ABSTRACT

LINDENMUTH, TRISHA KAY. Determination of Surface Acidity of Mixed Self-Assembled Monolayers. (Under the direction of Professor Edmond F. Bowden.)

The surface acidity of mixed self-assembled monolayers (SAMs) consisting of 7-carboxy-1-heptanethiol (C7COOH) and 8-hydroxy-1-octanethiol (C8OH) on gold electrodes was characterized by electrochemical impedance spectroscopy (EIS). The mole ratio of the two thiols was varied from 0-100% acid composition. The charge transfer resistance (Rct) increases as the pH of the solution containing the redox probe Fe(CN)6^3^-/4 increases. Titration curves, of Rct vs pH were obtained, from which the inflection point was determined as the pK1/2 value. Molar ratios of OH:COOH of 5:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:5 and a pure acid SAM gave pK1/2 values of 6.29, 6.54, 6.76, 6.89, 6.91, 7.11, 7.07, and 6.94, respectively. The pK1/2 values reported in this work are higher than the solution pKa value, which is ~4.7. This effect may be due to ion solvation, interfacial potential, hydrogen bonding and/or electrostatic interactions. The effect of ionic strength on the surface acidity was also examined. This work confirmed previous studies showing that the pK1/2 values of SAMs increase as ionic strength is decreased.
Determination of Surface Acidity of Mixed Self-Assembled Monolayers

by
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A thesis submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the Degree of Master of Science

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DEDICATION

This accomplishment is dedicated to my family….

….especially my Gram, whom I lost while I was completing my degree.
BIOGRAPHY

Trisha Kay Lindenmuth, the only child, was born on September 25, 1988, in Williamsport, Pennsylvania to Robin and Steven Lindenmuth. Trisha spent a few years of her life in South Williamsport, Pennsylvania, until her parents moved a few towns over to Montoursville, Pennsylvania. She received her secondary education from Montoursville Area High School in 2006. Trisha then enrolled at Lycoming College, where she graduated with a Bachelor of Science degree in Chemistry in 2010. In the summer of 2010, Trisha moved to Raleigh, North Carolina to pursue a Master of Science degree in analytical chemistry from North Carolina State University. Over the past two and a half years, Trisha studied chemistry and performed research under professor Edmond Bowden.
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CHAPTER 1

INTRODUCTION
1.1 Overview and Motivation

Although the work described in this thesis is focused on characterization of self-assembled monolayers (SAMs), the motivation for undertaking it arose from recent developments in protein electrochemistry. The first report of protein immobilization on a self-assembled monolayer was that of Tarlov and Bowden\(^1\) in 1991 wherein the adsorption of electroactive cytochrome c on gold electrodes modified by alkanethiolate SAMs terminated in carboxylic acid groups (i.e., COOH SAM’s) was described. Since then a considerable literature has emerged on cytochrome c electrochemistry on SAM-modified electrodes, as well as other proteins for which electron transfer (ET) is one of their primary functions\(^2-7\). The adsorption of cytochrome c on COOH SAM’s is driven primarily by electrostatic attraction. Cytochrome c is a cationic protein that binds readily to anionic domains. The heme edge side of the protein where ET takes place is dominated by a patch of 8 or 9 lysines\(^3\), which imparts excess positive charge to this side of the protein as shown is Figure 1.1. The ET-active heme edge side of cytochrome c
Figure 1.1: Electrostatic potential surface of the heme edge side of horse heart cytochrome c, PDB 1HRC. Blue denotes positive potential (primarily due to lysines) and red denotes negative potential. Figure prepared using DS Visualizer (Accelrys).

will thus have a strong attraction towards deprotonated carboxylate sites at the SAM surface, which results in adsorption in a preferred electroactive orientation\textsuperscript{3}. Clearly, the acid/base properties of COOH SAM’s will play a critical role in such interactions since the degree of ionization will be determined by solution pH. Proteins having well-defined binding sites for ET, such as cytochrome c, have enjoyed considerable success when employed in electrochemical investigations using COOH SAMs.

Less successful, however, have been electrochemical studies of the globin class of proteins, of which hemoglobin and myoglobin are the most common. For these proteins, oxygen binding and transport are the primary functions, although they do undergo secondary ET reactions. Over the past several years, the Bowden group has been investigating a
hemoglobin known as dehaloperoxidase (DHP)\textsuperscript{8,9}. DHP is a dual-function protein that not only binds and transports oxygen but also has a significant peroxidase function. Unlike cytochrome c, DHP and other globins do not have well-defined binding sites, as evidenced in Figure 1.2, which shows the electrostatic surface potential of the ET-active heme edge side of DHP. Although 4-5

\textbf{Figure 1.2:} Electrostatic potential surface of the heme edge side of dehaloperoxidase, PDB 2QFK. Blue denotes positive potential (primarily due to lysines) and red denotes negative potential arising from carboxylates. The two negative groups at the center of the image are the exposed heme propionates. Figure prepared using DS Visualizer (Accelrys).

lysines are located in the heme edge region, the positive charge resulting from them is partially offset by neighboring carboxylate groups, including two heme propionates visible at the center of the image. As a result, the binding of DHP to electrodes is much weaker and is
comprised of a distribution of orientations. D’Antonio was the first to achieve successful electrochemistry of DHP with the use of COOH SAMs. Interestingly, the types of COOH SAMs that had previously worked well with cytochrome c and other ET proteins did not work at all with DHP. Neither pure COOH SAMs nor mixed OH:COOH SAMs in which the acid chain length was longer than the alcohol chain length produced worthwhile results. Instead, D’Antonio found that mixed OH:COOH SAMs could be made to work as long as the number of carbons in each of the two thiol molecules was the same. Recent DHP research in the Bowden group has primarily made use of C_8OH:C_7COOH SAM/gold electrodes prepared from solutions of HS(CH_2)_7COOH and HS(CH_2)_8OH.

Accordingly, the present research was undertaken with the intention of characterizing the acid-base properties of C_8OH:C_7COOH SAM/gold electrodes, which are otherwise unavailable. This knowledge will provide additional insight into the interfacial interactions that govern the interactions between DHP and SAMs and therefore their electrochemistry. For example, with knowledge of the surface acid/base properties, it would be possible to estimate the degree of COOH ionization and therefore the anionic charge density at the surface for a given solution pH.

In the remainder of this chapter, both general and specific aspects of self-assembled monolayer research will be reviewed with an emphasis on acid-base properties of COOH SAMs and their determination.
1.2 Self-Assembled Monolayers (SAM)

Self-assembled monolayers (SAMs) have been reported and investigated for many years. Although first reported in 1946 by the Zisman group, intensive research on SAMs did not begin until the 1980’s. SAMs form when certain metal or metal oxide surfaces are exposed for some length of time to solutions containing characteristic types of molecules comprised of a chemical group with high affinity for the surface joined to a longer hydrophobic tail. The result of the self-assembly process is usually a stable molecular monolayer that coats the surface in a more-or-less uniform manner. There are many different variations of SAMs that can be prepared and the choice is commonly driven by the intended application. The choice of substrate is critical with many reports appearing over the past thirty years using gold and, to a lesser extent, silver and platinum.

Molecules that self-assemble into SAMs must have at least two parts and typically have three: the head group, the backbone (or chain), and the terminal group. The head group is the part that attaches to the solid surface and its choice thus depends on the nature of the surface being modified. The backbone is typically a saturated hydrocarbon chain whose length can be varied. A substantial portion of the stability of SAMs arises from the van der Waals and hydrophobic forces associated with chain-chain interactions. The head group and the chain are required in molecules that form SAMs. From a chemical perspective, it is the third part, however, the terminal group, that is most critical because its choice is based on the intended application and will largely define the chemical properties and reactivity of the resultant surface.
For electrochemical studies, SAMs of alkanethiolate/gold composition have proven to be the most useful and popular type by a large margin. This is due to the chemical inertness of gold metal and the excellent stability of the SAMs that form. Self-assembly is easily accomplished from solutions containing ω-functionalized alkanethiols. During the self-assembly process, a strong covalent bond forms between gold surface atoms and the sulfur atom of the alkanethiol. It is believed that loss of a hydrogen atom from the thiol group occurs during this reaction and that the sulfur atom becomes partially negatively charged\(^1\). As a result, it is more accurate to describe these structures as alkanethiolate SAMs rather than alkanethiol SAMs.
Figure 1.3: Simplified structure of a generic self-assembled monolayer on an electrode.

A wide variety of applications have been reported for alkanethiolate SAMs on gold across multiple fields of endeavor, of which a few examples are given here. For device fabrication, alkanethiols can be used as inks for “writing” molecules in SAMs. In molecular electronics, they can function as switches and transistors. In biomedicine, they have been employed as linkers or protective groups for the purpose of drug delivery. In sensors and biosensors, SAMs have been extensively utilized for the immobilization of proteins and other biomolecules. One of the main applications for COOH-terminated SAMs is, in fact, the immobilization of proteins for biosensor applications\textsuperscript{13}. 
1.3 COOH-Terminated Alkanethiolate SAMs

Investigation and characterization of COOH SAMs have been a focus for many research groups for more than two decades. Of particular interest is the fact that the surface charge can be altered by solution conditions, primarily pH. An important property in this regard is the pK$_{1/2}$ value, which is the key measure of surface acidity. It is defined as the solution pH value that results in deprotonation of one-half of the carboxylic acid groups to the carboxylate form$^{15,16}$. Although the terms pK$_{1/2}$ and pK$_a$ are often used interchangeably in the literature, their definitions are not identical. The term pK$_a$ should be reserved for acids dissolved in homogeneous solutions that are devoid of any electric field. The use of this term implies that each molecule can be described using the same value for the acid dissociation constant (K$_a$). When dealing with acids that are confined to electrode surfaces, however, the situation can be considerably more complex. Distributed pK$_a$ values are typically observed due to the microscopic heterogeneity of most electrode/solution interfaces. Furthermore, electric fields can affect the protonation state of acids localized at electrode surfaces. Accordingly, the term pK$_{1/2}$ is best used with interfacial systems and will be used henceforth.

The literature on pK$_{1/2}$ determinations of COOH SAMs has often been conflicting and inconsistent. When gold electrodes modified with alkanethiolate SAMs of the same nominal chemical composition have undergone characterization by various research groups, different pK$_{1/2}$ values have often been the result. The notion that different techniques may account for much of the inconsistency has often been proposed as an explanation. It is necessary, however, to consider structural variations in SAMs that can arise from differences in self-assembly procedures, substrate pretreatment, and solution conditions. For example, the
topography of the gold electrode (i.e., “roughness”) has been identified as a factor that can have a large influence on the acidity of the SAM surfaces, as described in the next section.

### 1.3.1 Pure COOH SAMs

A pure COOH SAM is self-assembled from a solution containing a single type of thiol molecule such as HS(CH₂)₁₀COOH. They are also referred to as homogeneous SAMs. The effect of microscopic surface roughness of the gold substrate on pKᵱ/₂ values of pure COOH SAMs has been considered by several groups. Leopold et al.¹⁷ investigated C₁₃COOH SAMs on gold electrodes spanning a wide range of surface roughness. From the results obtained along with a critical examination of the available literature, they concluded that a difference in pKᵱ/₂ value of up to 3 units could be attributed to the effect of roughness, with smoother surfaces yielding the more basic values. To rationalize these findings, a model was proposed that entailed extensive 2-dimensional hydrogen bonding among COOH groups on atomically smooth gold surfaces due to the close molecular proximity enforced by the regularity of the substrate surface. Such hydrogen bonding would stabilize the protonated states, causing a pKᵱ/₂ shift in the basic direction. On rough surfaces, SAMs would be more structurally disordered, with presumably disrupted hydrogen bonding between COOH groups. Burris¹⁸ subsequently examined COOH SAMs of different chain lengths and discovered that 3-mercaptopropionic acid pKᵱ/₂ values shifted ~2 pH units in the acidic direction due to increased surface roughness, consistent with Leopold et al. Scavetta et al.¹⁹ also investigated the effect of electrode surface roughness and found that rougher surfaces result in more acidic pKᵱ/₂ values on flexible electrodes as well.
The effect of chain length on pK$_{1/2}$ values for pure COOH SAMs has also been examined. It has been consistently found that longer chain lengths result in more basic pK$_{1/2}$ values. Dai$^{20}$ reported pK$_{1/2}$ values of 5.3, 7.3 and 7.9 for C$_2$COOH, C$_{10}$COOH, and C$_{15}$COOH SAMs, respectively. Liu$^{21}$ reported pK$_{1/2}$ values of 4.80 and 7.40 for C$_2$COOH and C$_5$COOH, respectively. One chain length that did not fit the trend, however, was CCOOH, which both groups characterized, obtaining values of 6.1 and 5.20. This chain length being the shortest possible, it is likely that CCOOH is a unique case due to the proximity of the acid group to the metal surface.

The influence of solution ionic strength on pure COOH SAMs was examined by Lu et al.$^{22}$ and Liu et al.$^{21}$, who both found that higher ionic strength leads to more acidic values of pK$_{1/2}$. It was proposed that at higher ionic strength, hydrogen bonding between COOH and COO$^-$ groups becomes diminished by the formation of ion pairs between COO$^-$ groups and cations in solution. As a result, the neutral acid groups near the ion pairs are more easily deprotonated, causing a lower pK$_{1/2}$ value. At low ionic strength, on the other hand, the scarcity of available counterions allows maximum hydrogen bonding between COOH and COO$^-$ groups to occur and, thus, a higher pK$_{1/2}$ value.

1.3.2 Mixed COOH SAMs

Mixed COOH SAMs are usually prepared by simultaneous self-assembly from a solution containing two different thiol molecules, one being terminated with COOH and other by OH or CH$_3$. They are also referred to as binary SAMs. Mixed SAMs offer considerably more flexibility in achieving desired chemical and physical surface properties
than do pure SAMs. For example, it has been found that cytochrome c exhibits significantly faster rates of ET on mixed COOH/OH SAMs for which the acid molecule is slightly longer than the alcohol\textsuperscript{1,4,7}.

In Creager and Clarke’s contact angle investigation\textsuperscript{23}, they measured pK\textsubscript{1/2} values for mixed COOH SAMs for which the second molecule was CH\textsubscript{3}-terminated. They maintained a constant length for the acid component while varying the length of the methyl component. They found that, as the CH\textsubscript{3} chain length became longer, the pK\textsubscript{1/2} values increased. Lu\textsuperscript{22} studied a mixed SAM composed of mercaptoacetic acid (MA) and 2-mercaptobenzothiazole (Mbz) and showed that when there is a smaller percent of acid, the pK\textsubscript{1/2} is more basic. They also showed that ionic strength had the same effect on mixed SAMs as it does for pure acid SAMs.

Tielens \textit{et al}\textsuperscript{24} investigated the mixing process of mixed OH:COOH SAMs of variable composition by XPS and by computational methods. They varied the COOH content from 0 to 100% mole fraction. They proposed that a SAM that is 50% acid and 50% alcohol will be perfectly mixed at the molecular level. For SAMs containing an acid mole fraction of less than 50%, they proposed that such SAMs will have the same stability as the 50% SAM due to the presence of perfectly mixed COOH/OH islands separated by pure OH regions. Then 50-75% will behave like a 75% SAM because it has perfectly ordered patterns of COOH surrounding OH. Finally the 75-100% SAM acts like a pure acid SAM.
1.4 Characterization Techniques for COOH SAMs

A variety of different techniques have been used to characterize SAMs and, in the case of acidic and basic SAMs, for determining pK$_{1/2}$ values. Some of the more commonly used methods include contact angle measurements, quartz crystal microbalance (QCM), infrared spectroscopy (IR), cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). In the first part of this section, faradaic electrochemical techniques based on the use of redox probes are considered. This is the approach that is followed in this thesis research with an emphasis on EIS measurements. In the second part of this section, a brief review of some of non-electrochemical techniques is provided.

1.4.1 Redox Probe Electrochemistry

Electrochemistry has proven to be a popular and useful approach for determination of pK$_{1/2}$ values for acidic SAMs. The most widely used techniques involve the use of diffusing redox probe molecules. The ET reactions of these molecules at the electrode surface can be highly sensitive to the presence of a SAM, which can inhibit the reaction by blocking the substrate electrode. For characterizing COOH SAMs, the most widely used probe has been ferri/ferrocyanide, whose high negative charge makes it very sensitive to the charge buildup resulting from deprotonation. At sufficiently high pH, excessive negative charge on the surface can act to “block” the anionic redox probe from reaching the electrode, resulting in complete inhibition of the reaction. At intermediate levels of pH and SAM ionization, ET can take place at reduced rates compared to a bare metal electrode due to partial inhibition of the reaction.
Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) have been the most widely used techniques in conjunction with redox probes. For CV, the cathodic peak current is usually monitored and plotted against pH to obtain a titration curve. For COOH SAMs, decreasing current occurs as pH is increased due to surface deprotonation. With EIS, the key parameter of interest is the charge transfer resistance, $R_{ct}$, which is plotted against pH to obtain a titration curve. One obtains $R_{ct}$ by fitting the response of an equivalent circuit, which includes $R_{ct}$ as one of its circuit elements, to electrochemical impedance data. Chapter 2 describes the EIS experiment and provides a detailed discussion of $R_{ct}$. In one report, Luo et al.\textsuperscript{25} found that cyclic voltammetry and EIS produced the same value of $pK_{1/2}$.

The nature of the redox probe has been investigated using EIS. Anionic probes have been found to result in more acidic values than cationic probes. Anderson’s group\textsuperscript{26} reported that the redox probe Ru(NH$_3$)$_6^{2+/3+}$ resulted in a $pK_{1/2}$ value that is ~2 units more basic than determined using Fe(CN)$_6^{-3/-4}$ for a C$_{10}$COOH SAM. They believe that the different applied potential used for the different probe molecules is the reason for the difference in results. Ru(NH$_3$)$_6^{2+/3+}$ has a more negative reduction potential than does Fe(CN)$_6^{-3/-4}$ which means that there will be relatively more negative charge on the gold surface in that case. If the electrode field due to the excess charge on the metal extends to the acid sites, the effect would be to shift the $pK_{1/2}$ to a more basic value. The authors propose that the actual $pK_{1/2}$ is between the two values they obtained for the differently charge redox probes.
1.4.2 Non-Electrochemical Techniques

Goniometers are used to measure contact angles at the interface formed between a SAM and a water droplet placed atop the surface. The resulting angle will depend on the hydrophobic/hydrophilic nature of the surface, which is dependent on the ionization state of COOH SAMs. Creager and Clarke\(^23\) were responsible for developing experimental protocols that produced well-defined titration curves and were among the first to publish extensive contact angle measurement data for acidic SAMs. Since then, the contact angle measurement has often assumed the role of a reference technique against which other techniques are compared. For example, in their investigations of 3-mercaptopropanioic acid SAMs, Dong and coworkers\(^27\) validated a new cyclic voltammetry technique by comparing CV results to contact angle results.

A quartz crystal microbalance (QCM) records the change in frequency of a gold-film coated quartz crystal resonator due to mass changes at the gold surface. When an acid SAM undergoes deprotonation and develops negative charge, cations will accumulate at the surface and cause an increase in mass, which lowers the frequency. This expected behavior was observed by Shimazu et al.\(^28\), who determined pK\(_{1/2}\) values for various lengths of COOH SAMs. Ward’s group, however, in an earlier paper, reported unexpected behavior in which the frequency change that occurred upon deprotonation fell in the opposite direction and was of far larger magnitude than predicted from counterion accumulation. They hypothesized the existence of a hydrogen-bonded structured water layer for the protonated state of the SAM, which increased its apparent mass. Leopold et al.\(^17\) later proposed that these dramatically different QCM observations were likely due to differences in gold surface topography. Xia
and coworkers\textsuperscript{29} have used QCM to quantify how the adsorption of cytochrome \textit{c} on COOH SAMs depends on the extent of ionization state as controlled by pH. It was found that higher pH gave rise to extensive adsorption whereas very little was observed at low pH.

Recently, Bouwstra\textsuperscript{30} and coworkers described a new technique for determining pK\textsubscript{1/2} values of amine and carboxyl terminated SAMs on silicon surfaces. They measured the fluorescence intensity due to the accumulation of fluorescent nanoparticles on the surface of the SAM as a function of pH. Electrostatic binding was realized by using positively charged nanoparticles with carboxyl SAMs and negatively charged nanoparticles with amine SAMs. This technique was compared to contact angle measurements and was claimed to be more sensitive and precise.
1.5 References


CHAPTER 2

ELECTROCHEMICAL IMPEDANCE SPECTROSCOPY (EIS)
2.1 Introduction

Faradaic electrochemistry has been used by several research groups in recent years as a means to determine the pK$_{1/2}$ values of carboxylic acid SAMs$^{1-11}$. The usual approach is to employ a negatively charged diffusing redox probe whose electrode kinetic behavior is sensitive to the ionization state of the surface. With but a few exceptions, ferri/ferrocyanide has been the probe of choice due to its high anionic charge and its well behaved electrochemistry. The simple idea is that, as a COOH SAM grows increasingly deprotonated at higher pH, the voltammetry of ferri/ferrocyanide becomes increasingly irreversible due to interfacial electrostatic repulsion. The two major faradaic techniques that have been employed to characterize systems of this type are cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Although CV is simpler to implement and the results easier to visualize, quantitative interpretation can be troublesome because the output signal reflects a combination of mass transfer and electron transfer limitations (see Chapter 4). On the other hand, because EIS can separate individual processes based on their frequency response, it is possible to isolate the kinetic limitation that shows sensitivity to the charged state of the SAM$^{12}$.

2.2 The EIS Experiment

The concept of impedance is usually credited to Oliver Heaviside, who introduced it into the analysis of electrical circuits in the 1870’s. Not too long thereafter, in 1894, Nernst first applied impedance analysis to physicochemical systems, initiating its usage in electrochemistry. Many scientists have since made major contributions to the development
of electrochemical impedance analysis, and it is currently a widely used technique in its modern format known as electrochemical impedance spectroscopy. A key historical contribution was made in 1901 by Warburg, who established the theoretical basis of mass transfer impedance due to linear diffusion. The complex circuit element that he introduced is known as the Warburg impedance. In 1947 Randles, as a result of investigating the kinetics of adsorption reactions at polarized mercury electrodes, proposed the seminal equivalent circuit that bears his name. Impedance techniques became increasingly popular for investigating redox systems beginning in the 1970’s and heterogeneous surfaces in the 1990’s\textsuperscript{13-14}.

EIS experiments reported on in this thesis made use of planar gold electrodes coated with either a COOH SAM or a COOH/OH mixed SAM and a solution phase that contained equimolar concentrations of ferricyanide and ferrocyanide, which function as redox probes. The ferri/ferrocyanide interconversion is a simple 1-electron transfer process:

\[
Fe(CN)_6^{3-} + e^- \leftrightarrow Fe(CN)_6^{4-} \quad \text{Eq. 1}
\]

The faradaic EIS experiment is implemented by applying a fixed DC voltage (\(E_{dc}\)) equal to the formal reduction potential (\(E^\circ\)) while superimposing a small amplitude sinusoidal voltage perturbation (\(E_{ac}\)) of amplitude \(\Delta E\):

\[
E = E_{dc} + E_{ac} = E^\circ + \Delta E \sin(\omega t) \quad \text{Eq. 2}
\]

where \(\omega\), the angular frequency, is equal to \(2\pi f\), with \(f\) designating the frequency in hertz. The experiment is conducted by sequentially applying the excitation waveform at individual frequencies over a suitably broad range. At each frequency of applied potential, an AC current having the same frequency and with a magnitude \(\Delta I\) will result. For circuits
composed entirely of resistors and capacitors, current will lead the voltage by a phase angle of $0^\circ$ to $90^\circ$. The AC voltage and the AC current are related through the impedance vector, $Z$, which is characterized by a magnitude $Z$ and a phase angle ($\phi$). Commonly, the impedance vector $Z$ is simply referred to as the impedance. Using complex notation, the impedance at a given frequency is commonly decomposed into real ($Z_{\text{Re}}$) and imaginary ($Z_{\text{Im}}$) components that correspond to resistive ($\phi = 0^\circ$) and capacitive ($\phi = 90^\circ$) impedance, respectively:

\[
Z_{\text{Re}} = R \quad \text{Eq. 3a}
\]

\[
Z_{\text{Im}} = \frac{1}{\omega C} \quad \text{Eq. 3b}
\]

where $R$ is resistance and $C$ is capacitance$^{12}$.

Typically, EIS experiments such as those described herein are conducted over a frequency range of sub-Hz to $\sim 10^5$ Hz. Impedance data are commonly analyzed using Bode plots, in which $Z$ is plotted versus log $f$, or Nyquist plots, in which $-Z_{\text{Im}}$ is plotted versus $Z_{\text{Re}}$. The Nyquist plot, also known as a complex plane impedance plot, is more prevalent in electrochemistry and this is due, in part, to the fact that the shape of the response is more revealing of the equivalent circuit used to model the interface. In order to extract physical and chemical information from EIS data, a valid equivalent circuit must be fitted to the data$^{15}$. 
2.3 The Randles Equivalent Circuit and $R_{ct}$

In this work, we utilized the Randles equivalent circuit shown in Figure 2.1, which is commonly used to model simple faradaic reactions that can be adequately described by Butler-Volmer electrode kinetics and semi-infinite linear diffusional mass transfer. In its simplest depiction, the circuit consists of four elements: the solution resistance ($R_s$), the double-layer capacitance ($C_{dl}$), the charge transfer resistance ($R_{ct}$), and the Warburg impedance ($Z_W$). $R_s$ arises from the uncompensated solution resistance between the working and reference electrodes, and is a purely Ohmic impedance. The double-layer capacitance ($C_{dl}$) reflects the structure of the electrode/solution interface whereby excess charge localized on the electrode surface gives rise to an oppositely charged layer of counterions that accumulate in the solution adjacent to the SAM. Thus, the electrode/solution interface can store charge in a manner analogous to a parallel plate capacitor. The double-layer capacitance provides a purely non-faradaic pathway for current. The series combination of $R_{ct}$ and $Z_W$, on the other hand, constitutes the faradaic current pathway for the ferri/ferrocyanide reaction (Eq. 1). The current from this reaction can be limited by both mass transfer and charge transfer processes. The Warburg impedance ($Z_W$) is a complex circuit element that is used to model semi-infinite linear diffusion. The Warburg impedance is applicable when using planar electrodes in quiescent solutions, as is the case in the present
Figure 2.1: Randles equivalent circuit.

The key parameter of interest for characterizing the acidity of a carboxylic acid SAM surface is the charge transfer resistance, which reflects the kinetic behavior of the redox probe. The ET kinetics for the reaction in Eq. 1 are assumed to follow Butler-Volmer theory and consist of the forward ET rate constant ($k_{et,f}$) for the reduction of ferricyanide and the back ET rate constant ($k_{et,b}$) for the oxidation of ferrocyanide. At the DC potential utilized in the EIS experiment, i.e., $E_{dc} = E^\circ^*$, the following relationship applies:

$$i_c = |i_a| = i_o$$  \text{Eq. 4}

where $i_c$ and $i_a$ are the respective cathodic and anodic components of the observed current (with cathodic current taken as positive), and $i_o$ is the exchange current. Because heterogeneous reaction rate is first-order and proportional to current, i.e., rate $= kC = i/nFA$, one can also write:

$$k_{et,f} C_{ferri(x=0)} = k_{et,b} C_{ferro(x=0)} = k^\circ C^* = i_o/nFA$$  \text{Eq. 5}
where $C_{\text{ferri}(x=0)}$ and $C_{\text{ferro}(x=0)}$ are the respective surface concentrations of ferricyanide and ferrocyanide, $C^*$ is the equimolar solution concentration value for the two redox species, and $k^\circ$ is the standard heterogeneous ET rate constant.

For a bare metal electrode equilibrated against an equimolar redox solution, the small amplitude limit of the exponential Butler-Volmer equation takes a simpler form in which overpotential ($\eta = E - E_{\text{eq}}$) and current are linearly related, thus giving rise to the well-known equation for $R_{\text{ct}}$ assuming a 1-electron reaction\textsuperscript{16}:

$$R_{\text{ct}} = \frac{RT}{F i_0} \quad \text{Eq. 6}$$

The exchange current is given by:

$$i_0 = FAk^0C^* \quad \text{Eq. 7}$$

$F$ is the Faraday constant and $A$ is the electrode area. When Eqs. 6 and 7 are combined, one obtains:

$$R_{\text{ct}} = \frac{RT}{F^2 A k^0 C^*} \quad \text{Eq. 8}$$

Based on this standard formalism\textsuperscript{16}, the charge transfer resistance is inversely proportional to the standard ET rate constant and the solution concentration of the redox probe. If one assumes that the nominal surface concentrations are essentially the same as the bulk concentrations, Eq. 8 can be readily invoked, and any variation in $R_{\text{ct}}$ can be ascribed to $k^\circ$. On the other hand, if surface concentrations should deviate significantly from their bulk values, Eq. 8 would no longer be applicable and a more complex theory would be required.

For carboxylic acid SAMs, as already noted, increasing pH causes a decrease in the rate of the ferri/ferrocyanide reaction and an increase in $R_{\text{ct}}$\textsuperscript{1,2,4,6-9}. And, as previously noted,
this phenomenon arises from increased electrostatic repulsion between the electrode and the ferri/ferrocyanide species due to the greater extent of COOH deprotonation that occurs at higher pH. We do not know how changes in surface ionization individually affect $k^\circ$ and redox probe surface concentrations for the mixed SAMs used in the present work. For example, it is possible that ET can occur across the SAM if it is well packed and pinholes can be neglected. On the other hand, ET may occur at pinholes that allow access of the redox probe to the metal substrate. This latter case was treated theoretically by Molinero and Calvo\textsuperscript{5}. By assuming that the same value of $k^\circ$ applies regardless of pH, they attributed all changes in $R_{ct}$ to changes in surface concentrations of the reactants arising from pH-dependent electrostatic repulsion. They derived a complicated equation for $R_{ct}$ based on Gouy-Chapman theory, Butler-Volmer theory, and Fawcett’s model for potential distribution across a SAM/metal interface\textsuperscript{17}. Qualitatively, the Molinero-Calvo equation gives rise to a sigmoidal relationship between $R_{ct}$ and pH (see Section 2.5), which is a useful result for estimating pK$_{1/2}$ values.

2.4 Nyquist plot for the Randles equivalent circuit

Figure 2.2 depicts the appearance of a Nyquist plot for electrochemical systems that can be modeled by the Randles circuit. In the limit of low frequency, the reaction will be electrochemically reversible and the value of $R_{ct}$ will be negligible. In this regime, the current is limited by diffusional mass transfer as represented by the Warburg impedance. This response gives rise to the linear arm in Figure 2.2, which has a phase angle of 45° due to equal resistive and capacitive contributions to the impedance. As frequency increases, the
current becomes increasingly controlled by ET kinetics, which is manifested as the semicircle in the Nyquist plot due to the parallel relationship of the $R_{ct}$ and $C_{dl}$ elements. In the limit of high frequency, $C_{dl}$ offers negligible impedance to charge flow, resulting in the observed impedance arising solely from the solution resistance.

![Nyquist plot](image)

**Figure 2.2**: Nyquist plot for the Randles equivalent circuit used in the present work.

Circuit element values can be estimated from a simple inspection of the Nyquist plot. Pure resistances will lie on the x-axis, i.e., impedances with $\phi = 0^\circ$. $R_s$ is found at the intersection of the high frequency end of the semi-circle with the x-axis. Where the lower frequency end of the semi-circle intersects the x-axis, the resistance value is $(R_s + R_{ct})$. Thus, the diameter of the semi-circle is equal to the charge transfer resistance\(^\text{16}\). The angular
frequency that gives rise to the impedance at the top of the semi-circle is equal to \((R_{ct}C_{dl})^{-1}\). Thus, knowing the values of \(R_{ct}\) and this frequency allows one to readily calculate \(C_{dl}\).

### 2.5 Using EIS to Determine pK\(_{1/2}\) of SAMs

Several groups have previously used EIS to characterize carboxylic acid terminated alkanethiolate SAMs and evaluate surface acidity\(^1-6,8-10\). The principal findings of these investigations were included in Section 1.3, which addressed acid/base properties of COOH SAMs. To determine pK\(_{1/2}\)'s value using EIS, the charge transfer resistance is usually plotted against pH, giving rise to a sigmoidal curve that is frequently referred to as a titration curve. The pK\(_{1/2}\) is commonly taken to be the inflection point of the graph. As mentioned above, for the case in which an anionic redox probe reacts at COOH SAM pinholes, the theoretical equation proposed by Molinero and Calvo\(^5\) predicts a sigmoidal dependence of \(R_{ct}\) on pH.

Recent investigations have examined whether the pK\(_{1/2}\) values obtained by EIS are independent of the choice of redox probe. The fact that more acidic values were obtained with anionic probes\(^3,4\) than with cationic probes raises important questions about the use of this technique in this application. Anderson\(^4\), for example, obtained a pK\(_{1/2}\) value for the C\(_{10}\)COOH SAM using the redox probe Ru(NH\(_3\))\(_6\)\(^{4+/3}\) that was 2 units more basic than was found using the Fe(CN)\(_6\)\(^{3/-4}\) probe. They explained this observation as a local interfacial pH effect related to the excess charge present on the metal surface. They noted that the standard potentials for Ru(NH\(_3\))\(_6\)\(^{4+/3}\) and Fe(CN)\(_6\)\(^{3/-4}\) are approximately +0.30 and -0.20 V, respectively, versus Ag|AgCl reference electrode, and that the potential of zero charge (PZC),
which they took to be 0.13 V, fell between them. The Stern model states that if the potential is held at a value more positive than the PZC, which would be the case for the Fe(CN)$_6^{3/-4}$ probe, a buildup of negative charge will occur in the solution near the electrode surface, which would cause the local interfacial pH to be more basic than the bulk pH. Since the pH near the interface is higher than the bulk solution, the observed pK$_{1/2}$ would be at a lower value than solution pH. The same argument can be made for when the potential is held at a value more negative than the PZC, which would be the case for the Ru(NH$_3$)$_6^{2+/3}$ probe. Based on results like these, one must consider that EIS-determined pK$_{1/2}$ values may be dependent on the DC potential that is used. Values determined using the Fe(CN)$_6^{3/-4}$ probe may thus be more acidic that the field-free value that would arise when there is no excess charge on the metal electrode, i.e., $E_{dc} = PZC$.

2.6 Additional EIS Characterization of SAM Modified Electrodes

Besides determining pK$_{1/2}$ values, EIS can be used to study other aspects of carboxylic acid SAMs including structural features and packing quality. For example, the impact of SAM defect sites on electron transfer rates have been examined by EIS$^{18}$. In another study, it was found that the structural quality of SAMs of different chain lengths is dependent on preparation protocol. Longer self-assembly times were required to achieve high quality SAMs when longer chain alkanethiols were used$^{19}$. More recently, the formation of SAMs on nanoparticles has been investigated for biosensor applications. Gold nanoparticles can be deposited on planar gold electrodes and then modified with SAMs. The SAMs that form are less ordered and have diminished interactions between chains, which is
similar to the behavior observed for rough electrode surfaces\textsuperscript{20}. In studies using cytochrome c, the rate at which the protein adsorbs on COOH SAMs from low ionic strength solution has been shown to be diffusion controlled\textsuperscript{21} and EIS has been used to measure electron transfer rate constants between the protein and COOH SAM modified electrodes\textsuperscript{22,23}. 
2.7 References


CHAPTER 3

EXPERIMENTAL
3.1 Electrochemical Cell

The electrochemical cell used in these experiments is a modified version of a basic design first utilized by Koller and Hawkridge\(^1\). Shown in Figure 3.1, this cell has been used extensively in the Bowden lab for many years. Electrolyte solution is introduced into the cylindrical cavity at the center of the glass body. The water jacket for temperature control was not utilized in the present work. The working electrode was a planar thin-film gold electrode (see below for details) positioned horizontally below the cylindrical solution compartment. An O-ring seal demarcated an exposed electrode area of 0.32 cm\(^2\). Electrochemical experiments were performed using a standard 3-electrode arrangement. The reference electrode (Microelectrodes, Inc.) was a Ag/AgCl (1 M KCl) type and the auxiliary electrode was a platinum wire twisted around the small diameter glass tube that housed the reference electrode wire and solution.

Figure 3.1: a) Front view of electrochemical cell. b) Top view of electrochemical cell.
3.2 Self-Assembled Monolayers on Gold Electrodes

3.2.1 Preparation of Electrodes

The evaporated gold electrodes used were purchased from Evaporated Metal Films. Each electrode consisted of a 1000 Å thick evaporated gold film on float glass with an intervening adhesion layer of titanium that was 50 Å thick. Each electrode was initially cleaned by 10-minute sonications in 1% Liqui-Nox solution and then twice in Milli-Q water. After assembling the cell, the gold electrode was electrochemically pretreated using repetitive potential cycling in an electrolyte solution composed of 0.01 M KCl and 0.1 M H$_2$SO$_4$. The electrode potential was scanned linearly from 0 V to 1.5 V and back to 0 V at a rate of 100 mV/s. This cycle was repeated for a total of 10 times. This electrochemical pretreatment method cleans and roughens the electrode surface through etching and re-deposition of gold. Following pretreatment the electrode was rinsed with Milli-Q water and then ethanol.

3.2.2 Self-Assembly

6-Mercaptohexanoic acid, 90% (C$_5$COOH) was purchased from Sigma-Aldrich and used to form SAMs for cyclic voltammetry (CV) experiments. 7-Carboxy-1-heptanethiol, >97.0% (C$_7$COOH) and 8-hydroxy-1-octanethiol, 98.0% (C$_8$OH) were purchased from Dojindo and used to prepare SAMs for electrochemical impedance spectroscopy (EIS) experiments. SAMs were self-assembled from solutions of alkanethiols dissolved in 95% ethanol. Total alkanethiol concentration was 1.0 mM. Each SAM was prepared by introducing 200 µL of an alkanethiol solution into an assembled cell containing a pretreated
electrode and then covering with parafilm and incubating for 18-24 hours at room temperature. After removing the alkanethiol solution before conducting an experiment, the cell was rinsed with ethanol and then Milli-Q water.

### 3.3 Redox Probe Experiments

#### 3.3.1 Cyclic Voltammetry (CV)

The redox probe used in CV experiments was 1.0 mM potassium ferricyanide, K$_3$Fe(CN)$_6$. After dissolving the compound in 0.2 M KCl, pH was adjusted to desired values by dropwise addition of 0.01 M HCl or 0.01 M NaOH. A Bio-Logic SP-200 potentiostat was connected to a laptop computer and controlled with EC-Lab software (also from Bio-Logic). Using a scan rate of 100 mV/s, CVs were acquired by scanning between 600 mV and -100 mV.

#### 3.3.2 Electrochemical Impedance Spectroscopy (EIS)

The redox probe solution for EIS experiments contained 0.50 mM K$_3$Fe(CN)$_6$ and 0.50 mM K$_4$Fe(CN)$_6$ in 20 mM potassium phosphate buffer (KP$_1$) and 0.50 M KCl. Impedance data were acquired using the same Bio-Logic SP-200 potentiostat model equipped with an optional built-in impedance module and EC-Lab software. The DC potential was set to 0 V versus the open circuit potential, which coincided with the solution equilibrium potential imposed by the ferri/ferrocyanide redox couple. The RMS amplitude of the AC potential perturbation was 10 mV, and the frequency was scanned over a range of 100 kHz to 10 mHz. A total of seventy-five points were obtained over this frequency range.
at logarithmic intervals, where points were recorded variable spacing over the frequency range. The other option for collecting data points was to collect a given amount per decade, but this did not give smooth lines on the semi-circle.

Phosphate buffer composition was used to control pH in the EIS experiments. Two solutions were prepared, one containing 20 mM monobasic KP$_i$ + 0.50 M KCl and the other containing 20 mM dibasic KP$_i$ + 0.50 M KCl. To make buffer solutions of lower pH, the dibasic solution was added dropwise to the monobasic solution while monitoring pH. For higher pH buffers, the monobasic solution was similarly added dropwise to the dibasic solution. The pH values were measured with a VWR SympHony SB80PI pH meter. All chemicals used for preparing solutions were purchased from Sigma Aldrich.

When the effect of ionic strength was briefly looked at, the only part of the experiment that was changed were the solutions used. Instead of using 20 mM monobasic KP$_i$ + 0.50 M KCl and 20 mM dibasic KP$_i$ + 0.50 M KCl, 2.0 mM monobasic KP$_i$ + 0.10 M KCl and 2.0 mM dibasic KP$_i$ + 0.10 M KCl was used.
3.4 References


CHAPTER 4

RESULTS AND DISCUSSION
4.1 Cyclic Voltammetry

Our initial attempts to characterize $pK_{1/2}$ values for carboxylic acid SAMs utilized cyclic voltammetry (CV). In the body of previous literature in which faradaic electrochemistry has been used for this purpose, CV has been the most widely used technique\textsuperscript{1-7}. Using ferricyanide or any other anionic redox probe, electron transfer kinetics have been found to be fastest at lower pH, becoming increasingly more irreversible at higher pH values because of the gradual deprotonation of the SAM, as discussed in Chapters 1 and 2. Such behavior is typically manifested by increased peak separation ($\Delta E_p$) and decreased peak currents\textsuperscript{1-5}. Figure 4.1 displays a set of ferricyanide CVs that were obtained at different pH values for a C\texttextsubscript{5}COOH SAM. The response was quasireversible at pH 2.80 with a peak separation of 109 mV. Increasing the pH stepwise up to 8.14 resulted in increased peak separation and decreased cathodic peak current, as expected. The anodic peak appears relatively unaffected by the pH, a behavior that has been seen in some previous studies\textsuperscript{2}, but in other studies the anodic peak behaved like the cathodic peak\textsuperscript{3-5}.

Although it is straightforward to recognize increasing irreversibility by inspection of CVs, it is not immediately apparent how this data is best used to establish a titration-like curve from which a $pK_{1/2}$ value can be determined. Some previous researchers have used cathodic peak current ($i_{p,c}$) and have estimated $pK_{1/2}$ values from plots of $i_{p,c}$ vs pH\textsuperscript{1,2}. Using this approach with the data shown in Figure 4.1 gives rise to Figure 4.2. It is obvious that fitting a sigmoidal curve to this data with an acceptable level of confidence is not feasible. Furthermore, reproducibility of CV experiments was not very good. Because of these major shortcomings, the use of cyclic voltammetry for determining $pK_{1/2}$ values was abandoned in
favor of electrochemical impedance spectroscopy, which can be more revealing of the underlying ET kinetic limitations as previously stated by Molinero and Calvo\textsuperscript{8}.

**Figure 4.1:** Cyclic voltammograms of 1.0 mM Fe(CN)\textsubscript{6}\textsuperscript{3-} in 0.2 M KCl at various pH values. The electrode is gold modified by a C\textsubscript{5}COOH SAM. Scan rate = 100 mV/s and electrode area = 0.32 cm\textsuperscript{2}.
Figure 4.2: Titration curve plotted from the cyclic voltammograms shown in Figure 4.1.

4.2 Electrochemical Impedance Spectroscopy

As described in Chapter 2, each EIS data point is acquired by analyzing the AC current that results from the application of an AC potential perturbation to an electrode interface whose DC potential is fixed at the formal potential of an equimolar ferri/ferrocyanide solution. Data were acquired over a frequency range of 10 mHz to 100 kHz and displayed in a Nyquist plot of $-Z_{\text{im}}$ vs $Z_{\text{Re}}$. A Randles equivalent circuit was fitted to the data. The blue trace in Figure 4.3 is a typical experimental response obtained from an EIS experiment. The data shown were obtained for a 3:1 C$_8$OH:C$_7$COOH SAM at pH 3.91.
Qualitatively, the response is as expected for a Randles circuit. The semi-circular feature arises from the parallel circuit elements, $R_{ct}$ and $C_{dl}$. The onset of the Warburg response due to semi-infinite linear diffusion is just discernible at the right terminus of the trace, which corresponds to the lowest frequency. To more fully establish the characteristic $\phi = 45^\circ$ Warburg response, it would be necessary to acquire data at frequencies below 10 mHz. Because diffusional aspects of the response were not relevant to the thesis aims, this was not done. The high frequency intercept located at the left terminus of the trace is not discernible on this plot because it is negligible in magnitude relative to the charge transfer resistance, the magnitude of which is equal to the diameter of the semicircle.

The red trace in Figure 4.3 is the Randles equivalent circuit that was fitted to the data. Fitting was accomplished using Z-Fit, a software program supplied by Bio-Logic with the potentiostat and impedance module. The fitting was performed in two steps. The first step utilized the Randomize option in Z-Fit, which uses random numbers for the initial values. From this coarse fit, estimates of the circuit element values were obtained. These numbers then became input values for obtaining a final fit using the Levenberg-Marquardt algorithm, a common approach for fitting nonlinear data. A chi-squared test was used to determine the number of iterations$^9$. 
Figure 4.3: EIS of a 3:1 SAM in a ferri/ferrocyanide solution at pH 3.91. The solution contained 0.50 mM K$_3$Fe(CN)$_6$, 0.50 mM K$_4$Fe(CN)$_6$, 0.50 M KCl, and 20 mM KP$_i$ buffer. The experimental data is the blue trace and the fitted curve is the red trace. Frequency ranged from 10 mHz at the right terminus to 100 kHz at the left terminus. Element values obtained from the Randles equivalent circuit fit: $R_s = 81.48$ Ω, $R_{ct} = 24,176$ Ω, $C_{dl} = 3.96$ μF/cm$^2$, $Z_{w} = 1,284$ Ωs$^{-1/2}$

Electrochemistry was found to be well behaved at pH values up to 8 or 9. At higher pH, however, results became much less reproducible and difficult to analyze. This observation is consistent with other literature reports$^2$-$^4$,$^8$,$^{10}$. Adsorption of hydroxide ion on the gold electrode at higher pH has been invoked to rationalize such behavior$^8$. Figure 4.4 gives an example of an EIS spectrum obtained at a pH of 7.97. Even though the fit satisfied
the chi-squared criterion, the quality of the fit is inferior to those that could be obtained at lower pH.

**Figure 4.4:** EIS of a 1:2 SAM in a ferri/ferrocyanide solution at pH 7.97. The experimental data is the blue trace and the fitted curve is the red trace. See Figure 4.3 for solution composition and frequency range. Element values obtained from the Randles equivalent circuit fit: $R_s = 116.3 \ \Omega$, $R_{ct} = 774,064 \ \Omega$, $C_{dl} = 4.01 \ \mu F/cm^2$, $Z_{wi} = 20,305 \ \Omega s^{-1/2}$
4.3 Determination of pK$_{1/2}$ Values

All pK$_{1/2}$ values reported in this thesis were determined for SAMs comprised of C$_7$COOH and/or C$_8$OH alkanethiolates. The mole ratio of OH to COOH was varied from 100% C$_7$COOH to 100% C$_8$OH. It should be pointed out that these ratios refer to the concentrations of COOH- and OH-terminated alkanethiols that were present in the self-assembly solution. The actual ratios in the SAMs can deviate somewhat from the solution ratios$^{16}$. To demonstrate the determination of a pK$_{1/2}$ value from EIS data, we shall use the example of the (3:1) OH:COOH SAM system. In Figure 4.5 an overlay of EIS spectra are shown for the pH range between 3.52 and 7.32. One readily observes the strong dependence of the charge transfer resistance (as approximated by the diameter of the semicircle) on pH. From pH 3.52 to pH 7.32, the value of R$_{ct}$ increased from 20,440 $\Omega$ to $4.02 \times 10^5$ $\Omega$. 
Figure 4.5: Overlay of EIS spectra for a 3:1 SAM over a pH range of 3.52 to 7.32.

Figure 4.6 displays the titration curve obtained from a single experiment performed on the (3:1) OH:COOH SAM. Unfortunately because of the unavailability of data at pH above 7.32, a complete sigmoid with two plateau regions could not be obtained. If that had been the case, determination of the pK$_{1/2}$ value would have been a simple exercise. Instead, we assumed that the pK$_{1/2}$ value coincides approximately with the inflection point of the curve, an approach that has been followed by several other authors$^{1,3,10,11}$. To determine the inflection point, a polynomial curve was first fit to the data as shown in Figure 4.6. The polynomial order was determined by using the F-test to fit the data in DataFit. The inflection
point was then determined by taking the second derivative of the polynomial and setting it equal to zero. For the data shown in Figure 4.6, a value of 6.53 was determined for \( \text{pK}_{1/2} \).

\[
y = -2,820x^5 + 68,395x^4 - 644,381x^3 + 2,980,029x^2 - 6,796,025x + 6,147,276
\]
\[
R^2 = 0.9991
\]

**Figure 4.6:** Dependence of \( R_{ct} \) on pH. The data are shown as blue diamonds; the black line is a polynomial fit. EIS data from a 3:1 SAM in a ferri/ferrocyanide solution from pH 3.52 to 7.32. See Figure 4.3 for solution composition and frequency range.

Consideration was given to the possibility of hysteresis that might arise due to the sequence in which pH values were tested. It was found that as long as the pH was kept below
8, results did not depend on the order. For example, Figure 4.7 displays a set of $R_{ct}$ vs pH data points that were acquired on a single electrode by altering pH in both directions. No detectable dependence on the manner in which pH values were sequenced is evident from thesis data. For SAMs that featured more basic pK$_{1/2}$ values (above 7), it was found that pH values as high as 9 could be used without experiencing any hysteresis. In most experiments, it was found to be most convenient to start measurements at the lowest pH, increase to higher pH, and then return to lower pH values.
Figure 4.7: Titration curve of electrochemical impedance spectral data for a 1:2 SAM over a pH range of 3.06 to 7.97 demonstrating the lack of hysteresis. See Figure 4.3 for solution composition and frequency range.

4.4 Dependence of pK$_{1/2}$ on SAM Composition

Electrochemical impedance spectroscopy was used to determine the pK$_{1/2}$ values of mixed SAMs that had different ratios of OH:COOH. EIS titration curves for a pure C$_7$COOH SAM and for mixed SAMs with C$_8$OH : C$_7$COOH compositions of 5:1, 3:1, 2:1, 1:1, 1:2, 1:3, and 1:5, are shown in Figure 4.8. Each curve represents a single experiment and was selected to be representative of the responses for a given composition. Broadly
speaking, the curves shift in the basic direction approximately 1 pH unit as the COOH content of the SAM increases. To generate Figure 4.8, the y-axis of this plot was normalized because the resistance values for different compositions were not the same. Since $R_{ct}$ vs pH titration curves lacked upper plateau regions corresponding to complete deprotonation, it was not possible to normalize $R_{ct}$ values to a maximum $R_{ct}$ value. Instead, the charge transfer resistance at each pH unit was divided by the $R_{ct}$ value at the inflection point.
Figure 4.8: An overlay of titration curves for various SAM compositional ratios, where the ratios are written as C₈OH:C₇COOH. (■) 5:1, (■) 3:1, (■) 2:1, (■) 1:1, (■) 1:2, (■) 1:3, (■) 1:5, (■) C₇COOH only. The y-axis is $R_{ct}$ normalized to the $R_{ct}$ value at the inflection point of each curve.

Values of $pK_{1/2}$ obtained for replicate experiments are shown in Table 1. Each entry is the average value of 3-8 replicate experiments. The $pK_{1/2}$ values span a range of ~1 pH unit, so the surface acidity of the SAMs is clearly affected by COOH content. Figure 4.9 shows
the values from Table 1 plotted versus the nominal %COOH of the SAM. The trend is clearly in the direction that pK$_{1/2}$ increases as the COOH content of the SAM increases. The only exception to this trend appears to be the pure COOH SAM, which has a pK$_{1/2}$ value that appears to fall below the trend. However, it’s also possible that the pure COOH SAM is not an exception due to its relatively large standard deviation. One can picture a trend line drawn for Figure 4.9 that increases and reaches a maximum level, including the pure COOH SAM. The apparent exceptional behavior of the pure COOH SAM may simply be a result of experimental uncertainty.
Table 1: pK$_{1/2}$ values and their respective standard deviations obtained from various OH:COOH molar ratios of the SAMs on gold electrodes, that vary from 0-100% acid.

<table>
<thead>
<tr>
<th>SAM Ratio</th>
<th>% COOH</th>
<th>pK$_{1/2}$</th>
<th>Standard Deviation</th>
<th># Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>C$_8$OH</td>
<td>0</td>
<td>N/A</td>
<td>N/A</td>
<td>4</td>
</tr>
<tr>
<td>5:1</td>
<td>16.7</td>
<td>6.29</td>
<td>0.12</td>
<td>4</td>
</tr>
<tr>
<td>3:1</td>
<td>25</td>
<td>6.54</td>
<td>0.05</td>
<td>4</td>
</tr>
<tr>
<td>2:1</td>
<td>33.3</td>
<td>6.76</td>
<td>0.34</td>
<td>4</td>
</tr>
<tr>
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<td>6.89</td>
<td>0.26</td>
<td>8</td>
</tr>
<tr>
<td>1:2</td>
<td>66.7</td>
<td>6.91</td>
<td>0.09</td>
<td>4</td>
</tr>
<tr>
<td>1:3</td>
<td>75</td>
<td>7.11</td>
<td>0.21</td>
<td>4</td>
</tr>
<tr>
<td>1:5</td>
<td>83.3</td>
<td>7.07</td>
<td>0.15</td>
<td>4</td>
</tr>
<tr>
<td>C$_7$COOH</td>
<td>100</td>
<td>6.94</td>
<td>0.25</td>
<td>3</td>
</tr>
</tbody>
</table>
Figure 4.9: Plot of $pK_{1/2}$ values versus the percentage of COOH thiol present in the self-assembly solutions used to form SAMs.

The majority of researchers\textsuperscript{1-6,9-13} have come to a consensus that COOH SAMs deprotonate at higher pH values than bulk alkanoic acids, which have $pK_a$ values near 4.7\textsuperscript{4}. The results reported in this thesis are in agreement with this general finding, given that $pK_{1/2}$ values ranging from 6.3 to 7.1 were determined. Several different reasons have been put forth in the literature to explain why basic $pK_{1/2}$ values occur. Before reviewing these, we note that no previous reports of $pK_{1/2}$ values for mixed OH:COOH SAMs are available. Creager and Clarke\textsuperscript{12} did study related mixed SAMs of CH$_3$:COOH composition. In their
study, only 1:1 compositional ratio SAMs were examined. They made four CH$_3$:COOH mixed SAMs in which the chain length of the CH$_3$ component was varied in one-carbon increments. They found that when the chain lengths of the COOH and CH$_3$ terminated molecules were about the same, the pK$_{1/2}$ was much higher than when the COOH group protruded beyond the CH$_3$ termini. They attributed this to two possible reasons: an ion solvation effect and the interfacial potential at the surface. The ion solvation effect arises from the differential effect of solution polarity on the COOH and COO$^-$ forms of the acid. For example, when the solution or surrounding environment becomes less polar, the COOH form of the acid becomes stabilized relative to the ionized form, and the pK$_a$ shifts in the basic direction. Thus, when COOH groups are located at the surface of the SAM, they are surrounded by aqueous solution on only one side and a low dielectric medium on the electrode side due to the presence of the alkane film. The effective polarity would be less than a carboxylic acid that was totally immersed in aqueous solution. Turning to the second reason given by the authors, the interfacial potential near the surface may be altered when excess charge accumulates. When a negative charge is built up near the surface the COOH groups have a harder time deprotonating and this makes the pK$_{1/2}$ values become more basic, a view also backed by Smith and White$^{14}$. 

Kakiuchi$^{15}$ argued that electrostatic interactions between the surface COO$^-$ groups cause a more basic pK$_{1/2}$. When COOH groups undergo deprotonation, repulsion arises between the resultant negative charges on the SAM. When this happens, it becomes increasingly difficult for new COOH groups to lose a proton because any new COO$^-$ groups that emerge at the surface will do so in an increasingly negative electric field. Hence, it will
take a higher solution pH to cause the deprotonation to occur. Also, hydrogen bonding between the surface COOH groups may affect the pK$_{1/2}$. Leopold$^{13}$ and Burris$^{11}$ both reported that pK$_{1/2}$ increases when the gold topography becomes more flat, and this was attributed to enhanced hydrogen bonding involving COOH groups, which stabilizes the proton and makes it more difficult to remove.

Although some or all of these effects could be important for understanding the results presented in this work, the observation that pK$_{1/2}$ undergoes a basic shift with increasing COOH content in OH:COOH mixed SAMs seems consistent with Kakiuchi’s argument. For lower concentrations of COOH groups at the SAM surface, electrostatic repulsion due to deprotonated COO$^-$ groups would have less of an effect due to their wider spacing. Thus, it would be easier for COOH groups to lose a proton, resulting in more acidic pK$_{1/2}$. However, when the amount of acid in the SAM is increased, more concentrated negative charge will build up as the SAM becomes deprotonated, resulting in a higher pK$_{1/2}$ value. It is also possible that hydrogen bonding may play a role unrelated to any effect arising from electrode topography, which was kept constant in this work. Although both COOH and OH groups are both capable of hydrogen bonding, the extent of hydrogen bonding involving the acidic proton may become more extensive as the structure of the film changes with increased COOH content, which would cause a basic shift in pK$_{1/2}$.

Previously D’Antonio et al$^{17}$ reported cyclic voltammetry results for adsorbed dehaloperoxidase (DHP) at pH using OH:COOH SAMs of different compositions. As the COOH content was increased, the surface formal potential and the electroactive surface
concentration both decreased. Future work would be required in order to determine if there is a link between these trends and the pK$_{1/2}$ trend established in this thesis.

### 4.5 Effect of Ionic Strength on EIS Response

As previously stated in Chapter 1, it has been established that ionic strength affects the surface acidity of COOH SAMs$^{2,4}$. So the effect of ionic strength was briefly examined for a 3:1 SAM to ascertain if a similar dependence applies to mixed OH:COOH SAMs. The pK$_{1/2}$ value for the 3:1 SAM was 6.53 at high ionic strength (0.50 M KCl, 20 mM KP$_i$). Figure 4.10 shows an overlay of EIS data at pH values of 5.03, 6.16 and 7.36 that were obtained at a lower ionic strength (0.10 KCl, 2.0 mM KP$_i$). At the lower ionic strength, R$_{ct}$ values increased several fold. Accordingly, in order to enable a visual comparison of titration curves at low and high ionic strength, the R$_{ct}$ values at lower ionic strength were arbitrarily scaled, and the result is shown as Figure 4.11.
Figure 4.10: Overlay of EIS spectra for a 3:1 SAM at pH values of 5.03, 6.16 and 7.36 at a lower ionic strength of a solution containing 0.50 mM K$_3$Fe(CN)$_6$, 0.50 mM K$_4$Fe(CN)$_6$, 0.10 M KCl, and 2.0 mM KP$_4$ buffer.
**Figure 4.11:** An overlay of titration curves for 3:1 SAMs obtained at low and high ionic strength, (■) high ionic strength and (■) low ionic strength. On the y-axis, $R_{ct}$ refers to the black symbols and $R_{ct}/4$ refers to the red symbols.

The limited extent of the titration data precluded the determination of a $pK_{1/2}$ value at the lower ionic strength. By examining Figure 4.11, however, it seems evident that the ionic strength effect for OH:COOH SAMs is qualitatively the same as previously reported for COOH SAMs. The lower ionic strength appears to shift the $pK_{1/2}$ slightly more basic. This may be due to increased hydrogen bonding of COOH groups at lower ionic strength as proposed by Lu$^4$. 
4.6 References


CHAPTER 5

FUTURE WORK
5.1 Future Work

Due to time constraints, the study of the effect of lower ionic strength on pK$_{1/2}$ was limited in scope. Some experimental details would need to be elaborated so that complete titration curves instead of just partial curves could be obtained. Quantifying the effect of ionic strength on all of the SAM compositions that were studied in this work would also be highly desirable. Ionic strength may affect each SAM composition differently due to different surface charge densities. For example one might shift in pK$_{1/2}$ a lot and another might shift only slightly or not at all. It would be beneficial to figure out the answers to these questions.

Another aspect deserving of attention is how gold topography affects the properties of these mixed SAMs. Leopold et al.\textsuperscript{1} proposed that smooth gold surfaces result in higher pK$_{1/2}$ values than do rough surfaces. This hypothesis has yet to be proven in controlled experiments. The values reported in the present work were all obtained on a gold surface roughened by electrochemical pretreatment ($R_f = 1.6$), so smoother substrates would need to be prepared to test this hypothesis. That was an original objective of this research, but a smoother substrate was not obtained. Potential candidates for smoother substrates include gold annealed in either a hydrogen flame or an oven, or gold-on-mica.

Creager and Clarke\textsuperscript{2} established how pK$_{1/2}$ is affected in CH$_3$:COOH SAMs by varying the length of the CH$_3$-terminated thiol while holding the COOH-terminated thiol length constant. They showed that the pK$_{1/2}$ would become more basic as the CH$_3$ chains became longer. It would be interesting to see how OH chain length alteration in C\textsubscript{n}OH:
C₇COOH SAMs affected surface acidity. Based upon what has already been seen in this work and theirs, it is likely that the same effect would be observed.

Anderson³ has already begun to look at how anionic and cationic redox probes affect the pK½ values of a C₁₀COOH SAM. A different experiment could be designed with this same concept for the OH:COOH SAMs used in this work. Since Fe(CN)₆³⁻/⁴⁻ was the redox probe selected in this work, only Ru(NH₃)₆³⁺/²⁺ would need to be used to see if there is a difference for mixed SAMs as well. Any other redox probe could also be implemented to see if there may be a difference in pK½ values of various anionic or cationic redox probes.

A final idea that could be explored would be to determine why DHP electrochemistry appears to be optimal for the 3:1 OH:COOH SAM at pH 6 compared to the other SAM compositions. It would be interesting to repeat the experiments that D’Antonio⁴ performed with CV but using EIS instead to obtain impedance data.
5.2 References


