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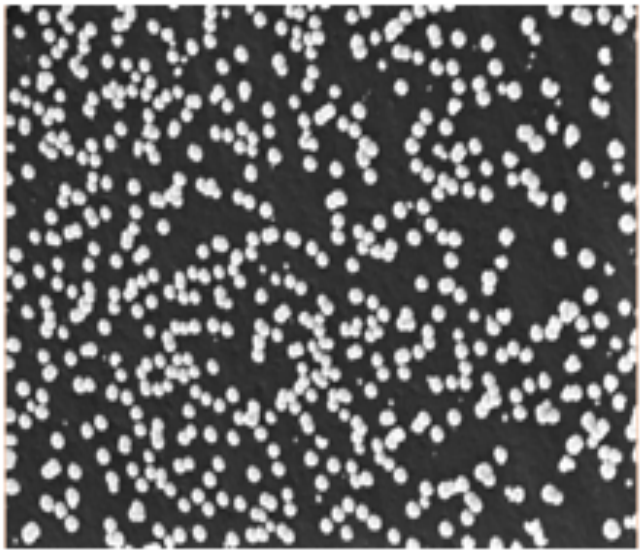

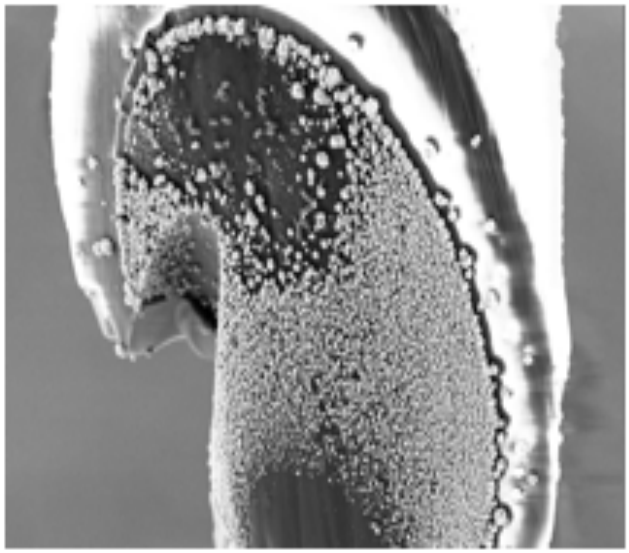

Fabrication of biosensors enabling glucose and glutamate detection

Characterisation and study of enzymatic electrochemical biosensor

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Abstract

Analytical methods are vital when it comes to gaining further understanding regarding compounds and their various functions in biological processes, such as processes in the human body. Electrochemical analytical methods are specific types of analytical methods, that can be employed in the usage of measuring analytes. One such method that utilize electrochemistry is biosensing. This project fabricated and studied how to achieve two types of enzymatic electrochemical biosensors that can be used for the detection of glucose and glutamate, respectively. These sensors consisted of carbon fibre microdisc electrodes, CFMDE, modified with platinum nano-particles, PtNP, and glucose oxidase, GOx, for glucose sensing and glutamate oxidase, GluOX, for glutamate sensing. In the process of obtaining these sensors, deposition parameters of PtNPs were first studied. Two different deposition methods were chosen to conduct the depositions, cyclic voltammetry, CV, and potential step. For both methods, the deposition time and the concentration of Pt were studied. The results showed that CV is more suitable than potential step to achieve PtNPs that are evenly distributed on the carbon surface. However, in terms of the diameter of the PtNPs, desired results were not achieved. The sensitivity of H_2O_2 for CFMDEs with CV deposited Pt was investigated by studying the change in current for different H_2O_2 concentrations. The measurements showed that a lower Pt concentration gave a higher sensitivity for H_2O_2. To show an indication that H_2O_2 is detected on the PtNP surface and not on the carbon surface, a comparing measurement was made between a Pt deposited CFMDE and a non-deposited CFMDE, both tested in 10 mM H_2O_2. CV was used in both measurements. This comparison indicated that H_2O_2 is detected on the PtNP surface. The final step of the fabrication process was immobilisation of enzymes onto the PtNPs. After immobilisation, sensitivity measurements of the analytes were done. These measurements showed poor reliability and reproducibility. However, commonly for both of them showed that the sensitivity decreased after a rather high concentration. Lastly some proposals and discussion for future work, to achieve maximal sensitivity, are presented.

Abbreviations

AuNP	Gold nano-particle
GABA	Gamma-aminobutyric acid
CFMDE	Carbon fibre microdisc electrode
CV	Cyclic voltammetry
Echem	Electrochemical
FeMeOH	Ferrocenemethanol
GluOx	Glutamate oxidase
GOx	Glucose oxidase
K_2PtCl_4	Potassiumplatinumchloride
NHE	Normal hydrogen electrode
NP	Nano-particle
PBS	Phosphate buffered saline
Pt	Platinum
PtNP	Platinum nano-particle
SEM	Scanning electron microscopy
TCA cycle	The citric acid cycle

Contents

1 Introduction

The ability and desire to understand the human body has always been of great interest to humanity. One prerequisite to obtain understanding for various processes in the body is to understand what functions different chemical substances have [1]. For example, what function does glutamate, a neurotransmitter [2], have in neuronal communication?

To understand these functions in detail there must be analytical methods that can monitor the compounds involved in different processes [3]. Analytical methods utilize different properties of an analyte, e.g. electroactivity. Biosensors can be used in order to detect and monitor different compounds, depending on their electroactivity [4].

The field of biosensors has grown since the first sensor was developed in the early 1960s [5] and it is still expanding. In 2004 the market value for biosensors was 5 billion dollars. The estimated market value in 2020 is set to 22.7 billion dollars [6]. A biosensor can be defined as a sensor that uses a specific type of biological component that reacts with an analyte which produces a measurable signal. This signal, e.g. a change in current is correlated to the analyte concentration [7].

There are many ways and strategies for obtaining a well-functioning biosensor. A big field area of biosensors is enzymatic biosensors where continuous development occurs in the purpose of obtaining well-functioning sensors. Enzymatic biosensors use enzymes to achieve specificity, since enzymes can distinguish between various substrates. Other benefits of biosensors, enzymatic biosensors included, are high spatial and temporal resolution [7].

There are several applications where enzymatic biosensors can be used. Two analytes that enzymatic biosensors can detect if proper enzymes are used include glucose and glutamate. The glucose level in blood is important to monitor for people with diabetes which is a widespread disease in developed countries [6]. Besides the blood, glucose is also found in the brain since it is an energy source for the brain. Glutamate is as stated above a neurotransmitter and is involved in among other functions the memory [8]. The ability to monitor this kind of compounds is vital and therefore is the development of biosensors that can detect glucose and glutamate of great interest.

1.1 Aim

The aim of this project is divided into two parts, with the overall purpose to fabricate enzymatic electrochemical biosensors that consist of carbon fibre microdisc electrodes, CFMDEs, modified with platinum nano-particles, PtNPs, and enzymes. The first part of the project studies what deposition condition can provide a desirable diameter and surface coverage of deposited PtNPs on the CFMDE surface. This is done in the purpose of conducting a future comparison between the properties of PtNPs and gold nano-particles, AuNPs. The second part fabricates and studies two types of enzymatic electrochemical biosensors that detect glucose and glutamtate, respectively.

2 Theory

2.1 Electrochemistry

A fundamental concept, which is used in electrochemical techniques, is that charges can be transferred from one species to another. Chemical species such as ions or molecules can undergo charge transfer reactions. These reactions are called redox reactions. When a redox reaction occurs, charge is being transferred between two species involved in the reaction. Redox reactions consist of an oxidation reaction and a reduction reaction, where the specie that is oxidized is donating electrons and therefore have its oxidation number increased. The specie that is reduced will have a decrease in oxidation number, meaning that the specie will gain electrons [9]. The half reaction for a reversible redox reaction is shown in equation 1, where ne is the amount of electrons [10].

$$Red <-> Ox + n\mathrm{e}^- \tag{1}$$

In different materials, such as wires made of different metals, the amount of charge that passes through a point in the wire is called electric current, I, and is measured in amperes, A. A prerequisite for the flow of charges, electrons, is that there is a closed circuit. Another prerequisite for an electric current is that there is a potential difference between for example the ends of the wire [11]. In terms of liquids, charge is being transferred through ions. Positive ions flow in one direction and negative ions flow in the opposite direction. A liquid can evidently be a part of a closed circuit so that the flow of charges is possible [12].

The potential difference or the work that is needed to move charge from one point to another is called the potential, E, and is measured in volts, V. Transformation of electric energy into heat of a circuit is called the resistance, R. The direction of the resistance is opposite relative to the current´s direction and is measured in ohms, Ω. The relation between current, potential and resistance is described by Ohm´s law [11], see equation 2.

$$I = \frac{E}{R} \tag{2}$$

A difference in potential entails that electrons move in one direction for a redox reaction. They move from a higher potential energy to a lower potential energy. A simile is that water in a waterfall flows in one direction. The potential difference is the driving force for the reaction and is called the electromotive force [13].

In terms of thermodynamics, the driving force or the probability for a redox reaction is formulated

by Nernst equation, see equation 3. Nernst equation describes that the equilibrium potential, E, of an electrode is dependent of the temperature, T, the gas constant, R, Faraday´s constant, F, and the number of electrons in the reaction, n. It also depends on the ratio of the activities between the oxidized and reduced species. However, since measurements of diluted solutions often are conducted, C_{ox} and C_{red} in equation 3 stands for the concentration of Ox and Red respectively at the electrode surface [14].

$$E = \mathrm{E}^0 + \frac{RT}{nF} ln \frac{\mathrm{C_{Ox}}}{\mathrm{C_{Red}}} \tag{3}$$

In the context of analytical methods, fundamental electrochemistry is used to study the electron transfer process between an analyte and an electrode surface. The setup for electroanalytical methods consists of two or three electrodes immersed into an electrolyte. Whether two or three electrodes are needed is a question of what kind of electrode is used as the working electrode. At the working electrode, the desired reaction occurs. A working electrode and a reference electrode are always used and in some cases a counter electrode is also needed. Why is that?

A reference electrode should be able to have a constant potential so that a desired potential can be applied to the working electrode. A reference electrode is by this property nonpolarisable, which means that its potential does not vary much with flowing current unless there is a big amount of current flowing through it. For microelectrodes, that are in dimensions of tens of micrometer down to nanometers [4], the current magnitude is in pA-nA [15]. This entails that the ohmic drop is small. In other words, the current passing through the reference electrode is not big enough to vary the potential. For macroelectrodes however, sufficient amount of current will pass through the reference electrode, which in turn means that the potential of the reference electrode will not be maintained constant. An ohmic drop will occur. This can be avoided by adding a third electrode, a counter electrode. Current will pass between the working electrode and the counter electrode and negligible current will pass through the reference electrode. A constant reference potential can be obtained at the reference electrode with this setup [4].

There are several different reference electrodes that can be used for electrochemical measurements. The standard potentials of reference electrodes are tabulated and are always given versus the normal hydrogen electrode, NHE, which is set to 0 V. However, this reference electrode is not common to use since it is hard to achieve a well-functioning NHE [16]. A more common reference electrode and also the one used in many of the measurements conducted in this project is the Ag/AgCl electrode (0.197 V vs. NHE). This electrode consists of metallic Ag coated with AgCl which immersed into a solution. The solution has a determined concentration of chloride ions [14].

What is the difference of applying a positive potential to the working electrode compared to

applying a negative one? Let´s say that a positive potential is applied (relative to the normal potential of the analyte) to a working electrode immersed into a solution. Ions in the solution will donate electrons to the electrode when they are in close contact with it. An oxidation of the ions will happen, as seen in 1. By applying a negative potential, also compared to the normal potential of the analyte, the opposite will occur. The ions in the solution will receive electrons from the electrode and undergo a reduction [14].

There are several electrochemical methods, for example potentiometry and voltammetry. In potentiometry the difference in potential is studied while in voltammetry the change in current is studied. In this project, only voltammetric methods were used and these will therefore be the only ones taken into account in the theory section [17].

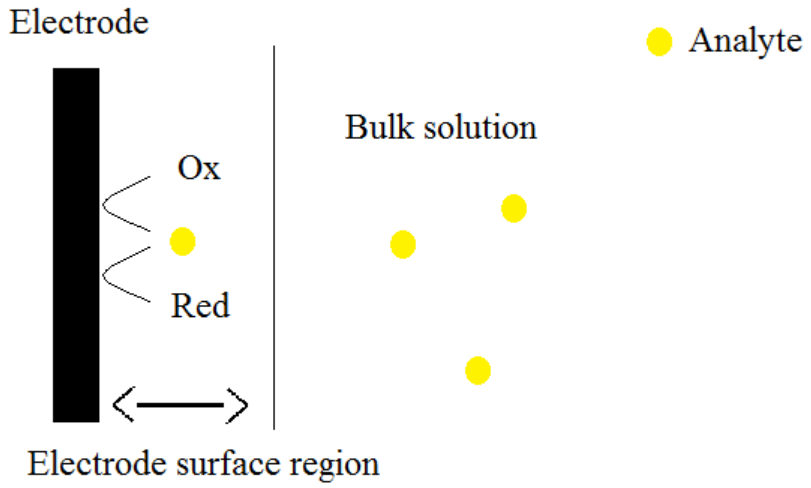


Figure 1: *Two different regions are shown, the bulk solution and the electrode surface area. When an analyte is in the electrode surface region, it can undergo a redox reaction with the electrode and get either oxidized or reduced.*

In figure 1, two regions are shown, the bulk solution and the electron surface region. The bulk solution represents the whole solution where the electrode is immersed, and where the analyte is not affected by the potential applied to the electrode. In order to achieve an electron transfer process between the analyte and the electrode, the analyte must be in the electrode surface region where a potential gradient exists. Thus, the analyte must reach the eletrode surface region via physical transfer. Physical transfer is affected by two factors, mass transfer and the electron transfer rate at the surface region [14].

Mass transfer is the movement of material from one location in a solution to another and can be branched into three categories. The first one is *migration* which means the movement of a charged material due to an electrical field. The second one, *diffusion*, includes movement affected by a

gradient of chemical potential. The third and last one is *convection* and occurs due to stirring or hydrodynamic transport. One such factor that can initiate convection is density gradients, called *natural convection*. Laminar and turbulent flow are two other factors contributing to convection, more specific that kind of convection is called *forced convection* [18]. Once the mass transfer has brought the analytes to the electrode surface region, electron transfer can occur.

2.1.1 Voltammetry

Common for all voltammetric methods is that they study the relationship between potential and current for an electrochemical reaction [4]. Thus, a potential is applied to an electrode and the obtained current is studied. Three voltammetric methods that are commonly used are cyclic voltammetry (CV), amperometry and potential step [17].

For CV, the potential is first altered from a starting potential to a determined one. The determined potential can be either more positive or more negative than the starting potential. Potentials are chosen by which analyte is studied. The current response obtained by the potential change is studied until it is stabilized. The potential is then changed back to the original potential and the current response is studied in the same way as described above. These two segments together form one CV cycle. The shape of a cyclic voltammogram with one cycle is presented in figure 2. The shape of CV curves is dependent of what kind of electrode is used. Figure 2 shows a CV curve for a microelectrode.

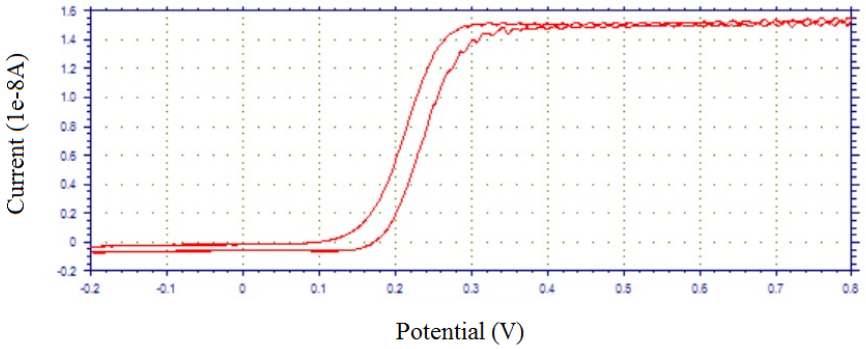


Figure 2: *A CV voltammogram for a microelectrode.*

It can be observed in the same figure that the current is stabilized after 0.4 V and remains stabilized until the curve returns back to the starting potential. This stabilized part of the current is called the limit current. If the area of the electrode is known, the limit current can be determined. The limit current can be calculated by using equation 4, where F is Faraday´s constant, n is number of electrons transferred in the reaction, D is a constant, C is the concentration and r is the radius of the electrode [18].

$$I = F4nDCr \tag{4}$$

As described in the section above a cycle in a CV measurement consists of two segments or curves. For the first curve, if the potential is chosen to be more positive, an oxidation of the analyte will occur. When the curve returns to the original potential, a reduction will occur [14].

Amperometry keeps a selected and suitable potential constant with respect to a reference electrode and measures the change in current with respect to time. The changes in current equals change in concentration at the electrode surface [14]. A redox reaction will occur and provide a signal and the signal will be proportional to the analyte concentration. Thus, amperomerty is a preferable method in order to determine unknown concentrations of an analyte. By using Faraday's Law, equation 5, the amount of analyte adsorbed expressed in moles can be calculated, where Q is the electric charge integrated from the peak, n is the amount electrons transferred per reaction, N is the amount of moles analyte reacted and F is the Faraday's constant [10].

$$Q = nFN \tag{5}$$

The potential step method is similar to amperometry. A chosen potential is held constant for an appropriate time and the current-time behaviour is observed when a sudden change in potential is set. The initial potential is chosen so that no redox reaction takes place. The method can be applied for different purposes. Figure 3 shows a potential step voltammogram for a microelectrode where the potential is held constant for 10 seconds and is then set down to -0.6. From this potential, a current response is shown. Eventually, a steady state level is reached. This is due to that the reaction is driven towards the reduced or oxidative species. This indicates a difference between the ox/red ratio at the electrode surface which means that the current is governed by mass transport caused by diffusion [10].

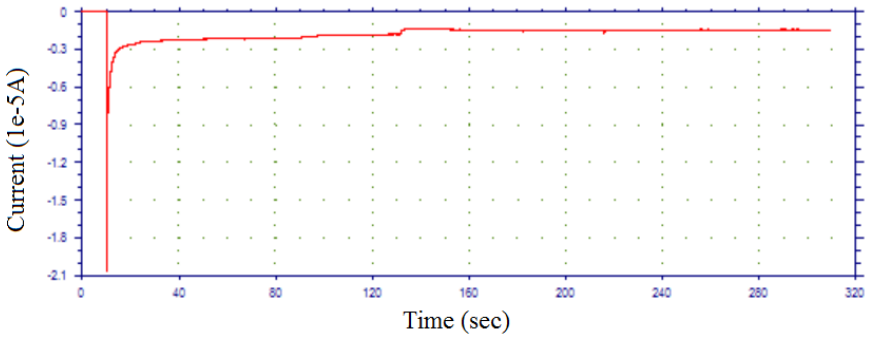


Figure 3: *A typical voltammogram for potential step.*

2.2 Neuroscience

Together, the 100 billion neurons in our brains creates one of the most complex machines known. The connections between the neurons make up complex networks that make them able to communicate in a well structured manner. Unlike other cells in the body, neurons are polarized and have a distinct morphological region, each with its own function. The neuron can roughly be divided into four parts, as can be seen in figure 4. At one end there are dendrites where connections from other neurons are received. The cell body contains all organelles important for the cellular function. In the axon, information is transmitted from one region in the neuron to the axon terminals, also called the pre-synaptic terminal. Information is transferred through the axon and is released in the synapse to an adjacent dendrite, also referred to as the post-synaptic terminal. This information transferred are expressed with chemical messengers, also known as neurotransmitters. Between the pro and pre-synaptic terminals there is a gap junction, called the synaptic cleft, where the communication between two neurons occurs and the neurotransmitters are received by receptors. The mechanism that appears when neurotransmitters are released is called exocytosis and occurs in sub-milliseconds. To better understand the neuronal activity inside the brain, electrochemical techniques can be used to measure the amount of neurotransmitters released. Thus, a further investigation for understanding of neuronal diseases can be performed [19].

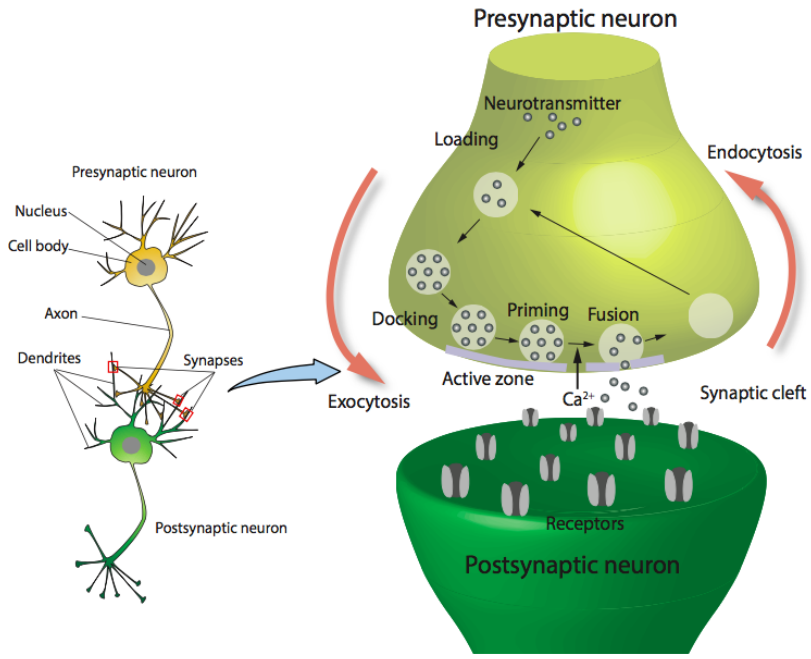


Figure 4: *A schematic picture of neuronal communication [8].*

As stated earlier, the neurons are polarized which indicates that a potential can be measured inside them. When holding a microelectrode connected to a recording device outside the cell body in the extracellular medium the potential will be zero. This because the extracellular medium is isopotential. If however the microelectrode penetrates the cell and is placed inside the membrane, a potential at -0.6 mV versus the outside will be recorded. This is called the resting potential and is constant if no stimulation occurs [19]. When the neuron is stimulated, an action potential can be recorded. When the action potential reaches the axon terminal a depolarization will be triggered, this mechanism is called exocytosis. Inside the neuron, there are vesicles containing neurotransmitters. Each vesicle contains a concentrated amount of some kind of neurotransmitter and when the action potential arrives, voltage-gated ion channels will open and let Ca^{2+} in which will increase the potential in the intracellular matrix. This depolarization forces the vesicles to dock to the presynaptic membrane and release neurotransmitters to the synaptic cleft. In the synaptic cleft they diffuse and bind to receptors at the postsynaptic membrane. The difference between the resting potential and action potential depends on the concentrations of potassium, sodium, calcium, and chloride ions inside and outside the cell. These concentrations are regulated between the membrane by ion pumps or ion channels whereas the ions are transported against their concentrations gradient [20].

There are approximately 100 neurotransmitters that have been identified today. Some of them have been well studied and they play a major role for our general functioning. Examples of these neurotransmitters include acetylcholine, dopamine, glutamate and gamma-aminobutyric acid, GABA. Using elelctrochemical techniques, neurotransmitters can be easily detected [8].

2.3 Glucose and glutamate

Glucose is necessary for the mammalian brain as it is the main energy source. In fact, 20 % of the glucose-derived energy produced in the body is consumed by the brain [21]. The energy demanded from glucose comes from the different glucose metabolisms, e.g. the citric acid cycle, TCA cycle. Besides providing energy (ATP), glucose also provides the generation of precursors for the synthesis of neurotransmitters that plays a major role for learning and long-term memory [22]. The neurontransmitter glutamate can be synthesized from the intermediate (i.e. alpha-ketoglutaric acid) of the TCA cycle [8].

Glutamate is a non-essential amino acid and one of the richest in the diet. It is found in high concentrations everywhere in the body. Besides beeing synthesized from the TCA cycle, glutamate is also synthesized in the glia cells from glutamine by the mitochondrial enzyme Glutaminase. Glutamate is one of those neurotransmitters that plays a critical role in learing and memory. So if

the concentration level of glutamate becomes abnormal, it could indicate that something is wrong with the glucose metabolism. This makes both glucose and glutamate interesting to analyse for further investigation of neurological diseases [8].

2.4 Biosensors

To be able to understand the utility of a biosensor, the difference between an electroactive and a non-electroactive analyte must be explained. Electroactive analytes can undergo redox reactions when they are in contact with an electrode. Once again, whether an oxidation or a reduction of the species occur can be controlled by what potential is applied on the electrode [4]. Non-electroactive analytes on the other hand can not undergo redox reactions under the same conditions as electroactive analytes. Since analysis of non-electroactive molecules, e.g. non-electroactive neurotransmitters, can be conducted with biosensors, these sensors are of great interest [7].

The main components of a biosensor are a transduction element and a sensing element. The sensing element, consisting of specific biomolecules, assists the production of an electroactive reporter molecule from a specific non-electroactive analyte [23]. The chemical signal, the reporter molecule, can be transformed into a measurable signal by the transduction element and then be correlated to the analyte concentration [7].

There are several types of biosensors and they are categorised by what biomolecules the sensing element consists of and what transducer element they use. Examples of biomolecules include enzymes and antibodies. For the first example, the biosensor can be classified as an enzymatic or catalytic biosensor. For the latter example, the biosensor can be classified as an immunosensor [23]. Examples of what transducing elements that can be used include electrochemical, optical and acoustic systems. The electrochemical transducer measures produced or consumed ions or electrons which then provides a measurable current or potential difference [5]. An electrochemical transducer is used in this project. A schematic illustration of a basic biosensor is presented in figure 5.

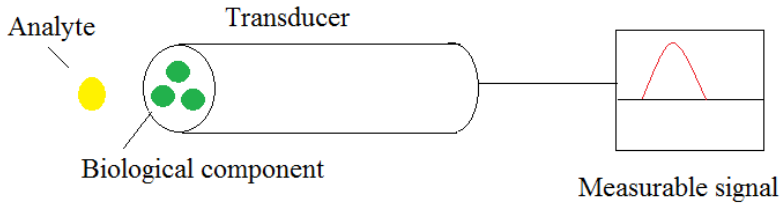


Figure 5: *A schematic illustration of a biosensor with its biological components and its transducer element. The reaction between an analyte and the biological component results in a measurable signal.*

Leland Clark is said to be the inventor of the biosensor, and created a biosensor in the early 1960s. He performed experiments where glucose oxidase, GOx, was entrapped between a Clark oxygen electrode, a negatively polarised platinum wire, and a semipermeable membrane. Glucose, the analyte, is non-electroactive and the consumption of it is related to the oxygen concentration. This biosensor detected the concentration of oxygen by a reduction of oxygen to water [5] [24].

Enzymatic electrochemical biosensors are common to use in brain research and consist of an electrode, enzymes as the sensing element and an electrochemical transducer [7]. For biological applications, the electrode is usually a carbon fibre microelectrode [14]. It is common to use carbon fibre that is in the range of 7-30 µm in diameter [25]. The carbon fibre is enclosed in a glass capillary and together they form an electrode. When the enzymes are deposited, the electrode becomes a biosensor. The biosensor has a tip on one side, which is where analyte encounters the sensors surface. On the other side of the biosensor is a pin, which is connected to a potentiostat. A potentiostat can maintain the potential constant between the working electrode (the biosensor) and the reference electrode [7].

Nano-particles, NPs, are deposited electrochemically onto the electrode surface to enhance the biosensor design. The NPs are a part of the transducer element and can catalyze the reduction or oxidation of the reporter molecule [26]. NPs also provide two major things for the enzyme immobilisation. Firstly, they provide a surface that the enzymes can be attached to, and secondly the curved surface that the particles produce can often keep the tertiary structure of the enzymes intact [5]. Examples of NPs that are suited for biosensors include AuNPs, and PtNPs [2], [6].

Enzymes are immobilised onto the NPs since they should be close to the electrode. When the enzymes are immobilised onto the NPs, the electrode becomes a biosensor. The function of the enzymes is to catalyze the reactions needed to transform the the analyte into the electroactive reporter molecule. Enzymes can be immobilised onto the NPs in multiple ways. Examples of immobilisation methods include entrapment of the enzymes under a semi permeable membrane

or entrapment within a polymer matrix. Another method is direct physical adsorption and is the method used in this project [10]. The surface of an enzymatic biosensor with its different layers is presented in figure 6.

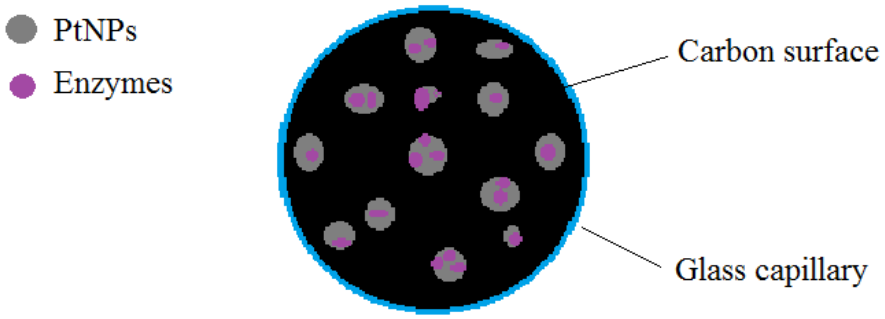


Figure 6: *A carbon surface of a biosensor modified with PtNPs and enzymes.*

As stated in the aim, the sensors that are studied in the project are glutamate and glucose sensors. The catalytic reaction of the analyte produces H_2O_2 by the enzymes GluOx and GOx, respectively, are shown in figure 7a and figure 7b.

(a) **(b)**

Figure 7: *Reaction formulas for a) the production of H_2O_2 from glutamate and for b) the production of H_2O_2 from glucose.*

Since the catalytic reaction of the analyte is very specific to a particular enzyme the availability of matching enzyme for a biosensor is very limited. For some analytes, there is only one enzyme needed to obtain an electroactive product, e.g. GOx for glucose. For acetylcholine, both acetylcholine esterase and choline oxidase are needed to achieve this product [7].

2.4.1 Deposition of NPs

In this project, CFMDEs have been fabricated using platinum, Pt, as the NPs. Therefore, the PtNPs are the NPs described and referred to in further sections. As mentioned recently in section 2.4, Pt is utilized to catalyze the reduction of hydrogen peroxide produced from the enzyme reaction. Pt is widely used due to its high activity when it comes to catalyzing electrochemical reactions, both in basic and acidic solutions [26].

Pt depostion onto a carbon surface can be performed using the impregnation-reduction method, where reduction is effected chemically or electrochemically, also called electrodeposition. Hereafter, electrodepostion is the method described and referred to. Electrodeposition has advantges when it comes to selectively orient particles onto the carbon surface. Though after evolution of a particle, further deposition continues on the same particle. Thus, a balance between time and potential, to achieve good size and even distribution, might be problematic to find [27]. The mechanism that occurs during electrodeposition is a redox reaction between the working electrode and the Pt ions. Pt that is used for deposition is usually dissolved in a salt solution and is reduced from Pt^{2+} or Pt^{4+} to Pt^{0}. Pt is reduced and deposited onto the carbon surface when a negative potential is applied to it. The rate of the deposition depends on the potential at the working electrode. The electrodeposition can be performed using both potential step and CV. A voltammogram using CV deposition is shown below in figure 8 where five cycles are used. Potential step deposition is shown in figure 3 in section 2.1.1.

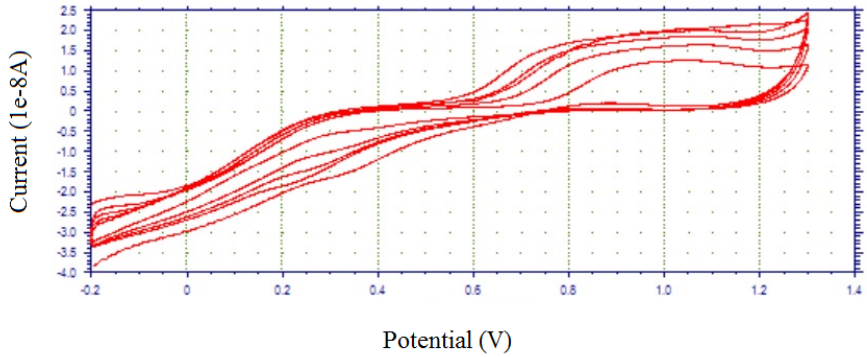


Figure 8: *Deposition curves showing CV deposition of Pt for 5 cycles.*

To ensure that a good quality of the electrode is provided after deposition, the active surface of Pt can be calculated. This surface can be determined using different methods such as electrochemical measurements, Echem, or visually by imaging and analyzing with a scanning electron microscopy, SEM [28] [29]. For this project, CV is used as the Echem measurement together with an acidic electrolyte. The measurement provides a voltammogram as the shown in 8. In SEM-analysis the electrode surface is imaged and characterized using a SEM instrument. The theory behind this instrument will be described in section 2.6.

When a CFMDE deposited with Pt immersed in an acid solution is reduced, hydrogen from the solution are adsorbed onto the surface and when Pt is oxidized the hydrogen is desorbed. By measuring the number of hydrogen adsorbed or desorb the number of adsorptions sites are stated. The desorption of hydrogen is proportional to the release of electrons, which can be seen in the reaction formula 6. Thus, the active surface can be determined [29]. The basic principle is that the

charge under the peaks for hydrogen adsorption or desorption in a voltammogram are assumed to correlate to one hydrogen atom adsorbed onto one platinum atom. By integrating the area under the curve, measured in Coulumb, the total amount of charge can be obtained [30]. The real area can then be calculated by dividing the charge with a conversion factor, Qm, see equation 7. The conversion factor is associated with a monolayer of the adsorbed compound of interest, for hydrogen the value is 2.1 $^{-12}$ μC/μm$_2$ [31].

$$\mathrm{H_{Ad}} - - > \mathrm{H^+} + \mathrm{e^-} \tag{6}$$

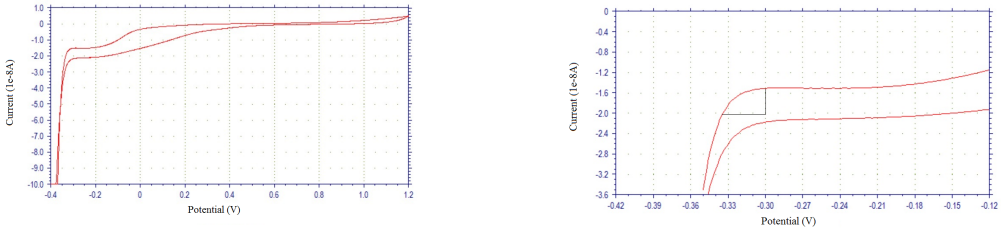
$$A_{pt} = Q_a/Q_m \tag{7}$$

Pt's active area is then determined by the ratio between $\mathrm{A_{pt}}$ and the area of the carbon surface, $\mathrm{A_{carbon}}$. Usually, the carbon surface is beveled down to a certain angle. Then $\mathrm{A_{carbon}}$ has to be calculated as an ellipse, using equation 8 below where r is the radius and x is the angle. The active surface of Pt can be calculated using 9.

$$A_{carbon} = r * (r/sinx) * \pi \tag{8}$$

$$Pt_{activesurface} = (A_{Pttotal}/A_{carbon}) * 100 \tag{9}$$

To perform this method, some assumptions have to be taken into account. First of all, it assumes that there is a precise relation between the adsorbed hydrogen and the charge. Thus, the adsorption has only appeared on the metal surface [30]. In addition to that the integration step it self can be a bit uncertain since where the hydrogen adsorption evolution initiates is sometimes hard to determine [31]. Also, no alteration at the surface is assumed to take place and the method´s reliability depends on the cleanliness of the electrode surface and also the solution. Cleanliness of the solution can be achieved by for example apply a $\mathrm{N_2}$ flow over if to avoid air exchanges with the environment, in this case oxygen [32]. However, the method has an inaccuracy about +-10% which is according to Trasatti and Petrii. In figure 9a, a CV curve for an active surface measurement is presented and in figure 9b the area that is integrated is zoomed in.



(a) *The entire CV curve.*

(b) *A zoomed part showing the hydrogen adsorption area.*

Figure 9: *A CV curve of an active surface measurement in acidic medium of a PtNP modified CFMDE.*

When this type of measurment is conducted, examples of reference electrodes that can be used include the Cu/CuSO4 and the Hg/Hg_2Cl_2 electrode. The Ag/AgCl electrode should not be used since there is a possibility that diffusion of Cl^- ions occur. Cl^- can adsorb to the Pt surface and thereby block adsorption sites. If blocking of adsorption sites occurs, an incorrect active surface will be obtained [32].

When calculating the active surface area of Pt from SEM images, the NPs must be recalculated from 2D to 3D. This calculation is conducted with the assumption that the NPs are shaped as hemispheres, other kinds of 3D shapes are not taken into account. The following equations were provided by Bergman and Wang. From the SEM-images the average diameter can be calculated using some kind of software. Using equation 10, the average area of the particle hemispheres is calculate, where d is the diameter of a particle [30].

$$A_{hemisphere} = 2 * \pi * (d/2)^2 \tag{10}$$

Since the average diameter is calculated from a picture taken from a zoomed part of the electrode, the whole coverage must be calculated by determining the total amount of particles on the surface. This is conducted by determining the amount of NPs and area in the SEM image using some software, such as ImageJ. From here on the number of NPs per unit area can be determined. Then the total amount of NPs on the surface can be determined by simply multiply the latter value with the total area of the electrode surface (see equation 11).

$$n_{particles} = (n_{particlesimage}/A_{image}) * A_{carbon} \tag{11}$$

Later, the area of platinum on the carbon surface is simply calculated by multiplying the total number of particles, $n_{particles}$ with A_{sphere} as seen in equation 12.

$$A_{Pttotal} = n_{particles} * A_{sphere} \tag{12}$$

From here on, platinum's active surface is determined using equation 13 presented below.

$$Pt_{activesurface} = (A_{Pttotal}/A_{carbon}) * 100 \tag{13}$$

2.4.2 Sensitivity determination of biosensors

One figure of merit that is important to investigate is the sensitivity of the NPs. The sensitivity describes the sensor´s or the CFMDE´s ability to distinguish between analyte concentrations [4]. The sensitivity of the PtNPs can be obtained by doing electrochemical measurements, e.g CV and multipotential steps, with various concentrations of an electroactive molecule. H_2O_2 is suitable since it is the produced reporter molecule for both glucose and glutamate. Both reduction and oxidation of H_2O_2 using multipotential step can be achieved. Figure 10 shows a multipotential step measurement where H_2O_2 is reduced. In the figure a constant potential is held for a certain time interval. Then the potential is changed and kept for some time. This can be repeated for more than one cycle, as in figure 10 [33].

Depending on which process is desired, oxidation or reduction, the potential must be increased or decreased compared to the starting potential. For the reduction of H_2O_2, the potential is changed to a lower potential. A potential of -0.5 V is suitable for the reduction of H_2O_2. The steady state current should decrease as the H_2O_2 concentration increase. In figure 10, the steady state current can be obtained just before the potential is shifted back to the starting potential. If wanting to oxidase H_2O_2, the potential must be changed to a higher one. The steady state current should increase as the concentration increase [33].

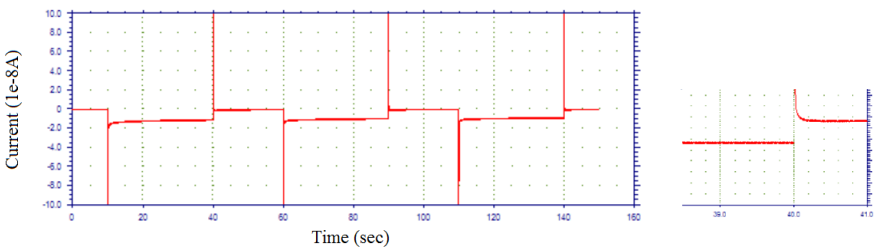


Figure 10: *Multipotential step measurement of a microelectrode in PBS solution. The steady state current can be obtained at for example t= 39 s (see zoomed picture).*

The current density can be calculated by dividing the steady state current of H_2O_2 with the Pt area. In a plot where the current density is plotted against the concentration of H_2O_2, the

sensitivity can be obtained since it is the slope of the plotted values. A steeper slope indicates a higher sensitivity and vice versa [4].

2.5 Electrochemical analyzer

An electrochemical analyzer/workstation is an instrument that can conduct several types of electrochemical measurements, e.g. CV measurements. The analyzer consists of a generator, a direct digital synthesizer, data acquisition circuitry, a potentiostat and sometimes a galvanostat [34]. It is coupled to a Faraday cage whose function is to minimize electrical noise during measurements [35]. In this project, the potentiostat of the electrochemical analyzer is used.

2.6 SEM

SEM is a useful and versatile instrument when characterizing and analysing the microstructure of solid samples. It provides two-dimensional scan images with a high spatial resolution. The sample is kept in a vacuum environment during analysis and the topology of a surface is determined using a focused electron beam at the sample [36].

3 Experimental

The experiments conducted are shown in a schematic illustration below (figure 11) to make it easier to follow the experimental steps and results obtained from them.

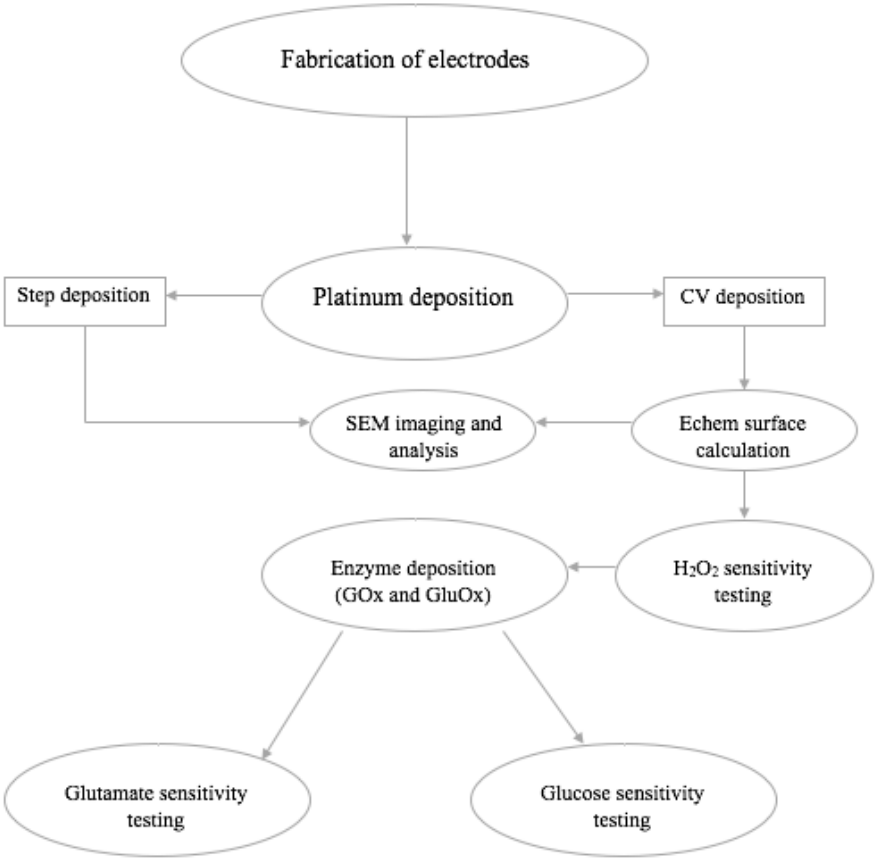


Figure 11: *A schematic illustration of all experimental steps conducted in this project.*

3.1 Instruments and softwares

CHI1030B software (CH Instruments, USA), Electrochemical analyzer (CH Instruments, USA) with a CS-3A Cell stand (a Faraday cage) (ALS Co., Ltd, Japan), ImageJ software, Micropipet puller (Sutter Instruments, USA), Microgrinder (Narishige, Japan), microscope (Leica micrsosystems, Germany), Scanning Electron Microscope (Zeiss, Germany).

3.2 Materials and chemicals

Carbon fibre 33 μm, Carbon stickers, Copper tape, Glass capillaries (borosilicate glass, OD:1.2 mm, ID:0.69mm), scalpel, beakers, Ag/AgCl reference electrode, Cu/CuSO4 reference electrode, Tungsten wire, Silver wire.

Epoxy glue (Epoxy Technology), FeMeOH 1 mM, glucose (Sigma Aldrich), GOx (Sigma Aldrich) glutamate (tillverkare), GluOx 1.7 μM (Sigma Aldrich), H_2O_2 30 % w/w (Fisher Bioreagents), H_2SO_4 95-95 % (Sigma Aldrich),KCl 3 M, K_2PtCl_4 (Sigma Aldrich), conductor paste (DuPont), PBS (Sigma Aldrich).

3.3 Fabrication and testing of CFMDEs

A 33 μm carbon fibre was inserted manually into a glass capillary tube with an inner diameter of 0.69 mm. The Micropipet puller, which has a filament heated to 585°C, was used to pull the capillary into two parts, and as a result two sharp microtips were obtained. The tips were then manually cut with a scalpel in a microscope next to the junction point, where the glass and fiber are fused together. They were then dipped into freshly made epoxy glue (two solutions, A and B, mixed together in the ratio 4:1) for 4 minutes. The CFMDEs were kept in an oven set to 100°C for about 24 hours. The tips were then beveled to an angle of 45° using the microgrinder. To ensure that the surface was flat enough, the limit current of the CFMDEs was tested using a potentiostat coupled to a Faraday cage. This test was done by immersing the CFMDEs one by one in a 1mM FeMeOH solution together with a Ag/AgCl reference electrode in the Faraday cage. Then CV with a low E at 0 V and a high E at 0.8 V (vs. Ag/AgCl), a scan rate of 100 mV/s and one cycle were used to determine if the electrode was flat enough. To make the electrodes conductive for this test, they were filled with 3 M KCl and a silver wire was inserted to create the electrical connection. The tips were also observed in a microscope to ensure that they were flat enough and that they were not broken. If an electrode showed a curve close to the desired one, an electrochemical washing was done. This was managed by set the parameters for CV to a higher scan rate at 100 mV/s and also an increase in high E to 1.2 V (vs. Ag/AgCl) for 10 cycles.

3.4 Pt deposition

A solution of x mM K_2PtCl_4 in a provided 0.5 M H_2SO_4 solution was prepared, where x = 0.25, 0.5, 1 or 2 mM. Before deposition, the electrodes were tested and beveled again and then electrochemically washed between 0-1.2 V with a scan rate of 100 mV/s and one cycle. Two different kinds of deposition methods were used, potential step deposition and CV deposition.

The potential step deposition was implemented for all the concentrations made except 0.25 mM. The potential was held at 1.1 V (vs. Ag/AgCl) for 10 seconds and then down to -0.6 or -0.4 V (vs. Ag/AgCl) at different times varied between 2-360 s. For this deposition method, approximately 20 different conditions were performed. The CV deposition was conducted with 0.5 mM and 0.25 mM respectively and had a potential ranging between -0.2 - 1.3 V (vs. Ag/AgCl) with a scan rate of 100 mV/s and 5 or 3 cycles. The parameters used for the different conditions used in CV deposition are presented in table 1. There were six CFMDEs made in total for every deposition condition. For each condition, three CFMDEs were made for SEM analysis and three were made for Echem measurements.

Table 1: *Pt deposition using CV with different conditions. For every deposition condition, six replicates were made.*

	Concentration K_2PtCl_4 (mM)	Scan rate (mV/s)	Number of cycles	Low E / High E (V)	Replicates
Condition 1	0.5	100	5	-0.2/1.3	6
Condition 2	0.5	100	3	-0.2/1.3	6
Condition 3	0.25	100	5	-0.2/1.3	6

3.5 SEM imaging and analysis

Before SEM-analysis, the PtNP deposited electrode was prepared by cutting the glass capillary in half. The capillary was then filled with conductive paste using a tungsten wire. This wire was then inserted into the capillary and attached to the inside of the capillary with the paste. The electrode was attached to a holder constructed for the SEM instrument using carbon stickers to achieve conductivity. Copper tape was used to cover the electrode and the tungsten wire. The only part that was uncovered with copper tape was the tip of the electrode. After this, the holder was attached into the SEM instrument, the door was closed and a vacuum system inside the instrument was obtained. The SEM-analysis was then conducted. The SEM images taken from the analysis were analysed in ImageJ where data on the particle sizes were given. From this data the average diameter was determined and platinum's active surface were calculated according to equations 10, 11, 12 and 13.

3.6 Echem measurement and calculation of the Pt active surface

A 0.5 M H_2SO_4 solution was prepared. A conductive PtNP deposited CFMDE was, togehter with a $Cu/CuSO_4$ reference electrode, immersed into the H_2SO_4 solution in the Faraday cage. The electrodes were then connected to the electrochemical analyzer (potentiostat) and a CV

measurement was conducted using a low potential of -0.3 V and a high potential of 1.1 V (vs. $Cu/CuSO_4$). The scan rate was set to 100 mV/s and number of cycles were 3. Integration of the hydrogen adsorption area was performed on one of the cycles and calculations of platinum's acitve surface were done according to equations 7, 8 and 9.

3.7 H_2O_2 sensitivity testing

200 ml of PBS solution was prepared by adding a PBS-tablet to a flask with 200 ml deionized water. A stock solution of 10 mM H_2O_2 solution was prepared by adding 102 μl of 30 w/w % H_2O_2 to 99.898 ml PBS. The solution was made fresh daily. 9.9 ml of PBS was added to a beaker which then was placed into the Faraday cage. A conductive PtNP modified CFMDE was immersed into the PBS solution in the Faraday cage. For all measurements, a Ag/AgCl reference electrode was used. A multipotential step measurement was conducted. After this, a CV measurement was done. Then 10 μl of H_2O_2 stock solution was added into the beaker. For this concentration, only the multipotential measurement was conducted. In table 2 there is a compliation of all measurements, including the two measurements described above, that were done on the same CFMDE. Table 3 and 4 show potentiostat settings used for both multipotential step and CV respectively. For concentrations 0.01 mM and 0.05 mM, pipette stirring was performed since the volume added was so small. For higher concentrations, the diffusion was considered to be enough.

Table 2: *Compilation of H_2O_2 measurements done on each electrode. *A beaker with 10 mM H_2O_2.**An arbitrary volume of PBS.*

V_{add} (µl×10^3)	V_{tot} (µl×10^3)	C_{H2O2} (mM)	Multipotential step	CV
0 (0.99 µl PBS)	0.99	0	Yes	Yes
0.01	1.00	0.01	Yes	No
0.04	1.04	0.05	Yes	Yes
0.10	1.14	0.10	Yes	No
0.40	1.54	0.48	Yes	Yes
0.60	2.14	1.00	Yes	No
2.20	4.34	2.50	Yes	Yes
6.60	10.34	5.00	Yes	No
0*	-	10.00	Yes	Yes
0 (only PBS)**	-	0	Yes	No

Table 3: *Potentiostat settings for reduction measurements using multipotential step.*

Setting	Value
E1 (V)	0
t1 (s)	10
E2 (V)	-0.5
t2 (s)	30
E3 (V)	0
t3 (s)	10
Number of cycles	3

Table 4: *Potentiostat settings for reduction measurements using CV. (*2 cycles for measurements containing H_2O_2. For measurements containing only PBS, number of cycles were 4.)*

Setting	Value
Low E/High E (V)	-0.5/0
Scan rate mV/s	10
Number of cycles	2*

After these experiments, a CFMDE was tested in 10 mM H_2O_2 with a multipotential step measurement, with the same potentiostat settings as in table 3. When the measurement was done, Pt was deposited onto the carbon electrode with condition 3. After deposition, a multipotential step measurement also with the same potentiostat settings was done.

3.8 Enzyme deposition

After the sensitivity measurements were finished, enzymes were immobilised onto the CFMDEs. CFMDEs with deposition condition 1 were immersed into a 2 mM GOx solution for about two hours. When not used, they were stored in PBS in a fridge. CFMDEs with deposition condition 3 were immersed into 1.7 μM GluOx solution, also for about two hours. These sensors were also stored in PBS in a fridge when not used.

3.9 Glucose and glutamate sensitivity testing

For glucose sensitivity experiments a 1 M glucose solution was prepared by dissolving 1.80 g of glucose in a provided solution of filtrated PBS containing NaN_3. Then 100 ml of 10 mM glucose solution was made by adding 1 ml of 1 M glucose solution to 99 ml of PBS. For glucose measurements, one of the sensors with immobilised GOx was attached to a holder in the Faraday cage and immersed into a beaker with PBS solution. The measurement was performed in the same way as the H_2O_2 measurements, see tables 2, 3 and 4 but glucose was added instead of H_2O_2. This procedure was repeated for the two other sensors with immobilised GOx.

For glutamate sensitivity experiments a 1 M glutamate solution was prepared by dissolving 1.69 g of glutamate in 10 ml PBS. Then 100 ml of 10 mM glutamate solution was made by adding 1 ml of 1 M glutamate solution to 99 ml of PBS. On all glutamate sensors, measurements were made in the exact same way as the glucose measurements and sensitivity measurements, see tables 2, 3 and 4. Thereafter, measurements with a positive potential were conducted for one of the glutamate sensors using the same concentrations as in 2. The potentiostat settings for these measurements are stated in tables 5 and 6.

Table 5: *Potentiostat settings for oxidation measurements using multipotential step.*

Setting	Value
E1 (V)	0
t1 (s)	10
E2 (V)	0.7
t2 (s)	30
E3 (V)	0
t3 (s)	10
Number of cycles	3

Table 6: *Potentiostat settings for oxidation measurements using CV step. (*2 cycles for measurements containing H_2O_2. For measurements containing only PBS, number of cycles were 4.)*

Setting	Value
Low E/High E (V)	0/0.7
Scan rate mV/s	10
Number of cycles	2*

4 Results

The results are presented according to the order in which the experiments were performed.

4.1 Testing of CFMDEs

Electrodes with a flat surface and that displayed a limiting current of 7-10 nA were considered as good. The limit current interval is obtained from equation 4. Electrodes with a very high limit current or abnormal shape of the curve were thrown away. An estimated 50 % of the electrodes made were in the acceptable limit current range, and were used for further experiments.

4.2 Pt deposition analysis

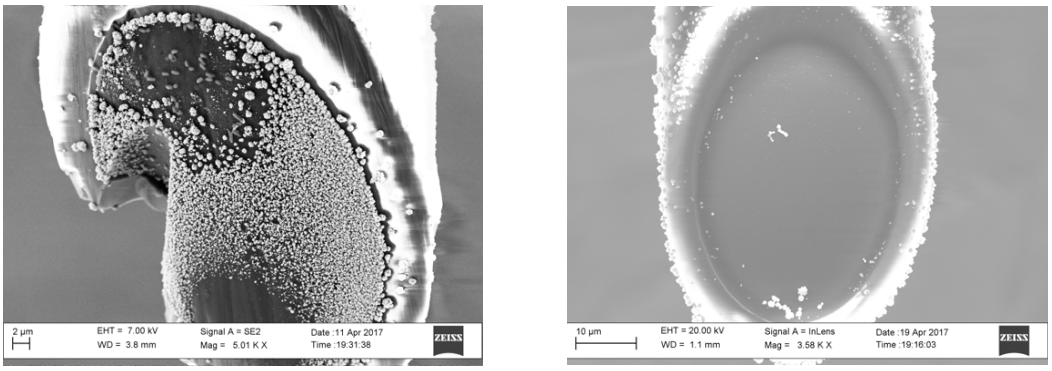
Two different deposition methods were conducted as described in the experimental section. For potential step deposition, only SEM analysis was done but for CV deposition both SEM and Echem analysis were made, which can be seen in the schematic diagram in figure 11.

4.2.1 CV vs. Potential step

Some selected SEM images from both CV and potential step depositions are presented in figures 12 to 15. The diameters were calculated from images using the software ImageJ. Diameters calculated for CV deposited CFMDEs are presented in table 7 together with the surface coverage. The calculated values in table 7 are mean values of the replicates.

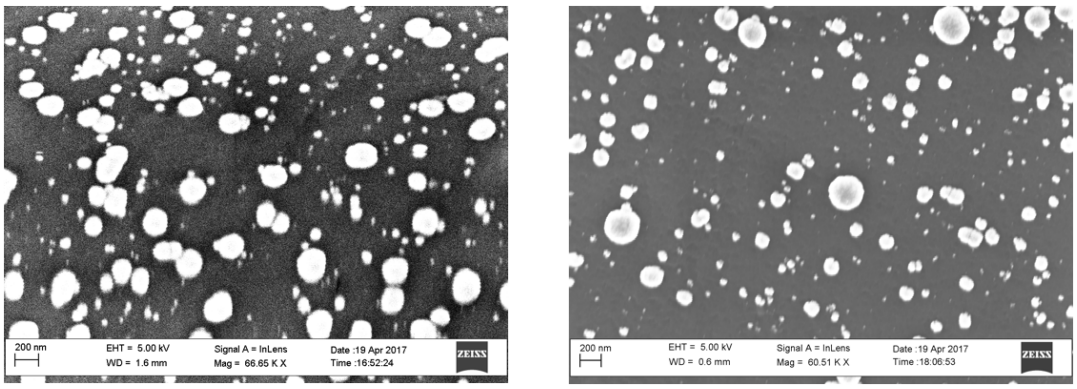
Table 7: *Calculated PtNP diameters plus the surface coverage obtained from SEM analysis. *One of CFMDEs deposited with condition 2 was broken, which was discovered when SEM analysis was conducted.*

	Condition 1	Condition 2	Condition 3
Replicates	3	2*	3
Surface coverage (%)	15	12	16
Diameter (nm)	107	61	105
Standard deviation (nm)	±29	±1	±23



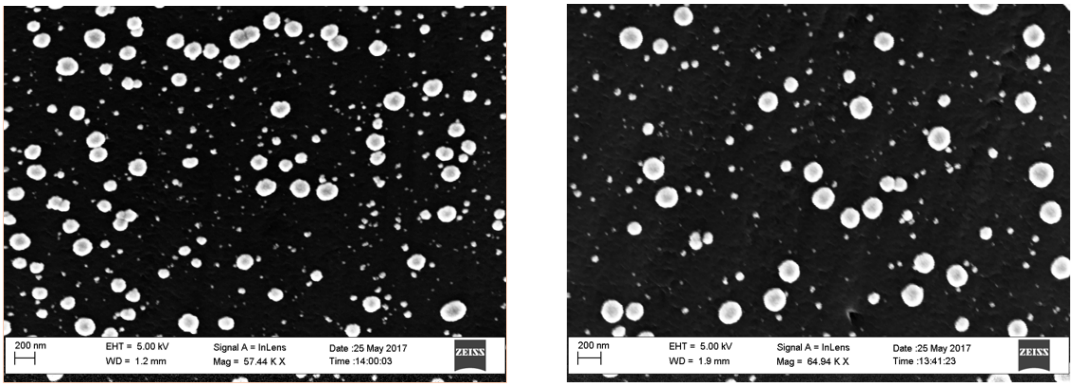
(a) **(b)**

Figure 12: *SEM analysis from potential step deposition where a) was deposited for 120s in in 1 mM K_2PtCl_4 at -0.6V and where b) was deposited for 60s in 1 mM K_2PtCl_4 at -0.4V .*



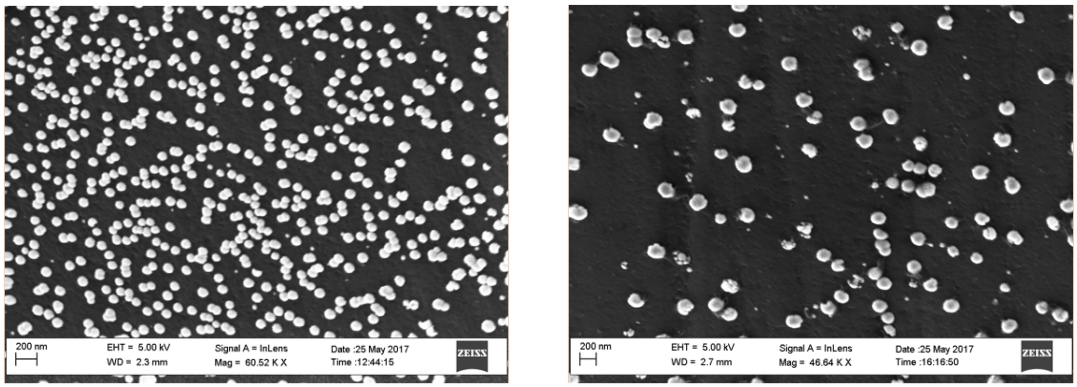
(a) **(b)**

Figure 13: *SEM analysis from CV deposition for condition 1 on two different electrodes where the average diameter size is 113 nm for a) and 138 nm for b). The standard deviation for the diameter for condition 1 was $\pm 29nm$*



(a) **(b)**

Figure 14: *SEM analysis from CV deposition for condition 2 on two different electrodes where the average diameter size is 60 nm for a) and 62 nm for b). The standard deviation for the diameter for condition 2 was $\pm 1nm$*



(a) **(b)**

Figure 15: *SEM analysis from CV deposition for condition 3 on two different electrodes where the average diameter size is 106 nm for a) and 134 nm for b). The standard deviation for the diameter for condition 3 was* $\pm 23nm$

To give an example of how the whole carbon surface can look like, a zoomed out picture of an CFMDE is shown in 16. This electrode has PtNPs that were deposited with condition 3.

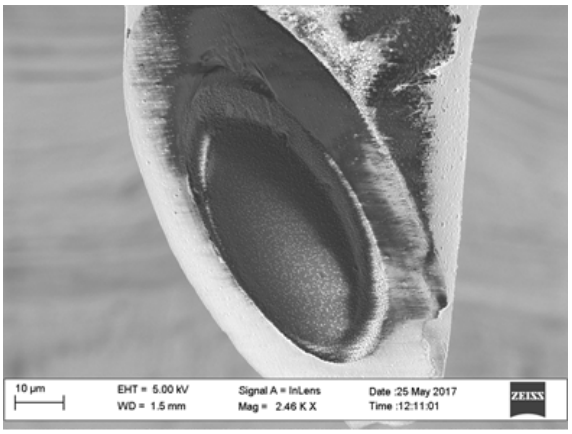


Figure 16: *SEM analysis showing the whole carbon surface of an electrode with PtNPs deposited with condition 3.*

4.2.2 Calculation of Pt's active surface

The area of the carbon surface was calculated using equation 8. The area calculation for a 33μm carbon fiber that is beveled to 45° is presented below, in 14.

$$A_{carbon} = (33/2) * ((33/2)/sin45) * \pi = 1208\mu m^2 \tag{14}$$

The shape of a graph obtained from a CV measurement for the calculation Pt's active surface can be seen in section 2.4.1. Pt areas for each of the CFMDEs are presented in table 8. The mean

values of the Pt active surface for each deposition condition (CV deposition) are presented in table 9. Equations used for these calculations are stated in section 2.4.1.

Table 8: *Pt areas obtained from SEM analysis and Echem measurements respectively. The Pt area for one of the CFMDEs analyzed with SEM was broken and the area could not be obtained.*

Condition number	1	1	1	2	2	2	3	3	3
A_{Pt} (μm^2) SEM	36	252	399	80	134	-	98	200	39
A_{Pt} (μm^2) Echem	1752	800	1338	1276	1771	65	182	2252	1614

Table 9: *The active surface of Pt from both Echem measurements and SEM analysis. The values presented are mean values for each deposition condition.*

Method	Condition number	Pt active surface (%)	A_{Pt} (μm^2)	Standard deviation (μm^2)
Echem	1	107	1297	±390
Echem	2	86	1037	±717
Echem	3	112	1349	±866
SEM	1	16	229	±149
SEM	2	8	107	±27
SEM	3	21	112	±66

4.3 H_2O_2 sensitivity measurements

As described in section 2.4.2, multipotential step measurements for various H_2O_2 concentrations were conducted to obtain the H_2O_2 sensitivity of the PtNPs. Data from these measurements are used to calculate current density. A sensitivity plot is presented in figure 17 where the mean values of the current density for each deposition condition is plotted against the H_2O_2 concentration.

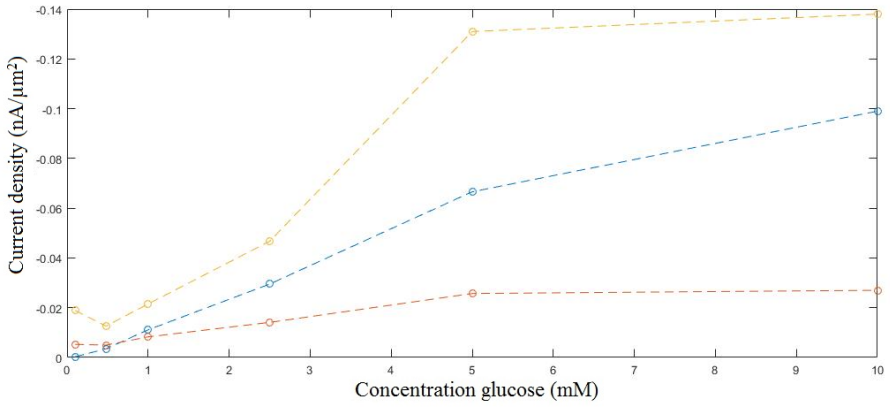


Figure 17: *H_2O_2 sensitivity measurements for three different deposition conditions where the yellow line represents condition 3, blue line represents condition 1 and orange line represents condition 2.*

Figure 18 shows multipotential step measurements in 10 mM H_2O_2 for a CFMDE (blue line) and

for the same electrode but with PtNPs deposited onto the surface (red line).

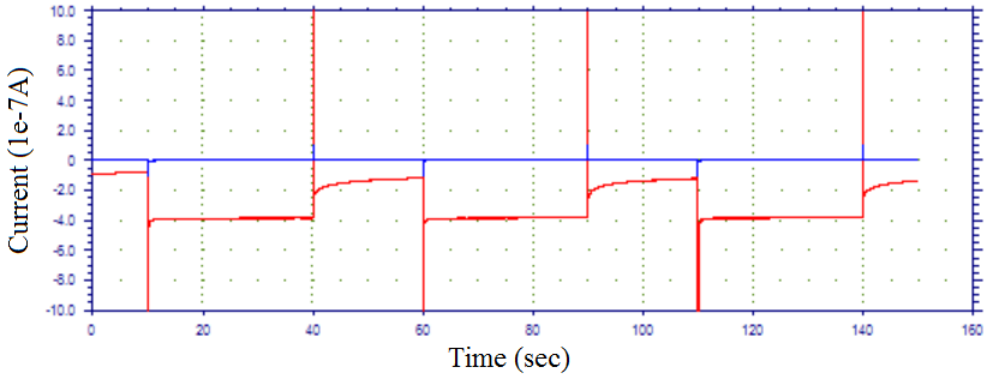


Figure 18: *Multipotential step measurements in 10 mM H_2O_2 for a CFMDE (blue line) and a PtNP modified CFMDE (red line).*

4.4 Glucose and glutamate sensitivity measurements

For glucose sensitivity measurements, only reduction of H_2O_2 was tested. Figure 19 shows a sensitivity plot where the mean values of the current densities are plotted against the different concentrations of glucose. The figure also shows a zoomed part of the first part of the curve.

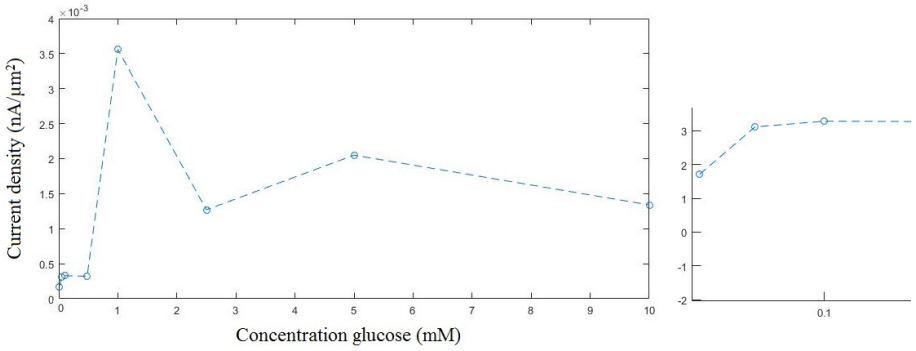


Figure 19: *A sensitivity plot where current density is plotted against glucose concentration. A zoomed part for concentrations 0.01, 0.05 and 0.1 mM is also shown.*

In terms of glutamate measurements, there were both reduction and oxidation of H_2O_2 tests conducted. For reduction measurements, figure 20 shows a sensitivity plot where the mean values of the current densities are plotted against the glutamate concentrations. It also shows a zoomed part of the sensitivity plot. The oxidation results showed no response for neither of the concentrations. Since this was the case, there are no results of these measurements presented.

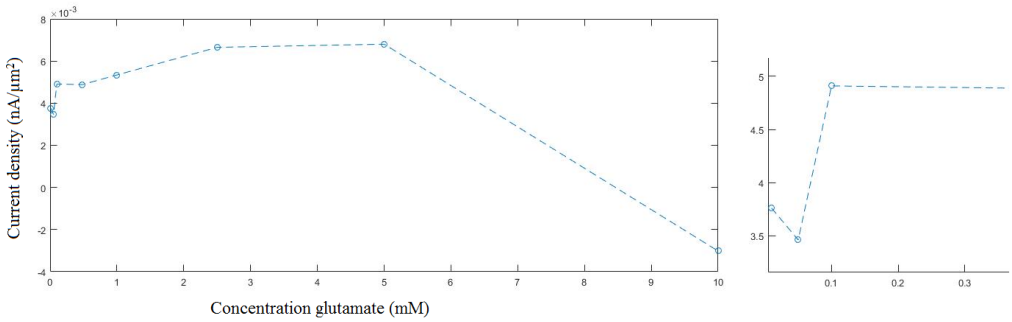


Figure 20: *A sensitivity plot where current density is plotted against glutamate concentration. A zoomed part for concentrations 0.01, 0.05 and 0.1 mM is also shown.*

5 Discussion

The factors of the surface characterization that were desired for the CFMDEs, and later the biosensors, include a PtNP diameter of 20-25 nm and a surface coverage of PtNP around 30 % . Furthermore, the criteria for the characterization of the fabricated sensors were to achieve good sensitivity for glucose and glutamate respectively. A diameter of 20-25 nm and surface coverage of 30 % were set due to previous work done by Wang et al. This for future comparisons between the properties of PtNPs and AuNPs.

5.1 CV deposition vs. Potential step deposition

In this experiment two different deposition methods were used and investigated for the deposition of Pt on CFMDEs. The first method performed was potential step deposition and many different parameters were varied and studied. Unfortunately, the results desired were never achieved and the decision to change focus to CV deposition was made. However, some correlations can be seen from the SEM analysis for the step depositions. Approximately 13 different time intervals between 2-360 s were examined, using three different K_2PtCl_4 concentrations (0.5, 1 and 2 mM). It was stated rather early that a high concentration of K_2PtCl_4 and a long deposition time provided big platinum particles collected in clusters (see figure 12a). Some CFMDEs that were deposited with a lower K_2PtCl_4 concentration and a shorter deposition time gave good size of the particles but they were not evenly distributed on the carbon surface (see figure 12b), while some other were evenly distributed on the carbon surface but the particles were very contrasting in size ranging between micro and nanometer in diameter. When trying out 0.5 mM for 5 minutes, a desired result was achieved with evenly distributed particles. But when trying this setting for three more CFMDEs, the results did not show any reproducibility. It is clear that neither of the settings inspected for the potential step deposition experiments gave consistent results. However if there was more time, the ideal parameters for platinum deposition using potential step deposition could have been stated.

For the CV deposition, the SEM images showed more interesting and desirable results as seen in figure 13. As presented earlier, in table 1, three different conditions were studied. The first condition studied was condition 1 and the SEM images presented evenly distributed PtNPs onto the surface with reasonable diameters, this is seen in figure 13. However, the particle sizes varied significantly and they were in general to big according to the desired size at 20-25 nm (see table 7).

In an article presented by Zahálková, they compared different deposition conditions for Pt using

CV [28]. In that article it was stated, among other things, that number of cycles can affect the particle size. Thus, the longer deposition time, the larger particles which also was stated from the step experiments in this project. With this argument in mind, condition 2 was studied where the number of cycles were decreased from 5 to 3. It is also understood from the step experiments and the article that a higher concentration provides more particles onto the surface, especially if a long deposition time is used. Because of this, condition 3 had a concentration of 0.25 mM instead of 0.5 mM.

Figure 14 shows SEM images taken from two electrodes deposited with condition 2. It is stated when both observing and calculating from ImageJ that the average diameter is decreased with nearly 50 % and thereby closer to the desired diameter as seen in table 7. It also has a standard deviation of +- 1 nm. However, the surface coverage is rather poor and significantly smaller than condition 1. In figure 15 two CFMDEs deposited with condition 3 is shown. When the concentration was reduced from 0.5 mM to 0.25 mM, it is shown that the surface coverage is larger compared to the other conditions. However, the average diameter is not much smaller compared to condition 1 but still the standard deviation is lower for condition 3. But when studying and comparing the images, it is clear that condition 1 has a major variation in particle sizes compared to condition 3 which may reduce the average diameter calculated from ImageJ.

5.2 Calculation of Pt's active surface

Since CV deposition was more promising in terms of getting more evenly distributed PtNPs on the carbon surface, here after and in further discussions, these electrodes are the only ones discussed. One purpose of doing Echem measurements is to measure the active surface of Pt with another method than SEM. As can be seen in the result section, in table 7 and 8, there are huge differences in Pt's active surface between the two methods. However, the standard deviations for all conditions, for both methods, are significantly large. This entails that the results are not reliable.

The ratios calculated from the SEM images are all much lower than the ones calculated from the Echem measurements. One reason for this might be that the carbon surface is not totally flat and has thereby more carbon for the platinum particles to deposit on. This can be seen clearly in figure 16 in the result section. The irregularity of the surface indicated that the exact surface area will be untrue for both methods, since both of them rely on that the carbon surface is always flat and has the same area.

Some error sources might have appeared from the assumptions taken for both methods. For the SEM analysis, the assumption that the particles were totally spherical was taken. In fact, from

neither of the methods, the exact geometry of the particles can be stated. For the Echem method, neither of the curves obtained were similar to the ones expected, according to other sources [37]. Another aspect to keep in mind when it comes to the Echem method the cleanliness of the electrode surfaces might be a factor since salt particles may not have dissolved enough before Echem testing. Additionally, a reason why Echem showed significantly higher surface area might be due to the fact that once one particle is positioned on the surface, further deposition will arise on that particle and a higher surface coverage will be obtained. If this happens, it will not show in the SEM images.

The adsorption curve was expected to be more significant and thereby easier to integrate. When these desired curves were not obtained, a cleaning of the electrolyte was done using N_2 gas to eliminate oxygen since this can be disturbing during adsorption. Though, no difference was seen. Thus, there might be many reasons for this difference between the results from these methods but Echem is the most reliable one and the method it self prevents that the electrodes break compared to SEM.

5.3 H_2O_2 sensitivity measurements

When conducting measurements in the purpose of doing a sensitivity plot, the desire is to get a concentration interval where the response to, in this case H_2O_2, is linear. The sensitivity plot, figure 17, shows three curves, one for each deposition condition. The plot presents the mean values of the current densities for each deposition condition. These values are plotted against the H_2O_2 concentrations. As described in section 2.4.1, the sensitivity is a property that describes, in this case, the CFMDE´s ability to distinguish between different analyte concentrations. A steeper slope in a sensitivity plot indicates a higher sensitivity for the chosen compound, in this case H_2O_2. Note that figure 17 does not include the concentrations 0.01 mM and 0.05 mM even though these measurements were conducted. This is because these concentrations did not give any response to H_2O_2. One reason for this can be that the solution was not stirred enough before the measurement started.

The current density is dependent on the Pt area which should theoretically be almost the same for all electrodes with the same deposition condition. Variations in Pt area can be one factor that contributes to the absence of a linear pattern in current density. The Echem measurements for the active surface of Pt detect all Pt on the electrode as described in the discussion above. Thus, in terms of evaluating these sensitivity measurements, the Pt area obtained from the Echem measurements are more reliable than the Pt area obtained from the SEM analysis. However, Pt that is deposited on the glass capillary should not contribute to the current response since glass is not conductive. Also to keep in mind is that Pt area is dependent of how integration is conducted.

If integration of Qa (see section 2.4.1) is done incorrect, the Pt area will be incorrect which entails that the current density will also be incorrect.

When observing figure 17 it is shown that condition 3 has the steepest slope which indicates that this condition has the highest sensitivity for H_2O_2 of all deposition conditions. Important is that even though condition 3 has the highest sensitivity, it can be observed that none of the curves are linear. However, this plot shows that a lower concentration of K_2PtCl_4, which in this case was 0.25 mM, results in a CFMDE with a high sensitivity for H_2O_2. However, as can be seen in figure 17, the curve for condition 3 decreases between the first two concentrations (0.1 and 0.48 mM).

The decrease can for example be due to experimental errors. Theoretically, the current response should become greater (more negative for a reduction reaction) when the concentration of an electroactive specie is increased. Two of the CFMDEs deposited with condition 3 had high platinum surface areas calculated from the Echem measurements. The third electrode had a much lower area and may have contributed to the fact that the mean values did not a linear response to H_2O_2. However since the individual CFMDEs did not provide a linear response to H_2O_2, there must be other errors that contribute to this pattern.

These errors can be applied for all deposition conditions. One error can be that H_2O_2 did not have time to diffuse enough before the measurement started. Another reason can be that PtNPs were lost on the way for some of the CFMDEs. Also, the volume can have an affect on the current response of H_2O_2. Why the volume might be an error source is since it was observed that when 10 mM H_2O_2 was measured in an almost full beaker, the current response followed the expected pattern. For another CFMDE, the beaker was only half full and did not follow the expected pattern. This was not thought of at the time so there is no documentation of what volumes were used for the 10 mM H_2O_2 measurements.

The other two conditions showed a lower H_2O_2 sensitivity compared to condition 3. Condition 1 indicates the second highest H_2O_2 sensitivity according to figure 17. Figure 17 also shows that this condition has the most linear curve of all the curves. This can be due to the fact that the condition 1 CFMDEs have areas that are quite similar to each other, as can be seen in table 8. The condition with the lowest H_2O_2 sensitivity is condition 2. For this condition there are two electrodes with a Pt area similar to each other. The third one has a Pt area that is around 4 % of the other two areas.

To show that H_2O_2 is reduced on the PtNP surface and not on the carbon surface, an experiment comparing the current response for a CFMDE and a CFMDE with Pt deposited with condition 3 was done and is shown in figure 18 in section 4.3. The difference in current response should also be clear if an oxidation of H_2O_2 was done instead of a reduction. This result show an indication

of that the PtNPs catalyse the reduction of H_2O_2 and that H_2O_2 is detected on the PtNP surface and not on the carbon surface. However, n=one for this measurement. To achieve reliable results, n should be equal to minimum three, but this is an indication of that H_2O_2 is detected at the PtNP surface.

5.4 Glucose and glutamate measurements

As described in section 2.4.2, when conducting reduction measurements of H_2O_2 the steady state current should decrease when the concentration of H_2O_2 is increased. Since the enzyme reactions result in H_2O_2, increasing glucose or glutamate concentrations should give an increase in H_2O_2 concentration. In both figure 19 and 20 there is first an increase in current density which indicates that it is also an increase in current when it should be a decrease. Then for higher concentrations, both curves decrease.

According Cargill et al. and McLamore et al. among others, the linear range of glucose and glutamate is at very low concentrations [6] [38]. However, there was no linear range observed for either of the analytes. Why the measurements were not successful can be of many reasons. This project did not have the time to do the research that is needed to distinguish what is causing the sensors not to respond in a predictable manner and not show a linear response detecting the analyte.

For the oxidation measurements of the glutamate biosensors, there is no current response for any of the glutamate concentrations. Why there was no response could be because the GluOx enzymes are no longer on the surface or that they have lost their activity. To make sure that the enzymes were inactivated, another glutamate sensor was tested in a 10 mM glutamate solution. This sensor did not give any response. A PtNP modified electrode was tested in PBS and 10 mM H_2O_2 to make sure that it was not due to an instrument error. Since this electrode gave response to H_2O_2, the conclusion was made that most likely the enzymes were either inactivated or lost. Further measurements were thereby stopped.

6 Conclusions

This degree project concludes that deposition of Pt using CV gives PtNPs that are more evenly distributed on the carbon surface than PtNPs deposited with potential step deposition. In terms of Pt deposition, the project also concludes that the lowest concentration used (0.25 mM) produces particles with more consistent diameters compared to condition 1 and 2. It is clear that the method development of deposition parameters that give desirable and reproducable results is time consuming. This deposition method needs to be studied further to achieve the results stated in the aim and beginning of the discussion.

In terms of the determination of the Pt active surface, the Echem method must be developed so that the curves obtained from the CV measurements are more similar to the ones found in the literature. To obtain reliable values of the Pt active surface calculated from the CV measurements, the carbon surface must be perfectly flat so that the calculations only result in Pt that is deposited on the carbon surface. If a perfectly flat surface is produced, the value of the Pt active surface calculated from the CV measurement should be more consistent if compared to the value calculated from SEM analysis.

This projects concludes that CFMDEs with Pt deposited with condition 3 gives the highest H_2O_2 sensitivity. This can be due to the fact that condition 3 produces the highest PtNP surface coverage. The project also concludes that H_2O_2 is detected on the PtNP surface and not on the carbon surface.

Regarding glucose and glutamate, the results presented in this project have rather low reliability due to the lack of response during experiments and expectations according to other sources. However, from the measurements conducted in this project it can be concluded that both the sensitivty for glucose and glutamate decreases after approximately 5 mM. More replicates has to be studied. In addition to that a verification that the enzymes actually are immobilised onto the surface and that they still are active has to be conducted.

7 Future aspects

To investigate further, an optimal deposition condition for PtNPs onto CFMDEs to achieve maxial sensitivity for glucose and glutamate should be developed. This project has initially followed the same guidelines made for CFMDEs with AuNPs. Noticeably, the two metals acts different and requires different treatments in the fabrication process. Thus, a further evaluation of platinum's characteristics might be of great interest since platinum has showed promising results in similar experiments. How the enzyme immobilisation should be conducted and how to get a good analyte response from the biosensors should also be investigated. If this could be resolved, these platinum enzymatic biosensors could contribute to research within neuroscience for further understanding of various kinds of degenerative neurological diseases.

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