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Polymer Nanocomposite-Based Electrochemical Sensors and Biosensors

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Abstract

Polymer nanocomposites (PNCs) play a significant role in modern day life and are widely studied for extensive properties which make them appealing to numerous applications. They are synthesized with scalable processing procedures with several nanoscale variations of fillers and forms leading to specific sensing applications. In this chapter, PNC-based electrochemical sensors and biosensors like DNA biosensors and immunosensors are discussed. These sensors related PNC applications uses nanofillers of various combinations like conductive polymers with graphene (Grp), carbon nanotubes (CNTs), and metal nanoparticles, which endow high electrical conductivity, effective surface area, and fast electron transfer rate. Currently, wearable devices based on electrochemical Sensors and biosensors have been of great interest in the detection of both physiological and environmental analytes.

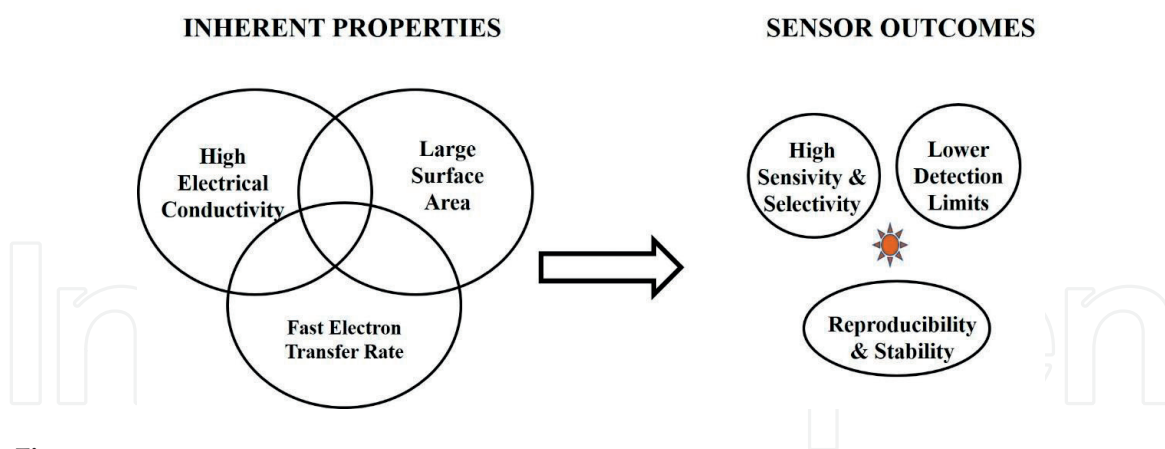
Keywords: polymer nanocomposites, electrochemical sensors, biosensors, DNA sensors, immunosensors

1. Introduction

Polymer nanocomposites (PNCs) have electrochemical properties as transducers which can be used for the manufacturing of electrochemical sensors and biosensors. They possess significant variations in responsiveness, synthesis, and morphology, which help in a significant level of variations in conductivity [1]. Apart from the economic aspect of the PNC-based sensors, the improved performance on the electronic side stands apart among its peers through the basal plane ratio of the nanofillers, method of doping, kinetic properties of the electrode, biological response and environmental impact [1]. The impact of nanofillers in PNCs plays a significant role in sensing, processing, and actuating capabilities of the electrodes of electrochemical and biosensing applications [2].

The “active states of PNCs” rests on three pillars: high electrical conductivity rate, large surface area and fast electron rate which leads to best electricidal sensor outcomes. PNCs helps in the material technological advancement of electrochemical sensors which have high sensitivity and selectivity, lower detection limits, reproducibility and stability as shown in **Figure 1**. All these increased used the PNCs in electrochemical sensor research which were manufactured through chemical synthesis or polymerization methods and could be easily scaled up for

PNC based Electrochemical Sensors and Biosensors

**Figure 1.**

Key properties of PNC based electrochemical sensors and biosensors. With permission from Elsevier [1].

various applications [3]. The electrochemical sensors along with the immunosensors and biosensors are becoming the norm of the day. Detections limits and sensing technologies are improved consistently due to developments happening in the unique properties of PNCs especially conductivity and electrochemical activity. The interactive fillers facilitate ion diffusion that impacts the sensing applications through intercalation into the PNC matrices leading to better stability of active electron transfer sites and detection limits. These active fillers help in reducing the layer thickness in PNC leading to ultrathin electrochemical detector technology. PNCs stand as an outstanding leader with significant advantages in large specific interaction surface area, reduced dimension of fillers and efficient electron transfer rate [3].

2. Electrochemical sensors

PNCs are widely used in the development of electrochemical sensors. The electrochemical sensors are based on three categories of PNCs. PNCs of conductive polymers and inorganic nanomaterials, PNCs of conductive polymers and Grp, and PNCs of conductive polymers and CNTs. Once interaction has occurred between the PNC-based electrochemical sensors and the target analyte, an electronic signal is detected by the transduction system. The applications of PNC-based electrochemical sensors different materials are shown in **Table 1**.

2.1 Polymer nanocomposites based on conductive polymers and inorganic nanomaterials

Metal and metal oxide nanoparticles have been extensively studied as electrochemical sensing materials due to such beneficial features as their small size; unique chemical, physical, and electronic properties; flexibility in fabricating novel and improved sensing devices; and good sensitivity to the ambient conditions are shown in **Table 1**. The assimilation of nanoparticles of metals into PNC matrices set the stage for enhanced electrocatalytic electrode detection leading to multiple modern-day applications. For example, a Zinc oxide nanoparticle intercalated into polypyrrole (ZnO-PPy) PNC showed excellent Xanthine detection by through xanthine oxidase enzyme immobilization [4]. A glassy carbon electrode (GCE) modified with ultrathin polypyrrole nanosheets decorated with Ag nanoparticles was fabricated for the detection of hydrogen peroxide (H_2O_2). The modified device showed

Sensory material	Analyte	Detection limit
PPy-ZnO-Pt	Xanthine	0.8 μM
PPy-Pt-GCE	Hydrogen peroxide	0.6 μM
PANI-TiO ₂ -GCE	Glucose	0.5 μM
PANI-NiCo ₂ O ₄ -GCE	Glucose	0.3833 μM
PANI-Grp-GCE	4-aminophenol	$6.5 \times 10^{-8} \text{ M}$
PANI-Grp-ITO	Artesunate	0.012 ng mL^{-1}
PANI-Grp-GCE	Lercanidipine	1.94 ng mL^{-1}
PANI-Grp-GCE	Nitazoxanide	2.2 $\mu\text{g mL}^{-1}$
PPy-Grp-GCE	Adenine	0.02 μM
	Guanine	0.01 μM
PPy-PIL-GO-GCE	Dopamine	73.3 nM
PEDOT-rGO-GCE	Dopamine	39.0 nM
PEDOT-Grp-GCE	Ascorbic acid	2.0 μM
PANI-Grp-Bi ₂ O ₃ -GCE	Etodolac	10.03 ng mL^{-1}
PANI-rGO-MIP-AuNP-GCE	Serotonin	11.7 nmol L^{-1}
PPy-MWCNT-ITO	Cholesterol	0.04 mM L^{-1}
PPy-MWCNT-GCE	Pemetrexed	$3.28 \times 10^{-9} \text{ M}$
PEDOT-CNT-CPE	Hydroquinone	0.3 μM
PEDOT-CNT-CPE	Dopamine	20.0 nM
PEDOT-CNT-CPE	Nitrobenzene	83.0 nM

Table 1.
Electrochemical sensors based on polymer nanocomposites [1].

high sensitivity toward the reduction of H₂O₂ [5]. Similar electrochemical sensor based on polypyrrole–platinum (PPy-Pt) PNC was fabricated for the detection of H₂O₂ [6]. Another voltammetric sensor based on a polyaniline-gold nanoparticle (PANI-AuNP) PNC deposited on GCE was used for the detection of epinephrine (EP) and uric acid (UA) [6]. Exploiting the advantages of PNCs, two GCEs modified with PANI-TiO₂ and PANI-NiCo₂O₄ PNC-based electrochemical sensors were developed for the detection of glucose [7]. TiO₂ nanotubes (TNTs) was intercalated into a PANI-TNT PNC composite for through hydrothermal method for the detection (a reported sensitivity of 11.4 $\mu\text{A mM}^{-1}$) of glucose (a reported sensitivity of 11.4 $\mu\text{A mM}^{-1}$) by the immobilization of glucose oxidase (GOD) [7].

2.2 Polymer nanocomposites based on conductive polymers and graphene

Graphene (Grp), an allotrope of carbon, has become the new material of interest and widely integrated into the sensor research from the beginning of this millennium due to its unique properties of electrical conduction and 2-dimensional existence. Grp-PNC-based electrochemical sensors are used for electroanalytical detection of target molecules with high precision of selectivity and sensitivity as shown in **Table 1**, which showed spectacular detection limits over a wide range. An electrochemical sensor fabricated for the detection of 4-aminophenol (4-AP) using a PANI-Grp-GCE-modified device showed a detection limit of $6.5 \times 10^{-8} \text{ M}$ and sensitivity of 604.2 $\mu\text{A mM}^{-1}$ [8]. A sensor was fabricated with a PANI-Grp-based PNC onto an ITO plate with immobilized horseradish peroxidase enzyme with a

sensitivity limit of $0.15 \text{ mA ng mL}^{-1}$ [9]. A PANI-Grp-GCE-based PNC sensor for the elimination of calcium antagonist lercanidipine in pharmaceutical formulations for medical purposes showed a detection limit in the range from 5 to 125 ng mL^{-1} [10]. The same PANI-Grp-GCE-based PNC sensor showed the detection of nitazoxanide compound which was an added advantage [11]. Electrochemical sensors based on PPy-based PNC are becoming popular these days due to their specific applications through their overoxidized form polypyrrole (PPyox). Fabrication of polypyrrole-graphene (PPyox/Grp) helped in the simultaneous detection of adenine and guanine through an electrodeposition method. PPy-Grp composite was electro-polymerized with pyrrole and graphene oxide (GO), followed by electrochemical reduction of GO composite. The electrochemical sensor's significant improvement in the sensing of adenine and guanine is due to the specific structure of the nanocomposite. The adenine and guanine showed strong π - π interactions, and cationic selectivity [12].

The detection of Dopamine (DA) using PNCs was the holy grail in neurochemical studies as it is a prominent neurotransmitter, which plays a role in neurological disorders such as Parkinson's disease and schizophrenia [13]. A poly(ionic liquid)-functionalized polypyrrole-graphene oxide (PIL-PPy-GO)-based PNC electrochemical sensor was fabricated by the polymerization of 1-vinyl-3-ethylimidazolium bromide on N-vinyl imidazolium-modified PPy-GO films. The PILs helped in changing the surface charge which dispersibility of the PIL-PPy-GO composite and helped in the detection of DA [14]. Another sensor used for the detection of DA was a PNC-based poly(3,4-ethylene dioxythiophene)-graphene oxide (PEDOT-GO) fabricated by electrodeposition showed significant sensing capabilities [15]. A one-step electrochemical redox synthesis process of PEDOT-Grp PNC film was done using simultaneous electrodeposition of PEDOT and electrochemical reduction of GO on a GCE with high detection sensing of the ascorbic acid molecules. In this sensor PEDOT-Grp thin film PNC mediated the electron transfer between AO and electrode interface resulting in significant improvement in electrocatalytic activity and sensitivity of ascorbic acid molecules [16]. Jain et al. [5] introduced the combination of Grp and a conducting PANI-Bi₂O₃ PNC, the synergic effect of which enhanced the performance of sensors used for the electrocatalytic oxidation of etodolac in pharmaceutical formulations.

In recent years, molecularly imprinted polymers (MIPs) with high selectivity, affinity, chemical stability, and easy preparation for the template molecule are a promising candidate for developing a new generation of recognition elements for sensors. A double-layered membrane-sensing interface was fabricated based on rGO-PANI nanocomposites and MIPs embedded with AuNPs for sensitive and selective detection of serotonin (5-hydroxytryptamine, 5-HT). The obtained sensor showed remarkable selectivity to serotonin against the interferences caused by ascorbic acid and other interferents with a good detection limit of 11.7 nmol L^{-1} [17].

2.3 Polymer nanocomposites based on conductive polymers and carbon nanotubes

PNCs based on conductive polymers helped in improving the sensing properties of the electrochemical sensors with enhanced selectivity and stability. Some of the popular CNT-based PNC reported in the literature are shown in **Table 1**. A PPy-multiwalled carbon nanotube (MWCNT)-toluene sulfonic acid-based PNC was fabricated for the detection of cholesterol with ITO-coated glass as the substrate for the sensor. The sensor showed high sensitivity and a fast response time of 9 s [18]. Sodium dodecyl sulfate-doped PPyox) with carboxylic acid functionalized MWCNT-modified GCE were reported for the detection of the anticancer drug

pemetrexed (PMX). The results showed that overoxidation of the PPy film conferred a negative charge density on the porous layer, which in turn enhances the adsorption of PMX [19]. Xu et al. fabricated a carbon paste electrode (CPE) modified with a PEDOT-CNT nanocomposite. They used this electrode for the analysis of hydroquinone, DA, and nitrobenzene [20].

3. DNA biosensors

PNCs are widely used these days in DNA biosensors. The medico biological field is growing leaping and bounds. In this era of 23 and me everything possible with DNA is bouncing through the boundaries of technology like DNA CRISPR editing, gene mapping. Biological agents for nefarious purposes and forensics. A basic DNA sensor work on a simple principle. You plant a DNA probe on a surface and this planted DNA chain hybridizes with its complementary pair. This hybridization technically called transduction can be detected optically and electrochemically. The electrochemical detection of transducers through electrochemical sensors leads us to DNA biosensors and are now extremely popular. The recent progress in the studies is summarized in **Table 2** and discussed in the section below.

The schematic illustration of the most popular DNA biosensor based on polyaniline-gold nanoparticle-chitosan-graphene sheet (PANI-AuNP-Cts-GS) composite with a functional capture probe for the detection of BCR/ABL fusion gene in chronic myelogenous leukemia (CML) is shown in **Figure 2**. The capture probe used a hairpin structure and was dually labeled with a 5'-SH and a 3'-biotin. The biotin electrode probe was used for the detection of streptavidin-alkaline phosphatase (AP) enzyme which in turn cause an electrochemical signal caused by the catalytic reduction of 1-naphthyl phosphate to 1-naphthol picked up by Diffuse Pulse Voltammetry (DPV) with a detection range of 10–1000 pM [21]. A DNA biosensor fabricated with PANI–AuNP PNC was used for the detection of Ag⁺. It works on the following principle: the electrochemical biosensor regenerates cysteine leading to the release of Ag⁺ from the cytosine to Ag⁺-Cytosine complex and reused again. The fabricated biosensor showed excellent selectivity with a good detection limit for silver ions [22]. Another DNA electrochemical biosensor was developed using polyaniline nanofibers (PANI-nf) enrapturing AuNPs making (PANI-nf-AuNP), a PNC. Gold was used as the electrode for the detection of *Staphylococcus aureus* DNA from the PANI-nf-AuNPs sensor, where the detection concentration varied from 150 × 10⁻¹² to 1 × 10⁻⁶ mol L⁻¹ [23]. A DNA biosensor based on the PANI-Fe₃O₄-CNT PNC was manufactured for sensing *Neisseria gonorrhoeae* through a DNA probe. The fabricated biosensor showed sensing in the range from 1 × 10⁻¹⁹

Sensor	Analyte	Detection limit
PANI-AuNP-GS-Cts-GCE	BCR/ABL fusion gene	2.11 pM
PANI-AuNP-Au	Silver ions	10 pM
PANI-AuNP-Au	DNA sequence associated with <i>S. aureus</i>	150 pM ⁻¹ μM
PPy-PANI-AuNP-Au	15-mer DNA oligonucleotides	1.0 × 10 ⁻¹³ M
PPy-PEDOT-AgNP-GCE	27-mer DNA oligonucleotides	5.4 ± 0.3 × 10 ⁻¹⁵ M
PANI-Fe ₃ O ₄ -CNT-ITO	<i>Neisseria gonorrhoeae</i>	1 × 10 ⁻¹⁹ M
PANI-AuNP-GSPE	microRNA-16	0.1 nM

Table 2.
Polymer nanocomposite-based DNA sensors [1].

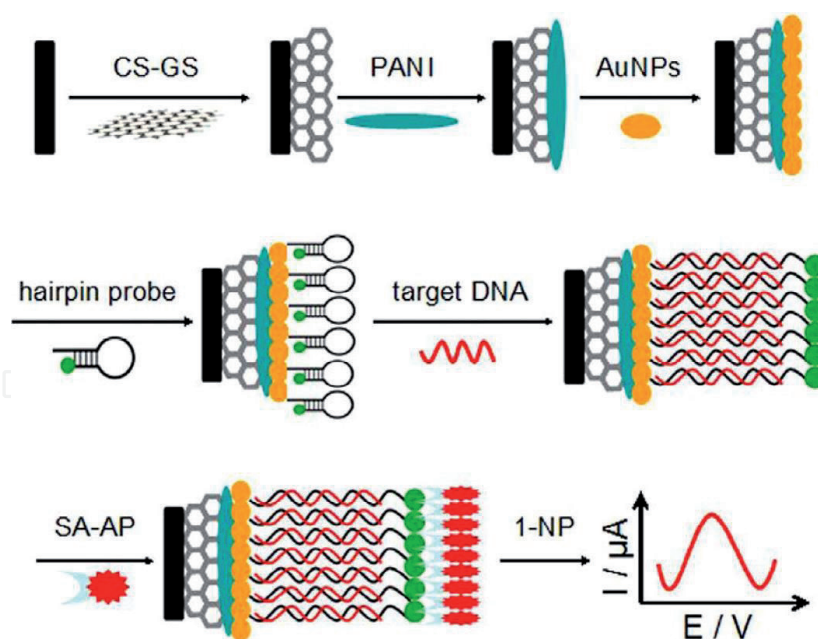


Figure 2.

Schematic illustration of the DNA sensor construction process. With permission from Elsevier [1].

to 1×10^{-6} M through DPV measurements [24]. The most recent DNA biosensor based on PANI-AuNPs PNC detected the microRNA-16 using a streptavidin-AP conjugate to biotinylated target sequences through transduction with a detection limit of 0.1 nM [25]. DNA biosensor made with polypyrrole-polyaniline-gold (PPy-PANI-Au) PNC responded to the target DNA through transduction, non-complementary and single- and double-base-mismatched target DNA-chains with a detection limit between 1×10^{-6} and 1×10^{-13} M [26]. Nanotube DNA biosensor based on polypyrrole and poly(3,4-ethylenedioxythiophene) (PPy-PEDOT) PNC, which was functionalized with Ag nanoparticles sensed DNA transduction through EIS detection. The DNA chains used for detection were thiol-capped on the modified sensor [27].

4. Electrochemical immunosensors

PNCs are superior candidates for the fabrication of electrochemical Immunosenors, where the antibodies are the probes which form ionic complexes with the corresponding antigen pair with a specific target. Electrochemical Immunosenors are becoming widely used in clinical diagnosis applications, doping or impurities or detecting biological components in the food industry and detecting the biomolecules of environmental origin and impact. The most widely reported are discussed in this section and shown in **Table 3**. The immunosenors based on CNT-PPy-goat IgGs showed the interaction between the goat IgGs and its anti-goat IgGs, which changes the charges at the sensor surface with changes in conductance level. The response time for the anti-goat IgG was 1 min [28]. A label-free impedance immunosensor for human chorionic gonadotropin (hCG) detection using a PPy-PPa-hCG-modified carbon ink electrode was fabricated by the deposition of a PPy-pyrrole-2-carboxylic acid copolymer. The hCG antibody was immobilized via the COOH groups of pyrrole-2-carboxylic acid, as a linker for covalent biomolecular immobilization. This immunosensor has a detection limit of the hCG antigen was in the range of 100 pg mL^{-1} to 40 ng mL^{-1} [29]. The next progress was PANI-AuNP hybrid electrochemical immunosensor with the gold electrode for the detection of prostate antigen (PSA). The immunosensor showed effective immobilization

Sensor	Analyte	Detection limit
CNT-PPy-microelectrode	Anti-goat IgG	0.05 $\mu\text{g mL}^{-1}$
PPy-PPa-carbon ink	hC	2.3 pg mL^{-1}
PANI-AuNP-Au	Prostate-specific antigen	0.6 pg mL^{-1}
Grp-DPB-AuNP-Au	Aflatoxin B1	1.0 fM
Au-PPy-GCE	Ofloxacin	0.03 ng mL^{-1}
Grp-AuNP-DPB-AuNPs-IL-GCE	Microcystin-LR	3.7×10^{-17} M
Grp-PANI-GCE	Estradiol	0.02 ng mL^{-1}
PANI-GO-CdSe-GCE	Interleukin-6	0.17 pg mL^{-1}
PEDOT-AuNP-ZnSe-Azure I-Pt	Alpha-Fetoprotein	1.1 fg mL^{-1}
PANI-AuNP-PWE	Carcinoembryonic antigen	0.50 pg mL^{-1}
	α -fetoprotein	0.80 pg mL^{-1}
pPPA-MWCNT-GCE	Prolactin	3 pg mL^{-1}
Pt(MPA)NP-PPy-ITO	C-reactive protein (α CRP)	4.54 ng mL^{-1}
PPy-PPa-rGO	Aflatoxin B1	10 fg mL^{-1}
PANI-Au-AMNP-NPG	Carbohydrate antigen 72-4	0.10 U mL^{-1}
AuNP-FC-PANI-GCE	Carcinoembryonic antigen	0.1 pg mL^{-1}

Table 3.
Electrochemical immunosensors based on polymer nanocomposite [1].

of anti-PSA with excellent sensing performance ($1.4 \mu\text{A M}^{-1}$) and detection limit (0.6 pg mL^{-1}) through effective electron transport [30]. For the detection of aflatoxin B1, an electrochemical immunosensor based on a Grp-CP-AuNP-IL composite film was used. The fabrication was in a five-part series mode as Grp-CP-AuNP-IL pattern. Poly(DPB), 2,5-di-(2-thienyl)-1-pyrrole-1-(p-benzoic acid) helped in the electrochemical stability as a CP. The covalent bonding through the antibody immobilization via carbonyl groups of the polymer helped in preventing the antibody loss, resulting in a detection limit of 1.0 fM [31]. For ofloxacin detection, an immunosensor was fabricated based on a dual-amplification mechanism resulting from Au nanoclusters embedded in the pre-synthesized PPy film as the sensor platform and multienzyme antibody-functionalized gold nanorods as the label. The electrochemical response was in the range of 0.08 and 410 ng mL^{-1} with a low detection limit of 0.03 ng mL^{-1} [32].

An electrochemical immunosensor was fabricated a PNC-based Grp-AuNP-poly-DPB-AuNP-IL for the detection of microcystin-LR through electrodeposition method on GCE. In this electrochemical sensor, the Grp-gold helped in the electron transfer of $[\text{Fe}(\text{CN})_6]^{3-}$, and the poly 2,5-di-(2-thienyl)-1-pyrrole-1-(p-benzoic acid)- gold nanoparticle (poly-DPB-AuNP) enhanced the electrical conduction and subsequent immobilization of the microcystin-LR antibody [33]. A Grp-PANI-based PNC electrochemical sensor for the estradiol using horseradish peroxidase-graphene oxide-antibody (HRP-GO-Ab) was designed where carboxylated GO serves the antibody carrier property while the horseradish peroxidase helped in catalytic hydrogen reduction on the electrode. This estradiol immunosensor detected the estradiol in tap water and milk samples, with average recoveries of 97.25% and 96.6%, respectively [34]. The electrochemical immunosensors with electrochemiluminescence (ECL) sensing property was achieved through Quantum dots (QDs). This was based on graphene oxide nanosheet-polyaniline nanowire-CdSe quantum dot (GO-PANI-CdSe) which detected human interleukin-6 (IL-6) [35]. A ZnSe QD-Azure

I-AuNP-PEDOT-modified Pt electrode electrochemical immunosensor helped in the detection of alpha-fetoprotein (AFP) through electrochemiluminescence (ECL) sensing (detection limit $\sim 1.1 \text{ fg mL}^{-1}$). The sensing mechanism was as follows: ZnSe QDs immobilize the antibody, the nanoAu-PEDOT facilitated the electron transfer, and Azure I did the catalytic reduction of redox dye with two active amino groups [36].

PANI-AuNP-modified paper working electrodes (PANI-AuNP PWEs) were fabricated for the simultaneous determination of two tumor markers, carcinoembryonic antigen (CEA) and AFP, in real human serum samples [37]. An electrochemical immunosensor for prolactin hormone was also constructed by immobilizing the antigen onto poly (pyrrolepropionic acid) CP and carbon nanotube (pPPA/CNT) hybrids deposited onto a GCE and labeled with AP enzyme with a reported detection limit of 104 ng mL^{-1} [38]. Polypyrrole (PPy)-based PNC was used to manufacture a bioelectrode for the detection of human C-reactive protein antigen (Ag- α CRP). This was made possible with the inorganic nanoparticles (3-mercaptopropionic acid (MPA)-capped Pt nanoparticles. First, the Ab- α CRP was immobilized covalently through specific carboxyl groups linkages through Pt(MPA)- NPs within the polypyrrole (PPy)-based PNC film by carbodiimide coupling. The resulted electrochemical immunosensor showed excellent fine probe orientation with a detection capacity of 10 ng mL^{-1} – $10 \mu\text{g mL}^{-1}$ [39]. Another label-free impedimetric immunosensor based on multifunctional PNCs was based on (polypyrrole–pyrrolepropylic acid–reduced graphene oxide (PPy-PPa-rGO)) for the detection of mycotoxin aflatoxin B1 [40]. An enzyme-free electrochemical immunosensor modeled on the *sandwich* pattern was used for the detection of carbohydrate antigen 72–4 (CA72–4). The sensing electrode was nanoporous gold (NPG) film and asymmetric multicomponent (AMNPs) nanoparticles based on PANI-Au was used as labels. The NPG helped in the creased immobilization of Ab1 on the electrode, while the PANI-Au AMNPs impacted on the reduction capability of the electrochemical immunosensor [41]. The doping of AuNPs and PANI films with potassium ferricyanide over a gold electrode was used to detect a carcinoembryonic antigen (CEA). This PNC-based biocompatible electrochemical immunosensor showed excellent conductivity and redox electroactivity. The detection of CEA was analyzed through electrode response of $[\text{Fe}(\text{CN})_6]^{3-}$ as the redox mediator [42].

5. Conclusion

PNCs have been explored for the construction of novel biosensors using PNC like materials as sensing elements. The efficient combination of different nanoscaled materials with good conductive polymers open a new avenue for utilizing novel PNCs as enhanced elements for constructing electrochemical sensing platforms with high performance. Health monitoring wearable tech like Fitbit or Apple Watch are all based on the PNC electrochemical sensors are of great interest in the health industry for the detection of physiological parameters of the human body. The progress of PNC-based wearable electrochemical sensors to analyses biochemical fluids other than blood such as interstitial fluids, sweat, tears, and saliva invoked interests in Silicon Valley echelons like Google, OrSense, and NovioSense which made the sector more interesting. Another significant challenge is the technical challenges to the wearability of the PNC-based material for manufacturing the same which includes analytical performance and biocompatibility. There is significant progress reported on the PANI-based wearable immunosensor used for epidermal pH monitoring. With all these advances the future of PNC-based devices is promising and applications shall reach out to commercial sensing applications like military, health-care and community fitness initiatives.

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