Truly Non Invasive Glucose Optical Sensor Based On Metal Nanoparticles Generation

2006

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TRULY NON INVASIVE GLUCOSE OPTICAL SENSOR BASED ON METAL NANOPARTICLES GENERATION

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

Spring term
2006

ABSTRACT

Diabetes is a disease that causes many complications in human normal function. This disease represents the sixth-leading cause of death in USA. Prevention of diabetes-related complications can be accomplished through tight control of glucose levels in blood. In the last decades many different glucose sensors have been developed, however, none of them are really non invasive. Herein, we present the study of the application of gold and silver nanoparticles with different shapes and aspect ratios to detect glucose traces in human fluids such as tears and sweat. This is to our knowledge the first truly non invasive glucose optical sensor, with extraordinary limit of detection and selectivity. The best proven nanoparticles for this application were gold nanospheres. Gold nanospheres were synthesized using chloroauric acid tri-hydrated ($HAuCl_4.3H_2O$) in solution, in the presence of glucose and ammonia hydroxide. The higher the glucose concentration, the higher the number of nanoparticles generated, thus the higher the extinction efficiency of the solution. The linear dependence of the extinction efficiency of the gold nanoparticles solution with glucose concentration makes of this new sensor suitable for direct applications in biomedical sensing. Our approach is based on the well known Tollens test.

DEDICATION

This work is dedicated to my family, especially to my husband Sergio Sotelo. Words are not enough to tell you how much your devotion and faith has meant to me throughout this entire process. I love you.

“Mother and Father, you once told me that no matter what you do with your life, you should always be the best at it. I have lived by those words since you told them to me; you are truly an inspiration in my life”.

ACKNOWLEDGMENTS

This work was made possible through the support of the University of Central Florida and my advisor Dr. Florencio E. Hernández.

I would like to acknowledge the members of my committee: Dr. Stephen Kuebler, and Dr. Andrés Campiglia. Their understanding and support guided me through this process. I would also like to thank my mentor, Dr. Florencio E. Hernández. Your influence in my life is a dissertation in itself. My deepest thanks extended to him.

I would like to acknowledge the support, assistance, and camaraderie of the members of Dr. Hernández's research group, Ion Cohanosqui and Carlos Toro and of Dr. Belfield's research group: Claudia Corredor, Katherine J. Schafer, Dr. Sheng Yao, Cicerón Yánez, and Carolina Yánez. I would also like to acknowledge my friends for ever Alma Morales and Marina Santos.

I would also like to thank to Dr. Kevin Belfield, my deepest gratitude for him. Acknowledge also extends to stock's room people, Jennifer Steele (Hello Katty girl), Kyle Parker and Nicole Kluger.

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CHAPTER ONE: INTRODUCTION

Diabetes is a disease defined as a condition caused when the body is unable to use insulin to process the glucose (sugar) in blood. Therefore, the level of glucose in blood is too high for normal health functioning. [1] This condition affects heart, kidneys, nerves, eyes, etc. An estimated 20.8 million people in the United States (7 percent of the population) have diabetes, a serious, lifelong condition. Of those, 14.6 million have been diagnosed, and about 6.2 million people have not yet been diagnosed. Each year, about 1.3 million people aged 20 or older are diagnosed with diabetes. This disease represents the sixth-leading cause of death in USA [1].

Prevention of diabetes-related complications can be accomplished through tight control of glucose levels in blood. In the last decades many different glucose sensors have been developed, still none of them are really non invasive. These include near infrared spectroscopy [2,3], optical rotation [4,5], colorimetric [6,7] and fluorescence detection [8-12].

In 2001 the Federal Drug Association (FDA) approved the Glucowatch [13]. This is the first step to the continuous and “non-invasive” monitoring of physiological glucose; however, people that uses Glucowatch have to monitor their glucose concentration by other blood sampling technique from time to time. Other emerging technologies include glucose monitoring skin patches; implantable glucose sensors coupled insulin pumps, and laser blood drawing [14]. These techniques are deemed less painful than finger pricking with a lancet or needle. Another recently

developed technology is the glucose sensing contact lenses [14]. This can monitor glucose levels in tears, and directly relate it with the existent blood glucose levels, but this sensor requires an excitation and detection device.

All the above methods still have limitations. The near infrared technique is limited by excessive background [15].The optical rotation technique results in low optical rotation and depolarization due to the tissue [15]. The enzymatic assays technique has the disadvantage of consuming glucose and generating high reactive species like hydrogen peroxide, which can be toxic and damaging to biological composites [15].The contact lenses methods can still be considered pseudo-invasive since the affected must wear contact lenses.

The goal of this study was to develop a truly non invasive glucose optical sensor based on spectral changes and extinction efficiency modification of metal nanoparticles. For this purpose we used the well known Tollens test to generate or modify noble metal nanoparticles. The sensing was done throughout monitoring the surface plasmon band spectral position and its extinction efficiency as a function of glucose concentration in aqueous solution. The gold nanoparticles based sensor showed a low limit of detection, extraordinary selectivity, high reproducibility and less interferences.

CHAPTER TWO: BACKGROUND

In order to design a sensor, one has to consider the element that is going to make the transduction suitable, the design of the matrix to incorporate that element, the capability of the sensor for producing accurate signals, its limit of detection, dynamic range, and reproducibility [14]. For glucose sensing, there is another important parameter to be considered; it has to be non invasive to avoid the painful frequent blood sampling. Unfortunately, at present, there is no available method for the continuous, non-invasive measurement of glucose concentration in human fluids.

There are different human fluids were glucose can be traced such as blood, saliva, urine and tears. In the case of blood, glucose is determined by finger pricking with a small needle, followed by glucose analysis using enzymatic assay. This is a painful test, thus many people are not willing to stick themselves several times per day. An alternative fluid for glucose sensing is urine, which allows for non invasive sensing of glucose. Urine sensors have to be rough to afford the strong dependence on the specific gravity, pH, temperature, the ascorbic acid concentration (more than 50mg/dl) and ketone bodies (more than 40mg/dl).[16] On the other hand, saliva is a choice, however, there are many variations in glucose and proteins concentrations, pH and composition that restrain the use of this fluid for glucose sensing. Tears are more suitable for glucose sensing because they contain a low concentration of proteins, their pH is extremely constant and the levels of glucose in tears are quite elevated.[14]

In order to settle for the existent limitations in glucose sensing methodologies, in this dissertation we have addressed the non invasive issue for glucose sensing, while keeping the sensitivity, selectivity and, reproducibility of the sensor. For this purpose we have proposed the use of noble metal nanoparticles and their exceptional optical and chemical properties.

2.1 Metal Nanoparticles

Noble metal nanoparticles have been used in many applications in the field of physics [17], chemistry [18], biology [19], material science [20] and medicine and their interdisciplinary fields [21], due their interesting optical and electronic properties [22].

Metal nanoparticles show properties that are often different from those of the bulk material [22]. The optical properties of metal nanoparticles are dominated by collective oscillation of conduction electrons resulting from the interaction with electromagnetic radiation. This property better known as the surface plasmon resonance is mainly observed in gold, silver and cooper, because of the presence of free conduction electrons. The electric-field of the incoming radiation induces the formation of a dipole in the nanoparticle. A restoring force in the nanoparticle tries to compensate for this effect, resulting in a unique resonance wavelength [22]. In silver and gold spherical nanoparticles a very strong absorption band with maxima at ≈ 420nm (see Figure 1) and ≈ 520nm (see Figure 2), respectively, can be observed. The oscillation wavelength depends on a number of factors including the particles size and shape, their composition, and the nature of the surrounding medium. For nonspherical nanoparticles, such as rods, the resonance wavelength depends on the orientation of the electric-field. Two oscillations; transversal and longitudinal (see Figure 2) are possible. Al-Sayed and co-workers recently described experimentally and

theoretically the presence of two absorption bands corresponding to these two modes of surface plasmon resonance in metal nanorods [22]. In the case of nanoprisms, three oscillations are present, corresponding to the out-of-plane quadrupole resonance, out-of-plane dipole plasmon resonance, and in-plane dipole plasmon resonance (longer wavelength band) (see Figure 3) [23].

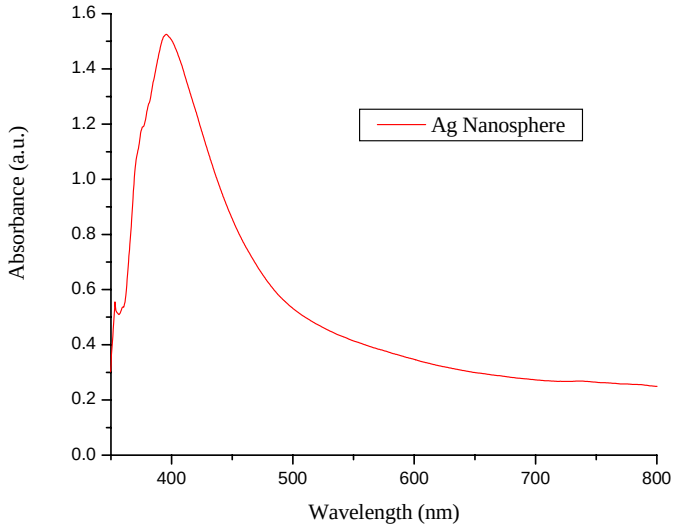


Figure 1: Absorption spectrum of Ag nanospheres

2.2 Application of Metal Nanoparticles in Glucose Sensing

There are few papers reported in the literature about glucose sensing using nanoparticles. Most of them are related to optical or redox properties of molecules attached to nanoparticles surfaces. For instance, the particle aggregation can result in further color changes due to mutually induced dipoles that depend on the interparticles distance and aggregates size [24].

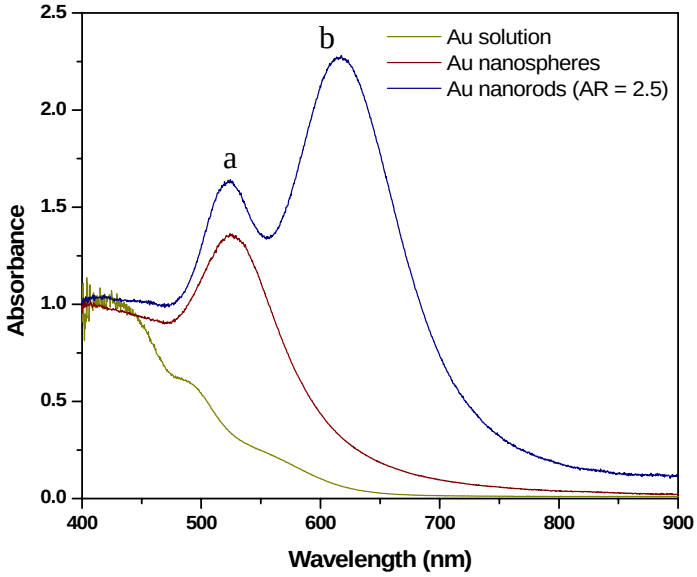


Figure 2: Absorption spectra of Au nanospheres (red line) and Au nanorods (blue) with transversal mode band (a) and longitudinal mode band (b).

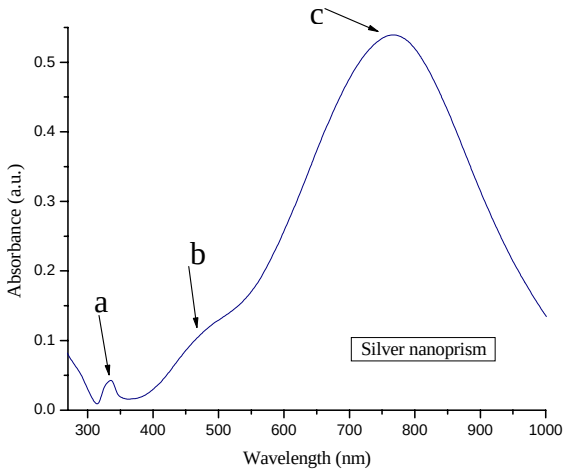


Figure 3: Absorption spectra of Ag nanoprisms: a) out-of-plane quadrupole resonance, b) out-of-plane dipole plasmon resonance, and c) in-plane dipole plasmon resonance

Lakowicz J.R. *et al*. [25], reported a glucose sensor based on the aggregation and dissociation of gold nanoparticles and, the changes in plasmon absorption induced by the presence of glucose.

The gold nanoparticles are first aggregated with concanavalin A (Con A), then, the addition of glucose competitively displaces Con A from Con A-aggregated dextran-coated gold colloids, reducing gold nanoparticle aggregation. Therefore, the surface plasmon resonance is blue shifted. This system can determine milimolar changes of glucose concentration in physiological fluids such as tears, urine, and blood.

Yang X. *et al*. [24] reported the use of gold nanoparticles/polyaniline composite to detect glucose. Water-soluble gold nanoparticles/polyaniline nanocomposites were synthesized using 3-aminophenylboronic acid, a reductive and protective reagent. The polyhydroxy compound poly (vinyl alcohol) (PVA) was used as a disperser, based on the covalent bond interaction between the boronic acid and diol groups. Because glucose molecules could competitively interact with PVA, the gold nanoparticles aggregate, causing a red shift of the SPR maximum with glucose concentration. This system can detect glucose concentrations in the micromolar range. However, given the affinity of boronic acid for ions as fluorides present in physiological fluids, [26] the sensitivity and selectivity of the sensor could be affected.

Beaudoin D. S. *et al*, [27] reported a glucose sensor based on the attachment of fluorescent glucose receptors (dipyrido1;3,2-a:2’3’-c3;phenazine (dppz)) to nanocrystal titanium dioxide. They were able to detect glucose at neutral pH. The detection involves fluorescence spectral changes, making the signal not reliable in human fluids because of the presence of heavy atoms that can work as quenchers.

Richard P. V., *et al*, [28] developed a glucose sensor based on a raman-active molecule on nanofabricated substrates using the surface enhanced raman spectroscopy (SERS) as the transduction signal. The authors used AgFON (silver film-over-nanosphere) substrates fabricated on glass and 1-decanethiol (1-DT). By subtracting the SERS spectrum of the 1-DT-AgFON substrate with glucose (they incubated 1-DT-AgFON substrate in a glucose solution for 10 min), from the 1-DT-AgFON substrate without glucose, they were able to determine quantitatively low glucose concentrations. In order to make the sensor effective in the presence of interfering proteins, the authors repeated the same experiment using (1-mercaptoundeca-11-y1)tri(ethyleneglycol) (EG3)-modified AgFON substrate instead of 1-DT-AgFON. EG3 was chosen as a partition layer because of its ability to reject nonspecific binding by background proteins and, its biocompatibility. The sensor demonstrated quantitative glucose sensing in the range 0-25mM with potential applications in implantable patches. However, the reproducibility of SERS is known to be poor [29].

There are also papers reporting glucose biosensors based on the oxidation of glucose (see Equation 1).

$$\beta\text{-d-glucose} + O_2 + H_2O \xrightarrow{\text{(glucose oxidase)}} \text{d-gluconic acid} + H_2O_2 \qquad (1)$$

The current produced from the decomposition of hydrogen dioxide on the electrode is used to quantify the glucose concentration. If more enzymes are used, or if the catalytic activity of the enzyme is enhanced, more H_2O_2 is produced, thus a higher response current is observed.

Haipeng Y., *et al*, [30] reported a glucose biosensor enhanced *via* nanosized SiO_2 using electrochemical responses. The authors used nanosized SiO_2 to increase the specific surface area of the sensor in order to increase the enzyme loading. They employed Platinum electrodes ($Pt/SiO_2/GOx$) to obtain a work range between 0.005 and 2.5mM.

Wei Zhao *et al*, [31] reported a glucose sensor based on multilayer membranes *via* layer-by-layer deposition of organic polymer protected prussian blue (P-PB) nanoparticles and glucose oxidase. P-PB can catalyze the electroreduction (Cyclic voltammetry) of hydrogen peroxide formed from enzymatic reaction at lower potential. They obtained a linear dynamic range from 0.10 to 11.0mM.

Xiang-Ling R, *et al*, [32] reported a glucose biosensor using the enhancement effect of silver-gold nanoparticles on the catalytic activity of immobilized enzymes. The current response was increased 1,000 folds for the same glucose concentration.

A more recent approach for glucose sensing is through the generation of metal nanoparticles on the surface of SiO_2 microparticles. [33] The aggregation of metal nanospheres produces a spectral change proportional to glucose concentration.

2.3 Generation of Metal Nanoparticles

There are many physical and chemical routes for the synthesis and generation of silver and gold nanoparticles reported in the literature. [34-38]

Physical methods are defined as those by which metal nanoparticles are directly generated from bulk gold. The nanoparticles are obtained by the generation of metal atoms in gas phase followed by a controlled condensation of the nanoparticles. For instance there is the well known metal-vapor synthesis [39] and, laser ablation and laser induced size reduction method. [40, 41]

The chemical routes uses metal salts as starting material and a reducing agent. [37] For instance, the Turkevich method is one of the most simple and applied procedures for the synthesis of gold nanoparticles employed now a day. Sodium citrate reduces [$AuCl_4^-$] in hot aqueous solution to give nanoparticles of 15-20nm. Citrate itself and its oxidation products (e.g. Acetone dicarboxylate) can act as protecting agents, if no other stabilizer is used. [37] One of the most popular modern methods for preparing gold nanoparticles of various sizes comes from Brust *et al*. [36]. It uses $NaBH_4$ as reducing agent, in the presence of alkanethiols to yield gold particles of 1-3nm. By varying the thiol concentration, the particles sizes can be controlled between 2 and 5nm. Thiol-stabilized gold nanoparticles have become available following a seeding growth approach starting with 3.5nm diameter particles. The particle size can be varied by changing the seed-particle-to-metal-salt ratio. [38] Gold nanoparticles in the size of about 10 to 30nm or more have been synthesized by the $NaBH_4$ method using mercaptosuccinic acid for stabilization. By varying the succinic acid to $HAuCl_4$ ratio from 2.5 to 0.5, particles of 10.2, 10.8, 12.8, 19.4 and 33.6nm were isolated as water-redispersable powders. [42] Another valuable route for the generation of gold nanoparticles by using “nano-reaction vessels”. Micelles can be considered appropriate chemical reactors. Due to their limited size, the control on growth by ligand concentration is not required. For example, diblock co-polymers exhibit regularly organized

micelles into which tetrachloroaurates can be transported, followed by a reductive step with hydrazine or $NaBH_4$ and, the formation of the nanoparticles. [43]

It is worthy to highlight that in the synthesis of metal nanoparticles, it is important to consider the stabilization of the particles to avoid coalescence effects. The stabilization can occur by electrostatic repulsion, steric hindrance, ligand molecules and nanocapsules embending. [18]

All the chemical methods described above have the same chemical principle, the reduction of Au (III), Au (I) or Ag (I) with a reducing agent. Because of silver and gold are highly electronegative, they can be generated from their salts by a huge number of reducing species. Classical reducers such as hydrides, hydrogen, hydrazine, and alcohols can be used to produce metal nanoparticles from salts. Also, the so-called “green chemistry” has evolved in recent years, opening new branches for the synthesis of metal nanoparticles using natural organic materials such as glucose. [35] The reduction of metal salts in solution using glucose, *i.e.* the application of the Tollen test, is the base of the work presented here. [44]

2.4 Tollens Test

The Tollens test is commonly used to detect aldehyde functionalities. Using a silver nitrate solution under basic conditions, a smooth deposit of silver metal on the inner surface of the test tube results when an aldehyde is present (see Figure 4). This is better known as the “silver mirror” test. [44, 45]

Figure 4: Tollens test

The Tollens test can be used to detect glucose because glucose can exist in an open-chain (acyclic) and ring (cyclic) form (see Figure 5). The cyclic chair form is the result of an intramolecular reaction between the aldehyde C atom and the C-5 hydroxyl group forming an intramolecular hemiacetal. An aqueous sugar solution contains approximately 0.02% of the glucose in the chain form, where the majority of the structure is in the cyclic chair form (see Figure 5).

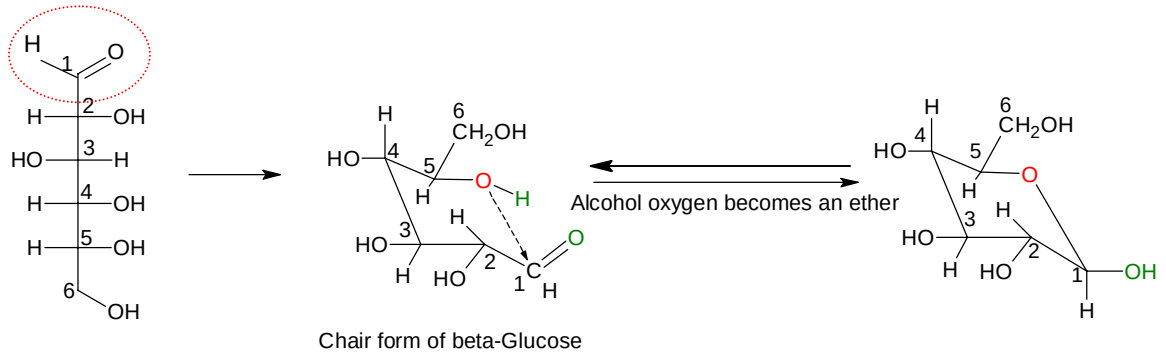


Figure 5: Structure of glucose

With 0.02% of glucose in the chain form, glucose can reduce Ag^+ ions to silver metal in solution. The standard-state half-cell potential for the reduction of Ag^+ ions is +0.800V and the standard-state half-cell potential for the oxidation of glucose is -0.050V. The two half-cell potentials are:

$2(Ag^+ + \cancel{e^-} \rightarrow Ag)$ E°red = 0.800V

$C_6H_{12}O_6 + H_2O \rightarrow C_6H_{12}O_7 + 2H^+ + \cancel{2e^-}$ E°ox = - 0.050V

$2Ag^+ + C_6H_{12}O_6 + H_2O \rightarrow 2Ag + C_6H_{12}O_7 + 2H^+$ E° = 0.750 V

The reaction is catalyzed with ammonia, producing a $Ag(NH_3)_2^+$ complex ion. This important step enhances the overall E^0 of the reaction because, while it reduces the half-cell potential reduction of the silver complex ion, it increases the reducing strength of glucose much more. The two half-cell potentials are:

$2(Ag(NH_3)_2^+ + \cancel{e^-} \rightarrow Ag + 2NH_3)$ E°red = 0.373V

$C_6H_{12}O_6 + H_2O \rightarrow C_6H_{12}O_7 + 2H^+ + \cancel{2e^-}$ E°ox = 0.600V

$2Ag(NH_3)_2^+ + C_6H_{12}O_6 + H_2O \rightarrow 2Ag + C_6H_{12}O_7 + 2NH_4^+$ E° = 0.973V

Based on the Tollens test, silver nanoparticles with different shapes and aspect ratios could be formed or transformed. Therefore, the detection of glucose traces in human fluids, using this tactic is an open possibility. [45-47] However, the use of silver for glucose sensing in human fluids could present some limitations because of the high content of NaCl in these fluids. [48] It is well known that $Ag^+(aq)$ and $Cl^-(aq)$ react to form a typical white solid of AgCl. This side reaction could introduce important interferences in the sensing system such as the unknown reduction of the initial amount of silver ions available in solution, scattering, and possibly instability due to photo-oxidation of the ions. Other promising metal with perhaps less interferences and negligible photo-oxidation that could be employed for glucose sensing is gold.

Considering the high stability of gold nanoparticles, this should be a more qualified metal to be used for this application. The two standard-state half-cell potential are:

$2(Au^{3+} + 3e^- \rightarrow Au)$ E°red = 1.400V

$3(C_6H_{12}O_6 + H_2O \rightarrow C_6H_{12}O_7 + 2H^+ + 2e^-)$ E°ox = 0.600V

$2Au^{3+} + 3C_6H_{12}O_6 + 3H_2O \rightarrow 2Au + 3C_6H_{12}O_7 + 6H^+$ E° = 2.000V

Based on the standard-state reduction potential, the Tollens test using gold is thermodynamically more favored than silver. Independently of the metal, our hypothesis was based on the generation of metal atoms that could aggregate to generate or transform metal nanoparticles and, on a recently developed mercury sensor based on metal nanorods. [49]

The metal nanorods based Hg sensor detects Hg upon wavelength changes on absorption spectra of Au nanorods as their aspect ratio changes with Hg concentration. The goal of this study was to demonstrate the analytical potential Au nanorods have for monitoring Hg in water samples. The outstanding selectivity and sensitivity of the method provide a unique way to determine Hg in water samples without previous separation and/or pre-concentration of the original sample. Through a selective amalgamation a the tips of the nanorods, a reduction of the effective aspect ratio of the nanoparticles takes place, inducing a blue shift of the maximum absorption wavelength of the longitudinal mode band.

The first article reporting on the absorbing properties of metal nanorods appeared a few years ago. Based on experimental evidence, Al-Sayed and co-workers [22] provided the theoretical

foundation to understand the two absorption bands typically observed in the UV-vis absorption spectra of nanorods. According to the authors [22] the two absorption bands correspond to the transversal and longitudinal modes of surface plasmon resonance (SPR). The transversal mode band belongs to the SPR along the short axis of the rod and appears at a shorter absorption maximum than the longitudinal mode band. The maximum absorption wavelength of the longitudinal mode - which corresponds to the SPR along the long axis of the rod - presents a linear correlation with the aspect ratio (length/diameter) of the nanorod. As the aspect ratio of the nanorod increases, the longitudinal mode band shifts to longer wavelengths. The same behavior is observed as the dielectric constant of the medium increases (ε_m) [22].

In this work, we wanted to study the analytical potential Ag and Au nanorods, nanoprisms and nanospheres have for monitoring glucose in aqueous solution. Glucose is a reducing agent that could grow nanorods or change the shape of nanoprisms, increasing their aspect ratio, thus inducing a red shift of the longitudinal mode band. Also, glucose can generate metal nanospheres increasing the extinction efficiency of the solution. Therefore, by monitoring the spectral shift of the longer wavelength band or the extinction efficiency of the SPR band of nanospheres, the glucose concentration could be determined in human fluids and correlated to the actual concentration in blood.

CHAPTER THREE: RESEARCH OBJETIVES

3.1 Major Goal

The development of a truly non-invasive glucose optical sensor based on nanotechnology, with low limit of detection and high selectivity.

3.2 Specific Goals

- Study of the spectral-shift of the longitudinal surface plasmon band of silver nanorods in the presence of glucose and an excess of $AgNO_3$
- Synthesis of silver nanospheres using glucose
- Synthesis of silver nanorods using glucose
- Study of the spectral-shift of the quadrupole surface plasmon band of silver nanoprisms in the presence of glucose and an excess of $AgNO_3$
- Study of the spectral-shift of the longitudinal surface plasmon band of gold nanorods in the presence of glucose and an excess of $HAuCl_4$
- Synthesis of gold nanorods using glucose
- Synthesis of gold nanospheres using glucose
- Study of the glucose concentration effect on the generation of and gold nanospheres
- Comparison between the different nanostructures and methods for glucose sensing
- Determination of the dynamic range of the glucose sensor based on absorbance-change
- Determination of the limit of quantization of the glucose sensor based on gold nanospheres

CHAPTER FOUR: EXPERIMENTAL

4.1 Chemicals

Analytical-reagent grade chemicals were used in all experiments. Hexadecyltrimethylammonium bromide (CTAB) ($C_9H_{42}BrN$), Hydrogen tetrachloroaurate III ($HAuCl_4$), silver nitrate ($AgNO_3$), Ascorbic acid ($C_6H_8O_6$), Trisodium citrate ($C_6H_5Na_3O_7.2H_2O$), Poly(vinylpyrrolidone) (PVP, weight-average molecular weight Mw~29000g·mol^{-1}), were purchased from Sigma-Aldrich. Sodium borohydrate ($NaBH_4$), sodium hydroxide (NaOH) and hydrogen peroxide (H_2O_2) were purchased from Fisher chemicals. Glucose was bought from Acros. All water used was obtained from a Barnstead infinity ultrapure water system.

4.2 Silver Nanoparticles

4.2.1 Synthesis of silver nanospheres

Silver nanospheres of 4nm of diameter were synthesized according to the wet chemical method developed by Jana *et al*. [46] A 20mL solution with a final concentration of 0.25mM $AgNO_3$ and 0.25mM trisodium citrate in water was prepared. While stirring vigorously the previous solution, 0.6mL of 10mM $NaBH_4$ was added all at once. Stirring was stopped after 30s. The solution was used 2h after preparation. After 5h, a thin film of particles appeared at the water surface.

4.2.2 Synthesis of silver nanorods

Nanorods of different aspect ratio were synthesized according to the wet chemical method developed by Jana *et al*. [46]. 3 sets of solutions were prepared containing 0.25mL of 10mM $AgNO_3$, 0.50mL of 100mM ascorbic acid, and 10mL of 80mM CTAB. Next, 0.5mL, 0.25mL and 0.125mL of 4nm seed solution (see section 4.2.1) were added. Finally, 0.10mL of 1M NaOH was added to each solution. After adding NaOH, the solution was gently shaken with the rest of the solution. Within 1-10 min a color change occurred.

4.2.3 Synthesis of silver nanoprisms

Nanoprisms of different aspect ratio were synthesized according to the wet chemical method developed by Métraux *et al*. [23] Briefly, an aqueous solution of silver nitrate (0.1 mM, 25mL), trisodium citrate (30mM, 1.5mL), poly(vinylpyrolidone) (PVP, weight-average molecular weight Mw~29000gmol^{-1}, 0.7mM, 1.5mL), and hydrogen peroxide (30wt.-%, 60μL) were combined and vigorously stirred at room temperature. Then, $NaBH_4$ (100mM, 100-250μL) was rapidly injected to the mixture, generating a colloid that was pale yellow in color. After 30 min, the colloid darkened to a deep-yellow color, indicating the formation of small silver nanoparticles. Over the next several seconds, the color of the colloid continued to change from yellow to red. The final color of the solution ranged from pink/purple to turquoise, depending on the $NaBH_4$ concentration used. The reaction occurred in the dark.

4.2.4 Synthesis of silver nanorods using glucose as reductant agent

The synthesis of silver nanorods using glucose was pursued using a modification of the wet chemical method developed by Jana *et al.* [46] 4nm silver nanospheres seed solution was prepared as indicated in the section for 4.2.1. Next, a set of solutions containing 0.25mL of 10 mM $AgNO_3$, 0 to 2mL of glucose (1 M), and 10mL of 80mM CTAB, 0.1mL of 4nm seed, and 0.780 mL aqueous ammonia (2%) were mixed. Finally, the solutions were gently shaken. Within 1-10 min a color change occurred.

4.2.5 Synthesis of silver nanospheres using glucose as reductant agent

A set of solutions containing 2mL of $AgNO_3$ ($25x10^{-5}$M), 0.20mL of aqueous ammonia (2%) and different volumes of glucose (1M) were prepared. Then, the solutions were gently shaken.

4.3 Gold Nanoparticles

4.3.1 Synthesis of gold nanospheres

Gold nanospheres of 5nm diameter were synthesized according to the wet chemical method developed by Jana *et al*. [50] A 20mL aqueous solution containing $2.5x10^{-4}$M $HAuCl_4$ and $2.5x10^{-4}$M trisodium citrate was prepared in round bottom flask. Next, 0.6mL of ice cold 0.1M $NaBH_4$ solution was added to the solution all at once while stirring. The solution turned pink immediately after adding $NaBH_4$, indicating particle formation. The solution was kept in the dark for 5h. The particle solutions were stable for few months.

4.3.2 Synthesis of gold nanorods

Nanorods of different aspect ratio (2-3.8) were synthesized according to the wet chemical method developed by El-Sayed *et al*. [47] First, a seed solution of gold was prepared as follow: a CTAB solution (5mL, 0.20M) was mixed with 5.0mL of 0.00050M $HAuCl_4$, to this stirred solution, 0.60mL of ice-cold 0.010M $NaBH_4$ were added. As a result, a brownish yellow solution was produced. Vigorous stirring of the seed solution was continued for 2min at 25^{o}C. After, for the growth of nanorods, CTAB (5mL, 0.20M) was added to (0.050, 0.10, 0.15, 0.20, 0.25mL) of 0.0040M $AgNO_3$ solution at 25^{o}C. Then, 5.0mL of 0.0010M $HAuCl_4$ were added, and gently mixed with 70μL of 0.0788M ascorbic acid. Finally, 12μL of the seed solution was added to the growth solution at 27-30^{o}C. The color of the solution gradually changed within 10-20min. The temperature of the growth medium was kept constant at 27-30^{o}C in all the experiments. The solutions were stable for many months.

4.3.3 Synthesis of gold nanospheres using glucose as reductant agent

Gold nanospheres were synthesized as follow: an aqueous solution of $HAuCl_4$ (2 mL, 2.5x10^{-4}M) was mixed with glucose (0.8 mL, 1M) and NH_4OH (0.2 mL, 2%). The solution turned pink immediately after warming for 12 min in a boiling water bath. The solution was stable for many months.

4.3.4 Synthesis of gold nanorods using glucose as reductant agent

Using a modification of the El-Sayed *et al.* [47] method, the synthesis of gold nanorods of three different aspect ratios was attempted using glucose instead of ascorbic acid as reducing agent. For this, a seed solution of gold was prepared as explained in section 4.3.2. Then, for the growth of nanorods, CTAB (5mL, 0.20M) was added to 0.15mL of 0.0040 M $AgNO_3$ solution at 25^{o}C. Then, 5.0mL of 0.0010M $HAuCl_4$ were added, and gently mixed with different concentrations of glucose (0 to 2 mL of glucose (1 M)), and 0.740mL NH_4OH (2%). Finally, 12μL of the seed solution was added to the growth solution at 27-30^{o}C. The color of the solution gradually changed within 10-20min. The temperature of the growth medium was kept constant at 27-30^{o}C.

4.4 Instrumentation

Absorption spectra of the solutions were obtained using an Agilent 8453 spectrophotometer, equipped with a deuterium and tungsten lamp, a diode-array detector, and wavelength range of 190 to 1100m.

CHAPTER FIVE: RESULTS AND DISCUSSION

5.1 Silver Nanoparticles

5.1.1 Change of the aspect ratio of silver nanorods with glucose

Silver nanorods of approximately 7.5 aspect ratio were treated with different glucose concentrations, in the presence of an excess of $AgNO_3$ and in basic conditions (NH_4OH, pH 11). Figure 6 shows the absorption spectra of these solutions after 30min. A very small shift in the maximum absorption wavelength of the longitudinal mode at different glucose concentrations was observed. Contrary to what it was expected, this is an indication of no aspect ratio variation throughout the reaction, as Ag^+ is reduced. However, as the glucose concentration was increased, the absorbance of the transversal mode band became stronger as an indication of silver nanospheres formation. This behavior can be explained by the stabilization of Ag nanoparticles by the gluconic acid generated throughout the reaction.[52] When glucose reduces silver ions, it transforms into gluconic acid. Because the generated gluconic acid is in the immediate vicinities of the silver metal atoms, and this acid behaves as a surfactant in the presence of metal nanoparticles, silver atoms prefer to form agglomerates, *i.e.* Ag nanospheres, rather than diffusing through the solution and attach to the sterically protected by CTAB, tips of the existing nanorods. Therefore, an increase of the SPR band at *ca*. 420nm was observed. To verify this hypothesis the direct synthesis of silver nanorods using glucose was attempted.

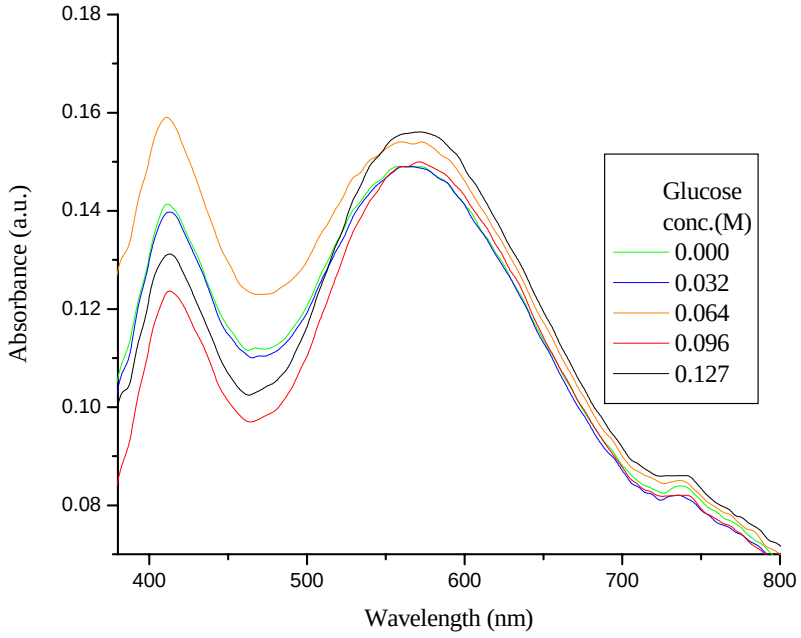


Figure 6: UV-vis. absorption spectra of silver nanorods in the presence of different glucose concentrations.

5.1.2 Generation of silver nanorods with glucose

The synthesis of silver nanorods using glucose was pursued using a modification of the wet chemical method developed by Jana *et al* [46] as explained in section 4.2.2 (glucose was used instead of ascorbic acid). The absence of the longitudinal mode band in all the spectra for different glucose concentrations corroborated the no formation of silver nanorods. The formation of nanospheres is evidenced by the presence of a strong surface plasmon resonance band centered at approximately 420nm. An increased in the concentration of glucose increases the absorbance of the final solution. The preferential formation of silver nanospheres is due to the presence of gluconic acid as the reaction goes through as explained in section 5.1.1.[52] Although, a change in the absorbance was observed when increasing the glucose concentration, the observed change seemed not to be directly proportional to glucose concentration. This can be explained by the high susceptibility to oxidation that silver presents in water and in the presence

of air. In order to confirm this last point, the direct synthesis of silver nanospheres using glucose was tried.

5.1.3 Generation of silver nanospheres with glucose

A set of solutions containing 2mL of $AgNO_3$ ($25x10^{-5}$M), 0.20mL of aqueous ammonia (2%) and different volumes of glucose (1M) were prepared. Then, the solutions were gently shaken. The spectra of the solutions, taken after 30min are shown in Figure 7. The formation of the surface plasmon band centered at *ca*. 420nm and the absorbance change with the glucose concentration is a probe of silver nanospheres formation. However, the change in absorbance is not linear with the glucose concentration. This no linear behavior is attributed to the oxidation of silver nanoparticles in aqueous medium and in the presence of air. Therefore, Beer's law does not apply to this system. In addition, perhaps the initial amount of glucose has an effect on the size of the nanoparticles generated as the different maximum wavelength position for different glucose concentrations showed. To finally elucidate the potential of silver nanoparticles for glucose sensing, silver nanoprisms shape changes were tried using the Tollens test approach.

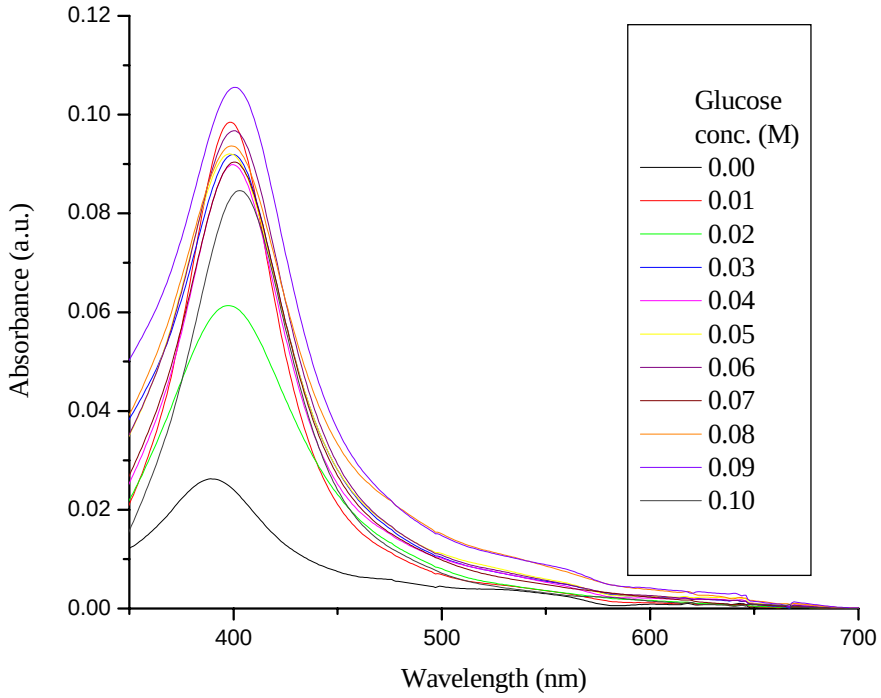
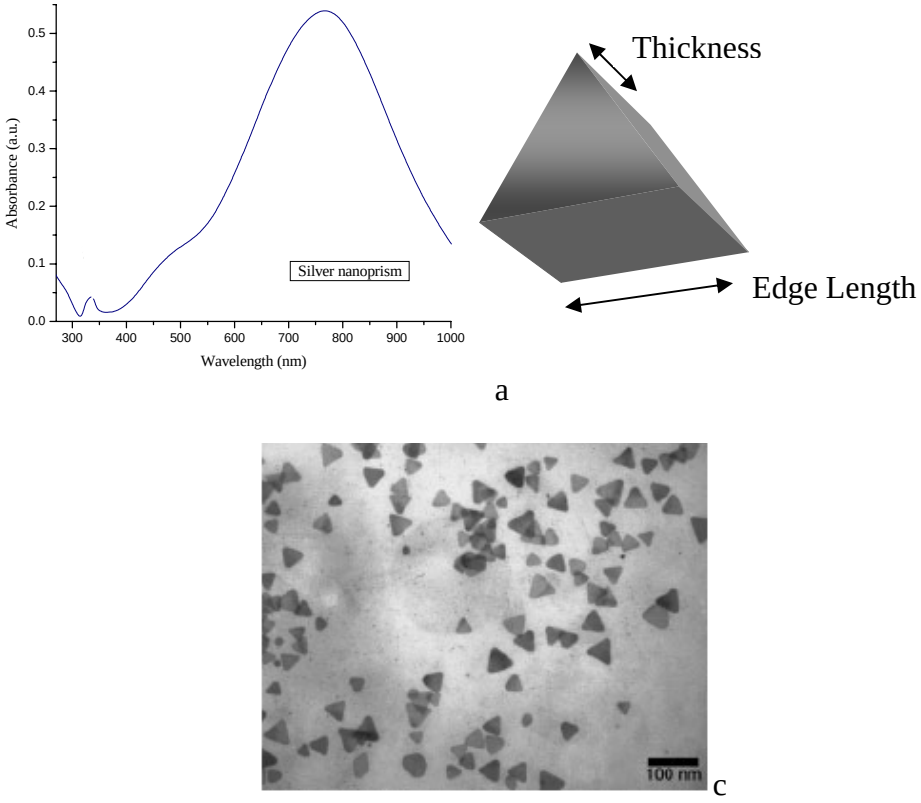


Figure 7: UV-vis. absorption spectra of silver nanospheres synthesized using different glucose concentrations

5.1.4 Change of the shape of silver nanoprisms with glucose

The UV-visible spectrum of silver nanoprisms is very sensitive to their size and environment [23]. Nanoprisms present three characteristics surface plasmon bands in the UV-vis spectral region corresponding to the out-of-plane quadrupole resonance, out-of-plane dipole plasmon resonance, and in-plane dipole plasmon resonance (see Figure 8).[23] The longer wavelength band corresponds to the in-plane dipole plasmon resonance. Because, the position of this band is very sensitive to the size and the environment of the nanoprisms, a spectral shift of the in-plane SPR band by the addition of glucose was expected [51].



a b c

Figure 8: a) UV-vis spectrum of nanoprims. b) silver nanoprism carton. c) TEM image of silver nanoprims [23].

In order to study the spectral shift for silver nanoprisms, a set of solutions containing 0.4mL silver nanoprisms, 0.29mL of aqueous ammonia (2%), an excess of $AgNO_3$, and different volumes of glucose (1M) were prepared, and then gently shaken. The spectra taken after 30 min are shown in Figure 9. No correlation between the glucose concentration and the spectral shift was observed.

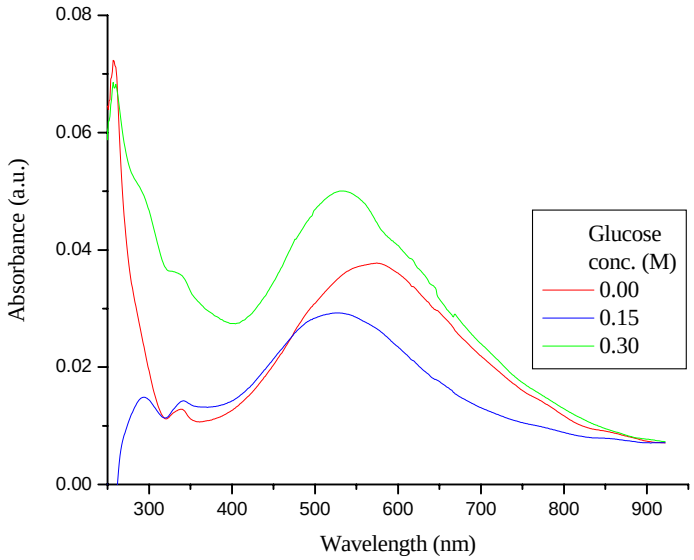


Figure 9: UV-vis. absorption spectra of silver nanoprisms in the presence of different glucose concentrations

In addition, silver nanoprisms solutions were no stable in the presence of glucose. Figure 10 shows the evolution depending of the time for a silver nanoprism solution where the glucose concentration was 0.15M. It can be seen a decomposition of the nanoprisms after 45 minutes. According to Figure 10, silver nanoprisms are transformed into silver nanospheres. This was evidenced by the extinction efficiency of the in-plane band at approximately 550nm and, the formation of a stronger typical SPR band for nanospheres at *ca*. 420nm. This interesting result could not be elucidated at this point based on the chemistry involved in the reaction.

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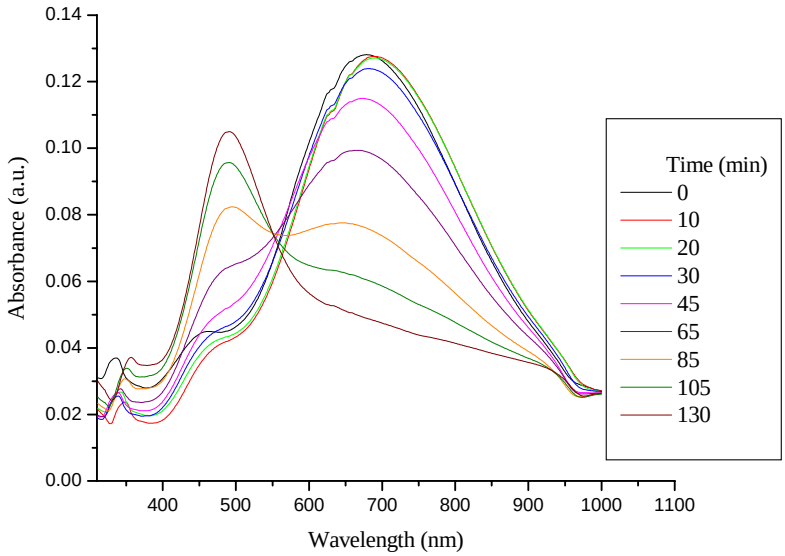

Figure 10: UV-vis. absorption spectra of silver nanoprisms in the presence of glucose at different times after the reaction was initiated.

All attempts using silver nanoparticles with different shapes failed for glucose sensing. Silver is not the best candidate for glucose sensing in human fluids because of the high content of NaCl. It is well known that NaCl precipitates Ag^{+} in the form of AgCl, a typical white solid. Also, the risk of having surface oxidation when using silver nanoparticles in aqueous media and in the presence of air discard this metal for this particular application. Therefore, more stable nanoparticles such as gold were tested.

5.2 Gold Nanoparticles

5.2.1 Change of the aspect ratio of gold nanorods with glucose

Two gold nanorod solutions were prepared using 0.2mL of gold nanorods with aspect ratio two, 0.20mL of ammonia hydroxide (2%), an excess of $HAuCl_4$, and glucose (1M). The blank was

0M in glucose while the other solution was 0.077M in glucose. The spectra of both solutions were taken after 30min. Figure 11 shows no appreciable spectral shift in the maxima absorption wavelength of the longitudinal mode of the glucose containing solution in comparison with the blank. Therefore, no change in the aspect ratio of these nanorods was produced.

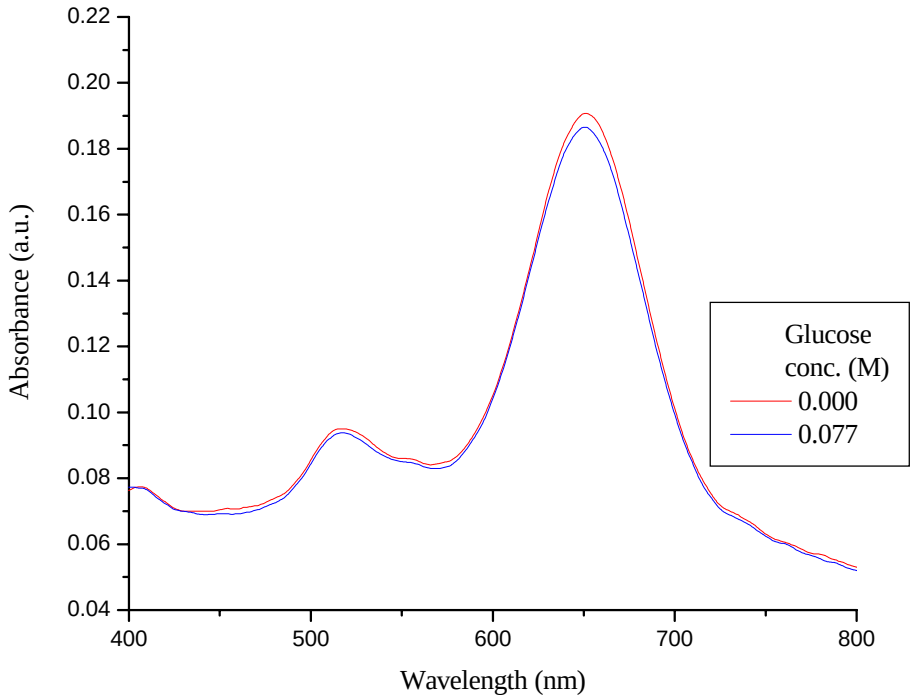

Figure 11: UV-vis. absorption spectra of gold nanorods with (—) and without glucose (—)

The experiment was repeated using different glucose concentrations. The spectra recorded after 30 min are shown in Figure 12. No representative spectral shift was observed in the maximum of the absorption of the longitudinal mode. However, as with silver nanorods, the transversal mode SPR band showed an increase in absorbance with the glucose concentration. This indicates the formation of gold nanospheres in solution when using glucose. The formation of gold nanospheres is due to the presence of the gluconic acid generated throughout the reaction. As in

silver, this acid can stabilize metal nanoparticles favoring the formation of nanospheres instead of increasing the length on the nanorods.[52] With gold the change in the absorbance seemed to increase linearly with glucose concentration. Probing this will confirm that silver nanoparticles are more susceptible to oxidation than gold in the presence of water and air. In addition, it could allow for monitoring low glucose concentrations in tears. Before getting to this part the direct generation of gold nanorods using glucose as reducing agent, was attempted.

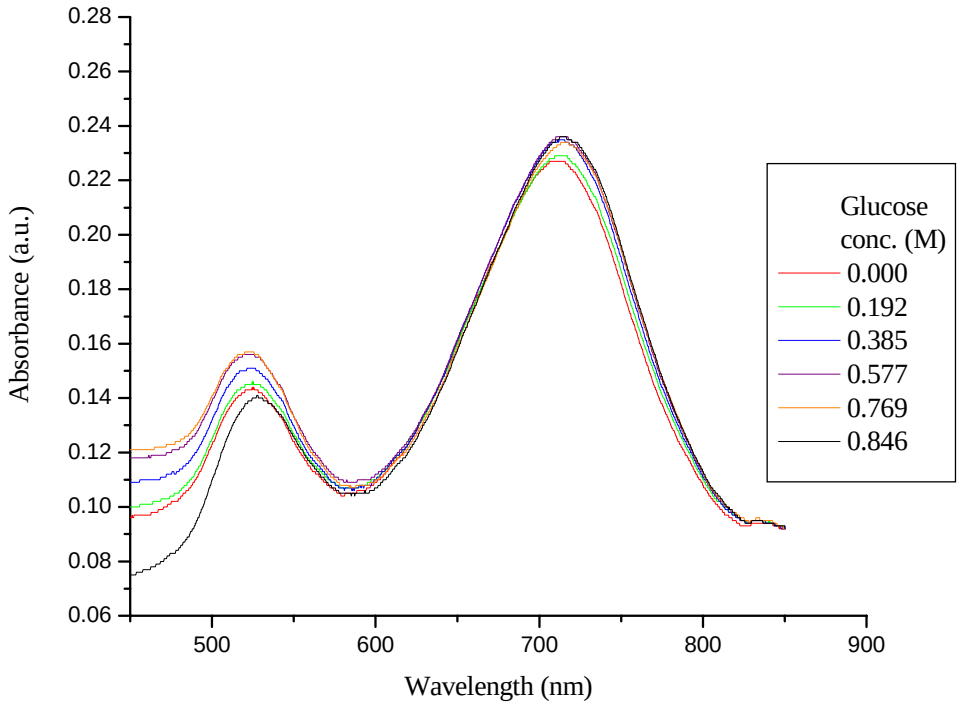

Figure 12: UV-vis. absorption spectra of gold nanorods in the presence of different glucose concentrations.

5.2.2 Generation of gold nanorods with glucose

Using a modification of the El-Sayed *et al* [47] method, the synthesis of gold nanorods with aspect ratio of three, was attempted using glucose instead of ascorbic acid as reducing agent. As in the case of the generation of silver nanorods using glucose, gold nanorods were not formed

The formation of gold nanospheres was obvious and the change in absorbance was linear with glucose concentration. We believe that the linear dependence is a consequence of the lack of oxidation of gold at the surface of the nanospheres.

5.2.3 Generation of gold nanospheres with glucose

Gold nanospheres were synthesized as described in the experimental section 4.3.3. Briefly, an aqueous solution $2.5x10^{-4}$M $HAuCl_4$ was mixed with aqueous ammonia (2%) and glucose (1M). The solution turned pink immediately after warming for 12min in a boiling water bath. The change in color of the solutions indicated the reduction of chloroaurate ions to form colloidal gold particles with typical SPR band centered at *ca.* 530nm. The absorption spectra recorded after 30min are shown in Figure 13. As the concentration of glucose increases, the absorbance of the SPR band increases, thus the amount of gold nanospheres produced is directly proportional to the glucose concentration added to the initial solution. Something very remarkable is the fact that as glucose is oxidized to gluconic acid, the latter acts as a surfactant stabilizing the nanoparticles in suspension [52].

Sastry M. *et al* [52] reported the same reduction of chloroaurate ions by glucose to form gold nanoparticles working at higher glucose concentration in a no basic conditions. Authors observed nanoparticles settled down after the period of experimentation. They attributed the precipitation to insufficient gluconic acid molecules covering the colloidal particle for stabilization. In the present work, the particles were not settling down after any period of time. It suggested that the reducing power of glucose was increased by the addition of NH_4OH, increasing the number of gluconic acid molecules that stabilize the nanoparticles.

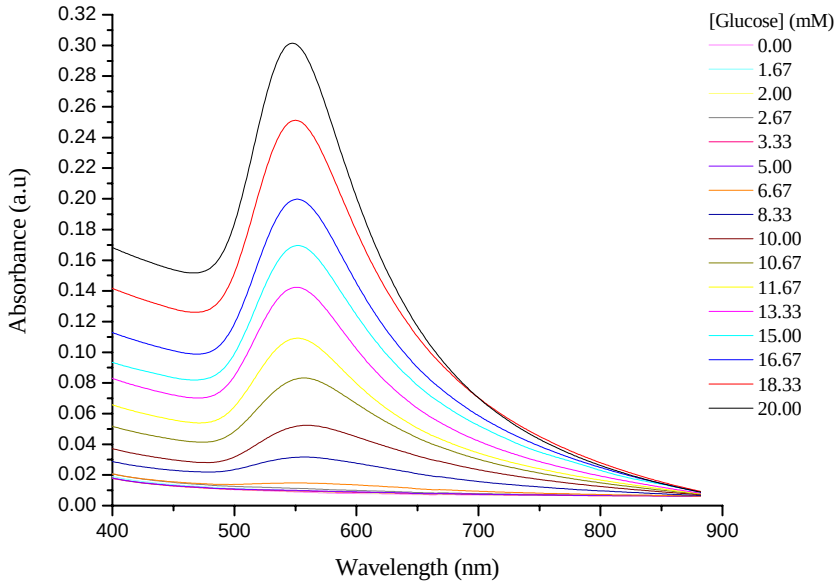

Figure 13: UV-vis. absorption spectra of gold nanospheres generated using different glucose concentrations. (Average on four trials).

Figures 14 show the average absorbance measured at the maximum of the spectra of gold nanospheres, generated with different glucose concentration in solution (average on four trials). This plot reveals the linear dependence of the absorbance with glucose concentration above 0.00667M. The increase in absorbance as the glucose concentration is increased is due to the increased number of nanoparticles generated in solution. The small spectral blue shift observed for high glucose concentration is due to the differences in size of the nanospheres.

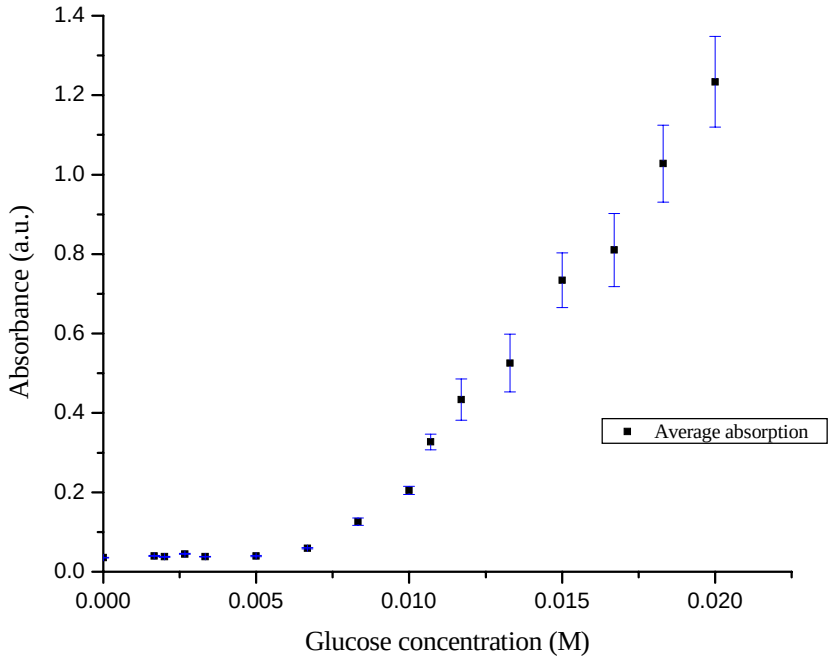

Figure 14: Maximum absorption of gold nanospheres generated at different glucose concentrations. (Average on four trials).

Figure 14 demonstrates that at low glucose concentration, *i.e.* below 0.00667M, the absorption of the solutions is constant and similar to the blank. If the glucose concentration is not high enough to produce gold nanospheres greater than *ca.* 3nm the absorbance of the smaller nanoparticles overlaps with the electron-hole transition band. [53] On the other hand, for concentrations greater than 0.00667M, a linear dynamic range up to 0.2M is observed.

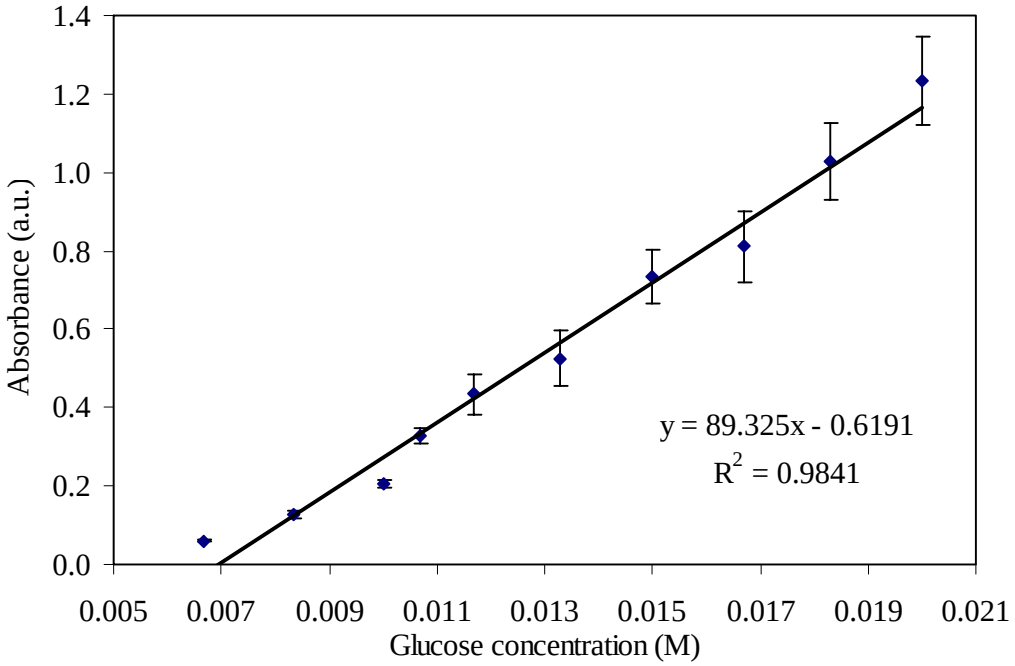

Figure 15: Plot showing the dynamic range of the UV-vis. absorption spectra of gold nanospheres generated at different glucose concentrations

The linear dynamic range shown in Figure 15 has been determined using 10 different glucose concentrations. The correlation coefficient (R equals to 0.9920, and R^2 equals to 0.9841) and the slope of the log-log plot (0.9868) are close to unity, demonstrating a linear relationship between the absorbance of gold nanoparticles in solution with glucose concentration. The limit of detection (LOD) was calculated with the following Equation (2), [54]

$$LOD = 3S_R/m, \qquad (2)$$

where m is the slope of the plot and S_R is the standard deviation of the blank ($S_R = \pm 0.00104$). The LOD was equals to 3.5 x 10^{-5} M, but Figure 14 reveals that the detection of glucose concentration below 0.00667M is not possible using gold nanospheres. In order to obtain a

realistic value, a limit of quantitation (LOQ) was estimated. Using the LOQ, the lowest glucose concentration that can be determined using gold nanospheres is be 7.5mM.

CHAPTER SIX: CONCLUSIONS

- Silver and gold nanoparticles were synthesized using glucose as reducing agent.

- Silver nanoparticles of different shapes: nanorods and nanoprisms, were not sensitive to changes in the presence of different glucose concentrations.

- Gold nanospheres were sensitive to glucose concentration changes.

- The absorbance of gold nanospheres SPR band increases directly proportional with glucose concentration.

- The gold nanospheres sensor can detect glucose concentrations as low as 7.5mM in aqueous solution.

- This work demonstrated a new approach for glucose sensing using gold nanoparticles, with low limit of detection and extraordinary selectivity.

CHAPTER SEVEN: FUTURE WORK

- Improve the sensitivity of the glucose sensor based on gold nanoparticles by controlling the size of the generated nanospheres.

- Test the sensor in human fluids and study of the matrix effects.

- Study of new metal nanostructures such as nanocubes and nanocages.

- Study of nanoparticles fluorescence for glucose sensing pumping in the UV region.

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