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ELECTRON TRANSFER KINETICS OF CATECHOLS ON MODIFIED GLASSY CARBON ELECTRODES

DISSERTATION

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

By
Stacy Hunt DuVall, B.S.

****

The Ohio State University
2000

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ABSTRACT

Catechols have been studied extensively for their biological significance and for fundamental electrochemical issues, and undergo a two electron, two proton oxidation on glassy carbon electrodes in the pH range 1 to 8. The oxidation rate of catechols on solid electrodes is dependent on surface condition. This work represents an effort to characterize catechol electron transfer on glassy carbon electrodes. Catechol oxidation kinetics on glassy carbon electrodes were studied by targeted surface modifications in order to gain insight into what factors control catechol oxidation. Catechol electron transfer kinetics were found to be enhanced on electrodes that had been cleaned with certain solvents and surfaces that had low oxide content from vacuum heat treatment. Modification of the glassy carbon electrode with an adsorbed monolayer of methylene blue, nitrophenyl, and trifluoromethylphenyl groups severely inhibited catechol electron transfer rates. Nitrophenyl and trifluoromethylphenyl were chemisorbed to the electrode surface via electrochemical reduction of the corresponding diazonium salt, producing a compact monolayer. Adsorption of the catechol to the electrode surface was found to be necessary for fast catechol electron transfer.

The relationship between catechol adsorption and fast catechol electron transfer kinetics was examined. Two general mechanisms were considered: a stepwise adsorption, electron transfer, desorption mechanism and a mechanism whereby catalysis
of catechol electron transfer occurs through an adsorbed layer of catechol. A stepwise mechanism was found not to be consistent with desorption studies. Different quinones were adsorbed to the surface to investigate the effect of changing the identity of the adsorbed quinone species on catechol electron transfer. While non-quinone monolayers blocked catechol adsorption and catechol electron transfer, an adsorbed layer of quinone can catalyze solution catechol electron transfer even though catechol adsorption is suppressed. This observation is discussed in light of several possible mechanisms for catechol electron transfer.

Diazonium modified surfaces were used on redox systems that represent commonly encountered problems in electroanalytical applications. The stability of diazonium modified surfaces with air exposure and upon potential cycling and potentiostatting was examined with cyclic voltammetry and x-ray photoelectron spectroscopy. Diazonium modified surfaces were found to be significantly more stable than bare glassy carbon surfaces.
To my parents, Donna and Harold Hunt,

And to my husband,

Aaron DuVall
ACKNOWLEDGMENTS

Yikes!

A chubby, bearded rock singer one said "What a long, strange trip it's been." He wasn't talking about graduate school, but the description serves just as well. Keep in mind that strange can be a very good thing!

This work would not have been possible without the assistance of many people. I wish to express my gratitude first to my adviser, Richard L. McCreery. He has provided encouragement and guidance during my time here, and without his thoughtful assistance and direction this thesis would not have been possible. Not only that, but the "boss" has a good sense of humor. After all, I can still graduate even though I can't write my name forwards and backwards at the same time after a few beers.

I must also thank my compatriots in the McCreery Group for making N&W 0130 and MP 3040 a fun and interesting place to work. Luck was on my side the year I joined the group since the guys who joined in Spring 1996 are good company in any situation: Lin Xia, who is smart and funny, Jeremy Ramsey, the guy who always has a pun on hand and has in-laws with super powers, and Kris Frost, who may not be a partner in crime, but at least a partner in mischief. I'm glad I can call you guys my friends. Thanks are also due to Srikanth Ranganathan, who has a sharp mind and a sharper sense of humor, Bill McGovern, who can lead conversations down interesting paths, Ilson Steidel, a guy
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no matter what I’ve chosen to pursue (Trombone? Sure! Chemistry? Sounds great!).
My sister Terri and I managed to outgrow sibling rivalry to become good friends; she is
there for me when I need her. Thanks, Sis. I owe so much to my family, and can never
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Finally, I thank my husband Aaron. This past year has been quite a roller coaster
ride, but we’ve made it! Thanks for being there for me, and for giving me your love and
friendship. This part of our life is done; lets go see what’s around the corner.

Veni, vidi, levi.

(I came, I saw, I polished.)
VITA

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CHAPTER 1

INTRODUCTION

Catecholamines function as neurotransmitters in mammalian nervous systems. Because of their vital role in brain function, catecholamines and their metabolites have been studied extensively (1-3). Catechols are also of interest for general electrochemistry; along with para-quinones, catechols represent a separate class of redox systems (4). Quinonoid redox systems have been studied on metal and carbon electrodes.

Using solid electrodes for electrochemical studies has become enormously popular (5). Solid electrodes have several advantages over mercury electrodes, such as mechanical stability, ease of use, and low toxicity. Solid electrodes also have the advantage that surface modification is possible, enabling specific and site-directed catalysis. However, the ability to modify surface chemistry walks hand in hand with surface reactivity, often making surface structure hard to determine. This has proven to be an especially difficult problem with carbon electrodes.

To maintain electroactivity while avoiding undesirable surface reactions on bare electrodes, electrode surfaces can be intentionally modified (6-8). These chemically modified electrodes are rational efforts to introduce catalytic activity, specificity, and to
prolong the life of the electrode. Before discussing electrode modifications and catechol
electrochemistry, a brief review of relevant electrochemistry basics is presented.

**Electrochemistry Basics**

**Electron Transfer Kinetic Theory**

The discussion of heterogeneous kinetics in this document is based on
information contained in Bard and Faulkner's "Electrochemical Methods" (9). For a
more complete discussion, the reader is referred to pages 86 to 116 of reference (9).

For the generalized reaction

\[ \text{Ox} + n\text{e}^- \rightarrow \text{Red} \]  

at equilibrium, the Nernst equation holds:

\[ E = E^{\circ} + \frac{RT}{nF} \ln \frac{C_{\text{Ox}}^b}{C_{\text{Red}}^b} \]  

where the superscript \( b \) denotes bulk solution concentrations in mol/cm\(^3\). By limiting the
discussion to the case where mass transport does not affect the current (i.e., where
observed current is under kinetic control), the rate of the forward reaction, \( \nu_f \), must be
proportional to the concentration of Ox at the surface, \( C^{\circ}(t) \). The superscript \( \circ \) denotes
the concentration at the surface, \( n \) is the number of electrons, \( F \) is Faraday's constant, and
\( A \) is the electrode area. For reduction

\[ \nu_f = k_f C^{\circ}_{\text{Ox}}(t) = \frac{i_c}{nFA} \]  

for the oxidation,

\[ \nu_b = k_b C^{\circ}_{\text{Red}}(t) = \frac{i_a}{nFA} \]  

The net rate of reaction is then
\[ v_f - v_b = k_f C_{ox}^e(t) - k_b C_{Red}^e(t) = \frac{i}{nF A} \]  

and the observed current is

\[ i = i_c - i_a = nFA[k_f C_{ox}^e(t) - k_b C_{Red}^e(t)] \]  

The standard rate constants for the reaction, \( k_f^o \) and \( k_b^o \), are:

\[ k_f = k_f^o \exp(-\alpha f E) \]  

\[ k_b = k_b^o \exp((1 - \alpha)n f E) \]  

For a system at equilibrium with \( C_{ox}^b = C_{Red}^b \), \( E = E^o \) and \( k_f C_{ox}^b = k_b C_{Red}^b \):

\[ k_f^o \exp(-\alpha n f E^o) = k_b^o \exp((1 - \alpha)n f E^o) = k^o \]  

Where \( \alpha \) is the transmission coefficient and \( k \) has units of cm/s. Physically, \( k^o \) is the value of \( k_f \) and \( k_b \) at \( E = E^o \), and provides a useful standard for comparison of different redox systems.

Combining expressions (1.7) and (1.8),

\[ k_f = k^o \exp(-\alpha n f (E - E^o)) \]  

\[ k_b = k^o \exp((1 - \alpha)n f (E - E^o)) \]  

With these expressions for \( k_f \) and \( k_b \), substituting into (1.6) gives

\[ i = i_c - i_a = nF A k^o [C_{ox}^e \exp(-\alpha n f (E - E^o)) - C_{Red}^e \exp((1 - \alpha)n f (E - E^o))] \]  

This is the Butler-Volmer equation, valid for electron transfer where mass transport does not control the observed current.

**Electrochemical Techniques**

Cyclic voltammetry is a potential sweep technique, which is useful for kinetic measurements. In potential sweep techniques, the potential of the electrode is swept over a range and the current is monitored as a function of potential. However, since many
things are happening to affect the current (mass transport, charge transfer) and the potential is a function of time, analytical solutions to the current-potential behavior are not available. Instead, numerical solutions are available to relate the observed separation in the anodic and cathodic peak potentials to the standard rate constant. Also, it is becoming increasingly more common to use digital simulation programs, such as Digisim (BAS, Inc.), to model experimentally observed voltammograms in order to obtain kinetic parameters. Digital simulation of voltammetry is discussed in more detail in Appendix B.

Figure 1.1 shows the simulated voltammograms of both diffusion (A) and adsorbed (B) species with the assumption that \( k^0 \to \infty \). For the case of diffusing species with soluble Ox and Red, the peak separation in the cyclic voltammogram is \( 57/n \) mV (\( n \) is the number of electrons). If electron transfer is "quasi-reversible," \( k^0 \) does not equal infinity, and the peak separation (\( \Delta E_p \)) is \( \text{not} \ 57/n \) mV. In the extreme case of irreversible electron transfer (when \( k^0 \) is very small) the reverse wave may not be observed. Most electrochemical systems fall into the quasi-reversible regime; therefore cyclic voltammetry is a useful technique for studying electron transfer kinetics.

Peak current in a cyclic voltammogram is a function of the square root of the scan rate for diffusing species, as is illustrated by the Randles-Sevcik equation, where \( D \) is the diffusion coefficient and \( v \) is the scan rate:

\[
i_p = 2.69 \times 10^5 n^{3/2} AD^{1/2} C^{1/2} v^{1/2}
\]  

(1.12)

Figure 1.1 also shows the voltammogram for an adsorbed species with fast electron transfer (\( k^0 \to \infty \)) whose adsorption follows a Langmuir isotherm. In this
Figure 1.1. Theoretical voltammograms.
situation, the peak current for both the anodic and cathodic processes are located at $E^\circ$.

Under the conditions of fast electron transfer ($k^\circ \to \infty$) and Langmuir adsorption behavior, the peak width at half peak current is $90.6/n \text{ mV}$. If these conditions are not met, however, the anodic and cathodic peaks will not be centered on $E^\circ$ and the peaks will broaden. In contrast to the voltammetry for diffusing species, peak current for adsorbed species is directly proportional to the scan rate:

$$i_p = \frac{n^2F^2}{4RT} vA \Gamma_{\text{ox}}$$

with $\Gamma_{\text{ox}}$ indicating the surface coverage of the adsorbed species in mol/cm$^2$. Plotting peak current vs. scan rate is useful for diagnosing adsorption. If there is a deviation from linearity in the plot, the observed current is not due solely to adsorbed species.

Integrating the area under the voltammetric peak and using the following relation can be used to determine surface coverage of the adsorbed species:

$$Q = nFA\Gamma_{\text{ads}}$$

where $Q$ is the charge, $n$ is the number of electrons, $A$ is the electrode area, and $\Gamma_{\text{ads}}$ is the surface coverage of the electroactive species.

Although the peak shape for purely adsorbed species is distinctive, when both adsorbed and diffusing species are present it becomes more difficult to diagnose adsorption. Adsorption complicates kinetic analysis since kinetic formulations for cyclic voltammetry assume that semi-infinite linear diffusion of electroactive species is the only method for transport of material to the electrode surface. The presence of adsorption may perturb the kinetics since peak separations of adsorbed species are generally very small (20 mV or less at moderate scan rates of 100 – 200 mV/s). The adsorption-
perturbed $\Delta E_p$ would then be smaller than the $\Delta E_p$ for diffusing species, implying faster electron transfer kinetics. A useful method for diagnosing adsorption from the voltammogram is semi-integration of the voltammogram (10, 11). The semi-integral or convolution of the current is defined by the following operation:

$$I(t) = \frac{1}{\pi v^2} \int \frac{i(u)}{(t-u)^v} \, du \quad (1.15)$$

or as the function generated by using the following operator on the current:

$$\frac{d^{-v^2}}{dt} i(t) = I(t) \quad (1.16)$$

If the current in the cyclic voltammogram is due solely from diffusing species, the semi-integral has a sigmoidal shape reminiscent of a steady state voltammogram (Figure 1.2 A). In contrast, if the current is due solely to adsorbed species the semi-integral has pronounced peak shape behavior, as seen in Figure 1.2 B. When the current is due to both adsorbed and diffusing species, the semi-integral has the shape of a sigmoid with a peak superimposed, Figure 1.2 C. The presence of adsorbed species is readily distinguished, and if necessary, more rigorous adsorption experiments can be performed based on the diagnosis of adsorption from the semi-integral. Several authors have also demonstrated the utility of semi-integrals in kinetic analysis (10-14).

Chemically Modified Electrodes

Bare solid electrode surfaces suffer from variability and instability in surface structure with use (5, 6). Often coupled with this variability in surface structure are undesirable surface reactions that either interfere with or make measurement of the desired reaction impossible. This variability has fueled the development of chemically
Figure 1.2. Theoretical semi-integrals.
Figure 1.2, continued. Theoretical semiintegrals.
modified electrodes (CMEs). The objective is to modify an electrode surface so that the modification controls the chemistry, not the base electrode material (6, 7). In other words, modification of electrode surfaces is carried out in a rational fashion in order to address the problem of surface variability, produce surfaces with desired properties, and create electrodes that are stable with time. A great deal of effort of many research groups has gone into the development of chemically modified electrodes, and some of those efforts are discussed below.

How the electrode is modified depends on the application and the particular chemistry of the modifier chosen. For a modification scheme to be effective, the immobilized substance must retain the properties of its solution analog, whether chemical or electrochemical in nature. These immobilization schemes can be divided into three general categories: physisorption, chemisorption, and film deposition. Briefly, physisorption is where the modifier is adsorbed to the electrode surface in a manner that does not include covalent bonding. Physisorption strength can range from weak, where the modifier readily desorbs from the electrode surface (not terribly useful) to strong or “irreversible” physisorption. Irreversible physisorption is often referred to as chemisorption (15-17). Chemisorption, as it will be referred to in this work, is bonding to the surface that involves covalent interactions. Lastly, film deposition is when a polymer layer, ranging from 2 to ~20,000 monolayer equivalents, is applied to the electrode surface. The polymer can either be bound through covalent interactions or cling tenaciously to the electrode surface due to attraction to the electrode and meager solubility in the electrolyte (6, 7, 18).
CMEs act in a variety of ways, including activation of a specific reaction, passivation of the electrode surface, redox mediation, catalysis, or action as a selector (6, 7). CMEs often perform analytical functions (e.g. acceleration of reaction rate, selectivity, or pre-concentration) or serve as probes of fundamental electron transfer issues. Self-assembled monolayers have been extremely useful in that regard. Although there exists a wide variety in types of modifications and the breadth of the literature on the subject is extensive, only three types of chemically modified electrodes will be briefly reviewed: polymer modified electrodes, self-assembled monolayer (SAM) electrodes, and modifications based on carbon electrodes.

**Polymer modified electrodes**

Polymer modified electrodes are popular for several reasons (6, 7, 18). Compared to bare surfaces, polymer coatings tend to yield stable surfaces for longer periods of time. In addition, the thicker layers may provide enhanced sensitivity, and can act as ion exchangers, redox mediators, and ionic or electronic conductors. Also, polymer modified electrodes can be split into additional categories based on redox polymers and conducting polymers. Redox polymers act by incorporating a redox active species in the backbone of the polymer. Conducting polymers incorporate ionic redox species by electrostatic binding, and should be differentiated from electrically conducting polymers. However, the complex structure of polymers is often not well understood, and study is still going on to understand how the polymers function. In particular, Bard, et al. have focused attention on the mechanism of charge transport in Nafion® films (19, 20), and White and Murray have investigated the rates of electron hopping in “polymer sandwiches” (21).
Polymer films have been useful for analytical applications. Dioxygen reduction has a large overpotential on electrode surfaces, and it is often difficult to drive the reduction of oxygen all the way to water on many electrodes. Polymer electrodes to catalyze oxygen reduction have been developed, mostly by incorporating polyazomacrocycles into the polymer film. While these films are effective for catalyzing oxygen reduction, sometimes the polymer films suffer from stability problems since one of the intermediates of oxygen reduction is hydrogen peroxide. The hydrogen peroxide attacks the polymer, rendering it useless after some time. Catalytic macrocycles include iron and cobalt porphyrins, nickel phthalocyanine, cyclam complexes with Ni(I), Co(II), and Co(III).

Khoo and Guo took a clever approach to the problem of surface renewability and polymer degradation by incorporating 2-methyl-8-hydroxyquinoline into carbon paste mixture as a renewable source of monomer. To produce a polymer coated surface, the electrode was cycled repeatedly to induce electropolymerization of the 2-methyl-8-hydroxyquinoline monomer. These polymer surfaces were then used for Cu(II) determinations. The polymer surface was regenerated after fouling by removing the surface layer of carbon paste and performing the electropolymerization again. Selective surfaces for various species, notably metal ions, have been developed by incorporating modifiers that target the selected ion. For example, crown ethers incorporated in polymer-modified electrodes have been used to determine neurotransmitters and metal cations. Ca\(^{2+}\) and Ba\(^{2+}\) were determined selectively at poly(crown ether ferrocene) films by cyclic voltammetry; the peak position of the free ferrocene/crown ether complex differed significantly from the bound ferrocene/crown ether complex.
Electrically conducting polymers have been used for DA and NADH catalysis. NADH oxidation has a substantial overpotential on unmodified electrodes (18, 28). Poly(3-methylthiophene) coated electrodes have been effective in reducing the overpotential for NADH (29). Also effective as NADH redox catalysts are conducting polymers with mediators such as ferrocyanide, chloranil, or 2,3-dichloro-1,4-napthoquinone (18). An oxidized polypyrrole film with ferrocyanide was found to be effective for catalysis of ascorbic acid oxidation (30). The polypyrrole film was used only at potentials where the film remained oxidized in order to prevent expulsion of the ferrocyanide from the polymer.

Ion exchange polymers as electrode modifiers have multitudes of uses. The polymer can function as an ion exchanger to electrostatically bind a redox couple in the polymer (18). The bound species can then act as a mediator for solution species. A poly(nickel phthalocyanine) film covered with a thin film of Nafion® was used to catalyze DA oxidation and reject ascorbic acid both in reagent solutions and spiked “real” samples (plasma and urine) (31). These electrodes were stable for at least one month with a 12% loss in activity. Poly(methyl methacrylate-co-hydroxylethyl methacrylate) was effective for binding heavy metal ions that were then analyzed by stripping for environmental batch analysis (32).

Nafion®, a commercially available perfluorinated-sulfonated polymer, is outstandingly useful for biosensor applications. Nafion®’s structure (18), shown below, has both cation exchange regions and hydrophobic regions.
Exploiting Nafion®'s properties has been a preoccupation of electroanalytical chemists for some time. Its uses range from acting as an ion-exchange medium (33, 34) to a matrix for immobilization of biological molecules (35). Nafion® provides a matrix for the positively charged catecholamine neurotransmitters to pre-concentrate in the polymer (14, 33, 36, 37). Since the polymer is negatively charged, the main interference for DA determination, ascorbate, is rejected at physiological pH. Nafion® has greatly facilitated catecholamine determination, both in vitro and in vivo (1, 14, 18, 36, 38-42).

The examples given here are only a few taken from the overwhelming body of literature available on polymer-modified electrodes. Polymers have many applications today, and new applications are promising, especially in the areas of microelectronics and biosensors.

**Self-Assembled Monolayers**

One of the most versatile methods for modifying electrode surfaces is by taking advantage of substances that undergo spontaneous adsorption and ordering. This process is referred to as self-assembly. Self-assembled monolayers (SAMs) have become extremely popular because of the wide range of modifications and relative ease of preparation of SAMs. What makes this type of modification so extraordinary is the degree of order exhibited by the adsorbed species (6, 43-46). Adsorbates have well defined head and tail groups and self-assembly occurs because the head groups have a
strong affinity for the substrate. The thermodynamically most stable configuration is one where there is a strong, ordering interaction between the tail groups. Most SAMs are based on thiols, disulfides, and sulfides adsorbed on the coinage (i.e. gold, silver, and copper (47)) metals with gold being the most popular (8). The most common type of SAM electrode is the gold/alkanethiol system. The interaction between the gold substrate and thiol is very strong, with the Au-S bond estimated to have bond strength on the order of 40-50 kcal/mol (8). Figure 1.3 is a schematic drawing of an alkanethiol SAM on a gold substrate. Note the degree of ordering in the system, with the tail groups oriented in a particular direction and at a certain angle (30° for alkanethiols adsorbed on gold). Mercury (48, 49) and platinum (50) have also been used, however, they do not form SAMs as compact as those formed on gold (8). Phospholipids also self assemble on mercury electrodes, providing a model for biological membranes (48, 51). SAMs are relatively easy to produce, and tend to be stable in aqueous solutions. Due to the variety of modifiers, many different types of surfaces modifications are available (6).

SAM electrodes have found wide application in studying the fundamentals of electron transfer (6). Since SAMs are well defined, it is possible to either create blocking layers of varying thickness (52, 53) or to tag electroactive groups to the ends of thiols of varying lengths (54, 55). The SAM is not meant to be a regulator of a specific chemical process; instead, the adsorbed layer merely acts as an inert spacer to prevent solution or tethered redox species from approaching the electrode surface. As long as the blocking monolayer is sufficiently free of pinholes and defects (so that tunneling current can be distinguished from current due to pinholes and defects), data about tunneling mechanisms can be obtained. Several groups have characterized the current-overpotential behavior at
Figure 1.3. Alkanethiol self-assembled monolayer on gold.
pinholes (sites where bare electrode is available) and defects (indentations in the monolayer where species can more closely approach the electrode) (53, 56, 57). The data about electron tunneling is in the form of the tunneling constant ($\beta$), reorganization energy ($\lambda$), and the maximum observed electrochemical rate constant ($k_{\text{max}}$). This data has been obtained for both solution species at “blocking” (8) SAMs and for SAMs tagged with electroactive species. One benefit of using SAMS tagged with electroactive species is that pinhole or defect currents become non-existent for tightly packed monolayers. However, kinetic heterogeneity is an issue in the “tagged” cases (6).

In addition to answering questions about fundamental electron transfer theory, such as that of Marcus (58), SAM electrodes have been useful for probing biological electron transfer. elKasmi et al (59) studied the electron transfer kinetics of cytochrome-c electrostatically bound to a mixed SAM with carboxylate and hydroxyl terminating groups, and compared the results to previous work on SAMs with terminating carboxylate groups only. The improved kinetics on the mixed monolayer was attributed to more favorable binding properties. The rate of iron reduction and release form horse spleen ferritin was investigated on gold electrodes modified with 3-mercaptopropanoic acid (60). Although the authors acknowledge that the short chain thiol does not pack tightly on the electrode surface, the modification is effective for “promoting” direct electron transfer at the electrode surface. The diffusion coefficient and electron transfer rate constant were determined for ferritin.

SAMs were used as a base from which to attach peroxidases to the electrode surface in an effort to characterize the direct electron transfer of heme containing catalyst (61). Large peroxidases, microperoxidase MP-11, and hemin were immobilized on the
SAM. The direct electron transfer and the reduction of hydrogen peroxide by the immobilized species were monitored. The authors found that the smaller species were more effective catalysts when bound to the electrode surface.

Biological quinones have also been the subject of extensive scrutiny. Ubiquinone reduction was examined on a phosphatidyl choline self-assembled monolayer on a mercury electrode. The phosphatidyl choline assembled with the polar groups towards solution, mimicking a biological membrane. The semiquinone radical was stable in the lipid layer (48). A self-assembled lipid layer on a mercury electrode was also used to study electron and proton transfer of vitamin K₁ (51). The authors found that the electron transfer kinetics of vitamin K₁, a napthoquinone derivative, were different from those of ubiquinone, and attributed this observation to differences in protonation of the semiquinone radicals.

SAMs are also used as bases for forming lipid bilayers, to more accurately model biological membranes (62-64). Membranes formed in this manner have greater stability than those formed by more traditional methods of bilayer membrane assembly. A SAM of an alkane thiol is assembled on a gold or mercury substrate, and the SAM is then exposed to lipids to form the bilayer lipid membrane (BLM). These membranes have been used as extremely effective blocking layers to study the electron transfer kinetics of Ru(NH₃)₆³+/²⁺ and benzoquinone (63). The authors attributed the higher activity of benzoquinone on the BLM surface to the greater hydrophobicity of benzoquinone compared to Ru(NH₃)₆³+/²⁺. A BLM saturated with ferrocene was used to study mediation across the membrane structure (62).
The wide variety of terminal functional groups that can be attached to alkanethiols provides a route to further surface modification. Neimz et al synthesized thiols with a terminal acid fluoride group. The acid fluorides on the surface were then used to derivatize the surface with various substituents by reaction with amine functionalities. Although no application of such surfaces was reported, the use of acid fluorides for surface synthetic routes was advocated based on the fact that the acid fluorides are sterically non-demanding compared to diimide linkers and the acid fluorides are reasonably stable (65). Carboxylate terminated alkanethiol SAMs on gold were modified by slow ion-ion collisions to form phenyl-terminated surfaces (66). The authors called this reaction an interfacial Kolbe reaction because of its superficial similarity to the Kolbe alkane synthesis, and suggested further surface modification such as introducing a ligand to the surface for preferential binding.

Huang et al attempted construction of a DNA electrochemical sensor (67). The DNA was coupled to the SAM by reaction with activated carboxylate groups on the SAM, in a method that parallels the reaction on oxidized glassy carbon (6). 9,10-Anthraquinone-2,6-disulfonate (AQDS) was then intercalated into the DNA helices to act as an electrochemical marker. The authors found that the DNA modified electrodes were better at blocking Ru(NH$_3$)$_6^{3+/2+}$ than the unmodified SAMs. However, the immobilization process was found to fragment the DNA. The authors suggest that sensors based on oligonucleotides would be more feasible. SAMs are good bases for polymer-modified electrodes; the many points of attachment lend stability to the polymer. Polyelectrolyte films have been deposited on a SAM of aminoethanethiol with the eventual goal of producing membrane based biosensors (23).
Derivatized SAMs have been the basis for creating electrodes with molecular recognition or ion recognition capabilities. Selectivity from charge effects has been attempted by polymerizing alkoxy silane SAMS on gold electrodes. The authors achieved nominal selectivity for Fe(CN)$_6^{3-}$ by this method (68). Electrostatic binding was used to discriminate between Ru(NH$_3$)$_6^{3+/2+}$ and anthraquinone-2,6-disulfonate on p-mercaptoaniline (69). Rubenstein et al were able to selectively bind Cu$^{2+}$ over Fe$^{3+}$ on a mixed 2,2'-thiobisethyl acetoacetate and octadecyl mercaptan SAM (70). The binding was based on the suitability of Cu$^{2+}$ (tetrahedral coordination) to bind to the tetradentate chelating center over Fe$^{3+}$ (octahedral coordination). Electrodes with cyclodextrin-modified SAMs are attractive, both in terms of selective sensors and synthetic modifications. β-cyclodextrin with a tethered anthraquinone was attached to gold electrodes by several attachment sites in an effort to increase the stability of the modified surface (71). The authors wished to characterize the electrochemical behavior of the bound anthraquinone with the inclusion of guest molecules into the cyclodextrin. Another group exploited the stereoselective properties of cyclodextrins in order to construct a cyclodextrin-SAM electrode (72). The modified electrodes were used to study the electrochemical behavior of 3,4-dihydroxyphenylalanine (DOPA) and various DOPA derivatives. The authors found the cyclodextrin-modified electrodes did exhibit some stereoselectivity; however, in the current incarnation, the electrodes were not selective enough to be practical. The authors stated further study was forthcoming.

Quinone-based SAMs have been used for both fundamental electron transfer studies as well as analytical applications. On platinum electrodes, SAMs based on 4-pyridylhydroquinone were studied with the electrochemical quartz crystal microbalance,
and found to undergo slow assembly (50). These surfaces were catalytic towards hydrazine. Cysteamine SAMs on gold electrodes were used to bind dopamine to the surface. This surface was catalytic for NADH oxidation, and remained active for over eight hours (73). The behavior of 2-mercaptohydroquinone SAMs was examined by voltammetry over a range of pHs; the strange behavior at intermediate pH was attributed to competing mechanisms caused by local pH changes (74). A tunneling constant for hydroquinone-terminated alkanethiols was determined and found to be comparable for that found for ferrocene-terminated alkane thiols (75).

SAMs produced by micro contact printing (µCP) techniques are promising for controlling and segregating surface properties. µCP allows the deposition of a monolayer of a thiol in delineated areas onto the prepared gold substrate. The µCP printing is done by preparing a poly(dimethylsiloxane) (PDMS) stamp with the desired features, and placing the stamp in a solution containing the thiol of choice. The stamp is then placed in contact with the gold substrate, leaving a pattern of the thiol on the surface. The surface is then placed in the “filler” thiol to complete the patterning process. Surfaces have been produced to study the interaction of surface groups with solvents by chemical force microscopy (76). The tip of the cantilever and the surface were coated with SAMs; the cantilever was coated with one type of SAM and the gold surface was patterned with alkanethiols that had either hydroxyl or methyl terminating groups. The adhesion and friction properties of unsymmetrical dialkyl sulfide SAMs on gold have been investigated with lubrication properties in mind (77). µCP was used to deposit patterns of lines or squares of the sulfide onto gold. The SFM probe tip was also coated with the sulfide.
To produce patterned, reactive or polymer surfaces on gold, μCP has been employed to lay down the reactive species in well-defined areas. In contrast to more common μCP methods, Yan et al produced a SAM of carboxylic anhydrides, then used a stamp to pattern a long-chain amine onto the SAM (78). The remaining active sites were reacted with a shorter-chain fluorinated alkylamine. Differences in surface patterns were observed with SEM and SIMS. The authors proposed these surfaces would have application in preparing structurally complex surfaces. Sayre and Collard prepared micron-scale polyaniline patterns on gold substrates by patterning SAMs on the electrode (79). Polyaniline would not deposit on the amine-terminated portion of the surface. Lackowski et al prepared micron scale regions of poly(acrylic acid) or poly(tert-butyl acrylate) by reaction with carboxylate terminated regions of a patterned surface (80). The remainder of the surface was covered with an inert alkanethiol. The polymer layers were approximately 25 nm thick, with lateral dimensions of 1 μm. Proposed applications for these polymer surfaces include etch resists, reactive interfaces, and corrals for isolating cells. Another group has used patterning techniques to produce etch resists. Huck et al used a five step procedure to produce patterned multilayers of polymers, first depositing poly(ethylene imine) onto the interchain anhydride portion of the SAM, and then either of two polymers with maleic anhydride components by reaction with the poly(ethylene imine) layer (81). These surfaces were either characterized or etched with a commercial gold etchant. The polymer multilayers were effective masks with few defect sites, and the breakdown voltages were comparable to commercially available polymers. These surfaces have potential for fabricating electronic devices.
There is no doubt that the popularity of SAM electrodes is due to the great variety of surface modifications available with SAMs. The ease of modification and wide variety of surfaces possible with SAMs makes control of surface properties relatively easy. This control opens up many possibilities for applications not just in analytical science, but the fields of biology, microelectronics, and nanotechnology.

**Carbon electrode modifications**

Carbon is attractive as an electrode material for several reasons, including low cost and rich surface chemistry (5). This rich surface chemistry provides opportunities for different surface modifications. Glassy carbon (GC) and carbon fiber electrodes have been particularly useful for producing modified surfaces. Glassy carbon is impermeable to gases or solvents, compatible with organic solvents, and is mechanically stable (i.e., GC can be polished, exposed to high vacuum, heated, and derivatized). However, carbon electrodes are difficult to prepare reproducibly (82, 83). Bare carbon electrodes tend to adsorb impurities from solution, fouling the electrode surface and degrading electroactivity. Modifying the surface can help circumvent many of the problems inherent in using bare carbon electrodes.

Several approaches have been taken to produce carbon electrodes with the desired surface properties. Carbon electrodes are popular substrates for polymer modifications (polymer electrodes are discussed above). Other modifications of carbon electrodes include physisorption of the catalysts, specific functional group modification, and chemisorption of the catalyst (6).

Carbon surfaces covered with a physisorbed layer have been used to probe electron transfer reactivity and for catalyzing otherwise unfavorable solution redox
reactions. Chen et al physisorbed bis-methylstyril benzene, anthraquinone-2,6-
disulfonate, and methylene blue to glassy carbon electrodes to use as blocking layers for
a selection of solution redox species (84, 85). With the aid of these blocking layers, as
well as other surface tests, classification of the redox systems was possible. Physisorbed
anthraquinone-2,6-disulfonate (AQDS) has been used as a probe to study surface
reactivity. Ta et al used AQDS to probe the surface reactivity of ordered graphite
electrodes with both voltammetry and scanning force microscopy (86). It was found that
the coverage of AQDS determined voltammetrically was far less than that determined
with scanning force microscopy, suggesting that although the AQDS is physisorbed to
the surface, a specific site is necessary for the AQDS to be electroactive. Swain et al
compared the electrochemistry of physisorbed AQDS on some of the most popular types
of carbon electrodes (87). The extent of AQDS adsorption on glassy carbon, highly
ordered pyrolytic graphite (HOPG), hydrogenated glassy carbon (HGC) and boron doped
diamond (BDD) was used to help determine the importance of surface functional groups
in promoting adsorption.

Catalyzing oxygen reduction is a problem that is of interest for analytical
applications, biosensors, and fuel cell development (6, 18). Complete oxygen reduction
to water (a four electron process) requires a large overpotential and it is often difficult to
drive the reaction past the production of hydrogen peroxide (a two electron process).
Certain metallated porphyrins are effective catalysts for oxygen reduction, and much
effort has gone into developing electrode modifications involving porphyrins. Although
many of these species are effective catalysts, electrodes are generally not stable with
time. The instability of the surface layer has been attributed to (for example) desorption
of the catalyst from the electrode and degradation of the catalyst by the reactive intermediate hydrogen peroxide. In an effort to identify more effective catalysts for oxygen reduction, Popovici et al (88) and Anson et al (24) have investigated porphyrins with different substituents. Anson et al found that by incorporating electron donating p-hydroxyphenyl groups, the cobalt porphyrin became more catalytic towards oxygen reduction than the unsubstituted porphyrin.

A very effective way of increasing the catalytic activity of porphyrins for oxygen reduction is to tether two porphyrins together, effectively creating a porphyrin “sandwich” with oxygen between the two porphyrins. Co(II) is the overwhelming favorite metal center of the porphyrins; this has been attributed to its more anodic potential (89). Several different attachment schemes for the "face to face" porphyrins include attachment via amide bridges (89), rigid aromatic pillars (90), and electrostatic interactions (91). By creating these face-to-face diporphyrins, oxygen is reduced directly by the four-electron process.

Chemisorption is a more stable way to attach species to the electrode surface than physisorption. Carboxylate groups on GC surfaces have been used to attach a variety of modifiers, both for catalytic purposes and to characterize electron transfer processes at the electrode surface. Amines on the modifier can be reacted with carboxylate groups on the surface that have been activated with a carbodiimide (6, 92, 93). Dopamine, fluorescein isothiocyanate, and several enzymes have been coupled to carbon electrodes with this method. Dopamine was used as an electrochemical probe of surface activity (93), while fluorescein isothiocyanate distribution was monitored by fluorescence microscopy (92, 93) in an effort to characterize the efficacy of the modification process.
Carbon surfaces have been modified with enzymes from the dehydrogenase family (94-96) and with glucose oxidase (97). For the dehydrogenase electrodes, the enzymatically-generated NADH is oxidized at the electrode surface, allowing the determination of a non-electroactive substance (such as glutamate). On the glucose oxidase modified electrodes, enzymatically generated hydrogen peroxide is monitored. Enzyme modified electrodes often have low stability, and in the case of NADH, poor electron transfer kinetics on the electrode surface. The Kuhr group is investigating modifications of the enzyme immobilization procedure that will allow both immobilization of the enzyme and preserve good NADH kinetics (94).

In addition to modifications that depend on a particular functional group for attachment to the electrode surface, general non-specific modifications are available. These modifications are attractive for their non-specific nature and strong binding. One of the earliest and most cited examples of chemical modification of carbon electrode surfaces to introduce a specific catalyst for NADH oxidation is the attachment of dopamine to a carbon surface (28). The surface was effective for NADH catalysis, however, it suffered from fouling by NAD\(^+\) upon continued cycling. Deinhammer et al used the oxidation of amine-containing species to form a reactive radical intermediate, which would then attack the carbon surface (98). Among the attached molecules were dopamine and biotin. The dopamine surface was catalytic for NADH oxidation, and the biotin-modified surfaces are expected to be useful for avidin-biotin linkages.

Perhaps the most versatile method for modifying carbon surfaces with a monolayer or less is with aryl radicals generated from the reduction of diazonium salts in
aprotic solvents (99). The reaction proceeds as below, with the electrochemical reduction of the diazonium salt to form an aryl radical and nitrogen. The aryl radical then attacks the glassy carbon, forming a covalently bonded derivative.

\[
\text{R}^+ + \text{e}^- \rightarrow \text{R}^+ + \text{N}_2 \rightarrow \text{GC} \rightarrow \text{R}
\]

Conceivably, if a diazonium salt of a desired modifier can be made, then the corresponding surface should be relatively easy to produce. Once produced, many of these surfaces are stable in air, solution, and under reasonable electrochemical conditions. Kuo found that for nitrophenyl-modified glassy carbon, the sample had to be heated to temperatures around 700°C to remove the nitrophenyl from the surface (100). Diazonium surfaces have been characterized with cyclic voltammetry (if the modification is electroactive) (99-102), X-ray photoelectron spectroscopy (99-102), and Raman spectroscopy (100-102). The ease of preparation and wide variety of modifiers available for this type of surface is similar to that of SAMs.

The diazonium surfaces have been used as a base layer for further modification. Allongue et al reduced a nitrophenyl layer on glassy carbon to produce a p-aniline surface; this surface was then reacted with epichlorohydrin (99). A biotin diazonium salt has been attached to a carbon electrode for use in avidin-biotin linkages (103) to bind alkaline phosphatase. Bourdillon et al used a phenylacetic acid modified surface to attach glucose oxidase to a carbon electrode through the carboxylate (104). This scheme for modification has several advantages over other methods: the high density of carboxylate
groups available for modification and fewer "handling" steps for the electrode, thus preventing excessive surface roughening which may adversely affect electron transfer.

Diazonium modified surfaces have been used for the differentiation of dopamine and ascorbic acid (105). The differentiation is based on the negatively charged carboxylate groups on the surface repelling the negatively charged ascorbate.

Nitrophenyl surfaces have been used by Chen and McCreery to help determine redox system classification of several inorganic systems (84), and by Yang and McCreery to study the electron transfer mechanism of phenothiazines and oxygen (106, 107). In the current work, diazonium-modified surfaces play a key role in investigating catechol oxidation.

**Quinones**

Both ortho- and para-quinones have a great deal of biological significance (4, 108). Para quinones function as links in the electron transport chain in respiratory functions, as vitamins, and in photosynthesis. Catechols and ortho-quinones function as neurotransmitters, hormones, and pigments. Figure 1.4 shows some of the most common quinones in biological systems. Ubiquinones, also called named "coenzymes Q," are found in many biological systems (bacterial, plant, and animal); the structure has a benzoquinone core with a long side chain. These quinones are found in the membranes of mitochondria and function in the respiratory electron transport chain. The vitamin K family has a napthoquinone core with a long side chain. Vitamin K functions in blood clotting by converting fibrinogen to fibrin in mammalian systems and in electron transport in bacteria. Plastoquinones are found in the membranes of chloroplasts in all oxygen producing photosynthetic organisms and function in electron transport in
Figure 1.4. Biological quinones.
photosynthesis. Tocopheryl quinones are not believed to be found in living organisms; however, the reduced forms of tocopheryl quinones, tocopherols, are the Vitamin E family.

Catechols, the reduced form of ortho-quinones, function as catecholamine neurotransmitters (1-3), hormones (1), and in melanin production (1, 3, 109). Figure 1.5 shows common catechols, including the ones studied in this work. Dopamine and other catecholamines are produced in mammalian systems from tyrosine by the following reaction:

A generalized reaction for the production of melanin pigments is shown in reaction below.
Catecholamines are metabolized in the body by the action of the enzyme monoamine oxidase. Below are the metabolic routes for dopamine and norepinephrine (adapted from (110)):
Figure 1.5. Catechols.
Figure 1.6. Schematic drawing of a neuron.
one neuron and the dendrite of the other is called the synaptic gap or synapse. Simply, axons can be thought as senders and dendrites as receivers. In the mammalian brain, there are $10^{10} - 10^{23}$ neurons that fire billions of times per second collectively; each neuron has hundreds of synapses (1, 3).

For a simplified discussion of neurotransmission, refer to Figure 1.7 (1, 3). The structures of note are the pre- and post-synaptic membranes (separated by 200-800 Å), the receptors on the post-synaptic membrane, and vesicles containing the neurotransmitter. The general process is as follows. An action potential of 100 mV in the axon of the first neuron fires, stimulating the release of neurotransmitters into the synapse. These neurotransmitters move toward the receptors on the post-synaptic membrane of the dendrite of the second neuron. The interaction of the neurotransmitter with the receptor causes changes in the permeability of the post-synaptic membrane to small ions. This generates a small potential change across the post-synaptic membrane of only a few millivolts for a few milliseconds. The magnitude of this potential depends on the amount of the neurotransmitter released, and this potential is either excitatory or inhibitory, determining whether or not a new action potential is propagated. The neurotransmitter-receptor complex must be deactivated so it does not continuously excite or inhibit the neuron. The complex can be dissociated by three modes: mass transport away from the synapse, enzymatic degradation, or re-uptake by transporters in the pre-synaptic membrane (1-3).

The procedure above describes the classic model of neurotransmission. Examples of neurotransmitter systems are the adrenergic or catecholamine system, the cholinergic
Figure 1.7. Synapse structure.
system, and serotonergic system (1). Dopamine neurotransmission is one of the most studied and well-understood systems in the brain.

Dopamine is found in many parts of the brain, notably in these fiber systems: nigrostriatal, mesolimbic, mesocortical, and tuberoinfundibular, as well as in the retina (2, 3). Dopamine accounts for fifty percent of catechoalamines in the brain, eighty percent of which is in the basal ganglia. Dopamine is involved in processes that control the ability to experience pleasure or pain, movement, and emotional response (1). Defects in the dopamine system have serious consequences. The loss of dopaminergic neurons in the nigrostriatal tract causes Parkinson’s disease (characterized by tremors, muscular rigidity, slowness in initiating movement, and stooped posture) (2). There is no cure for Parkinson’s disease; however, treatment options are available. Since dopamine cannot cross the blood-brain barrier, L-DOPA is administered. Once in the brain, L-DOPA is decarboxylated to form dopamine (1-3). Parkinson’s disease can also be treated by surgically with two procedures, palliodotomy and deep brain stimulation (111, 112). Other compounds, such as apomorphine, are also useful for mimicking the action of L-DOPA (2).

Other disorders are also associated with abnormalities in dopamine in the brain. There is a hypothesis that overactivity of some dopamine systems cause schizophrenia (1, 2). Also, deficiencies of dopamine and norepinephrine are thought to cause depression, whereas an excess of catecholamines may cause mania. Excess of L-DOPA has been found to induce mania (2).

Although the mechanisms of drug abuse and addiction are complex, with individuals varying a great deal and more than one neural pathway involved in addiction,
the role of dopamine in addiction has been established. Drugs can either act by directly stimulating dopamine receptors, blocking dopamine receptors, or preventing dopamine re-uptake by blocking dopamine transporters (2, 3). Cocaine is thought to act by binding to dopamine, norepinephrine, and serotonin transporters, thus increasing the time dopamine is available in the synaptic cleft (2, 3). Cocaine also blocks voltage gated Na⁺ channels, and is thought to act on other transporters in the cell membrane (2). Amphetamine also blocks dopamine reuptake by binding to dopamine transporters; however it differs from cocaine by causing the release of additional dopamine from neurons (2).

Catecholamines are among the most widely studied neurotransmitters, not only because of their biological significance, but because these compounds are easily oxidized (1, 5). Electrochemistry provides a useful probe of catecholamine activity, in vivo and in vitro.

**Electrochemistry of Quinones and Catechols**

The electrochemistry of p-quinones has been studied extensively, because of fundamental importance to electrochemistry and because of biological importance. The reduction of benzoquinone in aprotic solvents can be considered the simplest case:

\[
\text{quinone} \xrightarrow{-e^-} \text{semiquinone radical anion} \xrightarrow{-e^-} \text{hydroquinone anion}
\]

The reaction proceeds in two consecutive one-electron reductions, from the quinone to the semiquinone radical anion, and from the semiquinone to the hydroquinone anion.
The addition of proton donors and protic solvents has dramatic effects on the second reduction, indicating the importance of protons in the electrochemistry of quinones and catechols (113, 114).

In aqueous media, the electrochemistry of catechols and quinones becomes much more complicated. At pH lower than ~10, the formal potentials for the electron transfers are such that only one redox couple is observed, complicating the determination of kinetic variables. In addition to dealing with two electron transfers, there are also proton transfer reactions to consider. This is well illustrated by the nine-membered scheme of squares, described by Laviron in general and for benzoquinone/hydroquinone in particular (Figure 1.8) (115, 116). This theoretical treatment is based on the assumption that protonations are at equilibrium and that there is no significant disproportionation or dimerization of the radical intermediates. There are nine possible species, with six possible electron transfers and six possible proton transfers. Based on the pKₘₐₚ's of the species and a prediction of the fractional current contribution from each step, the orders of proton and electron transfers have been calculated for benzoquinone (115) and several catechols at carbon paste for moderate scan rate (100 mV/s) (12, 13) and carbon fiber for both moderate and fast (100 mV/s, 100 mV/s) (14) scan rates in the approximate pH range of 0 to 8. Such a plot is shown in Figure 1.9, with the predominant pathway at each pH marked. The order of proton and electron transfers changes with pH; for catechols, the mechanism for the oxidation shifts from e-H+e-H+ at low pH to H+e-H+e- at pH 6 to 8. However, at intermediate pH, the mechanism is not clearly defined, with e-H+H+e- appearing to dominate. For benzoquinone, the mechanism is similar; with the reaction
Scheme of Squares
Microscopic rates/potentials

Observables

Figure 1.8. Scheme of Squares.
sequence written for reductions, the orders are H+e-H+e- at low pH, e-H+H+e- for intermediate pH, and e-H+e-H+ for the higher pH range.

Although direct measurement of the microscopic electron transfer rates and potentials is not possible with current approaches, the formal potential and apparent rate for each electron transfer can be calculated. Wightman et al have used semi-integral analysis to determine the microscopic rates and mechanism described above, as well as the apparent rate constants and formal potentials of each electron transfer step (12-14). Table 1.1 summarizes the microscopic rate constants determined from these studies. It should be noted that semi-integral analysis is valid only when adsorption is not present; the presence of adsorption perturbs the semi-integral considerably.

At low pH, there are some inconsistencies with the model described above. The model calls for the formation of QH$_2^+$, which is thermodynamically unfavorable (108). Deakin and Wightman attributed the difficulty of fitting data at low pH to the problems associated with the formation of QH$_2^+$ (13). Perhaps interaction of QH$_2^+$ with surface groups stabilizes this species, resulting in the change in the pKa and creating more favorable conditions.

Much effort has gone into investigating the mechanism and surface interactions involved in dopamine and catechol oxidation, particularly on carbon electrodes. Dopamine is often used as a benchmark system to asses surface quality due to dopamine’s sensitivity to surface condition (5). Surface variability on carbon electrodes has been a very large problem, making correlations between surface activity and electron transfer difficult. Laser activation (117, 118), electrochemical pre-treatment (38, 119,
<table>
<thead>
<tr>
<th></th>
<th>$^a$k₁</th>
<th>k₂</th>
<th>k₃</th>
<th>k₄</th>
<th>k₅</th>
<th>k₆</th>
<th>$k_1^{app}$</th>
<th>$k_2^{app}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHBA (DA)</td>
<td>N/D</td>
<td>560</td>
<td>0.057</td>
<td>3.5</td>
<td>2.3</td>
<td>N/D</td>
<td>0.023$^c$</td>
<td>0.013$^c$</td>
</tr>
<tr>
<td>DOPAC</td>
<td>N/D</td>
<td>1500</td>
<td>0.063</td>
<td>11</td>
<td>8.2</td>
<td>N/D</td>
<td>0.026$^d$</td>
<td>0.012$^d$</td>
</tr>
<tr>
<td>4MC</td>
<td>N/D</td>
<td>780</td>
<td>0.063</td>
<td>24.1</td>
<td>9.3</td>
<td>N/D</td>
<td>0.026$^e$</td>
<td>0.028$^e$</td>
</tr>
<tr>
<td>BQ</td>
<td>N/D</td>
<td>160</td>
<td>1.6X10⁻³</td>
<td>1.15</td>
<td>0.11</td>
<td>N/D</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- k in cm/s
- not determined
- pH = 6.4
- pH = 6.61
- pH = 6.9

Table 1.1. Microscopic rate constants determined for catechols and quinones.
120), and Nafion® (14, 36-38, 40) coatings have been used to either clarify the surface chemistry or to provide surfaces with reproducible activity for dopamine oxidation.

**Objectives**

Understanding dopamine and catechol oxidation on carbon electrode surfaces is important for several reasons. From a fundamental electrochemistry standpoint, catechols are an important class of redox systems to be understood. From a more practical standpoint, once the mechanism for oxidation of catechols on carbon electrodes is understood, more effective analytical sensors can be made for dopamine and related catechols. As described in the previous sections, developing effective surfaces for dopamine oxidation would be of great assistance for studying neurological processes.

The objectives of this work are to characterize catechol oxidation on glassy carbon surfaces. The main focus of this work is on dopamine; other catechols are studied based on side chain charge and structure. This will be accomplished by preparing well-defined glassy carbon surfaces by established and new modification procedures. Several questions will be addressed: Is the catalytic site a specific functional group? Since adsorption of other quinones to carbon surfaces has been demonstrated, does adsorption play a role in catechol electron transfer kinetics? If a catalytic route is identified, can targeted surfaces for catechol oxidation be constructed?

Chapter Two deals with the response of catechols on carbon electrode surfaces prepared according to the "tree scheme" described by Chen and McCreery (84, 121). This series of surface modifications is useful in determining whether or not the surface state of the electrode is important in electron transfer and to pinpoint catalytic oxide functional groups. The pH range used in this chapter was 1 to 8 in an effort to prevent
unwanted side reactions of the catechols. The role of catechol adsorption and the identification of catalytic sites is the focus of Chapter Three. Possible analytical applications of the modified electrodes are discussed in Chapter Four. Investigation of quinone reduction in aprotic media is the subject of Appendix A. Simulation parameters used in Chapters Two and Three are discussed in Appendix B. Finally, spectroscopic data for synthesized diazonium salts is presented in Appendix C. Throughout the current work, cyclic voltammetry and X-ray photoelectron spectroscopy are used to study surfaces and electron transfer kinetics.

The over-reaching goal of this work is to elucidate the mechanism of catechol oxidation on glassy carbon electrodes and exploit this knowledge in order to make targeted catalytic surfaces. This work, while specific for catechols, represents part of the continuing efforts of the McCreery research group and a variety of other labs to construct analytically useful modified carbon electrode surfaces.
CHAPTER 2

CATECHOL KINETICS ON MODIFIED GLASSY CARBON SURFACES

Introduction

Glassy carbon surfaces are prone to oxidation, often acquiring 7% to 23% surface oxides with the most common pretreatment procedures (5, 83). In addition, polishing introduces debris, both carbon particles and polishing material, onto the electrode surface. Glassy carbon (GC) electrode performance varies a great deal with pretreatment procedure and electrode history (5, 42). Difficulty knowing the surface structure of glassy carbon electrodes translates into difficulty knowing the relationship between surface structure and redox system reactivity. It is the goal of this research group (84, 85, 117) and many others (14, 42, 92, 122) to define which surface variables are most important in the electron transfer activity of various redox systems. The objective is to define several universal properties of carbon surfaces and redox systems in order to understand the interconnecting variables that bind the two together. It is essential to have surfaces with well-defined properties for this purpose (5, 83, 123, 124).
Figure 2.1 is a schematic depiction of different carbon surfaces. Polished glassy carbon electrodes are not atomically smooth; instead, polished electrodes have roughness factors between 1.5 and 2.5 (125). In addition, glassy carbon undergoes oxidation upon exposure to the environment, with 7% to 23% of the surface covered with oxide groups (5, 82). These groups are most likely carbonyl groups, hydroxyl groups, ortho- and para-quinones, lactones, and carboxylate groups. A polished GC surface may or may not be active for electron transfer, with many different levels of activity in between.

Electrode treatments have been developed to address the problems associated with surface variability. These include ultraclean polishing (83), electrochemical pretreatment (ECP) (119, 126, 127), laser activation (117, 118, 128), and vacuum heat treatment (82). Polishing and ECP produce surfaces that are structurally ill defined; however, these procedures produce surfaces that are either reproducible or active for electron transfer. ECP produces a surface that has a layer of "graphitic oxide," a polymeric material rich in oxides (5). ECP GC and carbon fiber electrodes have been effective for promoting electron transfer kinetics for some redox systems (38, 83, 85, 119, 120). Fe^{2+/3+} and dopamine kinetics are accelerated on ECP surfaces; the increase in iron kinetics has been attributed to an increase in catalytic carbonyl groups upon oxidation, and adsorption of dopamine onto the negatively charged surface is thought to be responsible for DA's apparent increase in electron transfer kinetics.

Laser activation (117, 118, 128) and vacuum heat treatment (82) both produce surfaces that are better defined than polishing or ECP. In the case of laser activation, properties such as O/C ratio and electron transfer rate for benchmark systems depends on the treatment procedure (laser power, number of pulses). Under conditions of optimal
Figure 2.1. Schematic drawing of Carbon Surfaces. A. Polished/Solvent treated B. Monolayer modified. C. Low oxide.
electron transfer rate for Ru(NH₃)₆⁴⁺/²⁺, the O/C ratio decreased. ΔE_p for DA under the most advantageous conditions was 32 mV, a value that approaches the theoretical limit for a two-electron system (118). Vacuum Heat Treatment (VHT) is a rigorous treatment procedure where the electrode is heated under high vacuum conditions until oxide containing species are desorbed. It is difficult to rid a surface of all oxides, however, oxygen to carbon ratios of 0.02 are possible with this treatment (82, 84). The XPS spectrum of a VHT surface reveals only oxygen and carbon, indicating that the surface composition is relatively well defined. VHT is effective for improving the kinetics of some redox systems and is useful for producing surfaces with well-defined characteristics, but is an inconvenient and time-consuming process. Also, surfaces produced by VHT oxidize very quickly, limiting the usefulness of the surfaces to academic study.

The Tree

In an effort to characterize redox systems according to their behavior, Chen and McCreery organized a set of surface modifications into a flow chart for redox system behavior (84, 121). The rationale behind this effort was to systematically study the effects of surface modifications of GC electrodes in order to pinpoint the important surface variables for particular redox systems. The hope was that with this classification scheme, predictions about redox system behavior would be possible. The classification of 17 redox systems, mostly inorganic one-electron systems, was made using this classification scheme. Yang and McCreery extended the scheme to include several organic redox systems, such as phenothiazines, methylene blue, and methyl viologen, in an effort to judge the effectiveness of the scheme with organic redox systems (129).
Figure 2.2 is the flow chart for the classification scheme, also called the “tree" scheme. It can be broken up into several steps: monolayer sensitivity test, oxide sensitivity test, and individual oxide sensitivity tests.

The first surface the redox system in question was subjected to was the polished surface. Polished surfaces have been described above, and have been used as the base surface for comparison. Recently, solvent cleaning procedures (130, 131) have led to electrodes with “cleaner” surfaces and that are more active for some redox systems. Ascorbic acid, dopamine, and Fe(CN)$_6^{3/-}$ have faster electron transfer rates on solvent treated surfaces. Because of the cleaning effect on glassy carbon electrodes, these solvent treated surfaces have been adopted in this work as the base surface for comparison.

To determine whether or not a particular redox system is affected by the surface state of the electrode, a monolayer blocker is adsorbed to the electrode surface. This monolayer is not meant to catalyze the reaction in any way; instead, it is simply meant to prevent the solution species from approaching the electrode too closely. Both chemisorbed and physisorbed species are used as blockers.

If the kinetics of a redox system are significantly affected by the monolayer, the redox system is said to be “surface sensitive.” Then, if a redox system is found to be “sensitive" to the electrode surface, the next step is to try to pinpoint the nature of the interaction. To determine whether or not surface oxides are the catalyzing group, the surface oxides are removed as described above. If oxides are found to provide a catalytic route, then there are specific tagging groups available to block the oxides, and it may be possible to identify the catalytic oxide group.
Figure 2.2. Flow chart for surface characterization.

- **Surface sensitive?**
  - yes: Oxide sensitive? (low oxide)
    - yes: Specific group?
      - yes: Carbonyl? (DNPH)
      - no: Hydroxyl? (DNBC)
    - no: Specific group?
  - no: “tunnelers”
From this classification scheme, three general classes of redox systems have been defined. These classifications are outer-sphere, inner-sphere, not oxide catalyzed, and outer sphere, oxide catalyzed. Briefly, outer sphere systems are those that have very little change (a factor of 2 or 3) in electron transfer rate when the surface is blocked based on comparisons with alkanethiols (8). This class represents redox systems such as Ru(NH$_3$)$_6^{3+/2+}$ (84), methylene blue, and several phenothiazines (129). Inner sphere systems are those that show a significant reduction in electron transfer rate upon blockage of the surface. For these types of systems, either close contact or a specific surface interaction is necessary for a catalytic electron transfer route. For systems such as ascorbic acid and Fe(CN)$_6^{3-/-4-}$ (84), no specific surface interaction has been identified, however, these systems are sensitive to surface blockage. Such systems are classified as inner-sphere, not oxide sensitive. Catalytic sites on glassy carbon for aquated metal complexes, such as Fe$^{2+/-3+}$(aq), have been identified (85, 132). Blocking surface carbonyl groups with dinitrophenyl hydrazine causes a reduction in rate comparable to the reduction observed by completely blocking the surface for Fe$^{2+/-3+}$(aq). This type of redox system is referred to as inner sphere, oxide catalyzed.

This scheme for systematic redox system classification has been successfully used to identify redox system classification for several inorganic systems as well as some organics. In this chapter, the classification scheme was applied to dopamine (DA), 4-methylcatechol (4MC), 3,4-dihydroxyphenylacetic acid (DOPAC), and D,L-3,4-dihydroxyphenethyl glycol (DOPEG) in an effort to identify the mechanism for oxidation on glassy carbon.
**X-Ray Photoelectron Spectroscopy**

X-Ray Photoelectron Spectroscopy (XPS) is a useful spectroscopic technique for studying surfaces (133). Figure 2.3 is a schematic of the process involved in XPS. When soft (200-2000 eV) X-rays are focused on a sample, the photoelectric effect will cause core electrons to be ejected from the sample with a certain kinetic energy. Characteristic binding energies for the ejected electrons can be determined from the following equation (neglecting the work function for the spectrometer):

$$BE = h\nu - KE$$

where $BE$ is the binding energy (eV) and $KE$ is the kinetic energy of the ejected photoelectron. Because $BE$ is characteristic of a specific element, elemental analysis of the surface can be performed.

XPS is tremendously useful as a technique to study surfaces for several reasons. It is generally a non-destructive technique, allowing the sample to be subjected to further analysis. Although the penetration depth of X-ray radiation is fairly large (approximately a micron), the escape depth of electrons is only about 50 Å, limiting the analysis to the first few atomic layers. In this regard, XPS is very similar to electrochemical techniques. In addition to providing the elemental composition of the surface, XPS can also provide information on the oxidation state of the surface species based on chemical shifts in binding energy. XPS, in conjunction with other techniques, was used to characterize surfaces in this study.

**Experimental**

GC electrodes were either obtained from Bioanalytical Systems, Inc. (West Lafayette, IN) or disks cut from Tokai GC20 plates. Electrodes were polished according
Figure 2.3. Schematic of photoelectric effect.
to established procedures: first with P4000 SiC polishing paper (Buehler, Lake Bluff, IL), then with 1 μm, 0.3 μm, and 0.05 μm alumina powders (Buehler, Lake Bluff, IL) slurried with Nanopure water (Barnstead Nanopure System, Dubuque, IA). The electrode was polished for 2 minutes in each slurry, rinsed with Nanopure between slurries, and sonicated for 10 minutes in Nanopure water. The water was changed several times during sonication. An electrode treated in this manner will be referred to as a polished electrode. Polishing is the base treatment and precedes any other electrode treatment unless otherwise noted.

Solvent treatments consisted of either soaking a polished electrode in warm pyridine (Mallinckrodt, Inc.) or sonicating in a mixture of isopropyl alcohol (Mallinckrodt, Inc.) and activated carbon (Norit 221, Acros). For the pyridine treatment, a freshly polished electrode was placed in pyridine and heated to approximately 65°C. The heat was turned off and the electrode was allowed to soak in the pyridine for one hour. The electrode was then rinsed for 30s with water, dried and used. Isopropyl alcohol/activated carbon (IPA/AC) treated electrodes were prepared by sonicating the electrode in a 3:1 mixture of isopropyl alcohol and activated carbon for ten minutes then sonicating in water for an additional five minutes. The electrode was then rinsed, dried, and used.

Physisorbed layers were prepared by two methods. In the first, a polished or solvent cleaned electrode was placed in a high concentration solution of the adsorber for 10 minutes, rinsed copiously with solvent, rinsed with water, dried and used. The second
method consisted of putting a low concentration of the adsorber (10-50 μM) into the
analyte solution and allowing the electrode to soak in the solution for ten minutes before voltammetry.

Chemisorption was achieved by electrochemically reducing a diazonium salt in acetonitrile to produce an aryl radical, according to the procedure of Saveant, et al. Briefly, the GC electrode was reduced in a thoroughly degassed, 1 to 5 mM solution of the diazonium salt in acetonitrile (Mallinckrodt, Inc.) containing 0.1 M tetrabutylammonium tetrafluoroborate (TBATFB) (Acros Organics). The reduction of diazonium salt on glassy carbon is self-inhibiting in that the attachment of the aryl radical on the electrode surface blocks further reduction of the diazonium salt (99). The electrode is scanned negatively for 3 to 10 cycles, until the diazonium reduction peak disappears. Disappearance of the diazonium reduction peak indicated a monolayer had been adsorbed. The electrode was then sonicated for five minutes in acetonitrile, fifteen minutes in IPA/AC, and then five minutes in water (for aqueous work) or five minutes in acetonitrile (for non-aqueous characterization). Surface coverage was monitored by either voltammetry or X-ray photoelectron spectroscopy (XPS), whichever was more appropriate.

Low oxide surfaces were prepared by anaerobic polishing in degassed cyclohexane (Mallinckrodt, Inc.)/alumina slurries on bare glass plates (84, 121). An alternative method for preparing low oxide surfaces was vacuum heat treatment (VHT) (82). The electrode is heated to approximately 800°C by resistive heating with a tantalum heating stub. After 30 minutes at 800°C in UHV, the sample was cooled and an XPS spectrum was acquired.
Catechol solutions were prepared in 0.1 M H$_2$SO$_4$ or 0.1 M pH 7 PBS (phosphate buffered saline), consisting of 0.1 M phosphate buffer with 0.1 M sodium chloride and adjusted to pH 7 with KOH or HCl. Catechol solutions were 1 mM (except for DOPEG, 0.4 mM), and were thoroughly degassed with argon before use.

Cyclic voltammetry was performed with a BAS100B workstation in a three-electrode configuration. Peak separations ($\Delta E_p$) were calculated from the difference in anodic and cathodic peak potentials determined from the voltammetry using the BAS100B/W operating software (version 2.3). For aqueous solutions, a Ag/AgCl (3M NaCl) (BAS Inc, West Lafayette, IN) was used as the reference, while for non-aqueous solutions, a Ag/Ag$^+$ (0.01 M AgNO$_3$, 0.1 M TBATFB in acetonitrile) was used. A coiled Pt wire was used as the counter electrode. The scan rate was 200 mV/s unless otherwise noted. Electrode areas were determined by chronoamperometry of 1 mM Fe(CN)$_6^{3-}$ in 1 M KCl (diffusion coefficient 6.32 x $10^{-6}$ cm$^2$/s). Survey and regional XPS spectra were acquired with a VG Scientific ESCALAB MKII spectrometer with either an Mg or Al anode. Grams32 (Galactic) software was used to calculate peak areas, and instrumental sensitivity factors were used to calculate atomic ratios. Static contact angles for Nanopure water were determined with a conventional contact angle microscope (Rame-Hart) in air.

Fresh solutions were prepared daily, and each voltammogram was recorded on a fresh surface unless otherwise noted. Chemicals: Cyclohexane, isopropyl alcohol, pyridine, and acetonitrile (Mallinckrodt, Inc.) were used as received. Dopamine (DA) and D,L-3,4-dihydroxy phenyl ethylene glycol (DOPEG), and D,L-DOPA (Sigma) were used as received. Methylene Blue (Aldrich) was used as received. 4-methyl catechol
(4MC) and 3,4-dihydroxyphenyl acetic acid (DOPAC) (Sigma) were recrystallized from hot toluene and ethyl acetate/hexane, respectively. Antraquinone-2,6-disulfonate, disodium salt (Acros Organics) was recrystallized from hot water/ethanol.

Diazonium salts were prepared according to Dunker, et al (134) with some modification. The salts were characterized with MS and NMR. The synthesis and spectroscopic characterization of the diazonium salts is discussed in Appendix C.

Results
Surface Characterization

In order to determine surface structure/redox system reactivity relationships, it is necessary to start with well-defined surfaces (5, 84, 85). The surfaces described in the experimental section were characterized primarily with XPS, cyclic voltammetry, and contact angle microscopy. Table 2.1 details the contact angle measurements and surface composition determined by XPS for the surfaces used in this chapter.

Polished GC surfaces have oxygen to carbon (O/C) ratios ranging from 8 to 15%. This variability can be caused by differences in polishing procedure and conditions. Polished surfaces in the past have been used as baseline surfaces because these surfaces can be prepared with reasonable reproducibility. Recently, it has been found that subjecting a glassy carbon electrode to a solvent-pretreatment procedure can produce active surfaces for dopamine and ferricyanide electron transfer (131). Table 2.1 lists the O/C ratio and Fe(CN)$_6^{3-/4-}$ rate constants for polished GC surfaces and several surfaces that have been solvent treated. Treating the surface with a solvent increases the electron transfer rate constant of Fe(CN)$_6^{3-/4-}$ by a factor of two, which is comparable to the rate constants on ultra clean polished (83) and vacuum heat treated surfaces (82, 135).
<table>
<thead>
<tr>
<th></th>
<th>Contact angle, degrees</th>
<th>O/C atomic ratio</th>
<th>F/C atomic ratio</th>
<th>N/C atomic ratio</th>
<th>$k^0$ (cm/s) Fe(CN)$_6$$^{3-4-}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polished GC$^a$</td>
<td>30 ± 3 (N = 16)</td>
<td>0.12 ± 0.03</td>
<td></td>
<td></td>
<td>0.076 ± 0.007 (N = 3)</td>
</tr>
<tr>
<td>Pyridine-treated GC</td>
<td>31 ± 4 (N = 14)</td>
<td>0.07 ± 0.01</td>
<td></td>
<td></td>
<td>0.15 ± 0.02 (N = 3)</td>
</tr>
<tr>
<td>Low-oxide (anaerobic polishing) GC</td>
<td>59 ± 3 (N = 15)</td>
<td>0.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-oxide (VHT) GC</td>
<td>55 ± 7 (N = 6)</td>
<td>0.04</td>
<td></td>
<td></td>
<td>0.14, 0.07$^b$</td>
</tr>
<tr>
<td>MB (Γ = 328 pmol/cm$^2$)</td>
<td>45 ± 11 (N = 3)</td>
<td></td>
<td></td>
<td></td>
<td>0.078 ± 0.005$^b$</td>
</tr>
<tr>
<td>NP</td>
<td>41 ± 2 (N = 15)</td>
<td>0.21 ± 0.02</td>
<td>0.07 ± 0.01</td>
<td></td>
<td>0.01$^b$</td>
</tr>
<tr>
<td>TFMP</td>
<td>91 ± 4 (N = 21)</td>
<td>0.03 ± 0.006</td>
<td>0.30 ± 0.01</td>
<td></td>
<td>0.049$^c$</td>
</tr>
<tr>
<td>HGC</td>
<td>65</td>
<td>0.036</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. Tokai GC20 electrodes were used for the data in this table.

Table 2.1. Contact Angle, XPS, and Fe(CN)$_6$$^{3-4-}$ rate data for various GC surfaces.
The XPS spectra for polished, solvent cleaned, and low oxide GC are shown in Figure 2.4. The O/C ratio decreases from approximately 12% on polished GC to between 7 and 8% for both pyridine and IPA/AC treated GC. Treating the electrode in either warm pyridine or a mixture of isopropanol and activated carbon (IPA/AC) (131) presumably removed oxygen-containing contaminants from the electrode surface yielding an oxygen to carbon ratio of 7%. The XPS spectrum of pyridine treated GC did not contain a peak due to nitrogen, indicating that residual pyridine was not present on the surface. Figure 2.5 shows the XPS spectra of pyridine treated surfaces that were either rinsed with Nanopure water after treatment or sonicated in Nanopure water after treatment; the similarity of the spectra indicated that rinsing the electrode was enough to remove the pyridine.

The O/C ratio for IPA/AC surfaces was stable for several days in air, rising 2% in one week. These surfaces did not reach the previous level of 12% observed for polished glassy carbon, at least in the time range studied (Figure 2.6). Comparing the C 1s regional spectrum of polished GC and pyridine treated GC reveals some interesting differences (Figure 2.7). The shoulder on the peak towards higher binding energies attributed to carbon bonded to oxygen was less pronounced on the pyridine treated GC. The same narrowing of the C 1s peak was observed for IPA/AC GC. This was taken as further evidence that the solvent treatment was removing polar contaminants from the electrode surface.
Figure 2.4. XPS spectra of polished GC, pyridine treated GC, and low oxide GC. O/C values are indicated for each surface.
Figure 2.5. XPS spectra of pyridine treated GC. A. Rinsed  B. Sonicated in Nanopure water.
Figure 2.6. O/C ratio versus time for polished and IPA/AC treated GC upon exposure to laboratory air.
Figure 2.7. XPS spectra of polished (- -) and pyridine treated (—) GC.
The catechols studied all adsorbed to both polished and solvent-treated surfaces. The surface coverage of the catechols on polished and solvent-treated electrodes is listed in Table 2.2. Coverage was first diagnosed with semi-integral voltammetry for high concentration solutions (1 mM), and then studied at low concentrations (~10 μM) in order to determine surface coverage of the catechols. Figure 2.8 is an adsorption isotherm for dopamine on IPA/AC treated GC. While catechols do adsorb to polished surfaces, solvent-treated surfaces have much more catechol adsorbed. Figure 2.9 clearly demonstrates this. The amount of 4MC adsorbed increased ten-fold upon pyridine treatment based on the coverage determined voltammetrically.

Using a solvent treatment can often recover electroactivity after surface fouling. If an electrode has been exposed to laboratory air for an extended period of time, it exhibits slow dopamine kinetics (∆E_p ~ 600 mV). Upon solvent treatment, however, the ∆E_p for dopamine returned to nearly the same value as the freshly treated sample (∆E_p = 52 mV). Solvent treatment provides a fast and reproducible method to prepare active electrode surfaces, as well as a base surface for comparison to other modified surfaces.

By controlling the amount of active surface area available with physi- or chemisorbed molecules, the sensitivity of dopamine and the other catechols to surface condition can be assessed. That dopamine is sensitive to surface condition has been well established (5, 14, 18, 39, 117); however, the work herein represents a systematic effort to determine the cause of catechol sensitivity to surface condition.

Methylene Blue (MB) was adsorbed to act as an inert spacer. MB is a convenient blocking layer because it adsorbs well to carbon surfaces, and its E_{1/2} is negative of the E_{1/2}
Polished Pyridine-treated IPA/AC treated

<table>
<thead>
<tr>
<th></th>
<th>Polished</th>
<th>Pyridine-treated</th>
<th>IPA/AC treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>DA pH 1</td>
<td>58 ± 7&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td></td>
<td>215 ± 22</td>
</tr>
<tr>
<td>DA pH 7</td>
<td>79 ± 10</td>
<td>89 ± 4</td>
<td>282 ± 12</td>
</tr>
<tr>
<td>4MC pH 1</td>
<td></td>
<td></td>
<td>655 ± 8&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>4MC pH 7</td>
<td>53 ± 2</td>
<td>265 ± 8</td>
<td></td>
</tr>
<tr>
<td>DOPAC pH 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOPAC pH 7</td>
<td>27 ± 8</td>
<td>70 ± 2</td>
<td></td>
</tr>
<tr>
<td>DOPEG pH 1</td>
<td></td>
<td></td>
<td>507 ± 65&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>DOPEG pH 7</td>
<td>25 ± 2</td>
<td>93 ± 4</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>. $N \geq 3$

<sup>b</sup>. unless otherwise indicated, adsorption determined from a 10 μM catechol solution at 2 V/sec.

<sup>c</sup>. residual adsorption determined from 1 mM solution, 2 V/sec.

Table 2.2 Surface Coverage of Catechols (pmol/cm²).
Figure 2.8 DA adsorption isotherm on IPA/AC treated GC.
Figure 2.9. 10 μM 4MC on polished and pyridine treated surfaces. $v = 1 \text{ V/s}, \text{pH} = 7$. 
for the catechols. This makes it possible to determine the surface coverage of MB simultaneously with catechol kinetic measurements by integrating the area under the MB voltammetric peak. MB undergoes the following reactions in aqueous solution, involving two electrons and one or two protons:

\[
\begin{align*}
\text{H}_3\text{C}-&\text{N}-\text{CH}_3 \\
\text{N}-\text{CH}_3 \\
\end{align*}
\]

\[2\text{e}^- + 2\text{H}^+ \quad \text{pH} < 5.6\]

\[2\text{e}^- + \text{H}^+ \quad \text{pH} > 5.6\]

Figure 2.10 shows the adsorption isotherms for MB on polished and pyridine treated surfaces. Although there is some scatter to the data, the points can be fit to a Langmuir isotherm reasonably well (R^2 of 0.9328 for polished GC and 0.9396 for pyridine treated GC) allowing the determination of saturation coverage. Higher coverage of MB is possible on the pyridine surface (425 pmol/cm^2) compared to the polished surface (318 pmol/cm^2). The theoretical coverage of MB was determined from molecular areas for both flat (128 pmol/cm^2) and edgewise (191 pmol/cm^2) orientations (121). The values reported in this work are higher than the theoretical values, presumably due to the roughness factor of glassy carbon (1.5 to 2.5) (125).

Chemisorption of nitrophenyl (NP) and trifluoromethylphenyl (TFMP) groups on the electrode surface provides surfaces that are stable under reasonable experimental conditions and apparently defect-free (99, 102). Figure 2.11 is the XPS spectrum of NP.
and TFMP modified GC. Surface coverage for the monolayers was determined by integrating the area underneath the N 1s and F 1s peaks and ratioing these areas (corrected for instrumental sensitivity factors) with the C 1s peak area. The theoretical coverage (based on XPS peak areas) for NP is 9.1% (136) and TFMP is 20.9%, reported as N/C or F/C ratios on flat surfaces. Again, the experimental coverage was higher than that calculated, presumably due to surface roughness of glassy carbon. To ensure that a monolayer rather than multilayers of the NP or TFMP were adsorbed, the electron transfer kinetics of Ru(NH$_3$)$_6^{3+/2+}$ were assessed on these surfaces. The expected decrease in electron transfer rate by a factor of 2-5 was observed; if anything slower than that was observed, the surface was rejected as unsuitable.

Regional XPS spectra of the modified surfaces reveal the types of bonding on the surfaces. In Figure 2.11 B, the regional spectrum of N 1s for the NP surface is shown. The peak at binding energy 406 eV is due to nitrogen bonded to oxygen in the nitro group. The peak at 402 eV may either be due to physisorbed diazonium salt or the presence of amine groups on the surface as the result of reduction of some of the nitro groups. Upon sonication in IPA/AC or treatment with pyridine, the intensity of the 402 eV peak decreases significantly. This indicates that the 402 eV peak is due primarily to physisorbed diazonium species. The C 1s region of TFMP modified GC is shown in Figure 2.11 C. What is noteworthy about this spectrum is that a peak due to carbon bonded to fluorine is clearly visible at 294 eV, confirming the presence of TFMP on the surface.
Figure 2.10. MB adsorption isotherms on ( ) pyridine treated GC and (- -) polished GC.
Figure 2.11 A. XPS survey spectra of TFMP and NP modified GC.
Figure 2.11 B. N 1s region of the XPS spectrum of NP modified GC. C. C 1s region of the XPS spectrum of TFMP modified GC.
It is essential to thoroughly degas the TFMP diazonium solution before and during the derivatization process. If the solution is not degassed enough, the surface becomes oxidized and coverage of the TFMP is not as high as it is when properly prepared. Figure 2.12 shows the derivatization voltammetry for degassed and non-degassed TFMP solutions. It is interesting that in the non-degassed voltammetry, the derivitization peak does not fade. For the properly prepared surface, the O/C ratio is 3% whereas the O/C ratio on non-degassed surface is 10%, which is similar to the O/C ratio on polished GC. The F/C ratio decreases from 31% to 16% from the properly prepared surface. The reduction in TFMP coverage is likely due to a competing reaction between the TFMP radicals generated and oxygen in solution, resulting in fewer aryl radicals available to attack the electrode surface. The effects of low TFMP coverage on DA voltammetry will be discussed later, as well as the stability of TFMP surfaces with time.

As defined herein, low oxide surfaces are considered to exhibit an O/C ratio of 4% or less. Surfaces prepared by anaerobic polishing in cyclohexane had an O/C ratio of 4% (84). These surfaces were convenient because of their relative ease of preparation; however, cleanliness may be an issue. It was difficult to ascertain whether or not cyclohexane has contaminated the surface since cyclohexane does not contain elements with convenient XPS features. If the surface was not sonicated in water long enough after the cyclohexane polish, DA voltammetry was quite variable on these surfaces.

Vacuum heat treated (VHT) GC had O/C ratios of 2 to 4%. These surfaces are quite active toward catechol adsorption (DA, DOPAC, and HQ). VHT produces surface without significant perturbation of the microstructure of GC, in contrast to methods such as argon ion sputtering (84). However, the VHT is time consuming and cumbersome.
Figure 2.12. Comparison of the derivatization voltammetry of TFMP GC. A. Degassed solution. B. Non-degassed solution.
Also, the heating stub is prone to breakage, limiting its useful lifetime. The C 1s region of VHT GC and polished GC are shown in Figure 2.13. As with the solvent treated cases (Figure 2.7), the shoulder due to surface oxides is attenuated by heat treatment. VHT surfaces are useful for diagnostic purposes, but the instability of these surfaces towards oxidation and adsorption properties limits their analytical usefulness.

Interfacial capacitance measurements were performed on VHT and diazonium modified surfaces, and the values for the capacitance are listed in Table 2.3. It is important to note that capacitance varies with potential on carbon surfaces; however, the capacitances in Table 2.3 were determined in the same manner. In regions of a voltammogram with no Faradaic processes, the double layer capacitance for an electrode can be estimated by the following relationship: \( i = \nu C_{dl} \), where \( i \) is the observed current, \( \nu \) is the scan rate, and \( C_{dl} \) is the capacitance. For the data presented in Table 2.3, current was measured in 1 M KCl at 20 V/sec at 0 V vs Ag/AgCl. Thus, the observed values provide a reasonable relative comparison between surfaces. The measured interfacial capacitances of the solvent treated electrodes is markedly higher than that observed for polished surfaces, and the values observed on these surfaces compare well with what Ranganathan et al observed on commercial BAS electrodes (131). The higher values of capacitance are consistent with removal of adsorbed impurities from the electrode surface. Surfaces with high capacitance are generally more active towards electron transfer and adsorption (5, 117, 137, 138). The capacitance for the NP and TFMP surfaces are approximately half that observed for polished GC. TFMP surfaces have the lowest capacitance, which is expected since it is the most hydrophobic of the monolayers studied here.
Figure 2.13. C 1s regional spectrum of VHT (---) and polished (—) GC.
<table>
<thead>
<tr>
<th></th>
<th>$C_d/\text{cm}^2$ (μF)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polished</td>
<td>78.15</td>
<td>0.49</td>
</tr>
<tr>
<td>IPA/AC</td>
<td>103.64</td>
<td>0.60</td>
</tr>
<tr>
<td>Pyridine</td>
<td>89.56</td>
<td>0.39</td>
</tr>
<tr>
<td>MB</td>
<td>60.24</td>
<td>0.57</td>
</tr>
<tr>
<td>AQDS</td>
<td>72.06</td>
<td>1.08</td>
</tr>
<tr>
<td>NP</td>
<td>69.38</td>
<td>2.19</td>
</tr>
<tr>
<td>TFMP</td>
<td>19.17</td>
<td>1.26</td>
</tr>
<tr>
<td>2-AQ</td>
<td>66.60</td>
<td>1.52</td>
</tr>
</tbody>
</table>

Table 2.3 Capacitance of modified GC surfaces. Capacitance calculated from $i = C_d\nu$, $\nu = 20$ V/sec, current was measured at 0 V vs Ag/AgCl in 1 M KCl.
Electrode Kinetics

By systematically comparing the voltammetry of the catechols on the modified surfaces, some observations about the nature of the electron transfer properties of the catechols were made. Based on Chen and McCreery's "tree" scheme, there are three basic surface types: surfaces with high surface oxide coverage (polished and in this work, pyridine treated and IPA/AC treated GC), low oxide surface coverage (cyclohexane/alumina polished and VHT GC), and blocked surfaces (MB, NP, and TFMP covered GC). The voltammetric behavior of the catechols on these surfaces was assessed.

The benefits of treating the electrode surface with a solvent are shown in Figure 2.14. The $\Delta E_p$ for DA (and the other catechols) decreases on the solvent treated surfaces. Table 2.4 and 2.5 list how $\Delta E_p$ for each of the catechols changes on the modified surfaces at pH 1 and 7, respectively. The effects of pyridine and IPA/AC solvent treatment were very similar, both in their effect on catechol voltammetry and on the XPS spectra of the treated surfaces. Adsorption of the catechols was evident on polished, pyridine treated and IPA/AC treated GC, and the semi-integral of the background-subtracted voltammogram was used to diagnose adsorption. Semi-integrals for DA, 4MC, DOPAC, and DOPEG at pH 1 and 7 for both IPA/AC and polished surfaces are shown in Figure 2.15. The adsorptive behavior is indicated by a peak superimposed on a sigmoid, and is more pronounced on the solvent treated surfaces. To quantify the amount of adsorption on the electrode surfaces, low concentration voltammetry was performed. Table 2.2 lists the amounts of catechol adsorbed on the different surfaces. The differences in adsorption for one catechol, 4MC, are clearly illustrated in Figure 2.9.
<table>
<thead>
<tr>
<th></th>
<th>DA</th>
<th>4MC</th>
<th>DOPAC</th>
<th>DOPEG</th>
<th>DOPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polished</td>
<td>61 ± 8 </td>
<td>62 ± 7</td>
<td>61 ± 8</td>
<td>47 ± 4</td>
<td></td>
</tr>
<tr>
<td>Pyridine-treated</td>
<td>43 ± 2</td>
<td>47 ± 2</td>
<td>42 ± 4</td>
<td>40 ± 4</td>
<td></td>
</tr>
<tr>
<td>IPA/AC</td>
<td>36 ± 6 [39 ± 2]</td>
<td>39 ± 1</td>
<td>40 ± 2</td>
<td>37 ± 1</td>
<td>40 ± 3</td>
</tr>
<tr>
<td>Low oxide (anaerobic polish)</td>
<td>66 ± 4</td>
<td>81 ± 8</td>
<td>70 ± 6</td>
<td>66 ± 3</td>
<td></td>
</tr>
<tr>
<td>Low oxide</td>
<td>40 ± 2</td>
<td>48 ± 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MB</td>
<td>315 ± 7</td>
<td>256 ± 4</td>
<td>208 ± 8</td>
<td>267 ± 18</td>
<td>116 </td>
</tr>
<tr>
<td>NP</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>&gt;650</td>
<td>&gt;1000</td>
<td></td>
</tr>
<tr>
<td>TFMP</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>COOH</td>
<td>439 [463]</td>
<td>197</td>
<td>262 ± 2</td>
<td>262</td>
<td></td>
</tr>
</tbody>
</table>

a. N ≥ 3
b. values in brackets are for 0.1 M D₂SO₄ in D₂O
c. adsorption of catechols evident

Table 2.4. Voltammetric results for catechols at pH 1, scan rate = 200 mV/s.
<table>
<thead>
<tr>
<th></th>
<th>DA</th>
<th>4MC</th>
<th>DOPAC</th>
<th>DOPEG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polished</td>
<td>67 ± 10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62 ± 7</td>
<td>115 ± 6</td>
<td>47 ± 4</td>
</tr>
<tr>
<td>Pyridine-treated</td>
<td>47 ± 2</td>
<td>47 ± 2</td>
<td>75 ± 9</td>
<td>40 ± 4</td>
</tr>
<tr>
<td>IPA/AC</td>
<td>41 ± 5</td>
<td>59 ± 4</td>
<td>71 ± 5</td>
<td>41 ± 4</td>
</tr>
<tr>
<td>Low oxide (anaerobic polish)</td>
<td>88 ± 7</td>
<td>59 ± 2</td>
<td>118</td>
<td>89</td>
</tr>
<tr>
<td>Low oxide</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MB</td>
<td>193 ± 17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>210 ± 11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>274 ± 6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>253 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>NP</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>TFMP</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>&gt;800</td>
<td></td>
</tr>
<tr>
<td>COOH</td>
<td>114</td>
<td>172</td>
<td>530</td>
<td>264</td>
</tr>
</tbody>
</table>

<sup>a</sup> N ≥ 3
<sup>b</sup> adsorption of catechols evident

Table 2.5. Voltammetric results for catechols at pH 7, scan rate = 200 mV/s.
Figure 2.14 Comparison of Polished and IPA/AC treated GC A. DA B. 4MC
(---) polished GC (—) IPA/AC GC
Figure 2.14 continued. Comparison of Polished and IPA/AC GC C.
DOPAC D. DOPEG.
(- -) polished GC (—) IPA/AC GC.

82
Figure 2.14 continued. E. DA F. 4MC.
(- -) polished GC (—) pyridine treated GC.

Potential (mV vs Ag/AgCl)

Current Density (mA/cm²)
Figure 2.14 continued. G. DOPAC H. DOPEG.
(---) polished GC (—) pyridine treated GC.
Figure 2.15. Semi-integrals polished and solvent treated GC. A. DA B. 4MC (- -) polished (—) pyridine treated GC.
Figure 2.15. Semi-integrals polished and solvent treated GC. C. DOPAC D. DOPEG (- -) polished (—) IPA/AC GC.
Covering the electrode surface with an inert adsorbed layer had a dramatic effect on catechol voltammetry. Figure 2.16 compares the voltammetry of DA, 4MC, DOPAC, and Ru(NH$_3$)$_6^{3+/2+}$ on polished GC and NP modified GC at pH 7. Since Ru(NH$_3$)$_6^{3+/2+}$ is a well-behaved outer-sphere system, we expected and observed only small changes in $\Delta E_p$ upon modification. However, the catechol kinetics were severely inhibited on the NP surfaces, and for DA, oxidation and reduction were completely inhibited. The complete inhibition of electron transfer kinetics was unexpected in light of what has been previously observed on diazonium-modified surfaces. Although close contact with the electrode surface is prevented on diazonium-modified surfaces, an outer sphere electron transfer route should still be available. For example, carbonyl groups on glassy carbon surfaces are catalytic for the Fe$^{3+/2+}$ redox system. If these groups are blocked by diazonium modification, electron transfer still takes place for Fe$^{3+/2+}$, albeit more slowly. This slower rate represents the outer-sphere electron transfer rate for Fe$^{3+/2+}$. Electron transfer via an outer-sphere route has been observed for redox species on diazonium modified GC (84, 101, 105, 129) and for SAMs ((8) and references contained therein). In light of what has been observed for other redox systems on diazonium modified surfaces, the catechol's behavior is extremely unusual, since even if a catalytic inner-sphere route was blocked for the catechols there should still be some current observed due to an outer sphere mechanism. This inhibition of electron transfer was also observed for DOPEG at pH 7, and for all of the catechols at pH 1 as shown in Figure 2.17. Another unusual property of DA on NP modified surfaces was the appearance of several small adsorption type peaks in the voltammetry (Figure 2.18). These peaks were not diffusional in nature,
Figure 2.16. Comparison of NP(−−) and polished (→) GC, pH 7.
A. Ru(NH₃)₆⁺³⁺² 20 V/s B. DA, v = 200 mV/s.
Figure 2.16. Comparison of NP (--) and polished (—) GC, pH 7. C. 4MC D. DOPAC.
Figure 2.17. Comparison of NP (---) and polished (—) GC, pH 1.
A. DA  B. 4MC.
Figure 2.17. Comparison of NP(- -) and polished (—) GC, pH 1.
C. DOPAC  D. DOPEG.
Figure 2.18. Detail of DA ‘‘adsorption’’ peaks on NP GC, \( v = 200 \text{ mV/s} \)

A. \( \text{pH} = 1 \).  B. \( \text{pH} = 7 \).
and persisted even after the electrode was removed from the DA solution and placed in blank electrolyte. These peaks may have been due to DA that had partitioned into the NP layer.

Similar inhibition of catechol oxidation was observed on the TFMP surfaces, as shown in Tables 2.4 and 2.5. Although small adsorption-like peaks for DA were observed on the NP modified surfaces, no such behavior was observed on the TFMP modified surfaces. Since the fluorine from the TFMP groups provides a convenient marker for TFMP coverage, the effect of varying the surface coverage of the monolayer on DA voltammetry was monitored at pH 1. By changing the number of derivatization scans, the coverage of TFMP on the electrode surface was varied. Figure 2.19 illustrates this effect on DA voltammetry. As surface coverage of TFMP (as measured by the F/C ratio) increased, the $\Delta E_p$ for DA increased.

As discussed above in the surface modification section, by not degassing the TFMP derivatization solution the F/C of the TFMP surfaces decreased while the O/C ratio increased, indicating that surface coverage of TFMP decreased. The reduction in the coverage of TFMP was mirrored in the voltammetry for DA on the deaerated and non-degassed TFMP surfaces, as shown in Figure 2.20. $\Delta E_p$ was much smaller on the non-degassed surfaces. The same effect is seen on a surface that was cycled to extreme potentials (to 1.8V vs. Ag/AgCl) 10 times; the F/C ratio decreased to 10% and the $\Delta E_p$ for DA was 210 mV. Freshly prepared surfaces can also contain active sites at “pinholes” in the monolayer; DA voltammetry at pH 7 is shown in Figure 2.21A for such a case. The electrode was then rinsed and transferred to blank electrolyte; the residual
Figure 2.19. DA voltammetry on TFMP surfaces with varying coverage. Numbers represent the F/C ratio.

1 mM DA, $\nu = 200$ mV/s, 0.1 M $\text{H}_2\text{SO}_4$. 
Figure 2.20. Comparison of degassed (---) and non-degassed (—) TFMP surfaces.

1 mM DA, 0.1 M H₂SO₄, v = 200 mV/s
Figure 2.21A. TFMP GC with "pinholes." (---) 1 mM DA (--) residual DA pH 7 PBS, v = 200 mV/s.
Figure 2.21B. DA voltammetry on TFMP GC on (—) 0 to 1.8 V vs Ag/AgCl, 200 mV/s, 10 cycles (—-)
IPA/AC GC, 1 mM DA, 0.1 M H$_2$SO$_4$, $v = 200$ mV/s
DA adsorbed to the surface is shown in Figure 2.21B. Apparently, any free surface can act as an active site for DA oxidation.

MB adsorbed to the electrode surface also blocks catechol electron transfer, although not quite as completely as the chemisorbed monolayers. Figure 2.22 shows the effect of saturation coverage of MB adsorbed to GC compared to that of polished GC for the catechols. In contrast to what is expected, at pH 7 $\Delta E_p$ for DOPAC is larger than $\Delta E_p$ for DA. One would expect the opposite to be true since MB is a cation at pH 7, and should repel the positively charged DA and attract the negatively charged DOPAC. The effect of MB adsorption on DA voltammetry was studied in more detail by including low concentrations of MB in the DA solution, and it was found that increasing the MB coverage on the electrode surface increased the $\Delta E_p$ for DA as expected as shown in Figure 2.23A for a polished surface and Figure 2.23B for a surface that was initially treated with pyridine. This is represented graphically in Figure 2.24. For the pyridine treated surfaces at low MB solution concentration, there was still dopamine adsorption, evidenced by the semi-integral of the voltammetry and the small observed $\Delta E_p$.

The final class of surface treatments in the “tree” scheme was the low oxide surfaces. The effects of these treatments are shown in Tables 2.4 and 2.5, and are not very different from the polished and solvent treated surfaces. VHT surfaces, as noted above, tend to adsorb catechols as shown in Figure 2.25. VHT surfaces also have the fastest kinetics, based on $\Delta E_p$.

The voltammetry of hydroquinone (HQ) and benzoquinone (BQ) were also studied on the modified surfaces, with the results listed in Table 2.6. It is evident that HQ and BQ had similar behavior as the catechols on the modified surfaces. That is,
Figure 2.22. Catechols on polished (—) and MB adsorbed (—) GC, v = 200 mV/s, pH 7. A. DA. B. 4MC.
Figure 2.22. Catechols on polished (- -) and MB adsorbed (—)
GC, v = 200 mV/s, pH 7. C. DOPAC D. DOPEG.
Figure 2.23A. The effects of MB adsorption on polished GC on 1 mM DA, 0.1 M H$_2$SO$_4$, $\nu = 200$ mV/s.
Figure 2.23B. The effects of MB adsorption on pyridine treated GC on 1 mM DA, 0.1 M H$_2$SO$_4$, $\nu = 200$ mV/s.
Figure 2.24. Comparison of $\Delta E_p$ of 1 mM DA on polished (---) and pyridine treated (- - -) and pyridine treated (--- • ---) GC with MB adsorbed.
Figure 2.25 1mM DA, 0.1 M H$_2$SO$_4$, 200 mV/s
VHT GC (---), polished GC (--).
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<tr>
<td></td>
<td>HQ pH 1</td>
</tr>
<tr>
<td>Polished$^a$</td>
<td>61 ± 6$^c$</td>
</tr>
<tr>
<td>Pyridine-treated$^a$</td>
<td>53 ± 5</td>
</tr>
<tr>
<td>IPA/AC$^b$</td>
<td></td>
</tr>
<tr>
<td>Low oxide</td>
<td></td>
</tr>
<tr>
<td>(anaerobic polish)$^a$</td>
<td>58 ± 1</td>
</tr>
<tr>
<td>Low oxide</td>
<td></td>
</tr>
<tr>
<td>(VHT)$^b$</td>
<td></td>
</tr>
<tr>
<td>MB$^a$</td>
<td>200 ± 14$^d$</td>
</tr>
<tr>
<td>NP$^a$</td>
<td>&gt;800</td>
</tr>
<tr>
<td>TFMP$^b$</td>
<td>&gt;850</td>
</tr>
</tbody>
</table>

a. BAS electrodes  
b. Tokai GC20 plates  
c. $N \geq 3$  
d. adsorption of quinones evident from semi-integral

Table 2.6. HQ and BQ voltammetry
modifying the surface with NP and TFMP caused similar inhibition of HQ and BQ electron transfer kinetics while reduction of the O/C ratio had little effect on the voltammetry.

**Discussion**

After consideration of the data in Tables 2.4 and 2.5, it is evident that all of the catechols have the same general voltammetric behavior, regardless of side chain structure or electrostatic charge. Solvent treatment and oxide removal had the least effect on $\Delta E_p$ compared to polished surfaces. In fact, some of the decrease in $\Delta E_p$ may be an artifact of the perturbation in peak separation caused by the presence of adsorbed catechol species. The only system that shows a large change in $\Delta E_p$ upon oxide removal and pyridine treatment is DOPAC at pH 7; the decrease in $\Delta E_p$ for DOPAC observed is likely due to reduction in negatively charged surface oxide groups that could repel the anionic DOPAC. The most significant change on the VHT and solvent treated surfaces was the large increase in catechol adsorption on these surfaces.

The most dramatic effects on catechol oxidation are observed with the surfaces modified with a layer of NP or TFMP. Complete inhibition of electron transfer (in the potential range studied) when the blocking layer is only one molecule deep (~6.8 Å) is not expected, since electron tunneling through the layer should still occur. For Ru(NH$_3$)$_6^{3+/2+}$, a system known to undergo tunneling on monolayer covered surfaces, the decrease in rate is only a factor of 2-5. Even for a system such as Fe$^{3+/2+}$, which has a slow outer-sphere electron transfer rate (84, 85), a response was still observable on the monolayer-modified surfaces. For the fastest case of 4MC at pH 7 on NP modified GC, $\Delta E_p$ was 468mV, and evaluation of the relationship between $\Delta E_p$ and $k^0$ by the method of
Nicholson (139) implies a decrease in $k^0$ by a factor of approximately four orders of magnitude compared to polished that on a polished surface. This represents the fastest case, with the other catechols having $\Delta E_p$ or 600mV or greater and having concomitant decreases in electron transfer rate. This dramatic decrease in electron transfer rate is not observed for several other organic redox systems; methyl viologen, methylene blue, and several phenothiazines exhibit behavior that closely parallels that of Ru(NH$_3$)$_6^{3+/2}$ (129).

The unusual behavior of catechols and hydroquinone on the modified surfaces raises a puzzling question: Why do the catechols not tunnel through the inert spacer as do methyl viologen, methylene blue, phenothiazines, and Ru(NH$_3$)$_6^{3+/2}$? To gain insight into this problem, the observed results will be discussed in light of several hypotheses to explain possible electron transfer mechanisms. Figure 2.26 shows in graphic form two examples of electron transfer mechanisms; outer-sphere and inner-sphere, oxide catalyzed. Quinones definitely act as inner sphere systems, but the question remains: What makes quinones different?

Since the solvent treatments and VHT seem to remove adsorbed impurities and polishing debris, the decrease in $\Delta E_p$ can be attributed to faster kinetics and adsorption. The reduction of surface oxide coverage on the solvent treated surface is less than that seen on the VHT surfaces; because of this we say that surface oxide groups do not appear necessary for fast catechol electron transfer. Another question is whether or not there is a correlation between surface oxide coverage and catechol adsorption. In other words, is electrostatics a factor in catechol electron transfer? If surface oxides play a role in adsorbing catechols or catalyzing electron transfer of the catechols on the electrode surface, then removal of the surface oxides should result in either a decrease in
Figure 2.26. Redox mechanisms for outer sphere, inner sphere, and quinone electron transfer.
adsorption or retardation of electron transfer rate. However, on surfaces with low oxide coverage, such as VHT GC, fractured GC, and hydrogenated GC (HGC), DA kinetics are still fast. These surfaces, especially fractured and VHT GC, exhibit strong DA adsorption. There is no clear correlation between the presence of surface oxide groups and fast electron transfer kinetics for the catechols in the results presented here.

Two observations can be made about the electron transfer kinetics of catechols in general and DA in particular on the modified GC surfaces. One is that a tunneling mechanism is extremely slow on blocked glassy carbon surfaces, indicated by $\Delta E_p$ greater than 1V for some of the catechols. This implies that close association with the electrode surface is necessary for electron transfer. Secondly, adsorption of the catechols accompanied fast electron transfer on the active surfaces. On the MB and TFMP surfaces, as the active electrode area decreased ($\Gamma_{\text{mono}}$ increased), the electron transfer kinetics were slower. This leads to two possibilities: that adsorption of the catechols is necessary for electron transfer or that whatever is blocking catechol oxidation on the monolayer-modified surface also blocks fast electron transfer. These blocked sites may be sites for proton transfer or redox mediation, as discussed below.

There are other possibilities for the catalyzing mechanism for catechol electron transfer on glassy carbon electrodes. The suggested mechanisms include hydrophobic interactions, redox mediation by surface oxide groups (140), proton transfer to surface sites (127), and electrostatic attraction or repulsion (40). Referring to Table 2.4 and 2.5, there is no direct correlation between the observed peak separation for the catechols and the contact angle for the modified surfaces. For example, NP GC and polished GC have
similar contact angles, yet vastly different kinetics. This implies that hydrophobic effects cannot be the sole reason for changes in catechol electron transfer rate.

In a redox mediation mechanism, the $E_{\text{m}}$ of the mediated species would be expected to shift to near the $E_{\text{m}}$ of the mediating species \(^{(6)}\). If surface oxides were mediating the oxidation of the catechols, then some shift in $E_{\text{m}}$ should occur upon change in the oxide level of the GC surface. However, $E_{\text{m}}$ for the catechols remains the same regardless of oxide level. In addition, for mediated electron transfer an asymmetric wave shape would be expected since the redox mediation is effective in only one direction. The catechol waves do not exhibit the characteristic shape of a mediated electron transfer reaction; instead, the catechol waves are symmetric and remain so even upon change in oxide level. In short, there appears to be no definitive evidence for catechol oxidation being mediated by surface oxide groups.

Changing the pH from 1 to 7 changes the order of catechol oxidation from $e^+H^+e^-H^+$ to $H^+e^-H^+e^-\left(12, 13\right)$. If fast electron transfer depends on a proton transfer step, then one would expect changes in voltammetric current with direction of scan (i.e. oxidation or reduction) if the surface is modified. Modifications are expected to alter proton transfer sites, either by removal of oxygen or by blockage of general “sites”. However, significant changes in the voltammetry is not observed when oxides are removed, indicating that proton transfer is not an essential step. In fact, on carbon paste and platinum electrodes for catechols and quinones, respectively, protonations are assumed to be at equilibrium \((12, 13, 115, 116)\).

Finally, if electrostatic repulsion or attraction is controlling catechol oxidation, then very different effects should be observed depending on solution pH and surface
charge. For example, very different $\Delta E_p$ vs. pH trends should be observed for cationic DA and anionic DOPAC. However, it is observed that on surfaces with neutral modifiers such as NP and TFMP and the cationic modifier MB, the voltammetry of cationic DA, anionic DOPAC (pH 7), and neutral 4MC and DOPEG is very similar. Because these species have different charges and there are no drastic changes in voltammetry on the modified surfaces, it is difficult to conclude that electrostatic effects are the overriding factor in a catechol oxidation mechanism. Some small effects (approximately a factor of 2-3) are seen, but can be explained with what is expected with Frumkin corrections (141).

In fact, the opposite effect of what is expected is observed on MB surfaces concerning DOPAC and DA. At pH 7, DOPAC (anion) is slower on the positively charged MB surface than DA (cation). On surfaces where a thick film is involved, such as Nafion® modified electrodes or heavily oxidized electrodes, drastic effects are seen with changing catechol charge (14, 22, 33, 40, 119). In these cases, DA kinetics are fast while negatively charged analytes such as DOPAC, ascorbate, and Fe(CN)$_6^{3/-4}$ are rejected. However, such large effects are not observed in the current experiments, apparently because the modifications are monolayer rather than thick films. Finally, if surface oxides are removed or reduced (thus removing anything to attract cations and reject anions), as in on VHT, fractured, and solvent treated GC, the $\Delta E_p$ becomes independent of the charge on the catechol.

Conclusions

If the hypotheses discussed above do not adequately explain catechol oxidation on glassy carbon, then what is catalyzing catechol oxidation? Once again, the relevant observations for are:
- Catechols adsorb to polished, solvent treated, and VHT surfaces at both pH 1 and pH 7
- Catechol adsorption tracks $\Delta E_p$, with the fastest surfaces having the greatest amount of adsorption
- Changing the level of oxides on the surface does not correlate with the electron transfer kinetics
- Catechol oxidation is suppressed on monolayer-covered surfaces, indicating that a tunneling pathway is inefficient. This is unusual (compared to methyl viologen, methylene blue, phenothiazines); for phenothiazines on monolayer-covered surfaces, adsorption is suppressed and electron transfer is still fast.

Although it is dangerous to compare metal electrode surfaces with glassy carbon, some interesting observations may be relevant. On Pt electrodes with a layer of adsorbed cyanide, the electron transfer kinetics for benzoquinone are significantly decreased (4). Similarly, an oxide film on iridium electrodes inhibits quinone electron transfer, while adsorption of a $\sim$10% layer of sulfur atoms will almost completely restore quinone electron transfer (142). Considering these two cases, it seems more likely that the reduction of the electron transfer rate occurs due to blocking of quinone adsorption rather than a change in tunneling distance for outer sphere electron transfer.

Looking beyond the “tree” classification scheme, it is evident that adsorption of the catechols to the surface plays a key role in catechol oxidation. The question remains: Exactly why is adsorption necessary for fast electron transfer in catechols?
For other organic redox systems (methyl viologen, methylene blue, chlorpromazine), observable adsorption is not required for electron transfer. The role of adsorption in catechol oxidation is examined further in Chapter Three.
CHAPTER 3

THE ROLE OF CATECHOL AND QUINONE ADSORPTION IN CATECHOL OXIDATION ON GLASSY CARBON ELECTRODES

Introduction

Adsorption of p-quinones and catechols has been the subject of extensive research on metal (15, 17, 143, 144) and carbon (86, 87, 123, 135, 138, 145) electrodes. The interest stems from both fundamental issues and analytical issues. Investigation of properties such as adsorption orientation (17, 143, 144, 146-148), orientation-dependent redox potentials (15), and strength of adsorption (16) to the electrode surface indicate important factors that affect catechol and quinone interaction with the electrode surface. The advent of fast-scan cyclic voltammetric techniques led to intensive interest in the behavior of catechols on microelectrodes. Indeed, adsorption is essential for the analytical utility of carbon fiber electrodes used for some in vivo applications. In such applications, adsorption of the catechol occurs on a heavily oxidized surface or on a deposited anionic film, such as Nafion®. The anionic surfaces (oxidized and Nafion® coated electrodes) also serve to reject anionic interferences (14, 33, 36-38, 40).
Adsorption of quinones to metal electrode surfaces has received abundant attention. Most of the reported work involves Pt electrodes, with some studies on Ir, Rh, and Pd (15-17, 143, 144, 147). On clean platinum surfaces, the quinones have been found to adsorb irreversibly, requiring exhaustive oxidation of the bound quinone or displacement by a strong ligand such as iodine or sulfur to remove the quinone from the Pt surface. Interestingly, adsorption of the quinone to the Pt surfaces reduces the electroactivity of the quinone; this reduction in electroactivity of the quinone is not observed on disordered carbon electrodes (84, 87, 138, 145). In contrast to the results on disordered carbon, recent scanning probe microscopy studies suggest quinone adsorption takes place on the basal plane of ordered graphite materials that leaves the quinone in an electroinactive form (86). On Pt electrodes, the adsorbed layer of quinones actually inhibits fast electron transfer of quinones in solution (142, 148). Sub-monolayer coverage of either sulfur or iodine restores fast electron transfer kinetics for the solution quinone. The orientation of quinones on the Pt surfaces was found to depend on concentration, temperature, and identity of electrolyte ions.

On carbon electrodes, adsorption studies have been performed on electrochemically pretreated (ECP) electrodes (138), fractured glassy carbon electrodes (138), and Nafion® films (5, 18, 40). The Nafion® studies have focused on the ability of the polymer to pre-concentrate the cationic catecholamines into the polymer matrix. The main interest in the "adsorption" characteristics of these films is the analytical utility of such interactions. Electrochemically pretreated electrodes are effective for adsorbing catecholamines from solution, acting to pre-concentrate the catecholamines to enhance sensitivity (41, 119, 149). The increase in the amount of adsorbed catecholamine has
been attributed to electrostatic attraction to the negatively charged oxidized surface; however, adsorption of both cationic (dopamine, dihydroxy beznyl amine) and anionic (dihydroxyphenyl acetic acid, anthraquinone-2,6-disulfonate) quinones have called into question whether or not electrostatic interactions are the only factor involved (86, 138).

By fracturing glassy carbon in the analyte solution, a surface is obtained that presumably approximates the bulk properties of glassy carbon (138). Table 3.1 lists the amount of different catechols adsorbed on glassy carbon surfaces subjected to different surface pretreatments. Catechols were found to adsorb very strongly to these fractured surfaces, regardless of side chain charge (positive, negative, or neutral). Although the amount of adsorbed DOPAC does decrease on the ECP surface compared to the fractured surface, a substantial amount of the anionic catechol was still present on the ECP surface. This indicates that electrostatic interactions alone do not control catechol adsorption onto carbon electrode surfaces.

In Chapter 2, it was reported that fast catechol kinetics were accompanied by catechol adsorption to the electrode surface. Also, surfaces that blocked catechol adsorption severely hindered catechol oxidation kinetics. Based on these observations, it was concluded that catechol adsorption to the glassy carbon electrode surface is necessary for fast electron transfer. In this chapter, the relationship between catechol adsorption and the ease of catechol electron transfer is explored.

**Experimental**

Polishing and solvent-treatment procedures were as described in Chapter 2. Catechol solutions were prepared daily in either 0.1 M H\textsubscript{2}SO\textsubscript{4} or 0.1 M PBS (0.1 M phosphate buffer with 0.1 M NaCl added, “phosphate buffered saline”) adjusted to pH 7
<table>
<thead>
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<th>Polished(^a,b)</th>
<th>Pyridine-treated(^a)</th>
<th>IPA/AC treated(^a)</th>
<th>Fractured(^c)</th>
<th>Fractured + ECP(^c)</th>
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<td>58 ± 7</td>
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<td>93 ± 4</td>
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\(^a\) this work, calculated from voltammetry of 10 μM catechol solution at 1 to 5 V/s  
\(^b\) in pmol/cm²  
\(^c\) Allred, C. D.; McCleery, R. L. Anal. Chem. 1992, 64, 444-448  
\(^d\) this work, calculated from the residual voltammetry after immersion in 1 mM catechol

Table 3.1. Surface coverage for catechols on treated GC surfaces calculated from cyclic voltammetry.
with KOH or HCl. For studies involving the H/D isotope effect, 0.1 M D$_2$SO$_4$ in D$_2$O replaced the 0.1 M H$_2$SO$_4$. In addition to NP, TFMP, and COOH surfaces prepared from diazonium reduction, anthraquinone surfaces with two different points of attachment were prepared from the reduction of the corresponding diazonium salts, 9,10-anthraquinone-1-diazo-1-nium$_{\frac{1}{2}}$ ZnCl$_2$ (1AQ) (Aldrich) and 9,10-anthraquinone-2-diazo-1-nium tetrafluoroborate (2AQ). The 2AQ salt was prepared according to Dunker et al. from the amine precursor (134), and is described in more detail in Appendix C.

9,10-anthraquinone-2,6-disulfonate disodium salt (AQDS, Acros Organics), duroquinone (DUQ, Acros Organics), phenanthrenequinone (PQ, Aldrich), 1,2-naphtoquinone-4-sulfonate (NQS, Aldrich), and 4MC, and 6-hydroxydopamine (6DA, Sigma) were pre-adsorbed from solution by the following procedure: a freshly polished or solvent-treated surface was immersed in a solution of the quinone for 5 to 10 minutes, rinsed thoroughly with water, and immediately transferred to the catechol solution for voltammetry. Solution concentrations were as follows: AQDS, 10 mM, DUQ, saturated solution, PQ, saturated solution, and 4MC, NQS, and 6DA, 1 mM. For some experiments, AQDS was present in the catechol solution, as indicated in the relevant sections below.

Cyclic voltammetry was carried out using BAS 100W/B (Bioanalytical Systems, Inc), as described in Chapter Two. Digital simulation of voltammetry was accomplished by using Digisim version 2.0, a commercially available simulation program (Bioanalytical Systems, Inc). Survey and regional XPS spectra were acquired with a VG Scientific ESCALAB MKII spectrometer with either a Mg or Al anode. Grams32 (Galactic) software was used to calculate peak areas, and instrumental sensitivity factors.
were used to calculate atomic ratios. Electrode areas defined by Teflon o-rings were
determined by chronoamperometry, as described in Chapter Two. $\Delta E_p$ and surface
coverages are reported as averages ± standard deviations. Where a standard deviation is
indicated, 3 or more trials were used. The scan rate for catechol voltammetry was 200
mV/sec unless otherwise noted.

Results

Based on the conclusion reached in Chapter Two that catechol oxidation is
catalyzed by adsorption of the catechol on the electrode surfaces, the question arises:
What is the nature of the relationship between adsorption and fast catechol electron
transfer kinetics? The first possible mechanism that comes to mind is a stepwise
mechanism composed of adsorption of the catechol to the electrode surface, oxidation of
the catechol, then desorption of the o-quinone product. This mechanism is shown in
Figure 3.1 A. If one assumes that the electron transfer kinetics of the surface bound
species are fast, then the observed electron transfer kinetics are a function of the
catechol’s adsorption and desorption rates. It is evident from these and other studies
(138, 150) that catechol adsorption to the electrode surface is very rapid; the experimental
setup used in this work generally has the catechol solution in contact with the carbon
electrode 10 to 20 seconds before voltammetry takes place. During this time, almost all
observed catechol adsorption takes place. Because of the fast adsorption, the adsorption
kinetics of the catechols were not investigated; it was assumed to be sufficiently fast not
to be a rate limiting step. If the mechanism in Figure 3.1 A is to be considered viable,
then the desorption rate of the ortho-quinone oxidation product from the electrode surface
must be fast enough to support the observed current. If the desorption rate is not fast
Figure 3.1. DA oxidation mechanisms.
enough to keep up with oxidation, then a bottleneck will occur from all of the electrode
active sites being occupied by the ortho-quinone. If such a bottleneck occurred, the
current would dwindle to near zero.

To investigate the feasibility of a stepwise mechanism, the desorption rates of
both dopamine (DA) and dopamine ortho-quinone (DOQ) were estimated. An IPA/AC
treated GC electrode was soaked for ten minutes in a 1 mM DA solution, rinsed
thoroughly, and placed in a degassed blank electrolyte solution of 0.1 M \( \text{H}_2\text{SO}_4 \).
Voltammograms of this electrode were taken at time intervals ranging from 30 seconds to
5 minutes for approximately one hour. The surface coverage of DA or DOQ was
determined by integrating the area underneath the voltammetric peak to determine the
charge, then solving for coverage: 
\[
\Gamma = \frac{Q}{(nFA)},
\]
where \( \Gamma \) is the surface coverage, \( n \) is the
number of electrons transferred (2 for DA and DOQ), \( F \) is Faraday’s constant, and \( A \) is
the electrode area. For the DA desorption experiments, the potential was held negative of
DA’s \( E_{\nu} \) at 200 mV vs. Ag/AgCl (DA \( E_{\nu} = 511 \text{ mV} \) in 0.1 M \( \text{H}_2\text{SO}_4 \)), while for DOQ,
the electrode potential was held well positive of \( E_{\nu} \) at 800 mV to ensure that DOQ and
not DA desorption was monitored. Coverage versus time data is shown in Figure 3.2.
Although the coverage was monitored at long times, it should be pointed out that the time
scale of the voltammetric experiments in this work were generally less than 10 seconds.

The initial desorption rates were calculated as 0.3 pmol*cm\(^{-2}\)*s\(^{-1}\) for both DA and
DOQ from the initial slopes of Figure 3.2. However, a “required” desorption rate
calculated from the observed peak current for a 1 mM DA solution on IPA/AC GC is
4,000 pmol*cm\(^{-2}\)*s\(^{-1}\) \( (i_p = 750 \mu\text{A/cm}^2 \text{ at 200 mV/s}) \). Clearly, the desorption rates of DA
and DOQ are too slow to support the observed peak current by four orders of magnitude.
Figure 3.2. Desorption of DA and DOQ from IPA/AC treated GC.
This indicates that a stepwise adsorption/electron transfer/desorption cannot be responsible for the observed current. Instead, catalysis of catechol oxidation must be taking place by a different route.

If the stepwise mechanism shown in Figure 3.1 A is not the operating mechanism, then the alternative is catalysis through a static adsorbed layer of catechol. This general mechanism is shown in Figure 3.1 B. The question is now: what is so special about the catechol that allows the catalysis? Several possibilities exist, such as redox mediation with the surface bound catechol or physical interactions with the surface species that may lower the activation barrier for the electron transfer. Such a catalytic interaction was observed only when a catechol was adsorbed and to for MB, NP, and TFMP modified GC.

Does the adsorbed catechol need to be identical to the redox system in solution? To investigate the effect of the identity of the adsorbed quinone, a series of physisorbed and chemisorbed quinones were studied for their effect on DA kinetics and 4MC oxidation kinetics. Figure 3.3 shows the quinones used for this purpose. The physisorbed quinones used were duroquinone (DUQ), pheneanthrenequinone (PQ), 9,10-anthraquinone-2,6-disulfonate (AQDS), 1,2-napthoquinone-4-sulfonate (NQS), and 4MC. Chemisorbed quinones were attached via the reduction of the corresponding diazonium salt on clean GC were 2-anthraquinone (2AQ) and 1-anthraquinone (1AQ). Two conditions must be met in order for these experiments to be informative. The redox potential of the adsorbed quinone must be different from that of the solution catechol so that the coverage of the quinone can be monitored simultaneously with dopamine
Figure 3.3. Physisorbed and chemisorbed quinones.
kinetics. The second, and more important requirement is that DA does not displace the adsorbed quinone, thus leading to the same surface expected for clean GC in DA solutions.

**Physisorbers**

Quinones used for the physisorbed layers were chosen based on their $E_{\mathrm{h}}$ and adsorption properties on glassy carbon. Table 3.2 lists the $E_{\mathrm{h}}$ values of the physisorbed and chemisorbed quinones in acidic media, the observed coverage, and the effect of the adsorbed quinone layer on the electron transfer kinetics of the catechols.

Duroquinone adsorbed to solvent treated GC with coverage in the range of 433 to 527 pmol/cm². The theoretical coverage determined from molecular areas on a flat surface is 199 pmol/cm² (flat orientation) and 422 pmol/cm² (edgewise orientation) (17). As is often observed on GC surfaces, the measured coverage of DUQ is higher than the theoretical value and is attributed to the roughness of GC surfaces. Desorption rates of DUQ in blank electrolyte were measured in order to confirm that desorption of the DUQ was not likely for the duration of the experiment. The coverage vs. time data is shown in Figure 3.4, and the initial desorption rate for DUQ was calculated as 0.2 pmol·cm⁻²·s⁻¹. This indicates that no significant desorption of DUQ occurs on the time scale of the voltammetric experiments. Figure 3.5 shows the DA voltammetry on IPA/AC, MB, and DUQ surfaces in 0.1 M H₂SO₄. Despite the surface being blocked by an adsorbed layer preventing DA adsorption, only a slight increase in $\Delta E_p$ was observed for DA on the DUQ surface. This contrasted with what occurred on MB adsorbed GC (Figure 3.5) where a substantial increase in $\Delta E_p$ for DA was seen. Very similar results (Table 3.2) were observed for DUQ surfaces at pH 7. To confirm that no adsorbed DA was present
<table>
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<th>Surface treatment</th>
<th>E_{fs} (mV)</th>
<th>$\Gamma$ (pmol/cm²)</th>
<th>DA pH 1</th>
<th>DA pH 7</th>
<th>4MC pH 1</th>
<th>4MC pH 7</th>
<th>DOPAC pH 1</th>
<th>DOPAC pH 7</th>
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<td>433 to 527</td>
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<td>47 ± 8</td>
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- a. surface quinone in 0.1 M H₂SO₄
- b. $\Delta E_p$ of redox system in solution

Table 3.2. $\Delta E_p$ (mV, 200 mV/s) for catechols in solution on quinone-adsorbed surfaces.
Figure 3.4. Desorption of DUQ from IPA/AC treated GC.
Figure 3.5. DA voltammetry on IPA/AC (—), DUQ adsorbed (- -), and MB adsorbed (-.-) GC. 1mM DA, 0.1 M H$_2$SO$_4$, $\nu$ = 200 mV/s.
on the DUQ surfaces, low concentration voltammetry, semi-integral analysis, and residual adsorption checks were performed. Figure 3.6 shows the voltammetry and semi integrals of DA on DUQ-adsorbed surfaces for 1 mM, 100 μM, 25 μM, and 10 μM DA in 0.1 M H₂SO₄, and Table 3.3 gives the numerical values for peak separation of DA under these conditions for in both acidic and neutral media.

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<th>pH 7</th>
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<td>25 μM</td>
<td>39 ± 4</td>
<td>29 ± 3</td>
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<tr>
<td>10 μM</td>
<td>36 ± 7</td>
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Table 3.3. ΔE_p for DA (0.1 M H₂SO₄, 200 mV/s) on DUQ-adsorbed surfaces.

For low DA concentrations on IPA/AC GC, the voltammograms are expected to be dominated by adsorption current. The voltammograms for DA at low concentrations take on the peak shape that it observed for adsorbed species in cyclic voltammetry experiments. That is, the voltammetric peaks separation decreases and the peak shape changes from a symmetric wave to a tailing wave. This is indeed observed, and on DUQ adsorbed surfaces, the dopamine voltammetry shifts from adsorption-like behavior to diffusional behavior. The absence of DA adsorption is corroborated by the decrease in
Figure 3.6. A. 100 μM DA, 0.1 M H$_2$SO$_4$. IPA/AC (---) and DUQ (---) adsorbed GC. ν = 200 mV/s. Top: CV. bottom: semi-integral.
Figure 3.6. B. 25 μM DA, 0.1 M H₂SO₄. IPA/AC (--) and DUQ (—) adsorbed GC. v = 200 mV/s. Top: CV. bottom: semi-integral.
Figure 3.6. C. 10 μM DA, 0.1 M H₂SO₄. IPA/AC (--) and DUQ (—) adsorbed GC. v = 200 mV/s. Top: CV. bottom: semi-integral.
DA current (as would be expected for loss of adsorbed material) and the sigmoidal shape of the semi-integral.

AQDS was adsorbed to solvent treated surfaces, and its effect on $\Delta E_p$ was monitored for the four catechols. First, the effects of treating GC electrodes with various solvents on AQDS adsorption were investigated. Table 3.4 lists the values obtained for AQDS coverage on the various treated surfaces. Solvent treatments are listed in the formal of order of sonication. For example, the notation “ACN, H2O, IPA” means that the electrode was sonicated for 10 minutes in acetonitrile, followed by sonication for 10 minutes in Nanopure water, and finished by sonication in isopropyl alcohol for 10 minutes. ACN/AC, IPA/AC, and H2O/AC denote 3:1 mixtures of the solvent with activated carbon, and “purified ACN” is acetonitrile that has been stirred with activated carbon overnight and filtered through a sintered glass funnel before use. It was found that sonication or soaking the electrode in various solvents led to changes in AQDS coverage. Soaking the electrode in pyridine or sonication in IPA/AC was found to increase the coverage of AQDS compared to polished surfaces, at least doubling the coverage. Adsorption of impurities, inferred from the decrease in AQDS coverage, occurred even for reagent grade solvents with high purity. To maximize AQDS coverage so that no active surface was available for DA, a solvent pretreatment procedure such as pyridine or IPA/AC sonication was used before adsorption of AQDS to the electrode surface.
<table>
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<th>Surface Treatment</th>
<th>$\Gamma_{obs}$ (pmol/cm$^2$)</th>
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<td>polished GC</td>
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<tr>
<td>IPA</td>
<td>182 ± 13</td>
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<td>ACN, H$_2$O</td>
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<td>ACN/AC, H$_2$O</td>
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<td>&quot;purified&quot; ACN, H$_2$O</td>
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<td>H$_2$O/AC, H$_2$O</td>
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<tr>
<td>IPA/AC, H$_2$O</td>
<td>352 ± 24</td>
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</table>

Table 3.4. AQDS surface coverage on solvent treated GC surfaces.

The AQDS layer was an effective catalyst for almost all of the catechols, with only anionic DOPAC (pH 7) exhibiting slowed kinetics on the AQDS surface (Table 3.2) compared to polished or solvent treated GC. This slowing of the electron transfer rate for DOPAC can be rationalized in terms of electrostatic repulsion between the two anionic species. Competitive adsorption experiments with DA and AQDS, and 4MC and AQDS were carried out in 0.1 M H$_2$SO$_4$. The CVs for 100 $\mu$M 4MC with varying concentrations of AQDS in solution are shown in Figure 3.7 A. The data indicate that AQDS is able to displace 4MC from the surface as the AQDS solution concentration increased. However, it must be noted that it was not possible to displace 4MC completely under the conditions used in this experiment. The semi-integrals show evidence for some 4MC adsorption, even at the high AQDS concentration examined.
The coverage of 4MC and AQDS derived from these experiments has an inverse correlation (Figure 3.8), given approximately by:

\[ \Gamma_{AQDS} = \frac{A_{4MC}}{A_{AQDS}} \Gamma_{4MC} - \frac{1}{A_{AQDS}} \]  

(3.1)

where A is the area of the adsorbed AQDS or 4MC. A fit of the data to this equation does not produce a slope equal to the ratios of the areas; however, the inverse correlation between the two coverages is clearly evident. Similar results are observed for DA with the same conditions as shown in Figure 3.7 B.

The physisorbed quinone layers discussed to this point have been composed of p-quinones. Phenanthrenequinone (PQ) is an orthoquinone with a well-known propensity to adsorb to carbon electrodes (6, 150, 151). PQ coverage on solvent-treated electrodes did not exhibit one value; instead two different average values were observed for PQ, 471 ± 51 pmol/cm² and 1018 ± 143 pmol/cm². This change in surface coverage was apparently random, since the same adsorption procedure was used for each trial. Similar sharp changes in observed coverage of quinones on platinum electrodes has been observed and correlated with change in adsorption orientation from flat to edgewise (17). However, the change in orientation was also correlated with changes in bulk concentration for the quinones adsorbed on Pt electrodes. The surface of GC electrodes is rough, and orientation may not be well defined. The voltammetry of Ru(NH₃)₆Cl₂⁺⁺⁺ did not undergo significant change on the PQ-adsorbed surfaces (both high and low coverage), implying that a thick multilayer of PQ was not present. The effects of PQ adsorption on catechol oxidation are summarized in Table 3.2, and shown for 4MC in
Figure 3.7 A. Competitive adsorption between 100 μM 4MC and 0 μM (---), 0.1 μM (--), and 50 μM (—) AQDS. 0.1 M H₂SO₄, ν = 2 V/sec
Figure 3.7 B. Competitive adsorption between 50 μM DA and 0 μM (•••), 0.1 μM (•••), 1 μM (••••) and 20 μM (---) AQDS. 0.1 M H₂SO₄, ν = 2 V/sec.
Figure 3.8. Competitive adsorption between AQDS and 4MC.
Figure 3.9. Although $\Delta E_p$ on PQ surfaces was not as small on AQDS or DUQ surfaces, PQ clearly does not inhibit catechol oxidation as do MB and TFMP monolayers.

1,2-Napthoquinone sulfonate (NQS) was also adsorbed to GC electrodes. Unfortunately, two additional peaks complicated the voltammetry for NQS that could not be removed upon attempted purification of the NQS (Figure 3.10). Voltammetry in 1 mM NQS solution (0.1 M H$_2$SO$_4$) indicated the couple at 350 mV is due to the NQS species; the other couples are most likely due to polymerization products. DA adsorption was apparently suppressed on the NQS adsorbed surface. $\Delta E_p$ for DA did not have a large increase on the NQS surfaces. However, since the NQS voltammetry was difficult to interpret, it is difficult to assess the effect of NQS on DA voltammetry.

All of the physisorbed quinones assessed thus far have been in the oxidized state during catechol oxidation. Attempts of adsorb other catechols (such as 4MC or DOPEG) yielded results similar to those observed on solvent treated GC with only DA in solution. It is impossible to determine voltammetrically whether or not DA is competing for adsorption sites with the other catechols, so no definitive statements can be made about pre-adsorbing different catechols for DA voltammetry. Also, attempts to examine HQ oxidation on catechol-adsorbed surfaces were not successful, due to HQ's tendency to compete with DA and the other catechols for adsorption sites. A case of catalysis for which both the solution species and the adsorbed species were in the reduced state was desired, but a strong adsorber with $E_{1/2}$ positive of DA was not found.

Although DA adsorption was not apparent on GC surfaces with physisorbed quinones (as assessed by semi-integral, low concentration voltammetry, or residual adsorption), it must be pointed out that very little active surface (and thus very little
Figure 3.9. 4 MC voltammetry on PQ adsorbed GC. 1 mM 4MC, 0.1 M H$_2$SO$_4$. IPA/AC (---), 963 pmol/cm$^2$ PQ (—), 577 pmol/cm$^2$ PQ (- -) GC, $v = 200$ mV/sec.
Figure 3.10. A. 1 mM DA on IPA/AC (—) and NQS adsorbed (—) GC.
B. NQS adsorbed on IPA/AC GC. 0.1 M H₂SO₄, v = 200 mV/s.
adsorbed catechol) is necessary to catalyze fast electron transfer. This is exemplified by simulating electron transfer with the following model (5):

\[ k_{\text{obs}}^o = (1 - \theta)k_{\text{clean}}^o + \theta k_{\text{blocked}}^o \]  

(3.2)

where \( \theta \) is the fractional coverage \((\Gamma/\Gamma_{\text{saturation}})\) of the blocking layer. Simulations of voltammograms with \(k_{\text{obs}}\) indicate the quantitative effects of coverage of the electrode surface with a blocking layer. The rate constants are listed in Table 3.5, along with the observed \(\Delta E_p\) for a one and two electron transfer. If \(k_{\text{clean}}^o\) is very fast (chosen to be 0.5 cm/s for the simulations), very little active surface is required to produce a fast \(k_{\text{obs}}^o\) (as little as 5% active surface) even if \(k_{\text{blocked}}^o\) is very slow \((5 \times 10^{-6} \text{ cm/s})\). Because of the catalytic effect of small coverages of DA, it was very important to reduce displacement of adsorbed quinones by DA.

To prevent solution catechol from displacing the quinone layer from the electrode surface, anthraquinone was chemisorbed to GC at two attachment points, 1AQ and 2AQ. Figure 3.11 shows the voltammetry of the attached species in 0.1 M \(\text{H}_2\text{SO}_4\), from which surface coverage can be determined. As with DA and other quinones in this pH range, only one peak for the two electron reduction is observed. Coverage was determined to be 388 \(\pm\) 24 pmol/cm\(^2\) for 1AQ and 321 \(\pm\) 76 pmol/cm\(^2\) for 2AQ. This corresponds to compact coverage; the theoretical coverage for 2AQ calculated from molecular area is 310 pmol/cm\(^2\). Figure 3.12 shows the voltammetry of 2AQ modified surfaces; the voltammetry for 1AQ surfaces was very similar. The one electron processes can be resolved in acetonitrile since protons are not involved; however, it was not possible to resolve the individual reduction peaks for pH of 13 or higher. The stability of the 2AQ surface is discussed in more detail in a Chapter 4. Since no convenient XPS marker is
\[ k_{\text{obs}} = \theta k^0_{\text{blocked}} + (1-\theta)k^0_{\text{clean}} \]
\[ k^0_{\text{clean}} = 0.5 \text{ cm/s} \]

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a. \( k^0_{\text{obs}} \) from eqn (3.2)
b. \( \Delta E_p \) for one electron reduction, 200 mV/s (a + e \( \rightarrow \) b)
c. \( \Delta E_p \) for two electron reduction, 200 mV/s (c + 2e \( \rightarrow \) d)

Table 3.5. \( k_{\text{obs}} \) (cm/s) and \( \Delta E_p \) (mV) for one and two electron transfer from equation 3.2.
\[ k^0_{\text{clean}} = 0.5 \text{ cm/s} \].
Figure 3.11. 0.1 M H$_2$SO$_4$, $\nu = 200$ mV/s
A. 1AQ  B. 2AQ.
Figure 3.12. 2AQ GC in 0.1 M tetrabutylammonium tetrafluoroborate in acetonitrile, $v = 200$ mV/s.
available for AQ modified surfaces, voltammetry is the most practical way to quantify the surface coverage. However, there is a large difference observable in the O1s regional spectrum of 2AQ GC compared to solvent treated GC; a shoulder is observed at 532 eV (Figure 3.13).

Figures 3.14 and 3.15 illustrate the voltammetry of DA in 0.1 M H$_2$SO$_4$ on the 1AQ and 2AQ modified surfaces. DA kinetics were not severely retarded despite the presence of a compact layer on the electrode surface, similar to what was observed in the physisorbed cases. DA adsorption was suppressed below detectable amounts on the 1- and 2AQ surfaces, as illustrated in the semi-integrals on the anthraquinone-modified surfaces. The effect of a chemisorbed layer of AQ on DA and 4MC voltammetry is summarized in Table 3.2.

Although electrochemical pretreatment (ECP) produces surfaces that do not have well defined surface properties, ECP GC has been popular for producing surfaces with reproducible voltammetric properties (5, 123). Dopamine exhibits enhanced adsorption and kinetics on ECP GC; this enhancement of activity has been attributed to electrostatic attraction. For this work, ECP was carried out in 0.1 M H$_2$SO$_4$, 0.1 M PBS, and 1 M KOH by two methods, 1. five scans from 0 to 1.8 V (vs. Ag/AgCl) at 200 mV/s. 2. 30s at 1.8 V (vs. Ag/AgCl), followed by 30s at -0.6 V (vs. Ag/AgCl). The O/C ratio, $\Delta E_p$, and residual adsorption data for the different treatments is listed in Table 3.6. No clear correlations are available from the data; however, the dramatic increase in DA adsorption for DA on ECP surfaces in acid and base is worthy of note.

The role of electrostatics in catechol oxidation was assessed by chemisorbing a layer of benzoic acid at the 4 position on the surface by the reduction of the
Figure 3.13. O 1s region of 2AQ modified GC.
Figure 3.14. 1 mM DA, 0.1 M H₂SO₄, v = 200 mV/s. IPA/AC (—), 1AQ (---) GC. A. Cyclic voltammogram B. Semi-integrals.
Figure 3.15. 1 mM DA, 0.1 M H₂SO₄, v = 200 mV/s. IPA/AC (---), 2AQ (-----) GC. A. Cyclic voltammogram B. Semi-integrals
<table>
<thead>
<tr>
<th></th>
<th>O/C</th>
<th>DA</th>
<th>4MC</th>
<th>DOPAC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>H₂SO₄</strong></td>
<td>0.15</td>
<td>57±2&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>173±4</td>
<td>313±25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>766&lt;sup&gt;e&lt;/sup&gt;</td>
<td>169</td>
<td>90</td>
</tr>
<tr>
<td><strong>H₂SO₄/Reduced</strong></td>
<td>0.21</td>
<td>73±9</td>
<td>58±1</td>
<td>64±3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1064</td>
<td>700</td>
<td>485</td>
</tr>
<tr>
<td><strong>PBS</strong></td>
<td>0.13</td>
<td>107±5</td>
<td>45±2</td>
<td>80±15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>199</td>
<td>469</td>
<td>207</td>
</tr>
<tr>
<td><strong>PBS/Reduced</strong></td>
<td></td>
<td>58±8</td>
<td>53±2</td>
<td>44±4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>390</td>
<td>644</td>
<td>364</td>
</tr>
<tr>
<td><strong>KOH</strong></td>
<td>0.17</td>
<td>45±1</td>
<td>64±5</td>
<td>59±7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>636</td>
<td>188</td>
<td>327</td>
</tr>
<tr>
<td><strong>KOH/Reduced</strong></td>
<td>0.18</td>
<td>41±6</td>
<td>110±8</td>
<td>59±9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1108</td>
<td>275</td>
<td>400</td>
</tr>
</tbody>
</table>

**Table 3.6.** ΔE<sub>p</sub>, O/C ratio, and residual coverage of catechols on ECP surfaces.

- a. 5 scans for 0 to 1.8 V (vs. Ag/AgCl)
- b. 30 s at 1.8 V (vs. Ag/AgCl), followed by 30 s at -0.6 V (vs. Ag/AgCl)
- c. ΔE<sub>p</sub> in mV
- d. When a standard deviation is reported, N is three or more trials
- e. Residual adsorption observed in blank electrolyte, pmol/cm<sup>2</sup>
corresponding diazonium salt. These surfaces are referred to as 4-\text{-COOH}. This surface was better defined than the electrochemically pretreated surface, but retained the ability to form an anionic surface. Since the benzoic acid layer is not electroactive and does not have a good XPS marker, the presence of a compact monolayer of 4-\text{-COOH} was not clearly established. As discussed in Chapter 2, electrostatics play a part in the oxidation of charged catechols. Adsorption of the catechols to the COOH surface could not be determined; the semi integral of DA (or the other catechols) was ambiguous, and the low concentration data was not conclusive.

**Discussion**

It was established in Chapter Two that tunneling for the catechols through an inert monolayer such as NP and TFMP is slow. The large decrease in electron transfer rate on a monolayer modified surface compared to a solvent treated surface indicates that the catechols have high reorganization energy, making an outer sphere electron transfer route difficult. The large changes in bond length upon reduction of the catechol to the ortho-quinone also indicate that the catechols have high reorganization energy. Gaussian (152) was used to calculate structures for catechols; for 4MC the largest C-C bond length change was 0.1513 Å and the change in O-C bond length was 0.1492 Å (B3LYP/6-31G(d)) upon oxidation of the catechol to the ortho-quinone. Figure 3.16 shows the calculated and crystal structure data for 4MC. In contrast, species such as chlorpromazine which has a maximum change in bond length of 0.04 Å (129) or Fc/Fc° which has a maximum change in bond length of 0.04 Å (153, 154) do not have large changes in structure and consequently, rapid electron transfer kinetics.
<table>
<thead>
<tr>
<th>Bond</th>
<th>Experimental bond lengths (Å)</th>
<th>Calculated bond lengths (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.369</td>
<td>1.3797</td>
</tr>
<tr>
<td>2</td>
<td>1.373</td>
<td>1.3797</td>
</tr>
<tr>
<td>3</td>
<td>1.384</td>
<td>1.4059</td>
</tr>
<tr>
<td>4</td>
<td>1.384</td>
<td>1.3899</td>
</tr>
<tr>
<td>5</td>
<td>1.384</td>
<td>1.3987</td>
</tr>
<tr>
<td>6</td>
<td>1.384</td>
<td>1.3969</td>
</tr>
<tr>
<td>7</td>
<td>1.384</td>
<td>1.3987</td>
</tr>
<tr>
<td>8</td>
<td>1.384</td>
<td>1.3899</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bond</th>
<th>Experimental bond lengths (Å)</th>
<th>Calculated bond lengths (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.216</td>
<td>1.22</td>
</tr>
<tr>
<td>2</td>
<td>1.216</td>
<td>1.2183</td>
</tr>
<tr>
<td>3</td>
<td>1.541</td>
<td>1.5572</td>
</tr>
<tr>
<td>4</td>
<td>1.436</td>
<td>1.4734</td>
</tr>
<tr>
<td>5</td>
<td>1.366</td>
<td>1.3491</td>
</tr>
<tr>
<td>6</td>
<td>1.445</td>
<td>1.4715</td>
</tr>
<tr>
<td>7</td>
<td>1.366</td>
<td>1.3491</td>
</tr>
<tr>
<td>8</td>
<td>1.463</td>
<td>1.4734</td>
</tr>
</tbody>
</table>


Figure 3.16. Calculated and experimental bond lengths for catechol.
Based on the DA and DOQ desorption experiments, we concluded that the adsorption/electron transfer/desorption mechanism of Figure 3.1 A was not consistent with what was experimentally observed. It is intriguing that the catalysis of catechol oxidation can occur through an adsorbed layer of quinone, regardless of the identity of the quinone. This is clearly not the case for non-quinone monolayers. The quinone layers in this work were either in the oxidized or partially oxidized state during catechol oxidation. It was found in Chapter Two that electrostatics play a secondary role in affecting catechol electron transfer as shown by Φ-COOH monolayers; however, the dramatic effects on catechol oxidation observed on NP and TFMP surfaces are not dependent on side chain charge. Surfaces blocked with a non-quinone layer block catechol adsorption, which, as a consequence, blocked catechol oxidation. In contrast, if the blocking layer is a quinone instead of an inert spacer, the electron transfer kinetics of DA are not significantly affected. The DA electron transfer kinetics are fast on the quinone-adsorbed surfaces even though DA adsorption is suppressed. A listing of catalysts versus blockers for DA oxidation is given below in Table 3.7.

<table>
<thead>
<tr>
<th>Catalysts</th>
<th>Blockers</th>
</tr>
</thead>
<tbody>
<tr>
<td>AQDS</td>
<td>MB</td>
</tr>
<tr>
<td>DUQ</td>
<td>TFMP</td>
</tr>
<tr>
<td>PQ</td>
<td>NP</td>
</tr>
<tr>
<td>1- and 2AQ</td>
<td></td>
</tr>
<tr>
<td>DA, other catechols</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.7. Catalysts and Blockers for DA oxidation.
Semi-quantitative Model

Although several microscopic rate constants are defined in the "scheme of squares" model for catechol electron transfer (12-14), the data collected for this work does not permit the calculation of these constants. Instead, a semi-quantitative estimate of the two component rate constants can be modeled using digital simulation. The pertinent reactions are:

\[ \text{QH}_2 + 2\text{[SQ]} \rightleftharpoons \text{Q} \]

1. \(E^*_{\text{1}}, k^*_{\text{1}}\)
2. \(E^*_{\text{2}}, k^*_{\text{2}}\)

\(E^*_{\text{1}}\) and \(E^*_{\text{2}}\) for the simulations were determined from a potential-pH diagram (Figure 3.17) constructed from literature values (155-157), and were found to be qualitatively similar to that for BQ/HQ. In constructing the potential-pH diagram, the reactions that occur above pH 11 (such as cycloaddition, or the reversible addition of OH to the ring) were neglected. For the purposes of the simulations, \(k^*_{\text{1}}\) was assumed to be equal to \(k^*_{\text{2}}\). Other simulation parameters are \(\alpha = 0.5\) for each step and \(D_{\text{DA}} = 6 \times 10^{-6}\text{cm}^2/\text{sec}\). Protonations were assumed to be at equilibrium (12, 13, 115, 116). These simulations are discussed in more detail in Appendix B.

From the voltammograms in this work, \(k^*_{\text{1}}\) and \(k^*_{\text{2}}\) could not be independently assessed. However, by setting \(k^*_{\text{1}}\) and \(k^*_{\text{2}}\) equal to one another, a fair estimate of the magnitude of the effects of surface modification on the electron transfer rate for the catechols could be made. Table 3.8 lists the experimental \(\Delta E_p\), simulated \(\Delta E_p\), and rate constants for 4MC and DA at pH 7. The adsorbed quinone layers have minor effects on
Figure 3.17. Potential-pH diagram for catechol/ortho-quinone.
<table>
<thead>
<tr>
<th>Surface Treatment</th>
<th>$k^0_1 = k^0_2$ (cm/s)</th>
<th>Simulated $\Delta E_p$ (mV)</th>
<th>Experimental $\Delta E_p$ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>4MC pH 7</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IPA/AC</td>
<td>$&gt;0.5$</td>
<td>50</td>
<td>59</td>
</tr>
<tr>
<td>2AQ</td>
<td>0.1</td>
<td>93</td>
<td>90</td>
</tr>
<tr>
<td>DUQ</td>
<td>0.1</td>
<td>90</td>
<td>84</td>
</tr>
<tr>
<td>MB</td>
<td>0.036</td>
<td>166</td>
<td>162</td>
</tr>
<tr>
<td>NP</td>
<td>0.0025</td>
<td>455</td>
<td>468</td>
</tr>
<tr>
<td>TFMP</td>
<td>$&lt;1 \times 10^{-8}$</td>
<td>$&gt;1000$</td>
<td>$&gt;1000$</td>
</tr>
<tr>
<td><strong>DA pH 7</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IPA/AC</td>
<td>0.36</td>
<td>41</td>
<td>36</td>
</tr>
<tr>
<td>2AQ</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>DUQ</td>
<td>0.24</td>
<td>54</td>
<td>47</td>
</tr>
<tr>
<td>MB</td>
<td>0.024</td>
<td>194</td>
<td>193</td>
</tr>
<tr>
<td>NP</td>
<td>$&lt;1 \times 10^{-8}$</td>
<td>$&gt;1000$</td>
<td>$&gt;1000$</td>
</tr>
<tr>
<td>TFMP</td>
<td>$&lt;1 \times 10^{-8}$</td>
<td>$&gt;1000$</td>
<td>$&gt;1000$</td>
</tr>
</tbody>
</table>

Table 3.8. Simulated $\Delta E_p$ and $k^0$ and experimental $\Delta E_p$ for DA and 4MC on modified GC surfaces at 200 mV/s.
catechol kinetics compared to a layer of methylene blue (factor of 10) or a layer of TFMP (factor of \(1 \times 10^7\)). For DA and 4MC on IPA/AC treated surfaces, the calculated \(k^0\)'s may be artificially high due to perturbation of \(\Delta E_p\) values by catechol adsorption.

There are some potential problems with the simulation results. Both DA and 4MC simulated voltammograms do not fit the current envelope of the experimental voltammograms; an example is shown in Figure 3.18 for 4MC on a MB modified surface. The difference between the observed cathodic current and simulated cathodic current is larger for DA; however, this is due in part to the following chemical reaction that DA undergoes at pH 7 (shown in Chapter 1). The mechanism used here (sequential electron and proton transfer, redox potentials determined from the potential-pH diagram) does not agree well with experiment at pH 1. Extremely high values of \(k^0_1\) and \(k^0_2\) (\(\sim 10\) cm/s) are required to obtain the experimentally observed \(\Delta E_p\) for the catechols on the IPA/AC and quinone adsorbed surfaces. Deakin et al (13) also had difficulty fitting the scheme of squares model at low pH and attributed this to the formation of the high-energy \(\mathrm{QH}_2^+\) species. An alternate mechanism may be operating at low pH by which stabilization of the product occurs, perhaps changing parameters such as the one-electron formal potentials. A proton coupled electron transfer (PCET) has been proposed in aiding catechol oxidation (127).

**Possible Mechanisms**

If catechol oxidation is not taking place via a tunneling mechanism and the catalysis is not by a stepwise adsorption/desorption process (Figure 3.1A), then several other possibilities exist. One possibility is a mediation mechanism, either by redox self-exchange or redox exchange with the surface bound quinones. This type of mechanism is
Figure 3.18. Experimental (—) and simulated (- -) voltammograms of 4MC, pH 7 on MB adsorbed GC. 1 mM 4MC, v = 200 mV/sec.

\[ k_1^o = k_2^o = 0.036 \text{ cm/s} \]
represented by Figure 3.19, and has been established for NADH oxidation by surface quinones (28), porphyrin catalysis of oxygen reduction (22, 24, 88-91), and various reactions of enzyme modified electrodes (95). For the mediated reactions, the surface bound species undergoes fast electron transfer with the electrode, then electron transfer to the solution species. This type of reaction may certainly be considered feasible, since homogeneous exchange rates for benzoquinones are rapid (158). However, this type of redox mediation reaction for the quinone-modified surfaces may be discounted for the current work for the same reasons that redox mediation by surface oxides groups was ruled out in Chapter 2. For a redox mediation mechanism, the $E_{\nu}$ observed in the voltammogram should shift to that of the mediator (6). The modifier quinone’s $E_{\nu}$ are all situated approximately 200 to 600 mV negative of the $E_{\nu}$ for the catechols, and a shift was not observed for any of the catechols. Instead, the $E_{\nu}$ remains unchanged and equal to that of the solution phase catechol over a range of modifications. Additionally, the characteristic asymmetric wave shape of a mediated process is not observed for the catechols on the quinone adsorbed surfaces. While the oxidized quinone could conceivably oxidize the catechol to the ortho-quinone, the oxidized quinone would not be able to reduce the catechol ortho-quinone species. This factor, coupled with the unchanging $E_{\nu}$ values, rules out a redox mediation mechanism for the quinone modified surfaces. For the self-catalyzed catechol oxidations, it is not yet possible to rule out a self-exchange mechanism.

If the redox state of the adsorbed monolayer does not change, then some other catalysis that is not based on a redox reaction must occur. Such interactions may include formation of a quinhydrone-like species between the surface confined and solution
Figure 3.19. Redox mediation.
catechol or proton coupled electron transfer (PCET). Quinhydrone complexes are formed from a quinone and hydroquinone bound face to face, forming a symmetric complex of two semiquinones bound by hydrogen bonding. These complexes have been observed and characterized in the solid state; however, most complexes dissociate once placed in solution with very little remaining as the quinhydrone complex (159). However, if a similar interaction were to take place between the surface quinone and the solution catechol, the electron transfer may be aided by easing the proton transfer. That is, a more favorable, semiquinone-like geometry may be attained.

Proton coupled electron transfer is another possibility for a mechanism with adsorbed quinone catalysis. In PCET, the electron and proton transfer during the same event (160). Such a process has been observed for benzoquinone (161) and homogeneous and heterogeneous electron transfer to ruthenium complexes (127, 162). For these reactions, large H/D isotope effects were observed (~7 to 41.1). If a PCET mechanism is operating for catechol oxidation, it may be evident by a H/D isotope effect. Table 3.9 shows the $\Delta E_p$ for DA observed in 0.1 M H$_2$SO$_4$ in H$_2$O and 0.1 M D$_2$SO$_4$ in D$_2$O. While it is difficult to determine the exact H/D effect without knowing the correct mechanism, it appears that the effect is smaller ($k_{H_2O}/k_{D_2O} \sim 1.3$) than that observed for other redox systems that have PCET. Table 3.8 lists the observed $\Delta E_p$ for DA in H$_2$O and D$_2$O. Simulation parameters are listed in Appendix B.
<table>
<thead>
<tr>
<th>Surface Treatment</th>
<th>0.1 M H$_2$SO$_4$</th>
<th>0.1 M D$_2$SO$_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polished</td>
<td>61 ± 8</td>
<td>61 ± 7</td>
</tr>
<tr>
<td>IPA/AC</td>
<td>36 ± 6</td>
<td>39 ± 2</td>
</tr>
<tr>
<td>DUQ</td>
<td>51 ± 6</td>
<td>71 ± 3</td>
</tr>
<tr>
<td>COOH</td>
<td>439</td>
<td>463</td>
</tr>
</tbody>
</table>

Table 3.9. $\Delta E_p$ for 1 mM DA (200 mV/s)

In Chapter 2, a monolayer of $\Phi$-COOH groups was intentionally added to the surface in order to investigate the role of electrostatic interactions in catechol oxidation. While it was found that charge interactions were able to modulate catechol electron transfer kinetics, it was concluded that these interactions were not the driving force behind catechol oxidation. In a similar experiment, Downard et al observed that DA kinetics underwent a significant change upon modification of the carbon electrode surface with phenylacetate groups (105). In that case, the $\Delta E_p$ for DA on the phenylacetate surface decreased from 465 mV on a polished surface to 245 mV (conditions: PBS, 0.1 mV/s). 4MC did not have the same decrease as $\Delta E_p$ that DA on the phenylacetate surface, and $\Delta E_p$ for DOPAC doubled on the phenylacetate surface from 360 mV to 730 mV. The authors suggested that the charged surface was acting as an “ion gate” to DA. These results, as well as those reported herein, indicate that electrostatic interactions are a factor in catechol oxidation. However, the value of 465
mV for $\Delta E_p$ on a polished surface does raise some questions. In our experience, such a large $\Delta E_p$ indicates that the surface has already been subject to considerable fouling.

It was impossible to determine whether or not a full monolayer of the benzoic acid salt was deposited in the current work. However, while not catalytic for the neutral catechols (4MC, DOPEG), the $\Phi$-COOH surface did not severely inhibit catechol oxidation as was the case for the NP and TFMP surfaces. The electron transfer kinetics for the neutral catechols remained basically unchanged with different pH and the cationic and anionic catechols exhibited the shift in kinetics consistent with a Frumkin effect caused by local electrostatic interactions. The $\Phi$-COOH or $\Phi$-COO$^-$ surface groups may be providing a site for hydrogen bonding for the quinone that eases electron transfer.

**Conclusions**

As reported in this chapter, the role of adsorption in the electron transfer kinetics of catechols was investigated. By monitoring the desorption rate of DA and DOQ from the electrode surface, it was possible to rule out a stepwise adsorption/electron transfer/desorption mechanism for catechol oxidation. Instead, catalysis was found to occur through a static layer of adsorbed quinone. By varying the surface preparation, it was observed that identity of the adsorbed quinone was not a factor. In these cases, catalysis of catechol oxidation occurred despite blocked catechol adsorption, clearly in contrast to the cases of NP and TFMP. Electrostatic effects were not considered a main factor in catechol electron transfer since NP and TFMP modifications had the same large effects regardless of the charge on the catechol side chain. Since the characteristics of a mediated process were not observed on the quinone-modified surfaces, a redox mediation mechanism could be ruled out. Voltammetry was carried out in $D_2O$ in order to
investigate a proton coupled electron transfer mechanism; however, the observed H/D isotope effect was not as large as that observed for other cases where a PCET mechanism was operating. However, a likely possibility is a hydrogen bonding or bridging mechanism. Cukier and Nocera report that a H/D of 1.6 is expected if the proton does not move during electron transfer; however, the proton may still be bridging (160). Such an interaction is shown in Figure 3.20.

The results present in this chapter are consistent with the electron transfer behavior of catechols observed on other types of surfaces, such as fractured GC, laser activated GC, and solvent-cleaned GC. On these surfaces, both catechol adsorption and catechol electron transfer kinetics increase. On HOPG, catechol oxidation is inhibited; DA adsorption is weak or nonexistent. While ECP GC does not produce well-defined surfaces, a marked increase in adsorption on these surfaces was accompanied in most cases by fast electron transfer kinetics for the catechols. It must be stressed, however, that it is difficult to distinguish whether or not catalysis is due strictly to adsorption on ECP surfaces since surface oxides may be acting in parallel to catalyze catechol oxidation.

The identification of a catalytic “site” for DA oxidation could lead to the development for stable and specific analytical sensor. The stability of anthraquinone and other chemisorbed monolayers for possible analytical uses is the focus of Chapter 4.
Figure 3.20. Hydrogen bonding/bridging intermediates.
CHAPTER 4

ANALYTICAL APPLICATIONS OF DIAZONIUM MODIFIED SURFACES

Introduction

While carbon has many advantageous qualities as an electrode material, such as low cost, ease of preparation, and rich surface chemistry, carbon electrodes also have some properties that make their use in analytical applications more difficult (5, 6, 163). Glassy carbon surfaces are prone to fouling by adsorption of impurities, resulting in the degradation of electrode performance with time. Also, some redox systems cannot be studied on GC surfaces due to the anodic potential limit of glassy carbon. These properties can sometimes limit the use of glassy carbon electrodes for sensors and detectors.

Surface Modifications

To reduce the effects of surface fouling, variability, and extend potential limits, various surface treatments have been used to modify GC electrodes (6, 18, 163). Surface treatments can remove variability in electrode-to-electrode performance, improve electrode stability, and introduce selectivity to the surface. An example of how this has
been accomplished is the extensive work done on electrodes with polymer coatings (18, 22, 25, 29, 31-33, 35). Polymer coated electrodes have been used to catalyze reactions by incorporating mediating groups into the polymer backbone (22, 27, 30, 31, 164) or by rejection of interferences (14, 35-37, 40). Perhaps the most common polymer used for analytical applications is Nafion®, a perfluorinated-sulfonated polymer. Nafion® has been used both as a biocompatible matrix in which mediators or biological reactants can be immobilized and as a means to reject anionic interferences. The rejection of anionic interferences has been particularly useful in the study of neurological systems.

A more recent method of modifying glassy carbon electrodes is by the reduction of diazonium salts to produce a compact layer of the corresponding phenyl group onto the carbon surface (99, 102-105, 121, 129, 165). The reaction was discussed in detail in Chapter 2. The surface modification is expected to be stable since the interaction of the phenyl group with the electrode surface is covalent in nature. GC surfaces modified with diazonium salts have been extremely useful in studying the interactions between surfaces and redox systems in electron transfer reactions in the current work and in the work of many others (84, 102, 105, 129, 165). Aside from use in answering fundamental questions of electron transfer reactivity, diazonium modified surfaces are also being used for analytical applications (99, 103-105). A few examples are listed below.

Doward et al used phenylacetate modified GC and carbon fibers to differentiate between dopamine and ascorbic acid at physiological pH (105). Phenylacetate groups were also effective at suppressing DOPAC (anion at pH 7) voltammetric current. The reaction of surface bound amine groups generated by the reduction of nitrophenyl GC with epichlorhydrin was used to show that the diazonium surfaces could be further
modified (99). Allongue et al predicted that such reactions would find use in the field of carbon-epoxy composites. Finally, Dequaire et al used a biotinylated screen-printed carbon electrode for further enzyme modification (103). By generating the diazonium salt in situ from p-aminobenzoyl biocytin, the screen-printed electrodes could undergo the diazonium reaction without exposing the screen-printed electrodes to harsh, non-aqueous solvents. The biotinylated electrode was then coupled to an enzyme via an avidin-biotin linkage. One of the main advantages to using this coupling scheme is that the electrode is not subjected to as many handling steps as is common in other enzyme immobilization schemes. The authors also cite low cost and reusability as benefits to this type of surface modification.

**Redox Systems**

Some redox systems pose obstacles to easy and reliable electrochemical detection. This may be due to several factors. There may be a fundamental limitation, such as a redox potential that is either positive or negative of what may be conveniently monitored, or kinetic factors, where the slow electron transfer kinetics of the redox system may mean that detection occurs at large overpotentials. Another potential problem is fouling of the electrode by solution components or the products of the electrode reaction. Carbon electrodes are prone to adsorption, often losing maximum activity after short periods of time (5, 150). Finally, selectivity can be an issue for electrochemical detection. Sometimes, as in the case for catecholamines and ascorbic acid, the observed potentials for these species are very close to one another, making differentiation between the species difficult (5, 14, 22, 40, 55, 138).
Several systems can be considered representative of these problems. \( \text{Ru(bpy)}^{3+/2+} \) in 1 M KCl has a redox potential of approximately 1.1 V (vs. Ag/AgCl), which is near the upper limit observable on glassy carbon electrodes. Background current at this high potential can make observation of the \( \text{Ru(bpy)}^{3+/2+} \) couple difficult. Dihydronicotinamide adenine dinucleotide (NADH) can be oxidized directly on carbon electrodes; however, a high overpotential for oxidation and tendency for the oxidation products to foul the electrode surface make this system somewhat troublesome. Differentiation between ascorbic acid and dopamine at physiological pH can be problematic, and represent selectivity issues.

The oxidation of NADH to NAD\(^+\) has received a great deal of attention because of questions about its activity in biological electron transfer (109) and its uses in analytical sensors that rely on immobilized enzymes (18, 108, 166). NAD\(^+\) is a cofactor to over 300 dehydrogenases, and the enzymatically generated NADH can be detected at an electrode surface. Modified electrodes based on bound dehydrogenases have been used for the detection of substances that are not conveniently electroactive, such as alcohols (166-169), amino acids(96, 170, 171), and sugars. The reaction that occurs on these electrode surfaces is the two electron, one proton oxidation of NADH to NAD\(^+\), and is shown below.

\[
\text{NADH} \quad \leftrightarrow \quad \text{NAD}^+ + 2\text{e}^- + \text{H}^+
\]
Although the formal potential for the NAD$^+$/NADH couple is $-0.32 \text{ V vs. } \text{NHE}$ ($-0.087 \text{ V vs. } \text{Ag/AgCl}$) (172), the wave for the oxidation of NADH isn't observed until at least $0.4 \text{ V vs. } \text{Ag/AgCl}$ on GC surfaces. In many cases, the observed peak potential on carbon electrodes is much more positive of $0.4 \text{ V}$. The potential often moves more positive as the electrode is scanned repeatedly; the positive shift has been attributed to fouling of the electrode surface by NAD$^+$ (6, 28, 173). In addition, the processes used to immobilize enzymes on the electrode sensor often result in significant passivation of the electrode surface towards NADH oxidation (94, 170, 174).

Two approaches have been taken to reduce the high overpotential necessary to oxidize NADH on carbon electrodes. One method is to "activate" the surface for NADH oxidation. This has been done by electrochemical pre-treatment and laser activation. Laser activation has been used to pattern a carbon fiber electrode, with the objective that areas with enzyme modification alternate with laser-activated areas (170, 174). The rate of NADH oxidation on the laser activated areas is comparable to fast kinetics observed on electrochemically pretreated surfaces (94, 170).

The second, and more common option for enzyme-modified electrodes is to introduce a mediator either in solution or on the electrode surface. A mechanism for NADH oxidation by a mediating species is shown below.

\[
\begin{align*}
\text{ET} & \quad \text{Med}_{\text{ox}} \quad \text{NADH} \\
\text{Med}_{\text{red}} & \quad \text{NAD}^+
\end{align*}
\]
The NADH is oxidized by the mediator, which then undergoes electron transfer with the electrode. Mediators are chosen that will reduce the overpotential of the observed reaction. O-quinones, p-quinones, phenothiazines, and phenoxazine derivatives have been used as mediators for NADH oxidation ([175] and references contained within), as well as transition metal complexes (167). These mediators are often incorporated into a polymer matrix on the electrode surface. One of the earliest reported modifications effective for catalyzing NADH oxidation is the adsorption of dopamine onto the electrode surface (28). The ortho-quinones on the surface were effective in catalyzing NADH oxidation, however, NADH oxidation products quickly fouled the electrodes. In another attempt to make a stable and simple biosensor, the mediator, NAD$, and enzyme have been incorporated into a carbon paste electrode to make a "reagentless" biosensor (166, 168). After an initial loss of activity, the "reagentless" biosensor remained stable for eight days (166).

As discussed in Chapter 1, ascorbic acid is the main interference for the determination of dopamine and other catecholamines during in vivo detection. It is possible to resolve dopamine and ascorbic acid on fractured GC, but the ability to resolve the two peaks does not last, with adsorption of impurities from solution degrading electrode performance (138). By adding a layer of Nafion® to the electrode surface, anionic interferents are rejected and the cationic analytes (catecholamines) are pre-concentrated into the membrane (14, 22, 38, 40). Electrochemically pretreated (ECP) glassy carbon or carbon fiber electrodes also improve selectivity for catecholamines (38, 119, 120). However, Nafion® coated microelectrodes offer increased sensitivity for catecholamines without the slow response observed on the ECP electrodes (40).
In this chapter, diazonium surface modifications were used for several representative analytical problems. Diazonium modified surfaces are expected to be more stable with respect to oxidation in air and in solution, and should be effective for prolonging electrode lifetime by preventing adsorption of impurities. Ideally, the modified electrodes would remain stable for extended periods of time. Potential limits and surface stability were assessed for α,α,α-trifluoromethylphenyl (TFMP) and 2-anthraquinone (2AQ) modified GC surfaces. Surfaces modified with diazonium salts were then used for redox systems that represent problems sometimes encountered in electroanalytical applications. The observed potential of Ru(bpy)$_3^{2+}$ is nearly at the anodic limit of glassy carbon electrodes. By modifying the surface with a compact monolayer of TFMP, the couple should be observable without interference from background current. An attempt was made to produce electrodes that were active for NADH oxidation but were not prone to fouling by oxidation products. Finally, GC was modified with 2AQ and Φ-COOH groups in an effort to differentiate between ascorbic acid and dopamine at physiological pH.

Experimental

Electrode Preparation

Electrode preparation procedures and diazonium salt synthetic procedures are described in Chapters 2 and 3. GC surface treatments used in this chapter are polishing, solvent treatment (both pyridine and IPA/AC), and diazonium surface modification. Diazonium salts used were α,α,α-trifluoromethylphenyl diazonium tetrafluoroborate (TFMP), 9,10-anthraquinone-2-diazonium tetrafluoroborate (2AQ), and 4-diazonium benzoic acid tetrafluoroborate (Φ-COOH), prepared as described in Chapter 2.
Reagents

Dopamine (DA, Sigma), ascorbic acid (AA, Aldrich), and dihydronicotinamide adenine dinucleotide (NADH, Sigma) were used as received. Ru(bpy)$_3$(ClO$_4$)$_2$ was a gift from Professor Bruce Bursten. DA and AA solutions were prepared in either 0.1 M PBS or 0.1 M H$_2$SO$_4$, NADH solutions were 0.52 mM in 0.1 M PBS, and Ru(bpy)$_3^{2+/3+}$ solutions were prepared in 1 M KCl.

Methods

Voltammetry was performed using a BAS 100/W electrochemical workstation (Bioanalytical Systems, Inc., West Lafayette, IN). A Gamry potentiostat or BAS power module (Bioanalytical Systems, Inc., West Lafayette, IN) was used when necessary for long time-scale potential steps. XPS spectra were acquired with a VG Scientific Escalab MKII spectrometer with a Mg anode. Grams (version 4.02, Galactic) spectral analysis software was used to calculate peak areas, and all atomic ratios were corrected for instrumental sensitivity factors.

Results

The utility of diazonium surfaces for studying the effects of surface modifications on electron transfer rates has been well established (84, 105, 129, 165). It is the goal of the current chapter to investigate the stability of selected diazonium surfaces upon exposure to air and potential cycling or a potential step. In addition, the anodic and cathodic potential limits for TFMP surfaces were compared to the potential limits of polished and solvent treated surfaces. The effect of air exposure of 2AQ surfaces on DA
voltammetry was monitored. These surfaces were then used for several redox systems: Ru(bpy)$_3^{3+/2+}$, NADH, and DA and AA to illustrate the analytical utility of these modified surfaces.

**Surface Stability**

To determine the available potential range on polished, pyridine treated, IPA/AC, and TFMP GC, electrodes were scanned to high potentials in a several electrolytes: 1 M H$_2$SO$_4$, 0.1 M H$_2$SO$_4$, 0.1 M PBS (pH 7), and 1 M KOH. The anodic branch of these scans at 200 mV/s is shown in Figure 4.1. Table 4.1 lists the potentials where the current reaches 0.4 mA. The definition of anodic potential limit is somewhat arbitrary. This current represents approximately twice the peak current of 1 mM DA on IPA/AC GC at 200 mV/s. TFMP surfaces clearly offer a larger anodic potential limit over the range of pHs studied.

A stable TFMP surface in air or under potentiostatic control is defined as a surface that maintains high coverage of TFMP. DA oxidation is completely inhibited on a TFMP surface with F/C of 0.24; for a freshly prepared TFMP surface, F/C ratio can be from 0.29 to 0.32. TFMP surfaces were exposed to laboratory air for 28 days and XPS spectra were taken periodically during this time. The data for air exposure is listed in Table 4.2. Although the F/C ratio for the air-exposed surfaces decreased 4% from 0.31 to 0.27, these surfaces were still able to inhibit DA oxidation. The O/C ratio on these surfaces increased from 0.03 on a freshly prepared surface to 0.10 after 28 days. It is not certain whether these oxides represent actual oxidation of the GC substrate or reflect adsorbed oxide-containing impurities acquired during air exposure.
Figure 4.1. Anodic potential limits, \( v = 200 \text{ mV/s} \). A. 1 M H\(_2\)SO\(_4\)  
B. 0.1 M H\(_2\)SO\(_4\)
Figure 4.1. Anodic potential limits, \( v = 200 \text{ mV/s} \). C. 0.1 M PBS D. 1 M KOH
<table>
<thead>
<tr>
<th></th>
<th>polished</th>
<th>pyridine</th>
<th>IPA/AC</th>
<th>TFMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M H₂SO₄</td>
<td>1688 ± 19a</td>
<td>1721</td>
<td>1544</td>
<td>2143</td>
</tr>
<tr>
<td>0.1 M H₂SO₄</td>
<td>1777 ± 18</td>
<td>1616 ± 12</td>
<td>1702</td>
<td>&gt;2000</td>
</tr>
<tr>
<td>pH 7 PBS</td>
<td>1521 ± 7</td>
<td>1385</td>
<td>1394</td>
<td>2185 ± 12</td>
</tr>
<tr>
<td>1 M KOH</td>
<td>837 ± 16</td>
<td>776 ± 17</td>
<td>803 ± 30</td>
<td>1476 ± 43</td>
</tr>
</tbody>
</table>

a. Potential limits defined at 0.4 mA (1.48 mA/cm²), 200 mV/s

Table 4.1. Anodic potential limits (mV) at bare and TFMP surfaces.
Table 4.2. XPS data of TFMP surfaces exposed to laboratory air.

<table>
<thead>
<tr>
<th>t (days)</th>
<th>F/C</th>
<th>O/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.31 ± 0.01</td>
<td>0.03 ± 0.002</td>
</tr>
<tr>
<td>1</td>
<td>0.31 ± 0.022</td>
<td>0.052 ± 0.02</td>
</tr>
<tr>
<td>11</td>
<td>0.30 ± 0.02</td>
<td>0.092 ± 0.014</td>
</tr>
<tr>
<td>28</td>
<td>0.27</td>
<td>0.10</td>
</tr>
</tbody>
</table>
Although the potential limit experiments are informative, they do not evaluate the stability of the surface with voltammetric use. To evaluate surface effects of voltammetric scanning, modified TFMP surfaces were either cycled or stepped to a constant potential in 0.1 M H$_2$SO$_4$. Table 4.3 details the results of XPS analysis following voltammetry. Values without parentheses are F/C ratios, and values in parenthesis are O/C ratios. O/C ratios for solvent treated surfaces subjected to the same treatments are provided in brackets. For most of the treatments, the modified surfaces lost very little TFMP (and therefore retained the ability to inhibit DA oxidation). The O/C ratio did increase upon repeated scanning; however, the increase was not as large as for solvent treated GC. However, repeated scanning to extreme potentials (1.8 V) did result in significant reduction of the surface coverage of TFMP; the F/C ratio dropped from 0.31 to 0.10 and the O/C ratio rose from 0.03 to 0.14. The cycled surface exhibited a $\Delta E_p$ for DA of 220 mV (Figure 4.2), consistent with what was observed in Chapter 2 of partially derivatized TFMP surfaces. Also, potentiostatting a GC surface for 2 hours at 1.4 V was sufficient to reduce the F/C ratio to 0.18 and increase the O/C ratio to 0.09.

If the applied potential is limited to 1.2 V, TFMP surfaces retain high enough coverage on GC surfaces to still inhibit DA oxidation, even after 20 hrs (as shown in Figure 4.3). The TFMP surface did apparently oxidize to some extent; the O/C ratio increased to 0.13 after a 20 hr exposure to 1.2. Treatment with IPA/AC did decrease the O/C ratio from 0.13 to 0.8, indicating that some of the oxides were adsorbed impurities. The surface maintained its hydrophobicity upon the long treatment; in contrast, IPA/AC treated GC subjected to a 20 hr oxidation at 1.2 V had a contact angle of <5°. The IPA/AC GC's O/C ratio after the 20 hr treatment was significantly larger than that of
Table 4.3. XPS data for TFMP surfaces subjected to potential scans or steps.

<table>
<thead>
<tr>
<th></th>
<th>1 V</th>
<th>1.2 V</th>
<th>1.4 V</th>
<th>1.8 V</th>
<th>-0.6 V</th>
<th>-1 V</th>
<th>-1.2 V</th>
<th>-2 V</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.29 (0.03)</td>
<td>0.32 (0.03)</td>
<td>0.32 (0.04)</td>
<td>0.32 (0.03)</td>
<td>0.31 (0.03)</td>
<td>0.30 (0.03)</td>
<td>0.29 (0.04)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.27 (0.05)</td>
<td>0.32 (0.05)</td>
<td>0.32 (0.06)</td>
<td></td>
<td></td>
<td></td>
<td>0.25 (0.07)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.28 (0.05)</td>
<td>0.26 (0.07)</td>
<td>0.33 (0.06)</td>
<td></td>
<td>0.31 (0.06)</td>
<td>0.31 (0.06)</td>
<td>0.28 (0.10)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.28 (0.06)</td>
<td>0.25 (0.07)</td>
<td>0.28 (0.11)</td>
<td>0.10 (0.14)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>0.28 (0.06)</td>
<td>0.25 (0.12)</td>
<td>0.26 (0.11)</td>
<td></td>
<td></td>
<td></td>
<td>0.28 (0.08)</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.26 (0.12)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.31 (0.03)</td>
<td>0.32 (0.04)</td>
<td>0.32 (0.03)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 min</td>
<td></td>
<td></td>
<td></td>
<td>0.31 (0.08)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 min</td>
<td>0.30 (0.05)</td>
<td>0.32 (0.05)</td>
<td>0.25 (0.11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.25 (0.13)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5 hr</td>
<td></td>
<td></td>
<td></td>
<td>0.30 (0.08)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 hr</td>
<td></td>
<td></td>
<td></td>
<td>0.18 (0.09)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 hr</td>
<td></td>
<td></td>
<td></td>
<td>0.26 (0.13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. F/C
b. O/C
c. Bracket items are polished surfaces
Figure 4.2. DA voltammetry on a TFMP surface after 10 scans to 1.8 V. 1 mM DA, 0.1 M H₂SO₄, ν = 200 mV/s.
Figure 4.3. DA voltammetry on a TFMP surface after 20 hr at 1.2 V. 1 mM DA, 0.1 M H$_2$SO$_4$, v = 200 mV/s.

F/C = 0.26
O/C = 0.13
TFMP GC, 0.19 vs 0.13. TFMP surfaces remained stable after scanning to -1.2 V for 30 scans. However, by scanning more negative to -2 V, a large decrease in F/C was observed after only one scan. Small bubbles were observed on the TFMP surface along what are suspected to be scratches introduced during the polishing procedure; these bubbles were presumably hydrogen gas.

The stability of 2AQ surfaces upon exposure to laboratory air was monitored with respect to surface composition and dopamine kinetics. Both XPS spectra and DA voltammograms were taken periodically, with the results shown in Figure 4.4 and 4.5, respectively. These data are also tabulated in Table 4.4. For comparison, the theoretical O/C ratio estimated from a calculated geometry for 2AQ on a flat surface is 0.10. $\Delta E_p$ for DA increased on the 2AQ surfaces after exposure to laboratory air; however, the increase was not as dramatic as that observed for polished GC exposed to the same conditions. This implies that the 2AQ surface was better at preventing the adsorption of airborne impurities than IPA/AC treated GC. Both 2AQ and bare electrodes recovered their activity when treated with IPA/AC.

**Redox Systems**

TFMP, 2AQ, and -COOH surfaces were used for a selection of redox systems in order to investigate the modified surface's utility for analytical applications. The high formal potential of $\text{Ru(bpy)}_3^{3+/2+}$ in 1 M KCl places it on the edge of the convenient potential range of polished and solvent treated GC. To reduce interference from background current, $\text{Ru(bpy)}_3^{3+/2+}$ was examined on TFMP surfaces. Figure 4.6 shows the voltammetry of $\text{Ru(bpy)}_3^{3+/2+}$ (1M KCl, 20 V/s) on IPA/AC and TFMP GC. On the IPA/AC treated GC, background current obscures observation of the $\text{Ru(bpy)}_3^{3+/2+}$...
Figure 4.4. O/C ratio for 2AQ upon exposure to laboratory air.
Figure 4.5. $\Delta E_p$ for 1 mM DA (0.1 M H$_2$SO$_4$, 200 mV/s) after air exposure.
<table>
<thead>
<tr>
<th>Time (days)</th>
<th>$\Delta E_p$ (mV)</th>
<th>Time (days)</th>
<th>O/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>59</td>
<td>0</td>
<td>0.12</td>
</tr>
<tr>
<td>2</td>
<td>191</td>
<td>1</td>
<td>0.11</td>
</tr>
<tr>
<td>6</td>
<td>226</td>
<td>5</td>
<td>0.11</td>
</tr>
<tr>
<td>9</td>
<td>249</td>
<td>11</td>
<td>0.09</td>
</tr>
<tr>
<td>12</td>
<td>245</td>
<td>28</td>
<td>0.09</td>
</tr>
<tr>
<td>17</td>
<td>251</td>
<td>40</td>
<td>0.09</td>
</tr>
<tr>
<td>17*</td>
<td>63*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* a. after IPA/AC treatment.

Table 4.4. O/C ratio and DA $\Delta E_p$ for 2AQ surfaces. 1 mM DA, 0.1 M H$_2$SO$_4$, 200 mV/s
Figure 4.6. Voltammetry of 1 mM Ru(bpy)$_3^{3+/2}$ on IPA/AC treated and TFMP modified GC, 1 M KCl $v = 20$ V/sec.
voltammetric current difficult; the background is hard to remove effectively with subtraction (Figure 4.7). By treating the electrode with TFMP, the couple is observable without the interference from background current.

The oxidation of NADH at pH 7 was studied on IPA/AC, 2AQ, and TFMP GC. The products from the oxidation of NADH tend to adsorb to the electrode surface, reducing the activity of the electrode (28, 173, 176). Table 4.5 lists the peak potential of NADH oxidation on IPA/AC and 2AQ GC. Some reduction in the electron transfer kinetics is observed on the 2AQ surfaces; however, the shift is not great enough to cause difficulty in observing the peak. Figure 4.8A shows the voltammetry of NADH on IPA/AC GC. The peak current is not significantly different on the 2AQ-modified GC. Passivation of GC electrodes over time has been observed on surfaces repeatedly scanned in NADH solutions. A positive shift in NAHD peak potential of 81 mV occurred over a period of 1.5 hr on IPA/AC GC while the electrode was scanned once every thirty seconds. After four days of exposure to laboratory air, the same electrode had almost no observable current for the NADH oxidation (Figure 4.8B). In contrast, on 2AQ surfaces, no reduction in peak current upon electrode aging was observed, as shown in Figure 4.8C. After treating the aged electrodes in IPA/AC, a small increase in current was observed on the 2AQ GC while some activity was restored to the original IPA/AC GC. Although some electrode activity was restored on the IPA/AC treated GC, solvent cleaning is not practical for implanted sensors or on-line sampling.

TFMP modified GC was also examined for NADH oxidation. The oxidation peak on TFMP modified GC shifts 132 mV positive of the value on bare GC. However, the peak current remains essentially unchanged once the background is accounted for. Given
Figure 4.7. Background subtracted voltammograms for 1 mM Ru(bpy)$_3^{3+/2}$, 1 M KCl, $v = 20$ V/sec.
<table>
<thead>
<tr>
<th></th>
<th>IPA/AC $E_p$ (mV)</th>
<th>2AQ $E_p$ (mV)</th>
<th>TFMP $E_p$ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>443 ± 8</td>
<td>527 ± 3</td>
<td>575 ± 16</td>
</tr>
<tr>
<td>1 hr</td>
<td>498</td>
<td>545</td>
<td></td>
</tr>
<tr>
<td>1.5 hr</td>
<td>524</td>
<td>553</td>
<td></td>
</tr>
<tr>
<td>4 days</td>
<td>539</td>
<td>615</td>
<td></td>
</tr>
<tr>
<td>IPA/AC recovery</td>
<td>530</td>
<td>543</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.5. $E_p$ for NADH oxidation on bare and modified surfaces.
0.52 mM NADH, PBS, 200 mV/s
Figure 4.8. 0.52 mM NADH in 0.1 M PBS, pH 7, $v = 200$ mV/s.
A. IPA/AC and 2AQ GC
Figure 4.8. 0.52 mM NADH in 0.1 M PBS, pH 7. $v = 200$ mV/s.

B. IPA/AC GC
Figure 4.8. 0.52 mM NADH in 0.1 M PBS, pH 7. v = 200 mV/s.
C. 2AQ GC
Figure 4.8 0.52 mM NADH in 0.1 M PBS, pH 7. \( v = 200 \text{ mV/s} \).

D. IPA/AC and 2AQ GC
Figure 4.8 0.52 mM NADH in 0.1 M PBS, pH 7. \( v = 200 \text{ mV/s.} \)

E. IPA/AC and 2AQ GC after repeated scanning. One scan was taken every 30s.
the long-term stability of TFMP surfaces, TFMP GC should be a good choice for a stable sensor substrate. Figure 4.8D shows the voltammetry of NADH on IPA/AC, 2AQ, and TFMP GC.

The effect of repeated scanning on electrode performance is shown in Figure 4.8E. A scan was taken every thirty seconds for two hours. The 2AQ modified surfaces had less degradation in electrode performance than the IPA/AC GC electrodes upon repeated scanning.

The differentiation between catecholamines and AA on carbon surfaces has been the objective of many research groups due to the interest in monitoring neurotransmitters in vivo (14, 38, 39, 41, 120). Ascorbic acid is the main interference for electroanalysis of catecholamine in nervous tissue, being present in extracellular fluid at 100 times higher concentrations than the catecholamines (2, 38, 120). The most common approach used to reduce AA interference is to use carbon fiber electrodes that have been coated with Nafion® to exclude anion components and to run the voltammetry at fast scan rates.

In a previous chapter, it was reported that 2AQ on GC electrodes was catalytic for catechol oxidation. From previous studies it is known that AA is sensitive to the presence of a blocking layer of NP on the GC electrode surface, resulting in a large positive shift of 500 mV in the peak potential on monolayer modified surfaces (Figure 4.9). However, on surfaces with blocking layers, DA oxidation is also suppressed. Differentiating between DA and AA may be possible by exploiting 2AQ's catalytic activity for DA coupled with a blocking effect for AA caused by the 2AQ layer. The voltammetry of DA and AA on IPA/AC treated GC at pH 7 is shown in Figure 4.10. It is possible to resolve the two waves at slow scan rates on solvent treated surfaces when the concentration when
Figure 4.9. Voltammetry of 1 mM AA on solvent treated and NP modified GC, 0.1 M PBS, $v = 200 \text{ mV/s}$. 
Figure 4.10. Voltammetry of 50 μM DA and 100 μM AA, pH = 7, v = 200 mV/s.
the concentration of AA and DA are the same. However, it is no longer possible to
distinguish between DA and AA at low DA and high AA concentrations on a solvent
treated surface, as shown Figure 4.11. On a 2AQ modified surface (Figure 4.10), the
peaks of DA and AA overlap, making discrimination between the two difficult. The
problem lies in that the oxidation peak for ascorbate is shifted positive compared to its
position on IPA/AC treated GC; however, the observe shift is not as great as what is
observed on NP GC.

Increasing the scan rate for voltammetry on 2AQ GC yielded ambiguous results,
since the electrode area used in this study was relatively large. It was not possible to
increase the scan rate without encountering significant iR error, with accompanying
distortion of electrode response.

Carboxylate surfaces (φ-COOH) have been shown to slow the electron transfer
kinetics of DOPAC, an anion at pH 7, without slowing DA kinetics to the same degree.
By incorporating a carboxylate layer on the electrode surface, perhaps a large enough
shift in AA peak potential would be caused by electrostatic repulsion so that DA
oxidation could be observed in the presence of AA. Figure 4.12 shows the voltammetry
of DA, AA, and a mixture of the DA and AA on carboxylate modified GC at pH 1.
Figure 4.13 shows the same species and surfaces at pH 7. At pH 1, the surface is
protonated and the AA is not rejected. However, at pH 7 the surface and the AA are both
negatively charged, resulting in a severe attenuation of the AA peak. The reduction in
the AA peak allows the observation of DA without interference.
Figure 4.11. Voltammetry of 50 μM DA and 5mM AA on IPA/AC treated GC, 0.1 M PBS, ν = 200 mV/s.
Figure 4.12. Voltammetry of 1 mM DA and 1 mM AA on φ-COOH modified GC, 0.1 M H₂SO₄, v = 200 mV/s.
Figure 4.13. Voltammetry of 1 mM DA and 1 mM AA, on f-COOH modified GC. pH 7 PBS, $v = 200$ mV/s.
Discussion

Some conclusions about the suitability of diazonium modified GC surfaces can be made from the results presented in this chapter. The performance of the modified surfaces on the representative systems will be discussed in terms of advantages and disadvantages, namely surface stability and selectivity.

Surface stability is often an issue with solid electrodes. A GC electrode may be very active for the reaction of interest upon preparation or cleaning, but as time progresses the surface activity generally degrades, limiting its useful lifetime. TFMP and 2AQ modified surfaces are stable with air exposure for at least one month, and showed negligible change in their XPS spectra. TFMP GC in particular has some promising properties. TFMP surfaces were able to withstand exposure to air and high potentials without significant degradation. Only the application of extreme potentials (> 1.4 V) for long times damaged the surface to any appreciable extent. The anodic potential limit of TFMP surfaces were at least 500 mV higher than those observed for polished and solvent treated surfaces, extending the useful range of glassy carbon.

Ru(bpy)$_3$$^{3+/2+}$ has been used as a model redox system in several studies, and has a fast, outer-sphere rate comparable to that of Ru(NH$_3$)$_6$$^{3+/2+}$. Ru(bpy)$_3$$^{3+/2+}$ has been used to probe the effects of structure on reorganization energy for studies with self-assembled monolayers (177), has been incorporated into polymers for electrode surface modification (6, 18), and has been used to investigate the effects of carbon microstructure on electron transfer properties (178). In neutral media, the Ru(bpy)$_3$$^{3+/2+}$ couple has a redox potential of 1.1 V that is near the anodic potential limit of glassy carbon electrodes. Due to the variability in background at high potentials on polished GC, background subtraction is
often problematic and frequently gives rise to artifacts in the subtracted voltammogram. By using TFMP surfaces, the couple is easily observable despite its high formal potential. Both the extended potential range and low capacitance of TFMP modified GC aid in the observation of this couple.

The large number of dehydrogenases for various substrates makes this class of enzymes attractive for biosensor development, especially since the enzyme reaction requires the electroactive cofactor NAD\(^+\). The enzyme-generated NADH is related quantitatively to the amount of substrate, allowing the detection of what are often electroinactive analytes (95). Some examples of analytes determined with dehydrogenase-modified electrodes are alcohols, amino acids, and phenols. However, there are several drawbacks to using NADH as the detected species. The product of NADH oxidation, NAD\(^+\), undergoes further reaction and forms products that foul the electrode surface, resulting in poor electrode performance (173). Surface fouling can drive the potential of the oxidation past what is observable on carbon electrodes. In addition, the processes by which enzymes are immobilized can often leave electrodes with little intrinsic activity for NADH oxidation even before the electrode has had any use (94, 96, 170). Several strategies have been adopted to overcome these problems, including introduction of mediating species to shift the overpotential of the oxidation to more negative potentials and incorporation of electrode activation procedures.

It is not the intention of the current work to investigate the mechanism of NADH oxidation; the mechanism is fairly complex and some excellent work has been done to elucidate the mechanism (176, 179-181). Instead, the objective of these studies is to produce surfaces that are stable and not subject to fouling during the oxidation of NADH.
On polished GC, the oxidation of NADH involves two electrons and one proton. However, Yang observed a significant (~50%) reduction of peak current for NADH oxidation going from a polished surface to a nitrophenyl-modified surface (107). This indicates a shift in mechanism from a two-electron process to a one-electron process. However, on the 2AQ and TFMP surfaces examined in this work, no reduction in peak current was observed, only a shift in the peak position to more positive potentials. In addition, the 2AQ surface did not appear to be acting as an electron transfer mediator for NADH. Tse and Kuwana observed the expected shift in potential for a mediated reaction for NADH on an ortho-quinone modified surface (28). However, on the 2AQ modified surface considered here, the peak position for NADH remained well positive of that of the 2AQ on the surface.

A major problem with electrodes used for NADH oxidation is the lack of stability of these surfaces. An enzyme-modified surface often undergoes a rapid loss of activity before stabilizing at a reduced level of activity (94, 166, 170). A similar loss of activity on IPA/AC treated GC was observed after four days of exposure to laboratory air. 2AQ modified GC did not exhibit the same loss of activity after the four day exposure. Instead, the aged 2AQ electrode maintained almost the same level of activity as a freshly prepared 2AQ electrode. These results indicate that a 2AQ or TFMP surface is effective for preventing the adsorption of a passivating layer to the electrode surface, allowing the detection of NADH for longer periods than a bare, solvent treated electrode.

In enzyme-based biosensors, it is often possible to limit the electroactive components to the sensing system. However, for voltammetry in the extracellular fluid of nervous tissue, the electroactive catecholamines of interest are in a matrix of other
electroactive interferences, namely ascorbic acid and DOPAC, that must somehow be rejected (2, 14, 39). Adding a cation exchange polymer or electrochemically pre-treating the carbon electrode can lead to selective response for cationic catecholamines (2, 14, 38-40, 119, 120). Adsorption of the catecholamines to electrochemically pre-treated (ECP) carbon electrodes leads to slow time response when monitoring rapid changes in catecholamine concentration. Similarly, polymer films on electrode surface need to be thin to maintain fast time response, with the tradeoff of reduced sensitivity for a thinner polymer. Thicker polymer layers are able to pre-concentrate more catecholamines. In addition, variations in polymer layer thickness caused by the dip coating method used to deposit the polymer can lead to variations in electrode response. Despite these disadvantages, electrochemically pre-treating carbon electrodes and using cation exchange polymers such as Nafion® have been successful for monitoring catecholamines in vivo. Nafion® has been extremely successful for in vivo analysis by extending electrode stability to several hours and by rejecting major interferences (14, 22, 33, 37, 38, 40).

The introduction of a monolayer on carbon electrodes that allows the selective determination of DA in the presence of anionic interferents should have several advantages. These advantages are less variability in surface coverage than polymer-coated surfaces, modification of the surface without gross disruption of surface structure (as is the case for ECP surfaces), and enhanced stability imparted by the adsorption of a covalently bound surface layer. Two different diazonium modified surfaces were used to test for these advantages, 2AQ and carboxylate (Φ-COOH) modified GC.
It is possible to resolve AA and DA on solvent cleaned and fractured GC at slow scan rates (200 mV/s). However, near physiological pH an excess of AA can obscure the oxidation of DA even on solvent cleaned surfaces (Figure 4.11). Additionally, solvent cleaned and fractured GC are quickly fouled by adsorbed impurities, degrading the ability to resolve AA and DA. Modifying the GC surface with an inert monolayer such as TFMP to maintain surface stability is not practical since DA oxidation on TFMP surfaces is inhibited.

2AQ has been demonstrated to be catalytic for catechol oxidation in Chapter Three, and since it forms a compact layer on GC it was hoped that it would act as a surface blocker for interferences, namely AA. Previous studies have shown that AA is sensitive to surface condition, indicating that some interaction with the electrode surface is necessary. However, 2AQ was not effective at shifting the oxidation peak for AA enough to avoid interfering in DA oxidation. Quinones have been shown to catalyze the oxidation of ascorbic acid, so this is not entirely surprising. Since 2AQ was not effective for resolving DA and AA based on selective catalysis of DA oxidation, an approach based on electrostatic effects was attempted.

ϕ-COOH modified GC was effective for resolving DA and AA at pH 7. At this pH, the COOH groups are deprotonated and the surface negatively charged. The electrostatic repulsion between the AA and negatively charged surface was sufficient to allow the selective determination of DA in the presence of AA. That the discrimination is electrostatic in nature was confirmed by running analogous experiments at pH 1 where electrostatic interactions are not a factor. At pH 1, it was not possible to resolve AA and DA.
Conclusions

Diazonium surfaces have been shown to be effective for overcoming the difficulties associated with some representative systems. The advantages include:

- **Extension of potential limits.** TFMP surfaces have a larger anodic potential range (500 mV or greater) than polished or solvent treated GC. This property of TFMP surfaces was exploited to observe Ru(bpy)$_3$$^{3+/2+}$ oxidation at 1.1 V. An approximate doubling in signal to background for Ru(bpy)$_3$$^{3+/2+}$ on TFMP over GC was observed. TFMP modified GC also had more reproducible background subtraction qualities.

- **Surface stability.** 2AQ and TFMP surfaces were able to resist fouling during NADH oxidation, resulting in surfaces that were active for NADH oxidation after repeated cycling and extended exposure to laboratory air.

- **Selectivity.** By introducing an anionic surface group to the GC surface it was possible to keep AA from interfering with the determination of DA.

These examples show the effectiveness of using diazonium-modified surfaces for analytical applications. The stability of diazonium-modified surfaces presented here may be applicable to other diazonium-modified surfaces. There should be a wide variety of surface available from the diazonium reaction; the limiting factor is what sort of diazonium salt can be synthesized.
APPENDIX A

QUINONE REDUCTION AT GLASSY CARBON ELECTRODES IN ACetonitrile

Introduction

In aprotic solvents or in basic media above pH 11, proton transfers no longer complicate quinone reduction. Instead, the quinone undergoes two sequential electron transfers:

\[
\text{Catechol} \xrightarrow{-e^-} \text{catechol dianion} \xrightarrow{-e^-} \text{benzoate}
\]

The two waves are separated by approximately 700 mV in acetonitrile. When the proton transfers are no longer a factor in quinone electron transfer, the individual waves for each one-electron reduction can be observed with cyclic voltammetry. Under these conditions, the effects of surface modifications on each individual electron transfer can be observed.

Ideally, the two one-electron reductions are observable under these extreme conditions. However, there are some problems with the quinones when working at such high pH. Quinones tend to be quite unstable under these conditions and undergo
chemical reactions, most notably the reversible addition of hydroxide to benzoquinone at pH 13. The instability of the quinones under basic conditions makes examination of their electron transfer kinetics difficult. Quinones, on the other hand, exhibit two well-resolved waves in dry, aprotic solvents. The first reduction is often called electrochemically reversible, while the second reduction is quasi-reversible at moderate scan rates.

The second reduction wave is sensitive to the presence of proton donors and hydrogen bonding species. Upon addition of a proton donor, the second reduction wave shifts positive and the magnitude in the peak current decreases. As the concentration of proton donor increases, $E^{o}_{2}$ continues to shift to more positive potentials until it merges with the first reduction wave. The mechanism for this process is:

$$Q + e^- \rightarrow Q^- \quad (AA.1)$$

$$Q^- + H^+ \rightarrow QH^- \quad (AA.2)$$

$$QH^- + e^- \rightarrow QH^- \quad (AA.3)$$

$$Q^- + e^- \rightarrow Q^{2-} \quad (AA.4)$$

The potential for reaction AA.3 is the same as the potential for AA.1 (113, 114).

Occasionally, the peak heights of the second wave will decrease without a positive shift in potential. The decrease in peak height has been attributed to the formation of the following complex (114):

$$Q + Q^{2-} \rightarrow [QQ]^{2-} \quad (AA.5)$$

**Experimental**

GC electrode preparation procedures are discussed in detail in Chapter 2.

Quinone solutions were approximately 1 mM, prepared in 0.1 M tetrabutylammonium
tetrafluoroborate in acetonitrile. Solutions were degassed thoroughly before use since oxygen is a major interference in acteonitrile in this potential range. CV's were obtained with a BAS100B/W workstation (Bioanalytical Systems, Inc) as previously described. Scan rates were 200 mV/s. A Ag/Ag$^+$ reference electrode (Bioanalytical Systems, Inc.) was used for acetonitrile solutions. Standard rate constants were determined by fitting the $\Delta E_p$ of the experimental voltammogram to a simulated voltammogram using Digisim (Bioanalytical Systems, Inc).

Chemicals: Acetonitrile (Malinkrodt), isopropyl alcohol (Malinkrodt), activated carbon (Norit 221, Acros), tetrabutylammonium tetrafluoroborate (Acros), 9,10-anthraquinone (Aldrich), 1,4-napthoquinone (Aldrich), and benzoquinone (Aldrich) were used as received.

Diazonium salts were synthesized as described in Appendix C, and electrode surfaces were derivatized as described in Chapter Two.

Results and Discussion

Cyclic voltammetry of a series of quinones, benzoquinone (BQ), napthoquinone (NQ), and anthraquinone (AQ), were studied in acetonitrile on several modified surfaces at 200 mV/s. Table AA.1 lists the $E_{1/2}$, $\Delta E_p$, and $k^o$'s calculated for the quinones on the modified surfaces. These results are presented graphically for BQ in Figure AA.1, for NQ in Figure AA.2, and for AQ in Figure AA.3.

No major changed in electron transfer rate were observed upon solvent treatment of the GC electrodes. $k^o$ changed only by a factor of 2-3 on the bare surfaces. However, $E^{o'}$ for the second reduction shifted to more positive values upon IPA/AC treatment for BQ and NQ. The shift could possibly be due to residual IPA or water on the surface (4, 211
The shift in peak position can be seen in Figure AA.1 and AA.2. More dramatic changes in $k^o$ were observed on the TFMP GC surfaces. For the reduction of the quinone to the semiquinone radical, a factor of 7 decrease in $k^o$ going from polished surfaces to TFMP surfaces was observed. While this is certainly a noticeable effect, it is not nearly as dramatic as that observed for DA in aqueous solutions on TFMP surfaces. However, a factor of 42 decrease in the electron transfer rate for the reduction of the semiquinone radical of BQ was observed on TFMP surfaces. The decrease in electron transfer rate for the reduction of the semiquinone radical of NQ and AQ were not nearly as large, 13 and 12 respectively.

While it is not possible to directly compare data from catechol voltammetry in aqueous solutions and quinone voltammetry in acetonitrile, some interesting observations can be made. The reduction kinetics of the semiquinone radical anion has been reported as "quasi-reversible" in situations where proton donors or hydrogen bonding species have been added to solution (113, 114). Similar "quasi-reversible" kinetics for the second couple are also observed in this work on the TFMP modified surface. It is interesting to note that on TFMP surfaces in acetonitrile, the large decrease in benzoquinone kinetics that was observed for aqueous solutions (Table 2.6, $\Delta E_p \sim 650-700$ mV) is not seen. For the reactions discussed in this Appendix, proton transfers are not involved. The lack of proton transfer complications and the differences in solvent characteristics in acetonitrile may allow the electron transfer to the quinone species to occur at much faster rates than what is observed in aqueous solutions.

Howell and Wightman (182) observed that the electron transfer rate for the reduction of quinones to the semiquinone radical anion increased with increasing size of
the quinone on gold electrodes at fast scan rates (500 to 10,000 V/sec) in acetonitrile. The \( k^o \) for BQ, NQ, and AQ were measured as 0.39 ± 0.10 cm/s, 0.73 ± 0.12 cm/s, and 1.78 ± 0.35 cm/s, respectively. At the scan rate used in this appendix, no such differentiation in the rate constants measured for the reduction of the quinone to the semiquinone radical anion were possible, however, a size dependent increase in \( k^o \) for the reduction of the semiquinone radical anion to the dianion was observed on TFMP modified surfaces, with AQ having the fastest electron transfer kinetics on the TFMP modified surface.

Quinone, semiquinone, and dianion structures for BQ, NQ, and AQ were calculated using computational methods (B3LYP/6-31G(d)) in order to compare the effects of reduction upon structure. Table AA.2 lists the bond lengths calculated for the quinones as well as experimentally measured bond lengths for the quinone species. There is good agreement between the calculated bond lengths and the experimentally measured bond lengths. Figure AA.4 is a graphical representation of the calculated bond lengths, with the first bond length in the group of three representing the quinone species, the second the semiquinone radical anion, and third, the dianion. Table AA.3 has the differences in bond length for the respective structures, going from the quinone (Q) to semiquinone radical anion (SQ), SQ to the dianion (A), and from the quinone to the dianion (Q \( \rightarrow \) A). Also provided are the differences in the calculated bond lengths for 4MC to 4MC ortho-quinone and hydroquinone to benzoquinone. Table AA.4 provides comparisons between structures for species derived from 4MC.

The first notable difference between the protonated species (4MC, HQ to BQ) and the deprotonated species (BQ to \( Q^2^- \)) is that the change in bond length is much less for the
deprotonated species than it is for the protonated species. A large change in bond length is consistent with the slow electron transfer kinetics of the catechols in aqueous solutions. A large change in structure of the quinonoid species means that the reorganization energy for the electron transfer reaction is large, resulting in slower electron transfer kinetics. Since the magnitude of the changes in bond lengths is smaller, the faster kinetics of the quinone species in aprotic solvents is reasonable. However, it must stressed that direct comparisons of species in aprotic and aqueous solutions cannot be made due to differences in solvent polarity and solvation of the quinonoid species.

Conclusions

- The mechanism of quinone reduction in aprotic solvents is different from that in aqueous solutions, resulting in different observed voltammetry.

- The reduction of the semiquinone radical anion was more sensitive to surface modification by an inert monolayer than the reduction of the quinone for BQ, NQ, and AQ.

- The magnitude of the effect that the TFMP modified surface had on electron transfer kinetics decreased with increasing size of the quinones.
<table>
<thead>
<tr>
<th></th>
<th>BQ</th>
<th>NQ</th>
<th>AQ</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Polished GC</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E^{o^*}_{1}$</td>
<td>$-378 \pm 4$</td>
<td>$-576 \pm 2$</td>
<td>$-821 \pm 2$</td>
</tr>
<tr>
<td>$E^{o^*}_{2}$</td>
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<td>$-1204 \pm 5$</td>
<td>$-1351 \pm 9$</td>
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<td>$74 \pm 4, 0.016$</td>
<td>$73 \pm 11, 0.018$</td>
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<td>$\Delta E_p, k^o_2$</td>
<td>$79 \pm 3, 0.014$</td>
<td>$81 \pm 4, 0.012$</td>
<td>$72 \pm 10, 0.019$</td>
</tr>
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<td><strong>Pyridine GC</strong></td>
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</tr>
<tr>
<td>$E^{o^*}_{1}$</td>
<td>$-379 \pm 4$</td>
<td>$-574 \pm 8$</td>
<td>$-822 \pm 1$</td>
</tr>
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<td>$E^{o^*}_{2}$</td>
<td>$-950 \pm 14$</td>
<td>$-1219 \pm 10$</td>
<td>$-1351 \pm 3$</td>
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<td>$\Delta E_p, k^o_1$</td>
<td>$83 \pm 2, 0.011$</td>
<td>$80 \pm 3, 0.013$</td>
<td>$64 \pm 11, 0.032$</td>
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<td>$\Delta E_p, k^o_2$</td>
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<td>$82 \pm 3, 0.011$</td>
<td>$75 \pm 7, 0.016$</td>
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<td><strong>IPA/AC GC</strong></td>
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<td>$-575 \pm 2$</td>
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<td>$E^{o^*}_{2}$</td>
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<td>$64 \pm 4, 0.013$</td>
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<td>$\Delta E_p, k^o_2$</td>
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<td>$74 \pm 4, 0.011$</td>
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<td><strong>TFMP GC</strong></td>
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<td>$-1234 \pm 37$</td>
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<td>$\Delta E_p, k^o_1$</td>
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<td>$\Delta E_p, k^o_2$</td>
<td>$336 \pm 38, 0.00033$</td>
<td>$238 \pm 73, 0.0009$</td>
<td>$185 \pm 14, 0.0016$</td>
</tr>
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</table>

a. Potentials in mV  
b. $\Delta E_p$ in mV  
c. $k^o$ in cm/s

Table AA.1. Formal potentials, peak separations, and standard rate constants for quinones in 0.1 M tetrabutylammonium tetrafluoroborate in acetonitrile.
Figure AA.1. BQ in 0.1 tetrabutylammonium tetrafluoroborate in acetonitrile, \( v = 200 \) mV/sec.
A. Bare GC. B. Polished and TFMP GC
Figure AA.2. NQ in 0.1 tetrabutylammonium tetrafluoroborate in acetonitrile, \( v = 200 \text{ mV/sec.} \)

A. Bare GC. B. Polished and TFMP GC
Figure AA.3. AQ in 0.1 tetrabutylammonium tetrafluoroborate in acetonitrile, $v = 200$ mV/sec.
A. Bare GC. B. Polished and TFMP GC
Table AA.2. Calculated and experimental bond lengths for BQ, NQ, and AQ. Bonds closest to the quinone center are indicated.

<table>
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<tr>
<th></th>
<th>Quinone&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Semiquinone&lt;sup&gt;a&lt;/sup&gt;</th>
<th>dianion&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Quinone Exp</th>
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<tr>
<td>BQ</td>
<td>1.2249&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>1.3017</td>
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<td>1.294</td>
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<sup>a</sup> structures calculated using Gaussian98W, B3LYP/6-31G(d)
<sup>b</sup> bond length in Å
<sup>d</sup> Gaultier, J.; Christian, H. Acta Crystallographica. 1965, 18, 179.
Table AA.3. A. Differences in bond lengths from quinone to semiquinone anion, from semiquinone anion to dianion, and from quinone to anion. B. Comparison of differences in bond lengths for protonated and deprotonated species. Differences are absolute values in Å.

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<th>Q → SQ</th>
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</tbody>
</table>
Table AA.4. Differences in calculated bond lengths for 4MC and derived species, absolute values in Å. A. Deprotonated species. B. Protonated species.
APPENDIX B

DIGITAL SIMULATION OF CATECHOL VOLTAMMETRY

Digital simulations of voltammetric data in this work were done with a commercially available simulation program, Digisim version 2.0 (Bioanalytical Systems, Inc). A general description of simulation processes is given below, and is taken from Bard and Faulkner's "Electrochemical Methods," Appendix B (9). The reader is referred to that reference for a more complete description.

Although it possible to write exact differential equations that describe the transformation and movement of materials in reactions, it is usually not possible to solve these equations analytically. Digital simulations represent an effort to extract information such as current functions, concentration profiles, and potential transients from a model system that approximates a real system. This is done using numerical procedures called the method of finite differences. The general properties of this model system are discussed below.

To make the computation manageable, the solution is considered in terms of small, discrete volume elements. Within these volume elements the concentration of the
reactants and products remains constant; however, the concentration can vary volume
element to volume element. For the situation described here, the method of transport to
and from the first element (where the planar electrode surface is located) is linear
diffusion. This means that the concentration can only vary perpendicular to the electrode
surface. Algebraic expressions to describe other geometries have been written. The
system of boxes creates a discrete model of the solution consisting of an array of
concentrations. The size of the volume element, denoted $\Delta x$, is variable, with smaller $\Delta x$
better defining a continuous function.

This array of concentrations only defines the system for a limited time. Diffusion
will tend to equalize the concentrations in the volume elements, thus defining the next
array of concentration elements. The change in concentration in the next array is defined
by laws of reactions and diffusion, which are written as algebraic expressions and broken
up into time intervals defined at $\Delta t$. As with the concentration arrays defined by $\Delta x$,
smaller $\Delta t$ better approximates continuous systems. By iteratively solving the rate and
diffusion expressions, and evolving model of the system with time is obtained. From this
model, information about the concentration, current, and potential of the system can be
extracted.

Digisim version 2.0 uses implicit finite difference models to save computational
time. Digisim is able to simulate mechanisms in terms of one electron heterogeneous
reactions and first or second order homogeneous reactions. For the simulations used
here, the preset model parameters ($\Delta x$, $\Delta t$, etc) were used. User input parameters are the
mechanism used, $E^\circ$, $k^\circ$, and $\alpha$ for the heterogeneous reactions, diffusion coefficients for
the reacting species, concentrations, and voltammetric parameters (potential limits, electrode area, scan rate).

Simulation Parameters

As discussed in Chapter Three, the following mechanism can be used to simulate catechol voltammetry at pH 7.

\[
\begin{align*}
QH_2 & \rightarrow SQ + e^- & E^\circ'_{2}, k^\circ_{2} \\
SQ & \rightarrow Q + e^- & E^\circ'_{1}, k^\circ_{1}
\end{align*}
\]

(AB.1)

The numbering scheme used for the reactions follows that of Laviron (115, 116) and Deakin et al (12, 13). The model assumes that protonations are at equilibrium, and that the rate limiting steps are the electron transfers. \(E^\circ'_{1}\) and \(E^\circ'_{2}\), the formal potentials for each electron transfer, were obtained from a potential-pH diagram constructed from literature values, and are shown in Table AB.1.

<table>
<thead>
<tr>
<th>E^\circ' (mV)</th>
<th>DA</th>
<th>4MC</th>
</tr>
</thead>
<tbody>
<tr>
<td>E^\circ'_{1}</td>
<td>0.011</td>
<td>-0.048</td>
</tr>
<tr>
<td>E^\circ'_{2}</td>
<td>0.297</td>
<td>0.262</td>
</tr>
</tbody>
</table>

Table AB.1. Formal potentials for DA and 4MC used for simulations.

Other simulation parameters include scan rate = 0.2 V/sec, electrode area 0.27 cm², \(\alpha = 0.5\) for each electron transfer, and \(D_{QH_2} = D_{SQ} = D_Q = 6 \times 10^{-6} \text{ cm}^2/\text{s}\). For the purposes of
these simulations, $k^0_1$ was set equal to $k^0_2$, and these values were varied to match experimental $\Delta E_p$. From the voltammetric data presented in this work, $k^0_1$ and $k^0_2$ could not be independently assessed; however, setting the two equal to each other allows the magnitude of the effects of surface modification to be assessed. The data for the simulations is listed in Table 3.7 and again here in Table AB.2 for clarity. The simulated voltammograms for 4MC and DA are shown in Figures AB.1 and AB.2 respectively. As discussed in Chapter Three, the current waveshape observed for the simulated and experimental voltammograms are not a perfect match.

As discussed in Chapter Three, simulations carried out with the above mechanism at pH 1 do not yield reasonable values for the standard rate constants. To estimate the magnitude of the H/D isotope effect referred to in Chapter Three, simulations were carried out with the following model and parameters:

$$QH_2 \rightarrow SQ + e^- \quad E^o_2, k^o_2 \quad (AB.2)$$

$$SQ \rightarrow Q + e^- \quad E^o_1, k^o_1$$

$E^o_2 = E^o_1 = E^o$ observed for the two electron couple, 511 mV for 1 mM DA in 0.1 M $H_2SO_4$ and 543 mV for 1 mM DA in 0.1 M $D_2SO_4$. Other parameter simulations were the same as above. No effort was made to fit the current waveshape in the simulations to the experimental current; instead, $k^o_1$ and $k^o_2$ were varied until simulated $\Delta E_p$ matched experimental $\Delta E_p$. Rate constants determined in this manner are shown in Table AB.3.
Table AB.3. Simulated rate constants for modified GC surfaces in H₂O and D₂O.

\[
\begin{array}{|c|c|c|}
\hline
\text{Surface Modification} & k^\circ_H & k^\circ_D \\
\hline
\text{DUQ} & 0.035 \text{ cm/s} & 0.01 \text{ cm/s} \\
\text{COOH} & 1.2 \times 10^{-4} \text{ cm/s} & 9 \times 10^{-5} \text{ cm/s} \\
\hline
\end{array}
\]

The H/D kinetic isotope effect estimated in this manner is 1.3 for both DUQ and COOH modified GC. The limiting $\Delta E_p$ from the simulation was 42 mV, so estimation of the effect on IPA/AC GC was not possible. The implications of these values are discussed in Chapter Three.
Figure AB.1. Simulated and experimental voltammograms for 1 mM 4MC, pH 7, $\nu = 200$ mV/s.
Figure AB.1. Simulated and experimental voltammograms for 1 mM 4MC, pH 7, $\nu = 200$ mV/s.
Figure AB.1. Simulated and experimental voltammograms for 1 mM 4MC, pH 7, $\nu = 200$ mV/s.
Figure AB.2. Simulated and experimental voltammograms for 1 mM DA, pH 7, $v = 200 \text{ mV/s}$. 
Figure AB.2. Simulated and experimental voltammograms for 1 mM DA, pH 7, \( v = 200 \) mV/s.
APPENDIX C

PROCEDURE FOR THE SYNTHESIS OF DIAZONIUM SALTS AND SPECTROSCOPIC CHARACTERIZATION OF DIAZONIUM SALTS

Synthesis of Diazonium Salts

General Mechanism

Diazonium salts were prepared according to a procedure established by Duker et al (134) with some modification. Diazotation is accomplished by reacting primary aromatic amines with nitrous acid at 0°C to produce diazonium salts. While other amines may be used to prepare diazonium salts, the products are unstable and decompose readily, even at 0°C. The general reaction for the synthesis of aromatic diazonium salts is shown below:

\[
\begin{align*}
\text{R} & \quad \text{HONO} \quad 0^\circ \text{C} \\
\text{NH}_2 & \quad \text{N}^+\text{N}^{-}
\end{align*}
\]
A more specific mechanism follows (183):

\[
\text{Ar—NH}_2 + \text{HO—N=O} \rightleftharpoons \text{Ar—N}^+\text{H} \rightleftharpoons \text{Ar—N—H} \rightleftharpoons \text{Ar—N=N—OH} + \text{H}_2\text{O}
\]

Nitrous acid solution provides a source of nitrosonium ions, which add to the amine forming the N-nitroso species. The hydroxydiazo tautomer of the N-nitroso species yields the diazonium ion under acidic conditions.

If the temperature of the solution is allowed to rise above 4°C, the diazonium ion decomposes to form the phenol and N₂.

**Experimental**

The general procedure used for the synthesis of the various diazonium salts will be described first, followed by specifics for each individual salt. 0.1 mole of the amine precursor is dissolved in 40 mL of 40% fluoboric acid and the mixture is cooled to below 4°C in an ice bath. Fluoboric acid was used in order to provide tetrafluoroborate counter ion for the diazonium salt. 0.12 mol of sodium nitrite is then dissolved in 10 mL of Nanopure water and cooled in an ice bath to 4°C. The solution of sodium nitrite is then added drop wise to the amine mixture while stirring constantly. The sodium nitrite solution must be added slowly, or else the temperature of the mixture will rise rapidly. After the addition of the sodium nitrite, the mixture was stirred for 15 to 30 minutes in an
ice bath. The diazonium salt was then collected by suction filtration on a sintered glass filter. The diazonium salt was then washed with cold fluoboric acid, Nanopure water, and ether. The product was then recrystallized from acetonitrile and ether.

Specific procedures

4-nitrophenyldiazonium tetrafluoroborate: The product required two recrystallization steps.

α,α,α-trifluoromethylphenyl diazonium tetrafluoroborate: The sodium nitrite solution must be added very slowly; halfway through the addition the solid precursor will disappear. After the addition of the sodium nitrite solution, the mixture must be stirred for 40 to 45 minutes for complete reaction.

Benzoic acid-4-diazonium tetrafluoroborate: An initial heating step was required to disperse the 4-amino-benzoic acid evenly in the fluoboric acid. After the precursor was dispersed evenly, the solution was cooled and the general procedure followed.

2-anthraquinone diazonium tetrafluoroborate: The crude product must be recrystallized after the synthesis; very little diazonium salt is produced by this procedure.

Spectroscopic Data

The data for the various diazonium salts are given below, with the spectra following.

4-nitrophenyldiazonium tetrafluoroborate, NMR (d₆-DMSO, 400 MHz), MS (ES) calculated for C₆H₄N₃O₂ m/z 150.120, found 150.0 (M⁺).

α,α,α-trifluoromethylphenyl diazonium tetrafluoroborate, NMR (d₆-DMSO, 400 MHz), MS (ES) calculated for C₇H₄N₂F₃ m/z 173.121, found 173.1 (M⁺).

Benzoic acid-4-diazonium tetrafluoroborate, NMR (d₆-DMSO, 400 MHz), MS (ES) calculated for C₇H₅N₂O₂ m/z 149.13, found 149.0 (M⁺).
2-anthraquinone diazonium tetrafluoroborate, NMR (d₆-DMSO, 400 MHz), MS (ES) calculated for C₁₄H₇N₂O₂ m/z 235.229, found 235.1 (M⁺).

List of Spectra:

Figure AC.1A. 4-nitrophenyldiazonium tetrafluoroborate, NMR (d₆-DMSO, 400 MHz)
Figure AC.1B. 4-nitrophenyldiazonium tetrafluoroborate, MS (ES)
Figure AC.2A. α,α,α-trifluoromethylphenyl diazonium tetrafluoroborate, NMR (d₆-DMSO, 400 MHz)
Figure AC.2B. α,α,α-trifluoromethylphenyl diazonium tetrafluoroborate, MS (ES)
Figure AC.3A. 4-Benzolic acid diazonium tetrafluoroborate, NMR (d₆-DMSO, 400 MHz)
Figure AC.3B. 4-Benzolic acid diazonium tetrafluoroborate, MS (ES)
Figure AC.4A. 2-anthraquinone diazonium tetrafluoroborate, NMR (d₆-DMSO, 400 MHz)
Figure AC.4B. 2-anthraquinone diazonium tetrafluoroborate, MS (ES)
Figure AC.1A. NMR spectrum of 4-nitrobenzenediazonium tetrafluoroborate
Figure AC.1B. Mass spectrum of 4-nitrobenzenediazonium tetrafluoroborate
Figure AC.2A. NMR spectrum of α,α,α-trifluoromethylphenyl diazonium tetrafluoroborate.
Figure AC.2B. Mass spectrum of α,α,α-trifluoromethylphenyl diazonium tetrafluoroborate.
Figure AC.3A. NMR spectrum of 4-benzoic acid diazonium tetrafluoroborate.
Figure AC.3B. Mass spectrum of 4-benzoic acid diazonium tetrafluoroborate.
Figure AC.4A. Mass spectrum of 2-anthraquinone diazonium tetrafluoroborate.
Figure AC.4B. Mass spectrum of 2-anthraquinone diazonium tetrafluoroborate.
LIST OF REFERENCES


(100) Kuo, T.-C. Ph. D., The Ohio State University, Columbus, OH, 1999.


(107) Yang, H.-H. Ph. D., The Ohio State University, Columbus, OH, 2000.


(121) Chen, P. Ph. D., The Ohio State University, Columbus, OH, 1996.


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