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ELECTROCHEMICAL CAPACITANCE MEASUREMENTS TO STUDY MOLECULAR SURFACE INTERACTIONS

Nrutya Madduri
Clemson University, nmaddur@clemson.edu

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ELECTROCHEMICAL CAPACITANCE MEASUREMENTS TO STUDY MOLECULAR SURFACE INTERACTIONS

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Bioengineering

by
Nrutya Madduri
May 2012

Accepted by:
Dr Guigen Zhang, Committee Chair
Dr. Frank Alexis
Dr. Tzuen-Rong Jeremy Tzeng
Abstract

The behavior of biological molecules such as proteins at the electrode/electrolyte has been of considerable interest for the development of biosensors. Several investigative techniques including Potentiometry, Voltammetry, Amperometry, and Electrochemical Impedance Spectroscopy are being employed to study and analyze these molecular surface interactions. Investigative techniques such as cyclic voltammetry involve the application of a large potential to probe the electrode characteristics and capture the bulk membranous events to obtain the required measurements. This study looks to achieve two major objectives through the use of a novel technique, namely, the double layer capacitive method: 1) to analyze the electrochemical behavior of proteins adsorbed on Gold and Platinum metal electrode surfaces and 2) to study the adsorption and electro oxidation of glucose on Platinum surfaces. The change in the double layer capacitance value that varies upon adsorption of proteins is measured as an electrode-electrolyte interface characteristic perturbation upon application of a small potential. Bovine serum albumin (BSA) was used as model protein with Gold and Platinum working electrode surfaces. Results indicate that capacitance measurement is a sensitive and cost-effective method to probe the molecular surface interactions and can be exploited for the development of electrochemical biosensors. Also, the processes of adsorption and electro oxidation of glucose on a platinum electrode surface have been investigated. We recorded capacitance measurements of a Platinum working electrode before and after glucose adsorption in buffered solutions. We noticed that the obtained results explain the
adsorption phenomenon of glucose on platinum accompanied by capacitance changes. Therefore, with our approach we have been successful in studying the molecular surface interactions and capturing the surface events using our novel double layer capacitance measurements.
Dedication

I would like to dedicate this work to my parents, brother and my friends for their unwavering support and guidance.
Acknowledgments

I would like to sincerely thank my advisor, Dr. Guigen Zhang, for his guidance and immense involvement in my work. His mentorship and genuine interest in my personal growth as a graduate student is greatly appreciated. I am also grateful to the other committee members, Dr. Frank Alexis and Dr Jeremy Tzeng for being so considerate and supportive in the completion of this dissertation. I would also like to thank all the members of the Biosensors laboratory. I would like to specifically thank Rajan, for his constant guidance and support and also Andrew Zhang for all his help. I would like to thank all my friends at Clemson for their support. To all the staff in the Bioengineering department and the Institute for Biological Interfaces of Engineering for their help and cheerful disposition – Thank you so much! I would like to specifically thank Maria for all her help. Finally, I want to thank the financial support Professor Zhang provided me through his Grand Challenge Explorations grant from the Bill and Melinda Gates Foundation.
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title Page</td>
<td>i</td>
</tr>
<tr>
<td>Abstract</td>
<td>ii</td>
</tr>
<tr>
<td>Dedication</td>
<td>iv</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>v</td>
</tr>
<tr>
<td>List of Figures</td>
<td>viii</td>
</tr>
<tr>
<td>1. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Research Objective and Motivation</td>
<td>2</td>
</tr>
<tr>
<td>2. Literature Review</td>
<td>5</td>
</tr>
<tr>
<td>2.1 Introduction to Sensors and Biosensor</td>
<td>5</td>
</tr>
<tr>
<td>2.2 Bio sensing elements</td>
<td>8</td>
</tr>
<tr>
<td>2.3 Electrochemical Techniques</td>
<td>11</td>
</tr>
<tr>
<td>2.3.1 Voltammetry and Amperometry</td>
<td>12</td>
</tr>
<tr>
<td>2.3.2 Potentiometry</td>
<td>18</td>
</tr>
<tr>
<td>2.3.3 Electrochemical Impedance Spectroscopy</td>
<td>21</td>
</tr>
<tr>
<td>2.3.4 Double layer Capacitance measurements</td>
<td>35</td>
</tr>
<tr>
<td>3. Electrochemical Capacitance measurements to probe protein surface</td>
<td>37</td>
</tr>
<tr>
<td>interactions</td>
<td>38</td>
</tr>
<tr>
<td>3.1 Introduction</td>
<td>38</td>
</tr>
<tr>
<td>3.2 Gold Electrode Studies</td>
<td>41</td>
</tr>
<tr>
<td>3.2.1 Materials and Methods</td>
<td>41</td>
</tr>
<tr>
<td>3.2.2 Results and Discussion</td>
<td>44</td>
</tr>
<tr>
<td>3.3 Platinum Electrode Studies</td>
<td>50</td>
</tr>
<tr>
<td>3.3.1 Materials and Methods</td>
<td>50</td>
</tr>
<tr>
<td>3.3.2 Results and Discussion</td>
<td>53</td>
</tr>
<tr>
<td>4. Electrochemical Capacitance measurements to study glucose adsorption</td>
<td>63</td>
</tr>
<tr>
<td>onto Platinum surfaces</td>
<td>63</td>
</tr>
<tr>
<td>4.1 Introduction</td>
<td>63</td>
</tr>
<tr>
<td>4.2 Materials and Methods</td>
<td>65</td>
</tr>
</tbody>
</table>
Table of Contents (Continued) 

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3 Results and Discussion</td>
<td>68</td>
</tr>
<tr>
<td>5. Instrument Limitations and Challenges</td>
<td>78</td>
</tr>
<tr>
<td>6. Conclusions</td>
<td>80</td>
</tr>
<tr>
<td>References</td>
<td>81</td>
</tr>
</tbody>
</table>
# List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Schematic diagram of a Biosensor and its components</td>
<td>7</td>
</tr>
<tr>
<td>2.2</td>
<td>Various analytes used in Bio sensing and their reaction mechanisms</td>
<td>9</td>
</tr>
<tr>
<td>2.3</td>
<td>Illustrations of common Immobilization techniques</td>
<td>11</td>
</tr>
<tr>
<td>2.4</td>
<td>Input applied potential and its current response</td>
<td>14</td>
</tr>
<tr>
<td>2.5</td>
<td>Three electrode Amperometric set up and screen printed electrode</td>
<td>16</td>
</tr>
<tr>
<td>2.6</td>
<td>Input potential of a CV and its current response</td>
<td>18</td>
</tr>
<tr>
<td>2.7</td>
<td>Input and a phase shifted signal</td>
<td>22</td>
</tr>
<tr>
<td>3.1</td>
<td>Cyclic Voltammogram and Capacitance measurements of a Gold electrode in 0.5M Sulfuric acid</td>
<td>44</td>
</tr>
<tr>
<td>3.2</td>
<td>Open circuit potential (OCP) stabilization curve</td>
<td>45</td>
</tr>
<tr>
<td>3.3</td>
<td>CV’s of a Gold electrode in 0.001M KCl with and without BSA</td>
<td>46</td>
</tr>
<tr>
<td>3.4</td>
<td>Capacitance measurements of a Gold electrode in 0.001M KCl before and after protein addition</td>
<td>47</td>
</tr>
<tr>
<td>3.5</td>
<td>Capacitance measurements of a Gold electrode at increasing concentrations of BSA</td>
<td>49</td>
</tr>
<tr>
<td>3.6</td>
<td>Cyclic Voltammogram and Capacitance measurement of a Platinum electrode in 0.1M Sulfuric acid</td>
<td>53</td>
</tr>
<tr>
<td>3.7</td>
<td>Cyclic Voltammogram of a Platinum electrode in 0.01M PBS</td>
<td>55</td>
</tr>
<tr>
<td>3.8</td>
<td>Cyclic Voltammogram and Capacitance measurement of a Platinum electrode in 0.01M PBS</td>
<td>57</td>
</tr>
<tr>
<td>3.9</td>
<td>Cyclic Voltammograms of a Platinum electrode in 0.01M PBS before and after protein addition</td>
<td>58</td>
</tr>
</tbody>
</table>
List of Figures (Continued)

3.10 Capacitance measurements of a Platinum electrode in 0.01M PBS at increasing concentrations of BSA .......................................................... 59

3.11 Capacitance measurements of a Gold electrode in 0.01M PBS with nanoparticles ................................................................................. 61

4.1 Cyclic Voltammogram of a Platinum electrode in 0.1M Sulfuric acid .......... 68
4.2 Cyclic Voltammogram of a Platinum electrode in 0.01M PBS ..................... 69
4.3 Capacitance measurement of a Platinum electrode in 0.01M PBS ............... 70
4.4 Cyclic Voltammogram of a Platinum electrode in 0.01M PBS with 0.1M Glucose .................................................................................. 71
4.5 Capacitance measurement of a Platinum electrode in 0.01M PBS with 0.1M Glucose solution ................................................................. 72
4.6 Cyclic Voltammogram and Capacitance measurement of a Platinum electrode in 0.01M PBS ..................................................................... 73
4.7 Cyclic Voltammograms of a Platinum electrode in 0.01M PBS before and after addition of glucose ............................................................. 74
4.8 Capacitance measurements of a Platinum electrode in 0.01M PBS before and after the addition of Glucose .................................................. 76
CHAPTER 1

Introduction

Electrochemical biosensors have been studied for a very long time, and are the very first scientifically-proposed, as well as successfully-commercialized, biosensors used for multiple analyte detection [1]. They play a pivotal role in medical, clinical analysis, industrial and environment engineering.

Electrochemical biosensors are capable of achieving direct conversion of a biological event to an electrical signal, which makes them quite desirable and attractive for analyzing the content/concentration of a biological sample or analyte of interest. These sensors are bound together by a common feature that relies on the detection of an electrical property (i.e., resistance, current, potential, capacitance, impedance), which are detected and measured using different methods such as Potentiometry, Conductometry, or Amperometry [2].

Electrochemical biosensors require good understanding of both electrical and electrochemical properties, which is quite a challenge, and this makes it very interesting. Understanding the relationship between surface structure and reactivity is quite a challenge pertinent to these sensors. Knowledge about the fundamental processes that govern sensor response such as the chemical and physical properties should be very well studied. This would lead to the fabrication of sensors with superior selectivity, good chemical stability, higher sensitivity, and lower detection limits. The functions and mechanisms of various electrical processes that occur at the surface of the sensor or
inside the sensor membrane also need to be studied extensively, as they are of utmost importance to produce sensors of desired features and optimum characteristics. This often involves tailoring the chemistry of the surface layer so that it exclusively adsorbs the target molecule/ion in the presence of interferences. The bulk electrical conduction properties of the sensing device can also be altered by modifying the composition of the membrane. A range of conditions must be employed that could serve as an appropriate tool to analyze the sensor performance. This is achieved only by changing the interfacial properties or by modifying the composition of the membrane. Sensor novelty and improvement can be attained by gaining a thorough knowledge of how various parameters influence the interfacial reaction kinetics and response mechanisms.

Electrochemical sensors are the smallest of all the sensors, including optical and piezoelectric, which provides the electrochemical biosensors with an advantage of portability and simple instrumentation. Also, the sensitivity and response of the electrochemical sensors are higher than optical or piezoelectric sensors. Finally, electrochemical sensors are cost-effective. All these factors give us immense motivation to explore the field of electrochemical biosensors.

**Research Objective and Motivation:**

The main objective of our research is the electrochemical detection of biomolecular species. However, in order to detect the biomolecular species, a lot of factors need to be analyzed. The species to be detected are usually adsorbed onto the surface of the electrode, hence it is important to understand and analyze the adsorption phenomenon first. This adsorption phenomenon is in turn linked to how well we understand the
molecular surface events. Finally, to understand the molecular surface events, we need to capture all the surface events with high precision and sensitivity as that would help us analyze the surface events. Therefore, detection of bio molecular species is a complex, multistep task that could be achieved only if a thorough understanding of the various phenomenon related to it are analyzed first. In other words, the success of detection of the bio molecular species is completely dependent on how well we capture the surface events and the molecular surface interactions. Thus, our goal was to achieve a good understanding of the events that occur at the surface, as that eventually will help in detecting the species.

There are several transduction mechanisms being used to capture the molecular surface interactions, of which electrochemical transduction holds great importance.

In this thesis, Chapter 2 is basically a literature review section that begins by providing an introduction to sensors and biosensors, followed by a discussion of the various bio sensing elements that could be used. Finally, all the various techniques and methods employed to make electrochemical measurements are discussed. Some of the electrochemical detection techniques that have been discussed in this review include Voltammetry, Potentiometry, Amperometry, Electrochemical Impedance spectroscopy and Capacitance measurements. After having provided an overview of the several electrochemical detection techniques, I mention the advantages and disadvantages of each of the detection methods and will point out which of these techniques suits best for studying the molecular surface interactions and why.
Having discussed how our research has taken its shape, Chapters 3 and 4 would include all the experimental data and experimental procedures involved, including the results and discussion. Chapter 5 summarizes the research findings and derives conclusions from the above two chapters 3 and 4. Finally, recommendations and possible future studies are discussed.
CHAPTER 2

Literature review and Background

2.1 Introduction to Sensors and Biosensors:

Sensors convert signals of one type of quantity into an equivalent, another type of quantity, mostly an electrical signal. Their function is to register physical, chemical, or biological changes and convert them into a measurable/output signal [3]. The output is an electrical signal. Biomedical sensors take signals representing biomedical variables and convert them into electrical signals [4]. Biomedical sensors serve as an interface between a biological and electronic system. They operate in a way as to not severely affect either of the systems. Both the biological and electronic factors play a very crucial role in biomedical sensors. All sensors can be categorized into two groups, physical and chemical sensors.

Physical Sensors include geometric, mechanical, thermal, hydraulic, electrical and optical sensors. On the other hand Chemical sensors include gas, electrochemical, photometric and bio analytic sensors.

Physical sensors measure variables such as muscle displacement, blood pressure, core body temperature, blood flow, cerebrospinal fluid pressure and bone growth in case of biomedical applications, whereas chemical sensors are concerned with measuring chemical quantities. This chemical measurement involves the identification of the presence of particular chemical compounds, detection of the concentrations of various
chemical species and monitoring chemical activities in the body for diagnostic and therapeutic applications.

The most widely accepted definition of a biosensor [5] is: a self-contained analytical device that incorporates a biologically active material in intimate contact with an appropriate transduction element for the purpose of detecting (reversibly and selectively) the concentration or activity of chemical species in any type of sample.

Figure 1 represents a schematic diagram of a biosensor and its components [6]. A biosensor is comprised of three main components:

- A biological sensing element that enables a selective response to a particular analyte or a group of analytes thus minimizing the interferences from other sample components. Thus, the main purpose of a bio recognition element of biosensors is to improve the selectivity.
- Analyte or target of interest that needs to be detected.
- A transducer or the detector component that converts the signal into an electrical output.
The need for sample preparation and laborious procedures can be eliminated by using biosensors. However, the performance criteria of a biosensor are dependent on certain calibration characteristics, which include sensitivity, operational and linear concentration range, selectivity, stability, lifetime, detection limits, determination limits, sample throughput, steady state and transient response times [6]. There are certain desirable features which need to be fulfilled for a biosensor to be constructed:

- The biocatalyst being used must be highly specific, thereby improving the performance of the biosensor.
- The biosensor should be cheap, portable, small, and user friendly (should not be too complicated for use).
- Precision, accuracy, linearity, reproducibility are all important parameters that should be taken into account while looking at the response of the biosensor. The response of the biosensor is judged based on these parameters.

Figure 2.1. Schematic diagram of a biosensor and its components.[6]
• If an in-vivo or implantable biosensor is being designed then care needs be taken to avoid bio fouling. The sensor should be biocompatible and nontoxic.

• Real time analysis should be possible [7].

2.2 Biosensing elements

Numerous biosensing elements like enzymes, proteins, antibodies, nucleic acids, cells, tissues, or receptors are being used as biocatalysts or bio recognition elements to build a biosensor. As already discussed, the importance of a bio sensing element is to increase the specificity, by allowing only specific interactions and by avoiding interferences from other species other than the analyte of interest [8]. The bio recognition element is capable of sensing the presence, activity or concentration of a chemical analyte in solution. This recognition process could either be a binding process occurring in affinity ligand-based sensors, where the bio recognition element is, for example, an antibody, DNA segment or cell receptor; or a bio catalytic reaction (enzyme-based biosensor) [9].
In order to ensure appropriate sensing mechanism, the bio sensing element should be attached properly to the transducer. The process by which the sensing elements get attached to the surface of the transducer is termed as **Immobilization**. The technique of immobilization is further classified into five categories:

**Adsorption**: This is the simplest of all techniques that works well for shorter time frames. Two types of adsorption can occur, namely Physical adsorption or physisorption and Chemical adsorption or chemisorption. The first technique is weak and occurs through the formation of Vander Waals bond with hydrogen bonds. The latter technique involves the formation of covalent bonds and hence is much stronger than the first technique.

**Microencapsulation**: In this technique the biomaterial (enzyme) is trapped onto the transducer by an inert membrane. This provides a proximal contact between the biomaterial and the transducer separated by the membrane. Some examples of the membranes used are Cellulose acetate, polycarbonate, collagen, Teflon, Nafion and
polyurethane. This technique of Immobilization is very adaptable and reliable and a high degree of specificity is maintained. Variations in temperature, ionic strength, pH and substrate concentrations do not affect the stability of the system much. Contamination and infections can be avoided.

**Entrapment:** In this technique, the enzyme is entrapped in a polymeric gel made of a polymer such as Polyacrylamide. Apart from polymeric gels, silastic gels, nylon, starch gels, and conducting polymer matrices such as Polypyrrole can also be used to entrap the enzyme or the biomaterial. However there are a couple of problems associated with entrapping the enzyme within the polymeric gel, which include, large barriers being created that inhibit the diffusion of the substrate thereby slowing the reaction and hence the response time of a sensor. Secondly, there is a loss of enzyme activity through the pores of the gel. This can be avoided by crosslinking the gel with glutaraldehyde.

**Cross linking:** In this technique, the enzyme (biomaterial) is bound to solid supports using bi functional agents. This helps to stabilize the adsorbed enzymes. This method causes a lot of problems such as causing damage to the enzyme, limiting diffusion of the substrate. It also has a very poor mechanical strength.

**Covalent bonding:** Enzymes contain a few functional groups such as the nucleophilic groups like NH$_2$, COH, OH, C$_6$H$_4$OH, SH and imidazole which are not used for catalytic activity, these functional groups are covalently bonded to the transducer/support matrix.
Based on the nature of the biosensing element process, biosensors can be categorized into *Bio-catalytic sensors* and *Affinity sensors* [3]. Biosensors which make use of enzymes, whole cells or tissues as bio recognition elements to recognize the target analyte are termed as *Bio-catalytic sensors*. While designing a bio-catalytic sensor there are certain parameters that should be taken into account which include the storage and operational stability, origin and availability of the biological component. On the other hand, sensors based on antigen antibody interactions (a selective interaction method), DNA hybridization sensors, and Immunosensors are examples of Affinity biosensors.

### 2.3 Electrochemical Techniques

We discuss three electrochemical methods -- Amperometry/Voltammetry, Potentiometry, and Electrochemical Impedance Spectroscopy -- in this section. The principles of operation governing each of these methods are presented, along with a few biomedical applications.
2.3.1 Voltammetry and Amperometry

Voltammetric and amperometric techniques are characterized by applying a potential to a working (or indicator) electrode versus a reference electrode and measuring the current. The current is a result of electrolysis by means of an electrochemical reduction or oxidation at the working electrode. This current is limited by factors such as the mass transport rate of molecules to the electrode.

The term voltammetry is used for those techniques in which the potential is scanned over a set potential range. The current response is usually a peak or a plateau that is proportional to the concentration of analyte. Voltammetric methods include linear sweep voltammetry, cyclic voltammetry, hydrodynamic voltammetry, differential pulse voltammetry, square-wave voltammetry, ac voltammetry, polarography, and stripping voltammetry. These methods have a wide dynamic range, and are useful for low level quantitation.

In amperometry, changes in current generated by the electrochemical oxidation or reduction are monitored directly with time while a constant potential is maintained at the working electrode with respect to a reference electrode. It is the absence of a scanning potential that distinguishes amperometry from voltammetry. The technique is implemented by stepping the potential directly to the desired value and then measuring the current, or holding the potential at the desired value and flowing samples across the electrode as in flow injection analysis. Current is proportional to the concentration of the electro active species in the sample. Amperometric biosensors have additional selectivity in that the oxidation or reduction potential used for detection is characteristic of the
analyte species. The electrolysis current is limited by the mass transport rate of molecules to the electrode.

The basis for the amperometric and voltammetric controlled current experiments is that the current response is related to concentration of the target analyte. This is done by observing the electron transfer happening during the redox process of the analyte:

\[ \text{Ox} + \text{ne} \rightarrow \text{Re} \]

where Ox is the oxidized form of the analyte and Re is the reduced form of the analyte. If the system is in a thermodynamic equilibrium then the potential of the electrode can be used to control the concentration of the oxidized species \( C_{\text{OX}} \) and reduction species \( C_{\text{R}} \), governed by the Nernst equation:

\[
E = E^* + \frac{nF}{RT} \ln \frac{C_{\text{ox}}}{C_{\text{R}}}
\]

where \( E_0 \) is the standard potential, \( F \) is the Faraday constant, \( R \) universal gas constant and \( T \) temperature in Kelvin. The amount of current generated by the oxidation of the species is called Faradaic current, and it is directly related to the rate of the reaction.

Two of the commonly used amperometric measurements are chronoamperometry and cyclic voltammetry. In chronoamperometry the potential is raised from a value where there is no faradaic reaction to a potential where the surface concentration of electro-active species at the electrode surface become zero. The current-time graph is reflection of the concentration gradient at the electrode surface. The following figure depicts the applied potential and the current, chrono amperometric response.
Figure 2.4. Input applied potential and its current response.

Current in the electrode decays with time and is governed by Cottrell equation:

$$i(t) = \frac{nFACD^{1/2}}{\\Pi^{1/2}t^{1/2}}$$

where $A$ is the electrode area, $C$ the bulk concentration of the electroactive species, and $D$ is the diffusion coefficient. Chronoamperometry is one of the simplest techniques yet finds applications in biosensors. The advantages of this method include negligible charging current or lower background signal since it is operated at a fixed potential. The amperometric signal can be significantly improved by enhancing the mass transport using rotating electrodes or monitoring the flow conditions.

Amperometry involves the continuous measurement of the current resulting from the electrochemical oxidation or reduction of an electro active species in a biochemical reaction. It is usually performed by applying a constant potential to the working electrode. Pt, Cu, Au are the common working electrodes used. This constant potential is applied with respect to the reference electrode. However, if the currents are very low, then the reference electrode behaves as a counter electrode.
Clark oxygen electrodes perhaps represent the basis for the simplest forms of amperometric biosensors, where a current is produced in proportion to the oxygen concentration. This is measured by the reduction of oxygen at a platinum working electrode in reference to an Ag/AgCl reference electrode at a given potential.

**Electrochemical setup for Amperometry**: Most of the amperometric setups consist of a three electrode electrochemical setup. The three electrode set up includes a Main or working electrode, which is typically gold, platinum or a carbon metal electrode. The reference electrode is an Ag/AgCl electrode that helps maintain a constant half-cell potential. This is achieved as negligible current flows through the reference electrode and all the excess current passes through the counter or auxiliary electrode. A two electrode electrochemical setup, has only a working and reference electrode. As long as the current flow is low there is no adverse change caused in the reference electrode. The following figures represent a general three electrode electrochemical set up with a working, reference and counter electrode. The figure to the right represents an example of a screen printed electro chemical biosensor consisting of a) gold working electrode b) Silver/Silver chloride reference electrode and c) counter electrode.
Amperometric detection is commonly used with bio catalytic and affinity sensors because of its simplicity and low Limit Of Detection (LOD). Advantageously, the fixed potential during amperometric detection results in a negligible charging current (the current needed to apply the potential to the system), which minimizes the background signal that adversely affects the limit of detection. The most popular and widely used amperometric biosensor is an enzymatic glucose biosensor, which is based on the amperometric detection of glucose.

**Disadvantages of using Amperometric enzymatic biosensors:** One of the biggest drawbacks of using amperometric devices is their selectivity. Selectivity is expressed as the ratio of the output signal from analyte to the output signal of the interference species of similar concentrations. The various electro active species in biological fluids interfere with the main signal. Ascorbate, urate and acetaminophen are the common intereferents in an enzymatic glucose biosensor. Operational stability and shelf life of the biosensor are the other important sensor performance characteristics apart from selectivity that are of
utmost importance. The stability depends on factors such as the external or internal diffusion of the substrate and geometric factors of the electrode. Shelf life is limited by the enzyme stability and leaching of the enzyme from the immobilized matrix [10].

**Voltammetry:** In this electroanalytical technique, information about an analyte is obtained by varying a potential and then measuring the resulting current. It is hence considered as an amperometric technique. Cyclic voltammetry (CV) gives information about the redox potential and electrochemical reaction rates of analyte solutions. In a CV, the voltage is swept between two values at a fixed rate, however, when the voltage reaches $V_2$ the scan is reversed and the voltage is swept back to $V_1$. The scan rate, $(V_2 - V_1)/(t_2 - t_1)$, is a critical factor, since the duration of a scan must provide sufficient time to allow for a meaningful chemical reaction to occur.

The voltage is measured between the reference electrode and the working electrode, while the current is measured between the working electrode and the counter electrode. The shape of the voltammogram for a given compound depends on factors such as scan rate, electrode surface and catalyst concentration. For instance, increasing the concentration of reaction specific enzymes at a given scan rate will result in a higher current compared to the non-catalyzed reaction. The following figure depicts the input potential and its corresponding current response. The figure to the right is termed as a Cyclic voltammogram as it is plotted with the current versus potential.
2.3.2 Potentiometry

This technique involves the measurement of an electrical potential difference between two electrodes termed as the indicator electrode and the reference electrode. This measurement is done while the current is zero. The reference electrode is required to provide a constant half-cell potential, whereas the indicator electrode develops a variable potential depending on the activity or concentration of a specific analyte in solution. These two electrodes are separated by a permselective membrane. The change in potential is related to concentration in a logarithmic manner based on the Nernst equation. The use of a transducer, an ion-selective electrode (ISE) is a popular technique of potentiometry used in clinical industry, for fabrication of electrochemical sensors which make use of thin films or selective membranes as recognition elements.

The most common potentiometric devices are pH electrodes. The ion-selective electrode (ISE) for the measurement of electrolytes is a potentiometric technique
routinely used in clinical chemistry. Hence, potentiometry provides information about the ion activity in an electrochemical reaction.

For these measurements, the relationship between the concentration and the potential is governed by the Nernst equation, where $E_{\text{cell}}$ represents the observed cell potential at zero current. This is sometimes also referred to as the electromotive force or EMF. $E_{o \text{ cell}}$ is a constant potential contribution to the cell, $R$ the universal gas constant, $T$ the absolute temperature in degrees Kelvin, $n$ is the charge number of the electrode reaction, $F$ is the Faraday constant and $Q$ is the ratio of ion concentration at the anode to ion concentration at the cathode.

$$EMF \text{ or } E_{\text{cell}} = E_{o \text{ cell}}^0 - \frac{RT}{nF}\ln Q$$

The direct determination of the analyte concentration with the Nernst equation is termed as Direct potentiometry.

These ISE’s are mostly used to detect various ionic species such as Na+, K+, Ca2+, NH3+.

**Applications of Potentiometric and Amperometric sensors:**

**Amperometric glucose biosensors:** The working principle of these sensors is based on the fact that when a constant potential applied, there occurs oxidation or reduction of chemical or electroactive species at electrode surfaces. These oxidation and reduction events are captured using amperometric sensors. The very first amperometric biosensor found in literature was described by Clark and Lyons. They built a glucose sensor that involved the immobilization of glucose oxidase on the surface of the amperometric
electrode for $P_{O_2}$. In this design, the enzyme glucose oxidase was physically entrapped between the gas permeable membrane of the $P_{O_2}$ electrode and an outer dialysis membrane. The dialysis membrane was made of a low molecular weight cut off that could possibly allow only glucose and oxygen to pass, but not proteins and other macro molecules. The principle of measurement was oxidation of glucose, catalyzed by glucose oxidase.

$$\text{glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$$

It has been observed that the rate of decrease in $P_{O_2}$ is a function of the glucose concentration and is monitored by the $P_{O_2}$ electrode. Later to correct for the $P_{O_2}$ variations in the sample, a second oxygen working electrode without the enzyme is introduced, whose polarity is reversed, by making the platinum electrode positive, so that it leads to the oxidation of $H_2O_2$, which produces current that can be measured which is proportional to the glucose concentration. However a sufficiently high voltage of 0.7V is required to drive the oxidation of $H_2O_2$ to produce that current.

$$H_2O_2 \rightarrow 2H^+ + O_2 + 2e^-$$

**Potentiometric Urea sensors:** A popular example of a potentiometric sensor is a Urea based polyvinyl chloride ISE for ammonium ion, using ionophore non actin. The enzyme urease is immobilized at the surface of the ISE and this leads to the catalysis of the hydrolysis of urea to $NH_3$ and $CO_2$.

$$\text{urea} \xrightarrow{\text{urease}} 2NH_3 + CO_2$$
The product Ammonia is hydrolyzed to form NH4+ which is sensed by the ISE. The signal generated by NH4+ is proportional to the logarithm of the concentration of the urea in the sample.

The biocatalyst layer that is placed adjacent to the potentiometric detector depends on factors such as:

- Analysis of the substrate that is transported to the biosensor surface
- Process of analyte diffusion to the reacting layer
- Analyte reaction that occurs in the presence of biocatalyst, and
- Diffusion of reaction product towards both the detector and the bulk solution.

ISE based potentiometric devices also depend on the buffer capacity and the ionic strength of the sample.

### 2.3.3 Electrochemical Impedance Spectroscopy

Electrochemical Impedance is measured by applying an AC input voltage and measuring the AC output current produced in the electrochemical cell. In short, the input is a sinusoidal AC potential and the output measured is an AC sinusoidal current signal that can be analyzed as a sum of sinusoidal functions, as Fourier series. In these impedance measurements the output AC sinusoidal current that is measured in response to the AC sinusoidal potential, will have the same frequency as the excitation input signal, but will be out of phase. Hence the frequency is the same but the phase is shifted.
This phase shift occurs due to the capacitive and the resistive effects that are observed in the electrochemical cell, wherein the electrode electrolyte interface or the electrochemical interface behaves as a capacitor and the solution resistance and electron transfer events (occurring in the electrochemical cell) offer a resistive effect.

The excitation signal is expressed as a function of time:

\[ E_t = E_0 \sin(\omega t), \]

where \( E_t \) is the potential at time \( t \), \( E_0 \) is the amplitude of the signal and \( \omega \) is the radial frequency where \( \omega = 2\pi f \), \( f \) being the frequency.

In a linear system, the response signal, \( I_t \), is shifted in phase (\( \phi \)) and has a different amplitude, \( I_0 \). \( I_t = I_0 \sin(\omega t + \phi) \). These impedance results are often presented as Lissajous figures, Nyquist plots and Bode plots.
To get a complete data the measurements are done over a range frequency change (1 mHz- 1MHz). EIS is thus a non-destructive, steady state technique capable of probing the relaxation phenomena over a range of frequencies.

Equivalent circuits with capacitor and resistors are used to define the electrochemical system and this equivalent circuit model is fitted with the obtained data to get the resistor and capacitive values. In the early studies the equivalent circuits were simple (minimum number of components and single time constant) and measurement was done for only one or a few frequencies. But these assumptions still hold good if there is no electrochemical reaction occurring at electrode surface.

**EIS and electrochemistry:** Electrochemical impedance spectroscopy (EIS) is an experimental method for characterizing electrochemical systems. It involves the measurement of impedance over a range of frequencies. The frequency response, energy storage and dissipation properties of the electrochemical system are determined.

EIS measurements are widely used for sensor characterization [2]. Potentiometric, amperometric, electrochemical biosensors are all classified as electro analytical sensors that are used for the detection of wide range of analytes achieved by EIS. Most of these sensors are governed by the principles, working conditions and stability of the electrode-electrolyte interface. The sensor response or mechanism is dictated mostly by the events that occur at the electrochemical interfacial region. Hence, EIS is used which provides a great deal of information on the numerous fundamental interfacial processes/events occurring at the electrochemical interface which include adsorption/desorption, film formation, rate of charge transfer, electron transfer events, ion exchange, diffusion,
corrosion and corrosion rate and so on. EIS is capable of interrogating phenomena such as Double-layer capacitance, diffusion impedance, and determination of the rate of charge transfer and charge transport processes, and solution resistance. Corrosion mechanism, fouling and blocking phenomena have also been extensively studied using EIS [12]. Parameters are measured as a function of the frequency of the applied perturbation.

As already discussed, a majority of the electrochemical methods probe the electrochemical interface (electrode-electrolyte interface) by applying a large perturbation that drives the electrochemical reaction far from equilibrium. Hence, techniques such as EIS are used that involve only a small perturbation and records the events that occur at the near zero conditions or equilibrium conditions.

**Applications of Electrochemical Impedance Spectroscopy:** EIS techniques are widely used in enhancing the performance characteristics of Potentiometric and Electrochemical biosensors. Impedance spectroscopy has proven to be an efficient method to probe the interfacial properties of modified electrodes. This information is useful to understand chemical transformations and principles associated with conductive metal supports. The charge transfer processes that occur at the electrode-electrolyte or solid-solution interface depend on the nature of electrode surface and the structural properties of the electrical double layer. The adsorption and desorption phenomena that occur on conductive metal surfaces is expected to change the interfacial electron transfer events such as the capacitance and resistance. In other words, it is anticipated that the interfacial electron transfer properties such as resistance and capacitance would change upon adsorption or
desorption on metal surfaces. In most cases, the redox couple ferro ferri cyanide serves as a ‘probe’ for the insulating properties and the density of the adsorbed layer. Electron transfer is usually observed in the presence of a redox couple. The electron transfer and faradaic impedance are measured in the presence of a redox couple.

**EIS in Potentiometric biosensors:** Also termed as Ion selective electrodes, comprise of a membrane that is made up of glass, inorganic crystal or a plasticized polymer. A selective binding process occurs at the membrane electrolyte interface. The ion of interest that binds via this selective process is imparted with a certain potential which is decided based on the composition of the ISE.

Advantages of these sensors include long life, acceptable mechanical stability, simple instrumentation, low cost and suitability for continuous monitoring.

However, a few disadvantages include, low detection limits and leaching of the membrane components into the sample. Hence to overcome this drawback, EIS is used extensively to study the membrane leaching effects.

**EIS in Polymer based ISE membranes:** Research on these electrodes is being done to improve electrode sensitivities, selectivities and detection limits. In which case EIS is used for the characterization of the interfacial properties of various PVC based ISE’s. The impedance changes provided information about the variations in membrane selectivity. Through these studies, a link between membrane impedance and its potentiometric response characteristics was established. Mechanisms of adsorption, fouling and corrosions can be extensively studied using EIS. The membranes of these polymeric based sensors are chemically modified using surfactants and proteins. EIS is then used to
study and understand the adsorption and blocking phenomena in the presence of various levels and concentrations of surfactants and proteins, respectively. The impedance results showed that adsorption in the presence of surfactants increases and the extent and degree of adsorption depends on the concentration of proteins used to block the membrane. The impedance spectra measurements used in Potentiometric ISE’s are dependent on several factors such as the membrane thickness, ageing, composition and the electrolyte composition.

**EIS as a label free detection tool for Biosensors:** Another very interesting application of EIS is its use in label free detection for biosensors. In this particular application, an electrode surface such as gold conductive surface is chemically modifies by dipping the surface in SAM solutions that have the potential to completely block the surface and make it much more stable. However this depends on how well the SAM layer is immobilized onto the surface. The electrode surface is thus modified and the electrical and electrochemical changes that occur at the interface are detected as changes in capacitance, charge transfer. The changes can be detected by various techniques such as voltammetry, amperometry, or impedometry. To enhance the selectivity, a SAM layer is immobilized onto the surface of the electrode, followed by the detection of an analyte via an unlabeled electrochemical method, unlike fluorometric methods of detection where a fluorescent based label needs to be used that depends on the concentration of the analyte being used [13].

**Use of EIS in electrochemical Biosensors:** EIS in electrochemical biosensors is used to detect and study the molecular surface interactions at the semi conductive or conductive
surfaces. Several groups have worked on EIS to study/characterize cell culture monitoring, bio molecular screening, measurements of changes in the interfacial capacitance and resistance on SAM based and bilayer lipid based membrane surfaces, the enzyme based and micro-organism based biosensors.

**EIS in Enzyme sensors:** In this case, an enzyme is immobilized onto either a metallic electrode used in amperometry or an ISE employed in Potentiometry. Enzyme based Glucose biosensors are the very first and most popular commercialized biosensors that have helped monitor the blood glucose levels in diabetic patients. In these sensors EIS is used to evaluate the performance of an amperometric enzyme based biosensor coated with avidin loaded conductive polymer. Apart from this, EIS is used to study the electrochemical redox behavior of the enzymatic glucose biosensor. Different concentrations of glucose are used and the changes in the impedance that occur as a function of glucose concentration are recorded. Evaluation of sensor performance, investigation of the changes in resistance, capacitance and other electrical properties of the membrane at different stages of immobilization and solution treatment, monitoring of the capacitance changes that occur at the electrochemical interface in gold/platinum electrodes are all studied using EIS.

**EIS used in Immunosensors:** In these sensors, an antibody is immobilized onto the surface of an electrode like gold or platinum that is conductive, that provides a conductive support. The electrical properties of the interfacial region undergo changes when this antibody binds and reacts to the antigen. The surface organization and the immobilization of antibodies onto the electrode surface are quite crucial for sensor
fabrication. The sensor performance characteristics such as the sensor stability, sensitivity, selectivity, response time, depend on how well the antibody has been immobilized onto the electrode surface and how well it reacts or binds with the antigen leading to an antigen antibody complex. The formation of this antigen antibody complex leads to the formation of a blocking layer on a conductive support such as gold/platinum electrode surface which alters the electrical properties of the interfacial region, thereby altering the impedance. This occurs because a hindrance to the redox couple is caused due to the antigen-antibody complex formation formed by the antigens binding to the antibody surface. This hindrance is higher when compared to the one offered in the absence of antigens. As the hindrance offered by the redox couple of the faradaic reaction keeps increasing, the electron transfer resistance increases, resulting in a decrease in capacitance. However, the change in impedance that is observed is not significant enough, as the binding reaction of the antigen antibody complex is weak. Hence a signal amplification method to strengthen the detection signal is often required. Hence these changes in the impedance caused due to the formation of the antigen antibody complex are studied extensively using EIS by measuring the resistive and capacitive change that accompanies this reaction. In other words, EIS is used to study the molecular surface events that occur upon the formation of the antigen antibody complex on the electrode surface. Antibody immobilization or adsorption is studied just like protein adsorption is studied. For a successful performance of the immuno sensor, the covalent attachment of the antibody to the electrode surface is very pivotal. Based on this factor the extent of antibody immobilization can be studied using EIS. The better the covalent attachment
better would be the immobilization of the enzyme, formation of the antigen antibody complex formation that would finally lead to better sensor performance. EIS is also used to study the surface properties of the immune sensor, before and after the antibody immobilization apart from the extent of immobilization.

Controlling the thickness and the dielectric properties of the immune sensor surface is quite a challenge. It has been suggested that the use of conductive electro active polymers would actually help controlling the thickness. Thus, a need to understand the antibody-modified conducting polymer electrodes surfaces arises. The surface properties of a bare antibody immobilized electrode are a lot different from the surface properties of an antibody modified conducting polymer electrode. Hence, EIS is a very sensitive technique used to study these changes occurring on the conductive electrode surface.

**Use of EIS in DNA sensors:** In these sensors, a single stranded oligonucleotide is immobilized onto an electrode transducer surface and the sensor is exposed to a sample containing target of a DNA hybridization event. Hence, the immobilization of the single stranded DNA is quite a challenging task and this is studied using EIS. A few groups have reported that a lot of parameters such as the immobilization time, thickness, electrode treatment, oligonucleotide sequence length and concentration greatly effect, vary the electron transfer resistance and this change is detected using EIS. Thus EIS captures all the electron transfer resistance changes and the interfacial capacitive changes. In most of the EIS studies done for DNA sensors, the activity of the electrode surface is evaluated using an electro active prob. The advantage of using this electro active probe/indicator is that it serves as a reference point for the impedance studies.
**Use of EIS in Fuel cell diagnostics:** The performance of fuel cells can be drastically improved using EIS. The performance characteristics of a fuel cell depend on three fundamental sources of voltage loss in fuel cells: ‘kinetic’ losses characterized by charge transfer, ‘ohmic’ losses due to electron and ionic transfer and finally ‘mass transfer’ losses due to concentration. These three sources of polarization can be separated and quantified using EIS [14].

Impedance measurement techniques: Some of the techniques used to analyze and interpret the impedance spectroscopy data are AC bridges, Lissajous curves, Phase sensitive detection, Frequency response analysis, Fast Fourier Transform, Bode plots, and Nyquist plots [15].

**Advantages and drawbacks of EIS over other techniques:** The validity of the experimental EIS can be checked using Kramers-Kronig transforms. However this validation is possible only if the system is linear and interface is stable over the time span of sampling. However, a major disadvantage is that when the EIS experimental data is analyzed using plots, in most cases only Nyquist plots are used for interpretation leaving out Bode phase and Bode impedance plots and a final interpretation is done based just on the Nyquist plots.

**Future developments in EIS:**

*To reduce the time frame for the Impedance measurements:* It has been observed that the EIS measurements take a long time anywhere between 10 min to several hours to collect the Impedance spectrum. This recording time depends on the frequency range across which the measurement is being recorded and the Input range. Greater the potential and
frequency range, longer would be the measurement time. It also depends on the stability of the system. Hence, the long measurement time is a concern since this could lead to interpretation errors as the system may have changed during the time frame of the typical EIS study. Hence, research is being carried out to significantly reduce and minimize the time frame for the impedance spectra.

Localized electrochemical Impedance spectroscopy: Most of the impedance spectroscopy measurements that have been recorded so far have mostly been applicable to macroscopic conductive surfaces. Hence these spectroscopic results do not provide us with any information about the microscopic properties such as the surface defects and properties. In order to understand and study the impedance spectroscopic phenomenon occurring at the microscopic level, Localized Electrochemical Impedance Spectroscopy (LEIS) is used. LEIS is a very powerful tool that can interrogate and explore a lot of phenomena such as

- Corrosion studies and corrosion mechanisms which occur mostly at a microscopic level that include pit and crevice corrosion.
- Conduction properties of the surface modified electrodes sensors with defects such as impurities in films, localized oxidation, galvanic interactions between various components on a membrane surface.

Thus, LEIS is a very useful tool to interrogate the molecular surface interactions at a microscopic level. However a combination of LEIS coupled with other microscopic based techniques such as Atomic force microscopy will serve as a much more powerful tool to study the nano scale microscopic molecular interactions.
As already discussed in Chapter 1, the main goal of our research is the electrochemical detection of bio molecular species which depends on the precision of capturing the surface events. There is a need for us to analyze which of electrochemical techniques helps us capture the molecular surface interactions with a superior sensitivity. Having discussed the electrochemical detection techniques of Potentiometry, Voltammetry, Amperometry and EIS, it could be said that each of these techniques has its own share of advantages and drawbacks. However, of all the techniques I have discussed, which include Potentiometry, Amperometry and EIS, EIS seems to overcome most of the problems faced by the rest of the sensor techniques. All these electrochemical techniques depend on the fact that the success of a sensor is completely driven by factors such as the condition and stability of the membrane-electrode surface. In other words, a sensor response and outcome is primarily based on and driven by the surface structure and the electrochemical reactions that occur at the interfacial or electrode electrolyte interface. Hence, only a thorough understanding of the events and mechanisms occurring at this interfacial region would help us to achieve good sensor performance.

The electrochemical events that occur in an electrochemical reaction include electron transfer events, non-electron transfer events, and interfacial surface events. Of these, the surface events or the interfacial events that occur at the electrode electrolyte interface are of utmost importance, since they capture different phenomena such as adsorption and desorption, that occur during molecular surface interactions. Techniques such as Cyclic Voltammetry, Potentiometry and Amperometry all capture events of charge transfer that occur mostly in the bulk membrane regions of an electrochemical
cell. This implies that these techniques give us a lot of information regarding the electron transfer and non-electron transfer events that happen at the bulk membrane. However, these techniques lack the ability to capture the molecular surface events that occur at the interfacial region of the electrode. EIS has the potential to separate the bulk membrane processes from the interfacial events, and is capable of simultaneously measuring both the bulk membrane events and surface events, thereby enhancing the sensitivity of the sensor immensely. This makes EIS a sensitive technique to study molecular surface interactions.

Having said this, it goes without saying that no technique is perfect and each method has its own share of disadvantages, leaving immense scope for improvement and betterment, thus continuing the quest for science. Though EIS is a powerful diagnostic tool, it does suffer from serious drawbacks that are being looked into. I list some of these below.

- **Time frame:** EIS is a technique whose characterization is dependent on processes that are responsive at different rates of frequencies. These impedance measurements can take anywhere between 10 minutes to several hours. Now, this time span depends on the range of frequencies and the potential being applied. The higher the potential and larger the frequency ranges, the longer the measurement time. Unfortunately, during this large time span, the system of analysis could completely change. Thus reducing the time frame of measurements should be a method to enhance its performance.

- **Cost:** EIS is an expensive method.
- EIS data analysis/interpretation: EIS data analysis is a lengthy and complex process. When EIS experimental data is analyzed, in most cases only Nyquist plots are used for interpretation leaving out Bode phase and Bode impedance plots and a final interpretation is done based just on the Nyquist plots.

EIS does suffer from some drawbacks as mentioned above. Research is being carried out to overcome these drawbacks. But a remaining question to be addressed is: Is there a technique that has already been developed that could overcome all of these drawbacks? Our research group has embarked on a journey to find a better technique than EIS that offers more surface sensitivity, simplicity in terms of instrumentation, and would be much more cost-effective then EIS.

Ideally, we need a sensing technique that would be low-cost, faster, and more sensitive than EIS. Of the many drawbacks of EIS that have been mentioned, time frame and cost are serious concerns. We have been working on finding methods to reduce the time frame of measurements. We have noticed that one way we could possibly achieve this would be by recording Capacitance measurements. Capacitance measurements are also as sensitive as Impedance measurements. While impedance measurements are multi-point measurements taken over a range of frequencies, capacitance measurements are single point measurements that are taken at a single desirable frequency. This approach could make the measurement time much faster, as recording needs to be done only at a single frequency point. However, this requires a proper choice of frequency. Different electrochemical reactions occur at different frequencies. If we could determine the frequency at which our desired reaction occurs we could directly capture that event by
executing the measurement at that particular frequency. Thus our research finds its motivation in answering the above questions and in designing an attractive tool that could possibly be more sensitive than EIS. A basic introduction to Double layer Capacitance measurements is made in the following section.

2.3.4 Double layer Capacitance measurements:

Our lab focusses on developing electrochemical sensors for bio molecular species detection using double layer capacitance measurements. These capacitance measurements involve the measurement of the capacitance potentiostatically at the electrode electrolyte interface. The electrode electrolyte interface is an electrical double layer [16-20] that behaves as a capacitor where accumulation and buildup of charges occurs. The electrical double layer is explained as an array of charged particles and oriented dipoles which are thought to exist at every interface. The double layer consists of a layer of electrons, a layer of adsorbed ions, and a diffuse double layer consisting of an ionic atmosphere in which ions of one sign are in excess of their normal concentrations whereas those of the other sign are in defect. As we recede from the surface, the atmosphere of abnormal concentration of ions falls off rapidly. Finally, there exists at the interface a thin layer of neutral molecules, which exert an influence on the thermodynamic properties of the interface, irrespective of whether they are oriented or not. An electrode immersed in an electrolytic solution or in contact with a liquid could be described as a Capacitor that possesses the ability to store charge.[21] The capacitance that is measured at this double layer is termed as double layer capacitance, the value of which varies with the introduction of an analyte (protein) due to possible events such as adsorption. This in turn
depends on several factors such as electrode roughness, ionic concentration, and also on the composition and concentration of analyte. Since these measurements involve the application of a potential that probe the electrode-electrolyte interface characteristics, they possess the ability to capture the surface events with high sensitivity. Hence Capacitance measurements are one among the few techniques apart from EIS that allow simultaneous monitoring and measurements of both the bulk membranous electron transfer events as well as the surface events such as adsorption. While impedance measurements are multi-point measurements taken over a range of frequencies, capacitance measurements are single point measurements that are taken at a single desirable frequency. This approach could make the measurement time much faster, as recording needs to be done only at a single frequency point. This overcomes the limitation of time concern.

With this thought, we have carried out several experiments based on electrochemical capacitance measurements, experimental procedures, results of which would be discussed in detail in Chapters 3 and 4.

Chapters 3 and 4 represent the data and results for the Capacitance measurements carried out on Gold and Platinum electrode surfaces. In Chapter 3, we have Capacitance measurements carried out on both Gold and Platinum electrode surfaces using a model protein BSA. In Chapter 4, we discuss the adsorption of Glucose on Platinum electrode surfaces using Capacitance measurements.
CHAPTER 3:

Electrochemical capacitance measurements to study protein adsorption onto metal electrode surfaces

Behavior of biological molecules at the electrode/electrolyte interface has been of considerable interest for the development of biosensors. Most biosensors are made of electrodes interfacing with biological molecules in order to sensitively transduce the biological events. Such interactions with electrode-molecular interactions become important and hence are of considerable interest. Several investigation techniques are being employed to study and analyze these molecular surface interactions. Investigative techniques such as cyclic voltammetry involve the application of an electrical potential to probe the electrode characteristics and capture the bulk membranous events to obtain the required measurements. In the present study, novel capacitance measurements have been used to study the surface adsorption behavior of proteins adsorbed on metal electrode surfaces. The change in the double layer capacitance value that varies upon adsorption of proteins is measured as an electrode-electrolyte interface characteristic perturbation upon application of a small potential. Bovine serum albumin was used as model protein with Gold and Platinum working electrode surfaces. The results indicate that capacitance measurement is a sensitive and cost-effective method to probe the molecular surface interactions and can be exploited for the development of electrochemical biosensors. It is a highly sensitive technique that captures most of the molecular surface events. However,
it is bound by certain measurement and instrument limitations all of which would be discussed in detail.

3.1 Introduction:

In recent years, a lot of research is being carried out to study the surface adsorption behavior of proteins and electrochemistry plays a major part in this study [22]. Knowing the surface adsorption behavior of proteins on metal surfaces is of great importance in various applications such as development of biosensors, medical implant devices and antifouling [23-26]. Molecular surface interactions are mainly characterized by the adsorption behavior of proteins [27-29]. The study of adsorption phenomenon of proteins on electrode surfaces has been of considerable interest for some time, particularly in biomaterial science [22]. It is well documented that the adsorption process is a complex process, not driven or dominated by any single factor but a combination of several factors.

Protein adsorption process is considered as an irreversible process [30-33] by some but a reversible process supporting the Langmuir adsorption isotherm by others [34-36]. Whether protein adsorption is a reversible or an irreversible process is a debating topic [37, 38]. Surface adsorption of numerous proteins including Lysozyme, BSA, lactoglobulin, myoglobin, hemoglobin, cytochrome c, ribonuclease and a number of other biological molecules have been studied by electrochemical analysis for different biosensor applications [39-48].

Bio fouling is an adverse consequence of protein adsorption, in which a protein layer gets adsorbed onto the surface of a medical implant in the body. When a medical
implant is implanted in the body, care should be taken to avoid biofouling. Possible adverse effects of bio-fouling include inflammation [49], clot formation [50-52] and increased corrosion of metallic implants [43].

Many investigation techniques have been used for studying this complex interfacial phenomenon [53-66]. These techniques include scanning transmission X-ray microscopy, infrared reflection spectroscopy, electrochemical detection methods, neutron reflectivity, total internal reflection fluorescence, optical waveguide light mode spectroscopy, surface MALDI-TOF mass spectrometry, time of flight secondary ion mass spectrometry, electron spectroscopy for chemical analysis, radiolabelling, differential scanning calorimetry, surface Plasmon resonance, ellipsometry, ELISA, STM and AFM. Of these methods, electrochemical methods are very efficient and quite popular. Electrochemical methods can be further grouped into techniques include Cyclic Voltammetry, Potentiometry, Amperometry and Impedance Spectroscopy. All these electrochemical techniques are somewhat capable of providing information about the electrode surface.

The electrochemical events that occur in an electrochemical reaction include electron transfer events, non-electron transfer events, and interfacial or surface events such as adhesion, adsorption and desorption. Of all these events, the surface events that occur at the electrode electrolyte interface are of utmost importance, since they capture different phenomena such as adhesion, adsorption, and desorption, that occur during molecular surface interactions. Techniques such as Cyclic Voltammetry, Potentiometry and Amperometry all capture events of charge transfer that occur at the interface, but
they also capture events in the bulk solution such as mass transport. This implies that
these techniques give us a lot of information regarding the electron transfer and non-
electron transfer events. Because of this, these techniques lack the sensitivity to capture
the molecular surface events that occur at the interfacial region of the electrode. EIS has
the potential to separate the bulk solution processes from the surface events, and is
capable of simultaneously measuring both the bulk solution events and surface events,
thereby enhancing the sensitivity of the sensor immensely. Though EIS is a powerful
diagnostic tool, it does suffer from serious drawbacks that are being looked into. EIS is a
technique whose characterization is dependent on processes that are responsive at
different rates of frequencies. These impedance measurements can take anywhere
between 10 minutes to several hours. This time span depends on the range of frequencies
and the potential being applied. The higher the potential and the larger the frequency
range becomes, the longer the measurement time will be. Unfortunately, during this large
time span, the system of analysis could completely change. Thus reducing the time frame
of measurements should be a method to enhance its performance. In short, each method
has its own share of problems and disadvantages, leaving immense room for
improvement and betterment, thus continuing the quest for science. Ideally, for us we
need a method that is cost effective, faster and highly sensitive than EIS.

Do we have a technique better than EIS? In search of an answer to this question,
we hypothesized that a capacitive method may provide a better solution in studying
molecular surface interactions. To test this hypothesis, we carried out electrochemical
based capacitive measurement experiments along with voltammetry experiments to evaluate molecular surface interactions.

In our experiments we evaluated protein adsorption on Gold and Platinum electrode surfaces in various electrolyte solutions including Sulfuric acid, Potassium chloride and Phosphate buffer saline solution.

3.2 Gold electrode studies:

3.2.1 Materials and Methods:

Working electrode (a gold disc electrode) and diamond polish were purchased from BASi (West Lafayette, IN), 0.05 micron alumina polish and polishing pads were purchased from Beuhler (Lake Bluff, IL), an Ag/AgCl reference electrode was purchased from Fisher scientific (Pittsburgh, PA). Sulfuric acid, 99.999% pure was purchased from Sigma-Aldrich (St Louis, MO), Potassium chloride (crystalline) from Fisher scientific, Bovine serum albumin (BSA), 96% electrophoresis from Sigma (St. Louis, MO)

Experimental set up and methods:

All electrochemical measurements were taken using the Princeton Applied Research Versa stat MC Potentiostat (Oak ridge, TN). All tests were carried out in a three-electrode electrochemical cell consisting of a Gold disc working electrodes, Ag/AgCl reference electrode and a Platinum mesh counter electrode in respective. The electrolytic solutions used are Sulfuric acid, Potassium chloride (KCl) and Phosphate buffer saline solution (PBS).

Preparation and Cleaning of the Working electrode: The gold disc electrode was subjected to Piranha cleaning by dipping the electrode in Piranha solution (3:1 of
H₂SO₄:H₂O₂) for 2 minutes. The electrode was then polished using 0.1 micron alumina polish for about 3 minutes and ultra sonicated in Deionized water for 5 minutes.

Following the mechanical polishing/cleaning, the electrode was subjected to electrochemical cleaning by running voltammetric cycles in 0.5 M Sulfuric acid. The potential range across which CV measurements were taken was set from -0.3 V to +1.5 V and the scan rate was set at 100 mV/s.

**Capacitance measurements to study adsorption of BSA on gold electrode surfaces:**

Electrochemical measurements in the forms of cyclic voltammograms (CV) and capacitance measurements were recorded first in 0.1 M H₂SO₄ solution and later in 1 mM KCl solution. Protein adsorption studies with Bovine serum albumin were carried out in 1 mM KCl solution.

*CV and capacitance measurement for a Gold electrode in 0.5 M H₂SO₄ solution:*

Prior to taking the CV measurements for the Gold disc electrode the solution (0.5 M H₂SO₄) was purged with Nitrogen for 15-30 minutes. The potential range across which CV measurements were taken was set from -0.3 V to +1.5 V and the scan rate was set at 100 mV/s.

*CV and capacitance measurements in 1mM KCl solution:*

Following the experiments in sulfuric acid, CV measurements for of the Gold disc electrode were made in 1 mM KCl solution. Again, the KCl solution was Nitrogen purged for 15-30 minutes prior to measurements. The CV potential range was set from -0.4 V to +0.4 V and scan rate set at 100 mV/s. After the CV experiments, capacitance measurements were taken for the Gold disc working electrode in 1 mM KCl within the
same potential range from -0.4 V to +0.4 V under an alternating biasing potential of 20 mV in amplitude and 10 Hz in frequency.

**CV and capacitance measurements in 1mM KCl solution containing BSA:**

In addition to these experiments in blank KCl solution, Bovine serum albumin (BSA) protein was added to the KCl solution and similar experiments were performed in order to investigate how the capacitances change with the addition of BSA protein for some insight into the adsorption behavior of BSA. To do this, the Gold disc electrode after going through the same cleaning procedure was placed in the same electrochemical cell containing 1 mM KCl mixed with 0.1 mg/mL of BSA protein solution. Here BSA was used as a model protein, which is a widely used protein to study protein adsorption on gold electrode surfaces. Prior to electrochemical measurements, the solution was allowed to stabilize usually for 30 minutes as marked by a stabilizing open circuit potential (OCP) value. Figure 3.1 shows a typical OCP curve. Upon reaching a stable OCP value, the CV and capacitance measurements were made in a similar manner as discussed earlier. The obtained CV and capacitance results were given in Figure 3.2, Figure 3.3 and Figure 3.4.
3.2.2 Results and Discussion:

In Figure 3.2, a typical CV and capacitance-potential curve for the gold disc electrode in 0.5 M H₂SO₄ is shown. Several electrochemical features can be seen from these two curves. The blue curve represents the CV and the red curve represents the capacitance measurement of a gold electrode in sulfuric acid solution. As we follow the arrows on the curve, the electrochemical events begins with hydrogen evolution occurring around -70 mV. As we proceed further, we have only double layer charging that is seen from -70 mV to +800 mV. In this region, there are no major electron transfer events occurring. We notice only charging and discharging events. The next peak is the oxidation peak, where oxide formation occurs from +1.2 V to 1.6 V. Oxygen evolution commences
at 1.6 V. In the reverse/backward scan we have oxide reduction from 1.25 V to 0.85 V. All of these events are captured in the capacitance curve as well. The results we obtained are supported from the literature results [67, 68]. For example, Merrill and group have investigated the electrochemistry of gold in aqueous sulfuric acid solutions. CV of a gold electrode was recorded in nitrogen purged- oxygen free 50 mM sulfuric acid solution. Similar peaks of Hydrogen formation, double layer region, oxide formation, oxygen evolution and oxide reduction are all captured at similar potential values supporting the results we have obtained.

Figure 3.2 Cyclic Voltammogram and Capacitance measurements of a Gold disc electrode in 0.5M Sulfuric acid
Figure 3.3 Cyclic Voltammograms of a Gold electrode in 0.001M KCl with and without 0.1mg/mL of BSA
Figure 3.3 shows two CV curves, one before and one after the addition of 0.1 mg/mL of BSA, measured in 1 mM of KCl. In Figure 3.3, the red curve represents the CV without BSA and the blue represents the CV of a gold electrode with BSA. We notice that in both these curves, there is only charging and discharging events occurring. For both the curves, as we go in the forward scan direction, there is charging occurring and as we follow the reverse/backward scan, we observe discharging. The CV curve after the addition of proteins represents how the current charging and discharging behavior changes with the adsorption of a protein layer on the surface. Figure 3.4 shows the...
corresponding capacitance measurements. In Figure 3.4, one can clearly see a significant drop in capacitance over the entire potential range after the addition of BSA protein. We believe this drop in the capacitance is caused by the adsorption of BSA to the surface of the disc electrode. When a layer of protein is adsorbed on the surface of the gold electrode, the electron transfer resistance will increase and the capacitance will decrease. When a protein gets adsorbed on the surface of the electrode, it results in an increase in the electron blocking effect that increases the electron transfer resistance and impedance, thereby reduces the current and results in a drop in the double layer capacitance. Ying and group [69, 70] have investigated the adsorption of Human serum albumin (HSA) on a gold electrode surface by electrochemical and ellipsometric methods. For example, albumin adsorption onto gold was confirmed by the change of the open circuit potential of gold and by the ellipsometric parameter variation during albumin immobilization. The adsorption of albumin under applied potential was also investigated and it was found that both positive and negative applied potential promote albumin adsorption. They have reported that the effect of protein layer on the surface increased the electron blocking effect and have noticed an increase in the adsorbed protein in the positive potential range when compared to the negative range supporting our results.

**Effect of BSA protein concentration on capacitance:**

To examine if the observed capacitance change will be altered when the protein concentration is different, we repeated these experiments by adding different amounts of BSA protein each time. A protein stock solution was prepared and different protein
concentrations from 0.025, 0.05, 0.1, 0.3, 1 mg/mL were used. Figure 3.5 shows the obtained capacitance curves for the gold disc electrode at various BSA concentrations.

![Figure 3.5 Capacitance measurements of a Gold electrode at increasing concentrations of the BSA protein](image)

**Figure 3.5 Capacitance measurements of a Gold electrode at increasing concentrations of the BSA protein**

Indeed, as shown in Figure 3.5, the measured capacitance changes as protein concentration changes. Overall the capacitance curve shifts downward as protein concentration increases, pointing to a decreasing trend in capacitance as protein concentration increases. Intuitively, this fact suggests that higher concentration of BSA in the solution leads to more adsorption of the protein at the surface of the disc electrode. Moreover, the decrease in capacitance is much more prominent in the positive potential
range than in the negative potential range, owing possibly to the fact that BSA is a negatively charged protein resulting in a great amount of protein adsorption in the positive potential range than in the negative potential range.

In Figure 3.5 it is seen that the curve for the 1 mg/mL case almost overlaps with curve for the 0.3 mg/mL case, implying no further decrease in capacitance beyond 0.3 mg/mL concentration. This suggests that adsorption of BSA protein may reach its saturation level when the BSA concentration is 0.3 mg/mL. Similar protein saturation behavior and investigation of the concentration dependence of protein adsorption was reported by others [71].

Recall that the measured CV curves, shown in Figure 3.3, did not show much change in current measurements in the positive potential range before and after BSA was added. In light of this, the significant change observed in the capacitance measurements shown in Figure 3.5 thus indicates the sensitive nature of the capacitive measurements in discerning protein adsorption at the electrode surface.

### 3.3 Platinum electrode studies:

#### 3.3.1 Materials and Methods:

Working electrode (a Platinum disc electrode) and diamond polish were purchased from BASi (West Lafayette, IN), 0.05 micron alumina polish and polishing pads were purchased from Beuhler (Lake Bluff, IL), an Ag/AgCl reference electrode was purchased from Fisher scientific (Pittsburgh, PA). Sulfuric acid, 99.999% pure was purchased from Sigma-Aldrich (St Louis, MO), Albumin from Bovine serum (BSA), 96% electrophoresis from Sigma (St. Louis, MO). Phosphate buffer saline (composed of
Sodium phosphate, monobasic, monohydrate, crystal from J.T Baker (Phillipsburg, NJ), Potassium chloride from Fisher, Sodium chloride, crystalline from Fisher and sodium phosphate, dibasic, anhydrous from Acros, Belgium)

**Experimental set up and methods:**

All electrochemical measurements were taken using the Princeton Applied Research Versa stat MC Potentiostat (Oak ridge, TN). All tests were carried out in a three electrode electrochemical cell consisting of a Platinum disc working electrodes, Ag/AgCl reference electrode and a Platinum mesh counter electrode in respective. The electrolytic solutions used are Sulfuric acid and PBS.

*Preparation and Cleaning of the Working electrode:* The Platinum disc electrode was subjected to Piranha cleaning by dipping the electrode in Piranha solution (3:1 of H₂SO₄:H₂O₂) for 2 minutes. The electrode was then polished using 0.1 micron alumina polish for about 3 minutes and ultra sonicated in Deionized water for 5 minutes.

Following the mechanical polishing/cleaning, the electrode was subjected to electrochemical cleaning by running voltammetric cycles in 0.5 M Sulfuric acid.

**Capacitance measurements to study adsorption of BSA on Platinum electrode surfaces:**

Electrochemical measurements in the forms of cyclic voltammograms and capacitance measurements were recorded first in 0.5 M H₂SO₄ solution and later in 10 mM PBS solution. Protein adsorption studies with BSA were carried out in 10 mM PBS solution.

*CV and capacitance measurement for a Platinum electrode in 0.5 M H₂SO₄ solution:*
Prior to taking the CV measurements for the Platinum disc electrode the solution (0.5 M H$_2$SO$_4$) was purged with Nitrogen for 15-30 minutes. The potential range across which CV measurements were taken was set from -0.28 V to +1.15 V and the scan rate was set at 100 mV/s. The obtained CV and capacitance results were given in Fig 3.6

**CV and capacitance measurements in 10mM PBS solution:**

Following the experiments in sulfuric acid, CV measurements for of the Platinum disc electrode were made in 10 mM PBS solution. Again, the PBS solution was Nitrogen purged for 15-30 minutes prior to measurements. The CV potential range was set from -0.62 V to +0.9 V and scan rate set at 100 mV/s. After the CV experiments, capacitance measurements were taken for the Platinum disc working electrode in 10 mM PBS within the same potential range from -0.62 V to +0.9 V under an alternating biasing potential of 20 mV in amplitude and 10 Hz in frequency. The obtained CV and capacitance measurements are given in Figures 3.7 and Figure 3.8.

**CV and capacitance measurements in 10 mM PBS solution containing BSA:**

In addition to these experiments in blank PBS solution, Bovine serum albumin (BSA) protein was added to the PBS solution and similar experiments were performed in order to investigate how the capacitances change with the addition of BSA protein for some insight into the adsorption behavior of BSA. To do this, the Platinum disc electrode after going through the same cleaning procedure was placed in the same electrochemical cell containing 10 mM PBS mixed with 0.1 mg/mL of BSA protein solution. Here BSA was used as a model protein, which is a widely used protein to study protein adsorption on Platinum electrode surfaces. Prior to electrochemical measurements, the solution was
allowed to stabilize usually for 30 minutes as marked by a stabilizing open circuit potential (OCP) value. Upon reaching a stable OCP value, the CV and capacitance measurements were made in a similar manner as discussed earlier.

Figure 3.9 and Figure 3.10 show the results obtained from these experiments.

3.3.2 Results and Discussion:

![Graph](image)

**Figure 3.6 Cyclic Voltammogram and Capacitance measurements of a Platinum electrode in 0.5M Sulfuric acid solution**

Figure 3.6 shows the CV and capacitance measurement for a Platinum electrode in sulfuric acid solution. In the CV curve presented in Figure 3.6, we can point out several electrochemical events occurring at the Platinum and electrolyte interface such as oxide formation, oxide reduction, and hydrogen adsorption and desorption. For example, the scan starts at a potential of -0.28 V, in the forward direction. As we proceed further in
the forward direction, we notice the hydrogen adsorption and desorption peaks occur at potentials close to -0.25 V to -0.15 V. That region is termed as the hydrogen region. The hydrogen adsorption and desorption peaks are captured only in the hydrogen region. After the hydrogen region, the double layer charging region begins where only significant charging and discharging events are noticed. It starts at 0.1 V and ends at 0.4 V. The end of double layer charging region leads to oxygen formation that begins at 0.45 V, resulting in oxygen evolution at 1.15 V. The last event in the forward scan is the oxygen evolution. Beyond this potential value, the curve goes in the backward direction and oxide reduction happens between 0.7 V and 0.2 V and the CV ends at -0.28 V again in the backward scan branch of the CV curve [72].

In the capacitance curve shown in Figure 3.6, we can clearly see that both the hydrogen adsorption and desorption peaks as well as the surface adsorption and desorption events are all captured. Missing are the faradaic events like the oxidation and reduction of Platinum. This fact may suggest that the capacitive measurements are less sensitive to redox events.
Figure 3.7 Cyclic Voltammogram of a Platinum electrode in 0.01M PBS

From Figure 3.7, we note that various electrochemical events were captured at different potentials. For example, hydrogen desorption and adsorption peaks occur at potential values close to -0.4 V, to be precise at -0.45 V and -0.34 V. Hydrogen formation occurs at -0.6 V, Oxygen formation at 0.9 V and shoulder onset for oxygen formation starts at 0.245 V. In this figure, the region from -0.6 V to -0.2 V consists of the electro adsorption and desorption of Hydrogen. The -0.2 V to 0 V potential range corresponds to the double layer and discharging region. The electro adsorption of Oxygen takes place
beyond 0 V during the positive potential scan. The corresponding electro desorption of oxygen takes place within the potential range of 0.3 V to -0.1 V during the negative potential scan. These events are supported from the results obtained by Hudak and group. They have investigated the behavior of a Platinum working electrode exposed to a PBS solution subjected to Voltammetric conditions [73].

In Figure 3.8, the blue curve represents the CV curve and the red represents the Capacitance measurements. From figures 3.7 and 3.8, we notice that the Hydrogen adsorption and desorption peaks are captured at pretty much the same potential values for both the CV and Capacitance measurements. However, if observed carefully, there is a slight shift in the hydrogen adsorption and desorption peaks that occur during the capacitance measurement. This shift can be attributed to two important factors, which include the mass transport and the electrode kinetics. The mass transport and the electrode kinetics change for an electrochemical system with time. Hence, when we record the capacitance measurements after the CV measurements, although the peaks occur at almost the same potential values, there still is a slight shift in the potential values due to the change in the mass transport and electrode kinetics associated with the electrochemical system.
Figure 3.8 CV and Capacitance measurement of a Platinum electrode in 0.01M PBS solution
Figure 3.9 CV of a Platinum electrode in 0.01M PBS with and without BSA

Figure 3.9 represents the CV measurements of a gold electrode in KCl solution before and after the addition of BSA. The curves can be classified into three distinct regions. The left most region is the negative potential scan region, the middle region is the double layer region and the right most region represents the positive potential scan region. We observe that, in the negative scan region the current decreases after the addition of proteins, whereas in the positive potential region the current decreases a little after protein addition. Now, the noticeable decrease in current after the addition of proteins in the negative region can be explained by the fact that protein adsorption onto
the surface of the electrode has led to an increase in the electron transfer resistance thereby resulting in a decrease in the current confirming adsorption.

![Figure 3.10 CE measurements of a Platinum electrode in 0.01M PBS at various concentrations of BSA](image)

**Figure 3.10 CE measurements of a Platinum electrode in 0.01M PBS at various concentrations of BSA**

Figure 3.9 shows the two CV curves before and after the addition of BSA plotted together. Figure 3.10 depicts the capacitance changes for various concentrations of BSA. From the CV and capacitance curves shown in Figures 3.9 and 3.10, we can clearly see that the addition of the BSA protein has resulted in a drop in the values of capacitance measurements. We observe that as the BSA concentration increases, the coverage of the
Platinum surface with BSA also increases. This causes the blockage of Platinum surface sites, consequently resulting in a decrease in the Platinum-OH ads surface coverage. Hence we notice a decrease in the hydrogen adsorption and desorption charge in the hydrogen region. These results have been supported from studies carried out by Sasha Omanovic and group, to investigate the adsorption of BSA on Platinum surfaces [72]. For example, they have used techniques of cyclic voltammetry and EIS to explain the adsorption behavior of BSA on platinum electrode surfaces. They report how charge transfer resistance was used to determine the resistance due to protein adsorption, obtained using the cyclic voltammetry technique. They explain how the electrochemical techniques of CV and EIS can be used for the investigation of adsorption of proteins on a model metallic surface, platinum. The adsorption of the BSA protein onto Platinum surface is clearly evident in the hydrogen region, supported by the decrease in the hydrogen adsorption and desorption charge peaks for both the CV and the capacitance measurements. This behavior is well documented in other CV studies of protein adsorption [41, 43-45, 74]. For example, several proteins and other biological molecules such as alcohol dehydrogenase, fibrinogen, human serum albumin, cytochrome c, yeast alcohol dehydrogenase and lacto globulin have been used for adsorption studies. Most of these protein adsorption studies are done using only CV method, explaining how the redox events and current changes with the adsorption of protein. However, we report additional information regarding the change in capacitance measurements associated with protein adsorption making it a cost effective and sensitive method.
Additionally, apart from the BSA protein studies, we have used nanoparticles for the gold electrode in 10 mM PBS solution to confirm adsorption using Capacitance measurements.

The following results indicate the Capacitance measurements for a gold electrode in 10 mM PBS solution nanoparticles.

![Capacitance Measurements](image)

**Figure 3.11** Capacitance measurements of a Gold electrode in 0.01M PBS with nanoparticles
The above figure 3.11 confirms adsorption of the nanoparticles on the surface of the gold electrode. This is because we notice a drop in the capacitance after the addition of nanoparticles. With this experiment, we get to understand that capacitance measurements can be used for studying adsorption phenomenon for bio molecular species such as proteins and also for much smaller biomolecules such as nanoparticles, making it a very sensitive and versatile technique to capture surface events.
CHAPTER 4

Electrochemical Capacitance measurements to study glucose adsorption on Platinum electrode surfaces.

The processes of adsorption and electro oxidation of glucose on a platinum electrode surface have been investigated several years ago, as they are of special interest in fundamental and applied electrochemistry [75, 76]. Most of the investigation techniques used so far, for instance Cyclic Voltammetry, involve the application of a large potential to probe the electrode characteristics and capture bulk membranous events to obtain the required measurements. However, we have gone one step further and tried to analyze the adsorption of glucose on platinum surfaces using a different approach – capacitance measurements. These measurements involve the application of a small potential perturbation to probe the electrode-electrolyte interface characteristics and thereby effectively measure the changes in the double layer capacitance value that varies upon the adsorption of glucose. We recorded capacitance measurements of a Platinum working electrode before and after glucose adsorption in buffered media. We noticed that the obtained results explain the adsorption phenomenon of glucose on platinum accompanied by capacitance changes.

4.1 Introduction:

In recent times, the electro-oxidation and adsorption [77-81] of glucose on Platinum electrodes have been studied in connection with glucose sensors [82-86]. Prior
work explains the adsorption process of glucose on Platinum using Cyclic Voltammetry, a technique used to capture the bulk membranous events and electron transfer or faradaic events of an electrochemical reaction [73, 75, 87-89]. However, an electrochemical process is comprised of not just the electron transfer and faradaic events such as oxidation and reduction, but also the non-electron transfer or surface events that include phenomena such as adsorption, corrosion, and film formation. While techniques such as Cyclic voltammetry offer a great amount of information regarding electron transfer events, it has been studied that techniques such as Electrochemical Impedance spectroscopy and double layer capacitance studies provide us with information of the surface events such as adsorption. In fact, these techniques can offer simultaneous monitoring of both bulk membranous as well as surface events, offering superior levels of sensitivity [2].

However, the question to be addressed is, Can we explain the adsorption phenomenon of glucose on Platinum surfaces using Capacitance measurements? In search of an answer to this question and to see if we could actually study and explain the adsorption phenomenon of glucose on Platinum surfaces using capacitance measurements, we carried out preliminary experiments based on capacitance measurements that measure the double layer capacitance at the electrode electrolyte interface, along with the voltammetry experiments, hoping to attain a better understanding of the surface event/adsorption process.

Capacitance measurements are one among the few techniques that allow simultaneous monitoring and measurements of both the bulk membranous electron
transfer events as well as the surface events such as adsorption. Thus, we carried out experiments of glucose adsorption on Platinum electrode surfaces in buffered media. Our experiments are carried out in Phosphate buffer saline (PBS) solution, which serves as an accurate emulation of the living tissue. Cyclic Voltammetry and capacitance measurements are carried out in PBS solution that could capture current as well as the capacitance respectively across a wide potential range.

4.2 Materials and Methods:

Working electrode (a Platinum disc electrode) and diamond polish were purchased from BASi (West Lafayette, IN), 0.05 micron alumina polish and polishing pads were purchased from Beuhler (Lake Bluff, IL), an Ag/AgCl reference electrode was purchased from Fisher scientific (Pittsburgh, PA). Glucose from Fisher Scientific (Pittsburgh, PA), Sulfuric acid, 99.999% pure was purchased from Sigma-Aldrich (St Louis, MO). Phosphate buffer saline (composed of Sodium phosphate, monobasic, monohydrate, crystal from J.T Baker (Phillipsburg, NJ), Potassium chloride from Fisher, Sodium chloride, crystalline from Fisher and sodium phosphate, dibasic, anhydrous from Acros, Belgium)

Experimental Methods:

All electrochemical measurements were taken using the Princeton Applied Research Versa stat MC Potentiostat (Oak ridge, TN). All tests were carried out in a three electrode electrochemical cell consisting of a Platinum disc working electrodes, Ag/AgCl reference electrode and a Platinum mesh counter electrode in respective. The electrolytic solutions used are Sulfuric acid and PBS.
**Preparation and Cleaning of the Working electrode:** The Platinum disc electrode was subjected to Piranha cleaning by dipping the electrode in Piranha solution (3:1 of H$_2$SO$_4$:H$_2$O$_2$) for 2 minutes. The electrode was then polished using 0.1 micron alumina polish for about 3 minutes and ultra sonicated in Deionized water for 5 minutes. Following the mechanical polishing/cleaning, the electrode was subjected to electrochemical cleaning by running voltammetric cycles in 0.5 M Sulfuric acid.

**Capacitance measurements to study glucose adsorption on Platinum electrode surfaces:**

Electrochemical measurements in the forms of cyclic voltammograms and capacitance measurements were recorded first in 0.5 M H$_2$SO$_4$ solution and later in 10 mM Glucose solution. Glucose adsorption studies were carried out in 10 mM PBS solution.

**CV of Platinum electrode in 0.5M Sulfuric acid:**

Prior to taking the CV measurements in 0.5 M Sulfuric acid, the sulfuric acid solution was Nitrogen purged for 15-30 minutes. The potential range across which the current was measured is -0.28 V to +1.15 V. The scan rate is 100mV/sec. Figure 4.1, depicts the Cyclic Voltammogram of a Platinum electrode in 0.5 M Sulfuric acid.

**CV and capacitance measurements in 10 mM PBS solution:**

Following the CV in sulfuric acid, a CV of the Platinum electrode was carried out in 10 mM PBS solution. The PBS solution is also Nitrogen purged for 15-30 minutes prior to measurements. The potential range is -0.62 V to +0.9 V. The scan rate is 100 mV/s. Figure 4.2 depicts the Cyclic Voltammogram of a Platinum electrode in 0.01M PBS solution. Finally, Capacitance measurements of the Platinum working electrode are
recorded in 10 mM PBS at a potential scan of -0.62 V to +0.9 V, frequency of 10 Hz and amplitude of 20 mV. Figure 4.3 depicts the Capacitance measurements of a platinum electrode in 10 mM PBS solution.

Similar set of experiments have been carried out with the addition of glucose, to study the adsorption of glucose and measure how capacitance changes after the addition of glucose.

*CV and capacitance measurements in 10mM PBS solution with 0.1 M Glucose solution:*
The Platinum working electrode is subjected to similar procedures of polishing and cleaning in 0.1M Sulfuric acid, followed by a CV in 0.1 M Sulfuric acid. Following the CV measurements in sulfuric acid, 0.1 M of glucose solution has been added to 0.01 M PBS solution and allowed to stabilize for 30 minutes by monitoring the Open Circuit Potential (OCP). Once the OCP reached a stable value the CV and Capacitance measurements were recorded with similar experimental specification as mentioned earlier.

Figure 4.4 depicts the Cyclic Voltammogram CV of a Platinum electrode in 10 mM PBS solution with 0.1 M Glucose solution and figure 4.5 depicts the Capacitance measurements of the Platinum working electrode in 10 mM PBS with 0.1 M Glucose solution.
4.3 Results and Discussion:

Figure 4.1: Cyclic Voltammogram of a Platinum electrode in 0.1M Sulfuric acid
Figure 4.2: Cyclic Voltammogram of a Platinum electrode in 0.01M PBS solution
Figure 4.3: Capacitance measurements of a Platinum electrode in 0.01M PBS solution
Figure 4.4: Cyclic Voltammogram of a Platinum electrode in 0.01M PBS solution with 0.1M Glucose
Figure 4.5: Capacitance measurements of a Platinum electrode in 0.01M PBS solution with 0.1M Glucose

Discussion:

Figures 4.1 and 4.2 depict the Cyclic Voltammograms of the Platinum working electrode in 0.1 M Sulfuric acid and 0.01 M PBS solution respectively. It can be noticed that the same peaks are captured in both the CV’s, however at different potential values. From Figure 4.2, the peaks as pointed out correspond to the following electrochemical events:
Hydrogen desorption and adsorption peaks occur at potential values close to -0.4 V, to be precise at -0.45 V and -0.34 V. Hydrogen formation occurs at -0.6 V, Oxygen formation at 0.9 V and shoulder onset for oxygen formation starts at 0.245 V [73].

The following Figure 4.6 is obtained by plotting figures 4.2 and 4.3 together and depicts the CV and capacitance measurements of Platinum electrode in 0.01 M PBS solution.

![Figure 4.6: CV and Capacitance measurements of a Platinum electrode in 0.01M PBS solution without glucose](image)

The blue curve represents the CV curve and the red represents the Capacitance measurements. From Figure 4.6, we notice that the Hydrogen adsorption and desorption peaks are captured at the same potential values for both the CV and Capacitance measurements.
However, with the addition of glucose, we notice that both the CV and Capacitance curves look different from the curves without glucose.

Figure 4.7 is obtained by plotting Figures 4.2 and 4.4 together and depicts the CV curve of a Platinum electrode in 0.01 M PBS solution with 0.1 M Glucose solution and a CV curve in 0.01 M PBS solution without glucose. It points out the differences in the Cyclic Voltammogram that occur after the adsorption of glucose on Platinum surfaces.

![CV curves of a Platinum electrode in 0.01M PBS solution before and after Glucose addition](image)

**Figure 4.7: CV curves of a Platinum electrode in 0.01M PBS solution before and after Glucose addition**

The blue curve represents the CV without glucose solution, in blank PBS solution and the cyan curve represents the CV in PBS with glucose solution. In this figure, the region from -0.6 V to -0.2 V consists of the electro adsorption and desorption of
Hydrogen. The -0.2 V to 0 V potential range corresponds to the double layer and discharging region. The electro adsorption of Oxygen takes place beyond 0 V during the positive potential scan. The corresponding electro desorption of oxygen takes place within the potential range of 0.3 V to -0.1 V during the negative potential scan. Peak I corresponds to the electro desorption of adsorbed H atoms, Peak II is at the end of double layer region and peak III occurs in the potential range where the Platinum surface is partially covered with a monolayer of oxygen containing species. Correspondingly, Peak IV represents the partial electro desorption of the oxygen containing species covering the Platinum surface [87].

Finally, Figure 4.8 is obtained by plotting Figures 4.3 and 4.5 together and depicts the Capacitance measurements of a Platinum electrode in 0.01 M PBS with and without 0.1 M Glucose solution. It points out how the capacitance measurements have changed after the addition of glucose and upon glucose adsorption.
Figure 4.8: Capacitance measurements of a Platinum electrode in 0.01M PBS solution before and after the addition of glucose

The pink curve indicates the Capacitance measurements without glucose and the red line curve indicates the capacitance measurements in PBS solution with glucose solution.

From Figure 4.8, we notice that the Hydrogen adsorption and desorption peaks which have been very prominent in the PBS solution without glucose solution (red line curve), have completely been suppressed after the addition of glucose (pink curve). This would help us explain glucose adsorption.

These capacitance measurements depict that a prominent change has occurred after the addition of glucose in the hydrogen adsorption and desorption regions,
explaining that glucose adsorption occurs primarily in this region. It is found that glucose adsorption on Platinum surfaces is accompanied by dehydrogenation of adsorbed molecules [88]. Similar to other organic substances, the adsorption of glucose on a platinum electrode is chemical by nature and leads to a decrease in the hydrogen adsorption in the negative potential region. Hence we notice a suppression of Hydrogen adsorption and desorption peaks after the addition of glucose confirming the adsorption of glucose. Also, we notice a drop in the capacitance which would support the adsorption phenomenon, as the capacitance is known to decrease upon the adsorption of any analyte on the electrode surface.
CHAPTER 5

Instrument Limitations and challenges

From the discussion we had in the previous sections, we can say that double layer capacitance measurement is surely a cost effective and highly sensitive technique for studying the adsorption behavior of proteins on metal electrode surfaces. However, the efficiency of this technique is restricted by certain limitations. The first are limitations offered by the potentiostat-instrument. We have noticed that the recording times for the CV and Capacitance measurements offered by the potentiostat we use are different. The time taken to record a CV measurement is much faster than the time it requires to record a Capacitance measurement. Capacitance measurements are usually recorded at a much slower pace when compared to the CV measurement, although they are carried out at a similar potential range. The Versastat potentiostat offers an option to control the scan rate of a CV measurement. The scan rate is usually set to 100 mV/s. However, there is no option to control or set the scan rate while recording the capacitance measurement. Hence, the CV and Capacitance measurement are recorded at different scan rates, affecting the efficiency of the measurements. To record a CV, it takes a few minutes, around 1-2 minutes, whereas it takes 30-35 minutes to record a cycle of capacitance measurement. When compared to an EIS measurement, capacitance measurements are much faster. An EIS measurement is a multi-point measurement and is measured at several, frequencies. Hence it takes a minimum of 1-2 hours to finish an EIS measurement. Comparatively, Capacitance measurements are single point measurements,
taken at a single frequency value. Hence they are much faster than EIS measurements. However, if we could have a potentiostat that offers a way to control the scan rate of a capacitance measurement, similar to CV measurements, the efficiency and sensitivity of capacitance measurements can be enhanced and improved further.

The second challenge involves polishing the working electrode surfaces. It is extremely important that the working electrodes are polished well prior to recording the capacitance measurements. We have noticed that a slight change in the current density (in the order of a few microamperes) or difference in the surface area of the electrode surfaces affects the capacitance value immensely. Hence, we need to make sure that the electrode surface is polished well using the alumina and diamond polishes until a glossy, shining and smooth surface is obtained. Varying/inconsistent surface roughness causes a change in the surface area of the electrode. This in turn affects the value of the capacitance being measured. Thus, consistency is extremely crucial when it comes to polishing and controlling the surface area of the electrode.
CHAPTER 6

Conclusions

In the past, cyclic voltammogram studies have become the method of choice when it comes to explain protein adsorption and electro oxidation at metal electrode surfaces. As we argued in Chapter 2, most of the current approaches suffer from some drawbacks. To overcome this problem and help analyze the molecular surfacial interactions with superior sensitivity, we have developed a capacitive method. This capacitive method deals with the double layer effect and probes the molecular adsorption and/or adhesion characteristics. We have demonstrated that capacitance measurements are sensitive to capture the adsorption of biomolecules on the surface of the metal electrode. This work surely presents a promising new approach to bio sensing.
References


