T.C.
FATIH UNIVERSITY
INSTITUTE OF BIOMEDICAL ENGINEERING

THE DEVELOPMENT OF AMPEROMETRIC UREA BIOSENSOR BASED ON DENDRIMER MODIFIED ELECTRODES

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MSc. THESIS
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THESIS ADVISOR
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T.C.
FATİH ÜNİVESİTESİ
BİYOMEDİKAL MÜHENDİSLİK ENSTİTÜSÜ

DENDRİMER MODİFİYE ELEKTROT BAZLI AMPEROMETRİK ÜRE BİYOSENSÖR GELİŞTİRİMİ

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YÜKSEK LİSANS
BİYOMEDİKAL MÜHENDİSLİĞİ PROGRAMI

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Complete and wholehearted fidelity to GOD ALMIGHT, Father of our LORD JESUS CHRIST, sponsor of my life and to the “Memory Bearing the Name, the Late Mrs. KKORTO PAWEOH NYANGWEBAH”, Woman of Africa, Woman of the Tropical Forest (Warloyea-Woela) and Mother of the St. Paul River Bank.

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May 27, 2016 Jackson Narkpa NYANGWEBAH

(Researcher)

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ABBREVIATIONS

CV : Cyclic voltammogram

DNA/RNA: Deoxyribonucleic acid/Ribonucleic acid

Fc: Ferrocene

FAD : Flavin Adenine Dinucleotide

G : Generation Deoxyribonucleic acid

HCO_3: Carbonate

M: Molar

mol: Mole

CNTs: Carbon NanoTubes

NH_4: Ammonium

PAMAM: Polyamidoamine

pH: Potential Hydrogen

PBS: Phosphate Buffer Saline

Ur: Urease

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SUMMARY

THE DEVELOPMENT OF AMPEROMETRIC UREA BIOSENSOR BASED ON DENDRIMER MODIFIED ELECTRODES

Jackson Narkpa NYANGWEBAH

Biomedical Engineering Programme
MSc. Thesis

Advisor: Assoc. Prof. Dr. Mehmet SENEL

This study demontrates construction of the amperometric urea biosensors based on ferrocene polyamidoamine dendrimer with various generations (G1, G2 and G3) and carbon nano tubes (CNT). Pencile graphite electrode (PGE) has been modified by simple droping of the Fc-PAMAM (G1 to G3) and Fc-PAMAM (G3)/CNT nanocomposite, after which Urease enzyme was immobilized. In comparison of Fc PAMAM electrodes, G3 electrode showed best result, after which it was combined with CNT. Fc-PAMAM/CNT urea based biosensor showed very good selectivity towards urease with the detection limit of 0.05 mM, linear range of 0.2 to 1.65 mM, sensitivity of 1.15µA/mM and response time of 4s. In order to check for selectivity and reliability of the proposed biosensor analytical recover and real sample, the human blood analysis was conducted. The real sample investigation of the developed amperometric urea biosensor was held from the serum collected from human blood samples, with recovery of 95.8 to 103.3 %.

Keywords: Amperometric, Urea, Urease, Immobilization, Ferrocene, plyamidoamine

ÖZET

DENDRİMER MODİFİYE ELEKTROT BAZLI AMPEROMETRİK ÜRE BİYOSENSÖR GELİŞTİRİMİ

Jackson Narkpa NYANGWEBAH

Biyomedikal Mühendisliği Programı

Yüksek Lisans

Danışman: Doç. Dr. Mehmet Şenel

Belirtilen çalışma, ferrosen merkezli poliamidoamid dendrimerlerin 3 jenerasyon (Fc-PAMAM G1, G2 ve G3) türevleri ile çok duvarlı karbon nanotüp (KNT) bazlı oluşturulan amperometrik üre biyosensörlerini tanıtmaktadır. Kalem grafit elektrotları tek adımda basit damlama yöntemi kullanılarak münferit bir şekilde Fc-PAMAM(G1, G2 ve G3) ve Fc-PAMAM G3/KNT nanokompozit ile medifiye edildikten sonra üreaz enziminin yüzeye bağlanması sağlanmıştır. Diğer jenerasyonlarla kıyaslandığında Fc-PAMAM G3 elektrodunun en iyi performansı sergilemesinden dolayı, KNT ile bu jenerasyonda çalışılmıştır. Üreaz modifiye edilmiş Fc-PAMAM G3/KNT (Fc-PAMAM G3/KNT/Üreaz) biyoelektrodu 0.05 mM tespit limiti, 0.2-1.65 mM lineer aralığı, 1.15uA/mM hassasiyeti ve 4 s yanıt süresi gibi performans kriterleriyle çok iyi sonuçlar göstermiştir. Seçicilik ve güvenirlilik gibi özelliklerin sorgulanması için analitik geri kazanım ve kan numunelerinde ölçümler gerçekleştirilmiştir. Önerilen amperometrik üre biyosensörlerle yapılan insan kanındaki üre miktarının ölçüm sonuçlarına göre biyoelektrot, lineer aralığında %95-107 arasında geri kazanım göstermiştir.

Anahtar kelimeler: Amperometrik, Üre, Üreaz, İmmobilizasyon, Ferrosen, poliamidoamid.

CHAPTER 1

INTRODUCTION

1.1 Purpose of the Thesis

Urea is the bye-product of an amino acids production in the cytosol and the mitochondrial of the liver cells ordefined as the residues of protein breakdown products in most terrestrial mammals. Being a residue, it produces ammonia, which is a toxic substance for liver and kidney cells, therefore; the elevation of urea in human needs to be accurately and consistantly evaluated for the prevention of vital organ disease[1].The human systems function in a normal range of urea that present in the serum about 15 to 40 mg/dL(5 to 20 mg/dl, or 1.8 to 7.1 mmol urea per liter).On this note, the hyper-urea concentration in human lead to many body alnormaolities and medical dieases, such as the shrinking cells, tissues or organs due to the insufficient water,the digestive tract bleeding, cirrhosis, kidney damage and painful sensations in the body. And the hypo-urea level in the blood results in the reduction of body vitality and strength and form kidney stone [2]. Generally, there have been many laboratory detective methods used for the evaluation of this liver enzyme bye-product, urea in both clinical and research diagnoses. Despite of the prolong usage of their test establishment, the analysis and result process take lots of time and even as it's relate to method complexities. Meanwhile its alternative, the biosensors do creat numerous of benefits today to both clinical and research purposes, like the miniaturization of devices and their capability ofsignals data results [3]. Despite of variety of transducers that have been produced for urea evaluation, a novel urea biosensor is now proposed, that's based on ferrocene polyamidoamine dendrimer modified pencil graphite electrode. This nano amperometric urea biosensor meanly focuses on the evaluationof urea at a very low and at the initial elevation of urea level in the body having being constructed; this biosensor will use urease to catalyze the composition of urea in electrochemical environment. As it is proposed, the incorporation of carbon nanocomposites on the conducting polymer

Film gives the sensor viability, accurate result production, short processing time and more over less expensive for production.

1.2 Literature Survey

Urea defined asa by-product of proteins metabolism in the liver during the kerbs cycle.It is known as a protein residues in the body, which need to be monitored due to the related toxiccharacteristics associated to human body physiology. Due to its toxic(ammoniun) feature, which affects vital body organs(kidney, liver, etc.), the demands for the medical attention is required[4]. In clinical approach, urea evaluations are of vital importance in medical and biomedical settings to determine its biological concentration. Its high or low concentration provides health information for the organ (kidney)excretion orhepatotoxinproblems[5]. The normal urea level in blood serum ranges from 7 to 20 mg/dL (15 to 40mg/dL) (2.5 to 7.5mmol/L), where the elevationof its amount leads tohealth issues, such as acute or excretory bladder blockage, with shock, burns, fluid lost, and gastrointestinal bleeding. However, a reductionof urea contentleads tocirrhosis, nephritic syndrome and the reduction in body vitality and strength [6, 7].

Biosensors are defined as a converting instrucment which works with biorecognition elements (enzymes, nucleic acid (DNA or RNA), microorganisms, whole cells, antibodies) or biochemicaland electrochemicalpropared substance and physicochemical transducer to translates a biological signal into a measureable or counting signal.

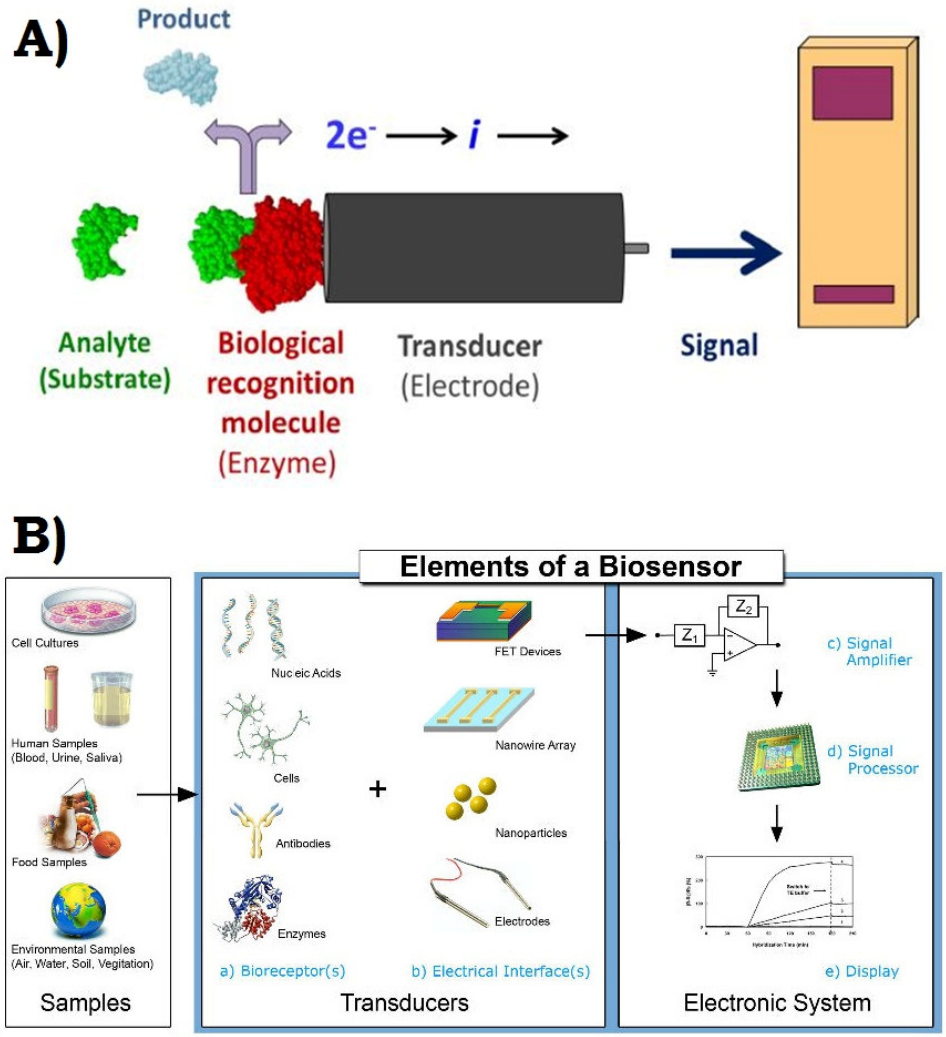

Figure 1.2 (**A)** presents the amperometric biosensing components [8],(**B)** shows various biosensor elements, sample, transducers and electronics system [9].

Amperometryis electrochemical method that conventionally uses the three electrodes configuration. In its method, a fixed working current is set with the modified electrode and the reference electrode, whichcorrelate bioelectrical signal,which is equivalent to the substrate composition present. And the signal is measured in the function of the modified electrode and measuring electrode. In the amperometric determination, the current signal is providedfrom the reaction of Redox[10]. As an example polyaniline–Nafion compoundwasprepared tofabricate amperometric urea sensor, which depends on polyaniline–perfluorosulfonated ionomer composite

electrodes, where the enzyme wasimmobilized to the constructed matrix (film). Due to this electrochemical composition, they carried out system modifying process and dropped coating. It provedthe biosensor sensitivity of the compound modified on the working electrode, showingdropped coating procedure as superior over the system modifying process[11]. The major purposes of the amperometric applications are rooted on the ability of the electron conduction from the active site of the recognition element tosensor within working of the transferrer (mediator) the endurance of the fixed applied potential. Urea biosensor is an example of amperometric instrument, whichconducts the breaking down of urea to HCO_3 and NH_4 [12]. Due to this process, urease converte the substrate(urea) into HCO_3 and NH_4substance, which are further liberated in the Krebs cycle[5]. Different biosensor matrices areevaluated for their inert with different functional groups to establish smooth redox reaction between substrate and biomolecule to produce electrochemical signals and the ability to amplify working signal to be desynchronized from interfering biological or chemical species signals [13].A number of well-established traditional methods have been used in determining urea elevation in human serum.Such as; spectroscopic, colorimetric, and the liquid high performance chrotomagraphy techniques [11].With the incorporations of synthesized chemical types, biorecognition elements and electrochemical matries,the amperometric method serves as alternative to the tradition methods. The amperometric urea biosensors are categorized into three phases. In the first phase, the biomolecule is attached or embedded within the filmthatcan be fixed to the sensing device. The second phase brings about the surface or medium adsorption of covalent fixation of the biomolecule active site is embedded within thesensing platform and allows the degradation of the partial-permeable membrane. In third phase, the biomolecules are attached directlyto the sensors and electronic device amplifies the signal [14]. Therefore, the enzyme immobilization presentsa major support for the fabrication of analytical amperometric urea biosensors, providing a well defined function, possessing good experimental and good shelf life, highly detectable and owns real time characteristics [15].

1.3 Urea Detection Methods

In the amperometric biosensors measurement, the reduction and oxidation current is acquire from the reaction of the biorecognition element and the targeted analytes on the transducer within the solutions. Due to the setting of fix amperometric applied potential, electron transfer mechanism can be deduced by its interaction with the catalyst molecules, which conductions are mostly influenced by mediator or the conducting polymers[14]. Scientists have worked efficiently with biosensors due to the many advantages they offer, such as specificity, speed, miniaturization and low cost effect for various applications [11]. In current discoveries for point-of-care testing, military detection, clinical diagnosis, food quality technology, environmental and biomedical monitoring, amperometric enzymatic biosensors plays a very important part in solving many challenges that were faced within the experimental and diagnostic procedures of the traditional techniques [16]. Normal level of urea composition in the human serum do allow well systemic functions of the kidney, hepatic system and the normal functioning of the gastrointestinal organs [11]. Since urea measurement being of a vital interest, biomedical engineers, researchers and clinical laboratory techniques have critically evaluated the hyper-urea concentration variation, that states between 180mg/dl and 480mg /dl to have been requiredfor the dialysis of the blood to remove toxic substances[17],and sometime related to many health problems; such as acute or chronic urinary tract obstruction, shock [18], liver failure [19], precisely, the elevation of human serum urea leading toan increasein mortality of acute ischemic stroke patients[20].An elevation of blood urea concentrations have been associated with chronic uraemic encephalopathy, other in hyper ammonaemia, acidosis and impaired calcium homeostasis latter state [21],renal deficiency [22], burns, dehydration, and gastrointestinal bleeding and the hypo-urea concentration has been notified of leading to nephritic syndrome, and cachexia [3, 20].Conventionally, there have been several analytical techniques used to monitor urea concentrations in the human blood serum. But there are two techniques commonly used for urea analysis, which include traditional techniques and electrochemical techniques. The traditional techniques although precise, but characterized by common pitfalls, such as pretreatment procedures, method complexity, the high costs associated with experimental procedures

and the delay observe between test time and the treatment time, which takes several hours, days and even weeks [11].

1.3.1 High Performance Liquid Chromatography (HPLC)

The High Performance Liquid chromatography method appliction in the detection of urea is mainly based on quantification. This procedure strongly analyzed size of particles arranged in a given column. Due to its technical complexity, inadequate sensitivity and involvement of expertise, the high performance liquid chromatography method hasn't been used for real time detection [23, 24].

1.3.2 Colourimetric Techniques

The colorimetric technique is widely used in the detection of urea concentration in a solution with the help of a color reagents. This analytical technique deeds with the conversion of absorbent spectra of a color gradient differentiation in the experimental chamber and results are counted as product that is proportional to urea present in the sample[25].

1.3.3 Flourometry

The fluorimetric technique, this is an analytical procedure which deals with the identification of molecules by detecting and quantifying the beam in a wavelength of the ultraviolet light emitted during fluorescence. There are several complications in this flow system, where the system need to be pre-concentrated, the analyte pretreated twice most probably and the medium transported to the detector [26].

1.3.4 Piezoelectric crystal

Piezoelectrtric crystal is the technique that deals with electric-charges that are accumulated in a given sample materials to develop a response, which is applied to produce a mechanical stress measurable respone. In piezoelectric urea detection, urea is catalyzed in the presence of Urease giving ammonia and carbon dioxide as a bye-products. The products of the reaction in turn exert a certain pressure on the piezoelectric detector, which converts the mechanical pressure into electrical signal that is counted. Its drawback for real time diagnosis is that, it produces very low sensors output and its crystal sample are prone to fracture[27].

1.3.5 Mass Spectrophotometry

Mass spectrometric technique; a mass spectrometric technique is a chemo analytical technique that aid in identify the quantities and type of chemicals present in a sample by measuring the mass ratio and abundance of gaseous ions. Mass spectrometric technique works by ionizing chemical compounds to generate charged molecules when bombarding it with electron that causes it breaking into charged fragments, this technique needs retreatment of samples in many experimental designs to achieve data accuracy, good linearity curve and a very little uncertainty in the determination of urea in real sample [28, 29].

1.4 Electrochemical Determination of Urea

There are three basic enzymatic urea biosensor techniques that are widely used for urea detection. In electrochemical bioanalysis, these techniques are based on different modes of measurements. Their modes of measurements includes:

1.4.1 Enzymatic conductometry

The conductometric technique, Conductometric transducers as a biosensing devices were constructed by Watson et al.. this work consisted of an oxidizing silicon adhesive disk with serpentine, interlock gold electrode pairs on one dimensional surface arrangement[30]. Conductometric biosensor techniques are the measurement of conductance, capacitance and admittance from an ion charge produce in a solution between the electrodes. In this reaction, an alternating potential is applied between two inert electrodes producing a change in the ionic strength of the sample, which can be monitored by conductometric devices. Common pit falls of this technique; its biosensors are limited due to the ionic background for the use of clinical sample and its limitation of measuring small conductive change in a solution with high ionic strength, of which it producesnarrow dynamic range and has low sensitivity [31, 32].

1.4.2 Enzymatic potentiometry

Potentiometric mode of measurement, one of the commonly used techniques for urea detection that based on ion charge selective characteristics. In this technique, the current potential changes based on different charges that are set between the two electrodes, the indicator electrode and reference electrode. Based on the potential difference, the

constant half-cell potential is required from the reference electrode, while the indicator electrode provides changing potential for analyte specificity in solution. This potential change causes a detection low response and high influence of interference signal, instead of having a fixed steady state potential values [14, 33].

1.4.3 Enzymatic amperometry

Amperometric urea technique is the analytical electrochemical technique that applied in most biomedical/clinical research for blood serum analysis, which takes advantage of electroactive chemical species that are involve with oxidation and reduction reaction. Conventionally, there are three electrodes system in the electrochemical amperometric working procedure, which includes the working electrode, the reference electrode and the counter electrode. During its reaction, the fixed applied current is usually set between the working electrode and reference electrode and the steady state signal is produced between the working electrode and the counter electrode,which are then measured and regulated by counter electrode. Its steady-state current generates is fixed and is proportional to the concentration of analyte in the solution. Its common drawback is, the factor affecting the transfer of electron from the catalytic site of an enzyme to the electrode surface, where electron landing affinity measurement is held [14, 31, 34]. Most amperometric urea detection techniques in research and clinical laboratories are based on mediation/conducting polymers, which are widely used. Mediators or conducting polymers are used to enhance the speed, sensitivity and the versatility of the biosensor in a polymer containing solution for precise measurement of analyte concentration. Because of the different behavior pattern of conducting polymers, they are widely used to perform high electrochemical conductivity, minimize compound interference signals that have affinities to electrons experimental setup [35].Based on these unique, characteristics of conducting polymers, scientists manipulate conducting polymers to yield different results and to enhance the speed, sensitivity and broad scope for biosensor experiment or for diagnosis [14].

In this proposed document, the development of amperometric urea biosensor, we work with ferrocene, polyamidoamine dendrimer (Generations) andcarbon Nanotube to modify the pencil griphite electrode using the covalent immobilization technique for urease binding to polymer in the fabrication of urea biosensors. The Ferrocene-

Pamamserves as mediator,conducting polymer film. But it was studied that the Fc-PAMAMA film electrons conduction was slightly unstable due to the weak interaction between the polymer film and ureasein the research. The composite of multi-wallcarbon nanotube was added to improve the electrons transfer capacity on the biosensor. In this paper, the carbon Nanotube plays major role in the promotion of electron transfer from the oxidation and reduction reaction and covalently immobilization of the urease to the polymer film, as similar work reported in[4].With the use of ferrocene-Pamam, carbon nanotube composite and urease, the developed amperometric urea biosensorhad low detectedlimitto urea concentration. Therefore keeping the enzyme activity stable, the biosensor precisely, efficiently had shorter response time, fast sensitivity, good reusability and a good shelf-life.

CHAPTER 2

BIOSENSORS

From the centuries of medical development, agriculture and environmental detection, the traditional methods of pathogens and chemical diagnoses have been the detective methods, which suffer analytical accuracy, specificity and lost real time sensitivity. These traditional experimental techniques are widely used in clinical and research laboratories. Due to the many challenges the present traditional methods (cell culture, microscopic detection, DNA- based method) are confronted with, an alternative, biosensors that provide researchers high sensitivity, low cost, reproducibility, simple, accurate and affordable for individuals, homes, industries and medical facilities have been emerged[36].Due to the biosensor integration, the device has been categorized into three generations. In its earlier development, the biocatalyst was based on the binding to or the entrapment of bio-recognition substance into the membrane that is held to the transducer surface. Based on the trapping technique, the first generation emerged. Ronkainen et al.. 2010, a glucose biosensor constructed to trapped glucose oxidase which then further more oxidized β-D-glucose to β-D-gluconolactone, follow by the proceeding reduction of flavin adenine dinucleotide to flavin adenine dehydrogenase (FADH2). To produce hydrogen peroxide, an oxygen was added to the flavin adenine dehydrogenase and another flavin adenine dinucleotide (FAD) was formed. In this electrochemical setup, a fix applied voltage accelerated the oxidation with the hydrogen peroxide production on the electrode surface to elaborate electrical signal. However, the first generation biosensor fabrications were marked by pitfall, that is; the active site of the enzyme and the flavin functional group were hidden deep beneath the enzyme, which causes obstruction for an electrons transfer, because of the confinement of the electrons, a high potential was required to accelerate the movement of electrons generated from the oxidation hydrogen peroxide to the sensor surface, thereby inducing many reduction and oxidation reactive species that form part of the blood matrix composition that cause noisy signals(artifacts) or signal interface,

like the case of paracetamol, ascorbic acid, uric acid, organic or inorganic elements present in the blood fluids[8]. Due to these electrons confinement, the second generation stands to result some of the shortcomings of earlier generation by the incorporation of chemically synthesized mediators, which nowregulates the movements of electrons in most electrochemical applications. For second generation biosensor construction, the oxidizing mediator produces flavin adenine dinucleotide (FAD) with a continuous self-reduction. In the same manner, the mediator is produced on the surface of the electrode and inducing a measurable signal [37]. The mediator is synthesized and sometimes diluted into a phosphate buffer saline (PBS) solution or in an electrolyte solution to conduct a quantitative electrons transfer from the active site of biomolecules to the surface of the electrodes. With the use of commonly synthesized mediators, such as; polyvinylimidazole [38], polyvinylpyridine [39], ferrocene-polyamidoamine (Fc-PAMAM) [40] , polypyrrole [41], these mediators provide speedy electron transfer during redox reaction and induce the redox platform for enzymes and sensor response [37]. It was really means of a covalent adsorption ofthe active biorecognition element to the transducer surface and the conducting polymers more permeable to be easily attached. Then in its third generation, an amperometric enzyme based biosensors have been divided into two main groups, which are the mediated based biosensors and the non-mediated biosensors. The biosensors based on mediator, which the second generation biosensors are part, have been widely used in purpose of upgrading the sensitivity and reliability of the first generation biosensors. It has been observed that few mediating molecules cause sample contamination or leakage outting through electron transfer mechanism [42]. Based on the improvement of biosensor usability and performance of the second generation biosensors, the non-mediated or mediator-free amperometric enzyme based biosensors are discovered in finding remedy. The third-generation amperometric enzyme based biosensors have the abilities to directly transfer electrons from the oxidizing or reducing amino acid residues or proteins to the sensing electrodes [14, 43].

2.1 Biological Recognition Elements

Biological Recognition Elementsin the study of biosensor, there have been several others matrix materials used as bio-recognition elements, namely antibodies, DNA/ RNA, enzymes, whole cell or tissues. The major function of the bio-recognition elements is to react with the targeted analyte that can improve the biosensor selectivity, which occurs according to its analyte proportionalities in the working environment. Due to the increase threats that are posed in the agricultural sectors, health and environmental areas, these bio-recognition elements are used based on their specificity to construct biosensor for fast detection of pathogens involve with food spoilage in the agro-industries[44], that cause disease outbreak in the health sector and that produce toxins or gaseous odors in environmental pollution. Bio-recognition elements are one the main component for biosensor fabrication. Since they are too important, the selection of bio-recognition elements must be done in careful view of the biosensor specificity to a target analyte concentration in complex medium[45].

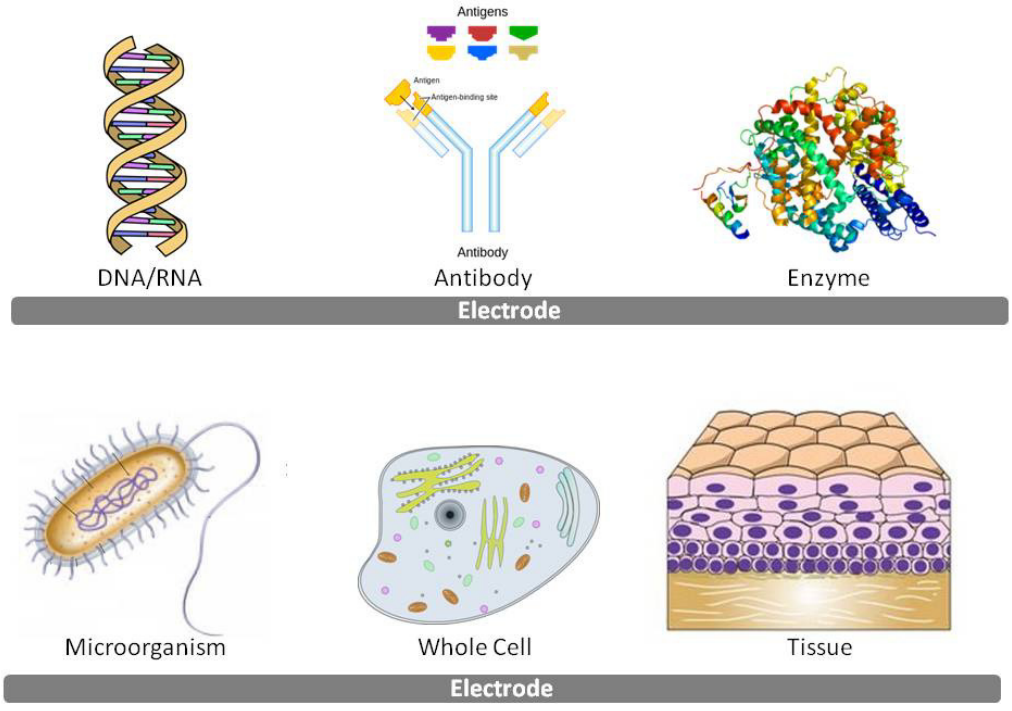

Figure 2.1 Biological recognition elements used in biosensors application

2.1.1 Antibodies

An antibody based biosensors are widely used in this age for most research and clinical laboratories. In most mammals, the white blood cell producesthe Y–shaped proteins called immunoglobulin which stage a defense against foreign bodies or antigens and protect the host body[46].There are several immobilization techniques used in binding antibody to the electrode surface for determination of good experimental result. These techniques include the direct adsorption of antibodies to the surface of the electrode [47] and covalent bonding of antibodies on the membranes or surfaces activated by ways of multifunctionalized terminal groups (Polyamidoamine(PAMAM), glutaraldehyde or Carbodiimides) and the entrapment of biological antibodies within a polymeric matrixes [48].

2.1.2 Deoxyribonucleic acid/Ribonucleic acid

DNA/RNA (Nucleic acids) based biosensors; Due to the exposure of living organisms to chemicals and toxins containing substance through food and water, there have been numerous medical cases in the world today. The deoxyribonucleic acid (DNA) is a biomolecules that carries genomics information that can be used in the fabrication, function and reproduction of living organisms and in the replication of viruses, that are most often involved with contaminants and pollutants. DNA as biological molecule is one of the biorecognition elements used in biosensor construction to evaluate and analyze chemical compositions and pollutants from the environment. Basically there two monitoring techniques for this DNA based electrochemical detection. They include the direct monitoring of analyte and Indirect monitoring technique of inhibitor, the direct monitoring technique deals with the signal produce from the reaction between biomolecules and substrate or analyte in solution. In the direct monitoring of DNA film or polymer film containing DNAenzyme modified on the working electrode, the production or consumption in reacting signals are measured from the proportional amount of analyte in the sample. Indirect monitoring technique is widely practiced with the use of biochemical receptor or activator, where the organic pesticides, heavy metals, fluoride, cyanide inhibits the DNAenzyme that are modified on the working electrode of the biosensors. However, the function of the DNAezyme based biosensor is

irretrievable. Due to the irretrievable ability of the biosensor, their biocatalytic functions are preserve when process chemically form. The technique does not require direct detection of substrate in a solution, rather detection is observed by the inhibition or obstruction of the biomolecule function on the biosensor [49].

2.1.3 Enzymes

Enzyme based biosensor; from the evolution of this great discover of biosensors, enzymes have been a component part of biosensor development. The biosensor based on enzyme is one of the widely used electrochemical techniques in the field of biomedical and clinical researches[50].An enzyme based biosensors having been discovered by prof. Leland C. Clark Jr. in 1962, the enzyme catalyzed reactions in biological environment have been one of the methods in diagnosing the human blood glucose and blood serum urea concentration[51]. From 1962 until now, the portable electrochemical enzyme based miniaturized devices have given the public easy access to self-diagnosis[52]. Enzymes as macromolecules is one of the receptors or biological recognition elements (Enzyme (mono-or multi-enzyme), Whole cells (micro-organisms, such as bacteria, fungi, eukaryotic cells or yeast) or cell organelles or particles and Tissue (plant or animal tissue slice)) that is incorporated unto the biosensor surface [49]. Enzymes are biological catalysts, which increases the rate of chemical reactions in a biological environment without being consumed. They are also protein and are folded into several dimensional shapes. There have been many works done in reported literature concerning enzyme based biosensors. On the basis the electrochemical enzyme based biosensors, techniques which are widely used, the enzymes are incorporated unto the sensors surfaces making continuous consumption of analytes.

2.1.3 Whole cells / Microorgnisms

The Whole cells/microbial cell biosensor is an analytical device that incorporates microorganisms to a transducer for fast, accurate and sensitive analysis of a specific analytes in medical research, environmental monitoring, defense and in quality control of food industries. Whole cell/microorganisms are one of the biological recognition elements, one of the component in biosensor design for its reliability and device efficacy[53]. There are many environmental threats and risk due to the uncontrollable use of industrial chemical compounds, which are released into the air and the soil.

These pollutants contaminate water sources or our ecosystems not only in industrial areas, but at other parts of our environments. Being exposed to these hazardous chemical compounds, humans and other wildlife suffer physiological gene alterations, reduced fertility, neurological disorder and common genetic mutation. On this basis, researchers have fabricated biosensor from the current technology that is dominating the hospitals and home diagnosticsfor the accurate evaluation of medical related cases, environmental contaminants and an oxygen shortage environs[52, 54],andwhole cell/ microbialbiosensors most often work with both chemcal and physical immobilization methods[14] .In many cases, the chemical immobilization technique, covalent binding doesn't suit for the whole cell to be immobilized on the biosensing surfaces. The simplest encounter is with the chemicals reaction to many different cell matrices, that which affects the morphology of the sensor and the survival of the immobilized cells on the sensing faces [55].

2.1.4 Tissues materials

Tissue based biosensors; Tissue is one of the biological recognition elements (bioreceptors). These biomolecules of the living organisms possess the quality characteristics, which are used in most detective or diagnostic studies[56]. In the most of the tissue biosensor based application, tissue slices, which are sources of enzyme used to catalyze an analyte in a given reactions. Over the years, the tissues based biosensors have offered many advantages, which have given it high attraction by the use of electrochemical biosensor techniques[57]. These advantages are: the high stability and high level of activity resulting from the maintenance of the enzyme in its natural environment, the biosensors long lifetime stability, a good reusability characteristics, this biosensor biomolecule (bioreceptor) has easy accessibility and application attractiveness, fast, less time consuming enzyme extraction and purification. The tissues based biosensors offer a minimum disadvantages, like the low detection specificity to the present of other chemicals that make up the organisms. In a given sample, the analyte detection takes time for the biosensor to produce a short response time due to the tissue enzyme interaction, that which produces artifacts signals (signal noise) or reaction barriers[58].

2.2 Electrochemical Immobilization Techniques

They are the major techniques that are performed in electrochemical biosensor applications. The immobilization techniquesare distinctive from other techniques based on the specificity of biological elements that are selected, the selective medium, the right analyte to be targeted and the scope of transducer [59]. These techniques have had numerous advantages due to their stability and good thermal functionalities, reproducibility, fast detection, reliability and low operational cost in the construction of biosensors [60]. Due the biosensor specificity of electrode modification deigns, immobilization techniques are categorized into two parameters, which include the physical and the chemical methods. Physical Immobilization technique includes adsorption and entrapment.

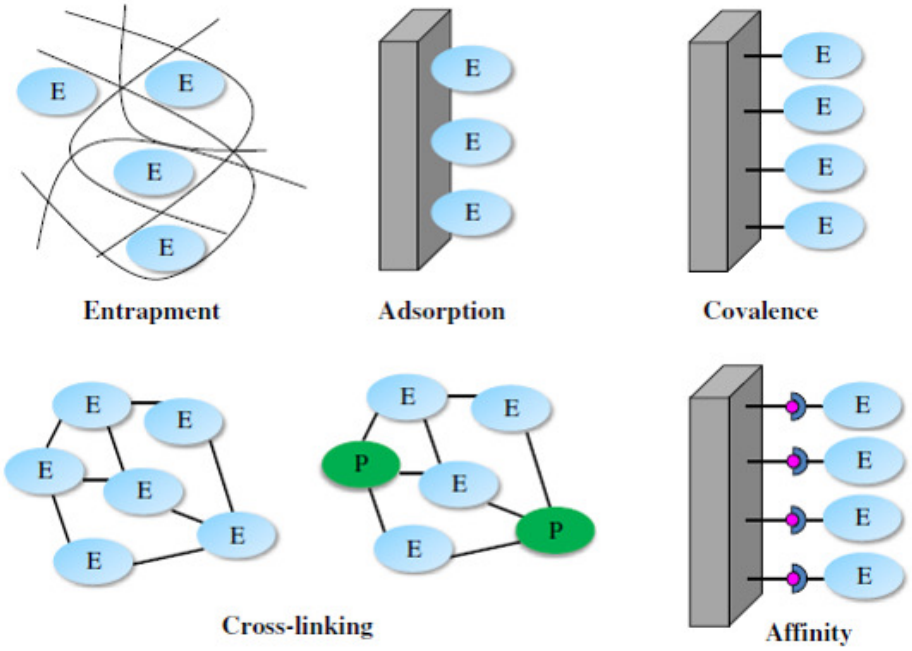

Figure 2.2 Schematic representation of the main different immobilization methods [61].

2.2.1 Adsorption

Absorption is one of the earliest and the most common technique practice in the immobilization of most biorecognition elements to biosensor faces. This technique is meanly based on Van der Waal's force interaction, electrostatic interactions and hydrogen binding interaction, where biological recognition elements are immobilized

on the basis of these weak binding forces [62]. Due to the adjustability of temperature, potential hydrogen and ionic strength, these weak forces easily adapt. This method can be applied to whole cell contain matrix. In this application, a microbial suspension is deposited directly to the face of electrode and incubated for a proper binding of cell or microbial components to polymer film on the electrode. Therefore is proceeded by washing out the unbind cell with buffer solutions. Meanwhile, cells are absorbed based on the face material polarity, charges, hydrophobicity and their hydrophilic interactions. Due to these properties, whole cell adsorption immobilized technique suffers faulty quality of shelf life stability [63].

2.2.2 Entrapment

An entrapment immobilization based technique is the encapsulation of biomolecules into any of these matrix types, where an enzyme can be entrapped into sol- gel, a silica gel, a polysaccharide or a carbon pastematrices or embedded into an electropolymerized films and or into an amphiphilic matrices [64], that form a polymer chains around the biological recognition elements. Due to this interaction of physical immobilization, in which, there have been three dimensional practices that are commonly used of entrapment procedures. It can be infers from the electropolymerized film construction[65]. Under an electrochemical polymerization procedure, there is a single step practice that contains a common and attractive procedure for the controlling of enzymes on the surface of the electrodes. In the electropolymerization, a medium containing a monomer, and an enzyme is prepared and a net applied current is set to the transducer. Within the medium, the monomer oxidizes during the reaction producing positive ions. These positive monomer ions are further reacted and producing dimers, forming polymers at the surface of the sensor. The polyaniline, polythiophene, polypyrrole, Hypoxanthine or Glucose, which are conducting polymers control film thickness and conductivity[66].

2.2.3 Covalent Binding

Covalentattachmentis one the immobilization method of the chemical methods, that is vastly used in most biosensor electrode modification. In this immobilizing procedure, the polymer film or electrochemical or supporting matrices are constructed and the biological recognition elements are attached to the supporting film or to the biosensor

surface [67]. To achieve biomolecule stabilization, an immobilization of several dimensional covalent attachment of enzymes should be associated with the activated conducting polymers, such as ferrocene or a multifunctional group (Polyamidoamine (PAMAM)), which provides several terminal points for covalent binding, process that is initiated by crosslinking agents (glutaraldehyde or carbodiimide) [68]. There are some common features of the covalent immobilization, which enhance the binding and functions of biomolecules to supporting matrices. These covalent features are; the activation of carboxylic groups, the activation of amino residues, the Chemisorption, etc. In covalent binding immobilization, biocatalysts are purposely attached to the biosensor surface initiated by conducting polymer of the functional groups in the supporting matrix for its catalytic activity. On the solid supporting film, the binding of enzymes is conducted by the early activation, which is controlled mostly by those reagents (carbodiimides or glutaraldehyde[64].

2.2.4 Cross-Linking

The activation of carboxylic groups; this carboxylic activator, carbodiimides influences attachment an enzyme amino acid residue unto a carboxyl groups as a supporting matrix. For immobilization efficacy, the carbodiimides are induced with the N-hydroxysuccinimide to give enzymatic biosensors a good detective response. For an insight, an immobilization of the enzyme cholesterol oxidase as an example was achieved by self-assembling of a thiol, 3-mercaptopropionic acid and carboxylic residues on the gold working electrode, where thecarbodiimide methiodide influences the attachment of the carboxylic residues to the 3-mercaptopropionic acid [69], the activation of amino residues, this procedure do work with the attachment of amino residue of a supporting matrix and carboxyl function of enzymereaction, which is influence by the Carbodiimides. With the use of carbodiimide coupling reaction, the immobilization Urease onto poly (N-3-aminopropyl pyrrole-co-pyrrole) film having free amino residue was developed to provide the amperometric urea biosensor a good response for urea detection [64].

2.3 Polymeric Mediators

Polymeric mediators of enzyme based biosensor is achemically synthezed co-polymer or polymers that contain functioal groups whichare electrochemical strategized

basically use to modify electrodes for experimental purposes and for ability of transferring electrons, attaching of biomolecules, improving electrodes that are less yielding to the present of interferent in giving low electrode potentials in a sample. In one of the working procedures, glucose was oxidized by glucose oxidase and produced hydrogen peroxides, where the analyte, glucose is detected with a working electrode modified with peroxidase. In this procedure, the polymer film was constructed with the polymer bonded to the mediator, which was further reduced on the working electrode by the oxidation of the enzyme (peroxidase) [70]. Polymeric mediators are defined as an artificial electron transferring agents which stimulate electrochemical reactions that involve the oxidation and reduction. Due to mediator ability to transfer, they are also involve in the speedy electron transfer mechanism, where analytes are catalyzed and mediators conduct electrons from the active site of the biomolecules to the surface of the working electrodes. To cause mediator stable for the proper transfer of electrons, there require several working environmental factors, which are enlisted as; the mediator chosen must possess low oxidation and reduction current, that is lesser than the active interferents in a given sample, the current of the oxidation and reduction precise mediator must produce an appropriate potential gradient for electron transfer between active site of the enzyme and surface of the electrode and the mediator oxidation and reduction potential should be more positive for oxidizing the biomolecules or highly negative for the reducing of biomolecules [70]. Advantages of the mediator, with the discovery of hydrophobic positive ion exchanged polymers, mediators in amperometric biosensor application have had tremendous advantages over the past years and these advantages include: the enzyme modification can be easily immobilized to polymer film on electrodes. Due to the negative ionic charges present on the polymer containing enzyme film, the negative biological interferent agents are obstructed of producing anion signal in the electrochemical solution. The polymer enzyme film serves as a promotion of ion exchange in the biological prepared sample [70, 71].In the 1960s, conducting polymers were discovered. One of the familiar electrically conducting polymers, polypyrrole was identified. From it identification period to the early 1970s, nothing was said about conducting polymers. Dated back in the 1977, Alan Heeger, Alan MacDiarmid and Hideki Shirakawa wrote about polyacetylene treated with iodine and discovering its ability to conduct electric signals. From the 1980, the understanding

and the development of polyheterocycles, such as polypyrrole, polythiophene, polyaniline, and poly(3,4-ethylenedioxythiophene, have boarding many fields of research in working with functional groups of aromatic conducting polymers, which possess good conductivities, a strong stabilities and easily synthesized[12].

2.4 Conducting Polymers

Coucting polymer is defined as the material contains an electron backbone that responsible in conducting many electrochemical behaviors in a given application. Because of the electrochemical character, they can electrically produce signals, has high electron affinity, it has low energy optical transmission, low ionization potential and their polymer chain contains single and double bonds[14].Based on the preparation conducting polymers, they are grouped into two broad categories, known as; the chemically prepared ones and the electrochemically category. Due to the polymerization procedure and material composition, these two categories of conducting polymers have both advantages and disadvantages. The chemical preparation produces condensation polymerization procedure; it is sometime called additional polymerization. Some of it advantages include; Larger-scale productive possibility, Post-covalent modification of bulk conducting polymer possibility, More options to modify conducting polymer backbone by covalent attachment, etc. and common disadvantages are; does not have the capability of producing a thin films, Synthesis of the conducting polymer contain highly complicated procedure, etc.The electrochemical preparation of conducting polymer was identified in the1970, when pyrrole black were isolated as a precipitate on a platinum electrode by exposing an liquid pyrrole medium and sulfuric acid to an oxidative potential. From this experimental method electrochemical polymerization in the 1970s and 1980, conducting polymers are widely used in many fields of studies today, such fields include; research biosensor, or biomedical, optical sensor application, clinical application, etc. There are conventionally three-electrode arrangement, working electrode, counter electrode, and reference electrodes in a sample according to its application of its specificity[12]. There are also some advantages and disadvantages in the electrochemical polymerization method, advantage include; capability of thin film synthesis, easily synthesis, synthetic or biomolecules are easily entrapped into conducting polymer film.Its disadvantages are; Post-covalent modification of bulk

conducting polymer is difficult, difficult to remove film from electrode surface[72]. The conductivity ability of conducting polymers it is influenced with several other factors such as the polar on length, the conjugation length, the overall chain length and their charge transfer ability to adjacent molecules. This mechanism was expressed that the large number of models is based on intersoliton hopping, the fast movement among the localized states, assisted in the arrangement of molecules during vibrations, intra-chain placement of molecules, variable range hopping in 3-dimensions and charging energy limited tunneling among the conducting domains [10]. The importance of conducting polymers in the field of research, biomedical and medical diagnosis has expressed vital interest with matrix biomolecules immobilization in biosensor applications. Because of the various geometry shape presented by conducting polymers, their chemical structure are flexible, which can allow the synthesis, chemical modeling and electrode modification attractive. Because of film thickness and modulate, the conducting polymer can change the enzyme activity, which have brought about the understanding of molecular interaction between the bioactive protein and simple composite or chemical complexes (dendrimers) [10].

2.5 Dendrimer

Dendrimer are known as hyperbranched macromolecules with central core which best defines it and identifying it a multifunctionalized group. Due to the hyperbranching structure, dendrimers are emerging synthetic structure possessing a treelike branching morphologies and have a monodisperse arrangement according to the generations [73]. Dendrimers are usually synthesized by the step-by-step building method, which characterizes the polymerization reaction. Its structure contain from a single central core with tertiary multifunctional terminal ends. Moreover, their sizes and their molecular mass are easily handle, thus giving them their polydispersing property, structural control, unique ornamentation of the physical characteristics and its classical polymeric property. Due to the step-by-step divergent synthesis, generations are formed when dendrimer layer is added. Because of these layer additions, dendrimers have produced about zero to twelve generations, which they own one to three dimensional structures. Dendrimer was first synthesized by F.Vögtle et al.. 1978, who named it “cascade structure” where the unidirectional step-by-step added layers of the six dendrimer units

within the cascade structure of a primary dendrimer was seem to be one of the best using dendritic groups.[74]. Then R.G.Denkewalter et al.. 1981 in his work, synthesized polylysine dendrimers in 1985, which was not published[75]. The word dendrimer, which means tree taken from a Greek word “déndros”, was given by D.A. Tomalia et al..1983 [76], when he first synthesized dendrimer and later published his work in 1985, at which the first synthesized polyamidoamine dendrimers were known. Due to controllable characteristics of Polyamidoamine, it has been used in many research field and point of care testing, clinical diagnostics within three major domains, such as, material characterization, biocatalytic interaction and biology or medicine. The process of producing dendrimer is based on various synthetic cores, for example, starting with the hexafunctional cores for production of the eighth generation or starting with the trifunctional core producing up to the twelfth generations. Due to the multi-terminal ends of dendrimers, there are many functional groups that can easily be immobilized to the dendrimers functional groups. The complexation of dendrimers range from one to several generations identified their chemistry for hold terminal or half terminal attachment. Due to the dendrimer complexation, the entire structure terminal groups are bonded to other functional groups or half of the terminal functional groups are also bonded. Due to the presence of various functional groups at the terminal ends dendrimers, this hyperbranched structure is able to incorporate different types of complexes, which can be found at different locations of the dendrimers. Like the ferrocene, the complexation of gadolinium or the bisphosphonate groups, generally gives rise to several branches that can be used also for new reactivity of dendrimers at one or more layers. The ferrocene derivatives contribute to the branches of dendrimer structure, where the macrocycles phthalocyanine develops the complexation of central core of the dendrimers [77].

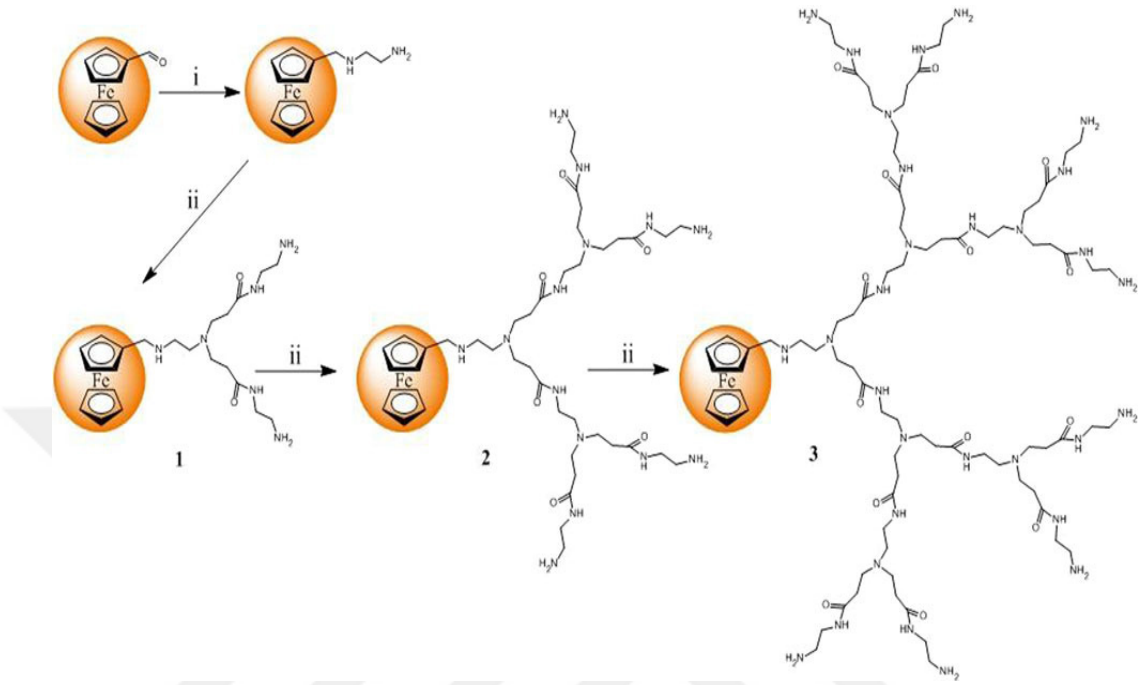

Figure 2.4 present the synthetic process of the ferrocene polyamidoamine dendrimer, whcih includes: i. Ethylenediamine and NaBH4 reduction, (ii) methyl acrylate, ethylenediamine in methanol, which demonstrate the step-by-step structural formation of the dendrimer first, second and third generations [78].

2.6 Carbon Nanotube Composites

From the discovery of Carbon nanotubes in 1991 by Iijima, there have been three different types of carbon groups. The carbon types include the diamond carbon type, graphite containing carbon and fullerene carbon. The diamond is arranged with four bonds of atom to the neighboring elements, which form three-dimensional structures. For graphite in the 1970s, the graphite stacks of the carbon nanofibers were then form. In Graphite carbon, the structure contains two-dimensional hexagonal carbon atoms sheet arrangement, where the carbon has an average distance of 1.42 Å. Relating to the ability of hydrogen uptake, the geometry of sheets, which contain 3.35Å are accessible to the outer and the inner layer for diffusivity of substance into the carbon nanostructure. The fullerene carbon was discovered in 1991, under which gave rise to the wide use of carbon carbon-nanotube today in many research application including biosensors, drugs delivery etc. Nanotubes are produced from the repeated graphite sheet that is folded cylindrically into tube. There are many forms of nanotube that exist,

which form a single-wall or multiwall nanotubes. The single wall nanotubes possess caps at its end that shows structural characterization of the fullerenes. The single wall nanotubes (SWNTs) developed structure from the Van der Waals forces that is found in the folded tubes. Due to the lengthiness of the nanotube, the diffusivity of the hydrogen on the inner layer of the tube can produce structural order that fills the entire tube volume, which most often appears as structural disorder within the tube or a sharp curve on the tube that may show like block layer due to the hydrogen diffusion. Because of its structural appearance like block, the cutting of the tubes to shorter pieces for experimental purposes can help convenient usage of the nanotube [79]. In the effort of S.E. Meibodi el al. 2014, an amperometric urea biosensor based on polyaniline multi wall carbon nanotube pencil graphite electrode was constructed. In this work, the carbon nanotube used with the electric conducting polymer, polyaniline created a feasible electrochemical ability to conjugate and form highly hydrophobic layer for electric and hydrophobic interaction with other compound. From the composition of the polymer film, the new nanocomposites were form to enhance the electric and the mechanical properties, which was intended to improve the stability and sensitivity of the urea biosensors [80].

CHAPTER 3

MATERIAL AND APPARATUS

Urea (reagent/analyte) was purchased from Sigma Aldrich, Urease (ur. enzyme, from Jack Bean Canavalia ensiformis plant) was purchased from Sigma Aldrich, Carbon Nanotube (CNT) was purchased from Sigma Aldrich, Ferrocene was purchased from Sigma Aldrich, polyamidoamine (PAMAM) was purchased from Sigma Aldrich, Ethanol isolate was purchased from Sigma Aldrich, Ascorbic Acid was purchased from Sigma Aldrich, Distilled Water (Fatih Distillation Millipore RiOs at 25 ^{o}c) system, crystal Sodium Phosphate (NaH_2PO_4 . $2H_2O$) was purchased from Merck Chemical company, crystal Sodium Phosphate ($Na_2H_2PO_4$. $2H_2O$) was purchased from Merck Chemical company, phosphate buffer saline (PBS) solution 10mM of pH of 8.0 was prepared from $NaH_2PO4.2H_2O$ and $Na_2H_2PO_4.2H_2O$ in Fatih physical chemistry Laboratory and all chemicals used in this project were of analytical reagent grade. Silver/Silver Chloride (Ag/AgCl) Reference Electrode, Platinum Wire Counter Electrode, Pencil Graphite working Electrode, Glass Cell (conventional three electrode configuration glass Cell), Micropipette, Ultrasonic cleaner (Bandelin Sonorex), Oven (Memmert GmbH+KG-D 91126 Schwabach FRG made in Germany), Magnetic stirrer (Heidolph stirrer made in Germany), pH meter (WTW Inolab pH-7110 made in Germany), Sartorius AX224 Auto-Scale (sarto@sarto.com.tr),Bench Mark ORBi-Shaker JR (Model BT-300), Refrigerator (BOSCH ELECTRONICS), Auto Lab. Electrochemical Analyzer (Potential and Galvanic static Ivium Tech. made in Tulsa, Oklahoma, United State of America) and Monitor (Computer).

3.1 Instrumentation

Sets of pencil graphite electrodes were prepared, polished and cleaned with ultrasonic cleaning for 60 seconds in ethanol solution. Prepared electrodes, which comprise of three generations of Ferrocene-polyamidoamine (Fc-PAMAM) dendrimer were coated and the electrochemical optimization and characterization were performed during the cyclic voltammetry using the auto lab Ivium, potentiostat.An autolab Ivium device is

equipped with the Ivium software and contained three-electrode-cell arrangement. The working electrodes with the electrochemically active areas of 2cm were activated and 1cm activated area was used, the platinum wire used as a counter-electrodeandsilver/ silver Chloride (Ag/AgCl) electrode placed in glass tube containing membrane filled with a saturated potassium Chloride was used as a reference-electrode. The electrochemical amperometric measurements, optimization and characterization experiments were performed in 15ml of 10mM phosphate buffer solution (pH- 8.0) prepared from $NaH_2PO_4.2H_2O$ and $Na_2H_2PO_4.2H_2O$. The autolab IVIUM TECH device was used for all electrochemical amperometric procedures. The zero to zero point eight volt (0.0 – 0.8 v) linear sweep of the cyclic voltammetry was set and at the applied potential of zero point three –five volt (0.35v) to obtain the all electrochemical measurements.

3.2 Methods

During the electrochemical procedure, three sets of pencil graphite electrodes were modified with 7.5µL of a synthesized ferrocene polyamidoamine (Fc-PAMAM) dendrimer with its concentration of 5mM. Two centimeters (2cm) each of the electrodes surfaces were prepared and activated in an isolate ethanol solution within the Ultrasonic cleaner. One centimeter (1cm) activated area of the electrodes was coated with the 7.5 µL of ferrocene polyamidoamine (Fc-PAMAM) dendrimer matrix. According to their molecular structure, these constructed polymer films were categorized into first, second and third generations. They were separately prepared with ferrocene combine to the hyperbranched structures to generations atthe same 5mM concentration on each electrode. The constructed polymer films were placed the Oven for three hundred(300) seconds (5minutes) at sixty degree Celsius (60 °C) for the sensor surfac incubation, then removed the sensors and allowed the fifteen hours(15hrs) sensor air dry incubation under twenty five degree Celsius (25 °C). After the incubation, six pairs of ferrocene-polyamidoamine electrodes were immerged in test tubes containing enzymes(Ur). These test tubes were then placed on the bench Mark ORBi-Shaker JR and these the modified electrodes were allowed to shake for 180 minutes. From the given time, enzyme was covalently immobilized to electrodes through porous surface of the polymer film. Upon electrodes removal and rinsed to disperse the unbind enzyme,were immerged in a

fleshly prepared 2ml of 10mM phosphate buffer solution (PBS) pH- 8.0 for the electrode preservation awaiting electrochemical amperometric measurement.

3.2.1 Preparation andModification of Pencil Graphite Electrodes

A sets of pencil graphite electrodes, working electrodes were measured and prepared according to each ferrocene polyamidoamine(Fc-PAMAM) dendrimer generation that had been previously reported [81]. The sets of electrodes were divided into three groups(G1, G2 & G3), which serve for each dendrimer generation. The first set of electrodes was measured and marked with one line each indicating for first generation. The second set of electrodes was measured and marked with two lines each presenting second generation and the same process was done for the third set of pencil graphite electrodes. These pencil graphite electrodes were cleaned with a set time of one minutes (1mins) each in 99% ethanol solution under an ultrasonic cleaning and allowed them to properly get dry. A 7.5µL ferrocene polyamidoamine (Fc-PAMAMA) dendrimer with the concentration of 5mM was casted on the prepared surface of the pencil graphite electrodes according to each prepared dendrimer generation. The modified pencil graphite electrodes were dried in the oven (Memmert) at sixty degree Celsius (60 °C) for ten minutes (10mins); the working electrodes were then taken and dried for fifteen hours (15hrs) overnight in a twenty five degree Celsius (25 °C). A fresh urease, enzyme was prepared, where the constructed polymer film electrodes were immerged into. The enzyme test tubes were anchored in rack and placed on the bench Mark ORBi-Shaker JR. The test tube rack was shaken for one hundred and eighty minutes (180 mins) to covalently immobilize the enzyme to the polymer film.

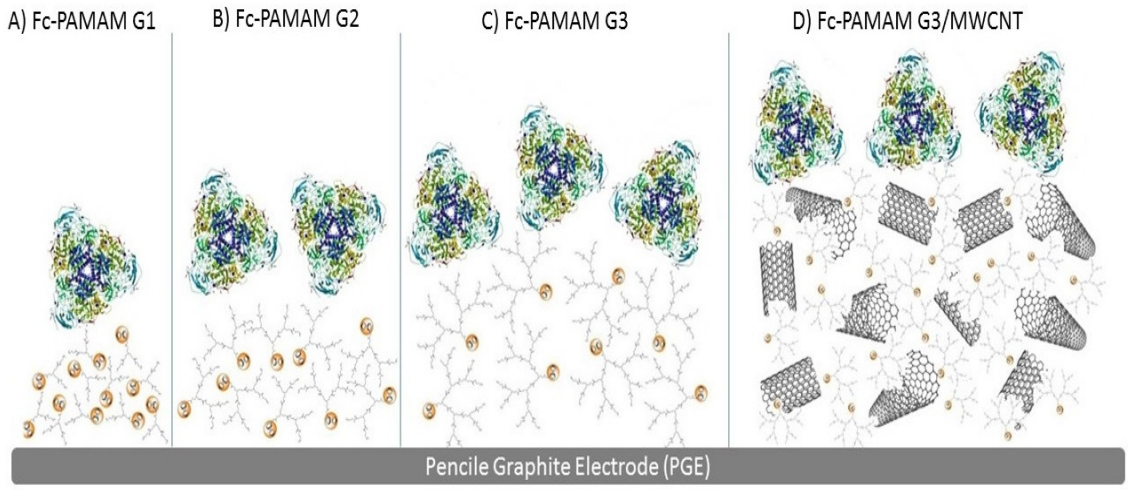

Figure 3.2.3 Shows the schematic view of the Fc- PAMAM Dendrimer Generational composition. A) Fc- PAMAMG1, B) Fc- PAMAMG2, C) Fc- PAMAMG3 and D)Fc-PAMAMG3/MWCNTs.

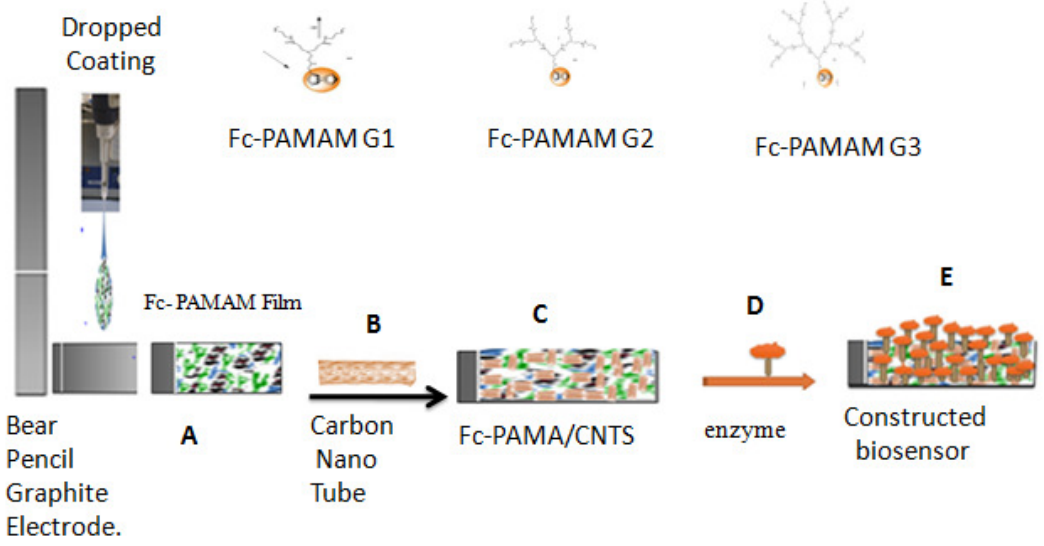

Figure 3.2.4 shows the progressive steps of the urea biosensor contruction. (A) The prepared pencil graphite bear electrode, (B) the dropped coating of synthesized ferrocene-PAMAM on the surface of the electrode, (C) Constructed film with Fc-

PAMAM dendrimers, (D) the addition of multiwall carbon nano composite to the polymer film, (E) Fc/PAMAM/CNTs modified working electrode, (F) the urease, enzyme covalent immobilization to the polymer film, (G) a biosensor with Fc/PAMAM/CNTs/Ur fully constructed.

3.2.2 Immobilizationof Ureaseon PGE/Fc-PAMAM Eectrodes

A twenty gram per one liter (20g/L) of urease was freshly prepared and placed in refrigerator. The Urease was immobilized onto the polymer film on the working electrode by the immerging of the working electrodes containing the constructed polymer film into the freshly prepared urease. The test tubes containing urease and the working electrodes were anchored in a test tube rack and placed on the Shaker. The test tube rack was shaken for one hundred and eighty minutes (180 mins) under a constant temperature, twenty five degree Celsius (25 °C) to conduct the physical covalent immobilization from the shaking interaction between enzyme and the polymer film. The purchased urease enzyme and the prepared enzyme were kept under a four degree Celsius (4°C).

3.3 Electrochemical optimization and characterization

3.3.1 Electrochemical optimization of PGE/Fc-PAMAM electrode

The electrochemical cyclic voltammgram were ran to determine the suitable working range of the amperometric urea detectors that are based on the ferrocene coupled with polyamidoamine (Fc-PAMAM) dendrimer modified on the pencil graphite electrodes. The optimization of ferrocene- polyamidoamine (Fc-PAMAM) dendrimer does establish the different behavior patterns in the oxidation and reduction peak currents. From the cyclic voltammetry, the linear sweep of ferrocene-polyamidoamine (Fc-PAMAM) dendrimer was set at a range of zero to zero point eight volt (0.0 – 0.8) with the applied potential of zero point third-five (0.35v). In this optimization procedure, there were three ferrocene polyamidoamine dendrimer generations and the working electrodes were studied at a scan rate of one hundred millivolts per second (100mv/s). The scan rate of the working electrode was ranged from 100mv/s to 500mv/s to determine the domains of direction in the cyclic voltammetric shift and the height of the oxidation and reduction peak currents. The

oxidation and reduction peak currents increase as the scan rate increases with a displayed peak potentials showing the slight directional change. As the scan rate increases, anodic peak potential or oxidation current increases and directionally shift toward highly positive end of the cyclic curve. To the opposite proximity of anodic peak current, the cathodic peak current was shifted toward a highly negative value.

3.3.2 Electrochemical characterization

The scans occurring at different rates ranging from 100mV/s to 500mV/s at the pencil graphite, working electrode of G1, G2 and G3 and the urease/ ferrocene polyamidoamine electrode of G1,G2 andG3 was well performed using the potentiostat to obtain the cyclic voltammetry. The electrode configuration was set up for the bio-analysis of ferrocene polyamidoamine electrode and urease ferrocene polyamidoamine(Fc-PAMAM) bioactive electrode. Using the three-electrode system couple with fifteen milliliters (15mL) phosphate buffer solution containing ten micro-molar(10mM) $NaH_2PO_4.2H_2O$ and $Na_2H_2PO_4.2H_2O$, the cyclic voltammetric response was obtained from the working electrode, oxidized ferrocene polyamidoamine(FC-PAMAM) electrode and oxidized ur/Fc-PAMAM on the pencil graphite electrodes. From potential range of 0.0 to 0.8 V at a scan rate of 100mV/s, the increase in the reduction current obtained for the ferrocene polyamidoamine (Fc-PAMAM) pencil graphite electrode gave flat curve. The cathodic current induces an increase when cyclic voltammetric curve was plotted for the working electrode of the three generations. The curves obtain from working electrode was been compared to the oxidize urease/ferrocene polyamidoamine on the working electrodes, in which the conducting polymer modified electrodes showed anodic cyclic voltammetric curves. Due to the low conductivity onthe working electrodes, the oxidation and reduction activity from the cyclic scan increases with the working electrode surface modification. Its cyclic voltammetric results show that with the low resistance, the polymer (Fc-PAMAM) can have ability to enhance electron transfer, that increases oxidation and reduction peak current in the three generations. It was then inferred that the increase in the scan rate increases the oxidation peak potential directionally shifting the oxidation peak moving towards the positive sides.

3.4 Operational Repeatability and Storage Stability

3.4.1 Repeatability

Sets of the pencil graphite electrodes were modified, dried and immobilized with urease. The reusability of the biosensors was worked out under room temperature with the electrochemical amperometric system. With the conventional electrode set up, the 0.35v applied potential and the 0.0 to 0.8v linear sweep, the constructed biosensors based on the ferrocene polyamidoamine (Fc-PAMAM) three generations was used fifteen consecutively times in different prepared sample for the detection of urea. A 15 mL of 10mM phosphate buffer saline (PBS) solution under a constant stirring condition in an electrochemical glass cell was used to establish the reusability of each the electrodes. With different time interval, the reusability of each of the working electrode was carried out. The reusability process of the amperometric mode of measurement was worked out with the use of 10mM phosphate buffer saline (PBS pH 8.0) solution.

3.4.2 Storage Stability

The operational stability of the modified pencil graphite electrodes was measured under an ambient amperometric electrochemical condition. In this experiment, the working electrodes were prepared and modified according to its dendritic generations. The amperometric procedures were conducted within the three electrode system within a continuous stirring environ. The analyte detection was held, in which the urease catalyzes urea from the oxidation and reduction reaction in the sample to form ammonium and bicarbonate ions. With 15ml of pH 8.0 of 10mM phosphate buffer saline (PBS pH 8.0) solution, subsequent measurement of biosensors were held after every 72 hours. The biosensors were kept in pH 8.0 phosphate buffer saline (PBS) solution and stored in 4°C temperature environment.

3.5 Amperometric Measurements

Amperometric measurements were done with the use of electrochemical amperometric conventional system. With the three electrodes configuration and an electrochemical glass cell, which comprise of the pencil graphite electrode as working electrode, platinum wire as counter electrode and Ag/AgCl (KCl solution) as reference electrode under a continuous stirred condition, a zero point three-five volts (0.35V) fixed applied

potential was set between the reference and working for the electrochemical amperometric analysis. With the given applied potential at room temperature, a 15 mL of 10mM phosphate buffer saline (PBS- pH 8.0) solution was used to detect a one molar (1M) urea concentration. And the amperometric measurement was proportional to the present of the analyte concentration in a sample, where the counter electrode regulates the potential generated biological signals.

3.6 Interferences

With the zero point three-five volts (0.35V) within fixed applied potential, set at a range of zero to zero point eight volt (0.0 – 0.8), uric acid , ascorbic acid, glucose and benzoate had insignificant interference on the urea biosensor construct with ferrocene, Polyamidoamnie dendrimer and carbon nanocomposte. This insignificant effect observed within a 10mM pH 8.0 PBS solution in the amperometric instrumentation under a constant room temperature and at a continue stirring electrochemical condition. Due to the present of the conducting polymers and nano composite, the signals (artifact) of these interferents (uric acid , ascorbic acid, glucoseand benzoate), that are commonly found in blood fluid were diminished as observed, analyzed and reported in the graphical presentation in our results.

3.7 Real sample analysis

Real sample experiments are perfromed using PGE/Fc-PAMAM/CNT proposed electrode to measure urea concenetration in human blood serum. Since linear range of the biosensor is not covering normal urea conncetration in human blood sample, the isolated real sample was diluted to meet linear range of the experiment. First experiments were preformed using healthy human blood serum, than healthy human blood serum was spiked with known amount of urea concentration 0.3, 0.6 and 1.2mM in order to see selectivity and reliabilty of proposed biosensor.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Electrochemical Analysis

4.1.1 Cyclic Voltammogram

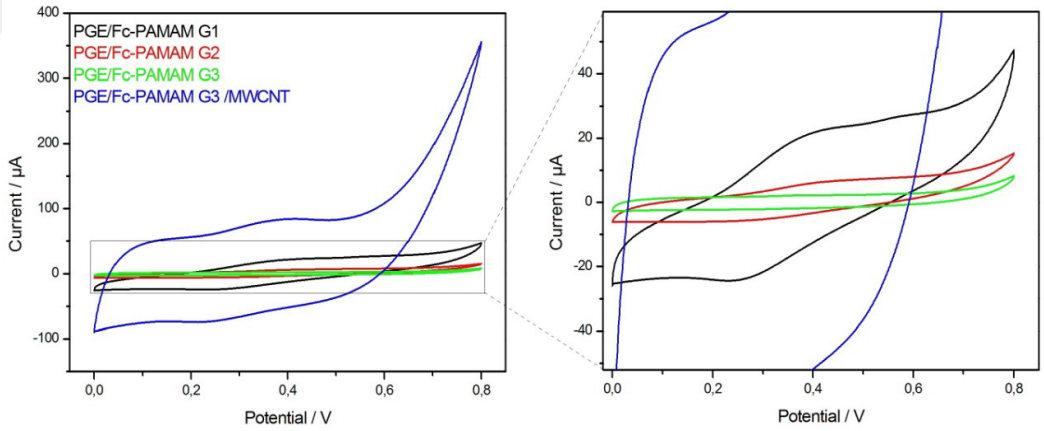

Fıgure 4.1.1 Cyclic voltammograms of Fc-PAMAM G1, Fc-PAMAM G2, Fc-PAMAM G3 and Fc-PAMAM /MWCNTs G3 with the scan rate of 100mVs^{-1} in 10 mM PBS(pH 8.0) solution.

Figure 4.1.1 Illustrates characterization of electrode surface modified with Fc-PAMAM G1, Fc-PAMAM G2, Fc-PAMAM G3 and Fc-PAMAM /MWCNTs G3 using cyclic voltammogram at 100mVs^{-1}within 10 mM PBS pH-8.0. The ferrocene units of Fc-PAMAM redox peak has been decrease with PAMAM generation increasingin G1 to G3, however addition of MWCNTs to Fc-PAMAM(G3) gave an accelerating electron transferresulted on the electrode surface. Applied potential of bio-sensing electrode of 0.35 V was selected due to the oxidation and reduction peak property of ferrocene mediator. The advantage of selecting mediators oxidation potential in amperometric measurementsas an applied potential of electrode, is to avoid or decrease the influence signals of biological interferent species in thesample.

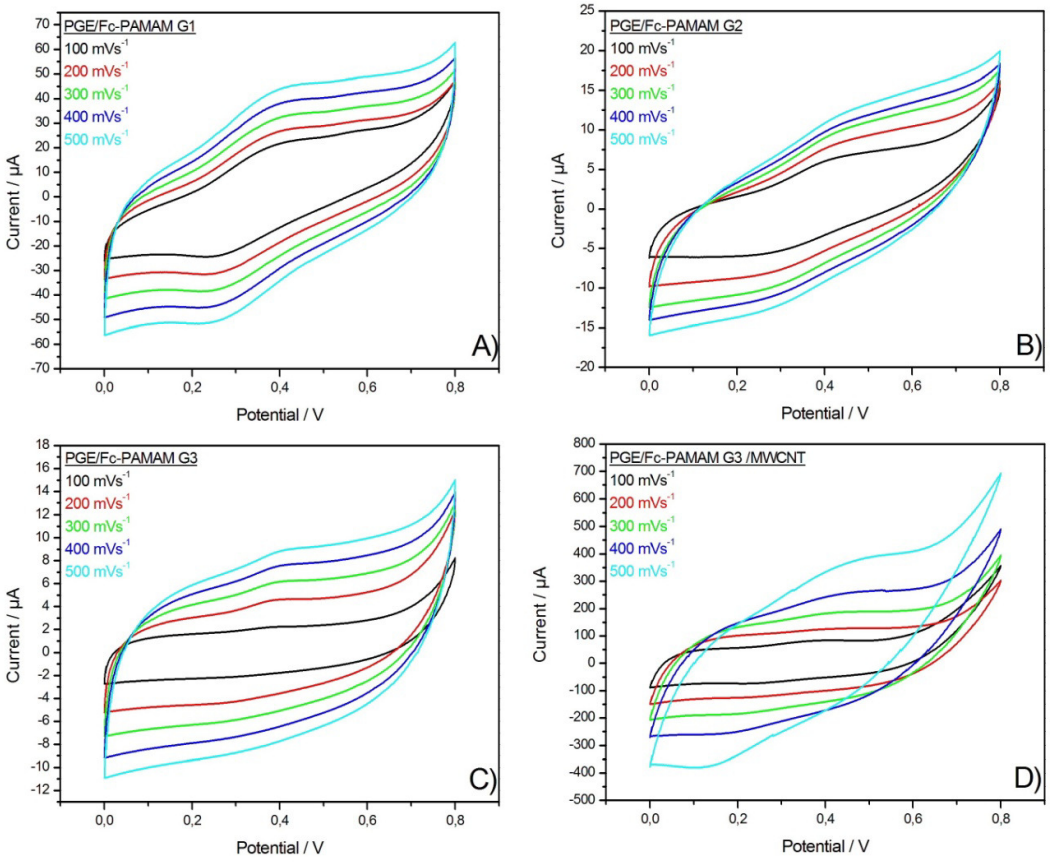

Figure 4.1.2 Cyclic voltammogram of the A) Fc-PAMAM G1, B) Fc-PAMAM G2, C) Fc-PAMAM G3, D) Fc-PAMAM/CNTs G3 10mM PBS, at 25º C,

In order to see number of oxidations stateas as well electron trasnfer rate behavior of cyclic voltammetry has been preformed, the CV reocrdes were preformed between 0.0 and 0.8 V with 10mM PBS with scane rates of 50, 100, 200, 300, 400 and 500 mVs^{-1} and an effect of the scan rate on the peak current was presented at the anodic current peak and cathodic current peak (Ipa and Ipc). Anodic peak potential (Epa) of the electrodes has been obtained at 0.35V as a results of oxidation of polymer and cathodic peak potential. The diffusion controlled process during forward and reverse scan reduction process of iron$(Fe)^{+III}$ at Fc-PAMAM coated electrodes can be seen from linear relation between scan rates and current. It was found out that the addition of CNTincreases the difference between reduction and oxidation current peak of CNT containing in the electrode as compared to the Fc-Pamam without MWCNTs.

4.1.2 Determination of Experimental Variable

4.1.2.1 Optimum pH

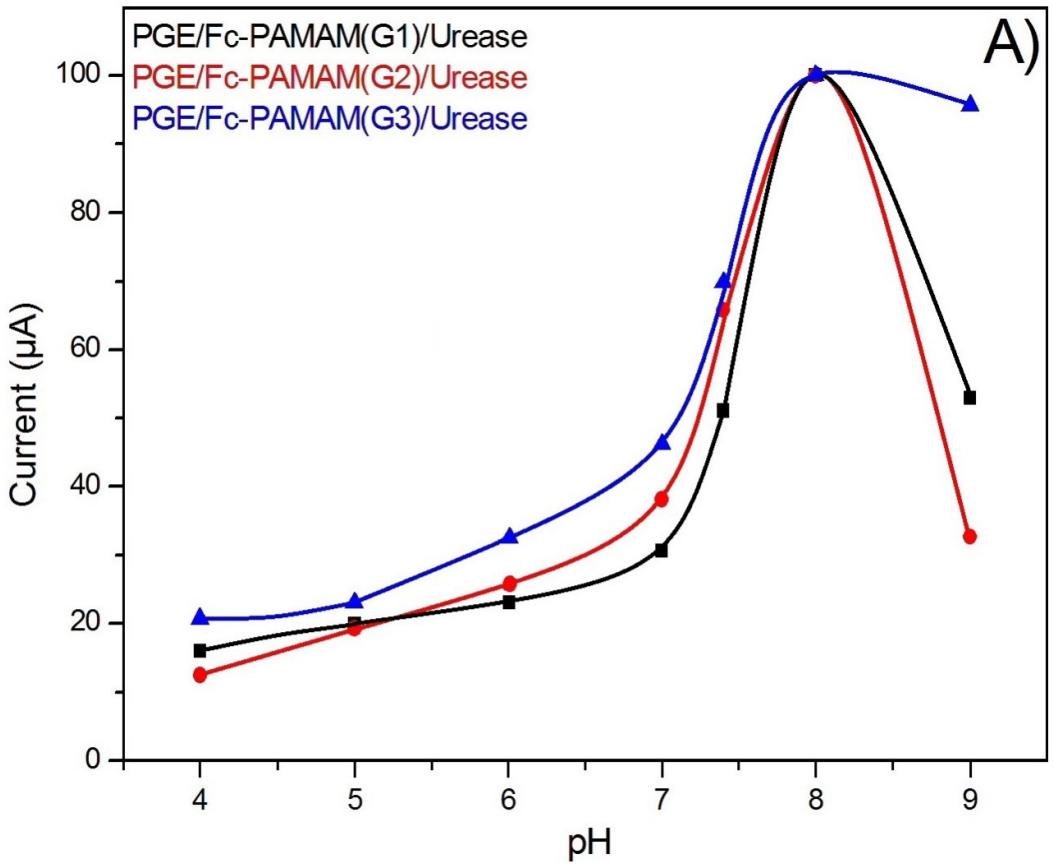

Figure 4.1.2.1 Effect of pH 0n the amperometric response of the Fc-PAMAM G1, Fc-PAMAM G2, Fc-PAMAM G3 bio-sensing electrodesat0.35V applied potential in 10mM PBS.

Figure 4.1.2.1 Illustrates the effect of the pH on amperometric current response of a proposed bio-sensing electrodes in the pH range of 4.0 to 9.0 prepared from10mM PBS at applied potential of 0.35V. The Fc-PAMAM G1/Urease, Fc-PAMAMG2/Urease and Fc-PAMAM G3/Ureasesuitably obtained pH 8.0 as an optimum pH, which is also reported by Pizzariello et al.,[33]. As shown from resulting current response of electrodes with low pH buffers, the signals start to increase as pH increases. At a low pH, the electrode shows malfunction effect due to the acidic environ, the potential of pH 8.0 presents aplateau and start to havea drastically current response reduction at a higher pH due to the based environ.

4.1.2.2 Optimum Temperature

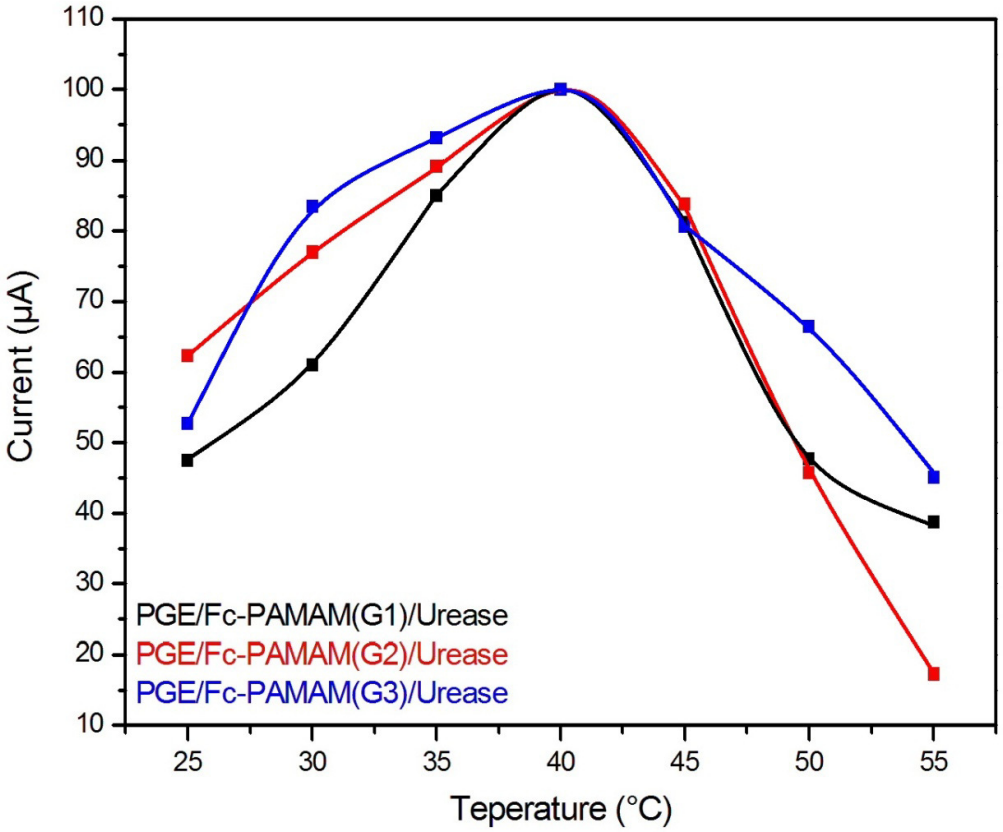

Figure 4.1.2.2 Temperature effect on the amperometric current response of a Fc-PAMAM G1, Fc-PAMAM G2 and Fc-PAMAM G3at 0.35V applied potential in a 10mM of PBS.

For the determination of optimum temperature, the enzyme electrode current response was recorded at various temperatures from 25°C-55°C range, at which time the electrodeswere susceptible 40ºC obtained as optimum temperature in a 1mM substrate of 10 mM PBS, pH 8. At an embientenvironmental temperature, the enzymes increases in reaction rate, in which maintains the physical bindings of redox reaction process. At higher temperature, the three dimensional shape of enzyme active site degrades and the enzyme starts to lose its full activity. In this work, the highest current response was obtained for all generations at 40°C and their current response graduallydecrease as the temperature further increases. An additional reason to the enzyme electrode current response decrement comes from the Van der waal's interaction between the dendrimer

polymer and enzyme molecules that eventually lead to the desorption of enzyme molecules from the electrode surface.

4.2. Amperometric Response of Electrodes

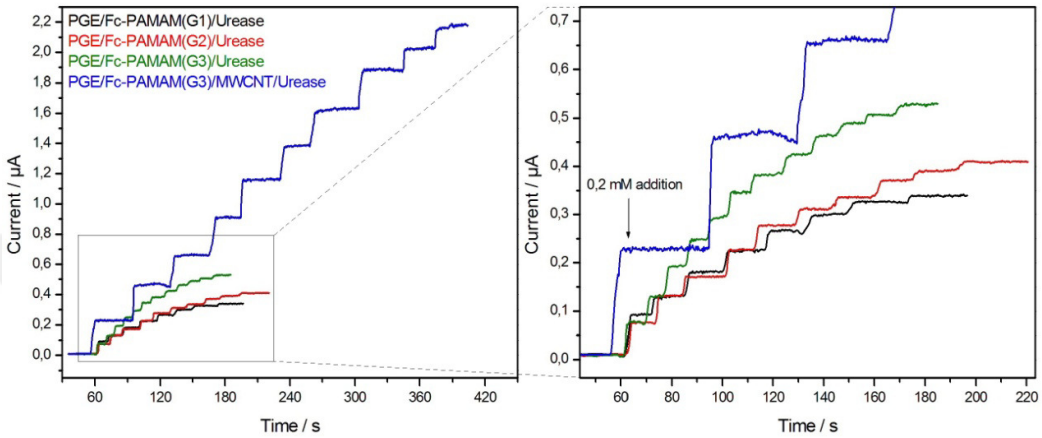

Figure 4.2 presents the amperometric responses of Fc-PAMAM G1, Fc-PAMAM G2, Fc-PAMAM G3 and Fc-PAMAM/CNTs G3 biosensor on the Fc-PAMAM dendrimers 5mM concentration at 10 mM PBS (pH 8.0) of applied potential of 0.35 V.

Amperometric current reponse of Fc-PAMAM based electrodes has been conducted by successful addition of 0.2mM urea. As it presented in the above figure 4.2 The electode Fc-PAMAM G3 containing CNTs shows high current response as compared to Fc-PAMAM G1 to Fc-PAMAM G3 electrodes. From this comparison, the bio-sensing electrode of the Fc-PAMAM /CNT have huge dominancy when compared to rest of the electrodes. The Fc-PAMAM /CNTshows a linear range of 0.2 to 1.8mM, detection limit of 0.05mM, sensitivity of 1.15µA/mM and response time of 4s. Reaching 1.8mM additional response was observed and showing a less activity from the electrode, which might be atributed to high analyte concentration in electrocehmical solution and leading to enzyme inhibition.

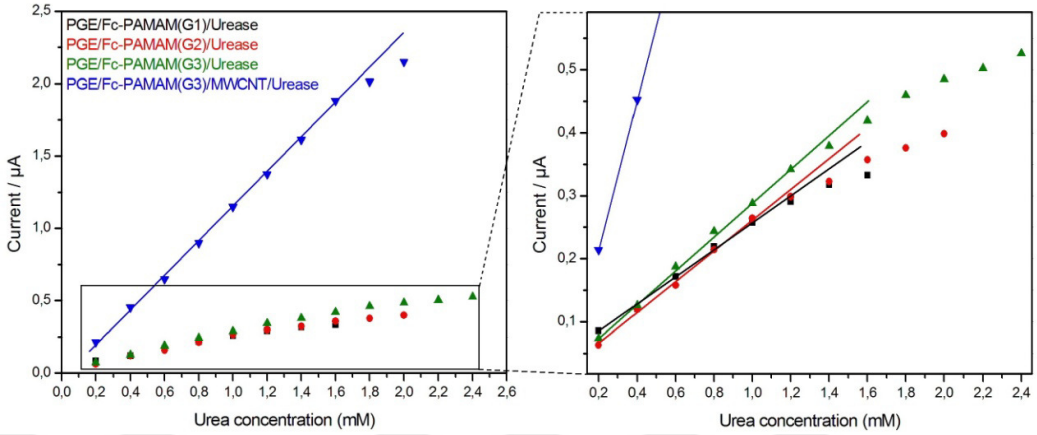

Figure 4.2.1 This shows the amperometric response of the Fc-PAMAM G3 , Fc-PAMAM G3 , Fc-PAMAM G3 , Fc-PAMAM/CNTs G3, biosensor Calibration curve of ferrocene-polyamidoamine dendrimer concentrations acquired from the urease immobilized electrodes in 10mM of PBS (pH 8.0)

Figure 4.2.1 Ilustrates calibration curves of Fc-PAMAM G1 to G3 and Fc-PAMAM G3 MWCNT based electrodes. Resulting calibration plot demonstrated linear range of electrodes for MWCNT based 0.2 to 1.8mM, Fc-PAMAM G3 0.2 to 1.4mM, Fc-PAMAM G2 0.2 to 1.2mM and Fc-PAMAM G1 based electrode form 0.2 to 1.2 mM. The response time of 4s at a linear range of 0.2 to 1.8mM, sensitivity of 1.15 µA/mM and low detectionlimit of 0.05mM. This values were acquire from the MWCNTs efficiency to increase electron transfer rate.

4.3. Reusability and Storage of Bio-sensing Electrode

4.3.1. Reusability

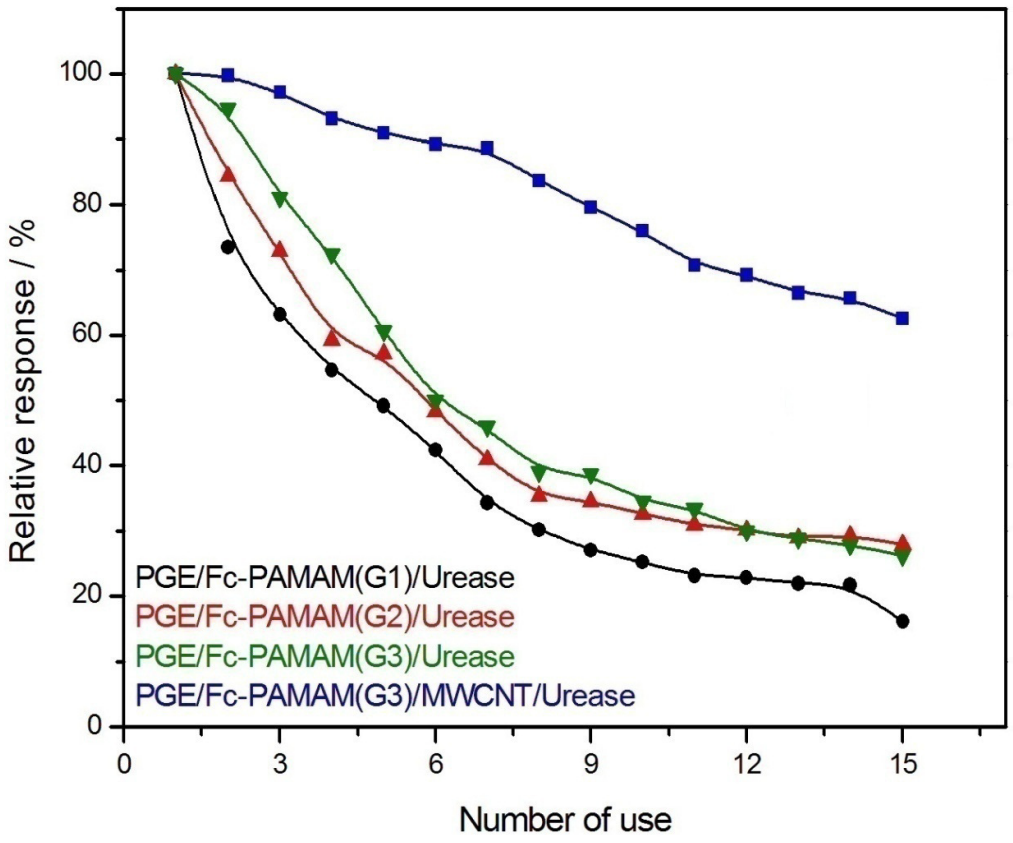

Figure 4.3.1 Operational stability of Fc-PAMAM G1, Fc-PAMAM G2, Fc-PAMAM G3 and Fc-PAMAM/CNTsG3 electrodes, 10mM PBS (pH 8.0)

The operational stability of enzyme electrode modified with each polymer and with CNT containing in the third generation of Fc-PAMAM polymer was determined in 10 mM PBS with a pH of 8, under 0.35V applied potential, and 1mM substrate addition, where the results are as shown in fig. 4.3.1.Having thesame number ofenzyme electrode,the number of electrode usage against relative response percentage is observed that highest operational stability percentage was generated fromFc-PAMAMG3/CNT enzyme electrode, where the first three measurements vary in 95% response area and the rest decreases by 60% in their responses. The Fc-PAMAM modified electrodes without CNT have a higher deduction in their responses as compared to CNT containing electrode, reaching 12% relative response at fifteen (15) measurements.

4.3.2 Storage Stability

Reusability

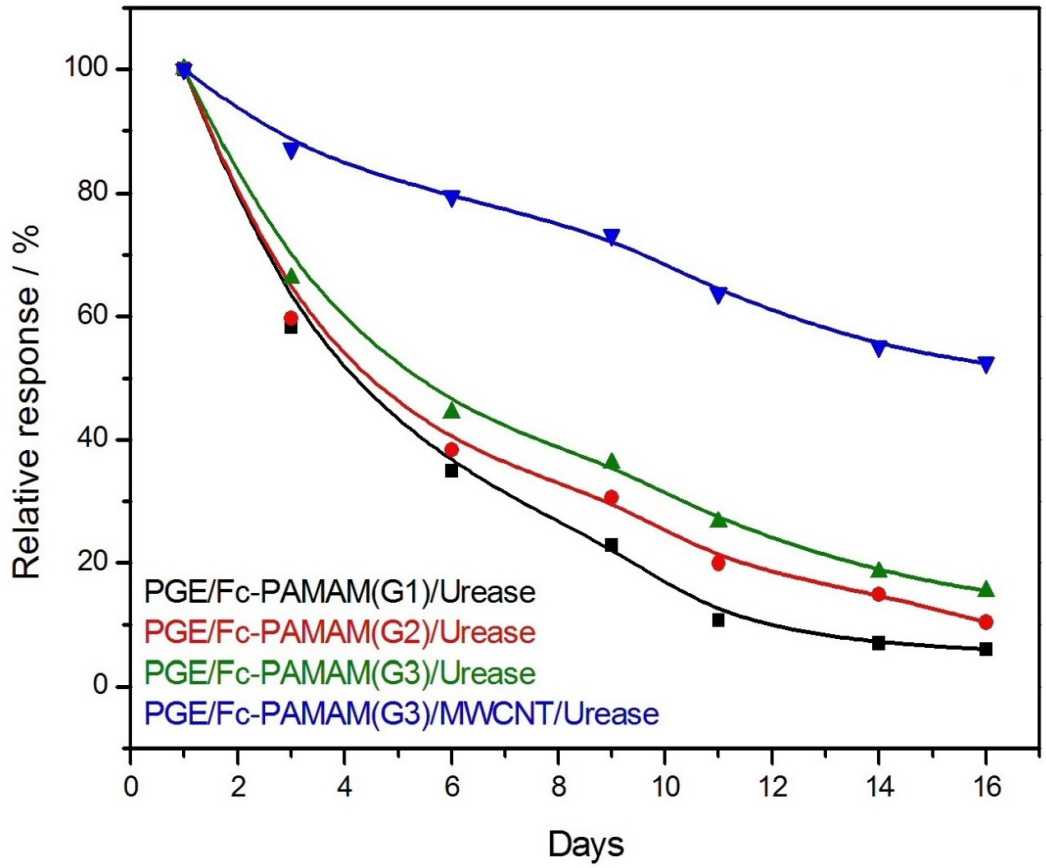

Figure 4.3.2 Storage stability of Fc-PAMAMG1, Fc-PAMAMG2, Fc-PAMAMG3 and Fc-PAMAM/CNTs G3 based bio-sensing electrodes

Storage stability experiments were performed at same conditions as in operational stability. The Storage stability was assessed in the course of 16-days, with the use ofsame enzyme electrode. Fig. 4.3.2, displays the current response of CNT-Fc-PAMAM (G3)/Urease electrode, which was decreased by 12% at 3rd day of measurements. In continuing measurement, the electroderesponse further more decrease to 50%, whereas PGE/Fc-PAMAM (G1-2-3)/Urease electrodes were able to retain the initial response for approximately 62% at 3rd day of measurements and later decrease in potential within the days of storage measurements, drawing down 10% response. The results from operational and storage stability experiments signify that the conductivity enhancement

at the CNT electrode surface provided a higher percentage current response when compared to PGE/Fc-PAMAM (G1-2-3)/Urease bio-electrodes.

4.4. Analytic Recovery

Table 4.4. Analytical recovery of PGE/Fc-PAMAM/MWCNT/Urease based bio-sensing electrode.

Urea added µM	Urea foundµMR	RSD (%)	Recovery %
0.50	0.53	2.3	106.0
1.0	0.95	1.95	95.0
1.5	1.44	2.54	96.0

Analytical recovery of Fc-PAMAM G3/CNT electrode has been performed using 0.5, 1.0 and 1.5 µM urea solution. After amperometric current response has recorded from working electrode, urea concentration was calculated from previously shown calibration curve. Fc-PAMAM G3/CNT fabricated biosensor demonstrated recovery in the range of 95 to 106% which is very good for electrode performance and it proves accuracy and reliability of proposed bio-sensing electrode.

4.5. Interferences

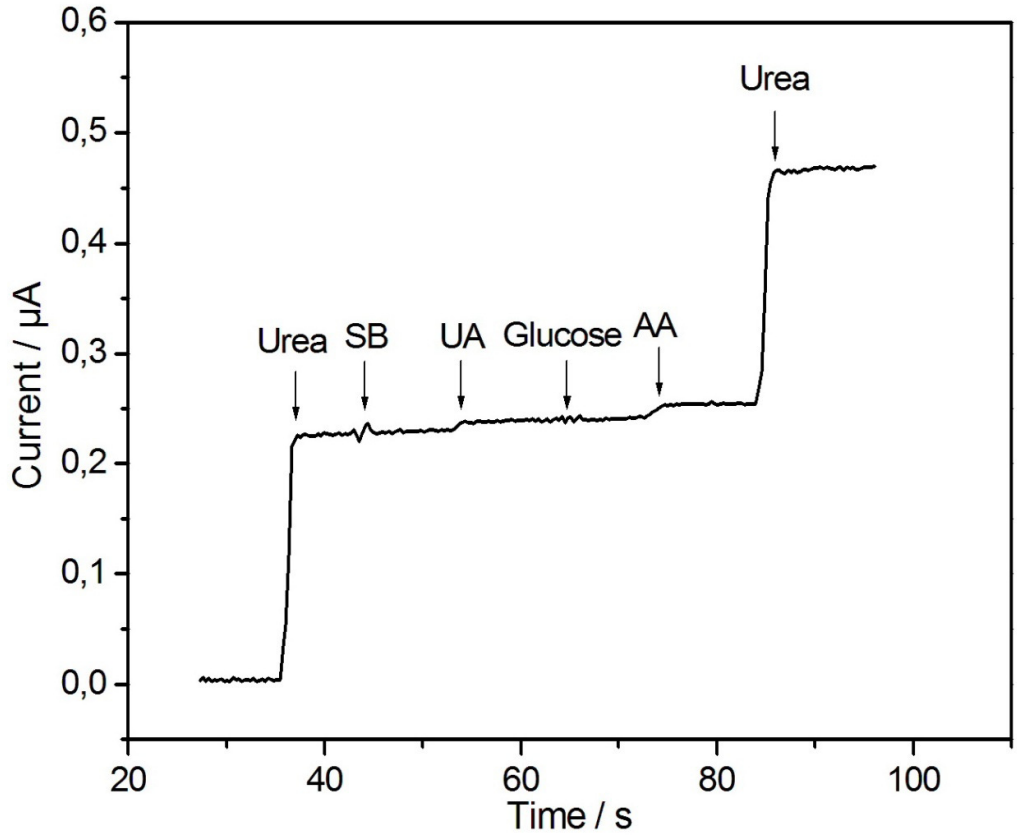

Figure 4.5 Interference effect of sodium benzoate (SB), uric acid (UA), glucose, and ascorbic acid (AA) on PGE/Fc-PAMAM G3/MWCNT /Urease electrodes in 10 mM PBS (pH 8.0) at applied potential of 0.35V.

Selectivity of constructed bio-sensing electrodes is of high significance. The prepared electrodes were subjected to possible interfering substances found human blood serum at 1mM of uric acid, ascorbic acid, glucose, and benzoic acid from the sodium benzoate solution. Fig.4.5 shows the results of interference study where there is no significant current response of Fc-PAMAMG3/CNTs/Urease electrodes as compare to the response towards 1mM of the substrate addition. The reaction conditions were established as has being for operational stability experiments, where the interfering substances were prepared in 10 mM PBS, pH 8.

4.6. Real sample application

Table 4.6 Determinaton of urea in human blood sample by PGe/Fc-PAMAM/CNTs based biosensing electrode.

Blood Sample	Urea mM	UreaDetected mMRSD	(%) Recovery	(%)
Sample 1	0	0.22	0 .51	-
Sample 2	0.3	0.513	0.97	103.3
Sample 3	0.6	0.792	0.34	98.3
Sample 4	1.2	1.35	2.65	95.83

NOTE: Recovery (%)=[$C_{(found)}$–$C_{(sample1)}$/ $C_{(added)}$] * 100, n=3

Real sample analysis of Fc-PAMAM/MWCNT based electrode has been conducted using human serum samples which were spiked with known concnetrations of urine. Table 4.6, illustrates results of fabricated electrodes current response on 0.3, 0.6 and 1.2 mM urea additions. It has to be shown that the results for the sample 1 which was not spiked with urea has been measured and obtained results have been excluded from other samples. Results show that the electrode gives good response towards urine with RSD (%) varies from 0.95 to 1.65 and recovery (%) from 95.83 to 103.3 %, implying that the selectivity and reliability of proposed biosensor was susceptible to. Theobtained real sample results can be attributed to electrode preparation method and the minimum influence interferent species. As well, it is important tonote that the blood sample has been pretreated and dilution in order to fit the linear range of the proposed biosensor.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

A new amperometric urea biosensor had been developed based on covalent enzyme immobilization method. This enzyme (ur) based biosensor was constructed from the synthesized ferrocene Polyamidoamine dendrimer and the multi-wall carbon nanotube, which form a conducting polymer film unto the surface of the pencil graphite electrodes by dropped coating procedure. The film therefore showed surface to materials biocompatibility due to the electrode porosity. With the used of electrochemical conventional electrodes (working, platinum and Ag/AgCl electrodes) configuration, at a 0.35V applied potential , 0.0 and 0.8V as linear sweep and with the scan rate of $100mVs^{-1}$, this biosensor was able to obtaina well-defined anodic and cathodic peaks in a 15mL of 10mM (PBS) pH 8.0 solution. There were distinguishable oxidation and reduction shifts, when the scan rate maximized from $100mVs^{-1}$ to $500mVs^{-1}$ reflecting the locations of the oxidation peak current shift from left to right and showing the direction of reduction current shift from right to the left. This reversibility of redox reaction mechanism implied the diffusion analyte molecule and biomolecule interaction. A suitable potential hydrogen (pH) working 10mM of PBS (pH 8.0), from where the given optimum pH current response, enzyme stability and activity were greatly improved in all the urease ferrocene-Pamam electrodes to 100% with a slight change of 5% in the fc-pamamG3/CNT/ Ur electrode, with 45% change in the fc-pamamG1/CNTs/ Ur electrode and a change of 65% in the fc-PAMAMG2/CNTs/ Ur electrode. The temperature range of 25ºC to 40ºC gives the biosensor stable current response, which was showing in all of the electrode current responses. The enzyme activity decreases when there was increase in temperature in all of the electrodes. Therefore, the increase in temperature to 45ºC led to 20% reduction in the Fc-PAMAM G1&G3/Ur/PGE, while the Fc-PAMAM G2/Ur/PGE had 15% current deduction. The amperometric response of fc-PAMAM dendrimer modified electrodes

was capable of detecting urea concentrations at the very outset like 0.05mM concentration at its elevated level in human serum. With the 7.5µL of 5mM concentration of the polymer, 15mL of 1M urea and fleshly prepared urease, stored at 4ºC, the biosensor provided a high sensitivity in a low detection limit and contained a broad sensing range, operational stability and maintaining its enzymatic properties during biosensor shelf-life. With fast and good response time, this biosensor presents a quality, affordable, real time detection. Therefore steady-state enzymatic current response of PGE/Fc-PAMAM G3/CNTs/Urease was achieved by (95- 98%) on catalytic function of urea concentration and the addition in the urea gave a tremendous increase in amperometric current signal, improving viability of the biosensor, meanwhile the amperometric current response to urea using the Fc-PAMAM dendrimer generations and the inclusion of multiwall carbon nanotube was linear in the urea range of 0.2 to 1.8mMand the limit of detection was 0.05mM. Meanwhile, the amperometric current response of the enzyme electrode reusability of the Fc-PAMAMG3/CNTs/Ur satisfied the electrochemical feasibility and influencing electrode sensitivity 80 to 100%, while the storage current response for the first six 80 to 100% of the sixteen days of active responses. Therefore, the The amperometric response time of 4s linear range of 0.2 to 1.8mM, sensitivity of 1.15 µA/mM and low detection limit of 0.05mM, which can be attributed to MWCNTs to increase electron transfer rate and influencing the expression the noisy signals that were generated from the interferent species (Uric acid, Ascorbic acid, Sodium benzoate and glucose) in the present of the signals produced form the urea been catalyzed. Therefore, the signals generated by the interferent species were negligible due its relative value.

REFERENCES

[1] Soares, J.C., A. Brisolari, V.D. Rodrigues, E.A. Sanches, and D. Goncalves,(2012), "*Amperometric urea biosensors based on the entrapment of urease in polypyrrole films",* Reactive & Functional Polymers, 72(2): 148-152.

[2] Tiwari, A., S. Aryal, S. Pilla, and S.Q. Gong,(2009), "*An amperometric urea biosensor based on covalently immobilized urease on an electrode made of hyperbranched polyester functionalized gold nanoparticles",* Talanta, 78(4-5): 1401-1407.

[3] Velichkova, Y., Y. Ivanov, I. Marinov, R. Ramesh, N.R. Kamini, N. Dimcheva, E. Horozova, and T. Godjevargova,(2011), "*Amperometric electrode for determination of urea using electrodeposited rhodium and immobilized urease",* Journal of Molecular Catalysis B-Enzymatic, 69(3-4): 168-175.

[4] Meibodi, A.S.E. and S. Haghjoo,(2014), "*Amperometric urea biosensor based on covalently immobilized urease on an electrochemically polymerized film of polyaniline containing MWCNTs",* Synthetic Metals, 194: 1-6.

[5] Massafera, M.P. and S.I.C. de Torresi,(2009), "*Urea amperometric biosensors based on a multifunctional bipolymeric layer: Comparing enzyme immobilization methods",* Sensors and Actuators B-Chemical, 137(2): 476-482.

[6] Suman, E. O'Reilly, M. Kelly, A. Morrin, M.R. Smyth, and A.J. Killard,(2011), "*Chronocoulometric determination of urea in human serum using an inkjet printed biosensor",* Analytica Chimica Acta, 697(1-2): 98-102.

[7] Hao, W., G. Das, and H.H. Yoon,(2015), "*Fabrication of an amperometric urea biosensor using urease and metal catalysts immobilized by a polyion complex",* Journal of Electroanalytical Chemistry, 747: 143-148.

[8] Putzbach, W. and N.J. Ronkainen,(2013), "*Immobilization Techniques in the Fabrication of Nanomaterial-Based Electrochemical Biosensors: A Review",* Sensors, **13**(4): 4811-4840.

[9] Veeradasan Perumal, U.H.,(2014), "*Advances in biosensors: Principle, architecture and applications",* Journal of Applied Biomedicine, 12: 1–15.

[10] Manju Gerard, A.C., beB.D. Malhotra,,((2002)), " *Application of conducting polymers to biosensors,"* Biosensors & Bioelectronics, 17 345–359.

[11] Dhawan, G., G. Sumana, and B.D. Malhotra,(2009), "*Recent developments in urea biosensors",* Biochemical Engineering Journal, 44(1): 42-52.

[12] Guimard, N.K., N. Gomez, and C.E. Schmidt,(2007), "*Conducting polymers in biomedical engineering",* Progress in Polymer Science, 32(8-9): 876-921.

[13] Svetla Ivanova, Y.I., Tzonka Godjevargova,(2013), "*Urea Amperometric Biosensors Based on Nanostructured Polypyrrole and Poly Ortho-Phenylenediamine",* Journal of Applied Biosensor 2: 12-19.

[14] Gerard, M., A. Chaubey, and B.D. Malhotra,(2002), "*Application of conducting polymers to biosensors",* Biosensors & Bioelectronics, 17(5): 345-359.

[15] Ramesh, R., P. Puhazhendi, J. Kumar, M.K. Gowthaman, S.F. D'Souza, and N.R. Kamini,(2015), "*Potentiometric biosensor for determination of urea in milk using immobilized Arthrobacter creatinolyticus urease",* Materials Science & Engineering C-Materials for Biological Applications, 49: 786-792.

[16] Wang, J.,(2006), "*Electrochemical biosensors: Towards point-of-care cancer diagnostics",* Biosensors & Bioelectronics, 21(10): 1887-1892.

[17] Chen, J.C., J.C. Chou, T.P. Sun, and S.K. Hsiung,(2003), "*Portable urea biosensor based on the extended-gate field effect transistor",* Sensors and Actuators B-Chemical, 91(1-3): 180-186.

[18] Kwon, M.S., S.W. Lim, and H.M. Kwon,(2009), "*Hypertonic Stress in the Kidney: A Necessary Evil",* Physiology, 24(3): 186-191.

[19] Batshaw, M.L., M. Tuchman, M. Summar, J. Seminara, and U.C.D. Consortium,(2014), "*A longitudinal study of urea cycle disorders",* Molecular Genetics and Metabolism, 113(1-2): 127-130.

[20] Zhang, Y., L. Churilov, A. Meretoja, S. Teo, S.M. Davis, and B. Yan,(2013), "*Elevated urea level is associated with poor clinical outcome and increased*

mortality post intravenous tissue plasminogen activator in stroke patients", Journal of the Neurological Sciences, 332(1-2): 110-115.

[21] Patassini, S., P. Begley, S.J. Reid, J.S. Xu, S.J. Church, M. Curtis, M. Dragunow, H.J. Waldvogel, R.D. Unwin, R.G. Snell, R.L.M. Faull, and G.J.S. Cooper,(2015), "*Identification of elevated urea as a severe, ubiquitous metabolic defect in the brain of patients with Huntington's disease",* Biochemical and Biophysical Research Communications, 468(1-2): 161-166.

[22] Hamilton, A. and C.B. Breslin,(2014), "*The development of a novel urea sensor using polypyrrole",* Electrochimica Acta, 145: 19-26.

[23] Clark, S., P.S. Francis, X.A. Conlan, and N.W. Barnett,(2007), "*Determination of urea using high-performance liquid chromatography with fluorescence detection after automated derivatisation with xanthydrol",* Journal of Chromatography A, 1161(1-2): 207-213.

[24] Gonzalez-Ruiz, V., A.I. Olives, and M.A. Martin,(2015), "*Core-shell particles lead the way to renewing high-performance liquid chromatography",* Trac-Trends in Analytical Chemistry, 64: 17-28.

[25] Rozin, R. and J. Almog,(2011), "*Colorimetric detection of urea nitrate: The missing link",* Forensic Science International, 208 (1-3): 25-28.

[26] Davletbaeva, P., M. Falkova, E. Safonova, L. Moskvin, and A. Bulatov,(2016), "*Flow method based on cloud point extraction for fluorometric determination of epinephrine in human urine",* Analytica Chimica Acta, 911: 69-74.

[27] Yang, Z.P., S.H. Si, H.J. Dai, and C.J. Zhang,(2007), "*Piezoelectric urea biosensor based on immobilization of urease onto nanoporous alumina membranes",* Biosensors & Bioelectronics, 22(12): 3283-3287.

[28] Chen, Y.Z., H.L. Teo, Q.D. Liu, and T.K. Lee,(2015), "*Developing a reference measurement procedure for free glycerol in human serum by two-step gas chromatography-isotope dilution mass spectrometry",* Clinical Biochemistry, 48(13-14): 897-903.

[29] Ayano, E., H. Kanazawa, M. Ando, and T. Nishimura,(2004), "*Determination and quantitation of sulfonylurea and urea herbicides in water samples using liquid chromatography with electrospray ionization mass spectrometric detection",* Analytica Chimica Acta, 507(2): 211-218.

[30] Soldatkin, A.P., A.V. Elskaya, A.A. Shulga, A.S. Jdanova, S.V. Dzyadevich, N. Jaffrezicrenault, C. Martelet, and P. Clechet,(1994), "*Glucose-Sensitive Conductometric Biosensor with Additional Nafion Membrane - Reduction of Influence of Buffer Capacity on the Sensor Response and Extension of Its Dynamic-Range",* Analytica Chimica Acta, 288(3): 197-203.

[31] D'Orazio, P.,(2003), "*Biosensors in clinical chemistry",* Clinica Chimica Acta, 334(1-2): 41-69.

[32] Jdanova, A.S., S. Poyard, A.P. Soldatkin, N. JaffrezicRenault, and C. Martelet,(1996), "*Conductometric urea sensor. Use of additional membranes for the improvement of its analytical characteristics",* Analytica Chimica Acta, 321(1): 35-40.

[33] Pizzariello, A., M. Stredansky, S. Stredanska, and S. Miertus,(2001), "*Urea biosensor based on amperometric pH-sensing with hematein as a pH-sensitive redox mediator",* Talanta, 54(4): 763-772.

[34] Singh, M., N. Verma, A.K. Garg, and N. Redhu,(2008), "*Urea biosensors",* Sensors and Actuators B-Chemical, 134(1): 345-351.

[35] Ahuja, T., I.A. Mir, D. Kumar, and Rajesh,(2007), "*Biomolecular immobilization on conducting polymers for biosensing applications",* Biomaterials, 28(5): 791-805.

[36] Francesca Cecchini, L.F., Serge Cosnier, Robert S. Marks,(2016), "*Vibrio cholerae detection: traditional assay, novel diagnostic techniques and biosensors",* Trends in Analytical Chemistry

[37] Zhu, Z.G., L. Garcia-Gancedo, A.J. Flewitt, H.Q. Xie, F. Moussy, and W.I. Milne,(2012), "*A Critical Review of Glucose Biosensors Based on Carbon Nanomaterials: Carbon Nanotubes and Graphene",* Sensors, 12(5): 5996-6022.

[38] Katrlik, J., I. Vostiar, J. Sefcovicova, J. Tkac, V. Mastihuba, M. Valach, V. Stefuca, and P. Gemeiner,(2007), "*A novel microbial biosensor based on cells of Gluconobacter oxydans for the selective determination of 1,3-propanediol in the presence of glycerol and its application to bioprocess monitoring",* Analytical and Bioanalytical Chemistry, 388(1): 287-295.

[39] Mao, L.Q. and K. Yamamoto,(2000), "*Amperometric on-line sensor for continuous measurement of hypoxanthine based on osmium-polyvinylpyridine gel polymer and xanthine oxidase bienzyme modified glassy carbon electrode",* Analytica Chimica Acta, 415(1-2): 143-150.

[40] L.B. Crepaldi, S.A.N., F.P. Cardoso, P. Ciancaglini, A.R. De Andrade,(2014), "*Ferrocene Entrapped In Polypyrrole Film and PAMAM Dendrimers as Matrix for Mediated Glucose/O2 Biofuel Cell",* Electrochimica Acta 136, 52–58

[41] Chong Sun, L.G., Daoying Wang, Muhan Zhang, Yuan Liu, Zhiming Geng, Weimin Xu, Fang Liu, Huan Bian,(2016), "*Biocompatible polypyrrole-block copolymer-gold nanoparticles platform for determination of inosine monophosphate with bienzyme biosensor",* Sensors and Actuators B 230: 521–527.

[42] Wang, B.Q. and S.J. Dong,(2000), "*Sol-gel-derived amperometric biosensor for hydrogen peroxide based on methylene green incorporated in Nafion film",* Talanta, 51(3): 565-572.

[43] Chen, S.H., R. Yuan, Y.Q. Chai, L.Y. Zhang, N. Wang, and X.L. Li,(2007), "*Amperometric third-generation hydrogen peroxide biosensor based on the immobilization of hemoglobin on multiwall carbon nanotubes and gold colloidal nanoparticles",* Biosensors & Bioelectronics, 22(7): 1268-1274.

[44] Byrne, B., E. Stack, N. Gilmartin, and R. O'Kennedy,(2009), "*Antibody-Based Sensors: Principles, Problems and Potential for Detection of Pathogens and Associated Toxins",* Sensors, 9(6): 4407-4445.

[45] Perez-Lopez, B. and A. Merkoci,(2011), "*Nanomaterials based biosensors for food analysis applications",* Trends in Food Science & Technology, 22(11): 625-639.

[46] B. Vijayalakshmi Ayyar, S.A.,(2013), "*ADVANCES IN ANIMAL AND VETERINARY SCIENCES •",* researchgate.net/publication/259267404.

[47] Skottrup, P.D., M. Nicolaisen, and A.F. Justesen,(2008), "*Towards on-site pathogen detection using antibody-based sensors",* Biosensors & Bioelectronics, 24(3): 339-348.

[48] Lucarelli, F., S. Tombelli, M. Minunni, G. Marrazza, and M. Mascini,(2008), "*Electrochemical and piezoelectric DNA biosensors for hybridisation detection",* Analytica Chimica Acta, 609(2): 139-159.

[49] Thevenot, D.R., K. Toth, R.A. Durst, and G.S. Wilson,(2001), "*Electrochemical biosensors: recommended definitions and classification",* Biosensors & Bioelectronics, 16(1-2): 121-131.

[50] Klos-Witkowska, A.,(2015), "*Enzyme-Based Fluorescent Biosensors and Their Environmental, Clinical and Industrial Applications",* Polish Journal of Environmental Studies, 24(1): 19-25.

[51] Marchenko, S.V., I.S. Kucherenko, A.N. Hereshko, I.V. Panasiuk, O.O. Soldatkin, A.V. El'skaya, and A.P. Soldatkin,(2015), "*Application of potentiometric biosensor based on recombinant urease for urea determination in blood serum and hemodialyzate",* Sensors and Actuators B-Chemical, 207: 981-986.

[52] Malhotra, B.D. and A. Chaubey,(2003), "*Biosensors for clinical diagnostics industry",* Sensors and Actuators B-Chemical, 91(1-3): 117-127.

[53] Thevenot, D.R., K. Toth, R.A. Durst, and G.S. Wilson,(1999), "*Electrochemical biosensors: Recommended definitions and classification - (Technical Report)",* Pure and Applied Chemistry, 71(12): 2333-2348.

[54] Nayak, M., A. Kotian, S. Marathe, and D. Chakravortty,(2009), "*Detection of microorganisms using biosensors-A smarter way towards detection techniques",* Biosensors & Bioelectronics, 25(4): 661-667.

[55] D'Souza, S.F.,(2001), "*Microbial biosensors",* Biosensors & Bioelectronics, 16(6): 337-353.

[56] Kuriyama, S. and G.A. Rechnitz,(1981), "*Plant Tissue-Based Bioselective Membrane-Electrode for Glutamate",* Analytica Chimica Acta, 131(Nov): 91-96.

[57] Sidwell, J.S. and G.A. Rechnitz,(1986), "*Progress and Challenges for Biosensors Using Plant-Tissue Materials",* Biosensors, 2(4): 221-233.

[58] Campas, M., R. Carpentier, and R. Rouillon,(2008), "*Plant tissue-and photosynthesis-based biosensors",* Biotechnology Advances, 26(4): 370-378.

[59] Chang, K., S.L. Deng, and M. Chen,(2015), "*Novel biosensing methodologies for improving the detection of single nucleotide polymorphism",* Biosensors & Bioelectronics, 66**:** 297-307.

[60] Erden, P.E. and E. Kilic,(2013), "*A review of enzymatic uric acid biosensors based on amperometric detection",* Talanta, 107: 312-323.

[61] Audrey Sassolas, L.J.B., Béatrice D. Leca-Bouvier,((2012)), " *Immobilization strategies to develop enzymatic biosensors,"*, Biotechnology Advances **30** 489–511.

[62] Trojanowicz, M.,(2006), "*Analytical applications of carbon nanotubes: a review",* Trac-Trends in Analytical Chemistry, 25(5): 480-489.

[63] Lei, Y., W. Chen, and A. Mulchandani,(2006), "*Microbial biosensors",* Analytica Chimica Acta, 568(1-2): 200-210.

[64] Sassolas, A., L.J. Blum, and B.D. Leca-Bouvier,(2012), "*Immobilization strategies to develop enzymatic biosensors",* Biotechnology Advances, **30**(3): 489-511.

[65] Uang, Y.M. and T.C. Chou,(2003), "*Fabrication of glucose oxidase/polypyrrole biosensor by galvanostatic method in various pH aqueous solutions",* Biosensors & Bioelectronics, 19(3): 141-147.

[66] Zhu, L.D., R.L. Yang, J.G. Zhai, and C.Y. Tian,(2007), "*Bienzymatic glucose biosensor based on co-immobilization of peroxidase and glucose oxidase on a carbon nanotubes electrode",* Biosensors & Bioelectronics, 23(4): 528-535.

[67] Durst, R.A., A.J. Baumner, R.W. Murray, R.P. Buck, and C.P. Andrieux,(1997), "*Chemically modified electrodes: Recommended terminology and definitions (IUPAC recommendations 1997)",* Pure and Applied Chemistry, 69(6): 1317-1323.

[68] Akyilmaz, E., I. Yasa, and E. Dinckaya,(2006), "*Whole cell immobilized amperometric biosensor based on Saccharomyces cerevisiae for selective determination of vitamin B-1 (thiamine)",* Analytical Biochemistry, **354**(1): 78-84.

[69] Shen, J. and C.C. Liu,(2007), "*Development of a screen-printed cholesterol biosensor: Comparing the performance of gold and platinum as the working electrode material and fabrication using a self-assembly approach",* Sensors and Actuators B-Chemical, 120(2): 417-425.

[70] Chaubey, A. and B.D. Malhotra,(2002), "*Mediated biosensors",* Biosensors & Bioelectronics, 17(6-7): 441-456.

[71] Mizutani, F., S. Yabuki, and Y. Hirata,(1996), "*Flow injection analysis of L-lactic acid using an enzyme-polyion complex-coated electrode as the detector",* Talanta, 43(10): 1815-1820.

[72] Nathalie K. Guimard, N.G., Christine E. Schmidt, ,((2007)), "*Conducting polymers in biomedical engineering",* Prog. Polym. Sci., 32: 876–921.

[73] Zhang, Z.X., W. Yang, J. Wang, C. Yang, F. Yang, and X.R. Yang,(2009), "*A sensitive impedimetric thrombin aptasensor based on polyamidoamine dendrimer",* Talanta, 78(4-5): 1240-1245.

[74] Christophe LarreÂ, B.D., Anne-Marie Caminade, and Jean-Pierre Majoral,, ,(1998,), " *Regioselective Gold Complexation within the Cascade Structure of Phosphorus-Containing Dendrimers",* Chem. Eur. J. , **4,**: No. 10.

[75] Sadhana R Shahi, M.S.K., Gopal S Karva, Prabhanjan S Giram, Ruchika R Gugulkar,,(2015), "*DENDRIMERS",* Int. J. Pharm. Sci. Rev. Res.,, 33(1)**,**: 187-198.

[76] DONALD A. TOMALIA, J.M.J.F.,(2002), "*Discovery of Dendrimers and Dendritic Polymers: A Brief Historical Perspective,"* J Polym Sci Part A: Polym Chem 40: 2719–2728.

[77] Anne-Marie Caminade, A.O., Régis Laurent, Cédric-Olivier Turrin, Jean-Pierre Majoral, ,((2016)

), "*Coordination chemistry with phosphorus dendrimers. Applications as catalysts for materials and in biology",* Coordination Chemistry, 308 478–497]. .

[78] Tugba Sagır, S.I., Mehmet Senel, ,((2013)), "*Ferrocene incorporated PAMAM dendrons: synthesis, characterization, and anti-cancer activity against AGS cell line,"* Med Chem Res, , 22: 4867–4876

[79] M. Hirscher, M.B., M. Haluska, A. Quintel, V. Skakalova, Y.M. Choi, U. Dettlaff-Weglikowska, S. Roth, I. Stepanek, P. Bernier, A. Leonhardt, J. Fink, ,((2002),), "*Hydrogen storage in carbon nanostructures,"* Journal of Alloys and Compounds 330–332: 654–658.

[80] Azam S. Emami Meibodi, S.H.,((2014)), " *Amperometric urea biosensor based on covalently immobilizedurease on an electrochemically polymerized film of polyanilinecontaining MWCNTs",* Synthetic Metals 194**,**: 1–6

[81] Senel, M., C. Nergiz, and E. Cevik,(2013), "*Novel reagentless glucose biosensor based on ferrocene cored asymmetric PAMAM dendrimers",* Sensors and Actuators B-Chemical, 176: 299-306.

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