2009

A Preliminary Study Of A Non-invasive Glucose Sensor Based On A Mercury Sensor

Erin Wood
University of Central Florida

A PRELIMINARY STUDY OF A NON-INVASIVE GLUCOSE SENSOR BASED ON A MERCURY SENSOR

by

ERIN LEIGH WOOD
B.S. CHATHAM COLLEGE, 2005

A thesis submitted in partial fulfillment of the requirements
for the degree Master of Science
in the Department of Chemistry
in the College of Sciences
at the University of Central Florida
Orlando, Florida

Summer term
2009

ABSTRACT

Diabetes mellitus is a potentially lethal disease that affects 7.6 percent of American people. In the US, it is recognized as the 6th leading cause of death. Failure to control blood glucose levels (BGL) in patients with either type of diabetes can lead to other serious complications as well, such as loss of limb, blindness and other health problems. Controlling and monitoring the BGL in post-op and intensive care patients in the hospital is also vital to their health. Currently the most reliable method of monitoring BGL is through an invasive procedure which monitors the amount of glucose in blood directly. A non-invasive glucose sensor would drastically improve the treatment of sensitive patients, and serve to improve the quality of diabetic patients' lives.

This glucose sensor is strongly based upon the mercury sensor developed by F.E. Hernandez and his colleagues. Glucose is used as a reducing agent to reduce mercury from Hg^{2+} to Hg^{0}, which will form amalgams with the gold nanorods in solution. The change in aspect ratio of gold nanorods leads to a change in the UV-Visible spectrum of the solution. The blue shift seen was measured and correlated with the glucose concentration of the system. The system was then tested varying conditions such as pH, temperature, gold nanorod concentration, and mercury concentration. A preliminary study of the kinetics of the reaction was also done.

The results showed a limit of detection of 1.58×10^{-13} and a linear dynamic range covering the concentrations of human tear glucose levels that are currently cited in the literature.

ACKNOWLEDGMENTS

I'd like to extend my gratitude to the University of Central Florida for supporting me as a graduate teaching assistant and as a graduate fellow. I'd like to think my advisor, Dr. Hernandez for encouraging this project and helping me to develop it. The lab group (Carlos and Leo, along with the others who visited briefly) was especially great and helped me through the process in more ways I can count. Carlos, Nikki, and Jenny in the stockroom have been indispensible in this process and I'll miss the great conversations I had with them.

I am forever indebted to my family for providing both the financial and emotional support I needed to complete this degree. Along with my family, my best friend and partner, Ti and his mother have also provided much needed support through the stress of being a graduate student.

Dr. Belfield and Dr. Campiglia have so graciously agreed to be on my board, even at the last minute, and I feel very grateful to have such a well rounded and knowledgeable board. And finally, I wish to thank those who have helped me develop throughout my academic career. While they are scattered throughout the country, the most notable ones are Dr. Viehland, Dr. MacNeil, Dr. Fisher (RIP), and Dr. Stilts at Chatham University (it will always be Chatham College to me!). At UCF some of my most influential professors have been Dr. Miles and Dr. Clausen. They have both come through for me in more ways than I could ever count and gone above and beyond their duties as professors to encourage my success.

TABLE OF CONTENTS

LIST OF FIGURES

1. INTRODUCTION

Diabetes mellitus is a common disease with no cure. Diabetes affects 7.6% of the American population with nearly 18 million people diagnosed with the disease. Another estimated 5.7 million people are living with undiagnosed diabetes. In 2007 over a million and a half American adults were diagnosed with diabetes. [1]. It is estimated that the frequency of diabetes is increasing rapidly world wide with an estimated 300 million people worldwide having the disease in the near future [2] Patients with diabetes must monitor their blood glucose levels (BGL) on a daily basis. Some must monitor their BGL up to 4-6 times a day. Failure to keep track of trends in BGL and uncharacteristic spikes or drops can lead to extremely serious complications such as loss of limb, eye sight, and nervous system damage

Diabetes is divided into two types. Type 1 diabetes affects 5-10% of all patients that exhibit this condition. Type 1, commonly referred to as juvenile diabetes, usually occurs at a young age, although this is not always the case. It is characterized by the body's immune system destroying pancreatic beta cells. This results in the patent losing the ability to naturally produce insulin. Insulin is the hormone that regulates glucose in the blood. Type I diabetes is often referred to as potentially curable. On the other hand incurable Type II diabetes is characterized by the way the body uses the produced insulin. Type II diabetes is usually onset later in life, however, it is also being seen in increased prevalence in younger people due to the increase in obesity. Type II diabetics may or may not be dependant on insulin pumps or injections, but they must monitor their BGL carefully in order to avoid complications related to the conditions of hyper and hypoglycemia. [3] There is also gestational diabetes, which is a condition similar to type

II diabetes that shows up in pregnant women. It may go away after deliverance of the child, however, the mother will forever be at increased risk of developing type II diabetes later in life.

However the need for monitoring blood glucose levels goes beyond diabetic patients. In a hospital setting, BGL levels are often monitored after surgeries, trauma, and in infants. The patients are often regarded as being at increased risk for the conditions of hyper and hypoglycemia, even if they are not diagnosed as diabetes. While the methods used for this are minimally invasive, they still require some degree of invasion. [4] Being able to non-invasively monitor glucose levels would increase the quality of care for these patients by a great deal.

Current non-invasive techniques, such as urine analysis rely on sensing the glucose concentrations in complex matrices such as interstitial fluid and urine. Because of the complexity of these matrices, there exists room for many false positives. [3-5] In fact, urine analysis itself is often regarded as too unreliable. Analysis for glucose in the urine is qualitative and used as a prescreening for diabetes and as a monitor of BGL control. There is, to the best of current knowledge, no non-invasive glucose monitoring technique that can be relied solely upon without any other calibrations (i.e. finger pricking). False positives and negatives will cause the user of these non-invasive techniques to confirm the results with a blood test before taking corrective action. In fact, the first non-invasive glucose sensor to achieve FDA approval in the US has since been discontinued. When Animas Corp.. bought out Cygnus, manufacturers of GlucoWatch, in 2007 they discontinued sales. Currently, there is no information on whether the GlucoWatch will be reinstated [6]. There was a host of problems associated with

GlucoWatch such as needing to use finger pricking for calibration, but it was a step in the right direction. Problems with the GlucoWatch and other potential non-invasive sensors will be discussed in the next chapter.

The method of glucose monitoring proposed herein will require no calibration, that is, no blood testing before use. Tears are a much less complex matrix than interstitial fluids, but still a rather complex matrix. Tears are primarily composed of water, but also have electrolytes, proteins, lipids, mucins, defensins, collectins, and other small molecules.[7] Glucose would be classified in tears under "small molecules". There is a lot of disagreement in the literature over the concentration of glucose in tears, and also the correlation of tear glucose to blood glucose. The median values are between 110 and 280 μM. [8]

Because of the low concentration of glucose in tears, it is imperative to have a sensitive method of detection. Gold nanorods, in particular, lend themselves to being used as sensors because of their UV-visible (UV-Vis) absorption. Gold nanorods have two absorption bands which correspond to the transversal and longitudinal dimensions. The transversal band is not sensitive to aspect ratio and matrix dielectric constant changes. The longitudinal band however, is very sensitive to changes in the aspect ratio and surroundings. [9] This band can be found anywhere between the visible (>550 nm) and the NIR region.

There have been several sensors based on gold nanoparticles including some very sensitive assays. Detection of glucose, viruses and other biologically important molecules has been reported in varying degrees of sensitivity. The reports of using nanoparticles as glucose sensors even [10, 11] however, they are based on aggregation of nanoparticles.

This method requires a functionalization of the surface of the nanoparticle which is specific to the analyte of interest. Because it is dependant on the aggregation, it is not quite so easy to control and depends on the smallest size of nanoparticles that can be made.

The glucose sensor proposed herein however is based on a mercury sensor developed in 2006 [12] which showed great sensitivity. It is well known that elemental mercury will form amalgams with bulk gold spontaneously. Hernandez et al, extrapolated this to the nanoscale. Mercury ions could be reduced with a strong reducing agent and form amalgams on the tips of the gold nanorods which will effectively change their absorption spectra. A blue shift occurred with mercury in the system and the system was sensitive to the parts per trillion levels. Instead of a strong reducing agent such as sodium borohydride, glucose would be used to reduce Hg^{2+} to Hg^{0} which could then form amalgams on the tips of the nanorods. The amount of amalgamation then is proportional to the amount of reducing agent, if mercury is in excess. Thus, the absorption spectra will exhibit a blue shift based on the amount of glucose present. Unlike aggregation based nanoparticles, the sensitivity of the sensor is not so reliant on size; however, it is reliant on the ability of the detector of the spectrophotometer to sense the subtle shifts of the plasmon resonance band.

This is only a preliminary study. There is much more work to be done on this project, which will be discussed in the final chapters of this thesis.

2. BACKGROUND

2.1 Current State of Non-Invasive glucose sensing

The ability to know what's happening inside of a human without opening them is a goal that many people have sought out. And while bones and tissues are now visible through the use of electromagnetic radiation, there has yet to be a way to tell what is in the blood or cellular fluid without physically extracting it. For most, this is not a problem. However for diabetics who must know constantly what their BGL this becomes a painful and tedious procedure which may lead to non-compliance. Over the past twenty or so years there have been a number of ideas proposed for non-invasive glucose sensing, however, there are currently none on the market in the USA today.

The ability to non-invasive glucose sensing (NIGS) is an incredibly attractive goal for diabetic patients. A typical diabetic will test their BGL four or more times a day. There have been several attempts to achieve this goal and most of them have failed for various reasons. This chapter will attempt to clarify some of them and assess their advantages and disadvantage.

Glucose sensing can be classified as continuous, semi-continuous, or non-continuous. As Jim Brauker wrote in his article "Continuous Glucose Sensing: Future Technology Developments" a "snapshot" cannot give a truly clear picture and had shown a photograph of a soccer ball midflight proposing the question "Which way is the ball going?" While it's possible to guess from a picture, it's impossible to know. Diabetics could see a moderately high or low number through a non-continuous method and not know if they're in for a period of more severe numbers, or if it is mediating back toward normal levels. The ultimate goal would be a glucose sensor which is both non-invasive,

and continuous, although perhaps tackling both issues has proven difficult as shown in the following examples

The GlucoWatch which was FDA approved in 2001 for adults and 2002 for children, offered a lot of hope to diabetic patients who relied on finger pricking techniques to check their glucose levels. The device measured the glucose levels in interstitial cellular fluid via electrical conductance. This is known as reverse iontophoresis. When a low level current passes through the skin it draws through ions from the interstitial fluid such as sodium. Through electroosmosis glucose is also carried. Discomfort from the electrodes was a common side-effect. Excitement from the GlucoWatch has slowly died down as it was realized that it still needs to be calibrated every day with a traditional BGL meter, and caused the user more discomfort. However, being able to continuously monitor ones BGL through the day while it was calibrated was an advantage to diabetic patients. Along those lines, there are also implantable sensors that will measure the glucose levels in interstitial fluid and send the data to a monitor. These implantable devices are meant to be used for only 1-2 days and disposed of. One final disappointment has been the lag-time between a spike in BGL and cellular fluid glucose, which has been cited to be up to half an hour. Other downfalls of the system were caused by the instrument shutting off randomly, and if the user was sweating it was unreliable. Also the two hour warm up time was frowned upon. [5] However when the company was purchased in 2007, the GlucoWatch has been taken off the market, and there is currently no news on if it will return.

Performance of diabetic testing devices is compared on a graph known as the "Clarke Error Grid". This graph plots the glucose values obtained from the device against

the traditional blood test glucose levels. Of course, a perfect match would form a curve with the formula y=x. Since no method is perfect, one talks about different "zones" on the CEG. While it is not too important to discuss the technicalities of this testing method, it is worth mentioning that as the letter increase it is "worse" for the sensor. Zone E, the highest error zone is the area where a patient may mistake hyperglycemia for hypoglycemia or vice versa. The GlucoWatch, in laboratory studies, had over 90% of the points in the clinically acceptable zones (A+B).

GlucoWatch is not the only non-invasive technique to be approved of and then later withdrawn from the market. Other such devices include the Pendra and Diasensor both were granted the CE mark. Diasensor used near IR (NIR) spectroscopy to sense glucose. [5] Problems with this include confoundation of signal with other NIR absorbing materials such as water, hemoglobin, proteins, and fats. Also the signal is made unreliable by hydration, blood temperature and temperature and amongst other factors. Diasensor was also declared inadequate at hypoglycemic detection, and was only a "snapshot" device. [6] The company that produced Diasensor is now out of business and the product has been taken off the market [5].

The other device, Pendra is based on impendence spectroscopy. This essentially measured the dielectric constant of interstitial fluids at varying frequencies. Glucose effects the sodium and potassium ion concentrations in the body, which will in turn cause a variance in the red blood cell potential. Even so, this is only estimated through the dielectric spectrum and not measured directly. It is adequate however to warn of high or low BGL. In the laboratory, Pendra performed quite well, but when it was released 4% of the points determined by human testing, fell in the most dangerous zone E of the CEG.

There are many other technologies being looked at today for NIGS. These can be spectrographic methods such as near IR, mid IR, fluorescence and Raman. There are also non-spectrographic methods such as metabolic heat methods being looked at. However, one of the downfalls of most of the methods is they do not look at how glucose interacts with a test matrix directly, but how glucose effects a very complex matrix such as cellular fluid, urine, or even blood. Because they are looking at such a complex matrix, there exists room for much error.

As stated in the previous chapter, tears are probably the most simple of the bodily fluids that can be tested. They also have the advantage that they are accessible readily without cutting open the body. Lachrymal fluid is a relatively simple matrix consisting of primarily water and salt. There are some other compounds that are present to a much lesser extent, such as glucose. Of important note though is that it seems collection method of tears will vary the glucose levels found. Also, tears will begin to decompose over time. Even bringing this into account they are perhaps most attractive option.

This is not the first research into a tear based glucose sensor. Lakowicz et al have investigated glucose sensors. They have proposed a contact lens which uses boronic acid derivatives which are florescent but can been quenched by glucose molecules. Due to the shift in florescence, the glucose levels can be detected. However this is minimally invasive at best, as the subject still needs to wear contact lenses. [19-21] One relied on the aggregation of nanoparticles that were functionalized with dextran which caused aggregation with concavilin A. As glucose could displace con-A, the aggregation would cease in the presence of glucose which would cause a blue shift in the plasmon resonance bands.

There was also an attempt by Yang et al [22] to use gold nanocomposites (gold nanoparticles and polyanaline) to sense glucose. The nanocomposites had a boronic acid protecting group, which like with the contact lenses was fluorescent in the absence of glucose but not in the presence. This suffered from problems such as quenching from the heavy atoms such as fluorine in human bodily fluids.

2.2 Gold nanoparticles

Like the two groups discussed above this project is focusing on the use of gold nanoparticles for sensing. There are numerous reports in the literature about the sensing capabilities of gold nanorods. [14, 25-27].

It is well documented that the bulk properties of materials at the nanoscale are different than the bulk materials. [9]. Of interest to this project is the appearance of plasmon bands in the UV-Vis spectra of gold nanorods. Gold nanorods can be thought of as two dimensional objects. Due to the ease of electron movement through the gold at this scale there is a strong absorption of electromagnetic energy. One corresponds with the flow across the diameter of the road, and this is usually at 535 nm. Also gold nanospheres exhibit one plasmon band at this wavelength. However, due to the longitudinal mode there is also a longitudinal plasmon band, which is very sensitive to the length of the nanorod. As the rod is drawn out longer, the plasmon band is red shifted, and can be seen even in the infrared wavelengths. Likewise shorter ones will absorb nearer to the transverse band. Because of this, the size of the nanorod is easily extracted from the UV-Vis spectrum so that TEM images are not needed.

Based on the mercury sensor published in 2004, it was known that the ideal nanorods would have a longitudinal band between 630 and 700, which corresponds to low aspect ratio nanorods (<3).

3. RESEARCH GOALS

3.1 Main Goal

To find a glucose sensor this has a linear dynamic range the covers the accepted biological glucose levels in tears.

3.2 Specific Goals

- To find the appropriate matrix for the gold nanorods to encourage to oxidation of glucose and reduction of Hg^{2+}
- Calibrate the system for glucose detection for biological levels in tears
- Study the effect of pH on the sensor's capability
- Study the effects of temperature
- Study the effects of time

4. EXPERIMENTAL

4.1 Instruments and chemicals

4.1.1 Chemicals

Silver nitrate (99% pure), hydrochloric acid, acetonitrile, and tetra chloroauric acid (99%) pure were purchased from Fisher scientific. Sodium borohydrate (99%), hexadecyltrimethyl ammonium bromide (CTAB, 98%+ pure), and L-Ascorbic acid (99%) were purchased from Sigma-Aldrich. All chemicals were used as received and not further purified. The water used for all synthesis was purified.

4.1.2 Instrumentation

All UV-Visible absorption measurements were taken with a USB-2000 Ocean Optics portable UV-Vis spectrophometer equipped with a white light LED and a spectral range of 450 to 750 nm. Also Agilent 8453 spectrophotometer equipped with tungsten and deuterium lamps and a range of 190 to 1100 nm. The LED of the Ocean Optics was passed through a neutral density filter to cut intensity across all wavelengths. A quartz cuvette with a pathlength of 1 cm was used unless otherwise noted. Both spectrophotometers were allowed to warm up for 20 minutes before using and blanked with either DI water or 0.1 M CTAB as appropriate. Cuvettes were washed with water in between trials, and acetone as needed. Blanks were run to check for instrument stability intermittently.

In trials that required the cuvette to be held at a constant elevated temperature a cuvette heater with a pathlength of 0.5 cm was used. This metal apparatus was controlled through a peltier-DC power supply

4.2 Generation of Gold Nanoparticles.

Gold nanoparticles were synthesized from a modified seed mediated method proposed by Jana et al [13] The modifications include elevating the temperature and adding hydrochloric acid to the growth solution [14].

All glassware was cleaned prior to use first by soaking in sulfachromic acid. This ensured there were no organic residues left on the glassware. The glassware was then washed with acetone and then tap water. It was then cleaned with *aquaregia* to ensure all traces of metals were removed. Glassware was then rinsed with purified water, and non-volumetric glassware was dried in the oven at 80°C. Volumetric glassware was left to air dry. Acid washed glassware was stored in a cool and dry cabinet which was not opened except for moving it. Clean glassware is essential to the successful synthesis of gold nanoparticles.

Gold nanospheres of 2-3 nm diameter, for use as seeds, were synthesized by adding 0.250 mL of 0.01M $HAuCl_4$, to 10 mL of 0.1 M CTAB. Then 0.6 mL of 0.01M $NaBH_4$ was added to reduce the gold in the solution. Successful reduction of the gold turned the solution from dark yellow-orange to brown. Upon aging the solution became pink. The seed solution was allowed to sit for at least 10 minutes before using to ensure that all of the sodium borohydride had decomposed.

The growth solution began with 10 mL of 0.1 mL CTAB and 0.500 mL of 0.01 M $HAuCl_4$. To this 0.01 M of silver nitrate was added in varying volumes to for size control. The volume most often used was 0.075 mL. This volume created nanoparticles of aspect ratio 1.6. This solution was allowed to sit for 5 minutes before 0.200 mL of 0.1 M hydrochloric acid was added. After this, 0.1 mL of 0.01 M L-Ascorbic acid was added to

the dark orange solution. 0.200 mL of 0.1 M HCl was added to the growth solution. This kept the pH slightly lower than typical synthetic methods, which helped when mercury (II) chloride was added in the subsequent experiments. Upon gentle agitation, the solution became colorless as Au^{3+} was reduced. 0.025 mL of the aged seed solution was quickly injected into the growth solution, and it was allowed to be heated at 70 degrees Celsius for 10 minutes. After 2 minutes, the solution became colored, indicating the formation of gold nanorods. After 10 minutes of heating, the reaction had come to completion and the gold nanorods were very stable for several months.

Before use in the experiments the gold nanorods were centrifuged. Centrifuging offers three main benefits to this experiment: concentration of gold nanoparticles, removal of excess CTAB and a more narrow size distribution. The calculated particle density of gold nanorods in solution is 10^{14} particles/mL, assuming 100% yield. The particle density in experimental solutions is calculated based on Beer's law.

4.3 Development of a testing solution

The first trials of the testing solution (everything except for glucose) were done similarly to that of a thesis work done by Marisol Garcia in 2006. [16] Her work showed that glucose was much better at reducing metals in a solution of pH 11. She achieved this pH through use of a 1% ammonium hydroxide solution. However, the addition of ammonium hydroxide to the testing solution of mercury and gold proved to form a precipitate, even without glucose. This precipitate was most likely mercury hydroxide. The reaction was then attempted with only gold nanorods, mercury, and glucose. While this reaction is slightly favorable, it was not seen most likely due to the slow kinetics of the reaction.

In order to increase the kinetics of reactions the solvent is often changed. A commonly used solvent is acetonitrile; however this was known to affect gold nanorods. [16] There are currently no literature articles, to the best of knowledge, that deal with a mixture of acetonitrile and water. Acetonitrile has a high dielectric constant and a high solvating capacity for $HgCl_2$ which made it an attractive option. It was also noted that as a solvent "the oxidizing properties of mercury ions should be manifested to a greater extent". [17] As long as the concentration of $HgCl_2$ was 0.001 M the volume of acetonitrile and $HgCl_2$ was kept the same. The basic experiment consisted of 1.0 mL of gold nanorods (10^{13} particles/mL), 0.200 mL of 0.001 M $HgCl_2$, and 0.200 mL of acetonitrile. The acetonitrile was added because mercury is much more easily reduced in acetonitrile than in water. A varying amount and concentration of glucose was added, and then the system was allowed to heat for 30 minutes at 80-90 degrees Celsius on a hot plate.

4.4 Effects of Glucose Concentration

The experiments to find the linear dynamic range began making a bulk testing solution and putting 1.300 mL into a 5 mL glass sample vial. If a white precipitate was noticed (insoluble mercury bromide because of excess CTAB) the solution was either heated, or diluted slightly with water and acetonitrile. Measurements were taken at 23 degrees Celsius, unless otherwise noted.

The first series of tests involved adding 0.100 mL of a variety of glucose concentrations to the solution and measuring the shift in the longitudinal plasmon band. A trial of adding small aliquots to the same testing solution over time to see if the band shifted upon each addition of glucose was also tried. After data was obtained the blue shift was plotted against the concentration of glucose added. Following this, the range of

glucose concentrations was narrowed, and a much more realistic volume of 0.010 mL of glucose solutions were added. It may be slightly unrealistic to hope for consistent collection of 0.100 mL of tears for one sample, but according to literature reports, 0.01 mL is very realistic. [7, 8] The solution was then heated to 80 degrees Celsius for 30 minutes after the addition of glucose. The solutions were allowed to cool to room temperature before measuring the spectra. Blanks were also taken with the same volume of water added as the volume of glucose added to the sample. One blank was taken for every 5 samples, as the hot plate could accommodate 6 vials.

There were over 40 blank samples in the end, and the last 40 (chronologically) were used to find the limit of detection of the system.

4.5 Kinetics

The kinetics of the system were monitored through the Ocean Optics spectrophotometer and the Ocean Optics InfoBase software suite which included an option for measuring the kinetics. It was known from the previous experiments that the reactions would be completed within one hour. A UV-Vis spectrum was taken every 30 seconds for 90 minutes to ensure that the reaction was truly completed. The cuvette was held at a constant temperature of 80 degrees Celsius during the measurements.

4.6 Varying the Conditions

Conditions were varied to see how the sensor would perform under different conditions. The conditions varied are the pH, the temperature that the vials were heated to, and the amount of gold nanorods in solution.

4.6.1 Varying the pH

The best sensors are those that can be used in a variety of conditions. It was discovered early on in experimental trials that this sensor cannot be used in basic conditions, which is a disadvantage. The primary reasoning for using the sensor in basic conditions was to push the reduction potential of gold from -0.05 V to 0.6 V. This would have made the reaction of gold reducing mercury extremely favorable, however, because mercury (II) chloride typically cannot be in bases because of precipitation reactions (such as $Hg(OH)_2$), or reduction reactions (such as ammonium hydroxide, the base of interest), it cannot be done.

However, it is slightly advantageous to have the pH at neutral or slightly lower than neutral. According to the literature, tears of healthy normal people are slightly more acidic than neutral (pH=6.94). The pH of the testing solution was natively just below this, with a median value of 6.5 +/- 0.4 pH units, measured with a Fisher Scientific brand pH meter. The acid used to alter the pH below these levels was carefully chosen to be hydrochloric acid based on the possible precipitation reactions that could happen .Chlorine anions were already present due to the mercury (II) chloride. Concentrated hydrochloric acid was diluted to 1.0 molar, and added to adjust the pH to 3 and 4.5, and no other alterations were made to the growth solution. A small amount (10 uL) of glucose was added to each sample vial, kept consistent for each pH value. The glucose

concentration of 0.025 M was chosen because it would produce a visible, but small shift. If the pH would have been found to affect the sensor, it would have been noticeable.

4.6.2 The effect of gold nanorod concentration

The effect of the concentration of gold nanoparticles relative to the solution was also studied. The synthetic technique used produced nanorods in a concentration of around $5x10^{14}$ particles/mL, assuming 100% yield. TEM images of other particles produced by the same method have shown a strong uniformity of shape and size, which is also confirmed through the UV-Vis spectra. While TEM images of these specific nanorods were not taken, it can be assumed that the same for these.

The absorbance intensity of the raw gold nanorods was noted before centrifuging. Using Beer's law, $A=\varepsilon bc$, the molar extinction coefficient was calculated. The gold nanorod solution was then centrifuged. The pellet at the bottom of the tube was then redispersed in a volume of water to bring the concentration down to the original concentration. To verify that this was true, the absorbance intensity was checked against the original solution. Following this the solution was diluted 1:1 in water. The resulting dilution was diluted yet again by one half to bring the concentration in the third solution to ¼ of the original. This solution was also diluted to be 1/8 of the original. No more dilutions were done due to the fact that the absorbance maxima were not distinguishable from the instrument noise with the Ocean Optics UV-Vis spectrophotometer.

4.6.3: The effect of maximum heating temperature

The reaction was not favorable at room temperature. Solutions were left over several days with no noticeable shifts. In order to make the reaction usable for nearly real

time glucose sensing it was found that the reaction needed to be heated. All reactions were heated to at least 80 degrees Celsius on a hot plate in the previous experiments. The effect of temperature was therefore studied to find an optimal temperature. For this experiment, temperatures below 80 degrees Celsius were controlled through a cuvette heater and a peltier-DC power supply. Temperatures above this were unattainable with this device. The samples that were heated to 80 degrees or above were done with a water bath and a hot plate.

The samples were heated for 45 minutes to ensure that the reactions had come to completion. Room temperature was measured to be 21.5 degrees Celsius. Both blanks and samples with analytes were left out at all temperatures. The other temperatures that were used were 40, 60, 80, and 100 degrees Celsius. The samples were allowed to cool completely to room temperature before the absorption spectra were taken.

5. RESULTS AND DISCUSSION

Gold nanoparticles were successfully synthesized via seed-mediated methods and characterized via UV-Visible absorption spectroscopy. The seed solutions spectrum is shown below in Figure 1. The peak around 515 nm indicates the solution is comprised of gold nanospheres of 2-3 nm size.

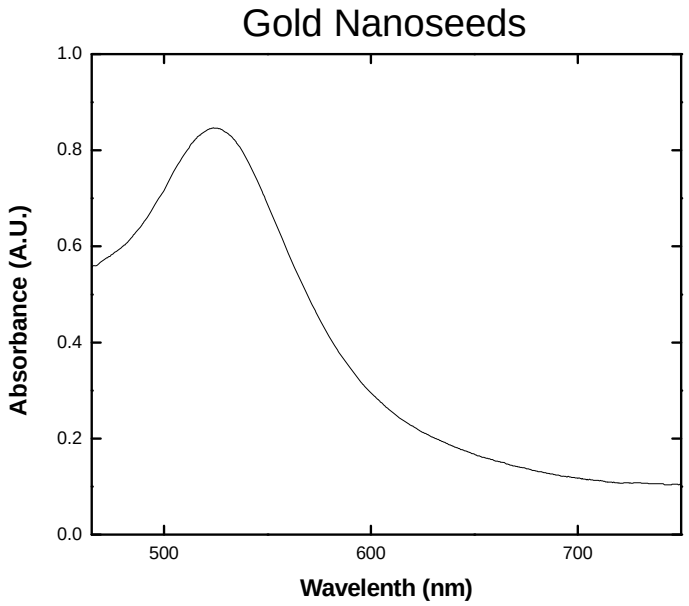

Figure 1: Absorption spectra of gold nanoseeds showing a plasmon resonance band around 530 nm, indicating a size of 2-3 nanometers.

Addition of the gold nanoseed solution to the growth solution generated gold nanorods within 10 minutes of heating at 70 degrees Celsius. Adding 0.075 mL of 0.01 M $AgNO_3$ to the solution, as was the typical procedure, generated gold nanorods of aspect

ratio 1.6 as shown in Figure 2. No TEM images were taken and the size was calculated using the equation proposed by Link and El-Sayed. [9]

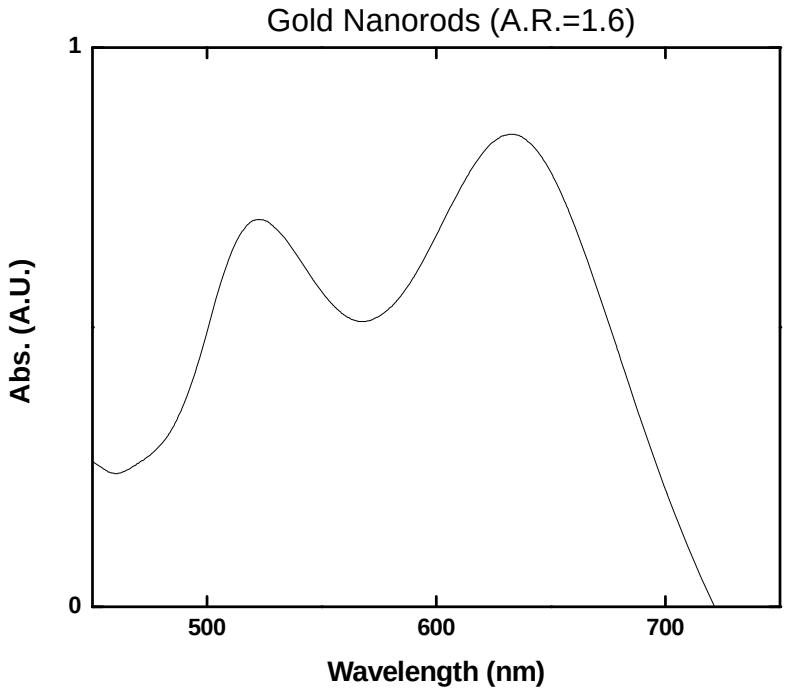

Figure 2: Gold nanorods used in most experiments with an aspect ratio of around 1.6 and good separation between the longitudinal and transverse bands. The nanorods were blue in color.

5.1 Linear Dynamic Range

It took several experiments to find the linear dynamic range of the system, and it perhaps is not completed yet. The first attempt at this was met with limited success. The r^2 value for the first attempt was 0.70, which showed a correlation, but not the strong one we had hoped for. The plot of shift vs. wavelength is shown below in Figure 3.

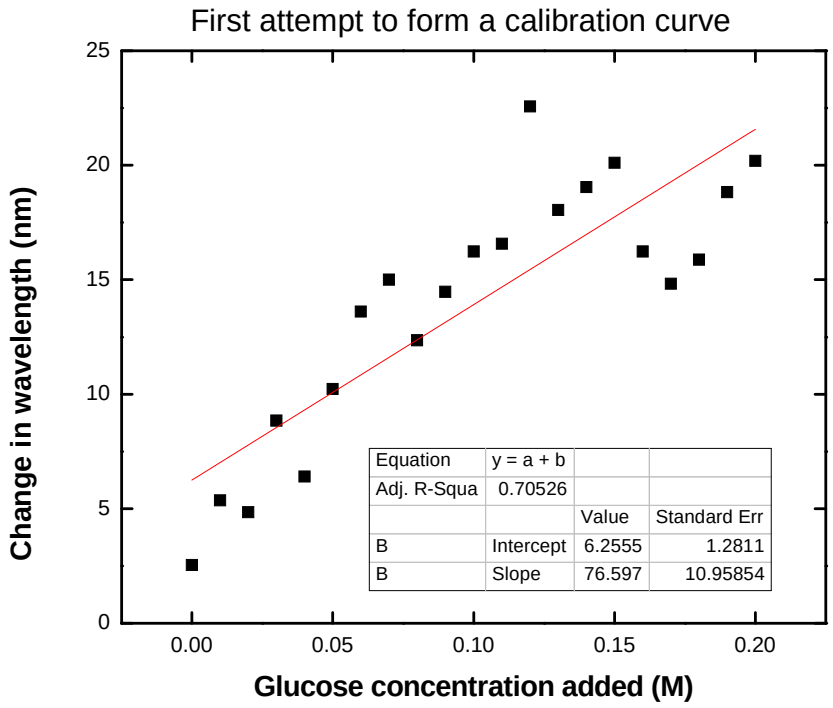

Figure 3: First attempt at a Calibration Curve showing a correspondence between glucose concentration added and the shift

It is important to notice that this is the concentration of glucose added, not the concentration of glucose in the system. It seems that there were a few outliers, and even though it was the average of three trials, anyone who has worked with gold nanoparticles will attest to their fickleness. The experimental trials had also been done on different days, and using the glucose solutions that had already been prepared. It was clear at this point the experiments needed to be repeated with much greater care.

A second, much more careful attempt, proved to have a much greater correlation constant r^2=0.99067, between 0.01 and 0.15 M glucose added. The experiments were completed over only two days, and fresh glucose solutions were made for each concentration. Serial dilution, as used in the previous experiment was not used. Instead, the glucose solutions were all diluted from one concentrated (2 M) stock solutions. The

results showed linear detection between $7.69*10^{-5}$M and $1.2x10^{4}$ M detection within the system. This calibration curve is show below in Figure 4.

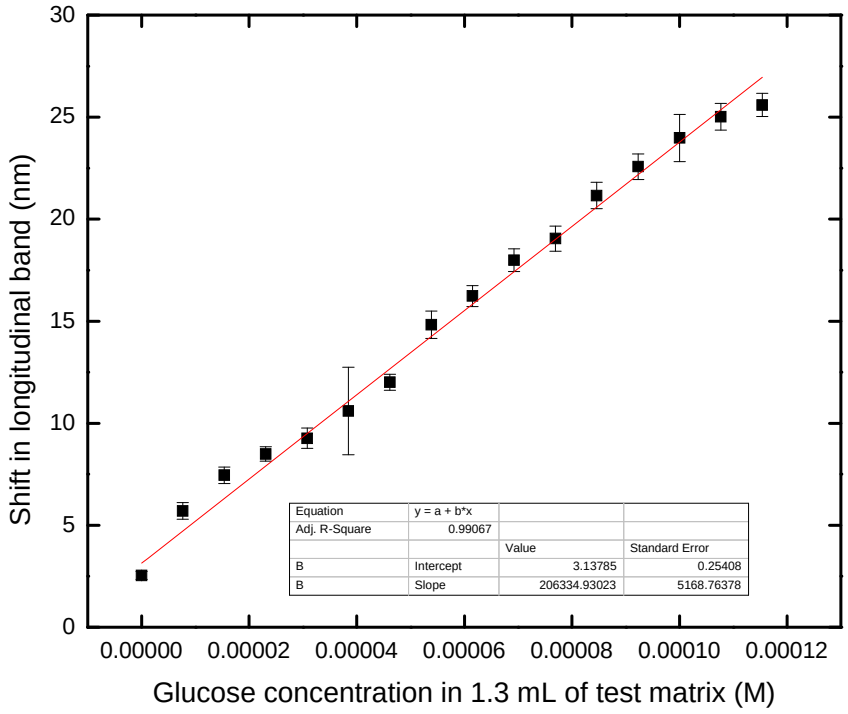

Figure 4: Linear Dynamic Range of the gold nanoparticle based glucose sensor showing an r^2 value of 0.99067 from 0-$1.2x10^{-4}$ M concentration.

At higher concentrations of glucose, the plot levels off. This is shown in the figure below. The reason for this is unknown, as up until this point these results had paralleled those of the 2004 mercury sensor. Glucose is not as strong of a reducing agent, and $HgCl_2$ is only partially hydrolyzed in the pH used (~6.0) so it is possible that the glucose simply cannot reduce the mercury ions enough to force all of the mercury in the system to be ionized according to Le Chatlier's principal. This problem, however, is easily resolved by using a dilution technique if a sample is above this saturation limit. The dilution factor can then be use to calculate the actual concentration of glucose in the undiluted sample

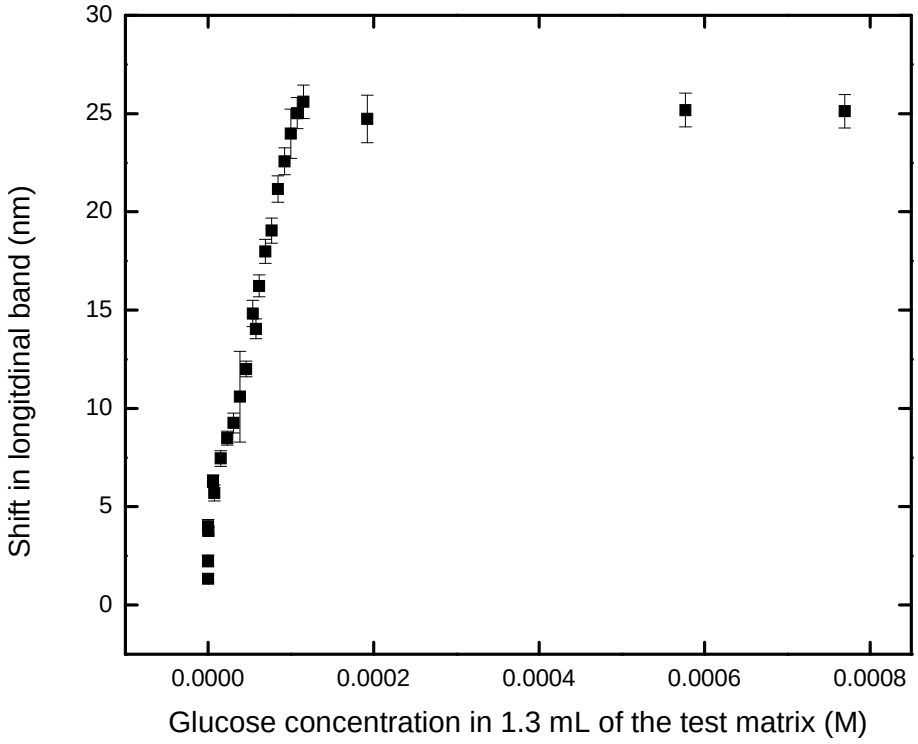

Figure 5: Expanded linear dynamic range showing the leveling off, or saturation, of the sensing capabilities of the glucose sensor.

.

It is also of interest to see all of the absorbance spectra plotted together, as shown in figure 5. It should be noted that this does not encompass the full range for clarity. Also the spectra are normalized so that the shift may be seen more clearly. It should be noted that addition of acetonitrile and mercury (II) chloride to the gold nanorod solution broadened the observed spectra slightly.

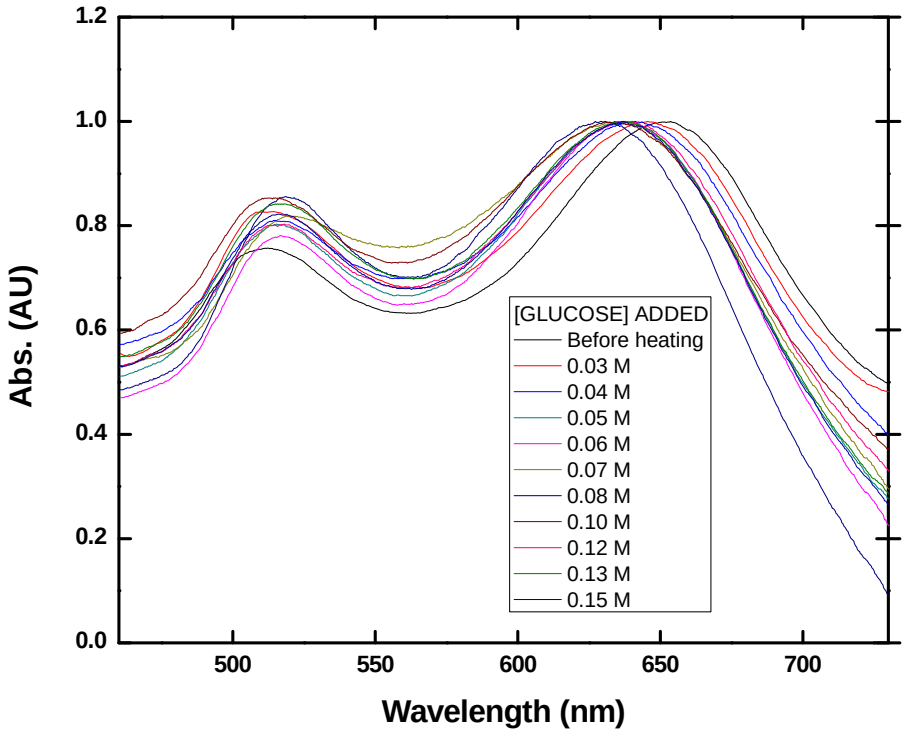

Figure 6: Absorbance of the gold nanorods with a variety of glucose concentrations (Legend shows concentration added in M). As the glucose concentration is added, the blue shift of the longitudinal band becomes greater.

The standard deviation of the system is comparatable, if not better than many glucose sensors reported in the literatre. . The standard deviation of the blank was found to be 0.0422. The limit of detection of the system is calculated as three times this value divided of the slope. This gives the system a detection of limit of 1.58×10^{-13} M. This system is then sensitive enough to detect extremely low levels of glucose in human tears, which can then be used to compute blood glucose trends.

5.2 Kinetics

As stated in the experimental portion, the kinetics of the system were monitored using the Ocean Optics InfoBase software every 30 seconds for 90 minutes. However, this was done very early on, and slightly different nanorods were used. The aspect ratio is nearly the same; however there is a much larger transverse band than the nanorods used in the rest of the experiments. However it was noted that the conclusions found here could be extrapolated to all sizes of nanorods. It was found, as seen in Figure 6 below that after 25 minutes the transverse band stopped shifting and thereafter (although all spectrum are not shown) the spectra were exactly the same.

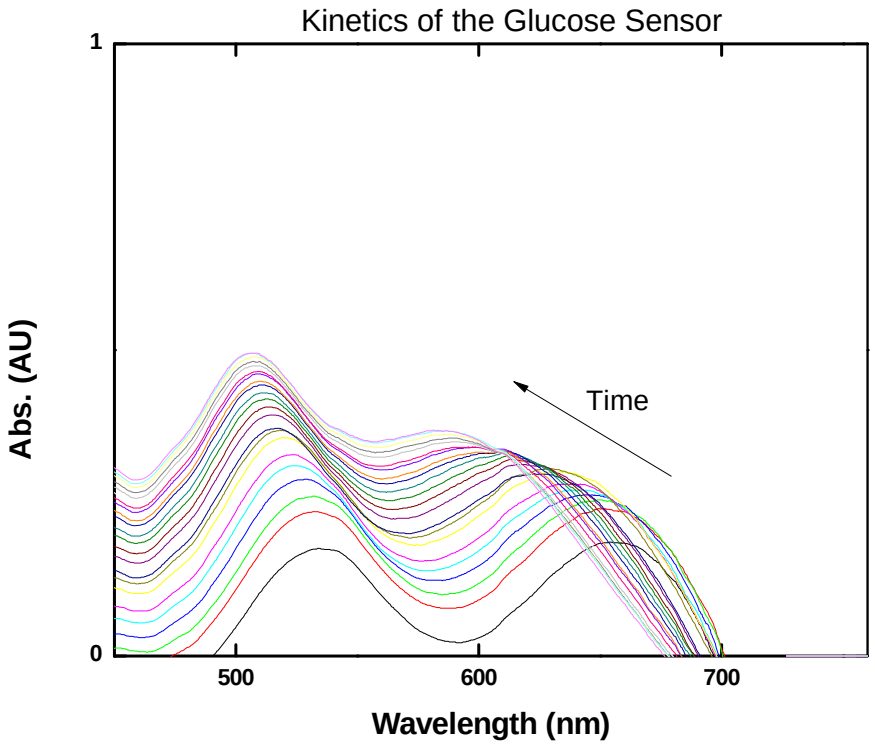

Figure 7: Kinetics of the reaction showing the blue shift over time, and ceasing of the shift after 20 minutes.

The observed blue shift is shown to increase as time goes on. The black line is the first spectrum taken at time=0, and the lighter grey, teal, yellow, and pink lines that overlap are at t=25 minutes to 26 minutes. After this all observed spectra overlapped these ones. Of interest in this plot is the intensity increase between the first measurement and taking the second (at t=30 seconds). This is because the cuvette was left open for all three trials and some solvent evaporated. Also of note is the fact that the transverse plasmon band increases drastically in comparison to the longitudinal. This is believed to be due to more complete amalgamation on some nanorods, causing them to appear more like spheres than rods.

5.3 pH variation.

It is unfortunate due to mercury (II) existing as a hydrated complex $Hg(OH)_2$ that the effect of strongly basic pHs could not be tested. However, as stated previously, the pH of tears is usually below neutral so that it is not such a problem. Even so, it is known that tears will become more basic as the eye is left open. It is not believed that such a small volume of a weak base will change the overall pH of the testing matrix, since it is over 100 greater in volume. Shown below are the plots of the absorption of gold nanorods in varying pHs before and after heating.

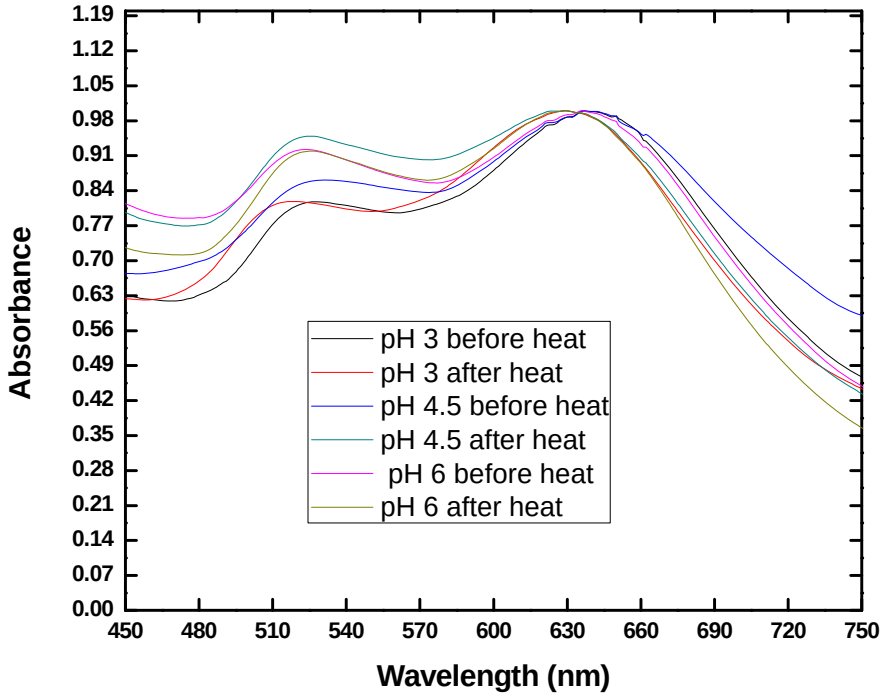

Figure 8: Spectra showing the stability of the sensor in a variety of pHs, covering 450-750 nm wavelength range.

Figure 8 shows only the longitudinal band normalized to show the effect of pH. It was concluded that increasing the pH has no effect on the system.

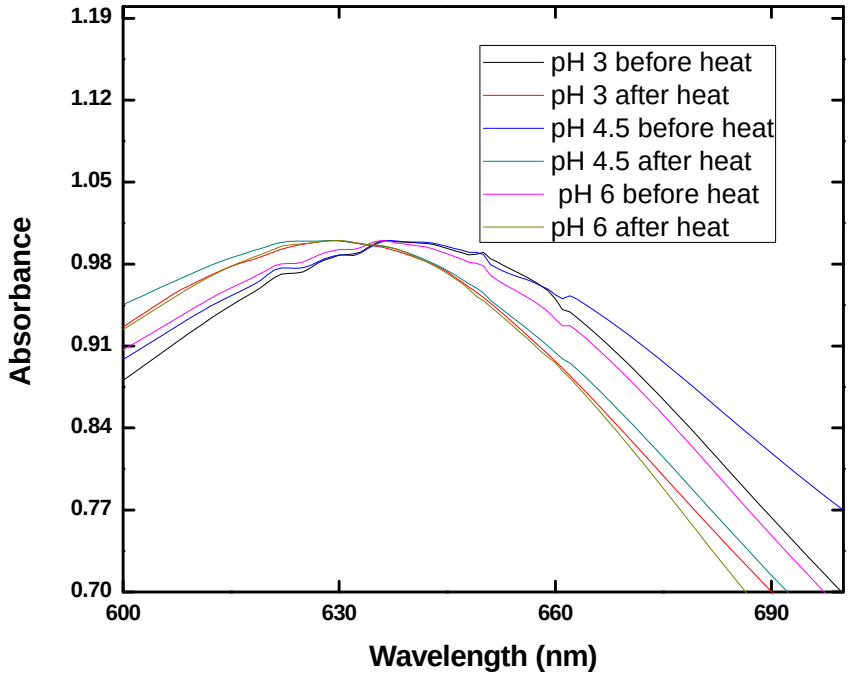

Figure 9: Zoom of the loigitdinal peak with pH variance, showing stability in a variety of pH environments

5.4 Effect of Temperature

The effect of the maximum temperature was studied. To ensure that the temperature was consistent, all vials of a trial were heated either in a water bath, or with the cuvette heater. The plots showing the effect of temperature are shown below in figure 9.

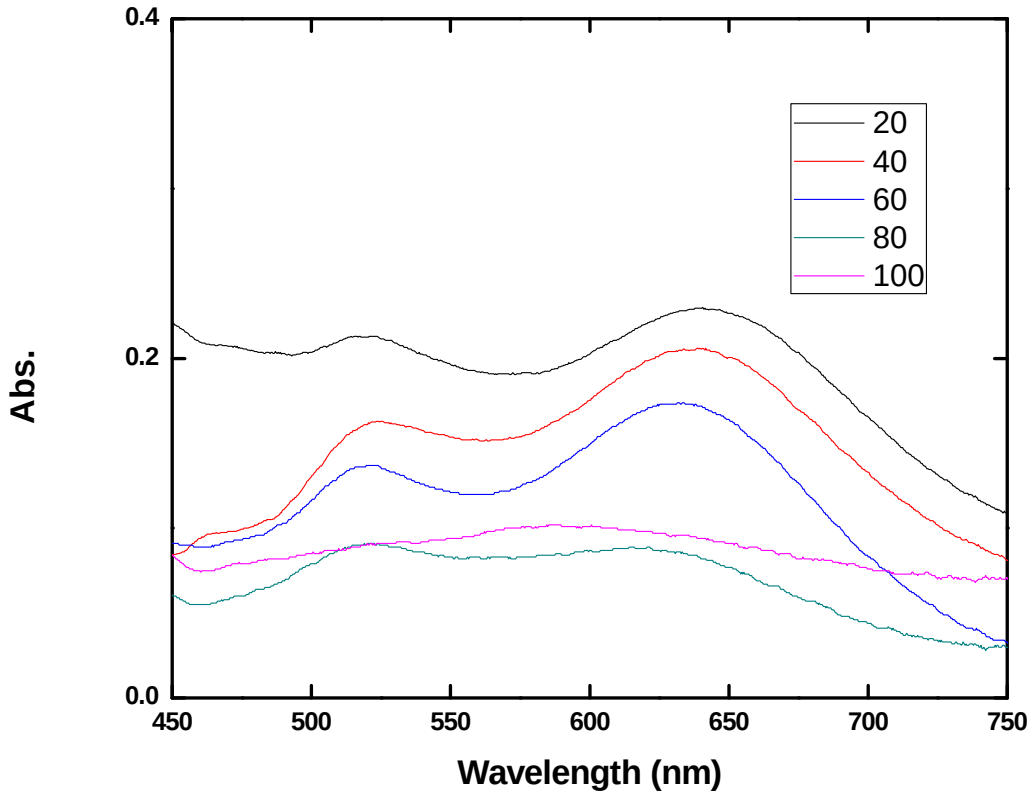

Figure 10: The effect of temperature. The system must be heated to at least 80 degrees Celsius for the expected shift to be seen. At temperatures exceeding this, it will scorch.

It is clear that the system needs to be heated to around 80 degrees Celsius. A concentration of glucose to shift the band to around 635 nm was chosen because either way the temperature affected it would be able to be seen at this wavelength. When heated to 100 degrees Celsius the spectra show a broad absorption band across all visible wavelengths. This was expected because the volatile substances because to leak out, even through a sealed vial and the solution then began to burn. This produced a visible brown-tar-like substance (most likely glucose) in the solution. Some trials had produced more of

this than others, so the three best trials were used to find the average for this plot. With the addition of acetonitrile to the testing solution, 100 degrees Celsius is too high.

At 60 degrees Celsius there is a shift that is seen, however it was not quite what was expected. If the solution had been heated longer perhaps the full shift would have been attained. All solutions were heated for only 20 minutes.

5.5 The effect of Gold Nanorod Concentration

It is expected that if the ratio of gold nanorods to mercury is changed one would see a more drastic shift. As shown below in Figure 10, this is exactly what is seen. As there is now more mercury per nanorod, the amalgamation can become more complete causing the aspect ratio of the nanorods to be lowered and the longitudinal band to be more redshifted.

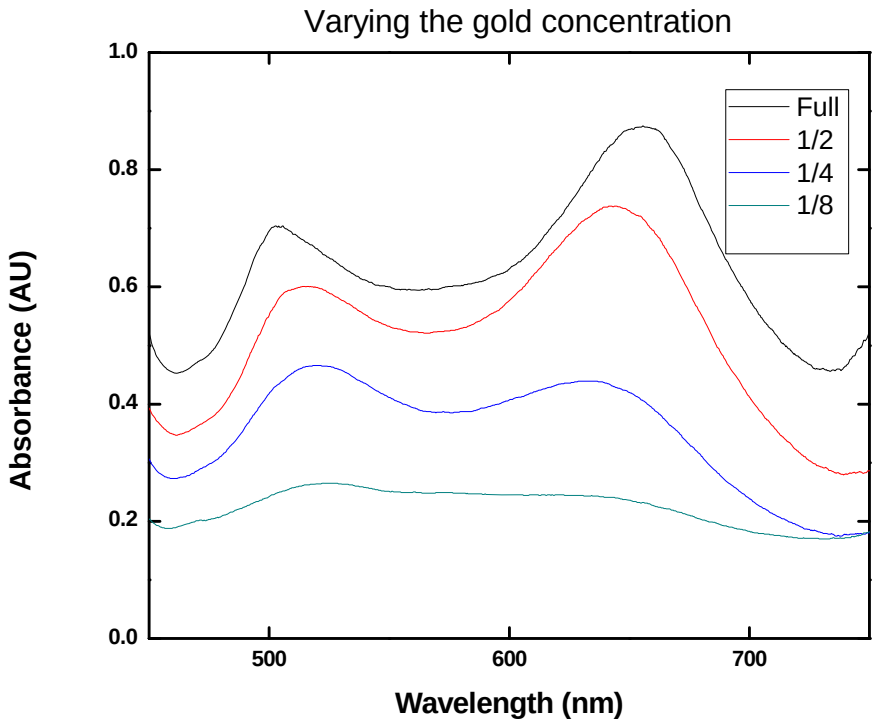

Figure 11 Effect of varying the gold concentration. As the gold to mercury^{2+} ratio is shifted to favor a higher concentration of mercury, a larger blue shift is noticed.

The concentrations of everything except for gold remained the same as in the pH and temperature trials. At each cut in concentration a blue shift of the longitudinal band is seen, as is the expected decrease in intensity. It should be noted that at 1/8th of the full concentration of nanorods ($5x10^{14}$/mL) the absorbance intensity is at the minimum the instrument is reliable at. With a more sensitive spectrophotometer, however, one may be able to decrease the gold concentration even more.

6. CONCLUSION

As a preliminary study, this shows a lot of promise. It was somewhat of a surprise that the system did not exhibit the same sensitivity as the mercury sensor it was modeled after. It does show a sensitive linear range throughout the concentration range of interest, that of human tears. There is much work to be done however, including optimizing the system for both cost and effectiveness. Tears are a much more complex matrix than glucose, and the system is ready to be tested at least on an artificial tear matrix. If the system shows selectivity toward glucose, it has to potential to be a truly non-invasive glucose sensor.

7. FUTURE WORK

Much is left to be done on this project. The linear dynamic range of the system needs to be expanded more, as there is still much argument in the literature over the tear glucose levels. Work is needed to be done to help the reaction proceed faster, so that results can become more instantaneous. The best non-invasive glucose sensor in the world is useless to a diabetic if they don't get the results for half an hour: this can mean life or death.

The system right now is also somewhat toxic, having both mercury and acetonitrile present. This could pose a problem from a regulatory point of view, both in approval of the product and waste collection. It would be advantageous to have a more non-toxic and greener solution if possible.

Testing with an artificial tear-matrix will need to be completed. This will show that the system is sensitive to only glucose, not to anything else. This may prove to be extraordinarily difficult, as it is known that salt can disrupt the electrostatic interactions that stabilize gold nanoparticles. There are several synthetic techniques available for gold nanorods, some of which have less sensitivity to the ionic strength of the matrix. This is not the most difficult hurdle.

The most difficult hurdle in the future of this sensor may be standardizing a method of sample collection. The methods of collecting lacriminyl fluid vary, and depending on the method, the concentrations of substances in tears may vary. This is part of the reason there is not a truly reliable report of tear content. Collecting tears with a glass capillary tube seems to be one of the gentler methods and would collect more than enough sample for a test.

The system shows tremendous promise as a glucose sensor. The process is stable and reproducible, with a linear range encompassing the most accepted glucose level in tears (based on collection with a glass capillary tube). The chemicals used for testing can be well contained within a strong plastic cuvette fitted with a septum to ensure they will not leak, and the cuvette will not break. This is a promising system that may help improve the quality of life for diabetics everywhere.

8. REFERENCES

1. National Diabetes Information Clearinghouse. URL http://diabetes.niddk.nih.gov
2. Pickup, J. C., F. Hussain, et al (2005) "In vivo glucose monitoring: the clinical reality and the promise." Biosensors & Bioelectronics **20**(10): 1897-1902
3. American Diabetes Association URL http://www.diabetes.org/
4. Ferrante do Amaral, C. E. and B. Wolf (2008). "Current development in non-invasive glucose monitoring." Med Eng Phys **30**(5): 541-9.
5. Tura, A., A. Maran, et al. (2007). "Non-invasive glucose monitoring: Assessment of technologies and devices according to quantitative criteria." Diabetes Research and Clinical Practice **77**(1): 16-40.
6. GlucoWatch URL http://www.mendosa.com/glucowatch.htm
7. Tiffany, J. M. "Tears in health and disease."(2007) Eye **17**(8): 923-926.
8. Baca, J. T., C. R. Taormina, et al. (2007). "Mass spectral determination of fasting tear glucose concentrations in nondiabetic volunteers." Clinical Chemistry **53**(7): 1370-1372.
9 Link, S. and M. A. El-Sayed (1999). "Spectral properties and relaxation dynamics of surface plasmon electronic oscillations in gold and silver nanodots and nanorods." Journal of Physical Chemistry B **103**(40): 8410-8426.
10. Ma, Y.; Li, N.; Yang, C.; Yang, X. *Colloids and Surfaces A: Physicochem. Eng. Aspects* 2005, 269, 1-6.
11. Aslan, K.; Lakowicz, J. R.; Geddes, C. D. *Analytical Biochemistry* 2004, 330, 145-155
12. Rex, M., F. E. Hernandez, et al. (2006). "Pushing the limits of mercury sensors with gold nanorods." Analytical Chemistry **78**(2): 445-451.
13. Jana, N.R.; Gearheart, L.; Murphy, C. J. *Langmuir* 2001, 17, 6782.
14. De Boni, L., E. L. Wood, et al. (2008). "Optical Saturable Absorption in Gold Nanoparticles." Plasmonics **3**(4): 171-176.
15. Garcia, Marsiol (2006) "Truly Non Invasive Glucose Optical Sensor Based on Metal Nanoparticles Generation" Submitted to the University of Central Florida, Master's Thesis
16. Ojha, A. K., G. Chandra, et al. (2008). "A study on adsorption of acetonitrile on gold nanorods by non-resonant Raman measurements and density functional theory calculations." Nanotechnology **19**(9): 095706.
17. Balyatinskaya, L. N. (1979). "The Solvation of Mercury Ions and the Formation of Their Complexes in Water and Non-aqueous Solvents." Russian Chemical Reviews **48**(4): 418-429.
18. Carney, L. G., T. F. Mauger, et al. (1989). "Buffering in human tears: pH responses to acid and base challenge." Investigative Ophthalmology Visual Science **30**(4): 747-754.
19. Badugu, R., J. R. Lakowicz, et al. (2003). "A glucose sensing contact lens: A non-invasive technique for continuous physiological glucose monitoring." Journal of Fluorescence **13**(5): 371-374.

20. Badugu, R., J. R. Lakowicz, et al. (2004). "Ophthalmic glucose sensing: a novel monosaccharide sensing disposable and colorless contact lens." Analyst **129**(6): 516-521.
21. Ma, Y.; Li, N.; Yang, C.; Yang, X. *Colloids and Surfaces A: Physicochem. Eng. Aspects* 2005, 269, 1-6.
22 De Block, C., J. Vertommen, et al. (2008). "Minimally-invasive and non-invasive continuous glucose monitoring systems: indications, advantages, limitations and clinical aspects." Curr Diabetes Rev **4**(3): 159-68.
23. Huang, X. H., P. K. Jain, et al. (2007). "Gold nanoparticles: interesting optical properties and recent applications in cancer diagnostic and therapy." Nanomedicine **2**(5): 681-693.
24 Huang, X. H., P. K. Jain, et al. (2008). "Plasmonic photothermal therapy (PPTT) using gold nanoparticles." Lasers in Medical Science **23**(3): 217-228.
25 Jain, P. K., X. H. Huang, et al. (2008). "Noble Metals on the Nanoscale: Optical and Photothermal Properties and Some Applications in Imaging, Sensing, Biology, and Medicine." Accounts of Chemical Research **41**(12): 1578-1586.
26. Lee, K. S. and M. A. El-Sayed (2006). "Gold and silver nanoparticles in sensing and imaging: Sensitivity of plasmon response to size, shape, and metal composition." Journal of Physical Chemistry B **110**(39): 19220-19225.
27. Waynant, R.W., Chenault, V M, (1998). "Overview of non-invasive fluid glucose measurement using optical techniques to maintain glucose control in diabetes mellitus." LEOS 12(2).