Electrode Development for a Lactate Biosensor

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**Abstract**

Implantable biosensors are an underdeveloped area of research which could provide many benefits to tumor detection. Utilizing an electrode that is selective toward the byproduct (H₂O₂) of many oxidase reactions enables a biosensor to be created through coupling of an enzyme layer. A biocompatible electrode with reactivity towards H₂O₂ is developed using Pt nanoparticles deposited on a titanium dioxide nanotube array. Titanium dioxide nanotubes were formed using anodic oxidation at varying potentials in an ethylene glycol based solution. Pt was deposited onto nanotube arrays of varying condition using cyclic voltammetry. Two electrodes with slight carbon deposits on the nanotubes showed reactivity with H₂O₂ at a potential around –0.277V.

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**Introduction**

Implantable biosensors have recently come under attention for their benefits in many applications. One such application is tumor detection. As a case study for this project, the most common, malignant type of brain tumor was studied. This cancerous brain tumor is called Glioblastoma Multiforme, which is also known as GBM. Current GBM detection methods include Magnetic Resonance Imaging (MRIs) and Computed Tomography (CT) Scans, but the problem with these techniques is that they require tumors to be partially developed to be detectable. Unfortunately for patients with GBM, because the tumor grows rapidly and is infectious, once it starts developing there is not much that can be done. In fact, the two-year survival rate according to the American Brain Tumor Association is 30% and the five-year survival rate is less than 10%. The key to increasing the survival rate is early detection.

One early detection method that is under development is continuously monitoring various substrates in the human body that change and develop as cancer cells grow. These substrates are known in medicine as biomarkers. One such biomarker that can indicate the presence of cancerous cells is lactate. As cancer cells grow, the lactate levels in the human body skyrocket. As a point of reference, a human healthy body has a lactate concentration from 0.3 to 1.3 millimolar (mM) (Phypers, 2006). Patients with GBM, however, can exhibit lactate levels as high as 40 mM (Hirschhaeuser, 2011). Therefore, continuously detecting lactate in the human body could be an early warning signal to for cancer.

One approach to monitoring lactate levels in the human body is through an amperometric, enzyme-coupled biosensor. An amperometric biosensor is an electrochemical device in which a voltage is applied to a sensing electrode and an electrical current produced by the induced oxidation/reduction reaction on the electrode's surface is recorded. In order to detect lactate, the inorganic electrode material must be coupled with an enzyme specific to breaking down lactate. Lactate is not electrochemically active on its own; but when it is in the presence of oxygen and the enzyme lactate oxidase, it is broken down into pyruvate and hydrogen peroxide. While lactate is not electrochemically active, hydrogen peroxide can be oxidized on an electrode's surface. This oxidation reaction produces a current, which is linearly proportional to the
hydrogen peroxide's concentration, which is equal to the lactate level. In summary, the current recorded by the biosensor is proportional to the detected lactate concentration.

For the sensor to work in the application of detecting brain tumors, the biosensor must be compatible with the human body. Titanium is a known biocompatible material currently used in the medical industry for prosthetics and implants. Titanium also has the capability, under anodic oxidation, to form highly ordered nanotubes. Nanotubes have been widely studied in electronics, and have been proven to have high conductivity. Titanium dioxide nanotubes, however, do not have a high sensitivity towards hydrogen peroxide. Platinum nanoparticles, on the other hand, have a high sensitivity to hydrogen peroxide. Depositing platinum nanoparticles into titanium dioxide nanotubes would produce an electrode both sensitive to hydrogen peroxide and biocompatible with the human body.

The purpose for this Major Qualifying Project (MQP), a Worcester Polytechnic Institute (WPI) senior graduation requirement, was to develop the inorganic half of an electrode for a lactate biosensor. Enzyme immobilization techniques were not researched. This MQP focused on two main goals. The first, was to synthesize adequately sized titanium dioxide nanotubes. The second, was to deposit platinum nanoparticles into the nanotubes. The platinum-deposited nanotubes were then tested at different concentrations of hydrogen peroxide to determine their sensitivity. This work produced two platinum-deposited nanotube electrodes capable of detecting hydrogen peroxide. Interestingly enough, the two successful electrodes did not have the most uniform nanotubes or the highest density of platinum nanoparticles, as was expected. Both successful electrodes had nanotubes with slight carbon deposits on the surface and a surprisingly low level of platinum nanoparticles. More research would need to be completed into why this phenomenon has occurred. The following report will go in-depth into the background of lactate biosensors, methods used in this MQP, results obtained through experimentation, and recommendations for further research.
Background
The purpose of this section is to provide background information on the key topics addressed in this Major Qualifying Project (MQP). The long-term goal for this work is to create an implantable biosensor capable of rapidly detecting tumors using various biomarkers. Past MQP's have explored this topic, focusing on using either glucose or glutamate as the detection analyte. This MQP will be specifically focus on detecting lactate. As a case study, this MQP will look at the most common type of malignant brain tumor, Glioblastoma Multiforme (GBM), to analyze how the tumor grows and effects the human body. The overall design of this MQP will be an amperometric, enzyme-coupled, platinum-deposited, titanium dioxide nanotube electrode biosensor. Basic background on GBM, lactate, amperometric biosensors, titanium dioxide nanotubes, and platinum nanoparticles is outlined below.

Glioblastoma Multiforme (GBM)
Due to the varying types of brain tumors and their effects in the human body, it is almost impossible to generalize "brain tumors" as one entity. Every brain tumor is different, so developing a biosensor to detect all types of brain tumors is infeasible with current technology. With this in mind, for the purpose of this work, the most common malignant brain tumor was researched. This tumor is called Glioblastoma Multiforme, which is more commonly known as GBM.

There are currently more than 120 known types of brain and central nervous system tumors categorized by the World Health Organization (WHO) today. Tumors are often used simultaneously with the word, “cancer,” but not all tumors are considered cancerous. Tumors are defined as abnormal growths of skin and tissue that occur in the body (American Brain Tumor Association). They are sorted into two general categories depending of the severity of the tumor; benign tumors and malignant tumors. Benign tumors are often considered the "more desirable" tumors, because they do spread throughout the body. They grow where they were initially developed and are often able to be removed and treated more easily. Malignant tumors, also called cancerous tumors, have the ability to spread throughout the body. Cancer cells grow uncontrollably, destroying healthy tissue, and are often difficult to fully remove once developed (American Brain Tumor Association).
GBM accounts for about 50% of tumors classified as gliomas (American Brain Tumor Association). A glioma is a type of tumor that starts in the brain or spinal tissue. More specifically this type of tumor originates from glial cells, also known and supportive tissue cells, located in the central nervous system. According to the American Brain Tumor Association, there are three types of glial cells that can produce tumors; astrocytes, oligodendrocytes, and ependymal cells. GBM originates from astrocytes and are amongst the glioma tumors categorized as astrocytomas. Astrocytomas can be recognized by their unique and distinctive star-shaped formation (American Brain Tumor Association).

Astrocytomas can be further categorized based on their severity and growth rate. GBM is classified as a Grade IV Astrocytoma, meaning that it is a rapid growing malignant tumor. In fact, GBM tumors are known to fully develop in as little as three months. Interestingly enough, although GMB is malignant, it does not typically migrate to other parts of the body (American Brain Tumor Association). This means that once it is detected, the probability of finding cancerous growths in other areas of the body is slim.

There are two types of GBM tumors, de novo GBM and secondary GBM. De novo, or primary, is the most common type of GBM. This type of tumor arises quickly and aggressively and comes with many symptoms. Secondary glioblastomas have a slower growth rate but are just as aggressive as de novo GBMs. Secondary glioblastomas tend to represent 10% of the cases of GBM and eventually increase their growth rate. Unfortunately, the survival rate is extremely low for glioblastomas. The two-year survival of GBM is 30%, and the five-year survival rate is less than 10% (American Brain Tumor Association). The low survival rate is directly related to the aggressiveness of this type of tumor.

Detecting GBM
The primary cause of GBM, like many cancers, is unknown. Unfortunately, current detection methods are only successful once the tumor is partially developed and the patient has started to display symptoms. GBM symptoms are primarily caused by increased cranial pressure and include intense headaches, nausea, and drowsiness (National Brain Tumor Society). These are common symptoms of other, less serious, complications and are often not looked into unless more serious symptoms develop. These more serious symptoms include memory, speech, or
visual problems and are often directly related to the tumor growing (National Brain Tumor Society).

According to the National Brain Tumor Society, “GBM was selected as the first brain tumor to be sequenced as part of The Cancer Genome Atlas, a national effort to map the genomes of the many types of cancer” because of the tumor’s lethalness (National Brain Tumor Society). The most common methods for detecting GBM include magnetic resonance imaging (MRI), computerized tomography (CT) scans, and magnetic resonance spectroscopy (MRS) scans. MRI and CT Scans use a contrast dye to make the tumor visible (called enhancement) and are solely used to determine if an unwanted growth exists. The issue with MRI's and CT scans is that they cannot accurately tell the type of growth a patient has. MRS scans, on the other hand, measure the chemical and materials found in the tumor, and can more accurately identify the type of growth. These chemicals help determine what kind of tumor, and whether the it is benign or malignant (National Brain Tumor Society).

The major downfall of current detection methods are that they all require substantial testing and time. As stated above, GBM tumors are fast growing and can develop in as little as three months (American Brain Tumor Association) so early detection is key to increasing the survival rate. If a continuous detection method could be developed, with a similar to the reliability of MRS scans, the survival rate of patients with GBM could increase substantially.

**Biomarkers**

A recent approach to detecting diseases and abnormalities in the human body, such as GBM, is using biomarkers. The World Health Organization (WHO) defines biomarkers as “any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease.” Essentially, each disease has its own unique chemical makeup that can lead to a diagnosis and predict how treatments will be received. It can also predict the disease's aggressiveness and progression (Nicolaidis, 2015). Known GBM biomarkers include O(6)-methlyguanine-DNA-methyltransferase (MGMT) promoter and deoxyribonucleic acid (DNA) methylation, loss of heterozygosity (LOH) of chromosomes 1p and 19q, isocitrate dehydrogenase (IDH) mutations, as well as many other substances and indicators (McNamara, 2013). This area of research is constantly changing as more cases of GBM arise.
Although many molecular signatures associated with GBM have been recorded in literature, no one unique signature has been found. Therefore, developing a biosensor to automatically detect specific types of cancer would be difficult to do with current knowledge and technology. There are certain biomarkers, however, that could be monitored that would act as "red flags," prompting doctors to look for problems (such as tumors). These such biomarkers include glucose, glutamate, and lactate. As stated earlier, the primary focus of this work is lactate. While doctors would have to use traditional techniques to accurately locate the problem, biomarkers could detect problems earlier than individuals would start feeling the symptoms. The earlier the problem is found, the better of a chance for survival.

**Lactate's Formation in Healthy and Cancer Cells**

Before designing a continuous monitoring system for detecting lactate, it is important to understand the role of lactate in the human body. Lactate is the conjugal base of lactic acid. Lactate and lactic acid are often used interchangeably, but they are different substances. In aqueous solutions, like the human body, lactic acid dissociates to lactate and H+ (Phyers, 2006). Therefore, lactate is more likely to be present in the human body than lactic acid.

Lactate has two common isomers, L-Lactate and D-Lactate. L-Lactate is the most common in the human body and is the only isomer produced by the human metabolism. Conversely, D-Lactate can be formed only through artificial means (Dhup, 2012). The structure of L-Lactate, as shown in Figure 1 is comprised of a hydroxyl group and a carboxyl group. The excess production of lactic acid can increase the concentration of protons in cells and affect the buffering capacity of the human body. This can lead to a process called lactic acidosis (Gunnerson, 2015).

![Figure 1 - Structure of L-Lactate](image)
Research has shown that normal lactate levels in the body range from 0.3 millimolar (mM) to 1.3 mM when people are at rest (Phypers, 2006), but can reach as high as 12 mM during intense exercise (Rassaei, 2014). Lactate buildup can cause declined muscle performance, which can lead to lactic acidosis. Lactic acidosis occurs when there are low levels of pH in tissues and blood of the human body and when cells do not receive enough oxygen. Plasma lactate levels record lactate production and lactate use (also known as lactate clearance). An imbalance in lactate production and lactate clearance, which leads to lactic acidosis, can be used to help determine different types of diseases (Gunnerson, 2015). In order to understand why lactic acidosis is so detrimental to the human body, it must first be understood how lactate is formed.

**Lactate Formation in Healthy Cells**

Lactate is a product of the glycolysis pathway. Glycolysis in its simplest form can be defined as the breakdown of glucose in the human body (Phypers, 2006). Glycolysis, however, is a very complicated process as shown in Figure 2 below. Glycolysis begins when glucose diffuses into a cell's cytoplasm and is in the presence of the enzyme phosphofructokinase (PFK). PFK breaks down glucose into pyruvate and two molecules of adenosine triphosphate (ATP). ATP is frequently referred to as "molecular currency" due to its high importance in cellular functions (Phypers, 2006). While ATP is a product of glycolysis, pyruvate is a substance that can be further broken down and is a key intermediate in several metabolic pathways (Phypers, 2006). The further breakdown of pyruvate is completely dependent on whether it's in the presence of oxygen.
Under aerobic (oxygen rich) conditions and in the presence of the enzyme pyruvate dehydrogenase (PDH), pyruvate is converted to Acetyl-coenzyme A (Acetyl CoA) which can eventually be converted to 36 molecules of ATP. Because ATP is the energy cells need to function, this is the desired metabolic pathway of pyruvate. Under anaerobic (oxygen depleted) conditions, however, pyruvate is broken down into the less desirable lactate by a process called lactic acid fermentation. High levels of lactate (lactic acidosis) are toxic to humans and must be purged from the body. Low levels of lactate under the right conditions, however, can be converted back into glucose and complete the glycolysis cycle again. The red box in Figure 2 above outlines how pyruvate, under anaerobic conditions and in the presence of the enzyme lactate dehydrogenase (LDH), is converted into lactate (Phypers, 2006). This is known as the fermentation of sugar. Figure 3 below provides an additional representation of the formation of lactate.
Once lactate is formed, typically in muscle cells, it and makes its way to the liver. In the liver, the lactate can be oxidized back into glucose, which is referred to as lactate clearance. Because the production of lactic acid occurs in anaerobic conditions, decreased levels of oxygen in the cells can lead to increased levels of lactic acid in the body. This can cause excessive lactate production and can overwhelm the body’s buffering capacity. An imbalance of lactate production in the cells and lactate clearance in the liver can lead to a condition called lactic acidosis (Gunnerson, 2015).

**Lactate Formation in Cancer Cells**
In cancerous cells, however, the breakdown of glucose does not occur as stated above. A German medical doctor by the name of Otto Heinrich Warburg is credited with researching this phenomenon in the 1920s and 1930s (Heiden, 2009). Warburg observed that whether or not oxygen was present in cancer cells, glucose tended to be converted almost entirely to lactate (Heiden., 2009). As stated above, lactate is toxic to the body. If it cannot be adequately purged, then lactic acidosis occurs and can cause complications. This phenomenon is known as the
Warburg effect (Heiden, 2009). Figure 4 below outlines how glucose is broken down in healthy cells and tumor cells.

Figure 4 - Glucose Breakdown in Healthy Cells (left) and it Tumor Cells (right) (Heiden, 2009)

Increasing lactate levels, therefore, could indicate cancerous cells are forming in the human body. In fact, Warburg was quoted saying, "Cancer, above all other diseases, has countless secondary causes. But, even for cancer, there is only one prime cause. Summarized in a few words, the prime cause of cancer is the replacement of the respiration of oxygen in normal body cells by a fermentation of sugar" (Heiden, 2009). Following the GBM case study for this MQP, lactate levels for individuals with GBM are known to be as high as 40mM (Hirschhaeuser, 2011). Determining a way to monitor the concentration of lactate in the human body could indicate the presence of cancer.
### Biosensors

Biosensors are a detection method that could be used monitor lactate levels in the human body. A biosensor is an analytical device made up of a physicochemical detector and a biological component (Evtugyn, 2014). Biosensors are used for the detection of biological analytes, such as lactate. Extensive research has been conducted on determining the feasibility of creating biosensors to detect analytes for a variety of different diseases. This section will outline a brief biosensor history, the basics of how these biosensors work, as well as current problems with biosensors.

### Biosensor History

The development of the first biosensor is closely associated with the names of Leland C. Clark and Champ Lyons. Clark and Lyons created the first enzyme based glucose biosensor at the Cincinnati Children's Hospital in 1962. The idea for the first biosensor, however, preceded the 1962 glucose biosensor by a few years. Clark first proposed a probe to measure oxygen concentrations in the blood using an "enzyme electrode" in 1956. There has been much advancement in the development of biosensors since Clark and Lyon's contribution (Evtugyn, 2014).

Over the years, several definitions have been proposed for biosensors due to the rapid advancement and wide range of biosensors. In a 1992 article, Nagel defined a biosensor as "a device that uses specific biochemical reactions mediated by isolated enzymes, immune systems, tissues, organelles or whole cells to detect chemical compounds, usually by electrical, thermal or optical signals" (Evtugyn, 2014). As biosensors developed, their definitions have also developed. A 1999 article defines a biosensor as, “An electrochemical biosensor is a self-contained integrated device, which is capable of providing specific quantititative or semi-quantitative analytical information using a biological recognition element (biochemical receptor) which is retained in direct spatial contact with an electrochemical transduction element” (Evtugyn, 2014).

For the purpose of this MQP, a biosensor will be defined as an analytical device that incorporates a biological sensing element integrated within a physicochemical transducer (Setford, 2005). Figure 5 below demonstrates the extremely simplified scheme of a biosensor assembly. The basic design of the transducer (1), the biological recognition element (2), and the desired analyte (3) are further explained in the section below.
Biosensor Structure

The primary structure of a biosensor includes two main elements, the biological element and the transducer element. These two elements are connected to an electronic display that converts the signal into a readable format for the viewer. The biological recognition element (number 2 in Figure 1 above) detects the desired analyte (number 3 in Figure 5 above). The desired analyte can be any biological element, from tissues to nucleic acids to proteins. This is a selective element that should only detect the desired analyte. The biological element is what drives the selection of the transducer element (Koyun, 2013).

The transducer converts the biological event into an electrical signal. The transducer element can be electrochemical or biological. Electrochemical transducers include nanotubes and nanoparticles while biological transducers include enzymes other biological components. Transducers can be combined to strengthen the biosensor's signal. The signal is then amplified and sent to a display where it is processed into a readable format for the viewer to use (Koyun, 2013). There are many variations, as displayed in Figure 6 below, to the structure of biosensors.
Ideal Biosensor
An ideal biosensor has many attributes. A successful biosensor will be specific and selective towards the desired analyte and have a short detection times. Ideally, the biosensor will be small. If the biosensor is implanted, it is critical that it is compatible with the human body and requires no maintenance by the user. Long-term stability of a biosensor is also important. If the biosensor is implantable, it will be invasive to remove and calibrate in a controlled environment, so longevity is important. The final component to an ideal biosensor is that it is affordable. The materials it is made of and fabrication methods must be cost effective so a profit can be made (Koyun, 2012).

Current Lactate Biosensors
There have been many research efforts over the past few decades to advance the performance of lactate biosensors. Lactate sensors are useful for a variety of different industries, the most common being food processing and sports medicine. Recent studies have also shown these lactate biosensors can aid in detecting elevated lactate levels in patients with brain tumors. An article entitled, “Lactate Biosensors: Current Status and Outlook” by Rassaei, Olthuis, and Tsujimura outlines three common types of biosensors that are actively studied. These three types include amperometric lactate sensors, potentiometric lactate sensors, and optical lactate sensors (Rassaei, 2013).
Rassaei explains that, “Amperometric enzyme sensors rely on the measurement of current on application of a potential between working and referenced cells,” “Potentiometric lactate sensors measure the electrical potential difference between working and reference electrodes through a liquid junction in the absence of a current,” and “Optical sensors rely on the depletion of a reactant or the formation of a product from enzymatic reaction on the sensing surface.” Amperometric sensors, however, are the most commonly researched sensor because they have a relatively simple design (Rassaei, 2013). An amperometric design will be used for this MQP.

**Amperometric Lactate Biosensors**

Amperometric biosensors hold the working electrode at a constant potential while measuring the current generated from a reduction/oxidation reaction of an electroactive species. The current measured is proportional to the bulk concentration of the electroactive species (Grieshaber, 2007). Amperometric sensors have a number of advantages over other types of sensors for biochemical applications. The constant potential enables some selectivity as specific molecules will only react with the electrode in certain potential windows. This type of sensor is able to have fast response times as current is able to be continuously measured. Many protein analytes such as lactic acid are not able to be a direct redox partner with the electrode and therefore be coupled with another mechanism to produce one that is. One such mechanism is the use of enzymes specific to the target analyte.

**Lactate Biosensor Enzyme Selection**

The most common enzymes used for amperometric lactate sensors are L-lactate oxidase and L-lactate dehydrogenase. These enzymes allow for an easy sensor design and simple enzymatic reaction (Rassaei, 2013). For the purpose of this MQP, L-lactate oxidase will be used because of its ability to break lactate down into hydrogen peroxide ($\text{H}_2\text{O}_2$).

Lactate is not electrochemically active on its own; but when it is in the presence of oxygen and the enzyme lactate oxidase, it is broken down into pyruvate and $\text{H}_2\text{O}_2$. While lactate is not electrochemically active, $\text{H}_2\text{O}_2$ can be oxidized on an electrode's surface. See Figure 7 below for the enzymatic reaction for a amperometric lactate sensor using the enzyme L-lactate oxidase.
How Biosensors Detect Lactate

The oxidation reaction of $\text{H}_2\text{O}_2$ that occurs on the electrode's surface allows the biosensor to detect the lactate concentration by the current it produces. The current given off by the electrode is linearly proportional to the $\text{H}_2\text{O}_2$ concentration. Because the enzymatic induced reaction (Figure 7) has a one-to-one molar ratio of L-lactate and $\text{H}_2\text{O}_2$, the current recorded by the biosensor is also proportional to the detected lactate concentration. Therefore, the lactate concentration is also linearly proportional to the current given off by the electrode (Zhang, 2008).

In practice, a calibration curve must be made for each electrode. To establish this calibration curve, a three electrode cell is set up in a PBS buffer solution. PBS is commonly used in biological research because its properties highly resemble the isotonic conditions of the human body (Gray, 1983). The three electrodes in this cell include a reference electrode, a counter electrode, and the working electrode. The working electrode is the electrode being tested. A constant potential is applied to the three electrode cell and increasing levels of known concentrations of $\text{H}_2\text{O}_2$ are added to the PBS solution for a set period of time. As more $\text{H}_2\text{O}_2$ is added, the absolute values of the current increases as shown in the right graph of Figure 8 below. A $\text{H}_2\text{O}_2$ concentration vs current graph can then be plotted. This would allow users of the electrode to know the $\text{H}_2\text{O}_2$ concentration, and thus the lactate concentration, based solely on the current given off by the working electrode (Zhang, 2008).
Biosensor Design for this MQP

According to Rassaei, "Most research in the development of amperometric L-lactate biosensors has focused on electrode materials, enzyme immobilization strategies, mediators and coenzymes, improving sensor stability, and lifetime." As stated in the "ideal biosensor" section of this report, these are the several components of the ideal biosensor. The research of this MQP is mainly focused around the electrode materials. Using titanium dioxide nanotubes and combined with different types of nanoparticles is the type of electrode that is being researched in this MQP (Rassaei, 2013).

Titanium is a well-known biocompatible material. It’s used in many surgical implants today not only because its high strength but also chemical resistance (Mirabolghasemi, 2013). Titanium does not corrode as easily as other metals. Titanium has recently come under interest because of its ability to form predictable and unique surface structures from the oxide layer.

Nanotube Arrays

One property of titanium, of particular interest to biosensing applications, is the ability to form self-assembling nanotube arrays. These nanotubes can be formed cheaply using anodic oxidation of the TiO₂ outer layer. A popular method of growing these tubes is through anodization of titanium foil in an electrolyte solution. By adjusting the electrolyte solution, potential applied, and length of time, the nanotubes order, diameter, and length can be adjusted (Mirabolghasemi, 2013).
Electrochemical Etching Mechanism of Titanium

Electrochemical etching of titanium in an ammonium flouride/ethyene glycol solution happens in 3 main steps. The first stage occurs just after the potential is switched on. Ti at the surface is oxidized as electrons are released into the electrolyte solution. Hydroxide ions form as a result of the electric field. These hydroxide ions combine with the oxidized titanium ions at the surface. The resulting complex is then dehydrated, leaving behind a layer of TiO$_2$ (Přikrylová, 2016).

\[
Ti \rightarrow Ti^{4+} + 4e^-
\]

\[
Ti^{4+} + 4OH^- \rightarrow Ti(OH)_4
\]

\[
Ti(OH)_4 \rightarrow TiO_2 + 2H_2O
\]

As the oxide layer forms, F$^-$ ions from the solution dissolve into the oxide layer forming a soluable titanium flouride complex. This complex is then released into solution. This process continuously happens as the oxide layer and etching happen simultaneously at the electrolyte interface (Přikrylová, 2016).

\[
TiO_2 + 6F^- + 4H^+ \rightarrow TiF_6^{2-} + H_2O
\]

The authors of the 2005 paper, "Fabrication of titanium oxide nanotube arrays by anodic oxidation" provided good visual representation of this etching phenomenon. Their description and visual representation are shown in Figure 9 below.
TiO$_2$ Nanotube Structure

TiO$_2$ can have different crystalline structures: the brookite, rutile and anatase phase. For this MQP, the anatase phase will be described as it is more desirable. The anatase unit cell is octahedral, where each titanium atom is surrounded by 6 oxygens (Alivov, 2009). A theoretical calculation performed by Enyashin & Seifert found the anatase phase to be stronger and most conductive (Alivov, 2009).

The nanotubes by themselves give the material increased conductance over plain titanium, but don’t significantly enhance its catalytic properties. In amperometric biosensors, it is desirable for the electrode to be selective toward the analyte. This is done by using catalysts to shift the potential window of the desired reaction. The honeycomb structure of the nanotubes mentioned above provide an ideal surface for attaching catalysts onto or in the tubes.
Use of Platinum
Platinum is widely used catalyst because of its high catalytic activity and high stability. It also is a known catalyst for hydrogen peroxide (H₂O₂), which is of particular interest to this MQP. However, its price has significantly increased because of its wide use in the expanding fuel cell market (Song, 2011). A biosensor utilizing the catalytic benefits of Pt would also benefit from more efficient forms of Pt.

Using Pt as nanoparticles in conjunction with TiO₂ nanotubes has been found to give an electrode with good sensitivity toward H₂O₂. Pt is expensive compared to other metals and maximizing the area of usable active sites of Pt means that less material can be used for the same catalytic response. Pt nanoparticles have been formed as small as 1.5nm (Isaifan, 2013) while TiO₂ nanotubes can have diameters of over 100nm. This enables Pt to be situated to the inside walls of the nanotubes. Using a smaller required amount of Pt and locating this catalyst inside the nanotubes will be cost beneficial as well as retain the biocompatibility of titanium.

![Figure 10 - Model of Pt nanoparticles inside TiO2 nanotubes (Kang, 2008)](image)

In 2008 Kang, Yang, and Cai formed a biosensor by depositing 50/50 mix of 20nm Pt and Au particles onto the surface of a titanium nanotube array. They reported Pt having a good catalytic response to H₂O₂ at an action potential of -0.2V. They also showed that introducing Au nanoparticles increased the response of the Pt decorated electrode but did not partake in catalytic activity towards the detection of H₂O₂. Additionally, they were able to demonstrate success in detecting glucose by layering glucose oxidase on top of the nanotubes. Because the group did not use any extra immobilization techniques, one of their downfalls was longevity of the sensor.
After 25 days of being stored in PBS, the sensor only showed 72.58% of the original response values (Qing Kang, 2008).

\[ H_2O_2 \leftrightarrow O_2 + 2H^+ + 2e^- \]

**Cyclic Voltammetry**

The most popular method for defining electroactive behavior of an electrode is cyclic voltammetry. Cyclic voltammetry involves linearly ramping the potential of the working electrode up and down between specified values. The resulting measured current vs potential relationship enables user to determine quick estimates of oxidation and reduction potentials as well as direct estimates of reaction reversibility (Nicholson, 1965)

Cyclic voltammetry graphs are interpreted by the observation of peaks. A non-polarizable electrode forms a double layer as potential is ramped up. As negative potential builds up in one electrode and positive in the other, charges in the solution separate and migrate toward the oppositely charged electrode. This movement of charge is measured in the circuit as current. As the potential is ramped high enough, charges in the solution have to travel farther and against larger like-charge gradients. This causes a mass transfer limitation on current for a given scan rate. When the potential scan direction is reverses, the built up charges are released causing a sharp spike in current. Electrodes that do not interact with the solution still measure current and take on a shape like the figure below.

![Cyclic voltammogram of an ideal double layer capacitor (Hu, 2008)]
Electrochemical reactions taking place on the surface of the electrode receive or donate electrons from the electrode. This causes measurable current. When the potential of the working electrode becomes more than that of the activation energy, the reaction begins to take place. This is shown by the positive peak in the figure below. In this example a reversible reaction is shown. The reactants are all used up as the potential is scanned up past the onset potential of the reaction. On the way down, the reverse reaction happens as the electrode potential falls below its threshold. A truly reversible reaction with similar diffusion rates of both reactants and products will have a potential gap of about 60mV for a one electron reaction. (Gary, 1983)

The reaction being considered in this MQP is not reversible. We expect to see a single reaction peak. A buffer solution is also required to maintain a constant potential. This reaction produces H\(^+\), which is reduced on the surface of the electrode much faster and within the operating potential window. H\(^+\) reduction to hydrogen could cause peaks.

\[ H_2O_2 \leftrightarrow O_2 + 2H^+ + 2e^- \]
Methodology Section
The purpose of this section is to describe the methods used during experimentation in this MQP. Our project focused on two main goals. The first was to create adequately sized titanium dioxide nanotubes (TiO₂ NTs) as the base electrode material. The second was to successfully deposit nanoparticles into the TiO₂ NTs to increase the detection capability towards H₂O₂. The methods used to synthesize the TiO₂ NTs, deposit nanoparticles, and test the H₂O₂ detection capacity are described in detail below.

Preparation of Titanium Dioxide Nanotubes
The procedure for preparing TiO₂ NTs was provided by previous experiments conducted by former WPI graduate student, Zanzan Zhu. Zhu's method outlined four steps; pretreatment to the surface of the titanium foil, chemical treatment, anodization, and annealing. For this MQP, the synthesis of all nanotubes followed Zhu's work. The cleaning process following the anodization step, however, was slightly altered due to discovering unwanted substances the foil's surface. These unwanted substances, mainly crystallized carbon, were visibly seen under a scanning electron microscope (SEM). Their identities were later confirmed using energy-dispersive X-ray spectroscopy (EDX). SEM and EDX images and reports are further explained in the Results section and the Appendices section. Also see the "Results and Discussion" section for observations into how the post-anodization cleaning methods can affect the successfulness of synthesizing nanotubes.

Pretreatment of Titanium Foils' Surface
The first step of fabricating the TiO₂ NTs was initial surface polishing and cleaning. The purpose of this step is twofold; first, the polishing ensures a smooth surface for growing nanotubes. Second, the cleaning removes any dirt or foreign substances from the titanium metal surface. To begin, a sheet of titanium metal was cut into several small foils with the size of 1.5 cm by 2 cm, which is slightly smaller than the platinum electrode used in the anodization process. Next, foils were manually polished with sandpaper in order of increasing grit (220-400-800 grit) for 60 minutes (20 mins for each sandpaper grit).
After, the foils were cleaned sequentially with acetone, ethanol, and deionized (DI) water using an ultrasonic cleaner for 15 minutes each. Acetone and ethanol are both well-known substances that remove oil, grease, and dirt from the surface of materials. They are ideal cleaners for industry because they are inexpensive, safe to use, and are not regulated as much as other cleaners. DI water was used to remove any residual dirt, acetone, or ethanol.

Next, the polished and ultrasonically cleaned foils underwent chemical treatment. A mixed acid solution composed of 5 mL DI water, 15 mL 70% nitric acid, and 5 mL 50% hydrofluoric acid was made, resulting in a solution with a 1:3:1 ratio by volume. The pretreated titanium foils were immersed in the mixed acid for 15 seconds to remove the oxidative layer at the surface. A brown gas (nitrogen dioxide) was released, showing that the oxide layer had been removed down to the titanium metal.
The foils were then immediately submerged in DI water to complete the polishing process. The foils were further rinsed with DI water to ensure complete removal of residual acid. The foils were subsequently blown dry with an inert gas (nitrogen) and immediately placed into the electrolyte solution for anodization.

**Anodization**

Anodization consisted of a two cell electrode system, with the titanium foil as the cathode and a platinum mesh electrode as an anode. The titanium foil and platinum mesh were carefully immersed in a continuously mixed electrolyte solution composed of 97% ethylene glycol by volume, 3% of water by volume, and a 1% by weight addition of ammonium fluoride (NH4F). The titanium foil and platinum mesh were placed into the electrolyte solution approximately 3.5 cm away from each other and connected to a power supply. To ensure even growth of nanotubes, the foil and the platinum mesh were physically oriented in parallel to each other.

![Figure 15 - Anodization of Foil](image)

All foils were anodized for 30 minutes at potentials ranging from 20 V to 35 V. Potentials were varied to test the relationship with nanotube diameter. From our literature review and conversations with Xiaolin Lu, a graduate student we worked in parallel with, we hypothesized that increasing the anodization potential would increase the nanotube diameter.
After anodization, the foils were thoroughly cleaned with DI water and ultrasonically cleaned for various times between 0 to 5 minutes using either water or acetone. Foils were dried with nitrogen gas. We experimented with various cleaning methods as we aimed to perfect our TiO$_2$ NT synthesis technique. Ethylene glycol proved to be difficult to clean off the foils. These cleaning techniques are outlined in the Results section below.

**Annealing**
Finally, foils were annealed at 400 Celsius for 2 hours in a tube furnace. Foils were left to cool at room temperature naturally before any testing or imaging was conducted. Completed foils were stored individually in plastic petri dishes in a cool, dry location to avoid contamination.

**Analysis under SEM**
Once the foils went through the four above steps, they were viewed under a scanning electron microscope (SEM) to determine the successfulness of the nanotube synthesis process. A total of 10 foils were determined to be adequate enough to move onto the nanoparticle deposition procedure. The successful foils were cut in half (into an A section and a B section) to double the number of usable electrodes for nanoparticle deposition experimentation. SEM images of all foils, successful and unsuccessful, are provided for review in the Appendix.

**Electrochemical Deposition of Platinum Nanoparticles**
Pt was electrochemically deposited using cyclic voltammetry in a three electrode setup (Figure X). Ag/AgCl was used as the reference electrode, a platinum coil as the counter electrode, and the TiO$_2$ NT foil as the working electrode. For this MQP, based on our literature review and conversations with WPI graduate Xiaolin Lu, we decided to focus on altering the electrodeposition solution (chloroplatinic acid), rather than adjust the potential range or scanning rate. Lu had previously conducted research and found a promising potential range and scanning rate. Potential versus current graphs were recorded and saved during deposition.
Electrodeposition Method A
A 1 millimolar (mM) concentration of Chloroplatinic Acid (H2PtCl6) was used as the deposition solution with potential range of –0.4 Volts (V) to 0.5 V for 3 cycles. The scanning rate was set at 0.01 V/s. Once deposition was complete, the TiO2 electrodes were rinsed off with DI water and blown dry with nitrogen gas. The detection capability for the completed foils was immediately tested via cyclic voltammetry (see section below).

Electrodeposition Method B
For Method B, all conditions were identical to Method A, except the 1mM solution of H2PtCl6 was brought to a 0.5 M concentration of sulfuric acid (H2SO4).

SEM and EDX Post-Deposition Imaging
After the deposition process and cyclic voltammetry testing was complete, select electrodes were imaged via SEM and EDX to analyze the electrode's surface.

Cyclic Voltammetry Testing
The completed platinum-deposited TiO2 NT electrode's detection capability was tested using cyclic voltammetry. Cyclic voltammetry (CV) is an electrochemical technique that measures the current produced in an electrochemical cell. Additional information regarding CV testing is provided in the background section above.
All CV tests were conducted with same three electrode cell setup used in the electrodeposition procedure explained above. The conditions, however, were varied. The scanning rate was significantly increased to induce the desired H$_2$O$_2$ decomposition reaction. The voltage range was slightly varied, to allow for comparison to literature data. Foils underwent CV testing at two different points, before nanoparticle deposition and once it was complete. CV tests were performed in 0.01M phosphate-buffered solution (PBS) with a pH of 7.4, replicating the pH of a healthy human body. PBS is a common buffer used in biological research because its ion concentrations closely match the isotonic conditions of the human body.

![Figure 17 - CV Testing Set-up](image)

**Pre-Nanoparticle Deposition CV Testing**
Prior to nanoparticle electrochemical deposition, the TiO$_2$ NT foils that were deemed acceptable to move onto the deposition process were immersed in 17 mL of 0.01M PBS to establish a baseline potential vs current graph. The potential applied to the foil ranged from $-0.4$ V to 0.6 V and ran for 3 cycles. Please note that this is a slightly different voltage range than during deposition. The scan rate was set at 0.1 V/s. After a consistent baseline was determined for each foil, the foils detection capability of hydrogen peroxide (H$_2$O$_2$) was tested.

Each foil's reactivity was tested for H$_2$O$_2$ concentrations of 2mM and 5mM. As explained in the background section, H$_2$O$_2$ is a byproduct of lactate when broken down with an enzyme. Based on the reaction, the H$_2$O$_2$ concentration is proportional (1:1 molar ratio) to the lactate concentration. To simulate how this electrode would function in the human body, 2mM of H$_2$O$_2$ detected would mean approximately 2mM of lactate is in the bloodstream. A healthy human body typically
contains about 0.3 to 1.3 mM of lactate (Phyers, 2006). Our sensors, therefore, should be designed to detect analyte levels in that range.

Post-Nanoparticle Deposition CV Testing
After electrochemical deposition, the foils went through the set of baseline PBS, 2mM and 5mM \( \text{H}_2\text{O}_2 \) cyclic voltammetry tests again using identical potential ranges, scanning rates, and cycles as the pre-deposition CV test procedure. Some foils were tested at higher or lower concentrations of \( \text{H}_2\text{O}_2 \), depending on their detection potential. For foils that showed detection potential (a reaction peak), time versus current tests were conducted. These tests measured the current produced by the electrode as increasing concentrations of \( \text{H}_2\text{O}_2 \) were added to the PBS solution.
Results
This purpose of this section is to report the results from the experiments conducted in this MQP. The objective of this MQP was to develop a nanoparticle deposited titanium dioxide nanotube electrode that could eventually be coupled with an enzyme as a biosensor that and detect lactate levels in the human body. The development involved trying to enlarge nanotube diameters, which was evaluated with scanning electron microscopes (SEMs). The effectiveness of the nanoparticle deposited electrode was determined using cyclic voltammetry and amperometric experiments.

Titanium Dioxide Nanotube Synthesis

Introduction
The first step of our research was to create viable titanium dioxide nanotube arrays. The starting point for our experimentation was evaluating the procedure developed by former WPI graduate student Zanzan Zhu for fabricating TiO$_2$ NTs. During experimentation, we collaborated with another graduate student, Xiaolin Lu, who was also continuing Zhu's work. Lu observed that Zhu's TiO$_2$ NT synthesis procedure would not create nanotubes at a diameter large enough to deposit platinum nanoparticles into.

Based on our literature review and Lu's suggestions, we varied the anodization potentials. Literature suggests that anodization potential has a positive correlation to tube diameter. Experimentation revealed that while increasing the anodization potential seemed to have a positive correlation to tube diameter, the reliability of making usable tubes decreased. A wide range of nanotubes were synthesized; from uniform nanotubes, to pinched nanotubes, to nanotubes with carbon deposits on the surface, to no visible nanotubes. These morphologies are described in the section below.

Titanium Dioxide Nanotube Morphologies
A total of five batches of four foils were made during the course of this MQP, totaling 20 TiO$_2$ NT foils. Foils were named in a 0A-0B format, where A is the batch number and B is the foil number of that batch. So, 03-02 would be the second foil made in batch 3.

Each batch of foils was viewed under a scanning electron microscope (SEM) before the next batch was made. This was most useful when determining a good post-anodization cleaning
procedure (see section below for observations). Additionally, using the SEM images, common morphologies of the synthesized nanotubes were defined. These morphologies are summarized in Figure 18 below.

Figure 18 - Nanotube Morphologies
(A) Uniform nanotubes, (B) Cracking of nanotubes, (C) Carbon Deposits on Electrode's Surface, (D) Pinching of nanotubes.

Based on past research of nanotube based electrodes, the desired morphology for an electrode is uniform and clean nanotubes (Figure 18A). During experimentation, however, other morphologies were also observed. Sometimes nanotubes would not form adjacent to each other. Instead, they would form a "crack," or space, between each other. This morphology is shown in Figure 18B above. Another phenomenon also occurred during the fabrication of the nanotubes. Often the tubes that formed cracks would grow into a center point, like the tubes had been "pinched." An example of severely pinched tubes is shown in Figure 18D.
Crystal-like nanoparticles also appeared on the surface of the foils, as shown in Figure 18C. These particles were found to be carbon-based upon and investigation using energy-dispersive X-ray spectroscopy (EDX). The carbon is thought to have come from the residual ethylene glycol electrolyte solution used in the anodization procedure. The carbon was believed to be crystallized during the annealing process. Due to the high density of carbon deposits in our early experiments, we looked into alternative post-anodization cleaning methods. Observations from these cleaning methods are described in section below.

Post-Anodization Cleaning Process

Carbon Deposits on Nanotubes
Once the foils completed the anodization process, the ethylene glycol/ammonium fluoride electrolyte solution (EG/NH4F solution) needed to be completely cleaned off the foil before moving to the annealing process. This was observed in our second batch of foils. During this batch, we rinsed the foils off with DI water, then dried the foils off with nitrogen gas and repeated the process 3 times. We then subjected Foil 02-01 & 02-02 to 2 minutes of ultrasonic cleaning with DI water. Foils 02-03 & 02-04 did not undergo this ultrasonic cleaning step. This was to determine if ultrasonic cleaning was necessary. As shown in Figure 19 below, significantly more carbon deposits formed on the foils that did not undergo ultrasonic cleaning.

Figure 19 - SEM Images for Foils 02-01 and 02-03

EDX analysis confirmed that the particles were mainly made up from carbon. We hypothesized that the carbon was coming from residual EG/NH4F solution on the foil's surface not properly cleaned after anodization. In our third batch, we took more care in washing our foils. We rinsed
the foils off with significantly more water and increased the ultrasonic cleaning time from 2 minutes to 5 minutes.

![SEM Images for Foils 03-03 and 03-04](image)

**Figure 20 - SEM Images for Foils 03-03 and 03-04**

As shown in Figure 20 above, the foils have significantly less carbon deposits than the foils from the previous batch. Something interesting that we observed, however, was that the nanotubes were not uniform. Rather, they displayed morphologies of cracking and pinching. It was also observed that as the anodization voltage increased, the tube uniformity decreased. The next section will explore this observation further.

**Effect of Voltage on Nanotube Fabrication**

As stated above, post-experimentation SEM images revealed that higher voltages seemed to yield less uniform nanotubes. The extent to the decreased uniformity, however, varied from slight cracking and carbon deposits to severe pinching or no nanotube formation. We carefully evaluated the SEM images for all foils and determined whether they were "acceptable" to move onto nanoparticle deposition, or if they "failed."

We observed that as the voltage increased, the stability of the tubes decreased. This is depicted in Figure 21 below. In 2008, Alivov, Fan, and Johnstone showed that voltages in the range of 10-240V could produce successful nanotubes by changing the anodization solution. Their nanotubes were created using a glycerol solution, and it was found that as voltage increased NH₄F concentration had to be decreased for successful nanotube formation (Alivov, 2008). Changing the solvent has other benefits as well. Hsu, Yang, Teng, & Leu have found that by increasing the
ionic diffusion coefficient of the electrolyte, a high $\text{H}^+$ concentration is kept at the bottom aiding in chemical etching, while a protective layer is kept around the walls of the tubes (Hsu, 2010).

Figure 21 - Acceptable and Failed TiO$_2$ Nanotubes in Relationship to Voltage

One paper had similar results to our observations during experimentation. In this study, increasing the voltage past 20V was found to lead to rapid degradation of the successfulness of the tubes. This paper hypothesized that raising the applied voltage, "leads to rapid dissolution of titania which results in thinning and breakage of the formed nanotubes" (Zhou, 2005). The applied electrical field forces the Ti-O bond of TiO$_2$ to polarize, which weakens the bonds and promotes the dissolution of the metal oxide (Zhou, 2005).

The stability was also hypothesized to be decreased by ultrasonic cleaning. Visible discoloration patches were observed after the ultrasonic cleaning, as shown in Figure 22 below. These are thought to be collapsed nanotubes. Due to the limited time of this MQP, we decided to produce more successful nanotubes at lower voltages (20 & 25V) and further studies at higher voltages were not continued.
Tube Sizes Increase with Voltage Increase

The primary reason for increasing anodization voltage was to increase the nanotube's diameter. This relationship was found in many articles we read during our literature review. For instance, one study found the average nanotube diameters at potentials of 25V, 50V, 70V and 90V to be 45nm, 55nm, 65nm, and 75nm, respectively (Galstyan, 2011). Anodization occurred for 30 minutes in this study. Although this study used glycerol instead of ethylene glycol as the anodization electrolyte solvent, we expected to see a similar trend because both solvents play the same role in etching of the TiO₂ layer.

Another anodization parameter that could be altered to see its effects would be changing the anodization time. In this experiment, we anodized our foils for 30 minutes. During our literature review, we found a study stating that increasing anodization time was found to have little effect on the inner nanotube size (Zhao, 2005). Anodization time primarily affects the length of the tubes (Zhao, 2005). Due to limited time, we kept a set anodization time and changed the anodization voltages.

Using the SEM images, a range of diameters was determined for each foil. As predicted, on average, larger voltages produced larger nanotubes. A visual interpretation is depicted in Figure 23 below.
Nanoparticle Deposition

Adding catalyst nanoparticles was the last step in preparing the electrodes. Based on literature review, we looked for foils with uniform nanotubes with limited carbon deposits on the surface. Due to the high likelihood of human error, we used foils we determined to be non-ideal for our first experimental run to get acquainted with the procedure. We chose Foil 03-02A, due to its high density of carbon deposits and non-uniformity of the tubes.

Foil 03-02A showed H₂O₂ detection using deposition Method A. Unexpectedly, the nanotubes with the "ideal" morphology (Figure 18A above) did not show sensitivity to H₂O₂ using Method A. A foil with similar nanotube morphology (Foil 04-03B) to Foil 03-02A was proven to also have H₂O₂ detection capability. Method B did not produce foils with H₂O₂ sensitivity. Further analyses of these results are provided in the subsections below.

Working Electrodes Using Nanoparticle Deposition Method A

Two foils that were subjected to Method A deposition (Foils 03-02A and 04-03B), showed catalytic activity toward H₂O₂. Both foils started with nanotubes of about 25nm in diameter and carbon deposits on the surface. The nanotube morphologies are shown in Figure 24 below.
Tests in PBS and \( \text{H}_2\text{O}_2 \) with the nanotubes prior to deposition confirmed that hydrogen decomposition did not occur in the desired potential window on TiO\(_2\) NT alone, as shown in Figure 25 below. Both electrodes display a similar pattern in PBS and \( \text{H}_2\text{O}_2 \) but at different magnitudes of current.

![Figure 24 - Images of foils resulting in successful electrodes](image_url)

![Figure 25 - CV graph of Foils 03-02A & 04-03B before nanoparticle deposition](image_url)
During deposition, CV graphs were recorded for all electrodes. Foils 03-02A and 04-03B showed nearly identical scans, as shown in Figure 26 below. This consisted of a continuously cathodic current, even in the positive potential window. CV nanoparticle deposition graphs of non-working electrodes are provided later in this report.

![Figure 26 - CV of the 3rd Deposition Scan by Method A](image)

After Pt nanoparticles had been deposited, the same CV test as before was run to confirm the deposition of platinum and to determine platinum’s effect on the electrode’s electrochemical properties. Pt was confirmed with EDX (provided in Appendix A and C) and caused the electrodes to exhibit enhanced electrochemical behavior toward the decomposition of $\text{H}_2\text{O}_2$. A cathodic peak was observed at -0.277V for foil 03-02A and -0.217 for foil 04-03B, as shown in Figure 27 below. Only one peak was observed for each electrode because the expected $\text{H}_2\text{O}_2$ decomposition reaction is irreversible.
To further investigate the electrode behavior of 03-02A and 04-03B, the electrode potential was held constant at -0.277V (for Foil 03-02A) and -0.217V (for Foil 04-03B) and current was measured over time. H$_2$O$_2$ was dropped in every 100 seconds to increase the solution concentration by 5mM. The H$_2$O$_2$ was dropped in at the opposite end of the container from the electrode. A stir bar could not be used, because the spinning magnetic field imparted too much noise on the graph. The resulting graphs for this test are provided in Figure 28 below for Foil 03-02A and Figure 29 for Foil 04-03B.
Figure 28 - Foil 03-02A: H2O2 was dropped into a PBS buffer solution to raise the H2O2 concentration by 5mM every 100 seconds.

Figure 29 - Foil 04-03B: H2O2 was dropped into a PBS buffer solution to raise the H2O2 concentration by 5mM every 100 seconds.
Signal instability decreased as concentration increased, but reaction with H$_2$O$_2$ is again confirmed. Plotting concentration versus current, a linear trend was observed as seen in Figure 30 (for Foil 03-02A) and Figure 31 (for Foil 04-03B). This trend was observed in (Kang, 2012) and indicates a good lack of mass transfer limitation of H$_2$O$_2$ within these concentrations. In a biosensor setup, linear response is important for quick and reliable calibration.

Figure 30 - Foil 03-02A Concentration H2O2 vs Current

Figure 31 - Foil 04-03A Concentration H2O2 vs Current
Unsuccessful Foils Deposited with Method A
Many of the foils which platinum had been deposited onto by method A did not show reaction to H\textsubscript{2}O\textsubscript{2}. Four such foils starting with similar morphology are 03-02B, 03-03A, 03-03B, and 04-01A. Surprisingly, 04-01 had the morphology we predicted to have positive H\textsubscript{2}O\textsubscript{2} detecting capacity results. Shown in Figure 31 below are the SEM images of all three nanotube arrays, with the middle image representing both 03-03 arrays. Remember, the A/B designation represents two individual halves cut from the same foil after imaging of nanotube formation.

Figure 32 - Foil 03-02, 03-03, and 04-01 SEM Morphologies
Under CV in PBS and 10mM H\textsubscript{2}O\textsubscript{2}, all of the unsuccessful foils exhibited similar behavior, as show in Figures 33 and 34 below. One interesting note is that electrodes from the same starting nanotube arrays differ in behavior after deposition. This suggests the deposition performed was not consistent from foil to foil. Some of these differences could come from different angles of electrode orientation in the testing/deposition solution or one side of the electrode having an unequal amount of disrupted nanotubes. The working Electrode 03-02A was provided as a comparison in Figures 33 and 34 below.
Figure 33 - Method A electrodes in PBS after Pt deposition

Figure 34 - Method A electrodes in PBS + 5mM H2O₂ after Pt deposition
As shown in the figures above, electrode 03-02A exhibits much different electrochemical properties than the other foils subjected to the same deposition method.

**Deposition Graph Comparisons**

An interesting observation that came up during experimentation was the ability to predict the functionality of the electrode solely based on the nanoparticle deposition graph. A limiting current appeared for positive voltages in electrodes that did not work. A linear trend occurred for the electrode that did work (03-02A). This is further explained below.

The deposition curves of these foils, as shown in Figure 35 above, are similar, but exhibit slightly differing characteristics than that of the 03-02A electrode (the curve also representing the 04-03B electrode which both showed a differentiable reaction peak for \( \text{H}_2\text{O}_2 \)), represented by the dotted line. A similar shape is shown amongst the foils in the cathodic region of the graph, but the main difference lies near the anodic end. Successful electrodes showed an increased negative current here.

![Figure 35 - 3rd scan of CV for Method A deposition](image-url)
Cutting the foils in half gave us more to work with but also gave a way to check the consistency of the deposition methods given that not all foils available had the same morphology. It was expected that identical foils would behave the same electrochemically. Electrodes 03-02 A and B did not follow this expectation. The differences first occur during deposition. In Figure 36, Pt was not deposited in the same way as the A foil. Figure 35 shows that the B foil had deposition similar to the other foils that did not respond to H₂O₂.

Changes in morphology have been shown to change electrochemical behavior to some extent. However, even though the morphology of the NT array was the same for electrodes 03-02A and 03-02B, an unknown difference caused much more drastic changes in Pt deposition and subsequent electrochemical behavior.

**Adding Strong Acid to Deposition Solution**
Several studies have found that adding a strong acid, like sulfuric acid, to the weak H₂PtCl₆ acid improves the deposition of nanoparticles. Since H₂PtCl₆ is a weak acid, some of it dissociates in
water forming the negatively charged complex: HPtCl$_6^-$. Adding a strong acid would cause an equilibrium shift for the complex back toward its associated state.

\[
H_2PtCl_6 \leftrightarrow H^+ + HPtCl_6^-
\]

This neutrally charged compound would then be unaffected by the electric field and cause more H$_2$PtCl$_6$ molecules to collide with the electrode surface. The abundant amount of H$^+$ would also cause hydrogen evolution which would compete with Pt deposition. However, gas bubbles forming on the surface of the electrode was thought to cause more nucleation sites.

Figure 37 - Foils 03-02 and 03-01 before Pt deposition

Figure 38 - Electrode 03-02A with Method A and Electrode 03-01A with Method B
The foil deposited with the solution of 0.5M sulfuric acid shows a higher density of nanoparticles as the research suggests. On average, the size of the nanoparticles on this foil is also much smaller. Some are as small as 15nm (measured with SEM images), which may be small enough to fit inside the tubes.

Smaller nanoparticle size was thought to increase reactivity with H2O2 because of volume to surface area gains. When subjected to a CV test with H2O2, this electrode did not exhibit a reaction peak shift, shown in the figure below.

![Figure 39 - CV plot of electrode 03-01A](image)

An experiment was devised to directly determine if adding sulfuric acid to the deposition mixture was beneficial on clean ordered tubes. Foils 01-01 and 01-02 were cut in half to give an A and a B foil after initial CV testing. The A foils were treated with Method A and the B foils were treated with Method B. Below is are the SEM images of the nanotube arrays of 01-01 and 01-02
SEM images of these electrodes were not available after deposition, however a CV graph of both electrodes under 2mM H₂O₂ does not yield a shifted reaction potential.

Figure 41 - CV graph of electrodes in PBS and 2mM H₂O₂

N₂ bubbling
The first foil deposited with method A was successful, but the subsequent 5 runs of deposition resulted in no indication of H₂O₂ detection. We analyzed our methods to find possible ways the 1ˢᵗ deposition could have been run differently. One technique which was used in CV testing is
bubbling oxygen out of the testing solution. Oxygen is a reactive species and to eliminate some of its possible interference, nitrogen gas is bubbled in the testing solution to remove it.

To find out if oxygen present in the 1mM H₂PtCl₆ deposition solution affected the deposition process, N₂ gas was bubbled into 17mL of 1mM H₂PtCl₆ for 20 minutes before immediately being used in deposition.

![Figure 42 - Deposition of foil 04-01A in N₂ bubbled solution](image)

The deposition curve predicted that this foil would not be sensitive to H₂O₂. This is confirmed with the CV test shown in Figure 43 below comparing reactivity to H₂O₂ before and after Pt deposition. No shifted potential reaction peak is observed for the post deposition electrode. EDX confirmed the presence of Pt, and the Pt nanoparticle density and average size did not differ greatly from non-N₂ bubbled methods. The SEM images and EDX report are shown in the Appendix B.
Figure 43 - Foil 04-01A tested in 5mM H2O2 before and after Pt deposition

**Lifespan Analysis**

Pt can degrade over time. It is especially susceptible to CO poisoning. Cyclic voltammetry was performed on the successful 03-02A electrode 3 days and 83 days after nanoparticles had been deposited.

Figure 44 - CV graph of electrode 03-02A under PBS solution an 10mM H2O2
Not only did the size of the peak decrease but the definition of the peak at -0.277V diminished as well. In the human body the effectiveness of this electrode would decrease faster given that this electrode was only exposed to the air and DI water.

Table 1 - Current values of electrode 03-02A at -0.277V under PBS and 10mM H2O2

<table>
<thead>
<tr>
<th>Days After 1st Test</th>
<th>3</th>
<th>83</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent of Starting Current</td>
<td>85.30%</td>
<td>43.40%</td>
</tr>
</tbody>
</table>

Table 1 above provides a summary of the lifespan analysis for Foil 03-02A. After 3 days, the starting current decreased substantially. In order for this biosensor to be a viable product, the stability of the electrode must be further researched.
Conclusions and Recommendations
Ordered nanotubes of predictable diameter were able to be formed with a 30-minute anodization process. Experiments were performed to look at cleaning methods, but a clean morphology was not consistent across all voltages of anodization. Ultrasonic cleaning with DI water was found to minimize carbon deposits, but also decreased the stability of the tubes.

Two trends were noticed as a result of increasing anodization voltage: 1. Increased tube diameter 2: Increased likelihood of pinching or cracking of the tubes under ultrasonic cleaning. Average nanotube size could not be achieved greater than 45nm. The higher anodization voltage caused instability under cleaning. To achieve larger diameter tubes the electrolyte solution will need to be changed. A study conducted by Hsu found that by increasing the ionic diffusion coefficient of the electrolyte, a high H⁺ concentration is kept at the bottom aiding in chemical etching while a protective layer is kept around the walls of the tubes (Hsu, 2010).

Two electrodes successfully detected H₂O₂ with a linear current response to H₂O₂ concentration. Both foils started with nanotubes of about 30nm in diameter and with carbon deposits on the surface. Pt was successfully deposited using cyclic voltammetry in a 1mM solution of chloroplatinic acid. However, two electrodes cut from the same foil, did not exhibit similar deposition and resulting electrochemical behavior. This leads us to believe an untested variable is affecting how the Pt is being deposited that may be unrelated to nanotube morphology. Some of these differences could be the result of different platinum nanoparticle structures. The catalytic activity of platinum can change depending on its molecular structure (Esparza, 2008). Foils can have differences in nanotubes across its surface area. The morphology appeared to be mostly consistent to the naked eye across both halves, but SEM images could not be obtained for the whole surface. Not readily visible structural differences could therefore not be completely ruled out. More research or expert analysis needs to be done in order to understand why this happened.

As predicted, adding acid to the Pt deposition solution increased nanoparticle density and decreased average size. Even though none of the foils treated with acid reacted to H₂O₂, the issue with Pt deposition may not have been related directly to the acid.
We recommend that more consistent foil preparation methods be tried to achieve more consistently clean nanotube arrays. Surface treatment by hand has a lot more margin for variances than using a machine to mechanically polish. Additionally, electrolyte changes could give greater improvements in nanotube stability. Finally, we recommend looking more in depth into the structure of platinum deposited on the foils to find out if there are any variances across the electrodes, and if so, what variables in CV deposition cause them.
References


Appendix

Appendix: A EDX Report of Foil 03-02A
Appendix B: EDX report of electrode 04-01A
Appendix C: EDX Report of Electrode 04-03B
Appendix A: EDX Report of Foil 03-02A

Comment: GG0302A-1
Appendix B: EDX report of electrode 04-01A

Comment: GG-03182016-0401A-1
Comment: GG-03182016-0401A-3
Comment: GG-03182016-0401A-5
Comment: GG0403-B-3