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ELECTROCHEMICAL AND SPECTROSCOPIC CHARACTERIZATION OF SELF-ASSEMBLED MONOLAYERS: ELECTRODE MODIFICATION FOR CARDIAC PACING APPLICATIONS

by

Mark Henry Schoenfisch

A Dissertation Submitted to the Faculty of the DEPARTMENT OF CHEMISTRY In Partial Fulfillment of the Requirements For the Degree of DOCTOR OF PHILOSOPHY In the Graduate College THE UNIVERSITY OF ARIZONA 1997
As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Mark H. Schoenfisch entitled \textit{Electrochemical and Spectroscopic Characterization of Self-Assembled Monolayers: Electrode Modification for Cardiac Pacing Applications} and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of \textit{Doctor of Philosophy}.

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I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

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DEDICATION

To Mom and Dad
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ABSTRACT

New biomaterials for permanent cardiac pacemaking electrode applications based on Au surfaces chemically modified with self-assembled monolayers (SAMs) have been developed. The research described herein focuses on four areas related to understanding the extraordinary pacing exhibited by modified pacemaker electrodes.

SAM-modified pacemaker electrodes were fabricated and tested in canines for chronic and acute cardiac pacing. In addition to having electrical properties suitable for pacing the heart, SAM-modified electrodes are proven superior to control electrodes in pacing performance. The data suggest that the biocompatibility of electrically conductive materials can be controlled at the molecular level with monolayer organic surface films.

The development of a small rodent model for studying cardiac pacing was explored as an alternative to using canines in clinical studies. Rodents, not previously used for such studies, were demonstrated to be excellent mammals for testing initial electrode modification strategies. Myocardial tissue resistance in a living mammalian heart was determined using chronoamperometry and cyclic voltammetry of Ru(NH$_3$)$_6^{3-}$.

Pacemaker systems represent complete electrochemical cells. Thus, modified pacemaker electrodes are simply examples of chemically modified electrodes, an area of electrochemistry which has been studied extensively over the past two decades. For these types of systems, the interfacial chemistry occurring in the vicinity of the SAM is crucial to its function. Therefore, investigations into the stability, order, and orientation of SAMs at the
metal electrode surface, and solvent behavior at the outer edge of the SAMs were undertaken. Such fundamental information is critical in understanding the biocompatibility of these modified pacemaker electrodes, and potentially, in understanding the mechanism for the pacing efficacy of the electrode modification. Surface Raman spectroscopy using an emersion approach was developed as an exceptional technique for probing the structural order and stability of SAMs on Ag and Au after exposure to solvent, electrolyte, and potential.

Finally, the stability of these SAM-modified pacemaker electrodes to air and mechanical stress was investigated. Raman spectroscopy, cyclic voltammetry and x-ray photoelectron spectroscopy were utilized to better understand the shelf-life of modified electrodes.
Chapter 1

Introduction: Covalent Surface Organochemical Modification of Pacemaker Electrodes for Cardiac Pacing Applications

Numerous biomaterials have been introduced as artificial implants since the first serious attempts to replace blood vessels during the 1940's.1,1,2 Eleven million people carry implanted medical devices, including cardiac pacemakers and artificial organs (hearts, blood vessels, hips, and knees).1,3 These devices have prolonged the life, and improved its quality, for many individuals. Although such applications are extraordinary engineering and medical feats, the difficulties and constraints of medical implants are not directly related to biofunctionality requirements. More important is the interaction between blood and the device in use, and the lack of complete biocompatibility. If it were not for the critical nature of these interactions, and the tendency for blood to interact unfavorably with virtually all foreign surfaces,1,1,2,1,4,1,6 artificial implants would initiate fewer complications and benefit greater numbers of people.

One device which is severely affected by the chemically complex environment in which it operates is the permanent cardiac pacemaker. Unlike classical biomaterial implants, a pacemaker must, in addition to withstanding the immune system's reactions, withstand an estimated 75 million cycles of heart beat per year and retain electrical conductivity at low impedance (so as to not deplete the power source) at a failure rate of
less than 0.016 ppb to reduce to less than 1 in 1000 the risk per year of death or brain
damage that would occur with just brief absence of heart beat. Yet, current pacemaker
technology is not exempt from thrombosis and the formation of scar tissue, which in
effect, decrease the efficacy of pacemaker therapy. Covalent surface modification of
pacemaker electrodes is a novel approach for producing surfaces that more resemble the
organic constituents of the local environment in heart muscle. Surface modification may
permit tissue integration of the electrode (true biocompatibility), creating a pacemaker
electrode with exceptional long-term performance and stability that is far superior to
currently available technology.

Cardiac Pacemaker Electrodes

General Description

Nearly 60 million Americans, more than 1 in 4, have one or more types of
cardiovascular disease. Abnormal heart rhythm is a symptom of heart disease or
premature development. Cardiac pacemakers are used to artificially stimulate heart tissue
and prevent the associated side-effects of abnormal heart rhythms, including dizziness,
fatigue, blackouts, or death. About 2 million pacemakers are in use world-wide, with ca.
130,000 implanted each year.

A pacemaker system consists of a two-component device: 1) a pulse generator
containing a sealed power source (typically a lithium iodide battery) and necessary
electronics for delivering intermittent voltage pulses, and 2) a metal electrode (lead) which
conducts the stimulus from the pulse generator to heart tissue. A conducting, helically
coiled and insulated wire, also known as the lead wire, is used to connect the electrode to
the pulse generator. The pulse generator is usually implanted in the abdominal wall or the
fascia of the pectoral muscle.

Two basic types of pacemaker leads are commonly used: passive- and active-
fixation. The tip of an active-fixation electrode consists of a helical metal screw that is
anchored to the heart via a piercing mechanism and traumatically disrupts heart tissue. A passive-fixation electrode consists of a hemispherical, porous metal surface, which
instead of piercing the heart, is sutured to heart tissue. This type of anchoring is regarded
as atraumatic, because the heart is not mechanically disrupted. Schematics of the two
general classes of pacemaker leads are shown in Figure 1.1.

In addition, electrodes can be either bipolar or unipolar. A bipolar lead has two
stimulating electrodes in the heart, while a unipolar lead consists of only one. Whether an
electrode is unipolar or bipolar has no significance on pacing performance as a function of
biocompatibility.

The material of which the pacemaker is composed must meet several requirements,
including electrochemical inertness, low electrical resistance/high conductance, resistance
to corrosion, and perhaps most important, low biological reactivity. Many early
pacemaker electrodes were made of stainless steel, but because steel corrodes rapidly in
the heart under pacing conditions, platinum (or platinum-iridium) became the material of
choice. Platinum is an inert metal, offering corrosion resistance in heart tissue.
Figure 1.1. Schematic of a) active-fixation and b) passive-fixation pacemaker electrodes.
However, platinum is expensive; therefore, other materials have been developed and studied as an alternative, including Elgiloy (an alloy of cobalt, iron, chromium, molybdenum, nickel, and manganese), a silver and steel combination, titanium, tantalum pentoxide, and activated vitreous carbon. Yet, platinum is still the most corrosion-resistant metal and remains the electrode material of choice by pacemaker manufacturers.

Pacing Mechanism

A pacemaker electrode and pulse generator represent a complete in vivo two- (unipolar) or three- (bipolar) electrode electrochemical system. The pacemaker electrode in contact with myocardial tissue represents the cathode. For a two-electrode system, the metal case of the pulse generator serves as the anode.

Heart tissue is excited by intermittent voltage pulses from the pulse generator through the electrode tip. An electric field gradient is created across stimulatable cell membranes. If the imposed potential difference is of large enough magnitude, the permeability of the membrane is changed, causing membrane depolarization, also known as an action potential. Neighboring heart cells are then stimulated by the initial depolarization of cells adjacent to the pacemaker lead, and a response propagates away from the electrode. A heart beat results after a sufficient volume of heart tissue becomes depolarized. Voltage pulses of 5 or 2.5 V are common.
Degradation of Bioelectrical Interface

One of the central problems with permanent pacemaker therapy is the degradation of the bioelectrical interface, the site at which the electrode contacts myocardial tissue. Chemical degradation is a constant factor common to all pacemakers. At the initial time of implantation, the electrode is in direct contact with electrically responsive myocardial cells. However, from that moment forward, the body responds to this foreign object through local immunological reactions, cell infarction (death), and intense inflammation, causing the electrode to become encapsulated in a thin layer of fibrous tissue immediately adjacent to the surface. Neither fibrous tissue nor infarcted tissue is sensitive to stimulation. Therefore, the efficiency of electrical stimulation decreases.

The effective electrode diameter (the distance between the electrode and the closest excitable tissue) of the pacemaker electrode is increased by the presence of a fibrous layer that forms around the electrode, creating what is referred to as a "virtual" electrode, as shown in Figure 1.2. Although conductive, scar tissue decreases the efficiency of the pacing process (the electric field strength necessary to stimulate the heart is directly proportional to the applied voltage and inversely proportional to the "virtual" electrode diameter) by increasing the pacing impedance. In severe cases of inflammation and scar tissue formation, complete failure of pacing can occur. Pacemaker failure results in potentially lethal rhythms with dangerous consequences and sometimes loss of life.

Even when degradation of the bioelectrical interface is slow, it is undesirable,
Figure 1.2. Schematic of the "virtual" electrode.
because more energy is required to stimulate the heart. Ultimately, battery life is shortened. Often, repeat operations are necessary to replace pacemakers with a high failure rate or systems with depleted power sources. In total, pacemaker operations cost Americans more than $4 billion annually. Even more critical, repeat operations are often performed as an emergency with life-threatening risks.

Pacing Threshold

The parameter that is commonly measured to establish pacing efficiency is called the pacing threshold. Threshold is defined as the minimal time period of constant voltage which just causes the heart to beat. As described in the previous section, pacing threshold (efficiency) is highly affected by scar tissue formation and the size of the "virtual" electrode. Early after implantation, threshold levels rise (i.e., additional energy is required to pace the heart) due to the formation of a non-stimulatable fibrous layer around the electrode. This behavior occurs regardless of electrode type (passive- or active-fixation). Most often threshold levels peak within 1-3 months after electrode implantation; however, for some patients (depending on individual immunological response), threshold can continue to rise for up to 6 months. The average increase is usually 3-5 times threshold values measured at time of implant. However, in 5-10% of cases, the threshold rises to 10 times or greater levels at implant. At such levels, pacing efficiency is extremely poor. Patient monitoring is intensified, because the chance of pacing failure is high. In addition, pacemakers are programmed to operate at higher voltages during the
initial weeks following implantation to counter elevated thresholds and ensure pacing. Higher programmed voltages significantly reduce overall battery life.\textsuperscript{1,18}

Over time, once inflammatory processes have run their course, the fibrotic layer becomes smaller and threshold decreases to values about 2-3 times that at implant.\textsuperscript{1,9} At this time, a stable interface forms in which the original pacing electrode is surrounded by a permanent layer of fibrous tissue that is also partly responsible for anchoring the device to the heart. A typical threshold versus implant time plot for commercially available pacemaker electrodes is shown in Figure 1.3.

In summary, the degree of threshold elevation (both magnitude and duration after implant) is a function of the "virtual" electrode diameter that forms around the electrode. Threshold is a highly variable parameter with pacemakers due to the diverse range of immunological responses to the electrode implantation among patients.\textsuperscript{1,19,1,20}

Previous Biocompatibility Strategies

Various electrode designs, sizes, and materials have been used in an effort to reduce acute and chronic threshold levels.\textsuperscript{1,14,1,21} One approach which has created considerable interest is the development of a steroid-eluting electrode for reducing inflammation and scar tissue formation after electrode implantation.\textsuperscript{1,22}

The first configuration of this technology was based on the incorporation of dexamethasone, an anti-inflammatory steroid, into the porous metal matrix of a passive-fixation electrode.\textsuperscript{1,14} After pacemaker implantation, the steroid is slowly eluted from the
Figure 1.3. Common threshold behavior for commercially available pacemaker electrodes.
electrode tip into adjacent tissue. These steroid-eluting electrodes have been shown to reduce threshold peaking and maintain low threshold chronically.\textsuperscript{1,23-1,27} However, as with other passive-fixation leads, the mechanical stability of these steroid-eluting electrodes, particularly in the atrium,\textsuperscript{1,28,1,29} remains a problem.

In addition, passive-fixation electrodes are generally the least suitable for infants/adolescents requiring permanent pacing (because of extensive heart growth) or patients having undergone heart surgery, due to high levels of mechanical complications. Such patients require active-fixation electrodes. The screw-in, anchoring mechanism of such electrodes provides better mechanical stability and a near zero dislodgement rate. However, active-fixation leads are reported to have higher acute and chronic thresholds than passive-fixation electrodes.\textsuperscript{1,30} The fibrous capsule layer is exacerbated by such leads, as a result of the anchoring mechanism which traumatically disrupts tissue and induces additional inflammation.

In an attempt to embody low acute and chronic thresholds (due to reduced inflammation) together with an electrode geometry possessing extremely low dislodgement rates, steroid-elution has been applied to active-fixation pacemaker electrodes.\textsuperscript{1,31} A steroid-loaded silicon plug (designed to elute a maximum of 1.0 mg of dexamethasone) was placed inside a dexamethasone coated-, helical, platinum electrode. Despite impressive acute pacing characteristics, comparable with those observed with passive-fixation, steroid-eluting leads, the study was suspended because of a high level of mechanical complications (mainly helix deformation). No information was collected on
the long-term efficacy of this approach. However, the researchers suggest that because short-term inflammation is reduced, chronic threshold rise would also be avoided.\textsuperscript{1,32-1,34}

To date, no steroid-eluting, active-fixation electrode has been successfully introduced to address the problem of maintaining the integrity of the bioelectrical interface. As a result, elevated acute and chronic thresholds are still common for patients requiring active-fixation technology.

An ideal interface between pacemaker electrode and biological tissue would be inert with respect to chemical degradation, corrosion, and the inflammatory cascades. Although the noble metals (including Au, Pt, and Ir) are resistant to chemical corrosion processes, such metals also stimulate thrombosis and fibrous tissue formation when used as pacemaker electrodes, as described above. A closer approximation to a biocompatible interface might be a surface that chemically resembles biological milieu. Covalent surface modification of pacemaker electrodes with organic, self-assembling molecules represents a strategy for creating surfaces that resemble heart tissue.

**Self-Assembled Monolayers**

Molecular self-assembly has become a popular surface derivatization procedure, mostly due to its simplicity, versatility, and ability for generating a well-defined interface.\textsuperscript{1,35-1,60} In this process, a molecular layer spontaneously forms on a metal substrate that is immersed in a solution containing the molecules as shown in Figure 1.4 for alkanethiols. The reaction is driven by the strong interaction between the substrate and
a surface-specific functional group of the adsorbate. Research on SAMs has exploited chemical interactions of alkanethiols, alkyltrichlorosilanes, and isonitriles with Au, Pt, and/or Ag surfaces.

The ability to conveniently change the structure, properties, and reactivity of a surface through the use of SAMs has led to the development of a new class of materials. Many interesting applications of these systems have been investigated, including adhesion, lubrication, biological membrane modeling, chemical sensors, electron transfer pathways, and biocompatibility/protein adsorption.

Modified-Pacemaker Electrodes

Organic thin films at metal substrates create unique interfaces which combine certain properties of the metal, such as mechanical stability and electrical conductivity, with organic properties of the film, for example, hydrophobicity. A system for which such a combination might be advantageous is active-fixation pacemaker electrodes. Recall, the degradation of the bioelectrical interface is a constant common to all pacemakers. It is hypothesized that organically modified surfaces more resemble local tissue, and thus, may be more biocompatible.

A modification approach pursued in this research utilizes a molecule bonding strategy in which the fundamental chemical nature of the pacemaker electrode surface is significantly altered and controlled by the covalent immobilization of alkanethiol self-assembled monolayers on Au-coated Pt(Ir) pacemaker electrodes.
Alkanethiol-modified, Au-coated pacemaker electrodes are attractive for a number of reasons. First, the chemisorption of organic sulfur compounds at metal surfaces generates versatile self-assembling systems. This is due to the strong metal-S bond created upon monolayer adsorption on Au, Ag, Cu, and Pt. For example, the attachment chemistry on Au results in films which are chemisorbed by 40-50 kcal/mol of energy. Films formed in this manner are dense enough to prevent direct contact of macromolecular matrix constituents with the metal surface. Thus, when implanted in the heart, the relevant bioelectrical interface is between the heart muscle and the organic terminal group of the SAM film, instead of the metal surface.

A second attractive feature of alkanethiol-modified, Au-coated pacemaker electrodes is based on the similar organothiolate bonding between Au-SAM films and the Au-thiolate bond of commonly employed Au-based anti-inflammatory drugs, examples of which are shown in Figure 1.5. This similarity may provide an in vivo beneficial effect, because even if chemical degradation of the bioelectrical interface occurs during chronic pacing, the Au-thiolate compounds released may have therapeutic, anti-inflammatory effects which could reduce inflammation and fibrotic tissue formation, improving pacemaker performance.

An additional feature of self-assembled monolayers which could be used to control the biocompatibility of biomaterials is molecular engineering of the terminal functional group, R (see Figure 1.6). A range of terminal functional groups ranging from very hydrophobic to hydrophilic to actual protein molecules could be covalently attached to the
Figure 1.5. Two examples of common Au-based anti-inflammatory drugs: a) gold sodium thiomalate and b) aurothioglucose.
Figure 1.6. Schematic of a self-assembled monolayer formed from alkanethiols on a metal surface. Note terminal functional group, R, which could be synthetically altered.
same in order to alter in vivo immunological responses. This control could alter the protein adsorption characteristics of these electrodes, and ultimately, inflammation and fibrous tissue formation. Protein adsorption is important in mechanisms of cellular adhesion to surfaces such as those responsible for initiating inflammation and the formation of scar tissue in the chronic phase of pacing.\textsuperscript{1,1} Whitesides has shown that organothiol surfaces modify protein adsorption characteristics depending on the chemical nature of the R group to which the biological medium is exposed.\textsuperscript{1,68} Ethylene glycol groups were observed to be particularly resistant to protein adsorption, an observation consistent with the literature on implant blood compatibility in which protein adsorption on a range of hydrophobic and hydrophilic polymer surfaces has been investigated.\textsuperscript{1,69-1,74} The ultimate goal of such a modification strategy would be that the modified-electrode be integrated with surrounding tissue, resulting in true biocompatibility rather than fibrous capsule formation.\textsuperscript{1,7,1,75}

One final feature that makes SAMs attractive for pacemaker electrode modification is that, since only monomolecular quantities of material are involved, limited toxicity is expected in the event that film corrosion/desorption from the surface occurs after implantation. In total, all of the above attributes are extremely attractive for molecular engineering of active-fixation cardiac pacemaker electrodes for permanent cardiac pacing applications.
Defect Structure

Any approach used as a surface modification strategy for pacemaker electrodes must result in a system that is conductive so that heart tissue can still be polarized at low impedance. At first glance, self-assembled monolayers may not seem to be good choices based on this criterion. When organothiol films are formed for long periods of time or when film formation occurs from relatively concentrated solutions of the organothiol, the film is quasi-crystalline in nature or very ordered, as shown in Figure 1.4. Such films are, in fact, not inherently conductive and exhibit excellent blocking properties towards electron transfer. Electron transfer, however, still occurs to a much lessened extent.

These films have been characterized as microelectrode arrays because of the presence of pinholes which effectively function as microelectrodes. The total area fraction of pinholes for very ordered, long chain SAMS (i.e., >dodecanethiol) has been estimated to be in the range of $10^{-2}$ to $10^{-5}$ of the total surface area covered by the organized molecular assembly. This fractional coverage corresponds to a ca. $10^{-12}$ to $10^{-15}$ moles/cm$^2$ surface coverage of pinholes, a very small surface concentration. However, if the film is produced during short times, from relatively dilute solutions, or on relatively rough surfaces, the film is less perfect (although still quite ordered relative to the liquid state), containing various types of "defects" which can be exploited to enhance conductivity.

A defect is considered to be any structural property of the film that allows ions or
redox species to approach closer to the electrode surface than the distance of an all trans extended film in an electrochemical environment. Defects are the result of incomplete film coverage at the surface or film disorder, attributed to substrate roughness, grain boundaries, film tilt boundaries, gauche conformers, and/or carbon contamination, as shown in Figure 1.7.

In addition, it should be noted that the extent of defect structure is also a sensitive function of alkane chain length of the molecules from which the SAM is formed.\cite{1.80, 1.81} Defects are more prevalent in films produced from shorter alkane chains. This behavior provides an important experimental variable that can be exploited to optimize the desired conductivity and blocking characteristics of these films. An important consideration for pacemaker electrode modification is that, although a greater defect structure enhances conductivity, a more defective film may be less able to provide an efficient blocking layer towards the metal surface from biological milieu. Therefore, less ordered films may be more susceptible to biochemical or electrochemical degradation during use in pacing applications.

Previous Work

Much of the interest surrounding SAMs arises from the unusually high degree of structural order that is present in these monomolecular films. Several analytical methods have been utilized for studying these highly ordered systems, including Raman spectroscopy,\cite{1.82-1.85} infrared spectroscopy,\cite{1.37, 1.45, 1.47, 1.86-1.91}
Gauche defects
Pinholes
Domain edges
Also: Grain boundaries and surface roughness

Figure 1.7. Schematic of defects in SAM films on metal surfaces.
Electrochemistry 

Cyclic voltammetry and AC impedance are the two most prominent electrochemical methods for characterizing the properties of alkanethiol-SAMs at metal surfaces. The most detailed information about defect sites in SAM films has been derived through capacitance and electron transfer data.

Capacitance studies probe the charge storing ability of SAM-modified electrodes. For a simple parallel plate capacitor model, the capacitance is directly proportional to the area of the plates and the dielectric constant of the medium between the plates. Capacitance is inversely proportional to the distance between the plates. In the electrochemical environment, the electrode can be considered one plate, the ions of closest approach are the second plate, and the SAM is the dielectric medium.
Finklea and co-workers were the first to measure the capacitance of alkanethiol films with several terminal functional groups in various electrolytes.\cite{1,5,6,7,16,76,92,94,95}

For bare Au, the capacitance was measured to be greater than 100 $\mu$F/cm$^2$, regardless of electrolyte. However, in the presence of a long-chain SAM, capacitance values dramatically decrease to 1-5 $\mu$F/cm$^2$.\cite{1,5,6,7}

A linear relationship between chain length and reciprocal capacitance has been reported by Porter and co-workers for short (C$_4$SH) to long (C$_{18}$SH) chain SAMs.\cite{1,45}

They found that the capacitance decreases as the distance between the ions of closest approach and the electrode increases (with SAM chain length). Interestingly, the capacitance behavior for shorter chain films was highly dependent on electrolyte. Specifically, Cl$^-$ and ClO$_4^-$ markedly increases capacitance values, suggesting ion penetration into the film.\cite{1,45} Porter hypothesized that the specific adsorption behavior of these ions at Au determine the extent of ion penetration.

Miller et al. have measured the capacitance of hydroxy-terminated alkanethiols at Au electrodes.\cite{1,93,1,114,8} They also observed a linear relationship between chain length and reciprocal capacitance in 0.1 M KCl. However, differences in differential capacitance data for C$_{12}$SH and 14-hydroxytetradecane SAMs on Au measured in 2.0 mM Fe(CN)$_6^{3-}$ were noted and linked to the large difference in the hydrophobicity of the two monolayer surfaces.\cite{1,93} Miller suggests that the surface hydrophobicity of the insulating monolayer induces dramatic changes in the concentration of redox species at the modified-electrode, resulting in different capacitance values as a function of terminal group.
Similar observations were made by Chidsey and Loiacono for alkanethiols with methyl-, carboxylic acid-, and nitrile- terminal functional groups at Au in various electrolytes. Greater capacitances were measured for nitrile and carboxylic acid terminated films. However, they correlate the larger capacitance and ion penetration to the additional disorder associated with non-methyl terminated SAMs through IR spectroscopy data.

Electron transfer studies have shown that solution-confined redox species can be reduced and oxidized at potentials similar to those observed in the absence of the film due to gross defects or pinholes, which expose regions of the electrode directly to the electrolyte solution. The magnitude of the Faradiac current is related to the area, size, and shape of the "exposed" electrode surface.

The behavior of octadecanethiol films on Au in 1.3 mM Ru(NH$_3$)$_6^{2+}$/1 M KCl has been reported by Finklea and co-workers. The magnitude and shape of the current-potential voltammogram indicate that ca. 1% of the electrode is exposed for their C$_{18}$SH SAMs at polycrystalline Au electrodes. The average diameter of the pinholes was calculated to be about 800 nm. A relatively short film deposition time of 1 hr was employed in these studies, suggesting a highly-defective film.

Films having negligible pinhole quantities (highly ordered) display different current-potential behavior. For these films, a large electron transfer overpotential is observed for solution-confined species. For example, Chidsey and Loiacono observe no Faradaic current for the reduction of Ru(NH$_3$)$_6^{3-}$ in 0.1 M NaClO$_4$ at Au electrodes.
modified with CF₃(CF₂)₇(CH₂)₂SH at the peak reduction potential for Ru(NH₃)₆³⁺ at bare Au electrodes. On the other hand, redox behavior for the Ru complex at carboxylic acid-terminated alkanethiols was almost identical to that observed at bare Au electrodes. These electron transfer results were correlated to the increased disorder present with -COOH terminated SAMs (inferred from rotational disorder observed in the ν(C-H) region by IR)

Creager et al. studied the blocking behavior of surfactants upon the redox behavior of 1 mM Ru(NH₃)₆³⁺ in 1 M KCl at octadecanethiol monolayers at Au electrodes. The addition of dodecyltrimethylammonium bromide to solution decreased the reductive current at C₁₈SH SAMs by a factor of four. No reduction inhibition was observed after surfactant addition to solutions for bare Au electrodes. A proposed mechanism suggested that the dodecyl chains of the tetraalkylammonium ions penetrated the films at defect sites, and subsequently, blocked Ru(NH₃)₆³⁺ diffusion into the film.

Electron transfer still occurs through a tunneling mechanism for completely defect-free films. The current is exponentially dependent on the distance between the redox species and the electrode. Any defect in the film, whether due to a film tilt boundary or gauche conformer, allows a redox species to approach closer to the surface without direct contact. The current for species within the film opening is vastly greater than the tunneling current of species held completely at the all-trans extended film edge. In conclusion, the above electrochemical experiments suggest that a large number of these defects exist in self-assembled monolayers at metal surfaces.
Vibrational Spectroscopies

Most of the preliminary spectroscopic work characterizing the packing and orientation of self-assembled monolayers has utilized IR spectroscopy. IR research suggest that SAMs are densely packed in a crystalline arrangement, with the alkane chains in an all-trans conformation. Comparisons of the frequencies in the $\nu$(C-H) region between adsorbed and solid and liquid alkanethiols have been used to deduce conformations of the C-C bonds, and indirectly, overall film order. An inherent weakness of IR spectroscopy has been its inability to provide information from any region other than $\nu$(C-H).

Raman spectroscopy has been proven as a very useful technique for investigating self-assembled alkanethiol monolayers, because vibrational information can be obtained from all locations within the molecule. Bryant et al. directly investigated the ordering, defect structure, and orientation of alkanethiol chains through spectral information obtained in the $\nu$(C-S), $\nu$(C-C), $\nu$(C-H), and $\nu$(S-H) frequency regions. It was concluded that the alkane chains of adsorbed alkanethiols at mechanically polished, smooth Au surfaces are mostly in the all-trans conformation based on C-C and C-S trans conformers within the chain and near the thiol head group, regardless of chain length.

Film Stability

Despite the tremendous interest in applications using alkanethiol SAMs,
there has been relatively little research directed toward understanding the stability of SAMs under various conditions. In certain applications, for example SAM-modified pacemaker electrodes, several factors may affect the stability of the alkanethiol, including mechanical stress due to the implantation procedure, oxidation due to oxygen-rich environments (heart tissue), immunological responses in blood milieu (adsorption/interaction of ions to large macromolecules, such as proteins, with the SAM), enzymatic degradation, as well as applied potential (for heart pacing).

Of the few reports on SAM stability which exist, most involve the study of film structure in various solvents. Sandroff and co-workers were the first to utilize surface enhanced Raman scattering (SERS) to investigate the effect of solvent on the structure of hexadecanethiol adsorbed on Ag island films. Their results indicate that H₂O has little effect on SAM conformational order, while CHCl₃ decreases the order, as evidenced by a decrease in the intensity of trans v(C=C) bands in the spectra. However, their conclusions are somewhat weak, because as with IR data of self-assembled monolayers, only spectral data from the v(C=C) region is presented and discussed; as with IR data, it is difficult to ascertain overall monolayer order from only one vibrational region.

Nuzzo and co-workers found using infrared and photoelectron spectroscopies that SAM films formed from organic disulfides are generally resistant to solvent rinses, with the exception of chlorobenzene where partial monolayer removal was noted for immersion times greater than 24 hours. In this same study, the SAM films formed from the disulfide could be partially (ca. 20%) removed by long exposure (24 hours) to
concentrated solutions of \( \text{CN}^- \) and completely removed by exposure to concentrated solutions of \( \Gamma^- \). However, thermal studies indicate that these films are stable to temperatures in excess of 150°C.\textsuperscript{146}

Porter \textit{et al.} were the first to study the effects of \( \text{D}_2\text{O}, \text{CD}_3\text{OD} \) and \( \text{CCl}_4 \) on the \textit{in situ} structure of dodecanethiol SAMs on Au using FTIR spectroscopy.\textsuperscript{1,156} Based on studies in only the \( \nu(\text{C-H}) \) region (from which indirect information about conformational order of the alkane chain can be obtained), they concluded that the effect of these solvents on the SAM structure is minor.

Anderson and co-workers also used \textit{in situ} FTIR spectroscopy in the \( \nu(\text{C-H}) \) region to study the effects of \( \text{D}_2\text{O}, \text{CD}_3\text{CN} \), and applied potential in 0.1 M NaClO\(_4\) on the structure of octadecanethiol SAMs on Au.\textsuperscript{1,157,1,158} Their results suggest that, under potential control (-0.5 V, 0.0 V, +0.5 V versus SCE) in aqueous 0.1 M NaClO\(_4\), the structure of the SAM does not change, presumably due to the inability of the electrolyte solution to permeate the hydrophobic organic monolayer. They did find that octadecanethiol monolayers disorder slightly in acetonitrile. However, the slightly disordered SAM reorganized to a more pseudo-crystalline environment with the application of potential.

Fritsch \textit{et al.} studied the electrochemical stability of dodecanethiol SAMs at Au in 0.1 M \( \text{N(CH}_2\text{CH}_2\text{CH}_2\text{CH}_2)_4\text{PF}_6 / \text{CH}_3\text{CN} \) and \( \text{CH}_2\text{Cl}_2 \) solutions using cyclic voltammetry.\textsuperscript{1,159,1,160} Several factors were found to contribute to the potential-dependent loss of these self-assembled organothiols from Au surfaces, including potential, solvent,
H₂O content, O₂, convection and Au substrate morphology. The SAM films were found to exhibit their greatest stability in the potential window between -1.0 V and +0.5 V (versus Ag/AgCl). Potential regions outside this window diminish SAM stability as observed by a decrease in SAM surface coverage. Fritsch and co-workers rationalize these observations by the formation of Au oxide at more positive potentials and the reduction of residual oxygen to form OH⁻ species which react with the SAM at potentials negative of -1.0 V.

Even less is known about the stability of SAMs in the ambient air environment. Some evidence for the slight air oxidation of the thiolate group to the sulfonate species in SAM films formed from alkanethiols has been reported. Hemminger and co-workers estimate that ca. 2% of an alkanethiol film is oxidized to the sulfonate species after exposure to the ambient laboratory environment for one week. These studies were based on LD-FTMS. Tarlov saw similar results for alkanethiol SAMs using secondary ion mass spectrometry. Other work has suggested that this oxidation process involves UV-photoionization. Hutt and Leggett report that the rate of photooxidation varies strongly with alkyl chain length and amount of disorder.

Whitesides and co-workers demonstrated that SAM films formed from ω-substituted alkanethiols are stable to both low dielectric constant media (Ar gas and air) and high dielectric constant media (water) for prolonged periods of time at room temperature using XPS, IR, ellipsometry and wetting.

Fritsch et al. recently observed non-UV induced air oxidation for dodecanethiol
SAMs on Au through exchange studies using LD-FTMS. They found that air oxidation for clean SAMs was rapid and complete in the dark and under normal laboratory fluorescent lighting. However, these observations have not yet been verified in other laboratories or with different analytical methods. A major problem with studying film oxidation is the poor reproducibility which exists among research groups. This variability is most likely due to the wide range of substrate preparation and SAM formation techniques employed.

The important conclusion to be drawn from previous work on chemical stability of SAMs is that these films maintain their chemical integrity under all but the most extreme chemical conditions. This feature predicts similar chemical stability under conditions of physiological pH and temperature that apply in pacing applications. Nonetheless, the stability of these films in ambient air, biological milieu, subsequent immune and enzymatic response of the body, as well as applied potentials has yet to be properly investigated.

Animal Model

The canine was chosen as the model for testing the performance of modified-pacemaker electrodes for several important physiological reasons. First, to date, the canine is the standard model for all pacemaker lead development because of expense, size, and ease of implanting commercially available electrodes. Other possibilities are larger animals; however, such alternatives are considerably more expensive and represent wasteful use of a less populous species. In order to use a smaller species (i.e., pig, rabbit,
or rat), significant down-scaling of the pacemaker electrode would be necessary to avoid puncturing the heart wall, which is significantly thinner relative to the heart of a human or canine. In addition, decreasing the electrode area (to accommodate a smaller species) results in markedly different pacing behavior due to a discontinuity in the equivalent pacemaker circuit. Finally, long-term pacemaker performance studies have never been described in a small animal model.

Research Objectives

The goals of this research are to create, study, and validate a class of electrically conductive biomaterials, alkanethiol self-assembled monolayers, for permanent cardiac pacemaking applications. Due to the organochemical nature of the modified surface and the anti-inflammatory action of Au(I)-S compounds, these biomaterials are expected to have enhanced biocompatibility which should result in considerably improved pacemaker performance and longevity. Much research has been undertaken in the study of self-assembled monolayers; however, there has been relatively little research performed toward understanding the reactivity or stability of SAMs used in electrochemical applications, such as those which exist in the use of modified-active fixation pacemaker electrodes.

Specific goals of this research include:

1) Develop a class of biomaterials for permanent active-fixation cardiac pacemaking electrode applications based on surfaces chemically modified
with self-assembled monolayers (SAMs).

2) Develop a small animal model suitable for pacing in order to limit animal usage and costs associated with chronic canine testing (e.g., to pre-test the pacing efficacy of the modified electrodes in a larger number of animals).

3) Quantitatively characterize the interstitial myocardial tissue resistance and perfusion efficiency in heart tissue using simple two-electrode potential sweep and potential step electrochemical measurements in a perfused living heart.

4) Investigate the conformational order and defect structure of alkanethiol films to better understand conductivity and stability issues for modified electrodes.

5) Develop methodology for acquiring Raman spectral data of alkanethiol SAMs on Ag and Au in an "emersed" environment to assess the effects of electrolyte and potential, as described in 7).

6) Investigate SAM stability under various conditions including the effect of solvent, aqueous electrolyte and applied potential on the in situ structure and conformational order of SAMs on Ag and Au surfaces using Raman spectroelectrochemistry.

7) Investigate the air and mechanical stability of SAM-modified electrodes using Raman spectroscopy and electrochemistry to evaluate the shelf-life of modified-pacemaker leads (i.e., whether the film changes during the time
after formation, but before implantation), as well as the effects of implantation on film quality.

In order to better understand the stability of SAMs under pacing conditions, as well as conditions existing for other electrochemical applications, a wide-range of alkanethiol chain lengths, electrolytes, and applied potentials were evaluated by Raman spectroscopy and electrochemistry. Due to the limited number of canines available, the pacing performance of only two SAM chain lengths, butanethiol and dodecanethiol, were investigated.
Chapter 2

Experimental

This chapter provides a detailed description of the instrumentation, materials, chemicals, and experimental methodologies used for acquiring the data presented in this dissertation. Additional experimental details are outlined in each chapter as appropriate.

Instrumentation

Pacemaker Testing

Pacing thresholds were assessed semi-weekly radiotelemetrically using a Medtronic Pulse Generator Programmer Model 9710A with Model 9742 software. Endocardial electrograms were printed on a Medtronic Model 9751A printer.

Raman Spectroscopy

Raman spectral data were acquired on a Spex 270M single monochromator with a 1200 gr/mm blazed grating at 630 nm. This instrument is designed to accommodate multiple laser sources and sample configurations. A block diagram for this system is shown in Figure 2.1.

Laser excitation at 514.5 nm was provided by a Coherent Radiation Innova 90-5 Ar⁺ laser. Excitation at 720 nm was provided by Ar⁺ laser pumping of a Lexel model 479
Figure 2.1  Block diagram for the Single Spectrograph Raman System.
Ti:sapphire laser tuned to 720 nm. Laser powers of 150 mW at the sample were consistently used for both excitation frequencies, and measured with a Newport Model 1815-C Optical Power Meter (Newport Corporation). Plasma lines at 514.5 nm excitation were removed using a 5150 Å (30 Å bandwidth) bandpass filter purchased from Pomfret Research Optics, Inc. Fluorescent lines from the Ti:Sapphire were removed with a 7200 Å (30 Å bandwidth) bandpass filter purchased from Barr Associates, Inc. The laser beam was focused onto the sample using a planoconvex lens (150 mm focal length). Incident light was polarized parallel with respect to the plane of incidence; proper polarization was achieved with a CVI Fresnel Rhomb model FR-2C425 (CVI Laser Corp.). The spot size of the laser beam on the metal surface was ca. 280 μm along the short axis of the ellipse.

Raman-scattered radiation was collected with a Nikon f/1.2 lens and focused onto the entrance slit (25 μm for plasma lines; 50 μm for 514.5 nm excitation; 200 μm for 720 nm excitation) of a Spex 270M single monochromator. A holographic SuperNotch Plus filter (Kaiser Optical Systems, Inc.) was used to remove Rayleigh scattering. Depending on the substrate (Ag or Au), detection was accomplished using either a Princeton Instruments (PI) or Photometrics (PM) charge coupled device (CCD). For experiments in which 514.5 nm excitation was utilized (Ag surfaces), detection was accomplished with a Tektronix TK-512T, 512x512 thinned, back-illuminated, CCD system (Princeton Instruments, Inc.) cooled with liquid nitrogen to -120°C. Images from this detector were processed using CSMA software, provided by Princeton Instruments. The CSMA files were converted to SpectraCalc™ files for the purpose of further data manipulation,
including calibration, background subtraction, and cosmic ray event removal. Experiments
with Au surfaces required detection with a Photometrics PM512, 512x512, frontside-
illuminated CCD system cooled to -110°C. The images acquired with this CCD were
processed with a Photometrics RDS2000 system equipped with a custom version of
SpectraCalc™.

Electrochemistry

Electrochemical measurements (cyclic voltammetry and chronoamperometry) were
made on a Bioanalytical Systems 100 B/W Electrochemical Workstation. For Au plating
of Pt-Ir pacemaker electrodes and spectroelectrochemical experiments, potentials were
controlled with an IBM Instruments EC/225 Voltammetric Analyzer. Charge was

X-ray Photoelectron Spectroscopy

XPS measurements were made on polycrystalline Ag and Au surfaces using a
Vacuum Generators ESCALAB MKII electron spectrometer equipped with a concentric
hemispherical analyzer and a channel electron multiplier detector. The base pressure of
the analyzer was ca. 5 x 10⁻⁹ torr. X-rays from the Al Kα, line at 1486.6 eV at a flux of
200 W were used as the excitation source. A take-off angle of 15° from the surface
normal was employed in order to give the highest possible signal contrast to
photoelectrons emitted from the metal/SAM interface. Electrons were collected in the
constant analyzer energy (CAE) mode with a pass energy of 50 keV. Integration times for high resolution spectra were 0.25 sec co-added 4 times for a total of 1.0 sec at an interval of 0.1 eV. Spectra were corrected for sample charging using the Ag 3d₅/₂ peak at 367.9 eV. Peak areas were calculated using a Shirley method background correction method. Relative peak ratios were then calculated using previously published photoionization cross sections and accounting for the transmission properties of the analyzer. Spectra shown here have been smoothed by a 5-point Savitzky-Golay function.

Atomic Force Microscopy

A Nanoscope® IIIa scanning probe microscope, from Digital Instruments Nanoprobe™ (Santa Barbara, CA) was used for thin film microscopic characterization. Tapping mode AFM images were generated with etched silicon nitride cantilevers (Digital Instruments Nanoprobe™ model number TESP) used as received. Ag and Au rod substrates (38.5 mm²) were mechanically polished, electrochemically polished, and electrochemically roughened (Au only) as described below. AFM images were acquired with a tapping frequency of ca. 300 kHz at a scan rate of 2 Hz. Images shown in this chapter were flattened (elimination of unwanted scan line features) but otherwise unmodified.

Mass Spectroscopy

Residual gas analysis of ambient laboratory air was performed with a Balzers
Prisma Model QMS 200 quadrupole-based mass spectrometer housed in a UHV chamber of base pressure of ca. $1 \times 10^{-4}$ torr. The spectra acquired were processed with Balzer software.

**Electrochemical Cells**

**Standard Cell**

Benchtop cyclic voltammetry was performed in a standard three-electrode electrochemical cell shown in Figure 2.2. A Pt wire wrapped around the Luggin capillary was used as the counter/auxiliary electrode. All potentials were measured and are reported versus a Ag/AgCl reference electrode. Working electrodes (ca. 10 mm in diameter) were made by cutting metal sections from a rod, by punching disks from a foil, or cutting metal wire pieces. Disk electrodes were Ag soldered onto brass stubs which were threaded to fit into a 10 mm brass electrode holder. Once attached to the electrode holder, the electrode and brass holder were wrapped with parafilm, exposing only the face of the metal surface to the electrochemical solution.

**Emersion Spectroelectrochemical Cell**

The spectroelectrochemical emersion cell is shown in Figure 2.3. The cell is made of glass with a Teflon lid. Solution is injected into a glass reservoir on the side of the cell occupied by the counter electrode (Pt wire) and pseudo-reference electrode (Ag wire). A Luggin capillary delivers solution to the working electrode surface. Rotation of the
Figure 2.2. Electrochemical cell used for benchtop cyclic voltammetry. 
Ref = Reference Electrode, WE = Working Electrode, 
CE = Counter Electrode.
Figure 2.3. a) Schematic of the spectroelectrochemical cell used for emersion studies. REF = Reference Electrode (Ag wire), CE = Counter Electrode (Pt wire), WE = Working Electrode (Ag or Au disk), b) front view of working electrode surface.
working electrode was controlled by a DynaOptic DC motor (12 V, 12 mm dia., 19 mm long, 13.552:1 gearhead ratio. Micro Dynamics, Laguna Hills, CA) at ca. 0.10 mm/sec (0.35-0.50 rpm). The cell was purged with N₂ gas in order to create an inert environment and retain constant solution vapor pressure.

**Materials**

All material was used as received unless noted otherwise. C₅SH (99%), C₆SH (99%), C₇SH (95%), C₈SH (97%), C₁₀SH (97%), C₁₂SH (98%), C₁₃SH (98%), and thiophenol (99%) were purchased from Aldrich.

Bulk polycrystalline Ag rod and foil (99.999%), Au foil (99.999%), and Pt foil (99.99%) were purchased from Johnson Matthey.

Ethanol (absolute) was purchased from Quantum Chemical Corp. Hexane (99.9%) was purchased from Fischer. NaF (99%) and NaSCN (98%) were purchased from Fluka. KCl (99.7%) was purchased from Mallinckrodt. NaCl (99.9%) was purchased from J.T. Baker Inc. NaOH (99.99%), H₂SO₄ (99.999%), and pyrene (99%) were purchased from Aldrich. H₂O₂ (30%) was purchased from VWR.

Hexaammineruthenium (III) chloride ([Ru(NH₃)₆]Cl₃, Ru 32%) was purchased from Johnson Matthey. Hydrogen tetrachloroaurate hydrate (HAuCl₄, 99.999%) and indene (99+%) were purchased from Aldrich.

N₂ (99.995%), O₂ (99.7%), and compressed air (medical grade=19-23% O₂ [99.7% purity], balance N₂ [99.995% purity] were obtained from U.S. Airweld (Tucson,
Ozone was prepared using a Boekel Model 135500 dry process cleaner (Boekel, Industries, Inc.) inside a glove bag purged with compressed air. A low pressure Mercury grid lamp enclosed in a reaction chamber generates an environment rich in ozone which is flushed into the glove bag by a stream of N₂.

Water was purified with a reverse osmosis (Milli-RO 10 Plus) system and then further purified with a Milli-Q UV Plus system (Millipore Corp.). Final resistivity of the ultrapure water was 18.2 MΩ/cm, and the total organic content was specified at less than 5 ppb.

Model 4057 active-fixation permanent pacemaker electrodes and Model 7071 DDDR pulse generators were provided by Medtronic, Inc. (Minneapolis, MN).

Procedures

Specific procedures that are related to a single type of experiment are described in the appropriate chapter. The following list consists of more commonly used experimental strategies. The surface preparation procedures are listed in chronological order, followed by Raman spectral data acquisition and pacing performance testing.

Surface Cleaning

Removal of previously adsorbed thiols from metal surfaces proved to be a critical problem for subsequent experiments. Initial investigations indicated that mechanical polishing does not completely remove previously adsorbed thiols, and thus, these surfaces
contain thiol contamination.

Different approaches were utilized to remove previously adsorbed thiols depending on metal substrate. Ag surfaces were cleaned of thiols by sanding with 1500 grit silicon carbide sandpaper before mechanical polishing. Thiol contamination on Au surfaces was removed by Piranha solution (1:1 H₂O₂ (30%)/H₂SO₄ (18 M)) immersion for 20 sec. (Caution! Piranha solution is a very strong oxidant and can spontaneously detonate upon contact with organic material!). Pt surfaces were cleaned by a reductive desorption process in which very negative potentials (-2.0 V versus Ag/AgCl) are applied for ca. 2 hr in 5.0 M NaOH.

Mechanical Polishing

Metal surfaces were mechanically polished to a mirror finish on a padded lapping wheel (Ecomet I, Buehler Ltd.). PSA-backed Microcloth polishing pads and 1.0, 0.3, and 0.05 μm agglomerated aluminas (powder form) were also purchased from Buehler. A mechanical electrode rotator designed by the Department of Chemistry Machine shop was used to create/maintain planar surfaces. First, the clean surface (see above) was attached to an aluminum stub which can hold up to 10 brass weights (17 g each) applying pressure and forcing the metal surface into the polishing pad while rotating at a constant rate. The initial polishing step involves polishing to a mirror finish with 1.0 μm alumina and 5 brass weights. This step can take up to several hours depending on the initial roughness of the substrate. Next, the metal surface is polished for 5 min with 0.3 μm alumina/3 brass
weights, and then 10 sec with 0.05 μm alumina/1 brass weight. The surface is then removed from the aluminum stub, rinsed with H₂O, and sonicated in neat EtOH for 10 sec to remove any trapped alumina (Branson 1210 Ultrasonic Cleaner). The entire procedure is repeated with 1 brass weight for ca. 15 min with 1.0 μm alumina, 30 sec with 0.3 μm alumina, and 10 sec with 0.05 μm alumina. Once again, the surface is sonicated for 10 sec in neat EtOH, rinsed with EtOH, and allowed to air dry.

AFM images for Ag surfaces after mechanical polishing following the above procedure are shown in Figure 2.4. The effect of repeating the mechanical polishing procedure is clearly seen by comparing the image roughness features. The depth of the scratches and pits for a Ag surface prepared by polishing without repeated cycling through 1.0, 0.3, and 0.05 μm Al₂O₃ slurries range from 6 to 14 nm (Figure 2.4a). In contrast, additional polishing by repeating the process produces a slightly smoother surface for which the depth of scratches and pits range from 8 to 10 nm (Figure 2.4b). Ag surfaces used for SAM studies presented in this dissertation were all prepared by repeated cycling in the variously sized Al₂O₃ slurries. Surface roughness factor (Rₚ) values for this protocol are ca. 1.6 (determined by Pb UPD as described in references 2.3 and 2.4).

Electrochemical Polishing

Pt surfaces were electrochemically polished in 1.0 M H₂SO₄ at +1.4 V for 10 min, -0.2 V for 30 sec, and then cycled 20 times from -0.2 V to +1.2 V at 200 mV/s. Au surfaces were electrochemically polished by 10 cycles in 1.0 M H₂SO₄ from -0.2 V to +1.2
Figure 2.4  AFM images of bare Ag surface after a) one cycle and b) two cycles of mechanical polishing and sonication in neat EtOH for 10 seconds.
V at 100 mV/s. Electrode cleanliness (free of thiol contamination) was established by the presence of hydrogen adsorption-desorption and oxide formation-reduction as shown in Figure 2.5. Electrodes were rinsed with 100% ethanol, allowed to air dry, and then transferred to thiol solutions for SAM formation.

Surface Roughening

Rough surfaces were necessary for alkanethiol studies on Au because of the inherently weak surface Raman enhancement provided by this substrate. Au surfaces were first mechanically polished and then roughened \textit{ex situ} (in a solution other than thiol) prior to alkanethiol solution immersion. Linear potential sweep ORCs were applied to Au electrodes in a 0.1 M KCl solution. The initial potential was -0.2 V and the potential sweep was reversed at ca. +1.20 V at a scan rate of 100 mV/s, resulting in ca. 10 mC/cm$^2$ of anodic charge passed per sweep. The electrodes were then removed from solution at open circuit potential, rinsed with TDI water and 100% ethanol prior to alkanethiol solution exposure.

AFM images of Au surfaces after mechanically polishing ($R_f = 1.5$, determined by Au oxide stripping in H$_2$SO$_4$ using 400 $\mu$C/cm$^2$),\textsuperscript{2.3,2.4} as well as surfaces after three ($R_f = 2.0$) and six ($R_f = 2.5$) roughening cycles are shown in Figures 2.6-2.8, respectively. As indicated in these images, three sweeps of electrochemical roughening results surfaces with greater roughness features than mechanically polished surfaces, although noticeably less than surfaces subjected to six sweeps. The depths of scratches and pits range from
Figure 2.5. Cyclic voltammogram for bare, mechanically polished a) Pt and b) Au electrodes. Sweep rate = 100 mV/s in 1.0 M H$_2$SO$_4$. 
Figure 2.6  AFM image of bare Au surface after mechanical polishing and sonication in neat EtOH for 10 seconds.
Figure 2.7  AFM image of bare Au surface after mechanical polishing / sonication with 3 cycles of electrochemical roughening in 0.1 M KCl at 100 mV/s.
Figure 2.8  AFM image of bare Au surface after mechanical polishing / sonnication with 6 cycles of electrochemical roughening in 0.1 M KCl at 100 mV/s.
10-26 nm for mechanically polished Au, 16-19 nm for three ORCs, and 24-29 nm for six ORCs. The data indicate that upon electrochemical roughening, the surface becomes more homogenous, as evidenced by the smaller range in depth profile. In addition, the nodules become noticeably larger.

In order to acquire surface Raman spectra of acceptable S/N for alkanethiols at relatively smooth Au, Au surfaces were first electrochemically roughened with three cycles before SAM formation to minimize surface roughness.

Thiol Film Formation

Alkanethiols were formed on clean, mechanically polished, smooth or roughened, polycrystalline Ag, Au, and Pt wires and disk surfaces. Disk surfaces were placed in a holder, wrapped with a layer of parafilm and teflon tape (so that only the metal surface of interest was exposed to alkanethiol solution), and then immersed in ca. 10 mM alkanethiol solutions made with 100% ethanol. Immersion times ranged from 3 to 24 h, depending on chain length. Short (<10 carbons) and long (≥10 carbons) alkanethiol molecules required 3-5 and 24 h, respectively, for self-assembly on Ag. Surfaces were handled in the ambient air environment prior to analysis (except for those used in Chapter 8) for less than 5 min, to minimize the possibility of contamination and oxidation.

Raman Spectral Acquisition

Spectra of neat liquid and solid samples were acquired in either sealed capillary or
NMR tubes. Solid alkanethiol spectra were acquired by cooling the sample in a capillary tube with liquid nitrogen and obtaining spectra with a series of 10 sec integrations to prevent laser warming. Acquisition times were commonly 1-5 min for liquid and solid spectra.

Spectra obtained from alkanethiol films at metal surfaces were obtained in air or within the spectroelectrochemical cell. An angle of incidence of 60° with respect to surface normal and parallel polarized light were used. Commonly, laser powers of 100-200 mW were used for 514.5 and 720 nm excitation. Integration times were 60 sec, co-added 5 times for a total of 300 sec to achieve reasonable signal-to-noise levels for 514.5 nm excitation (coupled with the PI CCD). Red excitation (720 nm) for Au experiments required integration times of either 5 or 10 sec, co-added 180 or 90 times for a total of 900 sec in order to achieve reasonable signal-to-noise levels.

Raw spectral data were calibrated using plasma lines (514.5 excitation) or neat indene bands (720 nm excitation). Sloping background due to instrumental response was removed by fitting the sloping baseline to fourth or fifth order polynomial equations supplied in the background subtraction program of SpectraCalc™.

Pacemaker Testing

Testing of control and organothiol-modified pacemaker electrodes was performed at the time of implant, semi-weekly (by radiotelemetry) during the period of chronic experimentation, and again at the time of explant with the assistance of Dr. Marc Ovadia.
and Evelyn (Gina) South Corso. Stimulation threshold was monitored for each active-fixation electrode with the following procedure. Cutaneous electrodes for electrocardiographic monitoring were connected to the awake canine. The atrial output was programmed to 2.5 V. The pulse width was varied to determine capture threshold (minimum time period at 2.5 V to cause heart beat), at which point electrocardiograms were recorded. The output was then set to 5 V. The pulse width was similarly reduced to determine capture threshold. The atrial settings were then programmed to be twice threshold until the next analysis date. The identical procedure was followed for ventricular monitoring.
Chapter 3

Fabrication and Pre-Clinical Testing of Modified, Active-Fixation Pacemaker Electrodes

Introduction

In an effort to improve the biocompatibility of cardiac pacemakers, a strategy involving chemical modification of the pacemaker electrode with self-assembled monolayers was investigated. As discussed in Chapter 1, electrodes modified with self-assembled monolayers possess several attributes which can be exploited so that (1) even though the bare metal surface is effectively blocked from intimate contact with biological milieu, electrical conductivity is adequately preserved through molecule-sized defects in the monolayer, $^{3,1-3,3}$ (2) the surface created chemically resembles normal biological constituents with extremely limited exposed metal surface area, and (3) the surface contains the known anti-inflammatory species Au(I).$^{3,1-3,8}$ The synthetic strategy employed for this modification is based on the well-characterized covalent attachment of thiol-containing compounds to Au surfaces through Au-thiolate bonding.$^{3,1,3,9-3,12}$ Many anti-inflammatory drugs are based on Au(I)-thiolate bonding, including the well-known anti-rheumatics.$^{3,4-3,8}$ Such drugs possess anti-inflammatory actions despite widely varying R groups on the thiolate ligand.

The pre-clinical data described in this chapter are for pacemaker electrodes modified with Au-alkanethiols terminated with methyl functional groups, as shown in
Figure 3.1. However, an advantage of alkanethiol-SAMs is the ability to molecular engineer the terminal group to represent an array of organochemical properties (i.e., hydrophobic versus hydrophilic). Alkanethiol SAMs with a variety of terminal groups ranging from chemically simple to elaborate are currently available or easily synthesized.\textsuperscript{3,11-3,14}

**Methodology**

Standard methods of self-assembly were used to chemically modify active-fixation pacemaker electrodes.\textsuperscript{3,11,3,9-3,11} Pt(Ir) pacemaker electrodes (Medtronic Models 4016, 4057, 4058, 4557, or 6957, Medtronic Inc., Minneapolis, MN) were initially cleaned electrochemically in 5.0 M NaOH, and then in 1.0 M H\textsubscript{2}SO\textsubscript{4}, as described in Chapter 2. Following cleaning, Au films were electrochemically deposited onto these electrodes in a solution of 5 mM HAuCl\textsubscript{4} / 0.1 M KCl under potentiostatic control. Prior to introduction to the HAuCl\textsubscript{4}/KCl solution, a small amount of the solution was introduced into the internal cavity of the helical pacemaker electrode using a small syringe. Au films were slowly deposited at +0.3 V while stirring the solution with the intent to obtain a smooth film. Current was monitored and deposition was allowed to proceed until 100 mC of charge had passed\textsuperscript{3,15} (i.e., ca. 788 ML assuming 728 µC/cm\textsuperscript{2} of Au deposited on a Pt(Ir) area of 0.172 cm\textsuperscript{2}). After formation, the Au films were characterized and further conditioned electrochemically by 10 potentiostatic cycles at 200 mV/s in 1.0 M H\textsubscript{2}SO\textsubscript{4} between -0.2 and +1.2 V. The electrode was then removed from solution, rinsed with
Figure 3.1. Schematic of a C_{12}SH SAM on a Au coated Pt(Ir) pacemaker electrode surface.
Milli-Q-UV H₂O, then neat ethanol, and immediately introduced into the alkanethiol solution for self-assembled monolayer formation.

Self-assembled monolayer films were prepared on both Pt(Ir) and Au-coated Pt(Ir) alloy pacemaker electrodes by immersion into an ethanol solution of ca. 10 mM alkanethiol for 30 min. Following film formation, the electrodes were removed from solution and rinsed with neat ethanol, then Millipore Milli-Q-UV H₂O, and finally inserted into a sterilization bag filled with nitrogen. Prior to implantation, pacemaker electrodes were sterilized for 5 h in ethylene oxide at room temperature.³¹⁶

After sterilization, electrodes were implanted by Marc Ovadia, MD, in canine hearts (greyhound and mongrel) as part of permanent dual-chamber cardiac pacing systems using surgical procedures similar to those employed for clinical implantation in man.

In pre-clinical (animal) experimentation, seven “control” metal permanent pacing electrodes, including five Pt(Ir) and two Au-coated Pt(Ir) leads were evaluated. Three “experimental” groups of alkanethiol-modified electrodes were studied including five C₁₂SH on Au-Pt(Ir) electrodes (substrate electrode Pt(Ir) as above with electrodeposited Au surface to which a monolayer film of C₁₂SH was covalently bonded), five C₄SH on Au-Pt(Ir) electrodes and lastly, six C₁₂SH on Pt(Ir) electrodes (Medtronic commercial electrodes upon which C₁₂SH monolayers were fabricated, differing from the previously described experimental electrodes in that the layer of Au was absent but the C₁₂SH was covalently bonded.) Four additional electrodes were implanted (two each of C₁₂SH on Au-
Pt(Ir) and C₄SH on Au-Pt(Ir) electrodes) from which no data were derived; one animal expired in the operating room under general anesthesia after successful implantation of the pacemaker because of anesthetic overdose, and one animal partially exteriorized the electrodes after tearing open its wound with its teeth. Testing at the time of implant (in the operating room) by Dr. Ovadia and staff indicated that the chemically-modified electrodes possessed electrical properties (i.e., electrode impedance and pacing performance) identical to control electrodes. In addition, these values were identical to human data with the same active-fixation (helix) electrodes.

Surveillance

Pacing performance as determined by stimulation threshold was monitored bi-weekly at 2.5 and 5 V for each electrode for 104 ± 13 months. Radiotelemetric surveillance in awake dogs, trained to cooperate with telemetry, was used to measure threshold. Due to excessive physical activity by two dogs, two electrode dislodgements occurred as determined by threshold rise and other surveillance data (including ultrasonographic imaging). Data from both a Au-dodecanethiol and Au-butanethiol electrode are censored for the corresponding dislodgment time periods for each dog.

Pacing Behavior: Control Electrodes

The typical threshold rise observed in most human patients after pacemaker implantation is also observed for each of our control electrodes. A threshold versus time
curve for a Pt(Ir) electrode, where an early phase threshold peak is clearly observed, is shown in Figure 3.2. In certain cases, a significantly larger threshold rise occurs, reflecting progressive inflammation and scar formation at the electrode-tissue interface. These immunological processes may lead to failure of the pacemaker because of limited maximal deliverable pulse duration and voltage of the power source. An example in which this failure occurs is shown in Figure 3.3. The threshold level is not measurable and exceeds the maximal settings of 5 V, 1.5 msec for this pacemaker system. The risk of pacemaker failure in the early stages after implantation is proportional to the magnitude of the threshold peak. At threshold levels above ca. 1/3 of the maximal pulse duration, ca. 0.4-0.45 msec, it is customary to intensify surveillance in humans. In addition, the pacemaker system must be programmed to operate at output voltages three times threshold in order to significantly decrease failure risk.

A less common observation made in at least one of the control electrodes is the appearance of a secondary threshold rise that occurred after the first rise appeared to resolve. Examples of this behavior are shown in Figures 3.3 and 3.4. Such behavior indicates that inflammation is not concluding, but still progressing. In most circumstances, physicians reoperate and replace the pacemaker electrode in order to avoid potentially dangerous outcomes from pacemaker failure (death).

As described in Chapter 1, after inflammatory responses have run their course, threshold declines as observed in Figure 3.2. The early phase of inflammation ends after threshold reaches a chronic plateau. Of note is the observation that threshold in the
Figure 3.2. Stimulation threshold measured at 2.5 V pulse amplitude versus time after implant for a Pt(Ir) control electrode (92D38A) in a greyhound atrium exhibiting early phase threshold rise. Implant duration 78 days.
Figure 3.3. Stimulation threshold measured at 5 V pulse amplitude versus time after implant for a Au-coated Pt(Ir) control electrode (91D146V) in a greyhound ventricle exhibiting failure (threshold $> 1.5$ msec). Implant duration 70 days. Inset: stimulation threshold for same electrode measured at 2.5 V.
Figure 3.4. Stimulation threshold measured at 2.5 V pulse amplitude versus time after implant for a Pt(Ir) control electrode (92D39A) in a greyhound atrium exhibiting secondary rise. Implant duration 70 days.
chronic phase is typically higher than that at time of implant. It is hypothesized that this deviation is the result of non-excitable scar tissue surrounding the electrode. In certain instances (ca. 14-20% of patients in human studies), threshold elevations in the chronic phase are observed. Such increases are related to micro-dislodgements and/or renewed inflammation.

Experimental Electrodes: C12SH on Au-Pt(Ir)

A typical stimulation threshold versus time after implant for C12SH SAMs on Au-coated Pt(Ir) electrodes is shown in Figure 3.5. Several striking observations are made when comparing the pacing behavior of the C12SH on Au-Pt(Ir) electrodes to that of the control group. Of greatest significance is the fact that an early phase threshold increase to ≥0.4 msec (considered significant) at 2.5 V amplitude output was observed in only 1 of the 4 experimental electrodes but in 6 of 7 controls. Severe threshold elevation to ≥1 msec was not observed in any experimental electrodes but was noted in 4 of the 7 noble metal control electrodes. The average stimulation threshold at peak maximum was 0.90 ± 0.18 msec for the control group but only 0.44 ± 0.13 msec for the C12SH Au-Pt(Ir) group. Additionally, subsequent threshold increases after the initial rise were not observed in any of the experimental electrodes, but these did occur in 3 of the 7 controls.

A typical measure of early phase inflammation duration is the latest day of threshold rise. This day was 25 ± 5.8 days for the controls but only 10.5 ± 2.4 days for the experimental group. The end of the acute phase, also known as the beginning of the
Figure 3.5. Stimulation threshold measured at 5.0 V pulse amplitude versus time after implant for a C1SH on Au-Pt(Ir) electrode (93D147A) in a greyhound atrium exhibiting very low threshold in early and chronic phases. Implant duration 108 days. Inset: stimulation threshold behavior for same electrode measured at 2.5 V.
chronic phase, was measured to be 67 ± 5.6 days for Pt(Ir) and Au-Pt(Ir) controls but 37 ± 2.9 days in the experimental group. In other words, after only 37 days, the C_{12}SH on Au-Pt(Ir) group reaches stable threshold values not significantly higher than those measured at time of implant. In contrast, thresholds of the control group remain higher than at implant (as shown in Figure 3.3), indicating significant scar tissue formation.

The late chronic phase is considered to be >5 weeks after implantation. Stimulation threshold levels during this time are a function of the amount of fibrous tissue residua and any ongoing inflammation. In the chronic phase, the minimum threshold at 5 V was 0.31 ± 0.19 for the control group but 0.08 ± 0.01 for the C_{12}SH on Au-Pt(Ir) experimental group. Noteworthy, the variance of the control electrodes is much greater than the experimental electrodes further indicating the suppression of normal inflammation response. The average threshold-time curves for both the control and experimental groups at 5 V and 2.5 V are shown in Figures 3.6 and 3.7. The C_{12}SH on Au-Pt(Ir) electrodes, already noteworthy for their lack of early phase threshold rise, display exceedingly flat and reproducible behavior in the chronic phase. Such behavior has never been observed before in pre-clinical or clinical lead surveillance.

The pacing behavior of these SAM-modified, active-fixation (helical) electrodes is remarkable in light of the fact that active-fixation electrode implantation traumatically disrupts heart tissue. This trauma induces protein adsorption, edema, and interstitial hemorrhage, which in turn trigger in amplificatory fashion the immunological cascade and local inflammation. Subsequently, the magnitude of the local inflammation determines the
Figure 3.6. Mean stimulation threshold at 5.0 V output amplitude versus time after implant for (♦) PtIr control electrode group and (■) C13SH on Au experimental group.
Figure 3.7. Mean stimulation threshold at 2.5 V output amplitude versus time after implant for (•) Pt(Ir) control electrode group and (■) C_{12}SH on Au experimental group.
duration of edema, cellular infiltration, and, ultimately, the amount of fibrous tissue formation. The data presented above imply that with C\textsubscript{12}SH on Au-Pt(Ir) experimental electrodes, scar tissue formation is dissociated from this tissue disruption during implantation. Although tissue disruption still occurs and is unavoidable (i.e., the electrode penetration into heart muscle is intrinsic to fixation, making this electrode class most resistant to mechanical dislodgement), inflammation and scar tissue are significantly reduced. The flat threshold behavior observed for the SAM-coated leads is *sui generis*, unique to only the experimental electrodes and not observed for any existing active-fixation technology.

This superior behavior suggests that the C\textsubscript{12}SH on Au may endow anti-inflammatory characteristics to the electrode-tissue interface, perhaps masking the electrode surface from the immune activation mechanisms and/or a more specific anti-inflammatory effect as a result of the Au(I)-thiolate moiety. The evidence for such anti-inflammatory action include the shortened duration of the early phase, the absence of secondary rises, and the extraordinarily low chronic threshold levels.

**Experimental Electrodes: C\textsubscript{12}SH on Pt(Ir) and C\textsubscript{4}SH on Au-Pt(Ir)**

In order to better understand the mechanism of improved pacing, the pacing behaviors of C\textsubscript{12}SH on Pt(Ir) and C\textsubscript{4}SH on Au-Pt(Ir) modified pacemaker electrodes were investigated. Typical stimulation threshold versus time after implant curves for these experimental electrodes are shown in Figures 3.8 and 3.9. C\textsubscript{4}SH on Au-Pt(Ir) was chosen...
Figure 3.8. Stimulation threshold measured at 2.5 V pulse amplitude versus time after implant for a C$_2$SH on Pt(Ir) electrode (94D32A25) in a greyhound atrium exhibiting early phase threshold rise. Implant duration 43 days.
Figure 3.9. Stimulation threshold measured at 2.5 V pulse amplitude versus time after implant for a C,SH on Au-Pt(Ir) electrode (94D29A25) in a greyhound atrium exhibiting early phase threshold rise. Implant duration 46 days.
to represent a short chain monolayer that less effectively masks the metal character of the
electrode relative to \( C_{12}SH \) on Au-Pt(Ir). \( C_{12}SH \) on Pt(Ir) contains the long chain
monolayer; however, it lacks the putatively anti-inflammatory Au(I)-thiolate.

As seen in Figures 3.8 and 3.9, both \( C_{12}SH \) on Pt(Ir) and \( C_4SH \) on Au-Pt(Ir)
modified pacemaker electrodes appear inferior to \( C_{12}SH \) on Au-Pt(Ir). In the acute phase,
significant threshold elevation (>0.4 msec) was apparent in 4 of 5 \( C_4SH \) Au-Pt(Ir)
electrodes and in 4 of 5 \( C_{12}SH \) Pt(Ir) electrodes. The peak magnitude of stimulation
threshold was similar to that observed for the control electrodes, and severe rises (>1.0
msec) were observed in 4 of 5 \( C_4SH \) on Au-Pt(Ir) and in 2 of 5 \( C_{12}SH \) on Pt(Ir)
electrodes. The latest day of threshold rise for both of these groups was later than that for
either the experimental or the control group, indicating inferiority to even the control
electrodes. Secondary rises were observed in 3 of 5 \( C_4SH \) on Au-Pt(Ir) electrodes and in
1 of 5 \( C_{12}SH \) on Pt(Ir) electrodes. After threshold finally subsided, both groups behaved
somewhat similar to \( C_{12}SH \) on Au-Pt(Ir); stimulation threshold reached levels lower than
control and remained quite flat.

**Mechanism**

The mechanism for improved pacing efficiency with \( C_{12}SH \) on Au-Pt(Ir) active-
fixation pacemaker electrodes versus control is not completely understood. Given the
complexity of the electrode-tissue interface, multiple effects from the alkanethiol-SAM
may be operative. It is hypothesized that the significantly diminished degree of acute and
chronic threshold could be the result of two classes of effects. First, the presence of a self-assembled monolayer significantly alters the interfacial chemistry of the pacemaker electrode-tissue interface (i.e., shielding or masking the metal and providing a lipophilic organochemical surface). Interfacial changes could be the result of one or more chemical events: a) changes in protein adsorption chemistry in the initial stages of the body's response to the implant that affect subsequent processes (i.e., platelet adhesion) and lead to fibrous tissue formation; b) partial or complete removal of the monolayer from the electrode surface after implantation resulting in therapeutic anti-inflammatory compounds (similar in structure to those used clinically\(^4\) which all include a Au(I)-thiolate bond despite wide diversity of ligand moiety) at high local concentration at the electrode-tissue interface; c) corrosion inhibition of the electrode by the self-assembled monolayer;\(^3\) or d) lubrication by the self-assembled monolayer that facilitates implantation such that the screw-in fixation is considerably less traumatic, generating less local edema and subsequent fibrous tissue formation.

Secondly, the presence of the alkanethiol-SAM significantly alters the electrochemical characteristics of the pacemaker electrode due to organic modification of the surface. Due to the large number of defect sites associated with these SAMs (purposely created by short immersion times to ensure conductivity), the electrode might behave as an array of microelectrodes. Larger electric field strengths and current densities could then be achieved. Such electric field and/or current density enhancements could result in an improved myocardial depolarization efficiency, leading to heartbeat at lower
output voltages. This would have the effect of reducing the size of the total electrode. A host of alternative covalent modification strategies could be employed (i.e., Pt-olefin, Pt-O-Si-R, or Pt-isonitriles and Au-isonitriles) if the efficiency of the masking can be made the limiting physicochemical characteristic which confers anti-inflammatory efficacy on the pacemaker electrodes. The potential for a reduction in the electrode size may lead to applications of these electrodes in all areas of bioelectrodes with practical applications in clinical cardiology and investigative and therapeutic neuroscience.

Impact on Pacemaker Technology

The most important impact of this study is that alkanethiol-SAM modification of cardiac pacemaker electrodes produces surfaces whose physicochemical properties are organic, while retaining electrical conductivity, and creating a stable electrode-tissue interface over long periods of time (notwithstanding the immunogenicity of the traumatic tissue disruption at implantation.) The use of n-alkanethiol self-assembled monolayers covalently bound to a Au coated Pt(Ir) surface attenuates acute and chronic threshold elevation which indirectly indicate avoidance of inflammation (acute) and scar (chronic).

The presence of either acute inflammation or chronic scar tissue in control electrodes results in a decrease in the electrical stimulation efficiency. Fibrous tissue, although conductive, physically removes the excitable myocardial tissue from the pacemaking electrode. Thus, the presence of this fibrous material (scar) has the direct effect of increasing the effective diameter of the pacemaking electrode (the so-called
"virtual" electrode effect\cite{18,23}, and decreasing the efficiency of the pacing process. Subsequently, additional power output is necessary to generate sufficient electric field (E) amplitude or current density field (J) to stimulate excitable myocardial tissue (removed from the electrode surface). The result of such pathological changes at the interface is dramatic pacing threshold elevation as a function of time after implant. Thresholds above 1.0 msec may result in total failure of cardiac pacing, and if not quickly detected, can result in death. If detected in a timely fashion, replacement operations are scheduled, sometimes as an emergency with life-threatening risks. In mild threshold elevation, increased power output leads to pacemaker power source depletion. In light of important and rapid recent improvements in lithium battery technology\cite{30}, which should minimize battery failure as a significant limitation of cardiac pacemaking in chronic implants, efforts to substantially increase the stability and longevity of the pacemaker electrode-tissue interface are essential. The introduction of a superior class of active-fixation electrodes, such as that represented by C_{12}SH on Au-Pt(Ir), may conversely necessitate improved power source technologies, since the reduction of thresholds (in the late chronic phase) to extraordinarily low levels (ca. 0.08 msec) would increase the potential longevity of the pacemaker system, and hence, of the battery. No battery currently available is this robust.

In addition, the results detailed above imply that for experimental C_{12}SH on Au-Pt(Ir) electrodes (but not control electrodes), an acceptable threshold shortly after implant predicts permanent (chronic) viability of the pacing system, and intensive surveillance only to the time that an initial threshold resolves may be sufficient for non-growing patients.
early in battery life. This point is of tremendous consequence suggesting assured survival of pacemaker patients with reduced surveillance.

Conclusions

The pacing performance of active-fixation pacemaker electrodes modified with C_{12}SH on Au are superior to commercially available electrodes. Furthermore, the data presented in this chapter suggest that both the Au(I)-S moiety and a film which adequately blocks biological milieu (long-chain monolayer) are necessary for enhancing the biocompatibility and pacing performance of modified electrodes. This research has important ramifications for other chronic bioelectrodes for which similar interfacial degradation processes are known to be limiting. Although the exact mechanism is not completely understood, the interfacial chemistry and electrochemical characteristics of the electrode are altered in a way that reduces the effects of implant inflammation and subsequent scar tissue formation.

Efforts to better understand the mechanism responsible for the improved pacing behavior realized with these covalently-modified noble metal bioelectrodes are narrowed to SAM stability in models of the pacing environment (i.e., electrolytes, applied potential, mechanical manipulation during implantation) and are discussed in the following chapters. The control of interfacial structure at the molecular level of electrically conductive materials with organic functionality opens new horizons for molecular engineering of biomaterial implants in living systems, including sensors and effectors of artificial organ
control systems.
Chapter 4

*In Situ* Electrochemistry of Ru(NH$_3$)$_6^{3+}$ in a Perfused Rat Heart

**Introduction**

Several decades of work have been devoted to quantitative definition of the fundamental electrical characteristics of cardiac tissue in an attempt to completely define the electrophysiology of the heart. This task is challenging due to the syncytial and anisotropic properties of cardiac tissue. The intracellular space of cardiac tissue behaves as if interconnected (syncytial) but with higher conductivity along the fiber axis than transverse to it. Similarly, the interstitial space also behaves as if interconnected and anisotropic with higher conductivity along the fiber axis.

Although many of the previous reports are theoretical modeling studies, experimental investigations have been undertaken on a variety of *in vitro* and *in vivo* systems. *In vitro* studies on chick myocardial cells and rabbit myocytes have been reported. Cardiac muscles excised from frog, rabbit, guinea pig, cat, sheep, and calf hearts have also been used. Whole rabbit hearts and guinea pig hearts in a Langendorff preparation are used frequently for such investigations. In this preparation, the excised heart is suspended in a bathing solution while the cardiac tissue is perfused from an external source through the aorta. Finally, *in vivo* studies in dog hearts have also been reported.

Most of this previous work has involved electrical stimulation of cardiac muscle...
and defibrillation by application of currents between two microelectrodes typically tens of microns in diameter. In many cases, potential changes between the stimulating electrode and a second electrode placed different distances from the stimulating electrode were monitored. In the case of sufficient excitation current, the potentials mapped in this case are the action potentials of the stimulated cardiac muscle. When viewed from the standpoint of electroanalytical methodology, these two-electrode experiments can be thought of as chronopotentiometric measurements, especially in the case of sub-threshold excitation. From such measures, quantitative values for intracellular resistivity on the order of 280 $\Omega$ cm$^{-1.18}$ and interstitial resistivity of 48 $\Omega$ cm$^{1.19}$ have been reported. Taking into account the typical diameter of a cardiac muscle fiber of ca. 15 $\mu$m$^{1.27}$ and packing density of ca. 3/7,$^{1.14}$ these values of resistivity translate to resistance per unit length values of ca. 10$^8$ $\Omega$/cm in both cases.$^{4,2}$

No previous investigation in cardiac tissue has utilized an electrochemical measurement based on Faradaic processes, nor has classical voltammetry been the focus of any effort in these cardiac environments. This fact is particularly noteworthy for studies in which perfused tissue has been used. Inherent in such studies is the unproven assumption that the perfusate adequately bathes the interior interstices of the cardiac tissue. Thus, we undertook this work with the belief that classical Faradaic-based electrochemical measurements in cardiac tissue might provide unique information about such systems.

Despite the dearth of electroanalytical measurement techniques in the cardiac electrophysiology literature, these tools have become commonplace in the study of other
types of living tissue and tissue cultures to better understand events of biological
importance. These studies have as their genesis the founding work of Adams and co-
workers in neurochemistry which led to new areas of study involving the application of
electroanalytical measurement methodology to living systems. Such neurochemical studies
have continued by probing small molecule dynamics in the brain, and using
amperometric and voltammetric measurements to characterize the micro-environment in
the vicinity of and inside of single cells. Previous application of common
electroanalytical techniques to the investigation of cardiac tissue is limited to recent
reports of in vivo potentiometric ion and gas-sensing microelectrodes. Such
measurements are extremely difficult in light of the physical activity of the muscle into
which the sensor is placed. This difficulty has been responsible in part for the fact that
voltammetric and chronoamperometric techniques have yet to be routinely applied to
cardiac tissue. Such measurements are desirable, however, in that they may provide unique
insight into tissue characteristics and basic quantitative electrochemical parameters
relevant to biostimulation electrodes.

Cardiac pacing is one area in which biostimulation is particularly significant (see
Chapter 1). Unipolar pacing devices are composed of two electrical components: a pulse
generator (power source) and a single pacemaker electrode permanently attached to or
adjacent to the myocardium. The pulse generator or power source is implanted under
the fascia of the upper-chest cavity external to the rib cage and connected through a
sealed, flexible conductor to the pacemaker electrode embedded in the myocardium ca. 20
cm away. This arrangement represents a two-electrode electrochemical system in which the pulse generator casing serves as a quasi-reference electrode and the pacemaker electrode (typically a Pt-Ir alloy) functions as the working electrode, periodically exciting the myocardium leading to a regenerative excitation wave and beat of the heart. A bipolar pacing system is slightly more complex than the unipolar pacing system. In this pacing arrangement, a similar power source is employed, but it is used in conjunction with two pacing electrodes inserted into the heart. In bipolar pacing, the current or voltage pulse is passed between the two pacing electrodes.

This work was undertaken with several goals. First, the suitability of routine potential sweep (cyclic voltammetry) and potential step (chronoamperometry) electroanalytical measurements in a rat model of perfused cardiac tissue was investigated. A secondary goal was to evaluate the use of data acquired from these experiments to estimate the inter-electrode resistance for a simple two-electrode configuration within the cardiac tissue intended to mimic a bipolar pacemaker circuit. Estimates of resistance were determined using chronoamperometry of double layer charging and cyclic voltammetry of a redox probe molecule, Ru(NH$_3$)$_6$$^{3-}$, perfused into the myocardium. Finally, the nature and quality of interstitial perfusion of cardiac tissue using a redox-active probe molecule that could be sensed voltammetrically in the interstitial space well separated from the perfusion source was studied.
**Methodology**

Pt (0.369 mm dia) and Ag (0.25 mm dia) wires (Johnson Matthey, 99.99%) were used as working and quasi-reference electrodes, respectively. Pt wire was chosen as the working electrode material because of its chemical inertness and ability to mimic the composition and diameter of pacemaker electrodes. The Ag wire serves as a quasi-reference electrode. 

Modified (Ca**- and albumin-free) Krebs buffer containing 0.00474 M KCl, 0.0948 M NaCl, 0.000950 M MgSO₄, 0.0249 M NaHCO₃, 0.00118 M KH₂PO₄, 0.0115 M glucose, was saturated with 95% O₂, 5% CO₂ and used as the perfusate. This medium thus served as the supporting electrolyte for these electrochemical experiments. The absence of Ca** prevents the heart from contracting, thereby permitting measurements in situ. All buffers were adjusted to pH 7.3, made isoosmolar to plasma (0.308 osmolar), and warmed to 37°C using a circulating water bath. Redox probe solutions of 1 to 5 mM Ru(NH₃)₆³⁺ were prepared using this buffer as the supporting electrolyte medium. Reagent grade chemicals obtained from Sigma were used as received. Water purified with a Millipore Q-UV system was used for all solutions.

**Procedure**

The protocol used for these experiments was approved by the Institutional Animal Care and Use Committee at the University of Arizona. Adult male Sprague-Dawley rats (ca. 400 gr) were exposed to a standard anesthesia (xylazine/ketamine/acepromazine) to
achieve a surgical plane of anesthesia prior to animal manipulation.

The desire for a "living" heart was achieved through the use of a modified Langendorff preparation of the rat heart. In our modification, aortic cannulation (connection of the perfusion source to the heart) was performed in situ in the beating heart to avoid ischemia (reduction of oxygen flow to the heart muscle), thereby preventing immediate death. Initially, the rodent was anesthetized via intramuscular administration of anesthesia. After surgical dislocation, rapid cervical dissection was performed to institute tracheostomy, with intermittent positive pressure ventilation. After 30-300 sec of ventilation with 100% O₂, median sternotomy was performed and the great vessels of the heart were carefully exposed. The aorta was cannulated with a metal catheter. The perfusion cannula was then attached to a syringe containing the modified Krebs buffer and the aorta was ligated as the oxygenated buffer was perfused. Ventilation was discontinued at this point, since the perfused modified Krebs buffer served as the O₂ source, keeping the heart alive.

The two electrodes, each ca. 4 cm long, were placed into the myocardium sufficiently far apart to ensure no contact between them. The Pt working electrode was inserted into the ventricle; the Ag quasi-reference was inserted at the atrium-ventricle junction via the atrium, and then back-looped through the ventricle for exposure of the reference electrode in the myocardium. Every effort was made to maintain the exposed electrode area constant from insertion to insertion in these experiments. The precision associated with the determination of current and solution resistance depends critically on
the constancy of surface area. Given the uncertainty associated with the physiological response of living cardiac tissue to foreign materials other than the metal of the electrode, we were reluctant to mask the electrode to a constant surface area in this initial set of experiments. Thus, the observed variance in these measurements can be attributed, in part, to small differences in electrode surface area exposed to the cardiac tissue.

Cyclic voltammetric and chronoamperometric measurements were performed in the following manner. Between each electrochemical measurement, fresh buffer solution or fresh buffer containing Ru(NH₃)₆³⁺ was mechanically introduced by perfusion at ca. 5-20 mL/min. For cyclic voltammetric measurements, potential scans were performed from +0.20 V to -0.80 V (all potentials are reported versus the Ag quasi-reference electrode) at sweep rates between 50 and 500 mV/sec. The current-potential responses were stored digitally for later processing. Chronoamperometric measurements were made with fresh buffer solution as the perfusate using the standard protocol available in the BAS 100/W workstation for estimating solution resistance. The details of this method are described below.

Data Treatment

Multiple electrodes were inserted into each heart preparation to minimize the number of rats used. Different working electrodes inserted in the same rodent heart are treated as different preparations and represent independent measurements. The standard deviations reported here come from independent measurements as defined in this manner.
In determining standard deviation values for $\Delta E_{r}$ and resistance, propagation of error was used. For determination of the standard deviation in all other measured parameters ($\Delta E_{p}$, $i_{pc}$, $i_{pc}/i_{pc}$, and $\Delta E_{rd}$), the method of population standard deviation was used.

Results and Discussion

Benchtop Cyclic Voltammetry Measurements

Before two-electrode cyclic voltammetric measurements on the redox probe species Ru(NH$_3$)$_6^{3+}$ were performed in the modified-Langendorff heart, the cyclic voltammetry of this system was characterized on the benchtop. The Ru(NH$_3$)$_6^{3+}$ redox species was chosen as the probe in these experiments for several compelling reasons: the Ru(NH$_3$)$_6^{3+}$-Ru(NH$_3$)$_6^{2+}$ redox couple exhibits Nernstian one-electron transfer in aqueous solutions, both redox forms are generally stable on the time scale of cyclic voltammetric experiments, the $E^0$ for this reaction is readily accessible at Pt electrodes in pH 7.3 solutions with minimal interference from other redox-active species commonly found in biological environments, and transport of Ru(NH$_3$)$_6^{3+}$ into the intracellular region of the cardiac tissue to any significant extent is unlikely on the time scale of the electrochemical experiments.

The solution cyclic voltammetry of this system acquired using both three- and two-electrode configurations on the benchtop demonstrates the suitability of this redox system for the intended measurements. The results from these measurements are summarized in
Tables 4.1 and 4.2. The cyclic voltammetry at a Pt disk electrode (ca. 0.20 cm²) in a three-electrode configuration in O₂-free, Ca²⁺-free Krebs buffer solutions containing 3.5 mM Ru(NH₃)₆³⁻ is shown in Figure 4.1. The shapes of the voltammograms associated with reduction of Ru(NH₃)₆³⁻ are consistent with simple one-electron Nernstian behavior based on the cathodic-anodic peak separation (ΔEₚ) of 58 ± 3 mV (n=4 independent measurements) for a sweep rate of 10 mV/s. This value compares favorably with the value of 59 mV expected for an ideal system. As seen in Table 4.1, ΔEₚ increases at faster sweep rates. Assuming that this system remains Nernstian under these conditions, this increase suggests the presence of a small amount of uncompensated solution resistance or slight polarization of the Ag quasi-reference electrode in this electrochemical system.

Figure 4.2 shows the cyclic voltammetry at a Pt wire (ca. 0.33 cm²) working electrode (necessary for in vivo myocardial electrochemistry) in a three-electrode configuration in O₂-free, Ca²⁺-free Krebs buffer. The shapes of these voltammograms are less ideal than for the Pt disk electrode. For example, a ΔEₚ of 68 ± 3 mV is observed at 10 mV/s for a Pt wire electrode as compared to 58 ± 3 mV for a disk electrode. As with the Pt disk electrode, ΔEₚ increases further with sweep rate also suggesting uncompensated solution resistance or quasi-reference electrode polarization.

Several experimental considerations of the rat heart studies prompted us to investigate a two-electrode configuration at faster sweep rates. The size constraints encountered when working with a rat heart (ca. 12 mm dia) clearly dictate a two-electrode configuration. Furthermore, such a two-electrode configuration best mimics a bipolar
Figure 4.1. Benchtop cyclic voltammetry as a function of sweep rate of 3.5 mM Ru(NH$_3$_6)$^{3+}$ at a Pt planar disk electrode in a three-electrode configuration.
Figure 4.2. Benchtop cyclic voltammetry as a function of sweep rate of 3.5 mM Ru(NH$_3$)$_3^{3-}$ at a Pt wire electrode in a three-electrode configuration.
Table 4.1. Sweep Rate Dependence of Cyclic Voltammetry for a Pt Planar Disk Working Electrode in Benchtop Three-Electrode Configuration

<table>
<thead>
<tr>
<th>Sweep Rate (mV/s)</th>
<th>$\Delta E_p$ (mV)</th>
<th>$i_{pc}$ (µA)</th>
<th>$i_{pa}/i_{pc}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>117 ± 2</td>
<td>254 ± 4</td>
<td>0.99 ± 0.01</td>
</tr>
<tr>
<td>300</td>
<td>85 ± 2</td>
<td>201 ± 8</td>
<td>0.99 ± 0.01</td>
</tr>
<tr>
<td>100</td>
<td>76 ± 2</td>
<td>132 ± 16</td>
<td>0.98 ± 0.03</td>
</tr>
<tr>
<td>50</td>
<td>75 ± 2</td>
<td>97 ± 6</td>
<td>0.97 ± 0.09</td>
</tr>
<tr>
<td>25</td>
<td>64 ± 3</td>
<td>62 ± 1</td>
<td>0.99 ± 0.05</td>
</tr>
<tr>
<td>10</td>
<td>58 ± 3</td>
<td>39 ± 1</td>
<td>0.99 ± 0.05</td>
</tr>
</tbody>
</table>
pacing arrangement. The temporal dependence of the Langendorff preparation as implemented in this work made the use of faster sweep rates (50-500 mV/s) a necessity. Thus, the behavior of the two-electrode configuration was investigated as well. The results of a series of cyclic voltammograms for the two-electrode configuration as a function of sweep rate are summarized in Table 4.2. At the slowest sweep rate investigated (50 mV/s) for this configuration, $\Delta E_p$ is 129 ± 4 mV, and increases from this value as the sweep rate increases. These results suggest that, in addition to the small uncompensated solution resistance detected in the three-electrode studies, an instability in the Ag wire quasi-reference electrode occurs, as expected, when current flows through it. The magnitude of this instability, determined as the difference between $\Delta E_p$ values for the three-electrode and two-electrode configurations, $\Delta E_{\text{ref}}$, is tabulated in Table 4.2. $\Delta E_{\text{ref}}$ becomes more pronounced with increasing sweep rate, and hence current, as expected. In both two- and three-electrode configurations, the anodic-to-cathodic peak current ratios are close to the ideal value of unity. This behavior is expected.

An initial concern for in vivo voltammetric measurements on Ru(NH$_3$)$_6$$^{3^-}$ was the potential electrochemical interference from the reduction of O$_2$, a necessary component for keeping the heart alive, in the modified Krebs buffer solution. Benchtop, two-electrode cyclic voltammetry on O$_2$-free and -saturated modified Krebs buffer solutions containing 3.5 mM Ru(NH$_3$)$_6$$^{3^-}$ at 100 mV/s is shown in Figure 4.3. The O$_2$-saturated voltammogram exhibits a slightly distorted, broadened cathodic peak shape due to the electrochemical reduction of O$_2$. A slight reduction in the $i_p/i_{pa}$ ratio, relative to the
Table 4.2. Sweep Rate Dependence of Cyclic Voltammetry for a Pt Wire Working Electrode in Benchtop Three- and Two-Electrode Configurations

<table>
<thead>
<tr>
<th>Sweep Rate (mV/s)</th>
<th>ΔE_p (mV)</th>
<th>i_pe (µA)</th>
<th>i_pe/i_pce</th>
<th>ΔE_p (mV)</th>
<th>i_pe (µA)</th>
<th>i_pe/i_pce</th>
<th>ΔE_rel (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>85 ± 2</td>
<td>314 ± 2</td>
<td>0.99 ± 0.01</td>
<td>202 ± 4</td>
<td>448 ± 8</td>
<td>0.93 ± 0.03</td>
<td>117 ± 5</td>
</tr>
<tr>
<td>300</td>
<td>75 ± 2</td>
<td>271 ± 5</td>
<td>0.99 ± 0.01</td>
<td>175 ± 3</td>
<td>348 ± 5</td>
<td>0.95 ± 0.04</td>
<td>100 ± 4</td>
</tr>
<tr>
<td>100</td>
<td>65 ± 2</td>
<td>162 ± 7</td>
<td>0.98 ± 0.03</td>
<td>148 ± 3</td>
<td>208 ± 3</td>
<td>0.94 ± 0.02</td>
<td>83 ± 4</td>
</tr>
<tr>
<td>50</td>
<td>71 ± 2</td>
<td>123 ± 6</td>
<td>0.97 ± 0.09</td>
<td>129 ± 4</td>
<td>176 ± 4</td>
<td>0.94 ± 0.03</td>
<td>58 ± 5</td>
</tr>
<tr>
<td>25</td>
<td>68 ± 2</td>
<td>84 ± 2</td>
<td>0.99 ± 0.05</td>
<td>NA^</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>68 ± 3</td>
<td>56 ± 2</td>
<td>0.99 ± 0.05</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^Data not taken.
Figure 4.3. a) Benchtop cyclic voltammetry as a function of sweep rate of $O_2$- free 3.5 mM Ru(NH$_3$)$_5^+$ at a Pt wire electrode in a two-electrode configuration; b) as in a) but in $O_2$- saturated solution; sweep rate 100 mV/s.
behavior observed in O_2-free solution is noted. This redox reaction is electrochemically irreversible at Pt resulting in no change in anodic peak shape. Despite the relatively minor distortion in voltammetric peak shape, the ΔE_p of 200 mV (15 mV greater than observed in the absence of O_2) suggests that the O_2 reduction provides only minimal interference in these benchtop measurements.

Surprisingly, our observations in living rat heart tissue indicate that the interference from O_2 reduction is even less. We conclude from this fact that the heart metabolizes the O_2 in the buffer solution upon introduction of the perfusate to the myocardial tissue, rendering it unavailable for reduction at the working electrode.

**Cyclic Voltammetry in the Rat Heart**

Cyclic voltammetric measurements on Ru(NH_3)_6^{3+}-free and Ru(NH_3)_6^{3+}-containing modified Krebs buffer solutions were performed at Pt wire electrodes in a two-electrode configuration in perfused rat hearts prepared as described above. Despite the non-idealities observed in benchtop experiments for a two-electrode arrangement at certain sweep rates, this configuration is the only one feasible given the small size of the rat heart. Sweep rates of 100-500 mV/s were used throughout these *in vivo* studies due to the limited lifetime of the modified Langendorff heart preparation (ca. 20-30 min).

Prior to *in vivo* cyclic voltammetric measurements on Ru(NH_3)_6^{3+}-containing solutions, it was necessary to prove that the modified Langendorff preparation did, in fact, result in "living" heart tissue. This was first established by potentiostatic sensing of the
heart beat. The beating of the heart can be followed in real-time during a potential sweep as shown by the current-potential response in Figure 4.4a. This voltammogram was taken immediately after electrode insertion into a heart cavity perfused with normal (i.e. Ca\(^{2+}\)-containing) Krebs buffer. The periodic (ca. 200 mV) bipolar current response superimposed on the background current-potential curve represents the polarization cycle normally associated with contraction of the heart. At a sweep rate of 500 mV/s, the frequency of these events suggests a heartbeat of 150 beats/min. This rate is about one-half that expected for a normal rodent heart (ca. 300 beats/min),\(^4\) but can be rationalized as a rate decrease due to sedation. Nonetheless, the tissue in this preparation can be considered to be "living" based on the criterion that the heart is still metabolizing nutrients leading to heart beat.

The voltammogram in Figure 4.4b was acquired in an identical manner except that the perfusate was a modified (i.e. zero-Ca\(^{2+}\)) Krebs buffer. The absence of the bipolar current events associated with heart beat is a response expected in the absence of Ca\(^{2+}\), which is essential for action potential transport. Suppression of the heart beat in this manner conserves energy, thereby minimizing depletion of the nutrients necessary to keep the heart alive. Thus, this modified Krebs buffer is used in all further measurements in order to prolong the life of the heart.

The cyclic voltammetry with Ru(NH\(_3\))\(_6\)^3+ in the perfusate exhibits various shapes dependent on the electrode placement within the heart. We hypothesize that each shape reflects a different type of electrode insertion into the heart as described below. The
Figure 4.4.  a) In vivo cyclic voltammetry at a Pt wire electrode in a two-electrode configuration in Ca\(^{2+}\)-containing Krebs buffer; sweep rate 500 mV/s; b) as in a) but in the absence of Ca\(^{2+}\).
criteria used to assign particular electrode insertion types were as follows: knowledge of
the nature of insertion; presence of perfusate leakage at point of insertion; in certain cases,
post-experimental dissection of the heart for establishing the anatomy of electrode
insertion; and finally, the shape of the voltammogram. The four types of insertions are
discussed in turn below.

Type 1 Electrode

For this insertion, the electrode is plunged into the myocardium perpendicular to
the plane of the epicardial heart wall. The shape of the voltammogram observed for a Type
1 electrode placement, shown in Figure 4.5, is consistent with mass transport dominated
by semi-infinite linear diffusion to a macroscopic electrode. This behavior is similar to that
observed in a conventional three-electrode electrochemical cell. The proposed insertion
geometry for this electrode type, shown schematically in Figure 4.6a, is that the wire spans
the entire width of the myocardium and extends well into the heart cavity (~6 mm) which
is filled with semi-quiescent perfusate solution. The anodic-to-cathodic peak current ratio
of the Type 1 voltammogram is 0.95 ± 0.03 at 300 mV/s (7 measurements in 3 rodent
preparations). The fact that this value is slightly less than unity suggests that some
hydrodynamic flow of redox product away from the electrode surface may be occurring in
this arrangement. Such fluid movement is most likely the result of a slight net flow of the
perfusate from the heart cavity into the myocardial tissue during the time scale of the
measurement. This explains why the response is dominated almost entirely by diffusion.
Figure 4.5. *In vivo* cyclic voltammetry at a Pt wire in a two-electrode configuration (sweep rate = 300 mV/s) for 3.5 mM Ru(NH$_3$)$_6^3^-$ perfusate for a Type 1 electrode insertion.
Figure 4.6. Schematics of a) Type 1, b) Type 2, c) Type 3, d) Type 4 working electrode insertions.
Little information about electrochemistry within the myocardium is obtained from this electrode geometry, since the majority of the response originates from redox solution within the heart cavity and not within the tissue. As a result, this electrode arrangement is unacceptable for investigation of electrochemistry within the cardiac tissue and was not further studied.

Type 2 Electrode

The Type 2 voltammetric response, shown in Figure 4.7, was observed after attempting to insert the working electrode into the myocardium without penetrating the heart cavity. The shape of the voltammogram for this preparation is strongly reminiscent of hydrodynamic mass transport in that a limiting current plateau is observed (superimposed on a Faradaic background) for reduction of Ru(NH$_3$)$_6$$^{3+}$. The magnitude of the limiting current is large relative to the peak current noted in the Type 1 response, consistent with hydrodynamic flow of redox reactant to the electrode surface. We postulate that this response is indicative of the electrode geometry shown schematically in Figure 4.6b in which the electrode pierces the inner heart wall creating a hole into which perfusate flows along the working electrode surface. In fact, this postulate is supported by several other observations. First, perfusate flow was observed at the insertion point for this electrode type. In addition, dissection of a Type 2 insertion verified electrode puncture of the endocardial wall. Redox product re-oxidation is not observed for a Type 2 electrode as the Ru(NH$_3$)$_6$$^{2+}$ flows away. This preparation is also deemed unacceptable, since it
Figure 4.7. *In vivo* cyclic voltammetry at a Pt wire in a two-electrode configuration (sweep rate = 300 mV/s) for 3.5 mM Ru(NH$_2$)$_3$ perfusate for a Type 2 electrode insertion.
provides no information about the electrochemistry of the myocardium.

It may be surprising, given the thickness of the endocardium in the left ventricle (ca. 6 mm), that the electrode could not be inserted without puncturing the inner wall. However, it is nearly impossible to know, before hand, into which ventricle (left or right) the electrode will be inserted. The right ventricle endocardium thickness is only ca. 2-3 mm.

Type 3 Electrode

In an attempt to measure a response originating solely from the cardiac tissue with no contributions from perfusate in the heart cavity, we tangentially inserted the working electrode into the outer edge of the myocardium as shown in Figure 4.6c. In this configuration, no direct path for perfusate between the heart cavity and the electrode exists. The resulting voltammogram, shown in Figure 4.8, comes only from Ru(NH$_3$)$_6^{3+}$ redox species in the myocardium that have access to the electrode surface by virtue of perfusion. The observation of redox activity in this configuration is quite significant in that it clearly establishes that the perfusate solution reaches this region of the myocardium. No previous studies of cardiac tissue have provided evidence as direct as this result that the myocardial tissue is efficiently perfused.

The shape of the Type 3 voltammetric response is similar to the Type 1 response and suggests that mass transport of Ru(NH$_3$)$_6^{3+}$ through cardiac tissue to the working electrode is dominated by diffusion. Despite the presence of an observable Faradaic
Figure 4.8. *In vivo* cyclic voltammetry at a Pt wire in a two-electrode configuration (sweep rate = 300 mV/s) for 3.5 mM Ru(NH₃)₃⁺⁺ perfusate for a Type 3 electrode insertion.
background in this voltammogram, the anodic-to-cathodic peak current ratio is estimated to be on the order of $0.93 \pm 0.05$ (12 measurements in 6 rat preparations.)

The relative dominance of diffusion in the Type 3 electrode response versus hydrodynamic mass transport in the Type 2 electrode is interesting in light of the fact that the electrode surface area exposed to the cardiac tissue is expected to be similar in each case. The relative contributions of these two means of mass transport in a given voltammetric experiment are a function of the solution flow rate, the potential scan rate, and the electrode length. Thus, the shape of the voltammetric wave can be controlled by varying these three experimental parameters. In fact, a similar dependence has been previously demonstrated in hydrodynamic voltammetry.\(^4\)\(^3\)

In the experiments described here, the electrode length remains generally constant; thus, the flow rate, controlled by electrode insertion type and scan rate, determines the dominant mass transport response observed. For a Type 3 insertion in which perfusate flow is minimized by tangential electrode insertion, diffusion as the major means of mass transport is further supported by the dependence of the cathodic peak current on potential sweep rate. Table 4.3 summarizes the results of the sweep rate studies which are plotted in Figure 4.9. The peak current increases linearly with the square root of sweep rate as expected for a voltammetric response dominated by diffusional mass transport. Despite the relatively low precision of these measurements, especially at faster sweep rates, the quality of the linear response is quite remarkable considering the nature of the mass transport environment expected in cardiac tissue.
Table 4.3 Sweep Rate Dependence for a Type 3 Electrode

<table>
<thead>
<tr>
<th>Sweep Rate (mV/s)</th>
<th>∆E_p (mV)</th>
<th>i_p (µA)</th>
<th>i_p/i_p</th>
<th>Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>213 ± 52</td>
<td>110 ± 33</td>
<td>0.93 ± 0.06</td>
<td>8 in 6 preps</td>
</tr>
<tr>
<td>300</td>
<td>241 ± 30</td>
<td>87 ± 50</td>
<td>0.93 ± 0.05</td>
<td>12 in 6 preps</td>
</tr>
<tr>
<td>100</td>
<td>207 ± 29</td>
<td>50 ± 24</td>
<td>0.89 ± 0.04</td>
<td>9 in 5 preps</td>
</tr>
<tr>
<td>50</td>
<td>180 ± 18</td>
<td>24 ± 8</td>
<td>0.83 ± 0.06</td>
<td>3 in 2 preps</td>
</tr>
</tbody>
</table>

*3.5 mM Ru(NH₃)₆³⁺*
Figure 4.9. Potential sweep rate dependence of $i_p$ for *in vivo* cyclic voltammetry in 3.5 mM Ru(NH$_3$)$_5^{3-}$ perfusate.
Table 4.4 summarizes the results of concentration dependence experiments, and a plot of the cathodic peak current at Type 3 electrodes as a function of perfusate Ru(NH$_3$)$_5^{3+}$ concentration is shown in Figure 4.10. This plot indicates a linear dependence of peak current on concentration further confirming the well-behaved nature of this electrochemical response.

An interesting point worth noting is that the Type 3 electrode response has no thin layer characteristics, as evidenced by the absence of a linear sweep rate dependence of peak current. Based on the known dimensions of the interstitial spaces in cardiac tissue (ca. 1 μm dia), such a thin layer response might be expected. Its absence may mean one of two things: 1) The insertion of the electrode is sufficiently disruptive to the integrity of the cardiac tissue that the interstitial structure is not maintained and the electrode sits in a "pool" of perfusate, and/or 2) a small hydrodynamic flow continues to bring redox species into the interstitial spaces sampled by the electrode, eliminating the depletion effects expected in a thin layer configuration. Based on experimental observations, we believe that the absence of thin layer characteristics is due mainly to the disruption of the tissue resulting in a "pool" of perfusate (visually observable), whose volume is greater than that expected for a thin layer configuration.

Type 4 Electrode

A fourth type of voltammetric response was observed twice when the working electrode was tangentially placed deep into the myocardium close to the inner cavity wall.
Table 4.4. Concentration Dependence for a Type 3 Electrode

<table>
<thead>
<tr>
<th>Concentration (mM)</th>
<th>$\Delta E_p$ (mV)$^a$</th>
<th>$i_{pc}$ (µA)</th>
<th>$i_{pc}/i_{pc}^o$</th>
<th>Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>210 ± 6</td>
<td>37 ± 5</td>
<td>0.85 ± 0.03</td>
<td>4 in 2 preps</td>
</tr>
<tr>
<td>3.5</td>
<td>241 ± 30</td>
<td>87 ± 29</td>
<td>0.93 ± 0.05</td>
<td>12 in 6 preps</td>
</tr>
<tr>
<td>5.0</td>
<td>300 ± 28</td>
<td>124 ± 37</td>
<td>0.92 ± 0.02</td>
<td>3 in 2 preps</td>
</tr>
</tbody>
</table>

$^a$ Sweep rate 300 mV/s
Figure 4.10. Ru(NH$_3$)$_6$$^{3+}$ concentration dependence of $i_\alpha$ for in vivo cyclic voltammetry at a sweep rate of 300 mV/s.
In one case, a small tear was observed in the inner cavity wall after dissection, shown in Figure 4.6d. The voltammetry for this Type 4 geometry produced a surprising response as shown in Figure 4.11. Two features of this response are immediately evident. First, the magnitude of the Faradaic current is very small relative to the other electrode types, suggesting extremely limited access of the Ru(NH$_3$)$_6^{3+}$ in the perfusate solution to the electrode surface. Secondly, the Faradaic response appears to be superimposed on an approximately linear background suggestive of resistive behavior. Only a small flow of perfusate at the electrode insertion point was observed in these cases. We interpret these observations to collectively suggest that perfusion of this portion of the myocardium is not as efficient as the portion resulting in a Type 3 response. Significantly, although only two Type 4 cases were investigated, both insertions turned out to be in the right ventricle. (The distinction between the right and left ventricles is not obvious from the outside of the heart.) Thus, our conclusions regarding perfusion efficiency may apply to the right ventricle myocardium.

**Estimation of Resistance from Cyclic Voltammetry**

Cyclic voltammetry of an interstitially-confined Nernstian redox species such as Ru(NH$_3$)$_6^{3+}$ should allow estimation of the resistance of the interstitial space in cardiac tissue. The basis of this method is that as the resistance between the two electrodes increases, $\Delta E_p$ should increase by an amount proportional to this resistance. $\Delta E_p$ is
Figure 4.11. *In vivo* cyclic voltammetry at a Pt wire in a two-electrode configuration (sweep rate = 300 mV/s) for 3.5 mM Ru(NH$_3$_)$_{6}^{3-}$ perfusate for a Type 4 electrode insertion.
described by equation (1):

\[ \Delta E_p = 59/n \text{ mV} + \Delta E_{ir} + \Delta E_{ref} \]  

(1)

Under ideal conditions, \( \Delta E_{ref} \) should be zero, and any increase in \( \Delta E_p \) beyond that expected for a Nernstian system should be due to an increase in myocardial resistance. However, as noted above, due to the use of a quasi-reference electrode and a two-electrode configuration in these studies, \( \Delta E_{ref} \) is not zero and also contributes to the \( \Delta E_p \) measured in myocardium.

\( \Delta E_{ir} \) for cardiac tissue can be determined from the difference between the measured peak separation (\( \Delta E_p \)), and the sum of the instability of the reference potential (\( \Delta E_{ref} \)) and the Nernstian peak separation (\( \Delta E_{Nernst} = 59/n \text{ mV} \)). This straightforward approach was utilized to generate the results shown in Table 4.5. \( \Delta E_p \) values for three sweep rates were measured \textit{in vivo} using 3.5 mM Ru(NH\textsubscript{3})\textsubscript{6}\textsuperscript{3+}. After subtracting the \( \Delta E_{ref} \) and \( \Delta E_{Nernst} \) values, \( \Delta E_{ir} \) was determined at each sweep rate. Using \( \Delta E_{ir} \) and the measured peak current, Ohm’s law was then used to estimate electrode resistance:

\[ R = \frac{\Delta E_{ir} i_p}{i_p} \]  

(2)

The resistance values calculated in this manner increase as the sweep rate decreases. This behavior is unexpected and suggests an additional contribution to \( \Delta E_p \). Although the nature of this contribution is unidentified at this point, several possibilities come to mind. In light of the inverse dependence of resistance on sweep rate, one might speculate that higher current levels (faster sweep rates) turn on modes of conduction other than ion transport through the interstitial space. These modes might involve cellular
Table 4.5. Myocardial Tissue Resistance from Cyclic Voltammetry

<table>
<thead>
<tr>
<th>Sweep Rate (mV/s)</th>
<th>ΔE_p (mV)</th>
<th>i_p (μA)</th>
<th>i_p/i_p</th>
<th>ΔE_rel (mV)</th>
<th>ΔE_Nernst (mV)</th>
<th>ΔE_irt (mV)</th>
<th>Resistance (Ω)</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>213 ± 52</td>
<td>110 ± 50</td>
<td>0.93 ± 0.06</td>
<td>117 ± 5</td>
<td>59</td>
<td>37 ± 52</td>
<td>336 ± 877</td>
</tr>
<tr>
<td>300</td>
<td>241 ± 30</td>
<td>77 ± 29</td>
<td>0.93 ± 0.05</td>
<td>100 ± 4</td>
<td>59</td>
<td>82 ± 30</td>
<td>1065 ± 560</td>
</tr>
<tr>
<td>100</td>
<td>207 ± 29</td>
<td>50 ± 23</td>
<td>0.89 ± 0.04</td>
<td>83 ± 4</td>
<td>59</td>
<td>65 ± 29</td>
<td>1300 ± 833</td>
</tr>
</tbody>
</table>
conduction mechanisms of the myocardium. Alternately, one might envision the involvement of additional mass transport resistance of $\text{Ru(NH}_3)_6^{3+}$ at low currents and slow sweep rates. The data in hand do not allow distinction between these or other possible contributions to $\Delta E_p$.

The resistance of the myocardial tissue can be estimated (ignoring this unidentified contribution) by averaging the results over all sweep rates. The average resistance is thus estimated to be $900 \pm 1333 \ \Omega$ for a ca. 4 mm inter-electrode separation in myocardium $(2.25 \times 10^4 \pm 3.33 \times 10^3 \ \Omega/cm)$. The large error associated with this resistance value is indicative of several contributions including the large range of resistance values encountered due to this unidentified contribution (which may be potential sweep rate-dependent), the variance in electrode surface are exposed to the cardiac tissue, and the fact that the environment in which these measurements were made is extraordinarily complex and difficult to duplicate from rodent to rodent.

Chronoamperometry of Double Layer Charging

In light of the uncertainty associated with the resistance estimates based on cyclic voltammetry, a second strategy for quantitative estimation of myocardial tissue resistance was used. This method involves measurement of uncompensated resistance using chronoamperometry of double layer charging in response to a small potential step perturbation. This approach is one conveniently programmed into the BAS 100/W software for routine determination of uncompensated solution resistance.$^{44}$ In this
measurement, the electrochemical cell is considered electronically equivalent to an RC circuit with the uncompensated resistance, $R_u$, in series with the double-layer capacitance, $C_d$. Since a Faradaic impedance is not considered part of the model, the test potential must be a value at which no Faradaic process occurs. A potential step to between +25 mV and -25 mV relative to a test potential is applied and the current sampled at 54 μs and 72 μs after the step, as shown in Figure 4.12. Assuming the expected exponential decay of the current characteristic of double layer charging, the initial current, $i_0$, is determined by extrapolation to zero time. Using Ohm's law, $R_u$ is calculated from this measurement as:

$$R_u = \Delta E / i_0 = 50 \text{ mV} / i_0$$

To reduce error, this measurement is performed 256 times by the computer, and the results are averaged. Using this method in a Type 3 electrode insertion in myocardial tissue at a test potential of 0 mV, the resistance is estimated to be $598 \pm 138 \Omega \ (1.50 \times 10^3 \pm 0.35 \times 10^3 \Omega/cm)$ for 37 measurements. Although all experiments ceased before the heart was visibly dead (determined by color), we noted a definite decrease in chronoamperometric resistance values during the course of the experiment. For this reason, only initial resistance values (measured during the first five minutes of the preparation lifetime) were employed for the chronoamperometric resistance determination.

**Limitations of the Modified-Langendorff Heart Preparation**

Although the modified-Langendorff heart preparation developed in this work is successful in enabling routine potential sweep and potential step experiments to be
Figure 4.12. Current-time response for chronoamperometric determination of uncompensated resistance. Sampling times of 54 and 72 μs indicated.
performed for relatively short times in living heart tissue, no method for assessment of
degradation of the modified Langendorff preparation has been developed. It has been
noted that crude indicators of heart viability are color and the absence of a voltammetric
response for $O_2$ reduction in the oxygenated perfusate. Interestingly, time-dependent
decreases in the chronoamperometric resistance measurements were observed in most
preparations. We attribute this decrease to the disruptive effects of prolonged perfusion on
the integrity of the cardiac tissue.

In addition, only one redox probe molecule of one charge type, $\text{Ru(NH}_3)_6^{3+}$, has
been studied to date. Given this fact, it is difficult to determine unequivocally whether or
not the anodic-to-cathodic peak current ratios for the voltammograms reflect mass
transport or other biochemical events selectively removing the $\text{Ru(NH}_3)_6^{2+}$ product.
Finally, no method for quantitative determination of the true electrode surface area
exposed in-situ has been developed. Thus, electrochemical measurements can only be
interpreted in a semi-quantitative manner at best.

Conclusions

The results presented here represent the first potential step and potential sweep
electroanalytical measurements made in living mammalian heart tissue. These
measurements, although carried out in an novel environment, show trends similar to
benchtop solution electrochemistry, as supported by both $\text{in vivo}$ concentration and sweep
rate studies. Both electrochemical measures of myocardial tissue resistance give similar
estimates of ca. <1000 Ω for inter-electrode distances of ca. 4 mm (<1.5 x 10³ Ω/cm). These values are considerably less (ca. five orders of magnitude) than other estimates of myocardial tissue resistance based on larger current flow involving depolarization of the myocardial cells. In these cases, ion transport in the interstices must be insufficient to carry the current.

Understanding the mechanism of current flow in myocardium is of considerable importance for cardiac pacing and defibrillation. The studies presented here suggest that electroanalytical measurements may have a role to play in understanding these phenomena.
Chapter 5

Surface-Confined Carbon Contamination in Self-Assembled Monolayers on Ag
Evaluated By Raman Spectroscopy

Introduction

The preparation, characterization, and application of self-assembled monolayers (SAMs) have generated much interest in the last decade (see Chapter 1). For many applications of SAMs supported on metals, the metals are exposed to the ambient atmosphere either during preparation or use. In this laboratory, SAMs have routinely been formed on polycrystalline metal surfaces prepared by mechanical polishing. This preparation procedure results in surfaces containing significant levels of unavoidable carbon contamination\(^5\).\(^1\), \(^5\).\(^2\)

Taylor has speculated that these impurities originate from the laboratory ambient, the polishing cloth, and/or the alumina used for polishing.\(^5\).\(^1\) Recent literature reports claim that carbon and oxide surface contamination is displaced upon thiol SAM adsorption.\(^5\).\(^3\)-\(^5\).\(^5\) However, evidence for carbon contamination has still been observed within SAM films using Raman spectroscopy, suggesting incomplete displacement by the adsorbing alkanethiol molecules and/or their incorporation into the SAM film. The spectral signatures of these contaminating species interfere significantly with the vibrational response of adsorbed alkanethiols, especially in the \(\delta(C-H)\) frequency region.
(1200-1600 cm\(^{-1}\)), preventing complete vibrational analysis.

The quantity and chemical nature of carbonaceous impurities remaining on polycrystalline Ag surfaces after preparation have previously been investigated in this laboratory using Raman spectroscopy and x-ray photoelectron spectroscopy (XPS)\(^{5.1}\). Raman spectroscopy was proven to be quite sensitive to surface-carbon contamination\(^{5.1}\). Very small amounts of contamination are easily detected due to the high Raman cross-section of these impurities and moderate surface enhancement levels at mechanically polished, polycrystalline Ag surfaces. Furthermore, the chemical nature of these impurities has been identified as graphite-like carbon and hydrocarbons\(^{5.1}\).

Several protocols for preparing Ag surfaces, including mechanical polishing (MP), \(\text{Ar}^-\) sputtering and two variants of chemical polishing (CP), were studied by Taylor and coworkers\(^{5.1}\). The resulting surfaces were characterized with XPS and Raman spectroscopy. Not surprisingly, \(\text{Ar}^-\)-sputtered Ag surfaces prepared in UHV were determined to be the cleanest surfaces prepared. However, exposure of these highly-active, sputtered surfaces to air during Raman spectral acquisition resulted in immediate carbon contamination (C/Ag ratio increased by a factor of 4)\(^{5.1}\).

CP surfaces were reported to be the cleanest surfaces prepared in the ambient environment. Taylor suggests that CP surfaces contain much less carbon and alumina contamination than MP surfaces, because chemical polishing removes impurities upon Ag dissolution by \(\text{H}_2\text{O}_2\)\(^{5.2}\). However, the cleanliness of CP surfaces works to the disadvantage of Raman spectroscopy for weakly scattering systems such as alkanethiol SAMs, since
surface enhancement is greatly diminished as a result of a much smoother surface.\textsuperscript{5,6} The effects of electrochemical perturbation on graphitic-carbon contamination on Ag have also been studied using Raman spectroscopy.\textsuperscript{5,7} The application of negative potentials (-1.2 V versus SCE) was shown to reduce the amount of carbon contamination on the surface, as evidenced by changes in the 1200-1700 cm\textsuperscript{-1} region. Mahoney and co-workers concluded that carbon impurities are diminished at these potentials based on the disappearance of the 1360 and 1580 cm\textsuperscript{-1} graphitic carbon modes and the appearance of intense hydrocarbon v(C-H) vibrations at ca. 2900 cm\textsuperscript{-1}. Quite interestingly, the graphitic carbon modes reappear when the potential is stepped back to -0.2 V (SCE), indicating that the form of carbon contamination changes as a function of potential.

One of the goals of some early fundamental research on SAMs performed by the author was to elucidate the effect of various solvents on the structure and integrity of alkanethiol SAMs. These preliminary studies were begun at approximately the same time as a concrete understanding emerged in this research group of the importance of carbon impurities in the surface Raman scattering of SAMs. Initial Raman spectral results quite clearly indicated that the form of these trapped carbon impurities is strongly affected by solvent exposure, specifically for contaminated C\textsubscript{3}SH SAMs on Ag. These studies prompted further investigation into such carbon impurities that are the subject of this chapter.
Spectroelectrochemical Emersion Cell

An emersion approach was utilized for acquiring surface Raman spectra of SAMs before and after exposure to solvents, electrolyte solutions, and potential. This approach is new and offers certain advantages over conventional spectroelectrochemical cells in which the SAM is separated from the window by a thin layer of solution. One important advantage is the minimization of bulk solution spectral interference as a result of electrode rotation through a solvent drop. This emersion procedure leaves a very thin layer of solution on the cross-section of surface that is spectroscopically sampled, allowing small spectral changes in the SAM to be more easily observed. Previous emersion studies on bare metal electrodes from aqueous and nonaqueous environments indicate that, upon emersion of the surface, at most a very thin layer of solution is maintained on the surface in which the molecules retain any preferred orientation they possess in situ. Electrochemical control of the surface potential is preserved through the contacting solution drop, and the reference and counter electrodes are placed in the solution reservoir from which the drop originates.

There are additional advantages which make using the spectroelectrochemical emersion cell desirable. The cell can be purged with N₂ so that a constant solvent vapor pressure is maintained and/or SAM exposure to the ambient environment is prevented. In addition, long laser exposure times (e.g., 5 min) and relatively high laser power (150 mW) can be used without inducing surface chemistry changes, since the electrode is constantly rotated through the laser spot.
Surface Raman Spectroscopy

Surface Raman spectroscopy has been used previously in this laboratory to characterize the self-assembly of organothiols at smooth, mechanically polished, and electrochemically roughened metal surfaces.\(^{5,13,5,14}\) Raman spectroscopy has proven to be a very useful technique for investigating SAMs, because, in contrast to surface FTIR measurements which are largely limited to the \(v(C-H)\) region, vibrational information can be obtained from all locations within the molecule. For example, conformational defect structure of the alkane backbone can be directly investigated by assessing intensities of both gauche (G) and trans (T) bands in the \(v(C-C)\) and \(v(C-S)\) regions.

The vibrational peak frequencies and assignments for \(C_3SH\), \(C_{12}SH\), and \(C_{18}SH\) SAMs are given in Table 5.1. Assignments are based on previous work by Bryant,\(^{5,13,5,14}\) and information in the literature.\(^{5,16-5,23}\) A more in-depth discussion of the vibrational assignments for \(n\)-alkanethiol liquids, solids, and SAMs in the 600-1300 cm\(^{-1}\) and 2800-3000 cm\(^{-1}\) spectral regions can be found in reference 5.15.

Raman spectroscopy is also useful for detecting the presence of carbon contamination at mechanically polished surfaces and within SAM films.\(^{5,1}\) As mentioned above, Taylor investigated carbon contamination at bare Ag surfaces as a function of surface preparation. Interestingly, considerable variability in both the quantity and chemical nature of carbon contamination occurs for a single preparation protocol, especially for surfaces prepared by mechanical polishing. In addition, the details of the polishing technique (e.g., polishing time, alumina concentration, surface pressure) have
Table 5.1. Raman Peak Frequencies (cm$^{-1}$) and Vibrational Assignments for C$_3$SH, C$_{12}$SH, and C$_{18}$SH on polycrystalline Ag.

<table>
<thead>
<tr>
<th></th>
<th>C$_3$SH</th>
<th>C$_{12}$SH</th>
<th>C$_{18}$SH</th>
<th>assignment$^*$</th>
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<tbody>
<tr>
<td>628</td>
<td>635</td>
<td>634</td>
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<td>v(C-S)$_G$</td>
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<tr>
<td>701</td>
<td>707</td>
<td>708</td>
<td></td>
<td>v(C-S)$_T$</td>
</tr>
<tr>
<td>783</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>842</td>
<td></td>
<td></td>
<td></td>
<td>CH$_2$ rock$_T$</td>
</tr>
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<td>1198</td>
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<td>CH$_3$ twist</td>
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<td>1352</td>
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<td>CH$_3$ wag</td>
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<td>1377</td>
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<td>2848</td>
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<td>v$_i$(CH$_3$)</td>
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<td>2880</td>
<td>2880</td>
<td>v$_i$(CH$_3$)</td>
<td></td>
</tr>
</tbody>
</table>

$^*$ Assignments taken from references 5.13-5.23.
been observed to influence the amount and chemical nature of carbon present at SAM-modified Ag surfaces. 5.1

Carbon Contamination in C3SH SAMs

Raman spectra in the ν(C-S) and ν(C-C) frequency region (ca. 600-1700 cm⁻¹) for C3SH SAMs on Ag with different forms of carbon contamination are shown in Figure 5.1. Several interesting observations can be made about these spectra. Very intense peaks are observed at 810 cm⁻¹, 910 cm⁻¹, and in the region from 1200-1600 cm⁻¹, that indicate the presence of carbon contamination. 5,2 Modes of this intensity at these frequencies are not observed in the Raman spectra of neat liquid or solid C3SH. 5,13 In fact, the δ(C-H) bands of C3SH (which should appear at 1285, 1325, 1373, 1424, and 1449 cm⁻¹ as shown in Table 5.1) are not observed because of the strong carbon contamination bands in the ca. 1200-1600 cm⁻¹ region. In addition, the intense band at 710 cm⁻¹ is not ν(C-S)ᵣ, but instead a contamination band which coincidentally overlaps with the ν(C-S)ᵣ band. Only one band, the ν(C-C)ᵣ mode at 1026 cm⁻¹, can unequivocally be assigned to C3SH.

Further evidence for such contamination has been observed by Taylor in deuterated systems. 5,2 These contamination bands appear at identical frequencies for deuterated alkanethiol SAMs on Ag, indicating species other than alkanethiols trapped within these films. The exact vibrational assignments for these bands will be discussed in greater detail by Taylor. 5,2

The differences between the C3SH SAM spectra in Figure 5.1 can be attributed to
Figure 5.1. Raman spectra of C₃SH SAM on Ag with varying amounts of carbon contamination from a) most to b) least. + denotes definitive C₃SH band. Integration times: 120 sec.
the nature of trapped carbon contamination. At longer surface polishing times, when extreme care is taken to prepare surfaces with minimal imperfections (specifically, scratches and entrapped alumina), the form of carbon impurity incorporated into the SAM structure is predominantly as small polyaromatic molecules. The intensity of these bands is a result of significant resonance Raman contributions. For surfaces which are prepared less carefully, the form of these impurities is more graphite in nature. As a result of perhaps, a different overall orientation, the intensity of the contamination bands decrease and the bands broaden considerably.

Examples of C$_3$SH SAMs in which the form of contamination varies from small aromatic molecules to graphitic-carbon are shown in Figure 5.1b, 5.1c, and 5.1d. Other than polishing time (related to the time required to remove surface imperfections), the same preparation procedure was used for each of these C$_3$SH SAMs. Although very sharp peaks are still observed in the ca. 1200-1600 cm$^{-1}$ region in Figure 5.1b, the intensities of the impurity bands are reduced by ca. 70%, suggesting either less contamination or contamination which is more graphitic like resulting in smaller resonance Raman contributions. If the contamination form is even more graphitic, such as for the C$_2$SH SAMs shown in Figure 5.1c and 5.1d, the shape of the contamination peaks broaden considerably. This behavior coincides with an increase in the intensity of the CH$_3$ rocking mode at 1026 cm$^{-1}$ and the appearance of vibrational bands associated with the SAM. To summarize, all mechanically polished Ag surfaces contain significant amounts of carbon contamination. We speculate that extensive mechanical polishing leads to surfaces for
which the predominant form of contamination is small polyaromatic molecules. On the other hand, mechanically polished Ag with greater surface imperfections exhibit graphitic-like contamination. In addition, the Raman cross-sections for these two forms of contamination is believed to be significantly different.

**Laser Power and Exposure Time**

The carbon contamination present in alkanethiol SAMs can be classified as film defects, similar to other imperfections that arise, for example, from inadequate immersion time, surface roughness, and/or grain boundaries. As described above, the nature of contamination occluded into SAMs is highly variable and primarily dependent upon surface preparation, similar to other SAM defects.

The chemical nature of such carbon impurities is sensitive to factors which also influence SAM structure, including temperature, laser exposure, and exposure to solvent. The effect of laser exposure time at 150 mW on the form of carbon contamination present in a C$_3$SH monolayer on Ag is shown in Figure 5.2. Initially, the form of contamination is predominantly small polyaromatic molecules as a result of extensive polishing. Long laser exposure times and high laser power graphitize these species, as indicated by the emergence of the two signature bands for graphite in the 1200-1600 cm$^{-1}$ region centered at ca. 1360 and 1570 cm$^{-1}$. Such bands have been labeled the "cathedral peaks" in the Raman spectrum of graphite. Similar photo-fragmentation of pyrazine to graphitic carbon at "rough" Ag surfaces after exposure to 100 mW of 514.5 nm radiation was observed by
Figure 5.2. Raman spectra of carbon-contaminated C$_7$H$_7$ SAM on Ag a) rotating through the laser beam (<1 sec laser exposure), b) stationary in the laser beam during the entire acquisition period. + denotes definite C$_7$H$_7$ band. Integration times: 120 sec.
Moskovits and coworkers using Raman spectroscopy.\textsuperscript{5,24} The amount of graphitic carbon was shown to increase as a function of laser exposure time. They claim that the decomposition of pyrazine to graphitic carbon at Ag is photocatalytic at 514.5 nm.\textsuperscript{5,24} The photo-decomposition of other aromatic compounds at Ar\textsuperscript{+} sputtered Ag surfaces at 406.7 nm radiation have also been reported, including pyridine, benzaldehyde, chlorobenzene, and 3-chloropyridine, further testifying to the conversion of these molecules to graphitic carbon after laser exposure.\textsuperscript{5,25-5,27}

As the carbon contamination becomes more graphite-like, several monolayer bands appear, specifically $\nu$(C-S)$_G$ and $\nu$(C-S)$_T$ bands at 630 and 703 cm\textsuperscript{-1} and a $\nu$(C-C)$_T$ band at 1085 cm\textsuperscript{-1}. This might be expected; photolysis of the polyaromatic species to graphitic carbon results in a diminished resonance enhancement of the carbon contamination such that the weakly scattering C$_2$SH bands appear.

Effect of Solvent and Electrolyte on Contamination

As described in Chapter I, the stability of SAMs upon exposure to solvent and electrolyte has important ramifications in the electrochemical community. Many proposed applications are critically dependent on SAM stability in an electrochemical environment. In order to better understand the effect of solvent on the structure of the SAM, surfaces were rotated through drops of different solvents while being analyzed with Raman spectroscopy. The effect of water on film structure was investigated first. As shown in Figure 5.3, the similarity of the Raman spectra for non-graphitized carbon-contaminated
Figure 5.3. Raman spectra of carbon-contaminated C₅SH SAM on Ag a) before, and after exposure to water for b) 5 min, and c) 30 min. + denotes definite C₅SH band. Integration times: 120 sec.
C$_3$SH monolayers before and during exposure to water indicates that water has relatively little effect on the composition of these films after 5 min. However, after 30 min of exposure, certain spectral differences are apparent. The intensity of all peaks are decreased, suggesting removal of aromatic-like carbon impurities from the film. Graphitization of the aromatic-like contamination is not observed, as evidenced by only a decrease in peak intensity, but not peak broadening. This behavior suggests that water may play a role in cooling the surface during laser exposure.

Exposure to aqueous 0.1 M NaCl or 0.1 M LiClO$_4$ has very different effects on film structure as compared to pure water. Examples of C$_3$SH SAMs on Ag after exposure to these solutions are given in Figure 5.4. Dramatic spectral changes are observed after a contaminated C$_3$SH SAM is exposed to aqueous electrolytes for only 5 min. The intensities of contamination bands decrease significantly as some of the small molecule aromatic species are extracted from the film. In contrast to pure water, electrolyte solutions lower the surface tension of water enabling it to penetrate the hydrophobic film and dissolve impurities. Similar observations are also made after exposure to other electrolytes including 0.1 M NaF, NaOH, NaSCN, and H$_2$SO$_4$. Significantly, very little graphitization of the aromatic contamination molecules is observed, indicating once again that excessive heating of the surface is averted.

The effects of nonaqueous solvents, specifically CCl$_4$ and CH$_3$CN, on the film structure of contaminated C$_3$SH SAMs were also investigated. A substantial change is observed in the Raman spectra of C$_3$SH SAMs on Ag after a 5 min emersion in CCl$_4$ and
Figure 5.4. Raman spectra of carbon-contaminated C$_2$H SAM on Ag a) before, and after exposure to 0.1M b) NaCl and c) LiClO$_4$ for 5 min. Integration times: 120 sec.
CH$_3$CN as shown in Figure 5.5. The bands due to the small aromatic molecule contamination broaden and their intensity decrease after nonaqueous solvent exposure, the band at 1125 cm$^{-1}$ completely disappears (no vibrational mode at 1125 cm$^{-1}$ occurs for either liquid or solid C$_3$SH), and several C$_3$SH modes appear.

These spectral changes suggest the accumulation of two effects: the extraction of the majority of these small aromatic molecules from the SAM and graphitization of the remaining molecules within the SAM. Interestingly, graphitization is promoted in the nonaqueous solvents, unlike after exposure to aqueous electrolytes. Most likely, the nonaqueous solvents are not adequate cooling media for the surface during laser exposure and heating. These observations further support faster graphitization kinetics of aromatic contamination at higher temperatures.

Longer Chain SAMs

The Raman spectra of C$_4$SH, C$_{12}$SH and C$_{18}$SH SAMs on Ag are shown in Figure 5.6. Similar carbon contamination is also observed in longer chain alkanethiol SAMs. However, the contamination in these films appears to be more graphitic in nature as evidenced by the intensity and breadth of the peaks in the 1200-1600 cm$^{-1}$ region. The greater van der Waals interactions between alkyl chains in these longer chain length SAMs may prevent significant intercalation of individual, small aromatic molecules. Thus, the aromatic contamination readily graphitizes into islands with laser exposure. This behavior was observed for C$_4$SH and longer SAM chain lengths on Ag for 150 mW of laser power
Raman spectra of carbon-contaminated C,S,SH SAM on Ag a) before, and after exposure to neat b) acetonitrile and c) CCl₄, for 5 min. Integration times: 120 sec.
Figure 5.6. Raman spectra of carbon-contaminated a) C$_{6}$SH b) C$_{12}$SH, c) C$_{18}$SH SAMs. Integration times: 120 sec.
and acquisition times of more than a few seconds. It is difficult to draw conclusions about changes in the Raman spectra of carbon contaminated, long chain SAMs upon solvent exposure, since the form of contamination is already graphitized.

**Conclusions**

Surface Raman spectra have been acquired for alkanethiol SAMs contaminated with various forms of carbon on smooth polycrystalline Ag surfaces. These spectra provide insight into SAM spectral response as a function of this carbon contamination. The carbon impurities exist predominantly as small polynuclear hydrocarbon species, which are intercalated into the SAM. In the presence of such aromatic impurities, the spectral response of the SAM is consumed by the high scattering of the contamination. Under these circumstances, the bands associated with C,SH are barely visible because the carbon contamination bands are extraordinarily intense because of resonance enhancement. As these aromatic molecules graphitize under laser exposure, the intensity of the contamination bands decease and C,SH bands are observed.

Overall, the results of this investigation suggest that the nature of carbon contamination is extremely sensitive to environment. Laser power, laser exposure time, SAM chain length, solvent, electrolyte and surface preparation affect the structure of contamination, which in turn affects SAM structural order. The following chapter describes an approach for minimizing carbon contamination so that the effects of solvents, electrolyte, and potential can be investigated for “pure” SAM films.
Chapter 6

Electrochemical Cleaning of Surface-Confined Carbon Contamination In Self-Assembled Monolayers on Ag Evaluated By Raman Spectroscopy and Cyclic Voltammetry

Introduction

Most of the self-assembled monolayer community utilizes metals exposed to the ambient atmosphere either during preparation or experimental use. These surfaces unavoidably experience contamination by carbon impurities, which become entrapped within the SAM during film formation as described in the previous chapter. Several cleaning protocols have been investigated for reducing carbon contamination before SAM formation including chemical polishing,\textsuperscript{6,1} Ar\textsuperscript{-} sputtering followed by annealing,\textsuperscript{6,2,6,3} and electrochemical polishing.\textsuperscript{6,4,6,5} Ar\textsuperscript{-} sputtering has proven to be the most effective method for preparing clean (carbon-free) surfaces.\textsuperscript{6,6} However, these surfaces experience significant contamination when exposed to the ambient environment,\textsuperscript{6,6} a necessary setting for most SAM preparation and analysis.

Films formed by other techniques, for example vapor deposition, might also contain such carbon inclusions after exposure to the ambient environment. When present, carbon contamination may alter SAM properties such as pinhole density. Fractions of the observed pinholes measured by cyclic voltammetry\textsuperscript{6,7,6,10} might actually be sites of carbon
inclusions. Electron transfer may preferentially occur through sites of contamination. Any method for removing carbon impurities might result in SAMs containing fewer defects and improved order. In order to prepare well-ordered SAMs of alkanethiols on mechanically polished metal surfaces, the conditions for and effectiveness of this "electrochemical cleaning" protocol are investigated in this chapter.

**Amount of Carbon Contamination**

X-ray photoelectron spectroscopy (XPS) was used to assess the amount of carbon contamination occluded in short and long chain length SAMs on Ag and Au. A typical XPS spectrum of a contaminated C$_{3}$SH SAM on Ag in the C$_{1s}$ and S$_{2p}$ binding energy region is shown in Figure 6.1. The N$_{C_{1s}}$/N$_{S_{2p}}$ peak area ratio (corrected for escape depth and cross-section) for C$_{3}$SH SAMs on Ag is 3.8 ± 0.3 (n=3 individually prepared surfaces), indicating that the amount of contamination in these films is quite small (nominally, N$_{C_{1s}}$/N$_{S_{2p}}$ = 3 for pure C$_{3}$SH films). Interestingly, C$_{3}$SH SAMs on Au are not as contaminated (N$_{C_{1s}}$/N$_{S_{2p}}$ = 3.3, n=1). Most likely, the additional electrochemical cleaning step involved for preparing Au substrates (cycling in 1.0 M H$_{2}$SO$_{4}$) removes surface contamination.

The N$_{C_{1s}}$/N$_{S_{2p}}$ peak area ratios for C$_{12}$SH SAMs on Ag and Au are 12.6 (n=1) and 11.0 (n=1), respectively, demonstrating that contamination is small in the long chain length films as well. These XPS measurements further support the high sensitivity of Raman spectroscopy to carbon contamination at Ag surfaces.
Figure 6.1. XPS survey spectrum of a "dirty" C$_3$H SAM on Ag.
Contamination Removal with Negative Potential

Alkanethiol Reductive Desorption

The reductive desorption behavior of alkanethiol SAMs has been widely reported.\textsuperscript{6,11-6,14} Previous results by Bryant in this laboratory established the electrochemical behavior of these films at mechanically polished Ag in 0.1 M KCl and 0.5 M KOH.\textsuperscript{6,14} The reductive desorption behavior of C\textsubscript{3}SH, C\textsubscript{12}SH, and C\textsubscript{18}SH SAMs at Ag in other aqueous electrolytes (NaF, NaCl, NaOH, NaSCN) has also been investigated and is discussed in Chapter 7. The following paragraph is a summary of the reductive desorption behavior of alkanethiol SAMs on Ag in 0.1 M NaF.

Reductive desorption for C\textsubscript{3}SH films occurs at potentials negative of ca. -0.9 V in NaF. This process is observed as a well-defined wave in the cyclic voltammetry of these films. For longer chain length alkanethiols (C\textsubscript{12}SH and C\textsubscript{18}SH) on Ag, a reductive desorption peak is not observed in any of the electrolytes studied. In these systems, reductive desorption of the film overlaps with solvent breakdown (ca. -1.4 V), preventing accurate determination of the reductive desorption potential. For both short and long chain length SAMs, however, alkanethiol vibrations are still observed in the Raman spectra of these molecules after subjecting the SAM-modified Ag surface to potentials negative of -2.0 V, suggesting that reductive desorption does not remove or displace the SAM completely from the metal surface (see Chapter 7).
C$_{3}$SH SAMs on Ag

As described in the previous chapter, Raman spectra of alkanethiol SAMs are cluttered with carbon contamination bands, especially in the 1200-1600 cm$^{-1}$ region. The spectral signature of these contaminating species interferes significantly with the vibrational response of adsorbed alkanethiol, especially in the $\delta$(C-H) region. An example of a contaminated C$_{3}$SH SAM on Ag is shown in Figure 6.2a. Notably, the form of carbon contamination within this film is predominantly graphitic-like as evidenced by the shape, intensity, and position of these peaks.

The emersion approach was used to expose the C$_{3}$SH SAM to 0.1 M NaCl. As shown in Figure 6.2b, there is little change in the Raman spectrum upon exposure to aqueous electrolyte; however, significant changes are observed at potentials negative of thiolate reductive desorption as shown in Figure 6.2c. In addition, the features due to graphite-precursor molecules are markedly reduced suggesting carbon impurity removal, similar to the effects of aqueous electrolyte exposure described in Chapter 5. This phenomenon is irreversible, since contamination bands do not reappear after the potential is returned to positive values, indicating complete removal of these contaminating species from the film. The loss of these carbon impurities is accompanied by a noticeable decrease in the $\nu$(C-S)$_{\alpha}$ to $\nu$(C-S)$_{\gamma}$ intensity ratio from ca. 0.22 to 0.12, suggesting that once these carbon impurities are removed from the film, an increase in order occurs, specifically near the headgroup. Identical observations are made in other aqueous electrolytes including 0.1 M NaF, NaOH, NaSCN, and H$_2$SO$_4$. 
Figure 6.2. Raman spectra of a C$_2$SH SAM on Ag in the following environments: a) N$_2$ (ex situ), and upon 0.1 M NaCl exposure at b) 0.0 V, c) -1.0 V, and d) -0.3 V. All spectra plotted on same intensity scale. Integration times: 60 sec x 5.
C_{12}SH SAMs on Ag

Similar behavior is observed with negative potential for longer chain length SAMs. The Raman spectrum from a C_{12}SH SAM on Ag is shown in Figure 6.3a. Once again, little change is observed in the Raman spectra of C_{12}SH SAMs upon exposure to 0.1 M NaF for up to 30 min (the longest time evaluated) as shown in Figure 6.3b. Application of a negative potential, but one positive of the reductive desorption potential for C_{12}SH (ca. -1.5 V), does not remove the carbon impurities either as shown in Figure 6.3c. Although the monolayer order increases slightly as evidenced by a small decrease in the ν(C-S)\_G to ν(C-S)\_T intensity ratio (0.39 to 0.35), the spectral response of the carbon impurities is still clearly evident in the 1200-1600 cm\(^{-1}\) region. Only after the potential is held negative of C_{12}SH reductive desorption (e.g., -2.0 V, Figure 6.3d) does the contaminant intensity in this region diminish and peaks due to the C_{12}SH SAM emerge. As a result of graphitic carbon removal, the ν(C-S)\_G to ν(C-S)\_T intensity ratio decreases from 0.31 to 0.17 and the peaks sharpen, indicating increased order within the SAM.

Finally, the overall intensities of the monolayer modes increase markedly upon application of very negative potentials. This behavior is attributed to surface roughening by water reduction through defect sites. To date, no reports on surface roughening through monolayer defect sights have appeared; however, hydrogen evolution at negative potentials (used to remove surface contaminants) has been reported to roughen Ag surfaces considerably.\(^6\)\(^{-15}\)\(^{-17}\) Surface roughening increases the area of the surface, and as a result, the surface coverage of the monolayer decreases. Nevertheless, the films appear
Figure 6.3. Raman spectra of a C$_{18}$SH SAM on Ag in the following environments: a) N$_2$ (ex situ), and upon 0.1 M NaF exposure at b) 0.0 V, c) -0.7 V, and d) -2.0 V. All spectra plotted on same intensity scale. Integration times: 60 sec x 5.
well-ordered, suggesting that the alkanethiol molecules remaining at the surface at negative potentials congregate together and form islands.

C\textsubscript{18}SH SAMs on Ag

Carbon contamination can also be present in C\textsubscript{18}SH SAMs on Ag as shown in Figure 6.4a. Exposure of the C\textsubscript{18}SH SAM to 0.1 M NaCl for up to 30 min does not affect the monolayer structure (similar to the C\textsubscript{12}SH). At potentials negative of C\textsubscript{18}SH reductive desorption (ca. -1.5 V), the spectral response due to these impurities completely vanishes. In addition, the $v(C-S)\text{G}$ to $v(C-S)\text{T}$ intensity ratio decreases from 0.43 to 0.16 and the modes attributed to the alkanethiol narrow significantly indicating increased order as a result of carbon removal.

The carbon removal and order increase is irreversible when the potential is returned to -0.1 V (Figure 6.4d). This observation is significant, because Mahoney and coworkers observed a similar decrease in the 1200-1600 cm$^{-1}$ region on bare-Ag surfaces at ca. -1.2 V which they attributed to graphite conversion to hydrocarbons.$^6$ However, the spectral features in this region reappeared, sometimes with greater intensity than originally, when the potential was returned to -0.2 V (SCE),$^6$ indicating the return of carbon contamination. For SAM-modified Ag surfaces, the carbon removal observed by negative potential application is permanent, even when the applied potential is again made positive.
Figure 6.4. Raman spectra of a C₁₈SH SAM on Ag in the following environments: a) N, (ex situ), and upon 0.1 M NaF exposure at b) -0.1 V, c) -1.5 V, and d) -0.1 V after -1.5 V. All spectra plotted on same intensity scale. Integration times: 60 sec x 5.
**Electrochemical Characterization**

Evidence for the quality of these SAMs can be obtained from electrochemical characterization (see Chapter 1). Capacitance values for these films were determined from linear potential sweep experiments in regions in which no Faradaic processes occur in 0.1 M NaF. The capacitive current is the product of the sweep rate, capacitance, and electrode surface area. The capacitance for C\textsubscript{3}SH, C\textsubscript{12}SH, and C\textsubscript{18}SH SAMs on mechanically polished Ag surfaces are given in Table 6.1. These values are approximately an order of magnitude higher than those reported for well-ordered SAMs on freshly evaporated Au surfaces.\(^6\)\(^1\)\(^8\)-\(^6\)\(^2\)\(^0\) (Capacitance values for SAMs on Ag have not been reported in the literature.)

Such discrepancy can be attributed to two factors. First, SAM formation on Ag may be different than on Au, as a result of dissimilar surface morphology (i.e., a larger number of grain boundaries), influencing capacitance values. More importantly, the graphitic-like contamination described above and in Chapter 5 might be conductive or influence the structure of the film such that non-Faradaic processes occur to a greater extent.

Cyclic voltammetry of Ru(NH\textsubscript{3})\textsubscript{6}\textsuperscript{3-} at alkanethiol SAM-modified Ag electrodes was used to estimate how much of the film remains on the surface after the negative potential application required to remove carbon contamination. Ru(NH\textsubscript{3})\textsubscript{6}\textsuperscript{3-} was chosen as the redox system, because SAMs are stable throughout the electrochemical window in which Ru(NH\textsubscript{3})\textsubscript{6}\textsuperscript{3-} redox chemistry occurs (see Chapter 7).
Table 6.1. Capacitance Measurements for SAM-Modified Ag Surfaces.

<table>
<thead>
<tr>
<th>Alkanethiol SAM</th>
<th>Capacitance ((\mu\text{F/cm}^2))^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>C\textsubscript{3}SH</td>
<td>43.6 ± 21.6</td>
</tr>
<tr>
<td>C\textsubscript{12}SH</td>
<td>27.2 ± 6.8</td>
</tr>
<tr>
<td>C\textsubscript{18}SH</td>
<td>16.9 ± 4.7</td>
</tr>
</tbody>
</table>

^ Standard deviations result from the analysis of a minimum of 3 samples.
Potential scans were performed between +0.050 V and -0.240 V at sweep rates of 100 mV/s. Prior to electrochemical cleaning of the SAMs, the cyclic voltammetry of Ru(NH$_3$)$_6^{3-}$ reduction at bare, mechanically polished and SAM-modified Ag surfaces was measured; these voltammograms in 1.0 mM Ru(NH$_3$)$_6^{3+}$/0.1 M KCl are shown in Figure 6.5. Short chain SAMs on Ag, such as C$_3$SH, only slightly impede the redox behavior of Ru(NH$_3$)$_6^{3-}$ due to relatively efficient electron tunneling across the film and electron transfer through the significant number of defects present in short chain SAMs. The shape of the cyclic voltammograms for C$_{12}$SH and C$_{18}$SH modified electrodes (Figure 6.5c,d) implies radial diffusion. Such behavior suggests that electron transfer occurs through pinholes and the modified-electrode functions as multiple microelectrodes. As expected, long chain alkanethiol SAMs block electron transfer more effectively than C$_3$SH.

Although both reductive desorption of alkanethiol SAMs and solvent breakdown occur at the negative potentials required for carbon contamination removal (-0.9 V for C$_3$SH and ca. -1.5 V for C$_{12}$SH and C$_{18}$SH SAMs), the spectral evidence suggests that these processes do not completely displace the SAM from the interface. For all chain lengths, vibrational modes due to alkanethiols are still observed after subjecting the surfaces to potentials negative of -2.0 V.

Cyclic voltammetry was used as a measure of the Ag surface exposed before and after this negative potential cleaning process under the assumption that the observed current for reduction of Ru(NH$_3$)$_6^{3-}$ is inversely related to the alkanethiol surface coverage. The parameter that is used as the indicator is the ratio of the peak reduction
Figure 6.5. Cyclic voltammograms in 1.0 mM Ru(NH$_3$)$_6^{3+}$/0.1 M KCl for a) mechanically polished bare Ag, and b) C$_3$SH, c) C$_{12}$SH, d) C$_{18}$SH SAMs on Ag. Sweep rate = 100 mV/s.
peak current for Ru(NH$_3$)$_6^{3+}$ at the SAM-modified electrode ($i_{SAM}$) to that measured at the bare Ag surface ($i_{Ag}$). Thus, the ratio of ($i_{SAM} / i_{Ag}$) x 100% is defined as the normalized percent electrochemically active surface area (%EAS). A bare Ag surface is 100% electrochemically active and able to reduce Ru(NH$_3$)$_6^{3+}$ at the maximum rate.

As shown in Table 6.2, C$_3$SH films are still quite conductive after film formation. Short chain films have a large %EAS (78.7 ± 13.6 %) due to relatively efficient electron tunneling through the SAM. As expected, longer chain length SAMs block electron transfer more effectively (0.22 ± 0.16 % for C$_{12}$SH and 0.11 ± 0.05 % for C$_{18}$SH). The magnitude of these values must be interpreted with care, since the bulk of electron transfer occurs through defect sights. As a result, the microelectrode array behavior (observed in Figure 6.5b-d) gives rise to inflated $i_{SAM}$ values as a result of radial diffusion contributions at low sweep rates. Therefore, the “true” %EAS values are actually smaller than shown in Table 6.2.

SAM-modified Ag surfaces containing carbon impurities were then “electrochemically cleaned” in an unstirred 0.1 M NaF solution for 120 sec at a potential negative of reductive desorption of the alkanethiol-SAM or water reduction (-1.1 V for C$_3$SH, -1.5 V for C$_{12}$SH, -1.8 V for C$_{18}$SH). A large increase in the Ru(NH$_3$)$_6^{3+}$ cathodic current is observed after this step, indicating the removal of carbon and alkanethiol. The cyclic voltammograms for C$_3$SH and C$_{12}$SH (shown in Figure 6.6) indicate significant removal of alkanethiol molecules from the surface after negative potential application. Interestingly, the voltammogram for C$_{18}$SH suggests microelectrode-like behavior at lower
Table 6.2. % Electrochemically Active Surface for SAM-Modified Surfaces after Various Treatments.

<table>
<thead>
<tr>
<th>Alkanethiol SAM</th>
<th>% Electrochemically Active Surface&lt;sup&gt;a&lt;/sup&gt;</th>
<th>After Immersion (electrolyte independent)</th>
<th>After Electrochemical Cleaning&lt;sup&gt;b&lt;/sup&gt;</th>
<th>After Re-Immersion</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;3&lt;/sub&gt;SH&lt;sup&gt;c&lt;/sup&gt;</td>
<td>78.7 ± 13.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>117.3 ± 24.4</td>
<td>84.1 ± 16.0</td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;12&lt;/sub&gt;SH</td>
<td>0.22 ± 0.16</td>
<td>54.0 ± 37.3</td>
<td>0.35 ± 0.19</td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;18&lt;/sub&gt;SH</td>
<td>0.11 ± 0.05</td>
<td>93.0 ± 24.4</td>
<td>0.26 ± 0.14</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> All values normalized to bare (unmodified) Ag surfaces. Standard deviations are reported for a minimum of 3 trials on each SAM type.

<sup>b</sup> The applied potentials are monolayer dependent: -1.1 V for C<sub>3</sub>SH; -1.5 V for C<sub>12</sub>SH; -1.8 V for C<sub>18</sub>SH. All potential application times are for 120 sec.

<sup>c</sup> High %EAS values represent efficient electron tunneling through short chains and defects.

<sup>d</sup> Standard deviations result from the analysis of a minimum of 3 samples.
Figure 6.6. Cyclic voltammetry in 1.0 mM Ru(NH$_3$)$_6$$^{3+}$ / 0.1 M KCl for a) C$_2$SH, c) C$_{15}$SH, d) C$_{18}$SH SAMs on Ag before and after negative potential treatment. Sweep rate = 100 mV/s.
monolayer coverages.

As shown by the data in Table 6.2, the removal of alkanethiol by a negative potential application beyond that of reductive desorption is evidenced by an increase of the %EAS. In fact, for C\textsubscript{1}SH SAMs, the %EAS after cleaning is greater than 100% indicating slight surface roughening as a result of this process. A similar elevation due to surface roughening is assumed to occur with the longer chain SAMs as well. For example, an increase from 0.11 to 93\% for C\textsubscript{18}SH does not imply 90\% of the SAM is removed, but rather a combination of surface roughening and alkanethiol desorption. Recall, such behavior was also observed in the Raman spectroscopy of these SAMs at negative potentials.

In conclusion, although negative potentials are effective for removing carbon impurities from SAMs on Ag, these potentials remove alkanethiol molecules as well. Therefore, the result of this negative potential treatment is an incomplete SAM film.

**Electrode Re-immersion**

An additional step was added to this cleaning protocol in an attempt to address the problem of alkanethiol monolayer depletion at negative potentials. In order to fill in and reanneal regions depleted of alkanethiol, the "cleaned" surface is re-immersed in thiol solution. A re-immersion time equivalent to that used in the original film formation procedure is necessary for creating highly-ordered monolayers as determined by Raman spectroscopic investigation of headgroup order. Shorter re-immersion times (ca. 1 h)
produce disordered films, evidenced by a significant intensity of the ν(C-S)$_G$ mode.

When done properly, electrochemical cleaning and re-immersion reproducibly results in SAM-modified interfaces which are highly-ordered and possess little or no carbon contamination on mechanically-polished surfaces. Raman spectra of C$_3$SH, C$_{12}$SH, and C$_{18}$SH SAMs on Ag before and after this electrochemical cleaning/re-immersion protocol are shown in Figures 6.7-6.9, respectively.

Several interesting and important spectral changes are observed in the spectra after cleaning and re-immersion. First, for SAMs of all chain lengths, the removal of carbon impurities (indicated by disappearance of the 1600 cm$^{-1}$ band) and a significant decrease in background in the 1200-1600 cm$^{-1}$ region allows resolution of several δ(C-H) modes not previously observed or reported for SAMs on mechanically polished Ag substrates. These modes are assigned in Table 6.3.

Of perhaps greater importance is the increase in monolayer order near the head group indicated by a decrease in the ν(C-S)$_G$ / ν(C-S)$_T$ intensity ratios tabulated in Table 6.4. This behavior suggests that carbon contamination affects film structure, specifically, monolayer order near the metal interface (head group) from which these impurities originate.

Further evidence for overall improved film order after electrochemical cleaning and re-immersion comes from the narrower band widths observed in the Raman spectra of “clean” SAMs relative to carbon contaminated SAMs. The full width at half maximum (FWHM) value for the ν(C-S)$_T$ band at 710 cm$^{-1}$ changes from ca. 34 to 27 cm$^{-1}$ for C$_3$SH,
Figure 6.7. Raman spectra of a C$_2$SH SAM on Ag a) after 3 h immersion in C$_2$SH/ethanol solution under N$_2$, b) after electrochemical cleaning with a 3 h initial immersion, 120 sec at -1.1 V in 0.1 M NaF and 3 h re-immersion in C$_2$SH/ethanol solution under N$_2$. Integration times: 60 sec x 5.
Figure 6.8. Raman spectra of a C$_{12}$SH SAM on Ag a) after 24 h immersion in C$_{12}$SH/ethanol solution under N$_2$, b) after electrochemical cleaning with a 24 h initial immersion, 120 sec at -1.5 V in 0.1 M NaF and 24 h re-immersion in C$_{12}$SH/ethanol solution under N$_2$. Integration times: 60 sec x 5.
Figure 6.9. Raman spectra of a C$_{18}$SH SAM on Ag a) after 24 h immersion in C$_{18}$SH/ethanol solution under N$_2$, b) after electrochemical cleaning with a 24 h initial immersion, 120 sec at -1.8 V in 0.1 M NaF and 24 h re-immersion in C$_{18}$SH/ethanol solution under N$_2$. Integration times: 60 sec x 5.
Table 6.3. Raman Peak Frequencies (cm\(^{-1}\)) and Assignments in the 1200-1600 cm\(^{-1}\) Region for C\(_3\)SH, C\(_{12}\)SH, and C\(_{18}\)SH SAMs on Polycrystalline Ag.

<table>
<thead>
<tr>
<th>C(_3)SH</th>
<th>C(_{12})SH</th>
<th>C(_{18})SH</th>
<th>assignment(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1212</td>
<td>1238</td>
<td>1285</td>
<td>CH(_2) twist</td>
</tr>
<tr>
<td>1215</td>
<td>1236</td>
<td>1294</td>
<td>CH(_2) twist</td>
</tr>
<tr>
<td>1259</td>
<td>1272</td>
<td>1325</td>
<td>CH(_2) twist</td>
</tr>
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<td>1295</td>
<td>1373</td>
<td>CH(_2) twist</td>
</tr>
<tr>
<td>1294</td>
<td>1378</td>
<td>1424</td>
<td>CH(_2) wag</td>
</tr>
<tr>
<td>1352</td>
<td>1377</td>
<td>1433</td>
<td>CH(_2) bend</td>
</tr>
<tr>
<td>1435</td>
<td>1449</td>
<td>1454</td>
<td>CH(_2) scissor</td>
</tr>
<tr>
<td>1453</td>
<td></td>
<td></td>
<td>CH(_2) scissor</td>
</tr>
</tbody>
</table>

\(^a\)Assignments taken from reference 6.22.
Table 6.4. *Ex situ* $I_{\nu(C-S)_{3}}/I_{\nu(C-S)_{2}}$ in the Absence and Presence of Carbon Contamination.

<table>
<thead>
<tr>
<th>SAM</th>
<th>After Initial Immersion</th>
<th>After Cleaning$^a$ and Re-Immersion</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{3}$SH</td>
<td>0.44 ± 0.12$^b$</td>
<td>0.24 ± 0.06</td>
</tr>
<tr>
<td>$C_{12}$SH</td>
<td>0.32 ± 0.17</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td>$C_{18}$SH</td>
<td>0.45 ± 0.12</td>
<td>0.27 ± 0.06</td>
</tr>
</tbody>
</table>

$^a$ Applying a negative potential beyond that of reductive desorption for 120 sec.

$^b$ Standard deviations result from the analysis of a minimum of 3 samples.
ca. 43 to 37 cm\(^{-1}\) for \(\text{C}_{12}\text{SH}\), and ca. 48 to 41 cm\(^{-1}\) for \(\text{C}_{18}\text{SH}\) following removal of contamination. Similar trends are observed when comparing the FWHM peak values of neat liquid and solid alkanethiols. Narrower bands are consistent with a more crystalline-like monolayer.

In addition, the Raman background intensity also decreases after the electrochemical cleaning/re-immersion protocol for all alkanethiol-SAMs studied. Such behavior suggests that the carbon contamination contributes significantly to the overall spectral background for SAMs on Ag. This might be expected due to the high Raman scattering and fluorescence efficiency of polyaromatic-like molecules.

Evidence for the formation of highly-ordered SAMs after re-immersion is also evident in the cyclic voltammetry and capacitance measurements of “clean” SAM-modified surfaces. Cyclic voltammograms of alkanethiol SAMs after electrochemical cleaning (before re-immersion) and after re-immersion are shown in Figure 6.10. As quantified in Table 6.2, the % EAS after re-immersion returns to values similar to those measured before negative potential treatment (after the initial immersion), indicating the reformation of a blocking layer for each chain length (complete alkanethiol monolayer). The small standard deviations in both the \(\nu(\text{C-S})_\text{G}\) to \(\nu(\text{C-S})_\text{T}\) intensity ratios and %EAS values indicate the extraordinary reproducibility of this electrochemical cleaning protocol. In addition, within standard deviation, clean films possess similar %EAS values compared to contaminated films suggesting that alkanethiol molecules replace sites previously occupied by carbon impurities. This observation suggests, quite significantly, that the carbon
Figure 6.10. Cyclic voltammetry in 1.0 mM Ru(NH$_3$)$_6^{3+}$ / 0.1 M KCl for a) C$_2$SH, c) C$_{12}$SH, d) C$_{18}$SH SAMs on Ag before (after negative potential treatment) and after re-immersion. Sweep rate = 100 mV/s.
contamination present initially must be largely non-conductive when entrapped within the SAM. In addition, the form of the contamination must be a precursor to graphite, since graphite is known to be conductive. These results suggest that carbon contamination, although detrimental for achieving exceptional monolayer order, does not change the dielectric properties of the film. Therefore, electrochemical estimates of pinhole density within SAMs remain reliable, regardless of contamination stemming from mechanical polishing.

Additional evidence for the formation of highly-ordered SAMs after electrochemical cleaning comes from capacitance measurements in 0.1 M NaF. The capacitance of "clean" SAMs is significantly smaller (ca. 5 times) than that for "dirty" SAMs as shown in Table 6.5, suggesting a larger number of defects for contaminated SAMs. The combination of the surface Raman spectroscopy, %EAS, and capacitance measurements clearly indicates that SAMs contaminated with carbon impurities are less ordered.

**Electrochemical Treatment Without SAM**

Koglin and co-workers reported the use of cetylpyridinium chloride as a surfactant for removing melamine, a stable heterocyclic structure, pre-adsorbed on Ag surfaces. The surfactant was shown to desorb from the surface with the melamine at negative potentials. Without the surfactant, melamine was not displaced from the surface at similar negative potentials. This report led us to question the role of the alkanethiol SAM in the
Table 6.5. Capacitance Measurements for “Clean” SAM-Modified Ag Surfaces.

<table>
<thead>
<tr>
<th>Alkanethiol SAM</th>
<th>Capacitance (µF/cm²)ᵃ</th>
<th>“dirty” SAM on Ag</th>
<th>“clean” SAM on Agᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₃SH</td>
<td>43.6 ± 21.6</td>
<td></td>
<td>9.1 ± 6.5</td>
</tr>
<tr>
<td>C₁₂SH</td>
<td>27.2 ± 6.8</td>
<td></td>
<td>5.1 ± 2.6</td>
</tr>
<tr>
<td>C₁₈SH</td>
<td>16.9 ± 4.7</td>
<td></td>
<td>4.2 ± 1.9</td>
</tr>
</tbody>
</table>

ᵃ Standard deviations result from the analysis of a minimum of 3 samples.

ᵇ Following electrochemical cleaning treatment: immersion, negative potential application beyond that of reductive desorption for 120 sec and re-immersion.
effectiveness of carbon contamination removal. Thus, an alternate electrochemical cleaning strategy involving negative potential application prior to initial SAM formation was investigated.

Mechanically polished, unmodified Ag surfaces were electrochemically pre-treated in 0.1 M NaF at -2.0 V for 5 min before initial alkanethiol-SAM formation. Raman spectra of bare Ag, before and after this treatment, and after C$_3$SH SAM formation are shown in Figure 6.11. The origin of graphitic-like contamination found in SAMs is clearly from the Ag substrate, as observed in the 1200-1600 cm$^{-1}$ of the Raman spectrum of bare Ag shown in Figure 6.11a. A few spectral changes are observed for bare Ag after negative potential application. The appearance of peaks at 801, 920, and 1047 cm$^{-1}$ suggest that some surface contamination is reduced to hydrocarbons; however, most of the graphite-like contamination is not changed or removed as evidenced by the intensity of the 1200-1600 cm$^{-1}$ bands. Not surprisingly, these impurities are trapped within the C$_3$SH film after formation as evidenced by dominant contamination modes. The C$_3$SH SAM formed after this pretreatment strategy (negative potential application before SAM formation) is not well-ordered (I [ν(C-S)$_G$] / I [ν(C-S)$_T$] of 0.59 versus 0.24 for electrochemical cleaning with SAM and re-immersion).

Although not shown, long chain SAMs formed after electrochemically pre-treating bare Ag electrodes also possess similar disorder and carbon contamination. These results strongly suggest that the alkanethiol SAM is a necessary component for removing carbon contamination. The alkanethiol SAM is proposed to serve as a surfactant, facilitating
Figure 6.11. Raman spectra of a) mechanically polished, bare Ag surface b) following electrochemical pre-treatment for 120 sec at -2.0 V in 0.1 M NaF, c) C\textsubscript{3}SH SAM on Ag formed after pre-treatment, d) C\textsubscript{3}SH on Ag from c) after standard electrochemical cleaning for 120 sec at -1.1 V in 0.1 M NaF. *denotes hydrocarbon bands. Integration times: 60 sec x 5.
removal of impurities after reductive desorption from the Ag surface at sufficiently negative potentials.

In summary, negative potential treatment of SAM-modified Ag in aqueous electrolyte effectively removes carbon contamination entrapped within SAMs. As evidenced by Raman spectroscopy and electrochemistry, the removal of carbon impurities is most effective at potentials negative of SAM reductive desorption where adsorbed alkanethiol molecules are also removed resulting in surfaces with less than monolayer coverage.

An effective, reproducible cleaning protocol was developed in which re-immersion of the surface into alkanethiol solution after electrochemical treatment results in carbon impurity-free SAM films with an exceptionally high-degree of structural order. Electrochemical treatment of unmodified (i.e., bare) Ag surfaces prior to initial alkanethiol formation does not effectively remove these carbonaceous impurities. Thus, the SAM serves the role of a surfactant in the removal of carbon contamination. A model for this electrochemical cleaning protocol is shown in Figure 6.12.

**Pyrene as a Model for Carbon Contamination**

In an attempt to test the proposed model described above, experiments using the model polynuclear aromatic hydrocarbon pyrene were undertaken. Toward this end, a mechanically polished Ag surface was spin-coated with 100 μL of a 1x10^{-6} M pyrene/EtOH solution and immersed into a C_{12}SH solution for 24 hr, resulting in pyrene
Figure 6.12. A schematic of the electrochemical cleaning protocol.
incorporation into the film. The Raman spectra of a Ag electrode before and after pyrene spin-coating, as well as after SAM formation and negative potential treatment are shown in Figure 6.13. As anticipated, the bare Ag spectrum contains significant carbon contamination as evidenced by the intense 1300 and 1570 cm\(^{-1}\) graphite "cathedral peaks". After pyrene spin-coating, several prominent pyrene modes are observed at 1128, 1195, 1235, 1250, 1379, 1500, 1595, and 1630 cm\(^{-1}\). These modes are assigned as aromatic \(\nu(C-C)\) and \(\delta(C-H)\) bending modes. The intensity of these pyrene bands are similar in magnitude to the carbon contamination bands observed in alkanethiol SAMs on Ag. Furthermore, several of the pyrene bands correspond directly to the commonly observed carbon contamination bands at 1128, 1500, and 1595 cm\(^{-1}\).

Comparison of the Raman spectra before and after alkanethiol immersion of the pyrene spin-coated surfaces suggests pyrene entrapment within the SAM, as evidenced by the distinct pyrene modes at 1235, 1379, and 1500 cm\(^{-1}\) and monolayer modes at 635, 707, 890, 983, 1063, and 1127 cm\(^{-1}\) in Figure 6.13c. These results are surprising, because pyrene is soluble in ethanol. (The Raman spectrum of a pyrene spin-coated electrode after 30 min of immersion in pure ethanol contains no evidence of pyrene.) These results suggest that the contamination at mechanically polished Ag is not loosely attached at the surface, but rather strongly adsorbed. As shown in Figure 6.13d, pyrene entrapped within a SAM film is easily removed after only a few seconds at -1.5 V in 0.1 M NaF, demonstrating the necessity of alkanethiol molecules and negative potential application for removing carbon impurities.
Figure 6.13. Raman spectra of a) mechanically polished, unmodified Ag surface, b) spin-coated with pyrene/EtOH solution, c) C$_{12}$SH SAM on Ag surface formed after pyrene spin-coating, d) C$_{12}$SH SAM on Ag surface after electrochemical cleaning: 120 sec at -1.5 V in 0.1 M NaF. *denotes bands assigned to pyrene. + denotes C$_{12}$SH monolayer bands. Integration times: a), c), d) 60 sec x 5, b) 12 sec x 5.
Contamination of SAMs on Au

Carbon contamination is also present in alkanethiol SAMs on Au. Raman spectra in the 1300-1700 cm⁻¹ region (720 nm excitation) for C₃SH, C₁₂SH, and C₁₈SH SAMs formed on mechanically polished Au with and without "electrochemical polishing" (described below) are shown in Figures 6.14, 6.15, and 6.16, respectively. Two peaks are observed in this spectral region. The band at 1440 cm⁻¹ has been assigned as the scissor CH₂ mode. As for SAMs on Ag, the features at 1370 and 1590 cm⁻¹ are assigned to graphitic-like contamination originating from mechanical polishing.

Significantly, for SAMs on Au, the contamination appears somewhat structured as evidenced by the weak intensity of the 1370 cm⁻¹ band relative to the 1590 cm⁻¹ and the narrowness of the peak at 1590 cm⁻¹. Raman spectra of highly-ordered graphite display a much larger 1590 cm⁻¹ relative to 1370 cm⁻¹ band. In contrast, the Raman spectra of SAMs on Ag with amorphous carbon contamination exhibit two broad features from 1300-1600 cm⁻¹.

The surface cleaning protocol used by the monolayer community for Au substrates involves "electrochemical polishing" in 1 M H₂SO₄ prior to SAM formation. In this procedure, the electrode potential is cycled between -0.2 V and +1.2 V resulting in the formation and reduction of Au oxide. As shown in Figures 6.14b, 6.15b, and 6.16b, these oxidation reduction cycles (ORCs) effectively clean the surface, and as a result, contamination-free SAMs are formed. Therefore, the negative potential application strategy described above is not necessary for fabrication of clean SAMs on Au, because...
Figure 6.14. Raman spectra of a C₃SH SAM on Au a) without and b) with electrochemical cycling in 1.0 M H₂SO₄ prior to C₃SH immersion. Integration times: 10 sec x 90. Spectra plotted on same intensity scale.
Figure 6.15. Raman spectra of a C_{12}SH SAM on Au a) without and b) with electrochemical cycling in 1.0 M H_{2}SO_{4} prior to C_{12}SH immersion. Integration times: 10 sec x 90. Spectra plotted on same intensity scale.
Figure 6.16. Raman spectra of a $C_{16}SH$ SAM on Au a) without and b) with electrochemical cycling in 1.0 M $H_2SO_4$ prior to $C_{16}SH$ immersion. Integration times: 10 sec x 90. Spectra plotted on same intensity scale.
the contamination is removed prior to film formation. Similar ORCs in H₂SO₄ might also clean Ag; however, such a strategy might result in undesirable surface roughening.

Conclusions

The utility of self-assembled monolayers depends critically on their reproducible and uncontaminated fabrication. In turn, these factors depend on many aspects of the metal surfaces onto which they are bonded, including surface roughness, the chemical nature of the metal bond, and surface cleanliness. Although carbon contamination is present at both Au and Ag surfaces following mechanical polishing, it can be removed from Au by electrochemically cycling in H₂SO₄ prior to film formation. The preparation of clean, smooth Ag is not as straightforward, largely because of the higher degree of malleability and reactivity associated with this metal.

An electrochemical cleaning protocol has been developed for reproducible formation of highly-ordered, films free of carbon contamination. Significantly, negative potential application only removes the surface-bound contamination on Ag in the presence of a SAM. The data suggest that the SAM functions as a surfactant for removing carbon impurities from Ag. As evidenced by electrochemical data, a considerable number of alkanethiol molecules are also removed from the interface with the contamination at negative potentials, indicating the strength with which the contamination is adsorbed.

Cyclic voltammetry indicates that the carbon contamination present in self-assembled monolayers is not-conductive; therefore, although carbonaceous contamination
results in slightly less-ordered films, as evidenced by Raman spectroscopy and capacitance measurements, the relative blocking properties of impure films are not drastically compromised.
Chapter 7

Effects of Electrolyte and Potential on the *In Situ* Structure of Alkanethiol Self-Assembled Monolayers on Ag

**Introduction**

With the ability to form extremely well-defined, contamination-free films as demonstrated in the previous chapter, the stability of alkanethiol SAMs in an electrochemical environment can now be investigated. The goal of these studies was to obtain a better understanding of alkane chain order, structural integrity of the metal-sulfur bond, and SAM retention at the surface under conditions mimicking those for which many electrochemical applications of SAMs, including pacemaker electrode modification, have been proposed.

Given the importance of SAM structure at the molecular level on macroscopic film properties, further work characterizing the structural behavior of these films under different conditions is warranted. Several variables were chosen for study including alkanethiol chain length, electrolyte (NaF, NaCl, NaSCN, NaOH and H$_2$SO$_4$), potential (open circuit potential to -2.0 V dictated by electrode and monolayer stability) and chemical integrity of the initial monolayer (presence or absence of small molecule graphite precursor inclusions). To date, no reports comparing the effects of solvent and potential for different SAM chain lengths have appeared. Thus, alkanethiols were chosen to
represent the full range of chain lengths commonly employed in SAM studies and applications. Electrolytes were chosen to investigate the influence of specifically adsorbing anions (SCN⁻ and Cl⁻) versus non-adsorbing anions (F⁻) and strong acid (H₂SO₄) versus strong base (NaOH) environments.

Raman spectroscopy was selected for evaluating the effects of potential and electrolyte on the structural stability of SAMs for the following reasons. First, water is a weak Raman scatterer allowing access to all spectral regions in the Raman spectrum containing information about SAM structure and integrity. In addition, Raman spectroscopy provides abundant spectral information about the structure of alkanethiol SAMs, including information in low frequency regions that is not easily accessible with FTIR. As described in previous chapters, evidence for the degree of conformational order near the thiol head group can be ascertained from the ν(C-S) stretching region. The intensity ratio of the ν(C-S)_g band to the ν(C-S)_r band can be used to evaluate the degree of alkane chain ordering near the thiol head group for these systems. One might speculate that this portion of the alkane chain would be more affected by potential due to its proximity to the electrode.

Electrochemical Environment Effects on SAM Structure

The electrochemical reductive desorption of alkanethiol SAMs has been reported. Porter and co-workers were the first to characterize the chemistry of the bound thiol head group and report the reductive desorption of n-alkanethiol SAMs on
evaporated polycrystalline Ag and Au surfaces in 0.5 M KOH, LiOH, and NaOH. The products of this process are proposed to be Ag or Au and thiolate species, RS⁻. Previous work by Bryant also established the electrochemical behavior of these SAMs at mechanically polished, polycrystalline Ag surfaces in 0.1 M KCl and 0.5 M KOH. Reductive desorption of a propanethiol film occurs at ca. -1.0 V. For longer chain alkanethiols such as C₁₂SH and C₁₈SH, no reductive desorption peak is observed at polycrystalline Ag, regardless of electrolyte. In these systems, reductive desorption of the film along with solvent breakdown occurs simultaneously at potentials negative of ca. -1.4 V. However, for both short and long chain alkanethiol SAMs, Raman scattering is still observed from the monolayers after subjecting the Ag surface to potentials negative of -2.0 V. These results suggest that the reductive desorption process does not necessarily imply complete removal of the monolayer from the interface in these systems even though the metal-sulfur bond may be completely severed.

The effect of pure water on film order of these SAMs was first investigated to compare with previous results from other research groups. Earlier FTIR results of Porter and Anderson indicate that water has no influence on the structure of these monolayers. Surface Raman spectroscopy confirms the finding that water exhibits little effect on monolayer structure, even after 60 min of exposure. Thus, these studies demonstrate that SAMs in pure water are stable for times greater than those used in our electrolyte and potential experiments.
C$_3$SH SAMs on Ag

The reductive desorption behavior of C$_3$SH SAMs on Ag was first investigated in each electrolyte by cyclic voltammetry. Cyclic voltammograms at bare Ag (for comparison) and for C$_3$SH SAMs on Ag in 0.1 M NaF, NaCl, NaOH, NaSCN, and H$_2$SO$_4$ are shown in Figures 7.1a-e and 7.1f-j, respectively. A thiolate reductive desorption wave is observed at ca. -1.0 V in 0.1 M NaF, NaCl, and NaSCN and at -1.1 V in NaOH solutions. No SAM reductive desorption is observed in 0.1 M H$_2$SO$_4$ in the potential window available on C$_3$SH-modified Ag. The potential at which water reduction begins at a C$_3$SH-modified Ag electrode is ca. -1.4 V in all electrolytes studied except H$_2$SO$_4$. The onset of water reduction in 0.1 M H$_2$SO$_4$ is, as expected, shifted positive to ca. -0.8 V. The cyclic voltammetry for C$_3$SH-modified Ag suggests that interesting structural changes may be observed with Raman spectroelectrochemistry at potentials where reductive desorption occurs.

Raman spectra in the v(C-S) and v(C-C) regions (ca. 600-1700 cm$^{-1}$) from C$_3$SH SAMs on Ag in a N$_2$ environment (ex situ) and in the presence of 0.1 M NaCl are shown in Figure 7.2, for open circuit potential (OCP, ca. -0.15 V in this medium), -0.9 V and -1.1 V.

The v(C-S)$_G$ and v(C-S)$_T$ (hereafter referred to as G and T) vibrations for C$_3$SH occur at 628 and 701 cm$^{-1}$, respectively. The G mode is visible in all spectra, indicative of disorder near the thiol headgroup as expected for short chain films. In addition, Bryant noted in past work that with inherently rough surfaces, a greater degree of alkane chain
Figure 7.1. Cyclic voltammograms for bare Ag (top) and C₃SH on Ag (bottom) in 0.1 M a), f) H₂SO₄, b), g) NaSCN, c), h) NaCl, d), i) NaF, and e), j) NaOH. Sweep rate = 100 mV/s. Surface area of electrode is 0.03 cm².
Figure 7.2. Raman spectra of a C\textsubscript{2}SH SAM on Ag in the following environments:
a) N\textsubscript{2} (ex situ), and in 0.1 M NaCl at b) -0.2 V, c) -0.9 V, and d) -1.1 V.
All spectra plotted on same intensity scale. Integration times: 60 sec x 5.
disorder is observed in the vicinity of the head group.7,7 Apparently, the alkanethiol molecules accommodate surface roughness by disordering near the head group, resulting in a small G intensity in all spectra, even for longer chain SAMs.

Small spectral changes resulting from exposure of the C3SH SAM to electrolyte and potential were quantified by determining the ratio of the G/T intensities (I_R) to the background intensity (B) before and after exposure. The effect of slight surface roughening at negative potentials is removed by ratioing I_R to B. These I_R/B values are shown in Figure 7.3 for each electrolyte. C3SH SAMs exposed to 0.1 M NaCl at ca. -0.15 V exhibit essentially no change in order relative to the ex situ spectrum as evidenced by little variation in I_R/B even at potentials negative of reductive desorption (ca. -1.0 V). C3SH SAMs remain stable at -1.1 V for at least 30 min, the maximum time investigated. Interestingly, no ν(Ag-Cl) mode is observed at any potential, suggesting that significant amounts of Cl^- do not penetrate the film and specifically adsorb at the Ag surface.

The response of C3SH-modified Ag to exposure to 0.1 M NaF or 0.1 M NaSCN is quite different as shown in Figures 7.4 and 7.5. In both of these solutions, initial electrolyte exposure at ca. -0.02 V does not affect the order of the SAM relative to the ex situ spectrum as evidenced by the I_R/B ratios shown in Figure 7.3. These ratios are invariant, even after 45 min of electrolyte exposure at -0.02 V. At -0.4 V, no significant changes are observed in the I_R/B ratios. However, as the potential approaches the reductive desorption potential, the monolayers disorder in each electrolyte as indicated by significant increases in I_R/B, going from 1.2 ± 0.3 to 3.5 ± 0.5 in NaF and from 1.1 ± 0.2
Figure 7.3. $\nu(\text{C-S})_a/\nu(\text{C-S})_b$ peak intensity to background intensity ratios with respect to potential for C$_3$H SAMs on Ag in 0.1 M aqueous electrolytes.
Figure 7.4. Raman spectra of a C\textsubscript{3}SH SAM on Ag in the following environments: a) N\textsubscript{2} (ex situ), and in 0.1 M NaF at b) -0.1 V, c) -0.4 V, d) -0.8 V, and e) -1.1 V. All spectra plotted on same intensity scale. Integration times: 60 sec × 5.
Figure 7.5. Raman spectra of a C\textsubscript{3}SH SAM on Ag in the following environments: a) \textit{N\textsubscript{2}} (ex \textit{situ}), and in 0.1 M NaSCN at b) 0.0 V, c) -0.6 V, d) -0.9 V, and e) -1.1 V. All spectra plotted on same intensity scale. Integration times: 60 sec x 5.
to 2.8 \approx 0.7 \text{ in NaSCN. Significantly, the absolute peak intensities decrease by about 40%}
at negative potentials in NaSCN. This attenuation is not reversible if the potential is again
made positive, suggesting that some of the SAM is removed from the surface at
sufficiently negative potentials, perhaps being replaced by SCN⁻.

Interestingly, the ν(C≡N) mode at 2100 cm⁻¹ is observed in NaSCN solution at all
potentials indicating SCN⁻ penetration of the C₃SH SAM as shown in Figure 7.6. This is
expected because of the strong tendency of this anion to specifically adsorb on Ag. At ca.
0.0 V the frequency of this peak is 2112 cm⁻¹, implying SCN⁻ adsorption⁷.⁸,⁹ and thiol
displacement. As the potential is made negative, the ν(C≡N) band decreases in intensity
and shifts from 2112 to 2093 cm⁻¹, suggesting SCN⁻ desorption from the electrode.
Therefore, two types of SCN⁻ species exist at the electrode interface, depending on
potential. If the potential is again made positive, as shown in Figure 7.6d, the position of
this band shifts to higher wavenumbers conveying readsorption. In summary, the data
clearly demonstrate the ease at which ions can penetrate and displace C₃SH. (Significantly,
no ν(C≡N) mode is observed for C₁₂SH or C₁₈SH SAMs at any potential implying solvent
and electrolyte penetration in the C₃SH SAM and not simply detection of an electrolyte
layer at the outer edge of the SAM).

The spectra in Figure 7.7 show the behavior of C₃SH SAMs in 0.1 M NaOH. As
with the other electrolytes, initial exposure of the SAM to NaOH solution at -0.06 V does
not change its order. After the application of potentials as negative as -0.8 to -0.9 V, no
significant changes in the Iᵣ/Iᵦ ratio are observed for at least 15 min. However, as the
Figure 7.6. Raman spectra in the \( \nu(\text{C} \equiv \text{N}) \) region of a \( \text{C}_2\text{SH} \) SAM on Ag in the following environments: a) \( \text{N}_2 \, (\text{ex situ}) \), and in 0.1 M NaSCN at b) 0.0 V, c) -1.1 V, and d) -0.02 V after c). All spectra plotted on same intensity scale. Integration times: 60 sec x 5.
Figure 7.7. Raman spectra of a C₃SH SAM on Ag in the following environments:
a) N₂ (ex situ), and in 0.1 M NaOH at b) -0.1 V, c) -0.8 V, and d) -1.1 V.
All spectra plotted on same intensity scale. * denotes peak due to oxidized
thiol product. Integration times: 60 sec x 5.
potential approaches and becomes more negative than the reductive desorption potential for C$_3$SH in 0.1 M NaOH (ca. -1.0 V), the integrity of the SAM is destroyed and the $I_R/B$ ratio increases substantially, approaching a value of 7. The solubility of alkanethiolates in aqueous NaOH solutions has led to their use in many reductive desorption studies.$^{7,8-7,4}$ This solubility is expected to be enhanced for short alkanethiols such as C$_3$SH. This fact probably leads to the catastrophic loss of C$_3$SH signal at these negative potentials.

Closer inspection of the spectra at these negative potentials reveals significant changes in the vibrational modes observed from species in the interface that originally derived from the C$_3$SH. The spectral features observed are, in fact, suggestive of oxidized thiolate headgroups under these conditions. Various oxidation states of the thiol headgroup are suggested in the spectrum acquired at -1.1 V by the peaks at 615 cm$^{-1}$ (assigned to a $\delta$(SO$_4^{2-}$) mode), at 975 cm$^{-1}$ (assigned to a $\nu_1$(SO$_4^{2-}$) mode), at 1040 cm$^{-1}$ (assigned to a $\nu_3$(SO$_4^{2-}$) mode), and at 1195 cm$^{-1}$ (assigned to a $\nu_4$(SO$_4^{2-}$) mode). Although the exact interfacial chemistry responsible for the observation of these species is unknown, these species could form from the reaction of products of residual oxygen reduction with the thiolate headgroup.$^{7,10-7,13}$ Such reactions could result in the creation of highly reactive R-SO$_3$ species which, due to their inherent instability towards disproportionation and other oxidation reactions, could be further oxidized. Finally, it should be noted that destruction of the monolayer at potentials near the reductive desorption potential is irreversible in NaOH solution.

The oxidation effects observed when the supporting electrolyte is 0.1 M H$_2$SO$_4$ are
even greater. Figure 7.3 displays no changes in $I_R/B$ ratios when comparing *ex situ* $N_2$ and *in situ* spectra after $H_2SO_4$ introduction at open circuit potential. The $C_3SH$ SAM is stable in $H_2SO_4$ solution for exposure times up to 30 min at ca. +0.2 V. However, oxidation and concomitant disordering of the monolayer occur at potentials negative of ca. -0.8 V, as seen in Figure 7.8. This result is unlike any other observed with the electrolytes studied here in which the SAM disorders only near the reductive desorption potential, if at all. Many of the bands observed in Figure 7.8c are indicative of oxidized thiolates similar to those observed in NaOH solution (e.g. bands at 615, 975, 1040 and 1195 cm$^{-1}$). The potential at which the instability in $H_2SO_4$ begins can be understood by considering the cyclic voltammetry of $C_3SH$ SAMs on Ag in 0.1 M $H_2SO_4$. As noted above, the cyclic voltammetry indicates that significant water reduction begins at ca. -0.8 V. Thus, the reduction of water, and perhaps the reduction of residual oxygen, and the onset of $C_3SH$ SAM instability are clearly coupled in this medium.

In summary, aqueous electrolytes have varying effects on the structural order of $C_3SH$ SAMs on Ag. None of the electrolytes disrupt the monolayer at ca -0.10 V for up to 30 min, the maximum time studied. All of the electrolytes studied except NaCl disorder the SAM to varying extents at potentials negative of thiolate reductive desorption. Among the electrolytes, NaOH and $H_2SO_4$ create the greatest disruption to the SAM through undetermined reaction chemistry probably at the thiol headgroup, but only at potentials at which thiolate reductive desorption or water or residual oxygen reduction occur. The spectral data suggest that such reactions in these respective media involve
Figure 7.8. Raman spectra of a C_2SH SAM on Ag in the following environments:
a) N_2 (ex situ), and in 0.1 M H_2SO_4 at b) +0.2 V, c) -0.4 V, and d) -0.8 V.
All spectra plotted on same intensity scale. *denotes peak due to oxidized thiol product. Integration times: 60 sec x 5.
oxidation of the thiol headgroup. Finally, the presence of a $\nu(C=\text{N})$ mode from adsorbed SCN$^-$ and a decrease in the intensity of monolayer bands are both observed at negative potentials indicating alkanethiol displacement by specifically adsorbed SCN$^-$. This is not surprising for such a short chain SAM, because the methyl groups alone do not form a sufficient blocking layer to the electrolyte solution constituents.

One additional observation was made for C$_3$SH SAMs in these electrolytes. No $\nu$($\text{O-H}$) modes from water are observed after emersion of the SAM from any of the electrolyte solutions at potentials positive of the reductive desorption potential. However, for potentials negative of reductive desorption, a weak, broad $\nu$($\text{O-H}$) band from water at ca. 3220 cm$^{-1}$ is observed in each electrolyte solution. The shape and frequency of this band are consistent with bulk H$_2$O.$^{7,14}$ indicating water near the Ag surface. Water penetration into the film at negative potentials beyond reductive desorption suggests that the integrity of the film is destroyed, consistent with the decrease in the intensity of monolayer peaks observed in all electrolytes except NaF.

C$_{12}$SH SAMs on Ag

The reductive desorption behavior of C$_{12}$SH SAMs on Ag was also investigated by cyclic voltammetry. Cyclic voltammograms in each electrolyte are shown in Figure 7.9. No reductive desorption wave is observed in any of the 0.1 M electrolyte solutions; reductive desorption of the film along with solvent breakdown occurs simultaneously at potentials negative of ca. -1.4 V. As with C$_3$SH, the onset of water reduction in 0.1 M
Figure 7.9. Cyclic voltammograms for C_{12}SH Ag in 0.1 M a) H_{2}SO_{4}, b) NaSCN, c) NaCl, d) NaF, and e) NaOH. Sweep rate = 100 mV/s. Surface area of electrode is 0.03 cm$^2$. 

Potential (Volts versus Ag/AgCl)
H$_2$SO$_4$ is shifted positive to ca. -0.8 V.

The potential-dependent stability of C$_{12}$SH SAMs on Ag upon exposure to the same five electrolytes studied above was also investigated using the spectroelectrochemical emersion approach. Exposure to these electrolytes at ca. -0.05 V does not drastically alter the monolayer structure. As expected, the van der Waals interactions for a longer chain alkanethiol prevent disordering, because of solvent is unable to sufficiently penetrate the film and reach the headgroup.

**Ex situ** spectra in N$_2$ and **in situ** spectra of C$_{12}$SH monolayers emersed from 0.1 M NaCl, NaF, NaSCN, NaOH, and H$_2$SO$_4$ are shown in Figures 7.10-7.14, respectively. Overall, these spectra indicate that the integrity of the C$_{12}$SH SAMs is retained, even at negative potentials. However, the bands in these spectra appear to sharpen as the potential is made negative. This effect is seen most prominently in the v(C-S)$_T$ mode whose full-width-at-half-maximum (FWHM) decreases from ca. 30 cm$^{-1}$ at +0.2 V to ca. 24 cm$^{-1}$ at -1.5 V in 0.1 M NaF (Figure 7.11), consistent with a more crystalline-like structure.

As shown in Figure 7.15, the I$_4$/B ratios appear to decrease slightly with increasing negative potential, consistent with the slight surface roughening (and hence, greater surface enhancement) that presumably accompanies water reduction (H$_2$ bubble formation) through defects in the SAM.$^{7,15,7,16}$ The fact that the intensities of all bands increase at negative potentials further supports this conclusion. Slight surface roughening most likely does occur for C$_3$SH SAMs; however, greater surface enhancements
Figure 7.10. Raman spectra of a C$_{12}$SH SAM on Ag in the following environments: 
a) N$_2$ (ex situ), and in 0.1 M NaCl at b) -0.1 V, c) -1.1 V, and d) -2.0 V. All 
spectra plotted on same intensity scale. Integration times: 60 sec x 5.
Figure 7.11. Raman spectra of a C\textsubscript{18}SH SAM on Ag in the following environments: a) N\textsubscript{2} (ex situ), and in 0.1 M NaF at b) +0.1 V, c) -0.8 V, d) -1.1 V, and e) -1.5. All spectra plotted on same intensity scale. Integration times: 60 sec x 5.
Figure 7.12. Raman spectra of a C\textsubscript{18}SH SAM on Ag in the following environments:

a) N\textsubscript{2} (ex situ), and in 0.1 M NaSCN at b) +0.06 V, c) -1.1 V, and d) -2.0 V.

All spectra plotted on same intensity scale. Integration times: 60 sec x 5.

Wavenumbers (cm\textsuperscript{-1})
Figure 7.13. Raman spectra of a C_{12}SH SAM on Ag in the following environments: a) N$_2$ (ex situ), and in 0.1 M NaOH at b) -0.2 V, c) -1.1 V, and d) -2.0 V. All spectra plotted on same intensity scale. Integration times: 60 sec x 5.
Figure 7.14. Raman spectra of a C$_{10}$SH SAM on Ag in the following environments: a) N$_2$ (ex situ), and in 0.1 M H$_2$SO$_4$ at b) +0.2 V, c) -0.4 V, d) -1.1 V, and e) -2.0 V. All spectra plotted on same intensity scale. Integration times: 60 sec x 5.
Figure 7.15. \( \nu(C-S)_n / \nu(C-S)_1 \) peak intensity to background intensity ratios with respect to potential for \( \text{C}_n\text{SH} \) SAMs on Ag in 0.1 M aqueous electrolytes.
manifested by improved signal are not easily observed, because short chain SAMs are unstable and depleted from the surface at negative potentials.

The increase in band intensity is irreversible for long chain SAMs, including C_{12}SH and C_{18}SH as discussed in the next section. Significantly, potentials beyond that of C_{12}SH reductive desorption (ca. -1.3 V) do not cause disordering as was observed for C_{3}SH SAMs. The spectra in Figures 7.10d, 7.12d, 7.13d, and 7.14e at -2.0 V clearly indicate that the stability of the SAM is maintained in all electrolytes even at considerably negative potentials.

For longer chain alkanethiol monolayers, additional evidence for overall SAM order can be extracted from the ν(C-H) region. The ν(C-H) region of alkanethiol molecules is quite complex and includes symmetric (ν_s) and antisymmetric (ν_a) stretches of the methyl and methylene groups and several Fermi resonance bands. An example of a C_{12}SH SAM in this region before and after exposure to 0.1 M NaF at several potentials is shown in Figure 7.16. The peak intensity ratio of the ν_a(CH₂) at ca. 2880 cm\(^{-1}\) to the ν_s(CH₂) at 2850 cm\(^{-1}\) is indicative of crystalline-like packing of the alkyl chains, implying the presence of all-trans conformational sequences in the alkane. This ratio for well-ordered C_{12}SH SAMs is approximately 1.1. As shown for C_{12}SH in 0.1 M NaF as an example (Figure 7.16), no change in this ratio is observed as a function of potential. Similar behavior was noted in each of the other electrolytes studied suggesting little structural perturbation at the outer edges of the monolayer. Significantly, the intensities of the bands in the ν(C-H) region increase as a result of the slight surface roughening and
Figure 7.16. Raman spectra in the v(C-H) region of a C$_{18}$SH SAM on Ag in the following environments: a) N$_2$ (ex situ), and in 0.1 M NaF at b) +0.2 V, c) -1.1 V, d) 0.0 V after c), and e) -1.5 V. All spectra plotted on same intensity scale. Integration times: 60 sec x 5.
increased enhancement observed at negative potentials in the $\nu(C-C)$ and $\nu(C-S)$ regions.

The weak water mode which appears at potentials negative of thiolate reductive desorption for C$_3$SH SAMs is not observed for C$_{12}$SH SAMs at any potential. This suggests that the amount of water penetrating C$_{12}$SH SAMs is smaller than the limit of detection using Raman spectroscopy. As expected, the van der Waals interactions for a longer chain alkanethiol prevent significant solvent penetration into the film.

C$_{18}$SH SAMs on Ag

The effects of electrolyte and potential on C$_{18}$SH SAMs on Ag were also investigated. Generally, the electrochemical and spectroscopic behavior is similar to that observed for C$_{12}$SH. As with C$_{12}$SH films, no reductive desorption wave is observed in the cyclic voltammetry of C$_{18}$SH films, regardless of electrolyte (Figure 7.17). The potential at which water reduction begins is consistently at ca. -1.4 V in all electrolytes, except H$_2$SO$_4$.

The stability of C$_{18}$SH SAMs as ascertained with Raman spectroscopy is summarized in Figure 7.18 as the potential-dependent I$_R$/B values after exposure to 0.1 M aqueous electrolytes and potential. Spectra of C$_{18}$SH monolayers on Ag in contact with 0.1 M NaCl, NaF, NaSCN, NaOH and H$_2$SO$_4$ are shown in Figures 7.19-7.23, respectively. As for C$_{12}$SH SAMs, no significant monolayer disruption, disorder, or headgroup oxidation are observed upon exposure to any electrolyte at ca. +0.10 V. However, as the potential is made negative, the relative intensities of the major modes
Figure 7.17. Cyclic voltammograms for C\textsubscript{18}SH Ag in 0.1 M a) H\textsubscript{2}SO\textsubscript{4}, b) NaSCN, c) NaCl, d) NaF, and e) NaOH. Sweep rate = 100 mV/s. Surface area of electrode is 0.03 cm\textsuperscript{2}.
Figure 7.18. $\nu(C-S)_p / \nu(C-S)_b$ peak intensity to background intensity ratios with respect to potential for C$_{18}$SH SAMs on Ag in 0.1 M aqueous electrolytes.
Figure 7.19. Raman spectra of a C₉SH SAM on Ag in the following environments: a) N₂ (ex situ), and in 0.1 M NaCl at b) 0.0 V, c) -0.9 V, d) -1.5 V, and e) -0.3 V. All spectra plotted on same intensity scale. Integration times: 60 sec x 5.
Figure 7.20. Raman spectra of a C$_{18}$SH SAM on Ag in the following environments: a) N$_2$ (ex situ), and in 0.1 M NaF at b) +0.1 V, c) -1.1 V, and d) -1.5 V. All spectra plotted on same intensity scale. Integration times: 60 sec x 5.
Figure 7.21. Raman spectra of a C_{10}SH SAM on Ag in the following environments:

a) N_2 (ex situ), and in 0.1 M NaSCN at b) +0.06 V, c) -1.1 V, and d) -2.0 V.

All spectra plotted on same intensity scale. Integration times: 60 sec x 5.
Figure 7.22. Raman spectra of a C$_n$SH SAM on Ag in the following environments:
a) N$_2$ (ex situ), and in 0.1 M NaOH at b) +0.2 V, c) -0.4 V, d) -1.1 V,
and e) -2.0. All spectra plotted on same intensity scale. Integration
times: 60 sec x 5.
Figure 7.23. Raman spectra of a C$_m$SH SAM on Ag in the following environments:
a) N$_2$ (ex situ), and in 0.1 M H$_2$SO$_4$ at b) +0.1 V, c) -1.1 V, and d) -1.5 V.
All spectra plotted on same intensity scale. Integration times: 60 sec x 5.
increase, the bands sharpen, and the I_R/B ratios decrease slightly, indicating slight surface roughening, similar to the effects observed with C_{12}SH SAMs. The most pronounced decrease in I_R/B ratio occurs in 0.1 M NaCl and 0.1 M NaSCN, the two electrolytes containing the strongest specifically adsorbing anions. In addition to slight surface roughening, this behavior may be the result of partial alkanethiol displacement by Cl^- or SCN^- . However, the absence of a v(C≡N) mode in the Raman spectra at all potentials makes this hypothesis unlikely.

The effects on absolute intensities due to negative potential application are consistently irreversible as shown in Figure 7.19 (0.1 M NaCl). After applying a very negative potential, a more positive potential has little effect on the spectral behavior consistent with slight surface roughening.

Further evidence for overall SAM stability is available in the v(C-H) region. The intensity ratio of v_s(CH_2) to the v_(as)(CH_2) modes remains constant at a value of ca. 1.1 throughout the potential region studied in each electrolyte. This behavior implies a reasonably ordered alkane environment throughout these experiments. An example of a C_{18}SH SAM in the v(C-H) region before and after exposure to 0.1 M NaOH and potential is shown in Figure 7.24. A slight overall intensity increase is observed in this region at negative potentials indicating surface roughening as noted above. This intensity increase is irreversible as evidenced by the spectrum acquired when the potential is again made more positive, as shown in Figure 7.24d.

No vibrational bands due to water appear in any of the electrolytes or at any of the
Figure 7.24. Raman spectra in the $\nu$(C-H) region of a C$_{18}$SH SAM on Ag in the following environments: a) N$_2$ (ex situ) and in 0.1 M NaOH at b) +0.1 V, c) -1.1 V, and d) 0.0 V after c). All spectra plotted on same intensity scale. Integration times: 60 sec x 5.
potentials studied suggesting that water does not penetrate the C_{18}SH monolayer at the emersion velocity used. This result is not surprising given the expected hydrophobicity of C_{18}SH SAMs. Furthermore, no vibrational bands indicative of a significant population of specifically adsorbed ions (e.g. Cl^- or SCN^-) at the Ag surface are observed. These results suggest that the strong van der Waals interactions between the alkane chains prohibit significant solvent and ion penetration.

Other Chain Lengths

Several alkanethiols (C_4SH, C_6SH, C_8SH, and C_{10}SH) with chain lengths intermediate between C_3SH and C_{12}SH were investigated to determine the transition chain length at which NaOH, NaF, and H_2SO_4 electrolytes affect SAM structural order. NaOH and H_2SO_4 were chosen for study due to the extreme disruptive effects observed for C_3SH (but not C_{12}SH or C_{18}SH) at negative potentials. NaF was chosen as a non-disruptive electrolyte.

As for C_3SH, initial exposure of C_4SH SAMs to NaF, NaOH, or H_2SO_4 at ca. 0.0 V does not change monolayer order, as shown by the Raman spectra in Figures 7.25-7.27, respectively. However, with application of potentials as negative as -1.5 V, the integrity of C_4SH is affected in each of the three electrolytes, analogous to the effects observed with C_3SH. The structural order of C_4SH is completely destroyed and the previously noted bands at 615, 975, 1040, and 1195 cm^{-1} appear. An overall decrease in the spectral intensities suggests that C_4SH is removed from the Ag surface after headgroup oxidation.
Figure 7.25. Raman spectra of a C$_2$SH SAM on Ag in the following environments:

a) N$_2$ (ex situ), and in 0.1 M NaF at b) +0.03 V, c) -1.1 V, and d) -1.5 V.

All spectra plotted on same intensity scale. Integration times: 60 sec x 5.
Figure 7.26. Raman spectra of a C$_2$SH SAM on Ag in the following environments: a) N$_2$ (ex situ), and in 0.1 M NaOH at b) -0.1 V, and c) -1.1 V. All spectra plotted on same intensity scale. Integration times: 60 sec x 5.
Figure 7.27. Raman spectra of a C$_2$SH SAM on Ag in the following environments: a) N$_2$ (ex situ), and in 0.1 M H$_2$SO$_4$ at b) +0.1 V, c) -0.8 V, and d) -1.1 V. All spectra plotted on same intensity scale. Integration times: 60 sec x 5.
at negative potentials in these media.

The integrity of C₆SH is maintained in 0.1 M NaF, but slightly damaged in 0.1 M NaOH and 0.1 M H₂SO₄ at -1.1 V, as shown in Figures 7.28-7.30, respectively. This behavior indicates the inability of the C₆SH to withstand negative potentials in aggressive environments, analogous to the other short chain SAMs studied. Interestingly, C₄SH and C₁₀SH SAMs are extraordinarily stable in all electrolytes as shown in Figures 7.31-7.33 and 7.34-7.36, respectively, similar to C₁₂SH and C₁₈SH. Thus, the transition chain length at which films retain structural order regardless of electrolyte or potential is somewhere in the vicinity of C₆SH to C₄SH.

The implications of this behavior are tremendous. First, many electrochemical applications using SAMs utilize longer chain lengths. For such systems, film order and thus, the ability to impede electron transfer, is retained at negative potentials beyond that of water reduction. In addition, these results confirm the expectation that SAM chain lengths longer than six carbon atoms more easily withstand aggressive environments. The larger van der Waals interactions of such longer chain SAMs prevent excessive solution penetration into the film, and thus, prevent headgroup degradation at negative potentials. This observation is important in understanding the improved pacing performance observed for C₁₂SH relative to C₆SH on Au-coated Pt(Ir) electrodes during the first few weeks of implantation. If the SAM can withstand the blood environment at negative potentials and adequately prevent protein interaction with the metal surface, inflammation due to thrombosis (an event initiated by protein adsorption) may be lessened.
Figure 7.28. Raman spectra of a C₇SH SAM on Ag in the following environments: a) N₂ (ex situ), and in 0.1 M NaF at b) +0.2 V, c) -0.8 V, and d) -1.6 V. All spectra plotted on same intensity scale. Integration times: 60 sec x 5.
Figure 7.29. Raman spectra of a C₆SH SAM on Ag in the following environments: a) N₂ (ex situ) and in 0.1 M NaOH at b) -0.1 V, and c) -1.4 V. All spectra plotted on same intensity scale. Integration times 60 sec x 5.
Figure 7.30. Raman spectra of a C₆SH SAM on Ag in the following environments:
a) N₂ (ex situ), and in 0.1 M H₂SO₄ at b) +0.24 V, c) -0.8 V, and d) -1.1 V.
All spectra plotted on same intensity scale. Integration times: 60 sec x 5.
Figure 7.31. Raman spectra of a C₆SH SAM on Ag in the following environments:
a) N₂ (ex situ), and in 0.1 M NaF at b) 0.0 V, c) -0.9 V, and d) -1.1 V. All plotted on same intensity scale. Integration times 60 sec x 5.
Figure 7.32. Raman spectra of a C$_2$SH SAM on Ag in the following environments: a) N$_2$ (ex situ), and in 0.1 M NaOH at b) 0.0 V, and c) -1.1 V. All spectra plotted on same intensity scale. Integration times: 60 sec x 5.
Figure 7.33. Raman spectra of a C\textsubscript{6}SH SAM on Ag in the following environments:
a) N\textsubscript{2} (ex situ), and in 0.1 M H\textsubscript{2}SO\textsubscript{4} at b) 0.0 V, c) -1.2 V, and d) -1.8 V.
All spectra plotted on same intensity scale. Integration times: 60 sec x 5.
Figure 7.34. Raman spectra of a C$_{10}$SH SAM on Ag in the following environments: a) N$_2$ (ex situ), and in 0.1 M NaF at b) +0.1 V, c) -1.1 V, and d) -2.5 V. All spectra plotted on same intensity scale. Integration times: 60 sec x 5.
Figure 7.35. Raman spectra of a C₆SH SAM on Ag in the following environments: a) N₂ (ex situ), and in 0.1 M NaOH at b) -0.1 V, and c) -1.5 V. All spectra plotted on same intensity scale. Integration times: 60 sec x 5.
Figure 7.36. Raman spectra of a C₁₀SH SAM on Ag in the following environments:
a) N₂ (ex situ), and in 0.1 M H₂SO₄ at b) 0.0 V, c) -1.2 V, and d) -2.2 V.
All spectra plotted on same intensity scale. Integration times: 60 sec x 5.
Conclusions

Surface Raman spectra of short and long chain alkanethiol monolayers on smooth polycrystalline Ag surfaces have been acquired as a function of potential during exposure to several aqueous electrolytes using a spectroscopic emersion approach. The spectra, particularly in the v(C-S) and v(C-C) regions, provide considerable insight into film order and the potential and electrolyte-dependent degradation of the thiol headgroup.

Exposure to aqueous electrolytes at positive potentials does not affect the structural order or chemistry of the thiol headgroup for any of the alkanethiols studied, regardless of chain length, in agreement with the results of others. More negative potentials result in an array of effects depending on the monolayer, electrolyte, and the magnitude of the potential. The short chain alkanethiols (ca. C\textsubscript{8}SH and shorter) are susceptible to disordering and headgroup oxidation at potentials negative of alkanethiol reductive desorption. These effects are most pronounced in NaOH and H\textsubscript{2}SO\textsubscript{4} media. After disruption of the SAM by negative potential, solvent penetration into the monolayer is indicated by the observation of vibrational bands for water.

Similar electrochemical instability was reported by Everett and coworkers for C\textsubscript{12}SH SAMs on Au in CH\textsubscript{3}CN and CH\textsubscript{2}Cl\textsubscript{2}. They observed that under the driest and most oxygen-free conditions, negative potentials in CH\textsubscript{3}CN and CH\textsubscript{2}Cl\textsubscript{2} (-1.7 V vs. Ag/AgCl) caused a 90% loss in alkanethiol surface coverage. Our results indicate that such behavior might be likely in highly acidic (H\textsubscript{2}SO\textsubscript{4}) or basic (NaOH) solutions for short chain alkanethiols.
Van der Waals interactions among the longer chain alkanethiols (ca. C₆SH and longer) prevent disordering, regardless of electrolyte or potential. As a result, little solution permeation occurs into the hydrocarbon monolayer, similar to electrochemical results obtained by Zhang and Anderson for C₁₈SH SAMs on Au after D₂O exposure. Overall, the results of these studies suggest that the utility of long chain SAMs in aqueous media and under electrochemical environments is not sacrificed.

Additional experiments for assessing the utility of SAMs for pacing and electrochemical sensing applications include other solvents (i.e., nonaqueous) and electrolytes (i.e., physiological buffer), longer exposure times to solution and potential (on the order of hours, days, months), and higher temperatures (similar to that of human blood). In addition, electrochemical experiments to assess changes in surface coverage in these environments are necessary for a better understanding of SAM stability under physiological conditions. Such experiments are now being studied in our lab and will be reported elsewhere.
Chapter 8

Air Stability of Alkanethiol Self-Assembled Monolayers on Ag and Au Surfaces

Introduction

As described in Chapter 1, organothiol self-assembled monolayers (SAMs) on Ag, Au, Cu, and Pt have been studied extensively because of their potential utility in a variety of applications including the study of corrosion inhibition and electron transfer phenomena, as platforms for chemical sensors, and as biomaterials. The ultimate utility of SAMs for each of these applications will be critically dependent on their stability. Although the structure of SAMs has been thoroughly characterized using a host of techniques, their stability in ambient laboratory air is has not been adequately characterized and has been somewhat controversial.

Air Oxidation

Much of the surface chemistry community believes that alkanethiol-SAMs are extraordinarily inert under ambient conditions. However, organothiol monolayers on Au exposed to air for prolonged periods (on the order of weeks) have been shown to oxidize to sulfinates and sulfonates, indicating their finite stability. Once oxidized, aromatic sulfonates have been claimed to subsequently desorb upon aqueous rinsing suggesting weaker surface affinity than the starting SAMs. In addition, it has been
established that oxidized SAMs rapidly exchange with thiols when placed in fresh thiol solutions.\textsuperscript{8,5,8,8,8,9} Li \textit{et al.} were the first to observe sulfonate formation resulting from air exposure of alkanethiol-SAMs \([\text{CH}_3(\text{CH}_2)_n\text{SH}, n = 3, 5, 7, 8, 11, 15, \text{and} 17]\) adsorbed on Au using laser-desorption Fourier transform mass spectrometry.\textsuperscript{8,4} Sulfonate species were detected from films on Au exposed to the ambient environment for a week. However, despite large sulfonate ion signals, the extent of oxidation could not be determined. Large sulfonate ion abundances were believed to be the result of a greater ionization efficiency for sulfonates over thiolates. No effort was made to study the rate of the oxidation process, perhaps because it was believed to be minimal.

Tarlov and Newman also detected alkanethiol-SAM oxidation on Au after prolonged periods of atmospheric exposure using static secondary ion mass spectrometry (SSIMS).\textsuperscript{8,5} Sulfonate species were detected from SAMs on Au \([\text{CH}_3(\text{CH}_2)_n\text{SH}, n = 7, 9, 11, 15, \text{and} 17]\) exposed to air for 11 days. However, the rate and extent of oxidation were not reported. Furthermore, it was suggested that the large sulfonate signal was the result of large SSIMS negative ion yields for oxidized sulfur. The sulfonate species formed in this process were shown to be displaced by thiols when re-immersed into thiol solution. No sulfonate species were detected with SSIMS from any SAMs formed in air-saturated thiol/ethanol adsorbate solutions if analyzed immediately following removal from solution. In fact, oxidation was not observed in SAMs stored in such solutions for periods up to 2 months suggesting that, if oxidation does occur in solution, sulfonates are
continuously displaced by thiol molecules.

Horn and coworkers used IR spectroscopy to study the aging of alkanethiol self-assembled monolayers. They found that over a period of ca. six months, the relative intensities of the v(C-H) bands changed indicating a tilting of alkanethiol molecules away from the surface normal. They ascribed the observed SAM restructuring to alkanethiol oxidation at the sulfur headgroup.

Recently, Scott and coworkers reported the exchange of C_{12}SH SAMs on Au with C_{10}SH using laser-desorption Fourier transform mass spectrometry (LD-FTMS) in the negative ion mode. Negligible exchange was observed when unoxidized C_{12}SH SAMs were soaked for 30 min in C_{10}SH solutions. However, abundant C_{10}SH exchange was detected for partially oxidized C_{12}SH SAMs. The detection of two thiol populations after soaking was indirectly interpreted in terms of sulfonate formation and displacement. In fact, the amount of C_{10}SH exchange was found to be dependent on the extent of C_{12}SH oxidation. These researchers concluded from these exchange studies that the ionization efficiencies of thiolates and sulfonates are approximately equal, suggesting the possibility of extensive SAM oxidation in air, contrary to the reports by Li et al. and Tarlov and Newman.

Photooxidation

UV-assisted oxidation of alkanethiol SAMs on Au has been more widely reported. Photooxidation of SAMs has received considerable attention recently
because of the potential for selectively patterning Au surfaces through this approach.⁸,⁹

Rieley et al. used near edge extended X-ray absorption fine structure and ultraviolet photoemission spectroscopy to study the photooxidation of C₈SH SAMs on Au (111)⁸,¹⁰. Incident UV radiation absorbed by the Au surface was found to produce O₂⁻ which can subsequently oxidize adsorbed thiolate species to the sulfonates. The mechanism by which O₂⁻ forms was proposed to be photoinduced interfacial electron transfer to adsorbed O₂. Extensive sulfonate formation was observed in regions of the surface exposed to UV light.

Hutt and Leggett studied the effects of UV irradiation of methyl terminated alkanethiols [CH₃(CH₂)ₙSH, n=2, 5, 7, 9, 11, 15, and 17] on Au using XPS.⁸,¹¹ They also observed significant thiolate oxidation to sulfonate species by "active" O₂ species as a result of UV exposure. The rate of photooxidation was found to vary strongly with alkyl chain length. Short-chain length SAMs were found to oxidize much faster than long-chain SAMs. Based on these results it was suggested that the oxidation rate correlates with the ability of the "active" oxygen species to penetrate the closely-packed alkyl chain structure and reach the S-Au interface.

Lewis, Tarlov, and Carron studied the photooxidation process of alkanethiol SAMs [CH₃(CH₂)ₙSH, n=5, 9, and 17] on electrochemically roughened Ag using surface-enhanced Raman scattering (SERS) and concluded that UV irradiation in air photochemically induces C-S bond scission.⁸,¹² The alkyl chain fragments subsequently desorb so that the surface-bound sulfur is exposed to the air and is oxidized. Similar
conclusions were observed for benzenethiolate and tetradecanethiolate monolayers on Au by Teuscher et al. using SERS and a quartz crystal resonator.12,13 The final oxidation product, adsorbed SO$_4^{2-}$, easily rinsed away with water. However, some uncertainty regarding the effects of surface morphology on sulfate-Ag affinity must be noted, because these two SERS studies were performed on SAMs on roughened surfaces.

Despite the interest in using SAMs for a variety of applications, few studies have thoroughly investigated alkanethiol oxidation in the ambient laboratory environment with spectroscopic techniques. The air stability of SAMs may be of considerable importance to the photopatterning community because of the need to selectively control oxidation. Thus, the goal of the studies presented in this chapter is to obtain a better understanding of SAM stability in ambient laboratory air without UV irradiation or light exposure. A sufficient time period between the fabrication of SAM-modified pacemaker electrodes and implantation is necessary, during which time air exposure may affect the integrity of the SAM. Surface Raman spectroscopy, XPS, and cyclic voltammetry are utilized to study the effects of air exposure on the structure of SAMs formed from three alkanethiols (C$_n$SH, n=3, 12, and 18) on mechanically polished, smooth, polycrystalline Ag and Au surfaces. The combination of these characterization tools provides a more detailed picture of the effects of air exposure on SAM structure and bonding than has previously been elucidated.
Sample Handling

Surfaces were prepared as described in Chapter 2. Rigorous precautions were taken to assure a dark, O$_2$-free **SAM formation environment** for these experiments by working in a N$_2$-filled glove bag. Air exposure of the alkanethiol solution during film formation was further prevented by a steady blanket of N$_2$ in the immersion cell. After emersion from the organothiol solution, surfaces were rinsed with 100% ethanol, allowed to dry in the N$_2$ environment of the glove bag, and transferred to a N$_2$-purged spectrochemical cell while in the N$_2$-filled glove bag. The electrochemical cleaning protocol described in Chapter 6 for Ag was also carried out in the glove bag. Thus, prior to the start of each experiment, the SAM-modified surfaces were never exposed to the ambient atmosphere.

Once films were formed, the surfaces were exposed to the ambient laboratory environment, N$_2$, O$_2$, compressed air, or O$_3$ in either the spectrochemical cell or a glove bag purged continuously with the gas of interest. O$_3$ was prepared using a Boekel Model 135500 dry process cleaner (Boekel Industries, Inc.) inside a glove bag purged with compressed air. A low pressure Mercury grid lamp enclosed in a reaction chamber generates an environment rich in O$_3$, which can be flushed into the glove bag by a stream of N$_2$. The SAM-modified Ag and Au surfaces were not exposed to UV light during O$_3$ production. All experiments were carried out in complete darkness.
SAMs on Ag

Surface Raman Spectroscopy

Surface Raman spectra for these experiments were collected in the spectrochemical cell described in Chapter 2. This cell has two advantages for studying surface oxidation: a controlled environment can be maintained by purging the cell with the gas of interest, and degradation of the sample by extensive laser exposure is prevented, because the surface is continuously rotated through the laser beam.

Raman spectra in the $\nu$(C-S), $\nu$(C-C), and $\delta$(C-H) region (ca. 600-1700 cm$^{-1}$) from C$_3$SH, C$_{12}$SH, and C$_{18}$SH SAMs on Ag before air exposure are shown in Figures 8.1a, 8.2a, and 8.3a, respectively. Several important attributes of the films are apparent from these spectra. First, the $\nu$(C-S)$_{\text{cis}}$ and $\nu$(C-S)$_{\text{trans}}$ modes for alkanethiols at ca. 630 and 705 cm$^{-1}$, respectively, indicate well-ordered SAMs near the thiol headgroup\textsuperscript{8,14,8,15} (peak assignments for alkanethiols on Ag are described in detail in references 16 and 17). Secondly, our films on Ag are free of carbonaceous impurities (a common problem associated with use of mechanically polished surfaces\textsuperscript{8,16}) as evidenced by the absence of broad bands in the 1200-1600 cm$^{-1}$ region. In addition, no evidence of oxidation is apparent.

A weak $\nu$(C-S)$_{\text{cis}}$ mode is visible in all spectra, presumably due to slight disorder introduced by metal grain and alkanethiol domain boundaries. In previous work, we have noted that with intentionally roughed surfaces, slightly greater disorder is observed in the vicinity of the head group.\textsuperscript{8,15} Apparently, the alkanethiol molecules accommodate surface
Figure 8.1. Raman spectra of C,SH SAM on Ag after exposure to the ambient environment for; a) 0 hr, b) 2 hr, c) 4 hr, and d) 18 hr. All spectra plotted on same intensity scale. * denotes peak due to oxidized thiol product. Integration times: 60 sec x 5.
Figure 8.2. Raman spectra of C₆SH SAM on Ag after exposure to the ambient environment for: a) 0 hr, b) 1 hr, c) 5 hr, and d) 15 hr. All spectra plotted on same intensity scale. * denotes peak due to oxidized thiol product. Integration times: 60 sec x 5.
Figure 8.3. Raman spectra of C₅SH SAM on Ag after exposure to the ambient environment for: a) 0 hr, b) 2 hr, c) 7 hr, and d) 18 hr. All spectra plotted on same intensity scale. * denotes peak due to oxidized thiol product. Integration times: 60 sec x 5.
roughness by disordering near the head group, resulting in the observed $v$(C-S) intensity in
the spectra for all chain length SAMs.

Nonetheless, we propose that these films are well-ordered and essentially
completely blocking of the underlying Ag surface. Further evidence for the quality of
these SAMs comes from electrochemical capacitance measurements. Capacitance values
can be deduced from linear potential sweep experiments on these films in regions in which
no Faradaic processes occur. For the films studied here, small capacitance values of $9.1 \pm
6.5$, $5.1 \pm 2.6$, and $4.2 \pm 1.9 \ \mu$F/cm$^2$ are measured for C$_3$SH, C$_{12}$SH, and C$_{18}$SH films.
These values are in good agreement with previously reported values for well-ordered
SAMs on freshly evaporated Au surfaces. $^{8,17-8.19}$ (Capacitance values for SAMs on Ag
have not been reported in the literature.) Thus, on the basis of these measurements and
the spectra shown in Figures 8.1a, 8.2a, and 8.3a. we conclude that our SAMs on Ag are
quite well-ordered.

One further noteworthy observation is that SAMs formed in air-saturated
alkanethiol solutions and emersed into the ambient environment also result in films free of
oxidation. This observation is in agreement with that reported by Tarlov and Newman in
which thiolate oxidation was not observed for SAM samples immersed in air-saturated
adsorbate solutions for up to 2 months. $^{8,5}$ These results suggest that the extra effort to
completely exclude air during immersion and sample transfer to the spectrochemical cell is
unimportant. However, for ultimate control of the surface chemistry of interest here, all
SAMs used in these experiments were formed in alkanethiol solutions from which air was
excluded, and transferred to the spectrochemical cell or XPS fast entry port in a pure N₂ environment.

SAM-modified Ag surfaces were exposed to the ambient laboratory air in the dark by removing the N₂ purge. As shown by the spectra in Figures 8.1b, 8.2b, and 8.3b, significant changes in the film indicative of oxidation are observed after only ca. 1-2 hr. The ν(C-S)G band at 630 cm⁻¹ broadens, and shifts to lower frequencies due to an oxidation band that grows in at ca. 615 cm⁻¹. In addition, a peak at 975 cm⁻¹ appears, and a broad background develops beneath the peaks in the 900-1200 cm⁻¹ region. These spectral changes clearly indicate sulfur headgroup oxidation to the corresponding alkyl sulfonates based on previous reports of SAM oxidation after UV exposure. Vibrational assignments for oxidized sulfur species are given in Table 8.1.

In an effort to correlate oxidation bands in the surface Raman spectra of alkanethiol SAMs to sulfates, spectra of solid Na₂SO₄ and NaHSO₄ were collected. The spectra of these inorganic salts are shown in Figure 8.4. Several bands in the spectra of these sulfate salts are clearly observed in the surface Raman spectra of SAMs on Ag, specifically the δ(SO₄²⁻) at 615 cm⁻¹, the ν₃(SO₄²⁻) at 875 cm⁻¹, the ν₁(SO₄²⁻) at 1040, and the νₛ(SO₂) at 1147 cm⁻¹. However, the oxidized SAM peaks at 975, 1195, and 1395 cm⁻¹ are not accounted for in the sulfate salt Raman spectra, suggesting sulfur headgroup oxidation to multiple oxidized species including, sulfonates, sulfinites, sulfites, and sulfates.

As the air exposure time for SAMs on Ag increases, the relative intensities of the
### Table 8.1. Sulfur Oxidation Mode Assignments

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<th>Mode</th>
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<tbody>
<tr>
<td>(\delta(\text{SO}_4^{2-} \text{ or } \text{R-OSO}_3^{2-}))</td>
<td>615</td>
</tr>
<tr>
<td>(v_s(\text{SO}_4^{2-} \text{ or } \text{R-OSO}_3^{2-}))</td>
<td>975</td>
</tr>
<tr>
<td>(v_s(\text{R-SO}_4\text{)})</td>
<td>1034</td>
</tr>
<tr>
<td></td>
<td>1127</td>
</tr>
<tr>
<td>(v_{as}(\text{SO}_4^{2-} \text{ or } \text{R-OSO}_3^{2-}))</td>
<td>1040</td>
</tr>
<tr>
<td>(v_s(\text{R-SO}_3\text{)})</td>
<td>1195</td>
</tr>
</tbody>
</table>
Figure 8.4. Raman spectra of solid a) Na$_2$SO$_4$ and b) NaHSO$_4$ in capillary tubes. Excitation wavelength is 514.5 nm. Integration times: 15 sec.
oxidation modes increase and discrete bands resolve, suggesting progressive oxidation. These changes are clearly seen in the spectra shown in Figures 8.1c,d, 8.2c,d, and 8.3c,d. These observations demonstrate that well-ordered alkanethiol-SAMs on Ag oxidize after only hours of air exposure without UV irradiation.

Several important conclusions come from comparison of the behavior of the three alkanethiols upon air exposure. First, oxidation proceeds most rapidly and completely for C$_3$SH. This behavior is apparent from the appearance and intensity of the oxidation bands at 615, 975, 1034, 1040, 1127, and 1195 cm$^{-1}$ after only 2 hours. The longer-chain alkanethiols (C$_{12}$SH and C$_{18}$SH) do not oxidize as quickly as C$_3$SH, presumably due to increased van der Waals interactions which slow penetration of the oxidant. Similar conclusions about the rates of UV-induced oxidation as a function of chain length were made by Laibinis and Whitesides, Hutt and Legett, and Lewis and coworkers.

At first glance, the intensity ratio of the $\nu$(C-S)$_G$ to $\nu$(C-S)$_T$ modes appears to increase with oxidation indicating disorder of the alkane chain near the headgroup as a result of sulfur oxidation. Although this effect would be reasonable, because the monolayer near the headgroup must reorganize to accommodate sulfur oxidation, a $\delta$(R-OSO$_3$)$^2$) mode at 615 cm$^{-1}$ which overlaps the $\nu$(C-S)$_G$ makes this conclusion tenuous. Curve fitting of spectra in the $\nu$(C-S) region suggests that as oxidation progresses, the alkyl chain in the vicinity of the headgroup indeed disorders. The $\nu$(C-S)$_G$/\nu(C-S)$_T$ peak area ratio for each chain length increases with air exposure time and oxidation.

The amount of disordering that accompanies oxidation is most pronounced for
C_{12}SH. The \( \nu(C-S)_{\alpha}/\nu(C-S)_{\tau} \) peak area ratio increases from 0.56 to 2.32 after exposure to air for 18 hours. In contrast, the \( \nu(C-S)_{\alpha}/\nu(C-S)_{\tau} \) peak area ratios for \( C_{12}SH \) and \( C_{18}SH \) SAMs increase from 0.13 to 0.40 and 0.12 to 0.30, respectively, after a similar air exposure time. These results are consistent with slower oxidation and less disordering of the longer chain length SAMs due to impeded penetration of the oxidant species.

Surface Raman spectra from alkanethiol SAMs on Ag exposed to air in the dark for 15 days are shown in Figure 8.5. All three monolayers are extremely oxidized. The broad feature at ca. 975 cm\(^{-1}\) suggests that the predominant oxidized species are derivatives of sulfate and sulfonate. Significantly, the presence of \( \nu(C-S)_{\tau} \) and \( \nu(C-C)_{\tau} \) peaks at 706 and 1026 cm\(^{-1}\) in Figure 8.5a, 718, 1030, and 1110 cm\(^{-1}\) in Figure 8.5b, and 723, 1058, 1101, and 1125 cm\(^{-1}\) in Figure 8.5c suggest that the alkane portions of these molecules are still present at the interface and somewhat ordered. Tarlov and coworkers reported that they did not observe any oxidation peaks in the Raman spectra of UV-exposed SAMs until the monolayer band intensities first decreased significantly.\(^8,12\) They suggest that the oxidation of the sulfur headgroup requires the removal of hydrocarbon chains to allow easier oxygen access. In contrast, no such decrease before oxidation is observed in our Raman spectra, suggesting that significant oxidation occurs without hydrocarbon chain removal, perhaps by penetration of the oxidant through SAM defect sites. Significantly, our results indicate that the mechanism for non-UV induced oxidation is different than UV-induced oxidation.
Figure 8.5. Raman spectra of a) C₃SH, b) C₁₂SH, and c) C₁₈SH SAMs on Ag after exposure to air for 15 days. All spectra plotted on same intensity scale. Integration times: 60 sec x 5. + denotes monolayer bands.
Effect of Application of Negative Potential

Selective removal of oxidized alkanethiol species on Ag can be achieved by application of negative potentials in 0.1 M NaF. An example of this behavior is shown in Figure 8.6 for C\textsubscript{3}SH films. The presence of sulfonate peaks after a 20 hr air exposure indicates extensive sulfur oxidation. Exposure of this SAM to a 0.1 M NaF solution for ca. 5 min at -0.05 V results in few spectral changes (Figure 8.6c), suggesting that the sulfonate species remain bound at the interface. However, extensive spectral changes are observed at -1.1 V (Figure 8.6d). The disappearance of the majority of bands due to oxidized species suggests that they are removed from the surface, presumably due to repulsion at these negatively charged surfaces. Bands due to the native alkanethiol species are still observed, however, suggesting that the oxidized species are less tightly bound to Ag than the thiolates. Similar conclusions regarding the weak affinity of sulfonates have been reported based on the displacement of oxidized sulfur species by thiols from solution.\textsuperscript{8,5,8,11}

The intense $\nu$(C-S)$_{\text{v}}$ and $\nu$(C-C)$_{\text{v}}$ bands at ca. 710 and 1030 cm$^{-1}$, relative to the $\nu$(C-S)$_{\text{G}}$ band at 630 cm$^{-1}$ indicate that the native alkanethiols are fairly ordered. The effects of other potentials and chain lengths were not investigated; nonetheless, this behavior implies sulfonates, although bound more weakly to Ag than thiolates, require significant activation (-1.1 V) for removal when incorporated into well-ordered SAMs.
Figure 8.6. Raman spectra of C₅SH SAM on Ag after a) 0 hr and b) 20 hr air exposure and in 0.1 M NaF at c) -0.05 V and d) -1.1 V. All spectra plotted on same intensity scale. * denotes peak due to oxidized thiol product. Integration times: 60 sec x 5.
Cyclic Voltammetry

Previous reports\textsuperscript{8.5, 8.8, 8.21} suggest rapid desorption of oxidized sulfur species from the surface when placed in a solubilizing solvent. In order to ascertain the extent to which such loss from our oxidized SAMs occurs, cyclic voltammetry of Ru(NH\textsubscript{3})\textsubscript{6}\textsuperscript{3-} was used as an indicator of the amount of exposed Ag surface before and after oxidation of the SAM. This experiment and the determination of %EAS values are described in Chapter 6.

As shown in Table 8.2, C\textsubscript{7}SH SAM/Ag surfaces are still quite active in electron transfer even in the presence of a well-ordered film. Short chain films exhibit a large %EAS (74.0 ± 3.3%), presumably due to electron transfer through defects and relatively efficient electron tunneling across the film. As expected, long chain alkanethiol SAMs block electron transfer more effectively.

Surprisingly, air oxidation appears to have little effect on the electron transfer blocking properties of these monolayers, regardless of chain length, even after 1 week of air exposure, soaking in ethanol for 30 min followed by copious rinsing with ethanol and water. In fact, the %EAS for a highly oxidized C\textsubscript{7}SH film decreases by almost a factor of 2, indicating impeded electron transfer following oxidation. The largely invariant %EAS values indicate that neither the SAM thiolate nor oxidized species desorb from the surface after extensive soaking and rinsing. This observation is consistent with the conclusions based on the surface Raman spectroscopy of these systems that the alkane portions of the monolayers remain at the surface. In total, these results suggest that the physical blocking characteristics of well-ordered alkanethiol SAMs are retained, even though they rapidly
Table 8.2 % Electrochemically Active Surface for SAM-Modified Ag Surfaces after Air Exposure.

<table>
<thead>
<tr>
<th>Alkanethiol-SAM</th>
<th>0 hr Air Exposure</th>
<th>3 hr Air Exposure</th>
<th>24 hr Air Exposure</th>
<th>1 wk Air Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₃SH-Ag</td>
<td>74.0 ± 3.30</td>
<td>78.0 ± 12.5</td>
<td>67.0 ± 7.00</td>
<td>39.3 ± 13.1</td>
</tr>
<tr>
<td>C₁₂SH-Ag</td>
<td>1.40 ± 0.20</td>
<td>1.50 ± 0.33</td>
<td>1.50 ± 0.33</td>
<td>3.20 ± 0.92</td>
</tr>
<tr>
<td>C₁₈SH-Ag</td>
<td>2.00 ± 0.45</td>
<td>2.60 ± 0.69</td>
<td>2.60 ± 0.96</td>
<td>2.79 ± 1.97</td>
</tr>
</tbody>
</table>

*After air exposure, SAM-modified Ag electrodes were immersed and stirred in ethanol for 30 min and rinsed with copious amounts of EtOH and H₂O before % EAS determination. All values normalized to bare (unmodified) Ag surfaces.

*The high % EAS for C₃SH represents efficient electron tunneling through short chain and defects, not exposed surface.

*Standard deviations result from the analysis of a minimum of 3 independently prepared SAM films.
oxidize in air.

In an effort to increase the solubility of the alkane chains (which may or may not be cleaved from sulfur), such that oxidized sulfur species might be washed away with water, similar cyclic voltammetry experiments were undertaken after soaking oxidized SAMs in hexane. In these experiments, alkanethiol-SAMs on Ag were exposed to the ambient environment for 1 week, immersed in neat hexane for 30 min, then rinsed copiously with hexane and water. As seen in Table 8.3, the %EAS values for each chain length remains unchanged relative to the asimilar treatment using ethanol.

The fact that oxidized sulfur species are not easily removed by solubilizing solvents contradicts previous reports of SAM oxidation.\textsuperscript{8, 8, 9} One possible explanation for this discrepancy might be the quality of the SAM formed initially. As described above, film quality is extremely sensitive to surface preparation and roughness and film formation conditions. We speculate that oxidized species formed in well-ordered films resist extraction when exposed to solubilizing solvents. Another possible explanation could be that air oxidation proceeds by a different mechanism than UV-induced oxidation, a theory which is further addressed by control experiments described below.

X-ray Photoelectron Spectroscopy (XPS)

XPS was used to confirm that alkanethiol SAMs readily oxidize upon air exposure, determine relative oxidation rates, and ascertain the extent of oxidation. A series of XPS spectra of the S 2p region acquired from C\textsubscript{3}SH, C\textsubscript{12}SH, and C\textsubscript{18}SH SAMs on Ag surfaces
Table 8.3. % Electrochemically Active Surface for SAM-Modified Ag Surfaces after 1 week of air exposure and stirring in hexane for 30 min followed by rinsing with copious amounts of hexane and water before % EAS determination. All values normalized to bare (unmodified) Ag surfaces.

<table>
<thead>
<tr>
<th>Alkanethiol-SAM</th>
<th>1 wk Air Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_3$SH-Ag</td>
<td>26.9 ± 11.4</td>
</tr>
<tr>
<td>$C_{12}$SH-Ag</td>
<td>3.80 ± 2.16</td>
</tr>
<tr>
<td>$C_{18}$SH-Ag</td>
<td>1.42 ± 0.61</td>
</tr>
</tbody>
</table>
before and after air exposure are shown in Figures 8.7, 8.8, and 8.9, respectively. Low x-ray fluxes (200 W) and short analysis times were used in collecting these spectra in order to minimize sample degradation or x-ray induced sample reduction. X-ray induced sample damage is a common problem during the XPS analysis of certain materials and can cause spectral changes with x-ray exposure time. A wide range of damage rates exist, depending on the material. In fact, the degradation index (a measure of x-ray damage after 500 min exposure to a 1.4 kW x-ray source) is ca. three times greater for sulphone polymers relative to sulfide polymers.

The SAM-modified surfaces, immediately after removal from thiol solution (no air exposure), exhibit a S 2p peak at a binding energy of 162 eV that is characteristic of thiolates on Au and Ag. Following air exposure, the 162 eV thiolate feature decreases in intensity and a new feature at 167 eV emerges. Tarlov et al. observed similar behavior with XPS after exposing C_SH SAMs on Au to UV irradiation in air; they assigned the 167 eV peak to sulfonate species formed by oxidation. The Raman results discussed above demonstrate that sulfonate and other oxidized sulfur species are formed by air exposure without UV irradiation. This suggests that the 167 eV peak represents a combination of oxidized sulfur species.

The time-dependent changes in the XPS spectra indicate that the oxidation rate varies as a function of chain length. The rates of oxidation were evaluated for each SAM by measuring the areas of the thiolate and sulfonate peaks after 1 hr, 4 hr, 24 hr, and 1 week of air exposure. Values of the thiolate and sulfonate S 2p peak areas normalized to
Figure 8.7. XPS spectra of S 2p region acquired from C\textsubscript{3}SH SAM on Ag after exposure to ambient lab environment for: a) 0 hr, b) 1 hr, c) 4 hr, d) 24 hr, and e) 1 wk. All spectra plotted on same intensity scale.
Figure 8.8. XPS spectra of S 2p region acquired from C₉SH SAM on Ag after exposure to ambient lab environment for: a) 0 hr, b) 1 hr, c) 4 hr, d) 24 hr, and e) 1 wk. All spectra plotted on same intensity scale.
Figure 8.9. XPS spectra of S 2p region acquired from C_{12}SH SAM on Ag after exposure to ambient lab environment for: a) 0 hr, b) 1 hr, c) 4 hr, d) 24 hr, and e) 1 wk. All spectra plotted on same intensity scale.
the thiolate peak area at $t=0$ for $C_{3j}SH$, $C_{12j}SH$, and $C_{18j}SH$ are given in Table 8.4 as a function of ambient exposure. Clearly, $C_{3j}SH$ SAMs are oxidized more quickly in the ambient laboratory air than the long chain monolayers on Ag surfaces. The oxidation rates observed with XPS are consistent with those observed using Raman spectroscopy.

If the oxidized thiol remains at the surface, the percentage of thiolate and oxidized sulfur species should combine to 100% assuming that the x-ray cross-sections are approximately equivalent for the two types of species. Such behavior is observed for $C_{12j}SH$ and $C_{18j}SH$ SAMs on Ag. In fact, for these longer chain SAMs, the sum of the two forms is generally slightly greater than 100%. This observation is rationalized on the basis of a slightly larger cross-section for the oxidized sulfur species relative to the thiolate sulfur species.

For $C_{3j}SH$ SAMs, however, ca. 10-20% of the film appears to be lost after oxidation. This observation suggests either that $C_{3j}SO_x$ are more volatile than $C_{12j}SO_x$ and $C_{18j}SO_x$ species, or that the smaller chain length SAM is more prone to x-ray degradation. Control experiments in which $C_{3j}SH$ films on Ag were exposed to a similar flux of x-rays for 15 min indicate an ca. 5% decrease in peak area for unoxidized SAMs and an ca. 12.5% decrease in total peak area (both 162 eV and 167 eV peaks) for partially oxidized films (3 hr air exposure). These results suggest that the decrease in total peak area is a result of x-ray degradation of the oxidized sulfur species. This degradation is most easily seen for $C_{3j}SH$ SAMs because of the extent to which they oxidize relative to the longer chain length SAMs in the time frame of the experiment.
Table 8.4. % of Thiolate and Oxidized Sulfur Species for SAMs on Ag After Air Exposure Time as Determined from XPS.

<table>
<thead>
<tr>
<th>Exposure Time</th>
<th>0</th>
<th>1 hr</th>
<th>4 hr</th>
<th>24 hr</th>
<th>7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C₃SH</strong></td>
<td>100</td>
<td>80.1</td>
<td>72.9</td>
<td>68.5</td>
<td>49.3</td>
</tr>
<tr>
<td><strong>C₁₂SH</strong></td>
<td>100</td>
<td>98.5</td>
<td>89.9</td>
<td>63.8</td>
<td>58.1</td>
</tr>
<tr>
<td><strong>C₁₃SH</strong></td>
<td>100</td>
<td>92.5</td>
<td>89.5</td>
<td>71.1</td>
<td>65.8</td>
</tr>
</tbody>
</table>

*The % thiolate was determined by the ratio of the peak area at 162 eV at time t to the original peak area at 162 eV.*

*The % oxidized sulfur was determined by the ratio of the peak area at 167 eV at time t to the original thiolate peak area at 162 eV.*
SAMs on Au

Surface Raman Spectroscopy

Most proposed applications involving SAMs utilize Au surfaces and are based on the belief that SAMs on Au represent inert interfaces. Therefore, air exposure experiments on ordered n-alkanethiol (C_nSH, n=3, 12, 18) SAMs on Au before and after exposure to the ambient air environment were performed and compared to the behavior observed on Ag.

The sensitivity of Raman spectroscopy for SAMs on Au is greatly reduced for several reasons. First, the maximum surface enhancement for Au surfaces is achieved with near-IR excitation. However, the surface enhancement for Au at 720 nm is much less than for Ag at 514.5 nm. In addition, monochromator throughput and CCD quantum efficiency at 720 nm is significantly reduced. Therefore, in order to improve signal levels in these experiments, Au surfaces were roughened slightly (3 ORC cycles as described in the experimental section providing weak surface enhancement).

As a result of increased spectrograph dispersion at 720 nm, three Raman spectral regions were collected for each film (versus one for SAMs on Ag) in order to obtain vibrational information from 600-1700 cm⁻¹. Although these experiments require three times the work for the same information compared to Ag, spectral resolution is greatly improved, further clarifying the oxidation behavior.

Surface Raman spectra for C_3SH, C_12SH, and C_18SH SAMs on slightly roughened Au surfaces are shown in Figures 8.10, 8.11, 8.12 respectively. The top spectrum in each
Figure 8.10. Raman spectra of C$_3$H$_3$ SAM on Au after exposure to the ambient environment for: a) 0 hr, b) 6 hr, and c) 20 hr. All spectra plotted on same intensity scale. * denotes peak due to oxidized thiol product.

Integration times: 10 sec x 90
Figure 8.11. Raman spectra of C₃SH SAM on Au after exposure to the ambient environment for: a) 0 hr, b) 6 hr, and c) 20 hr. All spectra plotted on same intensity scale. * denotes peak due to oxidized thiol product. Integration times: 10 sec x 90.
Figure 8.12. Raman spectra of C$_n$SH SAM on Au after exposure to the ambient environment for: a) 0 hr, b) 6 hr, and c) 20 hr. All spectra plotted on same intensity scale. * denotes peak due to oxidized thiol product. Integration times 10 sec x 90.
of these figures is from a freshly prepared SAM in the N$_2$-filled spectrochemical cell prior
to air exposure. Peak assignments for alkanethiols on Au are described in detail in
reference 8.15.

Evidence for oxidation similar to that observed on Ag is detected for alkanethiol-
SAMs on Au upon air exposure. Several bands due to oxidized sulfur species are
observed after 6 hr of exposure to the ambient environment for each alkanethiol SAM.
Specifically, new peaks at ca. 615, 850, 917, 980, and 1010 cm$^{-1}$ corresponding to
sulfonate, sulfonite, sulfate, and sulfite, species, respectively, appear in this spectral
region.$^8,^{20}$ Similar bands are observed in the Raman spectra of solid Na$_2$SO$_4$ and NaHSO$_4$
collected using 720 nm excitation, as shown in Figure 8.13.

Further evidence for sulfonate and sulfonite formation is observed in the $\nu$(C-C)
region. In particular, the broad peak at 1150 cm$^{-1}$ is another indication of sulfur oxidation
with air exposure. This peak is most likely due to the overlap of $\nu$(R-SO$_3^-$) and $\nu$(R-
OSO$_3^-$) bands at 1127 and 1195 cm$^{-1}$, respectively.$^8,^{27}$ The intensities of these new bands
increase with exposure time, similar to the behavior observed on Ag. Moreover, the
intensities of the bands at 1390 and 1590 cm$^{-1}$ due to graphitic-like carbon increase
significantly with air exposure. Such an effect was not observed for SAMs on Ag and may
be related to monolayer decomposition. Specifically, the significant increase in the
intensity of the 1590 cm$^{-1}$ band with oxidation suggests significant alkane chain
degradation to graphite-like species. This point is discussed further below.

The rate of oxidation as a function of chain length on Au is slightly different from
Figure 8.13. Raman spectra of solid a) Na₂SO₄ and b) NaHSO₄ in capillary tubes. Excitation wavelength is 720.0 nm. Integration times: 60 sec.
that observed on Ag. The least stable SAM on Au appears to be C_{12}SH. After only 6 hr of air exposure, significant sulfur oxidation occurs. In addition, the intensities of several monolayer modes decrease including the \nu(C-S)_{\tau} at 705 cm\(^{-1}\), CH\(_3\) rock at 890 cm\(^{-1}\), \nu(C-C) at 1064, 1106, and 1128 cm\(^{-1}\), CH\(_2\) wag at 1300 cm\(^{-1}\), CH\(_2\) twist at 1430 cm\(^{-1}\), and CH\(_2\) scissor at 1452 cm\(^{-1}\). These changes are even more pronounced after 20 hr of air exposure, suggesting either hydrocarbon chain degradation or considerable disorder induced by oxidation.

The differences between the oxidation behavior on Au and Ag are significant. First, the dramatic decreases in overall monolayer band intensities noted on Au are not observed on Ag. Furthermore, the emergence of the 1590 cm\(^{-1}\) graphite mode with oxidation is only observed for SAMs on Au. These data results imply possibly different mechanisms for SAM oxidation on these two metals. For SAMs on Au, the initial decrease in intensity of the monolayer bands implies that oxidation of the sulfur headgroup requires removal of the hydrocarbon chains to allow easier access by the oxidant species. Tarlov et al. observed similar behavior using SERS while studying UV-induced oxidation of alkanethiol SAMs.\(^8,12\)

As expected, the SAM most resistant to oxidation on Au is C_{18}SH. However, C_{18}SH also shows signs of oxidation after the surface is exposed to air for 6 hr, similar to the behavior observed for C_{12}SH on Ag.
XPS Analysis

XPS was used to confirm SAM oxidation on Au after air exposure. The XPS spectra of C$_3$SH, C$_{12}$SH, and C$_{18}$SH SAMs on Au are shown in Figures 8.14, 8.15, 8.16. The decreased S/N for SAMs on Au is the result of greater inelastic scattering processes by the Au. Relative values of the thiolate and sulfonate S 2p peak areas for SAMs on Au are given in Table 8.5. Oxidation similar to that on Ag is observed for SAMs on Au, albeit with certain significant differences. The oxidation rate is clearly a function of chain length; the short chain SAMs (e.g., C$_3$SH) oxidize more quickly than the longer chain C$_{12}$SH or C$_{18}$SH SAMs on Au.

Overall, SAMs formed on Au surfaces are less stable than those on Ag, as evidenced by a more rapid and complete loss of intensity of the S 2p peak at 162 eV due to the thiolate species for all chain lengths after air exposure. For SAMs on Ag surfaces, after 1 week of air exposure, the intensity of the S 2p peak is still greater than that of the peak at 167 eV. However, on Au, the peak at 167 eV disappears after relatively short exposure times. The thiolate species appear to be completely oxidized for both C$_3$SH and C$_{12}$SH SAMs on Au after only 24 hr of air exposure, and for C$_{18}$SH on Au after 7 days. Furthermore, the combined peak areas for these two S 2p bands do not sum to 100% for any chain length SAM on Au, clearly indicating partial loss of S from the surface upon oxidation. This loss may be in the form of SO$_3$. Finally, the data suggest that the oxidation rate of SAMs on Au is larger than that on Ag.

Of additional significance in the XPS data from SAMs on Au is the indication of
Figure 8.14. XPS spectra of S 2p region acquired from C₃SH SAM on Au after exposure to ambient environment for: a) 0 hr, b) 1 hr, c) 4 hr, and d) 24 hr. All spectra plotted on same intensity scale.
Figure 8.15. XPS spectra of S 2p region acquired from C$_{12}$SH SAM on Au after exposure to ambient environment for: a) 0 hr, b) 1 hr, c) 4 hr, d) 24 hr, and e) 1 wk. All spectra plotted on same intensity scale.
Figure 8.16. XPS spectra of S 2p region acquired from C$_{18}$SH SAM on Au after exposure to ambient environment for: a) 0 hr, b) 1 hr, c) 4 hr, d) 24 hr, and e) 1 wk. All spectra plotted on same intensity scale.
Table 8.5. % of Thiolate and Oxidized Sulfur Species for SAMs on Au After Air Exposure from XPS

<table>
<thead>
<tr>
<th>Exposure Time</th>
<th>Thiolate(^a)</th>
<th>Oxidized Sulfur(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C(_3)SH</td>
<td>C(_{12})SH</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>1 hr</td>
<td>15.0</td>
<td>55.2</td>
</tr>
<tr>
<td>4 hr</td>
<td>8.5</td>
<td>61.2</td>
</tr>
<tr>
<td>24 hr</td>
<td>NM(^c)</td>
<td>88.8</td>
</tr>
<tr>
<td>7 days</td>
<td>NM</td>
<td>72.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)The % thiolate was determined by the ratio of the peak area at 162 eV at time t to the original peak area at 162 eV.

\(^b\)The % oxidized sulfur was determined by the ratio of the peak area at 167 eV at time t to the original thiolate peak area at 162 eV.

\(^c\)NM denotes peak areas were not measurable.
multiple oxidation states of the S headgroup. This chemistry is most clearly demonstrated in the XPS data for C3SH on Au at long times in which shoulders at ca. 166.5 eV and 169 eV are observed on the central band at ca. 167.5 eV. Similar broadening of the 167 eV band is observed for C12SH and C18SH for long oxidation times. This behavior is consistent with the Raman spectral evidence for multiple states of oxidation discussed above.

Control experiments in which an oxidized (3 hr air exposure) C3SH SAM on Au is exposed to a similar flux of x-rays for 15 min display an ca. 14% decrease in total peak area (combined 162 eV and 167 eV peaks). The area of the S 2p peak at 162 eV for C3SH on Au (not exposed to air) decrease by only ca. 5%, similar to the behavior observed for SAMs on Ag. These results suggest that sulfonates are ca. three times more likely to degrade upon x-ray exposure than thiolates. This effect is more pronounced on Au because the SAM is oxidized to a greater extent. Therfore, a greater loss of sulfur is observed from Au than Ag. Oxidized C3SH SAMs on Au left under an ultrahigh vacuum environment for extended time periods (ca. 12 hr) without x-ray exposure manifest no peak area dimunition.

Cyclic Voltammetry

Cyclic voltammetry of Ru(NH3)6³⁺ at alkanethiol SAM-modified Au electrodes was also used to study the stability of sulfonates on Au. As shown in Table 8.6, oxidized alkanethiols on Au after 24 hr and 1 week of air exposure are not easily removed, even
Table 8.6. \% Electrochemically Active Surface for SAM-Modified Au Surfaces After Air Exposure.$^a$

<table>
<thead>
<tr>
<th>Alkanethiol-SAM</th>
<th>0 hr Air Exposure</th>
<th>24 hr Air Exposure</th>
<th>1 wk Air Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_3$SH-Au$^b$</td>
<td>$65.5 \pm 12.3^c$</td>
<td>$69.0 \pm 15.1$</td>
<td>$85.8 \pm 2.1$</td>
</tr>
<tr>
<td>$C_{12}$SH-Au</td>
<td>$9.39 \pm 7.60$</td>
<td>$8.92 \pm 6.17$</td>
<td>$9.71 \pm 1.94$</td>
</tr>
<tr>
<td>$C_{18}$SH-Au</td>
<td>$3.21 \pm 1.04$</td>
<td>$2.06 \pm 1.13$</td>
<td>$1.91 \pm 0.46$</td>
</tr>
</tbody>
</table>

$^a$ After air exposure, SAM-modified Au electrodes were immersed and stirred in ethanol for 30 min and rinsed with copious amounts of EtOH and H$_2$O before \% EAS determination. All values normalized to bare (unmodified) Au surfaces.

$^b$ The high \% EAS for $C_3$SH represents efficient electron tunneling through short chain and defects, not exposed surface.

$^c$ Standard deviations result from the analysis of a minimum of 3 independently prepared films.
when stirred in ethanol for 30 min and rinsed with copious amounts of ethanol and water. This observation is similar to that made for alkanethiols on Ag. Furthermore, the insulating properties of the film are maintained, despite oxidation. These results, in combination with those from the Raman spectroscopy and XPS experiments, suggest that although extensive degradation of the thiolate headgroup occurs via oxidation, the partially decomposed hydrocarbon chains remain at the surface and prevent electron transfer to Ru(NH₃)₆³⁺.

Control Experiments

In an effort to gain a better understanding of alkanethiol oxidation, several control experiments were undertaken. Surface Raman spectra for C₇SH SAMs on Ag were collected after exposure to different controlled atmosphere environments, including pure N₂, pure O₂, compressed air, ambient environments in another lab and a private residence, and an O₃-enriched air environment. C₇SH SAMs were selected for these experiments over longer chain lengths because of its limited stability toward oxidation.

As shown in Figure 8.17a, no oxidation bands appear if the SAM is exposed to a pure N₂ environment for 24 hr, the maximum time investigated. Such behavior is expected. Quite surprisingly, however, exposure of the C₇SH SAM to pure O₂ for a similar period (24 hr) only results in minimal oxidation (Figure 8.17b), as evidenced by the broadened background beneath the 890 cm⁻¹ monolayer band, and the shoulder on the ν(C-C) mode at 1026 cm⁻¹. Clearly, massive film degradation due to oxidation, similar to
Figure 8.17. Raman spectra of C$_{x}$SH SAM on Ag after exposure to a) 100% N$_2$ for 24 hr, b) 100% O$_2$ for 24 hr, c) compressed air for 24 hr, ambient lab environment in another building, d) on campus, and e) at private residence for 12 hr, and f) O$_2$/compressed air for 30 sec. * denotes peak due to oxidized thiol product. All spectra plotted on same intensity scale. Integration times: 60 sec x 5.
that observed in the ambient laboratory air after only 1-2 hours of exposure, does not occur. Similar observations were made with a water-saturated O₂ environment, clearly suggesting that neither O₂ nor water is not the oxidant in ambient laboratory air. As shown in Figure 8.17c, purging the cell with breathing-quality compressed air results in a similar outcome: no significant oxidation is observed, even if the O₂ is saturated with water vapor (data not shown).

At this point in our studies, the possible components of our ambient laboratory air were meticulously assessed. Ag surfaces modified with C₃SH SAMs were exposed to the ambient environment in different rooms in our building, another building on campus, and at a private residence. Transfer of these surfaces in N₂ took place at night, and at no time were the SAMs exposed to light. The Raman spectra of these surfaces after 12 hr of air exposure in another building and a private residence are shown in Figure 8.17d and 8.17e, respectively. As was observed in our laboratory, the SAM exposed to the ambient air in different rooms in our building and another building on campus (Figure 8.17d) oxidized extensively, as evidenced by the appearance of oxidation bands at 615, 875, 975, 1040, and 1147 cm⁻¹. Interestingly, only slight oxidation was observed for the C₃SH film stored overnight off campus as shown in Figure 8.17e, indicating a difference between the ambient environment in the university laboratory building and a private residence.

After a lengthy discussion with building engineers at our institution, it became apparent that the air handlers at the University of Arizona bring in 100% outside air for cooling (and heating when necessary) for all university buildings; no percentage of
building air is recycled. Particularly important for laboratory buildings is that significant air flow is maintained in order to continuously replace laboratory air which exits the building through the fume hoods. Herein lies the variance between the university and private residence ambient environment. In the private residence, the volume of air flow is considerably smaller.

Residual gas analysis of our laboratory air was performed with a Balzers Prisma Model QMS 200 quadrupole-based mass spectrometer. Air was leaked into a UHV system so that the base pressure increased to $5 \times 10^{-7}$ torr and the mass spectral response was monitored at m/z 48 as a function of time. As shown in Figure 8.18, O₃ is clearly found to be a small component of our laboratory environment (this experiment was repeated in another laboratory and O₃ was detected as well).

O₃ levels in Tucson and throughout the southwestern United States, where sunlight is abundant, are known to be higher than those in other parts of the country (O₃ formation is accelerated by ultraviolet light from the sun). For example, during the month of July in 1996, the average hourly O₃ level as monitored by the U.S. Environmental Protection Agency² was 68% higher than O₃ measured in central Iowa (0.041 versus 0.028 ppm). O₃ levels are also greater in cities such as Tucson which are surrounded by mountains which trap air pollution. For that same month, the average hourly O₃ level in Palm Springs, CA, was measured at 0.063 ppm. Naturally, O₃ levels in buildings for which a large percentage of air is continually recycled will be higher than those having lessened circulation (e.g., private residence) or recycled circulation.
Figure 8.18. Residual gas analysis by quadrupole mass spectrometer monitoring a) m/z 32 (oxygen) and b) m/z 48 (ozone).
To further test whether $O_3$ might be accelerating thiolate oxidation, a mixture of $O_3$ and compressed air was generated in a glove bag by a method described above in the experimental section. As shown in Figure 8.17f, exposure of the $C_3SH$ SAM to this environment for only 30 sec results in rapid and almost complete oxidation.

Similar observations are made for $C_{18}SH$ SAMs on Ag, as shown in Figure 8.19. However, as expected, the rate of oxidation is much slower for the longer chain length SAM. In addition, a progression of several oxidation modes are observed as a function of $O_3$ exposure time, as evidenced by increases in the intensity of sulfonate oxidation bands at 615, 725, 917, and 975 cm$^{-1}$. This observation suggests that $C_{18}SH$ adequately impedes $O_3$ penetration into the film in the time period studied.

Interestingly, a decrease in monolayer band intensities with the appearance of modes at 1390 and 1590 cm$^{-1}$ as a function of $O_3$ exposure time is also observed. This behavior, not previously observed on Ag (even after air exposure for 1 wk), suggests C-S cleavage and alkane chain degradation at sufficiently high oxidant concentrations.

Interestingly, sulfonite oxidation modes at 1034, 1040, 1127, and 1195 cm$^{-1}$ are never observed, suggesting that the intermediate oxidation states of sulfur are extremely unstable in this highly oxidizing environment.

$C_3SH$ and $C_{18}SH$ SAMs on Au were also exposed to the mixed compressed air and $O_3$ environment. The Raman spectra of $C_3SH$ and $C_{18}SH$ SAMs on Au before and after $O_3$ exposure are shown in Figure 8.20 and 8.21, respectively. The appearance of oxidized sulfur modes at 615, 850, 917, 975, 1010, and 1150, suggest considerable oxidation. As
Figure 8.19. Raman spectra of C_{16}SH SAM on Ag a) before, and b) after exposure to O_{2}/compressed air-filled glove bag for b) 5 min, c) 15 min, d) 40 min, and e) 1 hr. All spectra plotted on same intensity scale. * denotes peak due to oxidized thiol product. All spectra plotted on same intensity intensity scale. Integration times: 60 sec x 5.
Figure 8.20. Raman spectra of C₆SH SAM on Au a) before, and b) after exposure to O₂/compressed air for 30 min. Integration times: 10 sec x 60. * denotes peak due to oxidized thiol product. All spectra plotted on same intensity scale.
Figure 8.21. Raman spectra of C$_2$SH SAM on Au a) before, and b) after exposure to O$_2$/compressed air for 30 min. Integration times: 10 sec x 60. * denotes peak due to oxidized thiol product. All spectra plotted on same intensity scale.
was noted in the air exposure experiments, SAMs on Au appear to be less stable toward oxidation than SAMs on Ag. The combination of the observed decrease in monolayer bands by ca. 70% and the appearance of modes at 1390 and 1590 cm\(^{-1}\) indicate massive alkane chain degradation to amorphous carbon.

Finally, the stability of thiophenol (TP) SAMs on Ag was investigated. Garrell and coworkers have reported that monolayers formed from aromatic thiols on Au are significantly more robust and less prone to air and electrochemical oxidation than alkanethiol SAMs.\(^8,^8,^8,^29,^8,^30\) The Raman spectra for TP SAMs before and after O\(_3\) exposure is shown in Figure 8.22. The most interesting feature, an ca. 90% decrease in the intensity of the ring breathing and bending modes at 997, 1019, and 1070 cm\(^{-1}\), suggests O\(_3\) exposure causes extensive cleavage of the C-S bond of TP with concomitant removal of presumably benzene upon oxidation of this monolayer on Ag. The fact that the peak intensity ratio of these modes remains constant implies little variation in the orientation of remaining TP as oxidation and degradation occur. These data indicate that aromatic thiols on Ag are not stable in environments containing high levels of O\(_3\).

Based on the above control experiments, we propose that O\(_3\) is the primary oxidant in the ambient laboratory air environment that causes the rapid oxidation of alkanethiol SAMs on Ag and Au. This hypothesis may possibly explain the current discrepancy regarding air oxidation of SAMs in the community. Air oxidation studies taking place under ambient laboratory environments in which O\(_3\) levels are low will result in less thiolate oxidation. The O\(_3\) concentration in laboratory air in a given location is a
Figure 8.22. Raman spectra of a thiophenol SAM on Ag a) before, and b) after exposure to O₂/compressed air for 30 min. Integration times: 30 sec.
function of several factors including outdoor O₃ levels, fresh air circulation, and air flow.

In buildings in which partial air recirculation is a regular mechanism by which laboratory air is provided, the oxidation rate of SAMs will be less for a given O₃ level in the outside air.

**Oxidation Mechanism**

There have been few investigations of the reaction of O₃ with organosulfur compounds. The most notable study involved the infrared spectroscopic analysis of the reaction of ground state oxygen atoms, generated by visible photolysis of O₃, with dimethylsulfide and methanethiol in solid argon matrices. Oxygen atoms were shown to react in a stepwise mechanism with dimethylsulfide in argon matrices to produce dimethylsulfoxide and dimethylsulfone with no apparent activation barrier even at 10 K. Codeposition of methanethiol and O₃ produced oxidation bands which were assigned to -C-O-S- and -C-S-O- linkages, suggesting multiple oxidation products. Photolysis of these matrices increased product yield. When these reactions were carried out in the gas phase, HSO⁻ species were produced resulting in C-S bond cleavage.

The results of this study support our conclusions regarding the oxidation of alkanethiol SAMs by O₃. The ease with which methanethiol is oxidized at 10 K supports the high reactivity of O₃ with sulfur at room temperature. Mechanisms for UV-induced oxidation are proposed to occur by the interaction of sulfur with singlet oxygen, a product of O₃ decomposition. We propose a similar mechanism for air-induced oxidation.
without UV-surface irradiation.

XPS and Raman spectroscopy of oxidized SAMs suggest that these films on Ag are more stable than on Au. This enhanced stability on Ag is proposed to be due to the greater strength of the Ag-S bond. The Ag-S bond is expected to be more ionic than the Au-S bond based on the electronegativity differences between S and these two metals.\textsuperscript{8,15} Significantly, C-S bond cleavage is observed to a greater extent on Au than on Ag as evidenced by a decrease in intensity of the bands attributed to the monolayer and the appearance of amorphous carbon modes. Only after O\textsubscript{3} levels are dramatically increased, is similar behavior observed for SAMs on Ag.

The coexistence of thiolates and oxidized sulfur species, as evidenced by XPS and Raman spectroscopy, suggests that oxidation does not occur uniformly, but rather at specific localized areas. Defects which are inherent for SAMs formed on polycrystalline surfaces, are sites at which an oxidant could more easily penetrate the film and react with the sulfur headgroup. Therefore, film stability is critically dependent on the quality of the starting film. As shown by the variation in rate of different chain lengths, films containing a larger number of defects are more prone to oxidation.

Conclusions

The data in this chapter clearly support oxidation of alkanethiol self-assembled monolayers upon air exposure in the absence of light. However, even after significant oxidation, SAM films still generally retain their integrity. Therefore, the potential utility of
SAMs for many applications, including modified pacemaker electrodes, is not sacrificed.

Many factors affect alkanethiol oxidation, including chain length, initial film quality, nature of the metal substrate, and atmospheric O$_3$ levels. Raman spectroscopy and XPS indicate that the oxidation rate varies strongly with alkyl chain length. Long chain length SAMs oxidize much more slowly because of the inability of the active oxidant species to penetrate the closely-packed alkyl chain structure. Short chain SAMs oxidize at faster rates as a result of shorter distances between the outside of the SAM and the sulfur headgroup and the greater number of defects. Similarly, poorly formed films containing larger numbers of defects are less stable. SAMs formed on Au are significantly less stable than on Ag, contrary to previous literature reports, as a result of metal-sulfur bond strength. Finally, O$_3$ has been identified as the likely oxidant in air that causes alkanethiol oxidation. Conflicting reports on the stability of SAMs in air are probably due to a combination of the above factors. However, the concentration of O$_3$ in laboratory air (related to atmospheric O$_3$ levels and building air flow and circulation) is probably the limiting factor.

Raman spectroscopy is proven to be a superior tool for studying SAM structure and stability, because it provides information on all components of the alkanethiol, including the thiolate head group.
Chapter 9

Mechanical Stability of Alkanethiol Self-Assembled Monolayers on Au Electrodes

Introduction

Mechanical stability of SAMs is another important attribute in light of the expected mechanical stress imposed on these films by introduction into blood and during the "screw-in" implant procedure. It has not been resolved at this point whether the alkanethiol SAM withstands the mechanical stress of implantation, the abrasive environment of blood, and the electrochemical stress associated with cardiac pacing. Although alkanethiol monolayers on Au are considered to be robust, the cardiac pacing environment is extremely harsh. Previous chapters have addressed the stability of SAMs under electrochemical conditions (Chapter 7) and after exposure to the ambient lab environment (Chapter 8). Ru(NH₃)₆³⁺ was used to assess the exposed surface area after exposure to electrolyte and potential, and to study SAM retention after oxidation.

Although the average rate of interfacial electron transfer is significantly decreased at SAM-modified surfaces, particularly for the longer chain films, calculated %EAS values were used in a qualitative sense to assess whether a significant portion of the bare surface was exposed after each experiment. As discussed in Chapter 6, it is difficult to rule out rapid electron transfer at a few defect sites as the dominant contribution to the measured electron-transfer current.⁹⁻⁹⁷ Therefore, it is difficult to evaluate small changes in %EAS.
Self-assembled monolayers containing covalently attached electroactive molecules provide an additional avenue for studying small changes in alkanethiol surface coverage. Significant advantages are realized when freely diffusing redox species are replaced by covalently attached electroactive groups. First, the low mobility of densely packed hydrocarbon chains limits the diffusion of the attached electroactive sites to defects. Secondly, both the reduction and oxidation processes are exhaustive electrolyses of the solely surface-confined redox species, rather than steady-state as are currents obtained at alkanethiol modified-electrodes with redox couples in solution.

Several groups have studied the kinetics and thermodynamics of interfacial redox reactions.\textsuperscript{9.8-9.14} Ferrocene has been the most well-characterized, outer-sphere, electroactive moiety tethered to a surface via an alkanethiol to date\textsuperscript{9.8, 9.12-9.21} because of the ease with which it can be incorporated into SAMs. Chidsey et al. demonstrated that mixed monolayers containing low concentrations of ferrocenyldalkanethiols (Fc) relative to n-alkanethiols display thermodynamically ideal surface electrochemistry in 1 M HClO\textsubscript{4}.\textsuperscript{9.8} Their studies indicate that at low Fc surface coverages, the ferrocene groups are homogeneous, noninteracting, and extraordinarily robust over a wide range of potentials.\textsuperscript{9.8}

Recently, Everett and coworkers reported the use ferrocenyldalkanethiols for the study of the potential-dependent instability of mixed SAMs (C\textsubscript{12}SH and FcCON\textsubscript{2}(CH\textsubscript{2})\textsubscript{10}SH) on Au electrodes in nonaqueous solvents.\textsuperscript{9.21, 9.22} They demonstrated that small changes in alkanethiol surface coverage can easily be quantified, nondestructively, using the oxidation
and reduction of the bound ferrocenes.

The issue of mechanical stability of SAMs towards stress during tissue implant is addressed in this chapter using ferrocenylalkanethiols-modified Au wire electrodes. A series of experiments in which these electrodes are plunged into and removed from material which resembles heart tissue in order to mimic electrode implantation are described. Cyclic voltammetry in 1 M HClO₄ is used to evaluate the surface coverage of the film before and after these manipulations to elucidate the effect of mechanical stress on the integrity of the SAMs.

Methodology

Preparation of Wire Electrode

A 5 cm piece of Au wire of 0.5 mm dia (99.9985%, Johnson Matthey) was fastened with epoxy to a brass rod to create a working electrode approximately 2.5 cm in length. The exposed surface area was 0.47 ± 0.03 cm². "Piranha" solution was used to clean the electrode before each experiment. *(Caution! Piranha solution is a very strong oxidant and can spontaneously detonate upon contact with organic material!)* These surfaces were then electrochemically polished by at least 20 ORCs from -0.2 V to +1.2 V at 100 mV/s in 1.0 M H₂SO₄, rinsed with copious amounts of H₂O and ethanol, and then immersed into alkanethiol solution.
Ferrocenyldecanethiol Synthesis

Ferrocenyldecanethiol (FcCO₂(CH₂)₃₀SH, Fc = (η⁵-C₅H₅)Fe(η⁵-C₅H₅)) was synthesized by esterification of ferrocene carboxylic acid with 11-bromoundecanol, conversion of the bromide to a thioacetate with sodium thioacetate, and finally, mild hydrolysis of the thioacetate with sodium carbonate, as published in a procedure reported by Chidsey and coworkers. The identity and purity of ferrocenyldecanethiol was confirmed by ¹H NMR and GC/MS.

Monolayer Formation

Mixed thiol monolayers are formed by soaking Au wire in ethanol solutions of ferrocenyldecanethiol (FcC₁₁CO₂SH) and C₁₀SH at a 5 mM total thiol concentration. The mole fraction of FcCO₂C₁₁SH in the solution, denoted as Xₘ, was 0.2. After a 48 h immersion time, the wire electrodes were removed from solution and rinsed with copious amounts of ethanol and water prior to electrochemical experiments.

Cellulose Sponge and Beef

A 100% pure cellulose sponge was purchased and rinsed extensively with Millipore (deionized) H₂O before use. A small portion of the sponge (30 x 30 cm) was cut for easier handling. Pieces of beef stew meat were purchased from the supermarket and rinsed extensively with Millipore H₂O before use. Both the sponge and the beef were saturated with H₂O in a petri dish at all times during the experiment.
Cyclic Voltammetry of Mixed FcC$_{11}$CO$_2$SH and C$_{10}$SH SAMs

The cyclic voltammetry of a mixed monolayer of FcCO$_2$(CH$_2$)$_{11}$SH (Fc=$\eta^2$-C$_5$H$_5$Fe($\eta^2$-C$_5$H$_4$)) and CH$_3$(CH$_2$)$_9$SH (hereafter referred to as Fc and C$_{10}$SH) on Au formed from a $\chi$$_{Fe} = 0.2$ solution for 48 hr is shown in Figure 9.1. The electrode potential was scanned at 25 mV/s beginning at +0.30 V and reversing at +0.85 V in 1 M HClO$_4$. The peaks are symmetric about a potential of +0.56 V and exhibit no splitting; the full width at half maximum (fwhm) of the main peak is 90 mV. The expected linear relationship between anodic peak current of the main peak at +0.56 V and the sweep rate is shown in Figure 9.2, consistent with the surface bound Fc redox species.$^{9,23}$ Continuous cycling in 1 M HClO$_4$ for 15 min did not affect the observed voltammetric behavior, indicating that these SAMs are stable. These results agree with previously reported work for Fc SAMs on Au.$^{9,13,9,15}$

The shape of the individual peaks is not entirely symmetric reflecting a distribution of reactive states for ferrocene.$^{9,15}$ Previous reports suggest that such behavior reflects small differences in the local environment of the electroactive site.$^{9,11-9,13,9,15,9,18}$ For a single surface-bound species, the major and minor redox waves correspond to environments in which both a large number and small number of ferrocene groups congregate, respectively. Ferrocene groups are more difficult to oxidize when they interact with each other. Therefore, a more positive potential is required to oxidize these sites.

The number of adsorbed ferrocene molecules in the monolayer can be quantified
Figure 9.1. Cyclic voltammogram in 1 M HClO₄ of a mixed monolayer of FeCO₂(CH₂)₉SH and CH₃(CH₂)₉SH formed from an ethanol solution containing χₑ = 0.2 after 48 hr. Scan rate = 25 mV/s.
Figure 9.2. Potential sweep rate dependence of $i_{pa}$ for Au wire electrodes modified with a mixed monolayer comprised of FcCO$_2$(CH$_2$)$_{10}$SH and C$_{15}$SH. The potential was scanned between +0.30 and +0.85 V in 1 M HClO$_4$. 
from the anodic charge. The area under the oxidation peak is integrated and divided by the scan rate to obtain the amount of charge passed to oxidize ferrocene. To obtain Fc surface coverages, the anodic charge is divided by the area of the electrode and the charge of an electron.

For a $\chi_{Fe} = 0.2$ solution, the surface coverages observed for three immersion times are shown in Table 9.1. As previously reported, a loosely packed, mixed monolayer is quickly formed and slowly becomes denser with time. At 1 hr, the Fc coverage is slightly higher than at 24 hr, reflecting the presence of some ferrocene groups folded in among the poorly packed alkane chains. An Fc coverage of $(10.0 \pm 0.8) \times 10^{13}$ molecules/cm$^2$ after 48 hr represents saturation and is in close agreement with values obtained by Chidsey and coworkers.

**Effect of Mechanical Stress**

The effect of mechanical stress on the stability of mixed FcCO$_2$(CH$_2$)$_{11}$SH / C$_{10}$SH monolayers was investigated by determining changes in surface coverage after mechanical manipulation of the modified electrode. A cellulose sponge was chosen due to its similarity to biological tissue and the ease at which mechanical stress could be studied. Immediately after film formation, the SAM modified-Au wire was rinsed with ethanol and H$_2$O, and then examined by cyclic voltammetry in 1 M HClO$_4$. After a voltammogram was recorded, the electrode was again rinsed with H$_2$O, and then gently pierced through a cellulose sponge while twisting, to mimic the scenario during pacemaker electrode
Table 9.1. Fc Surface Coverage Values for Fc/C$_{10}$SH Mixed Monolayers on Au as a Function of Immersion Time

<table>
<thead>
<tr>
<th>Immersion Time</th>
<th>$Q_{\text{Fe}}$ ($\mu$C/cm$^2$)$^b$</th>
<th>$\Gamma_{\text{Fe}}$ ($\times 10^{13}$ molecules/cm$^2$)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hr</td>
<td>13.3 ± 0.5</td>
<td>8.3 ± 0.3</td>
</tr>
<tr>
<td>24 hr</td>
<td>12.4 ± 1.6</td>
<td>7.7 ± 1.0</td>
</tr>
<tr>
<td>48 hr</td>
<td>15.9 ± 1.3</td>
<td>10.0 ± 0.8</td>
</tr>
</tbody>
</table>

$^a$ Values determined using electrochemically determined surface area.

$^b$ Standard deviations result from the analysis of a minimum of 3 samples.
implantation. After removal, the electrode was rinsed with H₂O and examined again by cyclic voltammetry. The data presented below represent individual experiments for each mechanical manipulation.

The cyclic voltammetry of a FeC₁₁SH / C₁₀SH mixed SAM on Au in 1 M HClO₄ at a scan rate of 25 mV/s before and after gently piercing a H₂O- saturated cellulose sponge is shown in Figure 9.3. The shape of the voltammogram changes slightly after piercing the cellulose sponge a single time. More pronounced changes are observed in the voltammetry after 25 piercings. In addition to a reduction in both anodic and cathodic peak currents, the voltammogram shifts positive approximately 90 mV from +0.56 V to +0.65 V, the fwhm of both peaks increases from 90 mV to ca. 135 mV, and a splitting of 30 mV is observed between the anodic and cathodic peak potentials.

The positive shift in ferrocene oxidation potential and the splitting of the anodic and cathodic peaks are attributed to contamination of the surface by species originating in the sponge. Positive shifts in redox potential have been previously reported for ferrocenylalkanethiols in which the environment around the ferrocene is made more alkane-like by increasing the chain length of the diluent n-alkanethiol relative to the Fe in the mixed SAM.⁹ ¹¹ ¹² We speculate that piercing of the cellulose sponge results in adsorption of species on the SAM creating a lower dielectric “alkane-like” environment. In addition to cellulose monomers, plasticizers and/or other chemical additives in the sponge could be the culprit contaminants. Despite extensive rinsing of the sponge with H₂O prior to use, such contaminants could remain entrapped in the hydrophobic
Figure 9.3. Cyclic voltammetry for Fc/C₆SH mixed SAMs on Au in 0.1 M HClO₄; scan rate = 25 mV/s. The solid line is the sweep immediately after immersion. The dashed and dotted curves were obtained after gently piercing a H₂O saturated cellulose sponge 1 and 25 times, respectively.
environment of cellulose.

The observed peak splitting and broadening of the redox wave further indicate heterogeneous electron transfer kinetic complications that must arise as a result of this surface contamination. In addition to creating a more organic environment around the electroactive sites, contamination also causes the obstruction of defect sites through which electron transfer is known to be rapid for uncontaminated SAM films (Chapter 6). As a result, electron transfer only occurs via electron tunneling through the dielectric alkane barrier.\textsuperscript{9,24, 9.25}

Nonetheless, the tabulated ferrocene surface coverages indicate that the amount of ferrocene decreases by an average of 11.1 ± 4.0% and 26.0 ± 6.0%, for 1 and 25 pierces, respectively, as shown in Table 9.2. Assuming minimal surface roughening as a result of sponge piercing, the data indicate loss of the Fc group due to either mechanical or chemical stress. Significantly, identical behavior is observed for shorter immersion times (1 hr and 24 hr), demonstrating that the initial structural order of the monolayer plays a minor role in its resultant stability.

A similar experiment using a raw piece of beef was undertaken to more accurately model heart tissue which is considerably more malleable and chemically more complex than cellulose. As shown in Figure 9.4, piercing a piece of beef one time results in electrochemical behavior similar to that observed with the cellulose sponge described above. For meat, protein adsorption, instead of monomer contamination, is postulated to be the cause for a positive shift in the redox potential of Fc and the ca. 15 mV peak
## Table 9.2: Fc Surface Coverage Values for a Fc/C_{10}SH Mixed Monolayer on Au as a Function of Mechanical Stress

<table>
<thead>
<tr>
<th>Mechanical Stress</th>
<th>Average $\Gamma_{Fc}$ Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$O Rinse (control)</td>
<td>Negligible$^a$</td>
</tr>
<tr>
<td>Sponge Pierce $^b$ x1</td>
<td>-11.1 ± 4.0$^c$</td>
</tr>
<tr>
<td>Sponge Pierce x5</td>
<td>-19.5 ± 3.1</td>
</tr>
<tr>
<td>Sponge Pierce x25</td>
<td>-26.0 ± 6.0</td>
</tr>
<tr>
<td>Meat Pierce x1</td>
<td>-16.5 ± 4.3</td>
</tr>
</tbody>
</table>

$^a$ All surfaces were rinsed extensively with H$_2$O before and after mechanical manipulation. Control electrodes which experienced no mechanical piercing were rinsed with H$_2$O and displayed no change in $\Gamma_{Fc}$.

$^b$ After initial analysis by cyclic voltammetry, the wire electrode was pierced in a H$_2$O-saturated cellulose sponge or raw piece of steak, then rinsed with copious amounts of H$_2$O and again examined by cyclic voltammetry.

$^c$ Standard deviations result from the analysis of a minimum of 3 independent measurements.
Figure 9.4. Cyclic voltammetry for Fc/C_{10}SH mixed SAMs on Au in 0.1 M HClO₄; scan rate = 25 mV/s. The solid line is the sweep immediately after immersion. The dashed curve was obtained after piercing a piece of raw meat once.
splitting.

The data suggest that a significant fraction of molecules may be removed from the pacemaker electrode surface due to mechanical stress during implantation. In fact, the coverage of alkanethiol molecules depleted from the surface is probably greater for C\textsubscript{12}SH SAMs on Au-coated Pt(Ir) pacemaker electrodes, because instead of bulk, polycrystalline Au, an extremely rough layer of Au is electroplated onto the electrode. One would expect this metal substrate to be mechanically less stable than the surfaces used for the above experiments.

Conclusions

Ferrocenylthiols have been used to study the stability of SAMs after mechanical manipulation. This represents the first reported to date on the stability of SAMs after mechanical stress. Results are presented that reveal substantial loss of the Fc group after piercing H\textsubscript{2}O-saturated cellulose sponges or pieces of beef. In addition to depletion of surface ferrocene groups, positive shifts in anodic peak potential are attributed to a more organic environment created by surface contamination originating in the sponge or beef.

At this time, the exact mechanism of Fc loss is not well-understood. Hydrolysis of the ester linkage in a basic environment created in the sponge or meat, would also result in a decrease in Fc coverage. Further experiments involving n-alkanethiol SAMs on Au are necessary to elucidate the stability of SAMs following mechanical stress.

These studies have important implications for the mechanical stability of SAM-
modified pacemaker electrodes and other SAM-modified bioimplants. The results suggest a possibly greater depletion of surface alkanethiols for pacemaker implantation procedures involving repetitive electrode insertion and extraction which might be necessary to optimize electrode positioning. The effects of such action are not known at this time. Additional work is needed to establish the relationship between the removal of alkanethiols and Au during the mechanical stress associated with electrode implantation and the improved biocompatibility of the modified pacemaker electrodes.
Chapter 10
Conclusions and Future Directions

Overview of Problem

Active-fixation cardiac pacemaker electrodes possess biocompatibility problems which lead to excessive inflammation and scar tissue formation. Several attempts have been made to reduce the inflammation which occurs after implantation as well as prevent degradation of the bioelectrical interface; however, few have been successful. A new, successful strategy involves high-affinity covalent attachment of organic self-assembled monolayers (SAMs) through metal-S bonds. The fundamental chemical nature of the pacemaker electrode surface is significantly altered and controlled by the SAM, significantly improving pacing performance, albeit by a mechanism not completely understood. A critical factor in using SAMs for cardiac pacing applications is the stability of the SAM at the bioelectrical interface. Although the structure of SAMs has been thoroughly characterized in the past decade using several techniques, the stability of these films under conditions for which they are used has never been properly characterized. Perhaps a better understanding of the factors which determine SAM stability may lead to a more complete mechanism for improved pacing performance.
Objectives of Research

The objectives of this research were to better understand and characterize the improved pacing performance of alkanethiol-modified, active-fixation pacemaker electrodes via a comprehensive characterization of the stability of SAMs under conditions operable before and during pacing. The characterization of these organic films further contributes to the development of surface Raman spectroscopy as an excellent tool for analyzing self-assembled monolayers.

Summary of Research

The pacing performance of active-fixation cardiac pacemaker electrodes electrochemically plated with Au was substantially improved by covalent surface modification with C_{12}SH self-assembled monolayers. The SAM-modified electrodes proved superior to unmodified electrodes in maintaining the bioelectrical interface. Although the exact mechanism is not completely understood, the interfacial chemistry and the electrochemical characteristics of the electrode were altered in a way that enhances electrode biocompatibility. Different types of modification strategies suggest that both a Au(I)-S moiety (possibly providing anti-inflammatory qualities) and a long-chain organic film (preventing metal surface access to large molecules) work cooperatively to improve the pacing performance of modified electrodes. These results suggest that the biocompatibility of electrically conductive materials can be controlled at the molecular level with monolayer organic surface films.
Electrochemical measurements were made in a living mammalian heart in an attempt to estimate myocardial tissue resistance and define fundamental electrical characteristics of cardiac tissue. The results of these measurements demonstrated similar trends to those observed with benchtop electrochemistry in solution, despite the unique environment, as supported by both in vivo concentration and potential sweep rate studies. Myocardial tissue resistance was estimated to be $< 1.5 \times 10^3 \, \Omega/cm$ using cyclic voltammetry and chronoamperometry, significantly lower than previous reports. Perfusion studies implied that only interstitial contributions (and not intracellular transport) of redox molecules diffusing between cells to the electrode were measured using these electrochemical techniques.

Surface Raman spectroscopy using an emersion approach was developed as an excellent technique for probing the structural order and stability of self-assembled monolayers on Ag and Au. Alkanethiol SAMs on surfaces prepared by mechanical polishing were shown to contain graphitic-precursor-like contamination, changing the overall structure of the monolayer significantly. These carbon impurities, defined as an additional monolayer defect, were observed to be extremely sensitive to laser exposure, solvent, electrolyte, and potential. In addition, the order of the self-assembled monolayer was proven to be significantly affected by the amount of carbon contamination.

Several cleaning protocols were investigated for creating contamination-free films. The most effective cleaning protocol involved negative potential treatment of SAM-modified Ag electrodes. Potentials negative of alkanethiol reductive desorption were
found to completely remove carbon contamination and some alkanethiol molecules. Reimmersion of the "electrochemically cleaned" surface into alkanethiol solution after negative potential application reproducibly formed carbon impurity-free films possessing an exceptionally high degree of structural order.

The stability of alkanethiol SAMs on Ag in aqueous electrolytes at different potentials was also investigated using Raman spectroscopy. Such experiments serve as a simple model for understanding the stability of SAM-modified pacemaker electrodes under pacing conditions: electrolyte rich environment (blood) and constant voltage application (pacing system). Exposure to aqueous electrolyte at open circuit potential did not affect the structural order or oxidation state of the thiol head group for any of the alkanethiols studied, regardless of chain length. However, more negative potentials resulted in an array of effects depending on the monolayer, electrolyte, and the magnitude of potential. Short chain alkanethiols were shown to be susceptible to disordering and headgroup oxidation at potentials negative of alkanethiolate reductive desorption. Longer chain alkanethiols did not disorder at negative potentials up to -2.0 V, regardless of electrolyte, suggesting that the stability of well-ordered SAMs in aqueous media is dependent on alkanethiol chain length, electrolyte, and potential. These results provide insight into the greater efficacy of C₁₂SH on Au-Pt(Ir) over C₄SH on Au-(Pt) modified pacemaker electrodes. Perhaps the longer chain SAM is more better able to withstand the pacing environment.

Of comparable importance to the effects of electrolyte and potential was the
discovery that alkanethiol SAMs on Ag and Au readily oxidize after relatively short exposure times (ca. 2-5 hr) to the ambient environment, regardless of chain length. The predominant oxidant species in air was found to be O₃. Quite significantly, the data explain the controversy which exists in the community on SAM stability in air. XPS data indicate SAMs on Au are significantly less stable than SAMs on Ag, contrary to common belief. Cyclic voltammetric data indicate that even after ethanol immersion and rinsing, the blocking ability of the film is retained, suggesting that van der Waals forces between methylene units are strong enough to prevent removal of oxidized sulfur species. Quite relevant to SAM-modified pacemaker electrodes, these findings suggest that the dormant time between electrode fabrication and implantation should not significantly affect monolayer integrity.

Future Directions
Pacemaker Electrode Studies

Certain experiments remain to be done which may provide more insight to the improved pacing performance observed with SAM-modified pacemaker electrodes. Three areas of continued pre-clinical studies are necessary to further investigate the enhanced pacing performance observed with modified-pacemaker electrodes: alternative alkanethiol modification strategies, electrode characterization after explant, and evaluation of the pacemaker electrode implantation site. The following experiments are proposed under the assumption that a small animal model will successfully be developed to limit excessive
canine use.

To help determine the role of Au-alkanethiol SAMs in the improved pacing performance, three aspects of electrode modification should be investigated: the chemical nature of the terminal functional group, the degree of film order, and the need for the electroplated Au coating. To evaluate the importance of the functional group on pacing performance, the R-group of the alkanethiol SAM should be varied. The chemical nature of the terminal group on SAMs has been shown to alter protein adsorption at modified surfaces. To date, pacemaker electrode have only been modified with -CH\textsubscript{3} terminal functional groups resulting in hydrophobic interfaces. The pacing performance of hydrophilic surfaces should also be investigated. Initial experiments might include -OH or -COOH terminal groups. If similar pacing performance improvements are observed with Au coated -COOH SAMs, then alteration of protein adsorption is most likely not responsible for the improved biocompatibility. Modification strategies could then be extended to include SAMs with protein moieties to further assess the role of the terminal functional group.

Film quality is another important attribute of SAMs which remains to be optimized and related to pacing performance. Defects are necessary attributes for retaining electrode conductivity. The SAM-modified pacemaker electrodes described in Chapter 3 were formed for only short time periods on relatively rough Au surfaces in order to create defect densities large enough to ensure conductivity. However, no effort was made to optimize the conductivity of modified-pacemaker electrodes. The variables which affect
defect density and which should be properly characterized include SAM formation time, alkanethiol solution concentration, and the roughness of the underlying surface. The roughness of the Au layer can be controlled by deposition potential, Au solution concentration, and thickness of the Au coating, none of which have been properly characterized as of this time.

The stability of the alkanethiol-SAM in blood could be evaluated by implanting small SAM modified-Au disks into rat tissue. The disk should remain in the rat for several days or weeks allowing time for the full immunological response to take place. After removal, the modified-disk could be analyzed with surface vibrational spectroscopies to determine the presence and integrity of the SAM. Previous attempts to analyze explanted pacemaker electrodes using this approach have failed because of the difficult helical-electrode geometry; small disks would solve such complications.

In order to assess the effects of cardiac pacing on SAM corrosion, tissue samples from the region of the heart around the electrode implantation site should be analyzed using inductively coupled plasma/atomic emission spectroscopy (ICP/AES). These studies would allow the quantitation of dissolved electrode metal ions as a result of chemical or physical degradation of the interface during pacing. The extent to which such corrosion occurs is essential to developing a better understanding of SAM stability under pacing conditions, and the potential anti-inflammatory action of the Au-S moiety.
Fundamental Studies of SAM-Modified Electrodes

Another important issue which remains to be addressed for SAMs on Au is their stability after exposure to electrolyte and potential. Whether the behavior of Au-SAMs in an electrochemical environment follows Ag-SAMs, because of the difference in metal-sulfur bonding for the two metals. At the very least, a series of alkanethiols with varying chain lengths in different electrolytes (specifically adsorbing, nonadsorbing, and varying pH) should be investigated so that a systematic comparison can be made. In addition, these studies might provide extra insight to the mechanism of improved pacing performance for modified pacemaker electrodes.

The emersion approach using the spectroelectrochemical cell should be utilized for investigating the effects of nonaqueous solvents on alkanethiol film structure.

Tremendous interest for modifying electrodes with SAMs exists in the electrochemical community; however, very few studies on SAM stability in a nonaqueous environment have been reported (most likely due to the lack of adequate characterization tools).

Although the effects of acetonitrile and CCl₄ exposure on C₃SH structure was reported in Chapter 5, those films contained significant amounts of carbon impurities, which was proven to alter SAM stability. The structure of both SAM modified Ag and Au surfaces after solvent and potential exposure should be fully investigated.

Finally, the structure of SAM-modified electrodes with terminal groups other than -CH₃ need to be investigated. The chemical nature of the terminal group attached to the alkane chain can be varied over a range of functional groups in order to systematically
vary film hydrophobicity. Once characterized, a series of experiments involving SAMs with varying functional groups could be pursued including interfacial solvent structure and protein adsorption studies.

The solvent structure at the SAM-solution interface has important ramifications in the electrochemical community. SAM-modified electrodes have been used by many researchers to better understand heterogeneous electron transfer kinetics. A better understanding of the solvent structure at the interface would result in a more complete description of electron transfer.

The characterization of protein adsorption at chemically-modified surfaces using Raman spectroscopy might provide important insight to the fundamentals of blood biocompatibility and, perhaps, the mechanism of enhanced pacing performance for modified-pacemaker electrodes. Given the complexity of this phenomenon, initial studies will need to focus on amino acid adsorption on different alkanethiol SAMs as a function of terminal group. Adsorption characteristics for different amino acids are expected to be highly dependent on whether the surface is hydrophobic or hydrophilic.
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Chapter 9


