Polypyrrole Based Biofunctional Composite Layer for Bioelectrocatalytic Device System

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Received: 26 September 2018, Revised: 23 November 2018 and Accepted: 26 November 2018

DOI: 10.5185/amlett.2019.2279
www.vbripress.com/aml

Abstract

The involvement and development of intelligent polymers in enzymes as a catalyst have been interesting to develop processes that are environmentally benign, energy efficient, and selective towards their specific molecular applications. Polypyrrole based enzyme nanocomposites represents a continuous considerable redox organic polymer that have been increased in electromechanical devices in advance for surface functionalization. This review addresses the fundamental concepts of polypyrole and its composites role for enzyme immobilizing functioning and its bio functional bioelectrocatalytic system operating principle in biosensor and biofuel cells as well as its involvement in electron transfer mechanism as a bio electrode to create an advanced bioelectronics device from power generating system to extreme analyte detecting system. Copyright © VBRI Press.

Keywords: Polypyrrole, composite layer, enzyme bio functional, biosensor, biofuel cell.

Introduction

Conducting polymers and their nanocomposites [**1, 2**] have assumed to be of great importance in several functional electronic devices. Their ease of processing makes them useful in electromechanical concepts through the delocalized electronic states combined with the restriction on the extent of delocalization. These polymers are electrochemically active and their conductivity can be changed by means of doping/dedoping process in the presence of dopant group. Hence, a great number of sensing applications are designed by exploiting conducting polymers [**3**] due to their fast electron transfer characteristics due to delocalization of pi-electrons with in the conjugated frame work molecular system [**4**]. Among them polypyrrole (PPy) is being widely used in organic electronics owing to its ability towards chemical stability and high conductivity nature [**5**]. After the discovery of doped polyacetylene [**4**], continual use of PPy has been increased inherently among other conducting polymers due to its significant electronic (*e.g.* morphological and structural) property. Recent studies suggesting that due to its unique ease of synthesis route, high conductivity, good environmental stability and good mechanical properties [**6-8**], PPy in the form of films have been wildly used for sensor devices that detect various gases and volatile organic compounds. However, its sensitivity is better and selective with good response to these gases, showing larger response to polar than non-polar compounds [**3**].

Mostly the synthesis of PPy is carried out with two major ways (i) chemical methods which was supported by template assisted rigid heterocyclic process [**9**] (ii) electrochemical polymerization technique. PPy synthesized by electrochemically films have some attractive features, such as good conductivity and very high adherence to the substrates. Further, for electrochemical deposition of PPy from either aqueous or nonaqueous media, several techniques are available: constant potential electrodeposition, galvanostatic deposition, cyclic voltammetry and pulse voltammetry. The common factors that control an electro polymerization process are electrolyte anions, electrolyte solvents, pH of the aqueous solutions, polymerization temperature and current/potential values. Normally doping of PPy with large anionic detergents, it showed a much higher chemical stability than smaller anionic detergents. From chronoamperometric technique and voltammogram measurement result showed that the electro activity and film thickness of PPy increases with increase in dopant concentrations (in this synthesis acrylic sulfonic acid containing alkali salt solution was used as a dopant) [**10**].

Comparatively PPy has higher conductivity than some other conducting polymer family member groups such as polyaniline and as well as good environmental stability [**11**]. For these reasons, PPy has a continuous considerable attention. Thus, this review presents a detailed PPy functional contributions with enzyme and metal oxide for bioelectronics as biosensors and biofuel cell applications. Therefore, the increment in sensitivity and selectivity of PPy based functional bio electrode and its composites at optimum conditions in the presence of biomolecules like enzymes and apoenzymes as a bioelectrocatalyst [**12**] has been briefly reviewed. In addition, the further improvement by embedding with other active metal, carbon, and semiconductor particulate materials into PPy matrix to form a metal-polymer, carbon-polymer and semiconductor polymer composites would be explained. Thus, concepts in immobilizing enzyme either by directly (Direct Electron Transfer) or by indirect (Mediated Electron Transfer) mechanisms with a highly porous structure followed by a large effective surface area, good electronic conductivity and high catalytic activity would be briefly discussed from recent studied research.

Polypyrrole for enzyme immobilization

The involvement and development of enzymes in bio electrochemistry as a catalyst [**13**] have been interesting to develop processes that are environmentally benign, energy efficient, and selective towards their specific molecular applications. Commonly from the conventional prospective enzyme can catalyze a wide range of chemical transformations under relatively mild conditions. They can be efficient catalysts although the hallmarks of most enzymatic catalysts are their selectivity and specificity at converting substrate to product [**4**]. In bioelectrocatalytic system many of redox polymers containing bio composite functional material for energy conversion paradigm using enzyme immobilization have been successfully done, but the ready denaturation of immobilized enzyme/protein on the surface of the electrode is a common problem which leads to such modified electrodes suffering from a poor enzyme/protein activity and low reproducibility and stability. Considering these facts material compositing biosensors and some nonenzymatic sensor fabrication has been reported [**11**]. In addition to bioelectronics coating with biomolecule functionalized PPy has also extended to improve the biocompatibility of electrode (metallic) surfaces in implant material for biomedical application [**14**].

Jugović *et al*. reported that beyond biomedical applications, requirements in electrochemical biosensors are to reproducibly immobilize the biomolecules onto the biosensor while keeping their biological activity. Activity, reusability and sustainability of enzyme can also be enhanced using redox polymer as a conducting nanofiber as immobilization matrix. The porosity of conducting polymer is an important factor for the facile immobilization of biomolecules. One of the key steps in biosensor construction process is enzyme immobilization. Among many mechanisms for enzyme immobilization physical entrapment, adsorption, covalent binding, crosslinking, and doping are some of the most common and interactive bridge interactions [**15**].

Polypyrrole (PPy) can be very suitable as an immobilization matrix due to its simple preparation procedure, biocompatibility, chemical inertness, mechanical stability and limited permeability, which extends linear detection range. Immobilized enzymes exhibit better stability and durability than free enzymes in a solution system. Free enzymes are usually not stable and therefore their application in a sensing system is complicated. Further study from fluorescence results showed that, when glucose oxidase (GOx) self-encapsulation within polypyrrole, was successfully achieved and this process was monitored by registration of flavin adenine dinucleotide auto fluorescence spectra at different periods. The detailed data presented showed that at the same experimental conditions, encapsulation of glucose oxidase within polypyrrole was more stable than native enzyme. From fluorescence spectra reflection, unfolding of GOx from Penicillium *vitale* was investigated by monitoring with flavin adenine nucleotide (FAD) cofactor fluorescence result. **Fig. 1** shows PPy keeps GOx from denaturation and FAD from dissociations while GOx coated by a (PPy) shell (GOx/PPy-nanoparticles) and the ability of PPy to decrease the unfolding process of the enzyme was investigated. The stability of the GOx encapsulated within PPy was observed by the FAD fluorescence measurements [**7**].

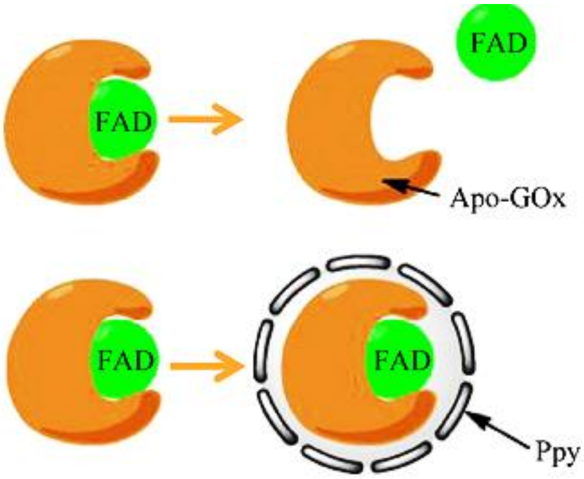

Fig. 1. Scheme of free GOx in solution and GOx/PPy nanoparticles. Reproduced with permission from Elsevier [**7**].

Note that direct immobilization of biomolecules on metal electrodes is hindered by different factors, most importantly distortion of their supramolecular structure. Therefore, to preserve the activity of an enzyme, their encapsulation with redox polymers into different matrices, is favored into various electrochemical procedures. Thus, study on encapsulation of enzyme laccase covered nanoparticles was fabricated by chemical interaction with modified magnetite nanoparticles. Subsequently, the enzyme coated magnetite nanoparticles were successfully incorporated into polypyrrole (PPy) matrix during galvanostatic electro polymerization. Therefore, a novel and efficient method for specific binding of laccase enzyme onto magnetite nanoparticles was reported [**16**]. Cesarino *et al.* aimed that immobilization of enzymes still does have questions, such as: the shortness of the enzyme lifetime on electrodes surface, and the loss of their activities when placed on electrodes with unmodified surface. In addition, there are some transport problems associated with the long paths between the sites of the biocatalyst and the electrode. In order to solve these problems, studies are focusing on different methods for the immobilization of enzymes on the electrode surface. To give such possible answer, a neurotransmitter biosensor has been developed by a simple electrocodeposition of multiwalled carbon nanotubes (MWCNT), PPy and laccase (Lac) on the platinum (Pt) electrode surface with a detection limit of 0.14µM after monitoring dopamine in urine [**13**]. Lalaoui and coworkers reported for efficient direct oxygen reduction by laccases. The formed bioelectrodes were formed by incubation of the MWCNT electrodes by poly (pyrrole-pyrene) in phosphate buffer (PB) solution containing laccase. The cyclic voltammetry results under argon and oxygen condition (**Fig. 2**) shows different response of bioelectrodes towards the bioelectrocatalytic reduction of oxygen [**17**].

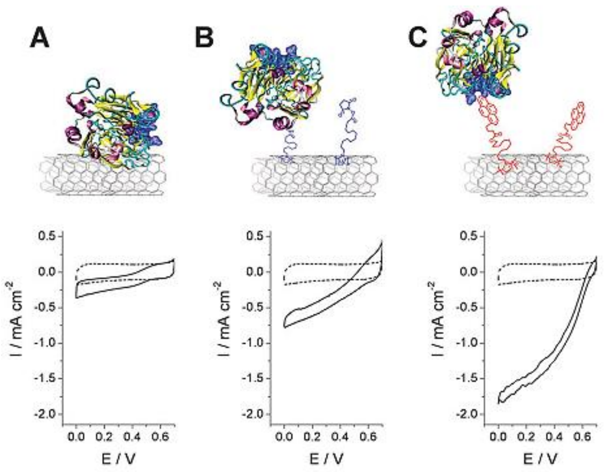

Fig. 2. Cyclic voltammograms of laccase-functionalized MWCNT electrodes under argon (dashed line) and under oxygen purging for (A) MWCNT/Lac (B) MWCNT/PPy-N-hydroxysuccinimide/Lac (C) MWCNT/poly (pyrrole-pyrene)/laccase. 0.1 M PB (pH 5.0); scan rate *v* $=0.01Vs^{-1}$. Reproduced with permission from Royal Society of Chemistry [**17**].

Electron transfer between polypyrrole-enzyme systems

The past researchers showed that bioelectrocatalysis can be either mediated or directed with in electrochemical reactions through electron transfer followed by biological catalysis. When an oxidoreductase enzyme interacts with a biological cofactor to transfer electrons between the active site and a substrate. Some of natural cofactors like FAD and others which were found to be effective in their native biological settings, have poor electrochemical properties at common electrode materials, such as carbon, gold, and platinum, and can be expensive. Therefore, to successfully transport electrons between enzymes and electrode surfaces, an intelligent polymer like PPy with natural cofactors are frequently replaced, altered, or removed completely, depending on the enzyme and the type of both biosensor and biofuel cell [**18**].

In mediated electron transfer (MET), molecules with multiple redox states are used to accept or donate electrons from the enzyme active site and to assist in transporting charge to or from the electrode surface [**18**]. For example, Hu and coworkers reported that the involvement of PPy as a mediated type bioelectrochemical sensor was developed using polypyrrole (PPy) immobilized ferricyanide (FC) as mediator and immobilized microorganism (*Pseudomonas aeruginosa (P. aeruginosa))* as a biosensing film for biochemical oxygen demand (BOD) fast detection in three-electrode system [**19**]. Shrestha *et al.* investigated that due its excellent electrocatalytic nature when polypyrrole (PPy) encapsulated with Prussian Blue (PB) composite was successfully prepared by mixing with carbon material (MWNTs), the PPy/PB/MWNTs, functionalized multiwall carbon nanotubes (*f*MWCNTs) in Nafion (Nf) doped with polypyrrole (PPy) [**20**] and Polypyrrole-Chitosan-Iron oxide (PPy/CS/Fe_3O_4) [**21**] nanocomposites shows improved electrochemical performance for glucose biosensor. Finally, system possesses enhanced electrochemical activity and good stability, which is promising for immobilizing different oxidase enzymes for other bioelectrochemical applications [**22**].

Bai and coworkers reported that laccase-mediated PPy dye wool fabric would have good electrochemical activity in terms of in situ polymerization using laccase in solution [**23**]. In this study a coloring dye methylene blue (MB) was chosen as a model for electron mediator dopant to fabricate hierarchically structured materials (HSMs) with tailored functional conducting polypyrrole micro particles (PPyMP). Subsequently, the PPyMPs were fabricated with a calcium carbonate ($CaCO_3$) template-assisted polymerization technique in aqueous media at room temperature, thus providing a new green chemistry for producing water processable functional polymers. The sacrificial $CaCO_3$ template guided the polymerization process to yield homogenous PPyMPs with a narrow size distribution. The porous nature of the $CaCO_3$ further allows the incorporation of various organic and inorganic dopants such as an electro catalyst and redox mediator for the fabrication of functional PPyMPs [**24**]. A comparative study by Cardoso *et al.* reported in Enzymatic Biofuel Cells as a biocathodes with species, namely porphyrin, ferrocene, osmium, and ruthenium complexes on immobilized carbon platforms entrapped in an electropolymerized polypyrrole matrix. Hence methanol/O_2 biofuel cell tests employing at bio cathodes showed that they provide higher output power in osmium and ruthenium complexes as compared with other systems [**25**].

On the other hand the participation of PPy in direct electron transfer (DET), presumes the absence of any freely diffusing or even immobilized mediators. More precisely, in direct bioelectrocatalysis the corresponding current-potential dependence is determined by thermodynamics of the catalyzed reaction. Based on this concept the first oxygen enzyme electrode made by immobilization of the enzyme laccase on carbon black electrode has been reported by reducing oxygen on the active site of the enzyme, and the electrons were donated from the electrode material [**12**]. More recently, Cai *et al.* developed the direct bioelectrocatalysis by glucose oxidase (GOx) using PPy nanoflake array which was synthesised by a secondary template (fir wood and ZnO microtube array) method and the result was confirmed by scanning electron microscopy and cyclic voltammetry. Compared with the traditional PPy nanoparticle electrode, the bioelectrocatalytic activation of GOx immobilised PPy nanoflake array electrode toward the oxidation of glucose has been significantly enhanced. Such instrumental result reflects an excellent properties with respect to low cost, facile preparation and good biocompatibility of the PPy nanoflake array that helps to set a bridge between proteins/enzymes and electrodes for a variety of biosensor applications [**26**].

Glucose oxidase is the most widely used enzyme in the field of biosensors because of its high specificity for a commercially important analyte, high turnover number and high stability. Hence Biosensors, prepared using glucose oxidase *via* glutaraldehyde enzyme electrode was formed by immobilization into electrochemically synthesized polypyrrole on glassy carbon electrode. The determined Michaelis-Menten constant value is significantly lower than that of free enzyme indicating enhanced enzyme efficiency when it is immobilized into electroconducting polymer matrix. This can be achieved by conductive polymers [**27**] because of a number of favorable characteristics among which is direct and easy deposition on the sensor electrode by electrochemical oxidation of the monomer. Although the application of pyrrole derivative within direct bioelectrocatalysis is common regarding to its composite. For example, study on pyrroloquinoline-quinone (PQQ) domain and a family 1-type carbohydrate-binding module direct electron transfer reaction) of pyranose dehydrogenase from *Coprinopsis cinerea* (CcPDH) was fabricated using some electron acceptors and a carbon electrode at variouspH levels [**28**].

Karyakin and coworkers investigated the frequent problem in direct bioelectrocatalysis and justify how to achieve an efficient electron exchange between the enzyme active site (its electron transport chain) and the electrode. This has two main factors i) the enzymes being macromolecules; have to be properly oriented on the electrode surface bringing their terminal redox group to the distance suitable for electron transfer. Random enzyme orientation obviously decreases the efficiency of bioelectrocatalysis, ii) electrochemical reaction of the terminal redox group has to be rather fast and occur at low overvoltages. This is not always the case of biological redox mediators. For instance, electrochemical conversions of NAD^+/NADH couple on platinum, gold and carbon electrodes require more than 1.0 V of overvoltage [**12**]. Therefore, this review summarizes how to improve efficiency of bioelectrocatalysis using electroactive/conducting polymers (PPy or its derivative) containing analogues of the enzyme substrates (usually acting as artificial donors-acceptors). Indeed, the latter having affinity to terminal redox groups of the enzymes can anchor them in an optimal orientation upon immobilization. Moreover, substrate analogues are expected to improve efficiency of the electron exchange between the enzymes either hydrogenases or cellobiose dehydrogenases electron transport chain and the electrode.

PPy embedded on novel metal platinum (Pt) can enhance DET because platinum is a well-known catalyst to design novel sensing systems and enhance the performance of bioanalytical assays due to its high surface area, nontoxicity, good biocompatibility and chemical stability, and also show fast electron communication features which provide a desirable microenvironment to enzyme for the direct electron transfer between the enzyme's active sites and the electrode. Pt nanoparticles (PtNP) on the electronically conducting polypyrrole can enhance conduction and charge transport properties compared to the conventionally synthesized polypyrrole film. The PtNP not only retain the bioactivity of the immobilized enzyme but also enhance the sensing characteristics such as sensitivity, selectivity and low detection limit of the fabricated amperometric enzymatic biosensors. Thus, PtNP based PPy multilayer nanocomposite electrode was fabricated which provides enzyme friendly environment, non-destructive measurement technique for total cholesterol, high sensitivity, reproducibility and shelf life of the biosensor for total cholesterol estimation [**29**].

Biosensors and biofuel biodevice application

The increasing industrialization and associated pollution of the environment call for fast and cost-effective analytical techniques to be used in different monitoring areas. The need for specific systems and tools for environmental monitoring and medical use has encouraged the development of new technologies and more suitable methodologies. In this circumstance, electrochemical biosensors and biofuel cells based on redox enzymes entrapment in PPy have emerged as suitable alternatives or complementary analytical tools. The technique of enzyme immobilization on PPy is an important aspect which needs to be considered when trying to enhance the overall operational performance of biosensors and biofuel cells [**30**].

There is a need for economical, simple and reliable methods to detect various analytes, because of its use in many research sectors such as food industry, biotechnology, clinics, pharmaceutical industry and environmental protection. Electrochemistry method is one of the most promising technique to analysis those analytes than other conventional methods such as spectrophotometry and chemiluminescence because of their low detection limit, high selectivity and high sensitivity [**11**]. Different methods of enzyme immobilization were tested and applied with GOx for constructing biosensors. The most important characteristics of *in vivo* application of the biosensors are stability and biocompatibility. In biosensor design PPy is the most exploited immobilization matrix for enzyme, such as GOx [**7, 15**], laccase [**31**] and fructose dehydrogenase [**30, 31**] that provides good stability and activity for suitable linear range practical application results.

German *et al.* reported that practical advantage of different nanomaterials for enhancing the electron transfer rate between the active centre of enzymes and electrodes, controlling size and surface morphology experimentally by adjusting the preparation conditions and helps to increase the efficiency of enzyme immobilization. To exploit this unique property an electrochemical biosensors based on gold nanoparticles were designed and applied for glucose detection. During enzymatic reaction electrons are transferred towards the positively charged electrode and steady state currents are registered. The reactions and electron transfer routes, which take place because of the catalytic action of GOx immobilised on bare carbon rod electrode (CR) modified with gold nanoparticles (GOx/Au-NPs/CR) electrodes. N-methylphenazonium methyl sulphate (PMS) serves as a redox mediator and re-oxidases the active site of GOx. In this case electron transfer via a reduced form of mediator ($PMSH_2$) may occur in two ways: directly to the CR electrode (I way) or in combination of $PMSH_2$ with Au-NPs (II way). GOx immobilised on the electrode in the presence of glucose and dissolved oxygen generated hydrogen peroxide and gluconolactone, which was hydrolyzed to gluconic acid. During this reaction the pH locally decreased close to the GOx active site while hydrogen peroxide concentration locally increased. Therefore, low pH value and high concentration of oxidisor near the enzyme created optimal conditions for the polymerisation of PPy that covers GOx [**32**].

Polypyrrole coated gold nanoparticles research has been extended based on immobilized enzymes due to its attractive attention because of their extraordinary selectivity. Enzymatic formation of PPy on the surface of graphite rod (GR) modified with electrochemically deposited gold nanoparticles (Au NPs) followed by adsorbed GOx. The polymer in this bio-nanocomposite (PPy/GOx/AuNPs/GR) electrode was applied in order to improve analytical characteristics and stability of developed biosensor. Hence, the developed biosensor is suitable for the determination of glucose in human serum samples [**33**]. Another study using gold nanoparticles electropolymerized with PPy was fabricated as a bioanode by immobilizing horseradish peroxidase (HRP) on PPy polymer matrices for best detection [**34, 35**]. Li *et al.* reported that this particular polymeric device also focused on novel polypyrrole nanotubes (PPy-NTs) derived from methyl orange (MO) was used as matrix to construct a highly responsive phenol bio electrode on polyphenol oxidase (PPO) via the cross-linking in the presence of glutaraldehyde on glassy carbon electrode surface [**36**].

Dervisevic and coworkers reported that the role of PPy to glucose biosensor for the design of chitosan/polypyrrole/gold nanoparticles hybrid bio nanocomposite platform was fabricated by an *in situ* chemical synthesis method on a glassy carbon electrode surface to enhance the electron transfer and to provide good enzyme affinity. Electrochemical studies carried out that the fabricated biosensor exhibited for significant added value from uric acid, ascorbic acid, glucose, and sodium benzoate act as a testing model. Hence, it is interesting that the resulting bio-nanocomposite xanthine biosensor was tested with fish, beef, and chicken real-sample measurements. Xanthine oxidase (XO) is widely distributed in mammalian tissue and it is a key enzyme in purine metabolism, which makes it an ideal biomolecule candidate for the design of amperometric biosensors. From amperometric detection of xanthine, it requires no complex sample preparation and provides fast analysis with lower cost through the electrochemical reaction indicated with the basic working principle [**37**].

On the other way, Tao *et al.* investigated a simple, cheap, highly sensitive and selective electrode for H_2O_2 detection has been developed using colorimetric method and has been used in buffer solution or even in living cells, which will expand the applications of PPy in medical diagnostics and biotechnology [**5**]. In addition, Samanta and coworkers reported the development of a generalized pH-sensitive drug delivery system can release any charged drug preferentially at the pH range of interest. Based on polypyrrole nanoparticles, synthesized *via* a simple one-step microemulsion technique. These nanoparticles are highly monodisperse, stable in solution over the period of a month, and have good drug loading capacity (~15 wt%). Result showed that PPy NPs can be tuned to release drugs at both acidic and basic pH by varying the pH, the charge of the drug, as well as by adding small amounts of charged amphiphiles. Moreover, these NPs may be delivered locally by immobilizing them in a hydrogel. Encapsulation within a calcium alginate hydrogel results in sustained release of the incorporated drug for more than 21 days. Such a nanoparticle hydrogel composite drug delivery system is promising for treatment of long-lasting conditions such as cancer and chronic pain which require controlled, localized, and sustained drug release [**8**].

Jaworska *et al.* developed a novel template less procedure-oxidation of pyrrole released either from poly (n-butyl acrylate) microspheres or from micelles of cross-linked alternating polymer (poly (maleic anhydride-alt-1-octadecene)) on a simple model step using chemical polymerization. The detailed electrochemical properties of PPy nanoparticles coated on glassy carbon electrodes were studied using voltammetric measurements. The result confirmed that high ion transport rate inside the polymer and minor influence of the kind of doping ion and electrolyte ions present in the solution. However, electrochemical impedance spectroscopy (EIS) measurements revealed a significant difference in the properties of PPy nanoparticles, depending on the synthesis conditions. In case of PPy synthesized in the presence of poly (n-butyl acrylate) microspheres EIS spectra were almost independent of kind of electrolyte ions and doping anion, pointing to exposed bulk capacitive properties. On the other hand, the obtained nanoparticles in the presence of alternating polymer micelles represent a typical constant phase element behavior with impedance significantly dependent on the kind of oxidizing (doping) anion. Due to their capacitive properties PPy nanoparticles obtained in the presence of poly (n-butyl acrylate) microspheres were applied as solid contacts in ion-selective electrodes. As shown in **Fig. 3**, in course of oxidation/reduction of the polymer ions entering the polymer have to transfer into the polymer nanoparticle. Due to typical size of nanoparticles the ions cover only the distance comparable with the nanoparticle diameter, in the range of 40 nm [**38**].

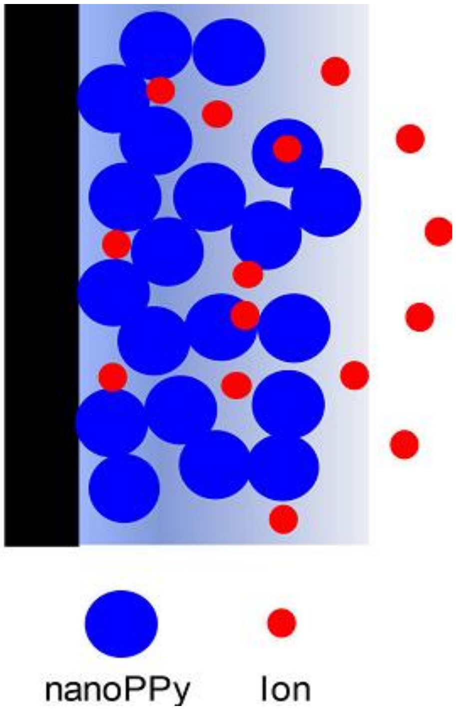

Fig. 3. Schematic of nano PPy layer on a GC electrode, big circles: PPy nanoparticles, small circles: electrolyte ions. Reproduced with permission from Wiley [**38**].

Decorating of PPy thin films by copper nanoparticles for glucose detection with lower detection limit of 3 µM for diabetic patients and helps to evade the clustering of blood was synthesized via, electrodeposition technique on stainless steel substrate. The electroactivity of PPy/copper oxide/stainless steel electrode was explored using cyclic voltammetric study and chronoamperometry under pH=7±5. The PPy/copper oxide sensor exhibited remarkable stability and reproducibility with good nonenzymatic current sensitivity [**39**]. However, the sensitivity efficiency of PPy in silver doped nickel oxide (Ag NiO) nanocomposites can offer the benefits of both doping as well as the best nanoparticle dispersion resulting from in situ polymerization technique. The formed nanocomposite was applicable for detecting gaseous ammonia [**40**]. A novel strategy to improve the sensitivity of antibiotics determination based on bioelectrocatalysis at molecularly imprinted polymer film on the surface of pyrolytic graphite (PG) electrodes, with pyrrole serving as the monomer and kanamycin as the template was developed by Lian and coworkers [**41**]. Interestingly, Ermiş and Tinkiliç developed a new sensing device based on molecularly imprinted technology at gold electrode for selective and sensitive detection for the determination of tyrosine (Tyr) in Biological Samples [**42**].

Bioelectronic device fabrication has been continued using electrospinning technique, which is one of the most straightforward and cost-effective methods for producing large surface area and high porosity nanofibers. According to the study, the involvement of PPy in this activity shows favorable thermal stability, high electrical conductivity and ingenious redox properties as well as help the environment to become more stable. In this system the contribution of polycaprolactone (PCL) [**43**] is as mixing species that enforces the mechanical property, in addition to its great biocompatibility and slow biodegradability during the electrospinning process. Therefore, the CuO/PCL/ PPy fibers were directly collected on the surface of indium-tin oxide (ITO) glass to thus form a nonenzymatic glucose sensor. Under the alkaline conditions of 0.01 M NaOH, the CuO/PCL/PPy/ITO-based sensor with its unique three-dimensional net structure presented reliable selectivity, good sensitivity, and reproducibility and stability to detect glucose in saliva [**44**].

On the other ways bioelectronics for efficient power generating devices based on enzymatic catalysis, known as biofuel cell is a rapidly developing research area today but one of the significant difficulties that overcome in this system is the rapid voltage drop which often seen when turning the powered devices on. Thus, biofuel cells have received significant attention in the last few decades due to its potential application as alternative energy sources and advantages over conventional fuel cells [**45**]. Slaughter and Kulkarni reviewed that polypyrrole as a good porous matrix in biofuel cell comprising composite bio electrode using Laccase *Trametes versicolor* and fructose dehydrogenase immobilized and adsorbed on naphthylated multi walled carbon nanotubes as the cathode and anode bioelectrocatalyst, respectively. The nanomaterials employed as the supports for the enzymes increased the surface area of the electrodes and provide direct contact with the active sites of the enzymes. The anode bioelectrode modified with conducting polymer layer exhibited significant pseudocapacitive properties providing superior performance with higher in energy mode during switching on/off powered device. In this device system three air-fructose biofuel cells connected in a series which was converted chemical energy into electrical giving 2 mW power and open circuit potential with 2 V [**30**].

Composites of noble metals and conductive polymers have a significant role for the construction of electrochemical and biofuel cells. For example, Ilčíková *et al.* reported that the utilization of gold (Au) in PPy composite was found to have beneficial effect on the electrochemical activity of electrodes enhanced electron transfer applied for construction of device. Due to the good electron conductivity, high surface area and biocompatibility, PPy modified interfaces could be used for fabrication of electrochemical biofuel cells (BFCs) as a bioanode electrode. The novel composites of gold nanoparticles and polypyrrole nanotubes (Au_x/PNT) were prepared and used as a platform for fabrication of bioelectrode interfaces in chitosan matrix with physisorbed oxidoreductases such as fructose dehydrogenase (FDH), laccase (Lac) and bilirubin oxidase (BOD). So, biocatalytic current density up to j = 2.45 mA cm^{-2} obtained for a bioanode based on direct electron transfer of FDH. Performance of biocathodes with immobilized Lac and BOD showed current density up to 232 µA cm^{-2} in the presence of a redox mediator [**31**]. In practice one of the most problems as a disadvantage to enzyme-based biofuel cells are low power density and a short lifetime. A number of strategies have been investigated to overcome these problems. For example, the stability of the enzymes and the efficiency of the biofuel cells are found to be enhanced by immobilization. Thus, glucose/O_2 biofuel cells with an improved power density were developed, using PPy nanowires containing glucose oxidase and 8-hydroxyquinoline-5-sulfonic acid hydrate as an anode. The PPy nanowire anode was made by electropolymerizing within the nanopores of an anodized aluminum oxide (AAO) template, and then dissolving the AAO template. The nanowire-type biofuel cell exhibited a higher power density than the film type biofuel cell by two orders of magnitude; this was likely due to an increase in surface area and enzyme loading. From the assembled biofuel cell was dipped in a 50 ml cell of solution containing 15 mM glucose in phosphate buffer saline (PBS) at 37 ºC (see **Fig. 4**) [**46**].

Periasamy *et al.* reported the high catalytic activity of PPy in microbial fuel cells (MFCs) device system. When Cu_2O/PPy composites coated linen texture (LT) papers possess enzyme activities of glucose oxidase (GO_x) and horseradish peroxidase (HRP) in alkaline solution to catalyze glucose in the presence of O_2 to form H_2O_2 and also showed enzyme mimic activity of horseradish peroxidase (HRP) to reduce as produced H_2O_2 at 0 V and -0.57 V vs. Ag/AgCl reference electrode, respectively [**47**]. Hence a good bioelectrocatalytic system, conducting polymer coated with stainless-steel wool (SS-W) was proposed for use as a low-cost anode for MFCs. In detail when polypyrrole (PPy) coated with polyaniline (PANi) the pristine SS-W, SS/PANi-W and SS/PPy-W anodes produced maximum current densities of 0.30 ± 0.04, 0.67 ± 0.05,0.56 ± 0.07 mA cm^{-2}, respectively, in air-cathode MFCs. Also, based on achieved power density, both SS/PANi-W and SS/PPy-W achieved 0.288 ± 0.036 mW cm^{-2} and 0.187 ± 0.017 mW cm^{-2}, respectively, which were superior to 0.127 ± 0.011 mW cm^{-2} obtained with pristine SS-W. Further, in comparison with SS-P based anodes, all SS-W based anodes gave improved power densities under similar experimental conditions by at least 70%. As a result, the observed enhancements of the bioelectrocatalytic performances in the prescence of redox polymers were well supported by physicochemical and electrochemical characterization [**48**].

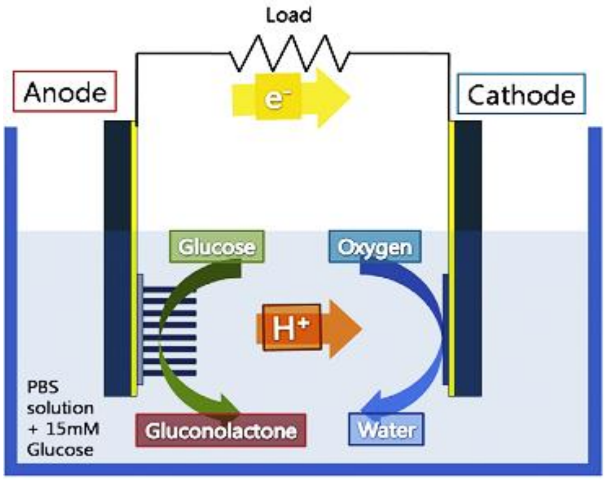

Fig. 4. Schematic illustration of glucose/O_2 biofuel cell. Reproduced with permission from Elsevier [**46**].

Currently a microbial fuel cell (MFC) is a noble green technology generating electricity from biomass and is expected to find applications in a real world. It is explained from the above thoughts; one of main hurdles to this purpose is the low power density. Thus, anodic modification with electropolymerized polypyrrole (PPy) has a dramatic power enhancement resulted from the PPy deposition onto the reticulated vitreous carbon (RVC) electrode. From the study a prototype MFC was constructed using *Proteus vulgaris* with maximum power density of 1.2 mW cm^{-3}. Accordingly, further power enhancement was possible by increasing the ionic strength of the solution to decrease internal resistance of the cell [**49**]. On the same year however, its low power density is a main limitation to be used as a new energy source. To overcome this limitation, the anode improvement in a mediator type MFC was focused using *Proteus vulgaris* as a biocatalyst. Fuel cell performance increased when the anode was coated with carbon black or polypyrrole. As a result, best performance was observed when PPy/carbon black (PPy/CB) composite material was coated on a carbon paper electrode. The obtained power density was 452 mW m^{-2} with in the similar system [**50**]. Later nanostructured polyurethane/graphite/polypyrrole composites were polymerized by chemical oxidization, which were used for anaerobic microorganisms grown and tested as anodes in MFC using municipal wastewater as fuel. The highest output power density in these cells was 305.5 mW/m^3 in the $FeCl_3$ oxidant system [**51**].

Conclusion and future perspectives

An Intelligent conducting polymer and their nanocomposites have several bioelectronics applications such as in biosensors and biofuel cells. This is due to their fast electron transfer characteristics from delocalization of pi-electrons in a conjugated system. Hence new advances in polypyrrole have been increased inherently among other conducting/or redox polymers due to its significant morphological and structural property. Novel immobilization of enzyme using intelligent polymer have been increased many times from the conventional prospective enzyme alone. Activity, reusability and sustainability of enzyme can also be enhanced using redox polymer as a conducting nanofiber as immobilization matrix and has also extended to improve the biocompatibility of electrode (metallic) surfaces in implant material for biomedical application due to its simple preparation procedure, chemical inertness, mechanical stability and limited permeability.

The applications of PPy have emerged as suitable complementary analytical tools that have an advancement implication in biosensor and biofuel cells biofunctional system. When the polymer is entrapped with other selective novel metals, conjugate it with other redox/smart polymers and carbonic nanomaterials as a composite contribute for enhancing electron transfer rate between the active centre of enzymes and electrodes, controlling size and surface morphology experimentally by adjusting the preparation conditions and helps to increase the efficiency of enzyme immobilization. Still low power density and a short life time in enzyme-based biofuel cell as well as denaturation of enzyme immobilization to a particular electrode in practice is one of the most problems as a disadvantage. Therefore, further research into these strategies as well as creating a stable biofunctional bioelectrode should yield more efficient to the near future and that will greatly improve the overall power generation and analyte detection performance.

Acknowledgements

The authors greatfully acknowledge Adama Science and Technology University for finanical suppport.

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