Adsorption of a Carboxylated Silane on Gold: Characterization and Application to PDMS-Based Electrochemical Cells

by

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Abstract

Integrated sensing and biosensing microfluidic systems often require sealing between polydimethylsiloxane (PDMS), glass, and gold interfaces. Studying substances that can self-organize onto glass and gold surfaces may achieve these goals and pave the way for new technological advances. Work presented in this thesis focuses on characterizing the adsorption of $N$-$[(3$-trimethoxysilyl)$propyl]ethylene-diamine triacetic acid (or TMS-EDTA) on Au and applying this knowledge to construct leak-free PDMS-based electrochemical cells.

First, surface analysis of TMS-EDTA-modified Au surfaces was conducted using various techniques. Water contact angle measurements and X-ray photoelectron spectroscopy confirm that the carboxylated silane can chemically modify Au surfaces. Atomic force microscopy studies indicate that a uniform surface coverage with monolayer thickness is formed. Infrared spectroscopy studies indicate that there is little evidence of siloxane cross-linking. Surface plasmon resonance results suggest that the carboxylates on TMS-EDTA-modified Au are available for streptavidin immobilization.

Second, electrochemistry was used to determine the Gibbs free energies of adsorption of TMS-EDTA on Au under aqueous conditions. Electrochemical differential capacitance measurements reveal that the potential-dependent free energies of adsorption are $\sim -20$ to $-30$ kJ/mol (for potentials between $-0.5$ and $0.2$ V) in the complex electrolyte solution used. Furthermore, at highly negative potentials ($\sim -1.1$ V), TMS-EDTA adsorbs minimally onto the Au surface.

Third, PDMS surfaces were functionalized to present primary amino groups, and glass or gold slides were functionalized to present carboxyl groups. Strong bonding was achieved by bringing the two surfaces in contact and reacting at room temperature. Shear tests reveal that the novel carboxyl-amine bonding strategy achieved a comparable bond strength as the conventional methods. Subsequently, TMS-EDTA was applied to construct leak-free PDMS-based electrochemical cells. Pressure leak tests were conducted to provide a more realistic measure of the bond strengths under aqueous conditions. A method to electrochemically remove the adsorbed TMS-EDTA layer off of the Au electrode, while maintaining the sealed cell chamber, was also developed.
Abstract

The characterization studies and fabrication strategy presented have led to the development of leak-free PDMS-based electrochemical devices that are suitable for sensing and biosensing applications.
Preface

Parts of this thesis have been published as three peer-reviewed journal articles:

- Article #1 (http://pubs.acs.org/doi/abs/10.1021/ac902926x) is published by American Chemical Society.¹ Drs. David Ng and Joanne Fox conceived of the initial idea. As first author, I designed, performed, and analyzed the research with some students. Jake Abbot and Cameron Lawson (high school students at the time) developed the initial protocol and tested the first Jell-O chip. Adrian Lee (undergraduate student at the time) created new experiments based on their techniques. I drafted the initial manuscript, and Eric Ouellet made contributions to the text and figures. Dr. Eric Lagally provided guidance throughout the manuscript submission.


- Article #2 (http://pubs.acs.org/doi/abs/10.1021/la1012582) is published by American Chemical Society.² As co-first authors, Eric Ouellet and I contributed equally to the work. I designed, performed, and analyzed all of the shear tests with technical advice from George Lee (Wood Science Department, UBC Faculty of Forestry). Tao Lin and Lee Ling Yang assisted with the sample preparation. The X-ray photoelectron spectroscopy (XPS) measurements were conducted in the Interfacial Analysis & Reactivity Laboratory (IARL) at Advanced Materials and Process Engineering Laboratory (AMPEL, UBC Vancouver) with the help of Dr. Ken Wong. The water contact angle measurements were conducted in the Life Sciences Institute (LSI, UBC Vancouver) with the help of Dr. Johan Janzen. I drafted the initial manuscript, and Eric Ouellet made significant contributions to the text and figures. Dr. Eric Lagally provided guidance throughout the research and manuscript submission.


*These authors contributed equally to the work presented.
Preface

• Article #3 (http://pubs.acs.org/doi/abs/10.1021/acs.jpcc.5b09915) is published by American Chemical Society. As first author, I designed the research together with Dr. Dan Bizzotto, and performed all of the experimental work and analyses as described. Isaac Martens contributed to the atomic force microscopy (AFM) experiments and the initial analysis of AFM contact mode imaging data. I drafted the initial manuscript. Isaac Martens and Drs. Előd Gyenge, Robin Turner, and Dan Bizzotto made valuable contributions to the manuscript revisions. Dr. Bizzotto provided guidance throughout the manuscript submission process.


Please see below for details regarding how these publications, along with additional unpublished experimental data, contribute to the construction of this thesis:

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# Nomenclature

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<thead>
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<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>3D</td>
<td>three-dimensional</td>
</tr>
<tr>
<td>AFM</td>
<td>atomic force microscopy</td>
</tr>
<tr>
<td>ATR</td>
<td>attenuated total reflectance</td>
</tr>
<tr>
<td>ATR-FTIR</td>
<td>attenuated total reflectance Fourier transform infrared spectroscopy</td>
</tr>
<tr>
<td>CE</td>
<td>counter electrode</td>
</tr>
<tr>
<td>CV</td>
<td>cyclic voltammetry</td>
</tr>
<tr>
<td>DiffCap</td>
<td>DiffCap buffer used for differential capacitance experiments</td>
</tr>
<tr>
<td>IHP</td>
<td>inner Helmholtz plane</td>
</tr>
<tr>
<td>IR</td>
<td>infrared</td>
</tr>
<tr>
<td>IRRAS</td>
<td>infrared reflection-absorption spectroscopy</td>
</tr>
<tr>
<td>MR</td>
<td>magnetic resonance</td>
</tr>
<tr>
<td>OHP</td>
<td>outer Helmholtz plane</td>
</tr>
<tr>
<td>PET</td>
<td>positron emission tomography</td>
</tr>
<tr>
<td>PM-IRRAS</td>
<td>polarization modulation-infrared reflection-absorption spectroscopy</td>
</tr>
<tr>
<td>POI</td>
<td>potential of interest</td>
</tr>
<tr>
<td>psi</td>
<td>pound per square inch or pound-force per square inch</td>
</tr>
<tr>
<td>PZC</td>
<td>point of zero charge</td>
</tr>
<tr>
<td>RAIR</td>
<td>reflection-absorption Fourier transform infrared spectroscopy</td>
</tr>
<tr>
<td>RE</td>
<td>reference electrode</td>
</tr>
</tbody>
</table>
### Nomenclature

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>SAMs</td>
<td>self-assembled monolayers</td>
</tr>
<tr>
<td>SCE</td>
<td>saturated calomel electrode</td>
</tr>
<tr>
<td>SE</td>
<td>secondary electrode</td>
</tr>
<tr>
<td>SPR</td>
<td>surface plasmon resonance</td>
</tr>
<tr>
<td>SPRi</td>
<td>surface plasmon resonance imaging</td>
</tr>
<tr>
<td>SSCE</td>
<td>silver/silver chloride electrode</td>
</tr>
<tr>
<td>UHV</td>
<td>ultra-high vacuum</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>WE</td>
<td>working electrode</td>
</tr>
<tr>
<td>XPS</td>
<td>X-ray photoelectron spectroscopy</td>
</tr>
<tr>
<td>11-MUA</td>
<td>11-mercaptoundecanoic acid</td>
</tr>
<tr>
<td>3-APTES</td>
<td>(3-aminopropyl)triethoxysilane</td>
</tr>
<tr>
<td>3-APTMS</td>
<td>(3-aminopropyl)-trimethoxysilane</td>
</tr>
<tr>
<td>3-MPTMS</td>
<td>(3-Mercaptopropyl)trimethoxysilane</td>
</tr>
<tr>
<td>BTMSE</td>
<td>1,2-bis(trimethoxysilyl)ethane</td>
</tr>
<tr>
<td>CoHex</td>
<td>hexaamminecobalt(III)</td>
</tr>
<tr>
<td>EDC</td>
<td>(N)-(3-(dimethylamino)propyl)-(N')-ethylcarbodiimide hydrochloride</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>NHS</td>
<td>(N)-hydroxysuccinimide</td>
</tr>
<tr>
<td>PDMS</td>
<td>polydimethylsiloxane</td>
</tr>
<tr>
<td>TMS-EDTA</td>
<td>(N)-[{(3-trimethoxysilyl)propyl}ethylene-diamine triacetic acid</td>
</tr>
<tr>
<td>(\alpha)</td>
<td>Frumkin lateral interaction parameter (unit: (\frac{kJ}{mol}))</td>
</tr>
<tr>
<td>(\Gamma)</td>
<td>surface excess of a particular species (unit: (\frac{mol}{m^2}))</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>surface or interfacial tension (unit: $\frac{N}{m}$ or $\frac{J}{m^2}$)</td>
</tr>
<tr>
<td>$\Gamma_i$</td>
<td>surface excess of species $i$</td>
</tr>
<tr>
<td>$\gamma_{LG}$</td>
<td>surface tension of the liquid-gas interface</td>
</tr>
<tr>
<td>$\Gamma_{max}$</td>
<td>surface excess at maximum surface coverage</td>
</tr>
<tr>
<td>$\gamma_{SG}$</td>
<td>surface tension of the solid-gas interface</td>
</tr>
<tr>
<td>$\gamma_{SL}$</td>
<td>surface tension of the solid-liquid interface</td>
</tr>
<tr>
<td>$\mu_i$</td>
<td>chemical potential of species $i$</td>
</tr>
<tr>
<td>$\nu$</td>
<td>frequency (unit: $s^{-1}$)</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>stored charge density (unit: $\frac{C}{m^2}$)</td>
</tr>
<tr>
<td>$\sigma^M$</td>
<td>excess surface charge density of the metal</td>
</tr>
<tr>
<td>$\theta$</td>
<td>the fraction of surface occupied by the adsorbing molecule</td>
</tr>
<tr>
<td>$\theta_C$</td>
<td>contact angle</td>
</tr>
<tr>
<td>$\epsilon$</td>
<td>dielectric constant of the medium (or relative permittivity)</td>
</tr>
<tr>
<td>$\epsilon_0$</td>
<td>permittivity of free space (unit: $\frac{C}{V\cdot m}$)</td>
</tr>
<tr>
<td>$\Delta G^\circ$</td>
<td>standard Gibbs free energy (unit: $\frac{kJ}{mol}$)</td>
</tr>
<tr>
<td>$\Delta G_{ads}^\circ$</td>
<td>standard Gibbs free energy of adsorption</td>
</tr>
<tr>
<td>$E_b$</td>
<td>binding energy (unit: eV)</td>
</tr>
<tr>
<td>$E_k$</td>
<td>kinetic energy (unit: eV)</td>
</tr>
<tr>
<td>$E_r$</td>
<td>recoil energy (unit: eV)</td>
</tr>
<tr>
<td>$A$</td>
<td>area of the dividing surface</td>
</tr>
<tr>
<td>$\varphi_{sp}$</td>
<td>spectrometer work function (unit: eV)</td>
</tr>
<tr>
<td>$A$</td>
<td>free molecules (or adsorbates) that can adsorb onto the surface</td>
</tr>
<tr>
<td>$A_{ad}$</td>
<td>adsorbed molecules (or adsorbates) on the surface</td>
</tr>
</tbody>
</table>
Nomenclature

\( C \)  
bulk concentration of a particular species (unit: \( \text{mol} \/ \text{L} \) or \( \text{M} \))

\( C_{\theta=0} \)  
capacitance of the electrode without the molecule of interest

\( C_{\theta=1} \)  
capacitance of the electrode completely covered by the molecule of interest

\( C_{dl} \)  
capacitance of the double layer

\( C_D \)  
capacitance of the diffuse layer

\( C_d \)  
differential capacitance (unit: \( \frac{C}{V \cdot m^2} \) or \( \frac{F}{m^2} \))

\( C_H \)  
capacitance of the Helmholtz layer

\( d \)  
spacing between the two plates (unit: \( m \))

\( E \)  
rational electrical potential with a reference at the point of zero charge

\( E_P \)  
peak potential

\( h \)  
Planck constant (6.626 \( \times \) 10\(^{-34} \) J \( \cdot \) s)

\( K \)  
thermodynamic equilibrium constant

\( k_a \)  
rate constant for adsorption

\( k_d \)  
rate constant for desorption

\( K_F \)  
Frumkin equilibrium constant (unit: \( \frac{L}{\text{mol}} \) or \( M^{-1} \))

\( K_L \)  
Langmuir equilibrium constant (unit: \( \frac{L}{\text{mol}} \) or \( M^{-1} \))

\( N_{free} \)  
number of free sites

\( n_i \)  
the number of moles in excess of species \( i \)

\( N_s \)  
total number of sites

\( R \)  
gas constant (8.314 \( \text{J} / (\text{mol} \cdot \text{K}) \))

\( r_a \)  
rate of adsorption

\( r_d \)  
rate of desorption

\( R_p \)  
reflectivity due to p-polarized radiation
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_s$</td>
<td>reflectivity due to s-polarized radiation</td>
</tr>
<tr>
<td>$S$</td>
<td>free vacant sites on the surface</td>
</tr>
<tr>
<td>$T$</td>
<td>temperature (unit: $K$)</td>
</tr>
<tr>
<td>$V$</td>
<td>voltage drop (unit: $V$)</td>
</tr>
</tbody>
</table>
Acknowledgments

My academic journey began when I was hired as an Undergraduate Research Assistant by Dr. Wun Chey Sin (of Dr. Christian Naus Lab) at Life Sciences Centre. I was able to conduct scientific research under her guidance and mentorship. Dr. Sin encouraged me to explore some research opportunities in the United States. In the summer of 2007, I secured a summer internship position at Washington University in St Louis with the Medical Physics Division, under the supervision and mentorship of Dr. Dan Low (now at UCLA). His advice for me was to pursue an Engineering degree to enrich my theoretical studies in Physics and Life Sciences. Dr. Sin and Dr. Low: I treasure the life experience you have shared with me. Thank you for being passionate about helping young students.

In 2008, I was hired by Dr. Eric T. Lagally as the Lab Manager, and officially started my M.A.Sc. degree in 2009 with the Department of Chemical and Biological Engineering (CHBE) at UBC. Dr. Lagally’s “open door” policy, scientific curiosity, encouragement to be innovative and learn from mistakes, and passion for Science and Engineering outreach have forever influenced my academic and personal life. Shortly after, I transferred to the PhD program. Dr. Lagally: I appreciate the opportunities you have opened up for me.

After the decommissioning of Lagally Lab in 2011, Dr. Jim Kronstad - Head of Michael Smith Laboratories (MSL) - and Dr. Peter Englezos - Head of CHBE - worked together to revive my PhD program. After consultation with Drs. Eric Lagally, Bhushan Gopaluni, Előd Gyenge, Robin Turner, Dan Bizzotto, and Jamie Piret, my PhD Supervisory Committee was formed:

- Előd Gyenge, Co-Supervisor (CHBE): Electrochemistry
- Robin Turner, Co-Supervisor (MSL, CHEM, ECE): Surface Plasmon Resonance & Electrochemistry
- Dan Bizzotto, Committee Member (CHEM): Electrochemistry & Physical Chemistry
- Jamie Piret, Committee Member (MSL, CHBE): Microfluidics & Bioreactors

Dr. Piret: Thank you for the technical advice. Dr. Gyenge: Thank you for the invaluable scientific and
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- Drs. Michael Blades and Robin Turner Lab members: Yan Tan, Chad Atkins, Georg Schulze, Kevin Buckley, and Stanislav Konorov
- Dr. Karen Cheung Lab members: Josiah To, Samantha Grist, and Yan Li
- Past Thunderbird’s Residence Life Team members: Fiona Hess, Jayden Beaudoin, Karen Ratchford, Lindsey Curtis, Margarita Iturriaga Bustamante, Poureya Bazargani, Sean Kim, Simi Toma, Yiwei Liu, Cristel Moubarak, Ken McClure, Jenny Huang, Yanlong Guo, Varune Rohan Ramnarine, and Emily Gao
- ATP Band members: Ales Horak, Tina Chou, Noriko Tanaka, and Hardy Hall
- MSL, AMPEL, and CHBE Faculty, Researchers, Staff, and Students

Last but not least, I would like to sincerely thank the unconditional love and support from my family: Harry, Sherry, Jack, Sharon, and Margaret. Thank you for everything! To my family around the world: I look forward to reuniting with all of you soon.
Dedication

To my Family...
Chapter 1

Introduction

1.1 Identifying the Problem

The interdisciplinary field of microfluidics develops miniaturized technologies for manipulating the flow and reaction of small amounts of fluids. Microfluidics has the potential to revolutionize modern biology and medicine because it offers the advantages of working with smaller reagent volumes and shorter reaction times, which greatly reduce the cost required for an analysis. Current efforts are being made to integrate an entire laboratory of analytical instrumentation onto a single chip to produce “lab on a chip” systems. Microfluidics has been applied to solve problems in diverse areas in both basic and applied sciences, and highly parallelized microfluidic systems are also being actively explored to improve existing technologies. Currently, highly integrated microfluidic chips with a thousand or more detection chambers can be produced and implemented easily.

Many types of materials have been introduced for microfluidic chip fabrication. Polydimethylsiloxane (PDMS) is quite attractive for the development of microfluidic applications not only due to its biocompatibility, but also because it is transparent, nontoxic, permeable to gases, thermally stable, and easy to handle, and it can be used to form submicrometer structures. The recent advancements in fast prototyping and mass production technologies, combined with the inexpensive nature of this material, have made this polymer quite attractive in a variety of fields. For longer than a decade, several PDMS-based medical devices have been under development and used in blood pumps, cardiac pacemaker leads, catheters, drainage tubing, implants, and contact lenses.

Despite its many advantages, however, a major area of concern with the use of PDMS is the bonding of such a polymer to other materials, as this step is crucial for the assembly of microfluidic devices. For instance, gold surfaces are frequently integrated into sensors and biosensors made of silicon and glass for the quantification of a variety of substances (see Figures 1.1A and 1.2A). Therefore, it is important to form a good seal simultaneously between PDMS, glass, and gold interfaces for these integrated microfluidic systems.
1.1. Identifying the Problem

A number of PDMS bonding strategies have been reported. The simplest method involves forming a reversible bond using clamps to maintain the seal. However, the use of clamps can be cumbersome when additional equipment or apparatus is required. Also, the force exerted by the clamp(s) may deform the PDMS and add internal stress to the device. Oxygen plasma treatment of PDMS surfaces has been widely used, whereby silanol groups are created on the PDMS surface and can form covalent siloxane bonds with some substrates (e.g., glass, quartz, or PDMS). However, other substrates (e.g., gold) will not bond by this method. For bonding PDMS to metals (such as gold and silver), mercaptosilanes have been widely used in the past and very few alternatives exist. As a result, an additional passivation layer (e.g., silicon dioxide or SU-8 photoresist) is often coated on top of the gold substrate, in order to use the plasma bonding strategy. Other methods that have been used make use of an adhesive layer on the substrate in order to bond the two surfaces together. Though this strategy may be effective, the sensor surface may become inaccessible due to the adhesive layer. Also, the use of either a passivation layer or an adhesive layer involves additional cleanroom time and requires specialized equipment. Developing a simple and an economical fabrication method, which leads to sensitive, stable, and reliable integrated systems still remains a challenge.

In the case of biosensors, a sensor surface (usually gold) is modified with biomolecular recognition elements (such as proteins, nucleic acids, or cells) to detect a specific target of interest. For example, microfluidic surface plasmon resonance (SPR) imaging biosensors often require the use of glass substrates partially patterned with a microarray of gold spots (see Figure 1.1A). The use of self-assembled monolayers (SAMs) on the sensor surface is common for the immobilization of recognition elements. Commonly, carboxylic acid-terminated alkanethiols (COOH–R–SH) are used to covalently attach biomolecules at sites of primary amines using the well-known \(N\)-hydroxysuccinimide (NHS) and \(N\)-(3-(dimethylamino)propyl)-\(N'\)-ethylcarbodiimide hydrochloride (EDC) coupling chemistry. However, due to the destructive nature of the existing bonding techniques (such as oxygen plasma or UV-ozone), these SAMs and biological recognition elements can be easily degraded or removed from the Au surface. In addition, most alkanethiol molecules are most soluble in organic solvents. Depending on the incubation time of the organic solvent and the design of the microfluidic chip, the PDMS material may swell, causing delamination from the surface and deformation of any channel structures molded into the PDMS. Therefore, it is challenging to form a strong bond between PDMS and glass while maintaining a compatible surface on the Au (inside the PDMS flow cell) for the covalent immobilization of biomolecules (see Figure 1.1B).

Microfluidic electrochemical devices often utilize glass substrates decorated with sputtered Au elec-
1.1. Identifying the Problem

Figure 1.1: (A) Photograph of a PDMS microfluidic chip bonded to a gold-patterned glass slide for SPR imaging applications (inset: a close-up of the marked region of the gold microarray; scale bar: 650 μm). Reproduced with permission from RSC Publishing. (B) Cross-sectional schematic view of an individual PDMS flow cell, such as the one indicated by the red region selected in (A). It is challenging to form a strong bond between PDMS and glass while maintaining a functional SAM on Au.
1.2 A Potential Solution

In general, it is well known that SAMs with thiols can modify Au or other metal surfaces, and that silane-based SAMs can modify glass, PDMS or metal oxide surfaces. For example, a carboxylic acid-terminated alkanethiol (11-mercaptopoundecanoic acid, or 11-MUA) has been widely studied due to its ability to attach strongly to gold and to covalently capture primary amine-terminated biomolecules. Similarly, a carboxylated silane (N-[3-trimethoxysilyl]propyl]ethylene-diamine triacetic acid or TMS-EDTA) has been widely used to modify oxide-, silanol-, and hydroxyl-containing surfaces and nanoparticles for heavy metal ion chelation, PET and MR imaging, rare-earth ion adsorption and separation, and bacteriophage immobilization.

Interestingly, there is a growing literature suggesting that some silanes can also modify Au surfaces, resulting in an expansion of the application space for silanes. However, the modification of Au substrates using TMS-EDTA (i.e., a carboxylated silane) has not been reported in the literature. If TMS-EDTA can modify both glass and Au, then it may offer distinct advantages over 11-MUA, which can only modify Au but not glass. More specifically, TMS-EDTA can potentially be used in a one-step, solution-based modification strategy for glass substrates that have been partially patterned with gold electrodes (to assist in both biosensing and sensor fabrication). To illustrate this concept, Figure 1.2C shows the cross-sectional view of the mixed Au/glass substrate across the WE inside the PDMS chamber. Functional carboxylates are potentially available on the Au electrode (via TMS-EDTA modification). This surface may then be used to immobilize biomolecules for biosensing applications. Moreover, Figure 1.2D shows the cross-sectional view of the device across all Au electrodes. After chemically modifying the surface of PDMS with (3-aminopropyl)-trimethoxysilane (3-APTM), TMS-EDTA can potentially be used to chemically modify both glass and gold surfaces for carboxyl–amine bonding with primary amine-
1.2. A Potential Solution

Figure 1.2: (A) Hypothetical PDMS-based electrochemical cell (with two Au electrodes sputtered on glass). Working electrode = WE, and secondary electrode = SE. (B) Top view of the device with the black dotted lines showing the cross sections observed in (C) and (D). (C) Cross-sectional view of the hybrid glass/gold substrate across the TMS-EDTA-modified WE inside the PDMS microfluidic cell chamber. (D) Cross-sectional view of the device, illustrating how TMS-EDTA can be used to chemically modify both glass and gold surfaces for carboxyl–amine bonding with 3-APTMS-modified PDMS. Reproduced with permission from ACS Publications.
terminated PDMS. Specifically, strong bonds between PDMS–Au and PDMS–glass could seal the cell chamber and prevent leaks.

1.3 Scope of the Thesis

The surface modification of Au by TMS-EDTA has not been previously characterized in any rigorous manner. It has therefore not been possible to exploit the utility of TMS-EDTA to achieve an optimal bond while maintaining a chemically modified or electrochemically compatible Au surface. Therefore, this thesis aims to first address existing knowledge gaps, and then apply the resulting knowledge to facilitate the design of a leak-free PDMS-based electrochemical cell. Thesis objectives include:

- Characterize the adsorption of TMS-EDTA on Au using different surface analysis techniques (Chapter 3)
- Demonstrate the feasibility of using TMS-EDTA-modified Au to capture biomolecules containing primary amino groups (Chapter 3)
- Quantify the Gibbs free energies of TMS-EDTA adsorption onto Au using electrochemical methods (Chapter 4)
- Assess the proposed carboxyl–amine bonding strategy (i.e., 3-APTMS-modified PDMS in contact with TMS-EDTA-modified glass or Au substrate) using shear tests (Chapter 5)
- Apply the knowledge obtained about TMS-EDTA adsorption on Au to develop leak-free PDMS-based electrochemical cells (Chapter 5)
Chapter 2

Literature and Background Review

This thesis project is multidisciplinary and involves numerous areas of research, including biosensors, surface science, microfluidics and electrochemistry. The objective of this chapter is to provide the reader with an introduction to the topics, theories, and techniques encountered in this work.

2.1 Introduction to Biosensors

Biosensor technology encompasses an expanding field used in diverse applications including healthcare diagnostics, forensic analysis, metabolic engineering, environmental monitoring, and food quality evaluation. The term “biosensor” indicates a sensing device that integrates three key elements (see Figure 2.1): 1) a biomolecular recognition element (i.e., a probe) that is selective towards a particular analyte molecule (i.e., a target), 2) a selective interaction event between the target and the probe, and 3) a transduction mechanism (i.e., physicochemical change such as current flow, heat transfer, or change in refractive index or mass) to convey the occurrence of this recognition event.

Optical (e.g., colorimetric, fluorescent, and plasmonic) and electrochemical (e.g., amperometric, potentiometric, and impedimetric) transduction mechanisms are often used. These sensing approaches have been applied in biosensors to analyze a wide range of biomolecular interactions. Typically, the biorecognition element (e.g., protein, peptide, or nucleic acid) is positioned physically adjacent to the transducer. As a result, one of the most important steps in the fabrication of a successful biosensor is the immobilization (or attachment) of the probe(s) onto the sensing surface. Many types of surfaces have been used as the substrate for biosensors, with glass and gold being examples of the most versatile ones. It follows that the strategy chosen for probe immobilization depends on the type of surface utilized.

Biosensors with silica-based (e.g., quartz or glass) substrates often exploit self-assembled monolayers (SAMs) of silanes for the immobilization of the probe. Conversely, gold surfaces often require a different immobilization approach. Gold is often used in biosensors because it is easily obtained...
2.1. Introduction to Biosensors

Figure 2.1: Schematic of a typical biosensor operation (left) and regeneration (right). Reproduced with permission from ACS Publications.
2.1. Introduction to Biosensors

Figure 2.2: Schematic of the three popular immobilization strategies for gold surfaces: physical, covalent, and bioaffinity immobilization. Modified and reproduced with permission from ACS Publications.

(both as a thin film and as a colloid), widely studied, chemically inert, and electrochemically active. In addition, patterning gold on glass is easily achieved using a combination of lithographic tools (e.g., photolithography and micromachining) and chemical etchants. In general, physical, covalent, and bioaffinity immobilization strategies are popular techniques used for the attachment of probes onto gold surfaces (see Figure 2.2).

Physical immobilization is the simplest method since the probe is directly attached to the surface without any chemical modification. Biomolecules (e.g., proteins) can adsorb on the surface via intermolecular forces (i.e., mainly polar and hydrophobic interactions, and ionic bonds for proteins). However, this type of immobilization often yields a surface with weakly attached and randomly oriented probes.

Alternatively, covalent immobilization often involves (1) modifying the surface with a SAM to form the desired terminal functional group(s), (2) activating the functional group(s) on the surface, and (3) covalently coupling the probe onto the surface (please see Section 2.1.1.1 for an example of a typical SAM for Au). This multi-step approach yields sensing surfaces with high stability suitable for prolonged use. However, it is also difficult to control the orientation of the probes using this method.

Lastly, bioaffinity immobilization offers the ability to control the orientation of the probes. In particular, the specificity between biotin and avidin/streptavidin has been widely exploited, since their interaction produces one of the strongest non-covalent bonds known in nature. Streptavidin is a tetrameric protein (i.e., it can interact with up to four biotins), is soluble in aqueous solutions, and is stable over wide tem-
2.1. Introduction to Biosensors

Temperature and pH ranges. Bioaffinity immobilization often involves the physical or covalent immobilization of streptavidin onto the surface, and biotinylated probes are introduced and allowed to bind. Sometimes a biotinylated surface may be prepared to achieve a better control of the streptavidin orientation. The streptavidin-biotin bond formed is highly resistant to organic solvents, detergents, denaturing agents, and extreme pH and temperatures. However, chemical modification of the probe with biotin molecules is required, which may be time-consuming or expensive. Nevertheless, this technique creates a strong and stable probe attachment with a better control over the probe orientation.

2.1.1 Chemical Surface Modification Using SAMs

The type of immobilization method chosen will affect the biosensor performance (i.e. accuracy, sensitivity, selectivity, and stability), due to changes in the probe’s coverage and orientation. Forming a reproducible SAM of high quality on a substrate is an active area of research since covalent immobilization of the probe is preferred over physical immobilization. Covalent immobilization may also be a critical component of bioaffinity immobilization. In this section, chemical surface modification of gold and glass surfaces by self-assembled monolayers (SAMs) will be discussed.

2.1.1.1 Thiols for Gold Modification

The molecule used to make a SAM typically has three key components: head group, spacer, and terminal functional group. Figure 2.3 shows a schematic diagram of an ideal SAM on a gold surface.

![Figure 2.3: Schematic diagram of an ideal SAM formed on a gold surface. The structure and characteristic of the SAM are highlighted.](image)

One of the most frequently used thiols for creating a SAM on gold is 11-mercaptoundecanoic acid
2.1. Introduction to Biosensors

(11-MUA). For 11-MUA, the head group is a thiol group, which can form a strong gold–sulfur (Au–S) bond. The spacer is a chain of saturated hydrocarbons (decane). The terminal functional group is a carboxylic acid (COOH). The 11-MUA SAM on Au can then be used to covalently immobilize probes containing primary amino groups. Thermodynamic studies reveal that the energy of Au–S bond is \(~15\) to \(30\) kcal/mol (or \(~62.8\) to \(125.5\) kJ/mol) for solution and gas-phase studies, respectively. A bond energy of \(50\) kcal/mol (209.2 kJ/mol) has also been reported. Therefore, the adsorption of molecules with thiol groups onto Au is usually considered chemisorption.

The self-assembly process can be performed in either a liquid environment or the gas-phase. Figure 2.4 shows the simplest model of this process: (i) initial physisorption, (ii) chemisorption of the molecules, (iii) nucleation of the upright phase, and (iv) formation of highly ordered layer. The most common strategy is to immerse a clean substrate in a dilute (\(~1\)–\(10\) mM) ethanolic solution of thiols for \(~12\)–\(18\) h at room temperature. A highly packed layer can be obtained within a few minutes, but more time is required to allow reorganization and formation of a layer with minimal defects. The mechanism of SAM formation is complex, and different experimental conditions (e.g., solvent, temperature, concentration, time, purity, and chain length) can influence the structure of the final SAM.

![Figure 2.4](image)

Figure 2.4: The simplest scheme of forming a SAM on Au: (i) initial physisorption, (ii) chemisorption of the molecules, (iii) nucleation of the standing up phase, and (iv) formation of highly ordered layer. Modified and reproduced with permission from RSC Publishing.

2.1.1.2 Silanes for Glass Modification

The term silane (SiH\(_4\)) has also been used to describe many of its derivatives. A list of commonly encountered functional groups on silicon is shown in Table 2.1.
Table 2.1: A list of common functional groups on silicon.

<table>
<thead>
<tr>
<th>Name</th>
<th>Chemical Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silanol</td>
<td>Si–OH</td>
</tr>
<tr>
<td>Silyleamine</td>
<td>Si–NH₂</td>
</tr>
<tr>
<td>Methoxysilane</td>
<td>Si–OCH₃</td>
</tr>
<tr>
<td>Ethoxysilane</td>
<td>Si–OCH₂CH₃</td>
</tr>
<tr>
<td>Chlorosilane</td>
<td>Si–Cl</td>
</tr>
<tr>
<td>Disilane</td>
<td>Si–Si</td>
</tr>
<tr>
<td>Disiloxane</td>
<td>Si–O–Si</td>
</tr>
</tbody>
</table>

Most functionalization methods used for glass involve silanes. After creating hydroxyl groups on the glass surface (more details in Section 2.1.2), the reaction with silanes typically involves four steps. The reaction of a methoxysilane with a surface containing hydroxyl groups is shown as an example in Figure 2.5. First, the methoxy groups (OCH₃) of the silane hydrolyze quickly in water environments to form reactive silanol groups. Then the silanol groups can condense with each other to form stable siloxane (Si–O–Si) bonds. Lastly, hydrogen bonding occurs between the silane and the hydroxyl groups of the surface, leading to subsequent covalent bond formation and the release of water molecules. It is important to note that silanes have also been observed to chemically modify many other types of surfaces, such as metal oxide and metal hydroxyl surfaces.

Figure 2.5: The reaction mechanism of (A) hydrolysis, (B) condensation and (C, D) bonding of silanes to a hydroxyl-containing surface. Modified and reproduced with permission from Elsevier.
Experimental factors (e.g., temperature, type of solvent, water content, sample purity, immersion time, and pH) will influence the reaction of silanes.\textsuperscript{105,106} In particular, acidic conditions catalyze the hydrolysis of silanes and reduce the self-condensation among the silanol groups. In contrast, under basic conditions, self-condensation occurs immediately after the hydrolysis reaction, which leads to the growth of three-dimensional molecular structures.\textsuperscript{107}

### 2.1.2 Polydimethylsiloxane (PDMS) and Biosensors

Recently, microfluidics has been frequently combined with biosensors to extend their capabilities. Advantages of microfluidics include decreased reagent volumes, increased sample throughput, decreased reaction time, and potential for automation.\textsuperscript{108} Typically, microfluidic devices are constructed with materials that form enclosed channels and cell chambers.

Due to advances in the microfabrication technologies in the semiconductor industry, first-generation devices were prepared with silicon wafers or glass.\textsuperscript{22} Glass is amorphous, electrically insulating, and optically transparent. Silicon wafers, on the other hand, are crystalline and opaque. Both of these materials were frequently used because of their resistance to organic solvents and high thermal conductivity. However, the hardness of these substrates limited their broad use. Some of the other challenges associated with silicon and glass include difficulty in bonding (e.g., high temperature and high pressure are normally required) and high cost of fabrication (e.g., clean room environment and dangerous chemicals such as hydrogen fluoride are involved, thus requiring expensive protective and waste disposal facilities). Furthermore, these substrates are not permeable to gas. Consequently, they cannot be used to culture cells. These challenges motivated the search for alternative materials for microfluidics. Various polymers were investigated, in particular elastomeric polymers, and polydimethylsiloxane (PDMS) emerged as the most popular elastomer used in microfluidics.\textsuperscript{22} The chemical structure of PDMS is shown in Figure 2.6.

![Figure 2.6: The chemical structure of PDMS.\textsuperscript{109} Reproduced with permission from Elsevier.](image_url)

PDMS is an excellent material for creating microfluidic devices because it cures at low temperatures,
2.1. Introduction to Biosensors

it is flexible, it is optically transparent, and it is non-toxic to biomolecules. Small channels can also be repeatedly reproduced by soft lithography using a mold with the desired features (see Figure 2.7). Due to its flexibility, PDMS can be easily removed from the mold. Subsequently, it can form reversible bonds to other materials via intermolecular (van der Waals) forces, or it can form irreversible bonds to glass or other PDMS after exposure to air or oxygen plasma by forming siloxane bonds.\textsuperscript{110,111}

![Figure 2.7: Scheme for producing PDMS chips using soft lithography. (A) A mold is made with the desired features. (B) Liquid pre-polymer material is poured onto the mold. (C) Mold with liquid PDMS is cured at low temperatures. (D) Solidified and flexible PDMS is peeled off and (E) placed on a rigid substrate for experiments.\textsuperscript{1} Reproduced with permission from ACS Publications.](image)

The surface of unmodified PDMS is hydrophobic. As a result, hydrophobic PDMS channels are susceptible to bubble formation and non-specific adsorption of hydrophobic molecules. One way to mitigate these challenges is via surface functionalization. Most of the functionalization methods applied to PDMS use silanes. This strategy is analogous to the standard glass-based surface chemistry as previously described.\textsuperscript{94} Prior to silanization, the surface is first treated to remove organic residues and to increase the number of hydroxyl (—OH) groups on the surface (i.e., silanols Si—OH). The most widely used method for PDMS (and glass) is using oxygen plasma.\textsuperscript{111,112} The literature also describes other methods such as exposing to UV-ozone and soaking in sodium hydroxide.\textsuperscript{94,113,114} This cleaning and oxidizing step is important for the effective silane functionalization.
2.1.3 An Example: Surface Plasmon Resonance (SPR) Biosensor

Surface plasmon resonance (SPR) is a powerful and versatile optical technique commonly used for analyzing biomolecular interactions. Typically, a glass substrate coated with a thin Au film is used for SPR applications. At a specific angle of incidence (i.e., resonance angle), a reduction in the intensity of the reflected light can be detected. When the refractive index of the interfacial region changes (e.g., due to protein adsorption on Au), the angle of incidence required for the resonance will change as well (see Figure 2.8). Therefore, by monitoring the change in the resonance angle (or reflected light intensity) as a function of time, the interaction between the analyte molecules (i.e., targets flowing in solution) and the biorecognition elements (i.e., probes immobilized on the gold substrate) can be examined and analyzed. SPR biosensors are commonly used to obtain binding kinetics and equilibrium constants for biomolecules, due to its inherent surface sensitivity and its virtually real-time response.

![Diagram of an SPR biosensor](image)

Figure 2.8: A typical setup of an SPR biosensor. Reproduced with permission from Nature Publishing Group.
2.1.3.1 Carbodiimide Activation Chemistry

Covalent immobilization is one of the most frequently employed strategies for the attachment of probes onto the gold surface used in SPR biosensors, especially via the carboxyl-amine coupling chemistry.  

Figure 2.9 shows a typical carboxyl-amine coupling mechanism using the carbodiimide reaction chemistry. First, the gold surface is modified either with a SAM or layer of dextran containing carboxyl groups. Then a mixture of \( N \)-hydroxysuccinimide (NHS) and \( N \)-((3-(dimethylamino)propyl))-\( N \)-ethylcarbodiimide hydrochloride (EDC) solution is injected over the surface to activate the carboxyl groups. Subsequently, the covalent coupling of biomolecules containing primary amino groups to the surface is achieved by forming amide bonds. The unreacted succinimide groups are usually quenched with ethanolamine. Modified and reproduced with permission from Springer.

![Chemical reaction scheme](image)

Figure 2.9: The chemical reaction scheme for the covalent immobilization of a probe onto the surface via carboxyl-amine coupling chemistry. (1) The carboxyl group is activated with EDC/NHS. (2) Covalent attachment of a probe by its primary amino group. (3) The unreacted active site can be deactivated with ethanolamine. Modified and reproduced with permission from Springer.

2.1.3.2 PDMS-Based SPR Biosensor

Most of the commercially available SPR systems, such as BIAcore 3000, have difficulties in performing high-throughput assays, concentration assays, and multiple interactions. Much effort has been devoted to address these issues by combining microfluidics with SPR imaging (SPRI) techniques to...
2.1. Introduction to Biosensors

decrease reaction time, reduce reagent consumption, allow concentration analysis, and increase assay throughput. In SPRi, usually a microarray of gold spots is formed on the glass substrate in order to create high-contrast SPR images, minimize cross-talk between adjacent sensing channels, and reduce background noise. SPRi usually measures at a fixed angle, where differences in reflectivity observed at the array interface are monitored over time with a camera. Microfluidic SPRi allows for multiplexed detection for high-throughput bio-analysis. Figure 2.10 shows the design of a PDMS-based microfluidic device for SPRi applications.

Figure 2.10: The design of a PDMS-based microfluidic device for SPRi applications. Reproduced with permission from RSC Publishing.

The PDMS microchip shown in Figure 2.10 could simultaneously monitor multiple analyte streams against different probes by using a parallel 264-chamber microarray. The addition of a dilution network and a system of valves and pumps illustrates the “lab on a chip” concept. Importantly, due to design complexity and the high pressure required, the PDMS microchip should be strongly bonded to the glass substrate so that the biomolecular interaction analysis could be performed on the Au spots. Modifying the Au spots with a SAM was not possible because of the harsh UV-Ozone cleaning/bonding method.
Therefore, physical immobilization of the proteins was utilized. However, the weakly and randomly adsorbed proteins could be easily washed off by the running buffer, thus affecting the SPR curves (as well as the binding kinetics and equilibrium constants) obtained.

2.2 Surface Analysis

The analysis of surfaces modified with self-assembled monolayers (SAMs) is an active area of research, since the characteristics of the adsorbed layer can affect the device performance. To better understand the process of SAM formation and the physical/chemical properties of the adsorbed layer, a wide range of surface-specific analysis techniques have been utilized. In the following sections, the techniques employed in this thesis are introduced.

2.2.1 Water Contact Angle

In nature, we observe that a small drop of water typically forms a somewhat spheroidal shape, since this shape has the lowest surface area for a given volume of water. This tendency to minimize surface contact area is characterized as surface tension $\gamma$. Surface tension is defined as a force per unit length (or energy per unit area) used to create a new surface.

When a drop of liquid is placed on a solid surface, a triple interface is formed between the solid, liquid and gas as shown in Figure 2.11, where $\gamma_{SL}$ is the surface tension of the solid-liquid interface, $\gamma_{LG}$ is the surface tension of the liquid-gas interface, and $\gamma_{SG}$ is the surface tension of the solid-gas interface. The angle between the solid surface and the tangent to the liquid surface is known as the contact angle $\theta_C$ (measured in the liquid phase).

$$\theta_C < 90^\circ \quad \theta_C > 90^\circ$$

![Figure 2.11: Illustration of contact angles formed by sessile liquid drop on a solid surface.](image_url) Modified and reproduced with permission from Springer.
2.2. Surface Analysis

At equilibrium, these tensions will be in balance according to Young’s Equation as shown eq 2.1:

\[ \gamma_{SG} = \gamma_{SL} + \gamma_{LG} \cos(\theta_C) \]  

(2.1)

It is important to note that cohesive forces exist between molecules in the liquid drop, and adhesive forces exist between the liquid molecules and the surface. For example, if there are polar groups on the surface (e.g., hydroxyl groups) and a water drop is used, there will be strong adhesive forces between the water molecules and the surface. This type of a surface is called hydrophilic and a low contact angle is observed (as shown by Figure 2.11 on the left). Conversely, if the surface consists mainly of non-polar groups (e.g., surface is covered with an organic layer), the surface is hydrophobic and a large contact angle is formed (as shown by Figure 2.11 on the right). As a result, contact angle measurements are used as a quick and simple technique to obtain qualitative information about the chemical nature of a surface.

The sessile drop method is commonly used, where measurements of the shape of a liquid drop sitting on a flat surface are made with a camera. Software can be used to analyze the digital images to determine the contact angle. Wettability is determined by the equilibrium contact angle \( \theta_C \). If \( \theta_C < 90^\circ \), the liquid is said to wet the solid (complete wetting occurs when \( \theta_C = 0^\circ \)). If \( \theta_C > 90^\circ \), the liquid does not wet the solid. In particular, the sessile drop method has been applied to study mixed SAMs of alkanethiols on gold\textsuperscript{135} and the hydrophobic recovery of PDMS\textsuperscript{136} by oxygen plasma and chemical treatment.

### 2.2.2 Atomic Force Microscopy (AFM)

Atomic force microscopy (AFM) is one of the most frequently used tools for imaging surface topography and measuring certain physical properties (i.e., hardness, friction, and thickness) of the surface with atomic-scale resolution. A diagram of the typical AFM setup is shown in Figure 2.12. A laser light source (with the help of some focusing optics) is reflected off the back of a cantilever, which has a sharp tip at the end. The small sharp tip used usually has a radius of \(~10\) to \(100\) nm.\textsuperscript{133} When the tip moves close to the sample surface, the tip–surface interaction can cause a deflection of the cantilever, which can be measured as a change in the deflected light and recorded by a detector.

Numerous AFM operation modes have been proposed to image the surface.\textsuperscript{137} As the cantilever is raster scanned across the sample, the topography of the sample is most commonly measured in either the constant-deflection or the constant-height mode.\textsuperscript{138,139} In the constant-deflection mode, the can-
2.2. Surface Analysis

teilever deflection is kept constant by extending and retracting the piezoelectric scanner. In the constant-height mode, the fixed end of the cantilever is kept at a constant height and the cantilever tip deflection due to sample-tip interaction is recorded. In this thesis, contact-mode imaging (i.e., imaging mode and lateral force mode) and force-distance curve were used.

Figure 2.12: Schematic diagram showing the setup of a typical AFM experiment.\textsuperscript{138} Reproduced with permission from Elsevier.

2.2.2.1 AFM Contact-Mode Imaging

In this imaging mode, the vertical displacement of the cantilever is controlled to keep a constant deflection of the probe as the tip scans laterally on the sample surface. This mode can be operated in either the attractive or repulsive regime. The variation of the displacement as a function of lateral position gives information about the surface topography. Lateral force (or friction force) mode is similar to the imaging mode. However, during the lateral scan of the tip over the surface, the twisting of the cantilever (due to the force between the tip and the surface) is also monitored. These techniques have been applied to study patterned SAM formation on Au.\textsuperscript{140}
2.2. Surface Analysis

2.2.2.2 AFM Force-Distance Curve

In a force-distance curve measurement, the displacement between the sample and the fixed end of the cantilever is changed, while the attractive (or adhesive) and repulsive forces between the sample and the cantilever tip are monitored (see Figure 2.13). At position A, the sample surface is far away from the cantilever tip, so the force is zero. As the tip is moved closer to the sample, the attractive force between the tip and the sample surface begins to pull the tip downward. At position B, the tip is in contact with the sample surface. As additional force is applied to the cantilever, the sample-tip interaction becomes repulsive as shown by position C. The tip is then retracted with the tip and sample still in contact through some range of force, as shown by position D. Finally, at a distance beyond the maximum adhesive force, the tip snaps out of contact with the surface (position E) and moves away from the sample (position F).\textsuperscript{141}

![Force-Distance Curve](image)

**Figure 2.13:** A schematic of force-distance curve.\textsuperscript{141} Reproduced with permission from Journal of Cell Science and Company of Biologists LTD.

The net interaction between the sample and the tip results from a sum of different forces. Before contact, long-range interactions (i.e., van der Waals and Coulomb forces) exist. Once in contact, chemical bonds may form between the tip and the surface. As the tip is removed from the sample, the pull-off force gives a measure of the adhesion force. The AFM force-distance curve has been applied to study the surface acid–base properties of SAMs with terminal carboxylic acid groups.\textsuperscript{142}
2.2.3 Infrared (IR) Spectroscopy

Infrared (IR) spectroscopy uses the fact that a molecule absorbs light at different frequencies in the IR region that are characteristic of its chemical bonding configuration and three-dimensional structure. For IR radiation to be absorbed, the molecule’s electric dipole moment must change (e.g., vibrations and rotations). Vibrations can involve a change either in bond angle (bending) or in bond length (stretching), such as symmetrical stretching or asymmetrical stretching. Even for a simple molecule, many different types of vibrations are observed.¹⁴³

Two methods for measuring IR spectra will be described: attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) and polarization modulation-infrared reflection-absorption spectroscopy (PM-IRRAS). Some applications of IR spectroscopy will be highlighted in Section 2.2.5.

2.2.3.1 ATR-FTIR

Traditional transmission spectroscopy is a simple method for the collection of IR spectra. Prior to experiments, a solid sample must be finely ground and diluted with IR transparent salt (e.g., potassium bromide) and pressed into a thin film (or pellet). As the beam passes through the sample, some of the light is absorbed and this method measures the percentage of IR light transmitted (or % Transmittance) at specific wavelengths. Liquid, solid, or gas samples can be analyzed using this approach.¹⁴⁴

In recent years, reflectance sampling techniques are becoming more popular, since they require less (or simpler) sample pre-treatment. In particular, attenuated total reflectance (ATR) spectroscopy is a non-destructive method that has been applied to study surfaces, films, and solutions. The sample may be directly placed on the ATR crystal (with some pressure applied) and the IR spectra can be quickly obtained (see reference for a typical setup).¹⁴⁵

ATR-FTIR employs the phenomenon of total internal reflection (TIR). TIR occurs when light is completely reflected at the interface between two optically different media (i.e., with different refractive indices) at an angle larger than the critical angle (with respect to the normal to the surface). This process creates an evanescent wave that travels along the boundary between the two materials. First, the IR beam is directed at a crystal of higher refractive index (at a particular angle of incidence). An evanescent wave, created by the internal reflection(s), extends into the sample held in contact with the crystal. In the spectral regions where the sample absorbs energy, the evanescent wave will be attenuated. Then the reflected radiation (with some sample absorption) is returned to the detector.¹⁴³ Many experimental factors can influence the measurements (e.g., number of reflections and quality of contact between the
2.2.3.2 PM-IRRAS

Infrared reflection-absorption spectroscopy (IRRAS) is a well-established technique frequently used to study monolayers and thin films deposited onto metallic surfaces. To overcome limitations of IRRAS (e.g., inability to detect ultra-thin films or lengthy experimental time periods), polarization modulation-IRRAS (PM-IRRAS) was introduced. The PM-IRRAS measurement depends on the polarization of the incident IR beam, the angle of incidence, and the optical constants of the thin film and substrate (as well as the molecule's orientation on the surface).

First, IR radiation illuminates the metal surface at a well-defined and controlled angle of incidence. A photoelastic modulator generates this radiation, which is either parallel (p-polarized) or perpendicular (s-polarized) to the plane of reflection.
(s-polarized) to the plane of incidence. When the beam is reflected from the surface, the electric vector experiences a phase change, whose magnitude depends on the polarization (see Figure 2.14).

At almost all angles of incidence, the radiation polarized perpendicular to the plane of incidence (i.e., s-polarized) undergoes a phase change of $\pi$ and the electric vectors sum to near zero at the sample, which results in a zero electric field strength. Therefore, no IR absorption occurs (see Figure 2.15). Near the grazing angle of incidence (i.e., about 88°), the radiation polarized parallel to the plane of incidence (i.e., p-polarized) undergoes a different phase change of about $\pi/2$ and the resultant standing wave is non-zero at the surface and is oriented along the surface normal. As a result, IR absorption is enhanced.\textsuperscript{144,147}

![Figure 2.15: Dependence of the absorption factor at the wavelength of maximum absorption on $\theta$ for a 1 nm layer of acetone on metal.\textsuperscript{147} Reproduced with permission from ACS Publications.](image)

It follows that the dipole transition moment of the adsorbed molecule(s) on the surface must have a component oriented along the surface normal in order to absorb the incident radiation. As a result, only the parallel component of reflectivity ($R_p$) will be influenced by these surface adsorbed molecule(s). In
contrast, the randomly oriented molecules in the beam path (e.g., water vapor and carbon dioxide in air) will influence both \( R_p \) and \( R_s \).

With a single detector, the sum of the reflectivity of the p- and s-components \((R_p + R_s)\) and the difference in the reflectivity \((R_p - R_s)\) may be obtained using a photoelastic modulator and a lock-in amplifier. The PM-IRRAS signal is given by the differential reflectivity \( \frac{\Delta R}{R} = \frac{(R_p - R_s)}{(R_p + R_s)} \), which is proportional to the absorbance. Therefore, the final PM-IRRAS spectrum contains signals/absorbance from only the surface adsorbed species with dipoles oriented along the surface normal.

2.2.4 X-ray Photoelectron Spectroscopy (XPS)

X-ray photoelectron spectroscopy (XPS) operates on the principle of the photoelectric effect (see reference for more information).\(^{148}\) Illumination of a surface with monochromatic X-ray radiation results in a primary excitation process that produces electrons (also called photoelectrons). These electrons may be ejected from the sample surface into the surrounding vacuum with minimal loss in kinetic energy. Therefore, the distribution of unscattered electrons as a function of their kinetic energies, which can be converted to binding energies, results in the XPS spectrum.

The energy of the overall process must be conserved and is described by eq 2.2, where \( h \) is Planck constant, \( \nu \) is frequency, \( E_b \) is binding energy, \( E_k \) is kinetic energy, \( E_r \) is recoil energy, and \( \phi_{sp} \) is spectrometer work function.

\[
h \nu = E_b + E_k + E_r + \phi_{sp} \tag{2.2}
\]

The two most important of these quantities are the kinetic energy \( (E_k) \) of the electron inside the spectrometer and the energy required to remove the electron from the initial state (i.e., binding energy \( E_b \)). Experimentally, the binding energy \( (E_b) \) can be approximated by \( h \nu - E_k \). Because the energy of the X-ray (with a particular wavelength) is known, the discrete \( E_k \) values measured can be correlated with the \( E_b \) values of different atomic levels. The resulting spectrum shows a peak corresponding to each energy level. For accurate binding energy assignments, energy contributions from the recoil energy \( (E_r) \) and the spectrometer work function \( (\phi_{sp}) \) must also be considered.

XPS is a surface-sensitive technique that measures the elemental composition, as well as the chemical and electronic states of the elements that exist on the sample surface. Quantitative information can also be obtained by counting the number of electrons that escape from the sample surface.
2.2.5 Surface Analysis of Silanes on Gold

One of the main goals of this thesis is to characterize the adsorption of a carboxylated silane (TMS-EDTA) on Au in order to better understand the physicochemical nature of this interface and thereby determine the properties relevant to its application to microfluidic electrochemical devices. Therefore, the literature related to the surface analysis of silanes on gold will be presented here. The adsorption of chlorosilane\textsuperscript{149} and mixed monolayers of silanes on gold,\textsuperscript{150} and the interaction of silanes with other oxide-free metal\textsuperscript{151} substrates have been reported. In the following sections, the use of some of the surface analysis techniques described above to investigate the adsorption of silanes on Au will be highlighted. Specifically, the adsorption of an ethoxysilane and alkylsilanes on Au will be discussed.

2.2.5.1 Amino-Silane for Gold Modification

Thin films of (3-aminopropyl)triethoxysilane (3-APTES) on aluminum oxide and planar gold substrates have been analyzed by contact angle measurements and IR techniques (see Figure 2.16).\textsuperscript{152} Contact angle measurement of 3-APTES on aluminum substrate showed a water contact angle of 65°. The wetting behavior was a result of several contributions, including amino, ethoxy, and silanol groups (as well as a high degree of siloxane cross-linking).

![Figure 2.16: Reflection-absorption Fourier transform infrared spectroscopy (RAIR) spectra of 3-APTES adsorbed on gold from methanol solution, as a function of hydrolysis and condensation reactions. (1) Freshly deposited 3-APTES at room temperature, (2) plus 4 min at 75 °C, (3) plus 6 min, (4) plus 6 min, (5) plus 20 min, and (6) after heating for 12 h at 75 °C.\textsuperscript{152} Modified and reproduced with permission from ACS Publications.](image)

Prior to modifying the gold substrate, normalized transmission IR spectra of 3-APTES were obtained to help with the assignment of band positions and modes. Subsequently, reflection-absorption
Fourier transform infrared spectroscopy (RAIR) was used to study the hydrolysis and condensation of 3-APTES on gold. Figure 2.16 shows the absorption IR spectra for two regions: the alkyl stretching region (3100–2700 cm\(^{-1}\)) and the fingerprint region (1800–900 cm\(^{-1}\)).

Freshly deposited 3-APTES (spectrum 1) shows Si–O modes with maxima at 1126 and 1091 cm\(^{-1}\) and some bands from the ethoxy groups (e.g., 2975 and 1390 cm\(^{-1}\)). The Si–O modes broaden and develop several maxima as the silane cross-links (spectrum 5). At the same time, the ethoxy modes decrease in intensity (e.g., at 2974 cm\(^{-1}\)). For spectrum 6, the film is extensively cross-linked (i.e., the Si–O region shows one major peak at 1150 cm\(^{-1}\) and a shoulder at 1050 cm\(^{-1}\)). Evidently, hydrolysis and condensation of the silane (i.e., siloxane cross-linking) were observed.

### 2.2.5.2 Alkylsilanes for Gold Modification

Recently, the adsorption of silane (Si\(_4\)) and methylsilane (CH\(_3\)--SiH\(_3\)) on gold has been studied.\(^{67}\) Furthermore, several alkylsilanes with longer hydrocarbon chains have also been studied by combining IR and XPS techniques.\(^{153–155}\) For the experiments with alkylsilanes, Figure 2.17 shows the IR transmission spectra for 3050–2750 and 2350–2000 cm\(^{-1}\) regions (the latter region can analyze the silicon-hydrogen stretch at 2150 cm\(^{-1}\)).

![RAIR spectra for monolayers of (A) octadecylsilane, (B) octylsilane, and (C) hexylsilane on Au. A solution IR spectrum of octylsilane (D) is included for comparison. Reproduced with permission from ACS Publications.](image)

The observed carbon-hydrogen stretching modes between 2850 and 3000 cm\(^{-1}\) are consistent with the alkyl chains. The silicon-hydrogen stretch at 2150 cm\(^{-1}\) is observed for the liquid alkylsilane (Figure 2.17).
2.2. Surface Analysis

However, this feature is absent in the other spectra. These results suggest that no silicon-hydrogen bonds remain after chemical adsorption.

Figure 2.18 shows the XPS spectra for C 1s electronic energy level (-288.5 to -278.5 eV), as well as Si 2p and Au 4f (-103 to -83 eV) core-levels. When compared to the Au reference (spectrum 4), all samples (spectra 1-3) show an increase in the C atoms (Figure 2.18A) and an increase in the Si atoms (Figure 2.18B).

The combination of XPS and IR data showed that all three silicon-hydrogen bonds reacted with the gold surface, and silicon formed bonds with three gold atoms on the surface. The proposed mechanism of alkylsilane adsorption on Au is shown in Figure 2.19.

Subsequently, the oxidation of alkylsilane-based monolayers on gold was studied. XPS spectra
of freshly prepared samples and samples treated with \( \text{O}_3/\text{O}_2 \) are shown in Figure 2.20. Exposing the freshly prepared sample (I) to ozone results in the oxidation of the alkyl chain. For example, there is an increase in O (Figure 2.20A) and loss in C (Figure 2.20B). Furthermore, the increasing shoulder at \( \sim -287 \) eV represents the formation of carboxyl groups in the monolayer.

![Figure 2.20: (A) O 1s and (B) C 1s core levels of an octylsilane monolayer on gold exposed to successive dose of O\textsubscript{3}/O\textsubscript{2}. Reproduced with permission from ACS Publications.](image)

These studies indicate that some silanes can be used to chemically modify Au surfaces. Furthermore, different silanes result in differing surface adsorbed structures. For example, 3-APTES modifies the Au surface with extensive siloxane cross-linking, forming multilayers. On the other hand, alkylsilanes adsorb onto the Au surface with little evidence of cross-linking, most likely forming monolayers. The combined use of the surface analysis techniques described above will help to elucidate the chemical and physical nature of the adsorbed TMS-EDTA layer on Au.

2.3 Thermodynamics of Adsorption

Generally, adsorption deals with the physical and/or chemical interaction of molecules, atoms, or ions (of gas, liquid, or dissolved solid) with a surface,\textsuperscript{156} and this process creates a layer of adsorbate contacting the surface. Solvent-based chemical modification is often employed to prepare a surface with self-assembled monolayer (SAM). As a result, the adsorption of a molecule for creating the SAM is in competition with solvent and other species in the solution. It is important to gain an understanding of the
molecule’s Gibbs free energy of adsorption,\(^{157,158}\) since this information will in turn provide an indication of the process spontaneity. The change in Gibbs free energy depends on the adsorbate/adsorbent bond strength (enthalpy change) and entropy change.\(^ {159}\) Depending on this strength, the adsorption process can be classified either as physisorption (e.g., weak van der Waals forces) or chemisorption (e.g., covalent bonding). For some species, adsorption may also occur due to electrostatic attraction.\(^ {160}\) Moreover, an understanding of the molecule’s adsorption process can potentially lead to the design of improved biosensors and devices,\(^ {96}\) since complex biological samples are often analyzed and multiple use of the devices may be required.

### 2.3.1 The Gibbs Adsorption Isotherm

For biosensors, the solid substrate (e.g., glass or gold) is often in contact with an aqueous solution. The interphase of such a system can be divided into three regions: two distinct bulk phases (i.e., solid and liquid) and a surface phase defined as the interphase region. Experimentally, it is difficult to determine the exact structure of this surface phase.

Josiah Willard Gibbs proposed an idealized model that defined the interphase region as having zero thickness. In this model, the chemical components of the two bulk phases remain unchanged except near the dividing surface. For a particular species \(i\), the quantitative measure of adsorption at the dividing surface is captured by the surface excess quantity \(\Gamma_i\) with respect to bulk (i.e., an arbitrary plane defined in the bulk solution) as shown in eq 2.3, where \(n\) is the number of moles in excess and \(A\) is the area of the dividing surface.

\[
\Gamma_i = \frac{n_i}{A} \quad (2.3)
\]

The Gibbs adsorption isotherm, given by eq 2.4, provides the simplest description of the properties of interphase. In this equation, \(\gamma\) is the surface tension, and \(\Gamma_i\) is the surface excess and \(\mu_i\) is the chemical potential for a particular species \(i\).

\[
-d\gamma = \sum_i \Gamma_i \ d\mu_i \quad (2.4)
\]

By definition, surface tension is equivalent to the Gibbs free energy per unit area of interface.\(^ {133}\) Equation 2.4 suggests that, for systems not at equilibrium, there is a natural tendency for the Gibbs energy at constant temperature and pressure to decrease. For example, a pure phase always assumes
2.3. Thermodynamics of Adsorption

A shape that creates the minimum surface area per unit volume. In addition, when a solution is in contact with another phase, the composition of the interphase differs from that of the bulk such that $d\gamma$ is minimized at constant temperature and pressure. From eq 2.4, it is evident that when the surface excess of a species is positive ($\Gamma_i > 0$), increasing the chemical potential of that species (e.g., by increasing its concentration in the bulk liquid phase) decreases the surface tension ($d\gamma < 0$). Conversely, when the surface excess of a species is negative, increasing the chemical potential of that species increases the surface tension. For examples, surfactants have a positive surface excess concentration and induce a decrease in surface tension; conversely, electrolytes have a negative surface excess concentration, and induce an increase in surface tension.

2.3.2 Langmuir Adsorption Isotherm

The interphase region (or surface phase) of a real system is different from the one proposed by Gibbs. In particular, if we assume that the molecules can only adsorb as a monolayer on the solid surface, then the surface coverage ($\theta$) can be defined as shown in eq 2.5 at equilibrium, where $\Gamma$ is the surface excess of a particular species and $\Gamma_{max}$ is the surface excess at maximum surface coverage. This concept is fundamental to the derivation of one of the simplest models for adsorption: the Langmuir adsorption isotherm. It is important to keep in mind that both Langmuir and Gibbs adsorption isotherms are ideal models.

$$\theta = \frac{\Gamma}{\Gamma_{max}}$$  \hspace{1cm} (2.5)

The Langmuir adsorption isotherm relates the surface coverage of a particular species ($\theta$) to its bulk concentration ($C$). This model assumes that the adsorbed layer is a monolayer and the species do not interact with each other. Furthermore, there is a one-to-one relationship between the species and the adsorption sites (and the free energies of all adsorption sites are assumed to be equivalent). The Langmuir adsorption process can be represented by eq 2.6, where $A$ represents the free molecules (or adsorbates) that can adsorb onto the surface, $S$ represents the free adsorption sites on the surface, and $A_{ad}$ represents the adsorbed molecules (or adsorbates) on the surface.

$$A + S \rightleftharpoons A_{ad}$$  \hspace{1cm} (2.6)

Using the definition that $(1 - \theta) = \frac{N_{free}}{N_s}$ (where $N_{free}$ is the number of free sites and $N_s$ is the total number of sites), the rate of adsorption ($r_a$) can be written as
2.3. Thermodynamics of Adsorption

\[ r_a = k_a[A][S] = k_a[A]N_S(1 - \theta) \quad (2.7) \]

and the rate of desorption \((r_d)\) can be written as

\[ r_d = k_d[A_{ad}] = k_dN_S\theta \quad (2.8) \]

(where \(k_a\) and \(k_d\) are the rate constants for adsorption and desorption, respectively). The dynamic equilibrium process can be represented by eq 2.9.

\[ k_a[A]N_S(1 - \theta) = k_dN_S\theta \quad (2.9) \]

Let us represent \([A]\) as \(C\) (i.e., the bulk concentration of the adsorbing molecule), we get eq 2.10.

\[ k_aC(1 - \theta) = k_d\theta \quad (2.10) \]

If we define the Langmuir equilibrium constant as \(K_L (= k_a/k_d)\), then we get eq 2.11.

\[ K_L = \frac{\theta}{C(1 - \theta)} \quad (2.11) \]

Solving for \(\theta\), we get eq 2.12 (i.e., the Langmuir adsorption isotherm).

\[ \theta = \frac{K_L C}{1 + K_L C} \quad (2.12) \]

2.3.3 Standard Gibbs Free Energy of Adsorption \(\Delta G^°\)

Adsorption is typically a spontaneous process, and is indicated by the negative Gibbs free energy change. The standard Gibbs free energy \((\Delta G^°)\) can be calculated at equilibrium using eq 2.13 under standard conditions, where \(R\) is the gas constant, \(T\) is the temperature in Kelvins, and \(K\) is the thermodynamic equilibrium constant.

\[ \Delta G^° = -RT\ln K \quad (2.13) \]

Experimentally, by changing the bulk concentration \(C\), different values for surface coverage \(\theta\) can be obtained. Then, the concentration-dependent surface coverage can be fit to Langmuir adsorption...
isotherm (eq 2.12) to obtain the equilibrium constant $K_L$ using non-linear least squares fitting. Consequently, the Langmuir adsorption free energy ($\Delta G_{ads}^*$) can be obtained.

### 2.3.4 Frumkin Adsorption Isotherm

One major drawback of the Langmuir adsorption isotherm is that it does not take into account the interaction among the adsorbed molecules. For instance, when negatively charged species are adsorbed on the surface, there may be electrostatic repulsion among the charged molecules. The Frumkin adsorption isotherm was developed to take these interactions into account.

The improved adsorption isotherm is shown in eq 2.14, where $K_F$ is the Frumkin equilibrium constant, and $\alpha$ is the Frumkin lateral interaction parameter.

$$
\theta = \frac{K_F C \exp(-\alpha \theta)}{1 + K_F C \exp(-\alpha \theta)}
$$

(2.14)

The neighbouring molecules may interact due to either attraction or repulsion. According to eq 2.14, positive $\alpha$ values indicate repulsion between the neighboring molecules. Negative $\alpha$ values indicate attraction between the neighboring molecules. When $\alpha = 0$, the Frumkin isotherm becomes identical to the Langmuir isotherm.

The concentration-dependent surface coverage can also be fit to Frumkin adsorption isotherm (eq 2.14) to obtain both $K_F$ and $\alpha$. The Frumkin $\Delta G_{ads}^*$ can also be obtained (using eq 2.13).

### 2.3.5 $\Delta G_{ads}^*$ in a Complex Environment

There are also many other adsorption isotherms proposed (e.g., Henry isotherm for low surface coverages and Temkin isotherm for uncharged and non-interacting species), but they do not adequately explain the complex TMS-EDTA system. Therefore, their discussion is beyond the scope of this thesis.

Historically, the adsorption isotherms have been derived for gas–solid systems. However, the equations derived above are also valid for liquid–solid systems, under specific conditions. It is important to note that for studies performed under aqueous conditions, water and components of the electrolyte may also adsorb onto the surface. As a result, the Langmuir equilibrium constant ($K_L$) obtained for a particular species may be affected by the displacement of water molecules and electrolyte. Similarly, the Frumkin equilibrium constant ($K_F$) and the lateral interaction parameter ($\alpha$) obtained for a particular species (under aqueous conditions) may also be affected. Therefore, the free energy of adsorption, $\Delta G_{ads}^*$, is uniquely specified for a particular liquid–solid interphase.
2.4 Electrochemistry

In this thesis, electrochemical techniques have been applied to study TMS-EDTA adsorption on Au under alkaline aqueous conditions, in the presence of chloride ions. Electrochemical differential capacitance is one of the best techniques to determine the standard free energies of adsorption in a complex aqueous environment, while also elucidating its dependence on the potential of the metal substrate. Therefore, the background theory of electrochemistry will be reviewed in the following sections. First, the electrode–solution interface, with an emphasis on the solution side of the interface, will be discussed.

2.4.1 Electrical Double Layer

Typically, electrochemistry involves the use of a metal (i.e., electrode surface) in contact with an aqueous solution containing a high concentration of ions. When excess charge (i.e., excess or deficiency of electrons) accumulates as a thin layer at the metallic side of the interface (assuming that the metal is a good electrical conductor), the counter-charge in solution is made up of an excess of either cations or anions near the electrode surface. The distribution of charged species and oriented dipoles that exists at the solution side of the interface is called the electrical double layer (or double layer).\textsuperscript{163} Several models of the double layer have been proposed and its historical development will be described below.

2.4.1.1 Helmholtz Model

In 1853, Hermann von Helmholtz proposed that charged electrodes (immersed in an aqueous solution of electrolytes) repel the ions of similar charge and attract the ions of dissimilar charge to their surfaces. As a result, two layers of opposite polarity (separated by a distance of molecular order) form at the metal–solution interface. This model is equivalent to a parallel-plate capacitor, which can be represented by eq 2.15, where \( \sigma \) is the stored charge density, \( \epsilon \) is the dielectric constant (or relative permittivity) of the medium, \( \epsilon_0 \) is the permittivity of free space, \( d \) is the spacing between the two plates, and \( V \) is the voltage drop.

\[
\sigma = \frac{\epsilon \epsilon_0}{d} V \quad (2.15)
\]

This model predicts that the potential profile changes linearly with distance. Also, the differential capacitance per unit surface area (\( C_d \)) can be derived as
2.4. Electrochemistry

\[
\frac{\partial \sigma}{\partial V} = C_d = \frac{\varepsilon \varepsilon_0}{d}
\]  \hspace{1cm} (2.16)

This model suggests that $C_d$ is a constant; however, this result is not typically observed. Experimentally, the $C_d$ values depend on both electrode potential and electrolyte concentration. Therefore, the Helmholtz model was revised.

2.4.1.2 Gouy-Chapman Model

Louis Georges Gouy in 1910 and David Leonard Chapman in 1913 independently proposed modifications to the Helmholtz model. Because the conductivities of the two sides of the capacitor are distinctly different, the Gouy-Chapman model suggests that a thicker layer of charge on the solution side of this double layer is required. This layer is known as the diffuse layer of charge, and it extends several nanometers into the solution.

These ions in solution interact electrostatically with the excess surface charge on the surface of the metal. These ions also move in the solution due to thermal motion. In this case, the potential profile changes non-linearly with distance. Evidently, an average distance of charge separation (or characteristic length) needs to replace $d$ in eq 2.16. This characteristic length also varies with electrode potential and electrolyte concentration. For example, when the electrode becomes more highly charged (or when the electrolyte concentration rises), the diffuse layer becomes more compact and $C_d$ also rises.

This model works well at low concentrations and at potentials near the point of zero charge (PZC), where the charge at the metal in contact with the electrolyte equals zero (i.e., if the charge of the electrode surface is zero, then there is no accumulation of oppositely-charged ions on the solution side). However, it fails for extreme potentials and high electrolyte concentrations. Hence, the Gouy-Chapman model also was revised.

2.4.1.3 Stern Model

One of the deficiencies of the Gouy-Chapman model is that the ions are assumed to be point charges. However, this view is not realistic because an ion has a finite size and cannot approach the surface any closer than the ionic radius. In 1924, Otto Stern suggested combining the Helmholtz model with the Gouy-Chapman model. This new model proposes that some ions adhere to the electrode as suggested by Helmholtz (called the Stern or Helmholtz layer), while some ions form a Gouy-Chapman layer (called the diffuse layer).\textsuperscript{164}
2.4. Electrochemistry

Equivalently, this model represents two capacitors in series: one for the Helmholtz layer \( (C_H) \) and the other one for diffuse layer \( (C_D) \). Mathematically, the interfacial capacitance derived from the Gouy-Chapman-Stern model can be represented by eq 2.17, where \( C_{dl} \) is the capacitance of the double layer (i.e., total capacitance).

\[
\frac{1}{C_{dl}} = \frac{1}{C_H} + \frac{1}{C_D}
\]  

(2.17)

2.4.1.4 The Role of the Solvent at the Interface

The Helmholtz/Stern layer accounts for the ion’s finite size and its ionic radius, but this model still has some deficiencies and has been further refined (e.g., by David C. Grahame\textsuperscript{165} in 1947, and by Bockris/Devanathan/Muller\textsuperscript{166} in 1963). Some of the most important modifications are summarized here.

Depending on the type of solvent, different ions in solution are solvated differently. The interaction between an ion and the surface depends on its degree of solvation. For ions that are strongly hydrated in aqueous solutions, the interaction with the surface is mainly electrostatic. On the other hand, the ions that are not hydrated (or partially hydrated) can be in direct contact with the electrode surface. These species are called specifically adsorbed ions.

In addition, the solvent molecules, such as water, has a strong dipole and will be influenced by the charge on the metal surface. In particular, water would act as a first solvation shell for the metal electrode and would exist in a fixed orientation. At some sites, the water would be displaced by the specifically adsorbed ions. Figure 2.21 shows a schematic representation of the electrical double layer of a negatively charged electrode.

It is important to note that the solution side of the double layer can be divided into several layers. The inner layer is the closest to the electrode and it contains solvent molecules and sometimes specifically adsorbed species. This inner layer is also called the compact, Helmholtz, or Stern Layer. The inner Helmholtz plane (IHP) passes through the centres of the specifically adsorbed species at a distance of \( \chi_1 \). In contrast, the solvated ions can only approach the metal to a distance of \( \chi_2 \), and the outer Helmholtz plane (OHP) passes through the centres of these solvated ions. Since the solvated ions interact with the charged metal via only long-range electrostatic forces, they are said to be non-specifically adsorbed. These non-specifically adsorbed ions are distributed, due to thermal motion, in a region called the diffuse layer (i.e., the region between the OHP and the bulk of solution).
2.4. Adsorption on an Electrode Surface

From Figure 2.21, it is evident that the specifically adsorbed species need to displace water molecules in order to be in contact with the electrode surface. It is important to note that the sum of charges on the metal and solution side should be zero. The solvent-based adsorption of self-assembled monolayers on Au undergoes a similar displacement process. In the following sections, the theory of adsorption on an electrode surface and use of differential capacitance measurements to obtain surface coverage and free energies of adsorption will be introduced.

2.4.2.1 Electrocapillary Equation

The energies of the electrode–solution interface can be obtained by measuring its surface or interfacial tension. The surface tension of an electrode in contact with an electrolyte depends on the metal–solution potential difference, as shown by the electrocapillary equation (eq 2.18). The surface excess on
the solution side is denoted by $\sum_i \Gamma_i d\mu_i$ (see Section 2.3.1). The surface excess on the metal side is expressed by $\sigma^M dE$, where $\sigma^M$ is the excess surface charge density of the metal and $E$ is the rational electrical potential with a reference at the point of zero charge (PZC).

$$-d\gamma = \sigma^M dE + \sum_i \Gamma_i d\mu_i$$  \hspace{1cm} (2.18)

Several equations can be derived from eq 2.18. One important relationship that follows from the electrocapillary equation is:

$$\Gamma_i = -\left( \frac{\partial \gamma}{\partial \mu_i} \right)_{E,\mu_j \neq i,T,P}$$  \hspace{1cm} (2.19)

Eq 2.19 can be used to determine the surface excess (e.g., the extent of adsorption at the interphase) of any species, while keeping the potential and the chemical potential of all other species constant. This equation indicates that measurement of changes in the surface tension as a function of chemical potential can yield Gibbs surface excess. Recall that surface excess can also be related to surface coverage as shown in eq 2.5 (see Section 2.3.2). However, experimentally it is not trivial to measure surface/interfacial tension on solid electrodes, so this information must be obtained indirectly from other measurements. As a result, we need to consider other relationships.

For a solution with constant composition (and at constant temperature and pressure), the partial derivative of $\gamma$ with respect to potential $E$ gives the excess surface charge density of the metal:

$$\sigma^M = -\left( \frac{\partial \gamma}{\partial E} \right)_{\mu_i,T,P}$$  \hspace{1cm} (2.20)

Eq 2.20 reveals that surface tension is a maximum at the point of zero charge (PZC), which is also called the electrocapillary maximum. At potentials more positive and more negative from the PZC, the surface tension decreases in a parabolic dependence fashion.\(^\text{163}\)

Subsequently, by measuring the change in charge density resulting from a small change in potential, one can measure differential capacitance. One definition is shown in eq 2.21, where $C_d$ is the differential capacitance. In other words, the ability of the interface to store charge in response to a perturbation in potential can be characterized by capacitance.

$$C_d = \left( \frac{\partial \sigma^M}{\partial E} \right)_{\Gamma_i,\mu_i,T,P} = -\left( \frac{\partial^2 \gamma}{\partial E^2} \right)_{\mu_i,T,P}$$  \hspace{1cm} (2.21)

Eq 2.21 relates the surface tension, or excess surface Gibbs energy, to the charge density $\sigma^M$ and...
2.4. Electrochemistry

the differential capacitance $C_d$. These relationships are purely thermodynamic and are not based on a model. The only assumption made in these derivations is that the interface is ideally polarizable (i.e., charge cannot cross the interface). In the next section, the use of capacitance measurements to obtain an estimate of surface coverage will be discussed.

2.4.2.2 The Parallel-Plate Model of Frumkin

Let us re-consider the Gouy-Chapman-Stern model of the double layer, as represented by eq 2.17 in Section 2.4.1.3. Assuming that measurements are taken in a concentrated solution of supporting electrolyte, the contribution from the diffuse layer capacitance can be neglected. Therefore, the double layer capacitance can be approximated by the capacitance of the Helmholtz/Stern layer. It is important to note that in this inner layer, the electrode is always solvated, and that the solvent molecules (and some ions) are in direct contact with the electrode surface. Adsorption onto a site on this surface requires the displacement of these solvent molecules and ions. Therefore, electrosorption is a competitive process.

For a system that experiences specific adsorption, the inner Helmholtz layer can be modeled as two capacitors in parallel: (1) the fraction of the surface representing the electrode without any adsorbate (with a capacitance of $C_{\theta=0}$), and (2) the fraction of the surface representing the electrode completely covered by the adsorbate (with a capacitance of $C_{\theta=1}$). The total capacitance is the sum of these capacitors in parallel. Therefore, when the electrode is partially covered with the adsorbate, the total capacitance of the electrode can be described as a weighted average as shown in eq 2.22.

$$C_H = C_{\theta=0} (1 - \theta) + C_{\theta=1} \theta$$  \hspace{1cm} (2.22)

Solving for $\theta$, we get eq 2.23.

$$\theta = \left( \frac{C_H - C_{\theta=0}}{C_{\theta=1} - C_{\theta=0}} \right)$$  \hspace{1cm} (2.23)

Recall from Section 2.3.2 that the surface coverage $\theta$ is defined as the fractional coverage of the molecule (i.e., $\theta = \Gamma / \Gamma_{\text{max}}$). Evidently, we can calculate $\theta$ with measured $C_H$ when both $C_{\theta=0}$ and $C_{\theta=1}$ are known. Experimentally, $C_{\theta=0}$ is the capacitance of the electrode in the presence of solvent molecules and ions (i.e., without the adsorbate). $C_H$ is the capacitance of the electrode with some adsorbate. Finally, $C_{\theta=1}$ is the capacitance of the electrode completely covered by the adsorbate (i.e., at maximum coverage).

From eq 2.16 (see Section 2.4.1.1), we see that the capacitance measured depends on the dielectric
constant $\varepsilon$ (if we assume $d$ remains constant). As a result, the Frumkin parallel-plate model can also be expressed in terms of $\varepsilon$. If we assume for $C_{\theta=0}$, the dielectric constant measured is that of water ($\varepsilon_{\text{water}}$). For $C_{\theta=1}$, we assume that the dielectric constant measured is that of the adsorbate ($\varepsilon_{\text{ads}}$). When the electrode is partially covered with the adsorbate, the measured $C_H$ essentially measures the average dielectric constant ($\varepsilon_{avg}$), which gives:

$$\varepsilon_{avg} = \varepsilon_{\text{water}}(1 - \theta) + \varepsilon_{\text{ads}}\theta \quad (2.24)$$

Most importantly, changes in the electrical double layer due to the adsorption of molecules on the electrode surface can be measured by differential capacitance measurements (since the dielectric constant $\varepsilon$ also changes, assuming $d$ remains constant). Using eq 2.23, an estimate of the surface coverage can be obtained.

Subsequently, the concentration-dependent surface coverage can be fit to either the Langmuir (eq 2.12) or the Frumkin adsorption isotherm (eq 2.14) in order to obtain the free energy of adsorption at a specific electrode potential. This process can be repeated for different electrode potentials. As a result, potential-dependent free energies of adsorption ($\Delta G^*_{\text{ads}}$) can be determined.

### 2.4.2.3 Parabolic Dependence of $\Delta G^*_{\text{ads}}$

For the simple Frumkin parallel-plate model, the slope of the $\Delta G^*_{\text{ads}}$ curve with respect to $E$ is linear. As a result, the $\Delta G^*_{\text{ads}}$ is parabolically dependent on $E$. This conclusion can be arrived at using an electrical analogue of charging a capacitor. The energy required to charge a capacitor is $\int_0^E C(\theta) E dE = \frac{1}{2} C(\theta) E^2$. The difference in energy between charging a capacitor with and without an adsorbed monolayer is $\frac{1}{2}(C_{\theta=0} - C_{\theta=1})(E - E_{\text{PZC}})^2$, where the potential is referenced to the PZC (in cases where it can be determined experimentally).

### 2.4.2.4 Pseudocapacitance

In reality, the excess surface charge density of the metal $\sigma^M$ depends on both the electrode potential ($E$) and the surface excess of a particular species ($\Gamma$), which is related to the surface coverage $\theta$. Taking the differential of $\sigma^M$, which is a function of two variables, yields eq 2.25.

$$d\sigma^M = \left(\frac{\partial \sigma^M}{\partial E}\right)_\Gamma dE + \left(\frac{\partial \sigma^M}{\partial \Gamma}\right)_E d\Gamma \quad (2.25)$$

Dividing eq 2.25 through by $dE$ yields eq 2.26, where $C_{dl}$ is the measured differential capacitance.
\[
\frac{d\sigma^M}{dE} = C_{dl} = \left( \frac{\partial \sigma^M}{\partial \Gamma} \right)_\Gamma + \left( \frac{\partial \sigma^M}{\partial \Gamma} \right)_E \frac{d\Gamma}{dE}
\] (2.26)

The first term of this equation (i.e., \( \left( \frac{\partial \sigma^M}{\partial \Gamma} \right)_\Gamma \)) is the capacitance of the interface from the double layer under conditions of constant coverage, as derived in eq 2.21. The second term (i.e., \( \left( \frac{\partial \sigma^M}{\partial \Gamma} \right)_E \frac{d\Gamma}{dE} \)) is the so-called pseudocapacitance.

This pseudocapacitance term is negligible when \( \frac{d\Gamma}{dE} \) is small, or at low coverages, which occurs at low adsorbate concentrations. However, this pseudocapacitance term becomes significant as the concentration increases (both \( \Gamma \) and \( \frac{d\Gamma}{dE} \) increases). For example, at more negative electrode potentials, water will adsorb more strongly onto the metal surface. As a result, water molecules will displace the adsorbate on the surface. This change in coverage will give rise to pseudocapacitance features, representing a peak in the capacitance-potential measurement due to the additional component \( \left( \frac{\partial \sigma^M}{\partial \Gamma} \right)_E \frac{d\Gamma}{dE} \). A similar trend is also observable at more positive potentials for stable systems.

The pseudocapacitance term represents the change in capacitance due to the amount of species adsorbed as a function of potential (\( \frac{d\Gamma}{dE} \)) multiplied by the electrosorption valency \( \left( \frac{\partial \sigma^M}{\partial \Gamma} \right)_E \). In this thesis, electrosorption is defined as specific adsorption because this process typically involves direct contact between an adsorbate and the electrode surface, which occurs when species penetrate the inner Helmholtz or Stern layer (see Figure 2.21). Specific adsorption of molecules or ions causes a change in the dielectric constant \( \epsilon \) of the inner layer, since the adsorbed water molecules are replaced by ions or molecules that may have a different dielectric constant (see eq 2.24). The electrosorption valency term shows that any change in \( \Gamma \) (and therefore a change in \( \epsilon \) of the inner layer) will effectively change the charge density of the metal, which results in a pseudocapacitance feature.

It is well known that chloride and hydroxide ions adsorb onto Au surfaces at certain potentials and will contribute to pseudocapacitance features. In addition to desorption/adsorption processes, pseudocapacitance peaks can also be caused by phase transitions of the adsorbed layer. Nevertheless, it is important to note that the Frumkin parallel-plate model does not account for the effects of pseudocapacitance (i.e., \( \left( \frac{\partial \sigma^M}{\partial \Gamma} \right)_E \) and \( \frac{d\Gamma}{dE} \)), which may limit the potentials that can be analyzed.

2.4.2.5 Electrodesorption

An adsorbed layer on the electrode can only exist on the surface in a limited potential range (i.e., the \( E \) region where \( d\gamma \) is a minimum), and the capacitance will be small due to the adsorbed layer (e.g., the adsorbed layer has a lower dielectric constant than the water molecules). At potentials outside of this
window, solvent molecules with a high dielectric constant (e.g., water) will displace the adsorbate (i.e., \( \frac{dt}{dE} \neq 0 \)), giving pseudocapacitance peaks at potentials near this boundary. In this region, some areas of the electrode are still covered with adsorbate molecules, and some areas of the electrode contain only the electrolyte (i.e., solvent-covered electrode). At extreme potentials, the layer may be completely displaced by solvent molecules, rendering a completely solvent-covered electrode. This phenomenon is called electrodesorption.

### 2.5 Integration of Microfluidics & Electrochemistry

In addition to studying non-faradaic processes, electrochemistry can also be applied to study heterogeneous electron transfer kinetics (typically at the ionic liquid/metal interface).\(^ {173} \) Electrochemistry has been applied in many fields, including corrosion science,\(^ {175} \) metallurgy,\(^ {176} \) fuel cells,\(^ {177} \) semiconductors,\(^ {178} \) self-assembly of coatings,\(^ {179} \) and electrochemical sensors.\(^ {180} \) In particular, there is a growing interest in combining microfluidics with electrochemical sensing due to two critical advantages: (1) suitability for miniaturization, and (2) inexpensive experimental setup.\(^ {108} \) For example, electrochemical systems often use inexpensive potentiostats to apply electric potentials for measuring currents. Furthermore, the advances in screen printing (i.e., to increase the manufacturing throughput and decrease the cost of electrodes)\(^ {181},182 \) have aided the development of point-of-care glucose\(^ {183} \) and alcohol\(^ {184} \) sensors.

An electrochemical cell typically consists of three electrodes: (1) a working electrode (WE), (2) a counter electrode (CE), and (3) a reference electrode (RE). In most cases, the WE and CE are directly immersed in the working solution and the RE is indirectly connected by a conductive salt bridge. For sensing applications, the electrode size, geometry, material, and surface structure are important factors to be considered.\(^ {108},185 \)

Most commonly, Au electrodes are patterned on a flat glass substrate for creating microfluidic electrochemical systems. Subsequently, a polymer-based structure (i.e., PDMS) with a microfluidic cell chamber is placed in contact with the hybrid glass/gold substrate, forming an enclosed cell chamber (see Figure 1.2 in Section 1.1).\(^ {186} \) The design of microfluidic electrochemical systems can have the REs connected via a salt bridge on chip,\(^ {187} \) or have the REs and CEs connected externally.\(^ {188} \)

A major problem associated with this type of design is the poor adhesion between gold and PDMS. It is simple to create a strong bond between glass and PDMS as described in Section 2.1.2. However, without treating the gold electrode, the electrolyte may leak at the PDMS–Au interface.\(^ {57} \) This leakage
issue will have numerous adverse effects on the electrochemical measurements.

2.5.1 Problems with Electrolyte Leaks

Some of the problems associated with electrolyte leaks at the PDMS–Au interface may include:

1. Electrolyte leaks will change the electrode surface area under measurement, which will in turn affect the current measured. For example, when the surface area of the electrode remains unchanged, the increased activity (or concentration) of an electroactive species will result in an increase of the current measured. However, an increase in the electrode surface area will also cause an increase in the current measured. Therefore, it would be difficult to determine the real cause of increased current when both of these factors are present during an electrochemical measurement.

2. Impedance is a standard measurement method used in electrochemical biosensors. This method relies on the modeling of an electrochemical cell with equivalent circuit elements like resistors or capacitors. For a system with a leak of electrolyte, the surface area increases (increasing the capacitance). More importantly, the small crack introduces another resistance that results in a variety of potentials on the electrode surface during current flow due to voltage drops.

3. Electrolyte leaks may also result in cross-contamination issues. For instance, solutions with highly different pH values sometimes need to be sequentially introduced and studied. However, any residual solution that remain in the unbonded areas at the PDMS–Au interface (e.g., cracks) may significantly change the pH of the working solution in the PDMS cell chamber. As a result, the electrochemical measurements may be adversely affected.

2.6 Summary

In this Chapter, the relevant literature and background theory related to biosensors, surface science, microfluidics and electrochemistry have been reviewed. Next, various surface analysis techniques will be applied to obtain physicochemical information about TMS-EDTA adsorption on Au. Electrochemical differential capacitance measurements will be used to determine the free energies of adsorption under a complex aqueous environment. Finally, the knowledge acquired will be applied to construct leak-free PDMS-based electrochemical cells.
Chapter 3

Surface Analysis of TMS-EDTA Adsorption on Gold

3.1 Synopsis

This chapter reports on the characterization of TMS-EDTA adsorption on Au via the use of four surface analysis techniques: (1) water contact angle to quantify the wettability of chemically modified Au, (2) X-ray photoelectron spectroscopy (XPS) to confirm the carboxylic acid attachment, (3) atomic force microscopy (AFM) to reveal the surface coverage, uniformity, roughness, and thickness, and (4) infrared (IR) spectroscopy to elucidate the chemical structure of surface-adsorbed species and the extent (i.e., presence or absence) of siloxane cross-linking. Related species (i.e., 11-MUA, EDTA, and 3-APTMS) were also analyzed to help with the data analysis. Finally, TMS-EDTA-modified Au was applied to develop a biosensor surface. Surface plasmon resonance (SPR) was used to test the amount and stability of immobilized streptavidin on TMS-EDTA-modified Au following carbodiimide activation.

3.2 Surface Analysis

3.2.1 Experimental Section

3.2.1.1 General Materials and Gold Surface Preparation

Detailed lists of the specific materials and reagents used are given below in each of the corresponding experimental sections. Here, the common materials and surface preparatory procedures are described. The chemical names and structures of the molecules used in this chapter are presented in Figure 3.1. All chemicals and reagents were obtained from commercial sources: N-[(3-trimethoxysilyl)propyl]ethylene-diamine triacetic acid, Na salt (TMS-EDTA; 50% in water) was from United Chemical Technologies (Bristol, PA); 11-mercaptoundecanoic acid (11-MUA; 95%), (3-aminopropyl)-trimethoxysilane (3-APTMS;
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Figure 3.1: Chemical structures of the molecules used in this chapter: TMS-EDTA, 11-MUA, EDTA, and 3-APTMS.

97%), ethylenediaminetetraacetic acid (EDTA; BioUltra, ≥99.0%), acetone (ACS reagent, ≥99.5%) and 2-propanol (ACS reagent, ≥99.5%) were from Sigma-Aldrich (Oakville, ON, Canada). Ethyl alcohol (de-natured) was purchased from UBC Zoology Stores (Vancouver, BC). Ultrapure water (~18.2 MΩ-cm) produced by a Milli-Q water purification system (EMD Millipore) was used.

The planar Au slides (see additional details in the corresponding experimental sections below) were cleaned by immersion in acetone, 2-propanol, and ultrapure water (repeated three times), followed by blow drying in an argon or nitrogen stream (Ultra High Purity 5.0, Praxair Canada Inc.). Subsequently, these planar Au surfaces were immediately immersed into different reagent solutions (specified below) for surface chemical modifications.

3.2.1.2 Water Contact Angle and X-ray Photoelectron Spectroscopy (XPS)

For these measurements, Au substrates were manufactured in-house. The glass slides were cleaned in a piranha solution (5:1 H₂SO₄:H₂O₂) at 80 °C for 15 min, rinsed with DI water, and dried in a N₂ stream. These slides were subsequently placed into a 200 °C oven for 15 min to eliminate residual moisture. A 20 nm chromium adhesion layer and a 200 nm gold layer were sequentially evaporated onto the
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clean glass slides in an electron-beam evaporator. After cleaning with organic solvents as described above, three Au slides were separately immersed for 2 hours in (1) aqueous solution of 10% TMS-EDTA (v/v), (2) ethanolic solution of 10 mM 11-MUA, and (3) ultrapure water (serving as reference surface). The surface-modified, gold-coated glass substrates were then rinsed with the respective solvent, dried under a N\textsubscript{2} stream, and analyzed immediately. The contact angle analysis was performed using the Low-Bond Axisymmetric Drop Shape Analysis (LB-ADSA) Plugin for ImageJ.\textsuperscript{189} The peaks in the XPS spectra were individually fitted assuming a Gaussian distribution.

3.2.1.3 Atomic Force Microscopy (AFM)

For AFM studies, cleaned planar Au slides (TA134, 5 nm Ti and 100 nm Au) from Evaporated Metal Films (Ithaca, NY) were used. An Agilent 5500 AFM equipped with a 90 μm scanner and SiN Nanoprobe tips (Digital Instruments, spring constant of 0.8 N/m nominal) were used for these measurements.

**AFM Contact Mode Imaging** For contact mode imaging, two types of surfaces were created. The first type was prepared by immersing the Au slide in an aqueous solution of 10% TMS-EDTA (v/v), and the second type was prepared by immersing the Au slide in ultrapure water (serving as Au substrate). After 2 hours of immersion, the surfaces were rinsed with ultrapure water, blown dry with argon gas, and analyzed immediately. A 512×512 pixel grid was measured over an area of 3×3 μm\textsuperscript{2} at 1 Hz per line with integral and proportional gains of 0.5 and 1, respectively (with a setpoint in the attractive regime). Topography and lateral force measurements were obtained for both TMS-EDTA-coated Au and clean Au substrate. Data processing was performed in Gwyddion 2.3.4 (Czech Metrology Institute) with median height matching and second-order polynomial baseline subtraction.

**AFM Force-Distance Spectroscopy** For force-distance spectroscopy, a third type of surface was prepared (in addition to the two surfaces used for contact-mode imaging) by immersing a cleaned Au slide in an ethanolic solution of 10 mM 11-MUA for 2 hours. For the three types of freshly prepared surfaces, approach and retraction force curves were obtained, and sampling was conducted at 64 points distributed in an 8×8 grid over the same scan size as contact mode imaging. The tip approach speed was 117 nm/s to allow for adequately high resolution sampling at 12.5 kHz. Data processing for the force curve measurements was conducted in MATLAB. Briefly, the 64 approach-only force curves were extracted, and the first derivative of the tip deflection (V) to distance (nm) was obtained. Subsequently, the maximum of these values was determined and the corresponding distance value was defined as the
3.2. Surface Analysis

interface of the gold (i.e., distance = 0 nm). The force-distance curves from these three samples were compared.

3.2.1.4 Infrared Spectroscopy

PM-IRRAS For polarization modulation-infrared reflection-absorption spectroscopy (PM-IRRAS) studies, planar Au slides (TA134, 5 nm Ti and 100 nm Au) from Evaporated Metal Films (Ithaca, NY) were cleaned using the protocol described above. Three types of Au substrates were created by overnight immersion in (1) aqueous solution of 10% TMS-EDTA (v/v), (2) aqueous solution of 10% 3-APTMS (v/v), and (3) ethanolic solution of 10 mM 11-MUA. The Au slides were blown dry with argon gas, and stored in a desiccator to remove excess moisture for at least two days prior to the experiments.

PM-IRRAS was performed using a Bruker-55 spectrometer with an external PMA 50 accessory. The IR beam, after passing through a ZnSe grid polarizer and a ZnSe photoelastic modulator (HINDS Instruments, PEM-90, modulation frequency of 50 kHz), was focused on the sample at an incident angle of 80° - 85°. The light reflected from the sample was then focused onto an MCT detector (model D313/x1, Infrared Associates Inc. Stuart, Fl, USA.). The PM-IRRAS signal is given by the differential reflectivity

\[ \Delta R = \frac{(R_p - R_s)}{(R_p + R_s)} \]

and the presented spectra resulted from the sum of 2048 scans (from at least two co-added spectra) recorded with 4 cm⁻¹ resolution. The final step of data processing involved baseline correction using spline interpolation of the experimental data points in MATLAB.

ATR-FTIR Three chemical samples (3-APTMS, EDTA, and TMS-EDTA) were analyzed by attenuated total reflectance Fourier transform infrared (ATR-FTIR). The spectra of the samples were recorded using a Perkin-Elmer Frontier Spectrometer (4 scans and 4 cm⁻¹ resolution, model equipped with a Universal ATR Sampling Accessory). The 3-APTMS and TMS-EDTA samples were separately prepared by pipetting ~1 mL of bulk solution onto glass slides containing KBr powder. The third sample was prepared by mixing solid EDTA with KBr powder on a new glass slide. The three powdered mixtures were sealed inside a desiccator with desiccants for at least two days before analysis in order to remove excessive water.
3.2. Results and Discussion

3.2.2 Water Contact Angle and X-ray Photoelectron Spectroscopy (XPS)

Water contact angle and X-ray photoelectron spectroscopy (XPS) were used to examine the feasibility of using TMS-EDTA to chemically modify Au substrates for creating a carboxyl-terminated Au surface. This surface was compared with 11-MUA-modified Au. 11-MUA is an extensively studied alkanethiol that has a well-defined orientation on Au (i.e., with terminating carboxylic acid groups).\(^{96}\)

Water contact angle measurements using the sessile drop method were performed on the surfaces of bare gold (Figure 3.2A), 11-MUA-modified gold (Figure 3.2B) and TMS-EDTA-modified gold (Figure 3.2C). For proof-of-concept, a single measurement was performed on each sample. The Au surfaces modified with TMS-EDTA and 11-MUA had water contact angle values that were more hydrophilic than the clean Au, confirming chemical modification (see Section 2.2.1).

XPS was used to further characterize the chemically modified Au surfaces. The results are shown in Figure 3.3. Figure 3.3A reveals that the gold surface modified with 11-MUA shows an increase in the quantity of carbon (C) in comparison to the bare gold control surface. The presence of some C atoms on the gold control surface may be from the organic solvents used or from the organics in air. The high resolution spectrum shows a C 1s peak at 284.7 eV with a shoulder peak at 288 eV, corresponding to the terminating carboxyl groups of 11-MUA (see Section 2.2.5.2).\(^{190,191}\) Similarly, Figure 3.3B shows an increase in the quantity of C for TMS-EDTA-modified gold substrate and a shoulder that corresponds to carboxyl groups (as compared to the bare gold-modified film). These results suggest that the carboxyl groups are still present on the TMS-EDTA-modified Au surface.

3.2.2.2 Atomic Force Microscopy (AFM)

Water contact angle and XPS results suggest that TMS-EDTA can be used to chemically modify Au and create terminating carboxyl groups. Characterization of the surface topography is needed to better understand the surface coverage and uniformity of TMS-EDTA adsorption on gold. Spatial characteristics such as film uniformity and film thickness are particularly important for biosensing and biosensor fabrication. For example, the uncontrolled deposition of silanes onto silicon and glass surfaces can result in films with a loose network structure, and the final morphology and thickness (typically 1 to 8 molecular layers) are sensitive to water content, pH, and temperature.\(^{60,192,193}\) To explore the thickness and uniformity of adsorbed TMS-EDTA layer(s) on Au, atomic force microscopy (AFM) was used (see Section 2.2.2).
3.2. Surface Analysis

Figure 3.2: Water contact angle measurements on (A) bare gold, (B) 11-MUA-modified gold, and (C) TMS-EDTA-modified gold.
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Figure 3.3: XPS high resolution spectra of the C 1s signal for (A) 11-MUA and (B) TMS-EDTA on gold (with spectrum for bare gold surface).
3.2. Surface Analysis

AFM Contact Mode Imaging

Topography (Figure 3.4A) and lateral force or friction (Figure 3.4B) measurements obtained from AFM contact mode imaging of a clean Au substrate and a TMS-EDTA-modified Au are shown. Histograms calculated from these topography and friction measurements were also determined (see Figures 3.5A and 3.5B). From Figures 3.4A and 3.5A, it is evident that the topography of the Au surface appears smoother when coated with TMS-EDTA. Similarly, from Figures 3.4B and 3.5B, the Au surface exhibits a higher lateral friction when coated with TMS-EDTA.

Subsequently, the same tip was used to re-scan the clean Au substrate (after scanning the TMS-EDTA-modified Au) to ensure comparability of the results. It is interesting to note that the friction of the bare Au substrate (after TMS-EDTA sample scan) has increased slightly (as compared with the first bare Au substrate scan). This result indicates that the silicon nitride tip may have been contaminated with adsorbed TMS-EDTA molecules. Nevertheless, it is evident from Figures 3.4 and 3.5 (as well as scans from multiple areas on the surface) that TMS-EDTA coats Au uniformly as measured over a large surface area (scan size of 3×3 μm²). Moreover, TMS-EDTA does not form large clumps on the Au surface (i.e., no patchy areas were observed) as commonly observed on silicon-based surfaces.194

AFM Force-Distance Curve

Next, AFM force-distance curve was used to provide a rough estimate of the relative thickness of the TMS-EDTA-coated surface, as compared to a control sample of 11-MUA-coated Au. In these measurements, the cantilever and the tip were moved directly toward the
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Figure 3.5: Topography (A), and lateral friction (B) distributions calculated from AFM contact mode images (scanned using the same tip for all images): cleaned gold substrate before sample scan (red), TMS-EDTA modified gold substrate scan (black), and cleaned gold substrate after sample scan (blue).
3.2. Surface Analysis

sample until a contact is made, and then retracted. The interaction between the tip and the sample was continuously monitored (see Section 2.2.2.2). To illustrate the procedure used for data analysis, the 64 approach-only AFM force curves (cantilever deflection vs piezo movement) for TMS-EDTA-modified Au substrate are shown in Figure 3.6A. First derivatives of the force curves are shown in Figure 3.6B.

Subsequently, the maximum of these derivatives was determined and the corresponding distance value was defined as the interface of the gold (i.e., distance = 0 nm) as shown in Figure 3.7A. The red curve shows the averaged values for the 64 samples. Figure 3.7B shows a zoomed-in view of the averaged and normalized values, with the blue line serving as the reference line. The same procedure was applied to analyze the force-distance curves of 11-MUA-modified Au and bare Au substrate.

Figure 3.8 shows the zoomed-in view of the interactions of silicon nitride tips with TMS-EDTA-coated gold (red), 11-MUA-coated gold (blue), and cleaned gold substrate (black). It is observed that the curve for 11-MUA-coated Au remained constant at a distance from 3 nm to ~1.6 nm, and started to increase from ~1.6 nm until the interface of the gold was reached (distance = 0 nm). This result confirmed that the 11-MUA on Au surface was a monolayer\(^{195}\) and validated this approach for determining the thickness of adsorbed layer of TMS-EDTA. From Figure 3.8, the force curves for the two chemically modified surfaces are indistinguishable (but distinctly different from the clean Au surface), suggesting that the thickness of TMS-EDTA on Au is similar to the 11-MUA monolayer.

The AFM results reveal that TMS-EDTA uniformly coats the planar Au surface and has a thickness comparable to a 11-MUA monolayer on Au, suggesting that TMS-EDTA adsorption is not multilayer in nature. Next, infrared spectroscopy was utilized to better understand the chemical nature of TMS-EDTA adsorption on Au.

3.2.2.3 Infrared Spectroscopy

Water contact angle and XPS results provided an initial analysis of the chemical nature of TMS-EDTA adsorption on Au, and suggested the presence of terminating carboxyl groups. However, the stability of the adsorbed TMS-EDTA layer in ultra-high vacuum (UHV), as required by the XPS measurements, might not be sufficient for quantitative analysis because of exposure to X-rays or low pressure. It is important to note that the solution-based chemical modification with TMS-EDTA should always be performed at room temperature, under atmospheric pressure. Furthermore, the TMS-EDTA-coated gold surface would not be exposed to UHV in future biosensor fabrication and applications. Therefore, TMS-EDTA adsorption on Au needs to be analyzed under ambient conditions in order to obtain a more accurate picture of the chemical structure of the adsorbed layer.
Figure 3.6: (A) An example of the 64 approach-only AFM force-distance curves for TMS-EDTA-modified Au. (B) First derivatives of the force-distance curves.
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Figure 3.7: (A) The maximum of the derivatives was defined as the surface of Au (i.e., distance = 0 nm). The red curve shows the averaged values for the 64 samples. (B) A zoomed-in view of the averaged and normalized values, with the blue line serving as the reference line.
3.2. Surface Analysis

**PM-IRRAS** Polarization modulation-infrared reflection-absorption spectroscopy (PM-IRRAS) was used to produce a spatially averaged picture of the chemical structure of the adsorbed layer on Au, under ambient conditions. All PM-IRRAS spectra presented have been baseline-corrected in MATLAB. An example of the baseline correction method applied for TMS-EDTA spectrum is shown in Figure 3.9. In Figure 3.9A, the raw PM-IRRAS signal (solid line) is plotted with the baseline curve determined from spline fitting (dotted line). The asterisks were the manually selected points for creating the baseline curve. In Figure 3.9B, the baseline-subtracted signal is shown. Subsequently, this signal was corrected for sample gain(s) to obtain the absorbance spectrum.

The amount of TMS-EDTA adsorbed and the chemical structure of the adsorbed layer (e.g., surface reactions that might change the chemical nature of the layer) could be inferred by comparison with other self-assembled monolayer (SAM) systems. In particular, 11-MUA SAMs have been studied using PM-IRRAS. This information supports the ability of PM-IRRAS to identify carboxyls and alkyl groups on the Au surface. In addition, the 3-APTMS-modified Au was also analyzed to provide some information regarding siloxane cross-linking on Au. Relative strengths of the absorbance will also provide a rough estimate of the extent of TMS-EDTA adsorbed on the surface, keeping in mind that the PM-IRRAS signal strength depends on the molecular orientation (see Section 2.2.3). The chemical structures of 3-APTMS, 11-MUA, and TMS-EDTA are shown in Figure 3.1. The PM-IRRAS spectra for Au modified with these substances are shown in Figure 3.10 for the alkyl stretching region (3200–2700 cm$^{-1}$).
Figure 3.9: An example of background correction method used for PM-IRRAS data analysis. (A) The raw signal of TMS-EDTA-modified Au (solid line) is shown with background curve determined from spline fitting (dotted line). (B) The background-subtracted signal is shown. Subsequently, this signal was corrected for sample gain(s) to obtain Absorbance spectrum.
3.2. Surface Analysis

Figure 3.10: The PM-IRRAS spectra for 3-APTMS on Au (black), 11-MUA on Au (red), and TMS-EDTA on Au (blue). Alkyl stretching region (3200–2700 cm\(^{-1}\)) and the fingerprint region (1800–800 cm\(^{-1}\)) are shown.

ATR-FTIR  Attenuated total reflectance Fourier transform infrared (ATR-FTIR) was used to provide supporting information regarding the chemical structure and the corresponding infrared (IR) bands of the bulk chemical compounds (see Section 2.2.3.1). In the literature, solid 11-MUA has been previously analyzed by ATR-FTIR and these results are used without confirmation in this study.\(^{199-201}\) The chemicals EDTA and 3-APTMS (see Figure 3.1) were analyzed to assist in identifying the IR bands of carboxylates and silanes, respectively. The ATR-FTIR spectra of 3-APTMS, EDTA, and TMS-EDTA were measured and presented in Figure 3.11. Alkyl stretching region (3200–2700 cm\(^{-1}\)) and the fingerprint region (1800–800 cm\(^{-1}\)) are shown.

PM-IRRAS and ATR-FTIR Analysis  First, TMS-EDTA and 11-MUA spectra will be compared and contrasted, which will then be followed by a similar analysis of TMS-EDTA and 3-APTMS spectra. Considering the 11-MUA spectra in Figure 3.10, this SAM shows an absorption that is characteristic of CH\(_2\) symmetric and asymmetric stretching bands (2850 and 2928 cm\(^{-1}\), respectively) as expected due to its long hydrocarbon chain.\(^{198}\) The absorption band around 1700 cm\(^{-1}\) is from the C=O stretch for 11-MUA on Au, but may also include adsorbed water, which may still be present on the surface even after extensive drying in the desiccator. The small peak at 1470 cm\(^{-1}\) was assigned to the CH\(_2\) deformation.\(^{198}\) Considering the TMS-EDTA spectra in Figure 3.10, the CH\(_2\) symmetric and asymmetric stretching bands (also evident from ATR-FTIR spectra as shown in Figure 3.11A) are also present as
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Figure 3.11: Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) transmission spectra of bulk TMS-EDTA (blue), EDTA (green), and 3-APTMS (red) mixed with KBr powder. (A) Alkyl stretching region (3200–2700 cm$^{-1}$) and (B) the fingerprint region (1800–800 cm$^{-1}$) are shown.
3.2. Surface Analysis

expected (2850 and 2928 cm\(^{-1}\), respectively), but a smaller absorbance than 11-MUA is observed. Signatures of CH\(_3\) asymmetric stretching mode\(^{198}\) are also evident as a shoulder at 2960 cm\(^{-1}\), supporting the conclusion that the methyl groups may still be present in the adsorbed layer (a small 2960 cm\(^{-1}\) shoulder is also visible in the 11-MUA spectrum, possibly due to the solvent used). The presence of the absorption at 1680 cm\(^{-1}\) is evidence that the carboxyl groups are still present in the adsorbed TMS-EDTA. These carboxyl groups are also present in the ATR-FTIR spectra of EDTA and TMS-EDTA, and are absent in the 3-APTMS spectra (as shown in Figure 3.11B). Furthermore, the 11-MUA SAM is known to form a thin monolayer on Au surface. The signal intensities of TMS-EDTA and 11-MUA spectra in Figure 3.10 are comparable (i.e., both have been multiplied by a factor of 4). This result suggests that the thickness of the TMS-EDTA layer is similar to that of the 11-MUA monolayer.

The PM-IRRAS spectra of 3-APTMS (Figure 3.10) have distinct features in the 1000–1200 cm\(^{-1}\) region (also evident from ATR-FTIR spectra as shown in Figure 3.11B), which are characteristic of Si–O–Si asymmetric stretching as well as Si–O–C bands\(^{196}\) due to polysiloxane formation (see Section 2.1.1.2). The extent of siloxane cross-linking for 3-APTMS is quite substantial, as shown by the large peak at 1130 cm\(^{-1}\) and the lack of CH\(_3\) features in the alkyl region (see Figure 3.10).\(^{152}\) This is evidence of hydrolysis of the methoxy groups and siloxane cross-linking (creating a significant amount of Si–O–Si absorption). These results are consistent with the previous observation that water plays an important role in the surface attachment of amino-silanes. More specifically, previous results suggest that in the presence of water the final multilayer film of amino-silanes on Au is composed of a cross-linked, two-dimensional siloxane network (due to complete hydrolysis).\(^{152}\) On the other hand, the presence of some polysiloxane formation in TMS-EDTA (also evident from Figure 3.11B) is not too surprising due to high pH values (see Appendix A), but the extent is significantly less than the 3-APTMS-coated Au sample (see Section 2.1.1.2). Furthermore, the intensities of the bands of 3-APTMS spectra are more than 5X that of TMS-EDTA. These results confirm the multilayer formation of 3-APTMS on Au,\(^{152,192}\) and suggest that the amines are adsorbed onto the Au surface and the silane groups are directed away, resulting in extensive cross-linking.\(^{202,203}\)

In these IR studies, the TMS-EDTA-coated gold surface shows a significant presence of carboxyl groups (similar to 11-MUA coated Au) and a lack of polysiloxane formation (in contrast to 3-APTMS coated Au). However, the orientation of adsorbed TMS-EDTA on Au remains unclear. Subsequently, surface plasmon resonance (SPR) was used to determine the chemical functionality of TMS-EDTA-modified Au, and thereby provide at least a rough indication of orientation.
3.3 Surface Plasmon Resonance (SPR)

The adsorption of TMS-EDTA on Au has been characterized via the use of four surface analysis techniques. Water contact angle, XPS, and IR results show that the carboxyl groups are present on TMS-EDTA-modified Au, and AFM results show that this surface is uniform. However, the orientation of adsorbed TMS-EDTA on Au still remains unclear. The orientation of the carboxyl groups is important for sensor fabrication and biosensor applications. In particular, if the carboxyl groups are oriented away from the gold surface, then they can potentially be used to covalently couple substances with primary amines, following carboxiimide activation. Therefore, SPR experiments (see Section 2.1.3) were conducted to demonstrate the feasibility of using TMS-EDTA-modified Au for coupling biomolecules and to determine its relative stability (compared to bare Au) when a stringent wash buffer was used. The quantity of biomolecules captured and the stability of this layer are important factors to be considered when developing a robust optical biosensor. Streptavidin was used as a model protein because it’s a common component of chemically selective films for biosensing applications.55

3.3.1 Experimental Section

For SPR studies, two planar Au sensor chips (SIA Kit Au) from GE Healthcare (Mississauga, ON, Canada) were cleaned as previously described. The Au chip was either immersed in (1) aqueous solution of 10% TMS-EDTA (v/v), or (2) ultrapure water (serving as a reference surface for physical immobilization). After 2 hours of immersion, the surfaces were rinsed with water and blown dry with argon gas and then glued onto the cassettes according to the manufacturer’s instructions. All SPR experiments were performed at 25 °C in an SPR biosensor (BIACORE 3000 apparatus) using these in-house assembled sensor chips. 1X PBS, pH 7.4, was prepared using Dulbecco’s phosphate-buffered saline (powder, no calcium, no magnesium) from Life Technologies (Burlington, ON, Canada) and used as the running buffer. N-(3-(Dimethylamino)propyl)-N’-ethylcarbodiimide hydrochloride (EDC; 98%) and N-hydroxyoxysuccinimide (NHS; 98%) were from Sigma-Aldrich (Oakville, ON, Canada). Streptavidin purified (S203) from Leinco Technologies, Inc. (St. Louis, MO) was used as the model protein. For the Au chip immersed in pure water, streptavidin (100 μg/mL, diluted in PBS) was injected over the surface for 10 min to facilitate physisorption. For the TMS-EDTA-modified Au chip, carboxyl groups were first activated with an aqueous mixture of 50 mM NHS and 200 mM EDC for 7 min, and streptavidin was injected over the surface for 10 min (see Section 2.1.3.1) to facilitate chemisorption. The stability of both streptavidin-covered surfaces was investigated by introducing five short injections (3 min each) of
3.4 Conclusions

The adsorption of a carboxylated silane (TMS-EDTA) on Au has been characterized using water contact angle, XPS, AFM, and IR methods. Water contact angle and XPS results provide an initial analysis of the chemical nature of TMS-EDTA adsorption on Au, and suggest the presence of carboxyl groups. AFM imaging strongly suggests that a thin and uniform coverage of TMS-EDTA on the gold surface is obtained. In IR studies, the TMS-EDTA-coated gold surface shows a significant presence of carboxyl groups (similar to 11-MUA-coated Au) and a lack of polysiloxane formation (in contrast to 3-APTMS-coated Au). Finally, the results from SPR experiments indicate that free carboxyl groups on TMS-EDTA-modified Au are available for the immobilization of streptavidin, after activation by NHS/EDC. Furthermore, this unconventional surface chemistry can withstand stringent regeneration conditions—a quality important for

3.3.2 Results and Discussion

Figure 3.12A shows the SPR sensorgram that indicates the immobilization of streptavidin on a bare Au chip, followed by five injections of 50 mM NaOH regeneration buffer. Figure 3.12B shows the immobilization of streptavidin on a TMS-EDTA-modified Au chip (using NHS/EDC coupling chemistry), followed by five injections of the same regeneration buffer.

As can be seen from Figure 3.12A, streptavidin immobilized on the bare gold surface resulted in a peak value of about 690 RU (Resonance Unit or Response Unit), and streptavidin washed off of the Au surface after NaOH injection resulted in a decrease of about 630 RU. Therefore, only about 9.1% of the signal for adsorbed streptavidin remained after five NaOH injections. In contrast, streptavidin immobilized on a TMS-EDTA-modified gold surface (Figure 3.12B) resulted in a peak value of about 1610 RU, and streptavidin washed off after NaOH injections resulted in a decrease of about 600 RU. Using a similar calculation, about 62.7% of adsorbed streptavidin remained on the TMS-EDTA-modified Au after the stringent NaOH wash. Thus, the TMS-EDTA-modified gold surface yielded both a substantial increase in the amount of streptavidin immobilized and a substantial decrease in the amount of streptavidin removed after NaOH injections. The results reported here suggest that TMS-EDTA-modified Au consists of free carboxyl groups that are able to react with primary amino groups. Previous publications demonstrating silanes interacting with gold surfaces have been reported, supporting this proposed orientation.149,204

3.4 Conclusions

a stringent regeneration buffer (50 mM NaOH). The NaOH regeneration buffer (NaOH 50) was from GE Healthcare. All buffer and sample injections were conducted at a flow rate of 10 μL/min.
3.4. Conclusions

Figure 3.12: (A) SPR sensorgram showing the immobilization of streptavidin on a bare Au chip, followed by five injections of the 50 mM NaOH regeneration buffer. (B) Streptavidin immobilization on TMS-EDTA-modified Au chip using NHS/EDC chemistry, followed by five injections of the same regeneration buffer.
3.4. Conclusions

developing robust biosensors. These results suggest that at least some of the carboxyl groups are oriented away from the surface. This orientation serves to facilitate the coupling reactions that are needed for the immobilization of biomolecules via NHS/EDC chemistry. The data presented so far show that TMS-EDTA adsorption on Au can be used to create a useful biosensing surface, but an estimate of its Gibbs free energy of adsorption onto Au is needed. Therefore, electrochemical differential capacitance was used to obtain this information as presented in the following chapter.
Chapter 4

Thermodynamic Studies of TMS-EDTA Adsorption on Au using Electrochemical Methods

4.1 Synopsis

The Gibbs free energies of TMS-EDTA adsorption on Au (in the presence of water and ions) are quantified in this chapter due to their importance to PDMS bonding and biosensing applications. Electrochemical differential capacitance is one of the best methods to determine the standard free energies of adsorption (see Section 2.3) in a complex aqueous environment, while also elucidating its dependence on the substrate potential (see Section 2.4). Therefore, in this chapter, a determination of the free energy of adsorption of TMS-EDTA onto Au (in an aqueous electrolyte similar to the deposition solution used previously) using electrochemical differential capacitance measurements is described.

4.2 Experimental Section

4.2.1 General Materials and Gold Surface Preparation

All chemicals and reagents were obtained from commercial sources: N-[(3-trimethoxysilyl)propyl]ethylene-diamine triacetic acid, Na salt (TMS-EDTA, pH ~11; 50–60 wt % water, 32–38 wt % TMS-EDTA salt, and 8–12 wt % sodium chloride) was from United Chemical Technologies (Bristol, PA). Sodium phosphate dibasic dihydrate (puriss. p.a., buffer substance), potassium chloride (puriss. p.a. ACS), and potassium hydroxide (pellets, 99.99% metals basis, semiconductor grade) were from Sigma-Aldrich (Oakville, ON, Canada). Ultrapure water (~18.2 MΩ·cm) produced by a Milli-Q water purification system (EMD Millipore) was used. Polycrystalline Au bead electrodes (diameter ~2 mm) were cleaned and flame
annealed by heating in butane flame until red hot, followed by rinsing with ultrapure water (repeated three times).

4.2.2 Electrochemical Measurements

4.2.2.1 Instrumentation

Electrochemical experiments were performed in a three-electrode glass cell with an Autolab electrochemical analyzer (PGSTAT 12, Eco Chemie B.V.) using NOVA 1.8 software. A scan/sweep controller (EG&G PAR 175) and an analog lock-in amplifier (EG&G model 5210) were used for cyclic voltammetry (CV) and differential capacitance experiments. The working electrode was a Au bead; the counter electrode was a Pt coil; and the reference electrode was a saturated calomel electrode (SCE). The reference electrode was connected to the electrolyte buffer solution via a salt bridge. The working solution was deoxygenated with argon and purged over the surface in all experiments.

4.2.2.2 Working Solution

A buffered working solution (an electrolyte containing 100 mM phosphate, 500 mM KCl, and 65 mM KOH at pH 11.6), hereafter called DiffCap buffer, was prepared and used as the electrolyte for all electrochemical differential capacitance measurements. This buffer was formulated to simulate the multi-component surface modification solution used for chemically modifying the Au substrates. A final working solution volume of 50 ± 0.1 mL was used in all experiments (after compensating for volume of solution used in salt bridge).

4.2.2.3 Differential Capacitance Measurements

To ensure high reproducibility and cleanliness of the three-electrode electrochemical setup, a cyclic voltammogram (CV) of the gold bead working electrode in DiffCap buffer was obtained before and after the addition of bulk TMS-EDTA concentrations for all experiments (see Figures B.1 and B.2 of Appendix B). Incremental concentrations of TMS-EDTA were then added to the DiffCap buffer. At each concentration, capacitance was measured for a range of 19 potentials (starting from —1.1 V and stepping positively to 0.2 V). At each potential, stirring was turned on for 60 s and turned off prior to measuring the capacitance (repeated six times). After 6 min of stirring, the potential-dependent adsorption of TMS-EDTA on Au was assumed to have reached an equilibrium (see Figures B.3 to B.9 of Appendix B). To desorb the TMS-EDTA layer (true for concentrations less than 50 mM) and to ensure similar starting conditions, a
4.3 Results and Discussion

−1.1 V potential was applied (for 30 s without stirring) before proceeding from one potential to the next (see Table B.1 of Appendix B). The resulting capacitance was determined assuming a series RC equivalent circuit. The capacitance values measured after 6 min of stirring were then averaged and used for further data analysis.

4.2.2.4 Data Analysis

MATLAB was used to analyze the concentration- and potential-dependent differential capacitance data for TMS-EDTA adsorption on a Au bead electrode. Using the estimation method based on Shepherd et al.’s study,\textsuperscript{205} the capacitance measured in DiffCap buffer solution at −0.9 V was normalized to the area of the Au bead electrode (calculated and estimated to be ∼0.25 cm\textsuperscript{2}), and a value of 16.4 μF/cm\textsuperscript{2} was obtained. All capacitance data were adjusted to account for the Au electrode area to ensure comparability of the results. Subsequently, differential capacitance values were converted to surface coverage (at each applied electrode potential) using the Frumkin parallel plate capacitor model\textsuperscript{206} as shown in eq 4.1 (see Section 2.4.2.2).

\[ C_\theta = C_{\theta=0}(1-\theta) + C_{\theta=1}\theta \]  

(4.1)

Here, \( C_\theta \) is the measured differential capacitance value at a particular TMS-EDTA concentration; \( C_{\theta=0} \) is the differential capacitance value of DiffCap buffer (i.e., zero TMS-EDTA coverage); \( C_{\theta=1} \) is the minimum differential capacitance value obtained from 200 mM TMS-EDTA (i.e., maximum TMS-EDTA coverage); and \( \theta \) is the calculated fractional surface coverage. Next, the concentration-dependent surface coverage at each applied electrode potential was fit to Langmuir or Frumkin adsorption isotherms to obtain potential-dependent free energies of adsorption (\( -\Delta G_{ads}^\alpha \)) and Frumkin lateral interaction parameters (\( \alpha \)),\textsuperscript{207} where positive or negative values of \( \alpha \) indicate a repulsive or attractive interaction (between adsorbed molecules), respectively (see Section 2.3).

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4.3.1 Open-Circuit Potential (OCP) During Surface Modification

From Figure 4.1, the presence of dissolved O\textsubscript{2} was found to set the potential of the substrate during the TMS-EDTA surface modification procedure. Specifically, the open-circuit potential (OCP) was measured to be ∼0.05 V and ∼−0.025 V (vs SCE) in the presence and absence of O\textsubscript{2}, respectively. In all of the
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Figure 4.1: The open-circuit potential (OCP) of bare Au bead electrode immersed in 10% TMS-EDTA (v/v) solution with (dotted curve) and without (solid curve) oxygen were determined.

previous surface analyses (see Chapter 3), the Au substrates were functionalized with an aqueous 10% TMS-EDTA (v/v) solution (prepared using the commercially available stock TMS-EDTA solution—pH \( \sim 11 \)—containing 50–60 wt % water, 32–38 wt % TMS-EDTA salt, and 8–12 wt % sodium chloride), in the presence of dissolved \( \text{O}_2 \). These conditions are important when determining the Gibbs free energies of TMS-EDTA adsorption under the typical wet chemical coating conditions. In other words, the electrolyte buffer must replicate the conditions of the aqueous 10% TMS-EDTA (v/v) solution in order to determine the relevant Gibbs free energies of TMS-EDTA adsorption on Au.

4.3.2 Initial Measurements of Au Electrochemistry in Working Buffer

For an accurate characterization of TMS-EDTA adsorption on Au, the electrolyte composition must be held constant. However, with every increase in TMS-EDTA concentration, \([\text{Cl}^-]\) also increased (the molar ratio of sodium chloride to TMS-EDTA salt of the stock solution was calculated to be \( \sim 2.26 \)). To minimize the effects of chloride competitive adsorption (contributed from the incremental addition of stock TMS-EDTA solution), 500 mM KCl was added to the 100 mM phosphate buffer. Furthermore, 0.2 g of KOH was added to the phosphate and chloride buffer solution to replicate the highly basic state of 10% TMS-EDTA (v/v) aqueous solution (pH = 10.88 ± 0.03). Consequently, the DiffCap buffer was
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Prepared and used in these electrochemical differential capacitance studies. The capacitance of the gold bead electrode in DiffCap buffer was measured at the start of each experiment and demonstrated high reproducibility (see four examples shown in Figure 4.2A). Figure 4.2B shows the averaged raw DiffCap Buffer data and the corresponding standard deviations. These standard deviation values were used to estimate the errors in subsequent fittings.

4.3.3 Data Treatment and Analysis

Differential capacitance measurements were performed on gold with incremental concentrations of TMS-EDTA (1 μM to 200 mM) added to the DiffCap buffer. At each concentration, a series of 19 electrode potentials were applied (changing from −1.1 to 0.2 V), and differential capacitance was measured after waiting for 6 min with stirring at each potential, which resulted in an experimental duration of approximately 2 hours. Therefore, further tests were conducted to test the stability of TMS-EDTA-coated Au electrode in DiffCap buffer for several hours. When compared with a bare Au electrode, the capacitance of TMS-EDTA-modified electrode appeared to be more stable in DiffCap buffer (due to a minimal decrease in capacitance) for the duration of the experiments (see Figure 4.3).

Triplicate measurements of lower concentrations of TMS-EDTA were conducted (an example is shown in Figure 4.4). Small differences were observed, indicating that the measurements were dependent on the area of the Au bead immersed in the buffer solution. Therefore, an area-normalization procedure for the Au bead electrode was required.

The area of the Au bead electrode was estimated using the method based on Shepherd et al.’s study, by comparing the capacitance of a Au bead electrode at −0.9 V in both 50 mM Perchlorate Buffer (basic pH) and DiffCap Buffer (see Figure B.10 of Appendix B). An electrode area of ~0.25 cm² was determined.

All of the subsequent capacitance data reported have been normalized to account for the Au electrode area (to ensure comparability of the results). Normalized capacitance for DiffCap Buffer is shown in Figure 4.5. In addition, the influence of each component (e.g., phosphate, hydroxide, and chloride ions) on capacitance measurements is shown.

4.3.4 Estimation of Surface Coverage

Figure 4.6 shows a selection of capacitance–potential curves of increasing TMS-EDTA concentrations in the DiffCap buffer. The curve labeled “Buffer” represents the capacitance of a Au bead electrode in
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Figure 4.2: (A) Raw capacitance data for DiffCap Buffer measurements from four independent experiments (from four different days). (B) Averaged raw DiffCap Buffer data and its corresponding standard deviations.
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Figure 4.3: The stability of freshly cleaned bare Au bead electrode (circle) and TMS-EDTA-modified Au bead electrode (square) in DiffCap buffer at open-circuit potential (OCP).

Figure 4.4: Raw capacitance data for three independent measurements (from three different days) of 20 μM TMS-EDTA in DiffCap buffer.
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Figure 4.5: Averaged and normalized electrochemical differential capacitance data at equilibrium (360 s with stirring) for individual DiffCap buffer components: (1) 100 mM phosphate (circle), (2) 100 mM phosphate and 65 mM KOH (square), and (3) DiffCap Buffer (triangle) — 100 mM phosphate, 65 mM KOH, and 500 mM KCl.

DiffCap buffer in the absence of TMS-EDTA. In general, as the concentration of TMS-EDTA increased, the measured capacitance value decreased, due to TMS-EDTA adsorbing onto the Au electrode displacing water, chloride ions, and hydroxide ions. At a potential of $-1.1 \text{ V}$, the capacitance values were similar (i.e., no significant decrease in capacitance) for the DiffCap buffer solution and for the solutions with TMS-EDTA at concentrations of less than 50 mM. This result indicates high reproducibility of the measurements and desorption of TMS-EDTA from the Au electrode at this potential. At TMS-EDTA concentrations above 50 mM, pseudocapacitance features at $-1.1 \text{ V}$ (related to the kinetics of desorption and adsorption process) were observed (see Section 2.4.2.4).

A constant minimum in capacitance was observed for potentials between $-1$ and $0.2 \text{ V}$ at 200 mM TMS-EDTA concentration (see Figure 4.6). The minimum value of capacitance at each potential was determined in order to calculate coverage (using eq 4.1). Assuming the curve for 200 mM TMS-EDTA corresponds to a maximum surface coverage ($\theta=1$), the fractional surface coverage ($\theta$) as a function of both potential ($E$) and TMS-EDTA concentration was calculated. For potentials less than $-0.6 \text{ V}$, pseudocapacitance artifacts were observed (see Section 2.4.2.4); hence this range of potentials did not fit the parallel plate model as stated in eq 4.1 (see Figure 4.7A) and these potentials were not analyzed. Therefore, the surface coverage was calculated in a limited potential window between $-0.5$
4.3. Results and Discussion

Figure 4.6: Capacitance–potential curves of increasing TMS-EDTA concentrations in DiffCap Buffer (select curves shown). For each concentration, capacitance was measured after each potential was held for 6 min with stirring. Series resistor–capacitor equivalent circuit was assumed for calculations.

and 0.2 V (Figure 4.7B). In general, as the concentration of TMS-EDTA increased, the calculated surface coverage increased. For potentials between $-0.2$ and 0 V, pseudocapacitance features due to Cl$^-$ or OH$^-$ adsorption (see Section 2.4.2.4) influenced the capacitance and the calculation of coverage (see Figure 4.5).

### 4.3.5 Determining the Gibbs Free Energies of TMS-EDTA Adsorption onto Au

With surface coverage data, the free energy of TMS-EDTA adsorption at each applied electrode potential was calculated by fitting to a Langmuir (dashed curve) or a Frumkin (solid curve) adsorption isotherm (see Figures B.11 to Figure B.16 of Appendix A). The results at selected potentials of $-0.5$ V (Figure 4.8A), $-0.25$ V (Figure 4.8B), and 0.1 V (Figure 4.8C) are shown. It was observed that at negative electrode potentials both Langmuir and Frumkin isotherms described the data adequately. Surface excess was likely smaller at more negative potentials since TMS-EDTA is negatively charged at high pH (see Figure A.1 of Appendix A). However, as the electrode potential became more positive, the Langmuir isotherm failed to adequately fit the experimental data. The surface excess may be greater at more positive potentials resulting in increased repulsion among the adsorbed TMS-EDTA molecules, for which the Frumkin isotherm better describes the experimental data.
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Figure 4.7: (A) The fractional surface coverage curves calculated from capacitance values using $C_\theta = C_{\theta=0}(1-\theta) + C_{\theta=1}\theta$. (B) A limited potential window is shown.
Figure 4.8: Adsorption of TMS-EDTA onto Au electrode in DiffCap Buffer, fit with Langmuir (dotted line) and Frumkin (solid line) isotherms, at applied electrode potentials of (A) $-0.5 \text{ V}$, (B) $-0.25 \text{ V}$, and (C) $0.1 \text{ V}$.
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Potential-dependent free energies of adsorption (Figure 4.9A) and lateral interaction parameters (Figure 4.9B) derived from the Frumkin isotherm analysis are shown. Figure 4.9A reveals that the spontaneity of TMS-EDTA adsorption on the Au electrode becomes more favorable as the applied potential (vs SCE) becomes more positive, similar to the adsorption of negatively charged chloride or hydroxide ions on Au.\textsuperscript{170,171} Potential-dependent free energies of adsorption were determined to be $\sim -20$ to $-30$ kJ/mol for the potential window shown ($-0.5$ to $0.2$ V). A positive Frumkin lateral interaction parameter from Figure 4.9B indicates repulsion among adsorbed TMS-EDTA and that this repulsion increases as the applied potential becomes more positive. These results can be explained as arising due to the surface excess likely being greater at more positive potentials, resulting in increased repulsion among the negatively charged TMS-EDTA molecules. However, for potentials between $-0.2$ and $0$ V, pseudocapacitance characteristics of the electrolytes were observed, and as a result, interpolated curves in this potential window are also shown (measured data shown with dotted lines and interpolation shown with solid lines in 4.9).

Nevertheless, it should be noted that TMS-EDTA adsorption was measured using the multi-component DiffCap buffer (100 mM phosphate, 500 mM KCl, and 65 mM KOH). The Gibbs free energy of adsorption of hydroxide on the Au(111) electrode was found to be $\sim -110$ to $-120$ kJ/mol from $\sim -0.1$ to $\sim 0.2$ V, respectively.\textsuperscript{171} For chloride, the Gibbs free energy of adsorption at the Au(111) electrode was found to be $\sim -100$ to $\sim -110$ kJ/mol within a similar potential range.\textsuperscript{170} Moreover, for hydroxide ions, adsorption occurs within the potential range from $-0.4$ to $0.2$ V, limited by desorption ($E < -0.4$ V) and gold oxide formation ($E > 0.2$ V).\textsuperscript{171} We also restricted our analysis to a similar potential range (between $-0.5$ to $0.2$ V). However, within this potential range, the adsorption of TMS-EDTA is in competition with water molecules, hydroxide ions, and chloride ions for the Au surface. In this complex system, adsorption is a displacement process; therefore, the adsorption free energy of TMS-EDTA on Au is likely more favorable than what the data presented here suggest. It is important to point out that the change in Gibbs free energy depends on the adsorbate/adsorbent bond strength (enthalpy change) and entropy change (see Section 2.3). If the entropy change of this system is assumed to be negligible due to the high electrolyte concentrations in DiffCap buffer, then the apparent Gibbs free energies of adsorption obtained may provide a rough estimate of the enthalpy change (i.e., strength of TMS-EDTA adsorption on Au).

Finally, recall that the typical wet chemical modification was achieved by immersing the Au substrate in a 10% TMS-EDTA (v/v) solution with dissolved oxygen. The open-circuit potential (OCP) of the Au substrate under these conditions (see Figure 4.1) was $\sim 0.05$ V (vs SCE). The free energy of adsorption...
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Figure 4.9: Potential-dependent (A) free energies of adsorption and (B) lateral interaction parameter $\alpha$, which are determined from Frumkin isotherm fitting. Positive $\alpha$ indicates repulsive interaction. Continuous interpolated values (solid line) are shown. Errors are estimated by robust fitting routine in MATLAB and represent 95% confidence interval.
at this deposition potential (obtained from Figure 4.9A) suggests that TMS-EDTA strongly modifies the Au surface. These results support the results that TMS-EDTA-modified Au could be used to capture streptavidin and could withstand stringent washes (see Section 3.3). Also, at highly negative potentials (i.e., $\sim -1.1$ V), TMS-EDTA adsorbs minimally onto Au (i.e., TMS-EDTA layer on Au may be electro-chemically removed).

4.4 Conclusions

Thermodynamic studies of TMS-EDTA adsorption on Au have been performed using electrochemical methods. Electrochemical differential capacitance measurements reveal that TMS-EDTA adsorption on Au is potential-dependent.

For potentials between $-0.5$ to $0.2$ V, the apparent Gibbs free energies of adsorption were determined to be $\sim -20$ to $-30$ kJ/mol in the complex electrolyte solution (measured under relevant conditions for use in future sensor fabrication and biosensor applications). These results suggest that at more positive potentials (i.e., at the potential typical for surface modification), adsorption is more thermodynamically favorable. Due to its negative charge, there is more repulsive lateral interaction among the adsorbed TMS-EDTA at these potentials.

At very negative potentials ($\sim -1.1$ V), there is minimal adsorption. Since the creation of a clean surface is essential for electrochemical sensing applications, this result suggests that the TMS-EDTA layer may be removed by applying negative potentials. The knowledge gained from these studies was valuable toward applying TMS-EDTA to construct robust PDMS-based electrochemical systems. These results will be reported in the next chapter.
Chapter 5

Fabrication of PDMS-Based Electrochemical Cells Using TMS-EDTA

5.1 Synopsis

The fundamental studies of TMS-EDTA adsorption on Au (presented in Chapters 3 and 4) provided valuable data for the rational design of integrated sensors and biosensors. The results suggested that TMS-EDTA can potentially be used in a glass or gold-coated glass substrate for developing leak-free microfluidic devices. First, shear tests were conducted to determine the feasibility of using TMS-EDTA to chemically modify either glass or gold slides (i.e., to form terminal carboxyl groups) in order to enable bonding to polydimethylsiloxane (PDMS) slabs chemically modified with 3-APTMS (i.e., to form terminal primary amino groups). The strength of this carboxyl-amine bonding strategy was compared with other chemical bonding methods. Subsequently, the proposed carboxyl-amine bonding strategy was improved and refined for electrochemical applications. Pressure leak tests were conducted on devices with PDMS cell chambers bonded to 3-electrode substrates to obtain a more realistic measure of the bond strength under aqueous conditions. Finally, a method to electrochemically remove TMS-EDTA from the Au surface (inside a bonded PDMS-based device) was developed.
5.2 Demonstrating the Feasibility of a Novel Carboxyl-Amine Bonding Strategy

5.2.1 Experimental Section

5.2.1.1 Materials

All reagents were from commercial sources and used as-received without further purification: N-[(3-trimethoxysilyl)propyl]ethylene-diamine tricetic acid, Na salt (TMS-EDTA; 50% in water) was from United Chemical Technologies (Bristol, PA); PDMS precursor and curing agent (Sylgard 184) were from Dow Corning (Midland, MI); sodium hydroxide (NaOH; certified ACS) was from Fisher Scientific (Ottawa, ON, Canada); (3-aminopropyl)-trimethoxysilane (3-APTMS; 97%), (3-mercaptopropyl)-trimethoxysilane (3-MPTMS; 95%), 1,2-bis(trimethoxysilyl)ethane (BTMSE; 96%), 11-mercaptopoundecanoic acid (11-MUA; 95%), N-hydroxysuccinimide (NHS; 98%), N-(3-(Dimethylamino)propyl)-N′-ethylcarbodiimide hydrochloride (EDC; 98%), acetone (ACS reagent, ≥99.5%), and 2-propanol (ACS reagent, ≥99.5%) were from Sigma-Aldrich (Oakville, ON, Canada); glass microscope slides were from VWR International (Mississauga, ON, Canada); nitrogen and argon (Ultra High Purity 5.0) were from Praxair Canada Inc. (Mississauga, ON, Canada); and ethyl alcohol (denatured) was from UBC Zoology Stores (Vancouver, BC, Canada). For all studies, ultrapure water (∼18.2 MΩ-cm) produced by a Milli-Q water purification system (EMD Millipore) was used. The chemical structures of the molecules used in the shear tests are presented in Figure 5.1.

5.2.1.2 Fabrication of PDMS Slabs (Planar PDMS Surfaces)

PDMS slabs with planar surfaces for shear tests were fabricated using standard soft lithography techniques (see Section 2.1.2) as shown in Figure 5.2. In short, a 10:1 (w/w) mixture of PDMS precursor and curing agent was prepared and poured onto a 5-inch aluminum weighing pan (VWR International; Mississauga, ON, Canada). After degassing, the pan was placed in an oven and PDMS was cured at 60 °C for 2 hours. Prior to being used, the 6 mm thick PDMS was peeled off of the aluminum pan and cut into 25.4 mm by 12.7 mm pieces with razor blades.

5.2.1.3 Fabrication of Gold-Coated Glass Substrates

The glass slides were cleaned in a piranha solution (5:1 H₂SO₄:H₂O₂) at 80 °C for 15 min, rinsed with ultrapure water, and dried in a nitrogen stream. The cleaned slides were subsequently placed into a 200
5.2. Demonstrating the Feasibility of a Novel Carboxyl-Amine Bonding Strategy

Figure 5.1: Chemical structures of the molecules used in shear tests: 3-APTMS, 3-MPTMS, BTMSE, TMS-EDTA, and 11-MUA.

Figure 5.2: Scheme for producing PDMS slabs using soft lithography. (A) A flat aluminum weighing pan is used as the mold. (B) Liquid PDMS is poured onto the mold. (C) Mold with liquid PDMS is baked in an oven. (D) Solidified PDMS is peeled off and (E) cut into slabs of appropriate sizes.
5.2. Demonstrating the Feasibility of a Novel Carboxyl-Amine Bonding Strategy

°C oven for 15 min to eliminate residual moisture. A 20 nm chromium adhesion layer and a 200 nm gold layer were sequentially deposited onto clean glass slides using an electron-beam evaporator (AMPEL Advanced Nanofabrication Facility at UBC Vancouver).

5.2.1.4 Cleaning of Glass or Gold-Coated Glass Substrates

The glass slides were cleaned by sequential immersions in acetone, 2-propanol and ultrapure water (with 10-min sonication for each). The gold-coated glass slides were cleaned by sequential immersions in acetone, 2-propanol, and ultrapure water (repeated three times, without sonication). Cleaned substrates were blown dry in a nitrogen stream.

5.2.1.5 Surface Functionalization of Glass or Gold-Coated Glass Substrate

The surface of the PDMS was modified with an amino-silane to create a layer of reactive primary amino groups. Briefly, PDMS slabs were exposed to UV-ozone (UVO-Cleaner, model 42, Jelight Co. Inc., CA) for 5 min and immediately immersed into a 10% (v/v) solution of 3-APTMS in ethanol for 1 h. The PDMS slabs were then washed three times in ethanol, followed by three times in ultrapure water, and then dried under N₂ for immediate use. Glass and gold substrates were cleaned as previously described, followed by exposing to UV-ozone for 5 min. Clean glass substrates were immersed in aqueous solution of 10% TMS-EDTA (v/v) or ethanolic solution of 10% BTMSE (v/v), whereas gold substrates were immersed in either ethanolic solutions of 10% 3-MPTMS (v/v) or 10 mM 11-MUA, or aqueous solution of 10% TMS-EDTA (v/v) for 2 h at room temperature. All substrates, except for TMS-EDTA, were washed three times in ethanol and three times in ultrapure water, and then dried under a N₂ stream. TMS-EDTA-modified substrates were washed three times in ultrapure water and dried under a N₂ stream.

5.2.1.6 Irreversible Bonding

Carboxyl-terminated substrates functionalized with TMS-EDTA or 11-MUA were subjected to carbodiimide activation (50 mM NHS and 200 mM EDC for 30 min), followed by drying under N₂. These substrates were then placed in contact with PDMS surfaces functionalized with 3-APTMS. Silanol-terminated surfaces (i.e., BTMSE, 3-MPTMS) were hydrolyzed for 1 h in a 1 M NaOH solution (see Section 2.1.1.2), followed by rinsing in ultrapure water and drying under N₂. These hydrolyzed surfaces were placed in contact with unmodified PDMS surfaces that were exposed to UV-ozone for 5 min. Control experiments consisted of bringing together the surfaces of both unmodified PDMS and clean glass
5.2. Demonstrating the Feasibility of a Novel Carboxyl-Amine Bonding Strategy

Slides that were exposed to either UV-ozone or oxygen-plasma for 5 min. For all bonding methods, the glass or gold slides were immediately brought into contact with the PDMS surfaces and allowed to bond at room temperature for 1 h.

5.2.1.7 Shear Tests of PDMS Slabs Bonded to Glass or Gold-Coated Glass Substrates

In total, four types of PDMS–glass bonding strategies and three types of PDMS–gold bonding strategies were analyzed. The strengths of various bonding strategies were measured by performing shear tests (Material Testing System, MTS 810, MTS Systems Corporation; Eden Prairie, MN) with the following procedural modifications (see Figure C.1 of Appendix C). The back of each PDMS-bonded substrate (i.e., the glass slide that was not bonded to PDMS) was epoxy-glued to a wood block, which was then clamped to a testing stage. In each test, the load cell was aligned and the PDMS slab was pushed off of the solid substrate (at a rate of 1 mm/min). The continuous displacement of the load cell applied a shear force on the PDMS material. The force curve was recorded against elapsed time (four measurements per second). The peak of each force curve represents the point of failure for the PDMS–substrate bond. The bond strength of each sample was calculated as the average of the highest force values in a 2-second time interval. Three elastic failure curves (from three independent samples) were obtained for each of the bonding method, and standard deviation was calculated using these values. The force values were then converted to pressures by dividing the PDMS–substrate contact area (see Tables C.1 and C.2 of Appendix C).

5.2.2 Results and Discussion

PDMS slabs were modified with ethanolic 3-APTMS in order to form terminal primary amino groups. Water contact angle measurements using the sessile drop method were performed on the surfaces of bare PDMS (Figure 5.3A), PDMS exposed to UV-ozone (UVO) for 5 min (Figure 5.3B), and 3-APTMS-modified PDMS (Figure 5.3C) to confirm the surface modification. A single measurement was performed on each sample for proof-of-concept. The PDMS surface modified with 3-APTMS showed a decrease in the water contact angle, which indicated that the surface became more hydrophilic than the bare PDMS and the UVO PDMS, confirming chemical functionalization (see Section 2.2.1).

To illustrate the concept of the carboxyl-amine strategy, a detailed scheme of the bonding method for 11-MUA-modified gold is presented in Figure 5.4. In short, PDMS surface was modified with 3-APTMS to form terminal primary amino groups (Figure 5.4A). Gold-coated glass (or simply gold) substrate was
5.2. Demonstrating the Feasibility of a Novel Carboxyl-Amine Bonding Strategy

Figure 5.3: Water contact angle measurements on (A) bare PDMS, (B) PDMS exposed to UV-ozone (UVO) for 5 min, and (C) 3-APTMS-modified PDMS.
5.2. Demonstrating the Feasibility of a Novel Carboxyl-Amine Bonding Strategy

modified with 11-MUA to form terminal carboxylic acid groups (Figure 5.4B). Finally, the terminal carboxylic acid groups on Au were treated with carbodiimide activation (i.e., NHS/EDC), followed by reacting with 3-APTMS-modified PDMS and allowing to form a bond by physical contact at room temperature (Figure 5.4C). The glass or gold substrates modified with TMS-EDTA were also treated with the same procedures.

For shear tests, gold substrates were modified with 11-MUA or TMS-EDTA, and glass substrates were modified with TMS-EDTA in order to create terminal carboxyl groups. Following carbodiimide activation, the solid (gold or glass) substrates were brought into contact with PDMS slabs modified with 3-APTMS after ~1 s. Strong bonding was observed after 1 h. A few other chemical bonding strategies were also studied for comparison. Glass substrates modified with BTMSE or gold substrates modified with 3-MPTMS were treated with 1 M NaOH for 1 h prior to bonding with UV-ozone-treated PDMS via silane cross-linking. Clean glass and unmodified PDMS treated with UV-ozone and oxygen-plasma were also bonded together, serving as controls.

The bond failure curves for TMS-EDTA-modified glass and 3-APTMS-modified PDMS are presented in Figure 5.5A. The peak value of each shear test curve represents the failure point of the PDMS–substrate bond. The small variation observed in the three replicates measured illustrates the reproducibility of this characterization method. The bond strengths of four different glass–PDMS bonding strategies are presented in Figure 5.5B. Figure 5.5C shows the results from three different gold–PDMS bonding strategies. In all instances, the bond failed at the PDMS–substrate interface by delamination due to the applied shear stress. However, it was difficult to determine the different modes of failure from these shear test results. Nevertheless, a thin layer of PDMS residue was left on the substrate, confirming the strong bonds formed. The pressure was calculated by dividing the peak force value (determined from the shear test) by the PDMS–substrate contact area (see Tables C.1 and C.2 of Appendix C).

To determine the validity of the shear test results, UV-ozone and oxygen-plasma bond strength values were compared with values reported in the literature for PDMS–PDMS devices. Typically, bonding methods using oxygen-plasma result in stronger bond strengths than those using UV-ozone. The results presented in Figure 5.5B follow this trend for PDMS–glass devices. In addition, the oxygen-plasma bond strength value is in close agreement with previously published values for PDMS–PDMS bonded devices. This result is expected due to the siloxane bonds formed (see Section 2.1.2).

From Figure 5.5, it was observed that the carboxyl-amine bonding strategy produced bond strengths that were comparable to those of conventional methods using UV-ozone treatment for glass substrates and 3-MPTMS treatments for gold substrates. The BTMSE-bonded PDMS–glass structure produced a
5.2. Demonstrating the Feasibility of a Novel Carboxyl-Amine Bonding Strategy

Figure 5.4: (A) Modification of PDMS with 3-APTMS to form primary amines. (B) Modification of gold with 11-MUA to form carboxylic acids. (C) Carbodiimide activation of the carboxylic acid groups, followed by irreversible bonding of PDMS to gold by physical contact at room temperature.
5.2. Demonstrating the Feasibility of a Novel Carboxyl-Amine Bonding Strategy

Figure 5.5: (A) Bond failure curves of TMS-EDTA-modified glass and 3-APTMS-modified PDMS slabs. The peak of each curve represents the failure point of the PDMS-substrate bond. (B) Bond strengths of four different PDMS–glass bonding strategies: UV-ozone treatment of both glass and PDMS surfaces (UVO); oxygen-plasma treatment of both glass and PDMS surfaces ($O_2$ Plasma); TMS-EDTA-modified glass with 3-APTMS-modified PDMS (TMS-EDTA & 3-APTMS); and BTMSE-modified glass with UV-ozone-treated PDMS (BTMSE & UVO). (C) Bond strengths of three different PDMS–gold bonding strategies: 3-MPTMS-modified gold with UV-ozone-treated PDMS (3-MPTMS & UVO); 11-MUA-modified gold with 3-APTMS-modified PDMS (11-MUA & 3-APTMS); and TMS-EDTA-modified gold with 3-APTMS-modified PDMS (TMS-EDTA & 3-APTMS). All samples were analyzed in triplicate. Error bars represent standard deviation.
comparable bond strength as oxygen-plasma-bonded glass and PDMS. The bonding method that utilized BTMSE did not employ the carboxyl-amine bonding strategy outlined above, but instead served as another demonstration of the solution-based chemical modification of glass for PDMS bonding. Additional control experiments were conducted by manual peel tests to determine the importance of forming both terminal carboxyl groups and terminal primary amino groups on solid substrate (i.e., glass or gold) and PDMS, respectively (see Table C.3 of Appendix C). The bonded structures were then completely immersed in water to determine its hydrolytic stability, and started to show signs of weakening after 2 weeks. It is known that for a short immersion time (i.e., 24 h), PDMS does not swell significantly in water.\(^{52}\) However, it has been reported that for a longer immersion time (i.e., 2 weeks), PDMS does indeed swell in water.\(^{211}\) The PDMS swelling may have caused the detachment of PDMS slabs from solid gold or glass substrates. As a result, it is difficult to determine the hydrolytic stability of the bonds between the PDMS slabs and the substrates over time. Nevertheless, devices kept at ambient conditions in air has not shown signs of weakening since 2009.

The carboxyl-amine bonding method presented here is similar to the approach developed by Lee and Chung, which employed epoxy-amine chemistry to bond PDMS–PDMS devices.\(^{212}\) However, the substrates reported here were modified with silanes or alkanethiols terminated with carboxyl groups, as opposed to epoxy groups. As a result, more specific covalent coupling with free primary amino groups can be achieved,\(^{213}\) because epoxy is known to react with other functional groups including alcohols, carboxylic acids, and acid anhydrides.\(^{214}\) Nevertheless, the important result obtained from the shear tests presented here was that TMS-EDTA could chemically modify either glass or gold substrates for carboxyl-amine bonding with PDMS modified with terminal primary amino groups. These results (and the results from SPR experiments in Section 3.3) suggest that TMS-EDTA-modified Au has at least some carboxyl groups available for reaction that are oriented away from the Au surface, resulting in irreversible coupling with substances containing terminal primary amino groups.

### 5.3 Applying TMS-EDTA to Construct Leak-Free PDMS-Based Electrochemical Cells

As mentioned in Section 2.5, there is a growing interest in creating microfluidic electrochemical sensors due to their inexpensive experimental setup and suitability for miniaturization.\(^{108}\) Typically, Au electrodes are sputtered onto a flat glass slide, and a PDMS chip is placed in contact with the solid substrate, form-
5.3. Applying TMS-EDTA to Construct Leak-Free PDMS-Based Electrochemical Cells

Inducing enclosed channels and/or cell chambers (see Figure 1.2 of Section 1.1). There are two separate and well-defined interfaces: PDMS–glass and PDMS–Au interfaces. It is simple to form either a strong PDMS–glass bond (e.g., using UV-ozone or oxygen-plasma) or a strong PDMS–Au bond (e.g., using 3-MPTMS). However, it is difficult to form both PDMS–glass and PDMS–gold bonds at the same time using conventional chemical bonding strategies. Therefore, aqueous solution may leak at the PDMS–Au interface with just a slight increase in fluidic pressure (e.g., during loading of aqueous electrolyte) when the gold surface is not chemically modified. It is important for these PDMS-based electrochemical devices to form a tight seal because the leakage problem will negatively impact electrochemical measurements and data analysis (see Section 2.5.1).

In Section 5.2, the shear tests were conducted on samples of either glass or gold substrates bonded to PDMS slabs. The positive results indicated the feasibility of bonding a substrate with both glass and Au surfaces (i.e., a mixed Au/glass substrate) to PDMS using the same carboxyl-amine chemistry with TMS-EDTA. The rest of this chapter is focused on the application of TMS-EDTA to develop leak-free PDMS-based electrochemical cell with a 3-electrode substrate (see Figure 5.6). Since most electrochemical sensing applications require the use and transfer of aqueous solutions, it is important to determine the maximum fluidic pressure that these bonded devices can withstand. Pressure leak tests with dyed water were conducted on devices bonded using the carboxyl-amine strategy (with the oxygen-plasma bonding method serving as a control). Finally, various electrochemical surface cleaning methods were tested to determine the best protocol that could remotely desorb TMS-EDTA from the Au surface within a bonded device.

Almost all aspects of the device fabrication process have been refined and improved in order to construct robust PDMS-based electrochemical cells. Detailed experimental procedures are summarized below, and some of the most important procedural modifications are highlighted here: (1) the PDMS precursor and curing agent was thoroughly mixed by hand and machine (instead of only by hand), (2) Ti (instead of Cr) was used as the adhesion layer between glass and gold, (3) a modified RCA cleaning method (instead of acetone, 2-propanol and ultrapure water) was used to clean all solid substrates (i.e., glass, gold-coated glass, and 3-electrode substrates), and (4) aqueous (instead of ethanolic) 3-APTMS was used to reduce PDMS swelling.
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Figure 5.6: Schematic of the fabricated 3-electrode device. (a) Top view of the PDMS-bonded device. (b) Cross-sectional view of the device through the center (i.e., down the length) of the device. (c) Enlarged view of the proposed carboxyl-amine chemistry between PDMS and the gold/glass substrate.
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5.3.1 Experimental Section

5.3.1.1 Fabrication of PDMS Cell Chambers

3D-Printed Molds Initially, 3D-printed molds were used to create the PDMS cell chambers with cylindrical side-walls due to its low cost and fast prototyping. The 3D-printed molds were designed in Solidworks. The designs were exported as .stl files and printed using the 3D printers in the Electrical and Computer Engineering Lightning Lab (UBC Vancouver). All molds were printed with the cell chambers in a positive-relief format (i.e. a negative of the actual chamber) and made of an acrylic-based plastic (VeroWhitePlus, Objet, Inc., MA). However, the PDMS structures created from the 3D-printed molds resulted in rough PDMS surfaces (since the surfaces of 3D-printed molds were uneven). As a result, the PDMS cell chambers with smooth surfaces were fabricated using a 2-step process as described below.

Step 1: Creating PDMS Cell Chambers with the 3D-Printed Molds Using the 3D-printed molds, the initial PDMS cell chambers were fabricated with the procedure described in Figure 5.7. In short, a 10:1 (w/w) mixture of PDMS precursor and curing agent was first mixed by hand and then by machine (Thinky AR-250, THINKY USA Inc., CA). The 3D-printed molds were surrounded with single-sided tape (3M Canada) on the rectangular perimeter in order to create sidewalls and to contain uncured liquid PDMS. Uncured PDMS mixture was then poured onto the 3D-printed molds and placed in a vacuum chamber to remove air bubbles. After degassing, the chambers were cured in an oven for 3.5 hours at 60-65 °C. Afterwards, the tape was removed and the cured PDMS structures were peeled off of the 3D-printed molds. The surface of the 3D-printed mold was rough, and as a result, the surface of the PDMS cell chamber produced was also rough. After making the initial PDMS cell chambers, the 3D-printed molds could be re-used by rinsing in 2-propanol, ethanol, and ultrapure water (repeated three times), and drying with in-house nitrogen gas.

Step 2: Creating PDMS Cell Chambers with Glass/PDMS Molds Figure 5.8 details the steps used to create the final PDMS cell chambers with new glass/PDMS molds. First, additional mixed and uncured PDMS was poured into the hollow chamber of a PDMS cell chamber (made using the protocol described in Section 5.3.1.1), which served as the negative mold in order to fabricate a PDMS disk with a volume of ∼50 μL. After degassing and baking the PDMS as before, the disks were removed from the PDMS negative molds. The solid PDMS disks were bonded to clean glass slides by air-plasma (Plasma Cleaner, model PDC-001, Harrick Plasma; Ithaca, NY) in order to create the final positive molds. Again, tape was used to create sidewalls around the PDMS/glass positive molds. Newly mixed and uncured
5.3. Applying TMS-EDTA to Construct Leak-Free PDMS-Based Electrochemical Cells

Figure 5.7: Step 1 of 2: Scheme for producing PDMS cell chambers from 3D-printed molds. (A) A 3D-printed mold is made with desired features. (B) Liquid PDMS is poured onto the mold. (C) Mold with liquid PDMS is cured. (D) Solidified PDMS is peeled off. The rough surface of the 3D-printed mold resulted in a rough surface on the PDMS cell chamber produced.

PDMS was poured into these molds, degassed in a vacuum chamber, and baked as before. Solidified PDMS cell chambers were peeled off of the molds, and cut with razor blades into appropriate sizes. Similarly, the PDMS/glass molds could be re-used by rinsing in acetone and cleaning using the same method as the 3D-printed molds (described above).

**Mold Designs** A few of the 3D-printed mold designs are shown in Figure 5.9A, and the glass/PDMS mold design is shown in Figure 5.9B. PDMS cell chambers with smooth surfaces were fabricated with these two types of molds using the 2-step process as described above. Each individual cell chamber was designed to have a radius of 3.8 mm and a height of 1.2 mm (i.e., a volume of $\sim50 \mu$L). Each PDMS cell chamber was hole-punched with a 0.75 mm PDMS biopsy hole puncher for solution injection.

5.3.1.2 Fabrication of Gold-Coated Glass Substrates

Glass slides were first rinsed with acetone, 2-propanol and ultrapure water, and then cleaned in a piranha solution (5:1 H$_2$SO$_4$:H$_2$O$_2$) at 80 °C for 15 min, followed by rinsing with ultrapure water and drying in a nitrogen stream. The cleaned slides were subsequently placed into a 120 °C oven for 5 min to eliminate residual moisture. A 10 nm Ti adhesion layer and a 200 nm gold layer were sequentially deposited onto clean glass slides using the electron-beam evaporator as described in Section 5.2.1.3.

5.3.1.3 Fabrication of the 3-Electrode Substrates

The 3-electrode substrate used Borofloat 33 as the glass wafer (4-inch diameter and 500 μm thickness, University Wafer; Boston, MA), Ti as the metal adhesion layer, and Au as the metal for the electrode.
5.3. Applying TMS-EDTA to Construct Leak-Free PDMS-Based Electrochemical Cells

Figure 5.8: Step 2 of 2: Scheme for producing the final PDMS cell chambers using glass/PDMS molds. (A) PDMS cell chamber made with the 3D-printed mold is used as the initial mold. (B) Liquid PDMS is poured into the hollow chamber. (C) Mold with liquid PDMS is cured and a small disk is produced. (D) Small disk is bonded to a clean glass slide by air-plasma. (E) Liquid PDMS is poured onto the new glass/PDMS mold. (F) Mold with liquid PDMS is baked. (G) Solidified PDMS cell chamber is peeled off and cut into appropriate sizes.
5.3. Applying TMS-EDTA to Construct Leak-Free PDMS-Based Electrochemical Cells

Figure 5.9: Molds used for creating PDMS cell chambers with smooth surfaces. (A) Designs of the 3D-printed molds for creating the initial PDMS cell chambers (Step 1), from which the small PDMS disks were created. (B) Small PDMS disks were then bonded to clean glass slides by air-plasma in order to create the final mold design. The final PDMS cell chambers were fabricated using these PDMS/glass molds (Step 2).
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These substrates were fabricated by photolithographically patterning a photoresist mask, depositing 10 nm Ti adhesion layer and 200 nm Au onto the glass wafer, and lifting off the sacrificial photoresist layer. The glass wafers were then diced into smaller (25.78 mm length and 10.8 mm width) 3-electrode substrates (see Figure 5.10). The Au electrode lead(s) had a width of either 770 μm or 500 μm.

5.3.1.4 Modified RCA Cleaning Method

Since gold and glass surfaces are present on the 3-electrode substrate, an adequate cleaning method must be used to simultaneously clean both types of surfaces. The protocol described here is based on a previously published cleaning/etching method. The glass, gold-coated glass or 3-electrode substrates were first cleaned by sequential immersions in acetone, 2-propanol, methanol and ultrapure water (with 15-min sonication for each). The substrate was then immersed for 15 min in a solution mixture called RCA (50 mL ultrapure water, 10 mL ammonium hydroxide, and 10 mL hydrogen peroxide). Methanol (certified ACS), ammonium hydroxide (reagent ACS), and hydrogen peroxide (certified ACS) were from Fisher Scientific (Ottawa, ON, Canada). After briefly rinsing the substrates with ultrapure water, they were placed in a 0.1 M nitric acid solution for 30 min. Nitric acid (certified ACS; 69-70%) was from VWR BDH Chemicals (Mississauga, ON, Canada). The cleaned substrates were rinsed with ultrapure water and blown dry in an argon stream.

5.3.1.5 Carboxyl-Amine Bonding Strategy Using TMS-EDTA

The carboxyl-amine bonding strategy using TMS-EDTA has been developed in Section 5.2. To avoid any ambiguity, the refined experimental procedures are summarized and reproduced here. All of the PDMS-based devices were fabricated using this protocol.
5.3. Applying TMS-EDTA to Construct Leak-Free PDMS-Based Electrochemical Cells

**Forming Terminal Carboxyl Groups on Glass, Gold-Coated Glass or 3-Electrode Substrate**  
Cleaned glass, gold-coated glass or 3-electrode substrate was exposed to UV-ozone for 5 min. The substrates were then immersed in an aqueous solution of 10% TMS-EDTA (v/v) for 2 h at room temperature. TMS-EDTA-modified substrates were washed three times in ultrapure water and dried under an argon stream.

**Carbodiimide Activation**  
Aqueous solutions of 100 mM N-hydroxysuccinimide (NHS) and 400 mM N-(3-Dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride (EDC) were prepared separately. An aqueous mixture of these two solutions (volume ratio of 1:1) was immediately prepared prior to use, in order to make a solution with a final concentration of 50 mM NHS and 200 mM EDC. This mixture was used to activate the terminal carboxyl groups on solid substrates.

**Forming Terminal Primary Amino Groups on PDMS**  
The surface of the PDMS was chemically modified with (3-aminopropyl)-trimethoxysilane (3-APTMS) in order to create a layer of terminal primary amino groups. First, the PDMS surface was cleaned using single-sided tape and exposed to UV-Ozone for 5 min. Then, the PDMS surfaces were immediately immersed into a 10% (v/v) solution of 3-APTMS in ultrapure water for 1 h. The PDMS surface was then washed three times with water and dried with argon for immediate use.

**Carboxyl-Amine Bonding**  
Solid substrates modified with TMS-EDTA were subjected to carbodiimide activation for 30 min, followed by a quick rinse with ultrapure water and dried with argon. The activated terminal carboxyl groups on the solid substrates were immediately brought into contact with terminal primary amino groups on 3-APTMS-modified PDMS surfaces and allowed to react at room temperature for 1 h (see Section 2.1.3.1).

5.3.1.6 Pressure Leak Tests

**Two Types of PDMS Bonding Strategies**  
The solid substrates (i.e., glass, gold-coated glass or 3-electrode substrates) were cleaned using the modified RCA cleaning method as described in Section 5.3.1.4. Two types of PDMS bonding methods were tested by conducting pressure leak tests: (1) carboxyl-amine strategy and (2) air-plasma strategy. First, PDMS cell chambers were bonded to the solid substrates using the carboxyl-amine bonding strategy as described in Section 5.3.1.5. After bringing the modified PDMS and the activated solid substrates into contact, irreversible bonding was observed within 1 h, but the pressure leak tests were conducted after 16 h. Second, the surfaces of both unmodified...
5.3. Applying TMS-EDTA to Construct Leak-Free PDMS-Based Electrochemical Cells

PDMS and clean solid substrate (i.e., glass, gold-coated glass or 3-electrode substrate) were exposed to air-plasma for 1 min and 15 sec and brought into contact to serve as control experiments.

**Supplies for Pressure Leak Tests**  All supplies were from commercial sources: green food-grade colour dye was from McCormick Canada (London, ON, Canada); plastic syringes (3 mL and 10 mL) were from Becton Dickinson (Mississauga, ON, Canada); dispensing tips (straight and right-angled 21 gauge and 22 gauge) with Luer lock attachment point were from Nordson EFD (East Providence, RI); plastic tubing (Tygon R-3603) was from Sigma-Aldrich (Oakville, ON, Canada); plastic (Tygon) microbore tubing (0.51 mm ID x 1.52 mm OD) was from Cole-Parmer Canada (Montreal, QC, Canada); and pressure sensor (HDIB002GUSMD8P5) was from First Sensor/Sensortecchnics (Mansfield, MA).

**Experimental Setup**  The experimental setup for pressure leak tests is shown in Figure 5.11. A 2 megapixel digital colour camera (A3250U, OMAX) with a 0.5X reduction (A3RDF50, OMAX) lens was mounted on a stereo zoom microscope (SZ6045, Olympus) and connected to a computer. OMAX TouView 3.7 was used to record images and videos of the pressure leak tests. Open-source hardware, Arduino ATmega328, was used to process the pressure sensor information and send the data to the computer. After placing the sample on the sample stage of the microscope, the syringe pump (KDS230, KD Scientific; Holliston, MA) was used to apply a continuous pressure to the syringe, which in turn applied a pressure to the solution-filled PDMS cell chamber.

The PDMS cell chamber of each sample was manually loaded by injecting green dye solution into the inlet at a low pressure. A 3-way valve was used to connect the 10 mL syringe and the pressure sensor with Tygon tubing. A third tubing (connecting the 3-way valve to the cell chamber inlet) was initially purged of air with ultrapure water using the syringe. Subsequently, the outlet of the cell chamber was blocked with a plug, while the inlet was connected to the 3-way valve by the third tubing (i.e., the water-filled tubing). After placing the connected device under the microscope, the syringe pump pushed ultrapure water from the syringe into the solution-filled cell chamber at a flow rate of 1 mL/min (while the pressure was monitored by the pressure sensor) until a leakage of the green dye was observed or the maximum pressure measurable was achieved. For samples that leaked, the pressure was recorded at the first sign of leakage (e.g., when the green dye emerges at either the PDMS–glass or PDMS–Au interface) as determined from the video images.
Figure 5.11: Setup used to evaluate the strength of the bond (via leak pressure tests) between PDMS and solid substrates (glass, gold, and mixed Au/glass substrates).
5.3. Applying TMS-EDTA to Construct Leak-Free PDMS-Based Electrochemical Cells

5.3.1.7 Electrodesorption of TMS-EDTA from Gold

Supply for Electrodesorption A commercial Ag/AgCl reference electrode (RE-5B) from BASi Inc. (West Lafayette, IN) was used as the silver/silver chloride electrode (SSCE) RE. The in-house fabricated Ag/AgCl RE was calibrated against the commercial SSCE RE. Hexaamminecobalt(III) chloride (CoHex) and sulfuric acid (ACS, 95.0-98.0%) were from Sigma-Aldrich (Oakville, ON, Canada). Potassium chloride and potassium hydroxide were from Fisher Scientific (Ottawa, ON, Canada).

Instrumentation Measurements were taken using either a PGSTAT30 potentiostat, a PGSTAT12 potentiostat, or a μAutolab potentiostat. NOVA (versions 1.10 and 1.11) software (Metrohm Autolab; Utrecht, Netherlands) was used to control the potentiostats, collect the data, and perform the data analysis. First, the PDMS-based 3-electrode device was bonded using the carboxyl-amine strategy as described in Section 5.3.1.5. The inlet of the PDMS cell chamber on the device was connected with tubing to the working solution of interest. The outlet tubing was initially connected to a syringe. By applying a suction pressure with the syringe, the working solution was drawn into the cell chamber. Subsequently, the outlet tubing was plugged with the in-house fabricated Ag/AgCl RE and filled with saturated chloride. The device was then connected to the potentiostat by DropSens μSTAT Cable Connector (DropSens; Asturias Spain). After inserting the connected device into a glass container, the glass opening was sealed by the Cable Connector. The container with the suspended PDMS-based device was deoxygenated with argon.

Electrodesorption The method to electrochemically desorb TMS-EDTA from the Au surface inside the PDMS-bonded 3-electrode device was developed. The cell chamber was initially filled with a solution of 750 μM CoHex (in a deoxygenated 100 mM phosphate buffer without chloride) and cyclic voltammetry (CV) measurements were conducted in an applied potential window of −0.5 V and +0.2 V. Then, the cell chamber was filled with 100 mM KOH (pH ~13). The electrode potential was held at −1.4 V for 0.5 sec, followed by holding the potential at open-circuit potential for 10 sec (repeated 200 times). The cell chamber was re-filled with the CoHex solution and CV was measured as before. Next, the cell chamber was filled with 1 M sulfuric acid and the potential was cycled between −0.15 V and +1.7 V. Finally, a CV of the CoHex solution was measured again.

For control experiments, a clean gold bead electrode was modified with aqueous 10% (v/v) TMS-EDTA solution for 2 h. Platinum was the CE and the commercial SSCE was the RE. The same surface cleaning protocols (i.e., pulsing to a negative potential in KOH and cycling in sulfuric acid) were applied.
Subsequently, the gold bead electrode was flamed with a butane torch and rinsed. CV measurements of the CoHex solution were conducted before and after each of the three cleaning protocols as before.

### 5.3.2 Results and Discussion

#### 5.3.2.1 Pressure Leak Tests with Bonded PDMS Cell Chambers

Pressure leak tests with PDMS cell chambers bonded to glass, gold, or mixed Au/glass substrates were conducted (an example of the experimental result is shown in Figure C.2 of Appendix C). The pressure sensor can detect up to 200 kPa (i.e., 2 bars or ~29 psi) of internal pressure (gauge) in the cell chamber. Prior to conducting the pressure leak tests, a hydrostatic pressure of 21 kPa was recorded. All of the pressures reported below (for samples that survived loading) have been subtracted by this value. Table 5.1 summarizes the results from these tests for air-plasma and carboxyl-amine bonding strategies.

<table>
<thead>
<tr>
<th>PDMS Bonding Strategy</th>
<th>Glass Substrate</th>
<th>Au Substrate</th>
<th>Mixed Au/Glass Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air-Plasma</td>
<td>&gt;179 kPa</td>
<td>On Loading</td>
<td>8±6 kPa</td>
</tr>
<tr>
<td>Carboxyl-Amine</td>
<td>146±6 kPa</td>
<td>38±9 kPa</td>
<td>50±5 kPa</td>
</tr>
</tbody>
</table>

For the air-plasma bonding strategy, the PDMS–glass devices did not leak (i.e., its leak pressure surpassed the pressure sensor’s detection limit). On the other hand, the bare Au substrates cleaned with air-plasma did not bond with plasma-treated PDMS chambers; solution started to leak upon loading (~3 kPa). For PDMS bonded to the substrate with both Au/glass, the solution only leaked via the PDMS–gold interface at the pressure of 8±6 kPa. These results are expected since the air-plasma method does not form a strong bond between PDMS and Au. Large variation in the measurements was observed due to weak PDMS–Au bond upon solution loading.

For the carboxyl-amine bonding strategy, PDMS–glass devices showed a leak pressure of 146±6 kPa. On the other hand, PDMS–gold devices showed a leak pressure of 38±9 kPa. For the mixed Au/glass substrate, solution also leaked via the PDMS–Au interface and a leak pressure of 50±5 kPa was obtained.

In general, the air-plasma bonding strategy creates a very strong bond for the PDMS–glass interface, but the bonding for the PDMS–gold interface is extremely weak. On the other hand, the carboxyl-amine bonding strategy creates a relatively weaker bond for the PDMS–glass interface, but the bonding for the PDMS–gold interface is improved. For the mixed Au/glass substrate, the carboxyl-amine bonding
5.3. Applying TMS-EDTA to Construct Leak-Free PDMS-Based Electrochemical Cells

Figure 5.12: (A) PDMS cell chamber bonded to the mixed Au/glass substrate using the air-plasma bonding strategy right after loading (i.e., at atmospheric pressure). (B) Initial leak pressure of the plasma-bonded device (i.e., 8 kPa). (C) Complete failure of the plasma-bonded device (i.e., 29 kPa). PDMS bonded to the 3-electrode substrate using the carboxyl-amine bonding strategy at (D) the atmospheric pressure after loading, (E) the leak pressure of plasma-bonded device (i.e., 8 kPa), and (F) the pressure of an initial solution leak (i.e., 49 kPa). Diameter of the cell chamber was 7.6 mm.

strategy creates a device that can withstand a fluidic pressure \( \sim 6X \) stronger than the air-plasma bonding strategy. Since the fluid pressure in microfluidic systems rarely exceeds \( \sim 5 \text{ psi} (\sim 34 \text{ kPa}) \),\textsuperscript{217} the seal obtained with this carboxyl-amine bonding strategy is adequate for most microfluidic applications (but may not be suitable for systems requiring high pressures).

Some representative images from the pressure leak tests of the 3-electrode devices are shown in Figure 5.12. Figures 5.12A and 5.12D show that upon loading of the solution, no leaks were observed for both plasma- and carboxyl-amine-bonded devices. At 8 kPa, the PDMS–gold interface of air-plasma-bonded device started to leak (Figure 5.12B), and the device failed completely at 29 kPa (Figure 5.12C). In contrast, the carboxyl-amine-bonded device remained leak-free (Figure 5.12E) at 8 kPa, and showed the indication of a leak at 49 kPa (Figure 5.12F). Figure 5.12F also shows the bulging of the PDMS cell chamber due to the increased fluidic pressure. These results demonstrate that the 3-electrode devices bonded using the carboxyl-amine strategy can create a strong seal around the PDMS cell chamber, primarily due to the increased PDMS–Au interaction.
5.3.2.2 Electrodesorption of TMS-EDTA from Gold

In Section 5.3.2.1, it has been demonstrated that the carboxyl-amine bonding strategy (i.e., using TMS-EDTA on mixed Au/glass substrate) can create a seal around the PDMS cell chamber. It is also evident that TMS-EDTA remains on the Au electrode after bonding (see Figure 1.2C). This layer may be suitable for the subsequent immobilization of biomolecules, as demonstrated in Section 3.3, for biosensing applications. However, this layer may also compromise electrochemical measurements. For example, the TMS-EDTA layer may impede the electron transfer between electroactive species in solution and the electrode surface. Consequently, a reproducible surface cleaning method needs to be developed in order to remotely remove the adsorbed TMS-EDTA and to prepare a clean Au surface (in the device after bonding) for effective electrochemical sensing. First, the electrochemistry of partially blocked electrodes needs to be reviewed.

**Electrochemistry of Partially Blocked Electrodes**  By adding a redox active species to the electrolyte, the quality of an adsorbed layer on the electrode surface can be indirectly inferred. For example, a bare electrode will show the faradaic currents that correspond to the oxidation or reduction of the particular species on the whole surface. When the electrode is covered with adsorbate molecules (e.g., an organic layer), this layer may act as a barrier against the electron transfer (redox) of the electroactive species. Two results can occur: total blocking or partial blocking (i.e., slowing down) of the electron transfer. This approach is commonly used as a general indicator of the cleanliness of the electrode surface.\(^{218}\)

For example, the oxidation and reduction of hexacyanoferrate(III) were studied by linear potential sweep and cyclic voltammetry (CV) on Au disk electrodes partially covered with photoresist.\(^{219}\) It was assumed that some sites were active and that some sites were inactive. It was observed that at a higher degree of coverage of the inactive photoresist (from \(\theta = 0.552\) to 0.815), the separation between the oxidation and reduction peak potentials was increased. Subsequently, a model for charge transfer at partially blocked surfaces was proposed.\(^{220}\) It was found that when the fractional coverage was small (i.e., not too close to maximum coverage), the electrochemical response was the same as the one for an unblocked electrode, but with a decreased apparent rate constant of electron transfer. The apparent electron transfer slowed when the coverage increased. The current peak in the CV also decreased with a further increase in adsorbate coverage. This reduction was due to decreased amount of electron transfer and smaller available area.

In another report, the potential of the reduction peak was used an indicator of the cleanliness of
5.3. Applying TMS-EDTA to Construct Leak-Free PDMS-Based Electrochemical Cells

When a layer is adsorbed on the Au electrode and it acts like a barrier to electron transfer, the potential of the reduction peak is more negative than for a bare electrode. In addition, it was observed that some electron transfer still occurred, due to defects in the adsorbed layer. Nevertheless, by monitoring shifts in the potential of the reduction peak, the extent of adsorbate (or contaminant) coverage of the electrode can be inferred.

**Electrodesorption and CoHex** There are many methods available for cleaning gold electrodes for electrochemical detection applications. However, the Au WE of the carboxyl-amine bonded devices cannot be directly accessed due to the enclosed PDMS cell chamber (see Figure 1.1B). For example, it would be difficult to employ the air-plasma or UV-ozone cleaning strategy. In addition, the solution used needs to be carefully chosen, since PDMS swells in a number of solvents (see Section 1.1). Therefore, several electrochemical cleaning methods were assessed to determine their ability to remove the adsorbed TMS-EDTA layer (detailed in Section 5.3.1.7).

The differential capacitance studies from Chapter 4 indicated that TMS-EDTA adsorption on Au is potential dependent. In particular, TMS-EDTA adsorbs minimally at highly negative potentials (\(-1.1\) V). Therefore, the effects of applying negative potentials to desorb TMS-EDTA were initially studied. Subsequently, a standard acid cycling cleaning method was also performed. Control experiments were conducted using a gold bead modified with TMS-EDTA for 2 h. In addition to the two cleaning methods described, flame annealing was used as the third cleaning method for the Au bead.

The reduction of hexaamminecobalt(III) (or CoHex) has been widely studied and has been found to be irreversible. Due to its slow electron transfer kinetics, CoHex can be used as an indicator of electrode cleanliness to test various surface cleaning strategies.

The cleanliness of the Au WE inside the PDMS cell chamber was monitored with CV scans of the aqueous 750 \(\mu\)M CoHex solution, by carefully interrogating its reduction peak potential \((E_P)\). The highly irreversible reduction reaction of CoHex results in a peak potential that is very sensitive to the cleanliness of the electrode surface. When compared to a bare electrode surface, the reduction \(E_P\) shifts to more negative values in the presence of a partially blocked electrode, which reduces the electron transfer rate constant. Figure 5.13A presents the CV scans of the gold bead electrode, and Figure 5.13B presents the scans of the carboxyl-amine bonded device.

From Figure 5.13B, the CoHex reduction \(E_P\) of the bonded device (activated and bonded using the NHS/EDC coupling chemistry) has a reduction wave at \(-0.3\) V (solid line). After repeatedly pulsing to the negative potential in KOH, the reduction \(E_P\) shifts to \(-0.13\) V (dashed curve). This shift in the
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Figure 5.13: CoHex cyclic voltammograms of TMS-EDTA covered Au. (A) Three different methods to clean a gold bead are shown: pulsing to negative potentials (-1.4 V) in pH 13 solution, CV in 1 M sulfuric acid, and flaming. (B) Two different methods to clean the Au within a bonded PDMS device are shown: pulsing to negatives potentials in basic solution and performing CV in acidic solution. Potential scan rate = 20 mV/s.
5.4 Conclusions

reduction peak potential indicates that the electrode surface is becoming clean. Subsequent cleaning by cycling in sulfuric acid yields an electrode surface that shows a CoHex reduction $E_p$ at $\sim -0.02$ V. This additional positive shift in the reduction peak potential indicates an improvement in the electrode cleanliness.

For comparison, Figure 5.13A shows the CoHex CVs for a TMS-EDTA-coated gold bead electrode cleaned using the same strategies as the bonded device. In general, the Au bead shows CoHex reduction peaks that are less negative than the bonded device. This result may be due to the lack of NHS/EDC activation step used for PDMS bonding. Nevertheless, the reduction $E_p$ values still indicate shifts in the positive direction after the hydroxide and sulfuric acid cleaning methods. Cleaning the Au bead by flaming yields a minor change in the $E_p$, showing that the overall cleaning procedure is quite effective.

These results demonstrate that cleaning the bonded device using sequential basic and acidic solutions yields a Au surface that is comparable to the flamed bead electrode. Pressure leak tests were also performed on the electrochemically cleaned devices. The leak pressures measured were comparable to the ones observed for devices without electrochemical cleaning ($\sim 50$ kPa). This result is expected because electrodesorption is a displacement (or replacement) process. At the PDMS–gold interface that is bonded, solvent molecules (e.g., water) cannot access the gold surface. Therefore, the TMS-EDTA layer on Au still remains strongly bonded to PDMS and creates a tight seal around the PDMS cell chamber. In other words, the strong PDMS–gold bond has been retained after this Au surface cleaning method.

5.4 Conclusions

First, TMS-EDTA was successfully applied to create bonded PDMS–glass and PDMS–gold structures using a novel carboxyl-amine bonding chemistry. Shear tests indicate that strong bonds, comparable to or better than the existing bonding techniques, can be achieved at room temperature and under mild conditions.

Second, pressure leak tests were performed on the bonded 3-electrode devices, in order to obtain a more realistic measure of the bond strength under aqueous conditions. The bonding at the PDMS–Au interface resulted in a 6-fold increase in fluidic leak pressure than air-plasma-bonded devices.

Finally, a method to electrochemically clean the Au WE inside the PDMS cell chamber was developed. Treatment by sequential basic and acidic solutions yields a clean Au surface that is comparable
to a flamed Au bead electrode.

Therefore, TMS-EDTA has been successfully applied to create leak-free PDMS-based electrochemical cells, suitable for subsequent sensing or biosensing applications.
Chapter 6

Concluding Remarks

The use of glass substrates partially patterned with a microarray of gold spots is often required for microfluidic surface plasmon resonance (SPR) imaging applications. It is not trivial to form a strong bond between polydimethylsiloxane (PDMS) and glass while maintaining a self-assembled monolayer (SAM) on Au (inside the PDMS cell chamber) for the covalent immobilization of biomolecules. On the other hand, glass substrates decorated with sputtered Au electrodes are often used for microfluidic electrochemical devices. During analysis, an aqueous solution of analyte(s) is injected into the PDMS cell chamber. However, without treating the gold electrodes, leaks can occur at the PDMS–Au interface, which can severely impact the device performance. Therefore, studying substances that can self-organize onto both glass and gold surfaces is required to address some of these challenges and to enable the development of new devices for advanced biosensing and sensing applications.

In this thesis, the adsorption of $N$-[(3-trimethoxysilyl)propyl]ethylene-diamine triacetic acid (or TMS-EDTA) on Au has been characterized and applied to construct leak-free PDMS-based electrochemical cells. In this Chapter, a summary of the key research findings will first be recapitulated. Second, major conclusions will be outlined. Finally, potential future work will be discussed.

6.1 Summary

In Chapter 3, the adsorption of TMS-EDTA on Au has been characterized using four complementary surface analysis techniques: water contact angle, X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), and infrared (IR) spectroscopy. The adsorption of a well-known alkanethiol (11-MUA) on Au has also been studied to help with the data analysis.

Water contact angle measurements indicate that TMS-EDTA can chemically modify the gold surface. The resulting surface, which is similar to 11-MUA-modified Au, is more hydrophilic than bare Au (Figure 3.2). Since the orientation of 11-MUA on Au is well defined (i.e., thiol interacts with Au to create terminating carboxyl groups), these results suggest that TMS-EDTA may also have some terminating carboxyl
6.1. Summary

groups on Au. XPS was used to confirm the presence of carboxyl groups. The Au surfaces modified with either 11-MUA or TMS-EDTA show an increase in the quantity of carbon (C) atoms in comparison to the bare gold surface. In particular, the high resolution spectrum shows an increase in the C1s peak at 284.7 eV with an additional shoulder peak at 288 eV, which indicates the presence of carboxyl groups on TMS-EDTA-coated Au (Figure 3.3).

AFM imaging was used to provide topographical and thickness information about TMS-EDTA-modified Au. The AFM results strongly suggest that a thin and uniform coverage of TMS-EDTA on the gold surface is obtained (Figure 3.4). The thickness of the TMS-EDTA layer on Au has been determined to be similar to the 11-MUA layer on Au (Figure 3.8), which is known to be of monolayer thickness. Next, IR studies were conducted to provide additional surface chemical information about TMS-EDTA adsorption on Au under ambient conditions. In addition to 11-MUA, an amino-silane (3-APTMS) was also studied to provide some insight into the extent of siloxane cross-linking (Figure 3.10). The TMS-EDTA-coated gold surface shows a significant presence of carboxyl groups (similar to 11-MUA-coated Au) and a lack of polysiloxane formation (in contrast to 3-APTMS-coated Au).

Subsequently, surface plasmon resonance (SPR) was used to provide some information regarding the orientation of TMS-EDTA on Au. SPR experiments were conducted to demonstrate the feasibility of using TMS-EDTA-modified Au for coupling biomolecules containing primary amino groups and to determine its relative stability (compared to bare Au) when a stringent wash buffer was used. The SPR results indicate that free carboxyl groups on TMS-EDTA-modified Au are available for the immobilization of streptavidin, after carbodiimide activation (i.e., using NHS/EDC activation). Furthermore, this unconventional surface chemistry can withstand stringent regeneration conditions (Figure 3.12).

In Chapter 4, the Gibbs free energies of TMS-EDTA adsorption on Au have been quantified using electrochemical methods. The TMS-EDTA-modified Au bead electrode appeared stable in DiffCap Buffer (100 mM phosphate, 500 mM KCl, and 65 mM KOH, pH 11.6). In other words, the buffer electrolyte did not appear to significantly displace the TMS-EDTA layer over the course of about 2 hours (Figure 4.3). The DiffCap Buffer was carefully formulated to ensure that the pH and the ionic concentrations remained relatively constant during all experiments (Figure 4.5).

Electrochemical differential capacitance measurements reveal that TMS-EDTA adsorption on Au is potential-dependent (Figure 4.6). Due to pseudocapacitance features, the adsorption isotherm data were analyzed in a narrow potential window (Figure 4.7). The Langmuir and Frumkin adsorption isotherms seem to adequately fit the data at more negative potentials. However, at more positive potentials, the Frumkin isotherm better describes the experimental data (Figure 4.8). These results are expected be-
6.1. Summary

cause TMS-EDTA is negatively charged. At more positive potentials, there is more lateral interaction among the adsorbed TMS-EDTA molecules, which is accounted for by the Frumkin adsorption isotherm.

For potentials between $-0.5$ and $0.2$ V, the potential-dependent Gibbs free energies of adsorption were determined to be $\sim -20$ to $-30$ kJ/mol in the complex electrolyte solution (Figure 4.9). In this complex system, adsorption is a displacement process (e.g., displacement of water molecules, as well as phosphate, hydroxide, and chloride ions); therefore, the adsorption free energies of TMS-EDTA on Au in a non-adsorbing electrolyte are likely more favorable than the values presented here. The open-circuit potential (OCP) was measured to be $\sim 0.05$ V and $\sim -0.025$ V (vs SCE) in the presence and absence of $O_2$, respectively (Figure 4.1). Therefore, the free energies of adsorption of TMS-EDTA on Au around these potentials are of particular importance (since typical surface modification is done in the presence of oxygen). These results suggest that at more positive potentials, adsorption is more thermodynamically favorable. In contrast, at highly negative potentials ($\sim -1.1$ V), TMS-EDTA adsorbs minimally onto the Au surface.

In Chapter 5, PDMS slabs were chemically modified with 3-APTMS to form terminating primary amino groups (Figure 5.3). To demonstrate the feasibility of a novel carboxyl-amine bonding strategy, irreversible bonding was first achieved between PDMS and 11-MUA-modified Au slides (see Figure 5.4). Similarly, TMS-EDTA was successfully applied to create bonded PDMS–glass and PDMS–gold structures using the same bonding strategy. This dual functionality (i.e., achieving PDMS bonding with both glass and gold) is not achievable by 11-MUA since it can only chemically modify Au surfaces. Shear tests indicate that strong bonds, comparable to (or stronger than) existing bonding techniques, can be achieved at room temperature and under mild conditions (Figure 5.5).

Subsequently, improved surface preparation procedures were developed to fabricate robust PDMS-based electrochemical devices. TMS-EDTA was applied to chemically modify a glass substrate partially sputtered with Au electrodes and to create a seal around the PDMS cell chamber using the carboxyl-amine bonding method (see Figure 5.6). Pressure leak tests were performed on the bonded 3-electrode devices, in order to obtain a more realistic measure of the bond strength under aqueous conditions. Plasma-bonded structures were fabricated and served as controls. The results show that the PDMS–glass bond (146±6 kPa) is stronger than the PDMS–gold bond (38±9 kPa) using the carboxyl-amine strategy (see Table 5.1). For the 3-electrode substrate, the increased bonding at the PDMS–Au interface resulted in a 6-fold increase in fluidic leak pressure (50±5 kPa) than air-plasma-bonded devices (8±6 kPa), which was shown to fail immediately upon loading of the aqueous sample (Figure 5.12).
6.2 Conclusions

Finally, the behavior of TMS-EDTA on Au electrode at highly negative potentials (i.e., minimal adsorption) was used as an advantage for preparing electrochemical sensors. After bonding the 3-electrode substrate with PDMS using the carboxyl-amine strategy, a method to electrochemically clean the Au WE inside the PDMS cell chamber was developed. Electrochemical cleaning using sequential basic and acidic solutions yields a clean Au surface that is comparable to the standard flamed Au bead electrode (Figure 5.13). Most importantly, the sealing around the PDMS cell chamber was still maintained after electrochemical cleaning. Therefore, the fundamental knowledge obtained about TMS-EDTA adsorption on Au has been successfully applied to create leak-free PDMS-based electrochemical cells, suitable for a variety of sensing and/or biosensing applications. This fabrication technology will allow the collection of more reliable data and enable a multiple use of the device.

6.2 Conclusions

Demonstrated in this thesis is the characterization of the the adsorption of a carboxylated silane (TMS-EDTA) on Au using many surface analysis techniques, resulting in a better understanding of this adsorption and future applications of this surface modification. The four complementary surface analysis techniques show that TMS-EDTA can be used to chemically modify the gold surface, and that the carboxyl groups are present on Au. Furthermore, a uniform surface of monolayer-thickness is formed with little siloxane cross-linking. This result is similar to alkylsilanes (i.e., monolayers) but different from the amino-silane (i.e., extensively cross-linked multilayers) on Au (see Section 2.2.5). The presence of three negatively charged carboxylates may contribute to this effect (i.e., the repulsion among the TMS-EDTA molecules may prevent extensive siloxane cross-linking).

The results from these initial experiments do not provide enough information about the orientation of TMS-EDTA adsorption on Au. The orientation of TMS-EDTA on Au has an effect on its utility in sensor fabrication and biosensing applications. Subsequently, SPR results show that when compared to physical immobilization (i.e., bare gold surface), TMS-EDTA plays a crucial role in capturing the large proteins onto the Au surface using the carboxyl-amine chemistry. These results suggest that at least some carboxylates are oriented away from the gold surface and are available for reaction.

The Gibbs free energy of TMS-EDTA adsorption on Au is also important for sensor fabrication and biosensing applications. Subsequently, electrochemical differential capacitance was used to quantify the potential-dependent Gibbs free energies of TMS-EDTA adsorption on Au in a complex electrolyte environment. The free energies of adsorption were determined to be $\sim -20$ to $-30$ kJ/mol for potentials
6.2. Conclusions

between −0.5 and 0.2 V, respectively. It is important to keep in mind that the adsorption of TMS-EDTA has been interrogated with the addition of molecules and ions (e.g., water molecules and phosphate, hydroxide, and chloride ions) that also compete for the Au surface. Therefore, the TMS-EDTA adsorption is likely more energetically favourable than the values presented here. More importantly, at more positive potentials, the adsorption is more favorable (accompanied by increased repulsive lateral interaction). At highly negative potentials, TMS-EDTA adsorbs minimally onto the Au electrode. This result suggests that the layer of TMS-EDTA may be electrochemically removed to recover a clean Au surface.

It is important to note that the change in Gibbs free energy depends on the entropy change and the adsorbate/adsorbent bond strength (enthalpy change). If we assume that the entropy change of this system is negligible (due to the high electrolyte concentrations in the working buffer), then the apparent Gibbs free energies of adsorption calculated may provide a rough estimate of the enthalpy change (i.e., the strength of TMS-EDTA adsorption on Au). The results indicate that the typical room temperature surface modification of Au using aqueous solution of 10% TMS-EDTA (v/v) creates a strongly adsorbed layer suitable for subsequent biosensor and sensor applications.

The unique ability of TMS-EDTA to bond PDMS with both glass and Au surfaces has been demonstrated. Shear tests show that the bond strength of PDMS–Au structure is slightly stronger than the bond strength of PDMS–glass structure. However, pressure leak tests show that the PDMS–glass structure produced stronger bonds. This discrepancy may be due to the increased friction observed for shear tests (as well as the inability to observe the different modes of failure using this method). Moreover, different solvents were used to prepare the 3-APTMS solution (i.e., ethanol for shear tests and water for pressure leak tests) for PDMS modification. Nevertheless, since the silane-coupling chemistry for glass is well understood, it is expected that TMS-EDTA reacts with glass to create a surface with three terminating carboxyl groups oriented away from the glass. Since the PDMS–gold bond from pressure leak tests is weaker, it is likely that the TMS-EDTA modification of Au creates a surface with some carboxylates that are oriented away from the Au and are available for coupling to primary amino groups.

Admittedly, the bond strength produced by the carboxyl-amine bonding strategy is not as strong as the air-plasma bonding method for PDMS–glass structures. However, the PDMS–gold bonding by TMS-EDTA resulted in a bond that can withstand 6X the pressure than the air-plasma strategy. Most importantly, the bond strength is above the maximum pressures typically encountered in microfluidic applications. In the literature, bonding between PDMS and gold may be achieved when additional clean-room time is used (see Section 1.1). However, the carboxyl-amine bonding strategy is the best method to bond a hybrid glass/gold substrate to PDMS at room temperature under ambient conditions, without
the use of expensive equipment.

Finally, due to its monolayer-thickness and little siloxane cross-linking, TMS-EDTA layer on Au WE inside the PDMS cell chamber can be remotely cleaned by applying electrochemical methods. The proposed device fabrication protocol and the WE cleaning strategy can be used to create leak-free PDMS-based electrochemical cells, suitable for a wide range of electrochemical and/or optical sensing/biosensing applications.

6.3 Future Work

Two types of problems have been identified in this thesis: (1) the inability to form strong PDMS−glass bonds while forming a functional layer on the Au surface (inside the cell chamber) for surface plasmon resonance imaging (SPRi) applications, and (2) the inability to form strong PDMS−glass and PDMS−gold bonds for creating leak-free electrochemical cells (see Section 1.1). TMS-EDTA has been demonstrated to address these challenges due to its unique ability to chemically functionalize both glass and Au surfaces. For SPRi applications, it is desirable for the TMS-EDTA layer to remain on the Au spot inside the carboxyl-amine bonded cell chamber in order to covalently immobilize biomolecules. For electrochemical devices, it is desirable for the TMS-EDTA layer on WE to be electrochemically removed for subsequent sensing applications. Evidently, the strategy employed is highly dependent on the application involved. For example, other researchers have applied this novel carboxyl-amine bonding strategy using TMS-EDTA to construct an integrated microfluidic biosensor.226 TMS-EDTA-modified Au was used for both PDMS bonding and covalent immobilization of antibodies for the detection of hormonal compounds. This result confirms the utility of TMS-EDTA in both biosensing and biosensor fabrication.

Many steps of the device fabrication process can be further investigated and/or optimized. Some ideas are presented below:

1. Throughout this thesis, the NHS/EDC activation time and concentrations have been kept constant. The NHS/EDC activation time198 and concentrations227 may have an effect on the efficiency of the carboxyl-amine coupling chemistry. Therefore, more studies could be performed to determine the optimal activation conditions for protein immobilization and bonding with 3-APTMS-modified PDMS.

2. The effects of removing the NHS/EDC activation step may also be investigated (e.g., to determine the extent of specific and non-specific immobilization of biomolecules).
3. In this thesis, PM-IRRAS experiments were conducted ex-situ. It would be interesting to combine electrochemistry with PM-IRRAS experiments (i.e., polarizing the Au surface in an electrochemical cell).

4. The effect(s) of increased temperature and pressure on the bond efficiency and the bond strength can be tested. Furthermore, sterilization is often required for some biological studies. It would be useful to test the bond strength after autoclaving the bonded device.

5. Aqueous and ethanolic 10% 3-APTMS (v/v) solutions have been used to chemically modify PDMS surfaces. Aqueous solution was used to reduce PDMS swelling. From the results obtained, aqueous solution seems to produce stronger bonds for glass slides, and ethanolic solution seems to produce stronger bonds for gold slides. Additional studies could be performed to determine the effect(s) of different solvents on the quality of the amino-silane layer produced and the subsequent bonding strength obtained.

6. The 3-electrode devices have different electrode widths (see Figure 5.10) that are bonded to PDMS. In general, it was noticed that leaks occurred more often for wider electrode widths. This results suggest that electrode width has an effect on the leak pressure of a particular electrochemical system. Since micrometre-scale electrodes are becoming more popular, solution leaks may not be a problem for ultrathin electrode widths. Pressure leak tests could be performed on different channel widths to determine this effect.

7. The glass and PDMS surfaces have been cleaned using UV-ozone for most of the surface modifications. The use of air-plasma to clean the surface and to prepare the surface for silane functionalization may be studied.

8. Currently, only a small range of potentials has been investigated for electrochemical sensing applications (Figure 5.13). Additional studies could be performed to determine the effects of increasing the potential range, which may expand the utility of this device fabrication strategy.

9. After bonding the PDMS cell chambers with the 3-electrode substrate modified with TMS-EDTA, it is evident that primary amino groups are still present on the PDMS surface. The primary amino groups make the PDMS surface less hydrophobic and more biocompatible. However, depending on the biosensing/sensing application pursued, the effects of primary amino groups on probe immobilization and biomolecular interaction need to be carefully analyzed. If necessary, the
primary amino groups may be reversibly blocked\textsuperscript{230} when the application demands a non-reactive PDMS surface.

Lastly, the reaction of TMS-EDTA with other materials (e.g., silver, platinum, copper, iron, aluminum, and polymer-based materials) can be explored to further expand the application space of this carboxyl-amine bonding strategy with PDMS. The adsorption of other functional silanes on Au and other materials can also be investigated. It is the belief of the author that the full potential of silanes has not been fully realized. More studies need to be performed in order to test this hypothesis.
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The aqueous 10% TMS-EDTA (v/v) solution is basic as indicated by Figure A.1 (prior to the addition of 1M HCl). The titration curve for adding 1M HCl to 10% TMS-EDTA (v/v) solution is shown. The derivative \( \frac{\Delta pH}{\Delta V} \) is plotted against average volume. The pK\(_{a}\) values of oxide-based nanoparticles modified with TMS-EDTA have been determined to be 4.17, 6.89, and 10.00.\(^{1,2}\) It is known that the pK\(_{a}\) values are different for solution-based and surface-adsorbed 11-MUA layer.\(^{23,24}\) Therefore, it is not surprising that the pK\(_{a}\) values are somewhat different. Nevertheless, at highly basic pH values (i.e., 11.6), the TMS-EDTA molecules are mostly deprotonated (i.e., negatively charged).

![Titration Curve of TMS-EDTA](image)

Figure A.1: Titration curve for adding 1M HCl to 10% TMS-EDTA (v/v) solution.
Appendix B

Appendix for Chapter 4

To ensure high reproducibility and cleanliness of the three-electrode electrochemical setup, full range CV of the DiffCap buffer (see Figure B.1) was obtained before the addition of any TMS-EDTA. Subsequently, double layer CV was obtained before and after the addition of bulk TMS-EDTA concentrations for all experiments (see Figure B.2 for two examples).

Once the system has been determined to be clean, electrochemical differential capacitance measurements were conducted. A total of 19 TMS-EDTA concentrations were investigated: Buffer (0 μM), 1 μM, 2 μM, 5 μM, 10 μM, 20 μM, 50 μM, 100 μM, 200 μM, 1 mM, 2 mM, 5 mM, 10 mM, 20 mM, 50 mM, 100 mM, 150 mM, and 200 mM. At each TMS-EDTA concentration, a total of 19 values of potential of interest (POI) were examined: −1.1, −1.0, −0.9, −0.8, −0.7, −0.6, −0.5, −0.45, −0.4, −0.35, −0.3, −0.25, −0.2, −0.15, −0.10, −0.05, 0, 0.1, and 0.2 V.

A portion of the algorithm used to collect the data (using NOVA 1.8 software) at a particular POI is shown in Table B.1. This algorithm was repeated for all 19 potentials from −1.1 to 0.2 V for a constant TMS-EDTA concentration. The potential-dependent capacitance measured at a particular TMS-EDTA concentration took about 2 hours and 40 minutes to complete. This process was repeated for all 19 concentrations, with triplicates for lower TMS-EDTA concentrations.

The raw differential capacitance data for DiffCap Buffer and some representative TMS-EDTA concentrations are shown in Figure B.3 to Figure B.9. Briefly, capacitance was measured for a range of 19 potentials (starting from −1.1 V and stepping positively to 0.2 V). At each potential, stirring was turned on for 60 s and turned off prior to measuring the capacitance (repeated six times). After 6 min of stirring, the potential-dependent adsorption of TMS-EDTA on Au was assumed to have reached an equilibrium (as determined by the 200 mM TMS-EDTA raw differential capacitance data).

The area of the Au bead electrode was estimated using the method based on Shepherd et al.’s study:

1. Capacitance curves of 50 mM Perchlorate Buffer (pH 12) and DiffCap Buffer (pH 12) were obtained (see Figure B.10).
Figure B.1: Full range CV of the DiffCap buffer was obtained.

Table B.1: The algorithm used to collect the electrochemical differential capacitance measurements (using NOVA 1.8 software) at one potential of interest (POI).

<table>
<thead>
<tr>
<th>Potential (V)</th>
<th>Time (s)</th>
<th>Stirrer</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1.1</td>
<td>30</td>
<td>Off</td>
<td>Desorption to ensure same initial conditions</td>
</tr>
<tr>
<td>POI</td>
<td>60</td>
<td>On</td>
<td>Adsorption with stirring at POI (1 min total)</td>
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<td>POI</td>
<td>10</td>
<td>Off</td>
<td>Allowing system to reach equilibrium</td>
</tr>
<tr>
<td>POI</td>
<td>10</td>
<td>Off</td>
<td>Measuring 200 data points (at 0.05 s intervals)</td>
</tr>
<tr>
<td>POI</td>
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<td>Adsorption with stirring at POI (2 min total)</td>
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<td>POI</td>
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<td>POI</td>
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<td>POI</td>
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<td>Off</td>
<td>Measuring 200 data points (at 0.05 s intervals)</td>
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<td>POI</td>
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<td>Off</td>
<td>Measuring 200 data points (at 0.05 s intervals)</td>
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<tr>
<td>POI</td>
<td>60</td>
<td>On</td>
<td>Adsorption with stirring at POI (5 min total)</td>
</tr>
<tr>
<td>POI</td>
<td>10</td>
<td>Off</td>
<td>Allowing system to reach equilibrium</td>
</tr>
<tr>
<td>POI</td>
<td>10</td>
<td>Off</td>
<td>Measuring 200 data points (at 0.05 s intervals)</td>
</tr>
<tr>
<td>POI</td>
<td>60</td>
<td>On</td>
<td>Adsorption with stirring at POI (6 min total)</td>
</tr>
<tr>
<td>POI</td>
<td>10</td>
<td>Off</td>
<td>Allowing system to reach equilibrium</td>
</tr>
<tr>
<td>POI</td>
<td>10</td>
<td>Off</td>
<td>Measuring 200 data points (at 0.05 s intervals)</td>
</tr>
</tbody>
</table>
Figure B.2: Double layer CV of the DiffCap buffer was obtained before and after the addition of bulk $TMS-EDTA$ concentrations for all experiments. Two $TMS-EDTA$ concentrations (20 $\mu$M and 20 mM) are shown.

Figure B.3: Raw differential capacitance data of DiffCap buffer.
Appendix B. Appendix for Chapter 4

Figure B.4: Raw differential capacitance data of 2 μM TMS-EDTA.

Figure B.5: Raw differential capacitance data of 20 μM TMS-EDTA.
Figure B.6: Raw differential capacitance data of 200 μM TMS-EDTA.

Figure B.7: Raw differential capacitance data of 2 mM TMS-EDTA.
Figure B.8: Raw differential capacitance data of 20 mM TMS-EDTA.

Figure B.9: Raw differential capacitance data of 200 mM TMS-EDTA.
Figure B.10: Capacitance curves of 50 mM Perchlorate Buffer (solid line) and DiffCap Buffer (dotted line) at pH 12 are shown. These curves were used to estimate the area of the Au bead in DiffCap Buffer.

2. The raw capacitance value of Perchlorate Buffer at $-0.9 \text{ V}$ was determined to be $\sim 4.26 \mu F$.

3. The capacitance value from Step (2) was divided by $17 \mu F/cm^2$ (a value proposed to be constant for the negative potentials of Perchlorate Buffer) in order to obtain an estimate of the area of the Au bead electrode.

4. An electrode area of $\sim 0.25 \text{ cm}^2$ was determined.

The adsorption of TMS-EDTA onto Au electrode in DiffCap Buffer, fit with Langmuir (dotted line) and Frumkin (solid line) isotherms, for applied electrode potentials between $-1.1$ and $-0.6$ are shown in Figures B.11 to Figure B.16. Although the data fitting converged to a numerical value for these potentials, the surface coverage data shown Figure 4.7A reveal that pseudocapacitance features adversely affect the data analysis. Therefore, the analysis was restricted to a range of potentials between $-0.5$ to $0.2 \text{ V}$.

Finally, the $R^2$ values from the Langmuir and Frumkin fittings are shown in Figures B.17 and Figure B.18.
Figure B.11: Adsorption of TMS-EDTA onto Au electrode in DiffCap Buffer, fit with Langmuir (dotted line) and Frumkin (solid line) isotherms, at applied electrode potentials of $-1.1 \, \text{V}$.

Figure B.12: Adsorption of TMS-EDTA onto Au electrode in DiffCap Buffer, fit with Langmuir (dotted line) and Frumkin (solid line) isotherms, at applied electrode potentials of $-1.0 \, \text{V}$.
Figure B.13: Adsorption of TMS-EDTA onto Au electrode in DiffCap Buffer, fit with Langmuir (dotted line) and Frumkin (solid line) isotherms, at applied electrode potentials of $-0.9$ V.

Figure B.14: Adsorption of TMS-EDTA onto Au electrode in DiffCap Buffer, fit with Langmuir (dotted line) and Frumkin (solid line) isotherms, at applied electrode potentials of $-0.8$ V.
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Figure B.15: Adsorption of TMS-EDTA onto Au electrode in DiffCap Buffer, fit with Langmuir (dotted line) and Frumkin (solid line) isotherms, at applied electrode potentials of $-0.7 \text{ V}$.

Figure B.16: Adsorption of TMS-EDTA onto Au electrode in DiffCap Buffer, fit with Langmuir (dotted line) and Frumkin (solid line) isotherms, at applied electrode potentials of $-0.6 \text{ V}$.
Figure B.17: The potential-dependent $R^2$ values from the Langmuir non-linear least squares fitting.

Figure B.18: The potential-dependent $R^2$ values from the Frumkin non-linear least squares fitting.
Appendix C

Appendix for Chapter 5

Three elastic failure curves (from three different samples) were obtained for each of the bonding method using shear tests, and standard deviation was calculated using these values. The force values were then converted to pressures by dividing the PDMS–substrate contact area. Table C.1 shows the results from shear tests of glass–PDMS bonding strategies.

Table C.1: Results from shear tests of glass–PDMS bonding strategies.

<table>
<thead>
<tr>
<th>Glass Substrate Modification</th>
<th>PDMS Modification</th>
<th>Force Recorded (N)</th>
<th>Bond Strength (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV-Ozone</td>
<td>UV-Ozone</td>
<td>87.4±10</td>
<td>271.2±30.9</td>
</tr>
<tr>
<td>Oxygen Plasma</td>
<td>Oxygen Plasma</td>
<td>149.1±12.5</td>
<td>462.2±38.3</td>
</tr>
<tr>
<td>TMS-EDTA</td>
<td>3-APTMS</td>
<td>92.5±5.6</td>
<td>286.6±17.2</td>
</tr>
<tr>
<td>BTMSE</td>
<td>UV-Ozone</td>
<td>163.5±21.9</td>
<td>506.9±67.9</td>
</tr>
</tbody>
</table>

Table C.2 shows the results from shear tests of gold–PDMS bonding strategies.

Table C.2: Results from shear tests of gold–PDMS bonding strategies.

<table>
<thead>
<tr>
<th>Gold Substrate Modification</th>
<th>PDMS Modification</th>
<th>Force Recorded (N)</th>
<th>Bond Strength (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-MPTMS</td>
<td>UV-Ozone</td>
<td>122.7±21.1</td>
<td>280.2±65.4</td>
</tr>
<tr>
<td>11-MUA</td>
<td>3-APTMS</td>
<td>103.1±14.6</td>
<td>319.7±45.3</td>
</tr>
<tr>
<td>TMS-EDTA</td>
<td>3-APTMS</td>
<td>104.9±6.3</td>
<td>325.3±19.4</td>
</tr>
</tbody>
</table>

Additional control experiments were conducted by manual peel tests to determine the importance of forming both terminal carboxyl groups and terminal primary amino groups on solid substrate (i.e., glass or gold) and PDMS, respectively. Table C.3 shows the results from these manual peel tests.

A schematic of the setup for shear tests is shown in Figure C.1. Since it was difficult to determine the different modes of failure from shear test results, pressure leak tests were conducted on devices with PDMS cell chambers bonded to 3-electrode substrates to obtain a more realistic measure of the bond strength under aqueous conditions. An example of the pressure leak test experiment is shown in Figure C.2. Each of the three electrodes failed at different pressures. The sharp drop in pressure near the end indicated that the solution had leaked beyond the PDMS–Au interface.
Table C.3: Results from manual peel tests of gold–PDMS bonding strategies.

<table>
<thead>
<tr>
<th>Gold Substrate Modification</th>
<th>PDMS Modification</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV-Ozone</td>
<td>UV-Ozone</td>
<td>No bonding observed</td>
</tr>
<tr>
<td>UV-Ozone</td>
<td>3-APTMS</td>
<td>No bonding observed</td>
</tr>
<tr>
<td>11-MUA</td>
<td>UV-Ozone</td>
<td>No bonding observed</td>
</tr>
<tr>
<td>TMS-EDTA</td>
<td>UV-Ozone</td>
<td>No bonding observed</td>
</tr>
<tr>
<td>UV-Ozone</td>
<td>TMS-EDTA</td>
<td>No bonding observed</td>
</tr>
<tr>
<td>TMS-EDTA</td>
<td>3-APTMS</td>
<td>Irreversible bonding</td>
</tr>
<tr>
<td>BTMSE</td>
<td>UV-Ozone</td>
<td>Irreversible bonding</td>
</tr>
<tr>
<td>3-MPTMS</td>
<td>UV-Ozone</td>
<td>Irreversible bonding</td>
</tr>
<tr>
<td>UV-Ozone</td>
<td>3-MPTMS</td>
<td>Irreversible bonding</td>
</tr>
</tbody>
</table>
Figure C.1: (A) A schematic of a typical sample used for shear tests: a PDMS slab bonded to a glass substrate is shown. (B) Top view of the PDMS-bonded glass substrate. (C) A side-view diagram of the shear test procedure.
Figure C.2: An example of pressure leak test experiment for carboxyl-amine bonded PDMS-based 3-electrode device.