Electrodeposition studies of gold nanoparticles and alginate hydrogel films.

Harikrishnan Nambiathan Nambiar
University of Louisville

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ELECTRODEPOSITION STUDIES OF GOLD NANOPARTICLES AND ALGINATE HYDROGEL FILMS

By

Harikrishnan Nambiathan Nambiar

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ELECTRODEPOSITION STUDIES OF GOLD NANOPARTICLES AND ALGINATE HYDROGEL FILMS

By

Harikrishnan Nambiathan Nambiar

A Dissertation Approved on

November 30, 2023

by the following Dissertation Committee:

___________________________________
Dissertation Director – Dr. Francis P. Zamborini

___________________________________
Dr. Richard Baldwin

___________________________________
Dr. Muriel Maurer

___________________________________
Dr. Gautam Gupta
DEDICATION

This dissertation is dedicated to my beloved amma, Manjula Nambiar

“And Miles to go before I sleep” – Robert Frost
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I want to extend my heartfelt appreciation to Dr. Frank Zamborini, my mentor, for providing exceptional guidance throughout my graduate studies. His patient mentorship not only enriched my interest in research but also played a crucial role in my success in a field that was initially unfamiliar to me. His timely support and encouragement proved invaluable, particularly during challenging times. Dr. Zamborini's encouragement to be involved in various aspects significantly improved my research abilities and honed my mentorship skills. It is truly an honor to consider myself a student of Dr. Zamborini, and I am grateful for everything.

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ABSTRACT

ELECTRODEPOSITION STUDIES OF GOLD NANOPARTICLES AND ALGINATE HYDROGEL FILMS

Harikrishnan Nambiathan Nambiar

November 30, 2023

This dissertation investigates the electrochemical deposition (ECD) and electrophoretic deposition (EPD) processes involving citrate (cit)-stabilized Au nanoparticles (NPs) and calcium alginate (Ca-Alg-Au NP) hydrogel films. The first part of the dissertation investigates the influence of NP size, surface area (SA) coverage, and ligand stabilizers on the electrochemical growth of Au on Au NPs, aiming to enhance our understanding of the growth process during the nucleation and growth of metal NPs. The growth kinetics depend on Au NP size (4 nm > 15 nm > 50 nm) for a constant SA coverage, as determined by the overpotentials for ECD. The growth rate increases with increasing SA, showing reversible kinetics for all NP sizes above a threshold SA. In addition, alkanethiol self-assembled monolayers (SAM)-coated Au NPs show a decrease in growth kinetics as the SAM chain length increases while showing the same NP size dependence as cit Au NPs. Faster growth kinetics on Au NPs relative to glass/ITO allows for a two-step EPD/ECD amplification for electrochemical detection of fM solution concentrations of Au NPs by anodic stripping voltammetry (ASV). This approach is sensitive, simple, fast, low cost, and has the potential for automation and portability, making it promising for Au detection for mining, electronic waste, and bioassays.
The second part of this dissertation explores the EPD of Ca-Alg and hybrid Ca-Alg-Au
NP hydrogel films utilizing Au NP-catalyzed electrooxidation of hydroquinone (HQ). This
approach induces a localized pH decrease at the electrode surface, facilitating Alg and Au NP
deposition followed by Ca^{2+} gelation. The catalytic action of Au NPs reduces the EPD potential,
presenting promising applications in sensors, biological studies, electrocatalysis, and energy-
related research. Electrochemical understanding in Ca-Alg-Au NP films involves investigating
the behavior of Fe(CN)$_6^{3-/4-}$ on Pt, Au, and glassy carbon electrodes coated with EPD-deposited
Ca-Alg and Ca-Alg-Au NP hydrogel films. Reversible electrochemistry with decreased Fe(CN)$_6^{3-}$
diffusion follows an initial equilibration time. The amide-functionalized hydrogel impeded
electrochemistry, likely due to hindered mass and ion transfer. Incorporating Au NPs initially
enhances Fe(CN)$_6^{3-}$ redox activity but hampers it at higher loads, indicating a significant change
in mass and ion transfer, likely altering hydrogel viscoelastic and fluid properties. Finally, EPD of
Ca-Alg and Ca-Alg-Au NPs in pipette tips and Pt/Ir scanning tunneling microscopy (STM) tips
enables electrochemical measurements (Fe(CN)$_6^{3-}$, Au ECD, Au ASV, and Au NP EPD) in
microscale hydrogel environments as small as 10 microns. Microscale electrochemistry differs
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CHAPTER 1: INTRODUCTION

1.1. Research Goals and Overview

The primary goals of this research were to study the effects of Au nanoparticle (NP) size and ligand stabilizer on electrochemical deposition (ECD) and electrophoretic deposition (EPD) processes with Au NPs and Au NP-Alginate (Alg) hydrogels. The dissertation delves into the effects of Au NP size and ligand stabilizer (alkanethiolates) on the kinetics of NP growth by ECD. This study led to the development of a two-step electrochemical amplification strategy to detect fM concentrations of Au NPs in solution for potential applications in chemical sensing, biosensors, and environmental monitoring. Another goal of the dissertation was to study the effect of Au NP size on the EPD of hybrid Alg-Au NP hydrogel films, which have potential applications in biotechnology, sensing, and catalysis. Finally, the electrochemistry of redox probes (Fe(CN)$_6$$_{3^-}$), ECD of AuCl$_4^-$ on Au NP, and anodic stripping voltammetry (ASV) of Au NPs was explored in bulk and spatially confined regions of Alg hydrogel and Alg-Au NP hydrogel films.

Chapter 1 of this dissertation provides background information about metal NPs, the EPD of metal NPs, size-dependent oxidation of metal NPs, ECD of Au on Au NPs, Alg hydrogels, the EPD of Alg, and electrochemical reactions in Alg hydrogel. Chapter 2 outlines the experimental procedures and several characterization techniques used in this research. Chapter 3 discusses the ECD of Au onto preformed Au NPs as a function of NP size. Chapter 4 describes the electrochemical growth of Au NPs functionalized with alkanethiol self-assembled monolayers.
Chapter 5 describes a two-step electrodeposition amplification detection technique to detect fM concentrations of Au NPs. Chapter 6 shows the size-dependent EPD of Alg catalyzed by Au NP-catalyzed electrooxidation of HQ to prepare Alg-Au NP hybrid hydrogel films. Chapter 7 discusses Fe(CN)$_6^{3-}$ redox reactions in Alg hydrogels in bulk and spatially confined environments. Chapter 8 explores the ASV of Au NPs, ECD of AuCl$_4^-$, and EPD of Au NPs on glass/indium tin oxide (ITO) electrodes using Alg hydrogels in bulk and microscale environments. Finally, chapter 9 summarizes the research that we have discussed throughout the dissertation and expands on the potential future directions.

1.2. **Motivation and Objective**

The motivation behind this research derives from the need to understand the kinetics of the growth mechanism of the ECD process for Au NPs. ECD is an electrodeposition technique that involves two steps; the nucleation step to form small metal clusters followed by the growth step that involves the growth of the initial nucleation sites. There has been a considerable amount of research regarding the nucleation and growth mechanism of ECD; however, isolated studies of the growth step are still underreported in the literature. Most of the research regarding ECD focused mainly on the nucleation and the early stages of the growth of the nucleation sites. However, these studies did not expand on the effects of the properties of initial nucleation sites in the growth process. Hence, the first objective of this research was to fully understand how the kinetics of the electrochemical growth process is affected by the properties of the nucleation sites (or here chemically formed Au NPs attached on the electrode surface), such as the size, number, and ligands attached to the NPs. Besides the properties of the NPs, we also correlated the effect of the scan rates and the concentration of metal ions in the electrolyte on the ECD growth step. As an extension of this research, we developed a simple two-step electrodeposition amplification technique to detect low concentrations of Au NPs. The objective of this research was to use the
knowledge derived from the early research to controllably grow low concentrations of Au NPs attached to the electrode surface as an essential amplification step, followed by using anodic stripping voltammetry (ASV) as the detection method to detect ultra-low concentrations of Au NPs. The motivation of this work was to demonstrate the use of simple and cost-efficient ASV analysis for the detection of ultra-low concentrations of Au NPs. The details of this process are described in the later chapters.

The objective of the next project was to understand the EPD of Alg using HQ electrooxidation catalyzed by Au NPs to prepare Alg-Au NP hybrid hydrogel. This work was motivated by previous research from our group, where it was observed that the HQ electrooxidation could be used to trigger the deposition of negatively charged citrate-capped Au NPs. It is also noted that the deposition of different sizes of Au NPs could be controlled by controlling EPD potential since the size of the NPs heavily influences the HQ catalysis, which will be elaborated on later in this chapter. This rationale was used to deposit Alg onto electrode surfaces using Au NP-catalyzed HQ electrooxidation. We expand on the role of Au NP size on the EPD of the hybrid Au NP-alg films. The motivation for the final research is derived from the recent increase in interest in sustainable semi-solid electrolyte systems for various applications in battery research, biomedical applications, and sensor fabrication. Hydrogels are key polymers that have shown promising results in this regard. Hence, the final objective was to understand the effects of Alg hydrogels as a medium, both in its bulk and spatially confined form, on some common electrochemical reactions. Spatially confined hydrogels allow for highly localized electrochemical patterning, sensing, and other measurements.

1.3. Introduction to Metal Nanoparticles (NPs)

Metal NPs display unique properties rendering them useful in a plethora of applications in the fields of electronics, medicine, catalysis, and sensors. The distinct nature of metal NPs
from their bulk counterparts can be attributed to its size of the NPs which ranges from 2 to 100 nm in one dimension (usually diameters). When the dimensions of the metals NPs are less than 2 nm, they are termed as metals nanoclusters (NCs). These metal NCs show unique optical and electrochemical behaviors due to its significantly smaller size, higher surface area and surface free energy in comparison to the larger NPs. Metallic NPs are typically created through chemical or electrochemical synthesis, which involves reduction of metal ions to metal atoms. This process occurs via a nucleation-growth mechanism in which the initial reduction of the metal precursors leads to the formation of metal nuclei or atoms. These nuclei then catalyze further reductions of the precursors, resulting in the formation of NPs. The size of these nanoparticles can be controlled by using ligands or capping agents and reducing agents during the reaction. The charge and steric properties of the ligands ensure the stability of the desired nanoparticle size through electrostatic repulsion or steric hindrance. The strength of the reducing agents can impact the number of initial nuclei formed during the reduction and can thus control the final size of the NPs.

At the nanoscale, metal particles have a higher surface area to volume ratio (SA/V) and, thus, a higher number of surface-active sites. This affects the optical, magnetic, thermal, chemical, and electrochemical properties of metal NPs leading them to have distinct behavior from their bulk forms. These properties are strongly influenced by the size, shape, and morphology of the NPs allowing them to be tunable for practical applications. For instance, bulk Au has a highly reflective yellow color, while at the nanoscale, Au exhibits size-dependent optical properties with a red-wine appearance. This is illustrated in Figure 1.1. The unique optical behavior of gold nanoparticles is a result of their localized surface plasmon resonance (LSPR). This refers to the excitation and oscillation of conduction electrons in resonance with light frequency. As the size of the Au NPs decreases, the number of electrons in the conduction band for resonance decreases as well, causing a shift in LSPR and altering the optical properties of the nanoparticles. This behavior is important for size discrimination of nanoparticles using
optical behavior and for applications in surface-enhanced raman spectroscopy (SERS). Other examples include the size of the metal NPs affecting the catalytic activities of the particles towards reactions such as CO$_2$ reduction,\textsuperscript{18} electroreduction of metals,\textsuperscript{1} and hydroquinone electrooxidation\textsuperscript{4} due to variability in the surface-active sites depending on their dimensions. Hence there is great interest in understanding the properties and impact of metal NP size on useful applications, which we will explore in this dissertation.

1.4. Introduction to Electrophoretic Deposition

Electrophoretic deposition (EPD) is a technique widely used in industries such as electronics, ceramics, and coatings to deposit charged particles on a substrate through an applied electric field.\textsuperscript{6,19,20} It is a versatile technique that offers advantages in terms of coating uniformity and material utilization. It traditionally has its application in the automobile industry for painting purposes.\textsuperscript{19} However, over the years, EPD has been used to deposit advanced materials including composites, functional films, biopolymers, and nanomaterials.\textsuperscript{20} EPD is a colloidal technique that often involves an initial electrophoresis step under an applied electric field followed by a deposition setup due to the accumulation of particles on the electrode surface.\textsuperscript{4,19,21} It is usually performed in a two or three-electrode electrochemical cell filled with the colloidal solution. This is illustrated in Figure 1.2. Under an applied potential, the charged particles migrate toward the electrode surface in an electrophoresis step. The size and the charge of the particles influence the electrophoresis process.\textsuperscript{4} When the migrated particles accumulate on the electrode surface, it leads to the deposition of the particles on the substrate. The deposition process is usually triggered by destabilization and/or neutralization of the charged particles at the electrode surface.\textsuperscript{4} The amount of charged particles can be determined using the following equation proposed by Hamaker:

\[ m = C_e \mu A E t \quad (1) \]
**Figure 1.1.** An illustration demonstrating the optical (LSPR) and physical properties (SA/V ratio) of bulk Au, Au NPs (diameters > 2 nm), and Au NCs (diameters < 2 nm).
Figure 1.2. Two steps in EPD: (a) migration under an electric field and (b) deposition due to accumulation on the electrode surface.
Where $m$ is the mass per area unit (g cm$^{-2}$) of deposited material, $Cs$ is the concentration of solids in the suspension (g cm$^{-3}$), $\mu$ is the electrophoretic mobility (cm$^2$ V$^{-1}$ s$^{-1}$), $E$ is the electric field strength (V cm$^{-1}$), $A$ is the surface area of the electrode (cm$^2$), and $t$ is the deposition time (s). Thus, the EPD parameters such as the concentration of particles in solution, the applied electric field, and time are easily adjustable, making the process more robust than other similar techniques. Hence, EPD offers several advantages including high uniformity, excellent reproducibility, dense packing, and predictable and controllable kinetics.$^{19}$ This behavior is due to the easily adjustable EPD parameters such as the concentration of particles in solution, the applied electric field, and time. EPD also requires a simple set-up, making it more robust than other similar techniques. It has been extensively used to deposit several materials, including metal NPs, to conductive surfaces.$^{21}$ For example, Mulvaney et al. demonstrated that EPD could be used to assemble thousands of single Au NPs into predefined patterned cavities on transparent conductive substrates with nanometer spatial resolution.$^{22}$

Charged metal NPs can be deposited onto electrode surfaces using EPD. The deposition of NPs is achieved by a simultaneous reaction at the electrode-electrolyte interface leading to a change in pH at the interface that would trigger the neutralization of NP surface charges leading to insolubility and deposition as illustrated in Figure 1.3a.$^{23}$ The most common reaction that would trigger localized pH change for negatively charged metal nanoparticles is water oxidation:

$$2H_2O (aq) \rightarrow 4H^+ (aq) + O_2 (g) + 4e^-$$

Some of the disadvantages of this reaction are that this electrochemical reaction requires high electrode potentials and the generation of $O_2$, which could interfere with the process. This has been overcome by using HQ electrooxidation (Figure 1.3b), to trigger the deposition of charged metal NPs at the electrode surface.$^4$ $^{24}$ For example, Sakurada et al. used HQ electrooxidation-assisted EPD to deposit zirconia particles onto palladium substrates in a bubble-free manner.$^{25}$
Figure 1.3. EPD is performed by generating protons at the electrode surface using:

(a) water oxidation or (b) HQ electrooxidation.
Allen and Zamborini employed HQ-mediated EPD to deposit citrate-stabilized Au NPs on a glass/ITO electrode at low potentials in a bubble-free manner. Using this method, they were able to selectively deposit smaller Au NPs from a solution mixture of small and large Au NPs. This is due to the increased catalytic activity of small-sized Au NPs towards HQ electrooxidation in comparison to larger ones.

1.5. Size-dependent Oxidation of Metal NPs

The size-dependent electrochemical oxidation of metal NPs was explored first by Henglein to describe the dependence of the standard redox potential on NP size and followed by Plieth developing equations to describe the shift in the reversible redox potential of small metal NPs depending on the size of the particles. The relationship between the shift in oxidation potential and NP radius, referred to as Plieth’s equation, is described as follows:

$$\Delta E_D = -\frac{2\gamma V_m}{zF\gamma/r}$$

Where $\Delta E_D$ is the shift in oxidation potential between the bulk metal and NPs of a particular size, $\gamma$ is the surface stress of the metal, $V_m$ is the molar volume of the metal, $z$ is the number of electrons transferred per oxidized metal atom, $F$ is Faraday’s constant, and $r$ is the metal NP radius. This equation predicts that the difference in the standard redox potential between NPs and bulk metal is proportional to the ratio of surface stress to the radius ($\gamma/r$). This means that as the size of the NPs decreases (radius) and the surface stress of the particles remain constant, leading to a larger shift in redox potential from its bulk form. Hence, the redox potential of small metal NPs shifts more negative to the redox potential of the bulk metals. It was observed that the NPs of radius < 20 nm have the most dramatic change in the electrochemical oxidation potential ($E_p$). When the NP radius is higher than 20 nm, the changes in oxidation potential is infinitesimal, where the metal NP is similar to the bulk metal. For example, Brainina et al. observed a shift of
the maximum current potential of Bi and Au oxidation into the area with more negative potentials while they transition from macro to nanoparticles. Toh et al. investigated the stripping of Au nanoparticles from a glassy carbon electrode. They observed a shift in oxidation potential that correlated with the size and coverage of Ag NPs according to Plieth’s equation. Ivanova and Zamborini were the first to show size-dependent electrochemical oxidation of Au NPs by using ASV and reported a ~200 mV negative shift in \( E_p \) as the size of the NP decreased from 250 nm to 4 nm diameter.\(^{28}\) The oxidation of Au NPs is caused by halides dissolved in an acidic electrolyte.

\[
\text{Au}^0 + 4 \text{Br}^- \rightarrow \text{AuBr}_4^- + 3 \text{e}^-
\]

Masitas and Zamborini also reported that the shift in \( E_p \) of ~850 mV for citrate-capped Au NPs of diameters less than 4 nm from bulk Au \( E_p \).\(^{30}\) Pattadar et al. demonstrated the size-dependent \( E_p \) of Au NPs whose diameter range from 0.9 nm to 50 nm, where a dramatic shift in \( E_p \) is observed when the size of the NPs dramatically decreases from ~450 mV for 1.6 nm to ~220 mV for 0.9 nm diameter.\(^{31}\) Besides size, the ASV, utilizing this size-dependent electrochemical oxidation behavior of Au NPs, can be used to determine NP coverage,\(^4\) aggregation state,\(^{23, 32}\) and surface area to volume ratio.\(^{11, 33}\)

### 1.6. Introduction to Electrochemical Deposition

Electrochemical deposition (ECD) is a technique commonly used to form solid structures on support materials that have applications in advanced energy storage and conversion capabilities, carbon dioxide capture and turnover, and fuel cell technologies.\(^{34-37}\) It is also a common technique to synthesize supported NPs with potential applications in electrocatalysis, electroanalysis, and sensing.\(^{38, 39}\) The reduced costs and versatility of the sizes and structures that can be attained by varying the parameters make ECD advantageous over alternative techniques.\(^{34}\) For this reason, ECD has been extensively used to form arrays of metal and alloy nanoparticles of
diverse sizes and shapes on conductive surfaces. For example, Glasscott et al. used droplet-mediated size-selective ECD to form ligand-free, uniformly distributed Pt NPs on an amorphous graphite electrode. This was achieved by confining the precursor metal salt to a water droplet suspended in oil. Ivanova and Zamborini used ECD to prepare different-sized Au NPs by controlling the deposition potential, also showing that the Au NPs oxidized at more negative potentials as the size decreased. Abdelmoti et al. grown Au nanorods or nanowires using seed-mediated electrochemical deposition (or growth) on MPTMS functionalized glass/ITO electrodes. Cepak and Martin prepared Au and Ag nanorods of diameter 40 and 90 nm with a variety of aspect ratios using ECD in organic solvents like hexafluoro-2-propanol and CHCl₃. Martin et al. fabricated multi-metal nanorods of Pt and Au by ECD in porous template membranes. Dick and co-workers demonstrated one-step direct electrodeposition of ligand-free PdPt alloy NPs from water droplets suspended in a water-in-oil emulsions system onto highly oriented pyrolytic graphite (HOPG). The ECD of NPs was controlled with respect to NP size and substrate coverage. The same group has also electrodeposited ligand-free Cu NPs onto a variety of substrates by nanodroplet-mediated ECD. The surfactants used in the process did not affect the growth kinetics of the NP; however, they impacted the morphology of the NP. Zhu et al. reported a two-step electrodeposition strategy to fabricate highly surface roughened Au nanoarrays on Si wafers that were used as SERS substrates. The first ECD step led to the formation of Au nanocones on the Au nanofilm-coated Si wafer, and Au nanothorns were grown on the upper part of the nanocones via a second ECD step in an electrolyte with many Au seeds. These complex nanostructures increase the surface roughness and hence can serve as high-performance SERS substrates. Ji et al. described the fabrication of trilayer asymmetric metal-dielectric-metal structures by sequential ECD of smooth Au, thin Cu₂O, and finally, thin Au on conductive surfaces, forming an effective optical cavity with angle-insensitive characteristics. By tuning the thickness of the deposited middle cavity layer, different colors of high brightness were achieved.
Traditionally ECD occurs by the well-established nucleation and growth mechanism, as shown in Figure 1.4.\textsuperscript{46, 47} The nucleation step involves the formation of active nuclei of metal atoms (or clusters) at sites randomly distributed over the electrode surface and occurs at an overpotential.\textsuperscript{48, 49} This is followed by a growth step where these nuclei then grow larger at a lower overpotential (more positive potential) by catalyzing the reduction of metal ions or complexes onto the nucleation site. The formation of initial nucleation sites requires higher activation energy and thus, higher overpotential compared to the continued growth of the formed nucleation sites.\textsuperscript{50} The growth step is also significant in the chemical seed-mediated growth of metal nanostructures, where metal NPs function as seeds (nucleation sites) to grow larger metal nanostructures of controlled shape and size.\textsuperscript{41, 51}

Several studies have been conducted to explore the nucleation and growth steps in metal ECD on various electrode substrates.\textsuperscript{48, 52, 53} For example, Ustarroz \textit{et al.} showed that the early stages of Pt electrochemical growth on carbon substrates might be affected by the aggregation, self-alignment, and partial coalescence of nanoclusters of \textasciitilde{}2 nm diameter.\textsuperscript{54} They studied this by using an optimized characterization approach that combined aberration-corrected TEM, electron tomography, and \textit{in-situ} ultrasmall angle x-ray scattering techniques. Pesika \textit{et al.} showed that tuning the ECD of Ag on an Ag electrode within the reaction-controlled/mixed-controlled regime resulted in thin films with varied roughness.\textsuperscript{55} At the reaction-controlled regime, where the current increases exponentially with applied overpotential, the island nucleation density can be systematically varied, and thin films with varied roughness can be formed. Macpherson and co-workers used identical location scanning transmission electron microscopy (STEM) to visualize the very early stages of nucleation at the single-atom level.\textsuperscript{56} This process involved potential-induced atom movement, atom clustering, and cluster transformation into crystalline NPs. Unwin \textit{et al.} investigated the nucleation and growth of Ag on the basal surface of HOPG at the nanoscale using scanning electrochemical cell microscopy (SECCM) and observed that it followed a nucleation-aggregative growth-detachment mechanism.\textsuperscript{48}
Figure 1.4. ECD mechanisms involve the first step being (a) nucleation, leading to the formation of initial nucleation sites, followed by (b) the growth process, which involves the growth of these nucleation sites.
According to this mechanism, initially formed critical nuclei aggregate into clusters, under a diffusion-controlled growth regime, in a process that lowers their surface tensions. When the NP reaches a critical size where the surface tension is sufficiently low, the NPs detach from the electrode surface and are transported into the electrolyte solution. Penner demonstrated the effects of the metal ECD rate on the particle size dispersion in an ensemble of NPs.\textsuperscript{57} The slow growth process on the array of NPs with no diffusional coupling present between neighboring NPs on the substrate effectively reduces the particle size dispersion. Yang et al. explored the initial stages of nucleation and growth in Pd electrodeposition using liquid cell TEM.\textsuperscript{58} They observed that the ECD could be best described by the electrochemical aggregative growth instead of 3D island growth. They also reported the effect of HCl as an added electrolyte on the change in the morphology of Pd electrodeposits from spherical to non-spherical clusters.

There has been a great deal of work exploring the nucleation process of ECD on diverse substrates to create metallic nanostructures, but little attention has been given to the growth step in ECD. Chapter 3 of this dissertation focuses on the electrochemical growth of Au on Au NPs, describing the effect of size and coverage of the Au NPs, as well as the concentration of H\textsubscript{3}AuCl\textsubscript{4}.3H\textsubscript{2}O, on the ECD growth kinetics.\textsuperscript{1}

### 1.7. Introduction to Calcium Alginate Hydrogels

Alginate is an extremely important family of natural polymers that are used to prepare hydrogels that can encapsulate sensitive biomolecules like proteins and nucleic acids and even living cells without them losing their bioactivity.\textsuperscript{59, 60} This is because of its stability at mild pH and temperature conditions. It is a naturally occurring anionic biopolymer derived from the cell walls of marine brown algae.\textsuperscript{61} Alg has several favorable properties, such as low toxicity, biocompatibility, relatively low cost, and mild gelation, which makes it suitable for many biomedical applications.\textsuperscript{62, 63} It consists of unbranched polysaccharides with 1→4 linked β-D-
mannuronic acid and its C-5 epimer α-L-guluronic acid as monomer subunits (Figure 1.5a). Because of the presence of carboxylate groups present in the polymer, Alg is a negatively charged pH shift polymer allowing it to be soluble in basic solution (pH > 7) and insoluble in acidic solution (pH < 7).\(^{64, 65}\)

Alg generally chelates with divalent cations like calcium ions to form hydrogels. Calcium ions, as cross-linking agents, are proposed to bind to guluronate blocks of the Alg chains.\(^{66}\) The guluronate block of one polymer is then crosslinked with the guluronate polymer of the adjacent polymer with the help of Ca\(^{2+}\) ions.\(^{59}\) The resulting structure is commonly referred to as the eggbox model of cross-linking (Figure 1.5b).\(^{59, 61}\) This cross-linking results in gelation and, thus, the formation of hydrogels. The Ca-Alg hydrogels are extensively used to immobilize enzymes, proteins, and nanoparticles at the microscale.\(^{6, 67, 68}\) Ca-Alg hydrogels are also a preferred matrix for the encapsulation of live cells, due to the immunoprotecting behavior of Alg.\(^{69, 70}\)

1.8. Electrophoretic Deposition of Calcium Alginate Hydrogels

To explore the electrochemical properties of Ca-Alg hydrogel, the hydrogel needs to be formed at the electrode surface. There are several methods available to form Alg hydrogels on conductive surfaces, one of the common being EPD. EPD allows the deposition of Alg in a controlled manner forming uniform thin films on the electrode surface.\(^{71}\) This method is also suitable for the co-deposition of biomolecules, NPs, or live cells, allowing them to encapsulate in the electrodeposited Alg films.\(^{72, 73}\) EPD is a versatile technique that allows spatiotemporally controllable deposition of thin film coatings on complex conductive surfaces. The EPD of Alg was first reported by Yokoyama et al., where the hydrogel is fabricated under the influence of an electric field (5 V) based on the electrolysis of water.\(^{74}\) In this system, the anodic reaction at the electrode triggers the ejection of protons due to water electrolysis resulting in low pH at the electrode surface.
Figure 1.5. (a) Structure of alginate and (b) Egg-box model of cross-linking of Ca2+ in alginate hydrogels.
The protons, then, transfer to Alg ions to form alginic acid, which is insoluble at low pH and attaches to the electrode. The anodic reactions are as follows:

\[
\begin{align*}
H_2O & \rightarrow (1/2) O_2 + 2H^+ + 2e^- \\
Na-Alg & \rightarrow Na^+ + Alg^- \\
Alg^- + H^+ & \rightarrow H-Alg \text{ (insoluble)}
\end{align*}
\]

Zhitomirsky and Cheong utilized this method to fabricate a nanocomposite of Alg, hydroxyapatite, TiO$_2$, and chitosan using EPD on a NiTi (50% Ni) shape memory alloy. The purpose of the Alg here is to ensure the electro-steric stabilization and charging of ceramics. Liu et al. fabricated an Alg-based hydrogen peroxide biosensor by performing the EPD of Na-Alg and horseradish peroxidase on Au electrodes. Ozawa et al. reported the use of EPD to fabricate Alg gel microwells on a patterned ITO electrode. Embryonic cells or hepatocellular carcinoma cells were co-deposited and cultured successfully in the gel microwells.

The Payne Group electrodeposited Ca-Alg hydrogels to glass/ITO in response to an anodic signal (localized pH drop) that triggers a localized release of calcium. In this method, the electrochemical oxidation of water at the anode, due to an applied electric field, induces a pH gradient with a locally high proton concentration at the electrode. The localized low pH triggers calcium release by the following reaction:

\[
\text{CaCO}_3 + 2H^+ \rightarrow \text{Ca}^{2+} + \text{H}_2\text{O} + \text{CO}_2
\]

This method was used to entrap and grow *E. coli* bacteria without losing their viability. The trapped cells could be liberated from the gels using sodium citrate that out-competed Alg for Ca$^{2+}$ binding, leading to disruption of the Ca-Alg hydrogel. Taira et al. achieved three-dimensional electrochemical printing of Ca-Alg/gelatin composite hydrogel using the above water hydrolysis triggered Ca$^{2+}$ release method. Mammalian cells were successfully cultured in the as-prepared 3-D composite hydrogels. Jin et al. used electrodeposition to form iron-ion-cross-linked Alg...
hydrogel with embedded drug-mimicking protein (bovine serum albumin) that has been designed for signal-triggered drug release. The electrochemical responsive nature of Alg films is based on the ability of iron ions to cross-link Alg depending on the oxidation state of iron ions. For instance, Fe$^{3+}$ cations result in the formation of hydrogel, while Fe$^{2+}$ ions have weak interaction with Alg, resulting in the hydrogel dissolution and triggering the release of the model protein. Ino et al. electrodeposited Ca-Alg hydrogels using the acidification by the water electrolysis induced by bipolar electrochemistry (BPE). The hydrogels were locally fabricated at target areas of the BPEs by controlling the position of the driving electrodes. The viability of the method was tested by successfully trapping living mammal cells in the electrodeposited hydrogels.

The EPD of Alg has been extensively used to prepare hydrogels on electrode surfaces for biomedical applications. For instance, Vigués et al. fabricated electro-addressable conductive Alg hydrogel for bacterial trapping by electrodeposition of graphite-doped Alg to Au screen printed electrodes. Jovanović et al. utilized electrodeposition to incorporate Ag NPs into Alg hydrogels. The resulting hybrid hydrogels were demonstrated to have antibacterial activity against Staphylococcus aureus. Ibarlucea et al. fabricated flexible Alg/CuO-modified Au electrodes using EPD for the electrochemical detection of ascorbic acid in artificial sweat. Ebrahimi-Nozari et al. used a combination of EPD and electrospinning to form heparin-loaded Alg and atorvastatin calcium-loaded polyurethane coatings on the stent luminal and abluminal stent surfaces, respectively. EPD was used to achieve asymmetric coating on stents and is considered a promising approach for dual drug release for multimodal effects in vascular stent application. Akshay et al. performed EPD of Alg/Bioglass composite coating on Mg-Ca alloy for metallic implant applications. Pupillo et al. utilized multiple electrodeposition steps to grow a composite coating containing Ag, alginic acid, and hydroxyapatite on Ti$_6$Al$_4$V alloy to provide antibacterial properties and osteointegration capability.
EPD of Alg is mainly performed via the electrochemical oxidation of water, which requires higher potential. This leads to the release of protons at the electrode surface, triggering a localized pH drop that neutralizes the negatively-charged Alg, leading to insolubility and deposition. A significant drawback with EPD at higher potentials is that it can limit proteins from getting entrapped in the electrodeposited hydrogels, causing severe problems for applications.\textsuperscript{85} Another problem with this approach is that the by-product of water oxidation, O\textsubscript{2} gas, can create bubbles and affect the morphology of the films.\textsuperscript{86} To overcome this problem, we have used Au NP-catalyzed electrooxidation of HQ to generate protons at the electrode surface that triggers the deposition of Alg.\textsuperscript{2} In this dissertation, chapter 6 elaborates on this approach and how this has been used to prepare hybrid Au NP-Ca-Alg hydrogel films.

\subsection*{1.9. Electrochemical Reactions in Alginate Hydrogels}

Hydrogels are generally viscoelastic, shapable, and ionically conductive, making them useful in several areas, such as conductive membranes, flexible backbones for bioelectronics, and solid gel electrolytes for energy storage devices.\textsuperscript{87, 88} For instance, in bioelectronics, redox reactions in hydrogels play a vital role as an electrical modality that bridges bio-device communication.\textsuperscript{89} Hydrogel thin films offer biocompatibility and can couple additional functionalities with the electrode measurement, which are routinely used in biosensing. Another avenue where hydrogel is being explored is in electrochemical energy storage devices.\textsuperscript{88} Here it is observed that the hydrogels function as immobilized liquid electrolytes that offer better mechanical integrity than liquid electrolytes and a much more desirable ionic conductivity and potential elimination of a separator since they could act as both electrolyte and separator at the same time.\textsuperscript{89} In addition to that, hydrogels can help widen the electrochemical stable potential window by reducing the amount of free water.\textsuperscript{88, 89}
Alg is widely used for hydrogel fabrication because of its good processability with ionic crosslinking, hydrophilicity, and abundant surface functional groups. It is considered an ideal electrolyte for energy storage devices due to its abundance and biodegradability. Alg hydrogels also show better cycling stability due to their neutral properties compared to the acid gel electrolytes. For instance, Guo et al. fabricated a micro-supercapacitor using Alg as a bio-hydrogel electrolyte. It is shown that Alg hydrogels, with MoO$_3$ nanorods as the active material of electrodes, outperformed the commonly used polyvinyl alcohol/H$_2$SO$_4$ hydrogels by widening the operating voltage window. Ji et al. constructed super strong, super stiff, and conductive Alg hydrogels via reconstructing processes that consist of anisotropic densification of pre-gel and a subsequent ionic crosslinking with rehydration. The resulting hydrogel was shown to exhibit high ionic conductivity without compromising the mechanical performance of the gel, and it acted as a stable gel electrolyte membrane for an aqueous supercapacitor. Dong et al. fabricated a cathode-stabilized zinc ion battery based on an alg hydrogel electrolyte coupled with a Na$^+$ pre-intercalated $\delta$-Na$_{0.66}$Mn$_2$O$_4$.13H$_2$O cathode. The battery delivered a high capacity of 305 mAhg$^{-1}$ at 0.1 Ag$^{-1}$, which is 10% higher than the batteries with an aqueous electrolyte. Silva et al. fabricated an assembly of a symmetric superconductor with Alg hydrogel as electrolyte and polyaniline films as electrodes. The supercapacitor with hydrogel showed better electrochemical performance compared to just polyaniline film. Du et al. used Alg hydrogel as electrolyte films to develop quasi-solid-state quantum dots sensitized solar cells with the TiO$_2$-sol interconnecting-modified photoanodes. Alg hydrogel electrolyte films possessed the crosslinking hydrogel network structure and exhibited perfect interfacial contact with modified TiO$_2$ surface, which results in the enhancement of ion transport and redox reaction of polysulfide couple.

Alg has also been used as an electrolytic medium in biomedical applications due to its non-toxic, biocompatible, and biodegradable properties. For instance, Chrouda devised an electrochemical sensor using Alg-decorated single-walled carbon nanotubes for the detection of heavy metal ions via direct electrocatalysis. Differential pulse ASV was used for the detection,
and the detection limits were found to be 0.1, 31, and 1 nM Pb$^{2+}$, Cd$^{2+}$, and Cu$^{2+}$, respectively. Márquez et al. reported using electrodeposited Alg as a matrix to develop an enzymatic biosensor for whole blood analysis. In the biosensor, horseradish peroxidase and glucose oxidase were co-deposited, and the activity of the system was analyzed by chronoamperometry using tetramethylbenzidine as the mediator. Pedrosa et al. reported the use of Alg hydrogel as an alternative to traditional electroencephalography (EEG) electrolytic gels. The Alg hydrogels are desired due to the reduced risks of gel leaking from electrode cavities and the ability for faster and easier cleaning after the clinical procedure. Kosik et al. modified Alg hydrogel by electrostatic binding of poly-L-lysine resulting in an electrolytic membrane that could provide advantageous conditions for connective tissue development.

Hence there is an increased interest in understanding the electrolytic behavior of Alg hydrogels, and there are only a few reports available discussing the electrochemical behavior within the hydrogels. Chapter 7 of this dissertation aimed at discussing the electrochemical reactions within the Alg hydrogel. In this chapter, we discuss electrochemical reactions of redox species like ferricyanide, ruthenium hexamine, and hydroquinone. We also go into detail about the EPD of Au NPs and the ECD of Au in Alg hydrogel electrolytes. We also briefly discuss anodic stripping voltammetry of Au NPs trapped within the hydrogel.

### 1.10. Summary and Accomplishments

Overall, this dissertation focuses on electrodeposition studies of Au NPs and Alg hydrogels. Our work elaborates on the growth kinetics of the ECD process in terms of the size and other characteristics of Au NPs used as nucleation seeds, as well as the scan rates. This work has led to a better understanding of the electrochemical growth process, which is less explored in the literature. The results from this research motivated us to develop the two-step electrodeposition amplification technique for the detection of ultra-low concentrations of Au.
NPs. This is significant as it demonstrates the detection of very low concentrations of Au NPs of the order of femtomolar using a high throughput and cost-effective analytical technique such as ASV. This overcomes the need for labor-intensive, expensive electron microscopy and many other techniques that are used for detection.

In addition, this dissertation focuses on the EPD of Alg using Au NP-catalyzed HQ electrooxidation. This work successfully expands on how the Au NP-catalyzed HQ electrooxidation improves the EPD of Alg by facilitating deposition at low potentials, which will overcome the problems associated with the general procedure. Finally, we also describe several electrochemical reactions performed in Alg hydrogels in both bulk and spatially confined forms.
CHAPTER 2: EXPERIMENTAL

2.1. Chemicals and Reagents

HAuCl₄·3H₂O was synthesized in our lab using the procedure described by B. P. Block by dissolving 99.9% metallic Au in aqua-regia, followed by multiple distillation and crystallization.⁹⁸ Acetone (Pharmaco-AAPER, ACS/USP grade), ethanol (Pharmaco-AAPER, ACS/USP grade), and 2-propanol (Sigma-Aldrich, ACS reagent) were used as solvents for cleaning indium tin oxide (ITO)-coated glass electrodes. Alginic acid sodium salt from brown algae (Sigma-Aldrich, medium viscosity), sodium borohydride (Sigma-Aldrich, ≥98.5 reagent grade), trisodium citrate salt (Bio-Rad laboratories), potassium bromide (Acros Organics, 99%), potassium perchlorate (Alfa Aesar, 99–100%), perchloric acid (Merck, 60%), potassium ferricyanide (Aldon Corp SE), potassium nitrate (Aldon Corp SE), hydroquinone (Alfa Aesar), tetrakis(hydroxymethyl) phosphonium Chloride or THPC (Acros Organics), triphenylphosphine monosulfonate or TPPS (Tokyo Chemical Industry), butanethiol (Sigma-Aldrich), octanethiol (Sigma-Aldrich), dodecane thiol (Sigma-Aldrich), hexadecanethiol (Sigma-Aldrich), octadecylamine (Thermo Scientific), tetradecylamine (Thermo Scientific), dodecylamine, hexadecane (Sigma-Aldrich), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (Sigma-Aldrich), N-hydroxysuccinimide (NHS) (Sigma-Aldrich), and hydrogen peroxide solution (Sigma-Aldrich, 30 wt %) were used as received. NANOpure ultrapure water (Barnstead, resistivity = 18.2 mΩ cm) was used for all aqueous solutions.
2.2. Substrate and Electrode Materials

Different substrates and electrode materials were used depending on the electrochemical experiments. The most used electrode material was indium tin oxide (ITO)-coated glass electrode. The slides were purchased from Delta Technologies Limited (Loveland, CO) and were made of unpolished float (soda lime) glass. Indium tin oxide is a transparent, conductive film coated on one side of the glass (SiO$_2$) with a surface roughness of < 0.2 mm/20 mm and a resistance of 8–12 Ω. Using a diamond cutter, the glass/ITO slides were cut into a rectangular shape with dimensions of 1.6 mm x 7 mm. After this step, the slides were cleaned by sonication for 30 min in acetone, ethanol, and isopropanol, successively. The glass/ITO slides were stored in isopropanol, and before their use, the slides were rinsed in isopropanol and dried under a fast flow of N$_2$ gas. Other substrates used in experiments include 2 mm diameter Pt, Au, and carbon disk electrodes (Delta Technologies Limited), and platinum-iridium (Pt/Ir) scanning tunneling microscopy (STM) tip. The disk electrodes were polished in 1 μm and 0.05 μm alumina powder on 1200 grit carbimet disks and microcloth polishing pads, respectively. The Pt disk electrodes were further cleaned by cycling in a solution of 0.1 M H$_2$SO$_4$ solution until the peaks (at -0.049 V and -0.145 V) related to hydrogen adsorption to Pt were distinctly visible (Figure 2.1.).

2.3. Au Nanoparticle (NP) Synthesis

2.3.1. Synthesis of Citrate-Stabilized 4 nm diameter Au NPs

Citrate-stabilized gold nanoparticles with a diameter of 4 nm were synthesized using the protocol established by Murphy and colleagues (refer to Figure 2.2a). In a 19 mL nanopure water solution under rapid stirring, 0.5 mL of 0.01 M HAuCl$_4$·3H$_2$O and 0.5 mL of 0.01 M trisodium citrate were added sequentially. Following this, 0.6 mL of ice-cold 0.1 M NaBH$_4$ was introduced as a reducing agent. The solution immediately turned orange, indicating the rapid reduction and formation of smaller-sized nanoparticles.
Figure 2.1. 60 CV cycles of a 2 mm Pt disk working electrode (WE) obtained in 0.1 M H₂SO₄ electrolyte at a scan rate of 100 mV/s. Ag/AgCl was used as the reference electrode (RE), and Pt wire as the counter electrode (CE).
2.3.2. Synthesis of Citrate-Stabilized 15 nm diameter Au NPs

A modified Turkevich method was used to synthesize 15 nm average diameter Au NPs (Figure 2.2b). Briefly, 0.5 mL of aqueous solution of 0.01 M HAuCl₄·3H₂O was added to 17.0 mL of nanopure water and heated until full rolling boil. Next, 2.5 mL of 0.01 M trisodium citrate solution was added, and the solution was stirred for 10 minutes. The solution turned bright red color, indicating the formation of Au NPs. Finally, the solution was removed from the hot plate and allowed to cool. Here citrate acts as both the stabilizer as well as the reducing agent. In comparison to NaBH₄, citrate is a weak reducing agent leading to slow reduction. This necessitates the use of boiling to achieve the formation of initial nucleation sites (fewer than 4 nm Au NPs) and larger-sized Au NPs. The final concentration of Au in the solution is 0.25 mM.

2.3.3. Synthesis of Citrate-Stabilized 50 nm diameter Au NPs using H₂O₂

The synthesis of 50 nm Au NPs is adopted from Wang and co-workers (Figure 2.2c). Briefly, solutions of 0.5 mL of 0.01 M HAuCl₄·3H₂O and 1.0 mL of 0.01 M trisodium citrate were added to 18.0 mL of 30 wt% H₂O₂ while stirring. Next, 0.5 mL of the as-prepared 15 nm Au NPs were added to the solution. The color of the solution turned to purplish pink, indicating the formation of Au NPs. Here 15 nm Au NPs are used as seeds to catalyze the reduction of HAuCl₄·3H₂O by H₂O₂, a weak reducing agent.

2.3. Modified Synthesis of Citrate-Stabilized 50 nm diameter Au NPs

A modified synthesis of 15 nm Au NP as mentioned in section 2.3.2 is used to synthesis H₂O₂ free 50 nm Au NPs. Briefly, 0.5 mL of aqueous solution of 0.01 M HAuCl₄·3H₂O was added to 17.0 mL of nanopure water and heated until full rolling boil. Next, 2.5 mL of 0.001 M trisodium citrate solution was added, and the solution was stirred for 10 minutes. The solution turned bright red color, indicating the formation of Au NPs was allowed to cool before use.
a. **Synthesis of citrate capped 4 nm diameter Au NPs**

\[ 8\text{AuCl}_4^- + 3\text{BH}_4^- + 9\text{H}_2\text{O} \rightarrow 8\text{Au}^0 + 3\text{H}_2\text{BO}_3^- + 24\text{H}^+ + 32\text{Cl}^- \]

\[
\begin{array}{c}
\text{Au}^0 \\
\text{Citrate} \\
\text{Continuous Reduction of Au}^{3+}
\end{array} \quad \begin{array}{c}
\text{4 nm Au NP}
\end{array}
\]

b. **Synthesis of citrate capped 15 nm diameter Au NPs**

\[ 2\text{AuCl}_4^- + \text{Na}_3\text{C}_6\text{H}_5\text{O}_7 + 2\text{H}_2\text{O} \rightarrow 2\text{Au}^0 + 3\text{CH}_2\text{O} + 3\text{H}^+ + 8\text{Cl}^- + 2\text{Na}^+ + 3\text{CO}_2 \]

\[
\begin{array}{c}
\text{15 nm Au NP}
\end{array}
\]

c. **Synthesis of citrate capped 50 nm diameter Au NPs**

\[ \text{AuCl}_4^- + 3/2\text{H}_2\text{O}_2 \rightarrow \text{Au}^0 + 3\text{H}^+ + 4\text{Cl}^- + 3/2\text{O}_2 \]

\[
\begin{array}{c}
\text{50 nm Au NP}
\end{array}
\]

**Figure 2.2.** Synthesis of (a) 4 nm, (b) 15 nm, and (c) 50 nm diameter citrate capped Au NPs. 4 nm Au NP synthesis is adopted from Murphy et. al., 15 nm Au NP synthesis is a revised version of the Turkevich method and 50 nm Au NP synthesis was adopted from Wang et.al.
2.4. Au NP Spectroscopic and Microscopic Characterization

Ultraviolet-visible (UV-vis) Characterization of Au NPs. UV-vis spectroscopy is used to characterize the size, shape and aggregation of Au NPs. Au NPs show absorption bands in the UV-vis region (300–700 nm) at a specific wavelength depending on the size of the Au NPs when they are present in the solution phase or while attached to the glass/ITO electrode. The absorption band redshifts to a more positive value as the size of the NP increases. This is due to the localized surface plasmon resonance (LSPR) behavior of Au NPs, which is influenced by the size of the NPs. As the size of the Au NPs increases, the LSPR band becomes sharp due to the collective oscillation of conducting electrons upon excitation with electromagnetic radiation. The UV-vis spectrum was obtained using a Varian Cary 50 Biospectrophotometer by scanning between 350 – 850 nm at a fast scan rate of 80 nm/s using water or bare glass/ITO as the blank.

Scanning Electron Microscopy (SEM). SEM can be used to image the Au NPs attached to the glass/ITO substrates. The size and shape of Au NPs are also characterized using SEM images. In this dissertation, SEM images of glass/ITO/Au NPs were obtained with a Thermo Fisher Scientific Apreo C LoVac field emission scanning electron microscope (FESEM) operating at an accelerating voltage of 20.0 kV using an in-lens Trinity T1 Detector.

2.5. Electrophoretic Deposition of Au NPs

EPD experiments were performed by following the procedure described by Allen and Zamborini to attach Au NPs directly to glass/ITO electrodes. The three-electrode setup (Figure 2.3a) consisted of a cleaned glass/ITO working electrode (area = 0.84 cm²), Pt wire counter electrode, and Ag/AgCl (3 M KCl) reference electrode connected to a CH Instruments (Austin, TX) 660E electrochemical workstation and placed in a beaker cell with the electrodes spaced approximately 1.5 cm from each other in a triangle arrangement. The conductive side of the glass/ITO working electrode faced toward the reference and counter electrodes. For the EPD of 4
nm diameter Au NPs, the beaker cell contained 24 mL of nanopure water, 1 mL of as-prepared Au NPs, and 5 mL of 0.1 M HQ. The EPD of 15 nm diameter Au NPs was performed in a solution containing 21 mL of nanopure water, 4 mL of as-prepared Au NPs, and 5 mL of 0.1 M HQ. The EPD solution for 50 nm diameter Au NPs contained 27.5 mL of Au NPs and 2.5 mL of 0.2 M HQ. The total volume of the EPD solution was 30 mL in all cases. The final concentrations of Au for 4, 15, and 50 nm Au NP EPD solutions were 8.33, 33.3, and 77.9 μM, respectively, and HQ was 16.7 mM in all cases. An electrode potential of 1, 1.2, and 2.2 V, respectively, for 4, 15, and 50 nm diameter Au NPs were applied to the glass/ITO working electrode and held for 90 s for 4 nm, 30 s for 15 nm, and 120 s for 50 nm diameter Au NPs. These conditions lead to different-sized Au NPs with the same total surface area (∼4 × 10⁻⁶ C). After EPD, the glass/ITO electrode was removed from the beaker cell and rinsed immediately with a copious amount of nanopure water before drying under a stream of N₂. After EPD, all glass/ITO/Au NP electrodes were placed in an ozone cleaner for 8 min to remove any stabilizer or impurities from the Au NP surfaces.

2.6. Electrophoretic Deposition of Alginate Hydrogel and Hybrid Ca-Alg-Au NPs

The experimental setup (Figure 2.3b) utilized three electrodes, including a glass/ITO working electrode (area = 0.84 cm²) or Pt disk electrode (area = 0.0314 cm²), Pt wire counter electrode, and an Ag/AgCl (3 M KCl) reference electrode arranged in a triangular pattern, with the electrodes spaced approximately 1.5 cm from each other and connected to a CH Instruments (Austin, TX) 700E electrochemical workstation.² The conductive side of the glass/ITO electrode faced the reference and counter electrodes. The electrodes were immersed in a solution containing 15 mL of 1% (w/v) Na-Alg solution, 6 mL of cit-Au NPs solution (0.25 mM Au), 5 mL of 0.1 M HQ, and 4 mL of H₂O (total volume was 30 mL). The final concentration of Na-Alg, Au NPs, and HQ was 0.50% (w/v), 50 μM (in terms of Au), and 16.67 mM, respectively. The 6 mL of cit-Au NPs solution was replaced with nanopure water for EPD in the absence of Au NPs. An electrode
Figure 2.3. Three-electrode setup for (a) the EPD of Au NPs and (b) the EPD of hybrid Alg-Au NP hydrogels.
potential between 0 and 1.4 V (up to 3.0 V in the absence of Au NPs) was applied to the glass/ITO working electrode and held for 30 min. After the EPD protocol, the glass/ITO electrode was removed from the beaker cell, rinsed immediately with copious amounts of 1% NaCl solution, and placed in a 1% (w/v) solution of CaCl$_2$·2H$_2$O for 30 min at 4 °C. The latter step causes the gelation of Ca-Alg-Au NPs film on the glass/ITO electrode. After this step, the electrode was allowed to air dry for 18 h before further characterization using UV–vis, ATR-FTIR spectroscopy, and dark-field microscopy (DFM). The film was airdried to remove excess water present within the hydrogel. After drying for 18 h, the hydrogel appeared to retain minimum amount of water within the gel. The experiments performed in Chapter 6 this protocol to perform electrochemical growth of Au on Au NPs.

### 2.7. Electrochemical Deposition of Au on Au NPs (Electrochemical Growth)

The three-electrode electrochemical cell (Figure 2.4a) consisted of a glass/ITO working electrode with and without the different-sized Au NPs, a Pt wire counter electrode, and an Ag/AgCl (3 M KCl) reference electrode.$^{102}$ The ECD was performed in a solution of 1 or 0.1 mM H AuCl$_4$·3H$_2$O in 0.1 M KClO$_4$ solution in a beaker cell using linear sweep voltammetry (LSV) from 1.0 to 0.0 V at a scan rate of 100, 10, 1 or 0.1 mV/s. For instance, Figure 2.4b shows the LSV showing the ECD of glass/ITO/15 nm Au NPs electrodes (2.2 NPs/μm$^2$) in 1 mM H AuCl$_4$·3H$_2$O + 0.1 M KClO$_4$ at a scan rate of 100 mV/s. ECD for amplification detection experiments were performed by holding the potential of glass/ITO/Au NP at 0.8 V for 1 min in a beaker cell containing a solution of 1 mM H AuCl$_4$·3H$_2$O in 0.1 M KClO$_4$ solution. The experiments performed in Chapter 3 and 5 followed this protocol to perform electrochemical growth of Au on Au NPs.
Figure 2.4. (a) Three-electrode setup for the ECD of Au NPs and (b) LSV showing the ECD of glass/ITO/15 nm Au NPs electrodes (2.2 NPs/μm²) in 1 mM HAuCl₄·3H₂O + 0.1 M KClO₄ at a scan rate of 100 mV/s. The scan begins at 1 V and ends at 0 V.
2.8. **Self-Assembled Monolayer (SAM) of Alkanethiols on Glass/ITO/Au NPs**

In chapter 5, the effect of ligands on electrochemical growth of Au on Au NPs is studied by using glass/ITO/Au NPs coated with a SAM of alkanethiols. The SAM of alkanethiols was formed on the glass/ITO/Au NPs by soaking the electrode in a 2 mM ethanolic solution of different alkanethiols for 20 h. The alkanethiols used in the experiments include 1-butanethiol (C4S), 1-octanethiol (C8S), 1-dodecanethiol (C12S), and 1-hexadecanethiol (C16S), and ethanol was used as a control.

2.9. **Electrochemical Analysis of Au NPs**

**Electrochemical Surface Area (SA) Characterization.** A three-electrode setup consisting of the ozone-cleaned glass/ITO/Au NPs working electrode, Pt wire counter electrode, and an Ag/AgCl (3 M KCl) reference electrode was used for SA characterization of Au NPs. The total SA of the deposited Au NPs was characterized by measuring the charge under the reduction peak after running an oxidation/reduction cycle (100 mV/s) from 0.0 to 1.4 V in 0.1 M HClO4. Integration of the Au oxide reduction peak near ~0.9 V on the return segment provided information about the total surface area of the Au NPs, allowing determination of the surface area and coverage for the different sized Au NPs. For example, Figure 2.5a shows the CV obtained in 0.1 M HClO4 solution of glass/ITO/Au NP (4, 15, and 50 nm) electrodes with the total constant Au surface area at ~4 × 10^6 C by integrating the cathodic peak at ~0.9 V.

**Anodic Stripping Voltammetry (ASV).** ASV was performed to characterize the size and coverage of Au NPs attached to the glass/ITO electrode. The electrochemical cell for ASV characterization consists of a three-electrode setup consisting of the ozone-cleaned glass/ITO/Au NPs working electrode, Pt wire counter electrode, and an Ag/AgCl (3 M KCl) reference electrode. LSV mode in the potentiostat is used to perform ASV by scanning from ~0.2 to 1.4 V at 10 mV/s in 0.1 M KClO4 plus 10 mM KBr electrolyte solution. The peak oxidation
potential ($E_p$), due to Au oxidation to AuBr$^{-}_2$ or AuBr$^{-}_4$, was used for qualitative size determination and for monitoring the aggregation of Au NPs. The integrated peak area from the ASV provides the coulombs of charge passed during the complete oxidation (stripping) of Au NPs, which provides a measure of the total amount of Au on the electrode surface and is an indicator of the total volume (V) of Au NPs on the electrode. Figure 2.5b shows the ASV obtained in 10 mM KBr in 0.1 M KClO$_4$ solution of glass/ITO/Au NP (4, 15, and 50 nm) electrodes with the total constant Au surface area. The $E_p$ associated with ASVs are indicative of the size of the Au NPs; for example, the $E_p$ for 4, 15, and 50 nm Au NPs are 0.712 V, 0.766 V, and 0.873 V, respectively.

2.10. Spectroscopic Analysis of Calcium Alginate (Ca-Alg) Hydrogels

**UV-vis spectroscopy.** Ca-Alg hydrogels deposited on glass/ITO were characterized by using UV-vis spectroscopy. UV–vis spectra were recorded using a Varian instrument model CARY 50 Bio UV–visible spectrophotometer by scanning between 350 to 900 nm wavelength range at a fast scan rate. The background was subtracted using bare glass/ITO as the blank. Figure 2.6a shows the UV-vis spectra of glass/ITO/Ca-Alg prepared by performing EPD in 0.5% Alg at 3 V (vs. Ag/AgCl) for 30 min, followed by Ca$^{2+}$ exposure for 30 min and air-drying for 18 h. The weak band observed at 475 nm is associated with Ca-Alg hydrogel.

**ATR-FTIR spectroscopy.** Infrared spectra were recorded using a PerkinElmer Spectrum Fourier transform infrared spectrophotometer with an attenuated total reflectance attachment (ATR-FTIR). The background was subtracted using bare glass/ITO as the blank. ATR-FTIR spectroscopy was used to characterize the Alg deposited on the glass/ITO electrode. Figure 2.6b shows the ATR-IR spectra of glass/ITO/Ca-Alg prepared by performing EPD in 0.5% Alg at 3 V (vs. Ag/AgCl) for 30 min, followed by Ca$^{2+}$ exposure for 30 min and air-drying for 18 h.
Figure 2.5. (a) CV obtained in 0.1 M HClO$_4$ electrolyte at a scan rate of 100 mV/s of Glass/ITO/Au NP (4, 15, and 50 nm) electrode, and (b) ASVs of glass/ITO/Au NPs (4, 15, and 50 nm) electrodes in 10 mM KBr + 0.1 M KClO$_4$ at a scan rate of 10 mV/s. The scan begins at 0 V and ends at 1.4 V.
Figure 2.6. (a) UV-Vis and (b) ATR-FTIR spectra of glass/ITO/Alg electrodes. The electrodes were prepared by performing EPD in 0.5% Alg at 3 V (vs. Ag/AgCl) for 30 min, followed by Ca\(^{2+}\) exposure for 30 min and air-drying for 18 h.
The peaks at 3345, 1600, 1424, 1263, 1213, 1081, and 1027 cm\(^{-1}\) correspond to the hydrogen-bonded O–H stretch, the asymmetric and symmetric stretching vibrations associated with the COO\(^–\) group (consistent with Ca\(^{2+}\)-carboxylates formed), the skeletal vibrations of Alg and alginic acid moieties, and the antisymmetric C–O–C stretching vibration of pyranose rings of the Alg polysaccharide, respectively.

2.11. Microscopic Analysis of Au NPs and Ca-Alg Hydrogels

**Darkfield Microscopy (DFM).** DFM is used to image the cross-section of the as-prepared glass/ITO/Ca-Alg or glass/ITO/Ca-Alg-Au NP hydrogel electrodes after drying the films for 18 h under ambient temperature and humidity.\(^2\) The darker section with the bright line above it in all images is the glass/ITO electrode as labeled on the images. The faint bright region above the bright line is the Ca-Alg or Ca-Alg-Au NP hydrogel film as labeled. All DFM images were obtained with an inverted Nikon Eclipse Ti microscope (Figure 2.7a) with a halogen lamp light source and a dark-field condenser (NA = 0.95–0.80) (Figure 2.7b) for sample illumination and a 10x objective (NA = 0.25) for imaging. Figure 2.8 shows the DFM images of glass/ITO/Ca-Alg (a) and glass/ITO/Ca-Alg-Au NP (b) prepared by EPD at 1 V for 30 min, followed by Ca\(^{2+}\) exposure for 30 min and air drying for 18 h.

**SEM Microscopy.** SEM was used to image and characterize the Ca-Alg attached to glass/ITO electrodes. The images were also used to characterize Au NPs trapped inside Ca-Alg hydrogel. SEM images of glass/ITO/Ca-Alg were obtained with a Thermo Fisher Scientific Apreo C LoVac field emission scanning electron microscope (FESEM) operating at an accelerating voltage of 20.0 kV using an in-lens Trinity T1 Detector.
Figure 2.7. A picture of (a) an inverted Nikon Eclipse Ti microscope and (b) the dark-field condenser (NA = 0.95–0.80) (Figure 2.8b) for sample illumination.
Figure 2.8. DFM images of (a) glass/ITO/Ca-Alg and (b) glass/ITO/Ca-Alg-4 nm Au NPs. The electrodes were prepared by performing EPD at 1 V (vs. Ag/AgCl) for 30 min, followed by Ca$^{2+}$ exposure for 30 min and air-drying for 18 h.
2.12. Two-step Electrochemical Amplification Detection of fM Au NP

**Electrophoretic Deposition (EPD) of Au NPs onto Glass/ITO.** The first step in the amplification detection of Au NPs, discussed in Chapter 4, is electrophoretic deposition (EPD). As-prepared Au NPs were directly attached to the glass/ITO electrodes using the hydroquinone (HQ)-mediated EPD described by Allen et al.\(^4\). The EPD is performed with a three-electrode setup consisting of a clean glass/ITO working electrode (area = 0.84 cm\(^2\)) (conductive side facing other electrodes), Pt wire counter electrode, and Ag/AgCl (3 M KCl) reference electrode placed in a beaker cell with the electrodes are arranged in a triangle formation spaced approximately 1.5 cm from each other. The electrode setup was connected to a CH Instruments (Austin, TX) 660E electrochemical workstation. The EPD of 4 nm diameter Au NPs was performed from a solution containing 5 mL 0.1 M HQ and 25 mL diluted Au NPs (refer SI) to obtain the desired concentrations (1.7, 17 and 170 nM) of NPs. The final volume of the EPD solution was 30 mL and the final concentration of the HQ was 16.7 mM in all cases. An electrode potential of 0.8 V was applied to glass/ITO working electrode and held for different times (30 s, 1, 3, 5 and 15 min) according to the experiment. Following the EPD, the NP deposited glass/ITO electrodes were rinsed with copious amount of nanopure water before drying under a stream of N\(_2\). Finally, the NPs were placed in an ozone chamber for 8 min to remove the citrate stabilizer as well as impurities from the NP surface.

**Electrochemical Deposition (ECD) of Glass/ITO/Au NPs.** The second step in the amplification detection of Au NPs is electrochemical deposition (ECD). The ECD is performed in a three-electrode setup containing a glass/ITO working electrode with and without the different-sized Au NPs, Pt wire counter electrode, and an Ag/AgCl (3 M KCl) reference electrode.\(^102\) It was performed in a solution of 0.001 M HAuCl\(_4\)·3H\(_2\)O in 0.1 M KClO\(_4\) solution in a beaker cell by holding the potential at 0.8 V for 1 min.
The Detection of amplified Au NPs using ASV. The Au NPs amplified by EPD and ECD were detected using ASV. As described earlier, ASV was performed on glass/ITO/Au NPs electrode by scanning the potential from 0.2 V to 1.2 V at a scan rate of 0.01 V/s in 0.01 M KBr plus 0.1 M KClO₄ solution. The peak current under the ASV curve is used as metric to detect Au NPs. This is because the peak current directly correlates the amount of Au NPs attached and grown on the glass/ITO electrode.

2.13. Fe(CN)₆³⁻ redox reactions of Pt/Ca-Alg, Au/Ca-Alg, and Carbon/Ca-Alg

As described section 2.9, an EPD was performed to form hydrogels at a Pt (2 mm radius), Au (2 mm radius) or Carbon (3 mm radius) disk electrode. ECD was performed by using a solution containing 20 mL of 1% Ca-Alg, 5 mL of 0.2 M HQ, and 5 mL of water at an applied potential of 1.4 V for a duration of 30 min. After the EPD process, the Alg-deposited electrode was cleaned using a solution made up of 1% NaCl. The hydrogel electrode was then soaked for 30 minutes in a solution containing 90 mM CaCl₂·2H₂O at a temperature of 5°C. As depicted in Figure 2.11, the last step of the procedure required running a CV test on a solution consisting of 4 mM Fe(CN)₆³⁻ and 1 M KNO₃ at a scan rate of 10 mV/s.

2.14. EDC coupling of Pt/Ca-Alg, Au/Ca-Alg, and Carbon/Ca-Alg

EPD was carried out on a Pt (2 mm radius), Au (2 mm radius) or Carbon (3 mm radius) working electrode by following the procedure described in section 2.6 using a suspension comprising 20 mL of 1% Ca-Alg, 5 mL of 0.2 M HQ, and 5 mL of water at an applied voltage of 1.4 V for a duration of 30 min. Following EPD, the deposited material was rinsed with a 1% NaCl solution. Subsequently, the hydrogel electrode underwent a 30 min soaking period in a 90 mM CaCl₂·2H₂O solution at 5°C. The next steps involved a 30 min immersion in a mixture of 4 mM
Figure 2.9. A general scheme illustrating the preparation of Pt/Alg electrodes and the Fe(CN)$_6^{3-}$ redox reactions with the electrodes.
1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 10 mM N-hydroxysuccinimide (NHS), followed by a 30-minute soak in 10 mM ethanolic solution of alkylamine solution. The alkylamines used for this step includes octadecylamine, tetradecylamine, and dodecylamine. The hydrogel electrode was then rinsed with ethanol before further analysis. The final step in process involved CV in a solution containing 4 mM Fe(CN)_6^{3-} and 1 M KNO_3 at a scan rate of 10 mV/s. This is illustrated in Figure 2.10.

2.15. **Ag/AgCl/Ca-Alg hydrogel Pipette electrodes for ECD and ASV of Au NPs**

The pipettes with Ca-Alg hydrogels were used to achieve spatially confined environments to perform ECD of Au on glass/ITO electrodes and ASV of Au NPs on glass/ITO/Au NPs electrodes discussed in Chapter 8. Figure 2.11a shows the preparation of Ag/AgCl/Ca-Alg containing KBr and KClO_4 pipette electrodes that are used to perform localized ASV of Au NPs attached to a glass/ITO electrode. To fabricate the electrode, 0.8 mL of a solution containing 10 mM KBr, 0.1 M KClO_4, and 1% Na-Alg was added to a regular Pasteur pipette (radius = 550 μm). Subsequently, the pipette was immersed into a solution consisting of 90 mM CaCl_2·2H_2O, 10 mM KBr, and 0.1 M KClO_4. Finally, the Ag/AgCl electrode was inserted into the pipette and securely sealed it with parafilm before performing ASV of glass/ITO/Au NP working electrode. Figure 2.11b shows the preparation of Ag/AgCl/Ca-Alg containing AuCl_4^- and KClO_4 pipette electrodes, which follows similar steps as described above. The pipette electrode was prepared by adding 0.8 mL of a solution containing 1 mM HAuCl_4·3H_2O, 0.1 M KClO_4, and 1% Na-Alg to the regular Pasteur pipette with a radius of 550 μm. Then, pipette was dipped into a solution containing 90 mM CaCl_2·2H_2O, 1 mM HAuCl_4·3H_2O, and 0.1 M KClO_4, followed by the insertion of Ag/AgCl electrode into the pipette and securely sealed it with parafilm before performing ECD of Au on the glass/ITO working electrode.
Figure 2.10. A general scheme illustrating the preparation of Pt/Alg-oleyl amide electrodes and the Fe(CN)$_6^{3-}$ redox reactions with the electrodes.
Figure 2.11. (a) Preparation of Ag/AgCl/Ca-Alg containing KBr and KClO₄ pipette electrodes to perform ASV of glass/ITO/Au NPs. (b) Preparation of Ag/AgCl/Ca-Alg containing AuCl₄⁻ and KClO₄ pipette electrodes to perform ECD of Au on glass/ITO working electrode.
2.16. Spatially Confined ECD using Pt/Ir/Ca-Alg

The spatially confined ECD of Au on glass/ITO was performed using a Pt/Ir scanning tunneling microscope (STM) probe (with tip diameter ~1 μm) with Ca-Alg hydrogel deposits serving as a reservoir for AuCl₄⁻ ions. This is discussed in Chapter 8. To create Ca-Alg hydrogel deposits on the Pt/Ir STM probe, an EPD process was carried out in a solution comprising 0.5% w/v Na-Alg and 33.4 mM HQ at 1.4 V for 15 minutes. Subsequently, the Pt/Ir/Alg assembly underwent a 30-minute immersion in an ice-cold solution of 90 mM CaCl₂·2H₂O before being utilized for ECD. Following the preparation of Pt/Ir/Alg electrodes, the hydrogel was enriched with AuCl₄⁻ and KClO₄ by soaking it in a solution containing 10 mM HAuCl₄·3H₂O and 0.1 M KClO₄, facilitating the ECD of gold on glass/ITO electrodes. In Figure 2.12a, the setup for ECD of Au on glass/ITO is depicted, utilizing Pt/Ir/Alg as the reference and counter electrode, while glass/ITO serves as the working electrode. Figure 2.12b illustrates a picture of the Alg hydrogel on the Pt/Ir STM electrode.
Figure 2.12. (a) The setup for ECD of Au on glass/ITO using Pt/Ir/Ca-Alg containing AuCl₄⁻ and KClO₄ as the reference and counter electrode, while glass/ITO serves as the working electrode. (b) A picture of the Pt/Ir/Ca-Alg electrode.
CHAPTER 3: SIZE-DEPENDENT ELECTROCHEMICAL METAL GROWTH KINETICS

3.1. INTRODUCTION

Electrochemical deposition (ECD) involves the reduction of simple metal ions or metal complexes to the metallic form onto a conductive electrode surface. It is widely used for coating purposes\textsuperscript{105} with applications in electronics,\textsuperscript{106} catalysis,\textsuperscript{107} Raman detection,\textsuperscript{38} and sensing.\textsuperscript{108} ECD can also be used to form arrays of metal and alloy nanoparticles (NPs)\textsuperscript{109, 110} of various sizes and shapes on electrode surfaces.\textsuperscript{34, 111, 112} For example, Glasscott \textit{et al.} used droplet-mediated size-selective ECD to form ligand-free, uniformly distributed Pt NPs on an amorphous graphite electrode.\textsuperscript{109} This was achieved by confining the precursor metal salt to a water droplet suspended in oil. Ivanova and Zamborini used ECD to prepare different-sized Au NPs by controlling the deposition potential, where the Au NPs oxidized at more negative potentials as the size decreased.\textsuperscript{113} Others have performed metal ECD in various templates, such as porous membranes,\textsuperscript{114} the step edges of highly-oriented pyrolytic graphite (HOPG),\textsuperscript{115} or lithographically-defined templates,\textsuperscript{116} to prepare one-dimensional metal nanorods and nanowires.

ECD traditionally occurs by the well-known nucleation and growth mechanism, where the formation of a supercritical (or stable) nucleus of metal atoms (or clusters) occurs first at an overpotential.\textsuperscript{46, 48, 117} These nuclei then grow larger at a lower overpotential (positive potential)
by catalyzing the reduction of metal ions or complexes onto the nucleus, known as the growth step. Metal ion (or complex) reduction to form the initial nucleation sites has a higher activation energy and, hence, requires higher overpotential compared to the continued growth of the formed nucleation sites.\textsuperscript{118} The growth step is the basis for the chemical seed-mediated growth of metal nanostructures, where pre-formed metal NPs act as nucleation sites, or “seeds”, for further growth of the seeds into larger metal nanostructures of controlled shape and size.\textsuperscript{51} Abdelmoti and Zamborini adopted the chemical seed-mediated growth method to synthesize Au nanorods (NRs) by an electrochemical seed-mediated deposition process in the presence of cetyltrimethylammonium bromide (CTAB).\textsuperscript{41}

Numerous studies have been conducted to understand the nucleation and growth phenomenon of metal ECD on various electrode materials.\textsuperscript{48, 52, 53} For example, Ustarroz \textit{et al.} showed that the early stages of Pt electrochemical growth on carbon substrates may be affected by the aggregation, self-alignment and partial coalescence of nanoclusters ~2 nm in diameter.\textsuperscript{54} Pesika and co-workers showed that by tuning the ECD of Ag on an Ag electrode within the reaction-controlled regime, where the current increases exponentially with applied overpotential, the island nucleation density can be systematically varied and thin films with varied roughness can be formed.\textsuperscript{55} Macpherson and co-workers used identical location scanning transmission electron microscopy to visualize the very early stages of nucleation at the single atom level, which involved potential-induced atom movement, atom clustering, and cluster transformation into crystalline NPs.\textsuperscript{56} Penner and co-workers discovered that the metal ECD rate affects the particle size dispersion in an ensemble of NPs.\textsuperscript{119} Slow growth on an array of NPs with no diffusional coupling present between neighboring NPs on the electrode surface greatly reduces the particle size dispersion. There has been a great deal of work measuring the overpotential needed to induce nucleation on various electrode surfaces and using ECD as a tool to create metallic nanostructures on electrode surfaces, but little attention has been given to the growth step kinetics in ECD.
3.2. RESULTS AND DISCUSSION

Experimental Design. In this work, we examine the ECD of Au onto chemically preformed Au NPs of varied sizes attached to indium tin oxide-coated glass (glass/ITO) electrodes. Scheme 1 shows the general experimental design of this work. First step of Figure 3.1 involves the electrophoretic deposition (EPD) of different-sized, chemically-preformed citrate-stabilized Au nanoparticles (NPs) onto a glass/ITO working electrode using our previously developed hydroquinone (HQ)-mediated method. The size of the Au NPs was controlled through chemical synthesis (4 nm, 15 nm, and 50 nm) while the coverage of Au NPs on the glass/ITO electrode was controlled by the EPD deposition conditions (Au NP concentration, deposition time, and deposition potential). After the EPD step, we measured the total surface area of the deposited Au NPs (Figure 3.2a) as mentioned earlier in section 2.8. Second step of Figure 3.1 involves the electrochemical deposition (ECD) of Au onto the glass/ITO/Au NP electrodes in 1 mM HAuCl₄·3H₂O and 0.1 M KClO₄ by sweeping the potential from 1.0 V to 0.0 V vs Ag/AgCl (3 M KCl) at varied scan rates. The peak potential and peak current provide kinetic and thermodynamic information about the ECD of Au onto the different-sized preformed Au NP nucleation sites (metal growth step).

ECD as a Function of Au NP Size with Constant Total Au Surface Area. Figure 3.2 shows linear sweep voltammograms (LSVs) of Au ECD on glass/ITO and glass/ITO/Au NP electrodes with different size Au NPs at different scan rates, including 100 mV/s (Figure 3.2b), 10 mV/s (Figure 3.2c), 1 mV/s (Figure 3.2d) and 0.1 mV/s (Figure 3.2e). The main cathodic peak in the LSVs ranging from about 0.80 to 0.25 V is due to the electrochemical reduction of AuCl₄⁻ to
Figure 3.1. The general experimental design involved in the ECD experiments.
metallic $\text{Au}^0$ at the glass/ITO and glass/ITO/Au NP surfaces according to the following electrochemical reaction:

$$\text{AuCl}_4^- + 3e^- \rightarrow \text{Au}^0 + 4\text{Cl}^- \quad E^0 = 808 \text{ mV vs. Ag/AgCl (3 M KCl)} \quad (1)$$

The electrochemical reduction of $\text{AuCl}_4^-$ on bare glass/ITO (a black legend in Figure 3.2b), at a scan rate of 100 mV/s, occurs at a peak potential of $268 \pm 19$ mV, which corresponds to the formation of Au nucleation sites at the bare glass/ITO electrode surface. Au nucleation on bare glass/ITO does not start until an overpotential of about $\sim 540$ mV, which is required to overcome the activation barrier for the initial reduction of $\text{AuCl}_4^-$ to form $\text{Au}^0$ nucleation sites on the glass/ITO surface. The $\text{AuCl}_4^-$ reduction to form $\text{Au}_x^0$ nucleation sites (small meal clusters) is a high activation energy process that requires more negative potential than required for $\text{AuCl}_4^-$ reduction onto $\text{Au}_x$ metal clusters (or Au seed NPs).\textsuperscript{46, 47}

$$x\text{AuCl}_4^- + (3x)e^- \rightarrow \text{Au}_x^0 + (4x)\text{Cl}^- \quad (2)$$

(nucleation = high activation energy = high overpotential = slow kinetics)

$$y\text{AuCl}_4^- + (3y)e^- + \text{Au}_x^0 \rightarrow \text{Au}_{x+y}^0 + (4y)\text{Cl}^- \quad (3)$$

(growth = low activation energy = low overpotential = fast kinetics)

As shown in Figure 3.2b, the presence of preformed Au NPs on the electrode surface catalyzes the $\text{AuCl}_4^-$ reduction, leading to ECD occurring at lower overpotentials for all sized Au NPs, which promotes the growth of the preformed Au NPs into larger Au NPs (equation 3). Hence the peak deposition potential for growth occurs at more positive potentials (equation 3) than that for nucleation on bare glass/ITO (equation 2). With the total Au surface area constant at $\sim 4 \times 10^{-6}$ C for the three different-sized Au NPs on the glass/ITO surface, the more positive ECD potential for the 4 nm Au NPs indicates faster charge transfer kinetics. The ECD rate increased with decreasing Au NP size as shown by the more positive growth potential as the size of the Au NPs decreased. The onset potentials for 4 nm, 15 nm, and 50 nm Au NPs at 100 mV/s were $928 \pm 4$ mV, $914 \pm 3$ mV, and $868 \pm 4$ mV, respectively. The peak potentials for 4 nm, 15 nm, and 50 nm
Figure 3.2. (a) CV obtained in 0.1 M HClO₄ electrolyte at a scan rate of 100 mV/s of Glass/ITO/Au NP (4, 15, and 50 nm) electrodes. LSVs (ECD) of Glass/ITO/Au NP electrodes obtained in 0.001 M HAuCl₄.3H₂O + 0.1 M KClO₄ at a scan rate of (b) 100 mV/s, (c) 10 mV/s, (d) 0.1 mV/s. The scan begins at 1.0 V and ends at 0.0 V vs. Ag/AgCl (3M KCl). EPD potentials and times for 4 nm, 15 nm, and 50 nm Au NPs are 1.0 V and 90 s, 1.2 V and 2.2 V and 120 s, respectively.
Au NPs at 100 mV/s were 702 ± 4 mV, 687 ± 8 mV, and 585 ± 26 mV, respectively. The smaller Au NPs required lower overpotentials compared to the larger Au NPs to initiate the electrochemical reduction of \( \text{AuCl}_4^- \) and growth of the Au NPs. All sizes exhibit more positive ECD potential than the nucleation peak potential of 268 ± 19 mV on bare glass/ITO. Interestingly, on bare glass/ITO, there are shoulder peaks at ~400 mV and 600 mV, indicating that there may be different sites on the ITO that have different nucleation kinetics.

In addition to the positive shift in reduction potential with decreasing Au NP size, the peak current associated with \( \text{AuCl}_4^- \) reduction increased with decreasing Au NP size, which was 5.7 x 10^4 A, 4.9 x 10^4 A, and 2.7 x 10^4 A, respectively, for the 4 nm, 15 nm, and 50 nm Au NPs, while the peak current due to nucleation and growth at the glass/ITO electrode was 4.7 x 10^-4 A. Kinetic limitations not only shift the peak potential negative but also decrease the peak current as the electron transfer rate constant decreases with increasing Au NP size and for Au nucleation and growth at bare glass/ITO. We do not believe the different currents are due to different electrode areas since the Au NP surface area of ~4 x 10^-6 C was kept constant, and the peak current should be proportional to the entire glass/ITO electrode area (0.84 cm^2) since the coverage of Au NPs is well above the nanoparticle spacing required for diffusional overlap (linear diffusion) at the scan rate employed (see Table 3.1).

<table>
<thead>
<tr>
<th>Electrode Condition</th>
<th>Scan Rate (mV)</th>
<th>Diffusion Layer Thickness (μm)</th>
<th>Onset Potential (mV)</th>
<th>Peak Potential (mV)</th>
<th>(^<em>)Peak or (^</em>)Limiting Current (A)</th>
<th>SA Coverage (C)</th>
<th>V Coverage (C)</th>
<th>Au NP Coverage (NP/μm^2)</th>
<th>NP Spacing (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass/ITO</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>8.94 x 10^3</td>
<td>647 ± 3</td>
<td>268 ± 19</td>
<td>(^*)4.72 (± 0.16) \times 10^4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>2.83 x 10^2</td>
<td>788 ± 9</td>
<td>673 ± 21</td>
<td>(^*)2.54 (± 0.21) \times 10^4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>8.94 x 10^2</td>
<td>801 ± 15</td>
<td>NA</td>
<td>(^*)5.01 (± 0.42) \times 10^3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.1</td>
<td>2.83 x 10^3</td>
<td>801 ± 16</td>
<td>NA</td>
<td>(^*)6.09 (± 0.22) \times 10^3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glass/ITO/4 nm Au</td>
<td>100</td>
<td>928 ± 4</td>
<td>702 ± 4</td>
<td>(^*)5.72 (± 0.69) \times 10^4</td>
<td>4.53 (± 0.10) \times 10^6</td>
<td>5.66 (± 0.12) \times 10^6</td>
<td>52.4 (± 1.1)</td>
<td>0.138</td>
<td></td>
</tr>
</tbody>
</table>
To confirm that the more positive ECD potential at smaller Au NPs is due to faster electron transfer kinetics, we performed ECD of Au onto glass/ITO and glass/ITO/Au NPs at slower scan rates. When the scan rate of the ECD process decreased to 10 mV/s, 1 mV/s and 0.1 mV/s, as shown in Figures 3.2c, 3.2d and 3.2e, respectively, using the same Au surface area as described earlier, the growth potential shifted positive for all three sized Au NPs and for Au nucleation and growth at the bare glass/ITO. When the scan rates were 10 mV/s and 1 mV/s, the onset and peak growth potentials are identical for 4 nm and 15 nm Au NPs and are just slightly more negative for 50 nm Au NPs and for nucleation and growth at the bare glass/ITO surface. At 0.1 mV/s, the growth onset potential is about the same at ~800 mV for all three sized Au NPs and

<table>
<thead>
<tr>
<th>NPs</th>
<th>10</th>
<th>2.83 x 10^2</th>
<th>931 ± 6</th>
<th>766 ± 8</th>
<th>9.56 (± 0.74) x 10^6</th>
<th>4.50 (± 0.26) x 10^6</th>
<th>5.63 (± 0.32) x 10^6</th>
<th>51.1 (± 4.0)</th>
<th>0.140</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>8.94 x 10^2</td>
<td>933 ± 4</td>
<td>793 ± 6</td>
<td>2.45 (± 0.11) x 10^6</td>
<td>4.41 (± 0.34) x 10^6</td>
<td>5.52 (± 0.43) x 10^6</td>
<td></td>
<td></td>
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<tr>
<td>0.1</td>
<td></td>
<td>2.83 x 10^3</td>
<td>860 ± 3</td>
<td>NA</td>
<td>7.39 (± 0.46) x 10^6</td>
<td>4.31 (± 0.06) x 10^6</td>
<td>5.39 (± 0.07) x 10^6</td>
<td>49.9 (± 0.7)</td>
<td>0.142</td>
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</table>

**Table 3.1.** ECD data for Glass/ITO and Glass/ITO/Au NP (4 nm, 15 nm, and 50 nm) electrodes obtained in 1 mM HAuCl₄·3H₂O + 0.1 M KClO₄ at scan rates of 100 mV/s, 10 mV/s, 1 mV/s and 0.1 mV/s.

To confirm that the more positive ECD potential at smaller Au NPs is due to faster electron transfer kinetics, we performed ECD of Au onto glass/ITO and glass/ITO/Au NPs at slower scan rates. When the scan rate of the ECD process decreased to 10 mV/s, 1 mV/s and 0.1 mV/s, as shown in Figures 3.2c, 3.2d and 3.2e, respectively, using the same Au surface area as described earlier, the growth potential shifted positive for all three sized Au NPs and for Au nucleation and growth at the bare glass/ITO. When the scan rates were 10 mV/s and 1 mV/s, the onset and peak growth potentials are identical for 4 nm and 15 nm Au NPs and are just slightly more negative for 50 nm Au NPs and for nucleation and growth at the bare glass/ITO surface. At 0.1 mV/s, the growth onset potential is about the same at ~800 mV for all three sized Au NPs and
~100 mV more negative for bare glass/ITO. Interestingly, the current reaches a steady-state value at 0.1 mV/s and the limiting current is a little smaller for the 50 nm Au NPs, even with a similar growth potential and similar Au surface area coverage. The reason for this behavior is not fully understood. At the slower scan rates diffusional overlap should be even more prevalent, resulting in linear diffusion, peak current proportional to the glass/ITO electrode, and a duck-shaped voltammogram. It is likely that convection becomes important at these very slow scan rates, which would lead to a steady-state limiting current (sigmoidal) rather than the expected duck-shaped voltammogram.

Table 3.1 shows the peak growth potentials and peak currents for all the electrodes studied with constant Au NP surface area and various scan rates (100, 10, 1.0, and 0.1 mV/s). In all cases, the Au NP surface area indicates a coverage that would lead to Au NP-Au NP spacings (equation 4) that are significantly shorter than the calculated diffusion layer thickness (equation 5) based on the scan rate and the diffusion coefficient of AuCl₄⁻ (D₀ ~ 1.0 x 10⁻⁵ cm²/s).

\[
\text{Average NP – NP spacing} = \frac{1}{\sqrt{N}} \text{µm} \quad (4)
\]

Where \( N \) is the coverage of Au NPs in NPs/µm². \( N \) is calculated from SA, which is the surface area coverage of Au atoms in terms of Coulombs that comes from the peak area under the Au reduction peak near 0.8 V in the CV in 0.1 M HClO₄ solution, using the following equations:

\[
\text{Volume (V) coverage before ECD} = \frac{\text{SA coverage from CV before ECD}}{\text{SA/V Ratio}} \quad (5)
\]

Where Volume (V) coverage is the total number of Au atoms on the electrode in terms of Coulombs of charge during complete oxidation, SA is the surface area coverage of Au atoms in terms of Coulombs that comes from the peak area under the Au reduction peak near 0.8 V in the CV in 0.1 M HClO₄ solution. The SA/V ratio for different-sized Au NPs comes from previous literature and is as follows: for 4 nm Au NPs, SA/V = 0.80; for 15 nm Au NPs, SA/V = 0.28; for 50 nm Au NPs, SA/V = 0.055.
Total Number of Au NPs per $\mu$m$^2$ ($N$) = \frac{V \text{ coverage before ECD}}{3 \times \left( \frac{F}{N_A} \right) \times n \times A} \quad (6)

= \frac{V \text{ coverage before ECD}}{3 \times (1.6 \times 10^{-19}) \times n \times (0.84 \times 10^8)}

Where 3 takes into account that the $V$ of Au atoms in Coulombs is based on 3 electrons per Au atom, $A$ is the electrode area in cm$^2$, $F$ is Faraday’s constant (96,485 C/mol e$^-$), $N_A$ is Avogadro’s number (6.022 x $10^{23}$ e$/\text{mol}$), and $n$ is the total number of atoms present in one Au NP. The values for $n$ are as follows: for 4 nm Au NPs, $n = 2,679$; for 15 nm Au NPs, $n = 141,285$; for 50 nm Au NPs, $n = 5,232,780$.

\[ \text{Diffusion Layer Thickness } \delta = 2\sqrt{Dt} \quad (7) \]

Where $d$ is the diffusion layer thickness (cm), $D$ is the diffusion coefficient (cm$^2$/s), and $t$ is time (s), which is $t = \text{voltage range/scan rate}$ (where scan rate is in V/s).

This would lead to conditions of linear diffusion at an array of electrodes, which would give peak currents proportional to the entire electrode area of 0.84 cm$^2$. The biggest difference in growth potential occurs at 100 mV/s, indicating that the kinetics for AuCl$_4^-$ are significantly faster at the smallest 4 nm Au NPs. At a scan rate of 10 mV/s, the peak growth potentials are very similar for 4 nm and 15 nm Au NPs at around 766 ± 8 mV and 765 ± 8 mV, respectively, which is close to the thermodynamically expected potential (808 mV). For 50 nm Au NPs, the peak growth potential is shifted slightly to 699 ± 11 mV. As expected, the nucleation and growth at bare glass/ITO has the highest overpotential with a peak potential of 673 ± 21 mV. When the scan rate is 1 mV/s, the onset and peak growth potentials follow similar trends with the 10 mV/s, where the peak growth potentials for 4 nm, 15 nm, and 50 nm Au NPs are 793 ± 6 mV, 797 ± 2 mV, and 746 ± 4 mV, respectively. The onset potentials for 4 nm, 15 nm, and 50 nm Au NPs are 933 ± 4 mV, 933 ± 3 mV, and 873 ± 13 mV, and the onset potential due to nucleation and growth at bare glass/ITO is at 801 ± 15 mV. At an even slower scan rate of 0.1 mV/s the $E_{1/2}$ growth
potentials were very similar for 4 nm, 15 nm, and 50 nm Au NPs (~800 mV) as compared to the 
$E_{1/2}$ for nucleation and growth of ~650 mV, which is still kinetically limited. The peak or limiting 
currents are also shown in Table 3.1 for the different sized Au NPs and bare glass/ITO at the 
different scan rates. The peak current is the highest for the 4 nm Au NPs at 100 mV/s, again due 
to faster electron transfer kinetics. The peak currents, or limiting currents in some cases, become 
similar for the 4 nm and 15 nm Au NPs at 10, 1.0, and 0.1 mV/s but a little lower for the 50 nm 
Au NPs for reasons that are not fully understood. The limiting current for the bare glass/ITO at 
0.1 mV/s is similar to the 4 nm and 15 nm Au NPs as expected since the entire glass/ITO 
electrode is in the same area and controls the current. The presence of a limiting current could be 
due to convection at slow scan rates, as mentioned earlier.

**ECD Under Constant Au NP Coverage Conditions.** We also performed ECD of Au on 
Au NPs at different scan rates while keeping a constant number of Au NPs on the glass/ITO 
surface (~1 Au NP/μm$^2$ area of the electrode). This is achieved by maintaining the total SA 
coverages of 4 nm, 15 nm, and 50 nm Au NPs before ECD (Figure 3.3a) at $9.49 \times 10^8$ C, $1.58 \times 10^6$ C, and $1.41 \times 10^5$ C, respectively (Table 3.2), which is estimated using equations 5 and 6. At a 
scan rate of 100 mV/s, the peak growth potentials for 4 nm, 15 nm, and 50 nm Au NPs are $616 \pm 
3$ mV, $638 \pm 3$ mV, and $650 \pm 24$ mV, respectively. Under these conditions, the ECD kinetics are 
more hindered at the smaller 4 nm Au NPs compared to the 15 nm and 50 nm Au NPs. We 
believe this is due to the lower overall surface area of the 4 nm Au NPs by about a factor of 14 
and 156 compared to the 15 and 50 nm Au NPs, respectively. Interestingly, the peak currents are 
still slightly larger for the smaller Au NPs, even though they exhibited higher overpotential for 
ECD.
Figure 3.3. (a) CVs obtained in 0.1 M HClO$_4$ electrolyte at a scan rate of 0.1 V/s of Glass/ITO/(4, 15 and 50 nm) Au NPs (1 NP per mm$^2$ of glass/ITO surface) before ECD; ECD of Glass/ITO/Au NPs electrode obtained in 0.001 M HAuCl$_4$·3H$_2$O + 0.1 M KClO$_4$ at a scan rate of (b) 100 mV/s and (c) 0.1 mV/s. The scan begins at 1.0 V and ends at 0 V.
The peak currents were $6.1 \times 10^{-4}$ A, $5.1 \times 10^{-4}$ A, and $3.6 \times 10^{-4}$ A for the 4 nm, 15 nm, and 50 nm Au NPs, respectively. With linear diffusion at all electrodes, the peak currents should theoretically be the same, proportional to the entire glass/ITO electrode area. When we decreased the scan rate to 0.1 mV/s at Au NP coverages of 1 NP/μm² (Figure 3.3c), the ECD showed electrochemically reversible kinetics with a growth potential ($E_{1/2}$) of ~0.80 V in all cases and limiting currents. The limiting current for growth was again slightly smaller for the 50 nm Au NPs compared to 4 nm and 15 nm Au NPs.

Coverage-Dependent ECD. The ECD results in Figures 3.2 and 3.3 interestingly show that 4 nm Au NPs display faster kinetics per Au surface area compared to larger Au NPs while the larger Au NPs display faster kinetics per NP compared to smaller Au NPs, due to higher surface area per larger NP. In order to investigate the coverage dependence further we studied the ECD for each size Au NPs as a function of coverage in terms of surface area and number of NPs per μm². Figure 3.4 shows the LSVs of 4 nm, 15 nm, and 50 nm Au NPs as a function of

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Trials</th>
<th>Peak AuCl₄⁻ Reduction Potential (mV)</th>
<th>Peak Reduction Current (A)</th>
<th>Total Au Surface Area (C)</th>
<th>Total Au Volume (C)</th>
<th>Au NP Coverage (NPs/μm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass/ITO/ 4 nm Au NPs</td>
<td>1</td>
<td>615</td>
<td>5.93 x 10⁻⁴</td>
<td>9.25 x 10⁻⁴</td>
<td>1.16 x 10⁻⁷</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>614</td>
<td>6.44 x 10⁻⁴</td>
<td>1.15 x 10⁻⁷</td>
<td>1.44 x 10⁻⁷</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>619</td>
<td>5.97 x 10⁻⁴</td>
<td>7.70 x 10⁻⁸</td>
<td>9.63 x 10⁻⁸</td>
<td>0.9</td>
</tr>
<tr>
<td>Average</td>
<td>616 ± 3</td>
<td>6.11 (± 0.28) x 10⁻⁴</td>
<td>9.49 (± 1.91) x 10⁻⁸</td>
<td>1.19 (± 0.24) x 10⁻⁷</td>
<td>1.1 (± 0.2)</td>
<td></td>
</tr>
<tr>
<td>Glass/ITO/ 15 nm Au NPs</td>
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<td>641</td>
<td>5.20 x 10⁻⁴</td>
<td>1.69 x 10⁻⁶</td>
<td>6.04 x 10⁻⁶</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>635</td>
<td>5.30 x 10⁻⁴</td>
<td>1.55 x 10⁻⁶</td>
<td>5.54 x 10⁻⁶</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>638</td>
<td>4.78 x 10⁻⁴</td>
<td>1.50 x 10⁻⁶</td>
<td>5.37 x 10⁻⁶</td>
<td>0.9</td>
</tr>
<tr>
<td>Average</td>
<td>638 ± 3</td>
<td>5.09 (± 0.28) x 10⁻⁴</td>
<td>1.58 (± 0.10) x 10⁻⁶</td>
<td>5.65 (± 0.35) x 10⁻⁶</td>
<td>1.0 (± 0.1)</td>
<td></td>
</tr>
<tr>
<td>Glass/ITO/ 50 nm Au NPs</td>
<td>1</td>
<td>623</td>
<td>4.18 x 10⁻⁴</td>
<td>1.23 x 10⁻⁵</td>
<td>2.24 x 10⁻⁵</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>669</td>
<td>2.59 x 10⁻⁴</td>
<td>1.45 x 10⁻⁵</td>
<td>2.64 x 10⁻⁵</td>
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<td></td>
<td>3</td>
<td>657</td>
<td>4.02 x 10⁻⁴</td>
<td>1.55 x 10⁻⁵</td>
<td>2.82 x 10⁻⁵</td>
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</tr>
<tr>
<td>Average</td>
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<td>3.60 (± 0.88) x 10⁻⁴</td>
<td>1.41 (± 0.16) x 10⁻⁵</td>
<td>2.56 (± 0.30) x 10⁻⁴</td>
<td>1.2 (± 0.1)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.2. Electrochemical deposition (ECD) data for indium-tin-oxide coated glass electrodes with Au NPs (Glass/ITO/Au NPs) having diameters of 4 nm, 15 nm, and 50 nm obtained in 0.001M HAuCl₄·3H₂O + 0.1 M KClO₄ at a scan rate of 100 mV/s and Au NP coverage of ~1 NP/μm².
coverage at 100 mV/s as indicated in Figures 3.4a, 3.4b and 3.4c, respectively. For all sizes, the peak ECD growth potential shifts positive as the coverage increases. Since the lowest coverage of Au NPs in the range of coverages has NP spacings close enough for diffusional overlap (linear diffusion) at this scan rate, the lack of electrochemical reversibility shows that the electron transfer kinetics are not able to keep up with mass transfer of AuCl$_4^-$ to the electrode surface.

Increasing the coverage of Au NPs increases the active area for electron transfer, which allows the electron transfer kinetics to keep up better with mass transfer. The electrochemical reversibility improves with increasing Au NP coverage. It has been observed that the overpotentials due to a catalytic process for surface-attached NP catalysts depends on the SA coverage of the catalytic sites present at the electrode surface.$^{123, 124}$ Due to different size-dependent electron transfer kinetics, there exists a different threshold coverage of Au NPs needed on the electrode surface to achieve electrochemical reversibility for the ECD of AuCl$_4^-$ onto Au NPs. The threshold in terms of total number of Au NPs is smaller for the larger Au NPs since the larger Au NPs have a larger total surface area per NP. If we use a target potential of 740 mV, the number of NPs is ~400 NPs/μm$^2$, ~40 NPs/μm$^2$, and ~8.5 NPs/μm$^2$ for the 4 nm, 15 nm, and 50 nm Au NPs, respectively (Figure 3.4d-3.4f), to reach that peak potential. More importantly, the threshold value occurs at a lower Au total surface area for the 4 nm Au NPs, confirming that the smaller Au NPs have more catalytic activity for ECD of Au per Au surface area, volume, and mass. Figure 3.4g shows a plot of the growth potential for all three Au NP sizes as a function of total Au surface area (Tables 3.3-3.5). A peak growth potential of ~743 mV corresponds to surface areas of 3.66 x 10$^{-5}$ C, 8.93 x 10$^{-5}$ C, and 9.84 x 10$^{-5}$ C for 4 nm, 15 nm, and 50 nm Au NPs, respectively. The peak deposition potentials reach a plateau with close to reversible kinetics at ~743 mV, indicating that the size dependent behavior diminishes at higher SA coverages due to
Figure 3.4. LSVs of (a) 4 nm, (b) 15 nm, and (c) 50 nm Au NPs as a function of coverage at 100 mV/s; A plot of the number of NPs per mm² area of glass/ITO surface vs peak deposition potential (V) for (d) 4 nm, (e) 15 nm and (f) 50 nm Au NPs; (g) A plot of SA coverage before ECD vs peak deposition potential (V).
reasons already discussed. This shows that the 15 nm and 50 nm Au NPs require ~2-3 times the surface area as 4 nm Au NPs to achieve the same electrochemical reversibility.

<table>
<thead>
<tr>
<th>SA Coverage Before ECD (C)</th>
<th>V Coverage Before ECD (C)</th>
<th>Coverage (NPs/µm²)</th>
<th>Peak potential (mV)</th>
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<tbody>
<tr>
<td>1.68 x 10⁶</td>
<td>2.10 x 10⁶</td>
<td>19.4</td>
<td>680</td>
</tr>
<tr>
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<td>3.36 x 10⁶</td>
<td>4.20 x 10⁶</td>
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<td>6.42 x 10⁶</td>
<td>8.03 x 10⁶</td>
<td>74.3</td>
<td>694</td>
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<tr>
<td>7.23 x 10⁶</td>
<td>9.04 x 10⁶</td>
<td>83.7</td>
<td>696</td>
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<td>1.19 x 10⁵</td>
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<td>709</td>
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<td>1.85 x 10⁵</td>
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<tr>
<td>1.18 x 10⁴</td>
<td>1.48 x 10⁴</td>
<td>1365</td>
<td>753</td>
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Table 3.3. Total experimental Au surface area (SA), calculated total volume (V) of Au, calculated coverage of Au NPs, and the measured peak reduction potential for AuCl₄⁻ at glass/ITO/4 nm Au NPs electrodes.

<table>
<thead>
<tr>
<th>SA Coverage Before ECD (C)</th>
<th>V Coverage Before ECD (C)</th>
<th>Coverage (NPs/µm²)</th>
<th>Peak potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.19 x 10⁶</td>
<td>7.82 x 10⁶</td>
<td>1.37</td>
<td>611</td>
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<td>2.28 x 10⁶</td>
<td>8.14 x 10⁶</td>
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<td>2.84 x 10⁶</td>
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<td>4.31 x 10⁶</td>
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<td>4.55 x 10⁶</td>
<td>1.63 x 10⁵</td>
<td>2.85</td>
<td>680</td>
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<td>1.34 x 10⁵</td>
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</tr>
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<td>2.25 x 10⁵</td>
<td>8.04 x 10⁵</td>
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<td>721</td>
</tr>
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<tr>
<td>8.93 x 10⁵</td>
<td>3.19 x 10⁴</td>
<td>56.0</td>
<td>744</td>
</tr>
<tr>
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<td>4.39 x 10⁴</td>
<td>77.1</td>
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Table 3.4. Total experimental Au surface area (SA), calculated total volume (V) of Au, calculated coverage of Au NPs, and the measured peak reduction potential for AuCl$_4^-$ at glass/ITO/15 nm Au NPs electrodes.

<table>
<thead>
<tr>
<th>SA Coverage Before ECD (C)</th>
<th>V Coverage Before ECD (C)</th>
<th>Coverage (NPs/µm$^2$)</th>
<th>Peak potential (mV)</th>
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<tr>
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<td>551</td>
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<td>3.17 x 10$^6$</td>
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<td>562</td>
</tr>
<tr>
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</tr>
<tr>
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<td>1.15</td>
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</tr>
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<td>2.40 x 10$^{-3}$</td>
<td>11.4</td>
<td>746</td>
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</table>

Table 3.5. Total experimental Au surface area (SA), calculated total volume (V) of Au, calculated coverage of Au NPs, and the measured peak reduction potential for AuCl$_4^-$ at glass/ITO/50 nm Au NPs electrodes.

Figure 3.5 shows the ECD of Au on glass/ITO/Au NP electrodes of different size with a total surface area coverage of ~4 x 10$^{-6}$ C. We performed ECD in a solution of 0.1 mM HAuCl$_3$·3H$_2$O and 0.1 M KClO$_3$ by sweeping the potential from 1.0 V to 0.0 V vs Ag/AgCl (3 M KCl) at a scan rate of 100 mV/s. Interestingly, with lower concentrations of AuCl$_4^-$, the peak growth potential (~730 mV) does not depend strongly on the size of the Au NPs. With lower AuCl$_4^-$, the number of Au NPs present at the glass/ITO surface is enough to support the flux of AuCl$_4^-$ ions during ECD. The concentration gradient, and hence the flux, is lower when the bulk metal ion complex concentration decreases. A high AuCl$_4^-$ concentration results in higher flux, or
Figure 3.5. ECD of Glass/ITO/Au NPs (4, 15 and 50 nm) electrodes obtained in 0.1 mM \text{HAuCl}_4 \cdot 3\text{H}_2\text{O} + 0.1 \text{M KClO}_4$ at a scan rate of 100 mV/s. The scan begins at 1.0 V and ends at 0 V.
lower mass-transfer resistance, which results in the resistance to electron transfer being the rate limiting step. This causes the kinetic limitations observed at 1 mM concentrations. With a lower concentration of AuCl₄⁻, the higher mass-transfer resistance is the rate limiting step and we do not observe electron transfer kinetic limitations. This leads to more reversible voltammetry at all NP sizes. The peak currents associated with ECD were 5.6 x 10⁻⁵ A, 4.1 x 10⁻⁵ A, and 3.2 x 10⁻⁵ A for the 4 nm, 15 nm, and 50 nm Au NPs, respectively (Table 3.6). It is also important to note that the peak growth potential with glass/ITO/Au NPs is more positive by ~400 mV compared to the peak nucleation and growth potential for glass/ITO even with the lower AuCl₄⁻ concentration. This again shows that the growth step occurs at much lower overpotentials compared to the nucleation step due to much slower nucleation kinetics compared to growth kinetics.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Trials</th>
<th>Peak Potential (mV)</th>
<th>Peak Current (A)</th>
<th>Total Au Surface Area (C)</th>
<th>Total Au Volume (C)</th>
<th>Au NP coverage (NPs/μm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass/ITO</td>
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<td>314</td>
<td>3.25 x 10⁻⁵</td>
<td></td>
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</tr>
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<td></td>
<td>2</td>
<td>321</td>
<td>2.75 x 10⁻⁵</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>373</td>
<td>2.97 x 10⁻⁵</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>336 ± 32</td>
<td>2.99 (± 0.25) x 10⁻⁵</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glass/ITO/4 nm Au NPs</td>
<td>1</td>
<td>723</td>
<td>5.37 x 10⁻⁵</td>
<td>4.71 x 10⁶</td>
<td>5.89 x 10⁶</td>
<td>54.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>732</td>
<td>5.98 x 10⁻⁵</td>
<td>4.54 x 10⁶</td>
<td>5.68 x 10⁶</td>
<td>52.5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>738</td>
<td>5.43 x 10⁻⁵</td>
<td>4.70 x 10⁶</td>
<td>5.88 x 10⁶</td>
<td>54.4</td>
</tr>
<tr>
<td></td>
<td>Average</td>
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<td>5.59 (± 0.39) x 10⁻⁵</td>
<td>4.6 (± 0.1) x 10⁶</td>
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<td>54 (± 1)</td>
</tr>
<tr>
<td>Glass/ITO/15 nm Au NPs</td>
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<td>4.02 x 10⁻⁵</td>
<td>4.87 x 10⁶</td>
<td>1.74 x 10⁵</td>
<td>3.05</td>
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<tr>
<td></td>
<td>2</td>
<td>745</td>
<td>4.35 x 10⁻⁵</td>
<td>4.42 x 10⁶</td>
<td>1.58 x 10⁵</td>
<td>2.77</td>
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<tr>
<td></td>
<td>3</td>
<td>755</td>
<td>3.88 x 10⁻⁵</td>
<td>4.54 x 10⁶</td>
<td>1.62 x 10⁵</td>
<td>2.85</td>
</tr>
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<td>Average</td>
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<td>4.08 (± 0.24) x 10⁻⁵</td>
<td>4.6 (± 0.2) x 10⁶</td>
<td>1.6 (± 0.1) x 10⁵</td>
<td>2.9 (± 0.2)</td>
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<tr>
<td>Glass/ITO/50 nm Au NPs</td>
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<td>744</td>
<td>3.40 x 10⁻⁵</td>
<td>4.90 x 10⁶</td>
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<td>0.42</td>
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<td>2</td>
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<td>3.05 x 10⁻⁵</td>
<td>4.36 x 10⁶</td>
<td>7.93 x 10⁵</td>
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<td>3</td>
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<td>3.04 x 10⁻⁵</td>
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<td>0.37</td>
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<tr>
<td></td>
<td>Average</td>
<td>743 ± 11</td>
<td>3.16 (± 0.20) x 10⁻⁵</td>
<td>4.5 (± 0.3) x 10⁶</td>
<td>8.2 (± 0.6) x 10⁵</td>
<td>0.39 (± 0.03)</td>
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</tbody>
</table>

Table 3.6. ECD data for Glass/ITO and Glass/ITO/Au NPs having diameters of 4 nm, 15 nm, and 50 nm obtained in 0.1 mM HAuCl₄·3H₂O + 0.1 M KClO₄ at a scan rate of 100 mV/s and constant Au NP surface area as indicated.
Figure 3.6 shows a linear sweep voltammogram (LSV) from 1.0 V to 0.0 V (vs. Ag/AgCl) at a scan rate of 100 mV/s of a glass/ITO/15 nm Au NPs electrode with a coverage of 2.2 NPs/µm² in a solution of 1 mM HAuCl₄·3H₂O and 0.1 M KClO₄ (Figure 3.6a) and the corresponding SEM image after the LSV (Figure 3.6b). Interestingly, the SEM image shows the presence of two particle size populations. There are those that grew greater than about 100 nm (indicated by yellow circles) and those that did not grow very large (indicated by red circles), remaining in the 15 to 50 nm size range. The original coverage of Au NPs based on the SA from the CV in 0.1 M HClO₄ was about 2.2 NPs/µm². The coverage of Au NPs from 6 different SEM images and close to 100 total NPs was 1.4 NPs/µm² when including both the larger and smaller Au NPs. Based on the SEM coverage being smaller, we conclude that all the Au NPs in the SEM images originated from 15 nm Au seed NPs that grew during the LSV, but that some of the Au NPs were more active and grew larger than others. This is consistent with the large size dispersity (often bimodal distribution) that is observed with an ensemble of metal NPs growing by ECD under conditions of linear diffusion with overlapping diffusion layers.¹¹⁹
Figure 3.6. (a) ECD of Glass/ITO/15 nm Au NPs electrodes (2.2 NP/mm$^2$) in 0.001 M HAuCl$_4$·3H$_2$O + 0.1 M KClO$_4$ at a scan rate of 100 mV/s. The scan begins at 1 V and ends at 0 V; (b) SEM images of Glass/ITO/15 nm Au NPs electrodes after ECD, yellow circles indicate the grown particles, and red circles indicate the particles that didn’t grow as much during ECD.
3.3. CONCLUSIONS

In summary, we describe the ECD of metallic Au from AuCl$_4^-$ precursor ions onto different sized Au NPs attached to glass/ITO electrodes by EPD. When the scan rate was 100 mV/s, the peak ECD potential increases positive (less overpotential) as the size of the Au NPs decreases provided the overall exposed Au NP surface area is constant and relatively low. However, when the scan rate is slow ($\leq$10 mV/s), the peak ECD potentials become mostly independent of the size of the Au NPs. The peak ECD currents follow a similar trend where it is larger for smaller Au NPs at a scan rate of 100 mV/s when the voltammetry is controlled by the electron transfer kinetics, while at slower scan rates the peak current is very similar for 4 nm and 15 nm Au NPs and a little smaller for 50 nm Au NPs and bare glass/ITO. Another major finding is that the electrochemical reversibility of the process depends on the Au NP coverage. Above a threshold Au surface area, the system displays electrochemical reversible voltammetry for all Au NP sizes. To achieve a peak potential of $\sim$740 mV, 15 nm and 50 nm Au NPs needed $\sim$2-3 times more surface area than 4 nm Au NPs. Similar electrochemical reversibility behavior occurred with ECD using low concentrations of AuCl$_4^-$, showing that the reversibility of the system depends on the AuCl$_4^-$ concentration relative to the Au NP surface area. If the number of catalytic reaction sites present at the electrode surface are enough to keep up with the AuCl$_4^-$ flux as determined by the scan rate and AuCl$_4^-$ bulk concentration, the system is electrochemically reversible. In conclusion, the kinetics of electrochemical growth at Au NP “seeds”, or “nucleation sites”, is influenced by the size of the NPs presents on the electrode surface, their number density (Au NP total surface area), and AuCl$_4^-$ flux. A better understanding of the details of size- and coverage-dependent Au NP growth by ECD will help researchers better control the growth of metal NPs on electrode surfaces for electrocatalysis, electrochemical sensing, electrosynthesis, and electrooptical applications.
CHAPTER 4: ELECTROCHEMICAL GROWTH OF GOLD
NANOPARTICLES FUNCTIONALIZED WITH ALKANETHIOL SELF-ASSEMBLED MONOLAYERS

4.1. INTRODUCTIONS

Electrochemical deposition (EDC) is a versatile and widely used technique for depositing metal coatings through the reduction of metal ions onto various substrates through the application of an electrochemical potential. This process is extensively used in various industries for coating, surface modification, and the fabrication of metallic structures. ECD follows a nucleation and growth mechanism, where initial nucleation sites are formed at an overpotential, and then further growth of these sites into larger metal structures occurs at a lower overpotential, known as the growth step. This process forms the foundation for seed-mediated growth of controlled shape and size of metal nanostructures using pre-formed metal nanoparticles (NPs) as nucleation sites, or "seeds." As detailed in Chapter 3, the kinetics of the electrochemical growth at Au NP “seeds” are influenced by NP size, their number density (total surface area coverage), and AuCl₄⁻ (precursor ions for growth step) flux. Understanding these factors is crucial for controlling the growth of metal NPs on electrode surfaces for various applications, such as electrocatalysis, electrochemical sensing, and electrosynthesis applications. The studies discussed in Chapter 3 avoid the impact of the ligands or capping agents attached to the NPs by
removing them through an ozone treatment process prior to the ECD step.\textsuperscript{132} Hence, it is important to understand the influence of the ligands attached to the NP surface on the ECD of Au on Au NPs.

There are several reports regarding the effect of the ligands on various electrochemical processes.\textsuperscript{135-137} For instance, Gevaerd \textit{et al.} showed the electrochemical activity of dodecanethiol (C12S)-capped Au NPs by performing CV using a glassy carbon electrode modified with such NPs in 0.1 M H\textsubscript{2}SO\textsubscript{4} solution.\textsuperscript{138} Out of the Au NPs prepared with varying Au: thiol ratios, those with the lowest amount of thiol on the surface showed the best electrochemical response. This is indicative of the influence of the C12S as a passivation agent towards the activity of the NPs. Taleb \textit{et al.} discussed the formation of Ag nano- and mesoporous structures by Ag ECD onto HOPG modified with Au NPs coated with C12S.\textsuperscript{139} The reduction of Ag\textsuperscript{+} at the Au/C12S interface led to the formation of nanostructures despite C12S being a passivating agent. This is the consequence of the surface curvature of the Au NPs decreasing the packing density and coverage of the C12S monolayer structure. Moreover, due to the hydrophobic nature of C12S, the thiols can diffuse on the NP surface to form bundles to minimize the hydrophobic interactions, leading to ECD of Ag occurring at the thiol-free region on the Au NP surface. She \textit{et al.} described the ECD of Au onto Au substrates modified by self-assembled monolayers (SAM).\textsuperscript{140} The binary SAM of ω-(4’-methyl biphenyl-4-yl)-propane thiol (MBP3) and octadecane thiol (ODT) led to the ECD of the Au microelectrode structure featuring line 15 µm wide and 3 mm long. Another single component, SAM of MBP3, resulted in the high deposition of Au structures. Thus, it is important to explore the effect of ligands on the ECD growth of Au (via AuCl\textsubscript{4}– reduction) on ligand-capped Au NPs.

This chapter explores the effects of the alkanethiol SAM attached to Au NPs during the growth of Au onto AuNPs by ECD. The alkanethiols used include 1-butanethiol (C4S), 1-octanethiol (C8S), 1-dodecanethiold (C12S), and 1-hexadecanethiold (C16S). This chapter expands on the effect of the ligand, specifically the alkyl chain length, on various-sized Au NPs, including
4 nm, 15 nm, and 50 nm diameter Au NPs. The effect of passivation from each ligand on ECD was explored to better understand ligand effects on the growth of Au NPs by ECD to fabricate controlled metallic nanostructures in the future.

4.2. RESULTS AND DISCUSSION

Experimental Set-up. Figure 4.1 outlines the experimental steps involved to study the electrochemical growth (ECD of AuCl\textsuperscript{4-}) of Au on thiol-capped Au NPs (4 nm, 15 nm, or 50 nm diameter). The first step involves the hydroquinone (HQ)-mediated EPD of chemically synthesized citrate-stabilized Au NPs onto a glass/ITO working electrode.\textsuperscript{4} The citrate coating was then removed using ozone treatment of the glass/ITO/Au NPs for 8 min. The surface area of the deposited Au NPs was measured by determining the charge of the reduction peak after performing cyclic voltammetry from 0 V to 1.4 V in 0.1 M HClO\textsubscript{4} at a scan rate of 100 mV/s.\textsuperscript{33,102} Following this step, a SAM of alkanethiols was formed on the glass/ITO/Au NPs by soaking the electrode in a 2 mM ethanolic solution of different alkanethiols. The alkanethiols used include 1-butanethiol (C\textsubscript{4}S), 1-octanethiol (C\textsubscript{8}S), 1-dodecanethiol (C\textsubscript{12}S), and 1-hexadecanethiol (C\textsubscript{16}S), and pure ethanol was used as a control. The final step involves the ECD of Au via AuCl\textsubscript{4-} reduction onto the thiol-capped Au NP electrodes (glass/ITO/Au NP/CnS, where n is the alkane chain length) in 0.001 M HAuCl\textsubscript{4}.3H\textsubscript{2}O and 0.1 M KClO\textsubscript{4} by sweeping the potential from 1.0 V to 0.0 V vs Ag/AgCl (3 M KCl) at a scan rate of 100 mV/s. The peak potential (E\textsubscript{p}) and peak current (i\textsubscript{p}) provide information on the growth kinetics of Au ECD onto thiol-capped Au NP nucleation sites.

Effect of Alkanethiol Soaking Time. In order to understand the impact of the alkanethiol layer on the electroactive surface of Au NPs, we performed linear sweep voltammetry (LSV) on glass/ITO/Au\textsubscript{4nm} NPs soaked in 2 mM ethanolic solution of C\textsubscript{8}S for varying times (0.5, 1, 3, and 20 h).
Figure 4.1. The general experimental design involved in the ECD experiments.
The LSV was performed by scanning the glass/ITO/Au\textsubscript{4nm}/C8S electrodes from 1 V to 0 V in a solution containing 1 mM HAuCl\textsubscript{4}.3H\textsubscript{2}O and 0.1 M KClO\textsubscript{4} at 100 mV/s. The glass/ITO/Au\textsubscript{4nm} electrodes were created using EPD in a solution containing 25 µM 4 nm Au NPs and 16.7 mM HQ at 1 V for 3 min. Figure 4.2 illustrates the LSV plots obtained for glass/ITO/Au\textsubscript{4nm}/C8S prepared by soaking in 2 mM ethanolic solution of C8S at 0.5, 1, 3, and 20 h. The major cathodic peak observed in the LSVs, ranging from approximately 0.80 to 0.25 V, is attributed to the electrochemical reduction of AuCl\textsubscript{4}\textsuperscript{−} to metallic Au\textsuperscript{0} at the surface of glass/ITO/Au\textsubscript{4nm}/C8S, according to the following electrochemical reaction:

\[
\text{AuCl}_4^{-} + 3\text{e}^{-} \rightarrow \text{Au}^{0} + 4\text{Cl}^{-} \quad E^0 = 808 \text{ mV vs. Ag/AgCl (3 M KCl)} \quad (1)
\]

When the soaking time of glass/ITO/Au\textsubscript{4nm} in 2 mM ethanolic solution of C8S increases, the cathodic peak resulting from the ECD of Au onto the glass/ITO/Au\textsubscript{4nm}/C8S electrode shifts to more negative potentials. This indicates that the ECD of Au onto the C8S Au\textsubscript{4nm} NPs is affected by the thiol layer formed at the surface of the NPs. This is evident from the shift in peak growth potential with an increase in soaking time. When the glass/ITO/Au\textsubscript{4nm} was soaked in an ethanolic solution of C8S for 0.5 h, as shown in the red plot in Figure 4.2, the $E_p$ for ECD was 0.686 V. As the soaking time increased to 1, 3, and 20 h, the $E_p$ shifted to 0.652 V (blue), 0.617 V (green), and 0.586 V (pink), respectively. Similarly, a general decrease in $i_p$ occurred as the soaking time in ethanolic alkanethiol solution increased. The $i_p$ values for 0.5, 1, 3, and 20 h soaking times were 0.590 mA, 0.512 mA, 0.374 mA, and 0.408 mA, respectively. The decrease in $E_p$ and $i_p$ correlated to the formation of SAMs on the Au NPs with higher coverage and order with increasing soaking time, leading to the better blocking of the electroactive NP surface. The Au-S bond between alkanethiol and Au NP is strong, leading to passivation of the electroactive surface of the Au NPs. Additionally, the alkyl group associated with the alkanethiols may sterically inhibit diffusion of the redox species towards the electroactive area of the Au NPs.\textsuperscript{141, 142} The shift in $E_p$ indicates irreversible kinetics towards the electrochemical reduction of AuCl\textsubscript{4}\textsuperscript{−} to metallic Au\textsuperscript{0} at the surface of glass/ITO/Au\textsubscript{4nm}/C8S.
Figure 4.2. LSVs (ECD) of Glass/ITO/4 nm-Au NP electrodes soaked in 2 mM ethanolic solution of C8S for 0.5 h (red), 1 h (blue), 3 h (green), and 20 h (pink) obtained in 1 mM HAuCl₄·3H₂O + 0.1 M KClO₄ at a scan rate of 100 mV/s. The scan begins at 1.0 V and ends at 0.0 V vs. Ag/AgCl (3M KCl). EPD potentials and times for 4 nm Au NPs are 1.0 V and 3 min, respectively.
Hence, the glass/ITO/Au\textsubscript{4nm} were soaked for 20 hours in the all alkanethiols in this study to ensure that the thiol had fully formed a well-ordered, high coverage assembly on the different-sized Au NPs. The formation of well-ordered assemblies of alkanethiols on Au NPs was further confirmed by measuring the electrochemically active surface area (ECSA) of Au NPs before and after soaking in alkanethiols for 20 h. This was achieved by performing CV on glass/ITO/Au NP electrodes before and after soaking in a 2 mM ethanolic solution of CnS in a solution of 0.1 M HClO\textsubscript{4} from 0 V to 1.4 V at a scan rate of 100 mV/s. A CV for glass/ITO/Au\textsubscript{4nm} and glass/ITO/Au\textsubscript{4nm}/C8S is shown in Figure 4.3. Before soaking in the alkanethiol solutions, the Au\textsubscript{2}O\textsubscript{3} surface reduction peak (refer section 2.9) was at 0.876 V with a charge (Q) under the peak of 37.5 μC, which is proportional to the total ECSA of the Au\textsubscript{4nm} NPs on the glass/ITO electrode.

The CV of the glass/ITO/Au\textsubscript{4nm}/C8S electrode soaked for 20 h in C8S had no visible reduction peak, confirming the passivation of the Au NP ECSA by the alkanethiol SAM, indicating a well-ordered assembly with high coverage.

**Effect of Alkanethiol Chain Length.** Since the ECSA of Au\textsubscript{4nm} NPs can be passivated by the alkanethiol SAMs, this will have a significant effect on the electrochemical reduction of AuCl\textsubscript{4} to Au\textsuperscript{0} at the Au NP surface. Figure 4.4 shows the effect of alkanethiol chain length of the SAM on the ECD growth process by showing LSVs of glass/ITO/Au\textsubscript{4nm} electrodes obtained in a solution of 1 mM HAuCl\textsubscript{4}.3H\textsubscript{2}O and 0.1 M KClO\textsubscript{4} after soaking in ethanol (red, control) and 2 mM ethanolic solutions of C4S (blue), C8S (green), C12S (pink), and C16S (orange) for 20 h.

The LSVs were scanned from 1 V to 0 V at a scan rate of 100 mV/s. The glass/ITO/Au\textsubscript{4nm} NPs were fabricated by EPD in a solution containing 25 mM 4 nm Au NPs and 0.1 M HQ at 1 V for 3 min. These EPD conditions led to an Au\textsubscript{4nm} NP surface Au reduction of ~35.9 μC (see Table 5.1), which was kept constant to avoid the impact of NP ECSA (coverage) on the growth process. Here, the alkyl chain length of the thiol has a significant impact on the ECD of Au on Au NPs. As the size of the alkyl chain increases, the $E_p$ for electrochemical growth of Au NPs shifts negative.
Figure 4.3. CV was obtained in 0.1 M HClO₄ electrolyte at a scan rate of 100 mV/s of Glass/ITO/4 nm-Au NP electrodes before (red) and after (blue) soaking in 2 mM ethanolic solution of C8S thiol for 18 hours. EPD potentials and times for 4 nm Au NPs are 1.0 V and 3 min, respectively.
Figure 4.4. LSVs (ECD) of Glass/ITO/4 nm-Au NP electrodes soaked in ethanol (red), 2 mM ethanolic solutions of C4S (blue), C8S (green), C12S (pink), and C16S (orange) and bare glass/ITO electrode (black) for 20 h obtained in 1 mM HAuCl₄·3H₂O + 0.1 M KClO₄ at a scan rate of 100 mV/s. The scan begins at 1.0 V and ends at 0.0 V vs. Ag/AgCl (3M KCl). EPD potentials and times for 4 nm Au NPs are 1.0 V and 3 min, respectively.
Moreover, the $i_p$ generally decreases with an increase in alkyl chain lengths. This is also evident from Table 5.1.

<table>
<thead>
<tr>
<th>Alkanethiols</th>
<th>Peak Potential (V)</th>
<th>Peak Current (mA)</th>
<th>SA Coverage (μC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4S</td>
<td>0.661 ± 0.028</td>
<td>0.578 ± 0.070</td>
<td>34.2 ± 4.7</td>
</tr>
<tr>
<td>C8S</td>
<td>0.533 ± 0.193</td>
<td>0.355 ± 0.026</td>
<td>37.5 ± 4.0</td>
</tr>
<tr>
<td>C12S</td>
<td>0.504 ± 0.066</td>
<td>0.348 ± 0.067</td>
<td>36.1 ± 2.3</td>
</tr>
<tr>
<td>C16S</td>
<td>0.396 ± 0.047</td>
<td>0.325 ± 0.124</td>
<td>35.9 ± 3.4</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.663 ± 0.077</td>
<td>0.552 ± 0.072</td>
<td>35.7 ± 0.7</td>
</tr>
</tbody>
</table>

Table 4.1. ECD data of Glass/ITO/4 nm-Au NP electrodes soaked in ethanol (red), C4S (blue), C8S (green), C12S (pink), and C16S (orange) and bare glass/ITO electrode (black) for 20 h obtained in 1 mM HAuCl₄·3H₂O + 0.1 M KClO₄ at a scan rate of 100 mV/s.

When the glass/ITO/Au₄nm was soaked in ethanol for 20 h (red plot), the LSV showed an $E_p$ of 0.663 ± 0.077 V and $i_p$ of 0.552 ± 0.072 mA, which has a low overpotential, indicating that the electrochemical growth kinetics are relatively fast. When the Au NPs are capped with C4S thiol monolayers, the LSV (blue plot) shows a similar LSV with average $E_p$ of 0.661 ± 0.028 V and average $i_p$ of 0.578 ± 0.070 mA. This suggests that when the alkyl chain length is as low as four carbon units, the effect on ECD of Au (growth) on Au NPs is minimal, and the process tends towards reversible kinetics. This behavior changed when the size of the alkyl chain was eight carbon units, as indicated by the LSV for glass/ITO/Au₄nm/C8S (green plot), which shows a negative shift in average $E_p$ to 0.533 ± 0.193 V and significantly lower average $i_p$ at 0.355 ± 0.026 mA. In addition to that, the voltammogram becomes significantly broadened, which is a further indication of sluggish charge transfer kinetics. The LSV of glass/ITO/Au₄nm/C12S and glass/ITO/Au₄nm/C16S showed average $E_p$ of 0.504 ± 0.066 V and 0.396 ± 0.047 V, respectively, and average $i_p$ of 0.348 ± 0.067 mA and 0.325 ± 0.124 mA, respectively, showing a continuation of the trend of more sluggish, slower kinetics with increasing alkane chain length. Interestingly, even with the very large negative shift in $E_p$ for glass/ITO/Au₄nm/C16S, the kinetics are still
faster than ECD of Au on bare glass/ITO (black plot) with no Au NP catalyst (nucleation sites or seeds), which shows $E_p$ and $i_p$ of 0.292 V and 0.485 mA, respectively. This shows that the activation energy for nucleation on bare glass/ITO is still larger than the activation energy for growth on C16S-coated Au$_{4nm}$ NPs. Overall, the thiolate binding to Au NPs and ordering of the increasing alkyl chain length results in higher overpotentials for ECD, indicating a need for higher reducing potential due to slower growth kinetics.

**Effect of Au NP Size.** Figure 4.5 shows the effect of Au NPs size on the ECD of Au in 1 mM HAuCl$_4$·3H$_2$O and 0.1 M KClO$_4$ onto glass/ITO/Au NPs/C8S, where the Au NP size was varied to be 4, 15, and 50 nm in diameter with constant ECSA (35.7 µC Au$_2$O$_3$ reduction). As the size of the Au NP increases, the $E_p$ shifts more negative in the order of Au$_{4nm}$ (0.587 V) > Au$_{15nm}$ (0.314 V) > Au$_{50nm}$ (0.188 V). This shows a clear increase in growth overpotential with increasing Au NP diameter, which indicates that the Au NP size still plays a role in the electrochemical growth kinetics apart from the alkanethiol layer surrounding the Au NP. As mentioned in Chapter 3, as the size of the Au NP decreases, the overpotential decreases (more reversible), indicating faster electrochemical growth kinetics with decreasing size of Au NPs. The same trend in size occurred for C8S-coated Au NPs. The exact reason is not clear, however. It could be due to the relative decrease in available electroactive surface area coverage for larger Au NPs in comparison to smaller Au NPs. This might be expected since the alkanethiol chains are more ordered on larger Au NP surfaces because they have less curvature.\textsuperscript{143} The kinetics could then be related to the number of exposed defect sites as a function of Au NP size. This is opposite to the kinetics of dissolution, which show higher rates on smaller Au NPs without alkanethiols but higher rates on larger Au NPs with alkanethiols. In that case the higher rates of dissolution on larger Au NPs was attributed to strong Au-S bonding with smaller Au NPs. For deposition, since the size trend is the same with and without alkanethiols, the Au-S binding strength does not seem to be a big factor in the growth of Au NPs by reduction of AuCl$_4^-$. However, it is interesting to observe that the effect of alkanethiols adds to the passivation of the process. The peak currents for C8S-capped 4 nm and
Figure 4.5. LSVs (ECD) of Glass/ITO electrodes with 4 nm (red), 15 nm (blue), and 50 nm (green) Au NPs soaked in 2 mM ethanolic solution of C8S for 20 h obtained in 1 mM HAuCl₄·3H₂O + 0.1 M KClO₃ at a scan rate of 100 mV/s. The scan begins at 1.0 V and ends at 0.0 V vs. Ag/AgCl (3M KCl). EPD potentials and times for 4 nm, 15 nm, and 50 nm Au NPs are 1.0 V and 3 min, 1.2 V and 5 min, and 2.2 V and 10 min, respectively.
15 nm Au NPs were 0.410 mA and 0.593 mA, respectively. When C8S-capped 50 nm Au NPs were electrochemically grown, the peak shift was akin to the nucleation events, as observed in Figure 4.4. When the ECD of Au on glass/ITO/C16S-capped 50 nm Au NP was performed, the peak growth potentials and current were observed at 0.188 V and 0.439 mA, respectively, which are closer to the peak nucleation potential and current on a bare glass/ITO electrode. This is consistent with the NP size-dependent kinetics of electrochemical growth studied in Chapter 3.

4.3. CONCLUSIONS

In conclusion, this study provides a better understanding of the electrochemical growth of Au on thiol-protected Au NPs and the significant impact of alkanethiol layers and NP size on the electrochemical growth kinetics. From the results, we hypothesize that the presence of the alkanethiolate layer reduces the ECSA of the Au NPs, leading to a negative shift in $E_p$ and decrease in $i_p$ for the electrochemical growth process. Longer soaking times in ethanolic alkanethiol solutions result in more extensive passivation of the electroactive surface, leading to higher growth overpotentials and irreversible kinetics. Furthermore, the alkane chain length of the thiol plays a critical role in the electrochemical growth kinetics. Shorter chain lengths have a minimal effect on the kinetics, whereas longer alkyl chains lead to large shifts in $E_p$ and $i_p$. This is attributed to the steric hindrance caused by the alkyl group, which decreases the diffusion of $\text{AuCl}_4^-$ to the electroactive NP surface but also reduces the number of active surface sites available to catalyze the ECD process. The impact of NP size is also evident, with larger Au NPs requiring higher overpotentials for electrochemical growth. We attribute this to better organized SAM assemblies on larger Au NPs due to less curvature and fewer defect sites. The combination of the C8S alkanethiolate SAM and NP size for 50 nm Au NPs resulted in kinetics for growth slower than electrochemical nucleation and growth kinetics on bare glass/ITO, whereas 15 nm Au NPs showed very similar kinetics as bare glass/ITO. In summary, this study highlights the
interplay between alkanethiolate SAMs, NP size, and electrochemical kinetics in the growth of Au by ECD on thiol-capped Au NPs. This information is crucial for the effective design and optimization of electrochemical processes involving nanoparticle-based materials.
CHAPTER 5: TWO-STEP AMPLIFICATION FOR fM DETECTION OF GOLD NANOPARTICLES BY ANODIC STRIPPING VOLTAMMETRY

5.1. INTRODUCTION

Gold is widely used in various day-to-day applications such as electronics, catalysis, sensors, jewelry, and drug delivery.\textsuperscript{144-146} Due to its widespread demand,\textsuperscript{147} Significant efforts have been made to explore ore deposits to meet the demands of the global supply chain.\textsuperscript{148, 149} Another way to maintain the Au market is to recycle gold from electronic waste generated yearly.\textsuperscript{150, 151} During gold exploration and recycling, Au is usually processed in the form of grains or nanoparticles (NPs).\textsuperscript{149, 152} The ability to detect low concentrations of Au in nanoparticle form has a huge impact on the process of identifying ores or electronics containing low concentrations of Au as well as potentially reducing the cost of processes.\textsuperscript{153} Hence, there is a need to develop efficient yet cheap analytical methodologies to detect economic elements such as gold at lower concentrations. The development of a new methodology to detect Au NPs requires an understanding of the basic nature of Au NPs. Au NPs have been extensively studied due to their unique properties that differ from their bulk counterparts.\textsuperscript{9, 14, 154} In nano form, Au has a higher surface area to volume ratio (SA/V), resulting in more surface-active sites.\textsuperscript{33, 155} This gives them unique optical, thermal, magnetic, chemical, and electrochemical properties that can be modified by size, shape, aggregation state, and coverage.\textsuperscript{23, 32, 104} Their properties can often be tuned by the NP size and shape to meet specific requirements. The detection of metal ions and metal NPs at low
concentration levels is important for environmental analysis, metal recovery, and chemical and biological sensing when using metal NPs as tags.\textsuperscript{144, 153}

There are several reports found in the literature regarding the detection of Au for its purpose in mining, sensors, recovery, and drug recovery. For instance, Palenik \textit{et al.} determined the presence of Au NPs (~5-10 nm in diameter) in Carlin-type pyrite deposits in Nevada using analytical and high-resolution TEM and high-angle annular dark-field (HAADF) imaging in STEM mode.\textsuperscript{156} The electron microprobe and secondary ion mass spectrometry analyses revealed the detection of Au content as low as 120 ppm, up to 0.84 wt\% in some regions of arsenian pyrite rim (1-20 μm). Mohamed \textit{et al.} fabricated a 4-aminoantipyrine solid sensor to detect low concentrations of Au(III) ions via colorimetric detection.\textsuperscript{151} 4-aminoantipyrine played the role of capping and reducing agent to form Au NPs that can be easily detected via colorimetry. The detection limit of this method was estimated to be 84 nM (in terms of Au atoms). Ogonczyk \textit{et al.} demonstrated a continuous electrochemical detection of Au NPs based on synchronous processes of their electrodissolution and electrocatalysis using a flow injection analysis system.\textsuperscript{146} This process detects Au NPs in a wide concentration range: $10^{-10} – 10^{-7}$ M. Ganesh \textit{et al.} developed a multi-walled carbon nanotube-reduced graphene oxide nanocomposite electrode using differential pulse voltammetry to detect gallic acid-capped Au NPs.\textsuperscript{157} The reduction of gallic acid-capped Au NPs in 0.1 M PBS buffer solution was used as the quantification signal, and the detection limit was observed to be at 2.57 pM in terms of Au NPs. Zuber \textit{et al.} studied the suitability of absorption and fluorescence for Au detection.\textsuperscript{148} The ability of Au NPs to catalyze the conversion of a fluorescent compound, I-BODIPY, to its derivative (H-BODIPY) was exploited for the detection. For both methods, the limits of quantification of Au NPs were dependent on the NP size (71 ppb of 5 nm and 24.5 ppb of 50 nm NPs for absorption and 74 ppb of 5 nm and 1200 ppb of 50 nm NPs for fluorescence).

Electrodeposition of Au NP can be used as a tool for detection purposes.\textsuperscript{158} For instance, Shiba \textit{et al.} selectively electrodeposited AuCl\textsubscript{4} (grown Au NPs) on sputter-deposited Au NPs
embedded in carbon film electrodes to form substrate for Se(IV) detection.\textsuperscript{159} The ppb level
detection of Se(IV) by ASV was observed with the template. The grown Au NPs shows higher
stripping currents without the potential shifts induced by the increase in Se(IV) concentration in
comparison to lower stripping currents observed with ungrown NPs. Dai and Compton performed
direct electrodeposition of AuCl\textsubscript{4} to glass/ITO electrode to modify the electrodes with Au NPs
that were used to detect 5 ppb level of As(III) using CV and LSV. Sarkar \textit{et al.} electrodeposited
AuCl\textsubscript{4} in a ZnO NPs in dispersion to glass/ITO electrode to fabricate SERS active substrates for
the detection of 1 nM of methylene blue.\textsuperscript{160} Wang \textit{et al.} electrodeposited A, Ag, and Pt to the
inner walls of carbon nanopipettes to detect glucose, H\textsubscript{2}O\textsubscript{2}, Cl\textsuperscript{−}, and H\textsuperscript{+} ions in micromolar
concentrations.\textsuperscript{161} The wide applications of NPs towards detection necessitates better
methodologies to improve the limit of detection of the analytes to be detected. This led to an
increased interest in single (or minimum) NP detection techniques. For instance, Anderson and
Zhang illustrated the single-particle immobilization and collision as powerful tools to detect Au
NPs on Pt nanoelectrodes.\textsuperscript{162} The detection of NPs was performed by fast-scan cyclic
voltammetry (FSCV) using hydrazine as redox species which get detected as increase in current
when the NP collide at the Pt nanoelectrode surface. The collision experiments led to the
detection of hundreds of NPs in a few minutes. Glascott \textit{et. al.} demonstrated the electrodeposition
of single Pt NPs inside aqueous nanodroplets as nanodroplets collide the Pt
ultramicroelectrodes.\textsuperscript{163} They quantified the growth kinetics of 100 single NPs by measuring
heterogenous rate constant for the growth of Pt NPs on Pt ultra microelectrode.

There are two types of electrodepositions used in this paper. The first one is
electrophoretic deposition (EPD), which is highly reliable and can deposit NPs with high
precision.\textsuperscript{19, 164-166} EPD enables the attachment of metal nanoparticles (NPs) to an electrode
surface for electrochemical analysis.\textsuperscript{4, 24, 155} Additionally, it can create thin films of metals with
specific surface characteristics and other biomaterials like proteins or hydrogels.\textsuperscript{2, 167, 168} This
highlights the significance of EPD as a method for capturing NPs on a conductive electrode surface. The second method is electrochemical deposition (ECD), which is a common method for growing nanoparticles (NPs) on a conductive surface.\textsuperscript{169-172} In ECD, metal structures are formed through the direct deposition of metal ion precursors onto the electrode surface via electroreduction, followed by growth through the deposition of metal on already-formed nucleation sites.\textsuperscript{169, 170, 173} Our group has demonstrated that the kinetics of metal growth can be controlled by manipulating the ECD potential and time, which is heavily influenced by the size and coverage of the initial nucleation sites.\textsuperscript{102} By selectively depositing metal onto the nucleation sites rather than the bare electrode, ECD can be used to grow existing nucleation sites without allowing growth on bare electrode surfaces. Anodic stripping voltammetry (ASV) is an accurate and cost-effective analytical tool for analyzing the presence of metal nanostructures on a conductive surface.\textsuperscript{103, 104} For example, by performing ASV of Au NPs on a conductive surface in a solution containing \( \text{Br}^- \) ions, the peak current, charge under the curve, and peak potentials can indicate the size, coverage, and nature (aggregated or isolated) of the NPs present on the conductive surface.\textsuperscript{104} The electrooxidation reaction of Au NPs is as follows:

\[
\text{Au}^0 + 4\text{Br}^- \rightarrow \text{AuBr}_4^- + 3e^- \quad (E^0 = 0.858 \, V \text{ vs. NHE}) \quad (1)
\]

This chapter describes the use of a combination of EPD and ECD, to deposit and amplify Au NPs and detect them using ASV. The double-step electrodeposition amplification method is a quick and cost-effective process that could potentially be automated and performed on-site with minimal skill required. By controlling the EPD and ECD potentials and times, NP concentrations as low as 17 fM (in terms of Au NP concentration) were detected.
5.2. RESULTS AND DISCUSSION

This chapter describes a two-step electrochemical amplification method for detection of Au NPs (as illustrated in Figure 5.1). Firstly, we electrodeposit the citrate-stabilized Au NPs (cit-Au NPs) onto a glass/ITO working electrode using hydroquinone (HQ)-mediated electrophoretic deposition (EPD), which is performed at 0.8 V (vs. Ag/AgCl) for various times and concentrations to detect lower concentrations of Au NPs. After EPD, the citrate capping agent and other impurities on the NP surface are removed by cleaning with ozone for 15 minutes. Secondly, we amplify the Au NPs through electrochemical deposition (ECD) of Au onto the glass/ITO/Au NPs. We perform ECD on glass/ITO/AuNPs in a solution of 0.001 M HAuCl₄·3H₂O and 0.1 M KClO₄ at 0.8 V (vs. Ag/AgCl) for various times (0.5, 1, 3, 5 and 15 min). The amplified Au NPs are then stripped from the electrode by scanning the potential from 0.2 V to 1.2 V (vs. Ag/AgCl) in 0.01 M KBr plus 0.1 M KClO₄ solution. The peak current and charge obtained from the ASV are proportional to the concentration of NPs present in the initial EPD solution.

Figure 5.2 compares ASVs performed in a solution of 10 mM KBr and 0.1 M KClO₄ of glass/ITO (without EPD) and glass/ITO/Au NPs (with EPD) after ECD amplification for 1 min at 0.7, 0.8, and 0.9 V (Frames a-c, respectfully). The ECD is performed in a solution of 1 mM HAuCl₄·3H₂O and 0.1 M KClO₄ and EPD in 170 nM 4 nm AuNPs plus 16.7 mM hydroquinone (HQ) at 0.8 V for 1 min. The glass/ITO (blank) was prepared by performing EPD from a solution containing 25 mL of nanopure H₂O and 5 mL of 0.1 M HQ followed by ECD at 0.8 V for 1 min. As previously reported, the rate of ECD is faster on Au NPs compared to bare glass/ITO, leading to preferential Au ECD on the 4 nm Au NPs deposited by EPD in the first step as compared to ECD on the bare glass/ITO. This is evident from the higher Au stripping current on the glass/ITO/Au NPs in comparison to that of glass/ITO. For example, at an ECD potential of 0.7 V, the ASV peak current (iₚ) from glass/ITO/Au NPs was 34 ± 3.9 μA as compared to 15 ± 6.8 μA for glass/ITO. At ECD potentials of 0.8 and 0.9 V, the ASV iₚ values were 4.2 ± 2.0 μA and 1.3 ±
Figure 5.1. A schematic representation of the two-step electrodeposition amplification detection of 4 nm diameter citrate coated Au NPs.
Figure 5.2. A comparison of ASVs of glass/ITO without EPD (blue) and with EPD (red) at ECD potentials of (a) 0.7 V, (b) 0.8 V, and (c) 0.9 V performed for 1 min in a solution of 1 mM HAuCl₄·3H₂O + 0.1 M KClO₄. (d) A bar graph comparing the ASV average peak currents (iₚ) obtained at the different ECD potentials for glass/ITO with and without EPD. The table in the inset shows the ratio of the average iₚ of glass/ITO with EPD to average iₚ without EPD at ECD potentials of 0.7, 0.8, and 0.9 V. The EPD of 4 nm Au NPs on the glass/ITO was performed from a solution of 170 nM Au NPs + 16.67 mM HQ at 0.8 V for 1 min. The ASV was performed in a solution of 10 mM KBr + 0.1 M KClO₄.
0.5 μA for glass/ITO/Au NPs, respectively, as compared to 0.32 ± 0.15 mA and 0.20 ± 0.04 mA on glass/ITO, respectively. Figure 5.2d shows bar graphs comparing ASV $i_p$ values for glass/ITO/Au NPs and glass/ITO at ECD potentials of 0.7, 0.8, and 0.9 V. At more negative ECD potential the $i_p$ increased for both glass/ITO/Au NPs and glass/ITO. For detection of Au NPs, it is desirable for the $i_p$ of glass/ITO/Au NPs to be much larger than glass/ITO, since glass/ITO would be the signal of the blank solution. The best condition is indicated by the largest $i_p$ (Au NPs)/$i_p$ (blank), which is shown in the table in the inset of Figure 5.2. The ratios follow the order of 0.8 V (14) > 0.9 V (6.5) > 0.7 V (2.3). The ratio of peak currents with and without EPD is highest at 0.8 V. Therefore, we chose to use 0.8 V ECD potential in further experiments.

Figure 5.3 illustrates the effect of varying EPD times (0.5, 1, 3, 5, 10, and 15 min) and Au NP concentrations (1.7, 17, and 170 nM) on the ASVs of the glass/ITO/Au NPs after ECD at 0.8 V for 1 min as already described. At low EPD times, such as 0.5 min (Figure 5.3 a) and 1 min (Figure 5.3 b), the ASV currents generally increase with increasing Au NPs concentration and are all below ~5 μA. Importantly, the $i_p$ values from glass/ITO (blank) are significantly lower compared to glass/ITO/Au NPs. This is due to the slow ECD kinetics on glass/ITO compared to glass/ITO/Au NPs. As shown in Figure 5.3 c, when the EPD time is increased to 3 min, the ASV $i_p$ is the highest for 170 nM Au NP concentration. This indicates that increasing the EPD times increases $i_p$ from the glass/ITO/Au NPs. This is due to more Au NPs depositing on the glass/ITO with longer EPD time. When we increased the EPD time to 5 min (Figure 5.3 d), the ASV $i_p$ for glass/ITO/Au NPs increased and correlated well with the concentration of Au NPs present in the EPD solution. At this EPD and ECD condition, the amplified Au NPs attached to the glass/ITO electrode are reflective of the concentration of Au NPs in the EPD solution. Hence the amplification through a combined EPD and ECD process can be employed to detect different concentrations of Au NPs using ASV analysis.
Figure 5.3. ASVs of glass/ITO/4 nm Au NPs prepared in EPD solutions with 0 nM (blank), 170 nM (red), 17 nM (blue), and 1.7 nM (green) Au NPs at 0.8 V for (a) 0.5 min (b) 1 min (c) 3 min (d) 5 min (e) 10 min and (f) 15 min. ECD is performed at 0.8 V for 1 min in a solution of 1 mM HAuCl₄·3H₂O + 0.1 M KClO₄. ASV is performed in a solution of 10 mM KBr + 0.1 M KClO₄.
This concentration-dependent behavior diminishes when the EPD time increases further. At 10 min (Figure 5.3 e) and 15 min (Figure 5.3 f) EPD times, the stripping currents reached a maximum value, irrespective of the concentration of Au NPs in the EPD solution. At high EPD times, there is significant time to achieve a high coverage of Au NPs on the glass/ITO electrodes. If the coverage is enough to cause overlapping diffusion layers for the array of Au NPs, then the amount of deposited Au by ECD will be similar and proportional to the entire area of the glass/ITO electrode instead of being proportional to the coverage of Au NPs on the glass/ITO. At lower Au NP coverage, the Au deposited by ECD is proportional to the area of the Au NPs and is therefore indicative of the amount of Au NPs in solution. The data indicate that the EPD time has a significant impact on the concentration-dependent \( i_p \) after the ECD amplification process.

<table>
<thead>
<tr>
<th>EPD Time (min)</th>
<th>Parameters</th>
<th>170 nM</th>
<th>17 nM</th>
<th>1.7 nM</th>
<th>0 nM (blank)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>Peak Current, ( i_p ) (( \mu A ))</td>
<td>3.9 ± 2.1</td>
<td>1.6 ± 0.4</td>
<td>0.98 ± 0.27</td>
<td>0.39 ± 0.25</td>
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<td>Peak Potential, ( E_p ) (V)</td>
<td>0.924 ± 0.020</td>
<td>0.946 ± 0.018</td>
<td>0.912 ± 0.032</td>
<td>0.961 ± 0.024</td>
</tr>
<tr>
<td></td>
<td>S/B (( i_p )sample/( i_p )blank)</td>
<td>9.9</td>
<td>4.1</td>
<td>2.5</td>
<td>-</td>
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<tr>
<td>1.0</td>
<td>Peak Current, ( i_p ) (( \mu A ))</td>
<td>4.3 ± 2.0</td>
<td>1.2 ± 0.7</td>
<td>2.1 ± 1.8</td>
<td>0.32 ± 0.15</td>
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<tr>
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<td>0.911 ± 0.027</td>
<td>0.944 ± 0.019</td>
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<tr>
<td></td>
<td>S/B</td>
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<td>3.7</td>
<td>6.6</td>
<td>-</td>
</tr>
<tr>
<td>3.0</td>
<td>Peak Current, ( i_p ) (( \mu A ))</td>
<td>16 ± 6</td>
<td>1.6 ± 0.2</td>
<td>2.0 ± 0.3</td>
<td>0.27 ± 0.14</td>
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<td>Peak Potential, ( E_p ) (V)</td>
<td>0.852 ± 0.018</td>
<td>0.935 ± 0.010</td>
<td>0.870 ± 0.033</td>
<td>0.934 ± 0.030</td>
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<tr>
<td></td>
<td>S/B</td>
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<td>5.9</td>
<td>7.2</td>
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</tr>
<tr>
<td>5.0</td>
<td>Peak Current, ( i_p ) (( \mu A ))</td>
<td>33 ± 4</td>
<td>14 ± 2</td>
<td>8.9 ± 2.9</td>
<td>0.44 ± 0.15</td>
</tr>
<tr>
<td>EPD Time (min)</td>
<td>Peak Current, $i_p$ ($\mu$A)</td>
<td>Peak Potential, $E_p$ (V)</td>
<td>S/B</td>
<td></td>
<td></td>
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<tr>
<td>---------------</td>
<td>-----------------------------</td>
<td>--------------------------</td>
<td>-----</td>
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<td></td>
</tr>
<tr>
<td>10</td>
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<tr>
<td></td>
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</tr>
<tr>
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<td>0.871 $\pm$ 0.007</td>
<td>20.4</td>
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<tr>
<td></td>
<td>0.47 $\pm$ 0.41</td>
<td>0.929 $\pm$ 0.036</td>
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<tr>
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<td>0.853 $\pm$ 0.032</td>
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<tr>
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<td>30 $\pm$ 2</td>
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<tr>
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<td>0.843 $\pm$ 0.011</td>
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<tr>
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<td>1.1 $\pm$ 0.2</td>
<td>0.895 $\pm$ 0.012</td>
<td>-</td>
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</table>

Table 5.1: $i_p$, $E_p$, and $S/B$ obtained from ASVs performed on glass/ITO/Au NPs prepared by EPD in solutions of 0, 1.7, 17, and 170 nM Au NPs at 0.8 V for 0.5, 1, 3, 5 and 15 min followed by ECD amplification at 0.8 V for 1 min in a solution of 1 mM HAuCl$_4$.3H$_2$O + 0.1 M KClO$_4$. ASVs were performed in a solution of 10 mM KBr + 0.1 M KClO$_4$.

Table 4.1 summarizes the ASV $i_p$ and peak potential ($E_p$) of the ECD-amplified glass/ITO/Au NPs prepared by different EPD times (0.5, 1, 3, 5, and 15 min) in solutions of different Au NP concentrations (0, 1.7, 17, 170 nM). The ASV $i_p$ values were low at shorter EPD times since the number of Au NPs deposited on glass/ITO increases with increasing EPD time. Unfortunately, the ASV $i_p$ of the ECD-amplified Au NPs at 0.5 min, 1 min and 3 min EPD times did not correlate well with the Au NP concentration in the EPD solution. For example, the $i_p$ values at 0.5 min EPD in 0 (blank), 1.7, 17, and 170 nM Au NP concentrations were $0.39 \pm 0.25 \mu$A, $0.98 \pm 0.27 \mu$A, $1.6 \pm 0.4 \mu$A, and $3.9 \pm 2.1 \mu$A, respectively. This is similar to the concentration independent ASV $i_p$ for EPD times of 1 and 3 min. The $i_p$ values observed for 1 min EPD in 0 (blank), 1.7, 17, and 170 nM Au NP concentrations following ECD amplification were
0.32 ± 0.15 μA, 2.1 ± 1.8 μA, 1.2 ± 0.7 μA, and 4.3 ± 2.0 μA, respectively, and that for 3 min EPD was 0.27 ± 0.14 μA, 2.0 ± 0.3 μA, 1.6 ± 0.2 μA, and 16 ± 6 μA, respectively. These results do not correlate well with concentration as the 1.7 nM solution sometimes gave a higher \( i_p \) than the 17 nm solution. When we increased the EPD time to 5 min, the \( i_p \) correlated better with the Au NP concentrations in the EPD solution. The \( i_p \) values observed for 0 (blank), 1.7, 17, and 170 nM Au NP concentrations were 0.44 ± 0.15 μA, 8.9 ± 2.9 μA, 14 ± 2 μA, and 33 ± 4 μA, respectively. For EPD times of 10 and 15 minutes, the \( i_p \) again loses its concentration-dependent behavior. This is due to a higher coverage of Au NPs on the electrode surface, which results in the ASV \( i_p \) being proportional to the entire electrode area instead of the sum of the electrode areas of the Au NPs attached to the glass/ITO following ECD, as previously observed in Figure 5.3F. This is due to diffusional overlap during ECD for the higher coverage of Au NPs.

Interestingly, the peak potential (\( E_p \)) associated with the ASV stripping peak depends on the Au NP concentration and EPD time to some degree. Generally, the \( E_p \) is smaller for 170 nM Au NP concentrations compared to 17 and 1.7 nM. This is because the \( E_p \) value is smaller for smaller Au NPs.\(^{113, 174, 175}\) Even though all Au NPs were 4 nm in diameter, the \( E_p \) from the ASV reflects the size of the Au NPs following ECD amplification. A higher concentration of Au NPs, such as 170 nM, leads to a higher coverage of Au NPs on the glass/ITO. Subsequent ECD leads to less growth for each Au NP because the AuCl\(_4^-\) ions responsible for the growth (through reduction of AuCl\(_4^-\)) is shared by all the Au NPs on the surface. With more Au NPs on the electrode surface, each Au NP will experience less growth (less mass transfer) compared to glass/ITO with fewer Au NPs on the electrode surface. The blank consistently showed the largest \( E_p \) value as this sample would have the fewest Au NPs on the glass/ITO electrode. Any Au NPs attached as an impurity or any nucleated Au NP would grow larger during ECD for the same time because those few particles would experience maximum mass transfer of AuCl\(_4^-\) ions to their surface for growth. Accordingly, the \( E_p \) values ranged from 0.823 to 0.867 V for 170 nM Au NP
concentration and EPD of 5 min or greater, while blank solutions under the same conditions showed $E_p$ values of 0.895 to 0.964 V. The $E_p$ is generally larger for the lower concentrations compared to 170 nM for the same reason. The $E_p$ also generally decreases with increasing EPD time for the same reason. The coverage of Au NPs increases with increasing EPD time, leading to less mass transfer per Au NP during ECD amplification. At 170 nM concentration, the $E_p$ was 0.924 and 0.823 V for 0.5 min and 10 min EPD, respectively, as an example of this.

The signal-to-blank (S/B) ratio determined by the $i_p$ of the ECD-amplified glass/ITO/Au NPs ($i_{p,\text{signal}}$) and $i_p$ of the ECD-amplified glass/ITO blank ($i_{p,\text{blank}}$) following EPD was correlated with the EPD time and concentration of Au NPs in the EPD solution. The following equation provides the S/B ratio:

$$S/B = \frac{i_{p,\text{sample}}}{i_{p,\text{blank}}} = \frac{\text{ASV peak current after EPD in Au NP solution and ECD amplification}}{\text{ASV peak current after EPD in blank solution and ECD amplification}}$$

The S/B allows comparison of the ECD-amplified signal following EPD of the Au NP samples compared to the ECD-amplified signal following EPD of a blank solution. A higher S/B is desirable in any analysis as this would lead to a lower detection limit. From Table 4.1, at 0.5 min EPD time, the S/B for 1.7, 17, and 17 nM Au NP concentrations were 2.5, 4.1, and 9.9, respectively, and at 1 min, the S/B were 6.6, 3.7, and 13.3, respectively. When the EPD time was 3 min, at lower concentrations, the S/B was < 10 (7.2 and 5.9 for 1.7 nM and 17 nM Au NP, respectively), but much larger for 170 nM (S/B was 58.0), showing more dramatic amplification. The S/B increased from 20.4 to 32.6 when the concentration increased from 1.7 nM to 17 nM, and the highest S/B was 77.0 for 170 nM at an EPD time of 5 min. The S/B for 1.7, 17, and 17 nM Au NP concentrations was 74.1, 43.4, and 49.3 for 10 min EPD time and 26.5, 27.2, and 21.9, respectively, for 15 min EPD time. S/B increased or stayed the same at 10 min and decreased as the EPD time increased to 15 min. From 0 to 10 min EPD, the S/B increased due to increased S. With 15 min, the S/B decreased due to the increase in B while S maxed out.
Figure 5.4a shows box plots of the ASV $i_p$ for different Au NP concentrations following EPD for different times and ECD amplification at 0.8 V for 1 min, which corresponds to the data described in Table 4.1. The $i_p$ generally increased with increasing concentration and increasing EPD time. At 3 min EPD, the amplification becomes significant for 170 nM Au NPs, but still low for 17 and 1.7 nM. At 5 min EPD time, $i_p$ exhibits good amplification for all concentrations and concentration-dependent $i_p$, showing the ability to detect Au NP concentration through EPD and ECD amplification. At higher EPD times (10 and 15 min), $i_p$ approaches a maximum value and the signal is no longer concentration dependent. The average $i_p$ seems to have decreased for 170 nM for 15 min compared to 10 min, but this may be the result of random error. The average $i_p$ for 170 nM is slightly higher than that for 17 nM and 1.7 nM at these times. It is also not clear if that is real or due to random error. The average $i_p$ generally appears to max out at 20-35 μA for all concentrations and the signal is independent of concentration. Figure 5.4b shows a general trend of increasing the S/B with EPD time from 0-10 min for most of the NP concentrations. As the EPD time increases, more NPs deposited on the glass/ITO electrode, resulting in increased ECD-amplified Au NPs stripped from the electrode. The S/B decreased at 15 min EPD time since the signal (S) maxed out and the blank signal (B) increased.

<table>
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<th>Concentration of Au NP (nM)</th>
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<th>NPs per μm²</th>
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Table 5.2. The total number of Au NPs and NPs per μm² obtained from SEM performed on the amplified glass/ITO/Au NPs prepared in EPD solutions with 170, 17, and 1.7 nM Au NPs at 0.8 V for 1, 5, and 15 min. ECD was performed at 0.8 V for 1 min in a solution of 1 mM HAuCl₄·3H₂O + 0.1 M KClO₄. ASV was performed in a solution of 10 mM KBr + 0.1 M KClO₄.
Figure 5.4. Bar graphs showing (a) ASV peak current ($i_p$) vs EPD time and (b) $i_{p_samp}/i_{p_b} vs EPD time measured from ASVs performed on glass/ITO/Au NPs prepared by EPD from solutions of 170 nM (green), 17 nM (blue), and 1.7 nM (red) Au NPs at 0.8 V for 0.5, 1, 3, 5 and 15 min followed by ECD amplification at 0.8 V for 1 min in a solution of 1 mM HAuCl$_4$·3H$_2$O + 0.1 M KClO$_4$. ASV was performed in a solution of 10 mM KBr + 0.1 M KClO$_4$. 
Figure 5.5 shows SEM images of the ECD-amplified glass/ITO/Au NPs prepared from EPD solutions containing different concentrations (1.7, 17, and 170 nM) of AuNPs and constant EPD time of 5 min. The SEM images qualitatively show an increase in the number of Au NPs deposited and ECD amplified as the concentration of Au NPs in the EPD solution increases. Table 4.2 shows the corresponding coverage data. For example, the number of Au NPs present on the ECD-amplified glass/ITO/Au NPs prepared from 1.7 nM EPD solution is 42 (Figure 5.5a and entry 4 in Table 4.2). When the concentration increased to 17 and 170 nM, the number of NPs deposited on the electrode increased to 207 and 583, respectively, as shown in Figures 4.5b and 4.5c (Entries 9 and 6, respectively in Table 4.2). This is consistent with the increase in \( i_p \) in the ASVs as the NP concentration in the EPD solution increased for 5 min EPD time and 1 min ECD amplification. This correlation is also shown in the calibration curves plotting average \( i_p \) vs. Au NP concentration in the EPD solution (left axis) and vs. the number of NPs per \( \mu \text{m}^2 \) from SEM (right axis), as shown in Figure 5.5D (average values in Tables 4.1 and 4.2). The number of NP per \( \mu \text{m}^2 \) is calculated by calculating the total number of NPs deposited and dividing it by the total area in \( \mu \text{m}^2 \) from 7 to 10 SEM images. At a concentration of 1.7 nM, the average ASV \( i_p \) is 8.9 ± 2.9 \( \mu \text{A} \) with 2.1 ± 0.4 NPs/\( \mu \text{m}^2 \). At 17 nM, it becomes 14 ± 2 \( \mu \text{A} \) and 6.3 ± 4.3 NPs/\( \mu \text{m}^2 \). The ASV average \( i_p \) increased at 170 nM to 33 ± 4 \( \mu \text{A} \) and a SEM coverage of 16 ± 4 NPs/\( \mu \text{m}^2 \). A comparison of both shows general agreement in the increase in ASV \( i_p \) and the number of amplified particles measured by SEM when the concentration of Au NPs in the EPD solution increased. For example, increasing the Au NP concentration from 1.7 nM to 17 nM resulted in an increase in ASV \( i_p \) by a factor of 1.6 (14/8.9) and increase in SEM coverage by factor of 3.0 (6.3/2.1). Increasing the concentration from 17 nM to 170 nM led to an increase in ASV \( i_p \) by a factor of 2.4 (33/14) and SEM coverage by a factor of 2.5 (16/6.3). The SEM data show good agreement between Au NP coverage and trends with the average ASV \( i_p \).
Figure 5.5. (a) 1.7 nM, (b) 17 nM, (c) 170 nM SEM Images. (d) Calibration Plot between ASV peak currents (in mA) (blue) or NPs/mm² (from SEM images) (red) vs concentration (in nM) for the amplified glass/ITO/4 nm Au NPs prepared by EPD performed at 0.8 V for 5 min in EPD solutions with no NPs (Blank), 1.7 nM, 17 nM, and 170 nM Au NPs, followed by ECD performed at 0.8 V for 1 min in a solution of 1 mM HAuCl₄·3H₂O + 0.1 M KClO₄. ASV is performed in a solution of 10 mM KBr + 0.1 M KClO₄.
Figure 5.6a plots the coverage of Au NPs in NPs per \( \mu m^2 \) and the average ASV \( i_p \) vs. the EPD time used for the analysis. Both the coverage and average \( i_p \) increased similarly with increased EPD time. When the EPD time was increased from 1 min to 5 min, the coverage increased from 0.78 \( \pm \) 0.25 to 2.1 \( \pm \) 0.4 (2.7x) while the ASV \( i_p \) increased from 2.1 \( \pm \) 1.8 \( \mu A \) to 8.9 \( \pm \) 2.9 \( \mu A \) (4.2x). Similarly, when the EPD time was increased from 5 min to 15 min, the coverage increased from 2.1 \( \pm \) 0.4 to 10 \( \pm \) 1 (4.8x) and the ASV \( i_p \) increased from 8.9 \( \pm \) 2.9 \( \mu A \) to 24.1 \( \pm \) 1.9 \( \mu A \) (2.7x). This shows that as the EPD time increases the coverage of Au NPs increased, leading to greater ASV \( i_p \). The values do not correlate perfectly because there could be a higher coverage of smaller Au NPs or a lower coverage but larger Au NPs. This makes perfect correlation between coverage and ASV \( i_p \) not possible, but the general trends show the capture and amplification process, providing important information on how to optimize it for the lowest detection limits. From the coverage in Au NPs per \( \mu m^2 \), we calculated the average Au NP-NP spacing using the following equation:

\[
\text{Average NP - NP spacing} = \sqrt{\frac{1}{N}}
\]

As the EPD time increased, more NPs deposited on the electrode, resulting in a decrease in the spacing between NPs (Figure 5.6 b). The average NP-NP spacing decreased from 0.462 to 0.347 \( \mu m \) as the EPD time increases from 1 min to 5 min, and further decreased to 0.313 \( \mu m \) when the EPD time increased to 15 min. This spacing is important to calculate because it indicates at what time there would be diffusional overlap during ECD and concentration-independent ASV \( i_p \) values. Based on a 1 min ECD amplification, the diffusion layer thickness should be 346 \( \mu m \) based on \((2Dt)^{1/2}\) and \(D_{AuCl_4^-} = 1.00 \times 10^{-5} \text{ cm}^2/\text{s}\). This is much larger than the Au NP-NP spacing even for the 1 min EPD, which was less than 1 \( \mu m \). Since there is diffusional overlap but the current did not max out at 1-5 min EPD coverages, that indicates that the amplified current is kinetically controlled and not controlled by mass transport.
**Figure 5.6.** (a) Calibration curve plotting Au NP coverage in NPs/mm² (from SEM images) (red) or ASV average iₚ (in mA) (blue) vs. EPD time (in min) for the glass/ITO/Au NPs prepared by performing EPD at 0.8 V for 1, 5, and 15 min in solutions containing 1.7 nM Au NPs. The ECD was performed at 0.8 V for 1 min in a solution of 1 mM HAuCl₄·3H₂O + 0.1 M KClO₄. The ASV was performed in a solution of 10 mM KBr + 0.1 M KClO₄. (b) Table showing the average NP-NP spacing of the amplified glass/ITO/Au NPs prepared at different EPD times (1, 5, and 15 min) following the above conditions.
The overall ECD rate is proportional to the Au NP coverage. When the Au NP coverage reaches the coverages at 10 – 15 min EPD, which is about 10 NPs/μm², then the ECD becomes mass transfer limited and the $i_p$ average maxes out. This informs us that the Au NP coverage must be below about 10 NPs/μm² with 4 nm Au NPs for concentration analysis by ASV using EPD and ECD amplification.

<table>
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<th>ECD Time (min)</th>
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<th>$E_p$ (V)</th>
<th>$i_p$ (μA)</th>
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The EPD and ECD amplification process followed by ASV analysis is capable of identifying concentrations of citrate-stabilized Au NPs as low as 1.7 nM under the described conditions. Unfortunately, the procedure fails to detect 0.17 nM compared to a blank solution, even when we employed EPD times as long as 5 hours and a 1 min ECD as before. We therefore attempted to lower the detection limit by extending both the EPD and ECD time. Figure 5.7 shows the results of ASV analysis after extending the EPD time to 30 min at 0.8 V on a solution of 1.7 nM Au NPs and also extending the ECD time at 0.8 V to 1, 5, and 10 min. The ASV average $i_p$ was $21.0 \pm 1.8 \mu A$ when the ECD time was 1 min and increased significantly to $138 \pm 6 \mu A$ and $184 \pm 24 \mu A$ for ECD times of 5 and 10 min, respectively (Figure 5.7a and Table 4.3). The $i_p$ amplification improved significantly with an increase in ECD time. Most importantly, the ASV of the glass/ITO after EPD in 0 nM Au NP solution (blank) using the same extended EPD and ECD times only showed a slight rise in peak current from $2.0 \pm 2.5 \mu A$ to $4.5 \pm 0.8 \mu A$. Extending the ECD time greatly enhanced the S/B for detection of Au NPs. Figure 5.7c shows the

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Table 5.3. The $i_p$ and $E_p$ obtained from ASVs performed on glass/ITO prepared by EPD in solutions with 0 nM (blank) and low concentrations of Au NPs at 0.8 V for 30 min. ECD was performed at 0.8 V for 1, 5, and 10 min in a solution of 1 mM H$\text{AuCl}_3\cdot3\text{H}_2\text{O} + 0.1$ M K$\text{ClO}_4$. ASV was performed in a solution of 10 mM KBr + 0.1 M K$\text{ClO}_4$. The EPD and ECD amplification process followed by ASV analysis is capable of identifying concentrations of citrate-stabilized Au NPs as low as 1.7 nM under the described conditions. Unfortunately, the procedure fails to detect 0.17 nM compared to a blank solution, even when we employed EPD times as long as 5 hours and a 1 min ECD as before. We therefore attempted to lower the detection limit by extending both the EPD and ECD time. Figure 5.7 shows the results of ASV analysis after extending the EPD time to 30 min at 0.8 V on a solution of 1.7 nM Au NPs and also extending the ECD time at 0.8 V to 1, 5, and 10 min. The ASV average $i_p$ was $21.0 \pm 1.8 \mu A$ when the ECD time was 1 min and increased significantly to $138 \pm 6 \mu A$ and $184 \pm 24 \mu A$ for ECD times of 5 and 10 min, respectively (Figure 5.7a and Table 4.3). The $i_p$ amplification improved significantly with an increase in ECD time. Most importantly, the ASV of the glass/ITO after EPD in 0 nM Au NP solution (blank) using the same extended EPD and ECD times only showed a slight rise in peak current from $2.0 \pm 2.5 \mu A$ to $4.5 \pm 0.8 \mu A$. Extending the ECD time greatly enhanced the S/B for detection of Au NPs. Figure 5.7c shows the
box plots of ASV $i_p$ vs ECD time with constant concentration of 1.7 nM and constant EPD time of 30 min. The S/B increased from 10 to greater than 40 for the longer ECD times. The lack of further increase in S/B between 10 and 5 min may be due mass transfer limitations slowing down the ECD of AuCl$_4$ along with the increased signal of the blank as more nucleation sites form with longer ECD times. Regardless of the limit, the data show that increasing the ECD time can significantly improve amplification and ASV detection of 1.7 nM Au NPs.

We next tested the ASV analysis using the protocol with 30 min EPD and 5 and 10 min ECD times for 0.17 nM and 0.017 nM Au NP solutions since it worked well on improving S/B for 1.7 nM Au NPs. The average ASV $i_p$ increased significantly above the blank signal for 0.017 nM, 0.17 nM, and 1.7 nM as shown in Figure 5.8a using an ECD time of 10 min. As shown in the box plots in Figure 5.8b and Table 4.3, the average $i_p$ values range from about 10 $\mu$A to 200 $\mu$A with increasing concentration and all can be distinguished from the blank signal plus 3 times the standard deviation of the blank ($i_{p,\text{blank}} + 3s_{\text{blank}}$), which is about 6-7 $\mu$A at the two different ECD times. The S/B ranges from 3 to greater than 40 as shown in the inset of Figure 5.8B, again showing the ability to distinguish concentrations as low a 0.017 nM Au NPs (or 17 pM). Since the 17 pM concentration is in terms of Au atoms and there are about 1700 Au atoms per 4 nm Au NP, the detection limit under these conditions is 10 fM in terms of concentration of Au NPs.
Figure 5.7. ASV Plots of glass/ITO after EPD at 0.8 V for 30 min of (a) 1.7 nM Au NPs and (b) 0 nM Au NPs (blank sample) and following ECD amplification at 0.8 V for 1 min (red), 5 min (blue), and 10 min (green). (C) Box plots showing average ASV $i_p$ (in mA) vs. ECD time (in min) for 1.7 nM Au NPs and 0 nM blank. The table in the inset shows the S/B for different ECD times.
Figure 5.8. (a) ASV plots of glass/ITO after EPD at 0.8 V for 30 min in 0 nM (black), 0.017 nM (red), 0.17 nM (blue), and 1.7 nM (green) Au NPs solutions followed by ECD amplification at 0.8 V for 10 min and (b) a box plot showing average ASV $i_p$ (in mA) vs. Au NP concentration using 5 min (red) and 10 min (blue) ECD times. The table in the inset shows the S/B at different Au NP concentrations and ECD times.
5.3. CONCLUSIONS

We demonstrated the detection of citrate-stabilized Au NPs using a combination of EPD and ECD followed by ASV analysis. We explored the influence of EPD and ECD potential and time on the resulting S/B for various Au NP concentrations. For 1.7 nM to 170 nM Au concentrations (in terms of Au atoms), the average ASV $i_p$ correlates with the Au solution concentration best at an EPD potential of 0.8 V for 5 min and ECD potential of 0.8 V for 1 min. SEM images showed good correlation between Au NP electrode coverage and ASV average $i_p$ values, but more importantly showed that the coverage should be below ~10 NPs per $\mu$m$^2$ for concentration-dependent signal under these conditions. The detection limit was reduced by increasing EPD times to 30 min and ECD times to 5-10 min, which resulted in the detection of 10 fM concentration in terms of Au NPs with good S/B. This approach is simple, fast, sensitive, low cost, and potentially automated and portable for Au detection at mining or electronic waste sites.
CHAPTER 6: ELECTROPHORETIC DEPOSITION OF HYBRID CALCIUM ALGINATE-GOLD NANOPARTICLE HYDROGEL FILMS VIA CATALYZED ELECTROOXIDATION OF HYDROQUINONE

6.1. INTRODUCTION

Metal nanoparticles (NPs) display unique optical, catalytic, and electrochemical properties, often different from their bulk metals.\textsuperscript{9, 10, 176} Properties, such as color,\textsuperscript{17} oxidation potential\textsuperscript{103}, and surface area-to-volume ratio (SA/V),\textsuperscript{33} are highly dependent on their size, shape, and aggregation state,\textsuperscript{23} making these properties tunable for specific applications. Since most of the chemical activity occurs at the surface of NPs, the high SA/V allows metal NPs to be more active on a per mole basis in catalytic and sensing applications compared to larger structures.\textsuperscript{14-16, 176}

In order to explore their unique properties and electrochemical applications, it is essential to attach metal NPs onto electrode surfaces. This can be accomplished by using various chemical assembly methods or electrophoretic deposition (EPD).\textsuperscript{4, 24}

EPD involves two processes occurring within a two- or three-electrode electrochemical cell. The first process is the migration of charged particles present in the solution/suspension toward the electrode surface under an applied electric field. The second process is the accumulation and deposition of the particles onto the electrode surface.\textsuperscript{6, 19, 20} EPD offers several advantages over other film formation methods, such as dense packing, high uniformity, predictable kinetics, and controllable kinetics by adjusting EPD parameters.\textsuperscript{177} It has been used to deposit many different
types of materials and has been extended to deposit charged metal NPs onto conductive surfaces.\textsuperscript{166, 178, 179} For example, Mulvaney \textit{et al.} demonstrated that EPD can be used to assemble thousands of single Au NPs per second into predefined patterned cavities on transparent conductive substrates with nanometer spatial resolution.\textsuperscript{22}

The most common mechanism for the deposition process at the electrode-electrolyte interface is charge reduction or neutralization.\textsuperscript{4, 19, 21} With this mechanism, a change in pH or ionic strength at the electrode-electrolyte interface leads to destabilization of the surface charge around electrostatically-stabilized NPs, which in turn leads to their deposition. The EPD of particles can occur upon protonation of their negatively-charged basic groups by the release of H\textsuperscript{+} (pH decrease) at the electrode surface, leading to insolubility and deposition. Release of H\textsuperscript{+} ions can occur by electrochemical water oxidation.\textsuperscript{4} The problem with this approach is that water oxidation also results in the generation of O\textsubscript{2}, which can create bubbles and affect the morphology of the film.\textsuperscript{4, 24} This has been overcome by using the oxidation of hydroquinone (HQ) or reduction of benzoquinone (BQ) to deposit negatively-charged basic or positively-charged acidic materials, respectively, by the generation or removal of protons at the electrode, respectively (see Figure 6.1 a, b). Zhou \textit{et al.} performed the EPD of chitosan using the electroreduction of benzoquinone on an Au electrode and constructed a glucose biosensor by entrapping glucose oxidase and multi-walled carbon nanotubes inside the chitosan film.\textsuperscript{180} Liu \textit{et al.} used HQ electrooxidation to co-deposit agarose and a pH-responsive small molecule gelator, fluorenyl-9-methoxycarbonyl-phenylalanine (Fmoc-Phe).\textsuperscript{181} Our group was the first to employ HQ oxidation to perform EPD of citrate-stabilized Au NPs (cit-Au NPs) at low potential in a bubble-free manner.\textsuperscript{4} We also observed that smaller cit-Au NPs catalyze the HQ oxidation better than larger cit-Au NPs, leading to a lower potential for HQ oxidation and selective deposition of smaller cit-Au NPs.

Alginate (Alg, Figure 6.1c) is a naturally occurring biopolymer derived from the cell walls of marine brown algae.\textsuperscript{61, 182} It has various properties, including biocompatibility, non-
toxicity, high availability, biodegradability, and good adhesive and mechanical properties.\textsuperscript{62, 63} Alg is a negatively-charged linear pH shift polysaccharide, which means that it is soluble in basic solution and insoluble in acidic solutions.\textsuperscript{64, 65} It is often used to form hydrogels in the presence of calcium ions.\textsuperscript{183} Ca-Alg hydrogels have been extensively used to entrap and immobilize enzymes, other proteins, live cells, and NPs at the microscale.\textsuperscript{6} Hence Ca-Alg has been successfully used as a matrix for preparing enzyme biosensors, providing good biocompatibility and a favorable environment to retain the enzyme’s bioactivity.\textsuperscript{64, 65, 67-69, 183-185} This requires a method to deposit films of Alg in a controlled manner followed by Ca\textsuperscript{2+} cross-linking or deposition of Ca-Alg in one step.

EPD is one method that can be used to immobilize Alg on a suitable electrode surface. Alg EPD allows the co-deposition of other biomolecules or NPs, incorporating them into the electrodeposited Alg films. Several groups have explored the EPD of Alg co–deposited with an enzyme or protein on the electrode surface. For instance, Cheong and Zhitomirsky fabricated a nanocomposite of Alg, hydroxyapatite, TiO\textsubscript{2} and chitosan using EPD on a NiTi (50\% Ni) shape memory alloy.\textsuperscript{75} Na-Alg was used here for the electro-steric stabilization and charging of ceramic NPs. Liu \textit{et al.} performed EPD of Na-Alg and horseradish peroxidase on Au electrodes to construct a Alg based hydrogen peroxide biosensor.\textsuperscript{76} The Payne group electrodeposited Ca-Alg on glass/ITO to form hydrogel films and used the method to entrap \textit{E.coli} bacteria without destroying its viability.\textsuperscript{183} The entrapped cells were observed to grow and could be liberated from the gels using sodium citrate that out-competed Alg for Ca\textsuperscript{2+} binding, leading to disruption of the Ca-Alg hydrogel. EPD of Alg is usually achieved via electrochemical oxidation of water (requires high potential), which creates a localized pH drop that neutralizes the negatively-charged Alg, leading to deposition on the electrode.\textsuperscript{75, 183} A major drawback with EPD at higher potentials is it can denature proteinsentrapped in the electrodeposited polysaccharides, causing serious problems for applications.\textsuperscript{186}
Figure 6.1. EPD through (a) H⁺ release by HQ oxidation and (b) H⁺ consumption by BQ reduction. (c) Chemical structure of alginate (A- or B). EPD occurs by protonation of COO⁻-groups.
In this chapter, we examine the EPD of Na-Alg and negatively-charged cit-Au NPs by proton generation through the oxidation of HQ followed by exposure to Ca\(^{2+}\) to form Ca-Alg/Au NP hybrid hydrogel films. This work is unique in that it combines both metal NPs and HQ into the EPD of a charged biopolymer or co-EPD of metal NPs and biopolymer. We observed that the co-EPD of Na-Alg and cit-Au NPs occurs on the electrode surface by Au NP-catalyzed generation of H\(^+\) via electrooxidation of HQ. This was previously used for the EPD of cit-Au NPs by NP neutralization. The addition of Na-Alg leads to co-EPD of both cit-Au NPs and Alg at low potentials for potentially valuable applications.

6.2. RESULTS AND DISCUSSION

Figure 6.2 shows the UV-Vis spectra of glass/ITO/Ca-Alg/Au nanoparticle (NP) films (4 and 15 nm diameter citrate (cit)-stabilized Au NPs) prepared by performing EPD from a solution of 0.5 % (w/v) Na-Alg, 16.67 mM hydroquinone (HQ) and 50 μM cit-Au NPs (or no Au NPs) at different potentials for 30 min. Figure 6.2a shows the UV-Vis spectra of the Ca-Alg hydrogels prepared without cit-Au NPs at potentials ranging from 0 to 1.2 V vs (Ag/AgCl). In the absence of cit-Au NPs, the deposition occurs at 1.2 V vs (Ag/AgCl) as indicated by the presence of a broad band at 475 nm with an absorbance value of 0.025 ± 0.002. This weak band is associated with the Ca-Alg hydrogel since the band at 475 nm (Figure 6.2a) is also observed when Ca-Alg hydrogel (Figure 6.2b) is prepared by performing EPD at 3 V vs (Ag/AgCl) for 30 min in the absence of HQ and cit–Au NPs. In the absence of cit–Au NPs (Figure 6.3a), the Alg deposited at an EPD potential of 1.2 V (vs Ag/AgCl). In contrast, in the presence of 4 nm cit–Au NPs (Figure 6.3b), the Alg deposited at potentials as low as 0.8 V (vs Ag/AgCl). The localized surface plasmon resonance (LSPR) band\(^{101}\) for the 4 nm diameter cit–Au NPs trapped in the hydrogel comes prominent at around 525 nm with a value of 0.054 ± 0.005 (Table 6.1).
Figure 6.2. (a) UV–vis spectra and (b) picture of glass/ITO/Ca-Alg prepared at 3 V for 30 min in the absence of HQ, followed by Ca$^{2+}$ exposure for 30 min and air-dried for 18 h.
A shoulder peak appeared at 476 nm with an absorbance of $0.051 \pm 0.003$ is related to the Ca-Alg hydrogel formed at the glass/ITO surface. At an EPD potential of 1 V (vs Ag/AgCl), the LSPR band appeared at 530 nm with an absorbance value of $0.065 \pm 0.010$ and the shoulder peak associated the hydrogel is observed at 478 nm with an absorbance value of $0.060 \pm 0.009$. The spectra show that as the EPD potential increases, the amount of 4 nm cit–Au NPs and hydrogel deposited by EPD increases. Moreover, the cit–Au NPs clearly catalyzed the EPD process since the threshold potential in the presence of 4 nm cit–Au NPs was 0.8 V (vs Ag/AgCl) compared to 1.2 V (vs Ag/AgCl) in the absence of the cit–Au NPs (Figure 6.3a). For 15 nm diameter cit–Au NPs (Figure 6.3c), the threshold potential required to initiate EPD was 1 V (vs Ag/AgCl). At this condition, a strong LSPR band associated with 15 nm diameter cit–Au NPs appeared at 525 nm with an absorbance of $0.064 \pm 0.009$ (Table 6.2). The shoulder peak at 479 nm associated with the Alg hydrogel had an absorbance of $0.042 \pm 0.004$. As the cit-Au NP size increased from 4 nm to 15 nm diameter, the threshold potential required to initiate EPD increased because the 4 nm diameter cit–Au NPs are more catalytic for HQ oxidation compared to 15 nm diameter cit–Au NPs.\(^4\)

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Table 6.1. UV–vis spectra data of glass/ITO/Ca-Alg–4 nm Au NPs.
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Table 6.2. UV–vis spectra data of glass/ITO/Ca-Alg–15 nm Au NPs.

Figure 6.4 illustrates the EPD of Alg and negatively charged cit–Au NPs on the glass/ITO electrode through the Au NP catalyzed electrooxidation of HQ. The electrochemical oxidation of HQ at the cit-Au NPs as they impact the glass/ITO generates a localized pH drop at the anode surface. As the concentration of protons increases at the electrode, the negatively-charged COO⁻ groups of Alg become neutralized (protonated to COOH). The neutralized Alg is insoluble in water, thus allowing it to deposit on the electrode surface.
Figure 6.3. UV–vis spectra of (a) glass/ITO/Alg; (b) glass/ITO/Alg–4 nm Au NPs; and (c) glass/ITO/Alg–15 nm Au NPs prepared at different potentials (vs Ag/AgCl) for 30 min, followed by Ca^{2+} exposure for 30 min and air-dried for 18 h. The EPD solution contained 15 mL 1% (w/v) Na-Alg solution, 6 mL cit-Au NPs solution, and 5 mL 0.1 M HQ.
Figure 6.4. EPD of Alg and cit-Au NPs using HQ
Since the excess protons on the electrode can also neutralize the COO\(^{-}\) groups of citrates, the EPD of cit–Au NPs will also occur simultaneously. We used this approach previously for the EPD of different sized cit–Au NPs by NP charge neutralization.\(^4\) Subsequent exposure to Ca\(^{2+}\) leads to the final Ca-Alg-cit-Au NP hydrogel films.

Figure 6.5 compares the UV-vis spectra of glass/ITO electrodes after EPD of Alg, 4 nm cit-Au NPs only, 15 nm cit-Au NPs only, 4 nm cit-Au NPs + Alg, and 15 nm cit-Au NPs + Alg at 1 V (vs Ag/AgCl) for 30 minutes. Solutions contained 50 μM cit-Au NPs, 16.67 mM HQ, and 0.50 % (w/v) Na-Alg (total volume is 30 mL) when all components were present and at the same concentrations when some components were absent. In the presence of Alg but no cit-Au NPs, the UV-Vis spectra of the glass/ITO electrode showed no significant absorbance. When the cit-Au NPs were present, the glass/ITO showed a strong peak for Ca-Alg hydrogel and a strong LSPR band associated with the NPs around 530 nm. The absorbance associated with the LSPR bands for 4 and 15 nm diameter cit–Au NPs were 0.065 ± 0.010 and 0.064 ± 0.009, respectively (Tables 6.1 and 6.2). A shoulder peak appeared around 478 nm alongside due to the Ca-Alg hydrogel with an absorbance of 0.060 ± 0.009 for the hydrogel with 4 nm diameter cit–Au NPs and absorbance of 0.042 ± 0.004 for 15 nm diameter cit–Au NPs. The shoulder peak at 476 nm had a larger absorbance with 4 nm cit-Au NPs compared to 15 nm cit-Au NPs compared to no cit–AuNPs. We also compared the EPD of 4 nm and 15 nm cit-Au NPs in the absence of Alg, where we substituted Na-Alg for 0.645 mM NaNO\(_3\) to keep the ionic strength of the EPD solution constant. The LSPR band for 4 and 15 nm diameter cit–Au NPs deposited at the electrode surface under this condition had absorbances of 0.045 ± 0.003 and 0.033 ± 0.004, respectively. The absorbance values are lower compared to the conditions with Alg present due to the overlap in Alg and cit-Au NP peaks when both are on the surface. The amount of cit-Au NPs deposited with and without Alg present appears to be similar. The spectra for cit-Au NPs without Alg also show a significant
Figure 6.5. UV–vis spectra of glass/ITO electrodes after EPD (1 V for 30 min) of Alg, 4 nm cit-Au NPs, 15 nm cit-Au NPs, 4 nm cit-Au NPs + Alg, and 15 nm cit-Au NPs + Alg as indicated, followed by Ca$^{2+}$ exposure for 30 min and air-dried for 18 h.
absorbance near 480 nm, indicating that the peak at 480 nm when Alg is present is a combination of cit-Au NPs and Alg.

Figure 6.6 shows dark field microscopy (DFM) images of the cross-section of as-prepared glass/ITO/Ca-Alg or glass/ITO/Ca-Alg-cit-Au NP hydrogel electrodes formed in the presence of 4 nm cit-Au NPs (Figure 6.6b), in the presence of 15 nm cit–Au NPs (Figure 6.6c), and in the absence of cit–Au NPs (Figure 6.6d) after drying the films for 18 hours under ambient temperature and humidity. The darker section with the bright line above it in all images is the glass/ITO electrode as labeled on the images. The faint bright region above the bright line is the Ca-Alg of Ca-Alg-cit-Au NP hydrogel film as labeled. The Ca-Alg-4 nm cit–Au NPs film is an orange color (Figure 6.6b) while the Ca-Alg-15 nm cit–Au NPs is more of a red color (Figure 6.6c) with a more intense LSPR band near 530 nm. The Ca-Alg film without any cit–Au NPs appears as a grey color (Figure 6.6d). Figure 6.6a shows a cross-section DFM image of glass/ITO without EPD of a Ca-Alg hydrogel film, confirming that the labeling of the Ca-Alg and Ca-Alg-cit-Au NP hydrogel films is correct in Figure 6.6, as opposed to that region being some type of image artifact. Figure 6.6e shows DFM images of Ca-Alg-cit-Au NP (4 nm) hydrogel films after air drying for 5 min, which shows a hydrogel of thickness greater than 200 μm. When it is compared to Figure 6.6b where the drying was for 18 h, it reveals that shrinking of the hydrogel film occurs due to water evaporation occurs much more after 18 hours versus 5 min. The thickness of the hydrogel films, after 18 h of preparation, was 72.4 ± 4.6 μm, 67.4 ± 4.7 μm, and 30.0 ± 2.5 μm for Ca-Alg formed with 4 nm cit-Au NPs, with 15 nm cit-Au NPs, and with no cit–Au NPs present, respectively. The presence of cit–Au NPs in the EPD solution results in thicker Ca-Alg films, consistent with the cit–Au NPs catalyzing the HQ electrooxidation and improving the depositions of the Ca-Alg-cit-Au NP films at the electrode surface. The thickness under these conditions is independent of the size of the cit-Au NPs, which is consistent with the similar UV-vis absorbance in Figure 6.5. under these conditions.
Figure 6.6. DFM Images of (a) glass/ITO, (b) glass/ITO/Ca-Alg – 4 nm cit-Au NPs, (c) glass/ITO/Ca-Alg – 15 nm cit-Au NPs and (d) glass/ITO/Ca-Alg prepared by EPD at 1 V for 30 min, followed by Ca^{2+} exposure for 30 min and air-dried for 18 h (e) glass/ITO/Ca-Alg–4 nm cit-Au NPs after air drying for 5 min.
Figure 6.7a shows ATR–IR spectra of the Ca-Alg hydrogels prepared in the presence and absence of cit–Au NPs at 1.0 V for 30 min and air-dried for 18 h. The ATR-IR spectra is consistent with the literature reported values. The peaks at 3345 cm⁻¹, 1600 cm⁻¹ and 1424 cm⁻¹, 1263 cm⁻¹ and 1213 cm⁻¹, and 1081 cm⁻¹ and 1027 cm⁻¹, are assigned to the hydrogen bonded O–H stretch, the asymmetric and symmetric stretching vibrations associated with the COO⁻ groups (consistent with Ca²⁺-carboxylates formed), the skeletal vibrations of Alg and alginic acid moieties, and the antisymmetric C–O–C stretching vibration of pyranose rings of the Alg polysaccharide, respectively. The peaks from 750–950 cm⁻¹ are associated with various vibrations of the carbohydrate moieties present in the Ca-Alg hydrogel. The absorbance of any peak follows the order of Ca-Alg-cit-Au NP (4 nm) > Ca-Alg-cit-Au NP (15 nm) > Ca-Alg. This is consistent with the UV-vis in Figure 6.5 and DFM in Figure 6.6, showing significantly greater deposition of Ca-Alg in the presence of cit-Au NPs due to cit-Au NP-catalyzed HQ oxidation. The absorbance from Ca-Alg in the presence of 4 nm cit-Au NPs is slightly higher than 15 nm cit-Au NPs, consistent with Figures 6.5 and 6.6. Figure 6.7b shows the ATR-IR spectra of the Ca-Alg hydrogel prepared using EPD with potentials 0.6 V, 0.8 V and 1.0 V, with the absorbance of all peaks (except for O-H stretching at 3345 cm⁻¹ due to the presence of water) following the order 0.6 V < 0.8 V < 1.0 V.

Spectra of the Figure 6.8 shows plots of Ca-Alg-cit-Au NP hydrogel film absorbance (at 525 nm and 475 nm) as a function of cit–Au NP concentration for both 4 nm cit–Au NPs and 15 nm cit-Au NPs as determined by UV–vis spectroscopy (Figures 6.8a and 6.8b), respectively. As the concentration of cit–Au NPs in the EPD solution increases, the absorbance at 525 nm associated with the LSPR band of the cit-Au NPs increases. For example, when the concentration of 4 nm cit–Au NP is 25 µM in the EPD electrolyte, the LSPR band (525 nm) has an absorbance value of 0.036 ± 0.007 and a shoulder peak at 475 nm (Figure 6.8c) at 0.035 ± 0.008.
Figure 6.7. ATR–IR spectra of (a) glass/ITO/Ca-Alg–cit-Au NPs (4 and 15 nm) as well as glass/ITO/Ca-Alg prepared by EPD at 1 V for 30 min, (b) glass/ITO/Ca-Alg–4 nm cit-Au NPs at 0.6 V, 0.8 V and 1.0 V for 30 min (30 min Ca$^{2+}$ exposure and 18 h air-drying).
When the concentration of 4 nm cit–Au NPs was 50 μM (2x), the LSPR peak and 475 nm peak (Figure 6.8c) increased to 0.065 ± 0.010 and 0.060 ± 0.009, respectively. At 75 μM 4 nm cit–Au NPs, the LSPR and 475 nm absorbance (Figure 6.8c) was 0.086 ± 0.017 and 0.077 ± 0.008, respectively. A similar trend occurred for the 15 nm diameter cit–Au NPs. The absorbances of the LSPR peak were 0.046 ± 0.006, 0.064 ± 0.009 and 0.129 ± 0.025 and absorbances of the shoulder peak (Figure 6.8d) were 0.033 ± 0.003, 0.042 ± 0.004, and 0.090 ± 0.030 for concentrations of 25 μM, 50 μM, and 75 μM 15 nm cit–Au NPs, respectively. This indicates that the EPD of Alg is heavily influenced by the concentration of cit-Au NPs within the electrolyte solution. This is likely due to the fact that during EPD the number of cit-Au NPs impacting the glass/ITO increases as the cit-Au NP concentration increases. More catalytic sites become available for HQ electrooxidation, which in turn increases the concentration of H⁺ formed at the electrode surface, resulting in the increased neutralization of Alg.

Figure 6.9 shows the plots of Ca-Alg-cit-Au NP hydrogel film thickness as a function of cit–Au NP concentration for both 4 nm cit–Au NPs and 15 nm cit–Au NPs as determined by DFM microscopy. The measured thickness, of the hydrogels formed under the above conditions sheds light on the influence of the cit-Au NP concentration on EPD. The hydrogel formed using 25 μM, 50 μM, and 75 μM 4 nm cit–Au NP (Figure 6.9a) had thicknesses of 51.3 ± 1.8 μm, 72.4 ± 4.6 μm and 75.2 ± 2.8 μm, respectively. The hydrogels prepared using 25 μM, 50 μM, and 75 μM 15 nm cit–Au NP (Figure 6.9b) had thicknesses of 65.8 ± 2.1 μm, 67.4 ± 4.7 μm and 73.9 ± 2.6 μm, respectively. The thickness data also shows that the concentration of cit–Au NPs in the EPD solution influences the final Ca-Alg hydrogel. Figure 6.9c and 6.9d show DFM images of Ca-Alg films prepared using 25 μM and 75 μM 4 nm cit–Au NPs. From the images it is evident that as the concentration of cit-Au NPs within the EPD solution increases, the number of NPs entrapped in the hydrogel increased as evidenced by the film color being more pronounced.
Figure 6.8. Plots of UV-Vis absorbance at 525 nm vs concentration of the cit-Au NPs in the EPD electrolyte for glass/ITO/Ca-Alg-cit-Au NPs containing (a) 4 nm cit-Au NPs and (b) 15 nm cit-Au NPs; plots showing the UV-vis absorbance at 475 nm vs the concentration of the cit-Au NPs in the EPD electrolyte for glass/ITO/Ca-Alg-cit-Au NP films containing (c) 4 nm cit-Au NPs and (d) 15 nm cit-Au NPs.
Figure 6.9. Plots of the thickness measured using DFM vs the concentration of cit-Au NPs in the EPD electrolyte for glass/ITO/Ca-Alg-cit-Au NPs containing (a) 4 nm cit-Au NPs and (b) 15 nm cit-Au NPs; DFM Images of glass/ITO/Ca-Alg-cit-Au NPs prepared by EPD at 1 V for 30 min using (c) 25 mM and (d) 50 mM 4 nm cit-Au NPs, and (e) 25 mM and (f) 50 mM 15 nm cit-Au NPs, followed by Ca\(^{2+}\) exposure for 30 min and air-dried for 18 h.
This also occurred with 15 nm cit–Au NPs, where the color of the hydrogel significantly increased with an increase in the concentration of cit-Au NPs in the EPD solution. Since the thickness of the films was not dramatically different from 50 µM to 75 µM (Figure 6.6 and 6f), but the absorbance increased dramatically, we conclude that a greater concentration of cit-Au NPs leads to greater density of cit-Au NPs within the film without a large increase in thickness.

The concentration of Na-Alg present in the electrolyte also influenced the EPD process (Figure 6.10). Figure 6.10a shows a plot of film absorbance at 525 nm (LSPR band) as a function of Na-Alg concentration with 4 nm diameter cit–Au NPs in the EPD solution. The film absorbances at 525 nm were 0.037 ± 0.008, 0.065 ± 0.010, and 0.070 ± 0.005 for concentrations of 0.25%, 0.50%, and 0.75% Na-Alg (w/v), respectively. Figure 6.10c shows the absorbances at 475 nm which were 0.034 ± 0.008, 0.060 ± 0.009, and 0.070 ± 0.005 for concentrations of 0.25%, 0.50%, and 0.75% Na-Alg (w/v), respectively. For 15 nm cit-Au NPs (Figure 6.10b), the absorbances at 525 nm were 0.023 ± 0.011, 0.064 ± 0.009, and 0.064 ± 0.008 while the absorbances at 475 nm (Figure 6.10d) were 0.020 ± 0.009, 0.042 ± 0.004, and 0.057 ± 0.014 for concentrations of 0.25%, 0.50%, and 0.75% Na-Alg (w/v), respectively. The film deposition increased with increasing Na-Alg concentration likely due to greater mass transfer of Na-Alg with increasing bulk concentration.

The thicknesses of the hydrogels prepared using different concentrations of Na-Alg and 4 nm diameter cit–Au NPs (Figure 6.11a) or 15 nm diameter cit-Au NPs (Figure 6.11b) showed a similar trend as observed in the UV–Vis spectra. The thicknesses were 38.6 ± 1.3 μm, 72.4 ± 4.6 μm, and 75.1 ± 1.3 μm for Ca-Alg hydrogel films with 4 nm cit-Au NPs prepared using 0.25%, 0.50%, and 0.75% (w/v) Na-Alg, respectively. The thicknesses for Ca-Alg films with 15 nm cit-Au NPs were 27.0 ± 4.7 μm, 67.4 ± 4.7 μm, and 82.3 ± 3.5 μm for 0.25%, 0.50% and 0.75% (w/v) Na-Alg, respectively.
Figure 6.10. Plots of UV-Vis absorbance at 525 nm vs concentration of Alg in the EPD electrolyte for glass/ITO/Ca-Alg-cit-Au NPs containing (a) 4 nm cit-Au NPs and (b) 15 nm cit-Au NPs; plots showing the UV-vis absorbance at 475 nm vs the concentration of Alg in the concentration of Alg in the EPD electrolyte for glass/ITO/Ca-Alg–cit-Au NP films containing (c) 4 nm cit-Au NPs and (d) 15 nm cit-Au NPs.
Figures 6.11c and 6.11d show DFM images for the Ca-Alg-cit-Au NP (4 nm) films prepared with 0.25% and 0.75% (w/v) Na-Alg, respectively. Figures 6.11e and 6.11f show the corresponding DFM images for Ca-Alg-cit-Au NP (15 nm) hydrogel films. In both cases the films are clearly thicker, and the color is more pronounced with the larger Na-Alg concentration. A higher concentration of Na-Alg leads to more film material but greater cit-Au NP density as well, as determined by the enhanced color. Interestingly, the thickness increase was not linear with concentration, suggesting that something else may be limiting the film growth. It may be that film density increases with larger Na-Alg concentration but that the thickness is similar. The long drying step may also affect the final film thickness.

Figure 6.12 shows the Ca-Alg hydrogel deposited on a 2 mm Pt disc electrode using the EPD of Na-Alg (in the presence and absence of 15 nm cit-Au NPs) using HQ electrooxidation at 1.4 V (vs Ag/AgCl) for 30 min. This method is suitable to selectively deposit Ca-Alg and Ca-Alg-cit-Au NP films on conductive surfaces, taking on the shape of the conductive material. Here, the film formation led to a thick hemispherical Ca-Alg-cit-Au NP hydrogel on the Pt disc electrode. The deposition occurred only on the conductive Pt surface, which could be used as a method to control the size and shape of the hydrogels formed or perform electrochemical experiments within the hydrogel, which will be the focus of future experiments.
Figure 6.11. Plots of the thickness measured using DFM vs the concentration of Alg in the EPD electrolyte for glass/ITO/Ca-Alg-cit-Au NPs containing (a) 4 nm cit-Au NPs and (b) 15 nm cit-Au NPs; DFM images of glass/ITO/Ca-Alg-cit-Au NPs prepared by EPD at 1 V for 30 min using (c) 0.25% and (d) 0.75% (w/v) Alg and 4 nm cit-Au NPs, and (e) 0.25% and (f) 0.75% (w/v) Alg with 15 nm cit-Au NPs (Ca$^{2+}$ exposure for 30 min and air-drying for 18 h).
Figure 6.12. Images of (a) Pt disk electrode, (b) Pt/Ca-Alg, and (c) Pt/Ca-Alg-cit-Au NP (15 nm) hydrogels formed on a 2 mm Pt disk electrode by EPD of Na-Alg in the presence of HQ at 1.4 V and 15 nm cit-Au NPs (c only) for 30 minutes followed by Ca$^{2+}$ exposure for 30 min.
6.3. CONCLUSIONS

In this chapter, we described the EPD of Ca-Alg and composite Ca-Alg-cit-Au NP hydrogel films using cit–Au NP-catalyzed electrooxidation of HQ. The oxidation of HQ leads to a local pH decrease at the electrode surface, promoting neutralization and deposition of Alg and cit-Au NPs simultaneously on the electrode surface. This approach has real significance as it can lower the EPD potential due to the catalysis of electrooxidation of HQ by cit-Au NPs. The threshold EPD potential was 0.8 V (vs Ag/AgCl) with Na-Alg and 4 nm cit–Au NPs compared to 1.0 V for 15 nm cit–Au NPs and 1.2 V or greater for Na-Alg with no cit-Au NPs. UV-Vis spectroscopy and DFM showed the role of cit-Au NPs in decreasing the potential and increasing the film thickness of Ca-Alg films. The concentration of cit-Au NPs and Na-Alg during EPD also plays a significant role in film deposition. ATR-IR spectroscopy showed characteristic peaks for the Ca-Alg hydrogel. The absorbance and thickness measured from UV-vis spectroscopy and DFM, respectively, as a function of cit-Au NP concentration and Na-Alg concentration revealed important trends. As cit-Au NP concentration and Na-Alg concentration increased, both absorbance and thickness increased. In the case of cit-Au NPs, the thickness did not increase as much as the absorbance, revealing that the films become denser and more loaded with cit-Au NPs with increasing cit-Au NP concentration versus increasing in thickness. With increasing Na-Alg concentration, the films become thicker with increasing cit-Au NP density indicated by the increase in color of the films. The absorbance is more dominated by the amount of cit-Au NPs due to its large extinction coefficient, whereas the thickness is controlled more by the Na-Alg concentration since it makes up a larger percentage of the film material. Our film deposition strategy has great promise for applications in sensors, biological studies, electrocatalysis, and energy relevant research.
CHAPTER 7: Fe(CN)$_6^{3-}$ ELECTROCHEMISTRY IN CALCIUM-ALGINATE HYDROGELS IN BULK AND SPATIALLY CONFINED ENVIRONMENTS

7.1. INTRODUCTION

Hydrogels play a crucial role in modern industry, specifically in the food, medical, pharmaceutical, cosmetic, and energy sectors. Their applications range from drug delivery, biosensors, and electrochemical devices to conductive solid media, energy storage, and conversion. This can be attributed to their tunable structure, mass transport properties, and ability to form biocompatible matrices that retain significant amounts of water. In addition to that, they can also interact with external stimuli such as temperature, pH, electromagnetic fields, salt, and solvent composition.

In recent years, there have been efforts to explore using hydrogels as potential solids or quasi-solid electrolytes for the abovementioned applications. The use of redox mediators such as Fe(CN)$_6^{3-}$ can be useful to study electron and mass transport of molecules in the hydrogel for its use as an electrolyte system for electrochemical cells and devices. Several reports explore the redox reactions of Fe(CN)$_6^{3-}$ in hydrogels and their potential applications. For instance, Yang et al. prepared a multifunctional gel polymer electrolyte (GPE) consisting of polyvinyl alcohol (PVA)-sodium alginate (SA)-K$_3$Fe(CN)$_6$-Na$_2$SO$_4$ for use as a supercapacitor, where K$_3$Fe(CN)$_6$...
served as a carrier donor, ionic crosslinking agent, and redox-active mediator.\textsuperscript{198} The GPE supercapacitor showed bending and stretching stabilities, self-healing, and anti-freezing properties with potential applications in flexible and wearable equipment. Han \textit{et al.} demonstrated the mass transport properties of Fe(CN)\textsubscript{6}\textsuperscript{3−} within agarose hydrogel using a macroelectrode and UME.\textsuperscript{201} They reported diffusion-controlled cyclic voltammetry (CV) in the hydrogels with diffusion coefficients of Fe(CN)\textsubscript{6}\textsuperscript{4−} decreasing with agarose concentration. Yin \textit{et al.} investigated the hydrophilic-hydrophobic nature of the microdomains in polysaccharide hydrogels, such as methylcellulose (MC) and κ-carrageenan, coated on glassy carbon electrodes using CV with ferri/ferrocyanide (Fe(CN)\textsubscript{6}\textsuperscript{3/4−}).\textsuperscript{202} CV was used to probe the hydrogel-electrode interface and the effective heterogenous electron transfer at the interface. Rousseau \textit{et al.} explored the application of Fe(CN)\textsubscript{6}\textsuperscript{3/4−} doped PVA hydrogels as a support material for miniature aqueous inner filling solutions used in ion-selective electrodes.\textsuperscript{203} The electrodes were equipped with hydrophilic cation-exchange membranes and plasticized ionophore-doped PVC membranes, and their potential was regulated by the Fe(CN)\textsubscript{6}\textsuperscript{3/4−} redox buffer. Buckingham \textit{et al.} reported the use of nanostructuring to increase the thermocapacitance performance of polyacrylate hydrogel containing Fe(CN)\textsubscript{6}\textsuperscript{3/4−}; specifically, nanostructuring the bulk of the hydrogel and modifying the electrode with carbon nanotubes and liquid crystalline graphene oxide.\textsuperscript{204} Increasing the electrolyte concentration and degree of hydrogel neutralization improved the thermocapacitance of the device. Russo \textit{et al.} developed a quasi-solid state electrolyte for thermoelectrochemical cells using polyacrylate hydrogels and Fe(CN)\textsubscript{6}\textsuperscript{3/4−}.\textsuperscript{205} This hydrogel electrolyte possessed improved electrochemical properties, mass transport, and thermocell performance while also providing desirable mechanical properties such as flexibility, elasticity, and leak prevention.

This chapter describes the oxidation/reduction of Fe(CN)\textsubscript{6}\textsuperscript{3/4−} on Pt, Au, and glassy carbon electrodes coated with calcium alginate (Ca-Alg) hydrogels by electrophoretic deposition (EPD).\textsuperscript{2} To modify the hydrogels and mass transport of the Fe(CN)\textsubscript{6}\textsuperscript{3/4−}, alkylamines have been
coupled to the carboxylate groups present on the hydrogel surface via an EDC amide coupling reaction (presumed to be covalent amide bonds). The experimental procedures for this chapter can be found in Chapter 2.

7.2. RESULTS AND DISCUSSION

**Fe(CN)$_6^{3-/4-}$ electrochemistry at Pt/Ca-Alg electrodes.** This section discusses the cyclic voltammetry (CV) of ferricyanide (Fe(CN)$_6^{3-}$) at working electrodes comprised of electrophoretically-deposited Ca-Alg hydrogels on Pt disk electrodes. Figure 7.1 shows the CVs of the Fe(CN)$_6^{3-}$ at Pt and Pt/Ca-Alg electrodes performed at a scan rate of 0.01 V/s. The electrochemical analysis was performed in a beaker cell that held a 4 mM K$_3$Fe(CN)$_6$ plus 1 M KNO$_3$ electrolyte solution. The setup consisted of a three-electrode configuration, including a 2 mm Pt or Pt/Ca-Alg disk working electrode, an Ag/AgCl (3 M KCl) reference electrode, and a Pt wire counter electrode. Figure 7.1a shows the CV of Fe(CN)$_6^{3-}$ redox reaction at a bare Pt electrode. The redox reaction shows reversible kinetics with $\Delta E_p$ of $\sim$63 mV and peak cathodic current ($i_{pc}$) and anodic current ($i_{pa}$) of 17.4 µA and 15.9 µA, respectively. The corresponding diffusion coefficients were calculated using the Randles-Sevcik equation as follows:

$$i_p = 2.69 \times 10^5 \times n^{3/2} \times A \times C \times \sqrt{D \times v} \quad (1)$$

$$D = \left( \frac{i_p}{2.69 \times 10^5 \times n^{3/2} \times A \times C \times \sqrt{v}} \right)^2 \quad (2)$$

Where $i_p$ is the anodic or cathodic peak current in A, $n$ is the number of electrons transferred in the reaction, $A$ is the electrode area in cm$^2$, $C$ is the concentration in mol/cm$^3$, $v$ is the scan rate in V/s, and $D$ is the diffusion coefficient in cm$^2$/s. Here, $D$ calculated from anodic ($D_a$) and cathodic ($D_c$) currents are $2.24 \times 10^{-5}$ cm$^2$/s and $2.65 \times 10^{-5}$ cm$^2$/s.
Figure 7.1b shows the CV of Fe(CN)$_6^{3-}$ at a Pt/Ca-Alg electrode performed at different times after soaking the electrode in the electrolyte solution. Pt/Ca-Alg was prepared by performing EPD on the bare Pt (2 mm diameter) disk electrode at 1.4 V for 30 min in a solution containing 20 mL 1 % (w/v) Na-Alg solution, 5 mL H$_2$O and 0.2 M hydroquinone (HQ). The electrophoretically-deposited Alg was then cross-linked with Ca$^{2+}$ by placing it in 90 mM Ca$^{2+}$ for 30 min. The CV shows that the electrochemistry at 3 min of soaking in the electrolyte did not show a cathodic current, but on the backward scan, the anodic current emerged with a peak current of 3.3 $\mu$A. As time increases, the cathodic and anodic currents increase in subsequent scans. For instance, after 6 min of Pt/Ca-Alg soaking in the electrolyte, $\Delta E_p$ is 70 mV, $i_{pc}$ is 5.1 $\mu$A, and $i_{pa}$ is 6.1 $\mu$A. After 15 min of soaking, $\Delta E_p$, $i_{pc}$, and $i_{pa}$ are 66 mV 7.6 $\mu$A and 8.0 $\mu$A, respectively. Correspondingly, the diffusion coefficients of the Fe(CN)$_6^{3-}$ also increase with the increase in soaking/scanning time as shown in Table 7.1. This is consistent with the hypothesis that the Ca-Alg film (~ 500 $\mu$m thick) slows down the Fe(CN)$_6^{3-}$ mass transport towards the Pt electrode surface. This is shown by the small peak currents that increase with time during subsequent scans, which remain lower than that observed in the absence of Ca-Alg on the Pt electrode (Figure 7.1a). The diffusion of Fe(CN)$_6^{3-}$ into Ca-Alg equilibrates after ~30 min of soaking/scanning, which is evidenced by the constant peak currents and diffusion coefficients after 30 min of soaking/scanning, where the constant $i_{pc}$ is 8.5 $\mu$A and constant $i_{pa}$ is 8.7 $\mu$A while constant diffusion coefficient is $\sim 6 \times 10^{-6}$ cm$^2$/s. The significantly low diffusion coefficient in the presence of Ca-Alg confirms that the hydrogel affects the mass transport of Fe(CN)$_6^{3-}$ to the Pt electrode surface. Additionally, $\Delta E_p$ also remains constant at 65 mV, indicating that the reaction kinetics are eventually reversible once the Fe(CN)$_6^{3-}$ equilibrates with the Ca-Alg film. This shows that the presence of Ca-Alg at the electrode does not slow down the electron transfer rate constant by a measurable amount. The redox reaction of Fe(CN)$_6^{3-}$ at the Pt/Ca-Alg hydrogel electrode is reversible with peak current limited by slower diffusion of Fe(CN)$_6^{3-}$ through the
hydrogel. Figures 7.1c and d show the control experiments where Pt and Pt/Ca-Alg CVs were performed in 1 M KNO$_3$ electrolyte solution. There was no observed Faradaic current, indicating that the peak currents in Figure 7.1a and b are due to Fe(CN)$_{6}^{3-}$ in 1 M KNO$_3$ electrolyte.

<table>
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<th>$D_c$ (cm$^2$/s)</th>
<th>$i_{pa}$ (µA)</th>
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<td>6.4 x 10$^{-6}$</td>
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**Table 7.1.** Peak currents and Diffusion coefficients associated with Pt/Alg electrodes at different soaking/scanning minutes.

**Fe(CN)$_{6}^{3-}$ electrochemistry at Pt/Ca-Alg-oleyl amide electrodes.** The electrodeposited Ca-Alg was modified in an attempt to alter the Fe(CN)$_{6}^{3-}$ diffusion through the hydrogel to the Pt electrode. In this case, we modified the Ca-Alg by amide coupling between oleyl amine and free carboxylic acid/carboxylate groups of the Ca-Alg hydrogel, as shown in Figure 7.2. We believe the amide coupling would occur on the outer surface of the hydrogel because of the very hydrophilic interior. The covalent bond between oleyl amine and the carboxylic acid of the Ca-Alg was performed by the very well-known EDC coupling. The electrophoretically-deposited Pt/Ca-Alg was soaked in an aqueous solution of 5 mM EDC and 10 mM n-hydroxy succinic acid (NHS) for 30 min, followed by exposure to ethanolic 10 mM oleyl amine for 15 min. The resulting Pt/Ca-Alg-oleyl amide electrode was cleaned by dipping it in nanopure water for a minute before CV analysis. Figure 7.3a shows the CVs of Fe(CN)$_{6}^{3-}$ redox reactions, performed at different times, at the modified Pt/Ca-Alg-oleyl amide electrode in a 4 mM K$_3$Fe(CN)$_6$ and 1 M KNO$_3$ electrolyte solution at a scan rate of 0.01 V/s. Notably, a slight peak of ~0.1 µA at approximately 0.25 V indicates a very small amount of Fe(CN)$_{6}^{3/-4+}$ electrochemistry compared to that without amide coupling (~8 µA).
Figure 7.1. Electrochemical redox reactions of Fe(CN)$_6^{3-}$ on (a) Pt electrode and (b) Pt/Alg electrode in a solution of 4 mM K$_3$Fe(CN)$_6$ + 1 M KNO$_3$ at a scan rate of 0.01 V/s and different times. Control reactions on (c) Pt electrode and (d) Pt/Ca-Alg electrode in a solution of 1 M KNO$_3$ at a scan rate of 0.01 V/s and different times. Pt/Alg is prepared by performing EPD on a 2 mm Pt disk electrode at 1.4 V for 30 min, followed by 90 mM Ca$^{2+}$ exposure for 30 min. The EPD solution contained 20 mL 1% (w/v) Na-Alg solution, 5 mL H$_2$O, and 5 mL 0.2 M HQ.
The hydrophobic oleyl chains at the hydrogel somehow block \( \text{Fe(CN)}_6^{3/4-} \) diffusion or electron transfer to the Pt electrode. The mechanism is not clear at this time. A bilayer structure at the outer surface of the hydrogel could block mass transfer of \( \text{Fe(CN)}_6^{3-} \) into the hydrogel, similar to the bilayer of a cell membrane. Alternatively, the double bond of the oleyl chain may form a bond with the Pt electrode surface by aligning towards the electrode, causing resistance to electron transfer with \( \text{Fe(CN)}_6^{3/4-} \). Both possibilities are depicted in Figure 7.2. Other possible mechanisms could exist as well. Notably, the voltammograms did not exhibit redox curves despite soaking in the electrolyte for 45 minutes, suggesting that the oleyl group is blocking the \( \text{Fe(CN)}_6^{3-} \) diffusion.

In order to demonstrate that amide formation is a result of EDC coupling, we conducted a control experiment where EDC coupling was carried out without EDC and NHS but instead with oleyl amine only. The resulting electrode was subjected to cycling in a solution of 4 mM \( \text{K}_3\text{Fe(CN)}_6 \) and 1 M KNO_3 at different time intervals after being soaked for the same amount of time (30 min). The obtained CVs closely resembled the redox reactions at the Pt/Ca-Alg electrode, proving that the formation of the oleyl amide bond through EDC coupling is necessary to block \( \text{Fe(CN)}_6^{3/4-} \) redox activity. Figure 7.3b displays the CVs for this control experiment, showing reversible behavior with a \( \Delta E_p, i_{pc}, i_{pa}, D_c, \) and \( D_a \) of approximately 0.072 V, 9.6 \( \mu \)A, 9.8 \( \mu \)A, 8.1 \( \times \) \( 10^{-6} \) cm^2/s, and 8.4 \( \times \) \( 10^{-6} \) cm^2/s, respectively.

**Fe(CN)_6^{3-} redox reactions at Pt/Ca-Alg-tetradecyl amide and Pt/Ca-Alg-hexadecyl amide electrodes.** Figure 7.4a shows the electrochemical \( \text{Fe(CN)}_6^{3-} \) redox reactions at Pt/Ca-Alg-tetradecyl amide performed in a solution containing 4 mM \( \text{K}_3\text{Fe(CN)}_6 \) and 1 M KNO_3 at a scan rate of 0.01 V/s. Pt/Ca-Alg-tetradecyl amide electrodes were prepared by performing EDC coupling on electrophoretically deposited Ca-Alg on a 2 mm Pt disk electrode using tetradecyl amine. After soaking in the electrolyte, CV scans were performed at various times, and it was noticed that \( \text{Fe(CN)}_6^{3-} \) diffused into the Ca-Alg hydrogels modified with tetradecyl amide.
Figure 7.2. (a) Pt/Ca-Alg-oleyl amide, inset shows an oleyl chain formed at the outer hydrogel surface. (b) Possible orientation of the double bond of the oleyl chain towards the Pt electrode, leading to the blocking of the Pt electrode to electron transfer with the Fe(CN)$_6^{3-/4-}$ redox probe.
Figure 7.3 (a) Electrochemical redox reactions of Fe(CN)$_6^{3-}$ on Pt/Ca-Alg-oleyl amide electrode (with EDC coupling) in a 4 mM K$_3$Fe(CN)$_6$ + 1 M KNO$_3$ solution at a scan rate of 0.01 V/s and different soaking times. (b) Control reactions on Pt/Ca-Alg-oleyl amine electrode (no EDC coupling) in a solution of 1 M KNO$_3$ at a scan rate of 0.01 V/s and different times. Pt/Ca-Alg is prepared by performing EPD on a 2 mm diameter Pt disk electrode at 1.4 V for 30 min, followed by 90 mM Ca$^{2+}$ exposure for 30 min. The EPD solution contained 20 mL 1% (w/v) Na-Alg solution, 5 mL H$_2$O, and 5 mL 0.2 M HQ. The Ca-Alg-oleyl amide bond was created by performing EDC coupling.
However, the diffusion was slower than that at the bare Pt electrode (Figure 7.1a). For instance, after 3 min soaking in the electrolyte, the CV shows an absence of cathodic current but the beginning of anodic current at 5.5 μA, indicating the slow diffusion of Fe(CN)₆³⁻ to the hydrogel. The cathodic and anodic peaks also increase when the soaking time is increased to 6 and 15 min. For 6 min of soaking, i_{pc} and i_{pa} are at 8.2 μA and 9.4 μA, while for 15 min of soaking, they are at 12 μA and 12 μA, respectively. The peak splitting, ΔE_p, is ~70 mV, indicating that the redox reaction follows quasi-reversible kinetics. When the soaking time is above 30 min, the diffusion of Fe(CN)₆³⁻ to hydrogels is equilibrating since the peak currents remain stable with a further increase in soaking time. The i_{pc}, i_{pa}, D_c, and D_a are at 14 μA, 14 μA, 2.6 x 10⁻⁶ cm²/s, and 2.8 x 10⁻⁶ cm²/s, respectively. The electrochemical Fe(CN)₆³⁻ redox reaction shows quasi-reversible kinetics with ΔE_p ~ 69 mV. If the amide is modified to a lengthy alkyl chain devoid of any functional groups, the diffusion of Fe(CN)₆³⁻ is slowed down without experiencing the full obstruction observed with the oleyl amide-linked Ca-Alg hydrogels. One probable explanation for this phenomenon could be the absence of a double bond that can coordinate with the Pt electrode and obstruct the redox reactions.

In Figure 7.4b, the modified Pt/Ca-Alg electrode undergoes reduction/oxidation of Fe(CN)₆³⁻/⁴⁺ when the alkyl amide bond on the hydrogel surface changes to a longer hexadecyl chain. The hexadecyl amide bond is formed by coupling hexadecyl amine with the carboxylic acids on the Ca-Alg hydrogel surface using the EDC coupling reaction. After soaking for 3 minutes in the electrolyte, the CV shows no cathodic current and a weak anodic current of 2.3 μA, which is consistent with slow Fe(CN)₆³⁻ diffusion through Ca-Alg, as previously discussed. When the soaking time exceeds 6 minutes, the redox behavior becomes constant with high peak splitting (ΔE_p > 70 mV), indicating that the reaction kinetics are not reversible. This could be because the hexadecyl chain is sterically rigid and causing high resistance to ion transfer through the hydrogel or directly blocking electron transfer between the Fe(CN)₆³⁻ and Pt electrode surface.
Figure 7.4. (a) CV of Fe(CN)$_6^{3-}$ on a Pt/Ca-Alg-tetradecyl amide electrode in a 4 mM K$_3$Fe(CN)$_6$ + 1 M KNO$_3$ solution at a scan rate of 0.01 V/s and different soaking/scanning times. (b) CV of Fe(CN)$_6^{3-}$ on a Pt/Ca-Alg-hexadecyl amide electrode in a 4 mM K$_3$Fe(CN)$_6$ + 1 M KNO$_3$ solution at a scan rate of 0.01 V/s and different times.
Fe(CN)$_6^{3-}$ redox reactions at Pt/Ca-Alg-oleyl amide electrodes after soaking in water and hexadecane. We also investigated whether soaking Pt/Ca-Alg-oleyl amide electrodes in water for 15 or 30 min can unblock Fe(CN)$_6^{3-}$ diffusion to the hydrogel by oleyl amide groups and enhance the redox activity of Fe(CN)$_6^{3-}$ with the Pt. First, Pt/Ca-Alg-oleyl amide electrodes were prepared using the previously mentioned methods. Then, the modified electrodes were soaked in nanopure water for 15 minutes (Figure 7.5a) and 30 minutes (Figure 7.5b) before performing CV in 4 mM K$_3$Fe(CN)$_6$ and 1 M KNO$_3$ at a scan rate of 0.01 V/s. The Fe(CN)$_6^{3-}$ could diffuse slowly through the hydrogel after being soaked in water, suggesting that the oleyl chains were partially unblocked. However, the redox activity of Fe(CN)$_6^{3-}$ still exhibits low currents and irreversible kinetics. The CVs also showed that as the soaking time in electrolytes increased, oleyl chains eventually blocked the redox activity of Fe(CN)$_6^{3-}$ due to a lack of redox species diffusion through the hydrogels. This suggests that oleyl chains were only partially unblocked briefly when the modified electrodes were soaked in nanopure water. The mechanism of this process is unclear at this time.

When Pt/Ca-Alg-oleyl amide electrodes are soaked in hexadecane for 15 min, as shown in Figure 7.5c, the Fe(CN)$_6^{3-}$ redox activity appears with successive CV scans. After 3 min of soaking, the first scan resulted in the absence of cathodic current and anodic current with $i_{pa}$ at 2.8 $\mu$A, indicating the slow diffusion of Fe(CN)$_6^{3-}$ through the hydrogel. As the soaking time increased to 6 min, the cathodic current started to emerge with $i_{pc}$ at 5.5 $\mu$A and $D_c$ at 4.2 x 10$^{-5}$ cm$^2$/s, followed by the anodic current with $i_{pa}$ of 6.6 $\mu$A and $D_a$ at 6.1 x 10$^{-5}$ cm$^2$/s. The soaking/scanning times in electrolytes higher than 15 min led to $i_{pc}$ at $\sim$10 $\mu$A, $i_{pa}$ at $\sim$9 $\mu$A, $D_c$ at 16 x 10$^{-5}$ cm$^2$/s, and $D_a$ at 13 x 10$^{-5}$ cm$^2$/s. Interestingly, the Fe(CN)$_6^{3-}$ electrochemistry follows irreversible kinetics with $\Delta E_p$ $\sim$140 mV. It is not clear why soaking in hexadecane leads to this type of electrochemical behavior.
Figure 7.5. CV of Fe(CN)$_6^{3-}$ on Pt/Ca-Alg-oleyl amide electrode in a 4 mM K$_3$Fe(CN)$_6$ + 1 M KNO$_3$ solution at a scan rate of 0.01 V/s and different soaking times after soaking in water for (a) 15 min and (b) 30 min, and (c) hexadecane for 15 min.
Fe(CN)$_6^{3-}$ redox reactions at Au, Au/Alg, and Au/Alg-oleyl amide electrodes. Figure 7.6a shows the CVs of Fe(CN)$_6^{3-}$ at a bare 2 mm diameter Au disk electrode in an electrolyte solution containing 4 mM K$_3$Fe(CN)$_6$ and 1 M KNO$_3$ at 0.01 V/s. The forward and backward scans lead to $i_{pc}$ at 3.5 $\mu$A, $D_c$ at $1.1 \times 10^{-6}$ cm$^2$/s, $i_{pa}$ at 3.7 $\mu$A, and $D_a$ at $1.2 \times 10^{-6}$ cm$^2$/s with $\Delta E_p$ ~61 mV, indicating reversible kinetics. When Ca-Alg is electrophoretically deposited onto the bare Au disk electrode (Figure 7.6b), the first CV, after soaking in 4 mM K$_3$Fe(CN)$_6$ and 1 M KNO$_3$ at 0.01 V/s, led to cathodic currents and anodic currents less than that observed at the bare Au electrode (Figure 7.6a) with $i_{pa}$ of 1.9 $\mu$A, $D_a$ at $0.31 \times 10^{-6}$ cm$^2$/s, $i_{pc}$ of 2.7 $\mu$A, and $D_c$ at 0.67 x $10^{-6}$ cm$^2$/s. The $\Delta E_p$ is ~127 mV, showing irreversible reaction kinetics or resistance to ion transfer. The higher peak currents for Au/Ca-Alg compared to bare Au compared to Pt/Ca-Alg compared to bare Pt suggest that electron transfer or ion transfer resistance is higher at the Au electrode. The cathodic and anodic currents increase as the soaking time in the electrolyte increases to 6 and 15 min, eventually leading to reversible kinetics ($\Delta E_p$ = 62 mV). The $i_{pc}$, $i_{pa}$, $D_c$, and $D_a$ after 6 min are 2.4 $\mu$A, 2.8 $\mu$A, 0.52 x $10^{-6}$ cm$^2$/s, and 0.67 x $10^{-6}$ cm$^2$/s, respectively, and after 15 min soaking, they are 3 $\mu$A, 3.1 $\mu$A, 0.80 x $10^{-6}$ cm$^2$/s, and 0.86 x $10^{-6}$ cm$^2$/s, respectively. Au/Ca-Alg electrodes after soaking in the electrolyte solution containing 4 mM Fe(CN)$_6^{3-}$ and 1 M KNO$_3$ for 30 min and above lead to $i_{pc}$, $i_{pa}$, $D_c$, and $D_a$ of ~3.2 $\mu$A, 3.2 $\mu$A, 0.89 x $10^{-6}$ cm$^2$/s, and 0.93 x $10^{-6}$ cm$^2$/s, respectively, with reversible kinetics ($\Delta E_p$ ~60 mV).

The electrodeposited Au/Ca-Alg was modified by performing EDC coupling with oleyl amine to form oleyl amide bonds with the carboxylic acids present at the outer surface of the Ca-Alg hydrogels. Figure 7.6c shows the CV of Au/Ca-Alg-oleyl amide in 4 mM K$_3$Fe(CN)$_6$ and 1 M KNO$_3$ at 0.01 V/s after soaking at different times. After soaking for 3 min, the forward scan led to no cathodic current, while the backward scan led to a weak anodic current, indicating the slow diffusion of Fe(CN)$_6^{3-}$ through the hydrogel towards the electrode surface. After 6 min of soaking, the Fe(CN)$_6^{3-}$ diffusion through the hydrogels improves, resulting in higher cathodic and
Figure 7.6. Electrochemical redox reactions of Fe(CN)$_6^{3-}$ on (a) Au, (b) Au/Ca-Alg, and (c) Au/Ca-Alg-oleyl amide electrodes in a solution of 4 mM K$_3$Fe(CN)$_6$ + 1 M KNO$_3$ at a scan rate of 0.01 V/s and different soaking times. Au/Ca-Alg was prepared by performing EPD on a 2 mm diameter Au disk electrode at 1.4 V for 30 min, followed by 90 mM Ca$^{2+}$ exposure for 30 min. The EPD solution contained 20 mL 1% (w/v) Na-Alg solution, 5 mL H$_2$O, and 5 mL 0.2 M HQ. The Ca-Alg-oleyl amide bond was then created by performing EDC coupling.
anodic currents even though the kinetics of the process are irreversible. However, the currents start to decline at soaking times higher than 15 min, indicating the hindrance of Fe(CN)$_6^{3-}$ diffusion through the hydrogel or resistance to electron transfer. This suggests that the unsaturated oleyl group binding the outer surface of the hydrogel is blocking the diffusion of the redox species or blocking electron transfer to Fe(CN)$_6^{3/-4-}$ by coordinating with the Au electrode. Alternatively, the oleyl amide bond alters the ionic conductivity of the Ca-Alg. This is evident from a lack of cathodic and anodic currents at higher soaking times in electrolyte solutions, as shown in Figure 7.6c. Restructuring of the film could lead to a hydrophobic interior and hydrophilic exterior, for example.

**Fe(CN)$_6^{3-}$ redox reactions at Carbon, Carbon/Ca-Alg, and Carbon/Ca-Alg-oleyl amide electrodes.** CVs of Fe(CN)$_6^{3-}$ were obtained on carbon, carbon/Ca-Alg, and carbon/Ca-Alg-oleyl amide electrodes. Figure 7.7a displays the CV of a bare 3 mm diameter carbon disk electrode in a solution containing 4 mM K$_3$Fe(CN)$_6$ and 1 M KNO$_3$ at a scan rate of 0.01 V/s from 0.6 V to -0.2 V. The CV has a cathodic and anodic wave with $i_{pc}$ at 7.3 μA, $i_{pa}$ at 7.5 μA, $D_c$ of 0.92 x 10$^{-6}$ cm$^2$/s, and $D_a$ of 0.97 x 10$^{-6}$ cm$^2$/s, respectively, and peak splitting $\Delta E_p$ of 65 mV, indicating fairly reversible kinetics. The CVs of carbon/Ca-Alg electrodes at different times after soaking in the electrolyte are shown in Figure 7.7b. The first CV scan resulted in $i_{p,c}$ of 6.8 μA, $i_{p,a}$ of 7.8 μA, $D_c$ of 0.81 x 10$^{-6}$ cm$^2$/s, and $D_a$ of 1.0 x 10$^{-6}$ cm$^2$/s, respectively. At the same time, the $\Delta E_p$ was ~102 mV, indicating irreversible kinetics or high mass transfer resistance. When the CVs were measured after 6 min soaking, $i_{p,c}$, $i_{p,a}$, $D_c$, and $D_a$ were nearly the same at ~8.6 μA, 8.0 μA, 1.3 x 10$^{-6}$ cm$^2$/s, and 1.1 x 10$^{-6}$ cm$^2$/s, respectively. Moreover, $\Delta E_p$ decreased to ~70 mV, signifying more reversible kinetics for the Fe(CN)$_6^{3/-4-}$ redox reaction. This suggests that from the second scan onwards, the diffusion of Fe(CN)$_6^{3-}$ through the hydrogel is favorable and exhibits consistent peak currents and peak splitting. One possible reason for this behavior is the
electrodeposition of a thin hydrogel film at the carbon electrode due to the decreased conductivity of carbon electrodes compared to the metal disk electrodes.

As discussed, we modified the carbon/Ca-Alg electrode through EDC coupling with oleyl amine to create carbon/Ca-Alg-oleyl amide. After soaking the carbon/Ca-Alg-oleyl amide electrode in a solution of 4 mM K₃Fe(CN)₆ and 1 M KNO₃, we conducted CV scans at various times (3, 6, 15, 30, and 45 min) from 0.6 V to -0.2 V, as shown in Figure 7.7c. The CVs show that after 3 minutes of soaking, little current was detected in the forward scan, while an anodic current with \( i_{pa} \) of 1.6 \( \mu \)A was observed in the backward scan, indicating slow diffusion of Fe(CN)₆³⁻ through the hydrogel. As soaking time increased to 6 and 15 minutes, both cathodic and anodic currents were detected at the carbon/Ca-Alg-oleyl amide electrodes. At this point, \( \Delta E_p \) was about 100 mV, suggesting the redox reaction was irreversible. Specifically, after 6 minutes of soaking, \( i_{pc}, i_{pa}, D_c, \) and \( D_a \) were 3.4 \( \mu \)A, 5.0 \( \mu \)A, 0.20 x 10⁻⁶ cm²/s, and 0.43 x 10⁻⁶ cm²/s, respectively, while after 15 minutes, \( i_{pc}, i_{pa}, D_c, \) and \( D_a \) were 8.0 \( \mu \)A, 9.6 \( \mu \)A, 1.2 x 10⁻⁶ cm²/s, and 1.6 x 10⁻⁶ cm²/s, respectively. When soaking time exceeded 30 minutes, both cathodic and anodic currents remained consistent with \( i_{pc}, i_{pa}, D_c, \) and \( D_a \) were 15 \( \mu \)A, 16 \( \mu \)A, 3.8 x 10⁻⁶ cm²/s, and 4.1 x 10⁻⁶ cm²/s, respectively, indicating that Fe(CN)₆³⁻ diffusion occurred through the hydrogel modified by the oleyl amide. This shows that carbon electrodes with Ca-Alg-oleyl amide do not completely block the redox reaction at the surface. While the peak splitting (\( \Delta E_p \)) of 154 mV indicated irreversible electron transfer kinetics, the high peak currents show that the electron transfer could be activated and that the Fe(CN)₆³⁻/⁴⁻ mass transfer through the hydrogel and to the carbon surface is not impeded. The more exposed electrode surface could be due to weak interactions between the unsaturated bond of oleyl amide and glassy carbon. Therefore, we can conclude that the blocking and unblocking of Fe(CN)₆³⁻ redox reaction at the electrode is affected by a combination of the interaction between the electrode and the oleyl group, as well as the steric
Figure 7.7. CVs of Fe(CN)$_6^{3-}$ on (a) Carbon, (b) Carbon/Ca-Alg, and (c) Carbon/Ca-Alg-oleyl amide electrodes in a solution of 4 mM K$_3$Fe(CN)$_6$ + 1 M KNO$_3$ at a scan rate of 0.01 V/s and different electrolyte soaking times. Au/Ca-Alg was prepared by performing EPD on a 3 mm diameter carbon disk electrode at 1.4 V for 30 min, followed by 90 mM Ca$^{2+}$ exposure for 30 min. The EPD solution contained 20 mL 1% (w/v) Na-Alg solution, 5 mL H$_2$O, and 5 mL 0.2 M HQ. The Ca-Alg-oleyl amide bond was created by performing EDC coupling.
hindrance of the alkyl chain attached to the hydrogel, decreasing mass transport and potentially ion transport.

**Fe(CN)$_6^{3-}$ redox reactions at Au/Ca-Alg-4 nm Au NPs electrodes.** We also investigated whether the presence of Au NPs can improve the electrochemistry of Fe(CN)$_6^{3-}$ with Au electrode. Figure 7.8a shows the CV of Au/Ca-Alg-4 nm Au NP electrodes prepared with different concentrations of 4 nm Au NPs (0, 5, 8.3, 25, 50, 75, 90 μM) in a solution containing 1 mM K$_3$Fe(CN)$_6$ and 1 M KNO$_3$ after 30 min soaking/scanning at a scan rate of 0.01 V/s from 0.5 V to -0.1 V. In the absence of Au NPs, the CV shows cathodic and anodic waves with $i_{pc}$ at 1.6 μA, $D_c$ of 3.4 x 10$^{-6}$ cm$^2$/s, and $i_{pa}$ at 1.7 μA, $D_a$ of 3.8 x 10$^{-6}$ cm$^2$/s, respectively, and peak splitting $\Delta E_p$ of 62 mV, indicating reversible kinetics. When the 4 nm Au NPs were introduced using 5 μM concentration of the solution, the CV showed somewhat lower cathodic and anodic waves with $i_{pc}$, $i_{pa}$, $D_c$, and $D_a$ were 1.4 μA, 1.6 μA, 2.8 x 10$^{-6}$ cm$^2$/s, and 3.5 x 10$^{-6}$ cm$^2$/s, respectively, with fairly reversible kinetics with $\Delta E_p$ of 65 mV. However, when the concentrations of Au NP in hydrogel increased, the corresponding CV showed an increase in cathodic and anodic currents along with an increase in the calculated diffusion coefficients while the electron transfer kinetics remained reversible due to $\Delta E_p$ of ~65 mV. This is highlighted in the Table 6.2. A possible reason for the increase in redox activity of Fe(CN)$_6^{3-}$ can be attributed to the presence of a larger number of electrodeposited Au NPs at the Au disk electrode. When the concentration of Au NPs increases at the electrode, it consequently increases the electroactive SA of the electrode. This led to an increase in the Fe(CN)$_6^{3-}$ redox reactions. This is illustrated in Figure 7.8b.

<table>
<thead>
<tr>
<th>Concentration of 4 nm Au NP (μM)</th>
<th>$i_{pc}$ (μA)</th>
<th>$D_c$ (cm$^2$/s)</th>
<th>$i_{pa}$ (μA)</th>
<th>$D_a$ (cm$^2$/s)</th>
<th>$\Delta E_p$ (V)</th>
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<tr>
<td>0</td>
<td>1.7</td>
<td>3.8 x 10$^{-6}$</td>
<td>1.6</td>
<td>3.4 x 10$^{-6}$</td>
<td>0.062</td>
</tr>
<tr>
<td>5</td>
<td>1.6</td>
<td>3.5 x 10$^{-6}$</td>
<td>1.4</td>
<td>2.8 x 10$^{-6}$</td>
<td>0.065</td>
</tr>
<tr>
<td>8.3</td>
<td>1.8</td>
<td>4.2 x 10$^{-6}$</td>
<td>1.5</td>
<td>3.4 x 10$^{-6}$</td>
<td>0.065</td>
</tr>
<tr>
<td>25</td>
<td>1.9</td>
<td>4.8 x 10$^{-6}$</td>
<td>1.6</td>
<td>3.7 x 10$^{-6}$</td>
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<td>5.2 x 10$^{-6}$</td>
<td>1.7</td>
<td>3.8 x 10$^{-6}$</td>
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</tr>
<tr>
<td>75</td>
<td>2.1</td>
<td>6.0 x 10$^{-6}$</td>
<td>2.0</td>
<td>5.5 x 10$^{-6}$</td>
<td>0.065</td>
</tr>
</tbody>
</table>
When the concentration of 4 nm Au NPs was increased further to 100 μM, as shown in Figure 7.8c, the CV curves initially showed high anodic and cathodic currents that declined as the soaking/scanning time increased. This behavior is consistent when the concentration of Au NPs is increased to 120 μM, as shown in Figure 7.8d. This indicates that at high concentrations of Au NPs, the Fe(CN)$_6^{3-}$ mass transfer is affected by the presence of Au NPs. This is possibly due to the formation of high-density hydrogel films that passivate the electrode surface. In addition to that, the high concentrations of Au NP embedded within the hydrogel slowed the diffusion of Fe(CN)$_6^{3-}$ within the hydrogel. This blocked Fe(CN)$_6^{3-}$ to reach the electrode, leading to the lack of reversible redox chemistry of Fe(CN)$_6^{3-}$. Figure 7.8e shows a picture of Au/Ca-Alg-4 nm Au NPs prepared using 120 μM of Au NPs that shows a thick blue film confirming the presence of a hydrogel film with a high density of Alg and 4 nm Au NPs.

**Fe(CN)$_6^{3-}$ redox reactions at Au/Ca-Alg electrodes in the presence of CaCl$_2$.2H$_2$O.**

The effect of CaCl$_2$.2H$_2$O toward Fe(CN)$_6^{3-}$ redox reactions on Au/Ca-Alg is investigated in Figure 7.9. Here, different concentrations (0, 50, 100 mM) of CaCl$_2$.2H$_2$O were introduced in the 1 mM K$_3$Fe(CN)$_6$ and 1 M KNO$_3$ before running the CV. The CV shows similar anodic and cathodic waves with $i_{pa}$ at 1.1 μA and $i_{pc}$ at 2.0 μA, irrespective of the concentration of CaCl$_2$.2H$_2$O. The calculated diffusion coefficients were also similar with $D_a$ and $D_c$ at 4.3 x 10$^{-6}$ cm$^2$/s and 5.7 x 10$^{-6}$ cm$^2$/s, respectively. The $\Delta E_p$ is 66 mV, indicating a fairly reversible kinetics. The results indicate that the Ca$^{2+}$ ions that can crosslink within the hydrogel don’t have any impact on the redox activities of Fe(CN)$_6^{3-}$.

**Table 7.2.** Peak currents and Diffusion coefficients associated with Au/Ca-Alg electrodes at different concentrations of 4 nm Au NPs.

<table>
<thead>
<tr>
<th>Concentration (μM)</th>
<th>$i_{pa}$ (μA)</th>
<th>$i_{pc}$ (μA)</th>
<th>$D_a$ (cm$^2$/s)</th>
<th>$D_c$ (cm$^2$/s)</th>
<th>$\Delta E_p$ (mV)</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>1.1</td>
<td>2.0</td>
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Figure 7.8. Electrochemical redox reactions of \( \text{Fe(CN)}_6^{3-} \) on Au/Ca-Alg-4 nm Au NP electrodes prepared using (a) 0 to 90 mM 4 nm Au NPs in EPD solution at 30 min of soaking/scanning. (b) A possible mechanism that shows how Au NPs increase the electroactive surface area. (c) 100 mM and (d) 120 mM 4 nm Au NPs in EPD solution at different soaking/scanning. The CV was performed in a solution of 1 mM \( K_3\text{Fe(CN)}_6 + 1 \) M KNO\(_3\) at a scan rate of 0.01 V/s. (e) A picture of Ca-Alg-4 nm Au NP prepared using 140 mM 4 nm Au NP in EPD solution.
Figure 7.9. Electrochemical redox reactions of Fe(CN)$_6^{3-}$ on Au/Ca-Alg electrodes at 30 min of soaking/scanning in a solution of 1 mM K$_3$Fe(CN)$_6$, 1 M KNO$_3$, and 0, 50, and 100 mM CaCl$_2$.2H$_2$O at a scan rate of 0.01 V/s.
Fe(CN)$_6^{3-}$ redox reactions using Ca-Alg-Fe(CN)$_6^{3-}$-KNO$_3$ pipette electrode. This section delves into exploring Fe(CN)$_6^{3-}$ redox reactions in a confined environment. Figure 7.10a shows a setup with Ca-Alg-Fe(CN)$_6^{3-}$-KNO$_3$ pipette electrode as a reference and counter electrode and glass/ITO as the working electrode where the pipette electrode creates the spatially confined regions. The Ca-Alg-Fe(CN)$_6^{3-}$-KNO$_3$ pipette electrode was prepared by fitting an Ag/AgCl electrode with a regular Pasteur pipette (tip diameter = 0.55 mm) followed by filling it with 1 % Alg + 4 mM K$_3$Fe(CN)$_6$ + 1 M KNO$_3$ while soaking in a solution containing 90 mM CaCl$_2$.2H$_2$O + 4 mM K$_3$Fe(CN)$_6$ + 1 M KNO$_3$. The setup was secured with a parafilm. The CV, in Figure 7.10b, shows a cathodic and anodic wave with $i_{pa}$ at 8.0 $\mu$A and $i_{pc}$ at 8.1 $\mu$A, respectively. The $\Delta E_p$ is ~ 450 mV, indicating an irreversible reaction kinetics. This is possible due to the ion transfer resistance through the hydrogel formed inside the pipette affecting the diffusion of Fe(CN)$_6^{3-}$ to the glass/ITO surface.

Fe(CN)$_6^{3-}$ redox reactions using Pt/Ir/Ca-Alg electrode. Here, the confined environment is created by electrodepositing Alg to a Pt/Ir STM tip, followed by soaking in a solution of 4 mM K$_3$Fe(CN)$_6$ and 1 M KNO$_3$ for 15 min before performing a CV scan. An illustration of the electrode setup is shown in Figure 7.11a, and the corresponding CV scan is shown in Figure 7.11b. The CV shows a cathodic and an anodic wave at $i_{pa}$ at 0.17 $\mu$A and $i_{pc}$ at 0.16 $\mu$A, respectively. The $\Delta E_p$ is ~64 mV, indicating a reversible reaction kinetics. Hence, it can be concluded that the Pt/Ir/ Alg containing Fe(CN)$_6^{3-}$ and KNO$_3$ electrode can be used to generate spatially confined areas to perform redox reactions.
Figure 7.10. (a) An electrode set up with Ca-Alg-Fe(CN)$_6^{3-}$-KNO$_3$ pipette electrode as a reference and counter electrode and glass/ITO as the working electrode. (b) Electrochemical redox reactions of Fe(CN)$_6^{3-}$ using the setup. Ca-Alg-Fe(CN)$_6^{3-}$-KNO$_3$ pipette electrode was prepared by fitting an Ag/AgCl electrode with a regular Pasteur pipette (tip diameter = 0.55 mm) followed by filling it with 1 % Alg + 4 mM K$_3$Fe(CN)$_6$ + 1 M KNO$_3$ while soaking in a solution containing 90 mM CaCl$_2$.2H$_2$O + 4 mM K$_3$Fe(CN)$_6$ + 1 M KNO$_3$. The setup was secured with a parafilm.
Figure 7.11. (a) A scheme showing an electrode set up with Pt/Ir/Ca-Alg containing Fe(CN)$_6^{3-}$ and KNO$_3$ electrode as a reference and counter electrode and glass/ITO as the working electrode. (b) Electrochemical redox reactions of Fe(CN)$_6^{3-}$ using the setup. Pt/Ir/Ca-Alg was prepared by performing EPD on a Pt/Ir STM tip at 1.4 V for 30 min, followed by 90 mM Ca$^{2+}$ exposure for 30 min. The redox species are populated by exposing the Pt/Ir/Ca-Alg to a solution of 4 mM K$_3$Fe(CN)$_6$ + 1 M KNO$_3$. The EPD solution contained 20 mL 1% (w/v) Na-Alg solution, 5 mL H$_2$O, and 5 mL 0.2 M HQ.
7.3. CONCLUSIONS

Chapter 7 explores the oxidation/reduction of Fe(CN)$_6^{3/-4-}$ on Pt, Au, and glassy carbon electrodes coated with Ca-Alg hydrogels through EPD. The CV data for the Fe(CN)$_6^{3-}$ redox reactions at Pt/Ca-Alg electrodes show the presence of Ca-Alg slows down Fe(CN)$_6^{3-}$ diffusion to the electrode surface, leading to reduced cathodic and anodic currents in subsequent scans relative to the same reaction in water. The hydrogels are modified to control mass transport using alkylamines via EDC amide coupling reactions. When Ca-Alg was modified with oleyl amide bonds, Fe(CN)$_6^{3-}$ redox activity was nearly completely blocked, possibly due to blocking of Fe(CN)$_6^{3-}$ mass transfer or blocking of electron transfer by alkene coordination with the Pt electrode. The modifications with tetradecyl and hexadecyl amides reveal slower Fe(CN)$_6^{3-}$ diffusion and irreversible kinetics, possibly attributed to steric hindrance or rigid chains affecting ion transfer. Soaking in water or hexadecane partially unblocks the redox activity, demonstrating the dynamic nature of the interaction.

Similar experiments are conducted on Au and glassy carbon electrodes, highlighting the impact of electrode material on Fe(CN)$_6^{3-}$ redox reactions. The CV of Au/Ca-Alg-oleyl amide electrodes exhibited time-dependent behavior, indicating the influence of the oleyl amide modification. While short soaking times showed slow Fe(CN)$_6^{3-}$ diffusion, longer soaking times resulted in declining currents, suggesting hindered diffusion or electron transfer, possibly due to the oleyl group blocking redox species diffusion or coordinating with the Au electrode. The CVs of the bare carbon electrode showed reversible kinetics, but the introduction of Ca-Alg led to irreversible kinetics, potentially due to the formation of a thin hydrogel film hindering mass transfer. EDC coupling with oleyl amine on carbon/Ca-Alg electrodes resulted in carbon/Ca-Alg-oleyl amide electrodes exhibiting time-dependent CV behavior. While short soaking times indicated slow Fe(CN)$_6^{3-}$ diffusion, longer times showed consistent currents, suggesting that the oleyl amide modification allowed Fe(CN)$_6^{3-}$ diffusion through the hydrogel without complete
blockage of the redox reaction. Finally, the incorporation of 4 nm Au nanoparticles on Au/Ca-Alg electrodes enhances Fe(CN)$_6^{3-}$ redox activity, with increased currents and diffusion coefficients as the nanoparticle concentration rises. This suggests a correlation between electrodeposited Au NPs and improved electrochemical behavior, possibly due to an increased electroactive surface area. However, the presence of different concentrations of CaCl$_2$.2H$_2$O in the electrolyte did not affect the Fe(CN)$_6^{3-}$ redox activity.

In the exploration of Fe(CN)$_6^{3-}$ redox reactions within confined environments, two distinct electrode setups were investigated. The Ca-Alg-Fe(CN)$_6^{3-}$-KNO$_3$ pipette electrode, featuring a spatially confined region, exhibited an irreversible reaction kinetics. This observation is attributed to ion transfer resistance through the hydrogel formed inside the pipette, influencing the diffusion of Fe(CN)$_6^{3-}$ to the glass/ITO surface. In contrast, the Pt/Ir/Alg electrode containing Fe(CN)$_6^{3-}$ and KNO$_3$, designed to create a confined environment by electrodepositing Alg onto a Pt/Ir STM tip, demonstrated a reversible reaction kinetics with a $\Delta E_p$ of approximately 64 mV. The capability of this electrode to generate spatially confined areas for redox reactions suggests its potential utility in controlled electrochemical processes.

This chapter provides valuable insights into the complex interplay of hydrogel modifications, electrode materials, and nanoparticle effects on redox reactions, contributing to the understanding of electrochemical processes at modified electrodes. These results shed light on how these reactions are influenced by the properties of the hydrogel and the effect of hydrogel modification with alkyl chains, which could serve as a potential mimic of biological cells and membranes. These preliminary results offer some valuable insight, but much more work is needed for applications in electrochemical sensing, bio-electrochemical technology, and energy storage.
CHAPTER 8: ELECTROCHEMICAL REACTIONS IN CALCIUM ALGINATE HYDROGELS IN BULK AND SPATIALLY CONFINED ENVIRONMENTS

8.1. INTRODUCTIONS

Hydrogels are materials composed of cross-linked polymers, which form gel structures that can absorb and retain water. These materials exhibit a wide range of structural and chemical properties, making them suitable for various applications as sensors and electrode materials. Hydrogels provide advantages to electrode materials by reducing the effects of natural convection in mass transport and allowing for the incorporation of nanomaterials into its structure for sensor applications. They have been shown to enable the diffusion of small ions, complexes, and various small nanostructures. When combined with miniature electrodes, hydrogels offer electrode setups that can investigate localized electrochemical events by voltammetry and potentiometry. Several studies have reported the use of electrodes modified with spatially confined gel layers in different electrochemical and electrocatalytic applications. For instance, Belmont-Hebert et al. developed a gel-integrated mercury-plated, iridium-based microelectrode array. The array can measure the concentration of lead and cadmium in natural water by using square-wave ASV. The agarose hydrogel that was used had a thickness of 275 µm, and it was able to detect Pb(II) and Cd(II) at concentrations as low as 50 pM. The agarose gel helped maintain the reproducibility of diffusion of the species, and the permeation of ions was completed in a few minutes.
To establish spatial confinement, there have been several methods reported that involve micropipette probes and microelectrodes integrated with hydrogels.\textsuperscript{214-216} Both micropipettes and microelectrodes offer localized detection of electrochemical events. For instance, Liu \textit{et al.} fabricated a gel probe to electrochemically map the topography and electrochemical activity of a protective coating on steel with a scratch and copper tape on aluminum alloy.\textsuperscript{217} The gel probe was prepared by electrodeposition of chitosan with gelatin on a Pt microelectrode. Dang \textit{et al.} developed an Ag/AgCl-gel micro-reference electrode to potentiometrically map the topography and the electrochemical potential of Cu tapes on Al plates.\textsuperscript{218} The probes were prepared by the electrodeposition of chitosan hydrogel on Ag micro-disk electrodes followed by Ag chlorination. Donnici and Daniele utilized micropipettes filled with a hydrogel composed of 2\% (w/v) agarose in water solutions to carry out voltammetric measurements of [Fe(CN)\textsubscript{6}]\textsuperscript{4-} and Ag\textsuperscript{+} ions confined within a limited contact region.\textsuperscript{219} They conducted a hydrogel capillary measurement to differentiate the presence of metallic silver deposited on a graphite-on-paper-based material.

In this chapter, we extend the range of applications of Ca-Alg hydrogels to include electrochemical processes such as ASV and electrochemical deposition (ECD) in a spatially restricted environment. The study delves into the impact of Ca-Alg on the ASV of Au NPs on glass/ITO surfaces, thus exploring the size-dependent stripping behavior of Au NPs. We have also extensively investigated Au ECD within confined Ca-Alg on glass/ITO electrodes to achieve microscale Au ECD. Additionally, we explored the feasibility of using Ca-Alg hydrogels on different electrodes, including pipettes, for spatially confined ASV of Au NPs as well as ECD of Au.

\section{RESULTS AND DISCUSSION}

\textbf{ASV of glass/ITO/AuNPs/Ca-Alg in bulk environment.} Figure 8.1 compares the effect of Ca-Alg on the stripping of 4 and 15 nm diameter Au NPs from a glass/ITO electrode. The
experiment was performed by co-depositing Alg and Au NPs on glass/ITO by EPD, then crosslinking with Ca$^{2+}$, and finally conducting ASV of the electrodes in 10 mM KBr plus 0.1 M KClO$_4$ at a scan rate of 10 mV/s using a 3-electrode set-up, which includes an Ag/AgCl reference electrode and Pt wire counter electrode. The ASVs of glass/ITO/Au$_{4\text{nm}}$ in the presence (red dotted line) and absence (red solid line) of Ca-Alg shows that Ca-Alg causes a positive shift in the peak potential for oxidation ($E_{p,\text{ox}}$), decrease in peak current for oxidation ($i_{p,\text{ox}}$), and broadened the peak significantly. The electrodes were prepared by performing EPD at 1 V for 5 min in a solution containing 16.7 mM HQ and 8.3 $\mu$M 4 nm Au NPs in the presence and absence of Alg (0.5 % w/v). A $i_{p,\text{ox}}$ of $\sim$3.18 $\mu$A is observed with an $E_{p,\text{ox}}$ at 0.723 V when the electrode is prepared without Ca-Alg. However, in the presence of the Ca-Alg, the stripping peaks were broad, with the $i_{p,\text{ox}}$ significantly lower at 1.77 $\mu$A with a positive shift in $E_{p,\text{ox}}$ at 0.801 V. This shows that in the Ca-Alg hydrogel film, the amount of Au NPs deposited at the electrode surface is lower than that in the absence of Alg and there is continuous Au NP oxidation as indicated by the diffusional tale on the stripping peak, especially for 4 nm Au NPs. This can be attributed to the ability of Alg to trap Au NPs within itself. Hence, the NP's migrating towards the glass/ITO during EPD become trapped within the growing hydrogel layer on the electrode, which decreases the population of Au NPs directly attached onto the glass/ITO surface. Additionally, this shows that the Ca-Alg allows ions, such as Br$^-$, to diffuse through and perform anodic stripping reactions at the electrode surface. It is important for the Ca-Alg to act as a hydrogel electrolyte for various electrochemical reactions to increase potential applications. Similar trends were observed when the size of the Au NPs was increased to 15 nm diameter (blue plot). Here, the EPD was performed at 1.2 V for 30 s from a solution containing 16.7 mM HQ and 33.3 $\mu$M 15 nm Au NPs in the presence and absence of Alg (0.5 % w/v). In the presence of Ca-Alg, the ASV resulted in a broad peak with $i_{p,\text{ox}}$ of 2.19 $\mu$A and $E_{p,\text{ox}}$ at 0.971 V, while the absence of Ca-Alg led to a sharp peak with $i_{p,\text{ox}}$ equal to 2.36 $\mu$A and $E_{p,\text{ox}}$ of 0.809 V.
Figure 8.1. ASVs of glass/ITO/Au₄nm (red solid line) and glass/ITO/Au₄nm/Ca-Alg (red dotted line) at a scan rate of 10 mV/s in 10 mM KBr plus 0.1 M KClO₄. The 4 nm Au NPs were attached to glass/ITO by EPD at 1 V for 5 min in a solution containing 16.7 mM HQ and 8.3 mM 4 nm Au NPs in the presence and absence of Ca-Alg (0.5 % w/v). The electrodes with 15 nm Au NPs (blue plots) were prepared by EPD at 1.2 V for 30 s from a solution containing 16.7 mM HQ and 33.3 mM 15 nm Au NPs in the presence and absence of Alg (0.5 % w/v). Crosslinking was performed after EPD to form Ca-Alg-Au NP hybrid hydrogel films.
ASV of glass/ITO/AuNPs using Ca-Alg Hydrogels in Spatially Confined Environments. As mentioned in the previous chapter, the Ca-Alg hydrogels are used to perform electrochemical reactions in spatially-confined environments. This section explores the ASV reactions of Au NPs in spatially confined environments created by Ca-Alg hydrogels. The first example shows the spatially confined stripping of Au NPs at a glass/ITO electrode using Ca-Alg hydrogels formed at the tip of the Ag/AgCl reference electrode. Figure 8.2a shows the set-up containing the Ca-Alg hydrogel enriched with KBr and KClO₄ on an Ag/AgCl electrode that functioned as both reference and counter electrode touching the glass/ITO/Au₄nm NPs working electrode. The Alg was formed at the tip of the Ag/AgCl electrode by adding 0.2 mL CaCl₂.2H₂O to an Ag/AgCl electrode, followed by soaking it in 1 % (w/v) Na-Alg solution for 15 min. The as-prepared electrode was rinsed with 1 % NaCl and subsequently soaked in a solution of 10 mM KBr and 0.5 M KClO₄. Figure 8.2b shows the ASV of a glass/ITO/Au₄nm NPs using Ca-Alg hydrogels formed at an Ag/AgCl reference electrode enriched with KBr and KClO₄ at a scan rate of 1 mV/s. The glass/ITO/Au₄nm were prepared by performing EPD at 1 V for 5 min in a solution containing 16.7 mM HQ and 8.3 µM 4 nm Au NPs. A sharp peak with an i_{p,ox} of ~1.26 µA was observed with an E_{p,ox} of 0.688 V. The E_{p,ox} is consistent with the presence of 4 nm Au NPs in the stripped regions of the glass/ITO/Au NP electrode. The low i_{p,ox} is due to the stripping of Au NPs in a confined part of the electrode. A significant drawback with this method is that the Ca-Alg formed at the Ag/AgCl electrode leads to a fairly large contact area, limiting the spatial confinement of the process. Figure 8.2c shows an Au film that was stripped using the Ag/AgCl/Ca-Alg soaked in 10 mM KBr + 0.5 M KClO₄ electrolyte. The grey spot under the circle is the stripped area, which had an i_{p,ox} of 1.96 mA.

The ASV of Au NPs in a confined region of a glass/ITO/Au NP electrode was also performed using Ca-Alg hydrogel deposited on a 2 mm diameter Pt-disk electrode (Pt/Ca-Alg).
**Figure 8.2.** (a) A setup consisting of an Ag/AgCl/Ca-Alg electrode soaked in KBr + KClO₄ electrolyte that functions as both reference and counter electrode touching the glass/ITO/Au₄nm NPs working electrode. The Ag/AgCl/Ca-Alg electrode was formed by adding CaCl₂·2H₂O solution to the inside of the Ag/AgCl electrode and soaking it in 1% Na-Alg solution for 15 min. The electrode was then rinsed with 1% NaCl and soaked in 10 mM KBr with 0.5 M KClO₄ for 15 min. (b) ASV of a glass/ITO/Au₄nm electrode using the Ag/AgCl/Ca-Alg electrode at a scan rate of 1 mV/s. The glass/ITO/Au₄nm NPs were prepared by performing EPD at 1 V for 5 min in a solution containing 16.7 mM HQ and 8.3 μM 4 nm Au NPs. (c) A grey spot on a Au film electrode resulting from ASV of the Au with the Ag/AgCl/Ca-Alg electrode that was soaked in KBr + KClO₄ electrolyte.
Figure 8.3 shows the set-up with Pt/Ca-Alg soaked with KBr and KClO₄ functioning as the reference and counter electrode and a glass/ITO/Au₁₅nm NPs functioning as the working electrode. The spatially confined region was generated by lightly touching the hydrogel at the glass/ITO/Au NP electrode. The hydrogel reference electrode was prepared by performing EPD in a solution containing 0.5 % w/v Na-Alg and 33.4 mM HQ at 1.4 V for 30 min, followed by soaking in a solution of 90 mM ice-cold CaCl₂·2H₂O for 30 min. The hydrogel was enriched with KBr and KClO₄ by soaking in a solution of 10 mM KBr and 0.5 M KClO₄ for 15 min. The glass/ITO/Au₁₅nm electrode was prepared by performing EPD at 1.2 V for 30 s from a solution containing 16.7 mM HQ and 33.3 μM 15 nm Au NPs. Figure 8.3b shows the ASV of the Au NPs in a confined region of the glass/ITO/Au₁₅nm electrode formed at the contact with the Pt/Ca-Alg electrode at a scan rate of 1 mV/s. The ASV shows an overlap of two peaks at Eᵯ,ox values of 0.637 V and 0.802 V, with the associated iᵯ,ox at 0.301 μA and 0.371 μA, respectively. The two peaks can be attributed to two dominant populations of Au NPs with varying sizes, which are ~4 nm and 15 nm Au NPs. Moreover, the low stripping currents indicate the spatial confinement of the stripping process. Hence, the spatially confined ASV using Ca-Alg hydrogels can detect the size dispersity in Au NPs attached to a glass/ITO electrode. One of the drawbacks is the poor reusability of the Pt/Ca-Alg electrode for the ASV of Au NPs due to the drying of hydrogel during the process (after 10 min of scanning).

In order to overcome the spatial confinement and drying problems of hydrogel, a setup using a glass pipette and Ag/AgCl was used to perform ASV reactions at glass/ITO/Au NPs. Figure 8.4a shows the electrode setup containing an Ag/AgCl/Ca-Alg pipette electrode acting as the reference and counter electrode and a glass/ITO/Au NP working electrode. The preparation of Ag/AgCl/Ca-Alg pipette electrode is described in section 2.15. The glass/ITO/Au NPs electrode was prepared by performing EPD in a solution containing 41.7 μM of 4 nm and/or 15 nm Au NPs and 16.7 mM HQ at 1.2 V for 30 s. Figure 8.4b shows ASVs performed with glass/ITO electrodes
Figure 8.3. (a) A setup consisting of a Pt/Ca-Alg electrode soaked in KBr + KClO₄ electrolyte, which functions as both reference and counter electrode touching the glass/ITO/Au₁₅nm NPs working electrode. The Pt/Ca-Alg electrode was prepared by performing EPD on the Pt electrode in a solution containing 0.5 % (w/v) Na-Alg and 33.4 mM HQ at 1.4 V for 30 min, followed by soaking in a solution of 90 mM ice-cold CaCl₂.2H₂O for 30 min, and finally soaking in a solution of 10 mM KBr plus 0.5 M KClO₄ for 15 min. The glass/ITO/Au₁₅nm NP electrode was prepared by performing EPD at 1.2 V for 30 s from a solution containing 16.7 mM HQ and 33.3 mM 15 nm Au NPs. (b) ASV of glass/ITO/Au₁₅nm NP electrode obtained using the Pt/Ca-Alg electrode soaked in KBr + KClO₄ electrolyte at a scan rate of 1 mV/s.
Figure 8.4. (a) A setup containing Ag/AgCl/Ca-Alg pipette electrode that functions as both reference and counter electrode touching the glass/ITO/Au NP working electrode. The Ag/AgCl/Ca-Alg pipette electrode was prepared by dipping the pipette in a solution containing 90 mM CaCl$_2$·2H$_2$O, 10 mM KBr, and 0.1 M KClO$_4$, followed by the addition of a solution of 10 mM KBr, 0.1 M KClO$_4$ and 1 % (w/v) Na-Alg solution to the pipette and inserting the Ag/AgCl electrode into the pipette, and sealing with parafilm. The glass/ITO/Au NPs were prepared by performing EPD in a solution containing 41.7 µM of 4 nm and/or 15 nm Au NPs and 16.7 mM HQ at 1.2 V for 30 s. (b) ASV of glass/ITO electrode containing 4 nm (red), 15 nm (blue) and a mixture of both sized Au NPs (green) measured using the Ag/AgCl/Ca-Alg pipette electrode containing the KBr + KClO$_4$ electrolyte at a scan rate of 10 mV/s.
containing 4 nm and/or 15 nm Au NPs using the Ag/AgCl/Ca-Alg pipette electrode at a scan rate of 10 mV/s. When the glass/ITO was coated with 4 nm Au NPs (red plot), the ASV led to a $i_{p,ox}$ of 87.5 nA at a $E_{p,ox}$ of 0.708 V, which is consistent with the desired spatial confinement with area 0.093 cm$^2$. The stripping of glass/ITO/Au NP (15 nm) (blue legend) led to a $i_{p,ox}$ of 132 nA at a $E_{p,ox}$ of 0.819 V. When the glass/ITO is deposited with both 4 nm and 15 nm Au NPs (green legend), the ASV contained two overlapping indicating the presence of both 4 and 15 nm Au NPs. The measured $i_{p,ox}$ at $E_{p,ox}$ values of 0.710 V and 0.809 V were found to be around 63.3 nA and 96.0 nA, respectively, corresponding to 4 nm and 15 nm Au NPs. This proves that the pipette-based hydrogel electrodes can be used to perform ASV of Au NPs in confined environments.

Figure 8.5a shows the ASVs of glass/ITO/Au$_{4nm}$ NPs using a single Ag/AgCl/Ca-Alg pipette electrode at 20 different locations on the working electrode at a scan rate of 10 mV/s. On average, the $E_{p,ox}$ was observed at 0.705 ± 0.009 with average $i_{p,ox}$ of 80.8 ± 37.7 nA. The individual ASV peak currents and potentials are listed in Table 8.1 below. The nearly uniform $E_{p,ox}$ shows the presence of a uniform size of Au NPs in different locations of the working electrode. The high standard deviation in the $i_{p,ox}$ is reflective of either the NP dispersity on the working electrode or different contact areas of the pipette on each measurement. Figure 8.5b shows the ASV of the whole glass/ITO/Au$_{4nm}$ electrode, prepared under the same conditions as before, in a solution of 10 mM KBr and 0.1 M KClO$_4$. This shows a $i_{p,ox}$ of 8.35 μA and a charge of 47.0 μC at a potential of 0.722 V. By using the charge under the ASV peak, the number of NPs present per area of the electrode can be measured using the following equation:

$$Number\ of\ NPs\ per\ \mu m^2(N) = \frac{\text{Charge\ under\ the\ ASV\ peak}}{3 \times \left(\frac{F}{N_A}\right) \times n \times A}$$
Figure 8.5. (a) ASVs of glass/ITO/Au$_{4}$NPs using Ag/AgCl/Ca-Alg pipette electrode at 20 different locations on the working electrode at a scan rate of 10 mV/s. (b) ASV of the whole glass/ITO/Au$_{4}$NPs electrode in a solution of 10 mM KBr and 0.1 M KClO$_{4}$.
\[ = \frac{47.0 \times 10^{-6}}{3 \times (1.6 \times 10^{-19}) \times 2679 \times (0.84 \times 10^8)} = 4.35 \times 10^2 \text{ NP/} \mu \text{m}^2 \quad (1) \]

Where F is the Faraday constant, n is number of atoms in a 4 nm Au NP, \( N_A \) is the Avogadro number, and A is area of the whole electrode which is 0.84 cm\(^2\). Under the above conditions, the average NP per \( \mu \text{m}^2 \) area present on the electrode is \( \sim 4.35 \times 10^2 \) NPs/\( \mu \text{m}^2 \). By using this value, the area of the stripped locations can be calculated by rearranging the above equation. For example, the area of location 1,

\[
A = \frac{\text{Charge under the ASV peak}}{3 \times \left( \frac{F}{N_A} \right) \times n \times N}
\]

\[
= \frac{0.58 \times 10^{-6}}{3 \times (1.6 \times 10^{-19}) \times 2679 \times (4.35 \times 10^2)} = 1.04 \times 10^2 \text{ cm}^2 \quad (2)
\]

By using the above equation, the area of each location is calculated and listed in Table 8.1. The total area of the stripped locations is calculated to be \( \sim 0.175 \text{ cm}^2 \) which means that the NPs present over the 0.175 cm\(^2\) of the glass/ITO/Au\(_{4 \text{nm}}\) NPs electrode (whole area = 0.84 cm\(^2\)) were stripped. The sum of the Coulombs from the areas measured are very close the Coulombs measured from the entire electrode. The total Q per area for the pipette is \( (0.489 \text{ } \mu \text{C} \times 20)/0.175 \text{ cm}^2 \), or 55.9 \( \mu \text{C/cm}^2 \), which is the same as that measured by the entire electrode, which is \( (47 \mu \text{C}/0.84 \text{ cm}^2 \), or 56.0 \( \mu \text{C/cm}^2 \). It is likely that contact area differences give the different Coulombs per area. Overall, we conclude that Ca-Alg hydrogels are suitable mediators for performing Br\(^-\) assisted stripping of Au NPs in confined environments.

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<tr>
<td>15</td>
<td>0.695</td>
<td>75.2</td>
<td>0.447</td>
<td>8.00</td>
</tr>
<tr>
<td>16</td>
<td>0.711</td>
<td>66.9</td>
<td>0.402</td>
<td>7.19</td>
</tr>
<tr>
<td>17</td>
<td>0.710</td>
<td>20.6</td>
<td>0.129</td>
<td>2.30</td>
</tr>
<tr>
<td>18</td>
<td>0.704</td>
<td>35.0</td>
<td>0.211</td>
<td>3.77</td>
</tr>
<tr>
<td>19</td>
<td>0.711</td>
<td>124</td>
<td>0.739</td>
<td>13.2</td>
</tr>
<tr>
<td>20</td>
<td>0.714</td>
<td>101</td>
<td>0.609</td>
<td>10.9</td>
</tr>
</tbody>
</table>

**Table 8.1.** The data showing the ASV peak current, peak potential, charge, and the calculated area of stripping with the pipette setup

**AVERAGE** | **80.8** | **0.489** | **Total: 0.175 cm²**

**STD DEV** | **0.009** | **37.7** | **0.233** |
ECD of Au on glass/ITO/AuNPs-Ca-Alg electrodes in bulk environment. Figure 8.6 compares the effect of Alg on the ECD of glass/ITO/Au NPs. This was achieved by performing an EPD of Alg on glass/ITO/Au NPs electrodes at 0.8 V for 15 min from a solution of 0.5 % (w/v) Alg and 33.4 mM HQ, followed by soaking the electrode in an in an ice-cold solution of 90 mM CaCl₂·2H₂O for 30 min. The ECD was then performed by performing LSV from 0 V to 1 V at a scan rate of 100 mV/s in a solution containing 1 mM HAuCl₄·3H₂O and 0.1 M KClO₄. Figure 8.6a compares the ECD of glass/ITO/Au₄nm NPs in the presence and absence of Alg. The glass/ITO/Au₄nm NPs were prepared by performing EPD at 1 V for 90 s in a solution of 8.3 μM 4 nm Au NPs and 16.7 mM HQ. In the absence of Alg, the ECD peak was observed at a peak potential of 0.642 V with a peak current of 0.471 mA. However, when the Alg was deposited over the glass/ITO/Au₄nm NPs electrode, the ECD peak became broader and the peak current reduced ~1.6 times to 0.293 mA, and the peak potential shifted to 0.572 V. This indicates that the reversibility of the process is affected by the hydrogel layer present over the working electrode. This can be attributed to the diffusion of AuCl₄⁻ to the electrode surface is affected by the hydrogel layer surrounding the Au NPs. When the size of the Au NP on the working electrode is changed to 15 nm Au NPs (Figure 8.6b), in the absence of Ca-Alg, the peak current due to ECD is 0.513 mA at a peak potential of 0.547 V while in the presence of Ca-Alg, the peak current is 0.298 mA and peak potential is 0.490 V. The glass/ITO/Au₁₅nm NPs were prepared by performing EPD at 1.2 V for 30 s in a solution of 41.7 μM 15 nm Au NPs and 16.7 mM HQ. The 15 nm Au NPs also show similar behavior as 4 nm Au NPs. However, the extent of decrease in peak current due to Ca-Alg hydrogel is higher for 4 nm Au NP in comparison to 15 nm Au NP. One reason for this behavior can be attributed to the ability of 4 nm Au NPs to catalyze the Ca-Alg EPD better than other sizes leading to a relatively thicker hydrogel film over 4 nm Au NP. Hence there is more difference in the effect of Ca-Alg towards ECD on 4 nm Au NP in comparison to 15 nm Au NP.
Figure 8.6. LSV of glass/ITO containing (a) 4 nm and (b) 15 nm Au NPs in the presence (blue) and absence (red) of Alg in a solution of 1 mM HAuCl₄·3H₂O and 0.1 M KClO₄ at a scan rate of 100 mV/s. The glass/ITO/Au₄nm NPs were prepared by performing EPD at 1 V for 90 s in a solution of 8.3 μM 4 nm Au NPs and 16.7 mM HQ. The glass/ITO/Au₁₅nm NPs were prepared by performing EPD at 1.2 V for 30 s in a solution of 41.7 mM 15 nm Au NPs and 16.7 μM HQ.
ECD of Au on glass/ITO electrodes using Ca-Alg Hydrogels in Spatially Confined Environments. The ECD of Au on glass/ITO electrodes can be achieved in spatially confined environments using the previously mentioned methods. One of the ways to achieve this is by using an Ag/AgCl/Ca-Alg pipette set up to explore the ECD of Au on glass/ITO electrode. The pipette was prepared by dipping it into a solution containing 90 mM CaCl₂·2H₂O, 1 mM AuCl₄⁻, and 0.1 M KClO₄. Then, a solution consisting of 1 mM AuCl₄⁻, 0.1 M KClO₄, and 1% Na-Alg solution was added to the pipette. Finally, an Ag/AgCl electrode was inserted into the pipette, and the pipette was sealed with parafilm. The ECD was later performed by forming the setup described in the Figure 8.7a. Here the pipette setup acts as the reference and counter electrode while the glass/ITO acted as the working electrode. When the hydrogel comes in contact with the glass/ITO electrode, the ECD of Au on the glass/ITO electrode is achieved by performing a chronoamperometry (CA) by stepping the potential from 1 V to 0.2 V for 900s. Figure 8.7b shows the CA plot obtained with the regular pipettes with a radius of 550 μm. The maximum current related to the ECD of Au on the glass/ITO electrode was 14.8 μA. Figure 8.7c shows the optical image related to the circular spot formed due to the ECD of Au on the glass/ITO electrode. Figure 8.7d shows a DFM image of the spot to show the Au deposits inside the spot. Figure 8.7e shows the SEM image of the Au spot prepared by using this method. The radius of the spot was estimated to be around 0.75 mm, which is close to the size of the pipette tip, which is around 0.55 mm.

The circular Au spot resembles the shape of the pipette tip. This means that if the size of the pipette can be controlled, the spot size due to the ECD of Au can also be controlled, thus improving the conformity of the ECD process. This can be achieved by melting the pipette tip under the flame (blue part of the flame). However, there is a necessity to theoretically estimate the radius of the pipette tip to compare with the spot size formed after the ECD process.
Figure 8.7. (a) A setup containing Ag/AgCl/Ca-Alg pipette electrode that functions as both reference and counter electrode touching the glass/ITO working electrode. (b) CA plot showing the ECD of Au on glass/ITO using Ag/AgCl/Ca-Alg pipette electrode containing AuCl₄⁻ and KClO₄ electrolyte. Chronoamperometry was performed by stepping the potential from 1 V to 0.2 V for 900s. (c) Optical, (d) SEM, and (e) DFM images of the Au spots were prepared by using this method.
This can be achieved by using a current vs potential (IV) plot across the pipette setup and
an external Ag/AgCl reference electrode in a solution of 1 M KCl, as shown in Figure 8.8a. Here
the current associated with the IV plot can be correlated to the radius of the pipette radius using
the following equation:

\[ R = \frac{V}{I} = \frac{\gamma \cot \theta \sqrt{2}}{\pi a} \]  

(3)

\[ I \propto a \]  

(4)

\[ I = ka \]  

(5)

\[ k = \frac{I}{a} \]  

(6)

Where \( R \) is the measured pipette resistance, \( \gamma \) is the resistivity of the electrolyte used, \( a \) is the
radius of the nanopore, \( \theta \) is the cone angle and \( k \) is a constant that depends on the current
measured (\( I \)) and the radius of the tip \( a \). The radius of the pipette tip used for this study is 0.55
mm. Figure 8.8b shows the IV plot related to this pipette. This can be used to correlate the pipette
tip radius to current measured from IV plot at constant potential. For instance, at 0.5 V, the radius
of the pipette tip, 0.55 mm, is directly proportional to the current measured which is 0.124 mA.
This correlation is used to estimate the constant \( k \) to be around 0.248 mA/mm by following the
equation 6. By using this \( k \) value, the change in the pipette radius after melting can be estimated
by measuring the currents at 0.5 V from the corresponding IV plots. Table 8.2 shows the
estimated tip radius \( a = I/k \) from the currents at 0.5 V in the IV plots for the pipette tips melted
under flame for different times.
Figure 8.8 (a) A set-up containing Ag/AgCl/KCl pipette as working electrode and Ag/AgCl as a reference and counter electrode placed in a solution of 1 M KCl. (b) A current vs. potential (IV) plot to measure the radius of the pipette tip.
Table 8.2. The data showing a correlation between melting time of pipette and the subsequent current measured at 0.5 V using IV-plots and the corresponding calculated tip radius.

<table>
<thead>
<tr>
<th>Melting Time (min)</th>
<th>Current at 0.5 V (µA)</th>
<th>Tip Radius (I/k) (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>70.1</td>
<td>0.2823</td>
</tr>
<tr>
<td>2</td>
<td>9.01</td>
<td>0.0363</td>
</tr>
<tr>
<td>3</td>
<td>2.03</td>
<td>0.0081</td>
</tr>
<tr>
<td>4</td>
<td>2.51</td>
<td>0.0101</td>
</tr>
</tbody>
</table>

Figure 8.9a shows the CA plot of the ECD of Au on glass/ITO electrode performed with the Ag/AgCl/Ca-Alg pipette setup where the pipette tip is melted for 1 min. The CA is performed by stepping the potential from 1 V to 0.2 V for 900 s leading to a peak current of 7.60 µA. The multiple peaks observed during the CA are indicative of the deposition of Au on the as-formed Au nucleation sites. The optical image of the Au spot is shown in Figure 8.9b, and the radius of the spot is measured to be around 311 µm which is close to the radius estimated from the IV plot, which is 282 µm, as shown in Table 8.2. Figure 8.9c shows the SEM of another Au spot prepared under similar conditions. The radius estimated by the SEM mage is around 370 µm. The apparent increase in spot radius in comparison to the pipette tip radius can be attributed to the pressure from the hydrogel tip at the glass/ITO surface. Figure 8.9d shows the DFM image of a different spot prepared under the same condition, which has a radius of 352 µm. Hence it can be concluded that the melting of the pipette tip led to the formation of narrow tips leading to electrodeposition in confined spaces. However, when the pipette tip was melted further, this led to poor or irregular ECD of Au to glass/ITO surface.
Figure 8.9. (a) CA plot showing the ECD of Au on glass/ITO using Ag/AgCl/Ca-Alg pipette electrode containing AuCl₄⁻ and KClO₄ electrolyte where the pipette tip was melted for 1 min. CA is performed by stepping the potential from 1 V to 0.2 V for 900s. (b) Optical, (c) SEM, and (d) DFM images of the Au spots were prepared by using this method.
The pipette tip melting further resulted in irregular size and deposition. Decreasing the pipette tip further also caused strain on the hydrogels, pushing them inside the pipette. Hence other methods were explored to attain better spatial confinity towards the ECD process.

To improve the spatial confinity, the ECD was also performed using Alg deposited on a 2 mm diameter Pt disk electrode. Here the electrode setup was prepared by performing EPD in a solution containing 0.5% w/v Na-Alg and 33.4 mM HQ at 1.4 V for 30 min, followed by soaking in a solution of 90 mM ice-cold CaCl$_2$.2H$_2$O for 30 min. The Pt/Ca-Alg electrode is then soaked in a solution of 10 mM HAuCl$_4$.3H$_2$O and 0.1 M KClO$_4$ for 15 min. The Pt/Ca-Alg setup functioned as the reference and counter electrode, while the glass/ITO was used as the working electrode, as shown in the picture in Figure 8.10. The ECD was performed using CA by stepping the potential from 1 V to -0.6 V for 1000s. The subsequent Au spots formed is shown in the picture 8.10. The diameter of the three spots was measured to be 1.74 mm, 1.68 mm, and 0.57 mm. Figure 8.10c shows the DFM image of one of the spots. One of the drawbacks of this method was the drying of hydrogel on the Pt electrode during the deposition. This leads to difficulties in forming deposits and affects the spot dimension. Another major drawback of this method is the formation of larger spots due to the bigger Pt disk sizes (2 mm). Hence it was difficult to achieve the spatial confinity using this method.

Another way to achieve spatially confined ECD of Au on glass/ITO is by using a Pt/Ir STM probe with Ca-Alg hydrogel deposits as a reservoir for AuCl$_4^-$ ions. To deposit Ca-Alg hydrogel on the Pt/Ir STM probe, an EPD was performed in a solution containing 0.5% w/v Na-Alg and 33.4 mM HQ at 1.4 V for 15 min. After performing EPD, the Pt/Ir/Ca-Alg was dipped in an ice-cold solution of 90 mM CaCl$_2$.2H$_2$O for 30 min before using it for ECD. Figure 8.11a shows the picture of the Ca-Alg hydrogel on the Pt/Ir STM electrode. The Pt/Ir/Ca-Alg is also characterized by Optical and DFM images, as shown in Figure 8.11b and c.
Figure 8.10. (a) A setup containing Pt/Alg electrode containing AuCl$_4^-$ and KClO$_4$ electrolyte that functions as both reference and counter electrode touching the glass/ITO working electrode. (b) a picture of the glass/ITO with the Au spots and (c) a DFM image of the Au spot after performing CA on the glass/ITO electrode by stepping the potential from 1 V to -0.6 V for 1000s.
Figure 8.11. (a) A picture of the Pt/Ir/Ca-Alg electrode. (b) Optical and (c) DFM images of the tip of the Pt/Ir/Ca-Alg electrode.
The hydrogel deposited at the tip is narrow with <10 μm width, which is ideal for spatially confined environments for the ECD process. After preparing the Pt/Ir/Ca-Alg electrodes, the hydrogel is enriched with AuCl₄⁻ and KClO₄ by soaking in a solution of 10 mM HAuCl₄·3H₂O and 0.1 M KClO₄ for performing ECD of Au on glass/ITO electrodes. Figure 8.12a shows the setup for ECD of Au on glass/ITO where Pt/Ir/Ca-Alg was used as the reference and counter electrode, and glass/ITO was used as the working electrode. Figure 8.12b displays the LSV performed using the setup to electrodeposit Au on a glass/ITO electrode by scanning from 1 V to -1 V at 100 mV/s. The LSV resulted in an onset deposition current at -0.045 V and a peak deposition current of 16.5 mA at -0.642 V. The peaks are due to the electroreduction of AuCl₄⁻ as shown below:

\[
\text{AuCl}_4^- + 3e^- \rightarrow \text{Au}^0 + 3\text{Cl}^- \quad E^0 = 0.808 \text{ V vs Ag/AgCl (3 M KCl)} \quad (7)
\]

Hence, it is necessary to apply negative potentials as low as -0.8 V to achieve adequate deposition of Au on glass/ITO using this setup.

The contact between the Pt/Ir/Ca-Alg containing AuCl₄⁻ and KClO₄ electrolyte and the glass/ITO was established by stepping potential from 0 V to -0.6 V using a CA method. This is evident from the Figure 8.13a where the plot indicates a sharp increase in current to 16.8 μA as result of the contact between the electrodes. After establishing the contact chronocoulometry (CC) was performed by stepping potentials from 0 V to -0.6 V with a maximum charge of 0.27 mC to electrodeposit Au to the glass/ITO surface. The resulted spot was characterized by optical and DFM images, as shown in Figure 8.13b and c. The radius of the spot was measured to be around 117 μm. However, on another trial under similar conditions, the CA showed a lower current of 4.5 μA due to contact, as shown in Figure 8.14a. This resulted in the Au spot with a radius of 79 μm, which is measured from the optical and DFM images in Figures 8.14b and c.
Figure 8.12. a) A setup containing Pt/Ir/Ca-Alg electrode containing AuCl$_4^-$ and KClO$_4$ electrolyte that functions as both reference and counter electrode touching the glass/ITO working electrode. (b) LSV was performed using the setup to achieve ECD of Au on a glass/ITO electrode by scanning from 1 V to -1 V at 100 mV/s.
Figure 8.13. (a) CA plot to establish the contact between the Pt/Ir/Ca-Alg containing AuCl$_4^-$ and KClO$_4$ electrolyte and the glass/ITO by stepping potential from 0 V to -0.6 V that resulted in peak current of 16.8 µA. (b) Optical and (c) DFM image of the Au spot formed by performing a chronocoulometry by stepping potentials from 0 V to -0.6 V with a maximum charge of 0.27 mC.
Figure 8.14. (a) CA plot to establish the contact between the Pt/Ir/Ca-Alg containing AuCl$_4^-$ and KClO$_4$ electrolyte and the glass/ITO by stepping potential from 0 V to -0.6 V that resulted in peak current of 4.5 $\mu$A. (b) Optical and (c) DFM image of the Au spot formed by performing a chronocoulometry by stepping potentials from 0 V to -0.6 V with a maximum charge of 0.24 mC.
Thus, the contact area between the hydrogel and the glass/ITO electrode can vary depending on the point of contact. However, the current measured through CA can be used to control the contact size and the size of the Au spot formed by ECD.

The necessity to control the spot size and control it by CA led to the use of a “contact-retraction” method to establish the spatially controlled ECD of Au on glass/ITO electrodes. In this method, after the initial contact of the Pt/Ir/Ca-Alg containing AuCl$_4^-$ and KClO$_4$ electrolyte and the glass/ITO electrode, the hydrogel was retracted using an XYZ positioner to decrease the contact area of the hydrogel at the electrode surface. This is demonstrated in Figure 8.15. This is then quantified using CA, where the current is correlated to the area of contact or the spot size. Since the formation of Au deposits was not allowed, the potentials were stepped down from 0.2 V to 0.1 V to inhibit nucleation and growth of Au. Here the current measured is equivalent to the charging current. Following the established contact, ECD of Au was performed by CC by stepping potentials from 0 V to -0.8 V to obtain a charge of 45 µC. Figure 8.16 demonstrates the CA, optical and DFM images of the spots formed at lesser charging currents. For instance, the charging current of 11.3 nA shown in Figure 8.16a resulted in an Au spot of radius 60 µm (Figure 8.16b and c). However, when the charging current is decreased to 1.54 nA (Figure 8.16d) through successive retraction of the hydrogel electrode, it leads to Au spots of radius as low as 30.8 µm, as demonstrated in Figure 8.16e and f. Hence it can be concluded that the charging current related to the hydrogel contact at the electrode can be correlated with the size of the spot formed due to ECD of Au at the contact area. This is demonstrated by plot that correlates the charging current with the spot radius measured from the optical images as shown in Figure 8.17, provided that the charging current is measured using CA by stepping from 0.2 V to -0.1 V. Here it is clear that as the charging current is decreasing the radius of Au spot also decreases. At higher charging currents, the spot sizes remain relatively same while the correlation become prominent at lower currents.
Figure 8.15. A scheme outlining the "contact-retraction" method used to control the contact area of Pt/Ir/Ca-Alg containing AuCl$_4^-$ and KClO$_4$ electrolyte on the glass/ITO electrode, allowing for spatially controlled ECD of Au on glass/ITO electrodes.
Figure 8.16. (a) CA plots to establish the contact between the Pt/Ir/Ca-Alg containing AuCl₄⁻ and KClO₄ electrolyte and the glass/ITO by stepping potential from 0.2 V to 0.1 V that resulted in peak currents of 11.3 nA. (b) Optical and (c) DFM images of the Au spot formed by performing a CC by stepping potentials from 0 V to -0.8 V with a maximum charge of 45 µC. (d) CA plots to establish the contact between the Pt/Ir/Ca-Alg containing AuCl₄⁻ and KClO₄ electrolyte and the glass/ITO by stepping potential from 0.2 V to 0.1 V that resulted in peak currents of 1.54 nA. (e) Optical and (f) DFM images of the Au spot formed by performing a CC by stepping potentials from 0 V to -0.8 V with a maximum charge of 45 µC.
Figure 8.17. A plot that correlates the charging current (nA) with the spot radius (μm) measured from the optical images of Au spots on glass/ITO electrodes provided that the charging current is measured using CA by stepping from 0.2 V to -0.1 V.
Hence, the size of Au deposits can be controlled by using the hydrogel retraction method. The radius of the spot can be correlated with the charging currents after the hydrogel is brought into contact with the glass/ITO electrode.

Similar behavior was observed when the glass/ITO working electrode with Au NPs to overcome the barrier of nucleation process during ECD. Figure 8.18 demonstrates the charging current, optical and DFM images of the spot formed on a glass/ITO/Au$_{4\text{nm}}$ NP electrode. Here the glass/ITO/Au$_{4\text{nm}}$ NP is prepared by EPD at 1 V for 30 s from a solution of 8.33 μM 4 nm Au NPs and 33.4 mM HQ. Here a charging current of 0.3 nA resulted in a bright spot of radius 11 μm. The increase in brightness of the spot is due to the growth of Au deposit on the Au NPs present on glass/ITO surface. Figure 8.19 shows a plot that correlates the charging current obtained using aforementioned method and the radius of the spot as measured from the optical images. As mentioned earlier, when the charging current is gradually decreasing, the contact area of the hydrogel also decreasing resulting in smaller spot radius. Hence this section successfully demonstrated the ECD of Au on spatially confined environments of glass/ITO working electrode.
Figure 8.18. (a) CA plots to establish the contact between the Pt/Ir/Ca-Alg containing AuCl₄⁻ and KClO₄ electrolyte and the glass/ITO/Au NP (4 nm) by stepping potential from 0.2 V to 0.1 V that resulted in peak currents of 0.3 nA. The glass/ITO/Au₄nm NP is prepared by EPD at 1 V for 30 s from a solution of 8.33 μM 4 nm Au NPs and 33.4 mM HQ. (b) Optical and (c) DFM images of the Au spot formed by performing a CC by stepping potentials from 0 V to -0.8 V with a maximum charge of 41 μC.
Figure 8.19. A plot that correlates the charging current (nA) with the spot radius (µm) measured from the optical images of Au spots on glass/ITO/Au$_{4nm}$ NPs electrodes provided that the charging current is measured using chronoamperometry by stepping from 0.2 V to -0.1 V.
8.3. CONCLUSIONS

In summary, chapter 8 expanded the applications of Ca-Alg hydrogels trapped at a conductive electrode surface to perform electrochemical processes such as ASV as well as ECD in a spatially confined environment. Initially the direct consequences of hydrogels towards ASV of Au NPs deposited on a glass/ITO surface was explored. Here it is observed that in the presence of Ca-Alg, the size dependent stripping behavior of Au NP was retained despite having the peak stripping peaks shifted to more positive direction and lower and broad stripping currents. Later the viability of Ca-Alg hydrogels trapped or deposited at the tip of Ag/AgCl electrode, Pt electrode, and Ag/AgCl/Ca-Alg pipette electrode containing KBr and KClO₄ electrolyte was studied to achieve the spatially confined ASV of Au NPs. The pipette electrode was the best electrode setup towards the localized stripping of Au NPs. This method detected the inhomogeneity in the NP population on the glass/ITO electrode making this method suitable for future spatially confined detection applications. Another electrochemical process that was explored extensively in chapter 8 is the ECD of Ca-Alg on glass/ITO electrodes using Ca-Alg hydrogels deposited at various electrode materials. Due to the success of pipette electrode set-up, initially the ECD of Au was explored with Ag/AgCl/Ca-Alg containing AuCl₄⁻ and KClO₄ electrolyte. ECD was performed to generate Au spots due to deposition and the spots were characterized by a combination of CA, optical, DFM and SEM imaging. When a regular pipette was used, the Au spots deposited resembled the pipette tip radius. The pipette tips were then melted to decrease the tip radius to achieve better spatial confinity which resulted in smaller spots. An IV plot is used to measure current which is directly proportional to the radius of the pipette tip in order to quantitatively control the Au spot size. This was successfully used to electrodeposit Au spots with decreased radius. However, the pipette tip sizes were irregular due to melting and the strain across the narrow tips led to the hydrogel pull inside the pipette. Pt/Ca-Alg containing AuCl₄⁻ and KClO₄ electrolyte were also used to deposit Au on glass/ITO, however the radius of the Au spots was big, and the hydrogels were drying during the ECD step. Another
method involving Pt/Ir STM probe containing Ca-Alg electrodeposits was used to achieve controlled spatial confinity towards Au electrodeposition. By using a retraction method using an XYZ positioner, the Pt/Ir/Ca-Alg containing AuCl₄⁻ and KClO₄ electrolyte was able to electrodeposit Au spots with radius of tens of microns on the glass/ITO electrodes. Hence Pt/Ir/Ca-Alg containing AuCl₄⁻ and KClO₄ electrolyte is a best setup to achieve ECD of Au on glass/ITO electrodes with spatial confinement.
CHAPTER 9: SUMMARY AND FUTURE DIRECTIONS

9.1. SUMMARY

This dissertation explores the influence of Au NPs' size and their ligand stabilizers on ECD and EPD processes, considering both Au NPs and Ca-Alg-Au NP hydrogels. The research led to the development of a two-step electrochemical amplification strategy for detecting femtomolar concentrations of Au NPs in solution. Furthermore, the dissertation delves into fabricating Ca-Alg and Ca-Alg-Au NP hydrogels through HQ-catalyzed EPD on diverse electrode surfaces. The electrochemical behavior of redox probes, EPD, and ECD was examined in both bulk and microscale regions of Ca-Alg hydrogel and Ca-Alg-Au NP hydrogel films.

Covering a spectrum of subjects related to the ECD of metal NPs, hydrogels, and their applications, the dissertation also discusses experimental procedures and characterization techniques. The findings in Chapters 3 and 4 encompass size-dependent growth kinetics of Au onto preformed Au NPs and the growth of Au onto alkanethiol SAM-functionalized Au NPs. The study investigates the influence of NP size, SA coverage, ligand stabilizers, and scan rates on the electrochemical growth of Au on Au NPs. It aims to provide a better understanding of the growth step in the nucleation and growth mechanism of ECD. This led to Chapter 5, which demonstrated a two-step electrodeposition amplification detection technique for detecting fM concentrations of Au NPs in solution with potential applications in environmental monitoring, chemical sensing,
and biosensing. Chapter 6 investigates the catalytic properties of Au NPs in the electrochemical oxidation of HQ, leading to the preparation of hybrid hydrogel films comprising Ca-Alg and Au NPs, which have potential applications in biotechnology, sensing, and catalysis. Here, hydrogels of varied thicknesses were fabricated by altering the size and concentration of Au NPs and the composition of Alg in the EPD solutions before Ca$^{2+}$ gelation. Finally, Chapters 7 and 8 discuss the electrochemistry of Fe(CN)$_6^{3-}$, EPD, ECD, and ASV in Ca-Alg hydrogels in bulk and microscale-confined environments. These studies are essential for understanding the fundamentals of electrochemical processes occurring in bulk and confined hydrogel materials, which is essential for developing hydrogel electrodeposition processes that have broad applications in thin-film manufacturing, sensors, solar cell production, environmental monitoring, catalysis, and nanoelectronics.

9.2. FUTURE DIRECTIONS

The dissertation work can be expanded to further explore the fundamentals of electrodeposition techniques and their potential applications. The electrochemical growth kinetics of Au on Au NPs with diameters of 4, 15, and 50 nm, studied in chapters 3 and 4, can be further expanded to study the growth kinetics on Au NCs with a diameter of less than 2 nm, also called atomically precise NCs. NCs exhibit unique properties different from both NP and bulk counterparts. Due to their smaller size and fewer atoms, NCs show less metallic behavior and alter their catalytic properties. Since Au nucleation has been shown for 1-5 atom clusters, it is important to understand the growth kinetics of very small Au NCs of this size regime.

A preliminary experiment was conducted to study the ECD of Au on Au NCs with diameters of 0.9 and 1.6 nm, stabilized with phosphine ligands, and the results were interesting. At a scan rate of 100 mV/s, the linear sweep voltammograms (LSVs) showed a negative shift in peak growth potential ($E_p$) and a decrease in peak current ($i_p$). THPC-capped 1.6 nm Au NCs
exhibited lower overpotential than 0.9 nm TPPS-capped Au NCs. In contrast to 4 nm Au NPs, 0.9 and 1.6 nm Au NCs displayed irreversible growth kinetics. This is intriguing because, at diameters less than 2 nm, Au NCs do not seem to catalyze the electrochemical reduction of $\text{AuCl}_4^{-}$ on Au NCs. This may be partly due to the decrease in the metallic behavior of the NCs, but the effect of the phosphine ligands on the electrochemical growth process still needs to be determined. The surface area (SA) coverage measurement of these Au NCs using CV cycles in 0.1 M HClO$_4$ was futile due to ripening issues with these clusters. Therefore, expanding the electrochemical growth studies to Au NCs with a controlled SA coverage on the electrode surface is essential without the effects of the capping ligands.

Additionally, the electrochemical growth kinetic studies can also be exploited to fabricate Au films with smooth surfaces. A preliminary experiment showed that the electrodeposition of Au on a glass/ITO electrode with a high coverage of 4 nm Au NPs at 0.7 V for 30 min in a solution of 0.1 M AuCl$_4^{-}$ and 0.1 M KClO$_4$ with stirring led to the fabrication of smooth Au film. The growth of Au on Au NPs in the presence of ligands, as mentioned in Chapter 4, can also be expanded to study how the electrochemical growth in the presence of diverse ligands can affect the surface morphology of the NPs. This is crucial for the development of controlled growth of Au on Au seeds, resulting in size- and shape-controlled metal nanostructures and porous nanomaterials.

As mentioned in Chapter 5, the two-step electrochemical amplification detection method can be used to detect Au NPs of concentrations less than fM. The potential and time for the two steps could be further optimized to detect Au NPs of concentrations below fM and extended to direct $\text{AuCl}_4^{-}$ detection or for detection of metal NPs used as tags in bioassays. Additionally, the electrochemical amplification detection method has the potential to detect other metal NPs as well as metal ions/complexes, making it useful for potential environmental monitoring and sensing applications.
Chapter 6 described the formation of thick hybrid Ca-Alg-Au NP hydrogel films by electrooxidation of HQ in the presence of Alg and citrate-stabilized Au NPs. In future studies, these films could potentially be used for spectroelectrochemical sensing, supported electrocatalysis, electrochemical sensors, and electrochemical energy generation or storage devices. The hybrid devices could be used as templates to fabricate porous metal structures on glass/ITO through a variety of EPD, ECD, and chemical processes. There are several potential challenges associated with this process that will require optimization of the Ca-Alg: Au NP ratio and variation of other conditions. Removal of the hydrogel template without disruption of the metal NPs is another challenge. The EPD of Ca-Alg was also preliminarily used to fabricate Ca-Alg-glutathione-capped Au NCs. These showed the possibility of forming hybrid hydrogels with < 2 nm diameter Au NCs. This also shows that this EPD technique can be further expanded to encompass biologically significant materials on the electrode surface for potential biosensing and biotechnology applications.

Chapters 7 and 8 focused on the electrochemical reactions of Fe(CN)$_6^{3-}$, EPD, ECD, and ASV using Ca-Alg and Ca-Alg-Au NP hydrogels in both bulk and confined environments. The initial study was reduced to Ca-Alg contact areas (spot sizes) of ~10 microns. To reduce this further, a tip-confined Ca-Alg-Au NP hydrogel is recommended. By using Pt/Ir STM tips with diameters in the range of a few nanometers, the contact area of the hydrogel on the electrode surface can be reduced to the nanoscale. Alternatively, pipette tips with nm radius openings can be used to minimize the Ca-Alg contact area. These techniques can significantly enhance the spatial resolution of the above-mentioned processes. The contact area of the hydrogel can be controlled using a series of "contact-retraction" methods, where the charging current measured using CA upon contact can be directly correlated to the contact area. As a result, when the electrode tip area decreases, the Ca-Alg hydrogel electrodes will also have reduced dimensions,
leading to contact areas on the nanoscale. This can expand the scope of the studies of highly localized electrochemical processes.

Some processes that can be explored using the hydrogel tip include ECD, EPD, and ASV (see Chapter 8). The spatially confined studies can provide incredible information on the electrochemical reactions occurring at a nanoscale level. This has tremendous implications for the development of electrochemical sensors, detectors, and thin films for other applications. For instance, the EPD performed using tip-confined Ca-Alg films has the potential to achieve single Au NP deposition on a glass/ITO surface. This can be achieved first by fabricating electrode nanotips with Ca-Alg-Au NP hydrogels with incredibly low concentrations of Au NPs. This is followed by soaking hydrogel nanotips in 0.1 M HQ for 15 min, followed by an EPD process at low potentials and short times using the hydrogel tip as a reference and counter electrode on a glass/ITO working electrode. Preliminary experiments showed the deposition of Au NPs on the glass/ITO surface. The aspirational single Au NP deposition could be achieved by controlling the parameters during the nano/micro-confined EPD process. Single NP EPD could be beneficial for fundamental single NP electrochemical studies and single NP devices.

Another potential application of the nanoscale Ca-Alg systems is improving the spatial resolution of ASV of Au NPs deposited at an electrode surface. An example of this at the micron scale is described in Chapter 8. Using pipettes filled with Ca-Alg, KBr, and KClO₄ allowed localized ASV of Au NPs that were deposited on a glass/ITO working electrode. This led to the detection of the inhomogeneity or homogeneity of the particle size distribution on the glass/ITO surface. If the spatial resolution of the process is expanded to the nm scale, this could further improve the resolution of the detection and help map the electrode surface coated with different-sized Au NPs, exposing the particle and size inhomogeneity on the electrode surface. Hypothetically, enhanced resolution can lead to the stripping of a single or a few NPs in the spatially confined contact area, depending on the size of the Au NPs. The spatial detection of Au
NPs on electrodes could be significantly improved using this micro/nano-confined ASV technique.

In conclusion, this dissertation thoroughly investigated deposition processes, including fundamental issues regarding ECD and EPD for both Au NPs and Ca-Alg-Au NP hydrogels. This dissertation covers various aspects of metal ECD, hydrogels, and their applications, focusing on experimental procedures, characterization techniques, and size-dependent growth kinetics. The findings of this dissertation can serve as a solid foundation for further research into the expanding field of electrodeposition techniques using Au NPs and Ca-Alg hydrogels, with an interest in the fundamentals of localized electrochemistry in hydrogels and applications.
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APPENDIX

LIST OF ACRONYMS AND ABBREVIATION

Alg: Alginate

APTES: 3-(Aminopropyl) triethoxysilane

ASV: Anodic stripping voltammetry

ATR-IR: Attenuated transmittance reflectance infrared spectroscopy

C12S: Dodecanethiol

C16S: Hexadecanethiol

C4S: Butanethiol

C8S: Octanethiol

CA: Chronoamperometry

Ca-Alg: Calcium Alginate.

CC: Chronocoulometry

CE: Counter electrode

Cit-Au NP: Citrate capped Au nanoparticle

CV: Cyclic voltammetry
DFM: Darkfield microscopy

ECD: Electrochemical Deposition

EDC: 1-ethyl-3-(3-dimethylamino propyl)carbodiimide

EPD: Electrophoretic deposition

FESEM: Field emission scanning electron microscope

HQ: Hydroquinone

ITO: Indium Tin Oxide

LSPR: Localized Surface Plasmon Resonance

LSV: Linear Stripping Voltammetry

Na-Alg: Sodium Alginate

NCs: Nanoclusters

NHS: N-hydroxysuccinimide

NPs: Nanoparticles

RE: Reference electrode

SAM: Self-assembled monolayers

SEM: Scanning electron microscopy

STM: Scanning Tunneling Microscopy

TEM: Scanning transmission microscopy

THPC: Tetrakis(hydroxymethyl) phosphonium chloride
TPPS: Triphenylphosphine sulphonate

UV-Vis: Ultraviolet-visible spectroscopy

WE: Working electrode

Au_{4nm} Au NP: 4 nm diameter Au NPs

Au_{15nm} Au NP: 15 nm diameter Au NPs

ECSA: Electrochemical surface area
Size-Dependent Electrochemical Metal Growth Kinetics

Author: Harikrishnan N. Nambiar, Francis P. Zambrini
Publication: The Journal of Physical Chemistry C
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Date: Mar 1, 2023
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customercare@copyright.com
CURRICULUM VITAE

Harikrishnan (Hari) Nambiathan Nambiar

Email: harikrishnan.nambiar@louisville.edu
Phone: (502) 262-2196

Education

University of Louisville  Louisville, KY, USA
Ph.D. Candidate in Analytical Chemistry EXP: Fall 2023
- Thesis: “Electrodeposition Studies of Metal Nanoparticles and Alginate Hydrogels”
- Advisor: Dr. Frank Zamborini

Mahatma Gandhi University  Kottayam, KL, INDIA
M.S. in Organic Chemistry* 2016
- Thesis: “Synthesis of Lactam-lactones employing (2S, 3S) - Tetrahydro-3-Hydroxy-5-Oxo-2, 3-Furandicarboxylic acids”
- Advisor: Dr. Ibrahim Ibnuusaud

Mahatma Gandhi University  Kottayam, KL, INDIA
B.S. in Chemistry* 2014
- Integrated B.S.–M.S. program with an emphasis on Interdisciplinary Studies

Research Experience

University of Louisville, Chemistry  Louisville, KY, USA

Electrochemical Deposition of Metal Nanoparticles

The impact of the size of the chemically formed nanoparticles (0.9 nm to 50 nm gold nanoparticles) used as nucleation sites, ligands attached to the nanoparticles, and the number of nanoparticles on electrochemical metal growth kinetics are studied. This work gives an insight into the impact of nucleation site properties on the electrochemical growth of metals.

A new two-step electrochemical amplification technique has been devised to detect lower concentrations of gold nanoparticles using anodic stripping voltammetry. This method enabled the detection of lower concentrations, as low as 17 femtomolar concentrations of gold nanoparticles.

Electrophoretic Deposition of Sodium Alginate

The electrophoretic deposition of sodium alginate using electrooxidation of hydroquinone catalyzed by gold nanoparticles is utilized to prepare hybrid alginate–gold hydrogels. The influence of the size and concentration of gold nanoparticles in the formation of hydrogels is explored. The electrophoretic deposition of alginate was achieved under lower potentials as opposed to higher potentials reported in the literature.

The electrochemical redox reactions of ferricyanide, ferrocene-methanol, and ruthenium hexamine trapped within alginate hydrogel deposited on a glass/ITO electrode are explored. This study elaborated on the robustness of alginate as a medium for electrochemical reactions.

The hydrogels deposited on micro tips are explored for their ability to be used as an electrolyte for electrochemical reactions.

Effect of Metal Nanoparticle Aggregate Structure on the Thermodynamics of Oxidative Dissolution

The electrochemical oxidation potential of 15 nm diameter citrate-stabilized gold nanoparticles aggregated by acid (low pH) is compared to those aggregated by tetrakis(hydroxymethyl) phosphonium chloride (THPC). This study explored the sensitivity of anodic stripping voltammetry to the details of the aggregate structure.

Mahatma Gandhi University, Chemistry Kottayam, KL, INDIA

Synthesis of Lactam-lactones using Hydroxycitric Acid Lactone.

The hydroxycitric acid lactone is isolated from natural sources and used to synthesize lactam-lactone, a key intermediate in the synthesis of corrinoid compounds.
Languages

English, Malayalam, Hindi

Software Skills

MS Office Package, Igor Plot, Sigma Plot, ImageJ, ChemDraw, Adobe Photoshop

Instrumental Skills

- **Electrochemical Techniques.** Linear Stripping Voltammetry, Cyclic Voltammetry, Chronoamperometry, Chronocoulometry, Electrochemical Deposition, Electrophoretic Deposition
- **Spectroscopic Techniques.** UV–Vis Spectroscopy, FT–IR Spectroscopy, Fluorescence Spectroscopy, NMR Spectroscopy, AAS spectroscopy
- **Microscopic Techniques.** SEM, TEM, Darkfield Microscopy, Raman Microscopy, Optical Microscopy
- **Separation Techniques.** HPLC, GC–MS
- **Glove Box.**

Undergraduate Advising

**Preeti Tanwani**
Present
May 2022 –
Thesis: “*Effect of Alkanethiol Ligands on Electrochemical Metal Growth Kinetics*”

**Landon Smith**
Aug 2021 – May 2022
Thesis: “*Electrophoretic Deposition of THPC Mediated Linear Aggregation of 15 nm Gold Nanoparticles*”

**Grayson Stringer**
May 2021 – Dec 2021
Thesis: “*Diffusion Studies of K₃Fe(CN)₆ through Amide Protected Alginate Hydrogel*”

**Gerald Leslie**
Jan 2021 – May 2021
Thesis: “*Preparation of Gold films using Electrochemical Deposition of Au on Au NPs*”

**Ismail Qadir**
Aug 2020 – May 2021
Thesis: “*Electrophoretic Deposition of Sodium Alginate via Glutathione Capped Gold Nanoparticle Catalyzed Electrooxidation of Hydroquinone*”

**Chloe Oldenick**
Jan 2020 – May 2020
Thesis: “*Electrochemical Deposition of Gold Nanoparticles*”
Awards

- Doctoral Dissertation Completion Award from the University of Louisville 2023
- Undergraduate Mentored Research Award from the University of Louisville 2022
- Graduate School Council Travel Award from the University of Louisville 2019

Community Services

- Chemistry Graduate Student Association (CGSA) President, University of Louisville, 2021–2022
- 42nd Derby Lecture Series by 2019 Nobel Laureate Dr. M. Stanley Whittingham Organizer, University of Louisville, KY (May 2022)
- GRC Power Hour, the Gordon Research Conference on Electrochemistry Organizer, Ventura, CA (September 2022)

Teaching Experience

University of Louisville, Louisville, KY, USA Instructor Jan 2020 – May 2020

- Instructed Chemistry 529 (Contemporary Methods of Synthesis and Analysis II) lectures and labs as a co-lead instructor to senior undergraduates. My responsibilities include preparing and leading lectures, labs, presentations, and exams.

Mentoring Jan 2020 – May 2023

- Mentored fellow graduate students (Miracle Amechi, Binu Thapa, and Fahad bin Halim) in the synthesis of different-sized gold nanoparticles, UV-vis spectroscopy, various voltammetry techniques, and scanning electron microscopy (SEM).

Graduate Teaching Assistant Aug 2017 – Dec 2020

- Lead Chemistry 343 and 344 organic chemistry labs to freshman-junior undergraduates.
- Lead Chemistry 527 analytical chemistry labs to senior undergraduates.

Publications


**Poster and Oral Presentations**

• Poster presentation at the *Gordon Research Conference on Electrochemistry* in Ventura, CA (September 2022). “Electrophoretic Deposition of Sodium Alginate Using Gold Nanoparticle Catalyzed Electrooxidation of Hydroquinone.”

• Poster presentation at the *Gordon Research Seminar on Electrochemistry* in Ventura, CA (September 2022). “Electrophoretic Deposition of Sodium Alginate Using Gold Nanoparticle Catalyzed Electrooxidation of Hydroquinone.”

• Poster presentation at the *National Meeting of the American Chemical Society* in Chicago, IL (August 2022). “Electrophoretic Deposition of Sodium Alginate Using Gold Nanoparticle Catalyzed Electrooxidation of Hydroquinone.”

• Virtual Oral presentation at the *Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy* (March 2021). “Electrodeposition of Gold on Gold Nanoparticles.”

• Poster presentation at the *University of Louisville Graduate Student Regional Research Conference* in Louisville, KY (February 2020). “Size-Dependent Electrochemical Deposition of Gold on Gold Nanoparticles.”

• Poster presentation at the *71st Southeastern Regional Meeting of the American Chemical Society* in Savannah, GA (October 2019). “Size-dependent electrochemical deposition of Gold on Gold Nanoparticles.”

• Poster presentation at the *Graduate Student Regional Research Conference at the University of Louisville* in Louisville, KY (February 2019). “Aggregation-Dependent Oxidation of Different-Sized Gold Nanoparticles.”

• Poster presentation at the *70th Southeast Regional Meeting of the American Chemical Society* in Augusta, GA (November 2018). “Aggregation-Dependent Oxidation of Different-Sized Gold Nanoparticles.”

**References**

**Dr. Frank P. Zamborini,**

Analytical Professor and Current Doctoral Degree Advisor

Chemistry Department, University of Louisville

Phone: (502) 852-6550

Email: f.zamborini@louisville.edu

**Dr. Muriel C. Maurer,**

Biochemistry Professor and a member of my doctoral dissertation committee

Chemistry Department, University of Louisville

Phone: (502) 852-7008

Email: muriel.maurer@louisville.edu
Dr. Andrea Gorce
Assistant Professor and instructor to my GTA courses
Chemistry Department, University of Louisville
Phone: (502) 852-2733
Email: andrea.gorce@louisville.edu