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INFLUENCE OF PHARMACEUTICAL MATERIALS ON THE SENSITIVITY OF PRUSSIAN BLUE-BASED SENSORS

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LIST OF ABBREVIATIONS

WE	Working Electrode
RE	Reference Electrode
CE	Counter Electrode
CV	Cyclic Voltammetry
GO_X	Glucose oxidase
FTO	Fluorine doped Tin Oxide Glass
PB	Prussian blue
SAMs	Self-assembled monolayers
DNA	Deoxyribonucleic acid
dsDNA	Double stranded DNA
RNA	Ribonucleic acid
$NADH^+$	Nicotinamide adenine dinucleotide hydrogen$^+$
NADP	Nicotinamide adenine dinucleotide phosphate
ITO	Indium tin oxide
pO_2	Partial pressure, oxygen electrode
SPE	Screen printed electrode
min	Minutes

LIST OF SYMBOLS

μ	Micro
A	Ampere
I	Current
s	Second
t	Time
C	Concentration
R^2	Linear dependency
ΔI	Change in Current
M	Molar concentration
V	Volts
L	Litre
cm^2	Centimetre square

INTRODUCTION

Electrochemical biosensors play an important role in the diagnosis of body fluids for both humans and animals. A number of sensors are made to detect biomarkers in urine and blood. Sometimes, the detection of some specific molecule requires implantation of the biosensor in the body. For diabetic patients, a constant monitoring of the hyper/hypoglycaemic events in the body is very crucial. Similarly, for confined brain areas, continuous neurotransmitter monitoring plays a great role. The successful study so far has been accomplished for the enzyme-based electrochemical biosensors in in-vivo [20].

The concept behind the construction of the glucose biosensor to study the impact of one particular material is based on the fact that the enzyme-based biosensors usually utilize oxidase enzymes and can detect electrons involved in redox reactions by reducing H_2O_2 at the electrode in many cases. [6].

The construction of enzyme-based glucose biosensor employs different types of electrodes which include rigid silicon or ceramic needles, metal wires, flexible polymer multichannel electrodes etc. [8,14,15,16]. These microelectrode biosensors have offered many opportunities in the pharmaceutical chemistry, biology and medicine. These electrodes can increase the response current and exhibit excellent anti-interferent properties [17]. Poor electrochemical conductivity, instable operational ability, high cost, high detection limit and the low response current are some of the problems with these electrodes. [7,8,14,15,16].

Prussian Blue (ferric hexacyanoferrate ($Fe_4^{III}[Fe^{II}(CN)_6]_3$)), on the other hand, has the potential to form electroactive layers onto the electrode surface after the electrochemical deposition [4]. The ability of Prussian Blue (PB) to convert the chemical signal into the electrical signal is very helpful in making biosensors and in the determination of the sensitivity. Prussian blue has been used widely as an “artificial enzyme peroxidase” in the construction of electrochemical amperometric biosensors because of its high activity and selectivity towards the reduction of H_2O_2 and O_2. [11,12].

In the present research scenario, the use of the glucose oxidase (GO_X) in the construction of glucose biosensors is becoming more popular [8],[9],[10]. From the last 25 years, glucose electroenzymatic biosensor has been a crucial clinical tool for detecting and monitoring diabetes. Majority of the glucose biosensors used in in-vivo applications, are based on the rate of glucose oxidase (GOx) catalysed oxidation of glucose in the presence of oxygen. In this the rate of the reaction is measured by observing the formation of hydrogen peroxide (H_2O_2) or the consumption of oxygen [8, 20]. Prussian Blue is usually involved in the formation of the biosensors for glucose owing to its catalytic activity in the reduction of hydrogen peroxide. However, there are other electrochemically active interferents, such as ascorbic acid, uric acid, L-cysteine, paracetamol etc in the biological fluids, which can easily interfere with the signals [17]. To avoid this interference, researchers came up with the idea to use the selective membranes that are permeable for hydrogen peroxide but non-permeable for interference species [1,18,19].

The purpose of this research project was to construct and examine the sensitivity of Prussian Blue-Based Sensors and to investigate the impact of some biological materials (Vitamin. C, Uric acid, L-Cysteine and Paracetamol) found in blood towards sensitivity of PB-based glucose biosensor by using electrochemical methods.

This research included:

(i) Construction of PB based biosensors by using the CV which includes deposition of PB on electrode (FTO) and then the stabilization of PB layer.

(ii) Investigation of the sensitivity and influence of interfering substances present in the blood along with glucose (ascorbic acid, L-cysteine, uric acid and paracetamol), on the sensitivity of PB biosensor by using chronoamperometry.

iii) The signals obtained by chronoamperometry were based on the principle that, when GO_X react with glucose ($C_6H_{12}O_6$) in the presence of oxygen it forms gluconic acid ($C_6H_{12}O_7$) and hydrogen peroxide (H_2O_2). It can be expressed in equation (a) as:

$$C_6H_{12}O_6 + O_2 \longrightarrow C_6H_{12}O_7 + H_2O_2 \quad \text{---(a)}$$

1. LITERATURE REVIEW

1.1 Clinical analysis of glucose

Glucose is not only the prime source of energy in the body but also is one of the major constituents of our food. The fluctuation in the normal blood glucose level in the body can cause serious health problems. For example, Hyperglycaemia is the condition related to high blood sugar level and is the cause of diabetes [21]. Higher concentration of glucose in the brain is associated with Alzheimer's disease [23]. Blood Glucose level is also the cause of the other body health problems like dyslipidaemia, hyperinsulinemia, glucose intolerance, diabetes-specific microvascular disease [22].

Normal concentration of glucose in the body ranges from 80 mg/dl to 120 mg/dl. Glucose has strong reducing properties which makes its concentration easy to measure. Thus, the estimation of circulating glucose became one of the earliest successful clinical tests. With micro glucose oxidase technology, it is now even more easy for the patients to measure their own blood glucose concentration, thus making it the most widely conducted test of the blood chemistry. With the better understanding of the blood glucose measurement, a lab technician will be able to accurately interpret the values thus avoiding drawbacks of the inaccurate testing [32]. The clinical analysis of glucose in blood has played an important role in the diagnosis and control of the disorders related to blood glucose level [24].

1.2 Clinical analysis methods for the determination of blood glucose level

1.2.1 Chemical Methods

To measure the total reducing capacity of the solution, Ferric and cupric reduction methods were used initially. Since blood and urine contains many other substances with the reducing properties, therefore these methods are considered non-specific nowadays [24]. A toluidine test is one of the chemical methods which is relatively specific. In this method the glucose and other aldohexoses are condensed with aromatic amines in hot acetic acid solution to give coloured glycosylamines [25]. Factors which may cause error in this method are the water content of the reaction mixture, sodium fluoride, haemoglobin and bilirubin [26].

1.2.2 Enzymic Methods

There are several specific methods for the determination of glucose in biological fluids. They use either glucose oxidase, hexokinase or more recently glucose dehydrogenase [24].

1.3 Solid-phase chemistries

The development taking place in the methods for measuring glucose can be termed as a unique example of the search for simple and specific methods of analysis. This progress has been further enhanced by the realisation that immobilisation of enzymes onto solid-phase

carriers can further extend the value of these techniques. The immobilisation of enzymes has brought three major benefits to the area of glucose analysis:

(i) Improved stability of the reagent enzyme;

(ii) Reduced use of expensive reagents;

(iii) Facilitated development of rapid glucose analyses [24,27].

There are different ways by which Immobilised enzymes have been employed. The first was in the development of the enzyme electrode [28]. A potentiometric system was described in 1973 by Nagy et.al. This potentiometric system was based on glucose oxidase and peroxidase immobilisation onto an iodide electrode [29]. Apart from this, spectrophotometric detection of the glucose, fluorescence monitoring, luminescent monitoring and continuous-flow immobilised enzyme systems by using dry reagent chemistry system have also been used for the detection of glucose [24,30,31].

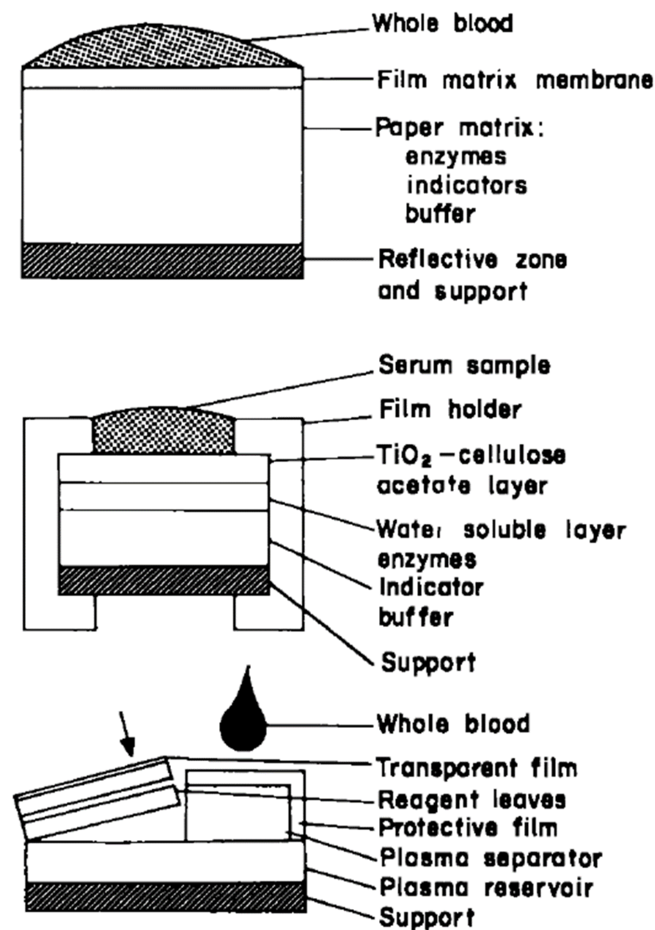


Fig. 1. Schematic representation of three types of solid phase reagent system for glucose measurement [24]

1.4 Types of biosensors

A biological material is the key component of a biosensor. This biological material can be an enzyme, a membrane receptor, an antibody, a signal transducer or a whole cell. This material is usually immobilized on the surface of a suitable transducer [33]. The determination of the stability, sensitivity and reliability of a biosensor's response can be done by functional integrity of the immobilized biological material and the accessibility of each individual biological setup within the biological component of the sensor and analyte [34]. Following are a few main types of biosensors.

1.4.1 Biosensors based on self-assembled monolayer

This method of making biosensor is based on deposition of one molecule thick layer of organic groups (thiol, disulphides or amines) on electrode through physical or chemical forces. To synthesis high electrical conductivity and water-based polymers, SEM is being used in the area of conjugated/conducting organic polymers [33,36].

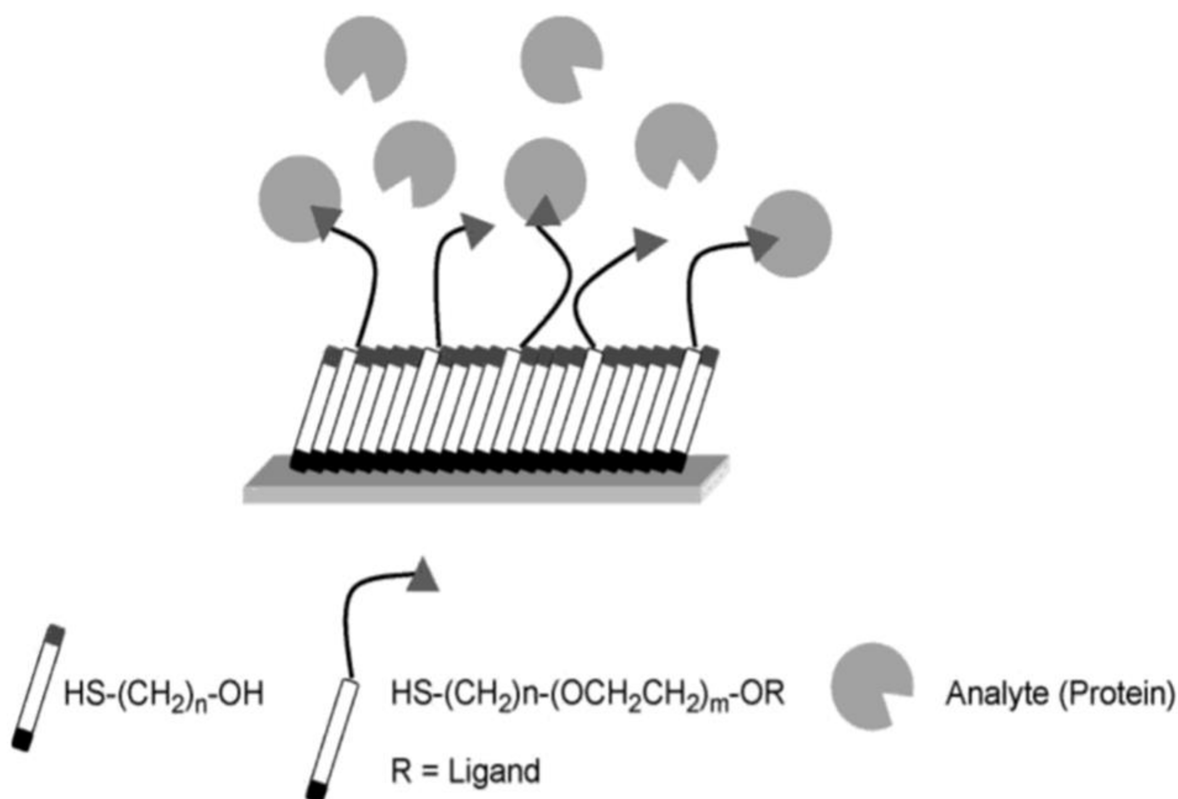


Fig. 2. Self-assembled layer-based biosensor, constructed by using chemisorption of mixed hydrolic-terminated alkyl-thiol and an oligo-thiol-terminated alkyl-thiol on protein resistant gold electrode with specific for a protein [33].

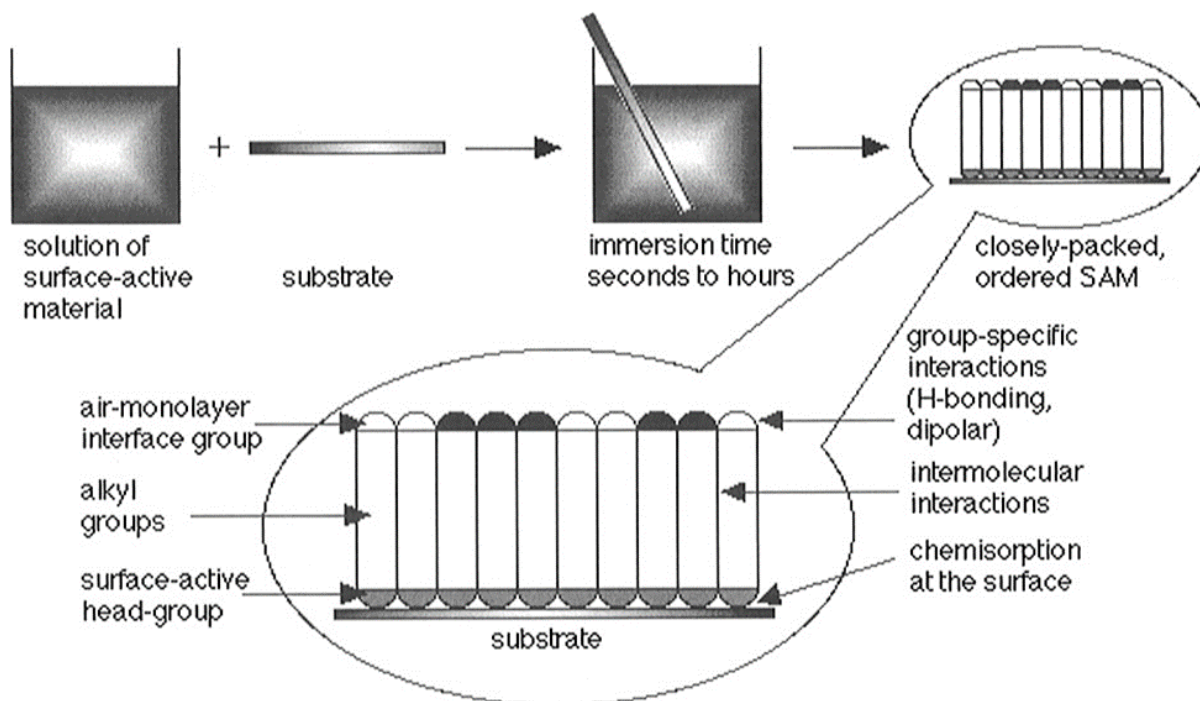


Fig. 3. Another SEM formed by simply immersing a substrate into a solution of the surface-active material [36].

1.4.2 Third generation biosensors

Third generation biosensor approach is based on direct electron transfer between enzyme, electrode and catalytic current generated in the presence of substrate [33,37].

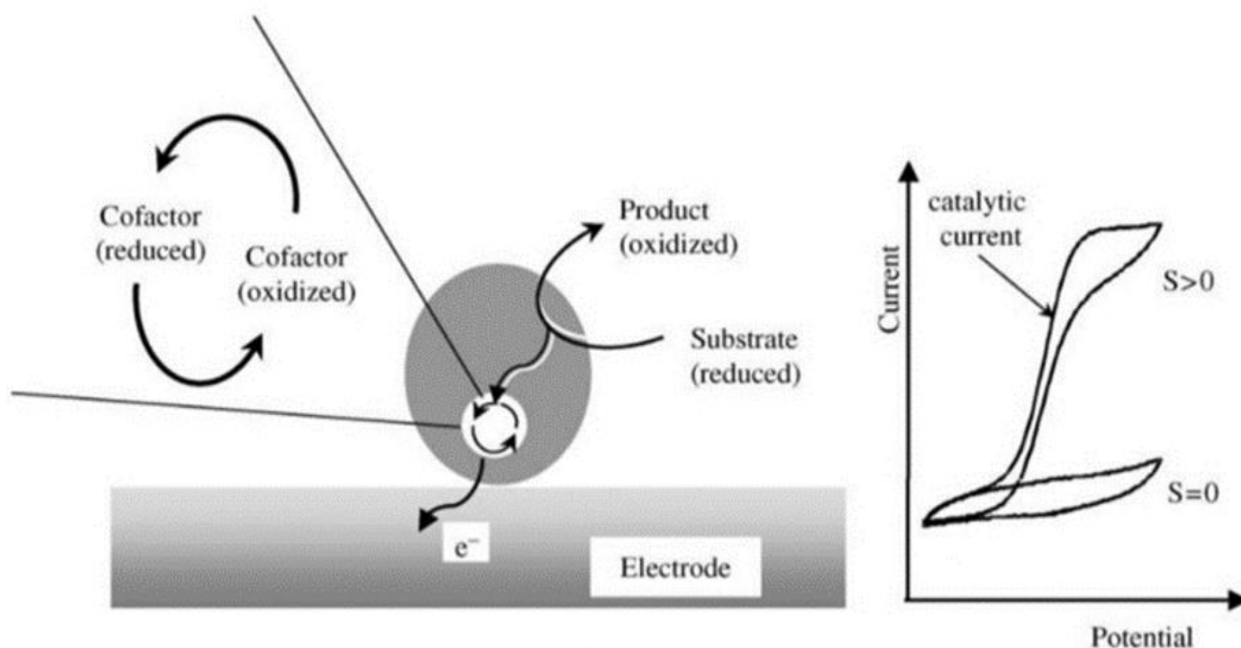


Fig. 4. General representation of third generation biosensors [33].

1.4.3 DNA based biosensor

DNA-based biosensors(genosensors) are based on the ability of the complementary nucleic acid strands to selectively form hybrid complexes. These biosensors are primarily used to study the biomolecular interaction mechanisms of compounds with double-stranded DNA (dsDNA) enabling the screening. [40,41].

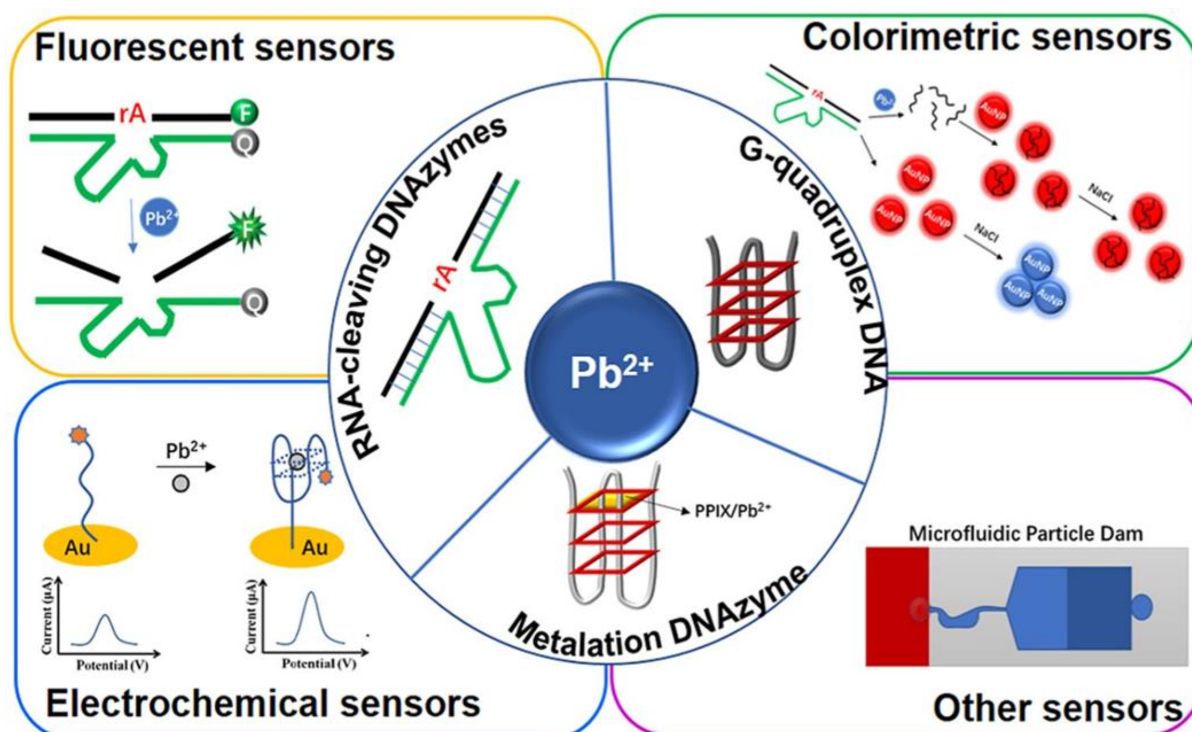


Fig. 5. Progress on DNA-based biosensors for Pb^{2+} detection (Schematic diagram) [42].

1.4.4 Enzyme-based biosensors

An enzyme biosensor, as the name suggests, consists of an enzyme and a transducer. The enzyme acts as a biological sensing agent. The transducer can be amperometric, potentiometric, conductimetric, optical, calorimetric, etc. These biosensors find their use in detecting various substrates [33,38]. By using these methods, the qualitative and quantitative analysis of many analytes of interest has become easier in the fields of biomedicine, food quality control, agricultural, pharmaceutical industry and environment [39]. The most crucial and most studied application of these types of biosensors is in the detecting and monitoring of the blood glucose level due to its high demand in medical field [33]. Further types of enzyme-based biosensors are discussed below-

1.4.5 Electrochemical Biosensors

Most important methodology used for constructing electrochemical biosensors is by using oxidoreductase enzymes investigated with amperometric technique [39]. By

using enzymes, like glucose oxidase, along with mediators and cofactors, such as nicotinamide adenine dinucleotide ($NADH^+$ and NADP), these biosensors can convert biological recognition element or analyte into current [43,44,54]. In 1962 Clark and Lyons Constructed the first blood biosensor by using an enzyme membrane on pO_2 electrode [46,47]. Enzyme based biosensor are being used to investigate certain substances in the blood, other body fluids and tissues. These substances can be carbohydrates (glucose), proteins (cholesterol) and amino acids (glutamate) [46]. In electrochemical biosensor the electrode surface of the biosensor provides the site for redox reaction between the analyte/ion and the electrolyte [48]. There are four major components of an electrochemical biosensor (1) a material required to form electroactive layers on the electrode, (2) material required for the stabilisation of electroactive substance, (3) a biological component, it can be enzyme, antigen, antibody or cofactors and (4) a material for the immobilisation of the biological component [46]. Traditionally an electrochemical electrode system involves a WE (mostly noble metal, sometimes FTO or ITO), an electrically stable RE (like Ag|AgCl, Ag|AgCl|KCl) and a CE (usually Ti,Pt) [4,46,49].

Table 1. Some electrochemical biosensors and their applications [59].

Method	**Target**	**Biological Element**	**Target Matrix**	**Transducer Element**
Amperometric	Cholesterol	Cholesterol oxidase	Human serum	Prussian Blue modified SPE
Amperometric	Lactate	Lactate oxidase	Wine	Prussian Blue modified SPE
Amperometric	Polyamines	Polyamine oxidase, spermine oxidase	Food	Prussian Blue modified SPE
Amperometric	Lysine	Lysine oxidase	Cheese	Pt electrode
Amperometric	Glucose	Glucose oxidase	Transdermal fluid	Transdermal microneedles
Amperometric	Glucose	Glucose oxidase	-	Gold nanoelectrode
Amperometric	Ethanol	Alcohol dehydrogenase	wine	Polyaniline doped modified SPE
Amperometric	Antioxidant capacity	Superoxide disumlase	Fruit juice and berries	Pt electrode
Amperometric differential	Antioxidant capacity+ ascorbate	Ascorbate oxidase	Fruit juice	Fullerene modified graphite
Amperometric inhibition	Atrazine	Tyrosinase	Drinking water	Carbon modified SPE
Amperometric	Oxygen profile	Biliribine oxidase	Microbial fuel cell	Pt electrode

1.4.6 Optical Biosensors

An optical biosensor is a sensing device which uses an optical transducer along with biological component [52]. Biological component can be enzyme, antibodies, receptors or antigens [52,53].

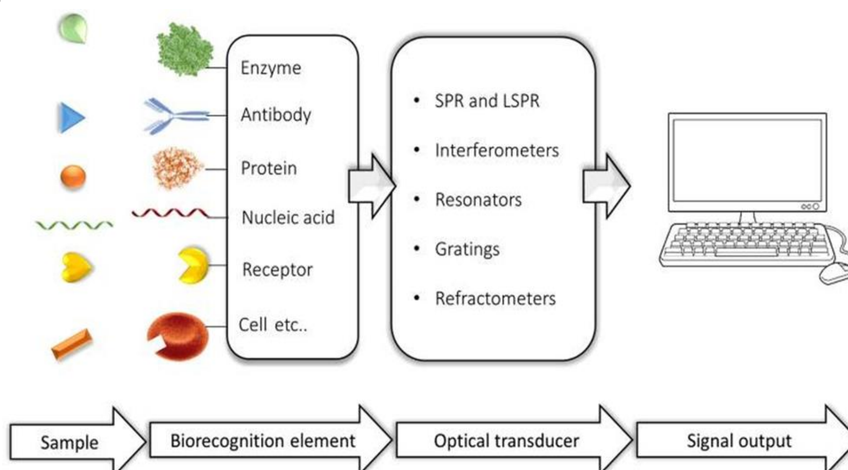


Fig. 6. Schematic diagram of optical biosensor [52].

1.4.7 Amperometric Biosensors

Amperometry is a technique in which WE is applied with constant potential (V) which gives a steady-state current (I) as signal [50,51,60]. Chronoamperometry is a type of amperometric technique which depends upon time, and we can obtain the dependence of current to time as a graph for further examination. Therefore, in the investigation of the biosensors, this technique is of the utmost use [60].

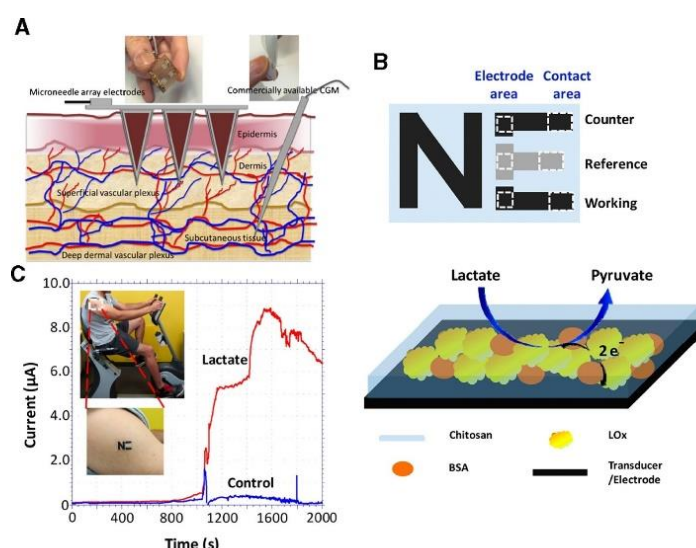


Fig. 7. Schematic diagram of two different amperometric techniques. A. Example of micro needle array electrodes versus continues glucose observation. B. Three electrode amperometric biosensor. C. Lactate monitoring by using three electrode system biosensors. [55].

1.4.8 Glucose Biosensors

Diabetes is a huge problem faced by industrialized nations. It affects approximately 5% of the worldwide adult population. In order to improve the lives of all diabetic patients, there is a dire need to develop an accurate, safe and painless method to monitor glucose level continuously. This could be achieved by developing an efficient glucose biosensor [24,33]. There are three approaches for constructing biosensors for the detection of glucose.

i. Through oxygen consumption detection [33].
ii. Through H_2O concentration detection [33].
iii. Through the use of non-physiological redox couple [33].

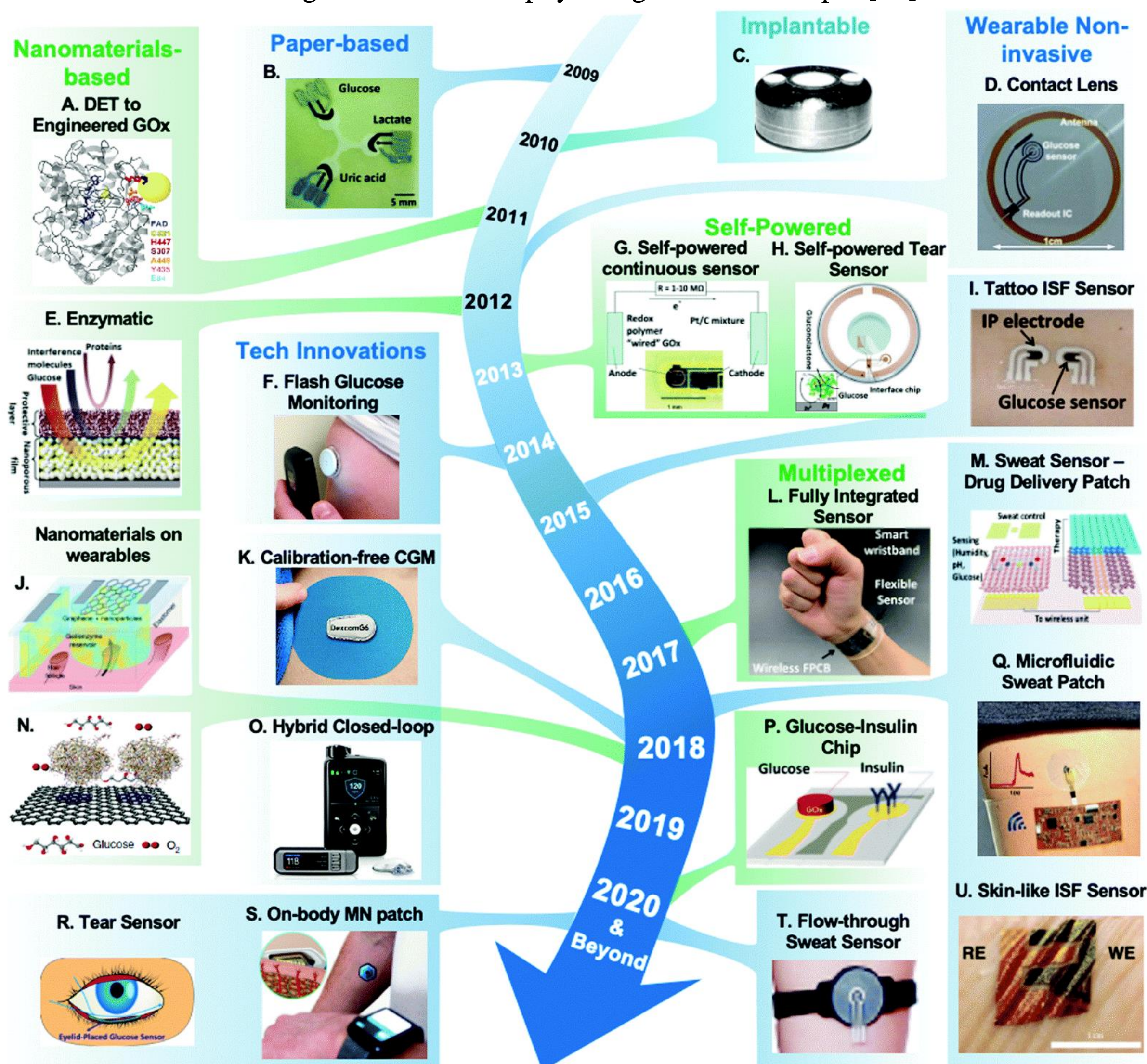


Fig. 8. Key concepts and the advancement in the use of different nanomaterials for the electrochemical detection of glucose [57].

1.5 Application of Prussian Blue (PB) in Glucose Biosensors

PB has many advantages over other materials to be used for constructing biosensors for glucose, for example when PB is electrochemically treated it can be deposited and form electroactive layers on the surface of electrode, and thus can help in converting the chemical

signal to electrical signal, it can also catalyse the reduction of H_2O_2 produced by the enzymatic reaction [4,56].

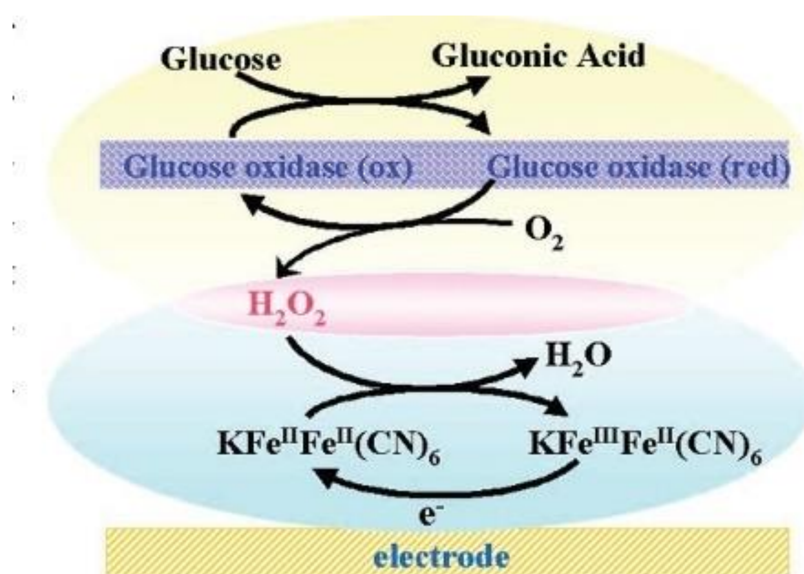


Fig. 9. Schematic representation of the application of PB for the multilayered biosensor [56].

1.6 Materials found in the blood which can impact the sensitivity of biosensor

There are many substances in the blood that can be co-existing with blood in addition to glucose, like uric acid, and some medications for example ascorbic acid, acetaminophen, L-dopa, tolazamide and paracetamol, these materials can overlap the signals and can interfere the detection of glucose [17,56,58].

Table 2. Some chemicals and their effect on changing blood glucose measurement [58].

Chemical	Effect
High oxygen	Decrease reading
Low oxygen	Increase reading
Uric acid	Decrease reading
Galactose	Increase reading
Xylose	Increase reading
Acetaminophen	Decrease reading
L-dopa	Variable
Tolazamide	Variable
Ascorbic acid	Variable
Icodextrin	Increase reading

1.7 Cyclic Voltammetry

It is an electroanalytical technique in which cycled potential is applied to study the current response of an electroactive material. This technique has wide applications in the field of material science like in the coating of sensors [61, 62].

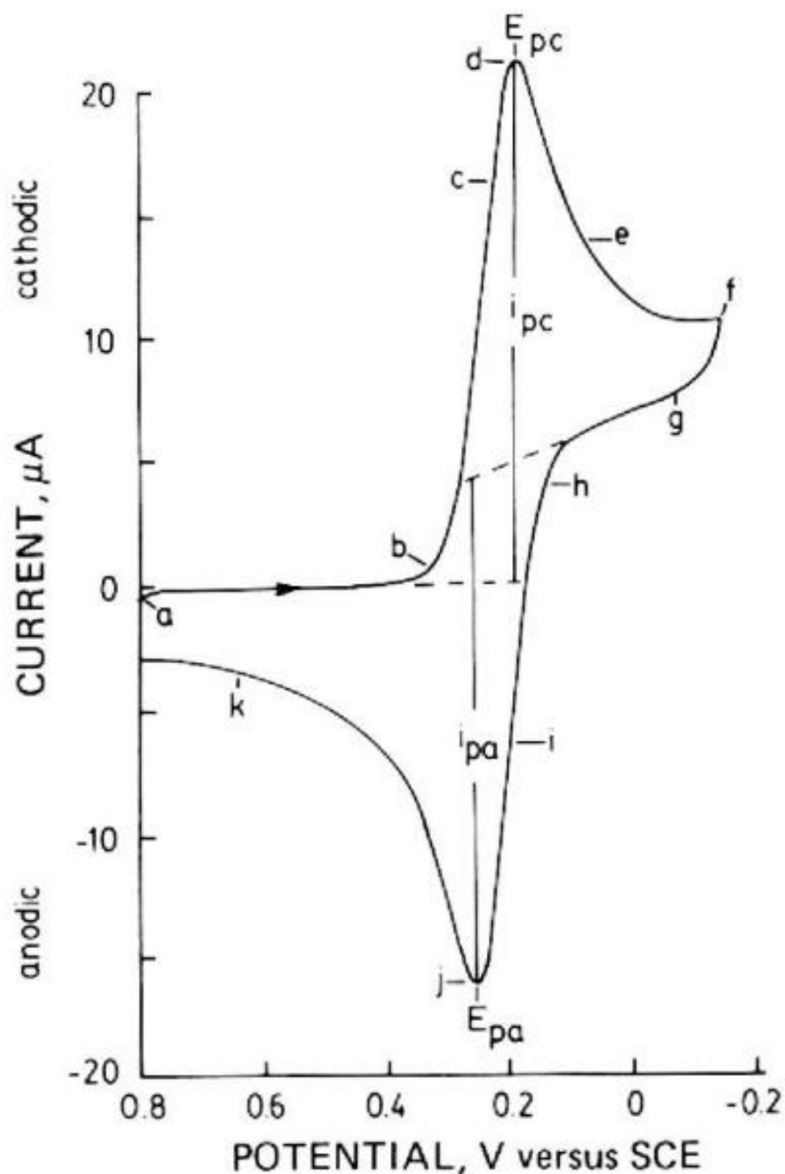


Fig. 10. A typical cyclic voltammogram 6mM $K_3Fe(CN)_6$ in 1M KNO_3.

Through this technique resulting current can be measured by cycling the potential of an electrode, suspended in non-stirring solution. This electrode refers as WE and the potential is controlled versus RE e.g. Ag|AgCl, this controlling potential is termed as excitation signal. A voltammogram is obtained by displaying current on y-axis and potential on x-axis [61, 62].

2. MATERIALS AND EQUIPMENT

2.1 Materials

I. $FeCl_3 \cdot 6H_2O$
II. $K_3[Fe(CN_6)]$
III. KCl
IV. Glucose ($C_6H_{12}O_6$)
V. Acetone- (purity ≥ 99.8%)
VI. Concentrated laboratory dish cleaning solution 'MICRO-90'
VII. 25% glutaraldehyde solution
VIII. Distilled water to prepare solutions- cleaned by Milli Q-plius-Millipore system (USA)
IX. Glucose oxidase- from ROTH (Karlsruhe, Germany)
X. NaH_2PO_4
XI. KOH

2.2 Solutions

Following solutions were prepared for the investigation:

I. 1 mM solution of $FeCl_3$
II. 1mM solution of $K_3[Fe(CN_6)]$
III. 0.1M, 0.1 M HCl
IV. K^+ containing phosphate buffer with pH 7 (used during electrochemical investigations and regeneration of biosensor was prepared in 1L volumetric flask by dissolving KCl (0.1M) and NaH_2PO_4 (0.01M) into millipore water)
V. 1M solution of glucose
VI. 0.2M solution of Vitamin C
VII. 0.2M L-cysteine
VIII. 0.1M Paracetamol
IX. 0.02M Uric acid

2.3 Equipment for electrochemical activity

I. μAUTOLAB- potentiostat/galvanostat from ECO-Chemie (Utrecht, Netherlands)
II. Magnet and magnetic stirrer- Carl Roth GmbH + Co. KG (Karlsruhe, Germany)
III. HI83141 analog pH/mV/°C meter equipped with HI1230B electrode from Hanna Instruments (Bedfordview, Republic of South Africa)
IV. Electrochemical cell
V. Analytical balance
VI. Mechanical pipettes
VII. Eppendorf

2.4 Electrochemical cell

I. Working electrode: FTO glass of 1 mm thickness and 3.22cm^2 area (from SIGMA-ALDRICH (Munich, Germany))

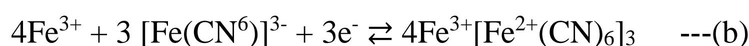
II. Reference electrode: Ag|AgCl|KCl electrode.

III. Counter Electrode: Titanium electrode.

3. METHOD AND INVESTIGATION

3.1 Deposition of PB on FTO

For electrochemical deposition with PB layer FTO-coated glass plate (glass/FTO) of 1 mm thickness was used as WE to construct the biosensor for this project. Before this deposition the surface of the glass/FTO plate was cleaned first by ultrasound as follow: (i) in 2% laboratory dish cleaning solution of 'micro-90' for 8 minutes, (ii) in acetone for 16 minutes, (iii) in deionized water for 16 minutes. When glass/FTO was cleaned, it was modified in the following sequence to deposit PB layer on it. An electrochemical cell was taken for this step, and the glass/FTO plate was hanged in an electrochemical cell containing 1mM of $FeCl_3$, 1mM of $K_3[Fe(CN)_6]$, 0.1M of HCl solution, by applying 40 potential cycles in cycling range between +0.4V and +0.8V at a scan rate of 40.0mVs^{-1}. This electrochemical deposition of PB caused by the reduction of Fe^{3+} and can be described in the equation (b):



$$4Fe^{3+} + 3\,[Fe(CN^{6})]^{3-} + 3e^{-} \rightleftarrows 4Fe^{3+}[Fe^{2+}(CN)_6]_3 \quad \text{---(b)}$$

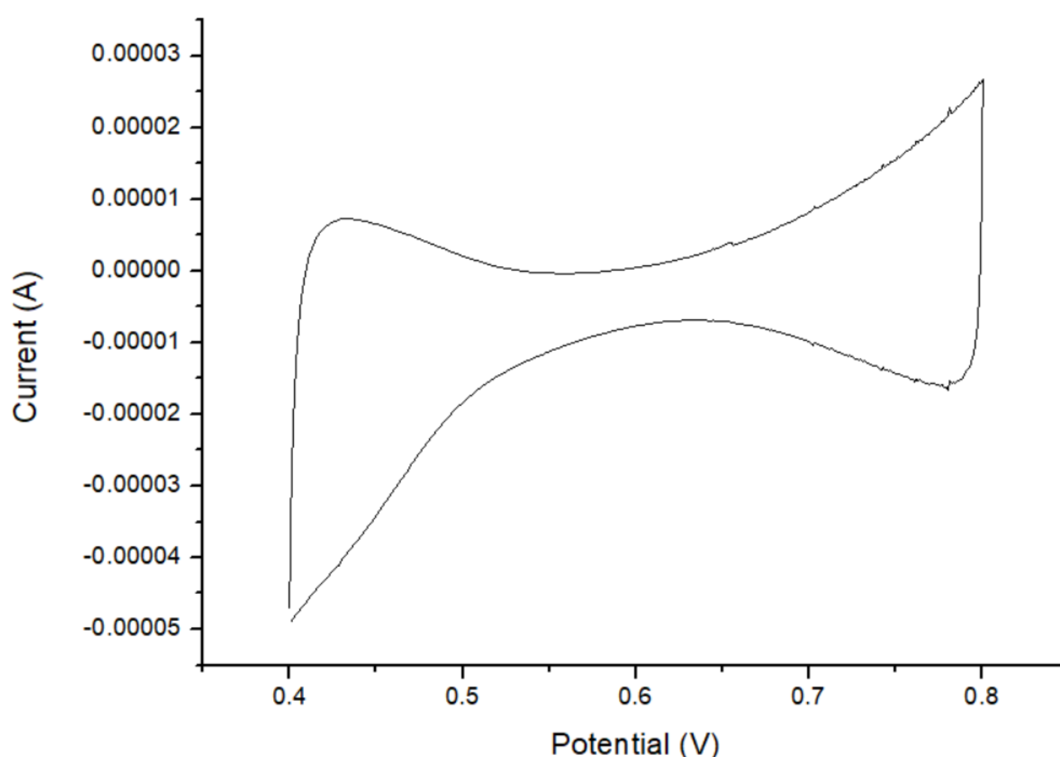
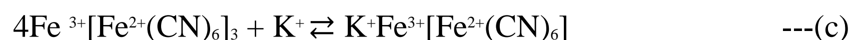


Fig. 11. Cyclic voltammogram of Prussian Blue deposition on (glass/FTO/PB) electrode, 1mM of $FeCl_3$, 1 mM of $K_3[Fe\ (CN)_6]$, 0.1 M of HCl, scan rate of 40 mVs^{-1}.

3.2 Stabilization of glass/FTO/PB biosensor

After the completion of this step, PB layer on the glass/FTO was electrochemically stabilized in 0.1M KCl and 0.1M HCl by applying 20 potential cycles, the potential cycling range was between -0.05V to +0.40V and the scan rate was 40.0mVs^{-1}. The electrochemical stabilization of PB in KCl solution occurs due to K^+ insertion to the crystal lattice of PB, this allows PB to be reversibly oxidized and reduced multiple times as follow (c):



$$4Fe^{3+}[Fe^{2+}(CN)_6]_3 + K^+ \rightleftarrows K^+Fe^{3+}[Fe^{2+}(CN)_6] \qquad \text{---(c)}$$

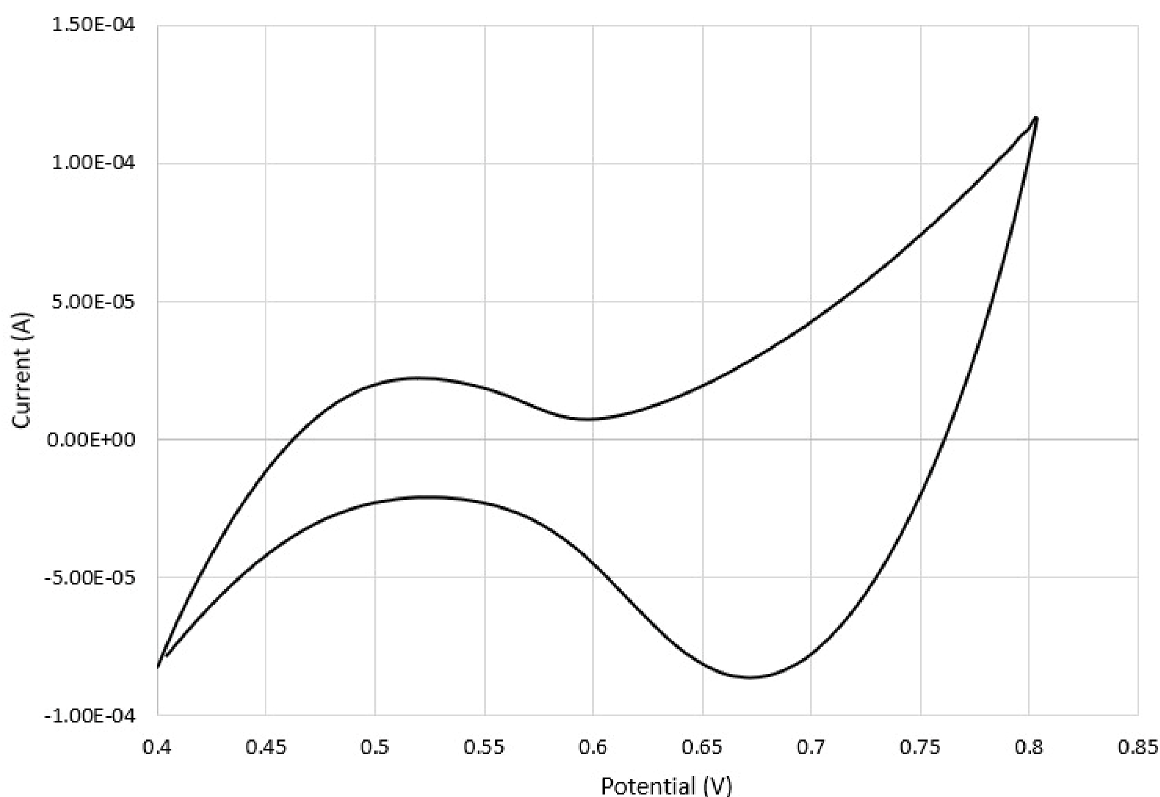


Fig. 12. Cyclic voltammogram for the stabilization of Prussian Blue modified (glass/FTO/PB) electrode, 0.1M KCl, 40mVs^{-1}.

3.3 Immobilization of Glucose Oxidase on Glass/FTO/PB electrode

5 mg/mL glucose oxidase solution was prepared by dissolving oxidase in the K+ containing phosphate buffer with pH 7 (0.1M KCl and 0.01M NaH_2PO_4) this solution was used for the immobilization of enzyme on glass/FTO/PB electrode. 10 µL of this solution was equally dispersed on the surface of 0.7 cm^2 geometric area of glass/FTO/PB electrode and then the deposited enzyme solution was dried at room temperature, glass/FTO/PB electrode with dried enzyme solution on its conductive surface was incubated above the 25% solution of glutaraldehyde for 15 min to cross-link glucose oxidase. The glass/FTO/PB/GO_X (glucose-oxidase) biosensor was designed.

3.4 Electrochemical investigation of Glass/FTO/PB-glucose oxidase Biosensor

Electrochemical investigation was done by using chronoamperometry, in this method glass/FTO/PB-glucose oxidase biosensor was immersed into electrochemical cell containing 10ml of K^+ containing buffer solution (pH 7, 0.1M KCl and 0.01M NaH_2PO_4) along with the magnet, the electrochemical cell was placed above the magnetic stirrer (the purpose of the stirrer was to continuously stir the solution after adding the samples). After setting the cell with the RE and counter electrode, constant potential of 0.05V was applied to the WE (glass/FTO/PB/GO_X biosensor) to measure current over time by adding analyte solution (glucose, ascorbic acid, l-cysteine, uric acid and paracetamol). Addition of analyte to buffer solution was done once the value of current became constant after initiating the experiment, every time when the current became constant. The glass/FTO/PB/GO_X Biosensor was investigated in the following two methods:

i. The glass/FTO/PB/GO_X Biosensor was investigated separately with all the analytes (glucose, ascorbic acid, l-cysteine, uric acid and paracetamol) one by one in pure.
ii. buffer solution.
iii. In second method the glass/FTO/PB/GO_X Biosensor was investigated by using different concentrations of glucose into pure buffer solution along with 10 µl of other investigating materials (ascorbic acid, l-cysteine, uric acid and paracetamol)

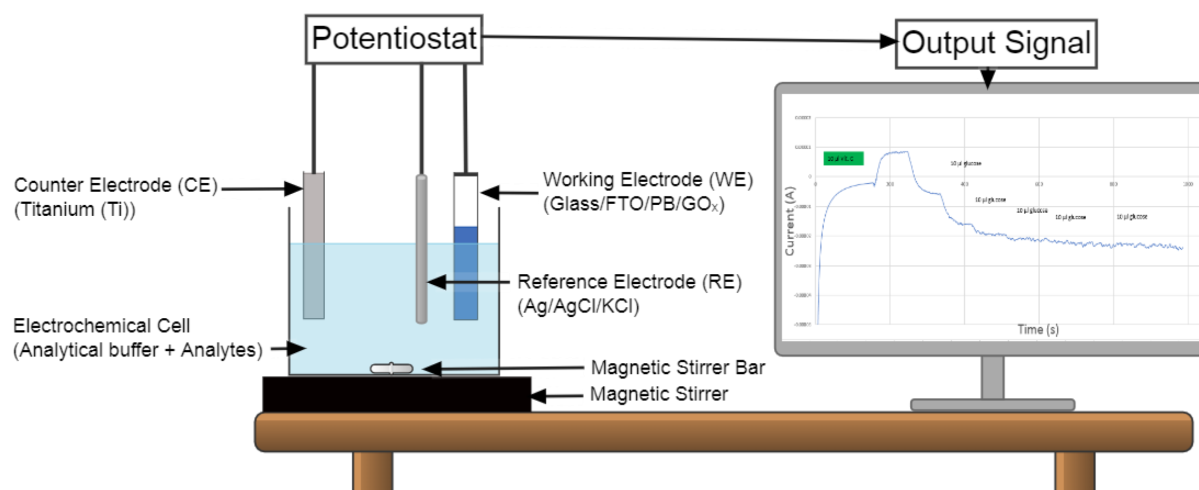


Fig. 13. Diagrammatic representation of the glass/FTO/PB/GO_X biosensor experiment.

The value of the change in current was noted every time after adding the analyte to evaluate the calibration curve, and when the current became constant another concentration of investigating material was added, this addition of analytes was continued until the glass/FTO/PB/GO_X Biosensor stopped giving response. And the chronoamperogram for each experiment was recorded for each of the experiment for further evaluation.

4. RESULTS AND DISCUSSION

4.1 First method – Investigation with ascorbic acid, l-cysteine, uric acid and paracetamol (without glucose)

4.1.1 Investigation with ascorbic acid

When the glass/FTO/PB/GO_X Biosensor was examined by adding 10µl of ascorbic at different intervals with constant stirring, after the addition of every concentration of ascorbic acid a change in current was observed, this response was measured by µAUTOLAB potentiostat and it can be seen in the chronoamperogram below.

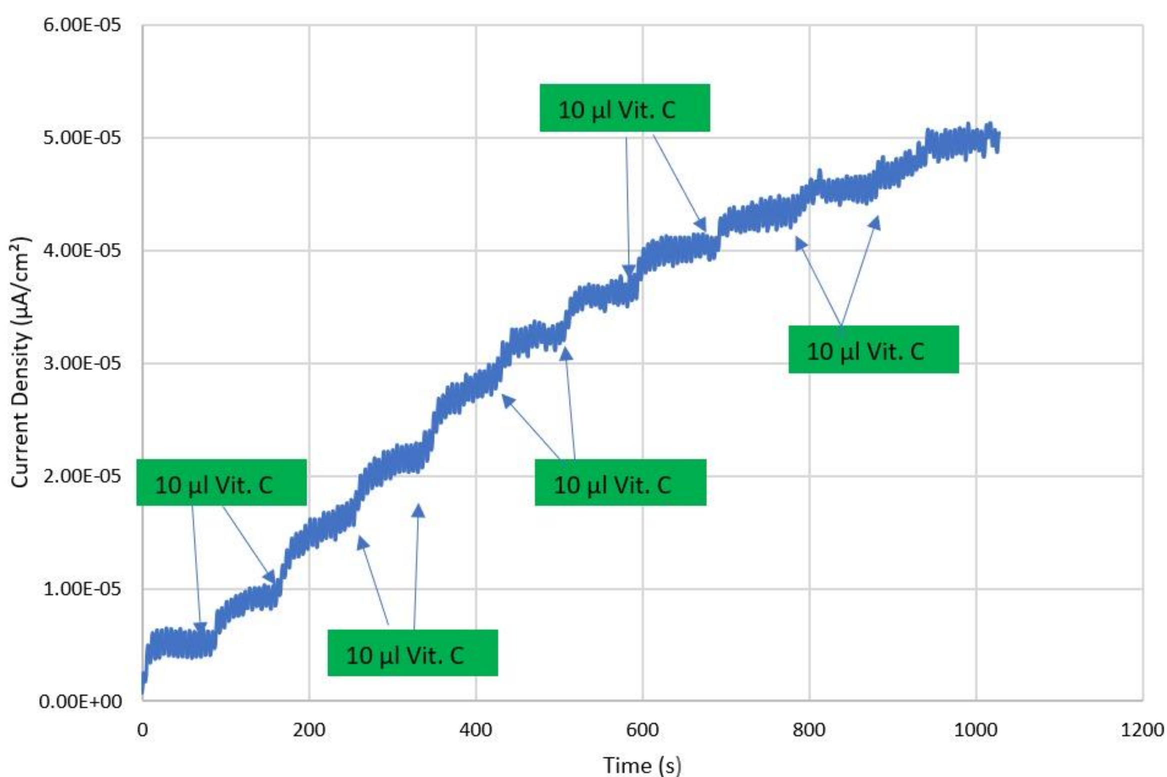


Fig. 14. Chronoamperogram of the experiment of Vitamin C. with glass/FTO/PB/GO_X biosensor against time and current density.

It can be clearly seen from the above graph that after the addition of ascorbic acid every time, the value of current increases. This change in current was due to oxidation of ascorbic acid by the glass/FTO/PB/GO_X biosensor.

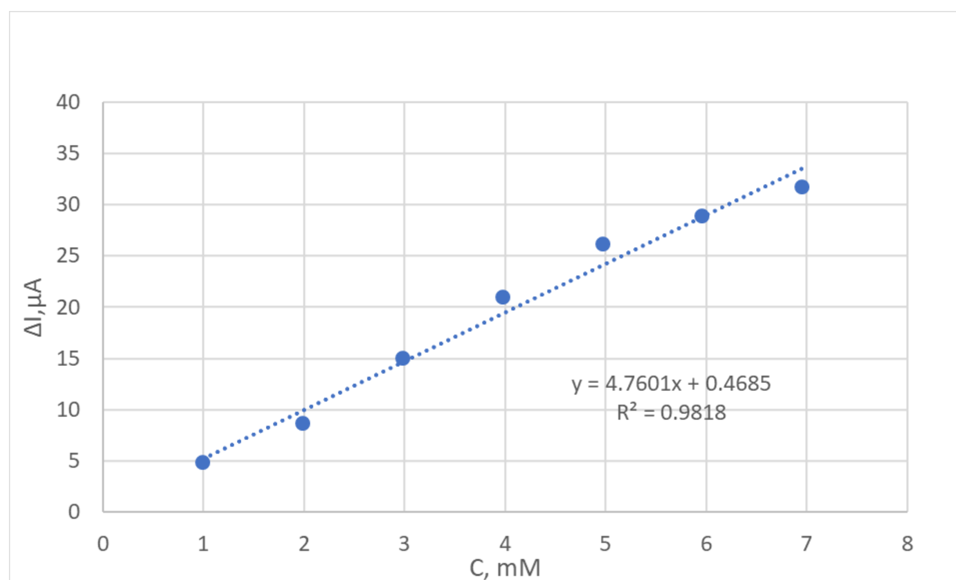


Fig. 15. Calibration curve of different concentration of Vitamin. C with the glass/FTO/PB/GO_X biosensor showing linear dependency between change in current (ΔI) on concentration (C).

The calibration curve further evaluates the sensitivity of glass/FTO/PB/GO_X biosensor and dependency against change in current (ΔI) on concentration (C). The sensitivity of the glass/FTO/PB/GO_X biosensor was 4.7601µA/mM for the ascorbic acid and it showed linear dependency ($R^2 = 0.9818$) between change in current (ΔI) and concentration (C).

4.1.2 Investigation with L-cysteine

During second investigation the glass/FTO/PB/GO_X Biosensor was examined by adding 10µl of L-cysteine at different intervals (every time when the value of the current became constant), the response of the glass/FTO/PB/GO_X Biosensor towards L-cysteine was also noticed by observing change in current due to the oxidation of L-cysteine by glass/FTO/PB/GO_X biosensor, this response can also be seen in the chronoamperogram below.

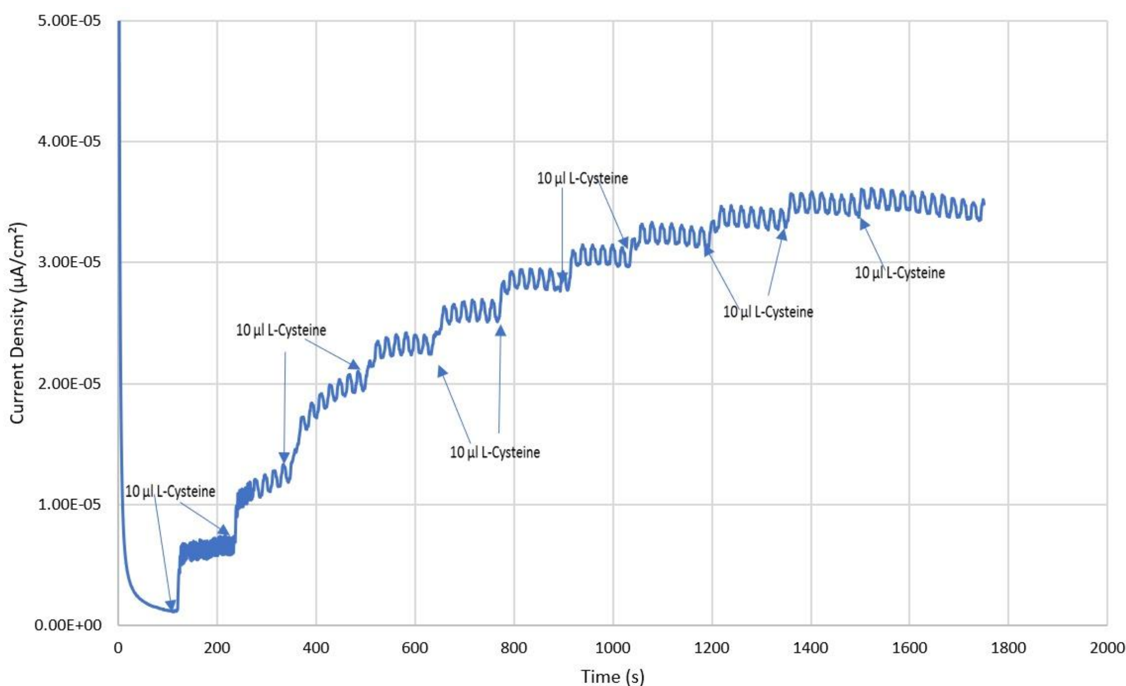


Fig. 16. Amperogram of the experiment of L-cysteine with glass/FTO/PB/GO_X biosensor.

It can be observed from the graph that every time after the addition of L-cysteine to the solution, the value of current changes, and the glass/FTO/PB/GO_X biosensor gives response upon the addition of L-cysteine by increasing the value of current. This change in current was also due to oxidation of L-cysteine by the glass/FTO/PB/GO_X biosensor.

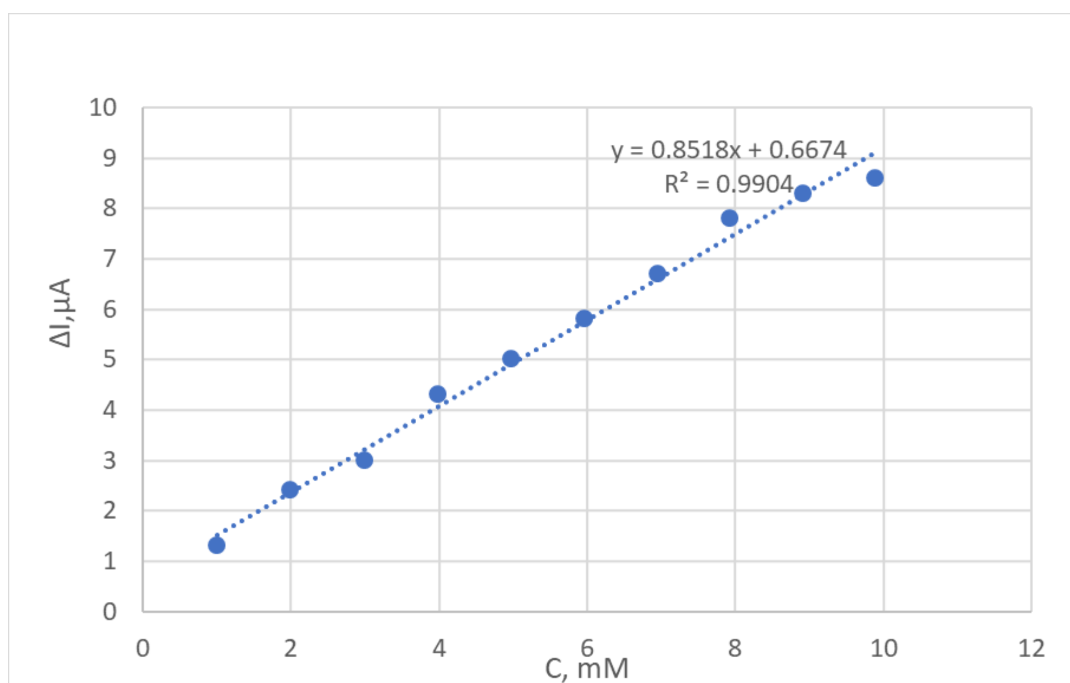


Fig. 17. Calibration curve of different concentration of L-Cysteine with the glass/FTO/PB/GO_X biosensor showing linear dependency of change in current on concentration.

The sensitivity of glass/FTO/PB/GO_X biosensor towards L-cysteine was about 0.8518μA/mM, and it showed linear dependency ($R^2 = 0.9904$) between change in current (ΔI) on concentration (C).

4.1.3 Investigation with paracetamol

Same technique was followed to investigate the impact of different concentrations of paracetamol towards the glass/FTO/PB/GO_X biosensor. The glass/FTO/PB/GO_X biosensor were remained nonreactive towards the paracetamol, this can be seen from the amperograms below.

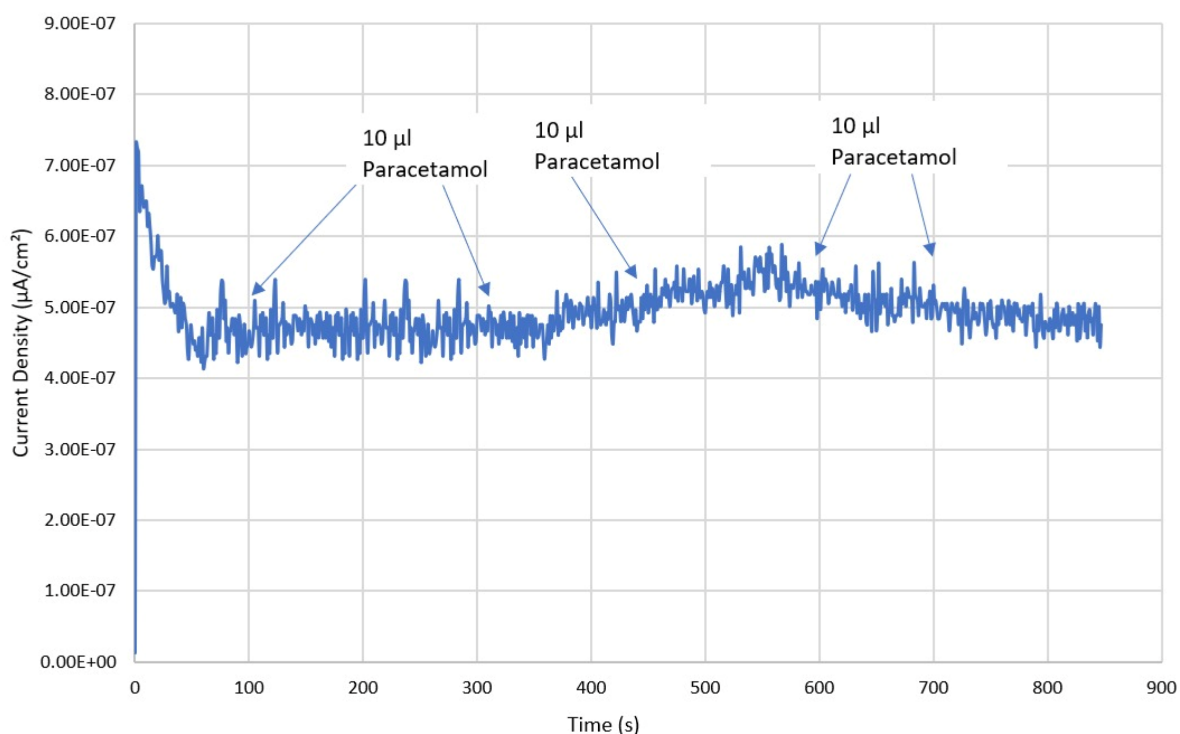


Fig. 18. Amperogram of the experiment of Paracetamol with glass/FTO/PB/GO_X biosensor.

It is clear from the graph that upon adding paracetamol solution to the cell no change in current was observed and the glass/FTO/PB/GO_X biosensor showed no response towards the sensor.

4.1.4 Investigation with uric acid

The glass/FTO/PB/GO_X biosensor was non-responsive towards uric acid, when it was treated with different concentrations of uric acid in different intervals of time, and no change in current was observed. The graph between the time and change in current density can be seen below.

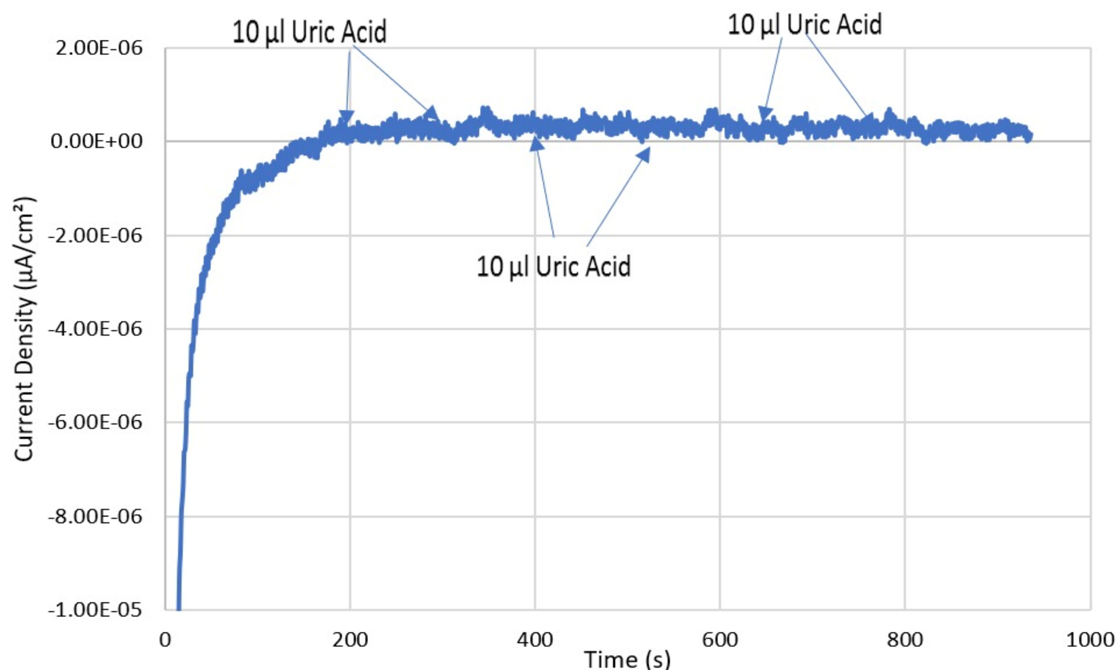


Fig. 19. Amperogram of the experiment of Uric acid with glass/FTO/PB/GO_x biosensor.

4.2 Second method – Investigation with ascorbic acid and l-cysteine (with glucose)

In the second way the glass/FTO/PB/GO_x biosensor was investigated with glucose and solutions containing glucose and interfering materials from the first investigated method (ascorbic acid and l-cysteine).

4.2.1 Investigation with glucose

When the glass/FTO/PB/GO_x was treated with glucose it gave response to the addition of different concentrations of glucose by showing decrease in current. This change in current was due to the reduction of H_2O_2. The addition of glucose was continued until the glass/FTO/PB/GO_x stopped responding to further addition. This investigation was done by constant stirring. The response of the glass/FTO/PB/GO_X biosensor was examined for period of 2 weeks to observe the effect of time on the sensitivity of the glass/FTO/PB/GO_X biosensor. This glass/FTO/PB/GO_X biosensor showed 50% response even after 2 weeks of its construction. The amperogram of this investigation was obtained between time and change in current density which can be depicted as follows.

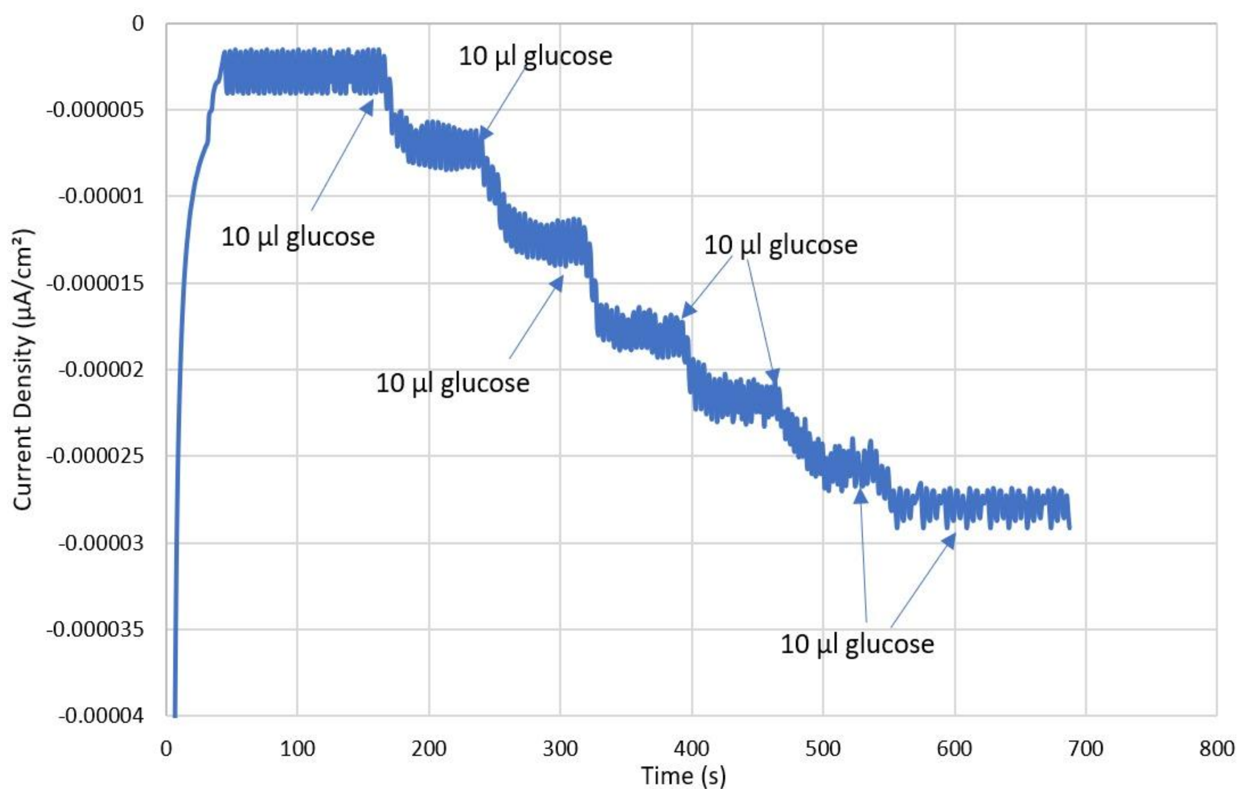


Fig. 20. Amperogram of the experiment of glucose with glass/FTO/PB/GO_X biosensor.

4.2.2 Investigation with Ascorbic Acid (Vitamin C) and glucose

For further evaluation the glass/FTO/PB/GO_X biosensor was investigated by adding 10µl of ascorbic acid in pure buffer solution and constant stirring was used. It was observed that upon the addition of ascorbic acid, the value of current increases suddenly which explains that the glass/FTO/PB/GO_X biosensor was responsive towards it. After the current became constant, the addition of glucose was started. Initially, the glass/FTO/PB/GO_X biosensor was responsive towards the concentrations of glucose. This response stopped after the addition of fourth concentration of glucose. The amperogram of this investigation was obtained between time and change in current density which is given below.

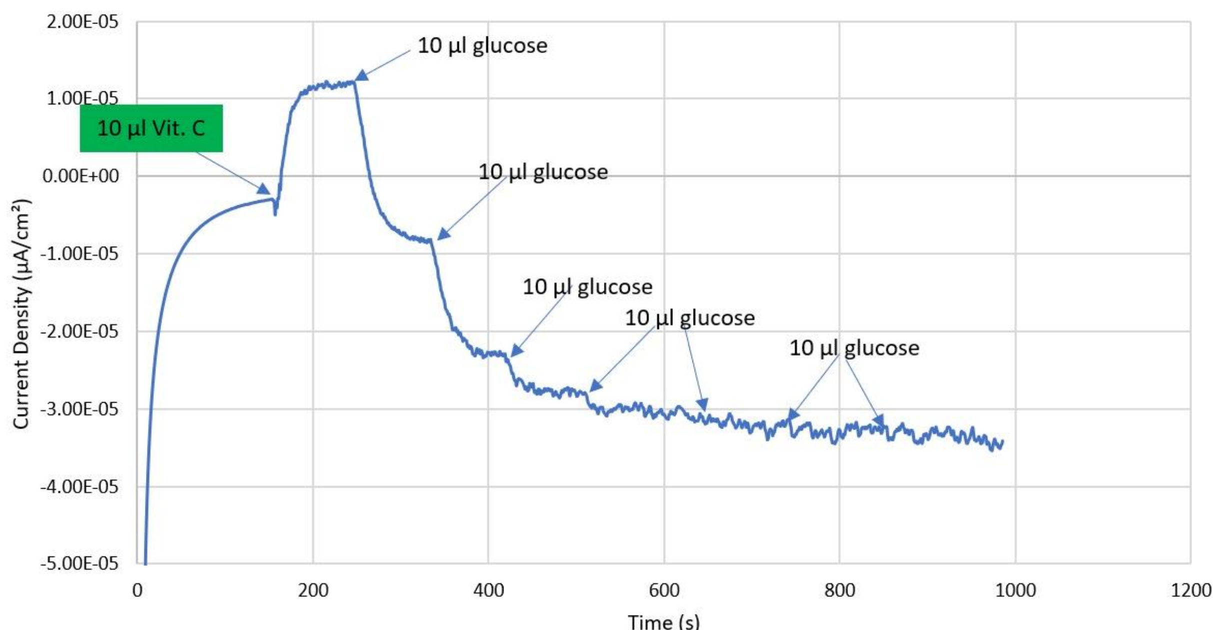


Fig. 21. Amperogram of the experiment of pure buffer solution + Vitamin C. and different conc. Of glucose with glass/FTO/PB/GO_X biosensor.

4.2.3 Investigation with L-cysteine and glucose

L-cysteine was added in the beginning of the experiment into the buffer solution with constant stirring. The value of current increased after the addition of L-cysteine which confirms the reactivity of the glass/FTO/PB/GO_X biosensor. When the current became constant, addition of 10µl of glucose was started and continued until the biosensor stopped giving response. The amperogram between time and change in current density is given below.

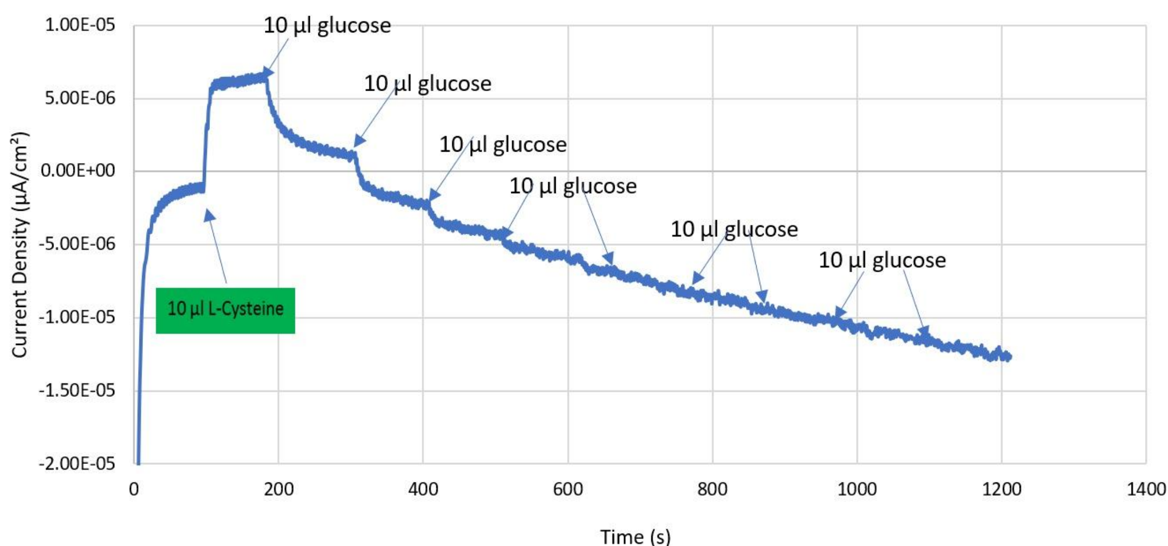


Fig. 22. Amperogram of the experiment of pure buffer solution + L-cysteine and different conc. Of glucose with glass/FTO/PB/GO_X biosensor.

4.2.4 Evaluation by using the calibration curves

The calibration curves were obtained by plotting the concentration of glucose on x-axis and change in current on y-axis for the above three experiments, which are depicted below.

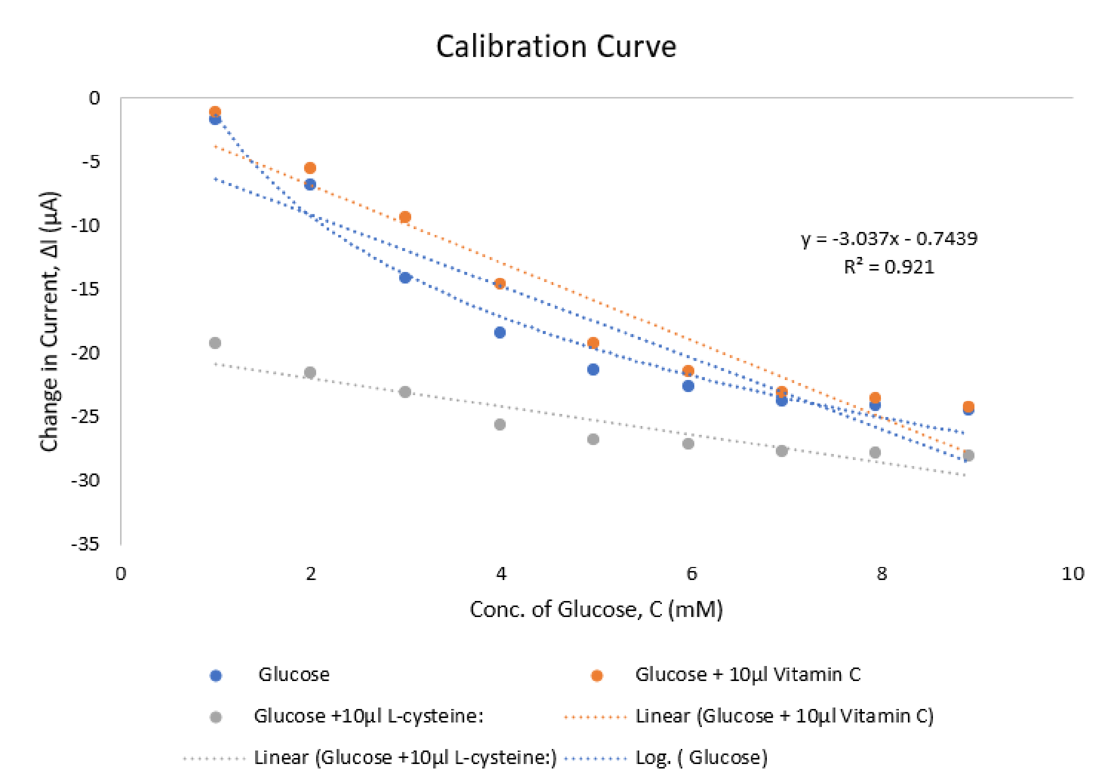


Fig. 23. Calibration curves of the investigation of glucose, different concentrations of glucose + 10µl of ascorbic acid (Vitamin C.) and different concentrations of glucose + 10µl of l-cysteine with glass/FTO/PB/GO_x biosensor.

It can be seen from the calibration curve that the glass/FTO/PB/GO_x biosensor showed linear dependency ($R^2 = 0.9$) between change in current (ΔI) and varying concentration range of glucose, ascorbic acid and uric acid in almost all the experiments. On the addition of ascorbic acid an l-cysteine the glass/FTO/PB/GO_x biosensor showed response by increasing current by reducing H_2O_2.

CONCLUSION

1. Electrochemical biosensors are major tools for the investigation of glucose. Therefore, PB can be used as a transducer for the constructions of such sensors. Amperometric techniques plays an important role in the investigation of the sensitivity of enzyme-based biosensor.

2. The interfering substances present in the blood were studied thoroughly in this research project. The main idea was to investigate the effect of sensitivity on the glass/FTO/PB/GO_X biosensor caused by ascorbic acid, L-cysteine, uric acid and paracetamol. The glass/FTO/PB/GO_X biosensor was treated separately with all the above-mentioned substances. The substances which showed interference with the glass/FTO/PB/GO_X biosensor were treated with glucose to co-study the interference. It was seen that among the above mentioned four substances only ascorbic acid and L-cysteine were found reactive towards the glass/FTO/PB/GO_X biosensor.

3. An interesting part of the investigation was observed when the glass/FTO/PB/GO_X biosensor was treated with ascorbic acid (without glucose). It showed a rise in the value of current every time after the addition of new concentration which depicts that the glass/FTO/PB/GO_X biosensors reactive to ascorbic acid. The sensitivity of the glass/FTO/PB/GO_X biosensor was 4.7601µA/mM. Upon drawing the calibration curve, a linear dependency ($R^2 = 0.9818$) was seen between change in current and concentration.

4. When the glass/FTO/PB/GO_X biosensors was treated with L-cysteine rise in the value of current was also observed. So, it can be evaluated that the glass/FTO/PB/GO_X biosensors faces interference due to the presence of L-cysteine in the solution. The overall sensitivity of the glass/FTO/PB/GO_X biosensors was 0.8518µA/mM. L-cysteine also shows linear dependency ($R^2 = 0.9904$) between change in current (ΔI) on concentration (C). Uric acid and paracetamol caused no interference to the glass/FTO/PB/GO_X biosensors if present in the solution.

5. The influence of ascorbic acid and L-cysteine with glucose were carried out for further evaluation. For this, the electrochemical cell was added with 10µl of interfering substances and different concentrations of glucose were followed. The glass/FTO/PB/GO_X biosensor showed linear dependency ($R^2 = 0.9$) between change in current (ΔI) and varying concentration range of glucose, ascorbic acid and uric acid.

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SUMMARY

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Influence of Pharmaceutical Materials on the Sensitivity of Prussian Blue-Based Sensors

Enzyme based electrochemical biosensor are very important for the diagnosis of body fluids. The need of implantable biosensors for the detection of glucose in the body is becoming crucial day by day. The major problem in the detection of blood glucose is caused by the interfering substances present in the blood.

Another problem is related with the choice of electrode used in previous researches. Despite of increasing the response current, the electrodes have poor electrochemical conductivity, instable operational ability and the cost is much high. Therefore, a project was designed to continue the research for the investigation of the effect of interfering substances on the sensitivity of enzyme-based biosensor for the detection of glucose.

To make enzyme-based glucose biosensor PB was used as a transducer. The electrochemical deposition was done by CV on FTO. It was followed by the dispersion of GO_X on the glass/FTO/PB. After the dispersion of enzyme, it was cross linked with 25% glutaraldehyde solution and glass/FTO/PB/GO_X biosensor was constructed.

In this research project four substances along with glucose were examined to see their influence on the sensitivity of the glass/FTO/PB/GO_X biosensors. These substances were ascorbic acid, L-cysteine, uric acid and paracetamol. Among above-mentioned substances ascorbic acid and L-cysteine were found reactive towards the glass/FTO/PB/GO_X biosensor, while uric acid and paracetamol showed no reactivity.

In future, using the information from this project, further investigation can be done to modify PB based biosensor for the identification of glucose. So, it can be use in practical implantation as a diagnostic tool in diabetes.

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A lot of help from the other people is required in a good project report, and this one too does not stand out as an exception. Thus, I extend my open and intense thanks to all those people who encourage me to start this research project and took their valuable time to help me in this task.

Before anyone else I would like to articulate my heartfelt thanks to my project supervisor and Associate Professor Aušra Valiūnienė. She bestowed me with lively and continuous interest from her part. This proved a great motivational force and encouragement for me to get boldly into an unexplored area before me.

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