

Review Article

Prospects of Organic Conducting Polymer Modified Electrodes: Enzymosensors

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Organic conducting polymer modified electrodes (OCPMEs) have emerged as potential candidates for electrochemical biosensors due to their easy preparation methods along with unique properties, like stability in air and being compatible with biological molecules in a neutral aqueous solution. OCPMEs are playing an important role in the improvement of public health and environment for the detection of desired analytes with high sensitivity and specificity. In this paper, we highlight the prospects of OCPMEs-based electrochemical enzymosensors.

1. Introduction

Various methods used for the determination of a variety of desired analytes (without use of biocatalyst) are gas chromatography, colorimetric and fluorimetric analysis, and so forth. However, these methods need sample pretreatment which stands as a major disadvantage in their versatility of applications. These methods cannot be used for field monitoring. Thus, devices were developed based on biocatalyst to analyze desired analytes of interest known as biosensors, which are of vital importance. Many biosensors have been developed for the determination of analytes in the biological samples, namely, spectrometry, potentiometry with application of pH sensitive electrode or an ion selective electrode or an ion sensitive field effect transistor, conductometry, coulometry, amperometry, and inductometry [1–13].

Biosensor is an analytical device, which incorporates biological sensing elements like enzymes, antibodies, receptors, aptamers, nucleic acids, cells, and so forth over electrodes. Electrode decides the sensitivity, selectivity, and reproducibility of the sensor/biosensor, with electronic transducer equipped with an electronic amplifier and was found to have applications in various fields, for example, clinical diagnostics, environmental monitoring, bioprocess monitoring, food, agricultural product processing, and so forth [14, 15]. According to IUPAC, biosensor specifically

defined as a self-contained integral device that is capable of providing specific quantitative or semiquantitative analytical information using a biological element [16]. Biosensor has three major components: (a) biorecognition element or bioreceptor, (b) an immobilization surface for immobilization of biomolecule, and (c) a transducer unit for conversion of biochemical reaction product into a recognizable signal. Thus, bioreceptor and transducer together may be referred to as biosensor membrane [17, 18]. The history of biosensor started in 1962 with the development of first enzyme-based glucose sensing device by Clark and Lyons using a thin layer of glucose oxidase (GOx) entrapped over an oxygen electrode via semipermeable dialysis membrane for the measurements of the O₂ consumed by the enzyme-catalyzed reaction [19]. Nowadays, biosensors are much more advanced than the Clark enzyme electrode, but are facing new challenges like the instability of desired biomolecules with an electrode. The redox reaction in terms of measuring the loss or formation of substrate or product, the use of a small mediator species that shuttles between the biomolecule and the electrode, or direct electron transfer (ET) between the biomolecule redox site and the electrode. Direct ET can be difficult to achieve, since the redox site of a biomolecule often buried deep inside the biomolecule. The modification of biomolecules or electrode surfaces using novel conducting materials as mediators and design of functional biointerfaces. Thus, highly conductive

organic transducers like CPs nanomaterials, sol-gel films, and self-assembled monolayers, and so forth are gradually emerging for the development of next-generation biosensor design for highly reliable, stable, and robust field-based biosensor devices [20, 21].

Chemical bonding in conducting polymers provides one unpaired electron, that is, π -electron per carbon atom in the backbone of the polymer. Carbon atoms are in sp^2p_z configuration in π -bonding and orbitals of successive carbon atoms overlap providing delocalization of electrons along the backbone of polymer. This delocalization provides the charge mobility along the backbone of the polymer chain and induces unusual properties such as electrical conductivity, low ionization potential, low energy optical transitions, and high electron affinity. The π bonds in conjugated polymers are highly susceptible to chemical or electrochemical oxidation or reduction. The origin of electrical conduction in conducting polymers has been ascribed to the formation of nonlinear defects such as solitons, polarons, or bipolarons formed during either doping or polymerization of a monomer. The conductive and semiconducting properties of these polymers make them an important class of materials for a wide range of electronic, optoelectronic, and biotechnological applications such as in rechargeable batteries, molecular electronics, electronic displays, solar cells, ion exchange membrane in fuel cells, diodes, capacitors, field-effect-transistors, printed circuit boards, chemical sensors, drug release systems, and biosensors. Conducting polymers can be used to transport small electronic signals in the body, that is, act as artificial nerves. Scientists have used films in a neurotransmitter as a drug release system into the brain [22–28].

The most commonly used organic conducting polymers and their potential applications are reported in the literature, including poly(acetylene)s, poly(pyrrole)s, poly(thiophene)s, poly(terthiophene)s, poly(aniline)s, poly(fluorine)s, poly(3-alkylthiophene)s, polytetrathiafulvalenes, polynaphthalenes, poly(p-phenylene sulfide), poly(paraphenylenevinylene)s, poly(3,4-ethylenedioxythiophene), polyfuran, polyindole, and polycarbazole. The polymer growth and stability are of the most important criteria for the stable device development. The growths and stabilities of poly(aniline)s, poly(pyrrole)s, and poly(azulene)s have been reported. Poly(thiophene)s shows excellent thermal and air stability, solubility, processability, and high conductivity when compared to other important class of conducting polymers. Conducting polymers can be reversibly doped and undoped using electrochemical techniques accompanied by significant changes in conductivity. The electrical conductivity of conducting polymers changes over several orders of magnitude in response to change in pH, applied potentials, or their environments. Grafting of organometallics to the backbone of the conducting polymer can also tune their physical properties. Potential applications of the conducting polymers have been reported in the development of sensors/biosensors, and emerged as one of the unique materials for the fabrication of electrochemical devices with improved response properties, including sensitivity and selectivity due to their electrical conductivity

or charge transport properties and biocompatibility. However, conducting polymer itself could be modified with biomolecules and acts as a biosensor. In addition, conducting polymers via electrochemical synthesis allows direct deposition of a polymer film on the electrode substrate followed by biomolecules immobilization. Thus, it is not only possible to control the immobilized biomolecules and film thickness but also to act as an electron promoter [29–39] as shown in Figure 1.

Ramaniviciene and Ramanavicius have reported an interesting review on the potential use of conducting polymers as electrochemical-based biosensors [40]. Malinauskas et al. have described the electrochemical aspects of conducting polymer based nanostructured materials for application to supercapacitors, energy conversion systems, batteries, and sensors [41]. Schuhmann et al. reported that functionalization of conducting polypyrrole films provides suitable surface for covalent linkage of enzymes after carbodiimide activation [42]. Gorton et al. have described the role of direct electron transfer between hemecontaining enzymes and electrodes as the basis of third-generation biosensors [43]. Schuhmann has classified biosensors based on (i) electron-transfer via conducting polymers, (ii) electron-transfer cascades via redox hydrogels, (iii) anisotropic orientation of redox proteins, and (iv) direct electron transfer between redox proteins and electrodes modified with self-assembled monolayers [44]. The interest in nanomaterials for biosensing applications has recently emerged. The size-dependent changes in the physical and chemical properties of materials make them different from that of their bulk counterpart. The change in physical properties makes nanomaterials an important candidate for technological development of biodevices. The large surface-to-volume ratio provides substantial changes in chemical properties. The fullerenes and carbon nanotubes can be a class of conjugated polymers due to semiconducting nature as well as possibility of p-type and n-type doping which can be made possible due to the presence of conjugated carbon structure with unusual molecular symmetries which makes them compatible with conducting polymers and promising materials from technological point of view. Ramanathan et al. have functionalized single wall carbon nanotubes with amino groups, which may covalently attach to polymers or biological systems such as DNA and carbohydrates [45]. Carrara et al. have utilized improved nanocomposite materials of poly(*o*-anisidine) (POA) containing titanium dioxide nanoparticles (TiO_2), carbon black, and multiwalled carbon nanotubes (MWNT) for biosensing applications based on electrochemical impedance spectroscopy [46]. Anzai et al. have described various techniques for the surface derivatization within biotin and avidin and for the coupling of the enzymes [47]. Nobs et al. have covalently bound NeutrAvidin to the surface of poly(dL-lactic acid) (PLA) nanoparticles with the aim of attaching targeting compounds such as proteins and other proteins such as antibodies could be coupled to the nanoparticles for active targeting. Furthermore, PLA nanoparticles are interesting candidates for active targeting with biotinylated antibodies using the biotin-avidin interaction in a two-step procedure [48]. Gref

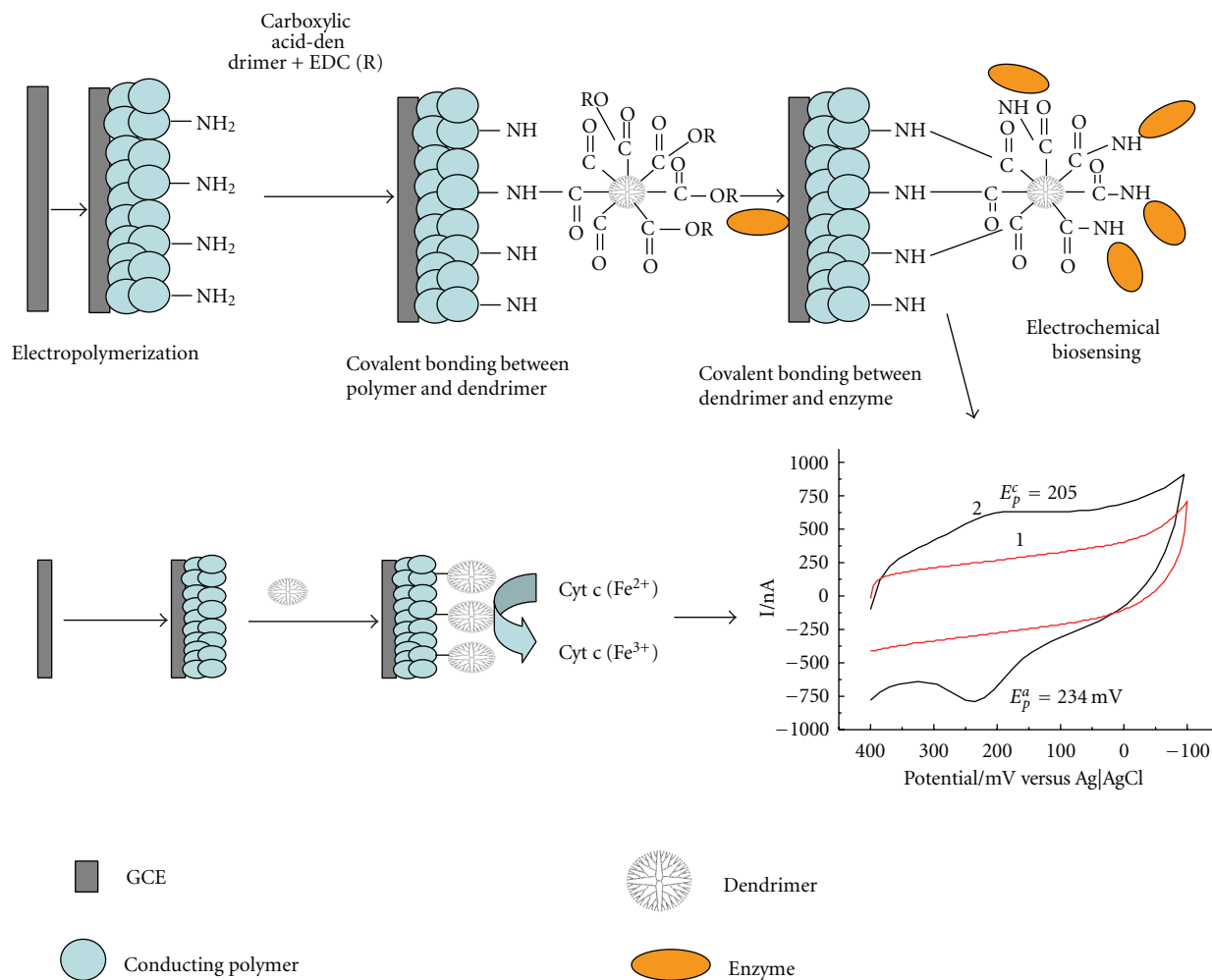


FIGURE 1: Shows the schematic presentation of enzymosensor using organic conducting polymer.

et al. have shown that biotin-poly(ethylene glycol)-poly(β -caprolactone) (B-PEG-PCL) can be helpful for studying the interaction between cells and functionalized nanoparticles with surface characteristics (ligand type and density, PEG layer density, and thickness) [49].

2. Enzymosensors

Enzymatic biosensors utilize the biospecificity of an enzymatic reaction, along with an electrode reaction that generates an electric current or potential difference for quantitative analysis. The biomolecules such as glucose, cholesterol, urea, triglycerides, creatinines, and pesticides are important analytes due to their adverse effects on health. Enzymatic biosensors utilize the biochemical reactions, that is, analyte and enzyme resulting in a product (hydrogen/hydrogen peroxide/hydroxyl/ammonium ion), that can be detected and quantified using a transducer (amperometric/potentiometric/optical thermal/piezoelectric). In general, many oxidoreductases including glucose oxidase catalyze the oxidation of substrates by electron transfer to oxygen to form hydrogen peroxide. These oxidoreductase enzymes

immobilized on conducting polymer surfaces, the H_2O_2 formed because of enzyme, and the corresponding analytes may be measured amperometrically. However, it has not been possible to discriminate between the direct electron transfers from the oxidation of hydrogen peroxide at polymer surface and that at the underlying electrode. Since conducting polymers are insoluble in aqueous solutions, electropolymerization are used to create a matrix for immobilization of enzymes at the electrode surface, and the sensor response obtained by the oxidation of hydrogen peroxide [50–57].

Measurement of a target analyte could be achieved by selectively converting molecular recognition occurring at analyte sensor interface from a nonelectrical domain to an electrical signal. The PANI in sensing due to its ability to reversibly oxidized and reduced through the application of electrical potential and electrical conduction in PANI is achieved through the formation of defects in the polaronic (one hole state) and bipolaronic forms (two hole state). When exposed to analytes, stoichiometry of the existing PANI energy levels is disturbed through the substitution of different species into the polymer lattice and causes a change in the electrical conductivity. The fabrication

of PANI-based electrode for biosensor application for its commercialization is a major challenge due to the aging effect, low electrochemical stability, and lack of deposition techniques. Wolter et al. have reported the continuous decrease in conductivity of PANI, which in turn influences its charge transfer properties and electrochemical stability, because of the oxidation/reduction of soluble electrochemical degradation products, including p-benzoquinone (PBQ) [58]. Rahmanifar et al. have speculated the formation of PBQ, which initiates cross-linking reaction between linear polymer chains that interrupts the delocalization of both the charge and electrons along the polymer chain leading to the decrease in conductivity and charge transfer of PANI [59]. Chandrakanthi and Careem have claimed that PANI has the highest environmental stability [60] and recognised as the only conducting polymer that is stable in air reported by Sergeeva et al. [61]. Luo and Do have reported PANI as an interesting material for sensor and biosensor interfaces since it acts as an effective mediator for electron transfer in redox or enzymatic reactions and can be used as a suitable matrix for immobilization of biomolecules [62]. Polyaniline is a semiflexible conducting polymer family due to its high conductivity and low cost, targeting for its applications in biosensors because of a number of useful properties such as direct and easy deposition onto the electrode, control of thickness, redox conductivity and polyelectrolyte characteristics, high surface area, chemical specificities, long-term environmental stability, and tuneable properties. An advantage of PANI in the field of biosensor are an impressive signal amplification and elimination of electrode fouling and exhibits two redox couples in the convenient potential range to facilitate an efficient enzyme-polymer charge transfer. Iribe and Suzuki have demonstrated the use of polyaniline as an enzyme switch, which yields “on” and “off” responses. PANI consists of reduced (benzenoid diamine) and oxidised (quinoid diamine) repeating units. The different redox forms of PANI are reported. Leucoemeraldine (LE) is fully reduced state. Pernigraniline (PG) is fully oxidized state with imine links instead of amine links. The emeraldine form of PANI, often referred to as emeraldine base (EB), is either neutral or doped, with imine nitrogens protonated by an acid. EB is regarded as the most useful form of PANI due to its high stability at room temperature and that its doped form (emeraldine salt; ES) is electrically conducting. LE and PG are poor conductors, even when doped with an acid. These forms have interconverted by chemical and/or electrochemical oxidation or reduction. The delocalized π -bonds available in this system are responsible for its semiconducting properties. When PANI has doped with an acid, a polaron formed through successive formation of bipositive species, bipolaron structure, and more stable polaron structure. This polaron structure is responsible for electrical conduction through hopping mechanism in its crystalline region and this hopping may be intrachain or interchain. In polaron structure, a cation radical of one nitrogen acts as a hole and this hole acts as charge carriers. The electron from the adjacent nitrogen (neutral) jumps to this hole and it becomes electrically neutral. Then, the holes start to move. In bipolaron structure, this type of movement

is not possible since two holes are adjacently located. In LE or PN structures, the electronic environments of all nitrogen atoms along the polymer chain are similar. Protons from a dopant can be attracted by any nitrogen atom and there may be a few (more than two) protonated nitrogen or free nitrogen atoms situated side by side across the chain. There is a less chance for chain regularity, creating less chance for the formation of a polaron. As a result, protonated LE or PN are insulating in nature. PANI's transport properties, electrical conductivity, or rate of energy migration, provide enhanced sensitivity. It is the best-known semiflexible rod CP system with chemical and structural flexibility surrounding its amine nitrogen linkages for effective binding and immobilization of biomolecules [63]. Besides this, Ding et al. have reported control over the shape and dimensions of PANI by varying synthesis or processing conditions is likely to result in desired physical and electrochemical properties for biosensing application [64]. Forzani et al. have reported nanostructures of PANI, for example, nanowires, nanospheres, nanorods, and nanotubes which have provided a new ground to improve its characteristics and offer the possibility of enhanced performance wherever a high interfacial area between PANI and its environment is important [65]. The morphology of nanostructured PANI plays an active role in enhancement of activity of desired catalyst. Sreedhar et al. have recently studied the activity profile of the oxidation of 4-bromothioanisole using PANI nanospheres (S), nanorods (R), nanotubes (T). These investigations have revealed that the activity varies as $S < R < T$, that has been attributed to enhanced surface area in case of PANI nanotubes (PANI-NT) [66]. The integration of nanostructures and biomolecules may lead to new hybrid systems that couple the recognition or catalytic properties of biomaterials with attractive electronic and structural characteristics. The use of PANI nanostructures also helps to overcome the processability issues associated with PANI. Mathebe et al. have fabricated H_2O_2 biosensor by immobilizing HRP on electropolymerized films of PANI by making use of electrostatic interactions between PANI backbone and enzyme. This electrochemically active PANI matrix serves as an efficient nondiffusional mediator, shuttling electrons between redox active centre of enzyme and the electrode surface [67]. Michira et al. have synthesized and characterized a novel anthracene sulfonic acid (ASA) doped PANI nanomaterial and have explored its application for amperometric biosensor for H_2O_2 and erythromycin. The studies reveal that this self-doped PANI/ASA matrix acts as an effective electron mediator as it provides direct electrical communication between the enzymes (HRP, cytochrome P450 3A4) and the platinum electrode [68]. Wang et al. have reported H_2O_2 biosensor constructed by cross-linking with HRP and PANI, electrochemically deposited in the presence of ionic liquid, using glutaraldehyde on F-doped tin oxide (FTO) [69]. Iwuoha et al. have examined amperometric behaviour of HRP in biosensor format where polyvinyl sulphonate (PVS) doped aniline, which electropolymerized onto the surface of glassy carbon electrode (GCE). Biomolecules are doped onto the polymer surface by electrostatic interactions with polymer backbone [70]. Kathleen et al. have reported

the influence of the thickness of PANI film deposited electrochemically onto screen-printed electrode (SPE) surface has been described in terms of its influence on a variety of amperometric sensor characteristics like time to reach steady state, charging current, catalytic current, background current, and signal/background ratio [71]. Lyons et al. have reported the sensitivity and limit of detection of polymer-based biosensor are dependent on the background current exhibited by polymer [72]. Mu and Xue have reported that if the background current of a sensor based on these PANI/PVS films is too large, then the contribution from the reduction of H_2O_2 by HRP is difficult to measure. High background currents could limit the contribution from the enzyme catalytic reaction, thereby decreasing sensitivity of the sensor. Therefore, thinner polymer films are preferable, especially where lower levels of analytes are to be detected [73]. The good biocompatibility of PANI nanostructure enables it to become a simple and effective platform for the integration of proteins/enzymes and electrodes, providing analytical access to a large group of enzymes for a variety of bioelectrochemical applications. Morrin et al. have described fabrication of uniform array of nanoparticulate PANI nodules using dodecylbenzenesulfonic acid (DBSA) as dopant for H_2O_2 detection using HRP as biosensing element. This effective biosensor format exhibits higher signal-to-background ratio and shorter response time. The authors show a comparison between the characteristics of the optimized nanoPANI/DBSA film with that of PANI/PVS film in terms of a platform for biosensing. The results indicate nanoPANI/DBSA film is a more efficient matrix in terms of protein immobilization and require concentration of protein sixfold lower than PANI/PVS layer for monolayer coverage [74]. Luo et al. have prepared a highly nodular, cauliflower-like nanostructured electrode through the electropolymerization of aniline on SPE with subsequent deposition of polystyrene (PS) nanoparticle template and further growth of PANI around these templates. The high sensitivity of this biosensor is probably due to the unique nanostructure on the electrode surface, as the cauliflower-like nanostructured PANI can contact with more enzyme molecules and thus can be more efficient in assisting electron transfer from the enzyme to the electrode surface [75]. Wang et al. have recently reported a glucose biosensor based on direct ET of GOx entrapped electrochemically into the inner wall of highly ordered PANI-NT synthesized using anodic aluminium oxide (AAO) as template. The direct ET from the GOx to the electrode (Pt) indicated by the generation of a pair of well-defined symmetrical redox peaks in CV of GOx-PANI-NT/Pt [76]. Xu et al. have reported a novel glucose biosensor, with high sensitivity and selectivity, fabricated by self-assembling GOx and Pt-DENs on nanofibrous PANI. The resulting biosensor exhibits excellent amperometric response to glucose and possesses biocompatible performance with the possibility of minimizing enzyme denaturing [77]. Xian et al. have reported the electrical contacting of gold nanoparticles (Au NP) with the glassy carbon through PANI nanofibers, which enables this nanocomposite structure to be used as an excellent matrix for electrocatalysis and enzyme (GOx) immobilization [78].

Zhou et al. have reported results of similar studies using platinum (Pt) microparticles for glucose detection and found the bioelectrode to be highly stable and reproducible [79]. Ramanathan et al. have utilized Langmuir Blodgett (LB) films of PANI for a glucose biosensor fabrication [80]. Recently, Nemzer et al. have demonstrated the role of PANI both as host polymer and redox indicator for glucose oxidase. Thus, this smart material provides a biosensing route that eliminates the need of redox dyes during optical detection of glucose [81]. Borole et al. have reported a comparative study of glucose sensor by entrapping GOx in PANI, poly(*o*-toluidine) (POT), and its copolymer. The amperometric response reveals that PANI-GOx electrode is much more preferable for use because of the comparatively fast response [82]. Xue et al. have developed a new glucose biosensor by in situ electropolymerization of aniline into microporous PANI-coated platinum electrode in the presence of GOx. The technology combines the advantage of microporous polymer material and electropolymerization in biosensor construction. Thus, prepared bioelectrode exhibits good selectivity, sensitivity, stability, and shows no apparent loss of activity after 100 consecutive measurements and intermittent usage for 100 days [83]. Shi et al. have reported that poly(aniline-boronic acid) wires generated on double standard DNA (dsDNA) template could facilitate electrical communication between electrode and GOx reconstituted on polymer wires. However, the operating potential for detection of glucose at this electrode is +0.5 V (versus SCE) and at this potential many other electroactive species commonly coexisting in biological fluids, such as ascorbic acid, uric acid, and 4-acetamidophenol, can get oxidized and severely affect the selectivity of sensors [84]. Zhao et al. have investigated PANI nanofibers as electrode material for direct ET of redox proteins/enzymes with GOx as a model. After immobilization on this nanofiber matrix, GOx remains in its natural structure and undergoes effective, direct ET reaction with a pair of well-defined, quasireversible redox peaks. The electrode displays good features in electrocatalytic oxidation of glucose with good reproducibility and stability [85]. Sukeerthi and Contractor have fabricated and compared the response of three different biosensor array structures of PANI [86], Contractor et al. have reported macrosensor (twin-wire electrode configuration) [87], Sangodkar et al. reported microsensors [88] and microtubular sensors for glucose, triglyceride, and urea. This microtubular sensor found to have the highest sensitivity for all the three analytes. This enhanced sensing characteristic in case of PANI microtubules attributed to shorter source-to-drain distance and more disordered structure of PANI that result in increased conductivity, transduction ability, and supports effective enzyme loading. Karyakin et al. have explored the advantages of PANI as potentiometric transducer for detection of bioanalytes like glucose, urea, and so forth. The potentiometric detection of enzyme activity based on the measurement of change in pH in the enzymatic layer on sensor surface has caused by oxidation of H_2O_2 produced by the enzymatic reactions [89]. Gaikwad et al. have fabricated potentiometric glucose biosensor by immobilizing GOx on electrochemically synthesized PANI film via glutaraldehyde. This bioelectrode

was found to perform well in terms of dynamic range of detection and short response time [90]. Wang and Mu have studied bioelectrochemical characteristics of ChOx immobilized onto an electrochemically deposited PANI film. These studies have revealed that the response current of the enzyme electrode at Triton X-100 concentration is of 1% [91]. Bayramoğlu et al. have proposed a new conductive fiber support based on PAN/PANI for immobilization of invertase enzyme and offers an economical protocol for reversible enzyme immobilization on conductive composite fiber [92]. Kim and Jeon have reported an amperometric superoxide microbiosensor by electropolymerization of aniline with concomitant doping of superoxide dismutase on the surface of Pt electrode. The amperometric response to superoxide anion was obtained at a potential of 0.75 V for oxidation of H₂O₂ generated from disproportionation of superoxide anion [93]. Shan et al. have developed a sensitive and stable amperometric phenol biosensor by coentrapping PPO with in situ polymerization of PANI in PAN matrix, and modified electrode was used to detect benzoic acid [94]. Castelletti et al. have developed a capillary electrophoresis (CE) biosensor for measuring ascorbic acid by coupling a PANI optical sensor and CE [95]. Langer et al. have developed a choline sensor by immobilization of choline oxidase (ChO) to nanoporous PANI layers [96]. Bartlett et al. have reported successful oxidation of NADH at PANI-coated electrode. The electropolymerization of PANI has accomplished in presence of PVS counter ions that gave rise to films that are both stable and electroactive at pH 7 [97]. Tian et al. have described an amperometric biosensor for the detection of H₂O₂ based on horseradish peroxidase/polypyrrole (PPy) membrane deposited onto the surface of ferrocenecarboxylic acid mediated derived sol-gel derived composite carbon electrode [98]. Yadav et al. have fabricated a highly sensitive, specific, and rapid electrochemical oxalate biosensor by covalently immobilizing sorghum leaf oxalate oxidase on carboxylated multiwalled carbon nanotubes and conducting polymer, polyaniline nanocomposite film electrodeposited over the surface of platinum (Pt) wire using N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxy succinimide (NHS) chemistry [99]. Tang et al. have fabricated a novel H₂O₂ sensor based on enzymatically induced deposition of electroactive polyaniline (PANI) at horseradish peroxidase (HRP)/aligned single-wall carbon nanotubes (SWCNTs) modified Au electrode. Thus, the synergistic performance of the enzyme, the highly efficient polymerization of PANI, and the templated deposition of SWCNTs provided an extensive platform for the design of novel electrochemical biosensors [100]. Chen et al. have fabricated multiwalled carbon nanotube (MWCNT)/polyaniline (PANI) composite film by electrochemical polymerization. The immobilized AChE catalyzed the hydrolysis of acetylthiocholine chloride to produce thiocholine, which engendered an irreversible oxidation peak. The enzyme activity was monitored by measuring the oxidation current of thiocholine and further detection of OP. The developed sensor provided a new promising tool for pesticide analysis and assay of enzyme activity [101]. Vatsyayan et al. have fabricated GCE/MWCNT-NF/CAT/PEI

electrode for hydrogen peroxide (H₂O₂) detection. Catalase (CAT) (Mr~90 kDa), immobilized on multiwalled carbon nanotubes-Nafion (MWCNT-NF) matrix and encapsulated with polyethylenimine (PEI) on glassy carbon electrode (GCE), showed a pair of nearly reversible cyclic voltammetric peaks for Fe(III)/Fe(II) couple with formal potential of about -0.45 V (versus Ag/AgCl electrode at pH 7.5). PEI significantly reduced the charge transfer resistance and stabilized the bioelectrode through electrostatic interaction [102]. Rajesh et al. developed highly sensitive biosensor for the direct and simultaneous determination of superoxide anion radical (O²⁻) and nitrite (NO²⁻) by incorporation of carbon nanotube (CNT) solubilized in nafion in polypyrrole (PPy) matrix on Pt electrode followed by immobilization of Cu, ZnSOD (SOD1) onto it. The modified electrode not only enhanced the immobilization of SOD1 but also promoted the electron transfer of SOD1 minimizing its fouling effect. This modified electrode is effective not only in detecting O²⁻ and NO²⁻ independently but also determining the concentration of O²⁻ and NO²⁻ simultaneously *in vitro* and in cancer cells [103]. Deng et al. have developed a novel strategy for fabricating the sensitive and stable biosensor that was present by layer-by-layer (LBL) self-assembling glucose oxidase (GOD) on multiwall carbon nanotube (CNT) modified glassy carbon (GC) electrode. GOD was immobilized on the negatively charged CNT surface by alternatively assembling a cationic poly(ethylenimine) (PEI) layer and a GOD layer. Therefore, the developed strategy is ideal for the direct electrochemistry of the redox enzymes and the construction of the sensitive and stable enzyme biosensor [104]. Du et al. have proposed a simple method to immobilize acetylcholinesterase (AChE) on polypyrrole (PPy) and polyaniline (PANI) copolymer doped with multiwalled carbon nanotubes (MWCNTs). The synthesized PAN-PPy-MWCNTs copolymer presented a porous and homogeneous morphology, which provided an ideal size to entrap enzyme molecules. Due to the biocompatible microenvironment provided by the copolymer network, the obtained composite was devised for AChE attachment, resulting in a stable AChE biosensor for screening of organophosphates (OPs) exposure. MWCNTs promoted electron-transfer reactions at a lower potential and catalyzed the electro-oxidation of thiocholine, thus increasing detection sensitivity. The developed biosensor exhibited good reproducibility and acceptable stability, thus providing a new promising tool for analysis of enzyme inhibitors [105]. Uang and Chou have investigated the pH effect on the electropolymerization in the presence of glucose oxidase on the characteristics of glucose oxidase/polypyrrole (PPy) biosensor [106]. Borole et al. have constructed glucose biosensors based on electropolymerized polyaniline, poly(*o*-toluidine) and poly(aniline-cotoluidine), respectively [107]. Seker and Becerik have electrochemically entrapped glucose oxidase into polypyrrole matrix along with *p*-benzoquinone in piperazine ethyl sulphate (PIPES) buffer [108]. It is revealed that application of the artificial network analysis applied to the nonlinear calibration plot can be used to predict the sensor failure detection. Ramanavicius et al. have prepared polypyrrole in the presence of glucose oxidase from *Penicillium vitale*, glucose, and oxygen

revealing that optimal conditions of glucose oxidase activity (pH 6.0) are similar to pyrrole polymerization reaction (pH 6.5) indicating possible biomedical applications of this conducting polymer [109]. Pan et al. have reported CNT-based glucose biosensor using immobilization of glucose oxidase in poly(*o*-aminophenol) and carbon nanotube composite through electrochemical polymerization onto gold electrodes [110]. Curulli et al. have studied the influence of electrolyte nature and its concentration on the kinetics of electropolymerization of monomers such as 1,2- and 1,4-diaminobenzene, 2,3- and 1,8-diaminonaphthalene, *o*-anisidine, and on the resulting morphology of nanotubules. Glucose oxidase (GOx), lactate oxidase (LOD), l-aminoacid oxidase (l-AAOD), alcohol oxidase (AOD), glycerol-3-phosphate oxidase (GPO), lysine oxidase (LyOx), and choline oxidase (ChOx) were immobilized on Prussian Blue (PB) layer supported on 1,2-diaminobenzene (1,2-DAB) nanotubes onto platinum electrodes [[111]. Callegavi et al. have fabricated the amperometric biosensors for lactate, phenol, catechol, and ethanol by incorporation of their respective enzyme in carbon nanotubes (CNT) matrix [112]. They have shown that the use of CNT electrode yields better results as compared to normal carbon paste electrode. The observed stability of the H₂O₂ sensor has attributed to the nano-tubular structure. It has shown that these nanostructured polymers could be utilized for fabrication of enzyme biosensors. Trojanowicz and Miernik have employed avidin-biotin interactions for the immobilization of glucose oxidase on bilayer lipid membrane (BLM) formed on polypyrrole and poly(*o*-phenylenediamine) electrodeposited onto platinum wire, respectively [113]. These BLM-based glucose biosensors resulted in stable and sensitive response with significant reduction from electroactive species such as ascorbic acid, cholesterol, and uric acid, respectively. Myler et al. have reported improved signal linearity of enzyme (glucose oxidase) ultra-microelectrodes fabricated via sonication and deposition of polysiloxane coating onto the working glucose oxidase/polyaniline electrode coated with insulating diaminobenzene [114].

He et al. have developed a sensitive hydrogen peroxide probe based on amplified fluorescence quenching ability of poly(9,9-bis(6-N,N,N-trimethylammonium-hexyl) fluorene phenylene (PPF-NMe³⁺, a cation conjugated polymer) and peroxyfluor-1 borate protective groups. The hydrogen peroxide probe has a detection range of 15–600 nM and can be used for glucose detection. Conducting a polymer-based self-regulating insulin delivery system has recently been reported [115]. Arslan et al. have fabricated a polysiloxane/polypyrrole/tyrosinase electrode by entrapment of tyrosinase in conducting matrix by electrochemical copolymerization for determining phenolic content of green and black tea [116]. Védrine et al. have fabricated an amperometric tyrosinase biosensor based on conducting poly(3,4-ethylenedioxythiophene) (PEDOT) for estimation of herbicides and phenolic compounds [117]. Böyükbayram et al. have made a comparative study of immobilization methods of tyrosinase (Tyr) on electropolymerized conducting and nonconducting polymers for application to the detection of dichlorvos

organophosphorus insecticide [118]. Jiang et al. have demonstrated that incorporation of polyvinylalcohol (PVA) onto a polymer film caused higher sensitivity than that of pure PPy sensor [119]. Rahman et al. have fabricated the amperometric biosensor for choline by immobilizing choline oxidase (ChOx) with and without horseradish peroxidase (HRP) onto carboxylated thiophene modified electrodes [120]. They have shown that the electrode with HRP has better performance and these biosensors have the response time of 5 s and selectivity to interfering species. Kan et al. have reported stronger affinity between uricase and polyaniline prepared by template process resulting in the increased stability of this polyaniline-uricase biosensor [121]. Haccoun et al. have fabricated a reagentless lactate biosensor using electrocopolymerized copolymer film of poly(5-hydroxy-1,4-naphthoquinone-co-5-hydroxy-3-acetic acid-1,4-naphthoquinone) [122]. These studies show that the presence of interferents like acetaminophen, glycine, and ascorbic acid does not influence the response of this mediated (quinine group) copolymer electrode. However, this biosensor can be used for l-lactate estimation upto 1 mM of l-lactate. Bartlett et al. have found that poly(aniline)-poly(anion) composites films can be utilized for electrochemical oxidation of NADHat around 50 mV versus SCE and pH 7 [123]. These results have implications towards the technical development of microelectrodes, biofuel cells, and amperometric biosensors. Asberg and Inganas have cross-linked horseradish peroxidase in highly conducting poly(3,4-ethylene dioxy thiophene) (PEDOT)/(polystyrene sulphonate) (PSS) dispersion using poly-4-vinylpyridine for estimation of hydrogen peroxide [124]. Grennan et al. have reported the horseradish peroxidase immobilized screen-printed electrodes based on chemically polymerized polyaniline/polyvinylsulphonate films, which can be used for the mass production of biosensors [125]. Jia et al. have fabricated horseradish peroxidase (HRP) biosensor by self-assembling gold nanoparticles to a thiol-containing sol-gel network of 3-mercaptopropyltrimethoxysilane (MPS) [126]. Ngamna et al. have immobilized horseradish peroxidase poly(2-methylsulphonated polyaniline-5-sulphonic acid)/l-lysine composite [127]. Zhou et al. have observed enhanced peroxidase activity in hemoglobin in sulfonated polyaniline on glassy carbon electrodes [128]. This result has been attributed to the facile interfacial transfer of hemoglobin mediated by sulphonated polyaniline. Lindgren et al. have immobilized horseradish peroxidase (HRP), sweet potato peroxidase (SPP), and peanut peroxidase (PNP) tobacco peroxidase (TOP) on graphite electrodes [129]. The peroxidase electrodes were used for estimation of H₂O₂. Dai et al. electrochemically immobilized cytochrome C on a NaY Zeolite modified electrode [130]. This electrode displayed excellent response to the reduction of H₂O₂ without the use of electron mediator and hence could be used for H₂O₂ detection. Brahim et al. have developed a p(2-hydroxyethyl methacrylate/polypyrrole) system containing glucose oxidase, cholesterol oxidase, and galactose oxidase [131]. The observed stability of 9 months for this system indicates that this biosensor can be subcutaneously used

to monitor glucose, cholesterol, and galactose. Bijanowska et al. have made a comparison of urea estimation in blood plasma and in dialysate for the physiological and the pathological range by enzyme field effect transistor (EnFET) based detection of pH and pNH_4 [132]. It is concluded that pNH_4 -based biosensors require predilution whereas for pH biosensors both for blood plasma and dialysate do not require dilution. Zhang et al. have discovered that stable mixed amphiphile (octadecylamine, ODA/behenic acid, BA)/urease Langmuir-Blodgett films can be formed onto the hydrolyzed surface of a pH-ISFET [133]. Rebr̄iev and Starodub have fabricated a urea biosensor based on ion-sensitive-field-effect transistor (ISFET) and photopolymeric membrane obtained by irradiation of a liquid mixture comprising vinyl pyrrolidone, oligo urethane methacrylate, and oligocarbonate methacrylate. This urea biosensor exhibited high sensitivity, response time [134]. Pinto and Schanze have demonstrated the application of conjugated polyelectrolytes (CPEs) based on pendent ionic sulphonate and carboxylate groups for estimation of protease activity. The sensor has been used for estimation of low enzyme concentrations and enzyme-catalyzed kinetics. This technique can be used to develop assays for kinases, phosphates, lipases and esterases, and so forth [135]. Ivanov et al. have used glassy carbon electrodes modified with polyaniline for the immobilization of cholinesterase using cross-linking technique [136] for the estimation of pesticides (trichlorfon, coumaphos, methiocarb, and aldicarb). Rahman et al. have covalently immobilized pyruvate oxidase onto nanoparticles comprising of poly-5, 2:5, 2-terthiophene-3-carboxylic acid, poly-TTCA (nano-CP) on a glassy electrode for amperometric detection of phosphate ions [137]. Qu et al. have reported an amperometric biosensor for detection of choline based on polyaniline multilayer film and layer-by-layer assembled functionalized carbon nanotube [138]. Biloivan et al. demonstrated that protein selective microelectrodes can be fabricated by immobilization of different proteinases such as trypsin, pronase E, and carboxypeptidase B on surface of platinum electrodes modified by conducting PPy [3,3-Co(1,2-C2B9H11)]₂ [139]. Lee and Cui have presented low-cost, transparent, and flexible ion-sensitive field-effect transistors (ISFETs) as pH and glucose sensors. Single-walled carbon nanotubes (SWCNTs) and poly(diallyldimethylammonium chloride, PDDA) are deposited by layer-by-layer (LbL) self-assembly between two metallic electrodes patterned on a polyethylene terephthalate substrate. The LbL self-assembly of nanomaterials and enzymes on the transparent and flexible substrate suggests various chemical and biological sensors suitable for *in vivo* application [140]. Tan et al. have reported the facile preparation of polymer-enzyme-multiwalled carbon nanotubes (MWCNTs) cast films accompanying *in situ* laccase (Lac) catalyzed polymerization for electrochemical biosensing and biofuel cell applications. The proposed biomacromolecule-immobilization platform based on enzyme-catalyzed polymerization may be useful for preparing many other multifunctional polymeric bionanocomposites for wide applications [141]. Xiao et al. have designed polymerized ionic liquid-wrapped

carbon nanotubes (PIL-CNTs) for the first time for direct electrochemistry and biosensing of redox proteins. The proposed biosensor (GOD/PIL-CNTs/GC electrode) displayed good analytical performance for glucose [142]. Haddad et al. have functionalized single-walled carbon nanotubes with biotin using either electropolymerization or formation of pi-stacking interactions for the construction of biosensors. A biotinylated glucose oxidase as a biomolecule model was immobilized on the biotinylated nanotubes [143]. Xie et al. have developed a novel general methodology for protein immobilization and third-generation biosensor, which involves Al^{3+} directed polyacrylamide (PAM) self-assembly into an ordered dendriform structure, easily immobilizing enzymes and nanoparticles. The Al^{3+} directed HRP/PAM/Pt/SWCNT film was one of ideal candidate materials for direct electrochemistry of redox proteins and find potential applications in biomedical, food, and environmental analysis [144]. Min and Yoo have developed a novel 3-dimensional single-wall carbon nanotube (SWNTs) polypyrrole (Ppy) composite as an electrode by chemically polymerizing polypyrrole onto SWNTs using a LiClO_4 oxidant. This tyrosinase SWNT/Ppy composite electrode was used for amperometric detection of dopamine in the presence of ascorbic acid [145]. Bhambi et al. have constructed a highly sensitive, amperometric uric acid biosensor based on PANI-carbon nanotubes for determination of uric acid level in serum [146]. Barik et al. have immobilized cholesterol oxidase (ChOx) covalently onto polyaniline-carboxymethyl cellulose (PANI-CMC) nanocomposite film onto indium tin oxide (ITO) coated glass plate using glutaraldehyde as a cross-linker for the detection of cholesterol [147]. Dhand et al. have fabricated ChOx/Glu/PANI-NT/ITO bioelectrode via electrophoretic technique using glutaraldehyde (Glu) as cross-linker for the cholesterol detection [148]. Dhand et al. have also prepared polyaniline nanospheres (PANI-NS) via morphological transformation of micelle polymerized camphorsulfonic acid doped polyaniline nanotubes in the presence of ethylene glycol and a PANI-NS (60–80 nm) film deposited onto an indium tin oxide (ITO) coated glass plate by coupling chemistry as a cholesterol biosensor [149]. Ansari et al. have fabricated hydrogen peroxide (H_2O_2) sensor via immobilizing horseradish peroxidase (HRP) onto polyaniline (PANI) cerium oxide (CeO_2) nano-composite film onto indium tin oxide (ITO) coated glass substrate using electrochemical technique [150]. Dhand et al. have reported nanocomposite film composed of polyaniline (PANI) and multiwalled carbon nanotubes (MWCNT), electrophoretically onto indium tin oxide (ITO) coated glass plate, used for covalent immobilization of cholesterol oxidase (ChOx) via N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) chemistry [151]. Pandey et al. have fabricated gold nanoparticles (AuNPs) via self-assembled onto electrochemically deposited polyaniline (PANI) films on indium tin oxide (ITO) coated glass plates, covalently immobilized glucose oxidase (GOx) in the near vicinity of gold nanoparticles using N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide

(EDC)/N-hydroxysuccinimide (NHS), chemistry between amino groups of PANI and COOH groups of GOx for the detection of glucose [152]. Matharu et al. have reported cholesterol oxidase (ChOx) linked Langmuir-Blodgett (LB) monolayers of polyaniline- (PANI-) stearic acid (SA) onto indium tin oxide (ITO) coated glass plates via glutaraldehyde (Glu) chemistry for cholesterol estimation [153]. Dhand et al. fabricated cholesterol oxidase (ChOx) via being covalently immobilized onto electrophoretically deposited conducting polymer film (on indium tin oxide (ITO) glass plate derived from nanostructured polyaniline (PANI) colloidal suspension using N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) chemistry [154]. Singh et al. have reported covalently immobilized cholesterol esterase (ChEt) and cholesterol oxidase (ChOx) on electrochemically prepared polyaniline (PANI) films [155]. Arora et al. have reported uricase immobilized using glutaraldehyde as cross-linker onto electrochemically synthesized polyaniline (PANI) films used to measure uric acid concentration in serum [156]. Sharma et al. developed an amperometric lactose biosensor by immobilizing lactase (EC 3.2.1.23) and galactose oxidase (GaO) (EC 1.1.3.9) in Langmuir-Blodgett (LB) films of poly(3-hexyl thiophene) (P3HT)/stearic acid (SA) for estimation of lactose in milk and its products to prevent "lactose intolerance." The reusability of electrode was found ten times with 3% loss in current response. The working electrode may be used for the estimation of lactose/galactose in food and biological fluids [157]. Sharma et al. have reported an amperometric biosensor to estimate galactose in human blood serum using monolayers of poly(3-hexyl thiophene) and galactose oxidase/stearic acid in chloroform and dispensed on to the air-water interface of Langmuir-Blodgett trough. The amperometric galactose biosensor was thus fabricated [158]. Singhal et al. have reported Langmuir-Blodgett films of poly(3-hexyl thiophene) prepared by simultaneous entrapment of glucose oxidase and transferred onto the indium tin oxide-coated glass plates [159]. Chaubey et al. have reported the electrochemical entrapment of polyaniline (PANI) onto sol-gel derived tetraethylorthosilicate (TEOS) films deposited onto indium tin oxide (ITO) coated glass utilized for immobilization of lactate dehydrogenase (LDH) for the lactate measurement [160]. Arya et al. obtained ChOx/FANB/ODT/Au self-assembled monolayer based bioelectrode to estimate cholesterol by surface plasmon resonance technique [161].

3. Conclusions

The electroactive property of organic conducting polymers makes them good candidates for highly selective, specific, stable, economic, and handy biosensing devices, which are widely used in biosensor industries. The growing need for rapid development of analytical devices requiring smaller sample volumes, decreased power consumption, and improved performance. So that the urgent need for reduction of shape and size dimensions of nanomaterials

display unique properties like increased surface area and enhanced electrical/optical properties, which make them suitable for numerous applications such as nanoelectronics, photovoltaics and chemical/biological sensing. In this review nanomaterials were incorporated in biosensor devices, it is possible to achieve enhanced sensitivity, improved response time, and smaller size. The nanomaterials geometries are particularly relevant, and specifically focus the increasing importance of inorganic, organic nanostructures, conducting polymer nanostructures with carbon nanotubes towards the development of various kinds of biosensors. The focus of this review is an in-depth analysis of the recent achievements in the concern field and the major challenges that are to be overcome for the widespread use of applications such as point-of-care diagnostics, food safety/control, and environmental monitoring.

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