Personal Account

THE CHEMICAL RECORD

DOI: 10.1002/tcr.201700101

Recent Progress in the Development of Conducting Polymer-Based Nanocomposites for Electrochemical Biosensors Applications: A Mini-Review

Maryam Naseri,[a] Lida Fotouhi,*[a] and Ali Ehsani*[b]

Abstract: Among various immobilizing materials, conductive polymer-based nanocomposites have been widely applied to fabricate the biosensors, because of their outstanding properties such as excellent electrocatalytic activity, high conductivity, and strong adsorptive ability compared to conventional conductive polymers. Electrochemical biosensors have played a significant role in delivering the diagnostic information and therapy monitoring in a rapid, simple, and low cost portable device. This paper reviews the recent developments in conductive polymer-based nanocomposites and their applications in electrochemical biosensors. The article starts with a general and concise comparison between the properties of conducting polymers and conducting polymer nanocomposites. Next, the current applications of conductive polymer-based nanocomposites of some important conducting polymers such as PANI, PPy, and PEDOT in enzymatic and nonenzymatic electrochemical biosensors are overviewed. This review article covers an 8-year period beginning in 2010.

Keywords: Conductive polymer, electrochemical characterization, biosensor, electron transfer

1. Introduction

1.1. Conducting Polymers and Conducting Polymer-Based Nanocomposites

Conducting polymers (CPs) are novel materials which attracted much interest due to their combined properties of organic polymers and electronic properties of semiconductors. The electrical conductivity of conjugated polymer has been reported for the first time in 1977 by Shirakawa et al.[1] Since then, CPs have been largely explored thanks to their unique electrical characteristics. CPs exert charge mobility along their π-electron backbones which is responsible for their unusual electronic properties such as low energy optical transitions, electrical conductivity, mechanical flexibility, high surface areas, and high electron affinity. Typical molecular structures of some CPs are summarized in Figure 1. Conductivity of the polymer is influenced by a number of factors including conjugation length, overall chain length, and charge transfer to adjacent molecules. They have been applied in electrocatalysis,[2] rechargeable batteries,[3] supper capacitors,[4] membrane separation,[5] optoelectronic,[6] extraction,[7] solar cells[8] and electrochemical biosensors.[9] CPs have also been used in design of molecularly imprinted polymers (MIP) and ion selective electrodes (ISE). The applications of CPs in design of MIPs are quite attractive due to their merits such as good control of film thickness, ease of fabrication and signal transduction. In this way, Ramanavicius et al.[10] have applied a molecularly imprinted polypyrrole (PPy) as sensitive impedimetric sensor for determination of theophylline. They investigated the impact of polymerization duration, the ratio of monomer, and template molecule on the capacitance of impedimetric sensor. Luo et al.[11] have prepared a water-dispersible molecular imprinted conductive polyaniline nanoparticles (PANI NPs) using polymeric micelle as nanoreactor and introduced an electrochemical sensor for detecting paracetamol, using the obtained molecular imprinted polyaniline nanoparticles as recognition element. They have demon-

[a] *M. Naseri, L. Fotouhi*
Department of Chemistry, Faculty of Physics & Chemistry, Alzahra University, Tehran, Iran
E-mail: lfotouhi@alzahra.ac.ir
[b] *A. Ehsani*
Department of Chemistry, Faculty of Science, University of Qom, Qom, Iran
E-mail: ehsani46847@yahoo.com
a.ehsani@qom.ac.ir

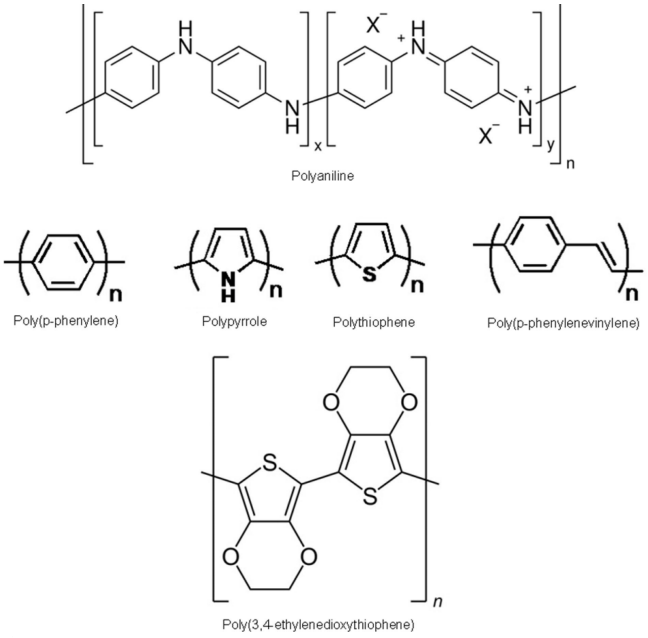

Figure 1. The chemical structures of typical conducting polymers.

strated that using polymeric micelle as nanoreactor offers an effective approach to overcome the problem of imprinting in aqueous system, whereas the electrically conductive PANI NPs, as the imprinting matrix, provides a path for the conduction of electrons from the imprinting sites to the electrode surface, and the imprinted PANI NPs assist large specific surface area owing to its nano-size, which could accommodate more effective recognition sites. Recently, Dutra et al.[12] have described a human cardiac troponin T graphene screen-printed electrode (SPE) based on electropolymerized-molecularly imprinted conducting polymer. They have reported that the use of CPs provides a contributing factor to improve the performance. Recently, much attention has been paid to conducting polymer-based ion-selective electrodes for the reason that CPs offer high stability of the signal and the possibility to externally adjust and control the redox state. For example, Ramanavicius et al.[13] have described a method for electrochemical modification of glassy carbon electrode (GCE) by poly-4-nitroaniline and application of reduced form of poly-4-nitroaniline for determination of Cu(II) concentration. They have proved that the reduced form of poly-4-nitroaniline displayed an attractive ability to efficiently accumulate trace of Cu(II) ions from water-based solution, offering a simple and reproducible way for determination of Cu(II) ions. In another work, Gyurcsanyi et al.[14] have applyed poly (3,4 ethylenedioxythiophene)/poly (styrenesulfonate) (PEDOT/PSS) films with 750 nm diameter interconnected pores as the intermediate layer between a GCE and a Ag^{+}-selective polymeric membrane. They have reported that the whole process from template deposition to the ready-to-use 3D polymer could be done within 3 to 5 h depending on the film thickness through the excellent control of the electropolymerization process by the amount of injected charge and the scalability of the layered structure. Recently, Bobacka et al.[15] have investigated a novel approach to signal transduction concerning solid-contact ion-selective electrodes with PEDOT as the solid contact. They have confirmed that the measured signal is amplified by increasing the thickness of the CP solid contact.

There are two common methods for the synthesis of CPs: electrochemical and chemical synthesis.[16] In traditional chemical synthesis, CPs are formed via oxidative synthesis under harsh oxidative conditions,[17] mild conditions where redox enzymes are applied,[18] or ionic-liquid/air interface.[19] For example, Ramanavicius et al.[20] have developed an environmentally friendly route to fabricate pure PPy NPs. They proposed a mechanism for PPy NPs formation by spectrophotometric measurements and atomic force microscopy (AFM) imaging, in which they use sodium dodecyl sulfate (SDS) and hydrogen peroxide as oxidants. The electrochemical synthesis has rapidly become the preferred method for synthesizing CPs due to simplicity and reproducibility. Electropolymerization is normally carried out in a single cell compartment where a three-electrode configuration is employed in an electrochemical solution consisting of a monomer and a supporting electrolyte. The polymerization can be carried out either potentiostatically where the potential is kept constant with a variation of the current with time, or galvanostatically by keeping the current constant thereby monitoring the electrode potential. The thickness and characteristics of the film can be easily controlled by varying either the potential or the current with time. Among galvanostatic, potentiostatic, pulse, and sweeping techniques which are employed in the polymerization of CPs, potentiodynamic techniques are more preferred because of their homogenous film production and strong adherence of the film on the electrode surface.

The field of the conducting polymer-based nanocomposites has expanded because of their potential use in various fields resulted from their high electrical conductivity, high electrocatalytic properties, and high chemical stability in aqueous solutions.[21] Figure 2 presents the number of papers published in the past few years on the conducting polymer-based nanocomposites. It has been shown that CPs could be useful matrices for the immobilization of NPs due to the porous structure of CP. Thus, the conducting polymer-based nanocomposite materials can be formed by incorporation of NPs into CPs. Furthermore, charge transfer between the substrate and the dispersed NPs is facile in the matrix of a CP and it results in improvement of conductivity of the hybrid

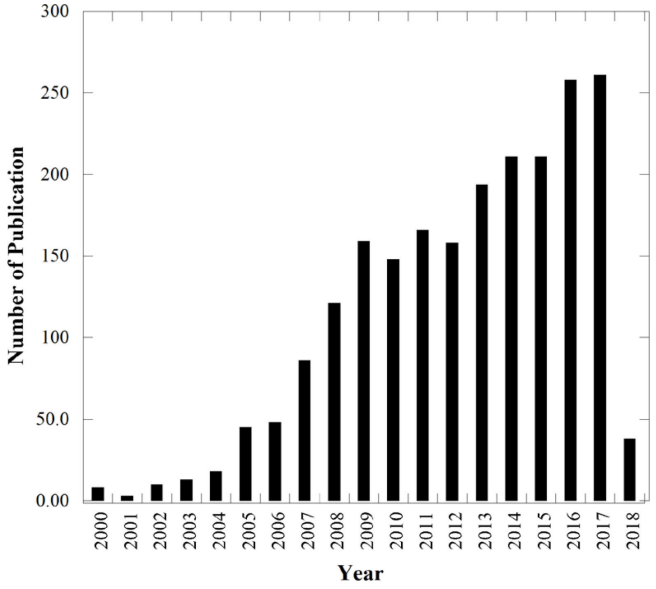

Figure 2. Numbers of papers published in the past few years on the conducting polymer-based nanocomposites, Data were obtained from Scopus-Analyse using the search input "conductive polymer nanocomposites".

systems. Moreover, the incorporation of NPs into CPs provides enhanced performance for both the CP and the NPs, and this can lead to different physical properties in compared with the constituent polymer and introduced NP. It is reported that the inherent stability and symbiosis between the two components used to prepare the nanocomposite material is often superior to the bulk components alone and combining CPs with NPs could be a useful approach to overcome some of the physical and chemical limitations, including the processability and mechanical and thermochemical stability of CPs.[22]

Nowadays, various kinds of NPs such as metals, metal oxides, and rare earth metal oxides have been employed to improve performance of CPs. Silva et al.[23] have introduced a one-step electrochemical deposition procedure to achieve an in-situ grown 3D-ternary composite consisting of polymerized PPy, electrochemically reduced graphene oxide (rGO), and nanoparticles of ZrO_2. They have reported that the incorporation of rGO and zirconia nanoparticles in the PPy matrix changed the morphological structure and improved the porosity of the active material, consequently increasing capacitance values by up to 100 %. Liu et al.[24] have applied electro-deposited Ag nanoparticles on the cellulose/PANI conductive hydrogels to obtain high capacitive performances. They argued that the loading of Ag nanoparticles had an obvious positive influence on the electrical conductivity and specific capacitance of the aerogels. Choudhary et al.[25] have reported an in situ synthesis approach to incorporate Eu_2O_3 into the polypyrrole matrix and investigated its electrochemical performance as supercapacitor electrode material. They have claimed that the incorporation of Eu_2O_3 markedly improved the pseudocapacitance, stability, porosity, and packing density of the PPy matrix. Kumar et al.[26] have introduced an approach based on electrochemical polymerization to synthesize PPy/CeO_2 nanocomposite on an AA2024 alloy surface. They have described that the loading of CeO_2 NPs enhanced the thermal stability and corrosion protection of the PPy matrix. In this way, Ramanaviciene et al.[27] have applied enzymatic polymerisation of PPy to increase linear detection range of glucose biosensors based on glucose oxidase immobilised on carbon rod electrode modified with gold NPs. They have also investigated the effect of the formed PPy layer on Michaelis-Menten kinetics, sensitivity, and linear glucose detection interval of biosensors.

1.2. Electrochemical Biosensors

Interest in the development and exploitation of analytical devices for quantification, detection, and monitoring of specific chemical species and reactions has led to the emergence of sensors. Sensors are the analytical device that consisted of an active sensing material with a signal transducer. Generally, they can be classified into two categories as chemical sensors and biosensors. This classification is done based on the terms of sensing aspects, where the biosensors can sense biochemical compounds such as enzymes, antibodies, nucleic acids, cells, and tissues.[28] Biosensors typically consist of two intimately associated elements: a bioreceptor and a transducer. They can be classified to five classes depending on the transducing mechanism: (i) resonant biosensors, (ii) optical-detection biosensors, (iii) thermal-detection biosensors, (iv) ion-sensitive field-effect transistor-based biosensors, and (v) electrochemical biosensors.[29] In the case of electrochemical biosensors, the signal detection occurs at the electrode/solution interface, which can be dynamic or static. These biosensors possess advantages over the others because they can detect materials without damaging the system. Clark and Lyons reported the first electrochemical biosensor by immobilization glucose oxidase (GOx) on the surface of an amperometric oxygen electrode via a semi-permeable dialysis membrane in order to directly quantify glucose concentration in a sample.[30] Since then, numerous electrochemical biosensors have been developed for determining various substances such as glucose,[31] cholesterol,[32] dopamine,[33] hydrazine,[34] hydrogen peroxide,[35] kanamycin,[36] and cysteine.[37]

Electrochemical biosensors can be divided into three generations as follows: (i) in the first generation, the product of reaction diffuses to the transducer and causes the electrical response. (ii) In the second generation, specific 'mediators'

between the reaction and the transducer are used in order to generate improved response. These biosensors involve two steps: first, a redox reaction between enzyme and substrate is reoxidized by the mediator, and in the second step the mediator is oxidized by the electrode. (iii) In third generation, the redox reaction itself causes the response without diffusion of product or mediator. The self-contained natures of these sensors facilitate the repeated measurements. The only disadvantage of electrochemical biosensors is the sluggish electron transfer of the biomolecules which limits electrochemical efficiencies of the sensors. Therefore, the development of an efficient, easy-to-use, and highly selective electrochemical biosensor has always been a challenge to the scientists.

The application of CPs in the bioanalytical sciences is of great interest by virtue of their compatibility which raises the possibility of using them in vivo biosensor application.[38] In biosensor application, CPs offer the most distinguishing features such as (i) tunable sensitivities by adjusting the synthetic variables like incorporated counter ions and polymerization temperature and (ii) fabrication of sensor array via electrochemical deposition or solution casting.[39] Despite of the advantages of conducting polymer biosensors, there are also some limitations for their practical applications. The stability and properties of conducting polymers may be affected by the ambient media like oxygen, acids, bases, and redox reactants.[40] Moreover, the physical properties of CPs could be a limitation for certain applications and must be considered when choosing which polymer to be employed. For example, it is necessary to maintain the conductivity of CP at pH values higher than 4 in bioelectrochemical applications. Although the conductivity of CPs such as polythiophene or polypyrrole demonstrates very little pH dependence, the conductivity of PANI is reduced as the pH is increased, leading to a decrease in proton doping. Furthermore, it is important to consider the effect of counterion incorporation into the polymer as a dopant during polymer synthesis on the pH stability of the CP.[22]

The conducting polymer-based nanocomposites have the ability to efficiently transfer electric charge produced by the biochemical reactions to electronic circuit. This unique property has been widely exploited for fabrication of biosensors. Keeping this in view, we have made efforts to present an updated review on the applications of the conducting polymer-based nanocomposites in the field of electrochemical biosensors. The presented work contains two main sections that introduced conducing polymer nanocomposites-based enzymatic and non-enzymatic electrodes.

2. Conducing Polymer Nanocomposites-Based Enzymatic Electrodes

Enzyme based biosensors have several problems relating to functioning of enzyme system such as loss of enzyme (especially expensive enzymes), maintenance of enzyme stability, and shelf life of the biosensors. The Immobilization of enzymes have been developed to overcome these problems and the procedure of biomolecule immobilization is a key step for the performance of the resulting electrochemical devices.[41] Immobilization of biomolecules can be carried out using many different procedures, such as adsorption, covalence, entrapment, cross-linking, or affinity. In order to assure greater sensitivity and stability of biosensors, intensive efforts have been carried out to develop successful immobilization strategies. The use of nanocomposites for the design of biosensing devices constitutes an exciting approach to improve the performance of detection platforms. They can increase the electrochemical reactivity of biomolecules and can promote the electron-transfer reactions of proteins. Table 1 summarizes recent studies on the application of conducting polymer-based nanocomposites in enzyme biosensors.

Gangopadhyay et al.[42] have introduced Au nanoparticles decorated polyaniline nanowires as a biosensing platform for immobilization of different biomolecules (GOx, ssDNA (single stranded DNA) and Lamin A antibody) for sensing of three respective analytes (glucose, complementary DNA strand and Lamin A protein). They immobilized biomolecules by covalent attachment of them on PANI nanowire decorated with gold nanoparticles. Au NPs on the surface of PANI nanowires have been functionalized with NH_2 functional group. Then glucose oxidase and Lamin A antibody have been covalently attached to the electrode surface by bonding with NH_2 moiety on AuNP. In contrast, the ssDNA modified with 5′-SH group was directly attached to the Au NPs without crosslinker because of the strong affinity of thiol group to Au NPs. Similarly, Ramanavicius and co-workers[43] have developed an enzymatic synthesized method for synthesis of two nanocomposite structures GOx/PANI and GOx/Au-NPs/PANI. According to Ramanavicius et al., the gold nanoparticles not only enhance the GOx activity and positively affect PANI formation, but they also may have the properties of an electron transfer agent, biomolecule protective agent or catalytic properties in an electrochemical reaction. Ramanavicius's team have evaluated the Michaelis constants of developed biosensors and illustrated that GOx/PANI and GOx/Au-NPs/PANI nanocomposites retained the catalytic activity of glucose oxidase and were suitable for the design of amperometric biosensors.

Another glucose enzyme biosensor has been fabricated by Pan's group[44] based on the PtNP/PANI hydrogel hetero-

Table 1. Several conducting polymer-based nanocomposites for enzymatic biosensors.

Nanocomposite	Enzyme	Immobilization method	Analyte	Type of Transducer	Detection limit (μM)	Linear range (μM)	Ref.
Au-PANI	glucose oxidase	covalent attachment	glucose	Chronoamperometric	1.0	$1.0–1.0\times10^3$	[42]
PtNP-PANI	glucose oxidase	adsorption	glucose	Chronoamperometric	0.7	1.0×10^3–2.0×10^4 0.1×10^2–0.8×10^2	[44]
GOx/Au-NPs/PANI	glucose oxidase	entrapment	glucose	Chronoamperometric	–	–	[43]
Pt/graphene/P(1,5-DAN)	β-galactosidase and glucose oxidase	cross-linking	lactose	Cyclic voltammetry	1.3*	0–60*	[45]
MWNT-PANI	glucose oxidase	covalent and adsorption effect	glucose	Chronoamperometric	1.0	$3.0–8.2\times10^3$	[46]
pTBA/AuZnO$_x$	glucose oxidase	chemical cross-linking	glucose	Potentiometric	1.7×10^4*	3.0×10^4–5.0×10^5*	[47]
AuNP/SNS-NH$_2$	glucose oxidase	covalent linking	glucose	Flow injection analysis	2.1×10^2	1.0×10^3–7.0×10^3	[48]
AuNPs-AgCl/PANI/gelatin	pyranose oxidase	entrapment	glucose	Chronoamperometric	–	0.5×10^2–7.5×10^2	[49]
ZnONRs/PPy	glutamate oxidase	adsorption	l-glutamate	Chronoamperometric	1.8×10^{-4}	0.2×10^{-1}–5.0×10^2	[50]

* μg/ml

structures. The fast response of the sensor was attributed to the 3D porous nanostructures that facilitated the diffusion of glucose and H_2O_2. The high sensitivity resulted from the 3D porous hydrogel matrix, which allowed for the immobilization of the enzyme and preserved the high activity of the enzyme and the catalytic effects of the Pt NPs for the oxidation of H_2O_2. Pt NPs played an important role as catalysts that enhanced the current output and the sensitivity of the glucose sensor. The diffusion distance of H_2O_2 molecules was greatly reduced when the high-density Pt NPs were homogeneously dispersed onto the surface of the 3D PANI hydrogel (Figure 3). Nguyen's team[45] has presented a lactose biosensor by co-immobilizing β-galactosidase (β-Gal) and glucose oxidase on microelectrodes pre-modified with Pt/graphene/ polydiaminonaphthalene (P(1,5-DAN)). P(1,5-DAN) has been employed due to its compatibility and conducting properties which facilitate the binding of biomolecules or shorten the distance between the electrode and enzyme active sites. Glutaraldehyde was served as a cross-linker for enzyme binding. The immobilization of enzymes was conducted by dispersing one drop of the β-galactosidase (β-Gal) and glucose oxidase mixture onto the surface of the Pt/graphene/P(1,5-DAN) electrode. Furthermore, the authors reported the reactions involved in electrochemical determination of lactose as follows:

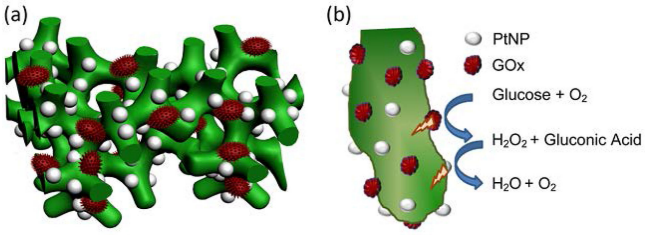

Figure 3. (a) Schematic representation of the 3D heterostructure of the PtNP/PANI hydrogel, in which the PANI hydrogel acts as a matrix for the immobilization of the GOx enzyme and homogeneous loading of PtNPs. (b) A 2D scheme showing the reaction mechanism of the glucose sensor based on the PtNP/PANI hydrogel heterostructure. Reprinted from[44] with permission from ACS Publications.

$$\text{Lactose} \xrightarrow[\beta-\text{galactosidase}]{H_2O} \text{Glocose} + \text{Galactose} \quad (1)$$

$$\text{Glucose} + O_2 \xrightarrow{GOx} \text{Gluconic acid} + H_2O_2 \quad (2)$$

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- \quad (3)$$

Recently, composite materials based on integration of carbon nanotube (CNT) and conducting polymers have gained growing interest owing to the properties of the individual components with a synergistic effect. In this respect, a glucose biosensor was constructed based on MWNT-PANI nanocomposite (MWNT = multiwall nanotube) films by Yuan et al.[46] Firstly, the MWNT-PANI nanocomposites which were formed during a chemically oxidative process, were immobilized on a GCE surface to

obtain conductive, redox, and porous nanostructured film. Then, Pt NPs were electrodeposited on the nanocomposites. Finally, GOx was adsorbed on the surface of Pt/MWNT-PANI film via covalent interaction with glutaraldehyde and the adsorption effect of Pt NPs. The GOD/Pt/MWNT-PANI/GC electrodes have indicated the best response to the addition of glucose concentration under the optimized conditions, because of the synergistic catalytic activity between MWNT-PANI nanocomposites and Pt nanoparticles.

The modification of conducting polymer structures with functional groups of –COOH and $–NH_2$ is received a great deal of attention for use it in electrochemical sensing substrates, because various molecules such as protein, enzyme, and DNA can be stably attached to the polymer layer through the formation of amide bond. In this regard, Shim et al.[47] have electrosynthesized poly (terthiophene benzoic acid) (pTBA) layered-AuZn alloy oxide (AuZnOx) on the screen printed carbon electrode (pTBA/AuZnOx/SPCE), and then FAD-glucose oxidase was immobilized onto pTBA/AuZnOx/SPCE to fabricate a glucose sensor. They have developed a glucose biosensor using an all-solid-state pH sensor fabricated via combining alloy oxide with a very stable conducting polymer bearing benzoic acid groups, because they have argued that it could enhance the stability, the proton transfer efficiency, and the enzyme binding property. Immobilization of GOx on the conducting polymer/AuZn oxide layer was carried out through the amide bond formation. They claimed that the fluctuation in the H^+ concentration by the enzyme reaction can be used to detect glucose by potentiometric method. Since the potentiometric method does not require applying any anodic potential, the proposed sensor was inert to interfering species. Furthermore, the sensor was applied to measure the glucose levels in whole blood human, which was in good agreement with the commercial glucose monitoring system. The same goal was pursued by Timur's team[48] who electrosynthesized the conducting polymer of 4-(2,5-di(thiophen-2-yl)-1H-pyrrol-1-yl) benzenamine (SNS-NH_2) and modified it with AuNPs. They used AuNP-SNS-NH_2 as a platform for immobilization of GOx and used it for glucose detection in both batch and flow injection analysis (FIA) modes. They suggested that the combination of SNS-NH_2 and Au NPs allows the immobilized enzyme to have higher bioactivity which results in fast, stable, and sensitive responses toward their substrates. This biosensing method was compared with the spectrophotometric method as the reference method for independent glucose analysis in beverages samples.

The flavoenzyme pyranose 2-oxidase (PyOx) catalyzes the C-2/C-3 oxidation of numerous sugars to their corresponding dicarbonyl derivatives and can be used instead of GOx in biosensor construction. Timur et al.[49] fabricated a novel PyOx biosensor based on AuNPs-PANI/AgCl/gelatin nanocomposite for the glucose detection. The presence of gelatin in matrix resulted in good stability of the sensor during the operational conditions. The amperometric biosensor based on PyOx/AuNPs-PANI/AgCl/gelatin composite exhibited good linearity in the operational conditions and also applied to analyze glucose content in real samples.

L-Glutamate (an amino acid), exists naturally in protein-rich foods. Since both too much and too little l-glutamate is harmful, l-glutamate is essential and also highly toxic at the same time. Therefore, the measurement of l-glutamate in biological materials is of great significance. An amperometric l-glutamate biosensor was fabricated based on immobilization of glutamate oxidase (GluOx) onto zinc oxide nanorods/polypyrrole composite.[50] The composite was electro-deposited onto a pencil graphite electrode and then, GluOx was immobilized onto this electrode by dipping into GluOx solution. The electrochemical impedance spectroscopy (EIS) introduced an effective method to follow electronic features of surface-modified electrodes. The EIS results indicated that the charge transfer resistance (R_{CT}) of composite was increased after immobilization of GluOx. This increase in R_{CT} has been attributed to the binding of enzymes onto composite. The presented biosensor was applied for determination of l-glutamic acid level in real samples and the result indicated a good correlation with the values obtained by standard colorimetric method.

In order to improve the sensitivity of acetylcholine (ACh) biosensor and facilitate the electron transfer, Jain's team[51] employed Fe_2O_3 NPs/PEDOT-rGO on fluorine doped tin oxide (FTO) (Figure 4). Acetylcholine and its metabolite choline (Ch) play vital roles in signal delivery from nerve cells

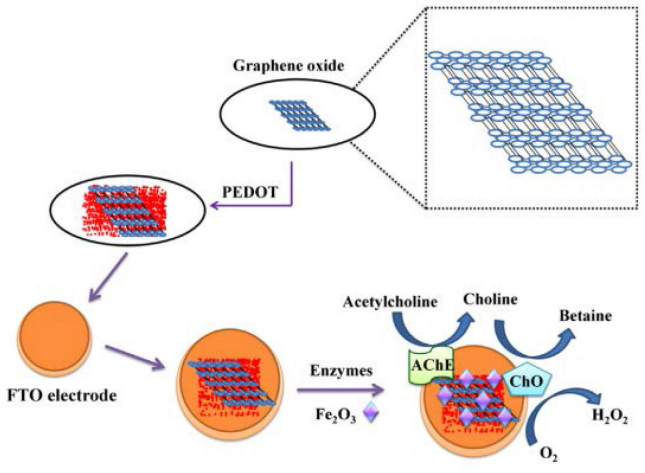

Figure 4. Schematic illustration of the AChE-ChO/Fe_2O_3/rGO/PEDOT nanocomposite onto the FTO-coated glass plate and subsequent recognition of ACh. Reprinted from[51] with permission from Elsevier Publications.

to the other cell types and functions as neurotransmitter and modulator. Since the several neural disorders like Parkinson diseases, Alzheimer's disease, and schizophrenia are associated with ACh, a sensitive, rapid, and accurate tool is required in clinical applications in order to ACh detection. The electrochemical sensing of ACh and Ch are carried out based on the following enzyme reactions:

$$\text{Acetylcholine} + H_2O \xrightarrow{AChE} \text{Choline} + \text{Acetate} \quad (4)$$

$$\text{Choline} + O_2 \xrightarrow{ChO} \text{Betaine aldehyde} + H_2O_2 \quad (5)$$

Jain's group has used cyclic voltammetry (CV) and electrochemical impedance spectroscopy techniques for characterization and detection. They have claimed that the fabricated biological sensor provides remarkable analytical features including a wide linear range, fair analytical recovery, multiple reusability, significant selectivity, and fast response.

Recently, Das et al.[52] have been electrochemically synthesized PPy and PPy–Ag nanocomposite from aqueous solutions containing pyrrole, KCl (system A); pyrrole, KCl, and SDS (system B); and pyrrole, silver nitrate, SDS (system C). They observed that SDS accelerated the polymerization rate showing a higher yield up to 0.0005 M and then decreased resulting from hindrance in electro-oxidation of pyrrole at higher anionic surfactant concentration. The characterization results indicated that potassium ion selective electrode constructed of PPy–Ag nanocomposite offered nernstian behavior with a maximum slope of 57 mV. The urease catalyzes the hydrolysis of urea to form the NH_4^+ cations. So, a cation-selective electrode could be applied to determination of the urea concentration. Based on this fact, Das's team has designed an enzyme electrode for the analysis of urea using ammonium ion selective electrode based on PPy–Ag nanocomposite-modified solid state electrode. For this purpose, they have immobilized the antibiotic nonactin within PVC matrix membrane over PPy–Ag nanocomposite film. Then, the urease-PVA membrane has been immobilized over the surface of nonactin-based ammonium ion-selective electrode. They have argued that the excellent responses of PPy–Ag nanocomposite film-based electrode could result from high concentration and better stability of urease on PPy–Ag nanocomposite-modified electrode. Moreover, it was confirmed that the excellent reproducibility of the presented urea biosensor was related to small drift in open circuit potential during subsequent measurements. Considering the advantages of the hollow carbon spheres (HCS) and PANI, Zhang' team[53] has fabricated an electrochemical biosensor for malathion detection using the hollow core-shell nanostructured nanocomposite of HCS@PANI. The presented biosensor has been designed based on the chemisorption/desorption process of acetylcholine esterase (AChE) which anchored onto HCS@PANI nanocomposite. In the presence of malathion, the electrocatalysis of AChE/HCS@PANI-based biosensor toward acetylthiocholine chloride (ATCl) has been inhibited after AChE was adsorbed onto the HCS@PANI nanocomposite. This fact has been employed to detect malathion concentration. It was reported that the AChE biosensor has exhibited a high sensitivity toward malathion with LOD of 0.71 ng mL^{-1} and 0.16 ng mL^{-1} of malathion within the 1.0–10 μg mL^{-1} concentration range using CV and SWV, respectively.

3. Conducing Polymer Nanocomposites-Based Non-Enzymatic Electrodes

Given the several disadvantages of enzyme-modified electrodes, such as their instability, the high cost of enzymes, and the complexity of immobilization, considerable attention has been drawn to the non-enzymatic electrodes. Non-enzymatic biosensors are based on current response of biomolecule redox directly at the electrode surface, and thus, the electrocatalytic activity of the electrode material is the key factor which affects both the sensitivity and the selectivity in the detection of biomolecule. Recent reports researches focused on application of conducting polymer-based nanocomposites in non-enzymatic biosensors are summarized in Table 2.

3.1. PANI-Based Nanocomposites

Polyaniline is a conducting polymer that is electrically synthesized in the presence of an acidic medium by anionic oxidation of the aniline monomer onto an electrode surface. Deposition of PANI on the electrode surface can be done either by chemical or electrochemical means. Generally, PANI exists in three oxidation states, namely the half oxidized emeraldine (ES), the fully reduced leucoemeraldine, and the fully oxidized pernigraniline (PNB) (Figure 5). Since a reversible acid-base reaction takes place between the half-

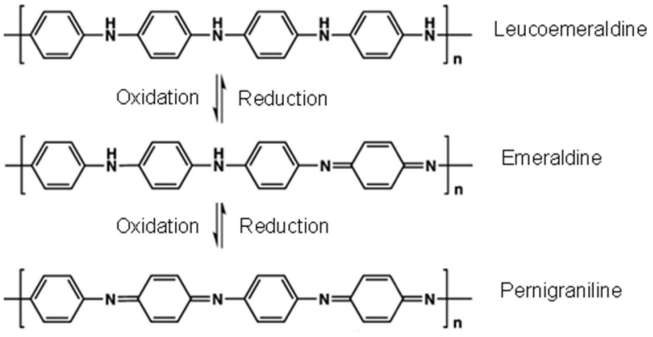

Figure 5. The oxidation states of PANI.

Table 2. Several conducting polymer-based nanocomposites for non-enzymatic biosensors.

Nanocomposite	Analytical method	Analyte	Linear range (μM)	LOD (μM)	Ref
PEDOT/RGO	Amperometric	dopamine	$0.1–1.7\times10^{2}$	3.9×10^{-2}	[84]
NiNPs/PEDOT/RGO	Amperometric	glucose	$1.0–5.1\times10^{3}$	0.8	[32]
PEDOT/IL	Amperometric	dopamine	$0.2–3.1\times10^{2}$	5.1×10^{-2}	[85]
G/PVP/PANI	Amperometric	cholesterol	5.0×10^{1}–1.0×10^{4}	1.0	[60]
Pd/PEDOT	Amperometric	hydrogen peroxide	$2.5–1.0\times10^{3}$	2.8	[87]
PEDOT/AuNPs	Flow-injection amperometry	cysteine	$0.5–2.0\times10^{2}$	0.5×10^{-1}	[86]
PPy/GQDs@PB	Amperometric	L-cysteine	$0.2–5.0\times10^{1}$ 5.0×10^{1}–1.0×10^{3}	0.1	[80]
Hb/AuNPs/PDDA-G	Amperometric	hydrogen peroxide	$6.0–1.0\times10^{3}$	0.4	[95]
PANI-GO	DPV	ascorbic acid	2.5×10^{1}–2.0×10^{2}	2.0×10^{1}	[58]
PANI-GO	DPV	dopamine	$2.0–1.8\times10^{1}$	0.5	[58]
PANI-GO	DPV	uric acid	$2.0–1.8\times10^{1}$	0.2	[58]
AuNPs@polyaniline	DPV	dopamine	1.0×10^{1}–1.7×10^{3}	5.0	[54]
AuNPs@polyaniline	DPV	ascorbic acid	2.0×10^{1}–1.6×10^{3}	8.0	[54]
Pt/MWCNTs-PANI	Amperometric	hydrogen peroxide	$7.0–2.5\times10^{3}$	2.0	[63]
Cyt c/G-PEDOT	Amperometric	hydrogen peroxide	$0.5–4.0\times10^{2}$	2.5×10^{-1}	[91]
PPy-PANi-Au	EIS	DNA	1.0×10^{-7}–1.0	1.0×10^{-7}	[71]
AuNPs@PPy	Amperometric	hydrogen peroxide	0.1×10^{3}–7.0×10^{3}	–	[73]
PPy/AuNPs/SWCNTs	DPV	epinephrine	4.0×10^{-3}–0.1	2.0×10^{-3}	[74]
PANIw-graphene	Amperometric	DNA	2.1×10^{-6}–2.1	3.2×10^{-7}	[61]
PANI/HRP/GE-CNT-Nafion/AuPt NPs	SWV	hydrogen peroxide	$0.5–1.0\times10^{2}$	1.7×10^{-1}	[64]
PANI/AuNPs	Amperometric	ascorbic acid	$3.0–2.0\times10^{2}$	5.0×10^{-1}	[55]
AuNPs-PANFs	Amperometric	hydrogen peroxide	1.0×10^{1}–1.0×10^{3}	–	[56]
$PEDOT_{SDS}$-nanoAg-MDB	Amperometric	dihydronicotinamie adenine dinucleotide	1.0×10^{1}–5.6×10^{2}	0.1	[82]
NiO/CuO/polyaniline	Amperometric	glucose	2.0×10^{1}–2.5×10^{3}	2.0	[67]

oxidized emeraldine base and emeraldine salt forms of PANI, PANI is very strong pH sensitive. PANI has captured the attention of researchers due to its relatively low cost, simple synthesis, good optical and electrical properties with excellent environmental stability. It finds potential applications in electronic, optical devices, such as light emitting diodes, sensors, electronic circuit boards, and bioelectrochemical sensing because of its electrochemical properties. Thanks to its excellent electrochemical properties, ease of synthesis and functionalization, high environmental stability, and low toxicity, PANI is a promising material for biosensor applications.

Li et al.[54] prepared AuNPs@PANI core-shell nanocomposites by one-step chemical oxidative polymerization of aniline using chloroaurate acid as the oxidant and Au NPs as the seed. The interface properties of the modified electrodes have been characterized by EIS. The characterization results have indicated that AuNPs@PANI nanocomposites offer excellent electric conductivity and accelerate the electron transfer. The electrocatalytic activity of the AuNPs@PANI nanocomposites were carried out by differential pulse voltammetry (DPV) for simultaneous determination of dopamine (DA) and ascorbic acid (AA) in human serum. The π–π interaction between phenyl structure of DA and PANI, which is π-rich in nature, made the easy arrival of DA molecules to the surface of electrode. In a similar study, Hu et al.[55] prepared polyaniline/gold nanoparticles nanocomposites using a simple self-assembly procedure and used polyvinyl pyrrolidone (PVP) as a stabilizing ligand of Au NPs. The presence of PVP caused both the stabilization of

the gold nano-particles and increase in affinity of the Au NPs for PANI. The authors have proposed that the strong redox electroactivity of PANI/AuNPs nanocomposites in neutral aqueous solutions resulted from the negatively charged groups of PVP associated with the Au NPs which changed the micro-environment in the PANI chains. The PANI/AuNPs nanocomposites films indicated electrocatalytic activity for the electrochemical oxidation of ascorbic acid and a good linear relationship between the oxidation currents and concentrations of AA was obtained (Table 2).

In another work, ultra-fine and uniform gold nanoparticles were fabricated on polyaniline nanofibers (PANFs) and used for H_2O_2 sensing.[56] The high surface area of dispersed PANFs played a key role in the formation of the uniform ultrafine Au NPs. The affinity between the protonated imine units of PANFs and $AuCl_4^-$ caused ultra-fine and uniform Au NPs selectively reduced on the surface of PANFs. The homogeneous distribution of Au NPs on PANFs resulted in a strong interaction between Au NPs and PANFs which enhanced the electroactivity of PANFs and the electrocatalytic activity of Au NPs for the oxidation and determination of H_2O_2. Recently, Chen et al.[57] have prepared an electrochemical immunosensor based on PANI-AuNPs and gap-based interdigitated electrode (IDEs) for detection of carcinoembryonic antigen (CEA). The combination of the special structure of IDEs and prominent properties of PANI-AuNPs could automatically improve the performance of sensor like selectivity, repeatability, and stability. In this work, IDEs have been applied as immune sensing platform instead of traditional metal electrodes and functionalized with carboxymethyl-β-cyclodextrin (CM-β-CD). Then, the adamantine-modified primary antibodies (ADA-Ab1) have been immobilized on the functionalized IDEs. The polyaniline-Au nanoparticles-labeled antibody (PANI-AuNPs-Ab2) as a signal tag has been captured onto the immunosensor and connected the microgap of IDEs after the immuno reaction between Ab1 and CEA. This could lead to decrease in resistance value of IDEs. Moreover, the bottom of the immuno platform which was decorated with CD, Ab1, and CEA was fully insulated and the electrochemical response only depended on the number of PANI-AuNPs-Ab2 which was exactly proportional to CEA. They reported that the presented immunosensor could detect CEA with a wide linear range from 0.1 ng/mL to 1000 ng/mL and low detection limit (0.007 ng/mL).

Graphene oxide (GO) and reduced graphene oxide as new members of carbon nanomaterial, have been applied for fabricating the polyaniline composites by in situ chemical or electrochemical polymerization, covalent or non-covalent functionalization, and self-assembly. For example, Viswanathan's group[58] has prepared polyaniline/graphene oxide fibrous nanocomposites via an in situ chemical polymerization method and investigated the electrochemical catalytic activity toward the electro-oxidation of ascorbic acid, dopamine and uric acid. The DPV measurements showed well-defined oxidation peaks at PANI-GO/GCE with peak potential value of −30 mV, 290 mV and 520 mV for AA, DA and UA, respectively (Figure 6). Both the CV and DPV measurements demonstrated that PANI-GO nanocomposite possess good electrocatalytic activity toward the oxidation of these three molecules, which was ascribed to the π–π interaction and electrostatic attraction between the target biomolecules and PANI-GO nanocomposite.

Wang et al.[59] introduced a facile in situ direct synthesis method by a charge transfer self-assembly technology at organic-aqueous interface and graphene-polyaniline nanocomposite were synthesized by simultaneous electropolymerization of G-aniline. Palladium nanoparticles were electrodeposited on the surface of G-PANI nanocomposite and the EIS results indicated that the electrochemical performance of Pd/G-PANI nanocomposite was affected by the content of graphene in G-PANI. The electrocatalytic activity of Pd/G-PANI nanocomposite was investigated by the selective electro-oxidation of hydroquinone (HQ) and catechol (CC) isomers as probe molecule. The good stability of Pd/G-PANI electrode was attributed to the less poisoning of the electrode surface and also the electron rich graphene nanosheets which can effectively prevent the loss of metal catalysts.

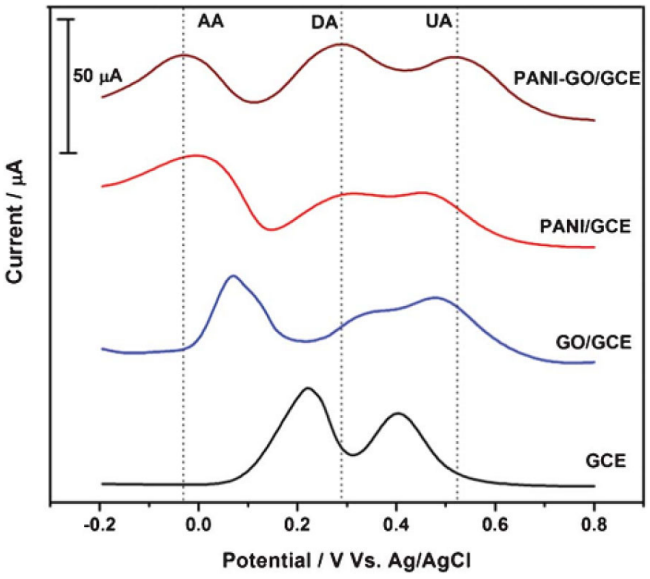

Figure 6. DPV curves at bare GCE, GO/GCE, PANI/GCE and PANI-GO/GCE in 0.05 M (pH 7.0) PBS containing ternary mixture of 1 mM AA, 1 mM DA and 1 mM UA. DPV settings: step potential: 5 mV; amplitude: 60 mV; pulse width: 0.05 s; sample width: 0.01 s and pulse period: 0.2 s. Reprinted from[58] with permission from the Royal Society of Chemistry Publications.

Rodthongkum et al.[60] prepared a nanocomposite of graphene, polyvinylpyrrolidone and polyaniline and used it for modification of paper-based biosensors. The G/PVP/PANI nanocomposites were fabricated on the paper-based biosensor via electrospraying method to further increase the surface area of the working electrode. The G/PVP/PANI-modified electrodes exhibited excellent electrocatalytic activity toward the oxidation of hydrogen peroxide. Based on this fact that H_2O_2 is generated from the enzymatic reaction between cholesterol and cholesterol oxidase, G/PVP/PANI-modified paper-based biosensors were used to measure the cholesterol in real samples such as human serum. The interference effect of ascorbic acid in the detection of cholesterol was eliminated by using an anionic surfactant due to an electrostatic repulsion between anionic surfactant and anionic AA at pH 7.4.

Another example is the integration of graphene and PANIw layer-by-layer for fabricating an DNA biosensor.[61] The authors suggested that the low interfacial electron transfer resistance of PANIw-graphene modified GCE was attributed to many small band gap of oxidized graphene and higher surface area of PANIw. The oligonucleotide probe was immobilized on the surface of PANIw/graphene/GCE through the formation of phosphoramidate bonds between the amino group of polyaniline and phosphate group of the oligonucleotides at 5'end (Figure 7). Daunomycin was selected as electroactive hybridization indicator of DNA biosensor; when hybridization happens, daunomycin is intercalated in the DNA duplex, which can show a good electrochemical response compared with single-stranded DNA.

Many essential bio-chemicals such as natural amino acids and simple sugars are chiral and significantly exist in only one isomeric form. The chiral purity of these bio-chemicals is important in stereo-specific synthesis, pesticides, food additives, and pharmaceuticals, where only one chiral molecule may interact satisfactorily and the other may be toxic or less active. In the field of analytical methods, the electrochemical sensors have attracted considerable attentions for chiral recognition because of their extreme specificities and sensitivities. In this aspect, Kant et al.[62] have conducted a study in which a novel chiral selective imprinted polyaniline-ferrocene-sulfonic acid film has been electrochemically fabricated on C-dots modified pencil graphite electrode for detection of L-ascorbic acid or D-ascorbic acid. Electrochemical oxidation of chiral L-AA is catalyzed by both C-dots and L-AA imprinted MI-PANI-FSA (imprinted polyaniline-ferrocene-sulfonic acid). C-dots are only involved in increasing the electro-active surface area of electrode but not in conduction of electrons. The EIS technique was employed for determination of L-AA and D-AA using L-AA selective MI-PANI-FSA-C-dots/PGE and D-AA selective MI-PANI-FSA-C-dots/PGE, respectively. The calibration plot was drawn between the concentration of L-AA and the change in R_{ct} ($\Delta R_{ct} = R_{ct}$ (MI-PANI-FSA-C-dots/PGE) $- R_{ct}$(MI-PANI-FSA-C-dots/PGE doped L-AA/D-AA)). The proposed sensor has been also applied for chiral detection of L-AA in real and commercial samples.

Yuan et al.[63] combined the advantages of MWCNTs-PANI composite and Pt nanoparticle films to fabricate a non-enzymatic amperometric sensor for hydrogen peroxide. MWCNTs-PANI composites were prepared by in situ electrochemical polymerization on the surface of a GCE and Pt nanoparticles were immobilized on the MWCNTs-PANI nanocomposites by potentiometric stripping analysis method. The synergistic catalytic activity between MWCNTs-PANI nanocomposites and Pt NPs made Pt/MWCNTs-PANI/GCE exhibit a high sensitive detection of H_2O_2 in disinfector real samples. In a similar study, Zheng's team enzymatically synthesized PANI on G-CNT-Nafion/AuPt NPs/GCE (Figure 8) and used it as a biosensor for determination of H_2O_2.[64] They have reported that the G-CNT-Nafion/AuPt NPs/GCE had greater R_{CT} compared to the GE-CNT-Nafion/GCE, which resulted from the deposition of AuPt

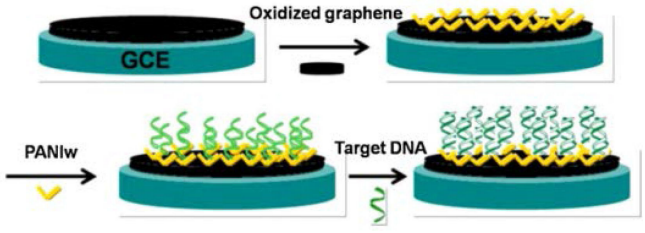

Figure 7. The assembly process of the electrode. Reprinted from [61] with permission from Elsevier Publications.

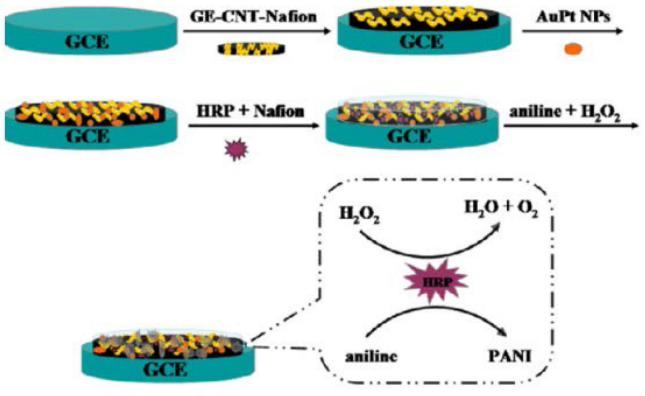

Figure 8. Scheme diagram of the H_2O_2 biosensor based on enzyme-catalyzed deposition of PANI on the designed modified electrode. Reprinted from[64] with permission from Elsevier Publications.

NPs on the electrode surface. When HRP was modified onto the G-CNT-Nafion/AuPtNPs/GCE surface, the observed RCT has been dramatically increased considering the hindrance of the macromolecular structure of HRP to the electron transfer. The diameter of the semicircular impedance spectrum has been decreased after incubating the HRP/GE-CNT-Nafion/AuPtNPs/GCE in an PANI growth solution which indicated the successfully polymerization of aniline on the designed electrode. The authors argued that this biosensor has a low detection limit due to the synergistic effect of G-CNT hybrid materials, AuPt alloy nanoparticles, PANI and the highly efficient polymerization of aniline under the enzymatic reaction.

Many researches on polyaniline-metal nanoparticles composites show enhanced sensing, electrical and catalytic capabilities, as compared with those of pure PANI. In this regards, Park's team[65] has fabricated a Ag-PANI-silica complex from a silver ion, aniline monomer, and silicate by using γ-irradiation at room temperature without using a colloidal stabilizer. The Ag-PANI-silica was simultaneously polymerized and reduced for the dispersed nano-Ag particles within individually PANI or silica molecules or a network of PANI and silica molecules. The Ag-PANI-silica/ITO electrode indicated electrocatalyic activity toward the reduction of H_2O_2. Recently, Zhao et al.[66] have fabricated a non-enzymatic hydrogen peroxide electrochemical sensor based on PANI/Cu nanocomposite film on a glassy carbon electrode. They have synthesized Cu NPs by using electro-deposition methods, and then fixed the metallic nanoparticles on the surface of GCE by PANI films to improve their electrochemical performance. The CV and amperometric results have confirmed that PANI/Cu nanocomposite showed improved electrocatalytic response of the electrode toward H_2O_2 compared to that of a bare electrode, polyaniline electrode, and Cu electrode due to the synergistic effects of polyaniline film and Cu NPs. It was reported that the prepared non-enzymatic electrochemical sensor demonstrated a low detection limit (1.0 to 500 μM), good reproducibility, and stability for H_2O_2 across a broad linear range (0.33 μM).

Nanocomposites of metal oxide-polyaniline have attracted much attention for their potential in sensor applications because it combines the advantages of metal oxides and organic polymers polyaniline. In this aspect, a non-enzymatic electrochemical glucose sensor has been developed based on NiO/CuO/PANI nanocomposite.[67] The mechanism for oxidation of glucose by NiO-based composite in alkaline solution was described by the following reactions:

$$NiO + OH^- \leftrightarrow NiO(OH) + e^- \quad (6)$$

$$NiO(OH) + Glucose \rightarrow Ni(OH)_2 + Gluconolactone \quad (7)$$

$$Gluconolactone \rightarrow Gluconic\ Acid \quad (8)$$

$$Ni(OH)_2 + OH^- \leftrightarrow NiO(OH) + e^- + H_2O \quad (9)$$

The sensor was used to detect the glucose concentration in real samples. The obtained results showed similar responses to those achieved by the spectrophotometric method in a medical diagnostic laboratory. Similarly, an electrochemical glucose sensor based on the core-shell structure of $NiCo_2O_4$@polyaniline nanocomposite was fabricated via a facile hydrothermal treatment followed by in situ polymerization coating process.[68] The high sensitivity of the nanocomposite was attributed to $NiCo_2O_4$@PANI core-shell network structure which provided large amount of highly conductive channels for electron transportation and simultaneously shortening the electron transportation pathway. Moreover, the authors claimed that the good selectivity of $NiCo_2O_4$@polyaniline nanocomposite was resulted from the PANI shell, which prevented the adsorption of poisoning intermediates at $NiCo_2O_4$ surfaces.

Recently, Ebenso et. al[69] have described successful modification of Au electrode with PANI, MWCNT, TiO_2 and RuO_2 nanoparticles and confirmed thriving modification of Au electrode using different spectroscopic, microscopic, and electrochemical techniques. They have declared that AuMWCNT-PANI-TiO_2 and Au-MWCNT-PANI-RuO_2 electrodes provide fast electron transport and better catalytic behavior toward the oxidation of epinephrine, also known as adrenaline, compared with other electrodes studied. Similar performance of both Au-MWCNT-PANI-TiO_2 and Au-MWCNT-PANI-RuO_2 electrodes in terms of epinephrine oxidation current have been attributed to the synergy between the metal oxide and MWCNT which improved the electrochemical properties of the electrodes. Moreover, Ebenso's group has employed the fabricated sensors for detection of epinephrine in a pharmaceutical sample. Similarly, Ghanbari and Ahmadi[70] have fabricated a sensitive non-enzymatic glucose sensor based on NiO/Au/PANI/rGO nanocomposite. They have illustrated electrocatalytic and electrochemical behavior of the modified electrode for the measurement of glucose by cyclic voltammetry and amperometric methods. It is revealed that the NiO/Au/PANI/rGO nanocomposite modified glassy carbon electrode exhibited remarkable electrocatalytic performance as a result of the large surface area and good conductivity of nanocomposite. Besides, the fabricated sensor has been used for determination of glucose in human plasma samples and the results have been compared with spectrophotometric method.

Label free electrochemical DNA sensing using conducting polymer modified surfaces attracts high interest owing to its simplicity in experimental design. In this direction, Wilson et al.[71] have fabricated a DNA hybridization sensor by

immobilization of DNA labeled at 5'end using 6-mercapto-1-hexhane (HS-ssDNA) on the PPy-PANI-Au surface. Combination of the polypyrrole-poly aniline (PPy-PANI) and Au nanoparticles has increased the conductivity and provided a good platform for the sensitive and selective discrimination of the completely complementary, non-complementary, single and double base mismatched targets in presence of the$[Fe(CN)_6]^{3-/4-}$. Authors declared that the enhancement of the hybridization efficiency resulted from the large number of thiol capped ssDNAs and the microdispersed Au nanoparticles in the PPy-PANI structure. The effect of varying concentration of target on the impedance behavior of the PPy-PANI-Au-S-ssDNA was investigated and a linear relationship was obtained between $\triangle R_{CT}$ (the variation of R_{CT} before and after target hybridization at each concentration) and log of concentration of DNA.

3.2. PPy-Based Nanocomposits

Polypyrrole is one of the most commonly investigated poly conjugated conducting polymers due to its different financial applications like chemical sensors, supercapacitors, biosensors, gas sensors, and corrosion protection.[72] Its monomer is environmentally stable, flexible and has lower oxidation potential. PPy can be synthesized either chemically or electrochemically. Electrochemical synthesis of PPy possesses high stable conductivity at a long period of time in comparison with chemical synthesis. Parameters such as applied current or voltage can control the location, morphology, thickness, and chemical composition of PPy. On the other hand, chemical synthesis includes the use of particular dopants during the synthesis. These dopants are used based on the fact that they have an ability to increase the conductivity of the polymer. Also, the oxidation of pyrrole has been carried out by photochemical and enzyme-catalyzed polymerization routes. Thanks to its good environmental stability, high biocompatibility, and very good electrical properties, PPy is one of the most promising materials for biological sensing applications.

Nanocomposites of polypyrrole and Au NPs have received considerable attention due to their potential application in many fields, such as electrocatalysis and electrochemical biosensing. For example, Song et al.[73] have reported a facile method for the fabrication of a novel three-dimensional (3D) dendritic AuNPs@PPy nanocomposits. In this way, AuNPs@PPy exhibited much improved electrocatalytic behavior toward H_2O_2 reduction than PPy due to the presence of 3D dendritic nanocrystal golds in the composites. The same goal was pursued by Lu et al., who introduced a fast and direct procedure for the fabrication of the PPy/AuNPs/SWCNTs nanocomposites modified gold electrode to detect epinephrine in presence of ascorbic acids and uric acids.[74] Recently, Ma et al.[75] have introduced an electrochemical non-enzymatic glucose sensor based on nickel-cobalt-sulfide nanosheet/polypyrrole nanowire (Ni-Co-S NS/PPy NW) core-shell structure on the nickel foam (NF). PPy NWs core have constructed a three dimensional micro/nano structure on the NF and the ternary Ni-Co-S NSs shell grown on the PPy NWs have acted as a catalyst for glucose electrooxidation. Electrochemical studies have demonstrated that the Ni-Co-S/PPy/NF electrode has exhibited a remarkable electrocatalytic activity to glucose, and two linear ranges (2 μM to 140 μM and 0.14 mM to 2 mM) with a low limit of detection (0.82 μM) resulting from the synergistic effects of Ni-Co-S, PPy and NF. In another work, Jain et al.[76] have fabricated a voltammetric sensor based on PPy/TiO_2 nanocomposite for detection of sulfamoxole (SLM). The PPy/TiO_2/GCE has been characterized by EIS and various voltammetric techniques. The EIS results have indicated that the interfaces between TiO_2 and PPy could markedly promote the charge transfer reactions in the composite film. They have reported that the outcomes of EIS measurements confirmed the results obtained from CV and hence, authenticates again the successful casting and fabrication of synthetic PPy/TiO_2 on bare GCE for the sensing application. Moreover, the fabricated sensor has exhibited a linear response for the oxidation of SLM over a concentration range from 1.25 $\mu g\,mL^{-1}$ to 12.50 $\mu g\,mL^{-1}$ with the detection limit of 1.24 $ng\,mL^{-1}$.

The condition of polymerization can be influenced by morphology and electrocatalytic behavior of the film. Moozarm Nia's group[77] have synthesized PPy via two different electrodeposition processes. Polymers with various lengths and non-homogenous was resulted from CV method (PPy_{CV}) by applying wide potential ranges which are not necessarily monomer oxidation. In contrast, in amperometry method (PPy_{amp}) monomers are exposed to oxidation potential very fast. These polymers have well-arranged chain structure with greatly high ratio of surface-to-volume that is useful for the decorating by Cu_xO particles. According to Moozarm Nia et al., PPy_{amp}/rGO/GCE has a lower resistance of charge transfer and also higher electrocatalytic activity toward glucose oxidation compared with PPy_{CV}/rGO/GCE. The accuracy of the sensor was assessed by applying of the sensor to real blood serum samples for determination of glucose. In another work, they have fabricated PPyNFs-AgNPs-rGO nanocomposite via two separate methods.[78] In the first method, graphene oxide reduction and polymerization of PPy was occurred in one step process by cyclic voltammetry (CVPPyNFs-AgNPs-rGO). In the second method, graphene oxide decorated with silver nanoparticles was electrochemically reduced on the electrode by an amperometric method. Then, pyrrole was electropolymerized on the surface of AgNPs-rGO electrode through amperometry

process in order to obtain nanofibers of polypyrrole (AMP-PPyNFs-AgNPs-rGO). The catalytic activity of AMP-PPyNFs-AgNPs-rGO composite increased compared to CVPPyNFs-AgNPs-rGO toward the reduction of H_2O_2 as a result of full reduction and polymerization of GO and PPyNFs, respectively. In presence of the PPy, the reduction of H_2O_2 could be increased as well:

$$2PpyNFs(s) + H_2O_2(l) \rightarrow 2PpyNFs^+(s) + 2OH^-(aq) \quad (10)$$

$$PpyNFs^+(s) + e^-(\text{applied potential}) \rightarrow PpyNFs(s) \quad (11)$$

Recently, Rajesh et al.[79] have demonstrated the electrochemical synthesis of poly (pyrrole-co-pyrrolepropylic acid) copolymer (PPy-PPa) over G-MWCNT hybrid film deposited on GCE to detect the protein antigen (cTnI). The pendant carboxyl groups of the copolymer have offered efficient biomolecular immobilization of protein antibody, antitroponin I, on the electroactive G-CNTs, which is suitable for fabrication of a low-frequency impedimetric immunosensor. The analytical performance of the bioelectrode has been investigated using RCT as a sensing element in EIS for the quantitative detection of cTnI. The EIS results have indicated that the RCT provided a good correlation with antigen concentration in the low frequency region (<1 Hz). Rajesh's team showed that the synergistic combination of physical and electrochemical characteristics of both the conducting polymer and 2D-graphene with pillared G-CNTs hybrid results in the detection of cTnI concentration as low as 1.0 pg mL^{-1} in spiked human serum, with a broad linear range (1.0 pg mL^{-1}–10.0 ng mL^{-1}).

Fang et al.[80] prepared a novel polypyrrole and graphene quantum dots @ Prussian Blue (PPy/GQDs@PB) nanocomposite on a graphite felt (GF) substrate at room temperature. Presence of GQDs resulted in increased conductivity of the composite and also enhanced reaction rate of the PB formation. The polypyrrole film electrodeposited on the surface of the electrode improved the electrochemical stability of the device. The PPy/GQDs@PB/GF electrode has indicated an efficient detection of L-cysteine and has been applied to analyze the commercially available L-cys capsule samples. Recently, Pilan et al.[81] have introduced an efficient strategy for development of biosensing platforms based on polypyrrole/sulfonated graphene nanocomposite materials. In this method, nanocomposite films have been prepared by polymerizing pyrrole in the presence of phenylsulfonyl-functionalized graphene as both support and sole dopant. Subsequently, the polypyrrole/graphene nanocomposite films have been modified with carboxyphenyl groups through electrochemical reduction of 4-carboxyphenyl diazonium tetrafluoroborate to permit covalent immobilization of glucose oxidase and also form an electrode blocking layer which hindered the oxidation of interfering substances. Since polyaryl films grafted via electrochemical reduction of aryl diazonium salts act as blocking layers which hinder the oxidation of interfering substances, Pilan' team have argued that the presented procedure could increase biosensor selectivity. It has reported that the biosensing platform based on polypyrrole/sulfonated graphene nanocomposite offered an improved performance like wide linear range (0.02–12 mM), good sensitivity, low LOD (0.02 mM), and adequate selectivity toward common interferes including ascorbic acid, paracetamol, uric acid, and cysteine.

3.3. PEDOT-Based Nanocomposits

Poly (3,4-ethylenedioxythiophene) has been considered as one of the most promising conducting polymers in electrochemical fields owing to remarkable conductivity, inherently well-defined structure, and stability. PEDOT offers high conductivity, stability, and biocompatibility in the doped state for its low band gap. With great environmental stability, conductivity, narrow electronic band gap and biocompatibility, PEDOT is one of the most promising candidates for constructing electrochemical biosensors. PEDOT conducting polymers have been used in biological and biomedical areas, such as biosensors and bio-interfaces because deposition of PEDOT on the electrode surface would effectively improve the electrochemical performance of the modified electrodes. Chen et al.[82] have employed the $PEDOT_{SDS}$-nanoAg electrode as a platform to immobilize electrochemically active mediator, Meldola Blue (MDB). In this method, the $PEDOT_{SDS}$-nanoAg-MDB electrode exhibited the strong electrocatalytic activity toward oxidation of dihydronicotinamide adenine dinucleotide (NADH) with decreasing overpotential (650 mV) and so, an amperometric NADH sensor was fabricated based on the PEDOTSDS-nanoAg-MDB electrode. In another work, Chen's group[83] constructed an enzyme-free peroxide biosensor based on MWCNT-PEDOT film modified electrode. Authors have also employed MWCNT-PEDOT film modified electrode to simultaneously determine AA, DA, and UA mixture by using DPV. It is noted that the conductivity of PEDOT can be dramatically increased by doping reduced graphene oxide. The same goal was pursued by Luo et al.[84] who prepared nanocomposite of reduced GO doped poly (3,4-ethylenedioxythiophene). Based on the characterization results, the PEDOT/RGO nanocomposite offered a large surface area and low electrochemical impedance indicating that it possesses excellent electrocatalytic property. The authors claimed that the electrochemical catalytic activity of PEDOT/rGO toward the oxidation of dopamine improved as compared to PEDOT/GO. This increase was attributed to the electrochemical reduction of GO, which can enhance the conductivity of the

GO sheets and hence the conductivity of PEDOT/rGO/GCE will be improved.

A big obstacle that restricts the broad application of PEDOT is that PEDOT conducting polymers have poor solubility in aqueous solution. In order to overcome this problem, polymerization of EDOT in organic solvents, especially ionic liquids (ILs) has been adopted. PEDOT conducting polymers could be polymerized with ILs to combine the unique properties of conducting polymers with ILs, and also to improve performance and lifetime of PEDOT. Recently, Luo et al.[85] have introduced a cost-effective electrodeposition method based on a composite of poly (3,4-ethylenedioxythiophene) doped with pure insoluble IL (1-ethyl-3-methylimidazolium bis (trifluoromethylsulfonyl) imide). A small volume of IL (less than 15 μL) containing EDOT monomers was used for an effective electrodeposition of PEDOT/IL. The consumption of expensive IL for the preparation of the PEDOT/IL composite was greatly reduced as compared with traditional three electrode systems in which IL was used as supporting electrolyte. Based on the excellent electrocatalytic activity of nanocomposite toward the oxidation of DA, an electrochemical sensor was fabricated that can easily distinguish between DA, AA, and UA.

Combining metallic nanoparticles and PEDOT conducting polymers not only enhances the charge transport ability, but it also effectively improves the electrochemical and catalytic properties of PEDOT conducting polymers. Cheng et al.[86] have fabricated a hybrid nanocomposite consisting of PEDOT and Au NPs onto a screen-printed carbon electrode. The PEDOT/AuNPs nanocomposite showed a great catalytic activity toward the oxidation of cysteine. The analytical performance of the PEDOT/AuNPs nanocomposite was achieved by flow-injection amperometry under optimized conditions and a linear calibration plot with a slope of 0.115 μA/μM was obtained. Du's group[87] has fabricated Pd/PEDOT nanospheres by one-pot green method without any surfactants and templates. The electrocatalytic activity of Pd/PEDOT nanospheres and PEDOT toward the reduction of H_2O_2 was investigated. In this method, Pd NPs anchored on the surface of PEDOT nanospheres as the platform for anchoring the Pd NPs play an important role as the main electroactive materials. The metal-based glucose sensors suffer from slow kinetics and surface fouling due to the adsorption of intermediates during glucose oxidation.

In similar work, Niu et al.[32] have constructed a non-enzymatic glucose sensor based on the NiNPs/PEDOT/rGO nanocomposite through a facile two-step electrodeposition process. The PEDOT/rGO offered a conductive 3D scaffold which was beneficial to the electrodeposition of Ni NPs and could overcome the drawbacks of metal-based sensors. According Niu et al., NiNPs/PEDOT/rGO nanocomposite has introduced excellent electrocatalytic activity toward glucose oxidation and a linear relationship has been obtained between the current response and glucose concentration. Also, Li et al.[88] have demonstrated a simple electrodeposition method to design an enzyme-free glucose sensor, in which $Ni(OH)_2$ NPs have been electrodeposited onto PEDOT/rGO/GCE. The presented enzyme-free glucose sensor has exhibited an ultrafast response time, a wide linear range (0.002–7.1 mM), a low detection limit (0.6 μM), excellent reproducibility, a good stability, a high sensitivity, and a high selectivity. The excellent performance of biosensor has been attributed to the synergistic effect of $Ni(OH)_2$ NPs possessing high electro-catalysis activity and to the PEDOT-rGO having good conductivity. Recently, an impedimetric biosensor for tumor marker alpha fetoprotein (AFP) based on Au NPs modified PEDOT/PEG (PEG = poly (ethylene glycol)) was fabricated through the immobilization of AFP antibodies.[89] Based on the target induced impedance change in PBS containing $[Fe(CN)_6]^{3-/4-}$, the EIS have been applied for the detection of AFP. It was reported that with the increasing of antigen concentration, the charge transfer resistance R_{CT} decreased, and the R_{CT} changes indicated a good linear correlation with the logarithmic value of target concentrations. The authors argued that the shortened incubation time for this biosensor resulted in the high biocompatibility and large surface area of the AuNPs/PEDOT/PEG matrix, which offered suitable micro-environment for the binding of AFP to its corresponding antibody. Also, the good selectivity of biosensor was ascribed to both the strong specific binding between AFP antibody and antigen and the antifouling property of the PEDOT/PEG composite film.

Recently, Xu et. al[90] have introduced a label-free electrochemical immunosensor for detecting carcinoembryonic antigen (CEA) based on three-dimensional gold nanoparticles/prussian blue-poly (3,4-ethylenedioxythiophene) nanocomposite. PB-PEDOT nanocomposite have been synthesized through a simple and green redox route by mixing Fe^{3+},$[Fe(CN)_6]_3^-$ and EDOT aqueous solution without applying any additional reductants or surfactants. In order to immobilize the CEA antibody, AuNPs were electrochemically deposited on PB-PEDOT nanocomposite. Figure 9 indicates the fabrication process of the immunosensor. In prepared nanacomposite, PB has introduced a couple of well-defined quasi reversible redox peaks at the potential range from −0.5 to 0.6 V (vs.SCE). Xu's team has confirmed that the conductive PEDOT not only facilitates interfacial electron transfer, but it also improves the stability of PB. It is also reported that the fabricated immunosensor possesses excellent analytical performance for CEA detection including a low detection limit, good selectivity, high stability, and good repeatability.

Once PEDOT conducting polymers have been doped with other functional materials, the surfaces of PEDOT

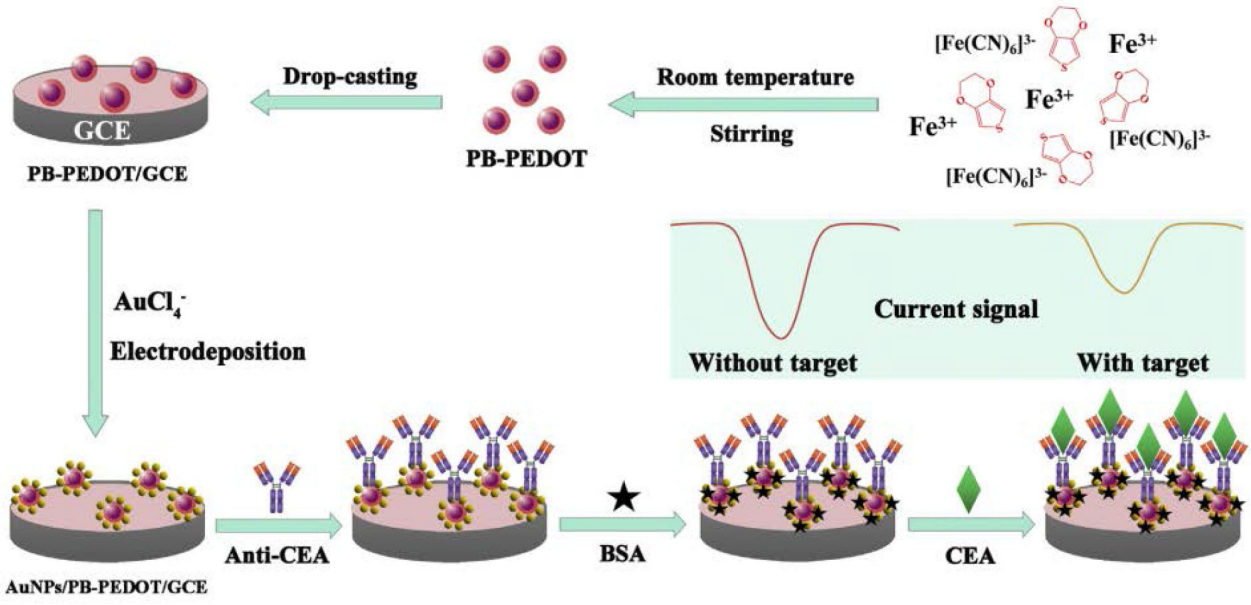

Figure 9. The fabrication process of the immunosensor. Reprinted from[90] with permission from Elsevier Publications.

composites could possess many functional groups, tending to be an ideal substrate to bond with multifunctional biomolecules for the fabrication of desirable biological systems. Xia et al.[91] prepared the G-PEDOT nanocomposite and immobilized cytochrome c (Cytc) on the G-PEDOT layer modified electrode by incubating it in phosphate buffer solution (PBS) containing Cytc. They have suggested that the G-PEDOT nanocomposite can provide a biocompatible and favorable microenvironment for Cytc immobilization and direct electron transfer between Cytc and the GC electrode. The Cytc/G-PEDOT electrode displayed quick electrochemical response toward oxidation or reduction of H_2O_2 and a sensor based on the Cytc/G-PEDOT was fabricated for the detection of H_2O_2 without interference of ascorbic acid, uric acid, and glucose. In another work, Hao et al.[92] have introduced the synthesis of ternary nanocomposite H-G/PEDOT (H = hemin) via a microwave-assisted method, in which PEDOT was polymerized with graphene oxide and induced with hemin on the H-G nanosheets without additional oxidants. They reported that the prepared H-G/PEDOT nanocomposite could combine the main excellent properties of the three components including the peroxidase-like activity of hemin, and a high-speed electron transfer ascribed to the presence of PEDOT and the graphene sheets. A biomimetic H_2O_2 biosensor has been fabricated based on the ternary hybrid H-GNs/PEDOT which indicated excellent performance such as low detection limit (0.08 μM), high dynamic response range (10^{-7} M to 10^{-5} M), high sensitivity, good selectivity, repeatability, reproducibility, and stability.

Lei et al.[93] have employed the potentiostatic method to induce the polymerization of EDOT in the GO aqueous solution on GCE without any other dopants. They have applied the prepared PEDOT/GO/GCE to the determination of acetaminophen (AP). Lei's team has reported that the encapsulated GO sheets by PEDOT could lead to a good electrocatalytic activity of the modified electrode toward the redox reactions of AP. Moreover, the sensor based PEDOT/GO films have exhibited good stability, low detection limit (0.57 μM), and acceptable sensitivity. Recently, Cui et al.[94] have introduced electrodeposition of PEDOT/GO onto the carbon fiber microelectrode (CFE) surface to increase the sensitivity and decrease the LOD of the CFE. They have argued that the signal enhancement was due to increased surface area and high affinity for the DA's oxidation product which catalyzed the electron transfer between DA and the electrode surface. However, the thicker PEDOT/GO coatings revealed higher sensitivities for DA, it displayed the drawback of slow adsorption and electron transfer kinetics which lacks the temporal resolution of the sensors necessary for real time in-vivo measurement. Furthermore, Cui's group has reported that the electrodeposited PEDOT/GO coatings are highly tunable. It means the coatings could be tailored toward any specific electroactive analyte or concentration range by varying deposition current, size and oxidation state of GO, etc.

3.4. Other Conducting Polymers

The incorporation of Au NPs into conducting polymers is of great interest because of their strong electronic interactions between Au NPs and the polymer matrixes. Recently, several reports have been presented on incorporating Au NPs into polymer matrixes to improve the electrocatalytic properties of Au NPs. Wang et al.[95] have assembled Au NPs on the surface of poly (diallyldimethylammonium chloride) functionalized graphene nanosheets (PDDA-G) film through electrostatic interaction to construct the biocompatible interface. PDDA has been employed due to its strong ionic property which offers excellent binding capability with graphene to form positive charge PDDA-G and can maintain the electronic structure of graphene. They have immobilized hemoglobin on the surface of AuNPs/PDDA–G film modified electrode. In this method, Hb/AuNPs/PDDA-G film-modified electrode showed a couple of well-defined and quasi-reversible redox peaks indicating the characteristic of heme Fe(III)/Fe(II) redox couples of Hb. According to Wang et al., both ΔE and the anodic or cathodic peak current of Hb/AuNPs/PDDA-G film-modified electrode were superior to both Hb/PDDA-G/GCE and Hb/GCE. AuNPs/PDDA-G film could provide a favorable microenvironment for the proteins and plays an important role in improving the electron exchange. Also, the electrocatalytic activity of Hb on the AuNPs/PDDA-G modified electrode toward reduction of hydrogen peroxide has been investigated by cyclic voltammetry and amperometric techniques.

A label-free electrochemical DNA aptamer sensor has been developed for the detection of kanamycin using self-assembled poly-[2,5-di-(2-thienyl)-1H-pyrrole-1-(p-benzoic acid)] on AuNPs (DPB (AuNP)).[96] Authors have investigated the role of the self-assembled poly-DPB (AuNP) nanocomposite in improving sensitivity of the sensor by modifying the aptamer sensor with electrodeposited Au NPs and poly-DPB. Compared with the aptamer sensor modified with electrodeposited nanocompsite, the sensitivity of the aptamer sensor based on self-assembled poly-DPB(AuNP) nanocomposite was improved about five times. The self-assembled poly-DPB(AuNP) enhanced the conductivity of the sensor platform and led to easy electron transfer for the signal transducer. Similarly, Zaijun et al.[97] have fabricated an electrochemical immune sensor for the detection of microcystin-LR based on G-AuNP/polyDPB/AuNP/IL composite film on the GC electrode. The graphene-gold nanocomposite enhanced electron transfer of the redox in aqueous solution to the electrode and the heterogeneous electron transfer rate constant was estimated by Laviron's model. The polyDPB and Au NP were electrodeposited on G-AuNP nanocomposite to immobilize microcystin-LR antibody and improve the electrical conductivity. An ionic liquid (1-isobutyl-3-methylimidazolium bis(trifluoromethane-sulfonyl) imide) was used to improve stability of the antibody. The difference in current before and after incubation with microcystin-LR, ΔI, increased linearly with the increase of microcystin-LR concentration. According to Zaijun et al. deareasing current after incubation is because the specific interaction between microcystin-LR and antibody produces an insulating bioconjugate, which partially blocks electron transfer of $[Fe(CN)_6]^{3-/4-}$ to the electrode.

Hao et al.[98] have synthesized polycarbazole/nitrogen-doped graphene (PCZ/N-G) composite by electropolymerization of carbazole on the N-GE/GCE for fabricating a novel electrochemical sensor for 4-nitrophenol (4-NP). The CV results have indicated that the PCZ/N-G/GCE offered the highest peak current and most positive reduction potential of 4-NP compared with the bare GCE, RGO/GCE, N-G/GCE and PCZ/GCE, which reflects that the PCZ/N-G composite has the best electrocatalytic activity toward 4-NP. This enhanced electrocatalytic activity toward 4-NP has been contributed to the synergic effect of PCZ and N-G with high conductivity and large surface area, which can greatly facilitate the electron-transfer processes between the electrolyte and electrode. The fabricated electrochemical sensor based on the PCZ/N-G/GCE has indicated the excellent performance such as wide linear range (8×10^{-7} M to 2×10^{-5} M) and low detection limit (0.062 μM) for the detection of 4-NP. Recently, Lei et al.[99] have fabricated a PLL/GO/GCE (PLL = poly (L-lysine)) by the electrochemical polymerization of L-lysine on the surface of GO sheets. The fabricated electrode has been employed as a sensory platform for the fast simultaneous detection of DA and UA. The electrochemical measurements have indicated that the PLL/GO/GCE provided an increase in the anodic potential difference and a remarkable enhancement in the current responses compared to the PLL/GCE, GO/GCE, and bare GCE for both UA and DA. They have argued that the high electrocatalytic activity of PLL/GO/GCE to the redox reaction of UA and DA is related to the good combination of PLL and GO and the synergistic effect of both composites. Moreover, using DPV in the linear concentration ranges of 0.5 to 20.0 and 0.5 to 35 μM the detection limits of 0.021 and 0.074 μM have been reported for the simultaneous detection of DA and UA, while 0.031 and 0.018 μM have been reported as the detection limits for the selective detection of UA and DA, respectively.

Similarly, Jiang et al.[100] have developed a glucose sensor based on PDDA functionalized graphene decorated with CuO NPs. It was noted that PDDA could effectively improve the stability, conductivity, and ability of graphene to be further decorated with more NPs. Also, PDDA-G/CuO could be directly coated on the surface of GCE due to its super dispersibility and specific surface area without the use of any

binder. The electrochemical performance of the PDDA-G/CuO/GCE has been studied for detection of glucose by CV and chronoamperometry. The excellent sensitivity and stability of PDDA-G/CuO/GCE toward oxidation of glucose has been attributed to the synergistic effects of highly electrocatalytic CuO and remarkably electron conductive PDDA-graphene. The introduced nonenzymatic glucose sensor have provided a high sensitivity and stability over 0.4 to 4000 μM glucose concentration range with a detection limit of 0.20 μM. Goyal et al.[101] have investigated the simultaneous determination of DA and 5-hydroxytryptamine (5-HT) in human biological fluids and pharmacological formulations by using graphene and poly 4-amino-3-hydroxy-1-naphthalenesulfonic acid (G/p-AHNSA) modified screen printed carbon sensor. In their work, the conducting polymer of p-AHNSA has been employed with G to overcome the aggregation of graphene sheets and enhance stability and sensitivity. The electrochemical behavior was demonstrated using CV, SWV, whereas the surface morphology of the modified sensor was studied by EIS and FESEM. They have claimed that G/p-AHNSA modified screen printed carbon sensor indicated a remarkable electrocatalytic activity toward neurotransmitters, DA and 5-HT considering the functional groups rich polymer film and the synergistic interaction between p-AHNSA and G via π–π stacking interaction which encouraged the electron transfer. It is revealed that the presented sensor facilitated the analysis of DA and 5-HT in the concentration ranges 0.05–100 μM and 0.05–150 μM with the detection limit of 2 nM and 3 nM respectively.

Atta et al.[102] have fabricated a promising modified electrode by distributing Pt or Pd nanoparticles into conducting polymer matrix of poly (3-methylthiophene) (PMT) and used it in the simultaneous determination of ascorbic acid and dopamine. The polymer film formation method, doping level of the polymer film, the amount of deposited metal, mechanism of its deposition and deposition potential have great impacts on the electroactivity of the polymer/metal hybrid electrodes. Authors showed that the Pd nanoparticle-modified PMT electrode can sense the increase of DA at micromolar concentration in the presence of AA at millimolar level which is typical of the physiological conditions. Also, Atta's team[103] have fabricated an electrochemical sensor based on poly(N-methylpyrrole) modified with Pd nanoclusters (Pd/PMPy). It is reported that PMPy has attracted much attention as a possible alternative to PPy for technological applications in spite of its lower conductivity. The authors have argued that the methods of formation of the polymer film and Pd_{nano} play key roles in controlling the electroactivity of hybrid electrode. The difference between the electrocatalytic activity of the two polymer films prepared under different electrochemical conditions (bulk electrolysis (BE) and CV method) was attributed to the larger surface area of $Pt/PMPy_{(CV)}$. The behavior of the composite electrode is a synergic phenomenon and is independent of the individual behavior of the polymer layer and Pd_{nano}. In fact, the electroactivity of $PMPy/Pd_{nano}$ depends on the way Pd_{nano} interacts with the polymer film. Although $Pt/PMPy_{(CV)}$ was more electroactive compared to $Pt/PMPy_{(BE)}$, bringing Pd_{nano} to the polymer film changed this picture and $Pt/PMPy_{(BE)}/Pd_{nano}$ electrode provided higher electroactivity. Ternary mixtures of AA, UA, and DA, epinephrine, norepinephrine or L–DOPA were distinguished by $Pt/PMPy_{(BE)}/Pd_{nano}$ electrode and it has been applied to the simultaneous measurements of AA, DA, and UA without interference.

Singh et al.[104] have synthesized Fe_3O_4/Polythiophene (Fe_3O_4/PT) hybrid nanocomposite to combine the magnetic and electrocatalytic properties of Fe_3O_4 and the electrical property of polythiophene. They have argued that using an external magnetic field could be a good solution to overcome the problem of processability of chemically synthesized polythiophene in electrode modification. By using Fe_3O_4, the nanocomposite could be easily immobilized on the electrode surface to get Fe_3O_4/PT nanocomposites modified electrode. In this method, they have firstly prepared oleic acid-capped magnetite nanoparticles (MNPs) by co-precipitation of iron (III) chloride and iron (II) chloride. Furthermore, the nanocomposite has been synthesized by oxidative polymerization of thiophene over oleic acid-capped MNPs. It was reported that the Fe_3O_4/PT nanocomposites modified electrode have potential application in sensing of H_2O_2 with good sensitivity, reproducibility, and stability. In another work, Lakouraj et al.[105] have synthesised poly (indole-co-thiophene) (In-co-T)@Fe_3O_4 nanocomposite by emulsion polymerization. Fe_3O_4 NPs have been prepared via coprecipitation reaction. Then, Poly (In-co-T)@Fe_3O_4 nanocomposite has been synthesized through emulsion copolymerization of indole and thiophene monomers using SDS as an emulsifier and ammonium persulfate as an oxidant in the presence of Fe_3O_4 NPs. The characterization results have indicated a synergic effect in thermal stability by good interaction between polymer chain and magnetic NPs and also an increment in the conductivity of the nanocomposites. Furthermore, Lakouraj's team has studied the application of Poly (In-co-T)@Fe_3O_4 nanocomposite as an applicant for construction of electrochemical biosensors. For this goal, a CPE modified with Poly (In-co-T)@Fe_3O_4 nanocomposite have been employed for immobilization of hemoglobin (Hb). They have reported that the functional groups of Poly (In-co-T) along with magnetic properties of Fe_3O_4 provided a good affinity for immobilization of hemoglobin. Recently, Chen et al.[106] have fabricated α-lipoic acid (ALA) sensor based on a functionalized multi-walled carbon nanotubes-polyindole/Ti_2O_3 (f-MWCNTs-PIN/Ti_2O_3) nanocomposite modified

GCE. The synthesized f-MWCNTs-PIN/Ti_2O_3 nanocomposite has been investigated by XRD, FT-IR, FE-SEM, TEM, TGA, DPV, and CV. Chen's team have reported that the prepared sensor offered a good performance including wide linear range (0.39–115.8 μM), low limit of detection (2 nM), good repeatability, and operational stability.

4. Conclusion

The construction and application of conducting polymer-based nanocomposites for using as biosensors with the aim of improving the performance of these sensing devices are the particular emphasis of this review article. We provide an overview of studies on conducting polymer-based nanocomposites with focuses on the electrocatalytic activity of some important CPs, such as PANI, PPy, and PEDOT in electrochemical sensing of biomolecules. Since there are some limitations like low environmental stability for practical applications of CPs, their combination with NPs could be a good candidate for biosensing platform. Conducting polymer-based nanocomposites with controllable, dynamic properties provide following advantages:

- They exhibit good catalysis and resistance to deactivation due to the highly synergistic action between CPs and nanomaterials.
- They offer high electronic conductivity, biocompatibility and environmental stability.
- They can significantly improve the sensitivity by facilitating electron transfer.

In the future, it is possible to design new nanocomposite conducting polymers and copolymers to develop new type of enzyme and non-enzyme based bio-electronic devices.

References

[1] H. Shirakawa, E. J. Louis, A. G. MacDiarmid, C. K. Chiang, A. J. Heeger, *J. Chem. Soc. Chem. Commun.* **1977**, 578–580.
[2] K. Dutta, S. Das, D. Rana, P. P. Kundu, *Polym. Rev.* **2015**, *55*, 1–56.
[3] a) A. Guerfi, J. Trottier, I. Boyano, I. De Meatza, J. Blazquez, S. Brewer, K. Ryder, A. Vijh, K. Zaghib, *J. Power Sources* **2014**, *248*, 1099–1104; b) Y. Zhang, Y. Zhao, A. Konarov, D. Gosselink, H. G. Soboleski, P. Chen, *J. Power Sources* **2013**, *241*, 517–521.
[4] a) M. Naseri, L. Fotouhi, A. Ehsani, H. M. Shiri, *J. Colloid Interface Sci.* **2016**, *484*, 308–313; b) A. Ehsani, H. M. Shiri, E. Kowsari, R. Safari, J. Torabian, S. Hajghani, *J. Colloid Interface Sci.* **2017**, *490*, 91–96; c) M. Naseri, L. Fotouhi, A. Ehsani, S. Dehghanpour, *J. Colloid Interface Sci.* **2016**, *484*, 314–319.
[5] a) T. Otero, J. Martinez, J. Arias-Pardilla, *Electrochim. Acta* **2012**, *84*, 112–128; b) C.-F. de Lannoy, D. Jassby, D. Davis, M. Wiesner, *J. Membr. Sci.* **2012**, *415*, 718–724.
[6] Y. Xia, K. Sun, J. Ouyang, *Adv. Mater.* **2012**, *24*, 2436–2440.
[7] H. Bagheri, Z. Ayazi, M. Naderi, *Anal. Chim. Acta* **2013**, *767*, 1–13.
[8] K. Saranya, M. Rameez, A. Subramania, *Eur. Polym. J.* **2015**, *66*, 207–227.
[9] M. Ates, *Mater. Sci. Eng. C* **2013**, *33*, 1853–1859.
[10] V. Ratautaite, S. D. Janssens, K. Haenen, M. Nesládek, A. Ramanaviciene, I. Baleviciute, A. Ramanavicius, *Electrochim. Acta* **2014**, *130*, 361–367.
[11] J. Luo, J. Sun, J. Huang, X. Liu, *Chem. Eng. J.* **2016**, *283*, 1118–1126.
[12] B. V. Silva, B. A. Rodríguez, G. F. Sales, T. S. Maria Del Pilar, R. F. Dutra, *Biosens. Bioelectron.* **2016**, *77*, 978–985.
[13] Y. Oztekin, M. Tok, H. Nalvuran, S. Kiyak, T. Gover, Z. Yazicigil, A. Ramanaviciene, A. Ramanavicius, *Electrochim. Acta* **2010**, *56*, 387–395.
[14] J. Szűcs, T. Lindfors, J. Bobacka, R. E. Gyurcsányi, *Electroanalysis* **2016**, *28*, 778–786.
[15] U. Vanamo, E. Hupa, V. Yrjänä, J. Bobacka, *Anal. Chem.* **2016**, *88*, 4369–4374.
[16] J. D. Stenger-Smith, *Prog. Polym. Sci.* **1998**, *23*, 57–79.
[17] E. Erdem, M. Karakışla, M. Sacak, *Eur. Polym. J.* **2004**, *40*, 785–791.
[18] A. Ramanavičius, A. Kaušaitė, A. Ramanavičienė, *Sens. Actuators B* **2005**, *111*, 532–539.
[19] P. Jha, S. P. Koiry, V. Saxena, P. Veerender, A. K. Chauhan, D. K. Aswal, S. K. Gupta, *Macromolecules* **2011**, *44*, 4583–4585.
[20] K. Leonavicius, A. Ramanaviciene, A. Ramanavicius, *Langmuir* **2011**, *27*, 10970–10976.
[21] aR. Gangopadhyay, A. De, *Chem. Mater.* **2000**, *12*, 608–622; bW. Lei, W. Si, Y. Xu, Z. Gu, Q. Hao, *Microchim. Acta* **2014**, *181*, 707–722.
[22] D. W. Hatchett, M. Josowicz, *Chem. Rev.* **2008**, *108*, 746–769.
[23] A. P. P. Alves, R. Koizumi, A. Samanta, L. D. Machado, A. K. Singh, D. S. Galvao, G. G. Silva, C. S. Tiwary, P. M. Ajayan, *Nano Energy* **2017**, *31*, 225–232.
[24] J. Tian, D. Peng, X. Wu, W. Li, H. Deng, S. Liu, *Carbohydr. Polym.* **2017**, *156*, 19–25.
[25] M. Majumder, R. B. Choudhary, A. K. Thakur, U. Kumar, *J. Electroanal. Chem.* **2017**, *804*, 42–52.
[26] A. M. Kumar, R. S. Babu, S. Ramakrishna, A. L. de Barros, *Synth. Met.* **2017**, *234*, 18–28.
[27] N. German, A. Ramanavicius, J. Voronovic, A. Ramanaviciene, *Colloids Surf. A* **2012**, *413*, 224–230.
[28] U. Yogeswaran, S.-M. Chen, *Sensors* **2008**, *8*, 290–313.
[29] A. Chaubey, B. Malhotra, *Biosens. Bioelectron.* **2002**, *17*, 441–456.
[30] L. C. Clark, C. Lyons, *Ann. N. Y. Acad. Sci.* **1962**, *102*, 29–45.
[31] A. A. Saei, J. E. N. Dolatabadi, P. Najafi-Marandi, A. Abhari, M. de la Guardia, *TrAC Trends Anal. Chem.* **2013**, *42*, 216–227.
[32] N. Hui, S. Wang, H. Xie, S. Xu, S. Niu, X. Luo, *Sens. Actuators B* **2015**, *221*, 606–613.

[33] Q. Huang, X. Lin, C. Lin, Y. Zhang, S. Hu, C. Wei, *RSC Adv.* **2015**, *5*, 54102–54108.
[34] Y. Liu, S.-S. Chen, A.-J. Wang, J.-J. Feng, X. Wu, X. Weng, *Electrochim. Acta* **2016**, *195*, 68–76.
[35] J. Ju, W. Chen, *Anal. Chem.* **2015**, *87*, 1903–1910.
[36] X. Qin, Y. Yin, H. Yu, W. Guo, M. Pei, *Biosens. Bioelectron.* **2016**, *77*, 752–758.
[37] M. Amiri, M. Salavati-Niasari, A. Akbari, *Microchim. Acta* **2017**, *184*, 825–833.
[38] T. Ahuja, I. A. Mir, D. Kumar, *Biomaterials* **2007**, *28*, 791–805.
[39] Deepshikha, T. Basu, *Anal. Lett.* **2011**, *44*, 1126–1171.
[40] L. Xia, Z. Wei, M. Wan, *J. Colloid Interface Sci.* **2010**, *341*, 1–11.
[41] R. A. Sheldon, S. van Pelt, *Chem. Soc. Rev.* **2013**, *42*, 6223–6235.
[42] A. D. Chowdhury, R. Gangopadhyay, A. De, *Sens. Actuators B* **2014**, *190*, 348–356.
[43] V. Mazeiko, A. Kausaite-Minkstimiene, A. Ramanaviciene, Z. Balevicius, A. Ramanavicius, *Sens. Actuators B* **2013**, *189*, 187–193.
[44] D. Zhai, B. Liu, Y. Shi, L. Pan, Y. Wang, W. Li, R. Zhang, G. Yu, *ACS Nano* **2013**, *7*, 3540–3546.
[45] B. H. Nguyen, B. T. Nguyen, H. Van Vu, C. Van Nguyen, D. T. Nguyen, L. T. Nguyen, T. T. Vu, L. Dai Tran, *Curr. Appl. Phys.* **2016**, *16*, 135–140.
[46] H. Zhong, R. Yuan, Y. Chai, W. Li, X. Zhong, Y. Zhang, *Talanta* **2011**, *85*, 104–111.
[47] D.-M. Kim, S. J. Cho, C.-H. Cho, K. B. Kim, M.-Y. Kim, Y.-B. Shim, *Biosens. Bioelectron.* **2016**, *79*, 165–172.
[48] S. Tuncagil, C. Ozdemir, D. O. Demirkol, S. Timur, L. Toppare, *Food Chem.* **2011**, *127*, 1317–1322.
[49] C. Ozdemir, F. Yeni, D. Odaci, S. Timur, *Food Chem.* **2010**, *119*, 380–385.
[50] B. Batra, M. Yadav, C. S. Pundir, *Biochem. Eng. J.* **2016**, *105*, 428–436.
[51] N. Chauhan, S. Chawla, C. Pundir, U. Jain, *Biosens. Bioelectron.* **2017**, *89*, 377–383.
[52] A. K. Pandey, P. C. Pandey, N. R. Agrawal, I. Das, *J. Appl. Polym. Sci.* **2018**, *135*.
[53] L. He, B. Cui, J. Liu, Y. Song, M. Wang, D. Peng, Z. Zhang, *Sens. Actuators B* **2018**, *258*, 813–821.
[54] L. Yang, S. Liu, Q. Zhang, F. Li, *Talanta* **2012**, *89*, 136–141.
[55] Q. Xu, J. Leng, H.-b. Li, G.-j. Lu, Y. Wang, X.-Y. Hu, *React. Funct. Polym.* **2010**, *70*, 663–668.
[56] C.-C. Hung, T.-C. Wen, Y. Wei, *Mater. Chem. Phys.* **2010**, *122*, 392–396.
[57] X. Li, M. Yu, Z. Chen, X. Lin, Q. Wu, *Sens. Actuators B* **2017**, *239*, 874–882.
[58] P. Manivel, M. Dhakshnamoorthy, A. Balamurugan, N. Ponpandian, D. Mangalaraj, C. Viswanathan, *RSC Adv.* **2013**, *3*, 14428–14437.
[59] Z. Zheng, Y. Du, Q. Feng, Z. Wang, C. Wang, *J. Mol. Catal. A* **2012**, *353*, 80–86.
[60] N. Ruecha, R. Rangkupan, N. Rodthongkum, O. Chailapakul, *Biosens. Bioelectron.* **2014**, *52*, 13–19.
[61] Y. Bo, H. Yang, Y. Hu, T. Yao, S. Huang, *Electrochim. Acta* **2011**, *56*, 2676–2681.
[62] I. Pandey, R. Kant, *Biosens. Bioelectron.* **2016**, *77*, 715–724.
[63] H. Zhong, R. Yuan, Y. Chai, Y. Zhang, C. Wang, F. Jia, *Microchim. Acta* **2012**, *176*, 389–395.
[64] Q. Sheng, M. Wang, J. Zheng, *Sens. Actuators B* **2011**, *160*, 1070–1077.
[65] H.-J. Kim, S. H. Park, H.-J. Park, *Radiat. Phys. Chem.* **2010**, *79*, 894–899.
[66] J. Liang, M. Wei, Q. Wang, Z. Zhao, A. Liu, Z. Yu, Y. Tian, *Anal. Lett.* **2018**, *51*, 512–522.
[67] K. Ghanbari, Z. Babaei, *Anal. Biochem.* **2016**, *498*, 37–46.
[68] Z. Yu, H. Li, X. Zhang, N. Liu, W. Tan, X. Zhang, L. Zhang, *Biosens. Bioelectron.* **2016**, *75*, 161–165.
[69] T. P. Tsele, A. S. Adekunle, O. E. Fayemi, E. E. Ebenso, *Electrochim. Acta* **2017**, *243*, 331–348.
[70] K. Ghanbari, F. Ahmadi, *Anal. Biochem.* **2017**, *518*, 143–153.
[71] J. Wilson, S. Radhakrishnan, C. Sumathi, V. Dharuman, *Sens. Actuators B* **2012**, *171*, 216–222.
[72] a) L.-X. Wang, X.-G. Li, Y.-L. Yang, *React. Funct. Polym.* **2001**, *47*, 125–139; b) A. Ramanaviciene, A. Ramanavicius, *Crit. Rev. Anal. Chem.* **2002**, *32*, 245–252.
[73] K. Xue, Y. Xu, W. Song, *Electrochim. Acta* **2012**, *60*, 71–77.
[74] X. Lu, Y. Li, J. Du, X. Zhou, Z. Xue, X. Liu, Z. Wang, *Electrochim. Acta* **2011**, *56*, 7261–7266.
[75] H. Dai, P. Cao, D. Chen, Y. Li, N. Wang, H. Ma, M. Lin, *Synth. Met.* **2018**, *235*, 97–102.
[76] A. L. Khan, R. Jain, *Ionics* **2018**, 1–16.
[77] P. M. Nia, W. P. Meng, F. Lorestani, M. Mahmoudian, Y. Alias, *Sens. Actuators B* **2015**, *209*, 100–108.
[78] P. M. Nia, F. Lorestani, W. P. Meng, Y. Alias, *Appl. Surf. Sci.* **2015**, *332*, 648–656.
[79] S. Singal, A. K. Srivastava, *Nano-Micro Lett.* **2017**, *9*, 7.
[80] L. Wang, S. Tricard, P. Yue, J. Zhao, J. Fang, W. Shen, *Biosens. Bioelectron.* **2016**, *77*, 1112–1118.
[81] C. Ott, M. D. Raicopol, C. Andronescu, E. Vasile, A. Hanganu, A. Pruna, L. Pilan, *Synth. Met.* **2018**, *235*, 20–28.
[82] A. Balamurugan, K.-C. Ho, S.-M. Chen, T.-Y. Huang, *Colloids Surf. A* **2010**, *362*, 1–7.
[83] K.-C. Lin, T.-H. Tsai, S.-M. Chen, *Biosens. Bioelectron.* **2010**, *26*, 608–614.
[84] W. Wang, G. Xu, X. T. Cui, G. Sheng, X. Luo, *Biosens. Bioelectron.* **2014**, *58*, 153–156.
[85] G. Sheng, G. Xu, S. Xu, S. Wang, X. Luo, *RSC Adv.* **2015**, *5*, 20741–20746.
[86] Y.-P. Hsiao, W.-Y. Su, J.-R. Cheng, S.-H. Cheng, *Electrochim. Acta* **2011**, *56*, 6887–6895.
[87] F. Jiang, R. Yue, Y. Du, J. Xu, P. Yang, *Biosens. Bioelectron.* **2013**, *44*, 127–131.
[88] L. Sheng, Z. Li, A. Meng, Q. Xu, *Sens. Actuators B* **2018**, *254*, 1206–1215.
[89] M. Cui, Z. Song, Y. Wu, B. Guo, X. Fan, X. Luo, *Biosens. Bioelectron.* **2016**, *79*, 736–741.
[90] T. Yang, Y. Gao, Z. Liu, J. Xu, L. Lu, Y. Yu, *Sens. Actuators B* **2017**, *239*, 76–84.

[91] G.-X. Wang, Y. Qian, X.-X. Cao, X.-H. Xia, *Electrochem. Commun.* **2012**, *20*, 1–3.
[92] W. Lei, L. Wu, W. Huang, Q. Hao, Y. Zhang, X. Xia, *J. Mater. Chem. B* **2014**, *2*, 4324–4330.
[93] W. Si, W. Lei, Z. Han, Y. Zhang, Q. Hao, M. Xia, *Sens. Actuators B* **2014**, *193*, 823–829.
[94] I. M. Taylor, E. M. Robbins, K. A. Catt, P. A. Cody, C. L. Happe, X. T. Cui, *Biosens. Bioelectron.* **2017**, *89*, 400–410.
[95] Q. Feng, K. Liu, J. Fu, Y. Zhang, Z. Zheng, C. Wang, Y. Du, W. Ye, *Electrochim. Acta* **2012**, *60*, 304–308.
[96] Y. Zhu, P. Chandra, K.-M. Song, C. Ban, Y.-B. Shim, *Biosens. Bioelectron.* **2012**, *36*, 29–34.
[97] L. Ruiyi, X. Qianfang, L. Zaijun, S. Xiulan, L. Junkang, *Biosens. Bioelectron.* **2013**, *44*, 235–240.
[98] Y. Zhang, L. Wu, W. Lei, X. Xia, M. Xia, Q. Hao, *Electrochim. Acta* **2014**, *146*, 568–576.
[99] Y. Zhang, W. Lei, Y. Xu, X. Xia, Q. Hao, *Nanomaterials* **2016**, *6*, 178.
[100] J. Yang, Q. Lin, W. Yin, T. Jiang, D. Zhao, L. Jiang, *Sens. Actuators B* **2017**, *253*, 1087–1095.
[101] M. Raj, P. Gupta, R. N. Goyal, Y.-B. Shim, *Sens. Actuators B* **2017**, *239*, 993–1002.
[102] N. F. Atta, M. F. El-Kady, *Sens. Actuators B* **2010**, *145*, 299–310.
[103] N. F. Atta, M. F. El-Kady, A. Galal, *Anal. Biochem.* **2010**, *400*, 78–88.
[104] B. Singh, R.-A. Doong, D. S. Chauhan, A. K. Dubey, *Mater. Chem. Phys.* **2018**, *205*, 462–469.
[105] M. Rouhi, M. Mansour Lakouraj, M. Baghayeri, V. Hasantabar, *Int. J. Polym. Mater.* **2017**, *66*, 12–19.
[106] R. Sasikumar, P. Ranganathan, S.-M. Chen, S.-P. Rwei, *Sens. Actuators B* **2018**, *255*, 217–225.

Received: November 6, 2017
Accepted: February 5, 2018
Published online on ■■ ■■, ■■■■

PERSONAL ACCOUNT