Advanced Materials Research Vol. 810 (2013) pp 173-216
© (2013) Trans Tech Publications, Switzerland
doi:10.4028/www.scientific.net/AMR.810.173

Biomedical perspectives of Polyaniline Based Biosensors

Amir Al-Ahmed[1], Haitham M. Bahaidarah[1]

and Mohammad A. Jafar Mazumder[2]*

[1]Center of Research Excellence in Renewable Energy, King Fahd University of Petroleum & Minerals, Dhahran-31261, Saudi Arabia

[2]Department of Chemistry, King Fahd University of Petroleum & Minerals, Dhahran-31261, Saudi Arabia.

Phone: (966) 3 86077836

*E-mail: jafar@kfupm.edu.sa (*Corresponding Author)

Keywords: Polyaniline, Biosensors, Transducer, Enzyme Kinetics, Immobilization.

Abstract. Electrically conducting polymers (ECPs) are finding applications in various fields of science owing to their fascinating characteristic properties such as binding molecules, tuning their properties, direct communication to produce a range of analytical signals and new analytical applications. Polyaniline (PANI) is one such ECP that has been extensively used and investigated over the last decade for direct electron transfer leading towards fabrication of mediator-less biosensors. In this review article, significant attention has been paid to the various polymerization techniques of polyaniline as a transducer material, and their use in enzymes/biomolecules immobilization methods to study their bio-catalytic properties as a biosensor for potential biomedical applications.

Introduction

Biosensors are becoming increasingly important as analytical tools because of their simplicity, specificity, fast and unique target recognizing ability, easy operation system, low sample preparation cost, repetitive measurements with miniature, and their "on site" usage [1-3]. Support for biosensors

as analytical devices is largely driven by the health care industry and to a lesser extent from the food quality, environmental monitoring and defense industries [1-4]. Typical biosensors, shown in Fig. 1 consist of three essential components namely, the biological detector, transducer and the output device.

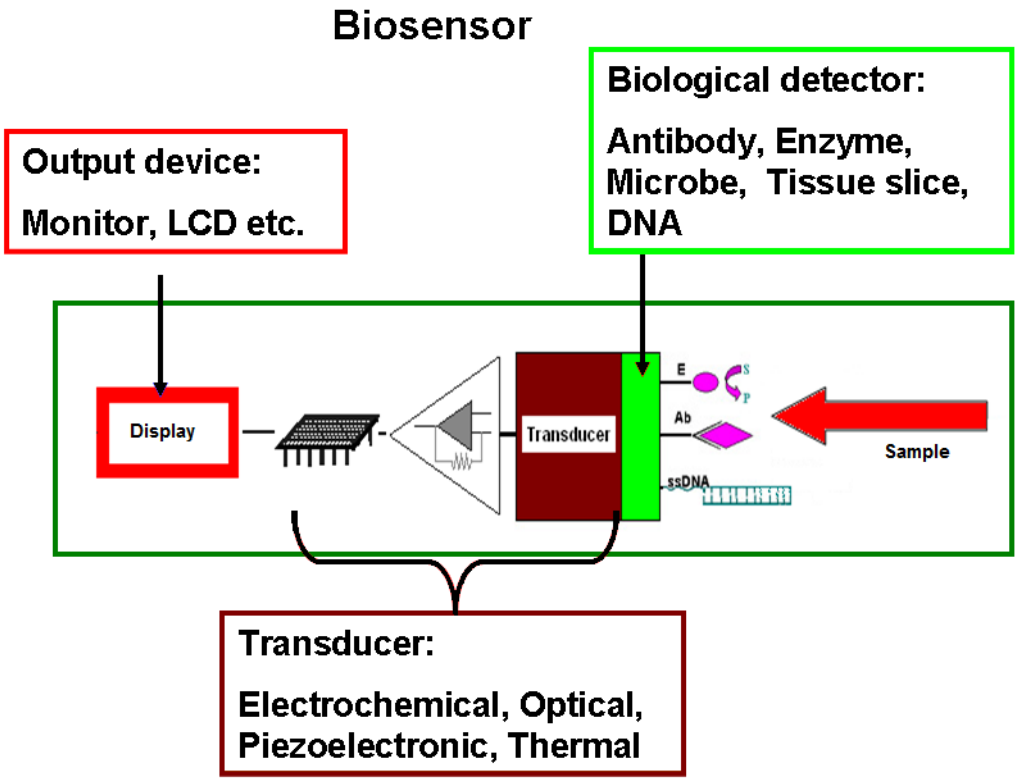

Fig. 1. Schematic representation of typical biosensors.

The biological detector recognizes a physical stimulus (or biochemical signal) that converted by the transducer into an electrical output which is amplified and displayed by an output device. The biological detector, receptor or biorecognition element is definitive, and is responsible for the selective recognition of the analyte that generates the physicochemical signal that being monitored at the transducer and ultimate sensitivity appeared to be on the bio-sensing device [4,5]. Single and multiple enzymes, organelles, whole cells or organisms, slices of animal and/or plant tissue contain many enzymes, various co-factors, antibodies, and antigens that may serve as receptors. Biosensor

can be classified or divided in many ways, which could specifically recognize as bio-recognition materials, and is responsible for its specific application.

An interesting immobilization concept is either to place the receptor in contact with, or integrated within the transducer. However, certain factors must be considered at the time of immobilization of the receptor, for example, it must retain substantial biological activity when attached and must remain tightly associated with the sensor surface whilst retaining its structural and functional credibility. In addition, the immobilized biological material must have long-term stability and a high degree of specificity towards analytes [1-4]. Transducer is one of the very important components of a biosensor. It converts the biochemical signal into an electronic one which can be amplified, stored and displayed. More often, transducer systems based on electrochemical, spectroscopic, thermal, piezoelectric and surface acoustic technology [3,6,7] need to be adapted in sensor assemblies depending upon the nature of their biochemical interaction with the species of interest. It is worthwhile to mention that in case of polyaniline based biosensors; researchers mainly used electrochemical transduction methods. Therefore, we have discussed this transduction methods in the text without further classification. However, successful biosensors must have certain beneficial features such as high specificity, adequate reproducibility, and accurate linear response over a wide analytical working range. In addition, biosensor should be cheap, compact and easy to handle, and non-interfering to the bio-catalytic reactions. Variety of bio-sensing devices that exploit enzymes, nucleic acids, cell receptors, antibodies and intact cells, in combination with electrochemical, optical, piezoelectric and thermometric transducers are readily available in various reference articles [9,10]. Within each permutation lies a myriad of alternative transduction strategies that have been applied to solve numerous analytical problems in health care, food and drink process industries, environmental monitoring, defense and security [1,2,11-14]. Owing to their attractive properties such as good biocompatibility, electrical conductivity, and stability, conducting polymers have been widely used for the immobilization of biomolecules and fabrication of sensors [15].

Electrically Conducting Polymers (ECPs) and Polyaniline (PANI) in Biosensors

The concept of using electrically conducting polymers in biosensors is based on the fact that it acts as both the immobilization matrix as well as the physicochemical transducer required to convert chemical signal (change of chemical potential of the microenvironment) into an electronic signal (change in electrical conductivity of the polymer). In 1976 Shirakawa, MacDiarmid and Heeger together with their talented research groups discovered the new class of organic polymers namely, the Electrically Conducting Polymers (ECPs) [16]. The ability to dope these polymers over a wide range of electrical conductivity from insulator through semiconductor to metal opened a new field of research and perspective [17-20]. Organic polymers are normally insulators. However, it can be presumed that an electrically conducting organic polymers show an unusual structure. ECPs such as, polyenes or polyaromatics contain a series of conjugated single (σ- bond) and double (π- bond) bonds, with π-electrons being highly delocalized along with easily polarizable polymer back bone. This ability of electronic delocalization of conjugated polymer provides a highway for charge mobility along with the polymer backbone [19-21]. Conjugated polymers have been widely used as a biosensor due to their distinct optical response in the presence of different analytes [22]. So far, a large number of ECPs have been prepared and investigated by different research groups. Some of the important and well studied ECPs are polyacetylene (PAC) [23], polypyrrole (PPY) [24], polyaniline (PANI) [24,25], polythiophene (PTH) [26,27] and poly(*p*-phenylene) [28,29]. Over the last two decades, electrically conducting polymers have established their unique position as an important constituent for various electronic and photonic devices due to their unusual characteristic properties. The earliest reported application of ECPs was the use of freestanding polymers in sensor devices that were designed to detect and measure the level of doping within the same material upon exposure to vapor-phase dopants. This simple sensor served to inspire the early pursuit of research into sensor applications of ECPs [20,21,30], and evolved many different schemes for using the transducer-active properties of electrically conducting polymers in chemical and biological sensors. This resulted in a rapidly growing academic and industrial research, patents, reference and product literature on ECPs

[31-33]. Furthermore, published works now appear in diverse forms and are being pursued by researchers of electrochemistry, analytical chemistry, material sciences, biochemistry and biotechnology [21,30-39]. The preparation of biosensors by immobilizing enzymes, immunochemical components, or other biorecognition species in the bulk or at the surface of the electrodeposited polymer was pioneered by Aizawa and Yabuki [40]. In general, chemical and biological sensors use electroconductive polymer films fabricated on metal or semiconductor electrode surfaces. The typical objectives of electrode modification with ECPs are to; (i) immobilize enzymes or biomolecules, (ii) shuttle electrons between the enzymes or biomolecules and electrode surface (ii) improve sensitivity, (iii) impart selectivity, and (iv) suppress the effect of interfering reactions [30]. The various techniques for incorporating enzymes into electropolymerizable conducting polymeric films help to localize the biologically active molecules onto an electrode surface of any size or geometry and are particularly appropriate in the fabrication of multi-analyte micro-amperometric biosensors.

The ECPs have considerable flexibility in their available chemical structure and can be modified as required by appropriate chemical modeling and synthetic methods. Hence, it is possible to modulate their electronic and mechanical properties. Since ECPs can directly be electrochemically deposited onto electrode surfaces, whilst simultaneously trapping biomolecules; it is possible to control the spatial distribution of immobilized enzymes, film thickness and modulate enzyme activity by changing the state of the polymer [41-54]. The ease with which ECPs are reversibly doped and undoped by electrochemical processes, accompanied by significant changes in their conductivity and spectroscopic properties, can be used as a signal for monitoring biochemical reactions. Furthermore, ECPs have the ability to transfer electric charge produced by a biochemical reaction to an electronic circuit. In an ECP based biosensor, the enzyme catalyzed redox reaction takes place in the bulk phase of this polymer layer on the electrode surface, and we get good detection capability and fast response from the biosensor [55-58]. Among the electrically conducting polymers, polyaniline is particularly different in nature due to its most highly conducting doped formed can be reached by

two complete different process i.e. protonic acid doping and oxidative doping. Protonic acid doping makes polyaniline, a unique ECP. It does not require any electron to be added or removed from the insulating material to make it conducting. In protonic acid doping of the emeraldine base unit for example, 1 M aqueous HCl; complete protonation of the imine nitrogen atoms gives the full protonated emeraldine hydrochloride salt. The reaction with a solution of chlorine in carbon tetrachloride also gives emeraldine hydrochloride. The same-doped polymer can be obtained by chemical oxidation (p-type doping) of leucoemeraldine base. This actually involves the oxidation of the σ- and π- systems rather than just the π-system of the polymer as is usually the case in p-type doping [19,21,59-61]. The synthesis of polyaniline is very simple; it involves aniline being chemically or electrochemically oxidized under acidic conditions. Because of the oxidation and subsequent dehydrogenation of two molecules of aniline, a quinone diimine forms. Multiple repetition of this process with simultaneous dehydrogenation affords emeraldine and nigraniline, which are long-chain molecules consisting of eight benzene rings with *para*-quinoid groups that are linked at the *para* position by nitrogen atoms. This converts to pernigraniline and finally aniline black [20]. In the middle of 1960s, polyaniline enjoyed a renaissance, and is currently experiencing a massive upswing in its fortunes regarding the development of electrically conducting polymers [20]. The main reason for the growing interest in polyaniline during the 1980s was attributed to its low cost, relatively easy production process, stability of the conducting forms and simple non-redox doping by protonic acids [19-21]. Unlike most other polyconjugated systems, the fully oxidized state of polyaniline is not conducting [19,21] and among the EPCs, polyaniline shows the best combination in terms of synthesis procedure, stability, response, and conductivity [62-67]. As a consequence, PANI and its derivatives, as well as its conducting composites are extensively used in biosensors and bioelectrochemical switches [30,62]. The structure of PANI is affected by the conditions employed during the synthesis such as concentration of monomer, pH of the electrolyte or solution, temperature, additives, and nature of doping agent. In the case of electro-synthesis, it also depends on electrode potential [30,62]. The conductivity of PANI films depends on the degree of

protonation along with the polymer back bone, and a PANI film grown in non-acidic medium usually shows conductivity in the insulating region [30,62], which limits its application in biosensors. However, this problem can be solved by using a separate dopant such as polystyrene sulfonic acid (PSS) [68], polyvinylsulphonate (PVS) [69] *etc*. These kinds of dopants are used to achieve electrical neutrality in the oxidized form of this polymer [1,2,30,62]. Similar to the chemical polymerization of aniline, the electrochemical polymerization using the cyclic technique also found to be very simple. Many biosensors have been fabricated and reported in which PANI and its derivatives served as the substrate layer; these biosensors showed excellent properties in terms of response; prolong work and storage life. A typical catalytic behavior of PANI in a biosensor, where PANI plays an important role of redox mediator between the electrode and enzyme (HRP) whilst at the same time holds the enzyme within its surface [62].

In this review article, we focused on different biosensors where polyaniline and its derivative have been used as a redox mediator for the immobilization of enzyme/ biomolecule.

Deposition of Polyaniline and its Derivatives on Electrode Surface

The surface, on which the biomolecule is immobilized, is responsible for retaining the structure of the biomolecule through hydrogen bonding or the formation of electron transition complexes. Synthesis of the electrode modifier and its eventual attachment to the electrode surface influences its electrochemical properties, retention of the biomolecules and general performance of the biosensor. Researchers therefore employed several methods of depositing polyaniline and its derivatives on the electrode surface. Among the chemical and electrochemical methods of polyaniline synthesis especially cyclic method is used for depositing polyaniline and its derivatives on the electrode surface. This could be due to the fact that : (i) the electrochemical reactions can be carried out at room temperatures, (ii) it allows direct, easy and controlled deposition of polyaniline on electrode, (iii) electrochemical synthesis can be used to prepare free standing and homogeneous and self doped films, (iv) by this method, it is possible to obtain copolymers and graft polymers, (v) by varying the

potential or the current with time, the thickness of the film can be controlled [1-4,70]. Singh and co-workers [71] electrochemically synthesized polyaniline on Indium-Tin-Oxide (ITO) coated glass plates using a three electrode cell configuration. The polyaniline layer formed on this electrode showed spongy and porous morphology. This porous structure was used as a support to entrap enzymes on the surface which was confirmed by the appearance of small and elongated globules/grains entrapped within the pores of polyaniline film after immobilization of cholesterol esterase and cholesterol oxidase. They further reported that polyaniline films grown at 1.1 and 1.2 V had a thickness of 10.9 μm and got scratched off from the surface of the ITO glass plate when cholesterol oxidase, cholesterol esterase or peroxidase was immobilized onto them, while the films prepared at 0.8 V were having thickness of 3.1 μm (too thin for immobilization of the enzymes). At 0.9 and 1.0 V they obtained a film thickness of 7.1 μm which was found to be suitable for immobilization of these enzymes. Furthermore, electrochemical polymerization through galvanostatic, potentiostatic and potentiodynamic allows for incorporation of a wide range of dopant ions since the reaction is carried out in the presence of a suitable electrolyte rather than a chemical oxidant [72]. Recent research has also made attempts to modulate electronic and mechanical properties of polyaniline in order to suit particular applications. One of the way in which this has been achieved by the addition of chemical reactants to preferentially protonate (dope) the imine nitrogen sites of the insulating polyaniline emeraldine base to yield an electrically conducting polyaniline emeraldine salt. Small anions such as Cl^-, NO_3^- and HSO_4^-, which can be incorporated into the film, can also be used to dope polyaniline. However, the polymer film formed with small anions appeared to be less stable and create an acidic environment inside its matrix. In general, for biosensor application, researcher prefers the doped polyaniline with neutral environment [70]. Therefore, large anion-like polyelectrolytes such as PVS, PSS etc are used in the synthesis of polyaniline. These large anions are not able to leave the polymer matrix and results to a stable polymer [70]. Moreover, polyaniline synthesized with these polyelectrolytes maintains charge neutrality during reduction and this poses an added advantage for immobilization of the biomolecule.

Otero and co-workers [73] showed that the presence of a polyelectrolyte in a polymerization solution results in increased growth rate, higher compactness and improved environmental stability of the synthesized film. In another case, Grennan and fellow researchers [72] used electrochemically deposited PVS doped polyaniline in 1 M HCl on screen printed carbon as well as glassy carbon electrodes. As reported by the authors in this particular example, the role of PVS was to maintain electrical neutrality in the oxidized form of the polymer and also to increase the structural conductivity and stability of the polymer over a broad range of pH. In both chemical and electrochemical syntheses of polyaniline, structure directing organic or inorganic molecules are added to the reaction mixture to get nano structured polyaniline, composites or copolymers. Self-directing of structural polymeric films can also be achieved by using template platforms. Mazur et al. [74] chemically deposited polyaniline and poly(2-methoxyaniline) into a polycarbonate membranes. During the synthesis process, polyaniline and poly(2-methoxyaniline) were obtained within the template. A wide range of chemically and electrochemically nanosized polyaniline, copolymer of polyaniline and composites such as polyaniline (PA), poly(*o*-anisidine) (POA) and poly(aniline-co-*o*-anisidine) (PA-co-POA) [75], polyaniline/poly(acrylic acid) [76], and *o*-ainophenol-aniline [77] have been synthesized and reported. Xian et al. [76] grew polyaniline/poly(acrylic acid) (Pan/PAA) on glassy carbon electrode coated with porous-sol-gel (PSG) which acted as film template. According to this method, the PSG template not only lead to the formation of a ordered monolayer film but also gave a less potential energy (ΔEp) for PSG/Pan/PAA electrode than Pan/PAA, and could behave as "molecular wires", thus finding an application as a nanoeletrode. Zhou et al. [78] used pulse galvanostatic method (PGM) when they electro-deposited polyaniline onto a stainless steel electrode. They found better nano fibers with large specific surface area, and in contrary better biosensor performance. They again deposited Pt microparticles onto this nano fibers followed by a protective layer of poly (m-phenylenediamine) (PMPD), which electrochemically form the solution of m-phenylenediamine and glucose oxidase (GOD). Using the PMPD as an anti-interferent barrier as well as a protective layer, it helped to get high level of selectivity and good biosensor response. In another

biosensor platform, Zou et al. [79] chemically synthesized polyaniline-Prussian Blue/multi-walled carbon nanotubes (PANI-PB/MWNTs) hybrid composites by spontaneous redox reaction. The PANI-PB/MWNTs modified electrode was found to be stable after scanning it in 0.1 M PBS of pH 6.5 for 50 cycles without decreasing peak current. Good stability of the hybrid composites was attributed to the method used in their preparation. Xian and co-workers [80] chemically synthesized AuNPs-conductive PANI by mixing $HAuCl_4$ and freshly distilled aniline (1 mol/L HCl), and stirring it continuously for 12 hours in presence of H_2O_2 which acted as oxidizing and reducing agent. The morphology of the resulting nanocomposite, studied under transmission electron microscopy (TEM) was found to be relatively uniform distribution of PANI nanofibres with diameters about 30-50 nm. On loading AuNPs on these PANI nanofibres, a relatively uniform PANI-metal nanocomposite with diameters between 60-80 nm was formed. By comparing the different depositing method, it is very clear that the nano fiber synthesized by PGM method has better surface morphology even than that of template method. Therefore, researcher could potentially use this PGM method to prepare the nano structured conducting polyaniline with better properties or even other conducting polymers to fabricate their biosensors.

Immobilization of Biomolecules on Polyaniline and its Derivatives

Immobilized biomolecules should retain their active functionality on the electrode surface or a matrix modified electrode. The immobilization matrix may be a membrane, gel, carbon, graphite, silica and polymeric films [1,3]. The immobilization of biomolecules (e.g.; protein molecules) should be performed under conditions that provide membrane like environment in which all the normal interactions of the biomolecules are preserved. The method chosen for immobilization of biomolecules should therefore prevent loss of their activity by avoiding the change of the chemical nature or reactive groups in the binding site of the biomolecule. Depending on the nature of biomolecules, the platform on which it is to be immobilized and the transduction method has been proposed and well accepted by researchers. Generally, active immobilization principles include

physical adsorption at a solid surface, cross-linking to a polymer network, covalent binding to the surface, and entrapment within a membrane, surface matrix, polymer, or microcapsule. Sol–gel entrapment, electropolymerization, self-assembled biomembranes, and bulk modification, among others, are also being used [1-4]. Immobilization leads to the changes in enzyme structure causing the immobilized enzyme kinetics, stability, and specificity of enzyme that differ from the enzyme in homogeneous solution. One of the methods on immobilizing the biomolecule is to entrap them directly into the polymer film during electrochemical polymerization. However, the biomolecule may be affected by monomers of the polyaniline or its derivatives and become denatured or inactive [81]. In another method, the biomolecule is electro statically attached onto the polymer film during oxidation of a pre-reduced polymer film in a buffer solution containing active biomolecules. Grennan et al. [72] for instance immobilized horseradish peroxidase (HRP) onto electrochemically polymerized polyaniline film by reducing the film modified electrode (either screen printed carbon or glassy carbon electrodes) at -500 mV followed by oxidation in presence of the enzyme at +700 mV. A similar method was used by Jiang et al. [82] to immobilize uricase onto polyaniline films prepared previously on platinum sheets by electrochemical polymerization. They reduced this polyaniline modified electrode at -200 mV in Britton-Robinson (B-R) buffer of pH 7.0 for 20 minutes. The reduced electrode was transferred into another buffer solution containing 0.75 mg cm^{-3} of uricase and oxidized at + 600 mV for 30 minutes. The biosensor fabricated by these authors lost only 18% of its activity after 157 days and 50% in 260 days. This relatively long stability of this biosensor suggests that the two-step method used in immobilizing the enzyme ensured a biocompatible microenvironment and no significant denaturation occurred. Above mentioned immobilization methods, amperometry was the transduction method that usually used for the analysis of various substrate samples [83,84]. In other immobilization procedures, the biomolecule, previously dissolved in bovine serum albumin (BSA) is mixed with a suitable cross-linker (usually glutaraldehyde) and physically adsorbed onto the surface of polymer modified electrodes. Glutaraldehyde is known to be a bifunctional compound mainly used in chemical modification of

proteins and polymers [85]. It is covalently links with amine (-NH_2) groups of lysine or hydrolysine in the protein molecules, creating a structure more stable than that attained by physical aggregation (Fig. 2).

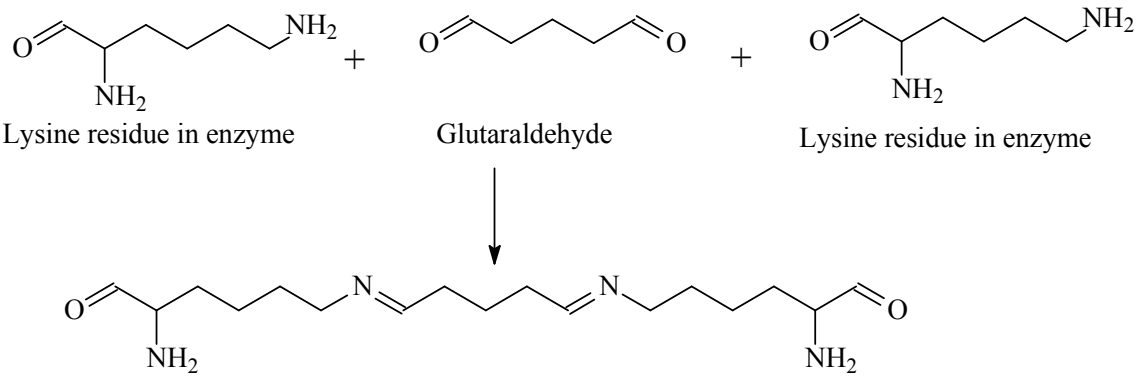

Fig. 2. Typical cross linking reactions between glutaraldehyde and lysine residue

The bonding results reinforce the compact tertiary structures, causing protein stabilization against pH inactivation for immobilizing the respective biomolecules. The cross linking mechanism of glutaraldehyde with lysine residues on the exterior of the proteins (enzyme) is shown in Fig. 2. In the electrochemical synthesis of polyaniline, a large number of free amine groups are present at the end of each polyaniline chain. These amine groups bind with a cross-linker glutaraldehyde, in which the C=O groups of the glutaraldehyde binds with the -NH_2 via condensation reaction releasing a water molecule and resulting in a covalent linkage of the biomolecule and polyaniline. Chenghong and Deng [86] successfully immobilized HRP enzyme on glassy carbon electrode using Bovine Serum Albumin (BSA)-Glutaraldehyde matrix crosslinker. This includes immobilization of glucose oxidase on gold nanoparticles-conductive polyaniline modified glassy carbon electrode [80], polyaniline-Prussian Blue multi-walled carbon nanotube modified glassy carbon electrode [79], polyaniline/poly(acrylic acid) modified glassy carbon electrode [76], cholesterol esterase, cholesterol oxidase and peroxidase on polyaniline modified ITO coated glass plates [71] and uricase on polyaniline modified ITO coated glass substrates [87]. An interesting two step template method of immobilizing enzymes was reported by few researchers [81]. Pan et al. [85] developed a

procedure, in which aniline was polymerized in presence of enzyme onto the electrode surface and then a suitable reagents (6.0 M hydrochloric acid or 4.0 M sulphuric acid) was used to fully hydrolyze the enzyme from the polymer layer over a period of time (~20 hours), removing the immobilized enzyme from the polymer film, and create spaces for subsequent enzyme attachment. A fresh enzyme solution was then used to immobilize enzyme on this modified electrode surface. By this template process, the insufficiency of common immobilization problems such as denaturing of enzymes as mentioned in one-step method could be overcome. Pan et al. [85] studied biosensor response and their stabilities using electro statically attached enzyme electrodes, and compare them with template process attached electrodes. They found that the response current of the template based biosensor reached its maximum value in 48 hours, then decreased only by 4.6% in 30 days time period, while electrostatic attachment based biosensor decreased approximately by 55.4% within the same studied period of time. This suggests that a template based biosensor was more stable and maintain its well electrochemical activity over the period of time.

Polyaniline in Biosensor Applications

Glucose Biosensor. Diabetes is one of the world's leading causes of human death and disability. Insulin deficiency and hyperglucemia causes blood glucose levels to become higher or lower than the normal range of 80 to 120 mg/dL or 4.4 to 6.6 mM; therefore this disease requires regular monitoring of blood glucose level. Amperometric biosensor based on glucose oxidase (GOD) enzyme played the leading role towards an easy to use compact blood sugar monitoring device [88] [89,90]. Since 1962, Clark and Lyons [91] proposed the very first concept of a glucose enzyme electrode; this biosensor research has experienced tremendous interest and development. Initial glucose biosensors used natural oxygen co-substrate, and detect the generated hydrogen peroxide. The bio-catalytic reaction involves the reduction of the flavin group (FAD) to ($FADH_2$) of the enzyme by glucose. This is followed by reoxidation of the flavin by molecular oxygen to regenerate the oxidized form of the enzyme GOD (FAD), and measuring the H_2O_2 generated. In late 1980s

different polymers, multilayers, mixed layers with transport properties based on size, charge or polarity have been used to construct glucose biosensors that are capable blocking interferences from coexisting electroactive compounds. If we categories GOD biosensors into different generations on the basis of their development, then the first generation were based on natural oxygen cofactors, the second generation on artificial redox mediators and third generation on direct electron transfer between GOD and electrode. Although many researchers claim the direct electron transfer between GOD and electrode is superior one, so far only a few turn out reliable results for mediator-less detection was crystalized till date [90,91]. The introduction of EPCs in the late 1980s caused a surge in sensor and especially, biosensor research largely because EPCs could serve as both an enzyme immobilization layer and as a redox mediator. The recent developments in nanotechnology also facilitated the use of nanostructured conducting polymers in biosensor technology along with carbon and gold nanoparticles [92]. Researchers used various electrode platforms such as, platinum [93-101] gold interdigited microelectrodes on oxidized silicon wafers [102], gold film covered with polycarbonate membrane [102], glassy carbon [76,79,80], stainless steel [78], and on passivated iron electrodes [103] to fabricate glucose biosensor. Borole et al. [95] compared different polymers such as PANI, poly (o-toludine) and poly(aniline-co-o-toluidine) copolymer based glucose biosensor. They electrochemically deposited these polymers and copolymers onto platinum electrode surface and found PANI-GOD biosensor having the fastest response time as compared to other two. Whereas, Dumont et al. [104] used polyaniline, polypyrrole, poly(o-phenylediamine) separately and polyaniline cross linked with p-phenylenediamine onto a platinum electrode surface. They found that the poly(o-phenylenediamine)/glucose oxidase based biosneosr gave better detection limit and response, and the PPY/GOD film based biosensor gave the most reproducible current responses. A biosensor array based on polyaniline was reported by Sangodkar et al. [101] for estimating glucose, urea and triglycerides in a single measurement. They polymerized aniline from the solution of aniline and GOD in acetate buffer to immobilize GOD at the time of polymerization and later deposited lipase and urease by adsorption method from their acetate buffer solution. This biosensor was able to

detect glucose, urea and triolein in a single measurement from a few micro liters of the mixture of the three. Karyakin et al. [105] reported the potentiometric detection of glucose, where trypsin and GOD immobilized within a PANI film covered with a Nafion protective layer had a detection limit of 10^{-7} M for paraoxon, which was lower than other reported potentiometric devices available in the market. The loading capacity of GOD was studied by Verghese et al. [106] for electrochemically polymerized PANI films using self-ion-exchange techniques. They exchanged tosylate and ferricyanide with chloride ion and found that it enhanced the porosity of PANI film which enables it to take more GOD in its surface. Gerard and Malhotra [107] also prepared two different PANI films electrochemically in presence of tosylate, ferricyanide, immobilized GOD and LDH by physical adsorption method. The results indicates that the self-ion-exchange in PANI-tosylate and PANI-ferricyanide film play an important role for better enzyme loading on PANI. Polyaniline was electrodeposited on a platinum electrode coated with microporous polyacrylonitrile film [96] from the solution of aniline and GOD in buffer to immobilized GOD during polymerization process. In another report, Sukeerthi et al. [102] used a gold electrode covered with isoporous polycarbonate and deposited GOD during electro polymerization of aniline from the solution of aniline and GOD in buffer. They also immobilized Lipase and Urease by the adsorption method onto the same electrode surface, and this biosensor was able to detect glucose, urea and triolein in a single measurement from the mixture of these three. PANI was also deposited on poly(acrylonitrile-co-acrylic acid) coated Pt electrode [100] and found detection limit of 1 μM glucose with fairly good stability with no significant decrease in the response after 45 days when studied at 4 °C in 0.1 M PBS. Xue et al. [98] dip-coated polyisoprene (PIP) onto a platinum electrode and electropolymerized aniline onto this PIP coated electrode at a constant potential (0.65 V) and immobilized GOD by electrostatic attachment technique. Garjonyte et al. [97] electrodeposited a Prussian Blue (PB) layer onto a Pt electrode followed and electrodeposition PANI over this PB layer. They immobilized GOD by the electrostatic attachment technique, and in another case, electropolymerization of aniline from the solution and GOD in PBS, and found the later one has better sensitivity. Chen et al. [108] constructed self-doped

PANI-PB bio-component film, which was fabricated via inverted crystal template technique using step-by-step electrodeposition. It was found that PB not only acted as a redox mediator, but also increased stability in neutral or weak alkaline solution. Zou et al. [79] prepared PANI-PB hybrid in typical chemical synthesis method and mixed multi-walled carbon nanotubes (MWCNTs) with this hybrid, and then dip coated onto the GC electrode by spin coating technique. The glucose biosensor they prepared with this electrode shows good stability with a Michaelis-Menten constant, k_m value of 5.1 mM and maximum current i_{max} of 249.5 μA. Myler et al. [109] have grown PANI film electrochemically from a solution containing aniline and GOD in buffer, which leads to a better enzyme attachment within the substrate layer. In another method, Xian et al. [80] prepared nanocomposite suspension using nano structured PANI and Au nano particle, and dip coated 3 μL of this suspension onto a GC electrode and immobilized GOD using gluteraldehyde. They put a protective Nafion layer onto this enzyme electrode. Their biosensor gave detection limit of 5.0×10^{-7} mol/L with a storage time of 2 weeks. Xianhua et al. [81] used a template process to prepare PANI based glucose biosensor by taking advantage of the linkage between uricase and GOD with the nitrogen sites of the quinodine ring in uricase and found good stability and detection limit. Xian et. al. [76] used porous sol-gel film based template process and electropolymerize aniline in presence of polyacrylic acid. They immobilized GOD onto this surface using gluteraldehyde and covered this bio-electrode with a protective Nafion layer to eliminate some interference. This glucose sensor was able to detect as low as 2.0×10^{-7} M of glucose with a very fast response time of 7 s. Ali Eftekhari [103] found good electrochemical properties such as better sensitivity and stability of PANI film grown on a passivated iron electrode on contrary to his glucose biosensor. Nano-structured PANI was grown on a stainless steel (SS) electrode by the pulse galvanostatic method (PGM), followed by the deposition of platinum micro particles using same PGM method [78], followed by the immobilization of GOD by electropolymerizing *m*-phenylenediamine (MPD) onto this SS/PANI/Pt (micro particle) electrode from a solution of MPD and GOD in buffer. The resultant glucose sensor showed a fast response time (nearly 7s) with good stability and reproducibility.

DNA Biosensor. Detection of appropriate DNA sequences has immense importance in diagnosing genetic or infectious diseases, environmental testing for bacterial contamination, rapid detection of biological warfare agents, forensic investigations and scientific exploration of genomics and proteomics. A tremendous development in nano and biotechnology creates opportunities for advance gene detection, which had enabled us to construct appropriate DNA based biosensor to use in the fields mentioned above. Initially, DNA biosensors were based on the application of electrochemical labels for indicating the immobilized single-stranded DNA's interaction with the target DNA present in the sample. However, the level free DNA sensors are mainly based on monitoring changes in electronic or interfacial properties accompanying DNA hybridization. The key factor for the successful fabrication of these kinds of sensors is to create an efficient interface between the nucleic acids and electronic transducer. Along with other materials polyaniline has been used to modulate the DNA interaction and for inducing electrical signals accrued from such interactions and for localization [110-112]. Wu et al. [113] used two different electrodes to construct a DNA biosensor. In the first case, they mixed PANI and graphite powder in paraffin oil to form a paste and was squeezed into a PVC tube to fabricate this electrode system. In second case, they electropolymerized PANI onto a glassy carbon electrode and then immobilized Calfthymus-DNA (CTDNA) and Rice-DNA on these modified electrode surface by immersing these electrodes in a tris buffer solution containing these DNAs. The resultant biosensors were able to monitor DNA hybridization and able to distinguish between single and double strand DNAs. L-DOPA (levodopa), a precursor of dopamine normally administrated to patients suffering from Perkinsion's disease and Dopa-Reponsive dystonia in order to boost the level of dopamine in the brain. L-DOPA is capable of crossing the blood-brain barrier into the brain where it is converted to dopamine. Bo et. al. [114] developed a novel DNA biosensor based on oxidized graphene and polyaniline nanowires (PANIw) modified glassy carbon electrode. The resulting graphene/PANIw layers exhibited good response for the complementary DNA sequences, which might be attributed to the effect of graphene and PANIw. Ma et al. [115] deposited self-doped polyanlineboronic acid on gold electrode surface and

immobilized single walled carbon nanotubes (SWNT) wrapped with ss-DNA. In another report, Ali et al. [116] used Nafion and carbon nanotubes on self-doped polyanilineboronic acid coated gold electrode surface to immobilize ss-DNA. Strong interactions between the polyaniline backbone and ss-DNA/SWNT were apparent; while ss-DNA/SWNT also showed good electrocatalytic reductive ability which greatly improved the overall electrochemical activity of the polymer. Both the biosensor showed good sensitivity and detection limit of dopamine. The results were found to be promising towards the molecular diagnosis of Parkinson's disease. Studies based on DNA hybridization was reported by some researchers has revealed that polyaniline or PANI nano particles served as both the DNA immobilizing layer, as well as the transducer. It is found that PANI nano particles contributed towards signal enhancement during hybridization process [112,117]. Highly organized PANI nanotube arrays [112] and PANI nanowires [117] were prepared on graphite electrodes using a nano porous layer of tin as a template. In both cases, the DNA-sensors were used to monitor the hybridization events between the immobilized probe DNA and target. Again, Arora et al. [87] reported an Escherichia coli detection biosensor based on the DNA hybridization detection technique; this technique helps to avoid PCR amplification. The same research group also reported a DNA biosensor based on the DNA hybridization detection technique, and immobilized five different 5′-biotin labeled probes ODNA on a PANI/Pt-electrode and found a moderate hybridization response time of 60s [118].

Uric Acid Biosensor. Determination of uric acid level in blood sample has importance for the patients suffering from gout or hyperuricaemia. The normal uric acid level in human blood ranges from 0.15 to 0.45 mM. Any further increase of uric acid concentration in human blood can lead to complications such as leukemia, pneumonia, etc. [119]. Biosensors can provide fast, inexpensive and reliable uric acid measurements when compared with other available methods. Yung-Chien et al. [120] electropolymerized aniline onto a gold plated ceramic electrode that had been coated with a layer of Nafion. They prepared two different electrode systems; the first system comprised of a GA

(Urease)/PANI-Nafion/Au/ceramic plate electrode; onto which urease was immobilized from a solution of urease and glutaraldehyde. The second electrode system consisted of a Nafion (Urease)/PANI-Nafion/Au/ceramic plate electrode, where they used solution of urease and Nafion in methanol to cast the enzyme layer. In comparison to the previously published methods, both bio-electrodes enhanced sensitivity and stability. A template procedure was reported by Jinqing and co-workers [121], they first electropolymerized aniline from the solution of aniline and uricase followed by refluxing PANI for 24 h in 6 mol/L HCl to remove the enzyme. After reducing the PANI film in Britton and Robinson (B-R) buffer, the electrode was immersed into another B-R buffer solution containing uricase. They found this template procedure resulted in better stability and affinity between uricase and substrate than for the conventional one or two step procedure. Pandey and co-workers [122] immobilized urease onto a tetraphenylborate (TPB) doped PANI substrate layer using two slightly different techniques from (i) a mixture of urease and polyvinyl alcohol in Tri-HCl buffer and (ii) an urease solution in Tri-HCl buffer. They reported a detection limit of 20 μM for urea with good stability. Cho et al. [123] electrodeposited PANI onto a GC electrode modified with a Nafion layer and immobilized urease (from a mixture of urease and glutaraldehyde) by cross-inking the enzyme onto the polyaniline surface. The biosensor showed good stability, a low detection limit and quick response time. Gluteraldehyde was also used by Kavita et al. [124] as a cross-linker between the amine groups of the electropolymerized polyaniline and amino groups of the enzyme (uricase). The working linear range for this uric acid biosensor extended from 0.01 to 0.6 mM, and showed good storage time and sensitivity. Karyakin et al. [125] dissolved chemically synthesized PANI in chloroform and sprayed this solution on screen printed and GC electrodes. They immobilized urease onto these modified electrode surfaces by casting method and later they put an additional layer of Nafion on this surface, and found good stability and comparable detection limit. Castillo-Ortega et al. [126] reported a biosensor based polyaniline and n-butyl-methacrylate composite films for estimating uric acid. They measured the change in electrical resistance with increasing uric acid concentration. They also used PANI-Poly (vinyl methyl ether) (PVME) and PANI-Poly (vinyl ethyl

ether) (PVEE) composites to construct a uric acid sensor. Jiang et al. [82] developed a polyaniline based uric acid sensing biosensor using the template method. The method depends upon the generation of H_2O_2, and its consumption by peroxidase and then measurement of dissolved oxygen concentration by biosensor. They reported a maximum current response i_{max} of 58.07 μA with an small apparent Michaelis-Menten constant, k'_m of 7.83 mMol.dm^{-3} in comparison to other similar method. The small k'_m value indicates the stronger affinity between the uricase and substrate. Pan et al. [77] copolymerized aniline and o-aminophenol in the presence of uricase followed by hydrolysis with HCl to remove uricase, followed by the immobilization of uricase using the electrostatic attachment technique onto a platinum sprayed quartz glass electrode. They prepared another set of the same electrode system using the conventional two step procedure and found a maximum current response i_{max} of 26.53 μA with a apparent Michaelis-Menten constant k'_m of 10.08 mmol.dm^{-3} for the first set of electrode. This Michaelis-Menten constant was lower than the electrode prepared using conventional two step process reported by the same research group [77]. This lower value of Michaelis-Menten constant indicates the stronger affinity between enzyme and substrate in case of electrode system prepared by the template process. They also found that the biosensor prepared by template process is more stable than two step method.

Peroxide Biosensor. Hydrogen peroxide is used as a bleaching agent in many industries; it is used in different pharmaceutical and cosmetic products, and present in vegetable oils and many food products. Most analytical techniques for peroxide determination such as chromatographic methods, chemical reduction, calorimetric and photometric methods are generally time-consuming or the instruments themselves are expensive and not very suitable for routine analysis. HRP based biosensors provide a relatively faster, sensitive and inexpensive means towards quantitative peroxide analysis [62,127]. In peroxide biosensors- different polymers, electroactive salts and nano particles (gold, carbon etc.) have been used to immobilize HRP onto the electrode surface and act as electron mediators between the electrode surface and enzyme. In peroxidase biosensors, researchers mostly

used electropolymerized polyaniline layers for enzyme immobilization and transduction on different electrode platforms, such as platinum [62,128,129], gold [130], glassy carbon [131-135], screen printed [72,136,137] and indium tin oxide (ITO) electrodes [138]. Radhapyari et al immobilized HRP based biosensors via glutaraldehyde onto the polyaniline modified electrode shows promising characteristics in detecting anticancer drug [139]. Iwuoha et al. [62] reported that the PANI film does not alter the Michaelis-Menten behavior of the HRP based bioelectrode. The Hill plot and their biosensor showed good efficiency for analyte detection when operated in the organic-phase mode (in acetonitrile medium) rather than in aqueous buffer. Luo et al. [135] dip coated a SiO_2 layer onto a GC electrode and electro polymerized a PANI nano film at constant potential +9.0 V on top of the SiO_2 layer. A comparison of the HRP/PANI/SiO_2/GC electrode with a HRP/PANI/GC was made, the SiO_2 containing electrode was found to be more sensitive with a response time of 2s, and detection limit of 1.8×10^{-7} M, based on the signal-to-noise ratio of 3. In another procedure, they dip coated carbon nanotubes over GC electrode and electro-polymerized aniline onto the modified GC electrode surface, followed by the immobilization of HRP onto same modified electrode by electrostatic attachment technique. Resulting biosensor provide good detection limit of 6.8×10^{-8} M and sensitivity of 44.3 μmM^{-1} [130]. Morrin et al. [137] dispersed chemically prepared polyaniline nanoparticles doped with dodecylbenzenesulphonic acid (nanoPANI/DBSA) in distilled water, and added HRP solution in PBS; they then dip coated the resultant nanoPANI/DBSA/HRP onto a screen printed electrode surface. In another technique, the same research group [132] electrodeposited DBSA doped PANI nanoparticles onto a GC electrode surface and immobilized HRP onto this modified surface. Grennan et al. [72] reported that the thickness of electropolymerized polyaniline films on a screen printed electrodes influences the biosensor's response towards H_2O_2 detection. A mesoporous PANI film was deposited onto the GC electrode [134] by a template process using Brij56; the biosensor showed good stability, reproducibility, and a wide linear range with a detection limit of 0.63 μM. Ngamna et al. [138] prepared a solution of HRP, BSA in poly-2-methoxyaniline-5-sulphonic acid (PMAS) with different concentration of HRP and mixed with poly(L-lysine)

hydrochloride (PLL). They dip coated this resultant material onto a UV treated ITO Mylar electrode surface; the biosensor gave maximum response at a concentration of 35 w/v% HRP with a detection limit of 0.1 nM for H_2O_2. Piletsky et al. [140] chemically polymerized aniline onto a highly porous polypropylene membrane and immobilized HRP onto this composite surface by absorption as well as by electrochemical techniques. They found that the quality of immobilization of HRP and activity of the electrode depended on the PANI film.

Cholesterol Biosensor. The presence of cholesterol in our food for example, some cooking oils, red meat, milk etc. is directly related to the increase in heart disease, coronary artery diseases, myocardial infarction, cerebral thrombosis, atherosclerosis and hypertension that we as a society are facing [141]. High cholesterol level in human blood is very dangerous that can create serious health issue for example problem in kidney, heart etc. Therefore, the blood cholesterol levels monitoring is an important issue in our everyday life. Biosensor can provide an easy, fast and inexpensive means for detecting cholesterol [71, 85]. Singh et al. [85] electropolymerized polyaniline onto an ITO coated glass electrode, followed by an immobilization of cholesterol esterase and cholesterol oxidase with the help of glutaraldehyde. In another case, they immobilized cholesterol esterase, cholesterol oxidase and peroxidase onto the similar electrode surface using glutaraldehyde [71]. These biosensors were characterized thoroughly by different experimental protocols such as loading of an enzyme onto the PANI surface, the effect of the working buffer's pH, working temperature, various interferents etc. Dhand et al. [142] electrophoretically deposited a nano structured polyaniline colloidal suspension on an ITO coated glass electrode, then they immobilized cholesterol oxidase using n-ethyl-n'-(3-dimethylaminopropyl carbodiimide) (EDC) as coupling agent and N-hydroxysuccinimide (NHS) as an activator to form a new covalent bond between the $–NH_2$ group of polyaniline and the -COOH group of cholesterol oxidase. Detection limit, stability sensitivity of these cholesterol biosensors are given in Table 1.

Table 1. Results obtained by researcher in PANI based cholesterol biosensor

Other polymer or material used	Polymerization technique	Linear range	Detection Limit	Response Time	Stability	Sensitivity	[Ref]
Gluteralde-hyde (GA)	Electrochemical	50-500 mg/dl	25 mg/dl	40s	6 weeks	$7.5 \times 10_{-4}$ nA/mgdl	[136]
Gluteralde-hyde (GA)	Electrochemical	50-500 mg/dl	25 mg/dl	-	6 weeks (at 4 ºC)	0.042 μA/mgdl	[137]
-	Chemical	25-40 mg/dl	25 mg/dl	-	11 weeks	7.76×10^{-5} Abs $(mg/dl)^{-1}$	[138]

Immunosensors. Immunosensors transduce antigen-antibody interactions directly into physical signals. The design and preparation of an optimum interface between the biocomponents and detector material is the key element in sensor development. This class of biosensors (immunosensors) has far reaching prospects in clinical applications. A conductometric immunobiosensor for detecting the Bovine viral diarrhea virus (BVDV) was reported by Thair et al. [143]. They used polyclonal and monoclonal antibodies for detection and PANI (synthesized in different acidic medium) as a transducer. Same research group [144] also developed an immunosensor electrode system on a conjugated pad to detect food borne pathogens. In this particular case, they used water soluble PANI with dehydrated antibody to prepare the PANI-antibody conjugate in a conductometric detection system. Pal et al. [145] prepared different polyaniline nanowires; i) doped with HCl, ii) doped with perchloric acid, and iii) emaraldine salt to fabricate biosensors to detect food borne pathogen Bacillus cereus. They found that the biosensor prepared by using emeraldine salt at a concentration of 0.25 g/ml gave the best performance in terms of sensitivity. Sai et al. [146] prepared a piezoelectric immunobiosensor by taking advantage of the covalent bond formation between the secondary amine groups of polyaniline and the aldehyde group of gluteraldehyde. They immobilized human immunoglobulin (HIgG) onto the modified electrode

and studied its activity by using FITC-targeted goat anti-HIgG antibodies (Ga HIgG), which were specific to Fc region of HIgG. The minimum detection limit was found to be 500 ng/mL or 3 nM IgG. Timur et al. [147] prepared three different biosensor electrodes based on PANI films. They immobilized Trametes versicolor laccases (TvL), Aspergillus niger laccases (AnL) and Agarieus bisporus tissues (AbT) onto the individual electrode. This electrode was able to detect phenolic compounds. The response time was 300 s for all the electrode system. Brief characteristics of these immunosensors are presented in Table 2. Owino et al. [148] electrodeposited PANI onto a Pt electrode from the solution of aniline and polystyrensolfonic acid in 1 M HCl. This electroactive layer successfully transfer electrochemical signals, and hold the AFB1 antibody (AFB1-Ab) on its surface. This Immunosensors was able to detect toxic aflatoxinB1 (AFB1) successfully at the 0.1 ppm/ml of aflatoxinB1 in the experimental solution.

Table 2. Some results of PANI based Immunosensors.

Other polymer or material used	**Polymerization technique**	**Liner range**	**Detect limits**	**Response time**	**Sensitivity**	**[Ref]**
a. Perchloric acid b. 4-hydro-benzenesulp-honic acid/ Phenylphos-phonic acid c. Hydrochloric acid/ Sulfobenzoic acid	Chemical	10^1 to 10^5 CCID/mL	a. 10^3 CCID/mL b. 10^5 CCID/mL c. 10^4 CCID/mL	-	-	[143]

	Chemical	10^1 to 10^5 CFU/mL	7.9 ×10^1 CFU/mL	10 min	10^1CFU/mL	[144]
Perchloric acid	Chemical	-	1.2× 10^1 CFU/mL	6 min	10^1 to 10_2 CFU/mL	[145]
Glutaraldehyde	Electrochemical	500 ng/mL - 25µg/mL	1 µg/mL	-	2.5 to 100 µg/mL	[146]

Polyaniline Based Other Biosensors. Choline is an essential nutrient required by the body for healthy cell membrane function. Amperometric acetylcholinesterase (AChE) biosensors show a promising alternative to the conventional methods due to their sensitivity, rapid response, good selectivity and miniature size [149]. AChE biosensors have shown satisfactory results for pesticides analysis [150]. Micro and nano layers of polyaniline was deposited onto a gold electrode surface by cycling the potential between -0.2 and +0.7 V and immobilizing Choline oxidase (CHOD) base onto the modified surface [151]. They observed that the required optimum enzyme immobilization time was found to be 45 minutes for a film thickness of 500 nm with a sensitivity of 5 µA/mM in amperometric mode, and 10 µA/mM in potentiometric mode. Fiorito and co-workers [152] prepared two sets of electrode systems; i) they used only an electrodeposited, self doped PANI film, and ii) a thin layer of PB was placed onto electrode surface prior to electrodepositing the self doped PANI layer; oxalate oxidase (OXO) was then immobilized onto both substrate using glutaraldehyde. The results showed that the second electrode system, with a thin biolayer of PB gave stable, sensitive and selective responses towards oxalate, with a detection limit ranging from 0.08 to 0.45 mmol.L^{-1}. Hu et al. [153] prepared a novel hypoxanthine biosensor using sodium montmorillonite-methyl viologen carbon paste on a chemically modified clay electrode. They immobilized xanthine oxidase (XO) onto the modified electrode surface, and then polymerized aniline from a solution containing aniline and

XO in sodium phosphate buffer. This biosensor was stable at pH 6.8-8.6 and temperatures 30-38 ^{o}C with a detection limit of 0.8 μM of hypoxanthine. Aniline was electropolymerized onto a polyacrylonitrile (PAN) coated Pt electrode at a constant potential (0.48 V), and polyphenol oxidase (PPO) [154] was immobilized onto this substrate layer using two different approaches (i) immersing the electrode in a solution of phosphate buffer containing PPO, (ii) electro-polymerized aniline from the solution containing PPO and aniline in phosphate buffer at constant potential (0.48 V). In second process, the enzyme is intercalated during polymerization. The biosensor exhibited good stability and high sensitivity since no apparent activity loss was observed after 100 consecutive measurements and intermediate usage for 8 months. Benzoic acid is one of the most extensively used food preservatives with a controlled use. Hence, biosensor appears to be a handy tool in food industry and show promise for the analytical determination of benzoic acid. A polyphenol oxidase (PPO) immobilized inhibition biosensor for phenol was used to detect benzoic acid using a polyaniline-polyacrylonitrile platform [155]. Qu et al. [156] modified GC electrode with multi-walled carbon nanotubes (MWCNTs) and then electrochemically deposited three layers PANI onto this modified electrode surface. They immobilized choline oxidase (CHOD) through cross linking with bovine serum albumin (BSA) using glutaraldehyde. This biosensor was able to detect as low as 0.3 μM choline (detection limit) with a fast response time of 3 s. Lactate is a metabolite formed from pyruvate in muscles and liver due to inadequate supply of oxygen. The normal range of lactate in blood is 0.5–2.5 mM and any increase of blood lactate level is a sensitive indicator of the patient's survival. Lactate levels give an indication of the oxygenation state of tissues and warning of an ischemic condition. The lactate sensor is mainly applied in critical cases, for example, during surgical operation and intensive therapy. These sensors have applications in sports, medicine and spatial medicine. Suman et al. [157] prepared a lactate biosensor by electrodepositing poly(aniline-co-fluroaniline) onto a ITO coated glass electrode. They activated this copolymer film with glutaralaldehyde and sprayed Lactate Oxidase (LAO) uniformly over the activated film from its sodium phosphate buffer solution. The lactate biosensor showed good stability with a detection limit

of 0.1 mM/L of lactate. Gerard et al. [158] electrodeposited PANI and immobilized lactate dehydrogenase (LDH) from its solution in buffer to detect pyruvate. This biosensor shows moderate response time of 90 s with good stability. Two different bioelectrode systems were developed and reported by Ali Eftekhari [159]. In first case, a mono layer of PANI deposited together with glycerol dehydrogenase (GDH) and β-nicotinamide adenine dinucleotide (NAD) onto an aluminum substrate electrode using electrochemical technique (potentiometric). In second case, only a thin layer of PANI was deposited onto the electrode surface followed by another PANI layer with GHD and NAD, however both electrodes showed similar properties with detection limits of 1.0×10^{-6} M for glycerol.

Conclusion

Polyaniline and its derivatives have been studied extensively for various biosensor applications. These materials play a crucial role as substrate layer to hold the enzymes/biomaterials while they facilitate electron transfer between enzyme and electrode surface. The PANI film thickness, enzyme immobilization techniques and use of supporting materials (dopant, crosslinker, conductive salt etc.) to immobilize enzyme are found to be important factors for obtaining an appropriate biosensor response on a particular electrode platform. All these biosensor systems that were prepared using PANI and its derivatives were successfully categorized using a range of instrumental techniques and most of them showed good detection limits, stability and fast response time compared with the data of other biosensors available in the literature. These confirms that PANI is a good substrate layer for the fabrication of different biosensors, and as a conducting polymer based biosensors especially PANI could see the light shade for commercialization in near future. Though some commercial biosensors are trying to use polyaniline, but not very specific data is available regarding PANI's use as a viable biosensor. Over all, much researches and efforts are still being required for preparing a real time deliverable, useable, and compact PANI based biosensor due to its low electrochemical stability and lack of well-optimized deposition techniques with the space for convenient use.

Acknowledgement

The authors of this article would like to gratefully acknowledge Center of Research Excellecne in Renewable Energy (CoRE-RE) and Department of Chemistry, King Fahd University of Petroleum and Minerals (KFUPM), Saudi Arabia for providing excellent research facilities.

References

[1] K. Radhapyari, P. Kotoky, M.R. Das, R. Khan, Graphene-polyaniline nanocomposite based biosensor for detection of antimalarial drug artesunate in pharmaceutical formulation and biological fluids, Talanta 111 (2013) 47-53.

[2] R.-S. Saberi, S. Shahrokhian, G. Marrazza, Amplified electrochemical DNA based on polyaniline film and gold nanoparticles, Electroanalysis 25 (2013) 1373-1380.

[3] V. Perumal, U. Hashim, Advances in biosensors: Principle, Architecture and Applications, Institue of Nano Electrical Engineering, University Malaysia Perlis (UniMAP), Perlis, Malaysia 2013.

[4] E. Iwuoha, A. Al-Ahmed, M. Sekota, T. Waryo, P. Baker, in : Jerry Atwood and Jonathan Steed (Ed.), Encyclopedia of Supramolecular Chemistry, 1:1, Taylor and Francis, New York, USA, 2007, pp. 1-18.

[5] C.R. Lowe, B.F.Y.Y. Hin, D.C. Cullen, S. E. Evans, L.D.S. Stephens, P. Maynard, Biosensors, J. Chromatogr. A 510 (1990) 347-354.

[6] B.M. Paddle, Biosensors for chemical and biological agents of defence interest, Biosens. & Bioelectron. 11 (1996) 1079-1113.

[7] G. Urban, A. Jachimowicz, F. Kohl, H. Kuttner, F. Olcaytug, H. Kamper, F. Pittner, E. Mann-Buxbaum, T. Schalkhammer, O. Prohaska, M. Schonauer, High-resolution thin-film temperature sensor arrays for medical applications, Sens. Actuators A 22 (1990) 650-654.

[8] J. Švorc, S. Miertuš, J. Katrlík, M. Streďanský, Composite Transducers for Amperometric Biosensors. The Glucose Sensor, Anal. Chem. 69 (1997) 2086-2090.

[9] W. Tan, M. J. Donovan, J. Jiang, Aptamers from cell-based selection for bioanalytical applications, Chem. Rev. 113 (2013) 2842-2862.

[10] X.-H. Zhao, L. Gong, X.-B. Zhang, B. Yang, T. Fu, R. Hu, W. Tan, R. Yu, Versatile DNAzyme- based amplified biosensing platforms for nucleic acid, protein, and enzyme activity detection, Anal. Chem. 85 (2013) 3614-3620.

[11] E. Kress-Rogers, Handbook of Biosensors and Electronic Noses: Medicine, Food and the Environment, CRC Press, Boca Raton, USA, 1996.

[12] C.L. Cooney, J.C. Weaver, S.R. Tannebaum, S.R. Faller, D.V. Shields, M. Jahnke, in: E.K. Pye, L.B. Wingard Jnr. (Eds:), Enzyme Engineering, Plenum, New York 1974, pp. 411-417.

[13] A.E.G. Cass, G. Davis, D.G. Francis, H.A.O. Hill, W.J. Aston, I.J. Higgins, E.V. Plotkin, L.D.L. Scott, A.P.F. Turner, Ferrocene-Mediated Enzyme Electrode for Amperometric Determination of Glucose, Anal. Chem. 56 (1984) 667-671.

[14] A.P.F. Turner, I. Karube, G.S. Wilson, Biosensors: Fundamentals and Application, Oxford University Press, Oxford 1987, pp.770.

[15] M. Ates, A review study of (bio)sensor systems based on conducting polymers, Mater. Sci. Eng. C 33 (2013) 1853- 1859.

[16] H. Shirakwa, E.J. Lowis, A.G. MacDairmid, C.K. Chiang, A.J. Heeger, Synthesis of electrically conducting organic polymers: halogen derivatives of polyacetylene, $(CH)_x$, Chem. Commun. 16 (1977) 578-580.

[17] A.J. Heeger, N.S. Sariciffici, E.B. Namdas, Semiconducting and metallic polymers, Oxford university press, Oxford, UK, 2010.

[18] J.C. Chiang, A.G. MacDiarmid, 'Polyaniline': Protonic acid doping of the emeraldine form to the metallic regime, Synth. Met. 13 (1986) 193-205.

[19] H. Naarmann, P. Strohriegel, in Handbook of Polymer Synthesis, Part- B (Ed: H.R. Kricheldorf), Marcel Dekker, New York 1992, pp. 1390.

[20] R. Kiebooms, R. Menon, K. Lee, in HandBook of Advance Electronic and Photonic Materials and Devices, (Ed: H. S. Nalwa), Academic Press, CA, USA 2001, Vol. 8 pp.32.

[21] R.V. Gregoy, in: T.A. Skotheim, R.L. Elsenbaumer, J.R. Reynolds (Eds.), Handbook of Conducting Polymers, Marcel Dekker, New York 1998, pp. 446.

[22] K. Li, B. Liu, water soluble comjugated polymers as the platform for protein sensors, Polym. Chem. 1 (2010) 252-259.

[23] M. Goh, S. Matsushita, K. Akagi, From helical polyacetylene to helical graphite: synthesis in the chiral nematic liquid crystal field and morphology-retaining carbonization, Chem. Soc. Rev. 39 (2010) 2466-2476.

[24] R. Rajesh, T. Ahuja, D. Kumar, Recent progress in the development of nano-structured conducting polymer/nanocomposites doe sensor applications, Sens. Actuators, B 136 (2009) 275-286.

[25] B. Guo, L. Glavas, A-C. Albertsson, Biodegradable and electrically conducting polymers for biomedical applications, Prog. Polym. Sci. (2013), (In press).

[26] H.N.M.E. Mamud, A. Kassim, Fabrication and characterization of organic semiconductor for electromagnetic interference shielding material, J. Polym. Eng. 31 (2011) 319-322.

[27] S. Zahn, G.S. Lal, W. F. Burgoyne, Jr, K.E. Minnich, A.F. Nordquist, L.M. Robeson, F.J. Waller, Fluorinated alkyl substituted-thieno[3,4-*B*] thiophene monomers and polymers therefrom, US Patent 7,572,879 B2, 2009.

[28] G.A. Snook, P. Kao, A.S. Best, Conducting-polymer-based supracapacitor devices and electrodes, J. Power Sources 196 (2011) 1-12.

[29] A. Ohira, T.M. Swager, Ordering of poly(p-phenylene ethylene)s in liquid crystals, Macromolecules 40 (2007) 19-25.

[30] A. Guiseppi-Elie, G.G. Wallace, T. Matsue, in: T.A. Skotheim (Ed.), Handbook of Conducting Polymers, Marcel Dekker, New York 1997, pp. 963-992.

[31] E. Stavrinidou, P. Leleux, H. Rajaona, M. Fiocchi, S. Sanaur, G.G. Malliaras, A simple model for ion injection and transport in conducting polymers, J. Appl. Phys. 113 (2013) 244501- 244506.

[32] M. Rehahn, T. Schwalm, S. Immel, S. Nickel, Method for producing (electro) luminescent, photoactive or electrically (semi) conducting polymers, US Paten Appl. 20130065358.

[33] N. Paul, M. Muller, A. Paul, E. Guenther, I Lauermann, P. Muller-Buschbaum, M.C. Lux Stiner, Molecular imprinted conducting polymers for trafficking of nerotransmitters at solid-liquid interface, Soft Matter 9 (2013) 1364-1371.

[34] M. Umana, J. Waller, Protein-modified electrodes. The glucose oxidase/polypyrrole system, Anal. Chem. 58 (1986) 2979-2983.

[35] A. Ivaska, Analytical applications of conducting polymers, Electroanalysis 3 (1991) 247-254.

[36] C.E.D. Chidsey, R.W. Murray, Electroactive Polymers and Macromolecular Electronics Bottom of Form, Science 231 (1986) 25-31.

[37] R.A. Hillman, Reaction and Application of Polymer Modified Electrodes in Electrochemical Science and Technology of Polymers (Ed: R.G. Linford) Elsevier, New York 1987, pp. 241.

[38] G.K. Candler, D. Pletcher, The Electrochemistry of Conducting Polymers in Electrochemistry, D. Pletcher (Ed), Vol.10, Royal Society of Chemistry, London 1993, pp.117.

[39] P.N. Bartlett, J.M. Cooper, A review of the immobilization of enzymes in electropolymerized films, J. Electoanal. Chem. 362 (1993) 1-12.

[40] M. Aizawa, S. Yabuki, H. Shinohara, in: G. Dryhurst, K. Niki (Eds.), Redox Chemistry and Interfacial Behavior of Biological Molecules, Plenum Press, New York 1988, pp. 173.

[41] S.B. Adeloju, G.G. Wallace, Conducting polymers and the bioanalytical sciences: new tools for biomolecular communications. A review, Analyst 121 (1996) 699-703.

[42] A. Heller, Electrical wiring of redox enzymes, Acc. Chem. Res. 23 (1990) 128-134.

[43] A. Boyle, E.M. Geniès, M. Lapkowski, Application of the electronic conducting polymers as sensors: Polyaniline in the solid state for detection of solvent vapours and polypyrrole for detection of biological ions in solutions, Synth. Met. 28 (1989) 769-774.

[44] G. Bidan, Electroconducting conjugated polymers: New sensitive matrices to build up chemical or electrochemical sensors. Sens. Actuators, B 6 (1992**)** 45-56.

[45] A.Q. Contractor, T.N. Sureshkumar, R. Narayanan, S. Sukeerthi, R. Lal, R.S. Srinivasa, Conducting polymer-based biosensors, Electrochim. Acta 39 (1994**)** 1321-1324.

[46] K.S.V. Santhanam, Conducting polymers for biosensors: Rationale based on models**,** Pure Appl. Chem. 70 (1998**)** 1259-1262.

[47] W. Schuhmann, Conducting polymer based amperometric enzyme electrodes, Mikrochim. Acta 121 (1995**)** 1-29.

[48] C. Kranz, H. Wohlschläger, H.L. Schmidt, W. Schuhmann, Controlled electrochemical preparation of amperometric biosensors based on conducting polymer multilayers, Electroanalysis 10 (1998**)** 546-552.

[49] P.N. Bartlett, P.R. Birkin, The application of conducting polymers in biosensors, Synth. Met. 61 (1993) 15-21.

[50] M.V. Deshpande, D. P. Amalnerkar, Biosensors prepared from electrochemically-synthesized conducting polymers, Prog. Polym. Sci. 18 (1993) 623-649.

[51] S. Cosnier, Biomolecule immobilization on electrode surfaces by entrapment or attachment to electrochemically polymerized films. A review, Biosens. Bioelectron. 14 (1999) 443-456.

[52] R.A. Bull, F.R. Fan, A.J. Bard, Polymae films on electrodes, J. Electrochem. Soc. 130 (1983) 1636-1638.

[53] T.W. Lewis, G.G. Wallace, M.R. Smyth, Electrofunctional polymers: their role in the development of new analytical systems, Analyst 124 (1999) 213-219.

[54] W. Lu, H. Zhao, G.G. Wallace, Pulsed electrochemical detection of proteins using conducting polymer based sensors, Anal. Chim. Acta 315 (1995) 27-32.

[55] M.S. Wrighton, Surface Functionalization of Electrodes with Molecular Reagents, Science 231 (1986) 32-37.

[56] H. Zhao, W.E. Price, G.G. Wallace, Effect of the counterion employed during synthesis on the properties of polypyrrole membranes, J. Membr. Sci. 87 (1994) 47-56.

[57] J.C. Cooper, E.A.H. Hall, Electrochemical response of an enzyme-loaded polyaniline film, Biosens. Bioelectron.7 (1992) 473-485.

[58] P.R. Teasdale, G.G. Wallace, Molecular recognition using conducting polymer: basis of an electrochemical sensing technology-plenary lecture, Analyst 118 (1993) 329-334.

[59] A.G. MacDiarmid, Synthetic metals: a novel role for organic polymers, Synth. Met. 125 (2002) 11-22.

[60] A.G. MacDiarmid, Polyaniline and polypyrrole: Where are we headed?, Synth. Met. 84 (1997) 27-34.

[61] J. Bai, S. Beyer, D. Trau, Conjugated polymers for Biosensor devices, Comprehensive Biomaterials 3 (2011) 529-556.

[62] E.I. Iwuoha, D. Saenzde de Villaerde, N.P. Garcia, M. R.Smyth, J.M. Pingarron, Reactivities of organic phase biosensors. 2. The amperometric behaviour of horseradish peroxidase immobilised on a platinum electrode modified with an electrosynthetic polyaniline film, Biosen. Bioelectro. 12 (1997) 749-761.

[63] W.-S. Huang, B.D. Humphrey, A.G. MacDiarmid, Polyaniline, a novel conducting polymer. Morphology and chemistry of its oxidation and reduction in aqueous electrolytes, Faraday Trans. 82 (1986) 2385-2400.

[64] A.J. Heeger, Semiconducting and metallic polymers: the fourth generation of polymeric materials, Synth. Met. 125 (2002) 23-42.

[65] E.W. Paul, A.J. Ricco, M.S. Wrighton, electrochemical potential and the fabrication of polyaniline-based microelectronic devices, J. Phys. Chem. 1985, 89 (8) (1985), 1441-1447.

[66] A.A. Pud, Stability and degradation of conducting polymers in electrochemical systems, Synth. Met. 66 (1994) 1-18.

[67] A.A. Syed, M.K. Dinesan, Review: Polyaniline—A novel polymeric material, Talanta 38 (8) (1991) 815-837.

[68] J. Luo, S. Jiang, Y. Wu, M. Chen, X. Liu, Synthesis of stable aqueous dispersion of grapheen/polyaniline composite mediated by polystyrene sulfonic acid, J. Polym. Sci., Part A: Polym. Chem. 50 (2012) 4888-4894.

[69] P.M. Ndangilli, T.T. Waryo, M. Muchindu, P.G.L. Baker, C.J. Ngila, E.I. Iwuoha, Ferrocenium hexafluorophosphate-induced nanofibrillarity of polyaniline-polyvinyl sulfonate electropolymer and application in an amperometric enzyme biosensor, Electrochimica Acta, 55 (2010) 4267- 4273.

[70] P.D. Gaikwad, D.J. Shirale, V.K. Gade, P.A. Savale, P.K. Kakde, H.J. Kharat, M.D. Shirsat, Potentiometric study of polyaniline film synthesized with various dopants and composite dopants: A comparative study. Bull. Mater. Sci. 29 (2006) 417-420.

[71] S. Singh, P.R. Solanki, M.K. Pandey, B.D. Malhotra, Cholesterol biosensor based on cholesterol esterase, cholesterol oxidase and peroxidase immobilized onto conducting polyaniline films, Sens. Actuators B 115 (2006) 534-541.

[72] K. Grennan, A.J. Killard, C.J. Hanson, A.A. Cafolla, M.R. Smyth, Optimisation and characterisation of biosensors based on polyaniline, Talanta 68 (2006) 1591-1600.

[73] T.F. Otero, V. Olazabal, Electrogeneration of polypyrrole in presence of polyvinylsulfonate. Kinetic study, Electrochim. Acta 41 (1996) 213-220.

[74] M. Mazur, M. Tagowska, B. Palys, K. Jackowaska, Template synthesis of polyaniline and poly(2-methoxyaniline) nanotubes: comparison of the formation mechanisms, Electrochem. Commun. 5 (2003) 403-407.

[75] D.D. Borole, U.R. Kapadl, P.P. Muhulikar, D.G. Hundiwale, Glucose oxidase electrodes of polyaniline, poly (o-anisidine) and their copolymer as a biosensor: a cooperating study, J. Mater. Sci. 43 (2007) 4947-4953.

[76] Y. Xian, F. Liu, L. Febg, F. Wu, L. Wang, L. Jin, Nanoelectrode ensembles based on conductive polyaniline/poly(acrylic acid) using porous sol-gel films as template, Electrochem. Commun. 9 (2007) 773-780.

[77] X. Pan, S. Zhou, C. Chen, J. Kan, Preparation and properties of an uricase biosensor based on copolymer of o-aminophenol-aniline, Sens. Actuators B 113 (2006) 329-334.

[78] H. Zhou, H. Cheng, S. Luo, J. Chen, W. Wei, Y. Kuang, Glucose biosensor based on platinum microparticles dispersed in nano-fibrous polyaniline, Biosens. Bioelectron. 20 (2005) 1305-1311.

[79] Y. Zou, L. X. Sun, F. Xu, Biosensor based on polyaniline–Prussian Blue/multi-walled carbon nanotubes hybrid composites, Biosen. Bioelectro. 22 (2007) 2669-2674.

[80] Y. Xian., Y. Hu, F. Liu, Y. Xian, H. Wang, L. Jin, Glucose biosensor based on Au nanoparticles–conductive polyaniline nanocomposite, Biosen. Bioelectro. 21 (2006) 1996-2000.

[81] X. Pan, J. Kan, L. Yuan, Polyaniline glucose oxidase biosensor prepared with template process, Sens. Actuators B 102 (2004) 325-330.

[82] Y. Jiang, A. Wang, J. Kan, Selective uricase biosensor based on polyaniline synthesized in ionic liquid, Sens. Actuators B 124 (2007) 529-534.

[83] D.F. Zielinska, F. Gnad, K. Schropp, J.R. Wisniewski, M. Mann, Mapping N-Glycosylation sites across seven evolutionarily distant species reveals a divergent substrate proteome despite a common core machinery, Molecular Cell 46 (2012) 542-548.

[84] M. Rehm, J.H.M. Prehn, Systems modeling methodology for the analysis of apoptosis signal transduction and cell death decisions, Methods 61 (2013) 165-173.

[85] S. Singh, P.R. Solanki, M.K. Pandey, B.D. Malhotra, Covalent immobilization of cholesterol esterase and cholesterol oxidase on polyaniline films for application to cholesterol biosensor, Analy. Chimica Acta 568 (2006) 126-132.

[86] C. Lie, J. Deng, Hydrogen peroxide sensor based on coimmobilized Methylene Green and Horseradish Peroxidase in the Same Montmorillonite-modified Bovine Serum Albumin-Glutaraldehyde Matrix on a Glassy Carbon Electrode Surface. Annal. Chem. 68 (1996) 3348-3349.

[87] K. Arora, N. Prabhakar, S. Chand, B.D. Malhotra, Escherichia coli Genosensor Based on Polyaniline, Anal.Chimica Acta 79 (2007) 6152-6158.

[88] N. German, A. Ramanavicius, J. Voronovic, A, Ramanaviciene, Glucose biosensor based on glucose oxidase and gold nanoparticles of different sizes covered by polypyrrole layer, Colloids Surf., A 413 (2012) 224-230

[89] J. Wang, Conducting polymers for biosensors: Rationale based on models Glucose biosensors: 40 years of Advance and Challenges, Electroanalysis 13 (2001) 983-988.

[90] J. Wang, Electrochemical glucose biosensors, Chem. Rev. 108 (2007) 814-825.

[91] L. Clark Jr., C. Lyons, Electrode systems for continuous monitoring in cardiovascular surgery, Ann. Ny Acad. Sci. 102 (1962) 29-45.

[92] V. Mazeiko, A.Kausaite-Minkstimiene, A. Ramanaviciene, Z. Balevicius, A. Ramanavicius, Gold nanoparticle and conducting polymer-polyaniline-based nanocomposites for glucose biosensor design, Sensor and Actuators B: Chemical, 2013, (In press).

[93] D.T. Hoa, T.N. Suresh Kumar, N.S. Punekar, R.S. Srinivasa, R. Lal, A.Q. Contractor, Biosensor Based on Conducting Polymers, Anal. Chem. 64 (1992) 2645-2646.

[94] J. Losada, M.P.G. Armada, A glucose amperometric sensor based on covalent immobilization of glucose oxidase in poly-2-aminoaniline film via chloranil on platinized platinum electrode, Electroanalysis 9 (1997) 1416-1421.

[95] D.D. Borole, U.R. Kapadi, P.P. Mahulikar, D.G. Hundiwale, Glucose oxidase electrodes of polyaniline, poly(o-toluidine) and their copolymer as a biosensor: a comparative study, Polym. Adv. Technol. 15 (2004) 306-312.

[96] H. Xue, Z. Shen, C. Li, Improved selectivity and stability of glucose biosensor based on in situ electropolymerized polyaniline–polyacrylonitrile composite film, Biosens. Bioelectron. 20 (2005) 2330-2334.

[97] R. Garjonyte, A. Malinauskas, Amperometric glucose biosensors based on Prussian Blue–and polyaniline–glucose oxidase modified electrodes, Biosens. Bioelectron. 15 (2000) 445-451.

[98] H. Xue, Z. Shen, Y. Li, Polyaniline–polyisoprene composite film based glucose biosensor with high permselectivity, Synth. Met. 124 (2001) 345-356.

[99] X.H. Xu, G.L. Ren, J. Cheng, Q. Liu, D.G. Li, Q. Chen, Self-assembly of polyaniline-grafted chitosan/glucose oxidase nanolayered films for electrochemical biosensor applications, J. Mater. Sci. 41 (2006) 4974-4977.

[100] D. Shan, S. Wang, Y. He, H. Xue, Amperometric glucose biosensor based on in situ electropolymerized polyaniline/poly(acrylonitrile-co-acrylic acid) composite film, Mater. Sci. Eng., C 28 (2008) 213-217.

[101] H. Sangodkar, S. Sukeerthi, R.S. Srinivasa, R. Lal, A.Q. Contractor, A Biosensor Array Based on Polyaniline, Anal. Chem. 68 (1996) 779-783.

[102] S. Sukeerthi, A.Q. Contractor, Molecular Sensors and Sensor Arrays Based on Polyaniline Microtubules, Anal. Chem. 71 (1999) 2231-2236.

[103] A. Efthekhari, Electropolymerization of aniline onto passivated substrate and its application for preparation of enzyme-modified electrode, Synth. Met. 145 (2004) 211-216.

[104] J. Dumont, G. Fortier, Behavior of glucose oxidase immobilized in various electropolymerized thin films, Biotech. Bioeng. 49 (1996) 544-552.

[105] A.A. Karkyin, O.A. Bobrova, L.V. Lukachova, E.E. Karyakina, Potentiometric biosensors based on polyaniline semiconductor films, Sens. Actuators B 33 (1996) 34-38.

[106] M.M. Verghes, K. Ramanathan, S.M. Ashraf, B.D. Malhotra, Enhanced loading of glucose oxidase on polyaniline films based on anion exchange, J. Appl. Poly. Sci. 70 (1998) 1447-1453.

[107] M. Gerard, B.D. Malhotra, Application of polyaniline as enzyme based biosensor, Current Appl. Physics 5 (2005) 174-177.

[108] X. Chen, Z. Chen, R. Tian, W. Yan, C. Yao, Glucose biosensor based on three dimensional macroporous self-doped polyaniline/Prussian biocomponent film, Analytical Chimica Acta, 723 (2012) 94-100.

[109] S. Myler, F. Davis, S.D. Collyer. S.P.J. Higson, Sonochemically fabricated microelectrode arrays for biosensors—part II: Modification with a polysiloxane coating, Biosens. Bioelectron. 20 (2004) 408-412.

[110] A. Ramanavičius, A. Ramanavičiene, A. Malinauskas, Electrochemical sensors based on conducting polymer—polypyrrole, Electrochim. Acta 51 (2006) 6025-6037.

[111] E. Katz, I. Willner, Integrated nanoparticle- biomolecule hybrid system: synthesis, properties and applications, Angew. Chem. Int. Ed. 43 (2004) 6042-6108.

[112] H. Chang, Y. Yuan, N. Shi, Y. Guan, Electrochemical DNA Biosensor Based on Conducting Polyaniline Nanotube Array, Anal. Chem. 79 (2007) 5111-5115.

[113] J. Wu, Y. Zou, Xiaolili, H. Liu, G. Shen, R. Yu, A biosensor monitoring DNA hybridization based on polyaniline intercalated graphite oxide nanocomposite, Sens. Actuators B 104 (2005) 43-49.

[114] Y. Bo, H. Yang, Y. Hu, T. Yao, S. Huang, A novel electrochemical DNA biosensor based on graphene and polyaniline nanowires, Electrochimica Acta, 56 (2011) 2676-2681.

[115] Y. Ma, S. R. Ali, A. S. Dodoo, H. He, Enhanced sensitivity for biosensors: Multiple functions of DNA-wrapped signal-walled carbon nanotubes in self-doped polyaniline nanocomposiyes, J. Phys. Chem. B 110 (2006) 16359-16365.

[116] S.R. Ali, Y. Ma, R.R. Parajuli, Y. Balogun, W.Y.C. Lai, H. He, A Nonoxidative Sensor Based on a Self-Doped Polyaniline/Carbon Nanotube Composite for Sensitive and Selective Detection of the Neurotransmitter Dopamine, Anal. Chem. 79 (2007) 2583-2587.

[117] N. Zhu, Z. Chang, P. He, Y. Fang, Electrochemically fabricated polyaniline nanowire-modified electrode for voltammetric detection of DNA hybridization, Electrochim. Acta 51 (2006) 3758-3762.

[118] K. Arora, N. Prabhakar, S. Chand, B.D. Malhotra, Ultrasensitive DNA hybridization biosensor based on polyaniline, Biosen. Bioelectro. 23 (2007) 613-620.

[119] H.A. Harper, Review of Physiological Chemistry, 16th edition, Lange Medical Publications, San Francisko, CA, 1977.

[120] Y.C. Luo, J.S. Do, Urea biosensor based on PANi(urease)-Nafion®/Au composite electrode, Biosens. Bioelectron. 20 (2004) 15-23.

[121] J. Kan, X. Pan, C. Chen, Polyaniline–uricase biosensor prepared with template process, Biosens. Bioelectron. 19 (2004) 1635-1640.

[122] P.C. Pandey, G. Singh, Tetraphenylborate doped polyaniline based novel pH sensor and solid-state urea biosensor, Talanta 55 (2001) 773-782.

[123] W.J. Cho, H.-J. Huang, An Amperometric Urea Biosensor Based on a Polyaniline-Perfluorosulfonated Ionomer Composite Electrode, Anal. Chem. 70 (1998) 3946-3951.

[124] K. Arora, G. Sumana, V. Saxena, R.K. Gupta, S.K. Gupta, J.V. Yakhmi, M.K. Pandey, S. Chand, B. D. Malhotra, Improved performance of polyaniline-uricase biosensor, Anal. Chim. Acta 594 (2007) 17-23.

[125] A.A. Karyakin, M. Vuki, L.V. Lukachova, E.E. Karyakina, A.V. Orlov, G.P. Karpachova, J. Wang, Processible polyaniline as an advanced potentiometric pH transducer application to biosensors, Anal. Chem. 71 (1999) 2534-2540.

[126] M.M. Castillo-Ortega, D.E. Rodriguez, J.C. Encinas, M. Plascencia, F.A. Méndz-Velarde, R. Olayo, Conductometric uric acid and urea biosensor prepared from electroconductive polyaniline–poly(n-butyl methacrylate) composites, Sens. Actuators, B 85 (2002) 19-25.

[127] E.I. Iwuoha, M.R. Smyth, M.E.G. Layons, Organic phase enzyme electrodes: kinetics and analytical applications, Biosen. Bioelectro. 12 (1997) 53-75.

[128] N.G.R. Mathebe, A. Morrin, E.I. Iwuoha, Electrochemistry and scanning electron microscopy of polyaniline/peroxidase-based biosensor, Talanta 64 (2004) 115-120.

[129] C.C. Chen, Y. Gu, Enhancing the sensitivity and stability of HRP/PANI/Pt electrode by implanted bovine serum albumin, Biosenos. Bioelectro. 23 (2008) 765-770.

[130] X. Kang, G. Cheng, S. Dong, A novel electrochemical SPR biosensor, Electroche. Communi. 3 (2001) 489-493.

[131] A. Mulchandani, C.L. Wang, H.H. Weetall, Amperometric Detection of Peroxides with Poly(anilinomethylferrocene).Modified Enzyme Electrodes, Anal. Chem. 67 (1995) 94-100.

[132] A. Morrin, O. Nagamna, A.J. Killard, S.E. Moulton, M.R. Smyth, G.G. Wallace, An amperometric enzyme biosensor fabricated from polyaniline nanoparticles, Electroanalysis 17 (2005) 423-430.

[133] X. Luo, A.J. Killard, A. Morrin, M.R. Smyth, Enhancement of a conducting polymer-based biosensor using carbon nanotube-doped polyaniline, Anal. Chim. Acta 575 (2006) 39-44.

[134] Q. Xu, J. J. Zhu, X.Y. Hu, Ordered mesoporous polyaniline film as a new matrix for enzyme immobilization and biosensor construction, Anal. Chim. Acta 597 (2007) 151-156.

[135] X. Luo, A. J. Killard, A. Morrin, M. R. Smyth, In situ electropolymerised silica–polyaniline core–shell structures: Electrode modification and enzyme biosensor enhancement, Electrochim. Acta 52 (2007) 1865-1870.

[136] A. Morrin, A. Guzman, A.J. Killard, J.M Pingarron, M.R. Smyth, Characterisation of horseradish peroxidase immobilisation on an electrochemical biosensor by colorimetric and amperometric techniques, Biosens. Bioelectron. 18 (2003) 715-720.

[137] A. Morrin, F. Wilbeer, O. Ngamna, S.E. Moulton, A.J. Killard, G.G. Wallace, M.R. Smyth, Novel biosensor fabrication methodology based on processable conducting polyaniline nanoparticles, Electrochem. Commun. 7 (2005) 317-322.

[138] O. Ngamna, A. Morrin, S.E. Moulton, A.J. Killard, M.R. Smyth, G.G. Wallace, An HRP based biosensor using sulphonated polyaniline, Synth. Met. 153 (2005) 185-188.

[139] K. Radhapyari, P. Kotoky, R. Khan, Detection of anticancer drug tammoxifen using biosensor based on polyaniline probe modified with horseradish peroxidase, Materials Science and Engineering: C 33 (2013) 583-587.

[140] S. Piletsky, E. Piletska, A. Bossi, N. Turner, A. Turner, Surface functionalization of porous polypropylene membranes with polyaniline for protein immobilization , Biotech. Bioeng. 82 (2003) 86-92.

[141] R. Ahmad, N. Tripathy, Y.-B. Hahn, Wide linear-range detecting high sensitivity cholesterol biosensors based on aspect-ratio controlled ZnO nanorods grown on silver electrodes, Sensors and Actuators B: Chemical 169 (2012) 382- 386

[142] C. Dhand, S. P.Singh, S.K. Arya, M. Datta, B.D. Malhotra, Cholesterol biosensor based on electrophoretically deposited conducting polymer film derived from nano-structured polyaniline colloidal suspension, Anal. Chim. Acta 602 (2007) 244-251.

[143] Z.M. Thair, E.C. Alocilja, D.L. Grooms, Polyaniline synthesis and its biosensor application, Biosens. Bioelectron. 20 (2005) 1690-1695.

[144] Z.M. Thair, E.C. Alocilija, A conductometric biosensor for biosecurity, Biosens. Bioelectron. 18 (2003) 813-819.

[145] S. Pal, E.C. Alocilja, F.P. Downes, Nanowire labeled direct-charge transfer biosensor for detecting Bacillus species, Biosens. Bioelectron. 22 (2007) 2329-2336.

[146] V.V.R. Sai, S. Mahajan, A.Q. Contractor, S. Mukherji, Immobilization of Antibodies on Polyaniline Films and Its Application in a Piezoelectric Immunosensor, Anal. Chem. 78 (2006) 8368-8373.

[147] S. Timur, N. Pazarlioğlu, R. Pilloton, A. Telefoncu, Thick film sensors based on laccases from different sources immobilized in polyaniline matrix, Sens. Actuators, B 97 (2004) 132-136.

[148] J.H.O. Owino, A. Ignaszak, A. Al-Ahmed, P.G.L. Baker, H. Alemu, J.G. Ngila, E.I. Iwuoha, Modelling of the impedimetric responses of an aflatoxin B_1 immunosensor prepared on an electrosynthetic polyaniline platform, Anal. Bioanal. Chem. 388 (2007) 1069-1074.

[149] D. Du, X. Ye, J. Cai, J. Liu, A. Zhang, Acetylcholinesterase biosensor design based on carbon nanotube- encapsulated polypyrrole and polyaniline copolymer for amperometric detection of organophosphates, Biosens. Bioelectron. 25 (2010) 2503-2508.

[150] D. Du, W. Chen, W. Zhang, D. Liu, H. Li, Y. Lin, Covalent coupling of organophosphorous hydrolase loaded quantum dots to caron nanotube/Au nanocomposite for enhanced detection of methyl parathion, Biosens. Bioelectron. 25 (2010) 1370-1375.

[151] J.J. Langer, M. Filipiak, J. Kecińska, J. Jasnowska, J. Włodarczak, B. Buładowski, Polyaniline biosensor for choline determination, Surf. Sci. 573 (2004) 140-145.

[152] P.A. Fioritio, S.I. Córdaba de Torresi, Optimized multilayer oxalate biosensor, Talanta 62 (2004) 649-654.

[153] S. Hu, C. Xu, J. Luo, J. Luo, D. Cui, Biosensor for detection of hypoxanthine based on xanthine oxidase immobilized on chemically modified carbon paste electrode, Anal. Chim. Acta 412 (2000) 55-61.

[154] H. Xue, Z. Shen, A highly stable biosensor for phenols prepared by immobilizing polyphenol oxidase into polyaniline–polyacrylonitrile composite matrix, Talanta 57 (2002) 289-295.

[155] D. Shan, Q. Shi, D. Zhu, H. Xue, Inhibitive detection of benzoic acid using a novel phenols biosensor based on polyaniline–polyacrylonitrile composite matrix, Talanta 72 (2007) 1767-1772.

[156] F. Qu, M. Yang, J. Jiang, G. Shen, R. Yu, Amperometric biosensor for choline based on layer-by-layer assembled functionalized carbon nanotube and polyaniline multilayer film, Anal. Biochem. 344 (2005) 108-114.

[157] S. Suman, R. Singhal, A.L. Sharma, B.D. Malthotra, C.S. Pundir, Development of a lactate biosensor based on conducting copolymer bound lactate oxidase, Sens. Actuators, B 107 (2005) 768-772.

[158] M. Gerard, K. Ramnathan, A. Chaubey, B.D. Malhotra, Immobilization of lactate dehydrogenase on electrochemically prepared polyaniline films, Electroanalysis 11 (1999) 450-452.

[159] A. Efthekhari, Glycerol biosensor based on glycerol dehydrogenase incorporated into polyaniline modified aluminum electrode using hexacyanoferrate as mediator, Sens. Actuators, B 80 (2001) 283-289.