A Novel Electrochemical Glucose Biosensor Based on TiO_2 Nanotube Arrays

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THESIS

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LIST OF ABBREVIATION

PB	Prussian Blue
PW	Prussian White
Au NPs	Gold Nanoparticles
Ag NPs	Silver Nanoparticles
AgO	Silver Oxide
PBS	Phosphate Buffer Saline
GOx	Glucose Oxide
$HAuCl_4$	Chloroauric Acid
1,2-DAB	1,2-diaminobenzen
SCE	Saturated Calomel Electrode
ISO	International Organization for Standardization
$[Fe\ (CN_6)^{3-}]$	Ferricyanide
TiO2	Titanium Dioxide
$C_6H_{10}O_6$	Glucono-d-lactone
FAD	Flavin Adenine Dinucleotide
GDH	Glucose-1-dehydrogenase
UV	Ultraviolet
WHO	World Health Organization
TEM	Transmission Electron Microscopy

LIST OF ABBREVIATION (Continued)

FESEM	Field Emission Scanning Electron Microscopy
EDS	Energy Dispersive X-ray Spectroscopy
XRD	X-ray Diffraction
H_2O_2	Hydrogen Peroxide
TNTs	Titanium Nanotubes
DI	Deionized
Ti	Titanium

SUMMARY

Diabetes mellitus is a widespread endocrine disorder of carbohydrate metabolism. It is a primary reason of morbidity and mortality and a main health problem. In this study, we developed two high performance glucose biosensors based on the immobilization of glucose oxidase (GOx) onto TiO_2 nanotubes (TiNTs) arrays modified by Prussian blue (PB) and Au and AgO NPs. Electrochemical Anodization followed by Ag electroplating process in the same electrolyte was used for synthesizing of Ag oxide deposited Titanium nanotubes. Deposition of Prussian blue (PB) particles was performed from an acidic ferricyanide solution. The surface morphology and elemental composition of the two fabricated biosesnors were investigated by scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) which exhibits successful deposition of Au and AgO nanoparticles as well as PB nanocrystals. Cyclic Voltammetry and Chronoamperometry were used to investigate the performance of the modified electrochemical biosensors. Findings reveal that the GOx/PB/Au/TiO_2 NTs electrode considerably enhanced the current response signal in glucose solution in compared to absent of glucose. In a same way, the GOx/PB/Ag/TiO_2 NTs electrode noticeably improved the current response signal in glucose solution in compared to absent of glucose. In addition, both electrodes exhibit significant increase in the current response signal as compared with the GOx/TNTs electrode. The results show that the developed electrochemical biosensors display good stability,

low detection limit as well as high reproducibility for the determination of glucose. Under the optimized conditions, the Amperometric response shows a linear dependence on the

concentration of Glucose with a detection limit down to 4.91 µM and 58.7 µM, sensitivity of 185.1 µA and 29.1 µM for Au NPs and AgO NPs modified biosensors respectively. Therefore, the reported study proposed a novel method for developing an enzymatic electrochemical glucose biosensor based on deposition of AgO NPs and PB nanocrystals on Titanium NTs.

Chapter 1: INTRODUCTION

Diabetes mellitus is a worldwide pathological disease threatening human health state globally(1). Lack of hormone insulin due to disorder function of pancreas or inappropriate insulin metabolism by the body lead to glucose level beyond the standard range of 80-120 mg/DL in blood plasma and diabetes problem(2). Numerous novel research attempts demonstrate the growing demand for sensitive and reliable glucose biosensors during the recent decades(3). Recently, metal oxide-based biosensor such as titanium oxide has been attracted considerable attention in detecting glucose level of body(4).

Titanium nanotubes (TNTs) have been considered as one common used nanomaterial in a variety of biosensor application due to their distinctive features and unique structures, e. g., large active surface area, great thermal and chemical stability, and excellent mechanical strength(5)(6). These inorganic nanostructures have been used as a suitable matrix for enzyme immobilization to stabilize the enzyme as well as retaining functionality of the enzyme(7).

Based on various research studies, some noble metals modified TiO_2 nanotubes demonstrate better catalyzation property due to their tremendous features such as biocompatibility and great conductivity(8). They can be considered as catalyzer to improve electrochemical reaction rate as well as electron transfer channel between enzyme and active surface of the electrode(8)(9).

Gold (Au) and Silver (Ag) nanoparticles are among the most promising metal nanoparticles for biosensing application due to many favorable attributes such as excellent conductivity, simplicity of fabrication and cost efficiency(9)(10).

Basic mechanism of glucose measurement is based on oxidization of β-D-glucose by dissolved molecular oxygen with catalyzing immobilized Glucose oxide (GOx) enzyme which results in generation of gluconic acid and hydrogen peroxide (H_2O_2)(11). During process of glucose measurements, generally a high working potential of 0.6 V is required for H_2O_2 reduction which result in electrochemically oxidation of some active species in blood plasma, e.g., uric acid and ascorbic acid which in turn cause an interfering amperometric signal current and decrease the overall selectivity and accuracy of glucose biosensor(12).

Due to top selectivity and electrocatalytic function of Prussian blue (PB) ($Fe_4(Fe(CN)_6)_3$) to H_2O_2 reduction, it has been known as an "artificial enzyme peroxide" in electrochemical reduction of hydrogen peroxide(13). Prussian blue as a mediator is generally employed to form an oxidase/peroxidase system with cooperation of enzyme glucose oxide. Hence, the electrochemical oxidation changes to a reduction process that occurs in a much less potential and significantly improve selectivity and sensitivity of the glucose biosensor(14).

In this research study, we have developed two high performance glucose biosensors based on immobilization of enzyme glucose oxide onto Au and Ag oxide (AgO) modified titanium nanotubes (TNTs), following by deposition of Prussian Blue (PB) as the electron transfer mediator. The surface morphology and elemental composition of the two fabricated biosesnors

were investigated by scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) which exhibits successful deposition of Au and AgO nanoparticles as well as PB nanocrystals. The electrochemical measurement of the developed biosesnors demonstrates high sensitivity and selectivity, excellent biological stability and low detection limit toward determination of glucose concentration.

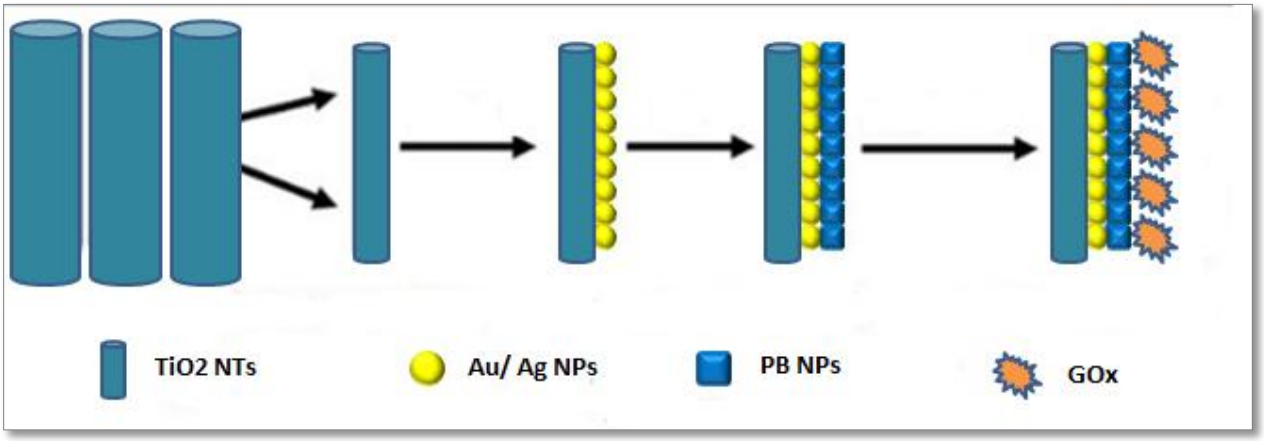

Figure 01 - General Schematic for preparing bienzyme electrode based on Tio_2 NTA

Chapter2: REVIEW OF LITERATURE

2.1. Introduction

Diabetes mellitus is considered as one of the most general endocrine disease of carbohydrate metabolism. Globally, it is a key health problem in many developed countries and a foremost reason of death and disability(15), (16) .Diabetes prevalence boost continuously. It is estimated that number of people who have diabetes will reach to 48.3 million in the US by 2050(17). According to the World Health Organization (WHO) diabetes caused 1.5 million deaths in 2012. In addition, the total numbers of people with diabetes from 108 million adults in 1980 have increased to an estimated 422 million adults in 2014(18).

Determination of glucose concentration in blood plasma is the main principle for diagnosing diabetes(19). Due to worldwide increasing rate of diabetic patients, developing of some appropriate clinical and industrial glucose-measuring devices is crucial. Development of biosensor technology with accurate response can play as a key factor for treatment and diagnosis of diabetes patients(20). Electrochemical signal-based biosensor represents many of essential requirements for glucose detection and control of diabetes. Measurement of a particular substrate can be achieved by specificity of the enzyme which is coupled with a signal transduction(21).

A biosensor can be defined as a “compact analytical device or unit incorporating a biological or biologically derived sensitive recognition element integrated or associated with a physiochemical transducer”(22). In general, a biosensor contains of three main parts: 1) the

molecular-recognition element which distinguish the objective molecules among a variety of samples including chemicals or body fluids. 2) a transducer which directly produce a respond signal in comparative to the analyte concentration. 3) data analysis system to convert and process the transduction signal(23)(24). Choosing the immobilized biological recognition elements such as variety of antibodies, enzymes and microorganism depend on the analyte. The recognition elements show particular binding affinity to the objective compounds which result in higher sensitivity and selectivity of the biosensor(22)(25).

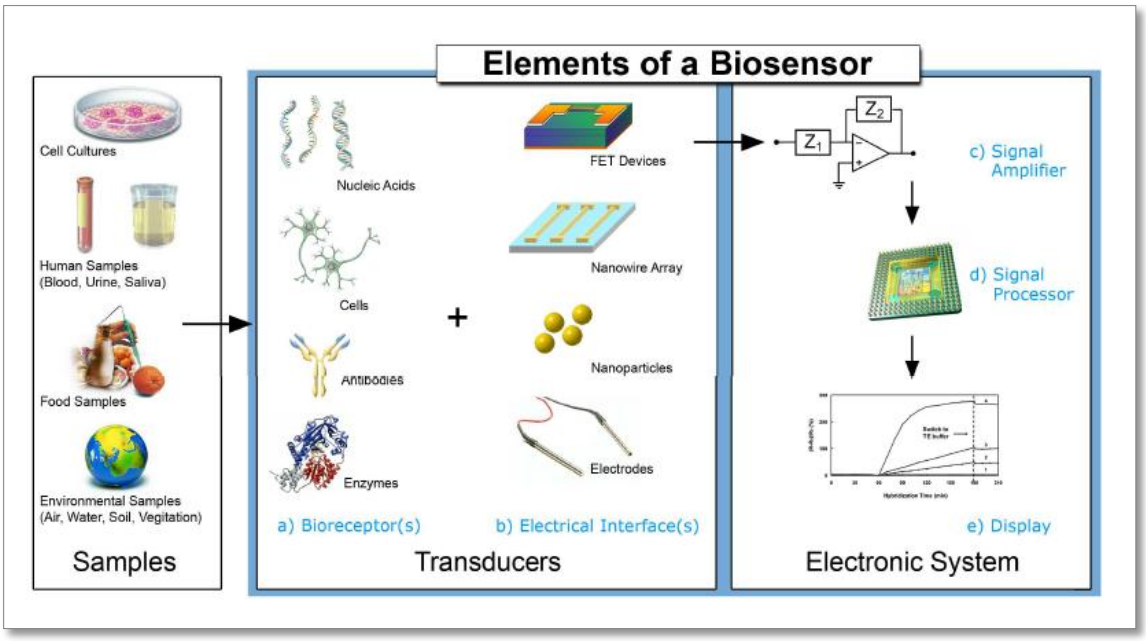

Figure 02 - Elements of a typical biosensor[26]

Electrochemical biosensors as the most common type of current glucose biosensors can firmly support their biosensing application due to their excellent properties such as sensitivity, stability, simplicity of operation and reasonable cost(21). The primary mechanism of electrochemical biosensing which is achieved by electrochemical techniques (e.g., amperometric and potentiometric) is based on biochemical reactions.

In general, adjustment of medium conductive properties between electrodes will result in generation of a measurable current during a chemical reaction process as shown in Fig3 (30). Despite all the mentioned merits of electrochemical biosensor, there are some drawbacks specially when it comes to the enzymatic reaction. The slow electron transfer efficiency between the biological recognition component and the electrode surface are considered as the most important disadvantage(28)(29). This is due to the incapacity of the enzyme to adjust itself satisfactorily with regards to the electrode surface for quick electron transfer and the location of the redox active site deep within the enzyme(28).

TiO_2 nanomaterials with specific properties such as biocompatibility and stability are considered as the unique candidates for biological elements immobilization(6)(27). This article will provide a general review of the highly developed research studies on electrochemical glucose biosensors that specifically focus on developments with functionalized titanium nanotubes and their applications in new generation of electrochemical glucose biosensing. Moreover, the most common experimental methods for detection and overcome the limitation related to coupling an enzyme with the signal transduction have been discussed.

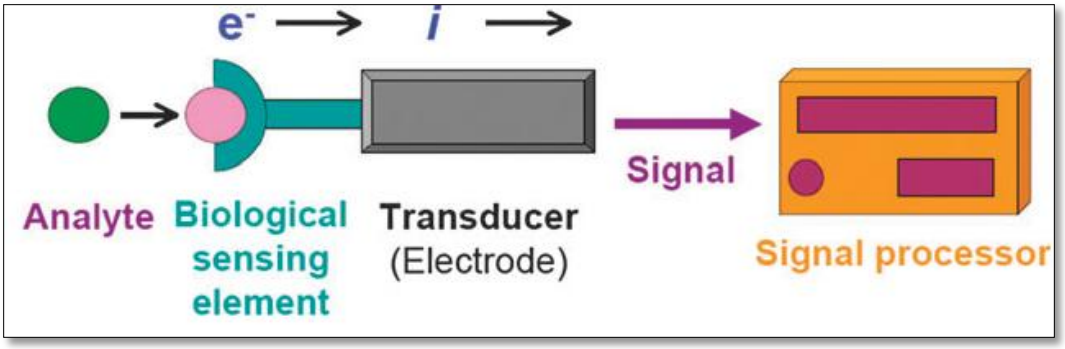

Figure 03 - Detection Process of Electrochemical Biosensor[30]

2.2. Electrochemical Principles of Glucose Biosensors

There are three main types of electrochemical glucose biosensors which include amperometric, potentiometric and comductometric(31)(32)(33). In an electrochemical biosensor, the electrode acts as the signal transducer where the chemical reaction take place and the recognition response is either an electrical current due to a redox reaction or change in electrode potential (amperometric and potentiometric sensor)(26). The most common commercial glucose biosensor which broadly studied during the last few decades is the enzymatic amperometric biosensor(34). In a typical amperometric sensor electron exchange between the electrode and analyte will result in generation of a measurable current(31)(34).

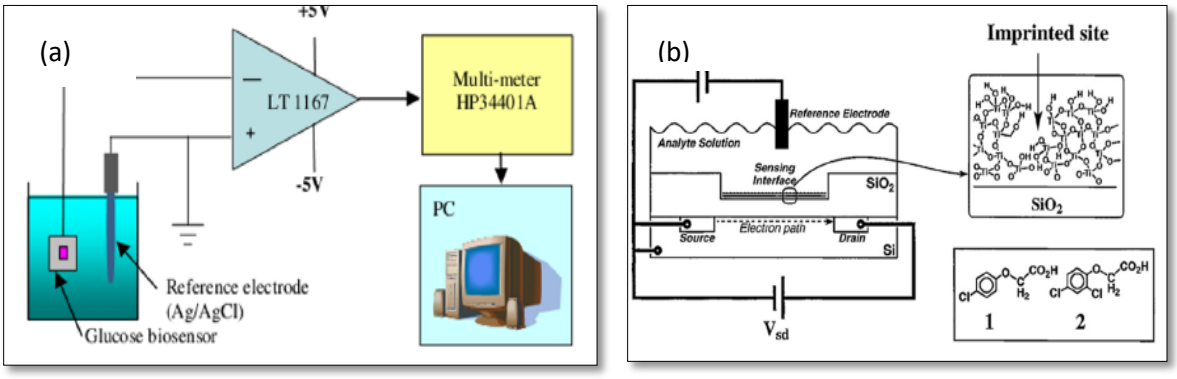

Figure 04 – (a)Amperometric and (b)Potentiometric Electrochemical Biosensors[35]

2.2.1. Historical Perspectives of Glucose Biosensors

The concept of electrochemical bio-sensing has its origin in 1962 by Clark and Lyons(23). Their work described the reaction between glucose and glucose oxidase with potentiometric electrochemistry for the electrochemical signal transduction to form an enzyme glucose biosensor. Since the pioneering work of Clark and Lyons, many studies has extended based on electrochemical glucose biosensor but the fundamental sensing system stay relevant today(21).

In first generation glucose biosensor, the oxygen is reduced to produce hydrogen peroxide in the presence of glucose and glucose oxide(2). The glucose concentration is proportional to oxygen reduction rate which is measured by increasing of hydrogen peroxide or decreasing the oxygen concentration(34)(36). However, the major problem with first-generation protocol was high positive overpotential of hydrogen peroxide. This high operation potential may results in

interference effect of some electroactive species such as uric acid and ascorbic acid which are common in biological fluids(34).

To overcome the aforementioned limits of first generation glucose biosensor, oxygen was replaced with employing artificial electron mediators such as ferricyanide, ferrocene and hydroquinone(34). The first studies on the electrochemical signal measurement of glucose using a redox mediated, GOx catalyzed reaction was considered in 1970(37). Ferrocenes fulfill all the requirement of an appropriate mediator, e.g., stability in oxidation and reduction, quick reaction with the enzyme, pH independent and no reaction with oxygen(38)(39). In second-generation of glucose biosensor, a faster electron transfer is occurred using a mediator as a redox electron acceptor for electron-shuttling between the electrodes and enzyme(40)(41).

In the third generation glucose biosensors, direct electron transfer between the electrode and enzyme produces an amperometric current signal. As a substitute for mediators, enhanced sensing performance is achieved by incorporating conductive and semi-conductive nano-materials with the enzyme based on charge-transfer matrices(42)(43). Viticoli et al. fabricated a third generation electrochemical biosensor based on functionalised TiO_2 thin films on Si substrates for the immobilization of different enzymes such as glucose oxide and horseradish peroxidase(44).

In four generation of glucose biosensors, some progress has been achieved in development of enzymeless glucose sensing based on direct oxidation of glucose(45)(46). Different semi-

conductive nanomaterials from Gold, Platinum, Carbon and Silver have been investigated to improve sensing mechanism of enzymeless glucose biosensors(47)(48). However, there are some remaining drawbacks due to the low sensitivity and poor selectivity relating to surface poisoning of the electrode which are considered as the obstacle in success of these electrochemical glucose biosensors(49).

2.2.2. Sensing Properties of Electrochemical Glucose Biosensors

The basic mechanism in glucose determination involves three enzymes: Glucose oxidase (GOx), Glucose-1-dehydrogenase (GDH) and Hexokinase(50)(51). The two enzymes GOx and GDH are commonly applied in self monitoring of blood glucose and the hexokinase assay is the measuring method for glucose determination using spectrophotometry in many medical laboratories(52)(53). The differences between the enzymes are related to cofactors, redox potential and glucose selectivity(54). GOx is considered as the main standard enzyme for biosensor due to its several favorable properties such as top glucose selectivity, cost-effective, great pH and thermal stability, and availability(54)(55). The enzyme glucose oxide (GOx) consists of one coenzyme molecule as flavin adenine dinucleotide (FAD) in the active site and two similar protein subunits. The FAD molecule firmly bound to the enzyme but not attached covalently(52)(55).

In development of Electrochemical Glucose Biosensor, the main concept is based on oxidization of β-D-glucose by dissolved molecular oxygen with catalyzing immobilized GOx enzyme which

results in generation of gluconic acid and hydrogen peroxide(11)(52). The redox cofactor of GOx, Flavin adenine dinucleotide (FAD) works efficiently as the electron acceptor and reduces to form $FADH_2$. Due to oxidation of $FADH_2$ by the dissolved O_2, Hydrogen Peroxide is produced, and the enzyme returns to its original state FAD(54)(55). The reaction of glucose ($C_6H_{12}O_6$) and O_2 which results in generating glucono-d-lactone ($C_6H_{10}O_6$) and H_2O_2, has shown as follows(56):

$$\text{Glucose} + \text{GOx} - \text{FAD}^{+} \longrightarrow \text{Glucolactone} + \text{GOx} - FADH_2$$

$$\text{GOx} - FADH_2 + O_2 \longrightarrow \text{GOx} - \text{FAD} + H_2O_2$$

The Enzyme cofactor is regenerated by reacting with dissolved oxygen, result in producing of hydrogen peroxides:

$$\text{Glucose} + O2 \xrightarrow{\text{GOx}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 \longrightarrow 2H+ + O_2 + 2e$$

The schematic diagram of glucose oxide (GOx) enzymatic reaction has been shown on figure 5.

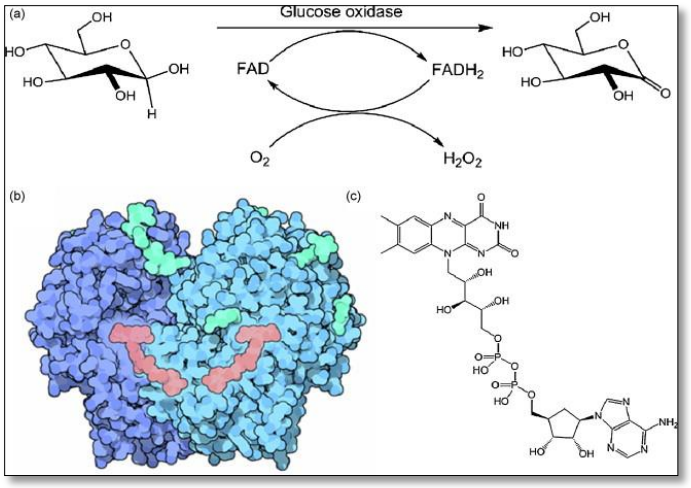

Figure 05 - Schematic diagram of glucose oxide (GOx) enzymatic reaction[57]

The generated Hydrogen peroxide is oxidized at anode electrode. The electrode identifies electron transfer flow which is proportional to glucose molecules numbers(58). This process will result in producing an amperometric signal current which can be measured easily by applying appropriate voltage.

If an artificial electron mediator (M) such as Ferricyanide or ferrocene is used in reaction, the reduced form of GOx ($FADH_2$) is oxidized while reduction of oxidized mediator will result in reduced form of the mediator(2)(37). Mainly the ferricyanide ($Fe\ (CN_6)^{3-}$) and enzyme GOx are exchanging electrons. A typical reaction of a mediator (ferricyanide ($Fe\ (CN_6)^{3-}$)) and reduced form of flavin group of Enzyme GOx ($FADH_2$), has shown as follows(37)(59):

$GOx - FADH2\ (reduced) + Fe(CN_6)^{3-} \longrightarrow GOx - FAD^{+}\ (oxidized) + Fe(CN_6)^{4-}\ (reduced)$

$Fe(CN6)^{4-}\ (reduced) \longrightarrow Fe(CN_6)^{3-}\ (oxidized) + e^{-}\ (given\ to\ the\ electrode)$

The released electron from the mediator (ferrocyanide $Fe(CN6)^{4-}$) is carried out to the electrode surface. This process produces a current signal which is measurable by applying certain amount of voltage(59).

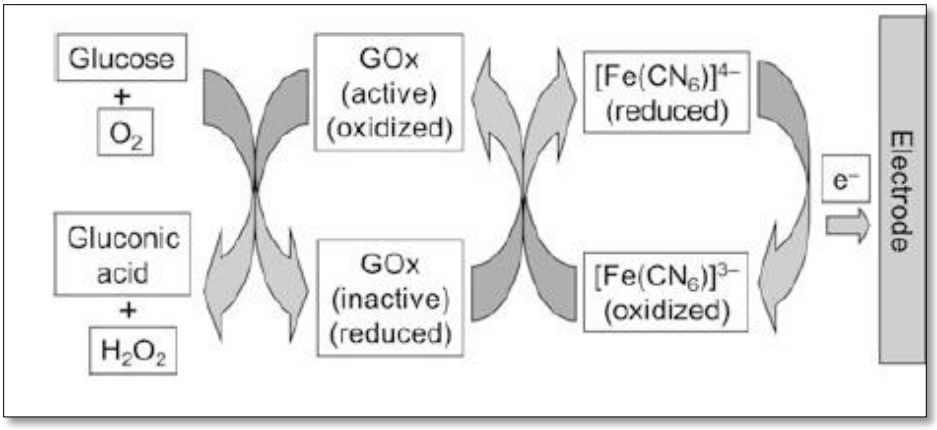

Figure 06 - Typical electrochemical detection of glucose[60]

On the whole, direct sweeping of the electron between electrode surface and enzyme is considered as a challenge. The main reason is conformational changes by adsorption on the

surface of electrode which result in decreasing of enzyme bioactivity. Therefore, it is essential to modify the surface of the electrode to achieve an accurate and stable response(61).

Titanium nanotubes (TNTs) are considered as an appropriate intermediate between the enzymes and electrodes. Due to their favorable attributes, e.g., top surface to volume ratio, high surface area, excellent mechanical strength and acceptable electrical conductivity, Titanium nanotubes (TNTs) application have been widely utilized in a variety of biosensor research studies(27)(62).

2.2.3. Glucose Sensor based on Titanium Oxide Nanotubes

TiO_2 nanomaterials as one sort of semiconductors found a broad interest in recent decades(27)(63). This is owing to the unique features of TiO_2 nanomaterials, e. g., biocompatibility, non-toxicity, resistive of photocorrosion, reasonable cost of fabrication and a potential interface for biomolecules immobilization(27). In addition, titanium retains the biocatalytic properties of the enzyme with coordination bonds between the enzyme amine groups(64).

Due to electron accepting attribute of Titanium, the electrons release can be carried out to the surface of electrode which results in simple detemination of the reaction(27). Distinctive physical and chemical properties of Titanium nanotubes in addition to their excellent large surface area provide an extensive attention in research studies of TiO_2 nanotubes structure, transduction function properties and simulation mechanism in biosensor application(65)(66).

Hence, TiO_2 nanotubes are considered as one of the effective intermediates in biosensor research studies(65)(67).

2.2.4. Measurement Principle of Electrochemical Model

Titanium oxide nanotubes electrode typically operated as the working electrode with corporation of reference and counter electrode in a three-electrode electrochemical work station as shown in Figure 3.1(68). In amperometric measurements, a redox reaction generally forms between objective molecules of the supporting electrolyte and electrode which result in generating a measured current using an electrochemical system workstation(69). During this mechanism, two modes of current signal measurement which called amperometry and voltammetry are carried out based on consent or variable applied potential. In general, the analyte concentration is corresponding to the peak values of the amperometric signal over linear range of applied potential in both mechanisms(31)(70).

Wang et al reported a Ti/TiO2 Nanotubes /Ni Composite electrode for amperometric detection of glucose. Using saturated calomel electrode (SCE) electrode as the reference and a platinum plate as the counter electrode, the Current Voltage measurement of Ti/TiO_2 NTs/Ni Composite electrode is shown in Fig7. Graph (a) shows CV measurements of Ti/TiO2 NTs/Ni electrode in 0.1 M NaOH solution in absence (red line) and presence (blue line) of 0.2 mM glucose concentration at a scan rate of 50 mV/s. The anodic peak current is clearly increased from 50 to 80 μA by injection of glucose, however the cathodic current has no considerable

changes. The anodic current signal in the positive scan is proportional to the glucose concentration, and with increasing the glucose concentration, an improvement of the anodic currents will be resulted, as shown in graph (b)(71).

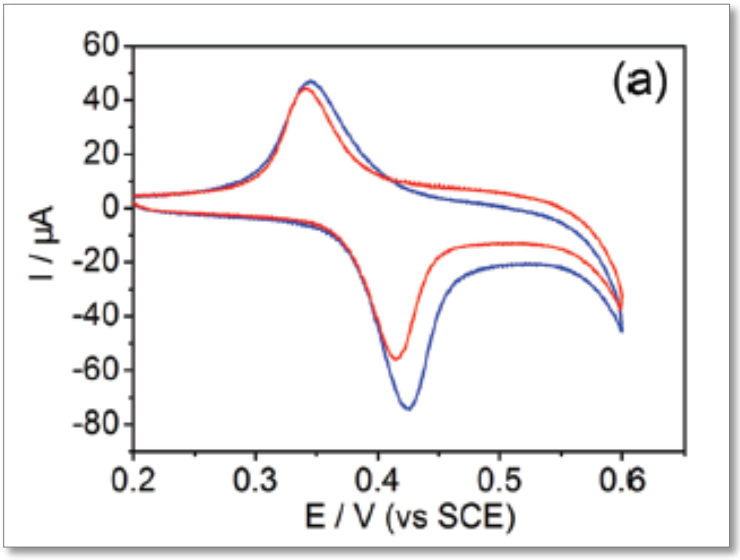

Figure 07 - (a) CV measurements of the Ti/TiO2 NTA/Ni electrode in the 0.1 M NaOH + 0.2 mM glucose (blue line) and 0.1 M NaOH (red line) solutions.(71)

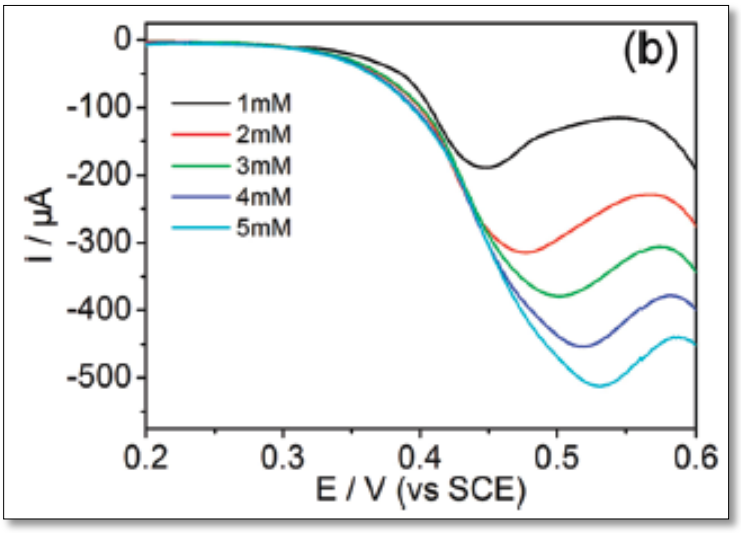

Figure 07 - b) Several concentrations of glucose: 1, 2, 3, 4, and 5 mM in positive line scans of the Ti/TiO2 NTs/Ni electrode in 0.1 M NaOH solution.(71)

2.2.5. Sensing Electrode of TiO2 Nanotubes Biosensor

Titanium nanotubes (TNTs) synthesizing from titanium substrate by electrochemical oxidation are among the most favorable nanostructures during the recent decades(72). Looking back into the TiO2 NTs history, Grimes's group research studies involved in novel synthesizing method and potential application of TNTs(73)(74)(75). A variety of synthesizing techniques are employed in fabrication of TiO_2 nanotubes electrodes, e.g., electrochemical anodization, physical and chemical vapor deposition, hydrothermal and rapid breakdown anodization(72)(76).

In general, TNTs are obtained by titanium sheet anodization in a Fluoride-contained solution at a specific applied potential. Different factors effect on morphology of titanium nanotubes such as anodization time duration, concentration of electrolyte components and anodization applied potential(72)(77). Furthermore, Schmuki's group has reported synthesizing complex nanotube arrays by employing biomedical alloys, e.g., Ti_6Al_7Nb, (71) and $Ti_{29}Nb_{13}Ta_{4.6}Zr$ (78)(79).which are highly ordered and show great vertical structure.

The morphology of titanium nanotubes crystallized structure is recognized as the amorphous state, rutile, anatase and Brookite phase which are shown in Fig8.(80)(81). Specific annealing treatment and pressure are essential in order to conversion of amorphous to anatase and rutile crystalline structure(80). For instance, amorphous can be transformed into anatase phase with heat temperature of 300−500 °C(78). Due to high chemical strength and electron transfer capacity, anatase structure is commonly utilized in biosensor development(83)(84).

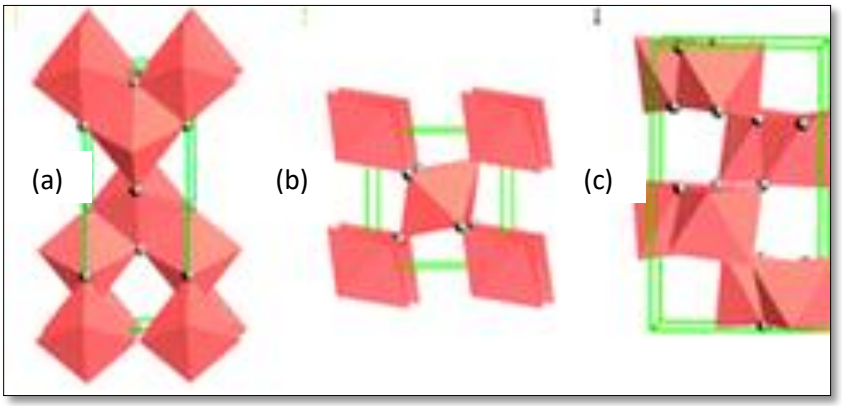

Figure08 - Various TiO2 structures: (a)Rutile and (b)Anatase have tetragonal crystal structure and (c)Brookite has an orthorhombic structure(85).

2.2.6. Electrochemical Glucose Biosensor Incorporating TiO_2 Nanotubes

Many research studies have brought into focus the TiO_2 nanotubes as the glucose biosensor matrix over the past decade(62)(86)(87). In compare to traditional electrodes, distinct properties, unique tubular features and high surface-to-volume ratio of TiO_2 NTs structures demonstrate facile heterogeneous electron transition kinetics in redox processes between the biomolecules from the solution to the active surface of the electrode(27)(88). Moreover, vertically aligned TiO_2 nanotubes are serving as a perfect "vessel" for enzyme immobilization in biosensor application(89).

However, in favorable electrochemical sensing of glucose, it is essential to obtain many required factors, e.g., sensitivity, quick response, accurate range of detection and stability, which

are hard to achieved by employing pure TiO_2 nanotube material electrodes(27). Hence, in addition to biomolecules including GOx or GDH enzymes as the key part of the glucose sensing, other mediator species such as polymer and noble metal have been utilized in TiO_2 nanotubes modification(90)(91). Many methods have been used in TNTs modification in order to achieve better direct glucose determination. For instance, silver nanoparticles modified TiO_2 nanotubes have shown a better performance in detection of glucose(92)(93).

Feng et al proposed a glucose biosensor based on photoreducing Ag nanoparticles on TiO_2 nanotubes following by GOx immobilization. As shown in Figure 9, Ag nanoparticles with average of 15 nm size were deposited on TiO2 NTAs surface uniformly. After optimization, high sensitivity of 0.39 μA has been achieved with liner range from 0.1 to 4 Mm toward H_2O_2 detection(93).

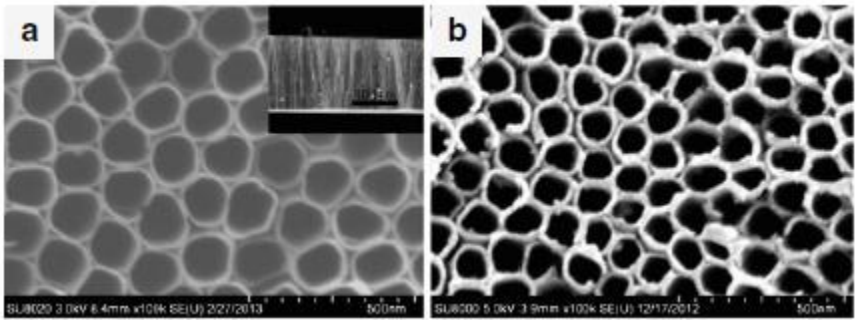

Figure09 - SEM morphologies of TiO_2 (a), Ag/TiO2 (b) NTAs in GOx/Ag/TiO_2 NTAs glucose biosensor(93)

In addition, Xie et al synthesized highly ordered Titanium NTs by anodization of the titanium foil. They reported a glucose biosensor based on GOx-pyrrole modified TiO_2 nanotubes with great reproducibility and high stability toward detection of glucose. After optimization, the proposed biosensor demonstrates high sensitivity of 45.5 μA and low limit of detection of 2 μM (94). The detection mechanism of GOD-modified TiO_2 NTs biosensor has been showed in Figure10.

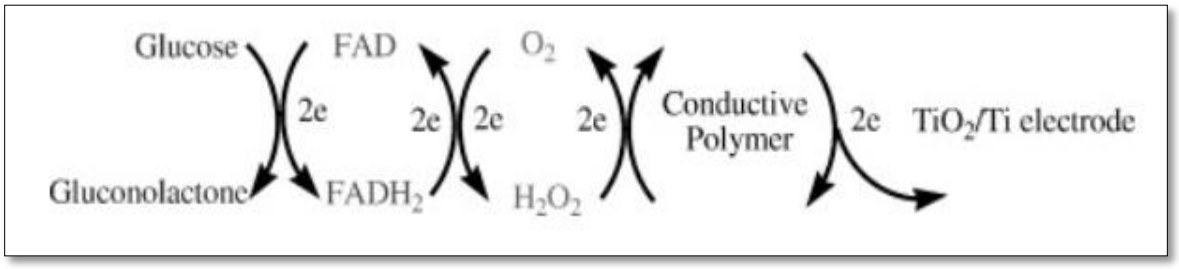

Figure10 - The amperometric detection mechanism of GOD-modified TiO_2 NTs glucose biosensor[94]

Benvenuto et al reported a high performance biosensor based GOx immobilization with Chitosan on TiO2 nanotubes modified by Au and Prussian blue. A thin film of Au was coated on the surface of TiO2 nanotubes by argon plasma technique, following electro-deposition of PB.

Their results demonstrate that the proposed glucose biosensor shows a high sesnsitivity of 36 μA and low detection limit of 5 μM under the optimum conditions(95). Figure11, presents the performance of several electrochemical glucose biosensors. The Ti/TiO2/Au/PB/GOx developed biosensor displays great performance in term of stability, sensitivity and linear range of detection.

Electrode modifier	Detection limit (μM)	Linear range (mM)	Stability	Detection potential (V)	Correlation coefficient	Reference
GCE/PB/GOx/DNA	5	0.050–0.40	90% (30 days)	0.1	N/A	[41]
AuE/PB/GOx	3.1	0.0060–1.60	73% (30 days)	0.0	0.9994	[35]
GCE/PB/MWCNT/GOx	12.7	0.00–8.00	85% (14 days)	0.0	0.995	[36]
Multilayer assembly of PB and poly(toluidine blue) with GOx	10	0.10–10.0	80% (21 days)	−0.1	0.998	[42]
Porous anodic alumina/PB/GOx	1	0.0050–8.00	85% (30 days)	−0.1	N/A	[47]
GCE/PB-modified/AuNP/GOx	0.69	0.0010–1.60	70% (14 days)	−0.05	0.9999	[15]
GCE/PBNWA/GOx	1	0.0020–10.0	82% (30 days)	−0.1	0.996	[44]
GOx on a PB-modified GCE/silica sol–gel outer layer	20	0.0–4.75	90% (45 days)	−0.05	0.9993	[46]
GCE/Chitosan-AuNP film/GOx	13	0.050–1.30	N/A	0.7	0.996	[45]
AuE/AuNP/GOx	8.2	0.020–5.70	70% (60 days)	0.3	N/A	[48]
Single-wall carbon nanohorns/nafion	6.0	0.00–6.00	N/A	0.3	0.993	[49]
Carbon nanotube/Au NP	6.7	0.01–10.00	92% (30 days)	0.35	0.993	[50]
NiO hollow Nanospheres/chitosan	47	1.00–7.5	85% (2 days)	0.35	N/A	[51]
Proposed biosensor	5.0	0.0150–4.00	90% (21 days)	−0.1	0.9993	This study

GCE – glassy carbon electrode, GOx – glucose oxidase, AuE – gold electrode, MWCNT – multi-walled carbon nanotubes, PBNWA – Prussian blue nanowire array, PB – Prussian blue, Au – gold, NP – nanoparticles, N/A – not available.

Figure11 - Comparison of the performance of different glucose biosensor(95)

2.3. Conclusion: Future Prospective and Challenges

With the advent of nanotechnology, numerous attempts have been made in investigation of molecular level glucose sensing devices(96). The innovation overcome some of the fundamental drawbacks of previous glucose biosensors e.g. kinetic limitation in electron transferring to the

glucose oxidase enzyme, reduction in the overpotential for H_2O_2 oxidation, or creating interfacial molecular architectures to optimize the signal transduction from the enzyme reaction(12)(97). Due to unique attributes of TiO_2 nanotubes such as large surface area and capability of enzyme immobilization, they have been considered as an attractive choice in scientific glucose sensing research studies(27). This Summery has reviewed recent developments on electrochemical glucose sensing incorporating TiO_2 NTs as well as fundamental concepts of electrochemical measurements in glucose biosensors.

Electrochemical glucose sensing incorporating modified TiO_2 nanotube materials still required more research and academic studies in order to achieve ultra sensitivity and faster detection response as well as developing reliable non-invasive continuous glucose biosensors and more accurate nonenzymatic biosensor for direct detection of glucose(27)(98). Last, increasing trend of diabetes prevalence will grow more research interest on novel idea developments in glucose biosensor studies. Novel research expertise as well as theoretical knowledge obtained through developments of TiO_2 NTs glucose biosensors can be taken as a valuable key in improvement of the whole biosensor technology.

Chapter3: MATERIALS AND METHODS

3.1. Chemicals and Instruments

Titanium sheets (0.25 mm thickness, 99.7 % purity), Potassium Ferricyanide, Potassium Chloride, 1,2-diaminobenzen (1,2-DAB) and Glucose oxidase (GOx) were purchased from Sigma Aldrich. Ferric(III) Chloride, Silver Flouride, ß-D(+) glucose were purchased from Fisher Scientific. All the chemicals were of analytical grade and were used without further purification. All the solutions were prepared with deionized water. Anodization of TiO_2 NTAs was performed using two electrodes with carbon rode and Ti foil as the cathode and anode respectively in a self-made electrochemical cell (KEITHLEY 2400).

3.2. Preparation of Au Nanoparticles Modified Titanium Nanotubes

3.2.1. Fabrication of Titanium NTs

Electrochemical anodization has been used for TNTs formation. Prior to anodization, pure Ti foil was sonicated in ethanol for 20 minutes following by rinsing with DI water. The anodization station was a self-made two-electrode electrochemical cell. The Ti foil used as the anode while a carbon rode electrode was served as the cathode. The anodization process was performed using power supplier at 60 VDC for 2 hours in a freshly prepared electrolyte containing 0.2 wt% NH4F, 10 vol % deionized water and ethylene glycol to form nanotubes on

the Titanium substrate surface. After nanotubes formation, the prepared sample was sonicated in isopropanol and deionized water each for 2 minutes.

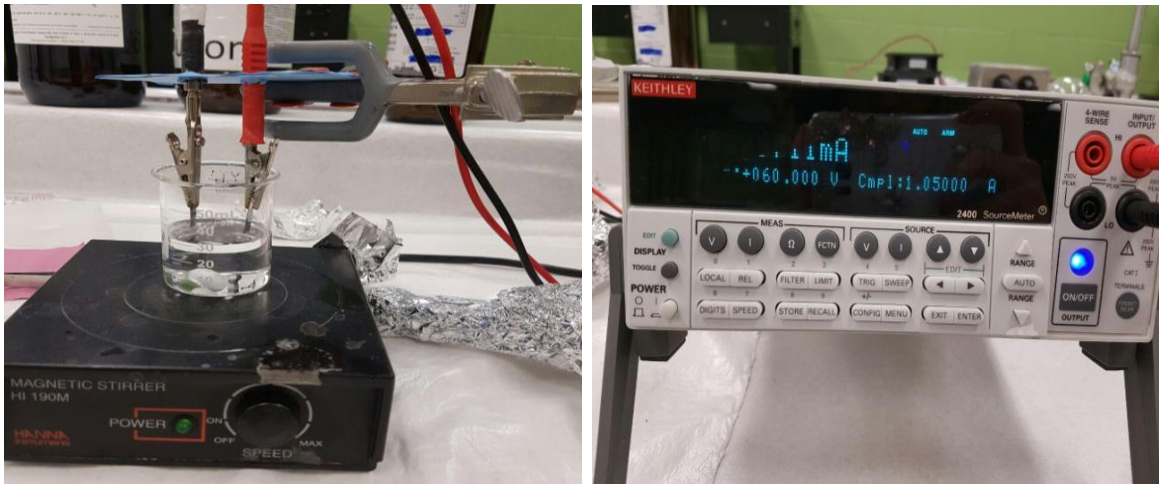

Figure12 - Working station for TiO_2 NTs Anodization

3.2.2. **Preparation of Prussian Blue/Au modified TNTs**

Colloidal AuNPs of about 10nm diameter was synthesized by sodium citrate reduction method following this protocol: 100 mL of 0.01% aqueous solution of $HAuCl_4$ was boiled under the strong stirring. Then, 2.5 mL of 1% trisodium citrate solution was added dropwise while

stirring. After around 15 minutes the solution turned into the deep red Figure 13(d) which representing AuNPs formation. The suspension was continued to stirring and cooling down to room temperature. The as-prepared colloidal AnNPs was stocked at 4'C until further use.

Figure13 - Preparation of Colloidal Au NPs by sodium citrate method

To fabricate Au modified TNTs electrode, TNTs were immersed in a 0.05% PDDA solution for 3 hours at room temperature. After rinsing in deionized water, the modified electrodes were immersed into 20 mL of the AuNPs colloidal solution (13nm) and incubated at 4℃ overnight. For decoration of the AuNPs modified TNTs electrode with PB NPs, the prepared sample was immersed in a acidic solution containing 0.1 mM K_3 ($Fe(CN)_6$) + 0.1mM $FeCl_3$ in the presence of 0.1M KCl overnight. Hydrochloric acid was used for adjusting the pH of aqueous solution to 1.6. As shown in Fig 14, deposition of PB nanoparticles was observed by forming a very thin dark blue film on the electrode surface.

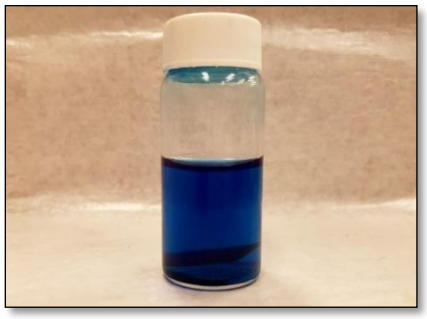

Figure14 - PB deposition process on Au NPs modified TiO2 NTs electrode

3.2.3. GOx Enzyme immobilization and fabrication of Glucose Biosensor

For enzyme GOx immobilization the electrochemical polymerization method was used to obtain GOx/PB/Au/TiO_2 NTAs sample electrode. The cyclic voltammetry was applied in a 0.01

M PBS solution electrolyte containing 30 mg ml of GOx and 10 mM o-Phenylenediamine (pH=7) at sweep voltage of 50 mV for 5-10 cycles. After rinsing with deionized water, the enzyme modified electrode was stocked at 4℃. Fig 14 has shown GOx immobilization process onto the modified TiO_2 electrode.

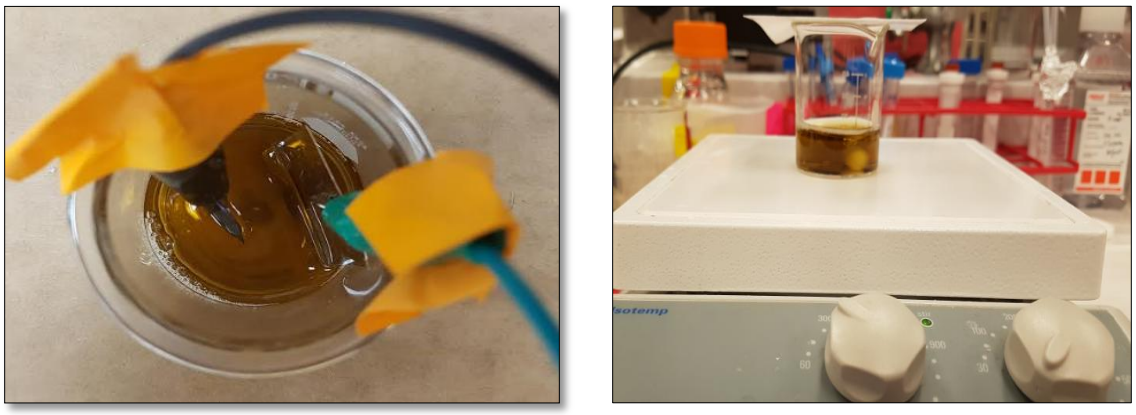

Figure15 - PB deposition process on Au NPs modified TiO2 NTs electrode

3.3. Preparation of AgO Nanoparticles Modified Titanium Nanotubes

3.3.1. Synthesize of AgO/TiO_2 NTs

Electrochemical anodization has been used for TNTs formation and Ag/TNTs electrode was prepared by following protocol: Firstly, pure Ti foil was sonicated in ethanol for 20 minutes following by rinsing with DI water. The anodization station was a self-made two-electrode

electrochemical cell. The Ti foil used as the anode while a carbon rode electrode was served as the cathode. The anodization process was performed at 60 VDC for 90 minutes in a freshly prepared electrolyte containing 0.686 g NH4F, 98 mL ethylene glycol and 2 mL deionized water to form nanotubes on the Titanium substrate surface.

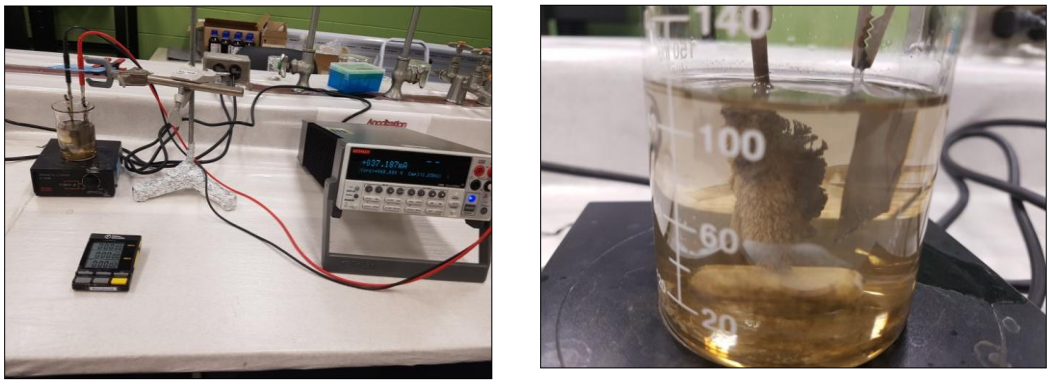

Figure16 - Synthesizing AgO/TiO2 NTs electrode by anodization TiO2 NTs following by electroplating of Ag NPs

Following formation of TNTs, deposition of Ag nanoparticles was performed by applying potential at 20 V for 60 seconds together with switching the electrodes. After deposition of Ag nanoparticles, the prepared sample was rinsed in isopropanol and deionized water each for 2 minutes.

3.3.2. Preparation of Prussian Blue/Ag modified TNTs

For decoration of the AgO NPs modified TNTs electrode with PB NPs, the prepared sample was immersed in an acidic solution containing 0.1 mM K3 ($Fe(CN)_6$) + 0.1mM $FeCl_3$ and 0.1 mM $HAuCl_4$ in the presence of 0.1M KCl with UV illumination for 10 hours. Hydrochloric acid was used for adjusting the pH of aqueous solution to 1.6. Photochemically deposition of PB nanoparticles was observed by forming a very thin dark blue film on the electrode surface.

3.3.3. GOx Enzyme Immobilization and Modification of Glucose Biosensor

Physical adsorption method was applied for immobilization of enzyme GOx to obtain GOx/Ag/TNTs electrode. Fresh enzyme solution was prepared in a 0.01 M PBS electrolyte containing 20 mg ml of GOx and 10 mM o-Phenylenediamine. Afterward, 50 μL of the solution was dropped onto the PB/Ag/TiO_2 NTs electrode and dried in the air following by keeping at 4℃ for 10 hours. After rinsing with PBS, the enzyme modified electrode was stocked at 4℃ when not in use.

3.4. Characterization

The surface morphology characterization of the both electrode samples was acquired with Field Emission Scanning Electron Microscope (FESEM0-JEOL JSM-6320F) and Scanning Electron Microscope (SEM, Raith 100). Elemental composition analysis of fabricated electrodes was investigated by energy-dispersive X-ray spectroscopy (EDS-Hitachi S-3000N VPSEM). Cyclic

voltammetry and Chronoamperometry measurements were performed with a three-electrode Electrochemical Workstation (Bio-Logic VMP3 equipped with EC-Lab software) comprising of an Ag/AgCl reference electrode, a Pt foil auxiliary electrode as well as two modified Titanium nanotubes electrodes (GOx/PB/Au/TiO_2 NTAs) and (GOx/PB/AgO/TiO_2 NTAs) as the working electrodes.

Chapter 4: RESULTS AND DISCUSSION

4.1 Titanium Anodization

Anodization is a procedure which results in forming an oxide layer on the surface of a metal. During this anodizing process, the metal is acted as an anode in a two electrode electrochemical cell. Titanium anodization process has been performed for preparation of TiO_2 nanotubes, using the electrolyte consists of Fluoride reagent, Ethylene Glycol and Dionized water (DI). A huge voltage drop takes place between two electrodes because of high resistivity of ethylene glycol. Addition of DI water increase the electrolyte conductivity and results in rapid formation of nanotubes. By applying the positive voltage to the working electrode the oxide layer will be formed on Ti surface. The reaction at metal/oxide surface shows as follow(99):

$Ti + 2H_2O \rightarrow TiO_2 + 2H_2$

The current decreased continuously by applying a constant voltage, because metal particles were trapped by oxygen in a self limiting procedure and formed the oxide layer which performs as an insulator.

In electrolytes with fluoride ions, the prepared oxide layer is permeable because of fluoride ions reaction with the oxide layer and soluble $(TiF_6)^{-2}$ formation. Accordingly the oxide layer forms and dissolves at the same time. The competition between oxide layer's forming and dissolving,

results in formation of some self organized TiO_2 nanotubes (Figure 17). The equation for dissolution of TiO_2 in presence of fluoride ions follows as below:

$TiO_2 + 6F + 4H \rightarrow TiF6 + H_2O$

$Ti^{4+} + 6F^- \rightarrow (TiF6)^{2-}$

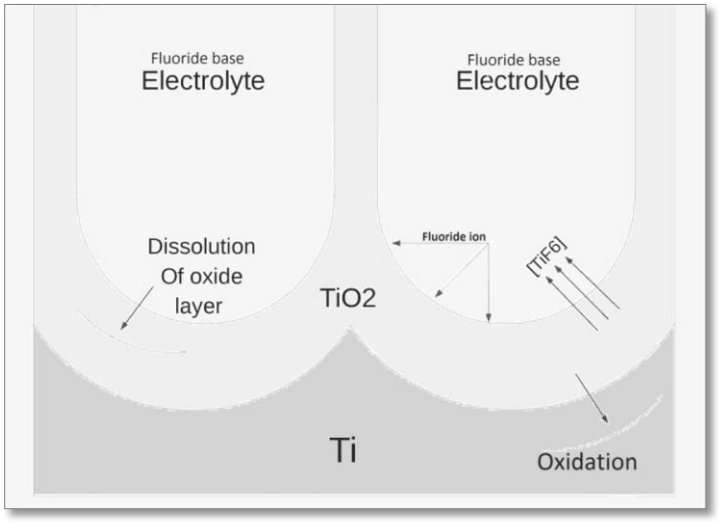

Figure 17 - Anodization of Ti. Metal oxide nanotubes in presence of fluoride, oxidation of the metal and etching the oxide layer occur at the same time and results in formation of the tubes(99)

4.2 Surface Morphology and Elemental Composition TiO_2 NTs

Most of the characterization about the surface morphology was obtained by scanning electron microscopy (FESEM-JEOL JSM-6320F). SEM imaging was performed in 2.5-5kV, with a

working distance about 4-6 mm for top view sample as shown in Figure 4.2. In addition to SEM information, EDX spectroscopy (EDS-Hitachi S-3000N VPSEM) also provided some information about the elemental composition of the sample. The EDS analysis shows containing elements in figure 18.

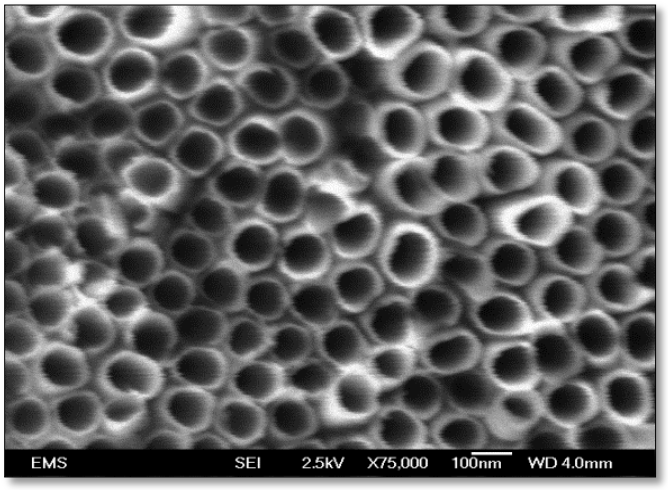

Figure 18(a) - Anodization in presence of fluoride ion for 2hours , 60V in ethylene glycol

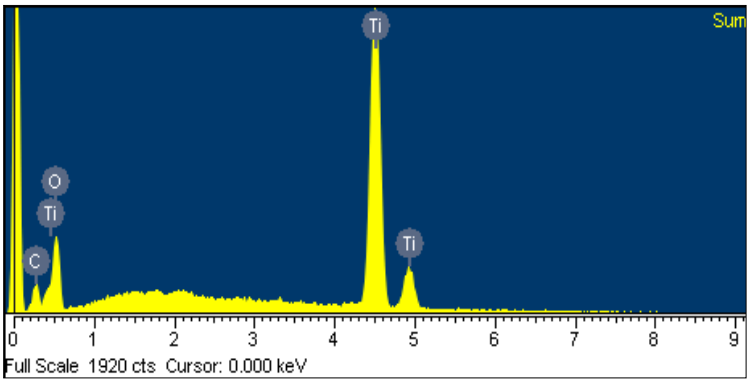

Figure 18(b) - EDX analysis of the fabricated Ti-TiO_2 layer

4.3 Deposition of PB and Au Nanoparticles on TiO_2 NTs Surface

Modification of TiO_2 NTs by metal oxide and other active catalysts with coordination polymers such as Prussian blue as a mediator with good electrochemical behaviour, can be applied in biosensor systems to speed up the electron transfer between enzyme and electrode.

Principally, TiO_2 NTs are charged negatively in a neutral solution. So electrostatic interaction was applied using polyelectrolyte PDDA to form positively charged PDDA-modified TiO_2 NTs. Consequently, the nanotube surfaces and walls absorbed negatively charged AuNPs by the PDDA bridges. Accordingly, photocatalytical activity and electronic conductivity of the TiO_2 NTs are improved by the attached AuNPs.

Experimental results indicated that PB would deposit instinctively on the surface of conducting substrates. For PB deposition, after incubating Au modified TiO_2 NTs in an acidic ferricynide

solution overnight, a thin layer of PB film was apparent on the electrode substrate in dark blue color, as shown in Figure 19.

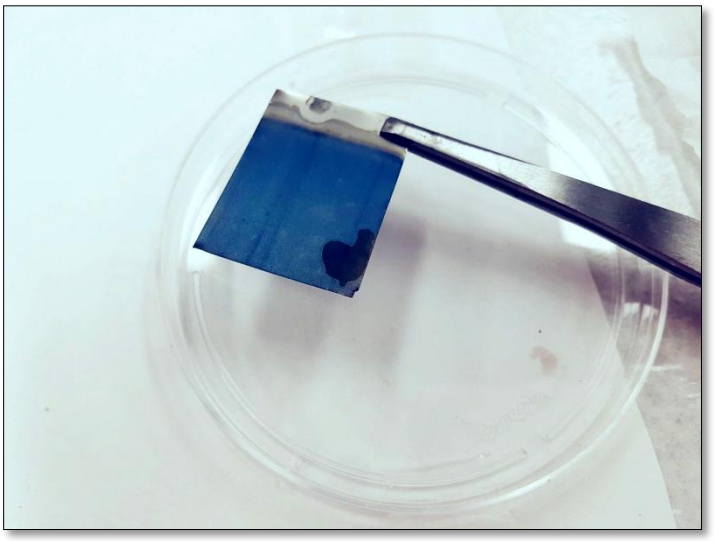

Figure19 - Dark blue PB thin film deposition overnight onto the modified electrode surface

Problems caused by PB dissolution in to solution will always be faced after a few scans at neutral pH due to the relativity weak arrangement between PB and bare electrode. To decrease PB leakage and enhance the stability of PB decorated nanotube electrode, a thin layer of poly (pDAB) as a nonconducting polymeric material was deposited on the PB/AuNPs/TiNT electrode surface.

4.4 Deposition of PB and Ag Nanoparticles on TiO_2 NTs Surface

In general electrochemical deposition or electroplating is a process in which metal ions in electrolyte deposit on the electrode surface by applying electric filled or current. The principles to control Electroplating are current/voltage source, temperature, plating duration, electrolyte concentration, the distance between electrodes and stirring. As the cell is depicted in figure 20, following formation of TNTs, Ag ions in the electrolyte solution attached onto the cathode (carbon rode) as the electron was provided for Ag deposition by the electric field. During deposition of Ag procedure, Ag dispersed into the electrolyte solution by losing electron on the cathode. With the electric field, Ag^+ ions in the solution moved to the TiO_2 NT, took up the electrons and resulted in Ag NPs formation(100).

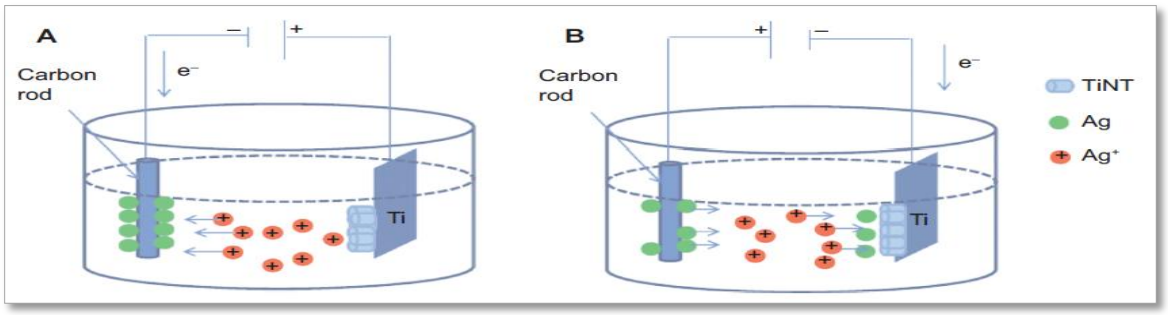

Figure20 - Schematic figure for the formation of AgO-deposited TiO_2 NTs. (**A**) Showing the anodization process of TiO_2 NTs. Ag ions migrated toward carbon rod cathode, which was negatively charged considering the Ti. Ag NPs were deposited on the carbon cathode as TiO_2 NTs were formed on positively charged Ti surface. (**B**) Electroplating process of Ag. (100).

The polarization was changed and Ag^+ ions migrated to the negatively charged Ti considering carbon rod. As a result, Ag NPs deposited on the surface of TiO_2 NTs (100).

For PB deposition, after incubating AgO modified TiO2 NTs in an acidic ferricynide solution overnight, a thin layer of PB film was apparent on the electrode substrate in dark blue color, as shown in Figure 1b. To decrease PB leakage and enhance the stability of PB decorated nanotube electrode, a thin layer of poly (pDAB) as a nonconducting polymeric material was deposited on the PB/AuNPs/TiNT electrode surface.

4.5 Surface Morphology and Elemental Composition PB-Au Modified TiO2 NTs and PB-Ag Modified TiO2 NTs Electrodes

The morphology of electrodeposited metal ions on surface of the nanotubes was investigated by Scanning Electron Microscopy (FESEM-JEOL JSM-6320F). Samples were kept on the holder and were partly covered by conductive tape and placed into the chamber with 10KV and 9mm for voltage and working distance parameters correspondingly. Accumulation of Ag and Au ions on the tubes makes its surface uneven, so SEM images are not as clear as pure nanotubes. To analyze the elements and to make sure that the deposited materials are Ag and Au NPs, elements spectrum of Energy-dispersive X-ray Spectroscopy (EDS) were used.

SEM images in figure 21(a) and 22(a) show that diffusion of gold, silver and PB nanoparticles on TiO_2 NTs surface. The nanoparticles deposition on the TiO_2 surface can also be confirmed by the EDS analysis showed in figure 21(b) and 22(b) Clearly, Au NPs, Ag NPs and nanocrystals of

PB can be detected on walls and entrance of the nanotubes. As the results of energy spectrum the C and Fe elements of PB can be observed.

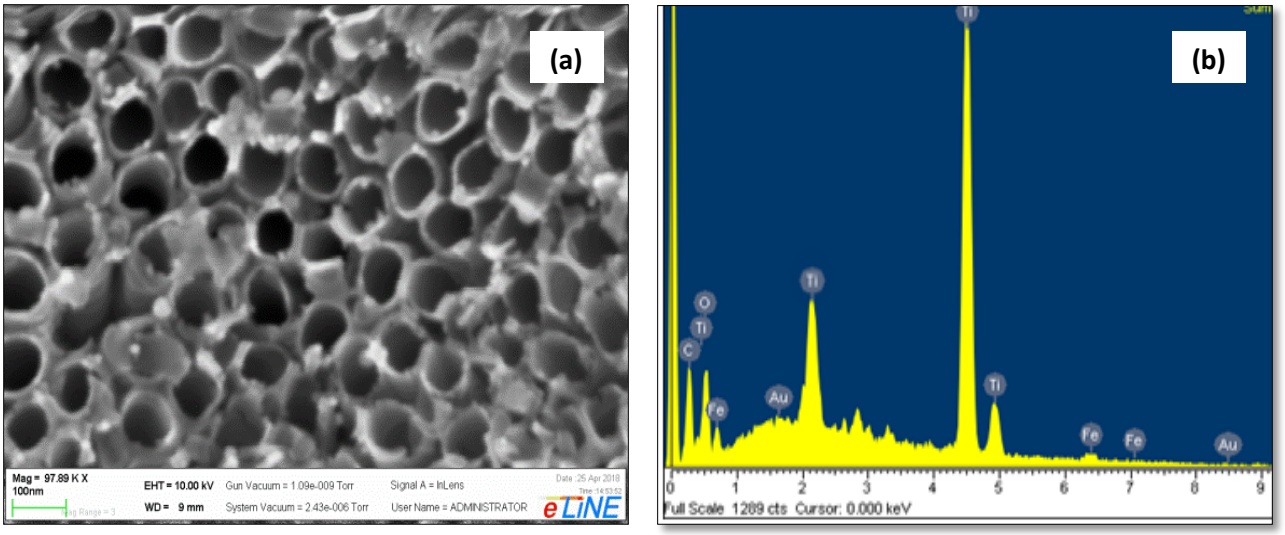

Figure 21(a) - SEM images of Au and PB nanoparticles deposited on to the TiO_2 NTs. (b) - EDS elemental analysis

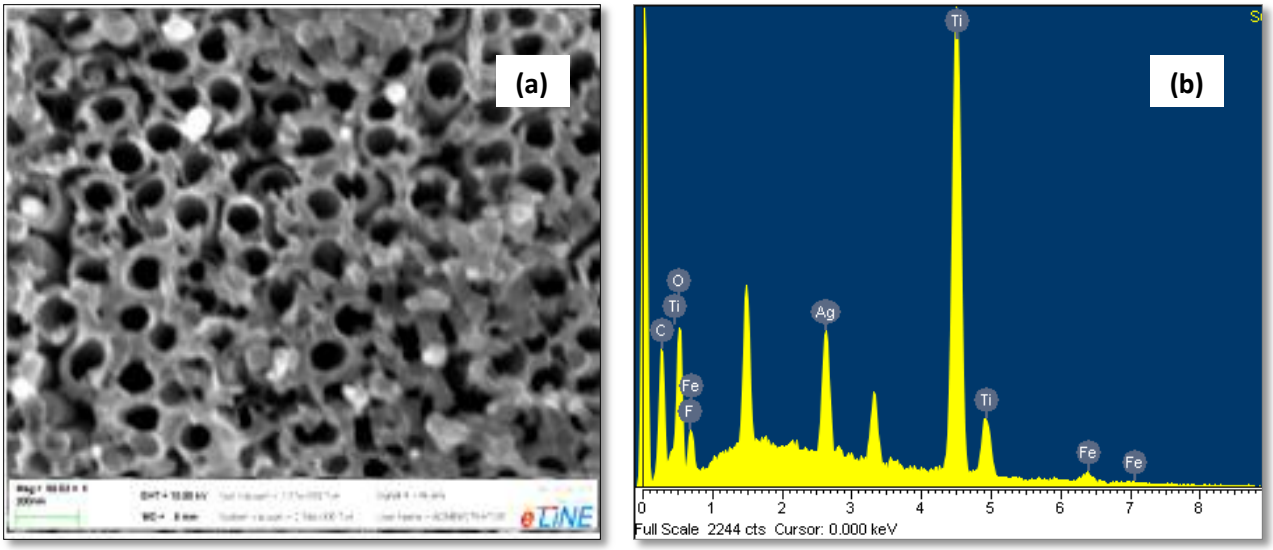

Figure 22(a) - SEM Images of Silver and PB nanoparticles deposited on to the TiO_2 NTs. (b) - EDS elemental analysis

4.6 Electrochemical polymerization of GOx on PB-Au Modified TiO_2 NTs Electrode

GOx enzyme were immobilized into the nanotubes surface with the electrochemical deposition of pDAB.. As shown in Figure23, the electron transfer between the electrode and the solution is slowed down due to nonconductive polymer. As a result, the anodic current reduces on every CV cyclic scanning with respect to the 1,2-DAB polymerization. This trend is consistent with the earlier studies. Figure24 indicate the development on electro polymerization procedure to form (GOX/ Au/pDAB)-PB/AuNP/TiNTs electrode with sweep rate of 50 mV s^{-1} for 1–10 cycles.

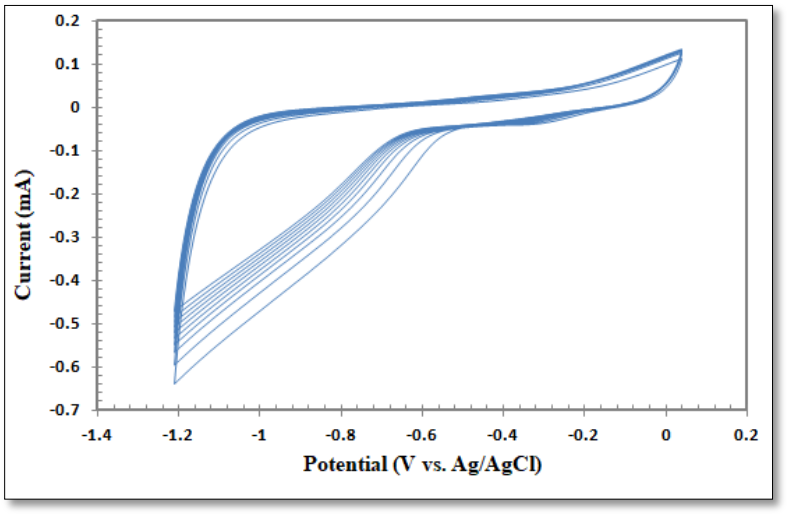

Figure 23 - Cyclic voltammograms of Phenylenediamine electrodeposition on PB/Au NPs/TiO_2 NTs electrode

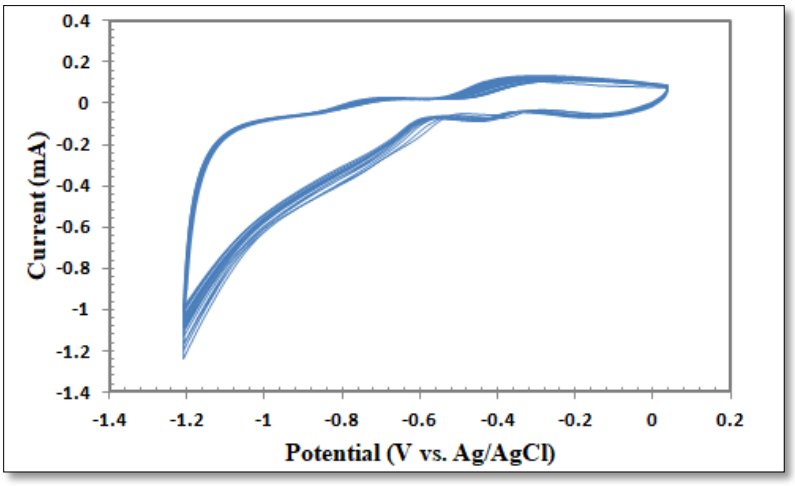

Figure 24 - Cyclic voltammograms of GOx electrodeposition onto the PB/AuNP/TiO_2 NTs electrode via the electropolymerization method of Phenylenediamine. Numerals show the electro polymerization cycles.

4.7 Cyclic Voltammetry Measurements of the PB-Au Modified TiO_2 NTs Electrode and PB-Ag Modified TiO_2 NTs Electrode

The electrochemical behavior of the modified TiO_2 NTs has been investigated by CV measurement as shown in Figure25. The PB/Au NP/TiO_2 NTs demonstrate a higher cathodic and anodic current response in compare to Au modified TiO_2 NTs and simple TiO2 NTs. Pure Ti electrode has been examined as well which exhibit very negligible electrochemical response activity in PBS solution. This can confirm the effect of Au and PB NPs to provide better electron transfer and improve redox reaction response of the electrode.

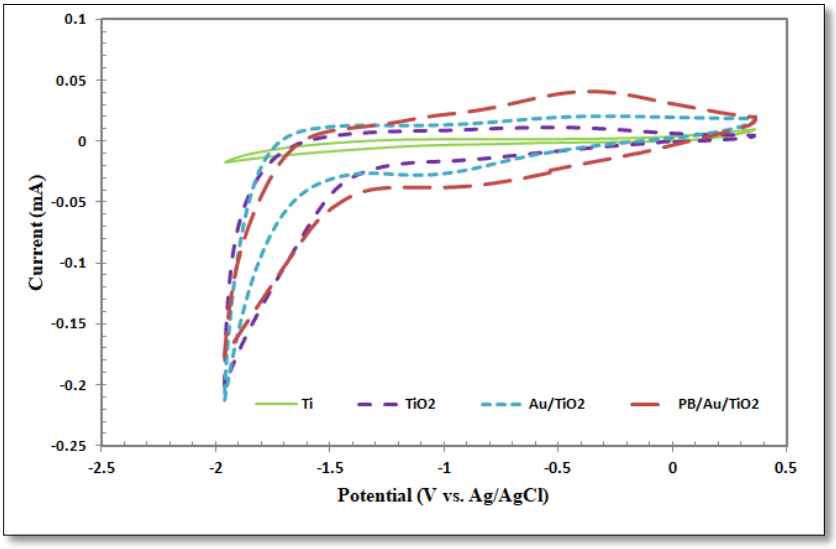

Figure25 - Cyclic voltammograms of the Ti electrode (green plot) in compare with TiO_2 NTs electrode (purple plot), Au/TiO_2 NTs electrode (blue plot) and PB/Au/TiO_2 NTs electrode (red plot) in PBS (pH 6.0).

The stability and electrochemical activity of the fabricated electrode was investigated using CV. As shown in Fig. 26, the GOx/PB/AuNP/ TiO_2 NTs electrode indicate cathodic and anodic currents in 0.01 M PBS solution (pH 6.0, containing 0.1 M KCl) at scan rate of 50 mV $s^{-}1$. By adding 10 mM glucose into the PBS solution, the quick increases in the cathodic currents come out as a result of hydrogen peroxide (H_2O_2) reduction which formed from the enzymatic reaction. Several reversible redox peaks located at -0.68 V caused from the transformation process between Prussian blue (PB) and Prussian white (PW) can be detected in the voltammograms.

The GOx/PB/AuNP/TiO_2 NTs based electrode indicates a higher current response to 10 mM glucose (curve c) in compare with the absent of glucose (curve b), signifying that GOx/Au/PB// TiO_2 NTs electrode shows a great biocatalytical response toward detection of glucose.

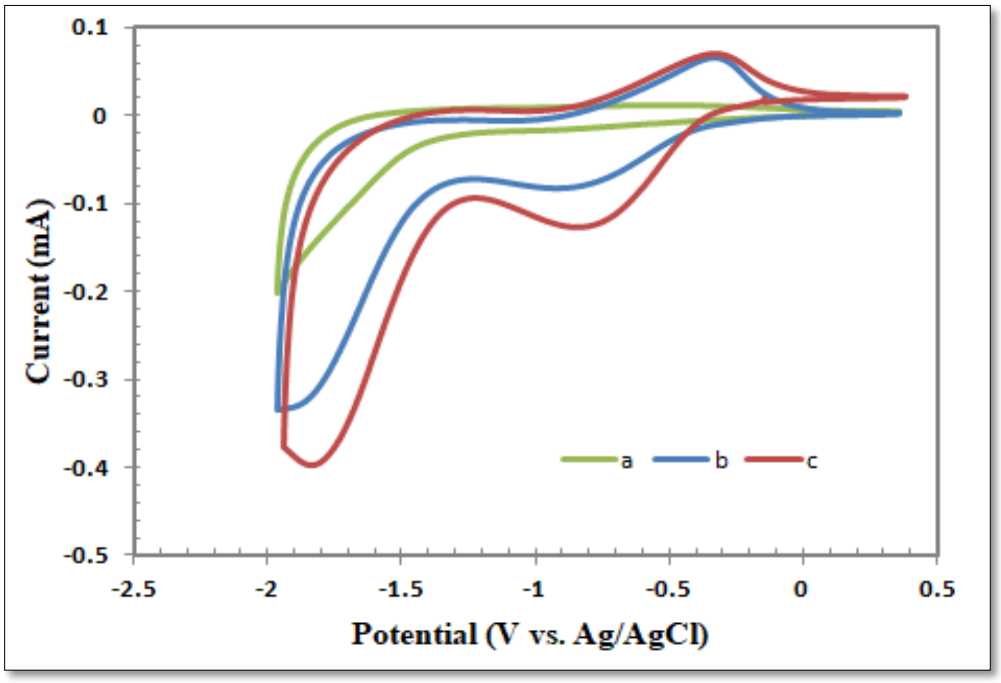

Figure 26 - Cyclic voltammograms of the TiO_2 electrode (curve a) in compare with PB/Au NPs/TiO_2NTs electrode in absence (curve b) and presence (curve c) of 10 mM glucose in PBS (pH 6.0).

In Fig. 27, the GOx/PB/Ag NPs/TiO_2 NTs electrode exhibit cathodic and anodic currents in 0.01 M PBS solution (pH 6.0, containing 0.1 M KCl) at scan rate of 50 mV $s^{-}1$. By adding 10 mM glucose into the PBS solution, the quick increases in the cathodic currents come out as a result of hydrogen peroxide (H_2O_2) reduction which formed from the enzymatic reaction. Several reversible redox peaks located at -0.29 V caused from the transformation process between Prussian blue (PB) and Prussian white (PW) can be detected in the voltammograms.

The GOx/PB/Ag NPs/TiO_2 NTs based electrode indicates a higher current response to 10 mM glucose (curve c) in compare with the absent of glucose (curve b), signifying that GOx/Ag NPs/PB/TiO_2 NTs electrode shows a great biocatalytical response toward detection of glucose.

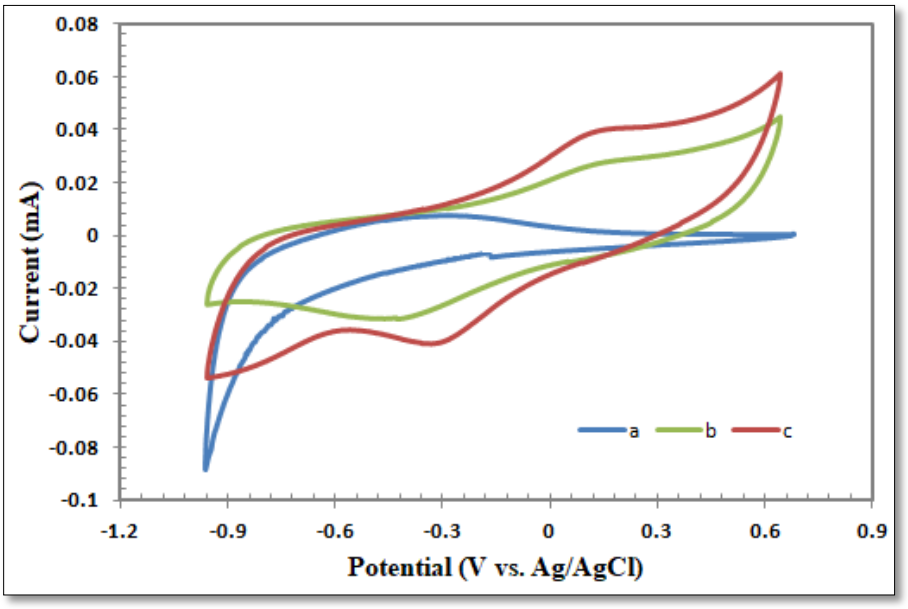

Figure 27 - a. GOx/PB/AgNP/TiO2 NTs based electrode in presence (curve c) of 10 mM glucose in PBS (pH 6.0) b. GOx/PB/AgNP/TiO2 NTs based electrode in absence (curve b) of 10 mM glucose in PBS (pH6.0) c. GOx-TiO2 NTs based electrode (curve a)

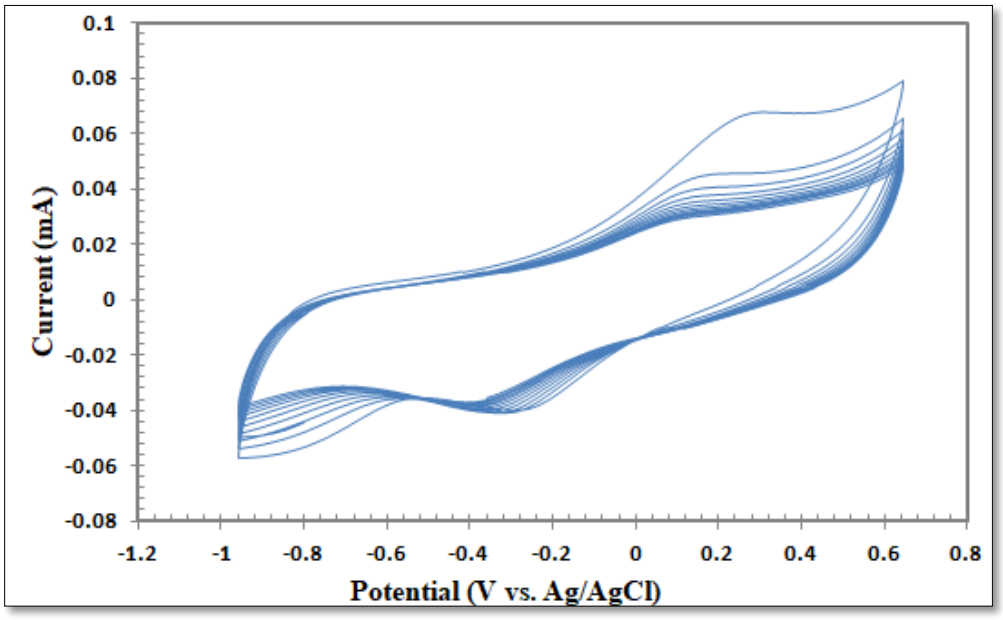

Figure 28 - Cyclic voltammetric behavior of the GOx/PB/Ag NPs/TiO2 NTs modified electrode in presence of 10 mM glucose in PBS on potential cycling between -0.956 to 0.644 at scan rate 0.05 V $s^{-}1$ (measured solution pH 6.0).

4.8 Chronoamperometry Measurements of the PB-Au Modified TiO_2 NTs Electrode and PB-Ag Modified TiO_2 NTs Electrode

Different concentrations of glucose were added to PBS solution to investigate effect of glucose concentration. Given that the electrochemical signal is caused by PB reduction on the electrode, the differences in cathodic response is detected in chronamperometric current. Figure 29 and 30 indicate the current response of the prepared electrodes on the successive increase of glucose to

the PBS solution at an applied voltage of -0.25 V. A linear increase in reduction current response according to the concentration increase has been measured in the range between 0.1–0.4 mM.

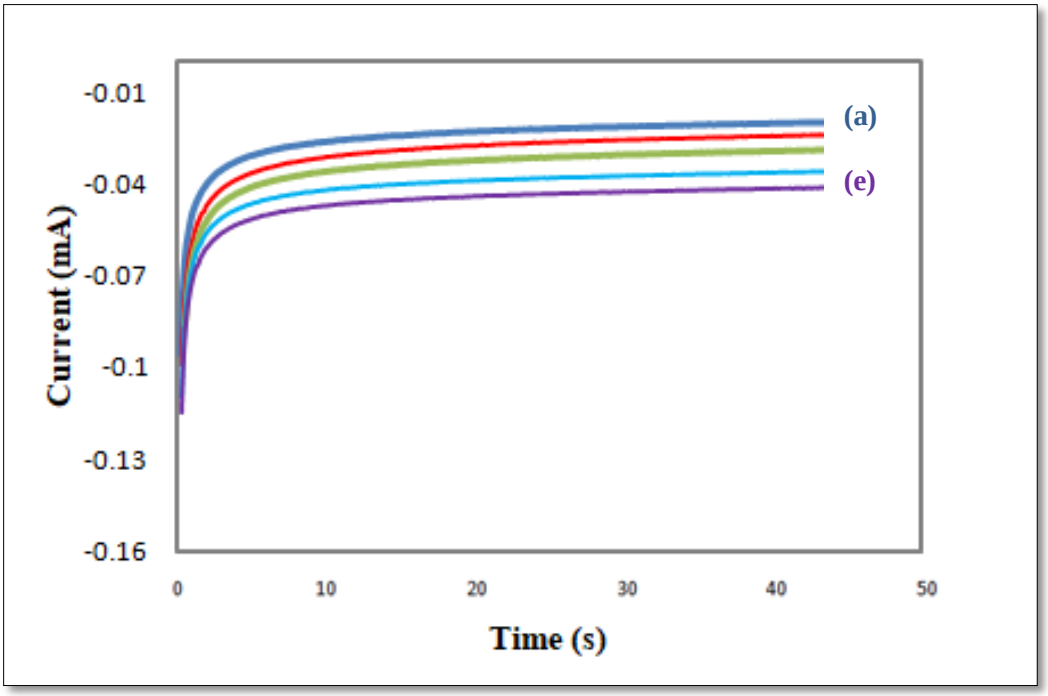

Figure 29 - GOx/PB/Au NPs/TiO_2 NTs modified electrode (a) in the absence and (b–e) successive addition of glucose at -0.25 V

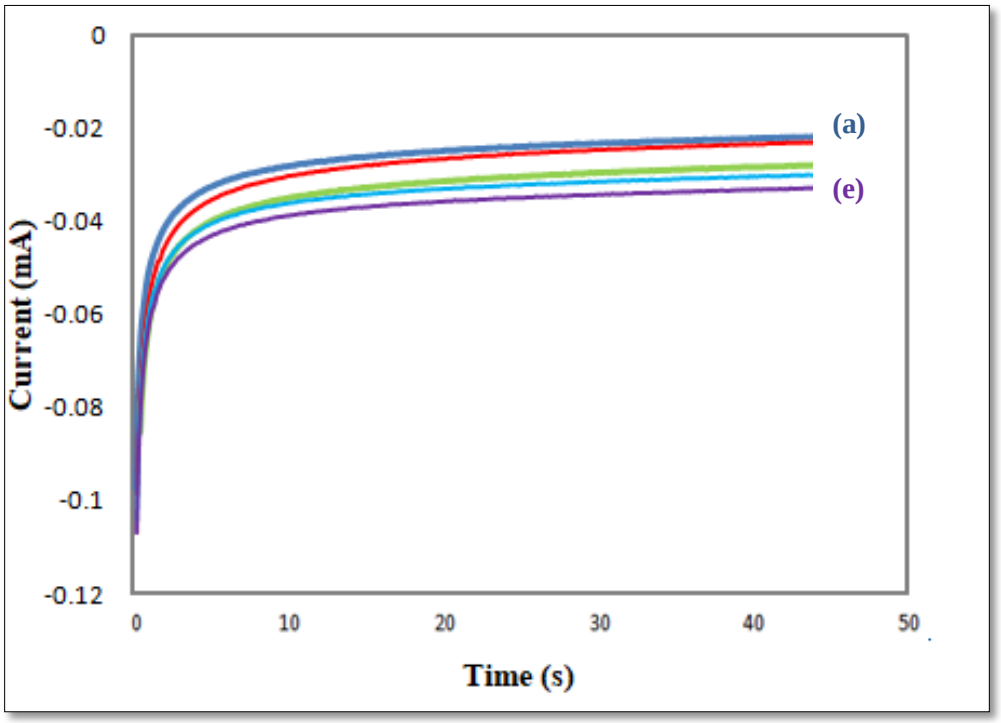

Figure 30 - GOx/PB/Ag NPs/TiO_2 NTs modified electrode (a) in the absence and (b–e) successive addition of glucose at -0.25 V

4.9 Optimization and Calibration Curve and Results of the PB-Au Modified TiO_2 NTs Electrode and PB-Ag Modified TiO2 NTs Electrode

In GOx/PB/AuNP/TiO2 electrode sample (Fig. 31), a linear increase in reduction current with the increase in concentration of glucose between the ranges of 0.1– 0.40 mM with a detection limit of 4.91 µM (3 times standard deviation of the blank) was detected. The linear regression equation was y = -0.1851x - 0.0552, where y represents the current in mA and x the glucose

concentration in mM; the R2 was 0.986. The sensitivity was 185.1 µA. For the GOx/PB/AgNP/TiO2 NTs electrode (Fig. 32), the resulted electrode shows an acceptable sensing performance. The linear range of prepared biosensor for the glucose measurement was found with a detection limit of 58.7 µM. The linear regression equation was: "y = -0.0291x - 0.0211, where y represents the current in mA and x the glucose concentration in mM; the R2 was 0.981. The sensitivity of such biosensor is 29.1 µA.

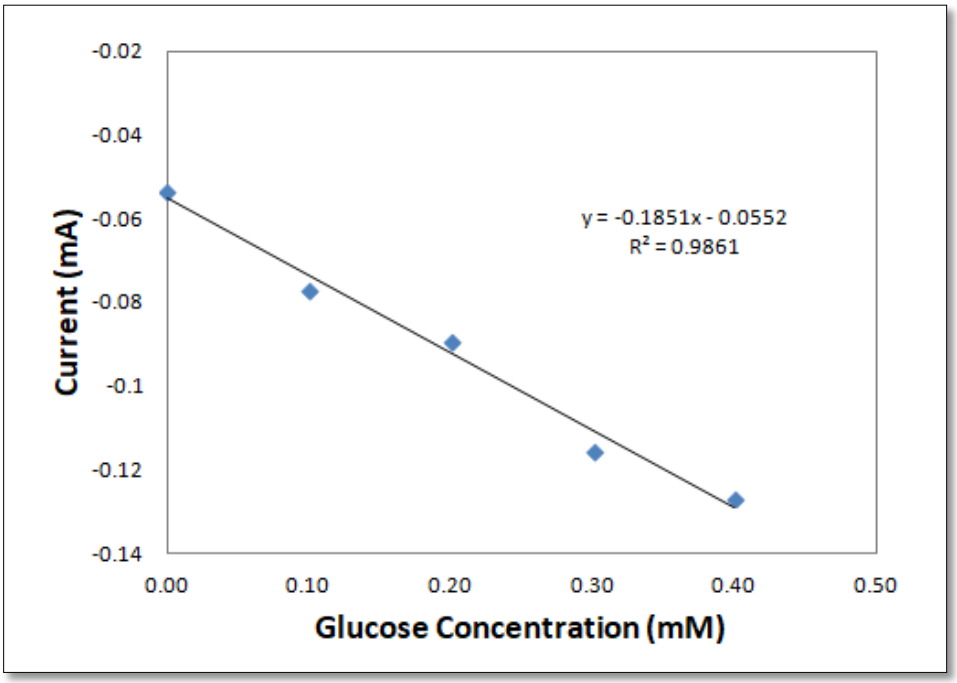

Figure 31 - GOx/PB/Au NPs/TiO2 NTs modified electrode (a) in the absence and (b–e) successive addition of glucose at -0.25 V

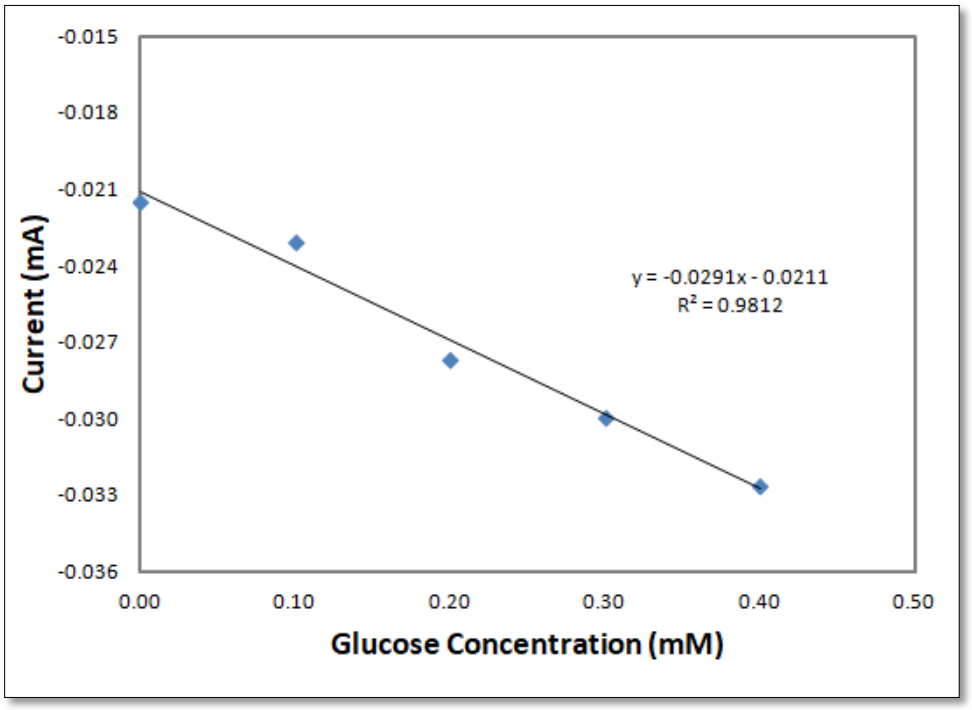

Figure 32 - GOx/PB/Ag NPs/TiO2 NTs modified electrode (a) in the absence and (b–e) successive addition of glucose at -0.25 V

Chapter 5: CONCLUSION AND FUTURE SCOPE

In this study, two high performance glucose biosensors based on immobilization of enzyme glucose oxide onto Au and Ag oxide (AgO) modified titanium nanotubes (TNTs), following by deposition of Prussian Blue (PB) as the electron transfer mediator was developed. The GOx/PB/Au/TiO_2 NTs electrode considerably enhanced the current response signal in glucose solution in compared to absent of glucose. In a same way, the GOx/PB/Ag/TiO_2 NTs electrode noticeably improved the current response signal in glucose solution in compared to absent of glucose. In addition, both electrodes exhibit a significantly increased the current response signal in compared to the GOx/TNTs electrode. Moreover, Low concentration of hydrogen peroxide demonstrates no interaction with Ag nanoparticles, which indicate in application of Ag NPs in glucose biosensors.

The surface morphology and elemental composition of the two fabricated biosesnors exhibit successful deposition of Au and AgO nanoparticles as well as PB nanocrystals. The results show that the developed electrochemical biosensors display good stability, low detection limit as well as high reproducibility for the determination of glucose. Under the optimized conditions, the reported biosensors exhibit a good linear response towards glucose concentration in the range of 0.1 to 0.4 mM with a detection limit down to to 4.91 µM and 58.7 µM, sensitivity of 185.1 µA and 29.1 µM, for Au NPs and AgO NPs modified biosensors respectively, which is acceptable in compare to other works.

In conclusion, the reported work indicate a novel method for developing an enzymatic electrochemical glucose biosensor based on assembling of AgO NPs and PB nanocrystals on Titanium NTs. Biocompatible effect of Ag NPs and excellent electrocatalytic properties of PB as well as large surface area of nanotublar TNTs structure, lead to enhancing the analytical performance of glucose biosensor in terms of high sensitivity, reliable selectivity, quick response time and great stability.

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