \) vs. \( (v^{1/2}) \) is linear with the slope of 99.40 \( \mu \text{A} \cdot \text{sec}^{1/2}/V^{1/2} \), which is twice the slope observed at pH 14, 49.31 \( \mu \text{A} \cdot \text{sec}^{1/2}/V^{1/2} \).

The decrease in the reverse wave in Figure 3.5 as the pH is lowered and the increase in the cathodic peak current in Figure 3.6 and 3.7 are likely to be caused by the instability of superoxide ion in acidic or neutral solution. Superoxide reactions have been studied in detail by various authors, often with pause radiolysis [137]. Bielski et al. concluded a two step mechanism:

\[
\begin{align*}
\text{O}_2^- + \text{H}_2\text{O} & \rightarrow \text{HO}_2^- + \text{OH}^- \\
\text{HO}_2^- + \text{O}_2^- & \rightarrow \text{HO}_2 + \text{O}_2
\end{align*}
\]  
(3-32)

They combined Equation 3-32 and 3-33 into a disproportionation:

\[
\text{O}_2^- + \text{O}_2^- + (\text{H}_2\text{O}) \rightarrow \text{O}_2 + \text{HO}_2^- + (\text{OH}^-)
\]  
(3-34)

The observed rate of decline of the total radical concentration \( [R] = [\text{O}_2^-] + [\text{HO}_2^-] \) was determined as follows:

\[
k_{obs} = \frac{7.61 \times 10^5 + 1.58 \times 10^{pH-3}}{(1 + 1.78 \times 10^{pH-5})^2}
\]  
(3-35)
At pH higher than 6, the first terms in both numerator and denominator become negligible and \( k_{obs} = 5 \times 10^{(12\cdot \text{pH})} \). The homogeneous decay of \( \text{O}_2^- \) in water is controlled by the reaction of \( \text{HO}_2^- \) and \( \text{O}_2^- \) and the pH dependence arises from the equilibrium of \( \text{HO}_2^- \) and \( \text{O}_2^- \). If one assumes that the modified electrode serves only to generate \( \text{O}_2^- \) from \( \text{O}_2 \) and does not adsorb or react with \( \text{O}_2 \) reduction products, the disproportionation of \( \text{O}_2^- \) depicted in Equation 3-34 should occur in homogeneous solution.

A commercial simulation program [138] was used to calculate voltammograms for oxygen reduction on methylphenyl modified GC, according to the following mechanism (Figure 3.8):

\[
\begin{align*}
\text{O}_2 + e^- & \rightarrow \text{O}_2^- \\
\text{O}_2^- + \text{O}_2^- + (\text{H}_2\text{O}) & \rightarrow \text{O}_2 + \text{HO}_2^- + (\text{OH}^-)
\end{align*}
\]

Table 3.2 lists the parameters used for the simulation. Note that literature values were used for the diffusion coefficient of oxygen and the rate of Equation 3-37, and the only adjustable parameters in the simulation were \( k^0 \) and \( \alpha \). \( \alpha \) was assumed to equal 0.5, \( D_o \) and \( C_o^* \) were the literature value of \( 1.65 \times 10^{-5} \text{ cm}^2/\text{s} \) and 1 mM, respectively [113,136].

Comparison of simulated voltammograms based on the Equations 3-36 and 3-37 and the parameters listed in Table 3.2 are shown in Figure 3.9. Figure 3.10 shows the comparison of the plot of \( (i_p) \) vs. \( (v^{1/2}) \) from experiment and simulation data. Both of the Figures show that the calculated results agree with the experimental result except that the
Figure 3.8. Schematic illustration of the mechanism of oxygen reduction on methylphenyl derivatized GC.
Figure 3.9. Comparison of voltammograms from experiment and simulation of 1 mM O₂ at (A) pH = 14, (B) pH = 12 on methylphenyl derivatized GC, 200 mV/sec, background subtracted.
Figure 3.9. (continued) Comparison of voltammograms from experiment and simulation of 1 mM O$_2$ at (C) pH = 11, (D) pH = 10 on methylphenyl derivatized GC, 200 mV/sec, background subtracted.
Figure 3.9. (continued) Comparison of voltammograms from experiment and simulation of 1 mM O₂ at (E) pH = 9, (F) pH = 7 on methylphenyl derivatized GC, 200 mV/sec, background subtracted.
Figure 3.9. (continued) Comparison of voltammograms from experiment and simulation of 1 mM \( \text{O}_2 \) at (G) pH = 4, (H) pH = 0 on methylphenyl derivatized GC, 200 mV/sec, background subtracted.
Figure 3.10. Comparison of $i_p$ vs $v^{1/2}$ from experiment and simulation for 1 mM O$_2$ at pH 14 on methylphenyl derivatized GC.
Figure 3.11. Comparison of voltammograms from experiment and simulation of 1 mM O₂ at pH = 14 on methylphenyl derivatized GC, 200 mV/sec, background subtracted.
Reaction | $E^0$ (V) | $k^0$ (cm/s) | K | $k_f$ (M$^{-1}$ s$^{-1}$) |
--- | --- | --- | --- | --- |
modified (3-36) | $b$-0.38 | 0.003 |   |   |
(3-37) |   |   | $[7.61 \times 10^5 + 1.58 \times 10^{(pH+3)}]^c$ | $[1 + 1.78 \times 10^{(pH-5)}]^2$ |
   |   |   | For pH $> 6$, $5 \times 10^{(12-pH)}$ |

$^b$ From the average of $E_{1/2}$ in this work. $^c$ ref.137.

Table 3.2.  Parameters for the simulation of the oxygen reduction.

calculated reverse current is larger. The adjustment of $\alpha$ value could not eliminate this difference, however, it affects the peak shape (Figure 3.11). Thus, the mechanism proposed for the decay of $O_2^{••}$ occurring in homogeneous solution is suitable. At higher pH, the product of oxygen reduction is superoxide radical. As pH decreases, the reaction rate of Equation 3-37 increases, which shifts the product to hydrogen peroxide. This mechanism explains the main experimental observations. First, as shown in Figure 3.6, the peak current at pH 7 is twice of the peak current at pH 14. Second, at pH 7, the plot of peak current ($i_p^c$) vs. $(v^{1/2})$ is linear on methylphenyl modified GC with twice of slope compared to the slope at pH 14 (Figure 3.7).

Although chemical reversibility for the $O_2/O_2^{••}$ redox system at pH $> 10$ is very unusual, it is not surprising once the consequences of the pulse radiolysis results are appreciated. At pH 14, $k_{obs}$ is predicted by Equation 3-35 to be 0.05 M$^{-1}$s$^{-1}$. For 1 mM $O_2^{•}$
•, the initial decay rate of the $O_2^*$ is predicted to be $5 \times 10^{-8}$ Ms$^{-1}$ and it would require about 30 minutes for 10% of the $O_2^*$ to decay via reaction 3-37. At pH 10, 10% of the $O_2^*$ reacts in about 200 msec, but that is slow enough to observe an anodic peak for $O_2^*$ oxidation (Figure 3-5).

The thermodynamic reduction potential for $O_2/O_2^*$ redox couple has been evaluated to be -0.33 V vs. NHE or -0.535 V vs. Ag/AgCl for 1 atm of $O_2$ and unit activity of $O_2^*$ [113]. The electrochemical observed $E_{1/2}$ occurs when the concentration of $O_2$ equals the concentration of $O_2^*$ assuming the same diffusion coefficient. In the experiment, the concentration of $O_2$ is 1 mM instead of unit activity. Thus, the predicted $E_{1/2}$ was shifted positively by $0.059 \times \log_{10} 0.177$, 0.177 V, to be -0.358 V vs. Ag/AgCl.

Several papers has been devoted to first electron transfer in the oxygen reduction. Chevalet et al. reported on mercury electrodes in the presence of surfactants such as quinoline which can form compact hydrophobic films, the second electron transfer of oxygen was selectively blocked and the superoxide radicals were yielded as a main product [139]. They observed $E_{1/2} = -0.319$ V vs. Ag/AgCl. Xu et al. observed a small reverse wave in tetraethylammonium hydroxide electrolyte and reported $E_{1/2} = -0.325$ V vs. Ag/AgCl [123]. Choi et al. reported that the oxygen reduction was reasonably chemically reversible in alkaline solutions at the Co(II)$_2$DSP.$4H_2O$ modified carbon electrodes and with $E_{1/2} = -0.21$ V vs. Ag/AgCl [135]. The observed $E_{1/2}$ in this study (-0.381 V vs. Ag/AgCl) agrees to the predicted value, with less than 30 mV difference.

In summary, a chemically modified surface with a methylphenyl monolayer
covalently bonded to GC surface blocks the adsorption of the superoxide radical without blocking the electron transfer rate constant. This electrode is inert except for electron transfer and acts only to reduce oxygen to superoxide radical. The pH dependence of the oxygen reduction can be explained by the spontaneous uncatalyzed second-order decay of the superoxide radical in aqueous solution reported by Bielski et al.[137].

**Conventionally Polished GC and AC/IPA Cleaned GC**

The methylphenyl monolayer intentionally blocks adsorption sites on GC, so the superoxide reactions ensue by established solution phase routes. The unmodified GC surface permits adsorption steps, thus catalyzes the reactions of superoxide radical [123]. Figure 3.12 shows the comparison of the voltammograms on polished and AC/IPA cleaned GC at various pH. It shows the oxygen reduction is sensitive to pretreatment on bare surfaces. AC/IPA treatment alters oxygen voltammetry significantly, presumably by removing impurities and increasing the number of adsorption sites. Ranganathan et al. showed that the AC/IPA treatment yielded usually clean and reactive GC surfaces, and very high physisorption of catechols, methylene blue, etc [49]. The polished and AC/IPA treated surfaces are compared because they provide insight into the role of adsorption in oxygen reduction. Of the GC surfaces studied here, the AC/IPA treated surface is the cleanest and should have the most adsorption sites.

Since the following reaction involves proton transfer, the cyclic voltammograms of the reduction of O₂ at different pH were compared. The voltammograms at scan rate 200 mV/sec on conventionally polished GC are shown in Figure 3.13. The
Figure 3.12. Comparison of voltammograms of 1 mM O₂ at (A) pH = 14, (B) pH = 10.5 on polished and AC/IPA cleaned GC, 200 mV/sec, background subtracted.
Figure 3.12. (continued) Comparison of voltammograms of 1 mM O$_2$ at (C) pH = 9, (D) pH = 7 on polished and AC/IPA cleaned GC, 200 mV/sec, background subtracted.
Figure 3.12. (continued) Comparison of voltammograms of 1 mM O$_2$ at (E) pH = 4, (F) pH = 0 on polished and AC/IPA cleaned GC, 200 mV/sec, background subtracted.
Figure 3.13. Voltammograms of 1 mM O₂ at different pH on polished GC, 200 mV/sec, no background subtracted.
Figure 3.14. Voltammograms of 1 mM O₂ at different pH on AC/IPA cleaned GC, 200 mV/sec, background subtracted.
voltammograms at scan rate 200 mV/sec on AC/IPA cleaned GC are shown in Figure

<table>
<thead>
<tr>
<th>0.2 V/sec</th>
<th>Polished</th>
<th>AC/IPA cleaned</th>
<th>MP modified</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>$E_p^c$ (mV)</td>
<td>$i_p^c$ (μA)</td>
<td>$E_p^c$ (mV)</td>
</tr>
<tr>
<td>14</td>
<td>-334</td>
<td>61.5</td>
<td>-294</td>
</tr>
<tr>
<td>10.5</td>
<td>-354</td>
<td>49.0</td>
<td>-334</td>
</tr>
<tr>
<td>9</td>
<td>-553</td>
<td>47.9</td>
<td>-349</td>
</tr>
<tr>
<td>7</td>
<td>-562</td>
<td>45.5</td>
<td>-440</td>
</tr>
<tr>
<td>4</td>
<td>-561</td>
<td>49.4</td>
<td>-440</td>
</tr>
<tr>
<td>0</td>
<td>-445</td>
<td>54.7</td>
<td>-395</td>
</tr>
</tbody>
</table>

Table 3.3  Peak potential and peak current at different pH on polished, AC/IPA cleaned and methylphenyl modified GC.

3.14. Table 3.3 lists the reduction peak potentials and reduction peak currents on polished surfaces, AC/IPA cleaned surfaces and methylphenyl modified surfaces at different pH. The dependence of peak currents and peak potentials on pH is shown in Figure 3.15 and 3.16 (the more positive peak if there are two peaks). As reported by Taylor and Humffray, the voltammograms exhibit a very usually pH dependence, with the more positive peak decreasing with decreasing pH, and a peak developing at about -0.55 V vs. Ag/AgCl which shifts very slightly with pH [127]. On AC/IPA pretreated GC surface, the relative size of the more positive reduction peak increases, as shown in Figure 3.12. Figure 3.17 shows that the relative magnitudes of the two reduction peaks observed at pH 10.5 depend on scan rate, with the more positive peak decreasing the
relative size as scan rate is increased. Dependence is more dramatic on polished GC.

Table 3.4 Peak potential, peak current, and peak ratio at different pH on polished and AC/IPA cleaned GC.
Figure 3.15. pH dependence of peak potentials of oxygen reduction on polished, AC/IPA cleaned and methylphenyl derivatized GC.
Figure 3.16. pH dependence of peak currents of oxygen reduction on polished and AC/IPA cleaned GC.
Table 3.4 lists the peak potential, peak current, and peak ratio for different scan rate at pH 10, 10.5, and 11.

The plot of \((i_p, v^{1/2})\) on polished GC at pH 14 is linear with a slope of 98.53 \(\mu A \cdot sec^{1/2}/V^{1/2}\) (Figure 3.3). The expected slope for a chemically irreversible reaction can be estimated from the following equation [7]:

\[
\text{Slope} = \frac{i_p}{v^{1/2}} = 2.99 \times 10^2 \times n \times (\alpha n_a)^{1/2} \times D_o^{1/2} \times A \times C_o. \tag{3-38}
\]

where \(n\) is the total number of electrons involved in the redox reaction, \(\alpha\) is the transfer coefficient for the rate determining heterogeneous electron transfer, and \(n_a\) is the number of electrons involved in that step (usually 1). Given \(n = 2\), and \((\alpha n_a) = .5\), the calculated slope is 121.95 \(\mu A \cdot sec^{1/2}/V^{1/2}\), larger than that observed on conventionally polished GC, 98.53 \(\mu A \cdot sec^{1/2}/V^{1/2}\). However, on the AC/IPA surfaces, the slope of the plot of \((i_p, v^{1/2})\) is 130.1 \(\mu A \cdot sec^{1/2}/V^{1/2}\) (Figure 3.3), which is almost the same as the predicted slope for irreversible two electron transfer reaction, 121.95 \(\mu A\) (Equation 3-38). Thus, it is reasonable to conclude that \(O_2\) undergoes chemically irreversible two electron transfer to hydrogen peroxide on AC/IPA cleaned GC.

Several conclusions are available from the results which lead to a plausible oxygen reduction mechanism on unmodified GC. First, the voltammetric peak currents imply an overall two electron reduction as expected from many reports that \(H_2O_2\) or \(HO_2^{-}\).
Figure 3.17. Comparison of voltammograms of 1 mM O$_2$ at pH = 10.5 on polished and AC/IPA cleaned GC, (A) 20 mV/sec, (B) 100 mV/sec, no background subtracted.
Figure 3.17. (continued) Comparison of voltammograms of 1 mM O₂ at pH = 10.5 on polished and AC/IPA cleaned GC, (C) 200 mV/sec, (D) 1 V/sec, no background subtracted.
Figure 3.17. (continued) Comparison of voltammograms of 1 mM O$_2$ at pH = 10.5 on polished and AC/IPA cleaned GC, (E) 2 V/sec, no background subtracted.
is the reduction product [114-125]. Even when two reduction peaks are appeared (as pH 9 or 10.5), their heights imply two electrons, rather than one or four. Second, both reduction potentials are nearly pH independent, although their relative magnitudes change markedly between pH 12 and 7. Third, the more positive peak potential for the initial oxygen reduction on unmodified GC is consistent with Xu’s et al. conclusion attributing it to reaction 3-39 [123]. Adsorption of O$_2^\cdot$ is more pronounced on AC/IPA treated GC (hence the more positive $E_p$), and is prevented altogether on methylphenyl modified GC. Fourth, the more negative of the two reduction waves observed near pH 10 exhibits a reverse wave, at around -0.43 V vs. Ag/AgCl. Consider the mechanism of reactions 3-39 to 3-43 (Figure 3.18):

\[
\begin{align*}
O_2 + e^- & \rightarrow O_2^\cdot_{(ads)} & (3-39) \\
O_2^\cdot_{(ads)} + H_2O & \rightarrow HO_2^\cdot_{(ads)} + OH^- & (3-40) \\
HO_2^\cdot_{(ads)} + e^- & \rightarrow HO_2^-_{(aq)} & (3-41) \\
HO_2^\cdot_{(ads)} + O_2^\cdot_{(ads)} & \rightarrow HO_2^- + O_2 & (3-42) \\
HO_2^\cdot_{(ads)} + HO_2^\cdot_{(ads)} & \rightarrow H_2O_2 + O_2 & (3-43)
\end{align*}
\]

Xu et al. examined the reduction of oxygen in base on several different carbon electrode surfaces and concluded that the mechanism involved adsorption of O$_2^-$ and associated enhancement of proton transfer from water to O$_2^\cdot$ (Equation 3-40) [123]. They proposed that adsorption of O$_2^\cdot_{(ads)}$ make O$_2^\cdot$ become a better base. Without adsorption, O$_2^\cdot$ is a relatively weak base ($pK_{eq}^{40} = pK_b = pK_w - pK_a \approx 14 - 5 = 9$). When O$_2^\cdot$ is adsorbed on
Figure 3.18. Schematic illustration of the mechanism of oxygen reduction on bare GC.
the surface, the pK\textsubscript{a} for O\textsubscript{2}•(ads) shifts to around 10 (pK\textsubscript{eq} = pK\textsubscript{b} \equiv 14 - 10 = 4), thus making reaction 3-40 more favorable. It is possible that HO\textsubscript{2}•(ads) could react with HO\textsubscript{2}• or O\textsubscript{2}•, possibly themselves adsorbed, to yield H\textsubscript{2}O\textsubscript{2} and O\textsubscript{2} (reactions 3-42 and 3-43).

However an important alternative arises when the nature of "HO\textsubscript{2}•(ads)" is considered. If HO\textsubscript{2}•(ads) is bound to a surface radical site, the product is electronically identical to an aryl hydroperoxide of the general formula R-O-O-H. Each oxygen has an octet of electrons and the t-butyl analog is stable in water. According to Das \textit{et al.}, the pK\textsubscript{a} of hydroperoxides ranges from 8.9 to 12.8, and is 8.9 for C\textsubscript{6}H\textsubscript{5}OOH [140]. If C\textsubscript{6}H\textsubscript{5}OOH is a reasonable analog of HO\textsubscript{2}•(ads), then the pKa of the O\textsubscript{2}•(ads)/HO\textsubscript{2}•(ads) acid base pair would explain the increased rate of reaction 3-40 for O\textsubscript{2}•(ads) compared to O\textsubscript{2}•(aq). Note that reaction 3-40 is the same as that concluded by Allen and Bielski for O\textsubscript{2}•(aq).

The observed rate constant for the overall reaction may be derived from the steady state approximation of two intermediates, O\textsubscript{2}•(ads) and HO\textsubscript{2}•(ads), for the above mechanism if reaction 3-42 and 3-43 are neglected and then adding the two equations of the steady state approximation.

\[
\begin{align*}
    k_{\text{obs}} &= \frac{k_{39}k_{40}k_{41}}{k_{40}k_{41} + k_{39}k_{41} + k_{39}k_{40}[OH^-]} \\
    \text{(3-44)}
\end{align*}
\]

where \(k_{39}\) and \(k_{39}\) are the forward and backward rate constants of the first electron transfer step (reaction 3-39), respectively; \(k_{40}\) and \(k_{41}\) are the forward and backward rate
constants of the proton transfer step (reaction 3-40), respectively, and $k_{41}$ is the forward rate constants of the second electron transfer step (reaction 3-41). The inverse of the observed rate constant is as follows:

$$
\frac{1}{k_{\text{obs}}} = \frac{1}{k_{39}} + \frac{1}{K_{39}k_{40}} + \frac{[OH^-]}{K_{39}K_{40}k_{41}}
$$

(3-45)

where $K_{39}$ and $K_{40}$ are the ratio of the forward rate constant to the backward rate constant for the respective reactions.

By replacing $k_{39}$, $k_{39}$, and $k_{41}$ with potential-dependent rate constants (Butler-Volmer equation) [7], Equation 3-45 becomes:

$$
\frac{1}{k(E)} = \frac{1}{k_{39}^0 \exp[-\alpha_{39}F(E-E_{39}^0)/RT]} + \frac{1}{k_{40}^0 \exp[-F(E-E_{39}^0)/RT]}

+ \frac{[OH^-]}{K_{40}k_{41}^0 \exp[F(\alpha_{41}E_{41}^0 + E_{39}^0)/RT] \exp[-F(1+\alpha_{41})E/RT]}
$$

(3-46)

where $k(E)$ is the apparent rate constant for the overall reaction at an applied potential of $E$; $k_{39}^0$ and $k_{41}^0$ are the apparent rate constants for the first step (reaction 3-39) and third step (reaction 3-41), respectively; $E_{39}^0$ and $E_{41}^0$ are the formal potentials for reaction 3-39 and 3-41, respectively, and $\alpha_{39}$ and $\alpha_{41}$ are the electron transfer coefficients of reaction 3-
39 and 3-41, respectively. As indicated from Equation 3-46, if the reaction 3-39 is the rate determining step (i.e., $k_{39} \ll k_{40}$ and $k_{41}$)

$$k(E) = k_{39}^0 \exp\left[-\alpha_{39} F(E - E_{39}^0)/RT\right]$$

(3-47)

or if the reaction 3-40 is the rate determining step (i.e., $k_{40} \ll k_{39}$ and $k_{41}$)

$$k(E) = k_{40}^0 \exp\left[-F(E - E_{39}^0)/RT\right]$$

(3-48)

or if the reaction 3-41 is the rate determining step (i.e., $k_{41} \ll k_{39}$ and $k_{40}$)

$$k(E) = \frac{K_{40} k_{41}^0 \exp[F(\alpha_{41} E_{41}^0 + E_{39}^0)/RT] \exp[-F(1 + \alpha_{41}) E/RT]}{[OH^-]}$$

(3-49)

As seen from Equation 3-47, when the reaction 3-39 is the rate determining step, the apparent transfer coefficient is $\alpha_{39}$; when the reaction 3-40 is the rate determining step, the apparent transfer coefficient is 1, and when the reaction 3-41 is the rate determining step, the apparent transfer coefficient is $1 + \alpha_{41}$. Therefore by evaluating the apparent transfer coefficient, the rate determining step for the reduction of oxygen can be determined.

A similar analysis was applied by Hu and Kuwana [45] and Deakin et al. [46] to the successive electron transfers accompanying ascorbic acid and catechol oxidation.
Semi-integral analysis was used to determine $\alpha$ as a function of potential. The comparison of semi-integral cyclic voltammograms of the reduction of oxygen at various pH on polished, AC/IPA cleaned, and methylphenyl derivatized GC is shown in Figure 3.19. For a totally irreversible reaction, the relationship between the semi-integral current, $I(t)$, and the potential, $E$, can be expressed as [7]:

$$E = E^0 - \frac{RT}{nF} \ln \left( \frac{D^{1/2}}{k^0} \right) - \frac{RT}{nF} \ln \left[ \frac{i(t)}{I_i - I(t)} \right]$$

(3-50)

where $i(t)$ is the current at the time $t$, $I_i$ is the semi-integral diffusional limiting current, $n$ is the number of electrons in the rate-determining step, and $\alpha$ is the apparent transfer coefficient. As shown in Equation 3-50, the apparent transfer coefficient can be evaluated from the slope of $\ln \left[ \frac{i(t)}{I_i - I(t)} \right]$ vs. $E$. As can be seen from Figure 3.19, there is a slope change in the rising portion of the semi-integral current for the AC/IPA cleaned GC at pH 10.5 and 9 and for polished GC at pH 9. This can be seen more clearly from the plot of $\ln \left[ \frac{i(t)}{I_i - I(t)} \right]$ vs. $E$ as shown in Figure 3.20. Table 3.5 lists the calculated $\alpha$.

At pH 14, the apparent transfer coefficient is about 1 on AC/IPA cleaned GC. This value suggests the rate determining step is reaction 3-40. As pH decreases, the reaction rate of reaction 3-40 increases, which shifts the rate determining step to the reaction 3-39. Thus, the apparent transfer coefficient is about 0.5 for the more positive peak. However, it is not clear why the values of the apparent transfer coefficient for the more negative peak and at pH 7 is lower than 0.5 since this work stresses basic pH.
Figure 3.19. Comparison of semi-integrals of 1 mM O$_2$ at (A) pH = 14, (B) pH = 10.5 on polished, AC/IPA cleaned, and methylphenyl derivatized GC, 200 mV/sec.
Figure 3.19. (continued) Comparison of semi-integrals of 1 mM O₂ at (C) pH = 9, (D) pH = 7 on polished, AC/IPA cleaned, and methylphenyl derivatized GC, 200 mV/sec.
Figure 3.20. Comparison of $\ln \left[ i(t) / (I_1 - I(t)) \right]$ vs $E$ for Figure 3.19, (A) $pH = 14$, (B) $pH = 10.5$. 
Figure 3.20. (continued) Comparison of $\ln [i(t)/(I_0 - I(t))]$ vs $E$ for Figure 3.19, (C) pH = 9, (D) pH = 7.
<table>
<thead>
<tr>
<th>$\alpha$</th>
<th>Polished</th>
<th>AC/IPA cleaned</th>
<th>MP modified</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 14</td>
<td>0.78</td>
<td>0.94</td>
<td>0.55</td>
</tr>
<tr>
<td>pH 10.5</td>
<td>$^d$0.39</td>
<td>$^e$0.29</td>
<td>$^d$0.52</td>
</tr>
<tr>
<td>pH 9</td>
<td>0.29</td>
<td>$^d$0.55</td>
<td>$^e$0.18</td>
</tr>
<tr>
<td>pH 7</td>
<td>0.29</td>
<td>0.23</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Note: $^d$ calculated between -0.2 and -0.35 V vs. Ag/AgCl.

Note: $^e$ calculated between -0.35 and -0.6 V vs. Ag/AgCl.

Table 3.5 Calculated $\alpha$ from the plot of ln $[i(t)/(I_1 - I(t))]$ vs. E.

$O_2$• adsorption, protonation, and disproportionation as presented by reactions 3-39 to 3-43 account for the chemically irreversible two electron reduction on bare GC, at least qualitatively. The second reduction peak observed near pH 10 is attributed to the reduction of oxygen without $O_2$• adsorption, as observed on the methylphenyl modified surfaces (reaction 3-36). As pH decreases and/or scan rate increases, the peak ratio for the more positive peak current to the more negative peak current is smaller. In other words, the reduction of oxygen without $O_2$• adsorption becomes the major reaction at lower pH and/or faster scan rate. It could be explained by the availability of the sites to adsorb $O_2$• is lower at lower pH and/or faster scan rate. The stability of $HO_2$•(ads) is proposed for the difference in the availability of sites with pH and/or scan rate. If the decomposition of $HO_2$•(ads) were slow, in other words, $HO_2$•(ads) is stable, then the surface radical sites might all be occupied by $HO_2$•(ads). In this case, no sites are available to
adsorb $O_2^\cdot -$ and the $O_2/O_2^\cdot -$ couple reverts to its chemically reversible behavior such as that on methylphenyl modified surfaces. This is the case at lower pH and/or faster scan rate, $HO_2^\cdot -(ads)$ builds up to the point where it blocks reaction 3-39. The reverse wave observed at about -0.43 V vs. Ag/AgCl at pH 10.5 is apparently due to the oxidation of $O_2^\cdot -(aq)$ on methylphenyl modified GC, but with faster electron transfer kinetics. At pH < 9, the oxygen reduction mechanism on AC/IPA or polished GC is similar to that on the methylphenyl modified GC, with $O_2^\cdot -$ degradation occurring primarily in homogeneous solution. At pH 7, 4, and 0, the differences in the reduction peak potential appears to be due to differences in the electron transfer rate for $O_2^\cdot -$ generation on the three surfaces (Table 3.3). On the other hand, at high pH and/or slow scan rate, $HO_2^\cdot -(ads)$ decomposition is fast enough to create adsorption sites for $O_2^\cdot -$ thus reaction 3-40 accelerates.

This proposed statement also explained the deviation from linear behavior for the plot of $(i_p)$ vs. $(v^{1/2})$ on the polished surfaces at pH 10.5 (Figure 3.21). At higher scan rate, there is not enough sites to adsorb $O_2^\cdot -$ since the mass transport of $O_2$ is faster compared to the decomposition of $HO_2^\cdot -(ads)$, thus the current is smaller. Of the three surfaces studied, the adsorption sites on polished GC is the intermediate between that on AC/IPA cleaned GC (most cleaned surfaces, with the most adsorption sites) and that on methylphenyl modified GC (no adsorption sites).

The nature of the adsorption site for $O_2^\cdot -$ is not obvious, with the only certainty being the ability to block the site with a methylphenyl monolayer. Previous reports indicate that anodization of the surface increases the activity for $O_2$ reduction but it is not
Figure 3.21. Comparison of $i_p$ vs $v^{1/2}$ for 1 mM O$_2$ at pH 10.5 on polished and AC/IPA cleaned GC.
clear that surface oxides are involved [123]. O\textsubscript{2} reduction is indeed accelerated by a surface oxidation [123] or adsorbed quinones [120], but reaction 3-40 appears to proceed on surfaces with low oxide coverage. For example, the AC/IPA treatment decreases the O/C ratio of polished GC [49] but oxygen reduction is faster on AC/IPA treated GC. A likely candidate for the adsorption site is a surface radical or "dangling bond". As noted earlier, adsorption of O\textsubscript{2}\textsuperscript{*} to a surface radical results in a relatively stable peroxide or hydroperoxide which is not a radical. The current results do not prove that a surface radical is the active site, but such an hypothesis is consistent with the observations.

In summary, the reduction of oxygen on polished and AC/IPA cleaned GC is two electron reduction. the reduction of hydrogen peroxide is not observable because the linearity of (i\textsubscript{p} \textsuperscript{5}) with (v\textsuperscript{1/2}) on the AC/IPA cleaned surface is consistent with an overall two electron reduction to HO\textsubscript{2}\textsuperscript{-aq}, with the first electron transfer being rate limiting for pH 14. AC/IPA treatment increased the adsorption of the superoxide radical. pH and AC/IPA treatment affect the ratio of the peak current at the pH where there are two peaks.

Conclusions

In conclusion, the reduction of oxygen is surface sensitive and pH dependent. When the surface is covered by an inert chemisorbed monolayer, the adsorption of superoxide radical is inhibited, thus, the reaction of superoxide radical with water is slowed down compared to the reaction rate of adsorbed superoxide radical with water. At pH 14, the cyclic voltammograms of the reduction of oxygen on the methylphenyl
derivatized GC is reversible due to the stability of the superoxide on the time scale of voltammograms. However, the reverse oxidation peak of the reduction of oxygen decreases as the pH decreases due to the homogeneous reaction of the superoxide with water increases with the decrease of pH.

When the GC surface is exposed by pretreatment with isopropanol and activated carbon, electrogenerated $\text{O}_2^\cdot$ adsorbs to form a species which is electronically similar to a deprotonated hydroperoxide, ROO'. This species is more basic than $\text{O}_2^\cdot$$_{(\text{aq})}$ and accepts a proton via reaction 3-40. The critical consequence of $\text{O}_2^\cdot$ adsorption is the change in pK$_a$ of $\text{O}_2^\cdot$$_{(\text{ads})}$. The result is a change in the rate determining step from $\text{O}_2^\cdot$ protonation (reaction 3-40) for pH > 12 to $\text{O}_2$ reduction (reaction 3-39) for pH < 10. As the pH is decreased to about 9, the adsorbed $\text{O}_2^\cdot$ protonates to form a relative stable hydroperoxide analog of ROOH. This species appears to occupy adsorption sites, thus preventing further $\text{O}_2^\cdot$ adsorption. As a result, the oxygen reduction reverts to its outer-sphere route without further adsorption. As a consequence, the oxygen reduction mechanism on clean GC is similar to that on the MP monolayer. Although, the surface is initially very active toward adsorption, an intermediate in the oxygen reduction process ($\text{HO}_2^\cdot$$_{(\text{ads})}$) rapidly blocks adsorption sites. Except for somewhat faster electron transfer, the clean surface behaves like the methylphenyl modified surface toward oxygen reduction.
CHAPTER 4

EFFECTS OF SURFACE MODIFICATIONS ON THE OXIDATION OF NADH ON GLASSY CARBON ELECTRODES

Introduction

Dihydronicotinamide adenine dinucleotide (NADH) and dihydronicotinamide adenine dinucleotide phosphate (NADPH) are the reduced forms of the pyridine coenzymes, nicotinamide adenine dinucleotide (NAD') (Figure 4.1) and nicotinamide adenine dinucleotide phosphate (NADP'). These coenzymes participate in a large number of enzyme catalyzed reactions in which they undergo a two-electron reduction to form the corresponding reduced forms. An important biological function of the pyridine coenzymes is their involvement in the electron-transport system for reduction of oxygen to water. In this coupled enzymic reaction chain, NADH is oxidized by riboflavin containing compounds which are known to form stable semiquinone intermediates [141]. Thus, NADH appears to be oxidized at the site in the electron-transfer chain at which transition from one-electron to two-electron control occurs. Accordingly, much effort has been directed toward establishing the mechanism of hydrogen transfer in the redox reactions of the pyridine coenzymes [142].
Figure 4.1. Structure of β-nicotinamide-adenine dinucleotide (NAD⁺) and its reduced form, NADH.
The electrochemical oxidation of NADH to NAD\(^+\) has been extensively studied on glassy carbon [42,143-150] and platinum electrodes [143,145,147,148,151,152] and some of those results are summarized here. In aqueous electrolytes, NADH is cleanly converted to enzymatically active NAD\(^+\) in a two-electron, one-proton process [145,152]. The mechanism of the conversion of NADH to NAD\(^+\) and the electrocatalytic oxidation of NADH are the two major topics studied in the past years.

Several mechanistic studies have been done on NADH or on model compounds [143,145,151,153-155]. It has been discussed in the literature whether the reaction is a two electron transfer or involves two successive one-electron transfer steps. Jaegfeldt used a more precise diagnostic test than those used earlier and showed that the evidence for consecutive one electron transfer steps became stronger at higher concentration [151]. Discussions on whether an acid-base reaction is involved in the rate limiting step have also been made [143,151]. Tests at higher NADH concentrations indicate a second order pH dependent chemical reaction, and the following scheme was proposed [151]:

\[
\begin{align*}
\text{NADH} & \rightarrow \text{NADH}^\bullet \rightarrow \text{NAD}^\bullet \rightarrow \text{NAD}^+ \\
\text{-e} & \quad \text{-H}^+ & \quad \text{-e} & \quad \text{-e}
\end{align*}
\]  

This scheme was tentative and did not explain all the experimental observations. Jensen and Elving concluded that there is still considerable uncertainty about the detailed nature of the electrochemical oxidation of NADH [156]. In a later work by Samec and Elving, they proposed that the oxidation of NADH at solid Pt and Au electrodes might occur.
through a mediated mechanism [143]. Two mechanisms were envisaged for the proton transfer path [143]. In one, electron transfer through the intermediate state located close to the surface oxygen atom and proton transfer are decoupled from each other, i.e., the proton bound to C (4) of NADH is not involved in the activated complex formed before electron transfer may occur. In the second mechanism, a bond is formed between the transferred proton and the surface atoms, and the intermediate state in the electron transfer path were presented by NAD* and OH*_{ads}(H_{2}O). The proposed mechanism is particularly interesting because it gives some hope that studies of chemically modified electrodes might help in the interpretation of reactions on metal electrodes.

It is generally found that the direct electrochemical oxidation of reduced nicotinamide coenzymes is electrochemically irreversible and requires a high overvoltage at bare electrodes. The high overpotential usually limit the use of bare electrodes for the direct electrochemical detection of NADH generated in enzymatic reactions. Therefore, there have been numerous attempts to enhance the electron transfer kinetics using various types of mediators. One way to increase the electron transfer rate and decrease the large overpotential is the introduction to the analyte solution of an electron transfer mediator that (i) in a first step reacts very quickly with NADH and (ii) in another step is reoxidized electrochemically at a potential substantially lower than that at which NADH is directly oxidized. The reaction scheme including the mediator is

\[
\begin{align*}
\text{NAD}^+ & \leftrightarrow M_{\text{red}} \\
\text{NADH} & \leftrightarrow M_{\text{ox}} \quad \downarrow H^+ 
\end{align*}
\]
where $M_{\text{ox}}$ and $M_{\text{red}}$ denote the oxidized and the reduced forms of the mediator, respectively.

Homogeneous electron transfer mediators have been successfully used for the detection of NADH. Ortho- and para-quinones, quinone imines, and phenylene diimines are the mediators which have been found to provide redox coupling between electrodes and NADH [157,158]. Chemical modification of electrode surfaces is another way to provide mediators and is a powerful tool for giving the electrode electrocatalytic properties [159,160]. It was shown in many instances that an electrode with a surface immobilized redox mediator displays similar or the same properties as those of the soluble mediator [160]. A more compact design of the NADH mediating system, mentioned above, would thus be to attach the mediator directly to the electrode surface; the mediator could then be reused for many cycles.

The introduction of the mediator functionality on an electrode can be achieved in either of two ways. (i) A drastic oxidation of a carbon electrode introduces in a rather unselective way oxygen containing functionalities on its surface [161]. In a later paper, it was clearly demonstrated that ortho-quinone groups were produced [162]. (ii) A mediator that is known to react quickly with NADH homogeneously in solution may be immobilized on the electrode surface. The attachment of the compound can be done in different ways: covalent binding to or adsorption on the electrode surface, inclusion into the electrode material or the introduction of the mediator into a polymeric backbone that is deposited on the electrode surface. As given in Table 4.1 (adapted from ref. 163), the initial reports involved attachments of molecules containing ortho-quinone groups [156]
<table>
<thead>
<tr>
<th>Mediator</th>
<th>Immobilization</th>
<th>$E^0_{\text{pH} 7}$ (V vs. SCE)</th>
<th>$\Delta E_p$ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>orthoquinone$^{164}$</td>
<td>covalent</td>
<td>0.170</td>
<td>200-250</td>
</tr>
<tr>
<td>orthoquinone$^{165}$</td>
<td>covalent</td>
<td>0.170</td>
<td>200-250</td>
</tr>
<tr>
<td>orthoquinone$^{166}$</td>
<td>polymer</td>
<td>0.160 (pH 6.35)</td>
<td>200-250</td>
</tr>
<tr>
<td>orthoquinone$^{167}$</td>
<td>polymer</td>
<td>0.170</td>
<td>200-250</td>
</tr>
<tr>
<td>orthoquinone$^{168}$</td>
<td>polymer</td>
<td>0.170</td>
<td>200-250</td>
</tr>
<tr>
<td>orthoquinone$^{169}$</td>
<td>adsorbed</td>
<td>0.170</td>
<td>200-250</td>
</tr>
<tr>
<td>orthoquinone$^{170}$</td>
<td>adsorbed</td>
<td>0.100</td>
<td>300</td>
</tr>
<tr>
<td>1.2-naphthoquinone$^{171}$</td>
<td>mixed with carbon</td>
<td>-0.150</td>
<td>500</td>
</tr>
<tr>
<td>N-methyl phenazinium$^{172}$</td>
<td>adsorbed</td>
<td>-0.160</td>
<td>500</td>
</tr>
<tr>
<td>N-ethyl phenazinium$^{172}$</td>
<td>adsorbed</td>
<td>-0.210</td>
<td>550</td>
</tr>
<tr>
<td>chloranil$^{173}$</td>
<td>mixed with carbon</td>
<td>0.050</td>
<td>350</td>
</tr>
<tr>
<td>phenylenediimines$^{174}$</td>
<td>mixed with carbon</td>
<td>0.100</td>
<td>300</td>
</tr>
<tr>
<td>Meldola Blue (phenoxazine)$^{175,177}$</td>
<td>adsorbed</td>
<td>0.175</td>
<td>550</td>
</tr>
<tr>
<td>3-β-naphthoyl-Nile Blue (phenoxazine)$^{176}$</td>
<td>adsorbed</td>
<td>-0.220</td>
<td>600</td>
</tr>
<tr>
<td>1,2-benzophenoxazine-7-one$^{178}$</td>
<td>adsorbed</td>
<td>-0.210</td>
<td>550</td>
</tr>
</tbody>
</table>

Table 4.1. Chemically modified electrodes for electrocatalytic oxidation of NADH (adapted from ref. 163).
followed by alkylphenazinium [172], para-quinone [173], phenlyenediimines [174], and phenoxazines [175-178].

In this chapter, the NADH oxidation on the nitrophenyl derivatized GC at pH 7 is described. In a previous chapter (Chapter 2), the surface effects on the electron transfer kinetics of methyl viologen and phenothiazines and on the adsorption of phenothiazines were discussed. The kinetics of these redox systems are not affected by the surface structure, only their adsorption is inhibited by the chemically modified surfaces. Since NADH oxidation is believed to involve adsorption, the effects of chemical modification should be pronounced. Our objective is an understanding of the effects of adsorption on carbon surfaces in NADH oxidation kinetics by studying carbon surfaces which were modified to suppress adsorption.

Experimental

Reagents

Tetrabutylammonium tetrafluoroborate (NBu$_4$BF$_4$), 50 % fluoboric acid, and 4-nitroaniline were purchased from Aldrich Chemical Company and were used as received. Sodium nitrite and NADH (grade III) were purchased from Sigma Chemical Company and were used as received. Phosphate saline buffer solution was prepared from 4/5 mole ratio of K$_2$HPO$_4$·3H$_2$O (reagent grade, J. T. Baker) to KH$_2$PO$_4$(reagent grade, J. T. Baker), NaCl (reagent grade, J. T. Baker), and adding NaOH (Millinckrodt) to the desired pH. The total concentration of the buffer components was 0.1 M and 0.5 M of NaCl was
added to adjust the ionic concentration. All electrolytes were made fresh before use.

**Synthesis of Diazonium Tetrafluoroborate Salts**

Diazonium tetrafluoroborate salts were synthesized according to the procedure described in Chapter 2.

**Electrode Materials and Polished Procedure**

Commercial glassy carbon (GC 20) electrodes from Bioanalytical Systems Inc. (MF2070) were used in this work. Preparation procedures for the electrodes were stated in the Chapter 2.

**Specific Adsorption**

Chemisorption of nitrophenyl radicals on GC surfaces was accomplished by the procedure described in Chapter 2.

**Electrolysis Procedures**

Electrolysis was performed in a three-electrode cell. The reference electrode was Ag/AgCl/3 M KCl with a glass frit, the auxiliary electrode was a platinum wire. A small volume of 1 mM NADH in pH 7 phosphate saline buffer (ca. 3 ml) was added to the cell and was saturated with argon (Linde Gas, pre-purified) for 20 min prior to electrolysis. The stirred solution was electrolyzed at 0.7 V vs. Ag/AgCl for 20 minutes. Argon was continuously passed through the cell during electrolysis.
Results

Conventionally Polished GC

Cyclic voltammograms of the oxidation of NADH were acquired in pH 7 phosphate saline solution. The typical voltammograms at different scan rate (0.02 V/sec to 102 V/sec) on conventionally polished GC are shown in Figure 4.2. The irreversible peak indicates there is a following chemical reaction for the oxidation product. The plot of peak current ($i_p$) vs. (scan rate, $v$)$^{1/2}$ is linear with a slope of 54.09 $\mu$A·sec$^{1/2}$/V$^{1/2}$ and a $r^2$ of 0.9999 (Figure 4.3). This shows that the peak current is due to a diffusion controlled process. The peak potential ($E_p$) exhibits a positive linear shift with increasing log ($v$) with a slope of 55.91, an intercept of 549.31 (mV), and a $r^2$ of 0.9941 (Figure 4.4).

Figure 4.5 shows the cyclic voltammogram of NADH oxidation at wider scan range (0.8 V - -1.4 V vs. Ag/AgCl). Around the potential of -1.05 V, a peak absent in the first segment shows up in the third segment. This is the reduction peak of NAD$^+$, the oxidation product of the NADH produced in the second segment. The peak potential agrees with the literature result of -1.0 V [179].

Figure 4.6 show the ultraviolet absorption spectra obtained by a 1/20 dilution of 1 mM NADH in pH 7 phosphate saline buffer solution before electrolysis and after electrolysis on polished GC. Before electrolysis, the NADH solution has two ultraviolet absorption maxima, one at 265 nm ($\epsilon = 15,820$ L cm$^{-1}$ mol$^{-1}$) and the other at 345 nm ($\epsilon = 6.282$ L cm$^{-1}$ mol$^{-1}$). After electrolysis, the solution has a single ultraviolet absorption maxima at 265 nm ($\epsilon = 17,373$ L cm$^{-1}$ mol$^{-1}$). The wavelength and $\epsilon$ agree with a earlier
Figure 4.2. Voltammograms of 1 mM NADH in 0.1 M phosphate buffer containing 0.5 M NaCl, pH = 7 at different scan rate on polished GC.
Figure 4.3. $i_p$ vs $v^{1/2}$ for 1 mM NADH in 0.1 M phosphate buffer containing 0.5 M NaCl, pH = 7 on polished GC and nitrophenyl derivatized GC.
Figure 4.4. $E_p$ vs log $v$ for 1 mM NADH in 0.1 M phosphate buffer containing 0.5 M NaCl, pH = 7 on polished GC and nitrophenyl derivatized GC.
Figure 4.5. Voltammogram of 1 mM NADH in 0.1 M phosphate buffer containing 0.5 M NaCl, pH = 7 on polished GC, 200mV/sec.
Figure 4.6. UV spectra of electrolysis of 1 mM NADH in 0.1 M phosphate buffer containing 0.5 M NaCl, pH = 7 (1/20 dilution) on polished GC and nitrophenyl derivatized GC.
report made by Braun et al. and Schmakel et al. [145,179]. The single band at 265 for the electrolyzed NADH solution is due to absorption by both the adenine and pyridine moieties. For NADH, absorption due to the pyridine moiety shifts to a longer wavelength so that two bands are seen, one at 265 nm and the other at 345 nm [179].

**Cyclohexane Polished GC**

Anaerobic polishing with cyclohexane/alumina produced a GC surface with low oxide coverage [36]. Typical voltammograms at different scan rate (0.02 V/sec to 102 V/sec) on cyclohexane polished GC are shown in Figure 4.7. Similar to the conventionally polished GC, the peak current ($i_p$) vs. ($v^{1/2}$) is linear and the peak potential ($E_p$) exhibits a positive linear shift with increasing log ($v$). Changes in surface oxide coverage had little observable effect on the observed voltammetry, implying that surface oxides are not important to kinetics.

**Nitrophenyl Derivatized GC**

A chemically modified surface with a nitrophenyl monolayer covalently bonded to GC surface was achieved by the reduction of nitrophenyl diazonium salt at -0.16 V vs. Ag/Ag⁺ for 10 minutes. Figure 4.8 shows the typical voltammograms of NADH oxidation at different scan rate (0.02 V/sec to 102 V/sec) on nitrophenyl derivatized GC. As on conventionally polished GC, an irreversible peak appears. The plot of peak current ($i_p$) vs. ($v^{1/2}$) is linear with the slope of 27.02 μA·sec⁻¹/V⁻¹/₂ and $r^2$ of 0.9961 (Figure 4.3). This shows that the peak current is due to a diffusion controlled process but
Figure 4.7. Voltammograms of 1 mM NADH in 0.1 M phosphate buffer containing 0.5 M NaCl, pH = 7 at different scan rate on cyclohexane polished GC.
Figure 4.8. Voltammograms of 1 mM NADH in 0.1 M phosphate buffer containing 0.5 M NaCl, pH = 7 at different scan rate on nitrophenyl derivatized GC.
with about half of the slope for the same plot on polished GC, 54.09 μA·sec^{1/2}/V^{1/2}. The peak potential (E_p) exhibits a positive linear shift with increasing log (v) with a slope of 56.13, an intercept of 770.02 (mV), and a r^2 of 0.9970 (Figure 4.4). Figure 4.9 shows the comparison of the cyclic voltammograms of NADH oxidation on the conventionally polished GC, cyclohexane polished GC, and nitrophenyl derivatized GC. The oxidation of NADH is slower on the nitrophenyl derivatized GC and the peak current is about half of the peak current on the conventionally polished GC.

Figure 4.10 shows the comparison of cyclic voltammograms of NADH oxidation at a wider scan range (0.8 V - -1.4 V vs. Ag/AgCl) on conventionally polished GC and nitrophenyl derivatized GC. Unlike on the conventionally polished GC, no peak shows up around the potential of -1.05 V on the nitrophenyl derivatized GC in the second segment. In other words, the oxidation product of the NADH on the nitrophenyl derivatized GC is not NAD^+.

Figure 4.6 show the ultraviolet absorption spectra obtained by a 1/20 dilution of 1 mM NADH in pH 7 phosphate saline buffer solution before electrolysis and after electrolysis on nitrophenyl derivatized GC. Before electrolysis, NADH solution has two ultraviolet absorption maxima, one at 265 nm (ε = 15,820 L cm^{-1} mol^{-1}) and the other at 345 nm (ε = 6,282 L cm^{-1} mol^{-1}). After electrolysis, the electrolyzed solution has two ultraviolet absorption maxima, one at 265 nm (ε = 33,037 L cm^{-1} mol^{-1}) and the other at 345 nm (ε = 7,035 L cm^{-1} mol^{-1}). The wavelength and ε of the electrolyzed solution agree with those reported earlier for the dimer (NAD)_2 by Schmakel et al. [179].
Figure 4.9. Comparison of voltammograms of 1 mM NADH in 0.1 M phosphate buffer containing 0.5 M NaCl, pH = 7 at (A) 20 mV/sec (B) 200 mV/sec on polished GC, cyclohexane polished GC, and nitrophenyl derivatized GC.
Figure 4.9. (continued) Comparison of voltammograms of 1 mM NADH in 0.1 M phosphate buffer containing 0.5 M NaCl, pH = 7 at (A) 2 V/sec (B) 102 V/sec on polished GC, cyclohexane polished GC, and nitrophenyl derivatized GC.
Figure 4.10. Comparison of voltammograms of 1 mM NADH in 0.1 M phosphate buffer containing 0.5 M NaCl, pH = 7 on polished GC and nitrophenyl derivatized GC, 200 mV/sec.
Discussions

The reversible redox behavior exhibited by the NADH/NAD⁺ couple under physiological conditions has prompted extensive electrochemical study of the reaction.

\[ \text{NADH} + \text{H}^+ + 2e^- \rightarrow \text{NADH} \]  \hspace{1cm} (4-2)

Moiroux and Elving studied the kinetics of NADH oxidation on various carbon electrodes and proposed an electron-proton-electron mechanism in which NADH was converted to its cation radical, which lost a proton to solvent giving a radical. This was in turn oxidized to NAD⁺ [148]. Moiroux and Elving also presented the evidence for an adsorption-controlled step [147]. Using the chemical modifications of the carbon surface which have been shown to suppress adsorption of simpler redox systems such as methylene blue and chlorpromazine [180], the effects of adsorption in the NADH oxidation was clarified.

In aqueous media, NADH shows a single diffusion controlled cyclic voltammetric anodic peak; scan reversal produces a cathodic peak due to NAD⁺ on conventionally polished GC. A cathodic peak complementary to the anodic peak, as expected for a reversible redox couple, was not seen at the highest scan rate used (102 V/sec). A conclusion can be drawn from this observation. The irreversible cyclic voltammogram indicates the oxidized product of NADH in the first electron transfer step undergoes a fast chemical reaction compared to the time scale in which cyclic voltammogram was taken.
Second, based on the previous reports, NADH undergoes a two electron oxidation to yield NAD$^{+}$ [143-150].

The diffusion coefficient of NADH can be evaluated from the characteristics of the voltammetric peak at the stationary GC, by using Equation 4-3 for an irreversible 2 electron transfer [7].

$$i_p = 2.99 \times 10^5 \times n \times (\alpha n_a)^{1/2} \times D^{1/2} \times A \times C^* \times v^{1/2}$$ (4-3)

where $n$ is the total number of electrons involved in the redox reaction, $\alpha$ is the transfer coefficient for the potential determining heterogeneous electron transfer, $n_a$ is the number of electrons involved in that step (usually 1), $D$ is the diffusion coefficient of the analyte, $A$ is the area of the electrode, $C^*$ is the bulk concentration of the analyte, and $v$ is linear scan rate. Given $n = 2$, $(\alpha n_a) = .5$, $A = 0.071$ cm$^2$, $C^* = 1$ mM, the slope of peak current ($i_p^a$) vs. ($v^{1/2}$) = 54.09 $\mu$A·sec$^{1/2}$/V$^{1/2}$ on conventionally polished GC (Figure 4.3), the calculated diffusion coefficient is $3.2 \times 10^{-6}$ cm$^2$s$^{-1}$. This result is compatible with the result previous reported ($2.2 \times 10^{-6}$ cm$^2$s$^{-1}$) [148].

On the nitrophenyl derivatized GC, the plot of peak current($i_p^a$) vs. ($v^{1/2}$) is linear with the slope of 27.02 $\mu$A·sec$^{1/2}$/V$^{1/2}$ (Figure 4.3) which is approximately half of the slope (54.09 $\mu$A·sec$^{1/2}$/V$^{1/2}$) on the conventionally polished GC. Since the experiment was done under the same conditions as the conventionally polished GC, the only difference is the surface structure of the electrode but not the diffusion coefficient. $A$, or
C°. Thus, according to Equation 4-3, n is the only parameter which can change the slope of the plot of peak current (i_p) vs. (v^{1/2}) if the change of (\alpha n_a) is neglected. Since the slope of the plot of peak current (i_p) vs. (v^{1/2}) on the conventionally polished GC is twice of that on the derivatized GC, the value of n on the conventionally polished GC is also twice of that on the derivatized GC. This means the mechanism of NADH oxidation changes from an irreversible two electron transfer process to irreversible one electron transfer process.

For an irreversible process, E_p is a function of scan rate given by Equation 4-4 [7]:

\[
E_p = E^0 - (RT/\alpha n_a F)(0.78 + \ln(D^{1/2}/k^0) + \ln (\alpha n_a F v/RT)^{1/2}) \tag{4-4}
\]

where F is the Faraday constant and \( k^0 \) is the heterogeneous electron transfer rate constant. Given \( R = 8.314 \text{ J/K\cdot mol} \), \( T = 298 \text{ K} \), and \( F = 96485 \text{ C} \) in Equation 4-4, it can be expressed as following equation:

\[
E_p = K + (0.0294/\alpha n_a) \times \ln (v) \tag{4-5}
\]

where \( K \) is \( E^0 - (RT/\alpha n_a F)(0.78 + \ln(D^{1/2}/k^0) + \ln (\alpha n_a F/RT)^{1/2}) \).

On the conventionally polished GC, the plot of peak potential (E_p) vs. log (v) exhibits a positive linear shift with increasing log (v) with a slope of 55.91, an intercept of 549.31 (mV), and a \( r^2 \) of 0.9941 (Figure 4.4). According to Equation 4-5, the
calculated $\alpha_n$ is 0.53. On nitrophenyl derivatized GC, the same plot ($E_p$ vs. $v$) also exhibits a positive linear shift with increasing log ($v$) with a similar slope of 56.13. Thus, the calculated $\alpha_n$, 0.52, on nitrophenyl derivatized GC is compatible to the calculated $\alpha_n$, 0.53, on conventionally polished GC. The obvious difference between the plot for two different surfaces is the intercept (770.02 mV for nitrophenyl derivatized GC and 549.31 mV for conventionally polished GC) which results from the different $k^0$ on the two different surfaces.

Cyclic voltammograms of NADH oxidation at wider scan range (0.8 V - -1.4 V vs. Ag/AgCl) provide the second evidence of changing redox kinetics of NADH. NADH shows a single diffusion controlled cyclic voltammetric anodic peak; scan reversal produces a cathodic peak due to NAD$^+$ reduction on conventionally polished GC. However, on nitrophenyl derivatized GC, there is no cathodic peak near the reduction potential of NAD$^+$ in the reverse segment (Figure 4.10). Based on this observation, we conclude the product of oxidation of NADH on the nitrophenyl derivatized GC is not NAD$^+$, which is the oxidation product of NADH on the conventionally polished GC.

Ultraviolet absorption spectra data support the conclusion that NADH oxidation kinetics change on the nitrophenyl derivatized GC (Figure 4.11). As shown in Figure 4.6, before electrolysis, NADH solution has two ultraviolet absorption maxima, one at 265 nm ($\varepsilon = 15,820 \text{ L cm}^{-1} \text{ mol}^{-1}$) and the other at 345 nm ($\varepsilon = 6,282 \text{ L cm}^{-1} \text{ mol}^{-1}$). After electrolysis on conventionally polished GC, the solution has a single ultraviolet absorption maxima at 265 nm ($\varepsilon = 17,373 \text{ L cm}^{-1} \text{ mol}^{-1}$). The wavelength and $\varepsilon$ agree with those reported earlier by Schmakel et al. [179]. The absorption of NAD$^+$ at 265 is
Figure 4.11. Ultraviolet absorption spectra data of NADH and its oxidation products (numbers in parentheses are literature value [169]).
due to absorption by both the adenine and pyridine moieties. For NADH, absorption due
to the pyridine moiety shifts to a longer wavelength so that two bands are seen, one at 265
nm and the other at 345 nm [179]. However, the electrolyzed solution on the nitrophenyl
derivatized GC has two ultraviolet absorption maxima, one at 265 nm ($\varepsilon = 33,037 \text{ L cm}^{-1}\text{ mol}^{-1}$) and the other at 345 nm ($\varepsilon = 7,035 \text{ L cm}^{-1}\text{ mol}^{-1}$). The wavelength and $\varepsilon$ of the
electrolyzed solution agree with those reported earlier for the dimer, (NAD)$_2$ [179]. The
molar absorptivity ($33,037 \text{ L cm}^{-1}\text{ mol}^{-1}$) of the dimer at 265 nm is about twice of the
molar absorptivity ($15.820 \text{ L cm}^{-1}\text{ mol}^{-1}$) of NADH is because the dimer has two adenine
moieties.

There are several possible reasons why the first electron transfer in the NADH
oxidation reaction is not inhibited on the nitrophenyl derivatized GC, but the second
electron transfer is. A strong candidate for the major factor involves inner sphere
reorganization energy. It has long been recognized that redox reactions requiring
significant changes in bond distances are often slow, due to the high reorganization
energy accompanying electron transfer. For example, Fe(H$_2$O)$_6^{3+/2-}$ has a slow
homogeneous electron exchange rate [108] and a small $k^0$ on oxide free carbon [89]
because the Fe-O bond distance must change by 0.13 Å between Fe$^{2+}$ and Fe$^{3+}$. Ferrocene
(Fc) and anthracene are much faster, because delocalization of the positive charge upon
oxidation leads to small changes in bond distances. For Fc/Fc$^+$, the bond lengths change
by at most 0.04 Å upon oxidation [109-111]. Molecular mechanics calculations based on
ab initio geometry optimization, UHF-3TO-3G basis set were used to establish bond
length changes for Fc/Fc$^{++}$ and NADH and its oxidation products. The maxima change in
Figure 4.12. Calculated bond distance of NADH and the intermediates produced during electrochemical oxidation.
bond length calculated for Fc/Fc$^{\ast}$ was 0.05 Å, while that for NADH oxidation was 0.04, 0.02 and 0.11 Å for the three reactions shown in (Figure 4.12. The large reorganization energy associated with large changes in bond length in the second electron reduction reaction is likely to contribute to the kinetics change observed for the NADH on the nitrophenyl derivatized GC.

In summary, the NADH is kinetically sensitive to surface modifications. Chemisorbed monolayers inhibit the second electron transfer process but not the first electron transfer process (Figure 4.13). The results indicate that adsorption of NAD$^+$ on the electrode surface lowers the activation barrier for the second electron transfer.
Figure 4.13. Schematic illustration of the mechanism of NADH oxidation on bare GC and nitrophenyl derivatized GC.
# APPENDIX A

## SPECTRAL DATA FOR DIAZONIUM SALTS

### LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>$^1$H NMR spectrum of phenyl diazonium fluoborate</td>
</tr>
<tr>
<td>A2</td>
<td>Mass spectrum of phenyl diazonium fluoborate</td>
</tr>
<tr>
<td>A3</td>
<td>$^1$H NMR spectrum of methyl phenyl diazonium fluoborate</td>
</tr>
<tr>
<td>A4</td>
<td>Mass spectrum of methyl phenyl diazonium fluoborate</td>
</tr>
<tr>
<td>A5</td>
<td>$^1$H NMR spectrum of trifluoromethyl phenyl diazonium fluoborate</td>
</tr>
<tr>
<td>A6</td>
<td>Mass spectrum of trifluoromethyl phenyl diazonium fluoborate</td>
</tr>
<tr>
<td>A7</td>
<td>$^1$H NMR spectrum of carboxyl phenyl diazonium fluoborate</td>
</tr>
<tr>
<td>A8</td>
<td>Mass spectrum of carboxyl phenyl diazonium fluoborate</td>
</tr>
<tr>
<td>A9</td>
<td>$^1$H NMR spectrum of ethyl phenyl diazonium fluoborate</td>
</tr>
<tr>
<td>A10</td>
<td>Mass spectrum of ethyl phenyl diazonium fluoborate</td>
</tr>
<tr>
<td>A11</td>
<td>$^1$H NMR spectrum of biphenyl diazonium fluoborate</td>
</tr>
<tr>
<td>A12</td>
<td>Mass spectrum of biphenyl diazonium fluoborate .................. 195</td>
</tr>
<tr>
<td>A13</td>
<td>'H NMR spectrum of aceto aminophenyd diazonium fluoborate ........ 196</td>
</tr>
<tr>
<td>A14</td>
<td>Mass spectrum of aceto aminophenyd diazonium fluoborate ........... 197</td>
</tr>
<tr>
<td>A15</td>
<td>Mass spectrum of butyl phenyl diazonium fluoborate ................. 198</td>
</tr>
</tbody>
</table>
Figure A1. $^1$H NMR spectrum of phenyl diazonium fluoborate
Figure A2. Mass spectrum of phenyl diazonium fluoborate
Figure A3. $^1$H NMR spectrum of methyl phenyl diazonium fluoborate
Figure A4. Mass spectrum of methyl phenyl diazonium fluoborate
Figure A5. $^1$H NMR spectrum of trifluoromethyl phenyl diazonium fluoborate
Figure A6. Mass spectrum of trifluoromethyl phenyl diazonium fluoborate
Figure A7. $^1$H NMR spectrum of carboxyl phenyl diazonium fluoborate
Figure A8. Mass spectrum of carboxyl phenyl diazonium fluoborate
Figure A9. $^1$H NMR spectrum of ethyl phenyl diazonium fluoborate
Figure A10. Mass spectrum of ethyl phenyl diazonium fluoborate
Figure A11. $^1$H NMR spectrum of biphenyl diazonium fluoborate
Figure A12. Mass spectrum of biphenyl diazonium fluoborate
Figure A13. $^1$H NMR spectrum of aceto aminophenyl diazonium fluoborate
Figure A14. Mass spectrum of aceto aminophenyl diazonium fluoborate
Figure A15. Mass spectrum of butyl phenyl diazonium fluoborate
References


138. Digisim, Bioanalytical Systems, West Lafayette, IN.


206


