2007

NANOSTRUCTURED SENSORS FOR IN-VIVO NEUROCHEMICAL RECORDING

Nagari Silpa
University of Kentucky, silpa.ngari@gmail.com

ABSTRACT OF THESIS

NANOSTRUCTURED SENSORS FOR IN-VIVO
NEUROCHEMICAL RECORDING

L-glutamate plays a vital role in central nervous system. It is a neurotransmitter associated with several neurological disorders like Parkinson's disease, epilepsy and stroke. Continuous and fast monitoring of this neurotransmitter has become a major concern for neuroscientists throughout the world.

A simple, sensitive, and reliable L-glutamate microsensor with short response time has been developed using ceramic-based microelectrode arrays with platinum recording sites. The electrodes were modified by electrodeposition of Platinum black (Pt-black) to detect hydrogen peroxide (H_2O_2) which was produced by enzymatic reactions of glutamate oxidase immobilized on the electrode surface. Modification of Pt electrodes with Pt-black has been adopted because the microscale roughness of Pt-black increases the effective surface area of the electrode and promotes efficiency of H_2O_2 electro-oxidation. The modified Pt recording sites were coated with m-phenylenediamine (mPD) and L-glutamate oxidase (L-GluOx). mPD acts as an barrier for extracellular interferents such as ascorbic acid and dopamine, thus increasing the selectivity of electrode for Glutamate (Glu). This modified microsensor was highly sensitive to H_2O_2 (686.3 ± 156.48 $\mu AmM^{-1}cm^{-2}$), and Glutamate (492.2 ± 112.67 $\mu AmM^{-1}cm^{-2}$) at 700mV versus Ag/AgCl reference. Deposition of Pt nano-particles on recording sites enhanced the sensitivity to H_2O_2 by 2 times and the sensitivity to glutamate by 1.5 times.

Keywords: Platinized Pt electrodes, Platinum black (Pt-Black), L-glutamate, H_2O_2, Glutamate microbiosensor.

Silpa Nagari

09/28/2007

NANO-STRUCTURED SENSORS FOR IN-VIVO NEUROCHEMICAL RECORDING

By

Silpa Nagari

Dr. J. Todd Hastings
Director of Thesis

Dr. YuMing Zhang
Director of Graduate Studies

9/28/2007

RULES FOR THE USE OF THESES

Unpublished theses submitted for the Master's degree and deposited in the University of Kentucky Library are as a rule open for inspection, but are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but quotations or summaries of parts may be published only with the usual scholarly acknowledgements.

Extensive copying or publication of the dissertation in whole or in part also requires the consent of the Dean of the Graduate School of the University of Kentucky.

A library that borrows this project for use by its patrons is expected to secure the signature of each user.

Name Date

_____

_____

_____

_____

_____

_____

_____

_____

THESIS

Silpa Nagari

The Graduate School

University of Kentucky

2007

NANO-STRUCTURED SENSORS FOR IN-VIVO NEUROCHEMICAL RECORDING

THESIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the College of Engineering at the University of Kentucky

By

Silpa Nagari

Lexington, Kentucky

Director: Dr. Todd Hastings, Assistant Professor of Electrical Engineering

Lexington, Kentucky

2007

MASTER'S THESIS RELEASE

I authorize the University of Kentucky
Libraries to reproduce this thesis in
whole or in part for purposes of research.

Silpa Nagari

09/28/2007

ACKNOWLEDGMENTS

This thesis would not have been possible without the support of Dr. J. Todd Hastings. Dr. Todd Hastings provided me with constant encouragement and guidance to complete this thesis work.

I thank

a) The Almighty for being with me all the time to complete my master's degree.

b) Dr.Vijay Singh and Dr. Janet Lumpp for taking out time to be in my defence committee.

c) Larry Rice and George Spiggle for helping me learn all the processes required for my thesis work.

d) My fellow lab-mates for their help throughout my research work.

e) Last but definitely not the least my parents for their constant support throughout my master's program.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF FILES

1. SilpaNagari_MSThesis.pdf

CHAPTER 1

INTRODUCTION

L-glutamate, also called Glutamic acid is the most common excitory neurotransmitter in the central nervous system (CNS) of vertebrates. L-glutamate is associated with wide range of brain disorders such as epilepsy, Parkinson's disease, schizophrenia and stroke. Because of its profound involvement in several neurological disorders, the molecular mechanisms regulating the release and uptake of glutamate are of considerable interest. Although amperometric biosensors have been around many years, production of much smaller microelectrode amperometric biosensors offers great utility for studying chemical signaling in the brain. With smaller size of the biosensors, we obtain better single neuron measurement and more accurate self referencing measurements. The miniaturization of biosensors faces two conflicting design requirements: maximizing sensitivity while minimizing size.

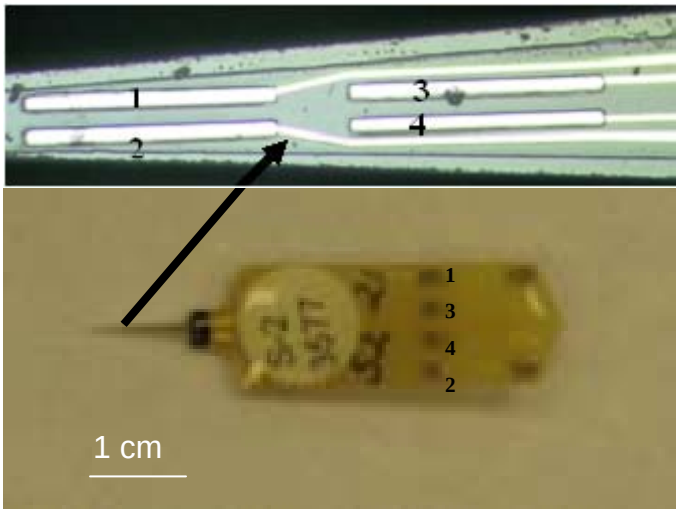
Presently intracranial microdialysis is most widely used for measuring L-glutamate and other neurotransmitter in the central nervous system (CNS) tissues *in-vivo*. However this technique has poor spatial resolution (1-4 mm) and poor time resolution (1-20 minutes) [11]. This is inadequate for sampling of neurotransmitters with fast kinetics such as L-glutamate. There is a need for some technique that can record glutamate dynamics, second-by-second, with a spatial resolution of microns. The terms L-glutamate or glutamate or glu are one and the

same in this document. To develop a sensitive, selective, and reliable L-glutamate micro biosensor is the objective of this project.

Figure 1.2 shows the configuration of electrode developed in this present study for L-glutamate (Glu) detection. Pt recording sites were coated with glutamate oxidase (GluOx) enzyme for rapid measurement of Glu. A layer of m-phenylenediamine (mPD) on recording sites acts as exclusion layer for anionic interferents found in CNS namely ascorbic acid and dopamine. When a glutamate molecule approaches the vicinity of the electrode, it is first decomposed by GluOx to produce hydrogen peroxide (H_2O_2), which in turn penetrates into the mPD layer and reaches the surface of the Pt nano-particles deposited on Pt recording surface (Pt/Pt-black) Figure 1.3. H_2O_2 is efficiently oxidized by the surface Pt/Pt-black recording sites at +0.7 V vs Ag/AgCl. The oxidation reaction of H_2O_2 generates current, which is amplified and digitized by FAST (Fast Analytical Sensing Technology) high speed electrochemistry instrument.

As seen in Figure 1.1, the electrode tip has four recording sites, each recording site is 15 µm wide and 333 µm long. These recording sites are connected to printed circuit board. The longitudinal distance between each recording site is 100 um and transverse distance is 30um. To avoid crosstalk between recording sites, they are shielded with insulator called polyimide. The four recording sites are connected to the electrode holder, which has separate connecting wires for each recording site. These electrodes can detect different neuro-transmittors depending

upon the type of enzyme immobilized on it. For example, glutamate oxidase, is applied on electrode to detect glutamate. Similarly, glucose oxidase is applied, to detect glucose.



(a)

(b)

Figure 1.1: Photograph of (a) 1 cm long ceramic based multisite microelectrode tip (b)multisite microelectrode bonded to a printed circuit board.

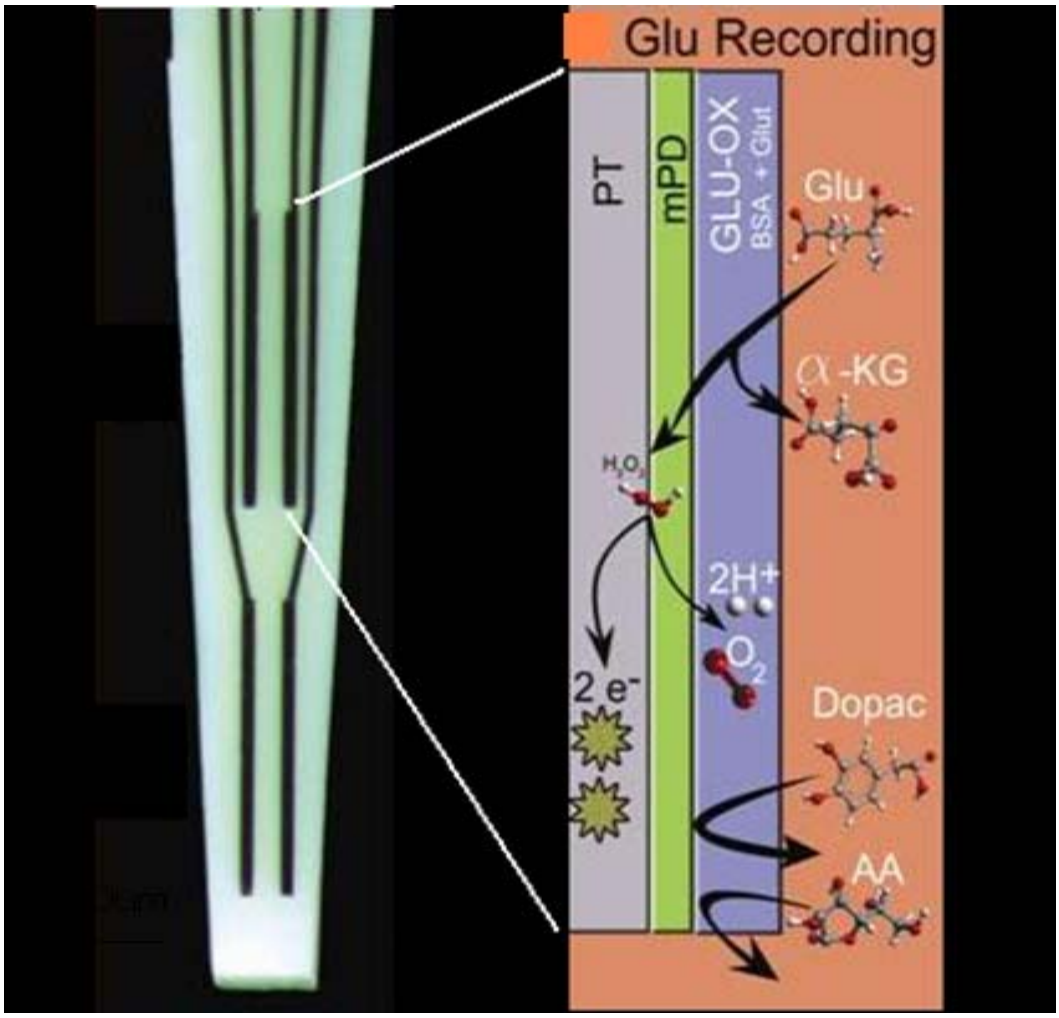


Figure 1.2: Schematic of the different layers present on the Pt recording site in order to measure glutamate.

HYPOTHESIS

Deposition of micro- and nano-structured platinum, or platinum-black (Pt-Black), on a platinum recording surface results in increased active surface area. This process, also called platinization, should result in higher amperometric sensitivity for microscale L- glutamate sensors. Several mechanisms may contribute to increased sensitivity. (1) Increasing the electrochemically active surface area ensures rapid oxidation of hydrogen peroxide produced by the immobilized

enzyme. (2) In some cases increased surface area may allow additional enzyme immobilization, and thus a higher amperometric response to L-glutamate. (3) Molecular oxygen produced by electrochemical reactions at the recording surface adheres to the electrode surface, and is a major noise source. [7]. This can be minimized by increasing electrode surface hydrophilicity, which is accomplished by roughening the electrode surface.

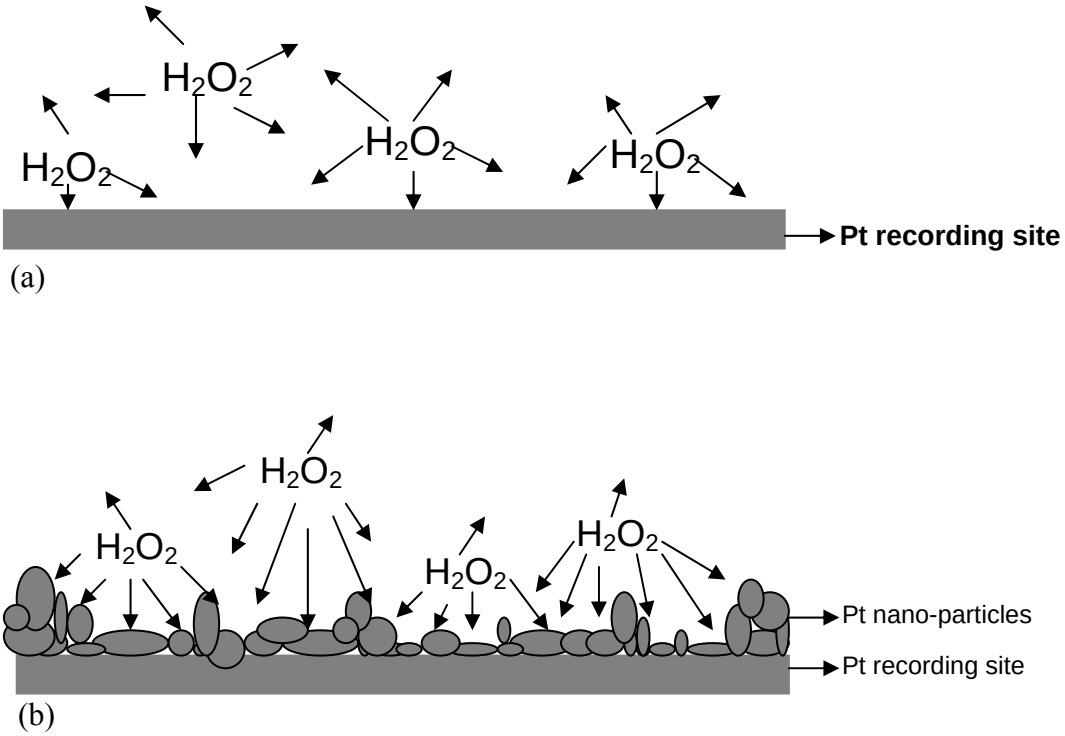


Figure 1.3: Schematic of H_2O_2 sensing process on (a) bare Pt electrode (b) high surface area electrode (Pt-black nanostructures deposited in the Pt surface). The addition of a high surface area coating increases the number of active sites for H_2O_2 oxidation.

CHAPTER 2

BACKGROUND

To date there has been only one other study on the effect of platinization on amperometric glutamate sensors. However, there have been several reports of platinization on glucose sensors.

A group at University of California, Hamdi et al [9] recently reported their work on platinization of L-glutamate microbiosensors. Platinum-black was deposited on a Pt wire electrode (~ 0.00122 cm^2) by cycling potential between -1.4 V and -2.0 V (vs. Ag/AgCl) at 500 mVs^{-1} in a plating solution of 1% chloroplatanic acid, 0.0025%HCl, 0.01%lead acetate in water. The solution was vigorously stirred during the deposition process. Pt-black modified microelectrodes were dip-coated with L-Glutamate oxidase (L-GluOx). A 80 ±10 $\mu AmM^{-1}cm^{-2}$ glutamate sensitivity was reported.

J.I.Reyes et al.[4] reported the effect of platinization on Pt wire (0.1727 cm^2) glucose biosensors. Potentiostatic platinization of electrodes was carried out from -50 to -250 mV vs Ag/AgCl reference in a mixture of 2 mM H_2PtCl_6, 1 mM $Pb(C_2H_3O_2)$, 0.1 M KCl to a charge density of 2 Ccm^{-2}. Glucose oxidase was immobilized in electrochemically generated Poly-o-phenylenediamine (PPD). The group reported glucose limit of detection to be 0.94 mM and glucose sensitivity of

platinized electrodes to be 192 $\mu Acm^{-2}mM^{-1}$, which is nearly 20 times higher than that of unmodified electrodes.

H.Olivia et al[5] reported the effect of platinum nano-particles deposition on boron doped diamond microfiber electrodes. Platinum nanoparticles deposition on their diamond microfibre electrode (8635 μm^2) was done by cyclic voltammetry in 0.1 M H_2SO_4 containing 100uM K_2PtCl_6, by cycling between the potentials of -0.2 and 1.2 V at 50 mVs^{-1}. Glucose oxidase was dip-coated on the platinized electrodes. They observed glucose LOD of 6.1 mM and glucose sensitivity of $1.158\mu AmM^{-1}cm^{-2}$.

Chi S.Kim et al [7] reported effect of platinization on Pt disk (1.2×10^{-2} cm^2) electrode. They carried out platinization under constant potential condition in 1% chloroplatanic acid solution containing lead acetate and sodium chloride. The platinization potential was in the range 0.0 to -0.25 V, and charge density was at 2 Ccm^{-2}. Glucose oxidase was electrodeposited on platinized electrodes. This group has shown glucose sensitivity of 41.66 $\mu AmM^{-1}cm^{-2}$, which is 30-40 times more than that similar of bare Pt electrodes.

J.Wang et al [8] observed increase in Glucose sensitivity by depositing Pt-Black on Pt thin film electrode surface (~0.01 sq.cm). Pt-Black was electrodeposited by cycling potential between -1.4 and -2.0 V at 500 mVs^{-1} in 1 wt% chloroplatanic acid and 0.1 M H_2SO_4. The estimated roughness factor of the electrode surface

was 63. Glucose oxidase enzyme was electrodeposited on Pt-Black electrodes in polypyrrole. This group has seen an increase in Glucose sensitivity from 0.7μA $mM^{-1}cm^{-2}$ to 29.17μA $mM^{-1}cm^{-2}$.

The comparison of microelectrode performance of different groups working on the same hypothesis is summarized in Table 2.1. Sensitivity normalized to electrode area is a useful figure of merit; however, sensitivity does not scale linearly with electrode area. Enhanced radial diffusion toward smaller electrodes results in improved normalized sensitivity.

Table 2.1: Reported enzyme-based microelectrode sensor performance reported by different groups.

Electrode type	**Enzyme coating**	**Electrode geometric surfacearea (cm^2)**	**Normalized Sensitivity ($\mu AmM^{-1}cm^{-2}$)**	**Reference**
Glutamate	Manual	4.995×10^{-5}	316.5*	Gerhardt's MEA
Glutamate	Dipping	1.22×10^{-3}	80	[9]
Glucose	Electropolymerization	0.173	192	[4]
Glucose	Dipping	8.635×10^{-5}	1.158	[5]
Glucose	Electropolymerization	1.2×10^{-2}	41.66	[7]
Glucose	Electropolymerization	1.0×10^{-2}	29.17	[8]

*Unplatinized

MEA: Microelectrode array

As can be seen from the Table 2.1, unmodified Gerhardt's microelectrode performance is several orders higher than that of modified microelectrodes of other groups. Gerhardt's microelectrodes are very close to be limited by mass transport of H_2O_2 molecules to the electrode surface.

2.1. MICROELECTRODE FABRICATION

The basic fabrication scheme for the ceramic-based microelectrodes is shown in Figure 2.1. The fabrication process was carried out by Thin Films Technology (Buellton, CA). 0.005 inch thick ceramic substrates were cleaned and then rinsed with deionized water. Substrates were then dried followed by O_2 plasma ashing. Photoresist was evenly applied to the cleaned ceramic substrate using a photoresist spinner. A photomask was used to pattern the bonding pads, connecting lines, and recording sites of the individual microelectrodes. The resulting microelectrode was composed of four 15 x 333 μm^2 recording sites in series. Substrates were then coated with an adhesion layer of 500 Å, titanium followed by a layer of 1500Å platinum. An ultrasonic acetone liftoff was used to remove the remaining masked areas from the ceramic wafer, thus exposing the recording sites, connecting lines, and bonding pads. To insulate the conducting lines the microelectrode surfaces, excluding the recording sites and bonding pads, were coated with polyimide using an additional mask.

The ceramic wafers were cut by a diamond saw to expose the individual microelectrodes. Microelectrodes for *in-vivo* use were further cut into a tapered

design. All multisite arrays were wire bonded to a printed circuit board carrier that allowed for easy handing and testing of the microelectrodes. The printed circuit board/microelectrode array was connected to instruments through a custom fabricated connector for microelectrode recording. An epoxy coating was used on the wire bonding sites to insulate the microelectrode substrate from the printed circuit board substrate.

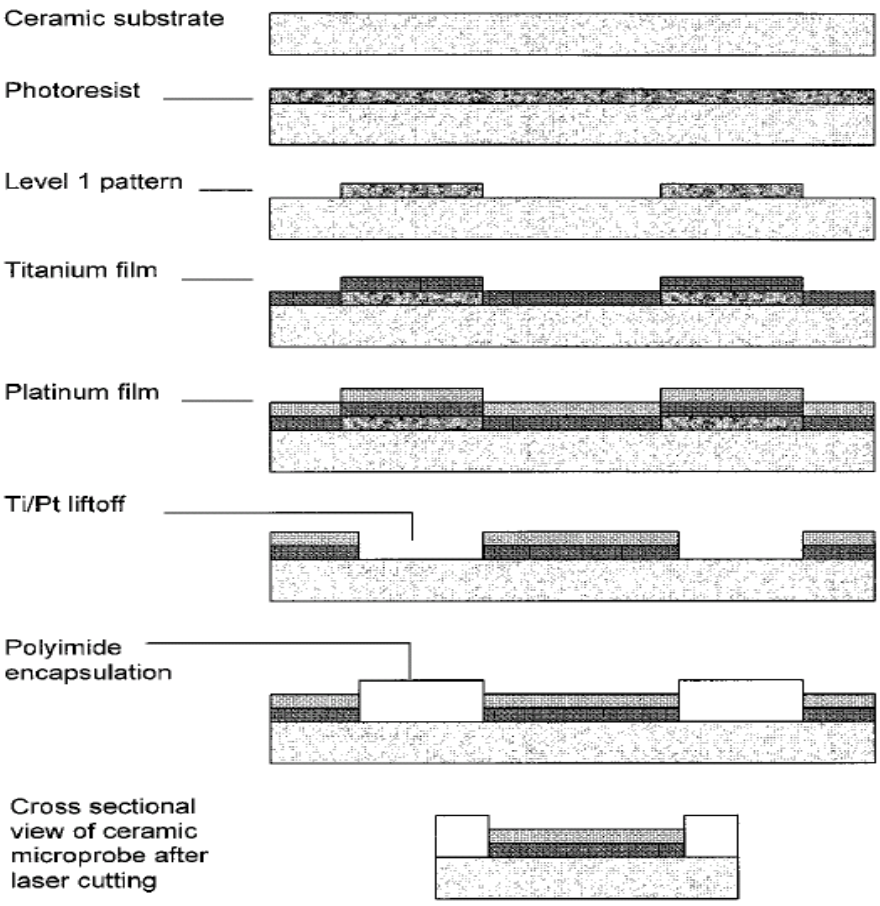


Figure 2.1. Schematic diagram illustrating the fabrication steps of the ceramic-based multisite microelectrodes.Reproduced from [12]

A photograph of the multisite microelectrode taken under an optical microscope is shown in Figure 2.2.

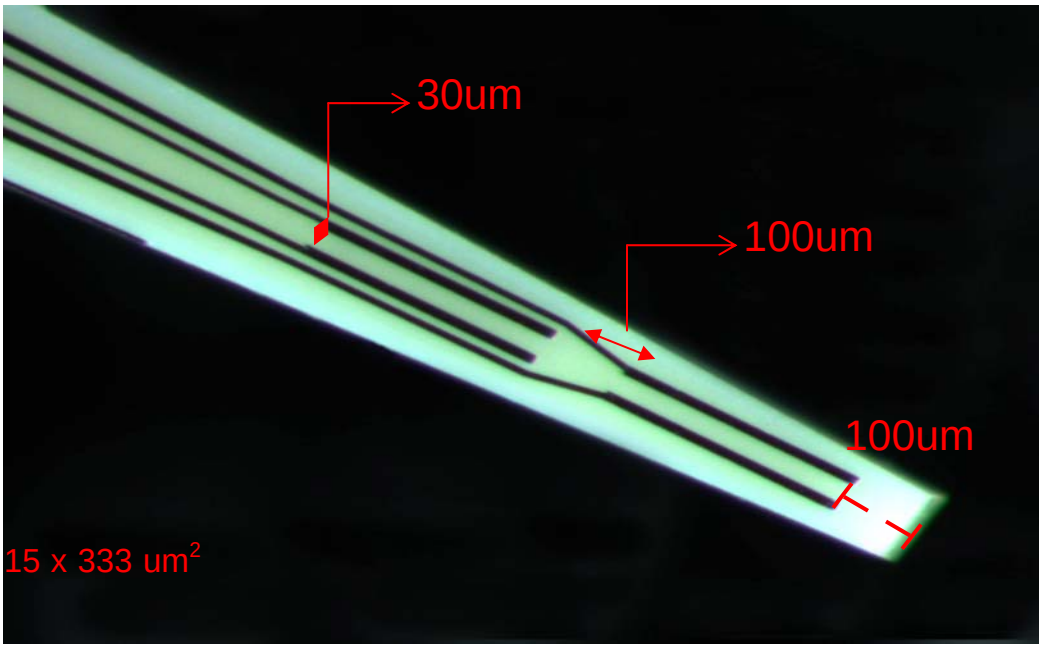


Figure 2.2. Photograph of Pt multisite microelectrode. The recording sites are 15µm wide by 333µm long.

2.2. OPERATION OF MICROELECTRODE ARRAYS

L-glutamate (Glu) is not an electroactive molecule. To make the rapid detection of Glu possible, we employed the use of an enzyme called glutamate oxidase (GluOx). GluOx catalyzes Glu into an electrically active reporter molecule – hydrogen peroxide. To obtain basal extracellular Glu concentrations, one pair of recording sites was configured for Glu detection. This was done by coating a pair of recording sites with GluOx. The other two recording sites do not contain

GluOx and serve as sentinel sites. The response of the sentinel sites (without enzyme), is subtracted from that of the measuring recording sites (with enzyme) in order to compensate for the signal produced by oxidation of electrochemically active interferents. The electrode recording sites with various layers present on it is shown in Figure 2.3. The decomposition of L-glutamate and production of H_2O_2 by glutamate oxidase is summarized in equations (2.1), (2.2) steps.

$$L\text{-}glutamate + H_2O + GluOx/FAD \rightarrow \alpha\text{-}kg + NH_3 + GluOx/FADH_2 \quad (2.1)$$

$$GluOx/FADH_2 + O_2 \rightarrow GluOx/FAD + H_2O_2. \quad (2.2)$$

$$H_2O_2 \rightarrow 2e^- + 2H^+ + O_2 \quad (2.3)$$

The generated hydrogen peroxide oxidizes efficiently at the Pt recording sites at +0.7 V (vs. Ag/AgCl reference) as shown in equation (2.3)

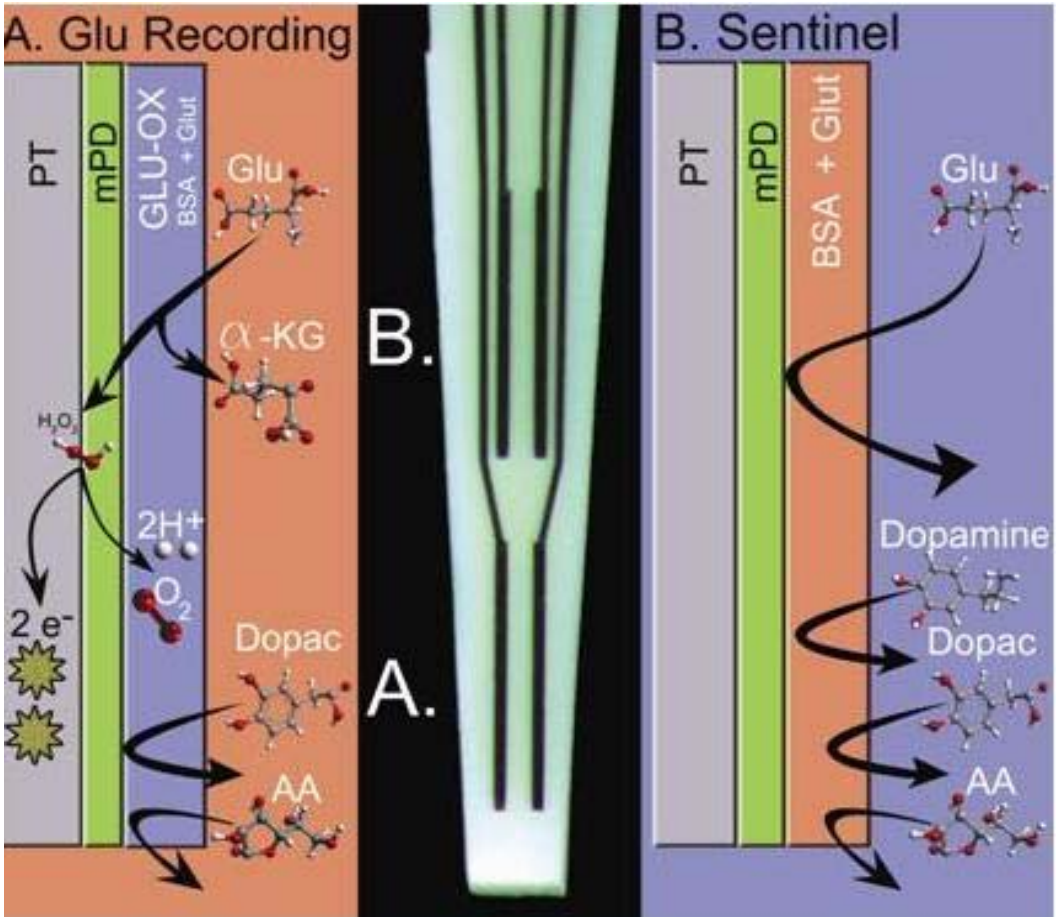


Figure 2.3. Schematic drawing of the different layers present on the microsensor in order to measure glutamate (A). Recording sites with GluOx (B). Sentinel sites without GluOx.

2.3. CURRENT ELECTRODE PERFORMANCE, WITHOUT Pt-black DEPOSITION

Pt-black is a powered form of metallic Pt. The performance of microelectrode arrays without any modifications on it is given in Table 2.2. Unmodified electrodes are the ones that do not have Pt micro- and nano- particles deposited on it. Here the value of N is the number of recording sites. Each electrode has four recording sites as discussed earlier. Sensitivity shown in the table is normalized to

area. Although, the sensitivity does not linearly increase with surface area. As seen from Table 2.2, the sensitivity of H_2O_2 is higher than that of glutamate. This is because glutamate has to go through several layers before being detected by electrode. Where as, for hydrogen peroxide injection *in-vitro*, the analyte (H_2O_2) need not pass through any layers to be detected by electrode.

Table 2.2: In-vitro performance of microelectrodes without surface modification.

Analyte	N	LOD (μM)	Normalized Sensitivity ($\mu AmM^{-1}cm^{-2}$)
Hydrogen peroxide	32	0.0185±0.0178	358.13±111.74
Glutamate	12	0.0157±0.0032	316.54±29.40

CHAPTER 3

METHODS AND EXPERIMENTS

3.1. REAGENTS

Chloroplatanic acid (H_2PtCl_6), ascorbate (AA), L-glutamate monosodium salt, glutaraldehyde (25% in water) and bovine serum albumin (BSA) were obtained from Sigma-Aldrich. L-glutamate oxidase was purchased from Seikagaku America, Inc. All solutions were prepared with distilled water, which was deionized (DI). Solutions used for intracranial injections were prepared in 0.9% saline, adjusted to PH 7.4 and filtered before use. H_2PtCl_6 and H_2SO_4 were stored at room temperature. Glutaraldehyde was stored at -20º C.

3.2. CLEANING OF MICROELECTRODE

The microelectrodes were cleaned before calibration procedure. The cleanliness of the electrode makes lot of difference on the electrode performance. They were first immersed in isopropyl alcohol for 5 minutes and then in deionised (DI) water for 5~10 minutes. These electrodes were then dried in desiccator (~60 minutes). Electrodes are also cleaned before platinization process with cyclic voltammetry. This procedure is described in Section 3.5.

3.3. CALIBRATION OF SENSORS FOR PEROXIDE SENSITIVITY

The micro electrode arrays (MEAs) were calibrated before platinum-black (Pt-Black) deposition using a FAST-16 high speed electrochemistry instrument (Quanteon, L.L.C, Nicholasville, KY, USA). Software called FAST (Fast Analytical Sensing Technology) was used for constant voltage amperometry. The software helps in simultaneous recording of all the four recording sites of MEA. Measurements were carried out at an applied potential of +0.7 V (vs Ag|AgCl reference). This potential is the ideal potential for H_2O_2 oxidation. Since the electric circuitry is exposed, one should coat the circuit board assemblies with epoxy for easier handling.

For calibrations, the microelectrodes were submerged in a solution of 0.05 M 40 mL Phosphate-Buffered Saline (PBS). PBS serves as an ionic medium for the transfer of electrons between the MEA and solution. In addition PBS solution is a good representative of cerebrospinal fluid (extracellular fluid) found in the brain. The solution was maintained at room temperature through out the calibration process. MEA is allowed to reach baseline (~25 minutes) in PBS solution under unstirred conditions. 40 μL of 8.8 mM hydrogen peroxide (H_2O_2) solution was added to PBS three times sequentially to produce final concentration of 8.8, 17.6 and 26.4 μM H_2O_2 solution. The solution was stirred slowly for 30 seconds immediately after each addition of H_2O_2. A 10 x 3 mm magnetic stir bar was used to stir the solution slowly without creating a vortex. This stirring of solution helps in mixing of added H_2O_2 with the solution in the beaker. With the electrolyte

movement, electrochemical noise increases [1] . To maintain a good signal to noise ratio (of ~30:1) the solution was not stirred through out the calibration process. Amperometric measurements were recorded for each addition. Sensitivity, limit of detection (LOD) and linearity for H_2O_2 are determined. It was observed that the average H_2O_2 sensitivity (of unmodified electrode) was 358.13±111.74 $\mu AmM^{-1}cm^{-2}$(n=32) and average H_2O_2 LOD was 0.018±0.017 µM (n=32).

The same electrodes were calibrated again after Pt-black deposition (procedure for Pt-black deposition is explained in Section 3.3), to see the effect of increased surface area on electrode sensitivity and limit of detection (LOD) of H_2O_2. The main difference observed was that platinized electrode took more time (60-90 minutes) to reach stable baseline. Possibly higher surface area Pt-black requires more charge to build up at the electrode surface. The current observed before H_2O_2 addition in PBS solution forms non-faradic current, which contributes to background noise. It was also observed that the base line current (non-faradic current) for platinized electrode is larger than that of bare electrode. This is because the non-faradic current is related to the electrode surface area. Since electrode surface area of the platinized electrode is larger than bare electrode, so is the non-faradic current [10].

The H_2O_2 sensitivity of platinized electrode was observed to be 686.31±156.35 $\mu AmM^{-1}cm^{-2}$(n=36) and their H_2O_2 limit of detection was observed to be 0.0461±0.088µM (n=36).

Figure 3.1: Photograph of microelectrode immersed in calibration chamber. The solution is maintained at room temperature for H_2O_2 calibration

3.4. DEPOSITION OF PLATINUM NANOPARTICLES ON RECORDING SITES

Electrodeposition of Platinum (Pt) nanostructures was carried out with the Gamry Reference 600 system under constant voltage conditions in 3%weight chloroplatinic acid solution (H_2PtCl_6) solution at room temperature. A three electrode setup was used for Pt nanoparticle deposition. The recording sites of the MEA were the working electrodes. (The working electrode is the one at which the electrochemical phenomenon being observed takes place.) Platinum clad niobium mesh was used as a counter electrode. (The counter electrode acts as sink or

source for electrons so that current can be passed from the external circuit through the electrolyte solution. In general neither its true potential nor current is ever measured or known.) Reference electrode was Ag/AgCl electrode dipped in sodium chloride (NaCl) solution. (Reference electrode potential is constant enough that it can be taken as the reference standard against which potential of other electrodes present in the cell can be measured.). Pt-black deposition was carried out until a charge density of 2 Ccm^{-2} was reached. This required approximately 40 seconds for each recording site. The research work of other groups [7] indicated that platinum deposition occurs when deposition potential is between -0.1V and -0.25 V. Therefore we chose a platinization potential of -0.12V. Moreover Chi.S.Kim et al.[7] reported that at a platinization potential of -0.12V Pt(110) planes are exposed, and enzymes are preferentially immobilized on 110 planes.

It was observed that a deposition charge density of more than 2 Ccm^{-2} resulted in peeling of the Pt layer. Higher charge densities result in greater Pt deposition. It is possible that additional intrinsic stress was responsible for peeling the underlying Pt layer. The plating solution was unstirred throughout the procedure. The electrodeposition was carried out at room temperature. Elliott et.al [6] reported that as the deposition temperature was increased the nanostructure deposition became non-uniform.

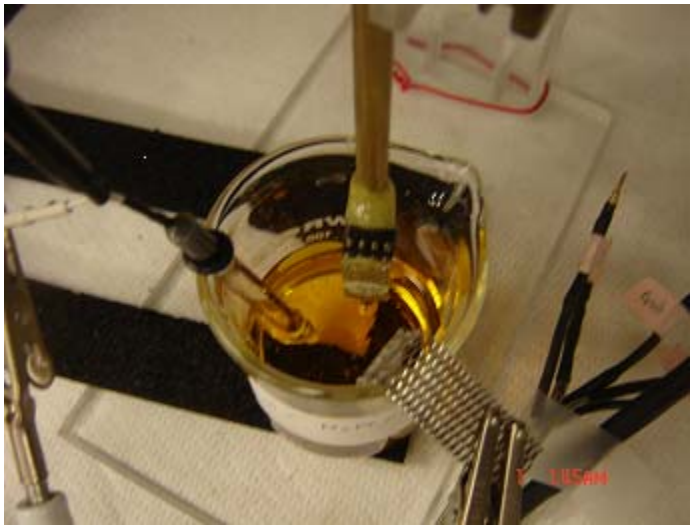


Figure 3.2: Photograph of Platinum-black deposition on Platinum recording sites.

3.5. CYCLIC VOLTAMMETRY

3.5.1. Before Pt-black deposition

The MEAs were subjected to cyclic voltammetry before Pt-Black deposition to clean the recording sites of any impurities that may have been deposited on the electrode surface during fabrication. A Gamry Reference 600 Potentiostat was used to obtain the cyclic voltammogram. Voltage was swept between 1.2V to -0.25V at 50 mV/s in unstirred 0.5 M H_2SO_4 solution for 15 cycles. Cyclic voltammetry of each site took about 9-10 minutes. During this procedure, Ag|AgCl (3.5M NaCl), MEA and Pt mesh were reference, working and counter electrodes respectively.

3.5.2. After Pt-Black deposition

The same produce as above was followed to obtain a stable cyclic voltammogram indicating the complete cleaning of the Pt active area and strong adherence of the platinum nanoparticles on the platinum recording site. Cyclic voltammetry at this stage cleans the recording sites of any platinum chloride that may have been deposited during the Pt-Black deposition process with chloro platanic acid (H_2PtCl_6).

3.6. GLUTAMATE OXIDASE (GluOx) COATING ON RECORDING SITES

The GluOx layer permits the detection of L-Glutamate (Glu) by decomposing it into α-ketoglutarate and H_2O_2. H_2O_2 diffuses through the mPD layer and contacts the Pt surface. H_2O_2 is oxidized at Pt surface at an applied voltage of +0.7V (vs. Ag/AgCl reference). Oxidation of H_2O_2 results in two free electrons per H_2O_2 molecule. These free electrons flow through electrochemical detection system and allow the recording of presence of Glu.

To make MEAs selective for Glu, they were coated with an enzyme solution. 10 µL of this solution was prepared with 1%(w/v)GluOx, 1%(w/v)BSA and 0.125%(w/w) glutaraldehyde. The coating was done at room temperature. To apply enzyme solution a micro syringe was used under dissecting microscope. Up

to 1 μL of solution was drawn into the 10 μL Hamilton syringe. A small drop of solution (~ 0.1μL) suspended at the tip of microsyringe needle was put in contact with the recording sites and then removed. The coating was allowed to dry for a minute. Three more coatings are applied at 1 minute intervals to achieve approximate thickness of about ~2 μm. Enzyme coating is generally applied to the two platinum (Pt) recording sites closest to the MEA tip [sites 1 and 2], thus configuring them to detect Glu. The other two Pt recording sites (sites 3 and 4) are coated with a solution having 1%(w/v)BSA and 0.125%(w/w) glutaraldehyde. Because of the absence of GluOx sites 3 and 4 (called sentinel sites) cannot detect Glu. This arrangement is called 'self referencing' and is particularly useful to acquire basal extracellular concentration. The response of the recording site without enzyme is subtracted from the measuring recording site (the one with enzyme on it) in order to compensate for the signal produces by oxidation of electrochemically active interferents. For optimal cross-linking of protein to the surface of the electrode, the coated MEA was set aside in clean dry place for about 5-7 days.

3.7. m-POLYETHYLENE DIAMINE (mPD) COATING ON THE Pt RECORDING SITES

In-vivo detection of glutamate generally suffers from some interference problems, especially due to the presence of ascorbic acid (AA), which exists in significant quantities and has a similar oxidation potential to that of enzymatically produced H_2O_2. To overcome this problem, the recording sites of MEAs were coated by

electropolymerizing m-polyethylene diamine (mPD). A layer of mPD acts as a restrictive surface to minimize access of anionic interferents (like dopamine and ascorbic acid) to reactive sites of the MEA. mPD excludes penetration of molecules through it based on the molecular size. During calibration and in-vivo recordings the hydrogen peroxide reporter molecule is small enough to diffuse through the mPD matrix where it is then oxidized at the Pt recording site when a contact potential of +0.7 V (vs. Ag/AgCl reference) is applied.

Generally electrodes were coated with mPD on the day of in-vivo recordings. Argon gas is bubbled through 0.5 M PBS solution to remove dissolved oxygen. This PBS solution that was bubbled was used for preparing 5 mM mPD solution (0.0905 grams of mPD in 50 mL of water). A plating potential of +0.5 V was applied for 15 minutes to plate the enzyme coated modified microelectrode. mPD layer of thickness approximately ~12-20 nm (molecular size of mPD) was formed.

3.8. CALIBRATION OF SENSORS FOR GLUTAMATE SENSITIVITY

Microelectrode arrays (MEAs) were calibrated in-vitro before in-vivo use. FAST-16 high speed electrochemistry instrument (Quanteon, L.L.C, Nicholasville, KY,USA) was used for calibration. Software called FAST (Fast Analytical Sensing Technology) was used for constant voltage amperometry. The software helps in simultaneous recording of all the four recording sites of MEA.

Measurements were carried out at an applied potential of +0.7 V (vs Ag|AgCl reference). This potential was chosen because it was observed from previous experiments that +0.7 V is ideal for H_2O_2 oxidation.

mPD coated microelectrode need not be soaked in 0.05 M PBS solution before calibration. The time period for which the microelectrode tip sits in mPD and calibration solution is sufficient for better diffusion of analytes through the mPD layer as well as for activation of the enzyme layer. PBS was heated for 9 seconds in microwave oven before submerging the electrode tip. PBS at slightly higher temperature than room temperature helps to activate the L-glutamate oxidase enzyme for physiologically relevant temperatures.

Electrodes were calibrated in 40 mL of 0.05 M PBS to mimic physiological conditions. The solution was maintained at 37ºC temperature throughout the calibration process. MEA was allowed to reach baseline (~60-90 minutes) in PBS solution under unstirred conditions. 20 mM AA, 20 mM L-glutamic acid (Glu) and 8.8 mM hydrogen peroxide solutions were made freshly before the calibration process. 500 µL of 20 mM AA (interferent) was added to PBS solution for a final concentration of 250 µM. When a new stable baseline was reached the interferent is recorded. Next, three 40 µL additions of 20 mM L-glutamate were added for final buffer concentration of 20, 40, and 60 µM L-glutamate. Analyte were recorded after each addition to create the calibration curve. After 3 additions of analyte, 40 µL of 8.8 mM (to produce 8.8 µM effective beaker concentration)

hydrogen peroxide was added to the solution to confirm operation (sensitivity) of all the recording sites. All the four sites respond to H2O2 addition, which confirms the sensitivity of all the four recording sites.

The solution was stirred slowly for 30 seconds immediately after each addition of AA, Glu and H_2O_2. A 10 x 3 mm magnetic stir bar was used to stir the solution slowly without creating a vortex. This stirring of solution helps in mixing of added interferent or analyte with the solution in the beaker. With the electrolyte movement, electrochemical noise increases [1]. To maintain a good signal to noise ratio (of 30:1) the solution was not stirred throughout the calibration process. Amperometric measurements were recorded for each addition. Sensitivity, limit of detection (LOD) and linearity for L-glutamate were calculated. The average Glu sensitivity (of modified electrode) observed was 492.29±112.69 $\mu AmM^{-1}cm^{-2}$ (n=6) and average Glu LOD observed was 0.171±0.095 µM (n=6). A ~1.5 times increase in glutamate sensitivity was observed when compared to unmodified electrode.

Figure 3.3 shows the photograph of microelectrode calibration chamber for glutamate calibration. The PBS solution in the beaker is kept at a higher temperature (37º C) than room temperature, using water bath. The higher temperature of the PBS solution serves two purposes. 1) It activates the enzyme layer 2) It mimics the temperature present inside the brain tissues, which is generally 37º C.

For the calibration process, as seen in Figure 3.3, two electrode set-up is used for calibration of electrode for glutamate. Ag/AgCl is used as reference electrode and the recording sites of the microelectrode are used as working electrode. The speed of the stir bar is kept at minimum, in order not to create vortex in the solution.

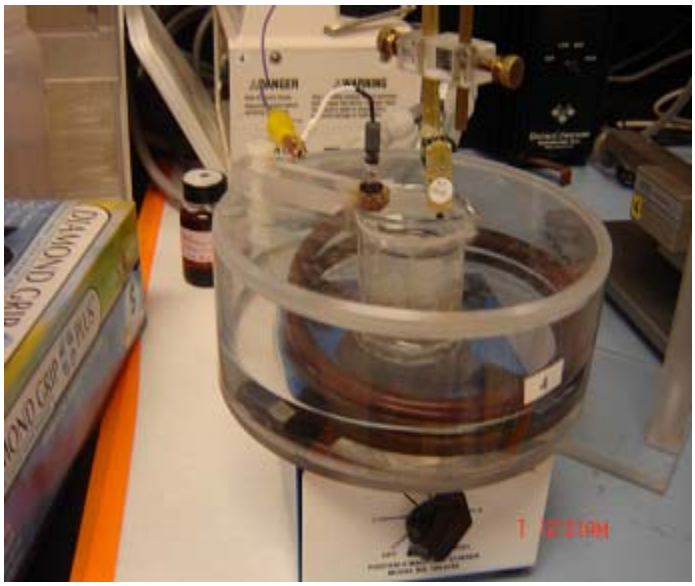


Figure 3.3: Photograph of microelectrode immersed in calibration chamber. The calibration solution is maintained at 37ºC for Glutamate calibration.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1. ELECTRODE PLATINIZATION

Electrode platinization at potentials -120mV vs Ag/AgCl reference resulted in a uniform Pt-Black deposit. As can be seen from the image Fig 4.1, there were more deposits at the boundaries of the recording sites because the electric field, and thus the deposition current density, is greater in these regions.

The electrodeposition of Pt nanoparticles can be summarized in two reactions as shown in equation (4.1) and (4.2). First Pt(IV) is decomposed to Pt(II) and then Pt(II) decomposes to metallic platinum. The formation of metallic Pt is efficient at -120mV. This potential was optimized through experiments. All the potentials mentioned in this document are with respect to Ag/AgCl reference electrode. This decomposition into metallic platinum on the smooth platinum surface, results in the deposition of Pt micro- and nano- particles on the smooth recording surface. Thus this increases the effective surface area of the electrode.

$$PtCl_6^{2-} + 2e^- \rightarrow PtCl_4^{2-} + 2Cl^{-1} \qquad \text{--------(4.1)}$$

$$PtCl_4^{2-} + 2e^- \rightarrow Pt + 4Cl^{-1} \qquad \text{--------(4.2)}$$

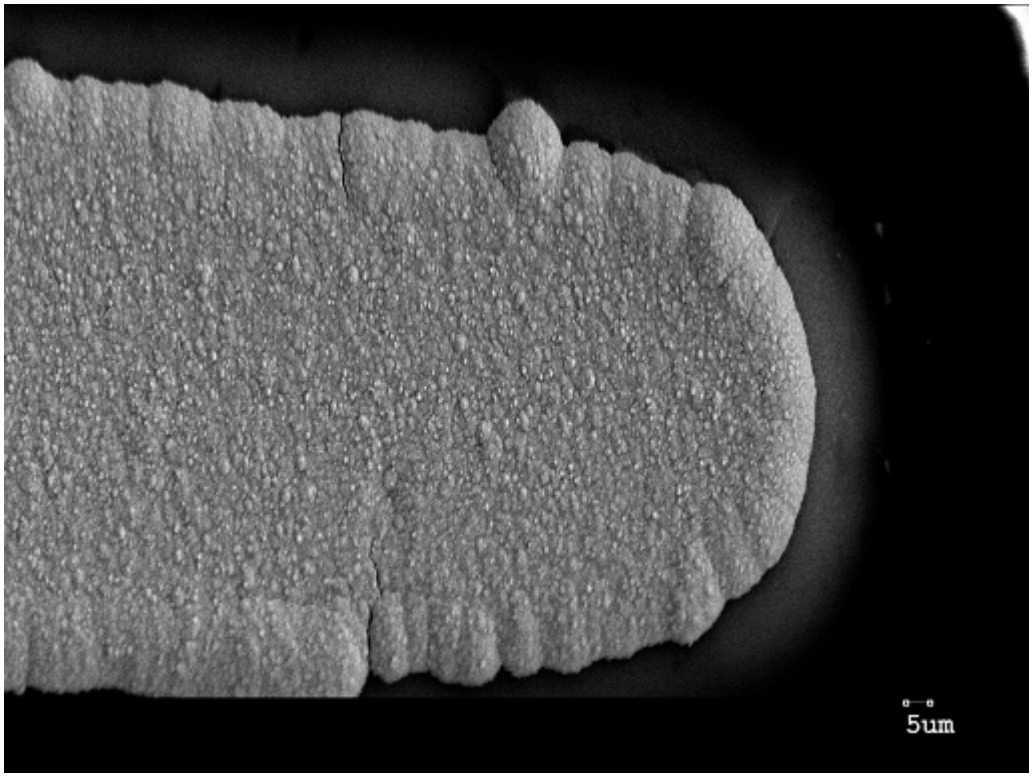
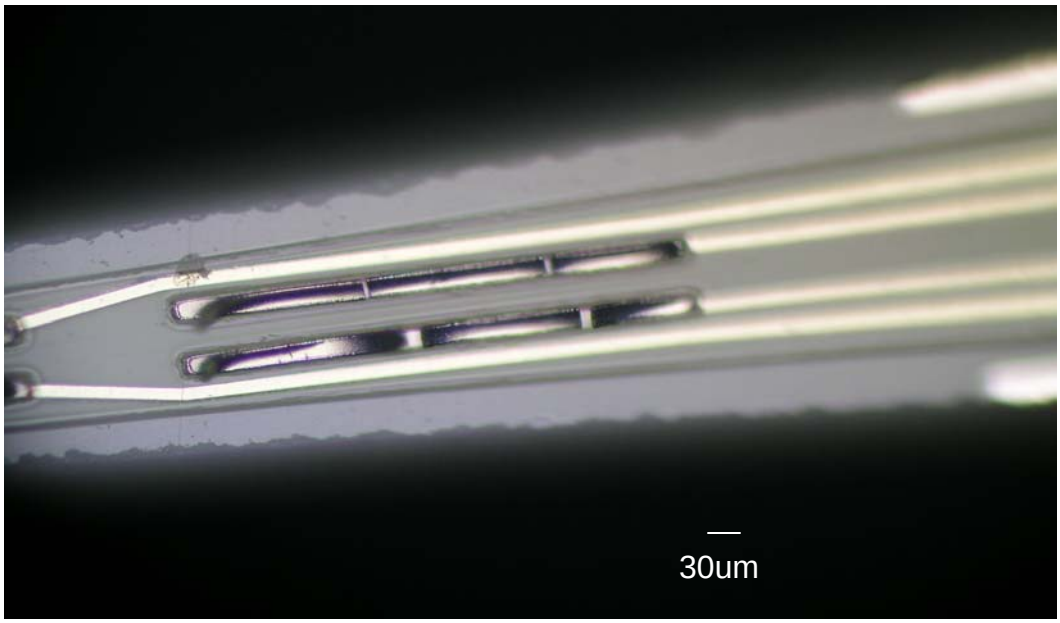
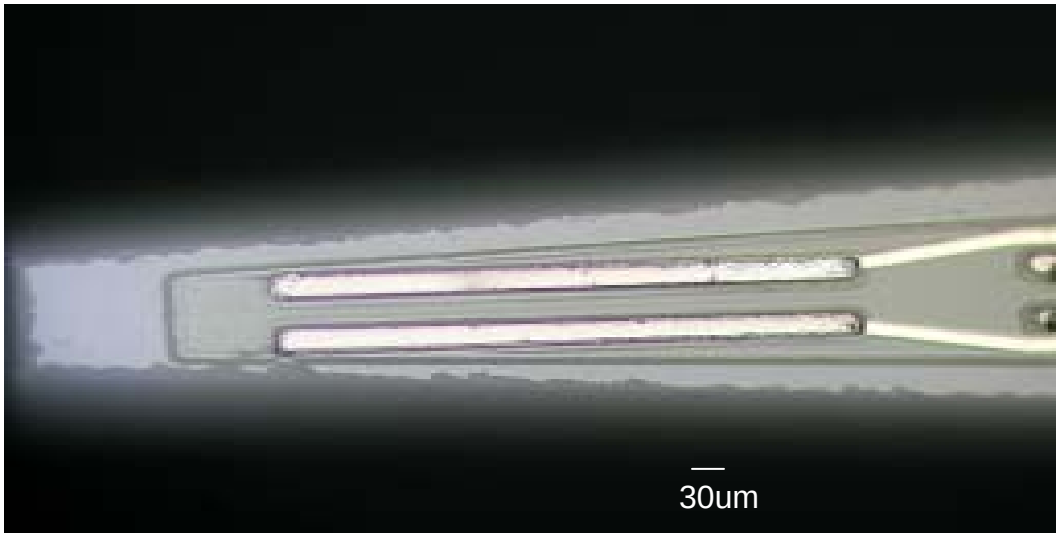


Figure 4.1: Scanning electron microscope image of platinized recording surface (15 x 333 um^2)

For Pt-Black, a deposition charge density of 2 Ccm^{-2} was applied. It required ~40 seconds for the total charge to reach 99μC. However platinization at higher charge density resulted in peeling off of the Pt film from the recording sites as shown in the Fig 4.2(a). Higher charge densities result in greater Pt deposition. It is possible that additional intrinsic stress was responsible for peeling the underlying Pt layer.



(a)



(b)

Figure 4.2: (a) Image of damaged recording site when charge of 5 Ccm^{-2} was passed. (b) Image of platinized recording site when a charge of 2Ccm^{-2} was passed for Pt-Black deposition.

The platinization potential of -0.12 V was chosen because at this potential, Pt(110) planes are exposed on the Pt-Black surface. Hydrogen atoms which are adsorbed at these planes (110) play crucial role during the crystallization of atomic platinum from Pt(II) ions.

At the beginning of the platinization process, there was a sudden decrease in the current, probably due to the formation of concentration gradients between the electrode surface and bulk of the solution. After about 2 seconds, as a result of platinum black deposition and increase in recording surface area, the platinization current increased and then stabilized with time. .

.

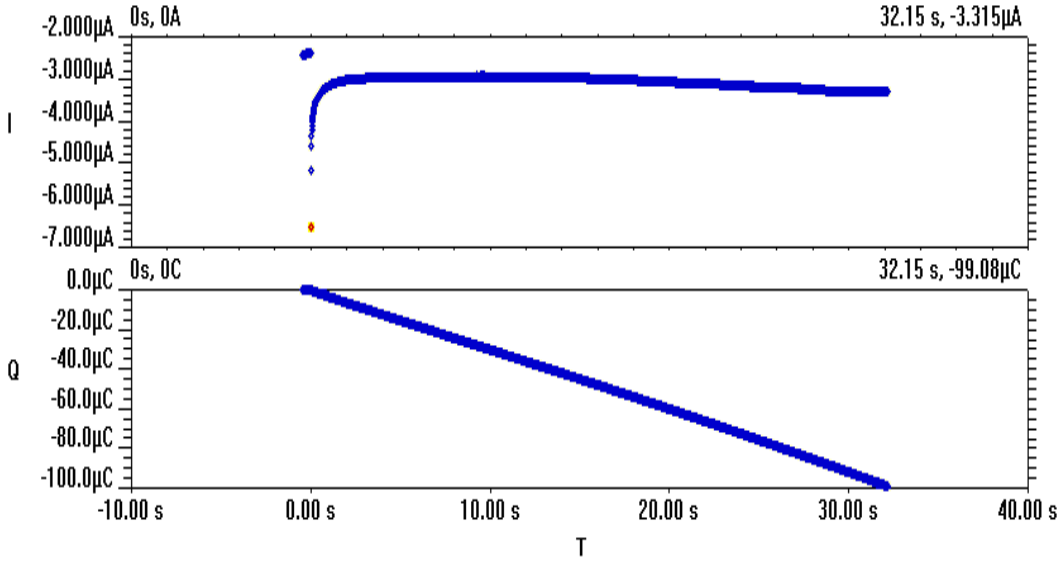


Figure 4.3: Plots of current and cumulative charge vs. time during electrodeposition.

4.2. CHARACTERIZATION OF PT-BLACK ELECTRODES

The surface morphologies of Pt-Black electrodes were characterized by scanning electron microscopy (SEM) using Hitachi 4300 field emission tool. For all the images 5KV and 14.3mm were set as accelerating voltage and working distance respectively.

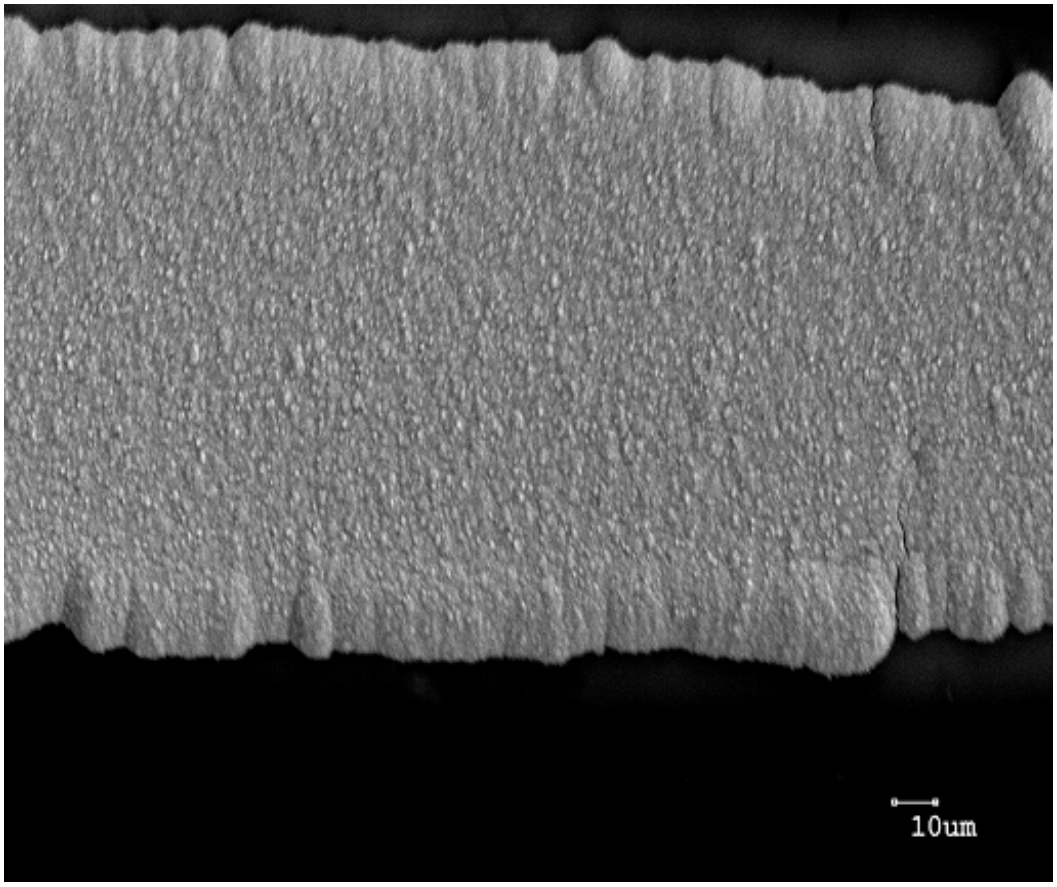


Figure 4.4: SEM image of nanostructured platinum deposited at -0.12V Vs Ag|AgCl reference, deposition temperature was 25ºC, and deposition charge density was 2Ccm^{-2}.

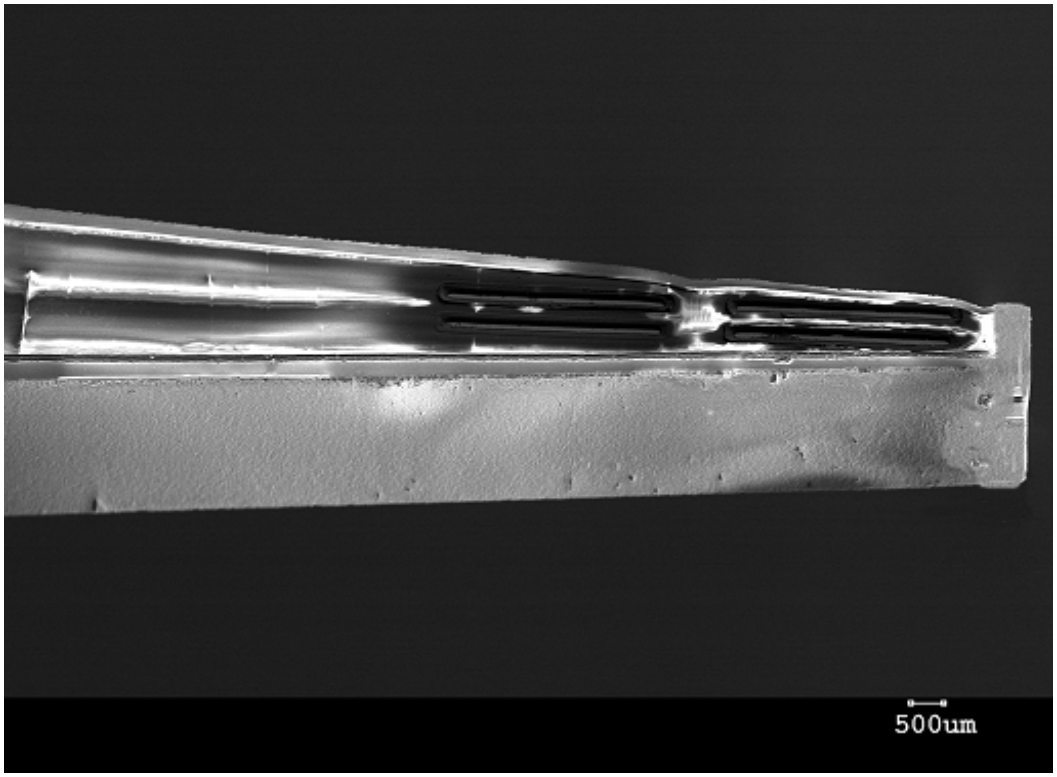


Figure 4.5: SEM image of microelectrode tip tilted by 60º. Variations in signal level are the result of charging of the ceramic substrate and polyimide coating.

4.3. EFFECTIVE SURFACE AREA AND ROUGHNESS FACTOR OF PT-BLACK ELECTRODES

The increased surface area of platinized electrode was estimated from the charge density of the hydrogen adsorption reaction between 100 mV and -250 mV as shown in the Figure 4.6. The surface roughness factor is defined as the ratio of the effective surface area (ESA) to the geometric area and was estimated by analyzing the cyclic voltammogram.

$$\text{Roughness factor} = \frac{\text{Estimated surface area}}{\text{Geometric surface area}}$$

$$\text{Estimated surface area} = \frac{\text{Total charge}}{Q^*}$$

Total charge is the charge under the hydrogen adsorption peaks, which corresponds to adsorption of one hydrogen atom on each metal atom of the surface. Q* is the charge associated with one-to-one hydrogen-Platinum correspondence per unit surface area. This value is based on the distribution of Pt metal atoms on the surface. It is estimated that Q* for Pt has a standard value of $210\mu Ccm^{-2}$, which corresponds to a $1.30x10^{15}$ $atomcm^{-2}$ for polycrystalline Pt electrode [5]. For a total charge of 904.9nC, the estimated surface area observed was $4.309\ x10^{-3}cm^2$ and roughness factor corresponding to this increased surface area was ~86.

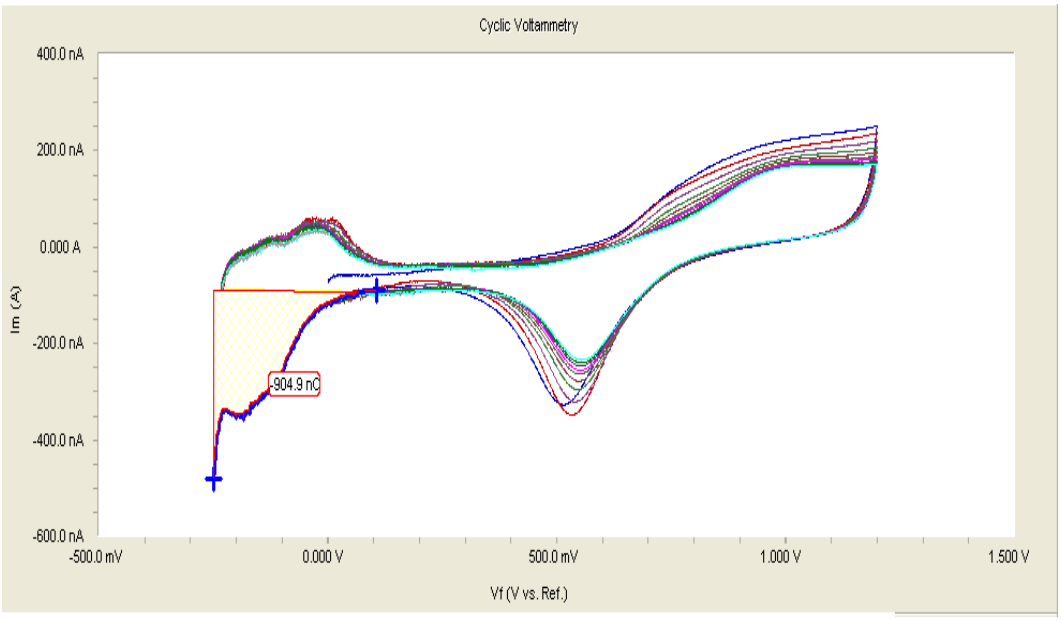


Figure 4.6: Cyclic voltammogram of platinized recording site. A charge density of 2Ccm^{-2} was used for Pt-black deposition. The charge in the shaded area depicts adsorption of one hydrogen atom on each Pt atom of the recording surface.

Increase in surface area was observed to be directly proportional to the charge density applied during platinum nano-particle deposition. This can be observed from the Table 4.1.

Table 4.1: Variation in surface roughness with charge density.

Applied charge density	Calculated roughness factor
2 Ccm^{-2}	86
10 Ccm^{-2}	368.9
11 Ccm^{-2}	436.6
12 Ccm^{-2}	441.2

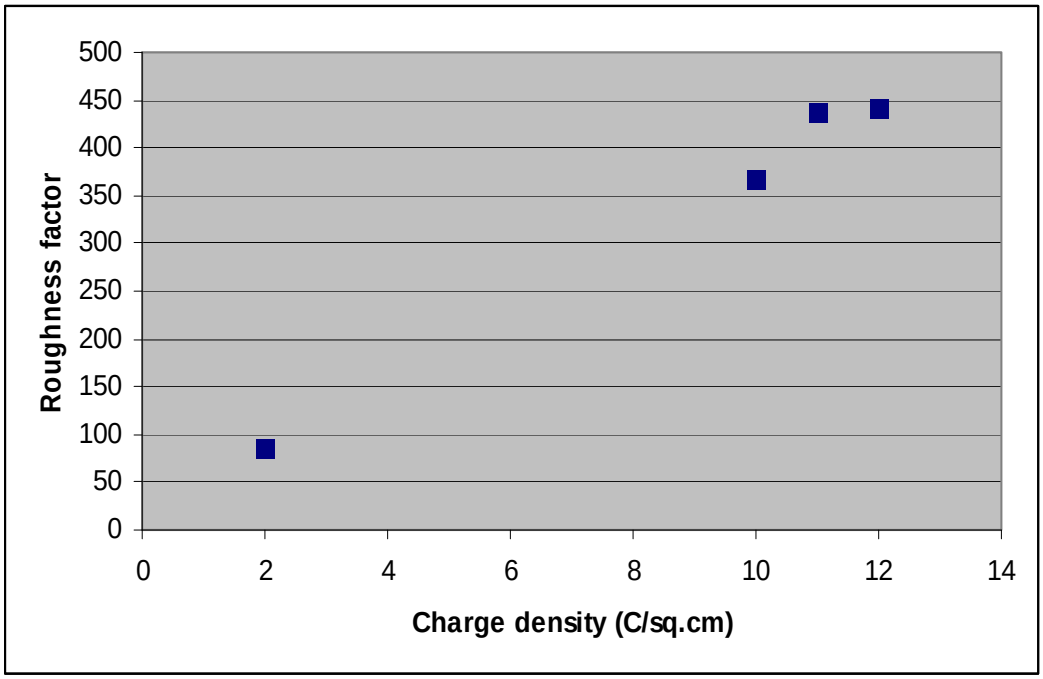


Figure 4.7: Effect of charge density on the roughness factor of electrode surface.

4.4. CYCLIC VOLTAMMETRY

Cyclic voltammograms of platinized electrodes in 0.5 M H_2SO_4 between 1.2 V to -0.25 V vs Ag/AgCl reference, at a scan rate of 50 mVs^{-1} showed typical profile for polycrystalline platinum. It was observed that if the scanning was done between 1.2 V to -0.3 V vs Ag/AgCl reference, huge currents passed through the electrode (over -10.00 µA), and this damaged the recording sites. A similar peeling off of the Pt layer was observed as shown in Fig 4.2(a). This drastic increase in the current below -250 mV was due to the formation of diatomic hydrogen (H_2).

Figure 4.8 depicts the cyclic voltammogram of smooth Pt recording site. The plot is flatter than that of rough surface as shown in Figure 4.9. This is because, in case of smooth Pt surface, there is no hydrogen adsorbed with Pt surface atoms.

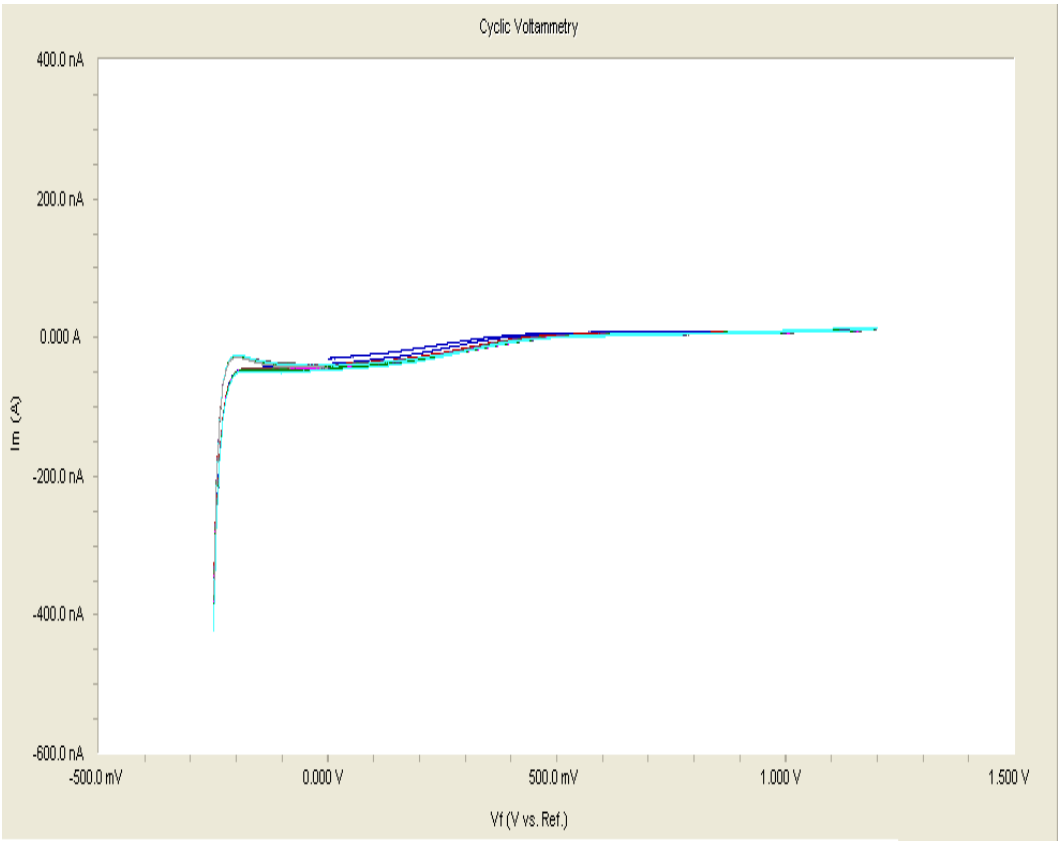


Figure 4.8: Cyclic voltammogram of unmodified recording site. The roughness factor of the recording site without any nanoparticle deposition was estimated to be between 2~3.

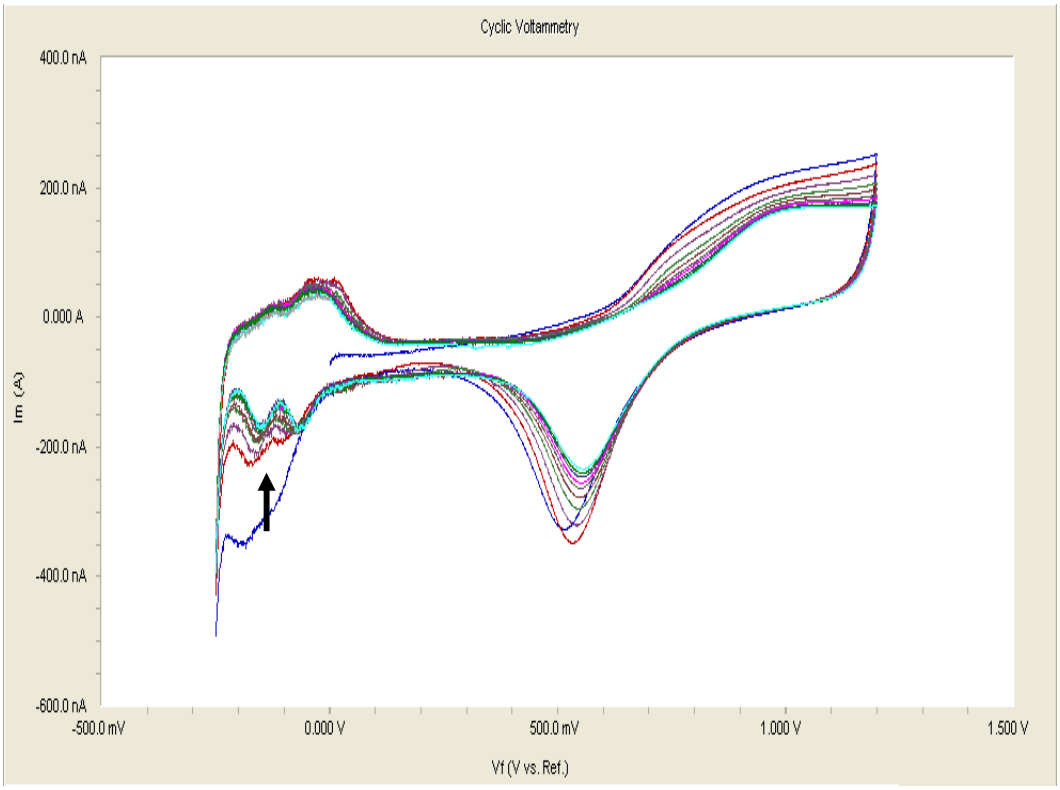


Figure 4.9: Cyclic voltammetry of platinized recording site. The charge density of 2 Ccm^{-2} was used for deposition. The calculated roughness factor of the recording site with nano-particle deposition was ~86. The voltammogram takes some time to stabilize and produce sharp adsorption peaks as shown by the upward arrow.

The recording sites where subjected to cyclic voltammetry before and after Pt-Black deposition. Cycling potential between 1.2 V and -0.25 V before Pt-Black deposition cleans the recording sites of any impurities that may have been deposited on the electrode surface during fabrication process. The voltammogram of the unmodified recording site is shown in Figure 4.8. Similarly cycling potential between 1.2 V and -0.25 V after Pt-Black deposition cleans the recording sites of any platinum chloride that may have been deposited during the Pt-Black deposition process with chloroplatanic acid (H_2PtCl_6). Voltammogram

(Figure 4.9) obtained at this stage is also useful to analyze the increased surface area (due to deposited Pt nano-particles). Table 4.2 indicates that cleaning the recording sites before Pt-Black deposition has considerable effect on the sensitivity and limit of detection of the electrode.

Table 4.2: Effect of cyclic voltammetry on electrode performance.

Electrodes not subjected to cyclic voltammetry before Pt-Black deposition.	**Electrodes subjected to cyclic voltammetry before Pt-Black deposition.**
H_2O_2 sensitivity of electrode increased by 2.3 times.	H_2O_2 sensitivity of electrode increased by 5.7 times.
H_2O_2 detection limit increased by 25 times.	H_2O_2 detection limit increased by 2.6 times.
Baseline standard deviation (non-faradic current) increased by 12 times.	Baseline standard deviation (non-faradic current) increased by 9 times.

The increase in sensitivity of the Pt-Black deposited electrodes when a compared to bare Pt electrode is both due to increase in electroactive surface area of recording sites as well as due to catalytic activity of Pt nano-particles. The effect of catalytic shape of Pt nano-particles can be calculated from specific activities

for H_2O_2 oxidation. [5]. Current density of H_2O_2 at 0.6V was used for calculating specific activities for H_2O_2 oxidation.

4.5. EFFECTS OF PLATINIZATION ON PEROXIDE RESPONSE OF ELECTRODE

Sensitivity of an electrode refers to its ability to measure the change in L-glutamate concentration. Sensitivity equates a change in current to a change in L-glutamate concentration. Figure 4.13 and Figure 4.14 clearly indicates increase in amperometric response (for peroxide) of platinized electrode when compared to unmodified electrode. From Figure 4.12(a), we can observe that there is 2 times increase in H_2O_2 sensitivity. The increased sensitivity of Pt-Black coated electrode is both due to increased electroactive surface area and better catalytic properties of the Pt(110) surface planes.

The increase in sensitivity of the electrodes is much smaller when compared to the increase in surface area of the electrode. There is ~86 times increase in surface area, but the increase in H_2O_2 sensitivity is only ~2 times. The electrode become diffusion limited rather than reaction rate limited. Once the recording surface area with Pt sites available for H_2O_2 oxidation equals geometric surface area of the recording site, any additional platinum site which is available deeper in the Pt-Black layer will not be reached by H_2O_2. Chi.S.Kim et.al [7] reported that during electrodeposition of platinum both Pt(100) and Pt(110) planes are exposed.

But enzyme molecules favorably deposit on Pt(110) sites. This explains why there is no proportionate increase in the electrode sensitivity with the large increase in the surface area (roughness factor of 86). Hence we may say that sensitivity of electrode is not just under surface area control, but also under diffusion (of H_2O_2 and Glu) control through the layers deposited over Pt recording site.

LOD refers to the smallest analyte concentration that can be detected by the electrode. From Figure 4.12(b) we see that there is increase in H_2O_2 LOD after Pt-Black deposition, which is not desired. One probable explanation for this increase in LOD is increase in baseline current (non-faradaic current) for platinized electrode. It was observed that baseline current for platinized electrode is ~0.125 nA which is nearly 10 times higher than that of unplatinized electrode (~0.012 nA). Non-faradaic current is directly proportional to electrode surface [10]. This explains why there is increase in background current with platinization. This increase in background current with increase in electrode surface area can be minimized by decreasing the electrode potential. Figure 4.10 and Figure 4.11 shows that sensitivity of the electrode remains constant when electrode potential is 0.7 V or 0.5 V, but the LOD is lower (and hence better) when the electrode potential is 0.5 V. Thus we may conclude that increase in background current due to increase in electrode surface can be reduced by decreasing the electrode potential from 0.7 V to 0.5 V.

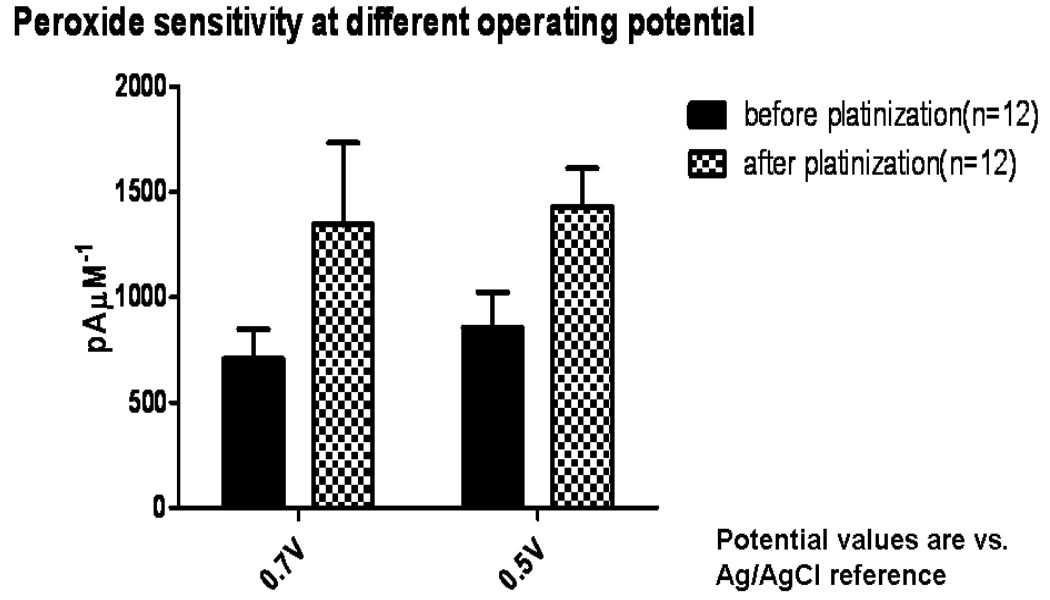


Figure 4.10: Comparative graphs depicting effect of electrode potential on electrode sensitivity.

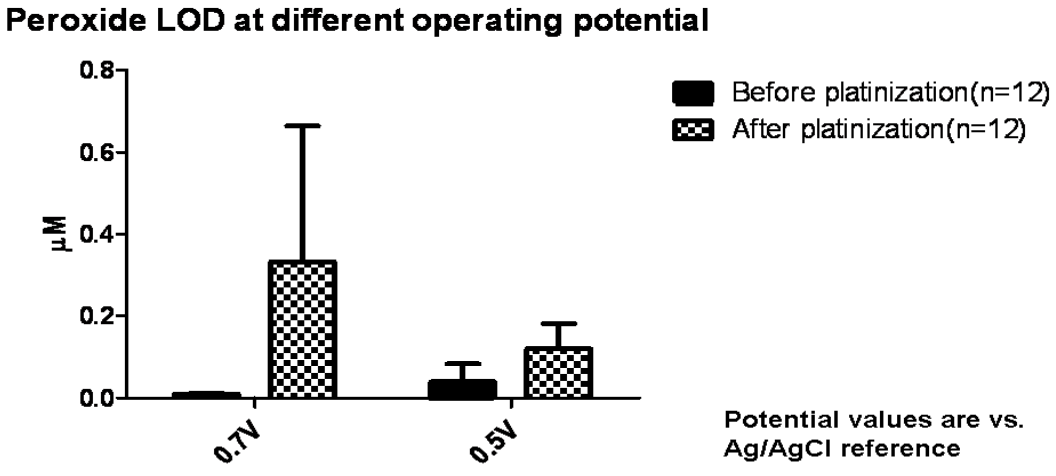
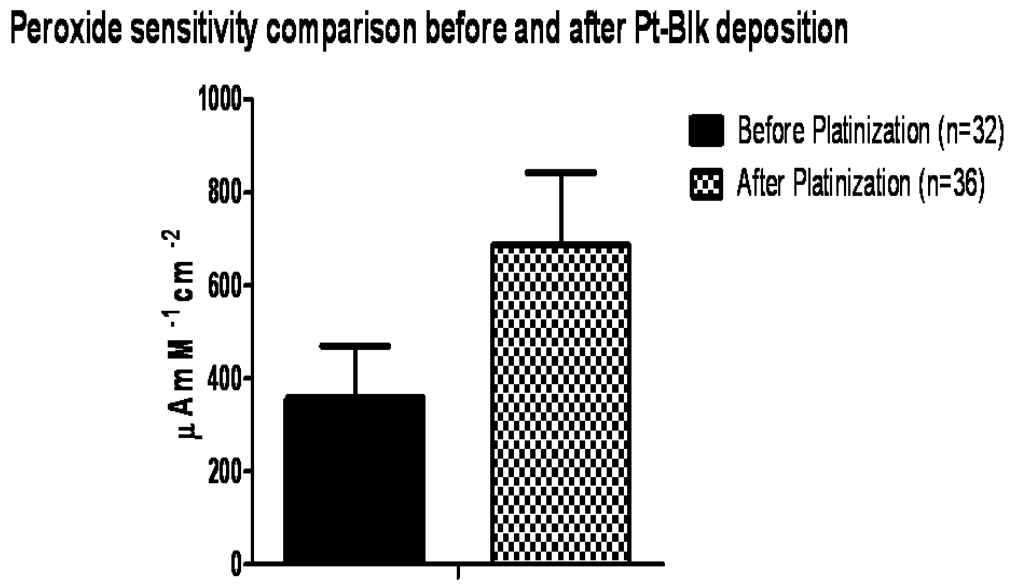
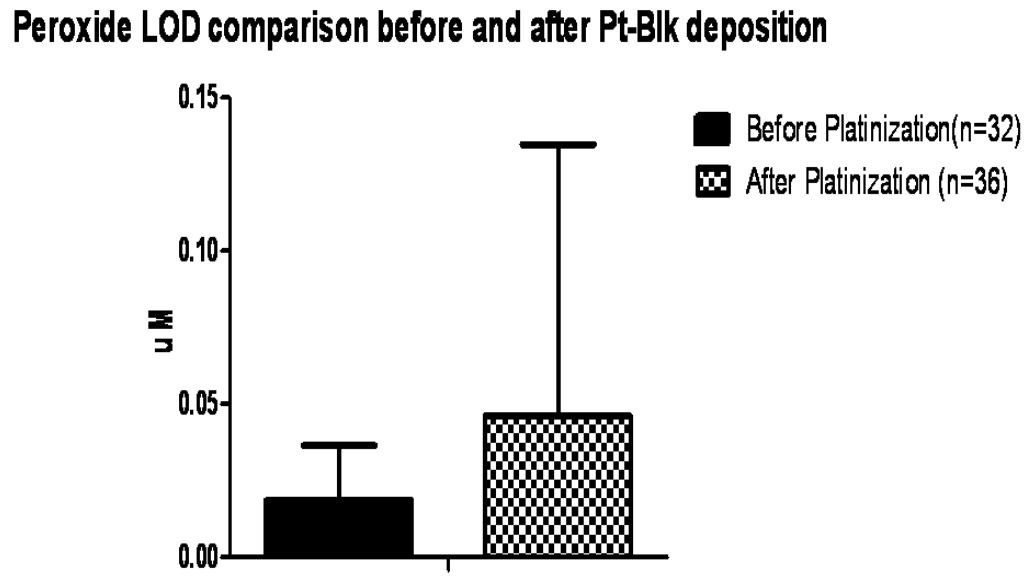


Figure 4.11: Comparative graphs depicting lower electrode potential improves the detection limit of the electrode.

One more reason for the background current may be due to the stirring of phosphate buffer solution (PBS). This cause of noise was minimized by stirring the solution only for 30 seconds immediately after H_2O_2 addition. It was observed that there is significant difference in H_2O_2 sensitivity and LOD values of platinized electrode when the solution is stirred and unstirred. (Figure 4.13). This might be the result of accelerated H_2O_2 transport to the electrode surface. But stirring or not stirring the solution did not seem to affect the electrode response to L-Glutamate (Figure 4.17).



(a)



(b)

Figure 4.12: Effect of platinization on electrode performance. (a) There is ~2 times improvement in H_2O_2 sensitivity. (b) There is ~2 times increase in H_2O_2 LOD.

As can be seen in Figure 4.13, the amperometric response for H_2O_2 of platinized electrode is ~ 2 times more than that of unplatinized electrode. This clearly indicates that the platinized electrodes respond better than unplatinized electrode. This is because of increase in reactive surface area and also due to increased catalytic property of platinized surface to oxidize H_2O_2.

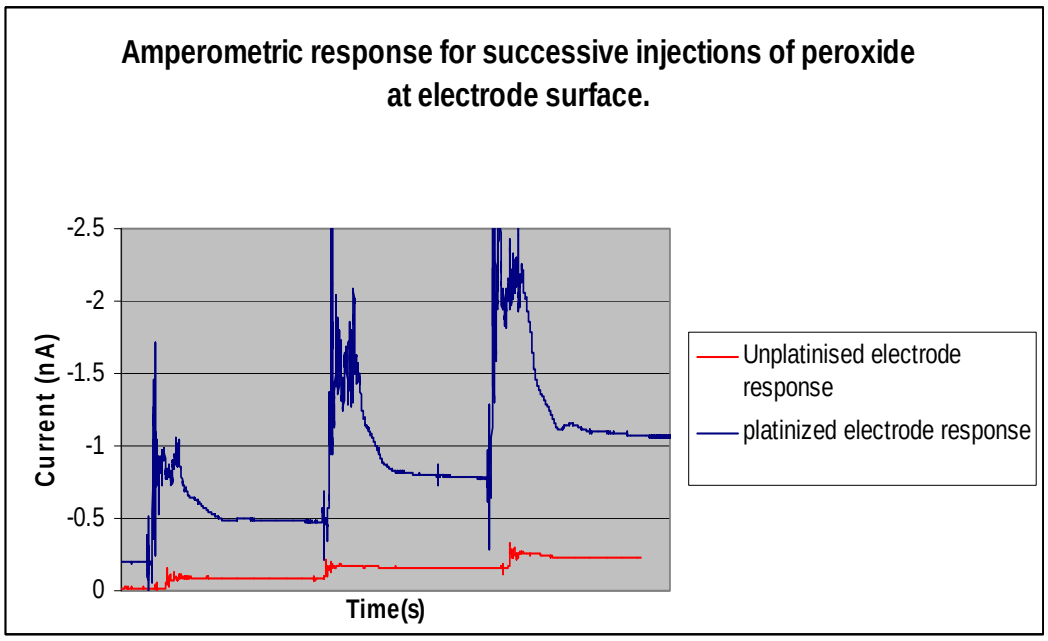


Figure 4.13: Amperometric response for successive injections of H_2O_2 at electrode surface. Applied potential was 700mV vs Ag/AgCl reference. Cell volume: 40mL. Injection volume: 40uL of 8.8mM of H_2O_2.

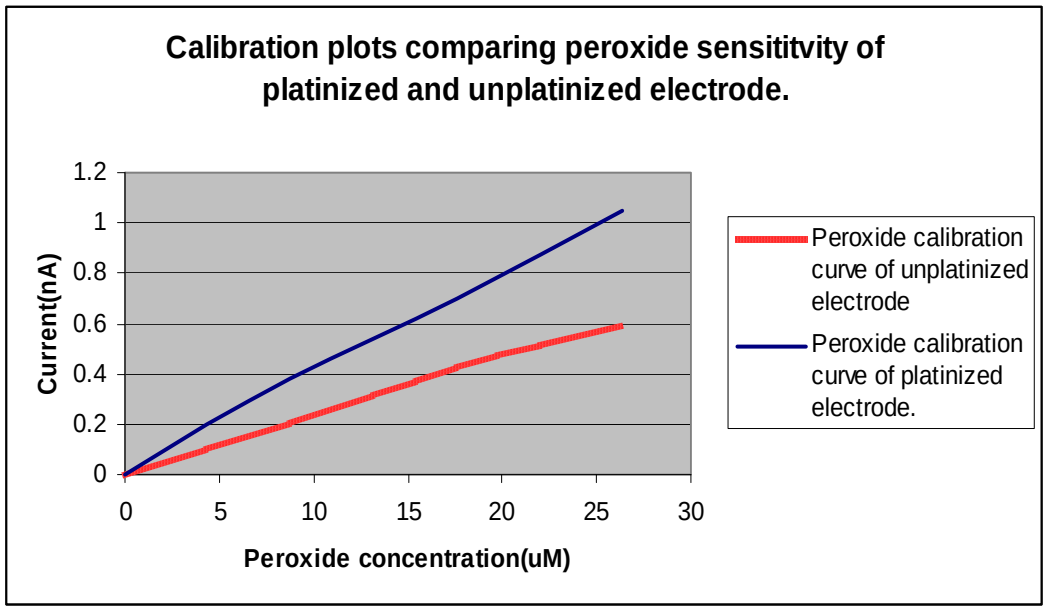


Figure 4.14: Calibration curves for bare platinum electrode and Pt-Black deposited electrode.

It was observed that improvement in peroxide sensitivity was about 5 times when peroxide sensitivity of electrode was initially smaller than average. Average peroxide sensitivity for unmodified electrode is 358.13$\mu AmM^{-1}cm^{-2}$. For example an electrode with small peroxide sensitivity (~159.68$\mu AmM^{-1}cm^{-2}$) benefited more from Pt-black deposition than an electrode that had higher initial peroxide sensitivity (~443$\mu AmM^{-1}cm^{-2}$). This again reiterates the fact that electrodes become diffusion limited rather than reactive surface area limited.

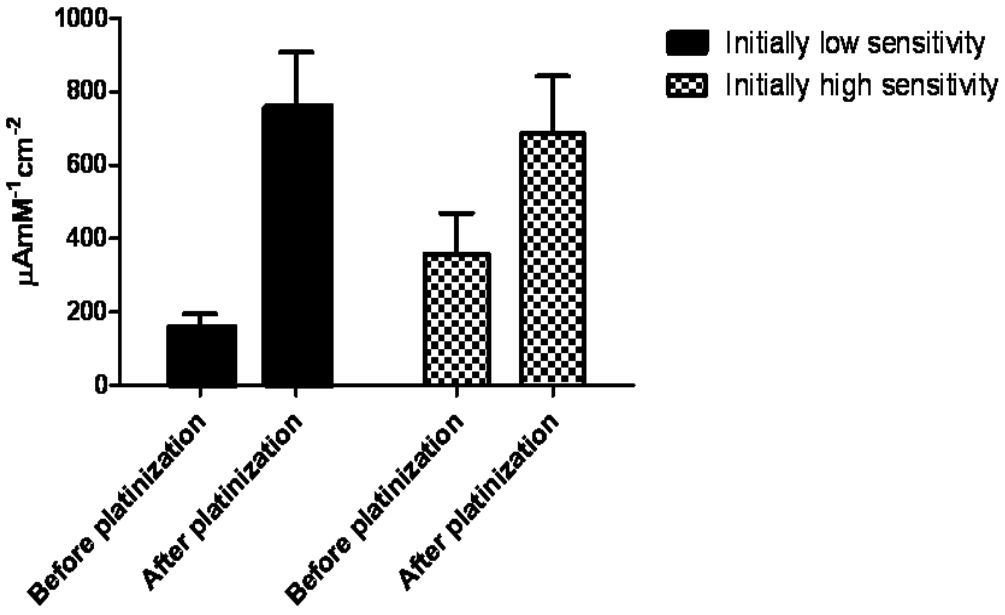


Figure 4.15: Effect of platinization on electrodes with different initial performance.

4.6. EFFECT OF PLATINIZATION ON L-GLUTAMATE RESPONSE OF ELECTRODE

To make MEAs selective for Glu, they were hand-coated with an enzyme solution. As indicated by Figure 4.17 and Figure 4.18 there was ~1.5 times improvement in Glutamate sensitivity. Hand coating of enzymes results in an inhomogeneous coverage which degrades reproducibility. In addition, hand-coating results in thick enzyme layer (~2um) formation on the recording sites. In the case of thick coating, most of the enzymatic reaction occurs in the outer layers of the enzyme. Because of that H_2O_2 generated by the enzymatic reaction was not completely captured at the Pt-Black surface and all the current corresponding to the H_2O_2 generation was not detected. (Figure 4.15).

Glutamate detection limit of platinized electrode was observed to be 0.171±0.095 μM which is ~10 times more than the detection limit of unplatinized electrode. Limit of detection (LOD) is given as

$$\text{LOD} = \frac{3\text{x baseline standard deviation}}{\text{sensitivity}}$$

We observed increase in LOD because of increase in baseline standard deviation (noise). The 1.5 times improvement in sensitivity does not offset the increase in noise.

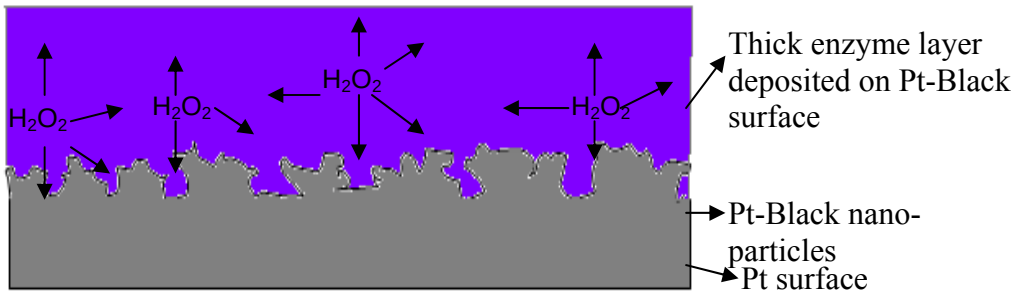


Figure 4.16(a): Schematic diagram depicting thick enzyme layer over Pt-Black surface. All the peroxide produced is not completely captured by the rough electrode surface

In future, electropolymerization of enzyme should be considered. It will produce thin enzyme layer over the platinized recording sites. Thin layer can easily cover all the contours of the Pt nanostructures and also allows for immobilizing larger amounts of enzyme than electropolymerization on bare Pt recording site. In addition thin film deposition of enzyme helps in decreasing the reaction time of the electrode to the detected glutamate. (Figure 4.16). However, the total enzyme loading may still be less for electrodeposited layer than for hand-coated layer.

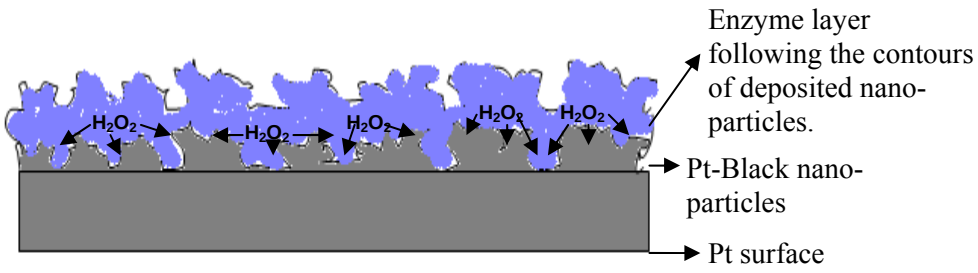


Figure 4.16(b): Schematic diagram of thin enzyme layer covering the contours of rough Pt-Black surface. Most of the peroxide produced by L-glutamate decomposition is captured by rough electrode surface.

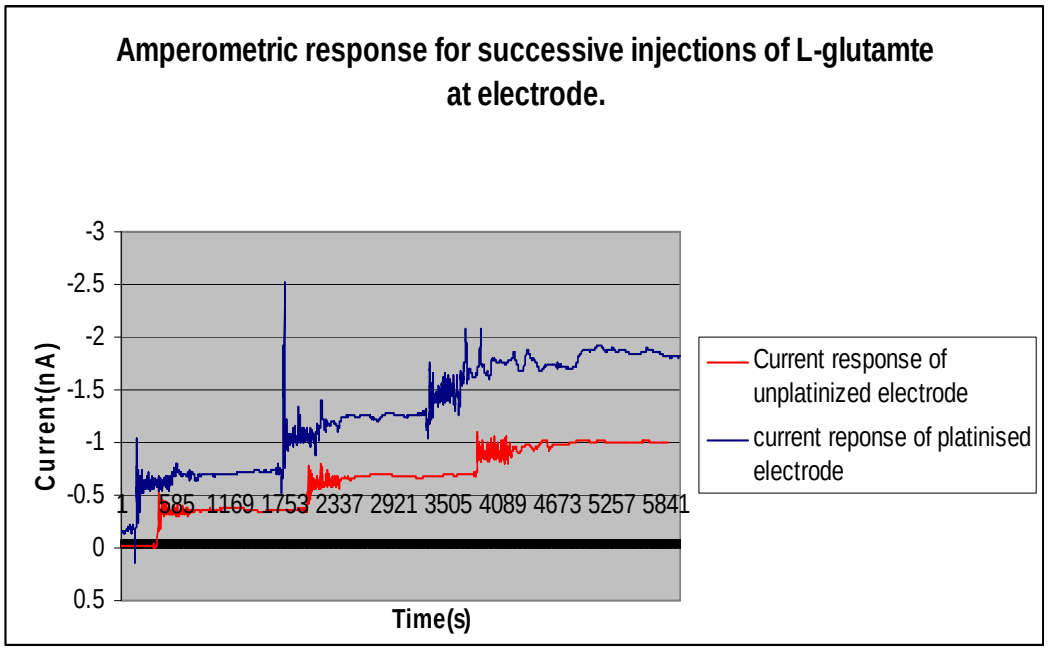


Figure 4.17: Amperometric response for successive injections of glutamate at electrode surface. Applied potential was 700 mV vs Ag/AgCl reference. Cell volume: 40 mL. Injection volume 40 µL of 20 mM of H_2O_2.

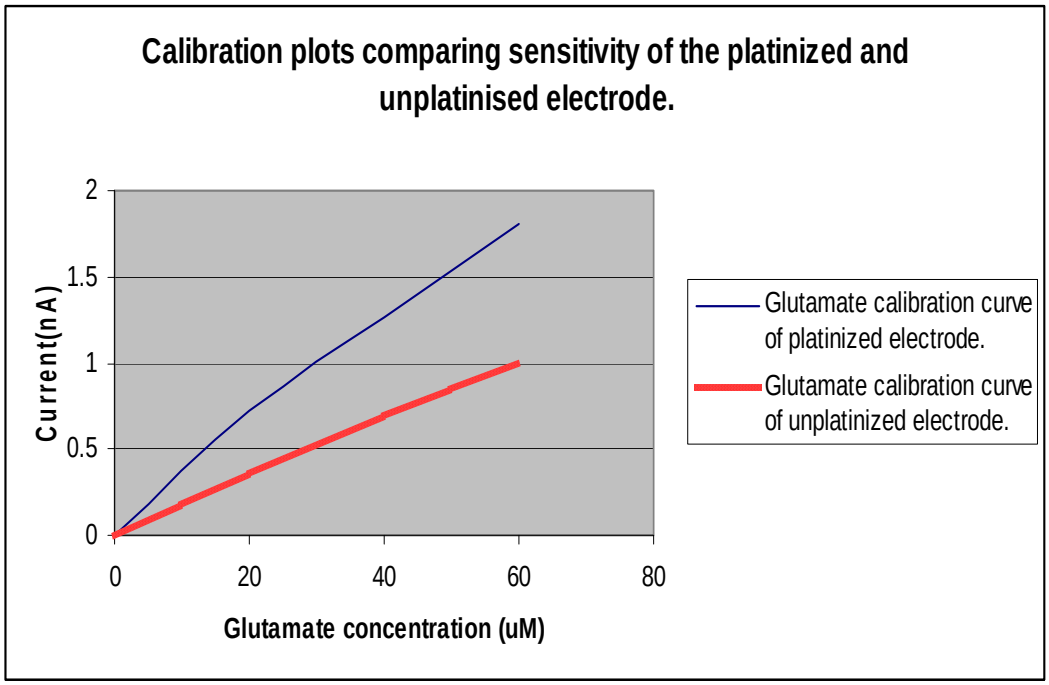
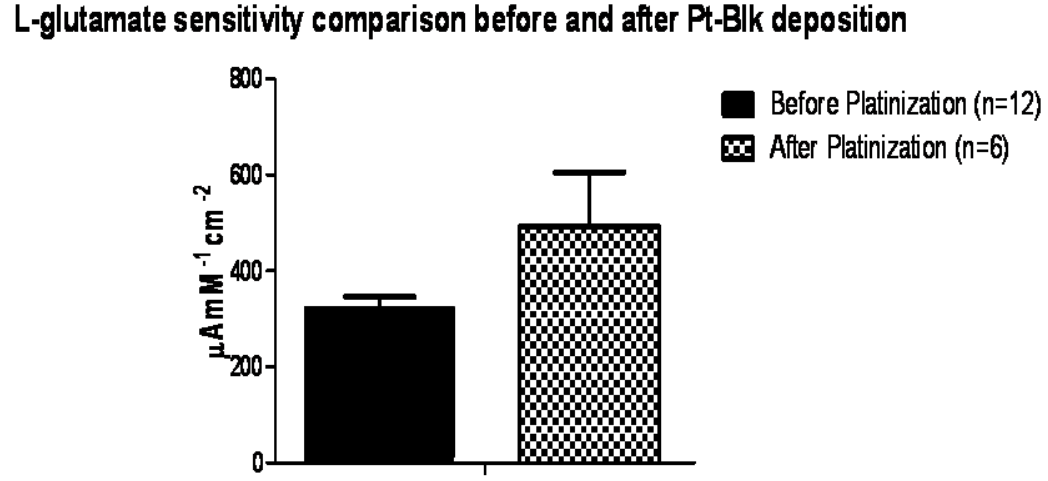
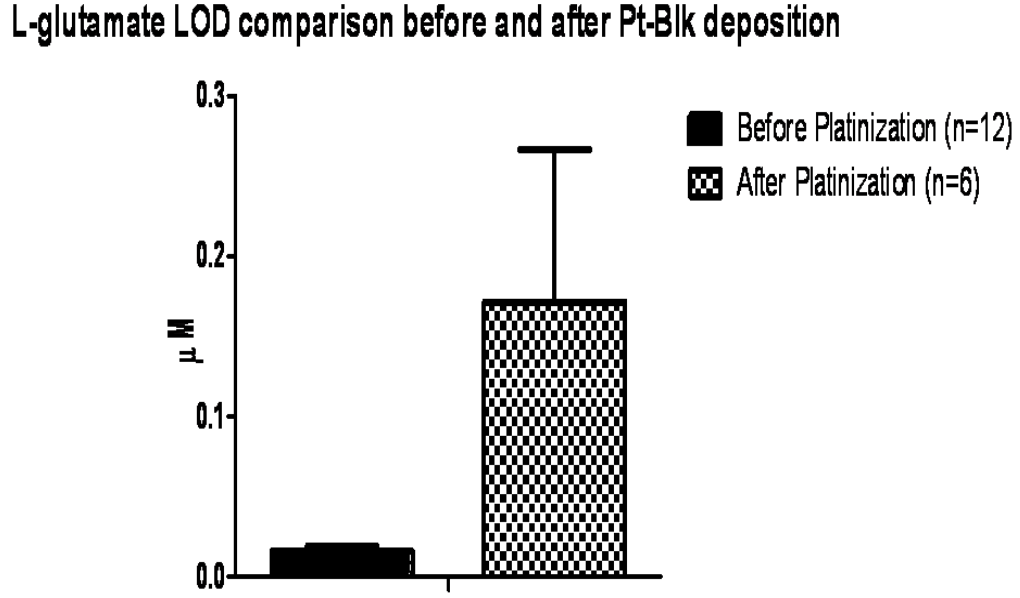


Figure 4.18: Calibration curves for bare platinum electrode and Pt-Black deposited electrode.

Sensitivity is given as change in current with change in analyte concentration. It is obtained from the slope of the plot between analyte concentration and current detected. As seen in Figure 4.18, the slope of the plot of platinized electrode is more than that of unplatinized electrode, which again indicates that the sensitivity of platinized electrode is more than that of unplatinized electrode.



(a)



(b)

Figure 4.19: Effect of platinization on electrode performance. (a) There is ~ 1.5 times improvement in Glutamate sensitivity. (b) There is ~10 times increase in Glutamate LOD.

CHAPTER 5

CONCLUSION

The micro-biosensor described here displayed an attractive combination of glutamate sensitivity of 492±112.69 $\mu AmM^{-1}cm^{-2}$ and glutamate detection limit of 0.171±0.095 μM, simplicity of design, and ease of fabrication. The microelectrode arrays also demonstrated mechanical stability when proper deposition conditions were used. Deposition of Pt nano-particles on recording sites enhanced the sensitivity to H_2O_2 by 2 times and the sensitivity to glutamate by 1.5 times. Given the thick enzyme matrix used here, the enhanced sensitivity is likely due an increase in active sites for H_2O_2 oxidation. The technique proved particularly useful for the electrodes that had poor initial peroxide sensitivity (~159.68 $\mu AmM^{-1}cm^{-2}$). For such electrodes, a ~4.7 times increase in peroxide sensitivity was observed. Nevertheless, the improvement in the performance of electrodes with high starting peroxide sensitivity (443.62 $\mu AmM^{-1}cm^{-2}$) was less significant.

Despite these improvements, the limit of detection (LOD) of glutamate micro-biosensor was determined to be 0.171±0.095 μM, which is ~ 10 times more than the LOD of unmodified electrodes(0.015±0.003 μM). This is due to the increase in background noise with increase in surface roughness. The origin for background noise and methods to reduce it need to be explored in future work.

FUTURE WORK

Electrodeposition of enzymes on platinized electrodes may be used in future work to improve the H_2O_2 capture by the increased surface area. Focus should be on how to reduce the background noise, with the increase in surface roughness. Due to variability in manufacturing of unmodified electrodes, sensor-to-sensor reproducibility was rather poor and needs to be addressed. By creating 3-dimentional pattern on electrode recording surface one can enhance active surface area and microelectrode performance.

REFERENCES

[1] A.Legat, Materials and Corrosion 2004, 55, No.10 'Effect of stirring on Electrochemical noise'.

[2] B.K.Day, G.A.Gerhardt et all, Journal of Neurochemistry 2006, 96, 1626-1635 'Microelectrode array studies of basal and potassium-evoke release of L-glutamate in the anesthetized rat brain'.

[3] John P.Bruno, Martin Sarter, Clelland Gash, Vinay Parikh, Encyclopedia of Sensors, 2006, Volume X, 'Choline- and Acetylcholine-Sensitive Microelectrodes'.

[4] J.I.Reyes, J.R.Powers et all, Journal of electroanalytical chemistry 575 (2005) 'Improved Platinization conditions produce a 60-fold increase in sensitivity of amperometric biosensors using glucose oxidase immobilisef in poly-o-phenylenediamine'.

[5] H.Olivia, B.V.Sarada, et.all, Electrochinica Acta 49(2004) 'Continuous glucose monitoring using enzyme-immobilized platinized diamond microfiber electrodes'.

[6] Joanne M.Elliott, George S.Attard et.all Chem.Mater 1999,11 'Nanostructured Platinum films: Effects of electrodeposition conditions on film properties'.

[7] Chi S.Kim, Seung M. Oh, Electrochimica Acta, Vol 41, No 15, 'Enzyme sensors prepared by electrodeposition on platinized platinum electrodes'.

[8] Jianjun Wang, Nosang V.Myung, et.al, Journal of Electroanalytical Chemistry 575 (2005),'Glucose oxidase entrapped in polypyrrole on high-surface-area Pt electrodes: a model platform for sensitive electroenzymatic biosensors'.

[9] Nase Hamdi, Jianjun Wang, Eric Walker et.al, Journal of Electroanalytical Chemistry 591(2006), 'An electroenzymatic L-glutamate microbiosensor selective against dopamine'.

[10] http://electrochem.usask.ca/chem322/Notes_2/Electrochem2.pdf

[11] Jason J.Burmeister et al, Journal of Neuroscience Methods 119 (2002) 'Improved ceramic-based multisite microelectrode for rapid measurements of L-glutamate in the CNS'.

[12] Jason J. Burmeister, Karen Moxon, Greg A. Gerhardt, Analytical Chemistry. 2000, 72, 187 -192.

[13] Md.Aminur Rahman, Nak-Hyun Kwon, Mi-Sook Won, Eun Sang Choe and Yoon-Bo Shim, Analytical Chemistry.2005, 77, 4854-4880.

[14] Joanne M. Elliott, George S. Attard, Philip N. Bartlett, Nicolas R. B. Coleman, Daniel A.S.Merckel, John R.Owen, Chem.Mater.1999, 11, 3602-3609.

VITA

The author Silpa Nagari, was born on January 20, 1983 in Andra Pradesh, India. In May 2005, she obtained her Bachelor of Engineering degree in Electronics and Communications Engineering from Osmania University, India. She enrolled in University of Kentucky's graduate school in Fall 2005. She worked as Research Assistant during her master's program at Center for Nanoscience and Engineering (CeNSE).