HHS Public Access

Author manuscript

Anal Chim Acta. Author manuscript; available in PMC 2016 August 05.

Published in final edited form as:

Anal Chim Acta. 2015 August 5; 887: 17–37. doi:10.1016/j.aca.2015.05.049.

Recent trends in carbon nanomaterial-based electrochemical sensors for biomolecules: A review

Cheng Yang[¤], **Madelaine E. Denno**[¤], **Poojan Pyakurel**, and **B. Jill Venton**[*]

Department of Chemistry, University of Virginia

Abstract

Carbon nanomaterials are advantageous for electrochemical sensors because they increase the electroactive surface area, enhance electron transfer, and promote adsorption of molecules. Carbon nanotubes (CNTs) have been incorporated into electrochemical sensors for biomolecules and strategies have included the traditional dip coating and drop casting methods, direct growth of CNTs on electrodes and the use of CNT fibers and yarns made exclusively of CNTs. Recent research has also focused on utilizing many new types of carbon nanomaterials beyond CNTs. Forms of graphene are now increasingly popular for sensors including reduced graphene oxide, carbon nanohorns, graphene nanofoams, graphene nanorods, and graphene nanoflowers. In this review, we compare different carbon nanomaterial strategies for creating electrochemical sensors for biomolecules. Analytes covered include neurotransmitters and neurochemicals, such as dopamine, ascorbic acid, and serotonin; hydrogen peroxide; proteins, such as biomarkers; and DNA. The review also addresses enzyme-based electrodes that are used to detect non-electroactive species such as glucose, alcohols, and proteins. Finally, we analyze some of the future directions for the field, pointing out gaps in fundamental understanding of electron transfer to carbon nanomaterials and the need for more practical implementation of sensors.

1.0 Introduction

Electrochemical sensors have been widely developed as an inexpensive, simple method to sensitively detect a variety of biological analytes. Carbon based electrochemical sensors are commonly used because of their low cost, good electron transfer kinetics, good chemical stability, and biocompatibility. Traditional carbon-based sensors include glassy carbon electrodes, carbon fibers, and pyrrolytic graphite. Recently, carbon nanomaterials have been incorporated into sensors. The feature sizes of the nanomaterials are 1 to 100 nm and they are advantageous because of their large surface-to-volume ratio and specific surface area. In addition, carbon nanomaterials have enhanced interfacial adsorption properties, better electrocatalytic activity, high biocompatibility, and fast electron transfer kinetics compared to many traditional electrochemical sensor materials.[1,2]

[*]corresponding author. PO Box 400319, Charlottesville, VA 22904, jventon@virginia.edu.

[¤]these authors contributed equally to this work.

Carbon nanotubes (CNTs) are rolled up sheets of graphene that exist as hollow tubes. There are a variety of CNT types, from single-walled (SWCNT) to double-walled (DWCNT) to multi-walled (MWCNT) that have varying thickness as well as different metallic/semiconducting properties. CNTs are usually acid treated to remove the end caps, which also creates defect sites and oxygen functional groups that are thought to aid in adsorption and electron transfer.[3,4] CNTs can be deposited on electrode surfaces through dip coating or they can be directly grown on surfaces. In addition, materials such as carbon nanotube fibers and yarns are now made from CNTs. CNTs are still widely used in electrochemical biosensors, but the field of carbon nanomaterial-based biosensors has rapidly expanded in recent years to include many other materials, including many forms of graphene. Thus, in writing this updated review we decided to include these many other carbon nanomaterials.

Graphene is considered the basic building block for graphitic materials. The majority of recent electrochemical studies involving graphene have been performed using reduced graphene oxide, which is an abundant, inexpensive source material.[5] The sp^3 hybridized carbons on the edge plane and defects on basal plane can be oxidized to provide functional groups and further enhance the electron transfer with biological molecules.[4] Specific doping of graphene or CNTs with nitrogen as a heteroatom has been used to introduce defects and increase biocompatibility.[6,7] Different forms of graphene can be used including graphene nanoribbons and carbon nanohorns (CNHs), horn-shaped aggregates of graphene layers about 80 nm in diameter.[8–11] Other 3D forms of graphene include graphene flowers, graphene foams, and graphene nanosheets.[12–15]

Strategies for incorporating carbon nanomaterials into biological sensors include directly growing materials on a substrate, drop casting, incorporating CNs into polymers, co-depositing CNs and metal nanoparticles, and using CNs in field-effect transistor (FET)-based devices to enhance conductivity. The direct growth of CNs on electrodes provides a more homogenous coating than conventionally used dip coating or drop casting methods, and direct growth may facilitate future batch fabrication of materials. Polymer coatings can modify the physical and chemical properties of carbon nanomaterials and aid in dispersing CNs for deposition.[16,17] However, the introduction of polymer has drawbacks including restricting diffusion, slowing temporal resolution, and decreasing conductivity.[18] Metal nanoparticles are often incorporated into polymers as an electron transfer mediator to improve the overall conductivity. Field-effect transistor based devices work by a completely different mechanism than traditional voltammetric sensors, but CNs can be advantageous in facilitating electron transfer and achieving high sensitivity detection.[19] This review concentrates on voltammetric, impedance, and FET-based strategies for many analytes, highlighting some of the advantages and disadvantages of each.

Other recent review articles have highlighted the advantages of carbon nanomaterials in electrochemical sensors.[18] Graphene-based electrochemical sensors were reviewed by Gan and Hu and their review covers a wide variety of biological analytes and approaches.[20] Liu et al reviewed surface modifications of graphene and compared graphene to other materials, including CNTs.[21] They and Lawal [22] have highlighted the advantages of graphene-based sensors and their applications. Zhan et al. focused more narrowly on graphene-based FET sensors.[23] Chen and Chatterjee compared a variety of nanomaterials

for biomedical applications, including carbon nanomaterials.[24] Similarly, Zhang et al. reviewed the use of nanomaterials from carbon, noble metals, and semiconductors in electrochemical sensors.[25] Balasbramanian and Kern focused on nucleic acids and proteins in their review and highlighted how CNTs and graphene can be useful for label-free detection.[26] Gao et al. presented the progress of functionalized CNT-based electrochemical sensors, covering organic, inorganic and organic-inorganic hybrid functionalized materials.[27] Similarly, Salavagione et al. reviewed the use of polymer composites with CNT and GO in various sensors including electrochemical biosensors.[28]

In 2010, we wrote a review on carbon nanotube-modified electrochemical sensors for biomolecules.[18] This review article expands on the topic, surveying all carbon nanomaterials and their use in electrochemical sensors for the detection of biological molecules. We have surveyed the literature from 2012–2014 and initially examined all relevant papers from journals with an impact factor greater than 2.0. However, because of the vast array of papers, not all could be included and we tried to emphasize studies that used novel approaches or were more recent. This review is organized by analyte, which allows a direct comparison of different types of approaches for the same molecule. The first section covers neurochemicals, and the focus is on dopamine which is a popular test compound for new sensors. The second section covers detection of hydrogen peroxide. The third section reviews detection of non-electroactive molecules using enzyme based sensors, and glucose is the primary focus as a test compound. The fourth section covers detection of proteins and the fifth section covers direct detection of DNA using CN-based sensors. Finally, the future challenges of the field are discussed, including making more implantable sensors, miniaturization, and future applications in the environment and the clinic.

2.0 Neurotransmitters/Neurochemicals

Neurotransmitters are chemical messengers that signal between neurons and other cells. *In vivo* measurements are challenging because the extracellular concentrations of neurotransmitters are low and concentrations can change rapidly.[29] In addition to neurotransmitters, many other electroactive neurochemicals are present in the brain and they can interfere with neurotransmitter detection. Electrochemical sensors are advantageous for monitoring neurochemicals because they offer high sensitivity and fast response times.

Carbon-based electrochemical sensors have been used widely for neurotransmitter analysis because the surface oxides facilitate electron transfer and readily adsorb neurotransmitters due to electrostatic interactions.[4] Carbon nanomaterial (CN) based sensors exhibit enhanced electrochemical properties and high biocompatibility.[2] The nanostructured surface provides a larger specific surface area, increased interfacial adsorption, and enhanced electrocatalytic activity. Thus, CN-based electrodes have rapid electron transfer, reduced electrode fouling, reduced overpotential, and increased sensitivity and selectivity for neurotransmitter detection.[30–33] Carbon nanotubes (CNTs), graphene, and their derivatives have been used for neurotransmitter detection, either by themselves or in conjunction with polymers or metal nanoparticles. For neurochemical studies, dopamine is the major test compound studied and it is often tested in combination with ascorbic acid and

uric acid which are interferents. Other electroactive monoamines studied include serotonin, norepinephrine, and epinephrine.

2.1 Dopamine, Ascorbic Acid, and Uric Acid

Dopamine (3,4-dihydroxyphenethylamine, DA), a catecholamine, modulates many aspects of brain circuitry and is implicated in several neurological diseases, including Parkinson disease.[34] The basal levels of extracellular dopamine are around 0.01–0.03 μM, while phasic release during a burst of neuronal firing can be 0.1–1 μM.[18] Dopamine is a cation at physiological pH.[35] Ascorbic acid, Vitamin C, is an anion at physiological pH that can undergo a two-electron transfer oxidation.[36] Ascorbic acid is one of the most abundant, low molecular weight antioxidants in the central nervous system (CNS).[37] Uric acid is the final product of purine metabolism and related to disorders such as hyperuricemia and Lesch–Nyhan syndrome.[38] Uric acid acts as an antioxidant in cerebrospinal fluid and is also an anion at physiological pH.[39,40] The extracellular levels of ascorbic acid [41,42] and uric acid are several orders of magnitude higher than dopamine in the brain.[38] Because dopamine, ascorbic acid, and uric acid have similar oxidation potentials and often coexist in biological samples, many studies have concentrated on the development of highly sensitive and selective methods for their simultaneous detection. Table 1 summarizes the limit of detection (LOD) of dopamine, ascorbic acid, and uric acid for all the sensors covered in this section.

2.1.1 Carbon Nanotube-Based Sensors—CNT-modified electrodes have been widely used for neurotransmitter detection with high sensitivity and selectivity. While dip coating or drop casting CNTs often results in an irreproducible surface due to agglomerations [3], directly growing vertically aligned CNTs can lead to more reproducible surfaces with CNT ends exposed. The ends of the CNTs have more defect sites that can be functionalized with oxygen containing groups to adsorb dopamine. The functional groups, such as carbonyls, phenols, lactones, and carboxylic acids, selectively adsorb cationic dopamine and repel the anionic ascorbate and uric acid at physiological pH.[4] The first strategy for fabricating vertically-aligned CNTs is to chemically self-assemble vertically aligned CNTs on substrates. Our group developed SWCNT-forest modified CFMEs by self-assembly of shortened, carboxylic acid functionalized SWCNTs on a disk CFME decorated with an iron hydroxide-Nafion film.[33] The immobilization was based on strong interactions between functional groups at the end of CNTs and the modified electrode surface, and alignment was driven by hydrophobic interactions between the sidewalls of CNTs. These aligned CNT sensors were used with fast-scan cyclic voltammetry (FSCV) at high temporal resolution to measure endogenous dopamine changes in *Drosophila melanogaster*, the fruit fly. The second strategy for vertically-aligned CNTs is to grow them on a sensor surface using chemical vapor deposition (CVD). This requires a solid phase buffer layer and catalyst to be deposited on the substrate. Xiang et al. used vertically-aligned, carbon nanotube sheathed carbon fibers (VACNT-CFs) for the detection of dopamine and ascorbate.[43] The VACNT-CFs exhibited good sensitivity and were used for *in vivo* measurements. Thus, multiple strategies for aligned CNTs have proven useful for implementation in real biological measurements, demonstrating this strategy is robust enough for practical use. Growing

aligned CNTs is easier, more reproducible, and more amenable to batch fabrication than chemical self assembly of CNTs.

Helical CNTs (HCNTs) have been used for simultaneous electrochemical determination of dopamine, ascorbic acid, and uric acid.[44,45] A HCNT has a 3D-helical structure with high specific surface area and good electronic properties due to the rupture of the basal plane on the end caps of CNTs that leads to a larger density of edge-plane like defects on the nanotube surface.[46] Poly(allylamine hydrochloride) (PAH) and poly(diallyl dimethylammonium chloride) (PDDA) were used to functionalize the nanotube surface and disperse the HCNTs. The PDDA-functionalized HCNT-modified GCE had a lower detection limit for dopamine than PAH-functionalized HCNTs because PDDA acted as an electron acceptor, enhancing the electrocatalytic activity of helical CNTs.

CNT yarns are made of multiple CNT fibers twisted together. Our group utilized a commercially-available CNT yarn to fabricate CNTYMEs for rapid measurements of dopamine.[47] Figure 1 shows that CNTYMEs exhibit nearly identical oxidation currents with increasing repetition rate while traditional carbon-fiber microelectrodes exhibit a large decrease in current at the 100 Hz repetition rate. The property of current being independent of scan rate was attributed to different kinetics of adsorption of dopamine and dopamine-*o*-quinone at CNT yarns. Future studies to assess how nanostructure relates to adsorption properties would be useful to understand what other carbon nanomaterials might have currents independent of waveform frequency. Schmidt et al. used CNT yarn microelectrodes (CNTYMEs) to detect rapid neurotransmitter fluctuations in brain slices using FSCV, which indicated that CNTYMEs are suitable for *in vivo* measurements.[48]

Carbon nanofibers (CNFs) are made of closed graphitic shells along the tube axis, rather than the concentric graphitic sheets rolled up into a tube characteristic of CNTs. CNFs grown by plasma-enhanced chemical vapor deposition (PECVD) have been used for neurochemical monitoring.[49,50] Rand et al. developed vertically-aligned CNFs for the simultaneous detection of dopamine and serotonin.[50] The physical characterization by FTIR and Raman indicated that the desirable electrochemical properties are due to the structure of the nanofibers and the presence of many defect sites along the sidewall. The relatively large diameter of CNFs makes the edge/basal plane ratio smaller than that of CNTs, which might limit the sensitivity of adsorption-controlled species.

CNT-based field effect transistors (FETs) have been extensively developed for biomolecules.[19] The depletion or accumulation of charge carriers caused by binding between the analyte and the CNT surface is strong enough that even a single charge at the surface can be monitored. FETs require high purity semiconducting CNTs but SWCNTs are usually a mix of semiconducting and metallic. Zhang et al. produced more than 90% semiconducting SWCNTs (s-SWCNTs) via *in situ* hydrogen etching.[51] Figure 2A,B shows representative scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images of the s-SWCNTs, which reveal the formation of uniform diameter and very straight carbon layer of the SWCNTs. Their s-SWCNT-based FET, with an attomolar LOD, had the lowest detection limit for dopamine of any CN-based sensor (Figure 2C). FET sensors typically need a long time for equilibration and are not able to

detect neurotransmitters in real time, which may limit their application for *in vivo* measurements.

2.1.2 Graphene Based Sensors—Graphene is a single layer of two-dimensional carbon with a hexagonal configuration of sp^2 hybridized carbon atoms. Since the first successful isolation of graphene from graphite in 2004 [52,53], graphene has attracted great interest in electrochemistry because of its large surface-to-volume area, high electrical conductivity, and fast adsorption kinetics.[2,54,55] Graphene does not contain metallic impurities [56,57] and has been produced by mechanical exfoliation of graphite, epitaxial growth of SiC, reduction of graphite oxide, and unzipping of CNTs.[58] Mechanical exfoliation of highly oriented pyrolytic graphite produces single or few layers of graphene, but is not suitable for mass production.[2] In comparison, chemical reduction of graphite oxide is popular for electrochemical sensors because it results in graphene with structural defects and functional groups, which benefits electrochemical detection of neurotransmitters.[59]

Recently, several novel graphene based materials have been reported. Du et al. developed a graphene flower-modified carbon-fiber electrode to simultaneously detect dopamine, ascorbic acid, and uric acid.[13] Graphene flowers were produced by a simple and “green” electrochemical method. The homogenous graphene flowers, with layers of petals, increased the surface area significantly and also accelerated electron transfer. However, further physical characterization of these flowers is needed to understand their properties. Dong et al. reported a 3D graphene foam as a monolithic, macroporous, free-standing working electrode with good sensitivity to dopamine due to high surface area and conductivity.[14] In addition, the oxidation of dopamine is easily distinguished from uric acid because of hydrophobic and π-π interactions between dopamine and the graphene foam. Recently, Hong et al. developed vertically-aligned ZnO nanowire arrays on 3D graphene foam.[60] The ZnO nanowires offered numerous active sites and their robust adhesion to the graphene foam facilitated electron conduction. The oxidation potentials of dopamine, ascorbic acid, and uric acid were separated due to different bandgap energies at the ZnO-graphene foam electrode. The sensor was used to detect uric acid levels in the serum of patients with Parkinson’s diease. Materials scientist continue to discover and characterize many novel forms of graphene, so studies that incorporate these new forms will continue to grow and research will be needed to compare the electrochemical properties of the new materials.

In a solution-gated graphene transistor (SGGT), the graphene channel is in contact with electrolyte instead of a gate insulator. Zhang et al. reported a SGGT with both channel and gate electrodes fabricated with graphene for dopamine, ascorbic acid and uric acid sensing.[61] Similar to other transistor-based devices, the detection limit was very low for dopamine and sensor selectivity was improved by Nafion modification on the gate. Thus, graphene is an alternative to CNTs for making transistor based sensors.

2.1.3 N-doped Carbon Based Sensors—The surface chemistry and electronic properties of carbon nanomaterials can be modulated by introducing heteroatoms such as nitrogen or boron.[7] Carbon and nitrogen have a similar atomic size and valence electron structure and thus N-doped carbon materials are easy to make.[6] Li et al. investigated dopamine sensing at N-doped graphene electrodes. They found that among the three

configurations of N-doped graphene (pyridinic-N, pyrrolic-N and graphitic-N types), the electrocatalytic activity towards dopamine redox was best at the pyrrolic-N doping sites because they were mainly located on the edge planes.[62] Yuan et al. reported N-doped carbon nanorods (N-CNRs) prepared by a direct carbonization method using polyaniline nanorods as the carbon precursor.[63] Because of the abundant defects on the surface, the porous structure facilitated fast mass transfer of the analyte and the high specific surface area provided more reaction sites. Although measurements of dopamine were obtained in presence of ascorbic acid, the buffer was pH 4.0, which is not physiological. Gai et al. used N-doped porous carbon nanopolyhedra (N-PCNPs) for simultaneous detection of dopamine, ascorbic acid, and uric acid. The narrow size distribution of N-PCNPs (*ca.* 200 nm) was advantageous to decrease noise and further increase sensitivity. However, there was only 1.67% nitrogen introduced into the N-PCNPs, compared to 25.8% of nitrogen in the N-CNRs in the previous study.[64] While one of the benefits claimed for N-doping is enhanced sensitivity, most of these studies did not directly compare N-doped and non-doped materials. Overall, LODs were not significantly better than other non-doped carbon nanomaterials. However, the N-doped defects increased the rate of electron transfer and therefore allowed better separation of dopamine from ascorbic acid and uric acid.

2.1.4 Polymer Coatings—Polymer coatings can modify the physical and chemical properties of carbon nanomaterials and facilitate CN deposition on a surface. The functionalization of CNs by polymer coating is mainly a non-covalent approach via van der Waals forces, π-π interactions, or adsorption/wrapping of polymer and surfactants.[17] The main advantages of polymers are the dispersion of carbon nanomaterials, the increased conductivity, and improved selectivity

Several conducting polymers have been used to modify CNT and graphene-based electrochemical sensors. Poly(3,4-ethylenedioxythiophene) (PEDOT) has been coated extensively on biosensors to improve sensor function or biocompatibility.[65] Luo group used PEDOT doped CNT [66] and PEDOT doped graphene [67] coated GC electrodes for the detection of dopamine. The authors stated PEDOT catalyzed the electrochemical reaction of dopamine, but gave no specific mechanism for the catalytic effect of PEDOT to dopamine. Moreover, the positive charge of PEDOT may limit the sensitivity to dopamine at physiological pH. Our group has used polyethylenimine (PEI)-CNT fiber electrodes for enhanced detection of neurotransmitters.[68] Wet spinning CNTs into fibers was performed using PEI as a coagulating polymer. Compared with poly(vinyl alcohol) (PVA) CNT fiber-electrodes[69], the PEI-CNT fiber electrodes had lower overpotentials and better detection limits for dopamine, likely because they were more conductive. The PEI-CNT electrodes did not require heat treatment for activation and were easily fabricated into microelectrodes. CNT fibers may attract future interest because of their easy sensor fabrication process and antifouling properties, which would be beneficial for *in vivo* neurotransmitter sensors.

The layer-by-layer (LBL) deposition of thin film polymers is a technique based on the electrostatic adsorption between oppositely charged molecules. Many different materials may be employed and the thickness and porosity of the films can be controlled by pH, ionic strength, and salt concentration of polyelectrolyte solution.[17] Manjunatha et al. assembled LBL MWCNT films based on electrostatic interactions between positively charged PDDA

and negatively charged polystyrene sulfonate (PSS) wrapped MWCNTs.[70] Dopamine, ascorbic acid, and uric acid were successfully separated using CV, DPV, and amperometry without electrode fouling. The conductivity at the modified electrode was better than the bare graphite electrode, which may contribute to the high selectivity. Dopamine was also detected at LBL assembled, oppositely charged phenylsulfonated carbon nanoparticles (CNPs) and functionalized silicate submicroparticles.[71] Some disadvantages of LBL sensor fabrication are that numerous modification steps for depositing the films leads to low reproducibility and many of these studies have relatively high LODs compared to other strategies.

Adding metallic nanoparticles (NPs) to carbon nanomaterials results in an increased surface area, heterogeneous electron transfer, and electrical contact between the redox center of biomolecules and the CN-modified electrode surface.[72] Du et al. fabricated LBL self-assembled graphene sheets and AuNPs on modified carbon-fiber electrodes (GR/Au/GR/CFE) for simultaneous detection of dopamine and uric acid.[73] The uniform and dense coating of size-homogenous AuNPs assembled between the two layers of graphene sheets increased the effective electroactive surface area and also enhanced the electron transfer. The main shortcomings of adding metal nanoparticles with carbon nanomaterials on sensors are the difficulty of construction and possible biocompatibility issues of metal nanoparticles.

Molecular imprinting is a powerful tool for the preparation of polymeric materials that specifically bind a target molecule. Molecularly imprinted polymers (MIPs) are tailor-made for a target molecule, giving them high chemical selectivity.[74] Carbon nanomaterials provide enhanced conductivity, increased surface to volume ratio, and maximized porosity in the MIPs. CNs also enhance binding to shorten the incubation and extraction time, improving the temporal resolution of detection. Liu et al. constructed a MIP-graphene-chitosan sensor (MIPs-GR/GCE) through electrochemical codeposition and removed dopamine from the MIP via electrochemical induced elution.[75] The cyclic voltammograms indicated a promising selectivity for dopamine over ascorbic acid, uric acid, and caffeine. Graphene enhanced sensitivity by enlarging the electrode surface area, creating more imprinted sites, and accelerating the electron transfer. However, the response time was not as fast as some other electrode designs. Similarly, Qian et al. reported a dopamine sensor based on molecularly imprinted polymer with CNTs.[76] Dopamine was imprinted on an oxygen-containing PPy via π-π stacking between aromatic rings and hydrogen bonds between amino groups of DA and oxygen-containing groups of PPy. The response time was about 2 minutes so more work is required to reduce the accumulation and elution time for real-time dopamine detection.

2.1.5 Indirect Detection using Enzymes—Enzyme-based electrodes can enhance the selectivity for dopamine detection over other analytes.[77] Tyrosinase catalyzes the oxidation of *o*-diphenols compounds, such as dopamine, to their respective *o*-quinone derivatives. The reduction of dopamine-o-quinone back to dopamine is then measured electrochemically. Carbon nanomaterials increase the overall conductivity, porosity, and active surface area while facilitating the immobilization of enzymes on the surface.[78] Canbay et al. reported a MWCNT/Nafion/cysteamine modified, tyrosinase-based dopamine biosensor.[79] The high porosity and conductivity of the MWCNT skeleton enhanced the

enzyme immobilization, improved electrochemical transduction to the enzyme, and ensured easy access to the enzyme active sites. However, the LOD for tyrosinase-based detectors is not as good as for direct detection of dopamine electrochemically.

Uric acid biosensors have been developed with uricase, which uses uric acid, oxygen, and water to produce hydrogen peroxide and carbon dioxide.[80] Quantification of uric acid was achieved by sensing the decrease in dissolved oxygen or measuring the amount of the enzymatically generated H_2O_2. Numnuam et al. reported an amperometric uric acid biosensor based on the change of the reduction current of dissolved oxygen.[80] CNT nanofibers incorporated with chitosan provided a polymer matrix to immobilize uricase, while an electrodeposited layer of silver nanoparticles catalyzed the O_2 reduction reaction. Enzyme electrodes benefit from the selectivity of the enzyme and carbon nanomaterials provide a large surface area for electron transfer. However, enzyme sensors are typically slower than direct electrochemical detection, which restricts their temporal resolution for *in vivo* measurements.

2.2 Serotonin

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter which regulates mood and sleep and is a major target for pharmaceutical treatments for depression. Serotonin is electrochemically active but more difficult to detect than dopamine because the oxidation process produces an insulating layer that fouls the surface of the electrode and decreases sensitivity over time.[31] Carbon nanomaterial-based sensors can alleviate surface fouling effects. Table 2 summarizes carbon nanomaterial based sensors for serotonin.

We observed that PEI-CNT fiber microelectrodes were resistant to surface fouling by both serotonin and its metabolite 5-hydroxyindoleacetic acid in brain slices.[68] Figure 3 shows that the current did not decrease for repeated injections of 1 μM serotonin at PEI-CNT fiber microelectrodes, in contrast to carbon-fiber microelectrodes, which had a 50% decrease after 25 injections. The resistance to serotonin fouling at CNT based electrodes is often attributed to the higher density of edge plane sites, but the addition of oxygen groups, while maintaining a high sp^2 conjugation, may also help reduce fouling.

Xue et al. used a one-step electron-deposition process to fabricate a serotonin sensor based on a double-layered membrane of rGO/PANI nanocomposite and AuNPs at a molecularly imprinted polymer. [81] The MIP-based sensor was successfully employed for the detection of serotonin in human serum samples without interference from ascorbic acid, uric acid, dopamine, and epinephrine. However, the long accumulation time (160s to reach the maximal current response) would limit the application for *in vivo* measurement. Kim et al. reported a chemically-reduced graphene nanosheet-modified GCE for the detection of serotonin.[15] Compared to the rGO/PANI/AuNP MIP sensor, this RG nanosheet/GCE sensor provided higher sensitivity and a faster response time (4 s) because of the fast mass transfer of serotonin. However, no measurements were made in biological samples to determine suitability for applications in real samples.

Another approach to detect serotonin is based on a carbon ionic liquid electrode (CILE). Traditionally, a CILE is fabricated packing a paste of graphite powder and ionic liquid into a

tube; however, CNs can be incorporated instead of graphite. Mazloum-Ardakani et al. used benzofuran-derivative functionalized MWCNTs and the ionic liquid 1-butyl-3-methylimidazolium hexafluorophosphat (BMIM–PF_6) modified GCE for simultaneous detection of norepinephrine and serotonin.[82] The same group then developed a sensor for simultaneous determination of isoproterenol and serotonin, in which TiO_2 NPs were introduced.[83] The detection limit was approximately ten times lower with TiO_2 because of the synergistic effects of CNTs with TiO_2 which enhance the electrocatalytic effect and support a mesoporous structure. To increase the selectivity of serotonin in the presence of ascorbic acid and uric acid, another study introduced Nafion into a $Co(OH)_2$-MWCNT-modified CILE.[84] Nafion made the sensor impermeable to ascorbic acid and uric acid and improved the selectivity to the cationic neurochemicals l-dopa and serotonin, which were detected in human serum. Advantages of CN-IL based microelectrodes include the electrocatalytic effects and the simple preparation procedure.

2.3 Epinephrine

Epinephrine, also known as adrenaline, is an electroactive catecholamine neurotransmitter regulating heart rate, metabolic shifts, and the “fight or flight” response.[18] Table 2 summarizes CN-based sensors for epinephrine. An oxidized, single-walled carbon nanohorn (SWCNH) modified-SPE was reported for the detection of epinephrine.[85] The electrode had a higher sensitivity towards epinephrine than dopamine, serotonin or norepinephrine, but all four neurotransmitters had similar oxidation peaks at 0.6 V. The increased sensitivity was due to a cyclicization reaction of epinephrine at oxidized SWCNHs, where the o-quinone of epinephrine underwent intramolecular cyclization via 1,4-Michael addition and was converted into easily oxidizable leucoepinephrinecrome. [86]

Other studies have combined polymers, carbon nanomaterials, and metal nanoparticles for sensitive and selective epinephrine detection. Thomas et al. reported a pristine MWCNT/SDS-modified CPE for the detection of epinephrine.[87] A ten-fold excess of ascorbic acid or two-fold excess of dopamine did not interfere in the quantification of epinephrine. Epinephrine was determined in spiked blood serum and adrenaline tartrate injections. The high sensitivity and selectivity were due to the low charge transfer resistance, high diffusion coefficient, and fast electron transfer rate of the sensor. Pradas reported a MIP-based sensor using MWCNTs bearing a ‘terminal monomeric unit’ (CNT-mer) for the fabrication of an epinephrine imprinted polymer-based pencil graphite electrode (CNT-mer dispersed MIP-modified PGE).[88] A low detection limit was achieved; however, a 210 s accumulation time was required, which limited the temporal resolution of this sensor. Further studies should focus on the restriction effect of the interlayer diffusion of analyte in the film.

Several other studies use nanocomposites of metal nanoparticles and polymers, such as graphene/AuNP/GCE [89], and MWCNT-$Ni(OH)_2$/GCE [90]. The lower detection limit for epinephrine at graphene/AuNP/GCE may be due to the more uniform diameter of AuNPs than the nickel hydroxide nanoparticles, leading to less noise. The addition of metal NPs and polymers is advantageous for enhancing electron transfer, increasing surface area, and selectivity based on charge.

2.4 Neurochemical Conclusions

Carbon nanomaterials, such as CNTs, graphene, and their derivatives have been widely used as electrode materials for neurochemicals because of their biocompatibility, electrocatalytic effects, enhanced sensitivity, and reduced overpotential. Graphene and its derivatives are becoming increasingly more popular because of the abundant, inexpensive source material and lack of metallic impurities. Polymer modifications of CNs are advantageous for enhancing selectivity for catecholamines and eliminating interference for ascorbic acid and uric acid. However, the introduction of polymers has drawbacks including restricted diffusion, slow temporal resolution, and reduced conductivity. Metal nanoparticles incorporated in polymers improve the overall conductivity of the polymer film and act as spacers to enhance the CNs suspension by inhibiting the aggregation. The fabrication of electrodes with nanocomposites can be cumbersome since adding polymer and metal nanoparticles modifications requires additional steps. MIPs and FET-based sensors have some of the lowest LODs [51]; however, there is a tradeoff of sensitivity and speed for dopamine sensing and MIPs and FETs tend to have slower response times.

While dopamine has been extensively used as a test compound, carbon nanomaterial based electrodes are useful for a variety of neurochemicals. Carbon nanomaterial-based electrodes alleviate surface fouling effects caused by serotonin and are promising for the selective detection of epinephrine. Uric acid and ascorbic acid can be directly detected at a variety of carbon nanomaterial electrodes and electrocatalytic effects help separate their peaks from dopamine. Biosensors are a good strategy for highly selective detection but they have only micromolar detection limits, so sensitivity will need to be improved in the future.

One shortcoming of many studies is the low numbers of electrodes that are made. Experiments in real biological samples require many electrodes that can be fabricated reproducibly, but this need is not typically addressed. An emerging strategy for batch fabrication of electrodes is direct growth of carbon nanomaterials on an electrode surface because it provides a more homogeneous coating than dip coating or drop casting methods. In the future, direct growth of CNs onto electrode substrates could be advantageous for easy and reproducible fabrication in large quantities.

Many studies have focused on sensor design and proof of principle detection, but only a few studies have been performed in real biological samples. More work is required to demonstrate that the advantages of carbon nanomaterial based sensors such as selectivity or resistance to electrode fouling extend into biological samples. For *in vivo* measurements, electrodes on the micrometer scale or even nanometer scale are advantageous. Conventional GCE and SPE substrates are too large for most *in vivo* experiments. In addition, conventional techniques such as DPV or CV are slow for real-time measurements and faster methods, such as FSCV and chronoamperometry are preferable. Sensor response time, sensitivity after implantation, and biocompatibility will need to be addressed for *in vivo* studies.

3.0 Hydrogen Peroxide

Hydrogen peroxide is a byproduct of many enzyme reactions, acts as a reactive oxygen species in the body, and is a byproduct of reactions in industrial processes. Direct detection of hydrogen peroxide is difficult using standard electrodes due to high overpotentials. Carbon nanomaterials, sometimes in conjunction with redox mediators such as Prussian blue, can lower the overpotential for hydrogen peroxide, simplifying detection. In addition, H_2O_2 is commonly detected indirectly by enzymes such as horseradish peroxidase or catalase. Carbon nanomaterial-based sensors for hydrogen peroxide are detailed in Table 3.

3.1 Direct Detection

Direct detection of hydrogen peroxide takes advantage of the use of carbon nanomaterials to lower the potential for detection. Lin et al. used poly(xanthurenic acid) and flavin adenine dinucleotide (FAD) and MWCNTs to improve the sensitivity for hydrogen peroxide.[91] After forming the polymer on the surface of the GCE, MWCNTs were added to create a larger exposed surface area. Compared to a GCE treated with polymer and FAD only, the polymer/FAD/MWCNT electrode had much higher sensitivity for hydrogen peroxide and the cathodic peak was shifted to higher potential. This sensor was not tested on real samples and was also sensitive to NADH, so this potential interference would limit the sensor to simple samples where NADH is not present.

Prussian blue (PB) is commonly used with carbon nanomaterials to decrease the potential for hydrogen peroxide detection. Husmann et al. fabricated a CNT paste electrode with PB to detect hydrogen peroxide.[92] PB was electrosynthesized on the surface and was visible by SEM as cubic structures on the CNT paste. With amperometry, the sensor response time was 5 s, and it was insensitive to most interferents except uric acid and ascorbic acid. This sensitivity to other analytes makes this sensor unsuitable for complex biological samples where uric acid and ascorbic acid may be present. In addition, the sensor lost sensitivity within the first 10 days of storage, although it was stable thereafter, so stability must be improved.

Combining ionic liquids with PB and MWCNTs, Zhu et al. developed a hydrogen peroxide sensor where a screen-printed carbon electrode was printed mixed with ionic liquid ($[Bmim]BF_4$) and then treated with the PB-MWCNTs and Nafion.[93] IL doped electrodes had higher sensitivity for hydrogen peroxide, approximately 2-fold higher than the non-IL doped sensors, and the fastest response time due to improved electron transfer. The cathodic peak for hydrogen peroxide on these electrodes occurred around 0 V. The sensor was tested in spiked milk samples and had good recoveries even when small concentrations of hydrogen peroxide were added. While interferent testing was performed, the interferents that were tested were not generally those that would be found in milk and interferents such as proteins or lipids were not tested.

Liu et al. used a different approach based on the structure and activity of some enzymes used for hydrogen peroxide [94]. They treated polyallylamine hydrochloride modified MWCNTs with an iron complex (Fe^{III}-DETPA). EDTMP/Fe^{III}-DETPA/PAH was evenly distributed along the MWCNTs and the redox activity of the Fe^{III}-DETPA complex was visible using

cyclic voltammetry. The Fe^{III}/Fe^{II}-DETPA acted as an electrocatalyst, as formation of Fe^{II}-DETPA allowed the reduction of hydrogen peroxide. This sensor was fast and had the widest reported linear range for hydrogen peroxide detection, likely due to high loading of Fe^{III}-DETPA on the surface and its fast electron transfer rate. This sensor design would be one of the most suitable for use in clinical or commercial settings due to the long shelf stability, high sensitivity, and good selectivity of the sensor for hydrogen peroxide.

3.2 Enzymatic Detection

Enzymes are highly selective and lower the detection potential for hydrogen peroxide. Hydrogen peroxide enzyme sensors use horseradish peroxidase (HRP) or catalase (CAT), which produce oxygen that is easily reduced. Direct electrochemistry of the redox center of the enzyme is also possible.

Using HRP is a common strategy for hydrogen peroxide biosensors. Kaçar et al. used HRP, MWCNTs, and Co_3O_4 nanoparticles on a GCE for hydrogen peroxide detection [95]. A coating of Co_3O_4/MWCNTs/gelatin solution was dried on the electrode surface, HRP was added, the electrode was treated with saturated glutaraldehyde, and then finally Nafion coated. The optimum working potential of the sensor was low, at −0.30 V. The low potential combined with Nafion made the sensor insensitive to common interferents. Wang et al. used a gold electrode modified with L-cysteine to immobilize SWCNTs, which were treated with sodium cholate and then HRP.[96] Using linear sweep voltammetry, the reduction current increased with the addition of H_2O_2 while oxidation current remained unchanged, showing that the SWCNTs were effective at shuttling electrons from the electrode to the redox center of HRP. By far, this was the most sensitive carbon nanomaterial based sensor for hydrogen peroxide, due to high surface coverage of the L-Cysteine–HRP–SWNTs and the direct electron transfer from the active site.

Catalase-based sensors have also been designed for H_2O_2 detection. Shamsipur et al. coated a GCE with MWCNTs, an ionic liquid ([bmim][PF6]), and then catalase.[97] Both the MWCNT only and MWCNTs/[bmim][PF6] modified sensors showed redox peaks corresponding to the catalase heme Fe(III)/Fe(II) redox couple, but the ionic liquid modified sensor had the highest signal due to improved enzyme immobilization and increased electron transfer rate. As scan rate increased, peak separations increased, indicating a quasi-reversible redox process of the redox center of catalase. Instead of using amperometric or voltammetric methods of detection with the sensor, electrochemical impedance spectroscopy (EIS) was used to determine the linear range and limits of detection. EIS avoids overpotentials common to other electrochemical methods and is highly sensitive.

CAT-based and HRP-based hydrogen peroxide sensors have comparable sensitivity. Differences in the reaction mechanism of CAT and HRP with hydrogen peroxide may influence the choice of one enzyme over another. Native CAT has high turnover number and produces oxygen which may be detected electrochemically. Additionally, catalase is exceptionally stable, and may be a better choice for sensors where shelf stability and ease of storage is important. HRP may be preferred as part of a multi-enzyme sensor or when cost and availability is a concern.

3.3 H_2O_2 conclusions

CN-modified sensors for the detection of hydrogen peroxide provide high sensitivity and selectivity. The most popular nanomaterials for these sensors were MWCNTs, which lowered the detection potential for hydrogen peroxide, increased sensitivity, and in the case of enzyme based sensors, “wired” enzymes to the surface of the electrode. Direct detection of hydrogen peroxide may be preferred due to simpler electrode design, but this method lacks the sensitivity that enzyme based sensors provide. Linear ranges of non-enzymatic sensors are generally wider because enzymes may become saturated at higher substrate concentrations, thus lowering the upper limit of the linear range for enzyme sensors. In addition to high sensitivity, enzymatic sensors provide more selectivity due to the specificity of the enzyme for hydrogen peroxide. The next section will highlight many enzymes that produce hydrogen peroxide as a byproduct, which is then directly detected at an electrode. Thus, hydrogen peroxide sensors can serve as a building block for enzyme sensors.

4.0 Enzyme Substrates

The electrochemical detection of non-electroactive species requires that they be converted into an electroactive analyte. Enzyme sensors, which use an enzyme to produce an electrochemically detectable signal, are useful for a variety of non-electroactive analytes. Typically, an electroactive product or changes in the redox center of an enzyme are monitored. Carbon nanomaterials are used to increase the electroactive surface area, improve sensitivity, improve electron transfer kinetics, and provide a point of attachment for enzymes. Direct immobilization by drop casting or chemical linkage is commonly used to anchor enzymes to carbon nanomaterials on the surface of the sensor.[96,98] Enzymes can also be immobilized in polymeric matrices such as redox polymers, or bioadhesive films.[99]

4.1 Glucose

Glucose biosensors typically use glucose oxidase (GOx) to produce hydrogen peroxide, which is detected by amperometry or voltammetry. Glucose oxidase has been immobilized directly on the surface of carbon nanomaterials via chemical linkage to nanotubes[100,101], graphite nanoparticles [102], or other carbon nanomaterials. Immobilization has also been reported in bioadhesive films such as chitosan [99], and other polymeric matrices. Table 4 summarizes carbon nanomaterial based biosensors for enzymatic substrates.

There are two main methods for chemical linkage of enzymes to carbon nanomaterials: glutaraldehyde (GAD) and 1-Ethyl-3-(3- dimethylaminopropyl)carbodiimide / N-hydroxysuccinimide (EDC/NHS) coupling. Zhu et al. used glutaraldehyde to chemically link GOx to carbon nanotube fibers.[100] Both the as–spun and annealed fibers had two linear ranges which are within the physiological range of blood glucose levels; however no biological samples were tested. EDC/NHS coupling links primary amines present in GOx to oxide groups on carbon nanomaterials. EDC/NHS coupling was used by Piao et al. to immobilize GOx on graphite nanoparticles (GN) on a GCE.[102] Glucose was detected in spiked urine samples with high recovery. However, the better clinical sample would be plasma or blood because there is a time lag between glucose consumption and urine output.

Additionally, urine is a far simpler sample matrix than plasma, so testing only in urine is not sufficient. Both GAD and EDC/NHS coupling immobilize enzymes efficiently, but GAD immobilization requires fewer steps and maintains enzyme activity because it couples to amino acids unlikely to be present in the active site.

Functionalized nanomaterials are used to promote interactions between the electrode and GO_X. Khodadedei et al. functionalized MWCNTs with amines using dielectric barrier discharge, drop cast them on the surface of a GCE, and then drop cast GOx on this surface. [101] Im et al. used oxyfluorinated, electrospun carbon fibers treated with polyacrylonitrile to immobilize GOx on a screen-printed carbon electrode.[103] These fibers had exposed nanoscale CNT features in addition to nanoscale porous areas. While both studies had a low K_M^{App} for GOx, indicating high specificity for glucose, the sensor by Khodadedei et al. had a lower K_M^{App} and faster response time. The functionalized nanomaterials facilitated immobilization of GOx without changing the conformation of the enzyme, enabling high sensitivity and rapid response times.

Enzyme immobilization can be performed by layer-by-layer assembly using redox active polymers. Muguruma et al. designed a LBL assembled glucose sensor using either metallic (mSWCNT) or semi-conducting (sSWCNT) CNTs that were drop cast onto a plasma polymerized acetonitrile film (PPF) with enzyme.[104] Using sorted SWCNTs, they determined the mechanism of the reaction was that CNTs facilitated direct electron transfer at GOx/sSWCNT sensors. Furthermore, they also developed a glucose dehydrogenase (GDH) based sensor, where sSWCNTs were more sensitive and less resistive. While no biological samples were tested, this work shows that sorted carbon nanomaterials may be useful in the future for creating highly sensitive sensors with known mechanisms of action.

A popular redox polymer and immobilization agent is chitosan (CS), which is biocompatible. Chitosan can be used to increase the suspension of carbon nanomaterials in aqueous solutions and to form linkages to enzymes. Liu et al. designed a glucose biosensor based on 3D graphene, with ferocene modified CS (Fc-CS), glucose oxidase, and SWCNTs that were electropolymerized on the surface, forming a hydrogel. [99] The modification of chitosan with ferocene improved electron transfer kinetics and provided a matrix for the immobilization of GOx. The response time was fast at 8 s, despite the method of immobilization. The sensor was tested on diluted serum samples, which is an improvement over other sensors which were not tested in serum.

4.2 Alcohols

Detection and quantification of alcohols such as methanol and ethanol is important in a variety of fields such as the food and beverage industry and clinical analysis. Alcohol oxidase (AOx) oxidizes primary alcohols, such as methanol and ethanol, to hydrogen peroxide, which is electrochemically detected. Alcohol dehydrogenase (ADH) is used for enzyme sensors, but it requires a NAD^+ co-factor.

Goswami's group developed ethanol sensors based on AOx, which unlike ADH, does not require NAD^+.[105,106] In their first paper, MWCNTs suspended in Nafion were coated onto a Au electrode, a coating of PEI applied, and the AOx immobilized on the surface.

[105] Increasing concentrations of ethanol increased the anodic peak due to the electrocatalytic oxidation of hydrogen peroxide and the cathodic peak due to AOx catalyzed reduction of oxygen. This design was improved by layer-by-layer immobilization of HRP in a sol-gel chitosan matrix followed by immobilization of ferrocene entrapped alcohol oxidase (FcAOx).[106] This HRP/FcAOx electrode had a larger linear range compared to unmodified AOx and a lower limit of detection because the FcAOx generated hydrogen peroxide efficiently and HRP selectively detected it with a low applied detection potential (−0.34 V vs Ag/AgCl).

Kowalewska et al. designed an ethanol specific biosensor using a bi-enzymatic approach with ADH and aldehyde dehydrogenase (AldDH), which uses the product of ADH to form acetic acid.[107] A CNT/PDDA modified electrode was immersed in ADH, then in AldDH, and coated with Nafion. The addition of AldDH further increased the oxidation current and lowered the peak oxidation potential, indicating the successful “wiring” of both enzymes to enhance ethanol oxidation. This design was not as sensitive as the AOx-based sensors and while they claimed ADH-based sensors are more stable, the shelf stability was comparable to the AOx sensors designed by Goswami et al.[108,109] Although selectivity for AOx is a concern, these sensors were sufficiently selective for ethanol over other alcohols and their high sensitivity and no cofactor make them more promising than ADH-based sensors.

4.3 Cholesterol

Cholesterol detection is important because high cholesterol causes increased prevalence of atherosclerosis and other illnesses. Cholesterol enzyme sensors use cholesterol oxidase (ChOx) to produce hydrogen peroxide for electrochemical detection, with carbon nanomaterials lowering the overpotential.

Zhang et al. designed a two enzyme electrode that combines ChOx and alkaline phosphatase (ALP).[110] When ALP is placed in a solution containing disodium phenyl phosphate, it produces phenol, which is electroactive. For the dual enzyme electrode, hydrogen peroxide produced by ChOx in the presence of cholesterol, decreased the sensitivity of the electrode for phenol, and produced a lower electrode response with DPV. While this method is sensitive, the disadvantage is that it requires the addition of an exogenous compound for cholesterol detection.

Barik et al. developed a polyaniline (PANI)/zinc oxide (ZnO) membrane-based junctionless carbon nanotube field effect transistor (JLFET) for the detection of cholesterol with ChOx.[111] Potassium-doped MWCNTs were used to improve electron transfer of the sensor, as they have high electrical carrier transport ability. The drain current (I_{DS}) was linear with respect to increasing concentration of cholesterol and interference was negligible from other compounds such as uric acid, urea, and glucose. The sensor was also stable at room temperature for 5 months, which is impressive for a biosensor. However, no biological, clinical or industrial samples were tested. Additionally, this sensor was not as sensitive as the ChOx/ALP based sensor, but covered a wider linear range.

4.4 Xanthine and Hypoxanthine

Xanthine and hypoxanthine are naturally occurring purines; xanthine is a product of purine degradation and hypoxanthine is a product of the deamination of adenine. Consuming xanthine and hypoxanthine leads to increased uric acid levels in the blood. Xanthine oxidase metabolizes xanthine to hypoxanthine and hypoxanthine to uric acid. Both reactions produce hydrogen peroxide, which is detected electrochemically.

Torres et al. developed a xanthine oxidase sensor for hypoxanthine based on a carbon film (CF) electrode modified with COOH-functionalized MWCNTs in a chitosan solution.[112] A solution of xanthine oxidase (XOD), bovine serum albumin (BSA), and glutaraldehyde was coated and dried on the surface. Modified electrodes lost 50% of their initial response within 14 days. The sensor was sensitive enough to detect hypoxanthine in sardine and shrimp samples. Using a combination of single-walled carbon nanohorns (SWCNHs), gold nanoparticles (AuNP), and xanthine oxidase, Zhang et al. designed a sensor for both hypoxanthine and xanthine.[10] SEM and TEM imaging showed that the SWCNHs displayed AuNPs over their surfaces and sides without loss of their original structure. The sensor was used to determine hypoxanthine in a fish sample, but was only tested by standard addition due to low sensitivity. The linear range and sensitivity were better for the MWCNT/chitosan/carbon film design, perhaps due to better immobilization with GAD and chitosan. This research illustrates that immobilization method and matterials can have a substantial impact on the performance of biosensors.

4.5 Enzyme sensor conclusions

Carbon nanomaterials have been successfully used to produce high sensitivity enzyme sensors for a wide variety of substrates due to their ability to catalyze electrochemical reactions, directly wire enzymes to sensors, and increase the surface coverage of enzymes. CNTs continue to be popular for wiring enzyme sensors, but newer strategies include using graphene nanoparticles and single-walled carbon nanohorns. Further developments in enzymatic biosensors will likely remain focused on amperometric detection, as it has fast response times and high sensitivity.

One of the major challenges for carbon nanomaterial based enzyme sensor continues to be the trade-off between sensitivity and selectivity. For example, polymer immobilization of enzymes (using chitosan and other polymers) is used to prevent fouling or interference from other compounds but often leads to longer response times and reduced sensitivity due to lowered conductivity. Other methods for reducing interference may also reduce sensitivity, such as using a dual-enzyme sensor or blocking the surface with bovine serum albumin to prevent non-specific interactions. EDC/NHS linkage to functionalized nanomaterials is an alternative to polymer immobilization that can facilitate fast response times

Another concern for enzyme-based sensors is the stability of sensors over time, both on the shelf and in real samples. Enzymes can degrade on the surface of an electrode, but a long shelf life is desirable for real-world applications. Stability tests performed in most of these studies focused on shelf stability in ideal storage conditions or after testing in simple buffered samples. However, stability in real biological matrices, such as serum, is also

important and sensor stability was rarely tested over time in real samples. Serum samples contain proteins and other compounds that can adsorb on the surface of the senso so biological stability may be worse than shelf stability. Thus, carbon nanomaterial-based biosensors need to be tested more in real-life samples in order for them to be routinely adopted for commercial or clinical use.

5.0 Protein and Biomarkers

Detection of proteins, bacteria, and biomarkers using carbon nanomaterials is a rapidly growing field. Protein detection can either be indirect or direct. Indirect methods use a coupled enzymatic reaction which produces an electrochemically detectable product. Direct methods monitor proteins after interactions with either an aptamer or antibody attached to the electrode surface. Alternatively, direct detection can employ molecularly-imprinted polymers engineered to have high affinity for the target protein. Due to the specificity of either the aptamer, antibody, or imprinted polymer, interference from other proteins or biomolecules is minimal. Carbon nanomaterials increase surface area for attachment of aptamers and antibodies while also providing a direct link between the sensor surface and the recognition element. Furthermore, the addition of carbon nanomaterials increases the conductivity and sensitivity of the sensor. Table 5 summarizes the carbon nanomaterial-based biosensors for proteins, biomarkers, and bacteria detection.

5.1 Cancer and disease biomarkers

5.1.1 Prostate Cancer—Prostate cancer is the most commonly diagnosed cancer and is a leading cause of cancer deaths in American men. Typical screening methods detect prostate specific antigen (PSA), a biomarker for prostate cancer but are prone to false positives and negatives. A new biomarker, osteopontin (OPN), is being investigated for prostate cancer and metastasis detection. Lerner et al. developed a carbon nanotube field effect transistor (NT-FET) treated with an antibody for OPN (anti-OPN) (Figure 4).[113] The addition of OPN to the device increased the on state current. No biological or clinical samples were tested using this sensor, and the mixture of proteins tested for sensor specificity and selectivity was not a good mimic for plasma or whole blood. However, exploring new biomarkers for prostrate cancer is important to finding a good marker that will accurately predict the disease.

Akter at al. developed a sandwich type electrochemical sensor for prostate specific antigen. [114] A gold electrode was treated with MWCNTs and AuNPs and then anti-PSA antibody was coated on to the surface, followed by incubation with PSA. 4-chloro-1-napthol was also added to the sample, which is a substrate for peroxidases. The product of the enzymatic reaction is a precipitate. The sensor was treated with either HRP-conjugated anti-PSA or MWCNTs treated with anti-PSA and HRP (Figure 5). Using CV and SWV, the MWCNT-HRP conjugates produced a lower signal, due to increased production of precipitate, which accumulated on the suface of the sensor. This decrease in current was used for quantification. The sensor detected PSA in spiked serum samples and was advantageous because it was more sensitive than the OPN sensor and was tested in biologically relevant samples.

5.1.2 α-fetoprotein—Another biomarker for cancer is α-fetoprotein (AFP), which is elevated in people with hepatocellular carcinoma, germ cell tumors, and metastatic liver cancers. Zhao et al. developed a sandwich type immunoassay for AFP that was free of enzymatic labeling on antibodies.[115] The sensor was based on anti-AFP immobilized on a chitosan-treated, screen-printed carbon electrode. The other part of the sandwich was formed by carbon nanohorns treated with PDDA, gold nanoparticles, and then anti-AFP. The sensor took about 30 min for the response to plateau. The AFP content in an unspiked human serum was measured using DPV and agreed with electrochemiluminescence values. Yang et al. developed another sandwich type device, based on a GCE treated with gold and graphene nanoparticles and then incubated with anti-AFP.[116] After incubation in AFP, the sensor was treated with carboxylated single-wall carbon nanohorns that were treated with anti-AFP, GOx, and HRP. AFP increased the resistance of the sensor due to the formation of precipitate. Without the use of the CNHs as a platform for the bioconjugates, the limit of detection was 100 times higher, demonstrating the importance of the CNHs for signal amplification. Real serum samples were tested, but in this case they used standard additions for quantitation. Both studies prove that sandwich assays can be a good strategy for high sensitivity, although the temporal response is slower than other sensor designs.

Using an ITO base that was split into two isolated sensors for two different proteins, Jia et al. designed a multiplexed sensor for carcinoembryonic antigen (CEA) and AFP.[117] The base was treated with RGO nanoparticles, AuNPs, and thionine on one side and Prussian blue on the other side. The thionine side was then incubated with anti-CEA and the Prussian blue side was incubated with anti-AFP. Cross talk between the two sensor plates was minimal and there was little interference from other compounds in serum. The concentration of CEA and AFP in unspiked serum samples determined by the sensor agreed with ELISA values.

Overall, the AFP sensors were sufficiently sensitive for clinically relevant detection of AFP and were tested in sample matrices that closely approximated real samples. The sensor proposed by Zhao et al. was the most sensitive for AFP. The multiplexed sensor proposed by Jia et al. may be more clinically useful, as it can analyze two cancer biomarkers at the same time with high sensitivity and selectivity.

5.1.3 C-reactive protein—C-reactive protein (CRP) is a biomarker for inflammation and can be used as an indicator of coronary heart disease risk. Gupta et al. developed a label-free electrochemical sensor for CRP using carbon nanofibers.[118] Vertically-aligned carbon nanofibers were grown using plasma-enhanced chemical vapor deposition on several poly(methyl methacrylate) (PMMA)-treated micropads on a silicon wafer. The fibers were functionalized with nitric acid and anti-CRP was immobilized using EDC/NHS coupling. With EIS, increasing concentrations of CRP increased the resistance to electron transfer of the sensor. Justino et al. used another approach for CRP: a CNT field-effect transistor.[119] A suspension of SWCNTs was dried on the surface of a FET and anti-CRP directly immobilized on the surface of the SWCNTs. Exposure to CRP led to a decrease in current due to fewer available holes. The linear range for the FET device is better than immunochemical assays and the sensor requires only a small sample volume for CRP detection. The FET sensor design is by far more sensitive for CRP than the nanofiber sensor

and has a linear range that is sufficient to detect CRP in clinically relevant levels. Unfortunately, neither sensor was tested in a biological sample for CRP detection.

5.2 Bovine Serum Albumin

Bovine serum albumin (BSA) is a common protein found in serum and is often used to block non-specific interactions between proteins and sensor surfaces. Using BSA as a model protein, Chen et al. designed a molecularly imprinted polymer (MIP) sensor.[120] Pyrrole and BSA were electrodeposited on a GCE treated with acryl chloride-functionalized MWCNTs and chitosan-coated magnetic nanoparticles. The electrode was washed to remove template BSA. Human serum albumin and bovine hemoglobin did not interfere and BSA was detected in diluted human serum; however no samples that naturally contain BSA were tested. While BSA is a test compound, there was little justification for the use of this sensor. A future application could be to detection of food adulteration; for example determining the type of animal meat samples come from.

5.3 Streptococcus

Group A streptococcal (*GAS*) infections are responsible for over 500,000 deaths per year. Shi et al. used an interdigitated gold electrode base treated with SWCNTs coupled to a piezoelectric quartz crystal (SPQC).[121] The electrode was then coated with an anti-*GAS* aptamer and the sensor evaluated using EIS. After adding *GAS* to the modified electrode, the resistance increased relative to the unreacted electrode. The frequency shift of the piezoelectric quartz crystal after the addition of *GAS* arises due to change in impedance, and was amplified by the IDE base. The addition of the IDE to the SPQC allows for increased sensitivity to changes in conductance and impedance of the solution. The best response was with an incubation time of 40 minutes, which while slow, was faster than the commonly used pour plate counting for *GAS* detection.

5.4 Protein Conclusions

Carbon nanomaterial-modified sensors for detection of proteins, biomarkers, and bacteria show great promise as they have comparable sensitivity to traditional immunoassays with lower cost and analysis time per sample. SWCNTs, MWCNTs, and SWCNHs were commonly used as carriers for enzymes and antibodies. As with many other biosensors, most studies are at the proof of concept stage and more rigorous analysis of real samples in real environments is needed.

The major difference between device designs was the use of direct or indirect detection. Indirect detection includes signal amplification by the addition of enzyme to the sensor, either as part of a sandwich or on the surface of the sensor. However, in the papers examined, the device design is complicated and the signal amplification did not dramatically increase the sensitivity of these sensors as compared to direct detection methods (Table 5). In sandwich sensors, long incubation times are required. Direct detection requires shorter incubation times and less complicated fabrication methods without sacrificing sensitivity or selectivity. Furthermore, direct detection encompasses more methods for recognition of analytes, such as molecularly imprinted polymers, which expands their use to proteins where antibodies are extremely expensive, unavailable, or impractical.

As demand for lower cost and faster analysis of samples for biomarkers increases, carbon nanomaterial based sensors are likely to replace ELISA and other immunoassays, as they are faster and more sensitive. These sensors have a small footprint and could easily be adapted for use in the food industry to detect pathogens or proteins, especially where concerns about food contamination or sourcing arise. Future medical applications include expansion to more biomarkers, especially cancer biomarkers and bacterial biofilm detection.

6.0 DNA

The discovery that DNA is electrochemically active has allowed direct detection of DNA and its bases by electrochemical sensors.[122] DNA sensors (or genosensors) have been widely used in biomedical and environmental research. For example, genosensors are used in the detection of food and environmental pollutants, determination of genetic diseases, and identification of viruses and bacteria. Electrochemical methods for DNA detection are less expensive, less labor intensive, faster, and more sensitive than other detection methods. Electrochemical genosensors can directly detect target DNA via direct oxidation of adenine and guanine bases or detect a signal change after DNA hybridization. Direct detection of purine bases is difficult because of their high oxidation potentials so carbon nanomaterials are beneficial for detection because of their electrocatalytic effects.

Indirect genosensors detect signal changes from electrostatic indicators such as $Ru(NH_3)_6^{2+/3+}$ or $Fe(CN)_6^{3-/4-}$ or intercalators such as daunomycin or methylene blue (MB). The $Fe(CN)_6^{3-/4-}$ redox reaction is typically coupled to EIS detection, where charge transfer resistance (R_{ct}) increases upon DNA hybridization as it forms an insulating film on the electrode surface. $Ru(NH_3)_6^{2+/3+}$ interacts with the negatively-charged phosphate backbone of DNA and generates signal proportional to hybridized DNA. Ruthenium complexes are advantageous as reversible redox indicators because they exhibit fast electron transfer kinetics, and unlike $Fe(CN)_6^{3-/4-}$, do not adsorb to carbon electrodes and affect the intercalation signal.[123] Table 6 summarizes detection limits of the different CN-based genosensors.

6.1 Test DNA

Many genosensors are developed around test DNA, in order to prove a concept works. Due to the low abundance of DNA in biological samples, signal amplification strategies have been extensively studied to develop more sensitive genosensors. By using LBL assembly of substrates that have individually shown to possess catalytic activities (DNAzyme and PtNP), Dong et al. fabricated a sensor to detect DNA by detection of the indicator tetramethylbenzidine (TMB). They prepared a LBL deposition of COOH-CNTs wrapped with PDDA and PtNPs functionalized with DNAzyme and probe DNA.[124] Detection was by a sandwich approach as the target probe hybridized with a capture DNA probe on a gold electrode and the reporter probe on the PtNP/CNT. The capture of the DNAzyme and PtNP near the surface catalyzed the oxidation of TMB and provided signal amplification for target DNA detection. This method provided selectivity for a given sequence and good signal amplification but was complex and required multiple steps to fabricate the sensor. Gutierrez et al. fabricated a GCE/CNT-GOx sensor where the probe DNA was incorporated by direct adsorption or LBL self-assembly using PDDA.[125] The GOx allowed for better dispersion

of CNTs while the PDDA assisted in adsorption and electrooxidation of dsDNA. Using the LBL method, the potential for guanine detection decreased and the sensitivity of the method was increased. These studies show that LBL methods are advantageous for producing signal amplification, but the sensors and detection strategies are complex and may be difficult to use in real samples.

Another strategy to achieve signal amplification is use of gold nanoparticles with CN-based genosensors. Dong et al. fabricated a chronocoulometric DNA biosensor where polydopamine was electropolymerized to MWCNT-COOH on a GCE, modified with AuNPs, and functionalized with probe DNA.[126] $Ru(NH_3)_6^{3+}$ was used as an indicator and the polydopamine was advantageous because it has good electrochemical response for biomolecules. The LOD reported for the sensor was better than a previous sensor that did not use polydopamine.[127] Huang et al. fabricated a 2D graphene analog molybdenum disulfide/MWCNT/AuNP electrode.[128] By using glucose oxidase as a redox marker and AuNPs, they were able to obtain signal amplification. Layered transition metal dichalcogenides such as MoS_2 have a large specific surface area, but low electrical conductivity so combining MoS_2 with graphene overcomes this conductivity problem. Another group electrochemically deposited AuNPs on vertically-aligned SWCNT arrays on a SiO_2/Si substrate.[129] The vertically-aligned SWCNT sensor performed better than random SWCNT arrays because of the edge plane sites that were available. Using EIS detection of $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ redox reaction this sensor had the lowest LOD reported for a test genosensor, because impedance spectroscopy is very sensitive. The electrode could be regenerated and reused after dehybridization of complementary DNA in hot water. Genosensors with AuNPs had some of the lowest LODs and wide linear ranges, up to twelve orders of magnitude.

Wipawakarn et al. developed a label-free sensor with carbon nanofibers.[130] Carbon nanofibers are less expensive to produce, more mechanically stable, and have higher surface-active groups to volume ratio than CNTs.[131] In addition, carbon nanofibers are easier to disperse and functionalize than CNTs. Although the linear range and LOD obtained were not better than CNT genosensors, the CNF sensor had good selectivity, reproducibility, and stability. Thus, sensitivity must be improved before CNFs will routinely replace CNTs in many genosensors.

Graphene is another form of carbon nanomaterial that is used in DNA sensors because of its 2D electrical conduction and absence of residual metallic impurities.[132] A RGO-based FET sensor was used by Cai et al., but instead of using a DNA probe, they used a peptide nucleic acid (PNA) probe.[133] PNA probes have rigid amino bonds and a neutral backbone, which decreases repulsion with the DNA molecules and lowers the background current. The RGO sensor performed better than a similar sensor with Si nanowires due to higher detection area, greater surface roughness, and greater carrier mobility of graphene. The PNA sensor was able to discriminate complementary DNA from a one-base mismatch. Xu et al. fabricated a FET array from graphene with 8 FETs on a SiO_2/Si substrate.[134] Site-specific immobilization of probe DNA on the FETs was achieved by using charge bias to attract or repel negatively charged probe DNA and hence the graphene acted as both the electrophoretic electrode and the gate for site-specific detection of DNA. While the

approach was label free and multiplexed, the FET sensor required a long hybridization time of an hour and was not easily regenerated. Graphene nanoflakes (GNFs) have also been explored because of their higher density of edge plane sites than CNTs. Immobilization of target DNA on vertical GNFs on Si wafers was used for the determination of native fish sperm and calf thymus DNA.[135] However, the prepared nanoflakes were fragile and adhered weakly to the electrode. Akhavan et al. used reduced graphene nanowalls (RGNW) and nanosheets (RGNS), which are made from vertical graphene oxide nanoflakes, for the detection of single bases, ssDNA, and dsDNA using DPV.[136] The RGNW electrode was more stable, had a wider detection range, and lower limit of detection (zeptomolar) than the RGNS. Although this method had a very good LOD and a wide linear range, it suffered from being sensitive to interferences from coexisting DNA and could only be used for detecting single nucleotide polymorphisms (SNPs) in short DNA sequences. Overall, graphene-based genosensors are simple to fabricate and have comparable LODs and linear range to more complex CNT-based sensors that incorporated polymers or AuNPs. Thus, graphene-based sensors are a good option for preparing ultrasensitive genosensors in the future.

6.2 Virus and Bacteria DNA

Viruses and bacteria cause disease and thus they are an important target for many genosensors. Electrochemical genosensors can provide rapid and cost effective detection of viral and bacterial DNA for medical diagnostics or control of bioterrorism. To detect hepatitis B virus (HBV), Nie et al. prepared a label-free MWCNT-doped poly(indole-6-carboxylic acid) (PICA)/GCE sensor that was simple to fabricate with only one step used to electrodeposit PICA-MWCNT.[137] The electron withdrawing COOH group on the polymer decreased the LUMO and band gap. Also, the PICA-MWCNT provided large surface area and large numbers of COOH group to enhance the sensitivity for NH_2-functionalized target DNA.

Wang et al. used a AuNP/SWCNT hybridization sensor to detect HBV and human papilloma virus (HPV).[138] The detection range was large for HBV and the LOD was low because of the incorporation of both AuNP and CNTs. The same group also developed a sensor without AuNPs that used DNA-wrapped CNTs, which in the presence of sequence specific target DNA formed a sandwich complex with a hairpin DNA capture probe immobilized on a magnetic bead.[139] The CNTs facilitated electron transfer between the electrode and redox indicator, ferrocenecarboxylic acid, in solution. However, the linear range was much smaller and detection limit higher for the second sensor that had no AuNPs. Bacteria were studied by genosensors and a GO-CHIT nanocomposite on ITO was used for the detection of *Salmonella typhi* specific DNA.[140] The hybridization time required for the sensor was only 60 s as chitosan improves hybridization. Most of the sensors developed for virus and bacteria detection have a smaller linear range and higher limit of detection than the sensors for test DNA, as the use of real samples decreases the sensitivity.

6.3 Genes related to diseases

Many genosensors target detection of DNA related to disease genes. Wang et al. fabricated sensors with either FePt-CNT or FePt-GO nanocomposite suspensions on GCE via a one-pot

polyol synthesis protocol.[141] The sensor was used for the nucleic acid hybridization detection of PML-RARA fusion gene (PML: promyelocytic leukemia, RARA: retinoic acid receptor alpha), which can be used to diagnose acute promyelocytic leukemia (Figure 6). The combination of FePt nanoparticles and GO created a sensitive, selective sensor that was reusable; however the hybridization time was long, in the range of hours. Yang et al. used a graphene oxide and poly(m-aminobenzenesulfonic acid nanocomposite (PABSA–R-GO) for PML-RARA detection.[142] Upon hybridization with target DNA, the probe DNA was released from the nanocomposite and increased the EIS signal. With this sensor, it is easy to switch target molecules and the graphene nanomaterial composite greatly increases the sensitivity for detection.

Li et al designed signal-on ECL detection of *rpoB* genes related to multidrug resistant tuberculosis using ruthenium (II) complex functionalized GO (Ru-GO). [143] Ferrocene (Fc) labeled, single-stranded probe DNA was adsorbed to the electrode by π-π stacking interactions, where Fc quenched ECL signal. In the presence of a complementary sequence, the Fc-dsDNA desorbed from the electrode and the signal increased. The sensor was simple to fabricate and use, could detect mutated genes (SNPs) in the presence of nine times higher wild type genes, and was more sensitive than previously reported PCR-free *rpoB* gene assays. Poh et al. explored nanoporous carbon-modified disposable electrical printed electrodes with a hairpin DNA (hpDNA) probe to detect synthetic oligonucleotides that are associated with Alzheimer's disease development.[144] Nanoporous carbons are carbon structures with pores between 0.2 – 100 nm. Nanoporous carbons have more oxygen groups and higher electron transfer rates than graphite or CNT.[145] The signal for EIS detection increased with increasing target DNA concentration. The oxidation of DNA bases also occured at lower potentials than a previous sensor with CNTs [145], although the present method did not have a wide linear range. Genosensors for disease related genes had the highest LOD as a group and the more sensitive sensors developed for test DNA should be applied in the future for studying disease-specific DNA. Futhurmore, there is a need to test the applicability of the sensors in more practical samples.

6.4 DNA Conclusions

Carbon nanomaterial-based electrochemical genosensors offer simple, cheap, and sensitive detection of DNA. Most routine methods for DNA analysis use sample amplification by PCR to obtain a detectable amount of DNA, but electrochemical methods provide direct detection without the time consuming PCR amplification, making them potentially useful for field or clinical applications. However, research has primarily concentrated on test samples and little work has been performed in real samples where interferents could potentially degrade the sensor.

Carbon nanomaterials enhance the sensitivity, selectivity, and efficiency of DNA detection. Carbon nanotubes are still the most widely used carbon nanomaterial for genosensors and functionalizing CNTs enhances their electrochemical properties and facilitates chemical reactions to the functionalized nanotubes. Graphene-based nanomaterials have a higher density of edge plane sites, absence of metallic impurities, more flexibility, and adjustable band energy compared to CNTs. Combining carbon nanomaterials with polymers and/or

other nanoparticles can lead to more sensitive genosensors. Conducting polymers such as PICA or PDDA create more controlled CNT deposition and decrease agglomeration, leading to attomolar detection limits. However, problems associated with miniaturization of the sensor, complex fabrication methods, and proper calibration have limited their widespread use. AuNPs and CNTs are often used together because AuNPs are biocompatible and provide signal amplification for DNA sensing. AuNP-CNT sensors with LODs at zeptomolar levels have been made and more work is in progress to increase the linear range and sensor reusability.

Most research to date has been on proof of concept studies that demonstrate different nanomaterials and composites can be used as genosensors. Future work will need to concentrate on shifting from detecting synthetic oligonucleotides to more complex real samples such as viruses, bacteria, or DNA in serum. These studies should concentrate not only on detection limits and linear range, but also on reducing sensing times. Inclusion of chitosan or linkers such as glutaraldehyde [146] improves hybridization efficiency and hybridization times as low as 60 s have been obtained. However, the sensors that require shorter hybridization times use linkers and indicators that complicate the process. In addition, the routine fabrication of either reusable or disposable sensors is necessary for clinical applications. While sensor miniaturization, portability, low cost, and rapid analysis are some of the potential advantages of electrochemical DNA sensors, no single sensor has realized all these advantages. Future studies should concentrate on making robust, reproducible sensors that will be useful for real-time analysis of DNA while keeping the cost of production and operation low.

7.0 Conclusions and Future Directions

Research in carbon nanomaterial biosensors has been growing in the past five years. This growth in sensor development has been spurred by the numerous new types of carbon nanomaterials. While past studies focused on CNTs, research has now been extended to many types of graphene materials. Newer materials just starting to be used include graphene nanoflowers, graphene foam, and carbon nanotube yarns.[13,47,48,147] Future research will undoubtedly utilize materials that are not readily available today. As the number of materials grows, it will be important to compare different types of nanomaterials to assess which ones are most advantageous for sensor development. While a few studies examined the differences between CNTs and graphene, most studies concentrated on a single carbon nanomaterial or a single electrode design. Thus, the literature is currently full of designs that work, but it is less known which materials are most optimal and how carbon nanomaterials and their properties might be optimized for future sensor development.

There are also future challenges for integrating a better fundamental understanding of carbon nanomaterials and practical applications of the nanomaterials. For example, new studies using scanning electrochemical microscopy have determined that the side walls of CNTs are electrochemically active.[148–151] These studies show that electrocatalytic activity is high even without defect sites. Some have suggested that defect sites might be responsible for most detection of molecules that adsorb, such as neurotransmitters [150], but other studies show high reactivity for neurotransmitters even at basal planes.[152] Most

current studies developing sensors for biological molecules still use forms of carbon nanomaterials that have defect sites and are functionalized. In addition, vertically-aligned CNTs, CNT fibers, and carbon nanofibers are popular for sensor development because they expose the ends of the nanomaterials which tend to have the most defect sites.[47–50,153,154] Thus, there is a dichotomy between the practical and the fundamental science that will need to be worked out in the future. New methods of assembling carbon nanomaterials in a rational design might help elucidate the best geometries and the extent to which electron transfer occurs at the edge plane or the basal plane in working sensors.

Another future challenge is making robust, reproducible sensors that can be used in the field for real samples. For clinical samples, this means batch fabrication of sensors that will allow similar results to be obtained every time. The sensor would need to be either completely reusable or disposable, and most of the current designs use solid electrodes, such as glassy carbon electrodes, that do not meet these guidelines. Instead, future research might focus on screen printed electrodes or grown carbon nanomaterials that could be low enough cost to be disposable. The other challenge for field implementation is instrumentation. Portable amperometric meters exist for glucose testing, but for other electrochemical techniques, small instruments would need to be developed that could be easily run by non-experts. Many electrochemical techniques or equilibration times for carbon nanomaterial sensors can be slow, so a focus on rapid detection would also be appropriate.[35,155] The range of applications for potential sensors is greater than just clinical testing. Environmental, industrial, and pharmaceutical applications are also promising and could use a range of sensors for fast monitoring of events.

For biosensors, another goal is developing implantable sensors that might continuously monitor biomolecules for human disease. A major issue with carbon nanomaterial-based devices for these types of applications is the possible toxicity of the materials. Currently, many studies of CNT toxicity have examined the effects of inhalation of CNTs and these studies suggest that CNTs aggregates may act similarly to asbestos.[156] MWCNTs have been shown to be more resistant to phagocytosis and longer CNTs are more difficult to clear than short CNTs.[157,158] Recently, there are many new geometries and types of carbon nanomaterials beyond CNTs that need to be tested and there may be materials that would be less toxic than others. If sensors can be made with nontoxic materials or in a way to minimize leaching, there is a large potential market for implantable sensors, especially for glucose. Biocompatibility and biofouling studies are also needed to drive the future of implantable sensors.

In conclusion, the field of carbon nanomaterial based biological sensors is growing quickly with the invention of many new carbon nanomaterials. Carbon nanomaterials have many advantages for electrochemistry including fast electron transfer rates, high aspect ratios, and resistance to fouling. While many new materials are still being developed, future studies will likely help narrow down which are the most effective for mediating electron transfer. Newer methods that allow growth of carbon nanomaterials directly on the electrode substrate[33,43] or fabrication of electrodes solely from CNs, such as CNT yarns and fibers, [47,48,153,159] might be helpful for making sensors of pure carbon nanomaterials. However, combinations of carbon nanomaterials, polymers, and metal particles will also

continue to be popular because of the synergistic effects of combining materials. In the future, advances in fundamental knowledge of new nanomaterials along with a focus on practical applications in real-world systems will drive the field and lead to breakthroughs in biosensing technology.

Acknowledgements

Research in the Venton lab was funded by the NIH (R01NS076875, R01MH085159, and R21DA037584) and the Dreyfus Foundation Teacher-Scholar program.

Abbreviations

Neurotransmitter

CN	Carbon nanomaterial
3,4-dihydroxyphenethylamine, DA	Dopamine
CFMEs	carbon-fiber microelectrodes
FSCV	fast scan cyclic voltammetry
CVD	chemical vapor deposition
VACNT-CFs	vertically aligned carbon nanotube sheathed carbon fibers
CNHs	carbon nanohorns
LSV	linear sweep voltammetry
HCNT	helical CNT
PAH	poly(allylamine hydrochloride)
PDDA	poly(diallyl dimethylammonium chloride)
CNTFs	CNT fibers
DPV	differential pulse voltammetry
CNTYMEs	CNT yarn microelectrodes
CNFs	carbon nanofibers
PECVD	plasma enhanced chemical vapor deposition
CNT-N	CNT nanoweb
MEAs	Multi-electrode arrays
CNT-FETs	CNT based field effect transistors
CRGO	chemically-reduced graphene oxide
s-SWCNTs	semiconducting SWCNTs
GNR	graphene nanoribbons
GNRred	reduced graphene nanoribbons

GNRox	oxidized graphene nanoribbons
GNS	graphene nanosheets
SGGT	solution-gated graphene transistor
N-doped graphene	nitrogen doped graphene
N-CNRs	Nitrogen-doped carbon nanorods
N-PCNPs	Nitrogen-doped porous carbon nanopolyhedra
HNCMS	Hollow N-doped carbon microspheres
H-GO-CNTs	hemin-GO-pristine CNTs complexes
PPy	polypyrrole
PEDOT	poly (3,4- ethylenedioxythiophene)
PANI	polyaniline
PEI	polyethylenimine
PVA	poly (vinyl alcohol)
PPyox	overoxidized polypyrrole
PABS	poly (orthanilic acid)
CPE	carbon paste electrode
LBL	layer-by-layer
PSS	polystyrene sulfonate
CNPs	carbon nanoparticles
AuNPs	gold nanoparticles
MIPs	Molecularly imprinted polymers
ILs	Ionic liquids
NADH	nicotinamide adenine dinucleotide
BMIM–PF_6	1-butyl-3-methylimidazolium hexafluorophosphate
CILE	Carbon ionic liquid electrodes
ERGO	electrochemically reduced graphene oxide
PDOP	polydopamine
DPASV	differential pulse anodic stripping voltammetry
EPPGE	edge-plane pyrolytic graphite electrode
SDS	sodium dodecyl sulfate
SPCE	screen printed carbon electrode
5-hydroxytryptamine, 5-HT	Serotonin

PGE	pencil graphite electrodes
PIL	polymerized ionic liquid

Enzyme Biosensors

EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
NHS	N-hydroxysuccinimide
GOx	glucose oxidase
ITO	indium-tin-oxide
GAD, GA	glutaraldehyde
GNRs	graphene nanoribbons
PB	Prussian Blue
EIS	Electrochemical impedance spectroscopy
CS, Chit	chitosan
Fc-CS	ferocene modified CS
GO	graphene oxide
ChOx	cholesterol oxidase
PPF	plasma polymerized acetonitrile film
mSWNCT	metallic single walled carbon nanotube
JLFET	junctionless carbon nanotube field effect transistor
ALP	alkaline phosphatase
DPP	disodium phenyl phosphate
KMWNTs	potassium doped carbon nanotubes
MNPs	magnetic nanoparticles
CAT	catalase
AOx	alcohol oxidase
ADH	Alcohol dehydrogenase
PEI	Polyethylenimine
FcAOx	ferrocene entrapped alcohol oxidase
AldDH	aldehyde dehydrogenase
XOD	xanthine oxidase

Proteins

BSA	Bovine serum albumin
CF	carbon film
SWCNH	single walled carbon nanohorns
PSA	prostate specific antigen
AFP	α-fetoprotein
PCT	procalcitonin
CEA	carcinoembryonic antigen
OPN	osteopontin
FET	Field Effect Transistor
HERs	Human epidermal growth factor receptors
ELISA	enzyme-linked immunosorbent assay
CRP	C-reactive protein
SPQC	Single piezoelectric quartz crystal
IDE	Interdigitated electrode
GAS	Group A streptococcus

DNA sensors

TMB	tetramethylbenzidine
PICA	poly(indole-6-carboxylic acid)
PML	promyelocytic leukemia
RARA	retinoic acid receptor alpha
HBV	hepatitis B virus
HPV	human papilloma virus
PNA	peptide nucleic acid
GNFs	graphene nanoflakes
RGNW	reduced graphene nanowalls
RGNS	reduced graphene nanosheets
SNP	single nucleotide polymorphism
hpDNA	hairpin DNA

REFERENCES

1. Pérez S, Farré Ml, Barceló D. Analysis, behavior and ecotoxicity of carbon-based nanomaterials in the aquatic environment. TrAC Trends Anal. Chem. 2009; 28:820–832.

2. Yang X, Feng B, He X, Li F, Ding Y, Fei J. Carbon nanomaterial based electrochemical sensors for biogenic amines. Microchim. Acta. 2013; 180:935–956.
3. Jacobs CB, Vickrey TL, Venton BJ. Functional groups modulate the sensitivity and electron transfer kinetics of neurochemicals at carbon nanotube modified microelectrodes. Analyst. 2011; 136:3557–3565. [PubMed: 21373669]
4. McCreery RL. Advanced carbon electrode materials for molecular electrochemistry. Chem. Rev. 2008; 108:2646–2687. [PubMed: 18557655]
5. Brownson, DaC; Munro, LJ.; Kampouris, DK.; Banks, CE. Electrochemistry of graphene: not such a beneficial electrode material? RSC Adv. 2011; 1:978.
6. Deng S, Jian G, Lei J, Hu Z, Ju H. A glucose biosensor based on direct electrochemistry of glucose oxidase immobilized on nitrogen-doped carbon nanotubes. Biosens. Bioelectron. 2009; 25:373–377. [PubMed: 19683424]
7. Sheng Z-H, Zheng X-Q, Xu J-Y, Bao W-J, Wang F-B, Xia X-H. Electrochemical sensor based on nitrogen doped graphene: simultaneous determination of ascorbic acid, dopamine and uric acid. Biosens. Bioelectron. 2012; 34:125–131. [PubMed: 22342696]
8. Martín A, Hernández-Ferrer J, Vázquez L, Martínez M-T, Escarpa A. Controlled chemistry of tailored graphene nanoribbons for electrochemistry: a rational approach to optimizing molecule detection. RSC Adv. 2014; 4:132.
9. Zhu S, Li H, Niu W, Xu G. Simultaneous electrochemical determination of uric acid, dopamine, and ascorbic acid at single-walled carbon nanohorn modified glassy carbon electrode. Biosens. Bioelectron. 2009; 25:940–943. [PubMed: 19733474]
10. Zhang L, Lei J, Zhang J, Ding L, Ju H. Amperometric detection of hypoxanthine and xanthine by enzymatic amplification using a gold nanoparticles-carbon nanohorn hybrid as the carrier. Analyst. 2012; 137:3126–3131. [PubMed: 22624146]
11. Liu F, Xiang G, Yuan R, Chen X, Luo F, Jiang D, et al. Procalcitonin sensitive detection based on graphene–gold nanocomposite film sensor platform and single-walled carbon nanohorns/hollow Pt chains complex as signal tags. Biosens. Bioelectron. 2014; 60:210–217. [PubMed: 24813909]
12. Zhu C, Guo S, Fang Y, Dong S. Reducing sugar: new functional molecules for the green synthesis of graphene nanosheets. ACS Nano. 2010; 4:2429–2437. [PubMed: 20359169]
13. Du J, Yue R, Ren F, Yao Z, Jiang F, Yang P, et al. Novel graphene flowers modified carbon fibers for simultaneous determination of ascorbic acid, dopamine and uric acid. Biosens. Bioelectron. 2014; 53:220–224. [PubMed: 24140872]
14. Dong X, Wang X, Wang L, Song H, Zhang H, Huang W, et al. 3D graphene foam as a monolithic and macroporous carbon electrode for electrochemical sensing. ACS Appl. Mater. Interfaces. 2012; 4:3129–3133. [PubMed: 22574906]
15. Kim SK, Kim D, Jeon S. Electrochemical determination of serotonin on glassy carbon electrode modified with various graphene nanomaterials. Sensors Actuators B Chem. 2012; 174:285–291.
16. Maleki N, Safavi A, Tajabadi F. High-performance carbon composite electrode based on an ionic liquid as a binder. Anal. Chem. 2006; 78:3820–3826. [PubMed: 16737243]
17. Mittal, V. Surface Modification of Nanotube Fillers. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA; 2011.
18. Jacobs CB, Peairs MJ, Venton BJ. Review: Carbon nanotube based electrochemical sensors for biomolecules. Anal. Chim. Acta. 2010; 662:105–127. [PubMed: 20171310]
19. Lin C-H, Hsiao C-Y, Hung C-H, Lo Y-R, Lee C-C, Su C-J, et al. Ultrasensitive detection of dopamine using a polysilicon nanowire field-effect transistor. Chem. Commun. (Camb). 2008:5749–5751. [PubMed: 19009069]
20. Gan T, Hu S. Electrochemical sensors based on graphene materials. Microchim. Acta. 2011; 175:1–19.
21. Liu J, Liu Z, Barrow CJ, Yang W. Molecularly engineered graphene surfaces for sensing applications: A review. Anal. Chim. Acta. 2014; 859:1–19. [PubMed: 25622601]
22. Lawal AT. Synthesis and utilisation of graphene for fabrication of electrochemical sensors. Talanta. 2014; 131:424–443. [PubMed: 25281124]
23. Zhan B, Li C, Yang J, Jenkins G, Huang W, Dong X. Graphene field-effect transistor and its application for electronic sensing. Small. 2014; 10:4042–4065. [PubMed: 25044546]

24. Chen A, Chatterjee S. Nanomaterials based electrochemical sensors for biomedical applications. Chem. Soc. Rev. 2013; 42:5425–5438. [PubMed: 23508125]
25. Zhang L, Wang J, Tian Y. Electrochemical in-vivo sensors using nanomaterials made from carbon species, noble metals, or semiconductors. Microchim. Acta. 2014; 181:1471–1484.
26. Balasubramanian K, Kern K. 25th anniversary article: label-free electrical biodetection using carbon nanostructures. Adv. Mater. 2014; 26:1154–1175. [PubMed: 24452968]
27. Gao C, Guo Z, Liu J-H, Huang X-J. The new age of carbon nanotubes: An updated review of functionalized carbon nanotubes in electrochemical sensors. Nanoscale. 2012; 4:1948. [PubMed: 22337209]
28. Vera S. Chemical sensors based on polymer composites with carbon nanotubes and graphene: the role of the polymer. J. Mater. Chem. A. 2014:14289–14328.
29. Richfield EK, Penney JB, Young aB. Anatomical and affinity state comparisons between dopamine D1 and D2 receptors in the rat central nervous system. Neuroscience. 1989; 30:767–777. [PubMed: 2528080]
30. Hocevar SB, Wang J, Deo RP, Musameh M, Ogorevc B. Carbon nanotube modified microelectrode for enhanced voltammetric detection of dopamine in the presence of ascorbate. Electroanalysis. 2005; 17:417–422.
31. Swamy BEK, Venton BJ. Carbon nanotube-modified microelectrodes for simultaneous detection of dopamine and serotonin in vivo. Analyst. 2007; 132:876–884. [PubMed: 17710262]
32. Takmakov P, Zachek MK, Keithley RB, Walsh PL, Donley C, McCarty GS, et al. Carbon microelectrodes with a renewable surface. Anal. Chem. 2010; 82:2020–2028. [PubMed: 20146453]
33. Xiao N, Venton B. Rapid, sensitive detection of neurotransmitters at microelectrodes modified with self-assembled SWCNT forests. Anal. Chem. 2012; 84:7816–7822. [PubMed: 22823497]
34. Robinson DL, Venton BJ, Heien MLAV, Wightman RM. Detecting subsecond dopamine release with fast-scan cyclic voltammetry in vivo. Clin. Chem. 2003; 49:1763–1773. [PubMed: 14500617]
35. Baur JE, Kristensen EW, May LJ, Wiedemann DJ, Wightman RM. Fast-scan voltammetry of biogenic amines. Anal. Chem. 1988; 60:1268–1272. [PubMed: 3213946]
36. Pisoschi AM, Pop A, Serban AI, Fafaneata C. Electrochemical methods for ascorbic acid determination. Electrochim. Acta. 2014; 121:443–460.
37. Rice ME, Russo-Menna I. Differential compartmentalization of brain ascorbate and glutathione between neurons and glia. Neuroscience. 1997; 82:1213–1223. [PubMed: 9466441]
38. Dutt VV, Mottola HA. Determination of uric acid at the microgram level by a kinetic procedure based on a “pseudo-induction” period. Anal. Chem. 1974; 46:1777–1781. [PubMed: 4412774]
39. Serra PA, Susini G, Rocchitta G, Migheli R, Dessanti G, Miele E, et al. Effects of sufentanil on the release and metabolism of dopamine and ascorbic acid and glutamate release in the striatum of freely moving rats. Neurosci. Lett. 2003; 344:9–12. [PubMed: 12781909]
40. Amaro S, Urra X, Gómez-Choco M, Obach V, Cervera Á, Vargas M, et al. Uric acid levels are relevant in patients with stroke treated with thrombolysis. Stroke. 2011:28–33. [PubMed: 22033997]
41. Goldstein DS, Holmes C. Neuronal source of plasma dopamine. Clin. Chem. 2008; 54:1864–1871. [PubMed: 18801936]
42. Tallaksen CM, Bøhmer T, Bell H. Concentrations of the water-soluble vitamins thiamin, ascorbic acid, and folic acid in serum and cerebrospinal fluid of healthy individuals. Am. J. Clin. Nutr. 1992; 56:559–564. [PubMed: 1503069]
43. Xiang L, Yu P, Hao J, Zhang M, Zhu L, Dai L, et al. Vertically aligned carbon nanotube-sheathed carbon fibers as pristine microelectrodes for selective monitoring of ascorbate in vivo. Anal. Chem. 2014; 86:3909–3914. [PubMed: 24678660]
44. Cui R, Wang X, Zhang G, Wang C. Simultaneous determination of dopamine, ascorbic acid, and uric acid using helical carbon nanotubes modified electrode. Sensors Actuators B Chem. 2012; 161:1139–1143.
45. Zhang B, Huang D, Xu X, Alemu G, Zhang Y, Zhan F, et al. Simultaneous electrochemical determination of ascorbic acid, dopamine and uric acid with helical carbon nanotubes. Electrochim. Acta. 2013; 91:261–266.

46. Tang NJ, Zhong W, Au CT, Gedanken A, Yang Y, Du YW. Large-Scale Synthesis, Annealing, Purification, and Magnetic Properties of Crystalline Helical Carbon Nanotubes with Symmetrical Structures. Adv. Funct. Mater. 2007; 17:1542–1550.
47. Jacobs CB, Ivanov IN, Nguyen MD, Zestos AG, Venton BJ. High temporal resolution measurements of dopamine with carbon nanotube yarn microelectrodes. Anal. Chem. 2014; 86:5721–5727. [PubMed: 24832571]
48. Schmidt AC, Wang X, Zhu Y, Sombers La. Carbon nanotube yarn electrodes for enhanced detection of neurotransmitter dynamics in live brain tissue. ACS Nano. 2013; 7:7864–7873. [PubMed: 23941323]
49. Zhang DA, Rand E, Marsh M, Andrews RJ, Lee KH, Meyyappan M, et al. Carbon nanofiber electrode for neurochemical monitoring. Mol. Neurobiol. 2013; 48:380–385. [PubMed: 23975638]
50. Rand E, Periyakaruppan A, Tanaka Z, Zhang Da, Marsh MP, Andrews RJ, et al. A carbon nanofiber based biosensor for simultaneous detection of dopamine and serotonin in the presence of ascorbic acid. Biosens. Bioelectron. 2013; 42:434–438. [PubMed: 23228495]
51. Li W-S, Hou P-X, Liu C, Sun D-M, Yuan J, Zhao S-Y, et al. High-quality, highly concentrated semiconducting single-wall carbon nanotubes for use in field effect transistors and biosensors. ACS Nano. 2013; 7:6831–6839. [PubMed: 23883135]
52. Novoselov KS, Geim aK, Morozov SV, Jiang D, Katsnelson MI, Grigorieva IV, et al. Two-dimensional gas of massless Dirac fermions in graphene. Nature. 2005; 438:197–200. [PubMed: 16281030]
53. Novoselov KS, Geim AK, Morozov SV, Jiang D, Zhang Y, Dubonos SV, et al. Electric field effect in atomically thin carbon films. Science. 2004; 306:666–669. [PubMed: 15499015]
54. Sanghavi BJ, Wolfbeis OS, Hirsch T, Swami NS. Nanomaterial-based electrochemical sensing of neurological drugs and neurotransmitters. Microchim. Acta. 2014
55. Thomas T, Mascarenhas RJ, Nethravathi C, Rajamathi M, Kumara Swamy BE. Graphite oxide bulk modified carbon paste electrode for the selective detection of dopamine: A voltammetric study. J. Electroanal. Chem. 2011; 659:113–119.
56. Wang Z, Liu J, Liang Q, Wang Y, Luo G. Carbon nanotube-modified electrodes for the simultaneous determination of dopamine and ascorbic acid. Analyst. 2002; 127:653–658. [PubMed: 12081044]
57. Liang M, Zhi L. Graphene-based electrode materials for rechargeable lithium batteries. J. Mater. Chem. 2009; 19:5871.
58. Geim AK, Novoselov KS. The rise of graphene. Nat. Mater. 2007; 6:183–191. [PubMed: 17330084]
59. Bu Y, Dai W, Li N, Zhao X, Zuo X. The graphene nanopowder for electro-catalytic oxidation of dopamine and uric acid in the presence of ascorbic acid. J. Energy Chem. 2013; 22:685–689.
60. Yue HY, Huang S, Chang J, Heo C, Yao F, Adhikari S, et al. ZnO nanowire arrays on 3D hierachical graphene foam: Biomarker detection of parkinson's disease. ACS Nano. 2014; 8:1639–1646. [PubMed: 24405012]
61. Zhang M, Liao C, Yao Y, Liu Z, Gong F, Yan F. High-Performance Dopamine Sensors Based on Whole-Graphene Solution-Gated Transistors. Adv. Funct. Mater. 2014; 24:978–985.
62. Li S-M, Yang S-Y, Wang Y-S, Lien C-H, Tien H-W, Hsiao S-T, et al. Controllable synthesis of nitrogen-doped graphene and its effect on the simultaneous electrochemical determination of ascorbic acid, dopamine, and uric acid. Carbon N. Y. 2013; 59:418–429.
63. Yuan D, Yuan X, Zhou S, Zou W, Zhou T. N-Doped carbon nanorods as ultrasensitive electrochemical sensors for the determination of dopamine. RSC Adv. 2012; 2:8157.
64. Gai P, Zhang H, Zhang Y, Liu W, Zhu G, Zhang X, et al. Simultaneous electrochemical detection of ascorbic acid, dopamine and uric acid based on nitrogen doped porous carbon nanopolyhedra. J. Mater. Chem. B. 2013; 1:2742.
65. Vreeland RF, Atcherley CW, Russell WS, Xie JY, Lu D, Laude ND, et al. Biocompatible PEDOT:Nafion Composite Electrode Coatings for Selective Detection of Neurotransmitters in Vivo. Anal. Chem. 2015; 87:2600–2607. [PubMed: 25692657]

66. Xu G, Li B, Cui XT, Ling L, Luo X. Electrodeposited conducting polymer PEDOT doped with pure carbon nanotubes for the detection of dopamine in the presence of ascorbic acid. Sensors Actuators B Chem. 2013; 188:405–410.

67. Wang W, Xu G, Cui XT, Sheng G, Luo X. Enhanced catalytic and dopamine sensing properties of electrochemically reduced conducting polymer nanocomposite doped with pure graphene oxide. Biosens. Bioelectron. 2014; 58:153–156. [PubMed: 24632460]

68. Zestos AG, Jacobs CB, Trikantzopoulos E, Ross AE, Venton BJ. Polyethylenimine carbon nanotube fiber electrodes for enhanced detection of neurotransmitters. Anal. Chem. 2014; 86:8568–8575. [PubMed: 25117550]

69. Vigolo B, Pénicaud A, Coulon C, Sauder C, Pailler R, Journet C, et al. Macroscopic fibers and ribbons of oriented carbon nanotubes. Science. 2000; 290:1331–1334. [PubMed: 11082056]

70. Manjunatha R, Suresh GS, Melo JS, D'Souza SF, Venkatesha TV. Simultaneous determination of ascorbic acid, dopamine and uric acid using polystyrene sulfonate wrapped multiwalled carbon nanotubes bound to graphite electrode through layer-by-layer technique. Sensors Actuators B Chem. 2010; 145:643–650.

71. Celebanska A, Tomaszewska D, Lesniewski A, Opallo M. Film electrode prepared from oppositely charged silicate submicroparticles and carbon nanoparticles for selective dopamine sensing. Biosens. Bioelectron. 2011; 26:4417–4422. [PubMed: 21641787]

72. Jackowska K, Krysinski P. New trends in the electrochemical sensing of dopamine. Anal. Bioanal. Chem. 2013; 405:3753–3771. [PubMed: 23241816]

73. Du J, Yue R, Ren F, Yao Z, Jiang F, Yang P, et al. Simultaneous determination of uric acid and dopamine using a carbon fiber electrode modified by layer-by-layer assembly of graphene and gold nanoparticles. Gold Bull. 2013; 46:137–144.

74. Gu L, Jiang X, Liang Y, Zhou T, Shi G. Double recognition of dopamine based on a boronic acid functionalized poly(aniline-co-anthranilic acid)-molecularly imprinted polymer composite. Analyst. 2013; 138:5461–5469. [PubMed: 23884110]

75. Liu B, Lian HT, Yin JF, Sun XY. Dopamine molecularly imprinted electrochemical sensor based on graphene–chitosan composite. Electrochim. Acta. 2012; 75:108–114.

76. Qian T, Yu C, Zhou X, Ma P, Wu S, Xu L, et al. Ultrasensitive dopamine sensor based on novel molecularly imprinted polypyrrole coated carbon nanotubes. Biosens. Bioelectron. 2014; 58C: 237–241. [PubMed: 24657643]

77. Njagi J, Chernov MM, Leiter JC, Andreescu S. Amperometric detection of dopamine in vivo with an enzyme based carbon fiber microbiosensor. Anal. Chem. 2010; 82:989–996. [PubMed: 20055419]

78. Valcárcel M, Cárdenas S, Simonet BM. Role of carbon nanotubes in analytical science. Anal. Chem. 2007; 79:4788–4797. [PubMed: 17542556]

79. Canbay E, Akyilmaz E. Design of a multiwalled carbon nanotube-Nafion-cysteamine modified tyrosinase biosensor and its adaptation of dopamine determination. Anal. Biochem. 2014; 444:8–15. [PubMed: 24090870]

80. Numnuam A, Thavarungkul P, Kanatharana P. An amperometric uric acid biosensor based on chitosan-carbon nanotubes electrospun nanofiber on silver nanoparticles. Anal. Bioanal. Chem. 2014; 406:3763–3772. [PubMed: 24718436]

81. Xue C, Wang X, Zhu W, Han Q, Zhu C, Hong J, et al. Electrochemical serotonin sensing interface based on double-layered membrane of reduced graphene oxide/polyaniline nanocomposites and molecularly imprinted polymers embedded with gold nanoparticles. Sensors Actuators B Chem. 2014; 196:57–63.

82. Mazloum-Ardakani M, Khoshroo A. High sensitive sensor based on functionalized carbon nanotube/ionic liquid nanocomposite for simultaneous determination of norepinephrine and serotonin. J. Electroanal. Chem. 2014; 717–718:17–23.

83. Mazloum-Ardakani M, Khoshroo A. Electrocatalytic properties of functionalized carbon nanotubes with titanium dioxide and benzofuran derivative/ionic liquid for simultaneous determination of isoproterenol and serotonin. Electrochim. Acta. 2014; 130:634–641.

84. Babaei A, Taheri AR, Aminikhah M. Nanomolar simultaneous determination of levodopa and serotonin at a novel carbon ionic liquid electrode modified with Co(OH)2 nanoparticles and multi-walled carbon nanotubes. Electrochim. Acta. 2013; 90:317–325.
85. Valentini F, Ciambella E, Conte V, Sabatini L, Ditaranto N, Cataldo F, et al. Highly selective detection of Epinephrine at oxidized Single-Wall Carbon Nanohorns modified Screen Printed Electrodes (SPEs). Biosens. Bioelectron. 2014; 59:94–98. [PubMed: 24704763]
86. Chen S-M, Chen J-Y, Vasantha VS. Electrochemical preparation of epinephrine/Nafion chemically modified electrodes and their electrocatalytic oxidation of ascorbic acid and dopamine. Electrochim. Acta. 2006; 52:455–465.
87. Thomas T, Mascarenhas RJ, D' Souza OJ, Detriche S, Mekhalif Z, Martis P. Pristine multi-walled carbon nanotubes/SDS modified carbon paste electrode as an amperometric sensor for epinephrine. Talanta. 2014; 125:352–360. [PubMed: 24840456]
88. Prasad BB, Prasad A, Tiwari MP, Madhuri R. Multiwalled carbon nanotubes bearing "terminal monomeric unit" for the fabrication of epinephrine imprinted polymer-based electrochemical sensor. Biosens. Bioelectron. 2013; 45:114–122. [PubMed: 23455050]
89. Cui F, Zhang X. Electrochemical sensor for epinephrine based on a glassy carbon electrode modified with graphene/gold nanocomposites. J. Electroanal. Chem. 2012; 669:35–41.
90. Babaei A, Sohrabi M, Afrasiabi M. A Sensitive Simultaneous Determination of Epinephrine and Piroxicam Using a Glassy Carbon Electrode Modified with a Nickel Hydroxide Nanoparticles/Multiwalled Carbon Nanotubes Composite. Electroanalysis. 2012; 24:2387–2394.
91. Lin K-C, Li Y-S, Chen S-M. Electrochemical determination of nicotinamide adenine dinucleotide and hydrogen peroxide based on poly(xanthurenic acid), flavin adenine dinucleotide and functionalized multi-walled carbon nanotubes. Sensors Actuators B Chem. 2013; 184:212–219.
92. Husmann S, Nossol E, Zarbin AJG. Carbon nanotube/Prussian blue paste electrodes: Characterization and study of key parameters for application as sensors for determination of low concentration of hydrogen peroxide. Sensors Actuators B Chem. 2014; 192:782–790.
93. Zhu X, Niu X, Zhao H, Lan M. Doping ionic liquid into Prussian blue-multiwalled carbon nanotubes modified screen-printed electrode to enhance the nonenzymatic H2O2 sensing performance. Sensors Actuators B Chem. 2014; 195:274–280.
94. Liu H, Cui Y, Li P, Zhou Y, Zhu X, Tang Y, et al. Iron(iii) diethylenetriaminepentaacetic acid complex on polyallylamine functionalized multiwalled carbon nanotubes: immobilization, direct electrochemistry and electrocatalysis. Analyst. 2013; 138:2647–2653. [PubMed: 23486722]
95. Kaçar C, Dalkiran B, Erden PE, Kiliç E. An amperometric hydrogen peroxide biosensor based on Co3O4 nanoparticles and multiwalled carbon nanotube modified glassy carbon electrode. Appl. Surf. Sci. 2014; 311:139–146.
96. Wang YF, Du J, Li YY, Shan DL, Zhou XB, Xue ZH, et al. A amperometric biosensor for hydrogen peroxide by adsorption of horseradish peroxidase onto single-walled carbon nanotubes. Colloids and Surfaces B-Biointerfaces. 2012; 90:62–67.
97. Shamsipur M, Asgari M, Maragheh MG, Moosavi-Movahedi AA. A novel impedimetric nanobiosensor for low level determination of hydrogen peroxide based on biocatalysis of catalase. Bioelectrochemistry. 2012; 83:31–37. [PubMed: 21880554]
98. Chatterjee S, Chen AC. Functionalization of carbon buckypaper for the sensitive determination of hydrogen peroxide in human urine. Biosens. Bioelectron. 2012; 35:302–307. [PubMed: 22459585]
99. Liu J, Wang X, Wang T, Li D, Xi F, Wang J, et al. Functionalization of Monolithic and Porous Three-Dimensional Graphene by One-Step Chitosan Electrodeposition for Enzymatic Biosensor. ACS Appl. Mater. Interfaces. 2014; 6:19997–20002. [PubMed: 25384251]
100. Zhu ZG, Garcia-Gancedo L, Flewitt AJ, Moussy F, Li YL, Milne WI. Design of carbon nanotube fiber microelectrode for glucose biosensing. J. Chem. Technol. Biotechnol. 2012; 87:256–262.
101. Khodadadei F, Ghourchian H, Soltanieh M, Hosseinalipour M, Mortazavi Y. Rapid and clean amine functionalization of carbon nanotubes in a dielectric barrier discharge reactor for biosensor development. Electrochim. Acta. 2014; 115:378–385.
102. Piao Y, Han DJ, Seo TS. Highly conductive graphite nanoparticle based enzyme biosensor for electrochemical glucose detection. Sensors Actuators B Chem. 2014; 194:454–459.

103. Im JS, Yun J, Kim JG, Bae TS, Lee YS. The effects of carbon nanotube addition and oxyfluorination on the glucose-sensing capabilities of glucose oxidase-coated carbon fiber electrodes. Appl. Surf. Sci. 2012; 258:2219–2225.
104. Muguruma H, Hoshino T, Nowaki K. Electronically Type-Sorted Carbon Nanotube-Based Electrochemical Biosensors with Glucose Oxidase and Dehydrogenase. ACS Appl. Mater. Interfaces. 2015; 7:584–592. [PubMed: 25522366]
105. Das M, Goswami P. Direct electrochemistry of alcohol oxidase using multiwalled carbon nanotube as electroactive matrix for biosensor application. Bioelectrochemistry. 2013; 89:19–25. [PubMed: 23000393]
106. Chinnadayyala SR, Kakoti A, Santhosh M, Goswami P. A novel amperometric alcohol biosensor developed in a 3rd generation bioelectrode platform using peroxidase coupled ferrocene activated alcohol oxidase as biorecognition system. Biosens. Bioelectron. 2014; 55:120–126. [PubMed: 24368229]
107. Kowalewska B, Kulesza PJ. Toward More Efficient Bioelectrocatalytic Oxidation of Ethanol for Amperometric Sensing and Biofuel Cell Technology. Anal. Chem. 2012; 84:9564–9571. [PubMed: 23066916]
108. Harreither W, Trouillon R, Poulin P, Neri W, Ewing AG, Safina G. Carbon Nanotube Fiber Microelectrodes Show a Higher Resistance to Dopamine Fouling. Anal. Chem. 2013; 85:7447–7453. [PubMed: 23789970]
109. Baldrich E, Gómez R, Gabriel G, Muñoz FX. Magnetic entrapment for fast, simple and reversible electrode modification with carbon nanotubes: application to dopamine detection. Biosens. Bioelectron. 2011; 26:1876–1882. [PubMed: 20378329]
110. Zhang HF, Liu RX, Zheng JB. Selective determination of cholesterol based on cholesterol oxidase-alkaline phosphatase bienzyme electrode. Analyst. 2012; 137:5363–5367. [PubMed: 23019566]
111. Barik MA, Dutta JC. Fabrication and characterization of junctionless carbon nanotube field effect transistor for cholesterol detection. Appl. Phys. Lett. 2014; 105:053509.
112. Torres AC, Ghica ME, Brett CMA. Design of a new hypoxanthine biosensor: xanthine oxidase modified carbon film and multi-walled carbon nanotube/carbon film electrodes. Anal. Bioanal. Chem. 2013; 405:3813–3822. [PubMed: 23263517]
113. Lerner MB, D'Souza J, Pazina T, Dailey J, Goldsmith BR, Robinson MK, et al. Hybrids of a Genetically Engineered Antibody and a Carbon Nanotube Transistor for Detection of Prostate Cancer Biomarkers. ACS Nano. 2012; 6:5143–5149. [PubMed: 22575126]
114. Akter R, Rahman MA, Rhee CK. Amplified Electrochemical Detection of a Cancer Biomarker by Enhanced Precipitation Using Horseradish Peroxidase Attached on Carbon Nanotubes. Anal. Chem. 2012; 84:6407–6415. [PubMed: 22793977]
115. Zhao C, Lin D, Wu J, Ding L, Ju H, Yan F. Nanogold-Enriched Carbon Nanohorn Label for Sensitive Electrochemical Detection of Biomarker on a Disposable Immunosensor. Electroanalysis. 2013; 25:1044–1049.
116. Yang F, Han J, Zhuo Y, Yang Z, Chai Y, Yuan R. Highly sensitive impedimetric immunosensor based on single-walled carbon nanohorns as labels and bienzyme biocatalyzed precipitation as enhancer for cancer biomarker detection. Biosens. Bioelectron. 2014; 55:360–365. [PubMed: 24419078]
117. Jia X, Liu Z, Liu N, Ma Z. A label-free immunosensor based on graphene nanocomposites for simultaneous multiplexed electrochemical determination of tumor markers. Biosens. Bioelectron. 2014; 53:160–166. [PubMed: 24140831]
118. Gupta RK, Periyakaruppan A, Meyyappan M, Koehne JE. Label-free detection of C-reactive protein using a carbon nanofiber based biosensor. Biosens. Bioelectron. 2014; 59:112–119. [PubMed: 24709327]
119. Justino CIL, Freitas AC, Amaral JP, Rocha-Santos TAP, Cardoso S, Duarte AC. Disposable immunosensors for C-reactive protein based on carbon nanotubes field effect transistors. Talanta. 2013; 108:165–170. [PubMed: 23601885]

120. Chen HJ, Zhang ZH, Luo LJ, Yao SZ. Surface-imprinted chitosan-coated magnetic nanoparticles modified multi-walled carbon nanotubes biosensor for detection of bovine serum albumin. Sensors Actuators B Chem. 2012; 163:76–83.

121. Shi X, He F, Lian Y, Yan D, Zhang X. A new aptamer/SWNTs IDE-SPQC sensor for rapid and specific detection of Group A Streptococcus. Sensors Actuators B Chem. 2014; 198:431–437.

122. Palecek E. Oscillographic polarography of highly polymerized deoxyribonucleic acid. Nature. 1960; 188:656–657. [PubMed: 13732209]

123. Chen PH, McCreery RL. Control of electron transfer kinetics at glassy carbon electrodes by specific surface modification. Anal. Chem. 1996; 68:3958–3965.

124. Dong X-Y, Mi X-N, Zhang L, Liang T-M, Xu J-J, Chen H-Y. DNAzyme-functionalized Pt nanoparticles/carbon nanotubes for amplified sandwich electrochemical DNA analysis. Biosens. Bioelectron. 2012; 38:337–341. [PubMed: 22776177]

125. Gutierrez F, Rubianes MD, Rivas GA. Adsorption and Electrooxidation of DNA at Glassy Carbon Electrodes Modified with Multiwall Carbon Nanotubes Dispersed in Glucose Oxidase. Electroanalysis. 2013; 25:1135–1142.

126. Dong X, Lu X, Zhang K, Zhang Y. Chronocoulometric DNA biosensor based on a glassy carbon electrode modified with gold nanoparticles, poly(dopamine) and carbon nanotubes. Microchim. Acta. 2013; 180:101–108.

127. Wang J, Li S, Zhang Y. A sensitive DNA biosensor fabricated from gold nanoparticles, carbon nanotubes, and zinc oxide nanowires on a glassy carbon electrode. Electrochim. Acta. 2010; 55:4436–4440.

128. Huang K-J, Liu Y-J, Wang H-B, Wang Y-Y, Liu Y-M. Sub-femtomolar DNA detection based on layered molybdenum disulfide/multi-walled carbon nanotube composites, Au nanoparticle and enzyme multiple signal amplification. Biosens. Bioelectron. 2014; 55:195–202. [PubMed: 24384259]

129. Li L, Wang S, Yang T, Huang S, Wang J. Electrochemical growth of gold nanoparticles on horizontally aligned carbon nanotubes: A new platform for ultrasensitive DNA sensing. Biosens. Bioelectron. 2012; 33:279–283. [PubMed: 22236779]

130. Wipawakarn P, Ju H, Wong DKY. A label-free electrochemical DNA biosensor based on a Zr(IV)-coordinated DNA duplex immobilised on a carbon nanofibre|chitosan layer. Anal. Bioanal. Chem. 2012; 402:2817–2826. [PubMed: 22297858]

131. Vamvakaki V, Fouskaki M, Chaniotakis N. Electrochemical Biosensing systems based on carbon nanotubes and carbon nanofibers. Anal. Lett. 2007; 40:2271–2287.

132. Pumera M. Electrochemistry of Graphene: New Horizons for Sensing and Energy Storage. Chem. Rec. 2009; 9:211–223. [PubMed: 19739147]

133. Cai B, Wang S, Huang L, Ning Y, Zhang Z, Zhang GJ. Ultrasensitive label-free detection of PNA-DNA hybridization by reduced graphene oxide field-effect transistor biosensor. ACS Nano. 2014; 8:2632–2638. [PubMed: 24528470]

134. Xu G, Abbott J, Qin L, Yeung KYM, Song Y, Yoon H, et al. Electrophoretic and field-effect graphene for all-electrical DNA array technology. Nat. Commun. 2014; 5:4866. [PubMed: 25189574]

135. Shang N, Kumar A, Sun N, Sharma S, Papakonstantinou P, Li M, et al. Vertical graphene nanoflakes for the immobilization, electrocatalytic oxidation and quantitative detection of DNA. Electrochem. Commun. 2012; 25:140–143.

136. Akhavan O, Ghaderi E, Rahighi R. Toward Single-DNA Electrochemical Biosensing by Graphene Nanowalls. ACS Nano. 2012; 6:2904–2916. [PubMed: 22385391]

137. Nie G, Bai Z, Chen J, Yu W. Simple Label-Free Femtomolar DNA Detection Based on a Nanostructure Composite Material: MWNT-Doped Poly(indole-6-carboxylic acid). ACS Macro Lett. 2012; 1:1304–1307.

138. Wang S, Li L, Jin H, Yang T, Bao W, Huang S, et al. Electrochemical detection of hepatitis B and papilloma virus DNAs using SWCNT array coated with gold nanoparticles. Biosens. Bioelectron. 2013; 41:205–210. [PubMed: 22947516]

139. Nie H, Yang Z, Huang S, Wu Z, Wang H, Yu R, et al. DNA-Wrapped Carbon Nanotubes as Sensitive Electrochemical Labels in Controlled-Assembly-Mediated Signal Transduction for the Detection of Sequence-Specific DNA. Small. 2012; 8:1407–1414. [PubMed: 22392696]

140. Singh A, Sinsinbar G, Choudhary M, Kumar V, Pasricha R, Verma HN, et al. Graphene oxide-chitosan nanocomposite based electrochemical DNA biosensor for detection of typhoid. Sensors and Actuators B-Chemical. 2013; 185:675–684.

141. Wang L, Zheng X, Zhang W, Quan X, Hu Q, Wu W, et al. One-pot synthesis of carbon-decorated FePt nanoparticles and their application for label-free electrochemical impedance sensing of DNA hybridization. Rsc Adv. 2013; 3:9042–9046.

142. Yang T, Guan Q, Guo X, Meng L, Du M, Jiao K. Direct and freely switchable detection of target genes engineered by reduced graphene oxide-poly(m-aminobenzenesulfonic acid) nanocomposite via synchronous pulse electrosynthesis. Anal. Chem. 2013; 85:1358–1366. [PubMed: 23256634]

143. Li F, Yu Y, Li Q, Zhou M, Cui H. A homogeneous signal-on strategy for the detection of rpoB genes of Mycobacterium tuberculosis based on electrochemiluminescent graphene oxide and ferrocene quenching. Anal. Chem. 2014; 86:1608–1613. [PubMed: 24422505]

144. Poh HL, Bonanni A, Pumera M. Nanoporous carbon as a sensing platform for DNA detection: The use of impedance spectroscopy for hairpin-DNA based assay. Rsc Adv. 2012; 2:1021–1024.

145. Poh HL, Pumera M. Nanoporous Carbon Materials for Electrochemical Sensing. Chem. Asian J. 2012; 7:412–416. [PubMed: 22162295]

146. Singh R, Dhand C, Sumana G, Verma R, Sood S, Gupta RK, et al. Polyaniline/carbon nanotubes platform for sexually transmitted disease detection. J. Mol. Recognit. 2010; 23:472–479. [PubMed: 20101643]

147. Ma Y, Zhao M, Cai B, Wang W, Ye Z, Huang J. 3D graphene foams decorated by CuO nanoflowers for ultrasensitive ascorbic acid detection. Biosens. Bioelectron. 2014; 59:384–388. [PubMed: 24755255]

148. Kim J, Xiong H, Hofmann M, Kong J, Amemiya S. Letters to Analytical Chemistry Scanning Electrochemical Microscopy of Individual Single-Walled Carbon Nanotubes. 2010; 82:1605–1607.

149. Byers JC, Güell AG, Unwin PR. Nanoscale electrocatalysis: visualizing oxygen reduction at pristine, kinked, and oxidized sites on individual carbon nanotubes. J. Am. Chem. Soc. 2014; 136:11252–11255. [PubMed: 25061694]

150. Dumitrescu I, Unwin PR, Macpherson JV. Electrochemistry at carbon nanotubes: perspective and issues. Chem. Commun. (Camb). 2009; 7345:6886–6901. [PubMed: 19904345]

151. Güell AG, Meadows KE, Dudin PV, Ebejer N, Macpherson JV, Unwin PR. Mapping nanoscale electrochemistry of individual single-walled carbon nanotubes. Nano Lett. 2014; 14:220–224. [PubMed: 24274402]

152. Patel AN, Tan S, Miller TS, Macpherson JV, Unwin PR. Comparison and reappraisal of carbon electrodes for the voltammetric detection of dopamine. Anal. Chem. 2013; 85:11755–11764. [PubMed: 24308368]

153. Viry L, Derré A, Poulin P, Kuhn A. Discrimination of dopamine and ascorbic acid using carbon nanotube fiber microelectrodes. Phys. Chem. Chem. Phys. 2010; 12:9993–9995. [PubMed: 20623074]

154. Koehne JE, Marsh M, Boakye A, Douglas B, Kim IY, Chang S-Y, et al. Carbon nanofiber electrode array for electrochemical detection of dopamine using fast scan cyclic voltammetry. Analyst. 2011; 136:1802–1805. [PubMed: 21387028]

155. Hermans A, Keithley RB, Kita JM, Sombers La, Wightman RM. Dopamine detection with fast-scan cyclic voltammetry used with analog background subtraction. Anal. Chem. 2008; 80:4040–4048. [PubMed: 18433146]

156. Poland, Ca; Duffin, R.; Kinloch, I.; Maynard, A.; Wallace, WaH; Seaton, A., et al. Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. Nat. Nanotechnol. 2008; 3:423–428. [PubMed: 18654567]

157. Murphy, Fa; Poland, Ca; Duffin, R.; Al-Jamal, KT.; Ali-Boucetta, H.; Nunes, A., et al. Length-dependent retention of carbon nanotubes in the pleural space of mice initiates sustained

inflammation and progressive fibrosis on the parietal pleura. Am. J. Pathol. 2011; 178:2587–2600. [PubMed: 21641383]

158. Murphy FA, Poland CA, Duffin R, Donaldson K. Length-dependent pleural inflammation and parietal pleural responses after deposition of carbon nanotubes in the pulmonary airspaces of mice. Nanotoxicology. 2013; 7:1157–1167. [PubMed: 22812632]
159. Harreither W, Trouillon R, Poulin P, Neri W, Ewing AG, Safina G. Carbon nanotube fiber microelectrodes show a higher resistance to dopamine fouling. Anal. Chem. 2013; 85:7447–7453. [PubMed: 23789970]
160. Lerner MB, D'Souza J, Pazina T, Dailey J, Goldsmith BR, Robinson MK, et al. Hybrids of a genetically engineered antibody and a carbon nanotube transistor for detection of prostate cancer biomarkers. ACS Nano. 2012; 6:5143–5149. [PubMed: 22575126]

Biographies

Highlights

1. We review the types of carbon nanomaterials used in electrochemical sensors
2. Different materials and sensor designs are compared for classes of biomolecules
3. Future challenges of better sensor design and implementation are assessed.

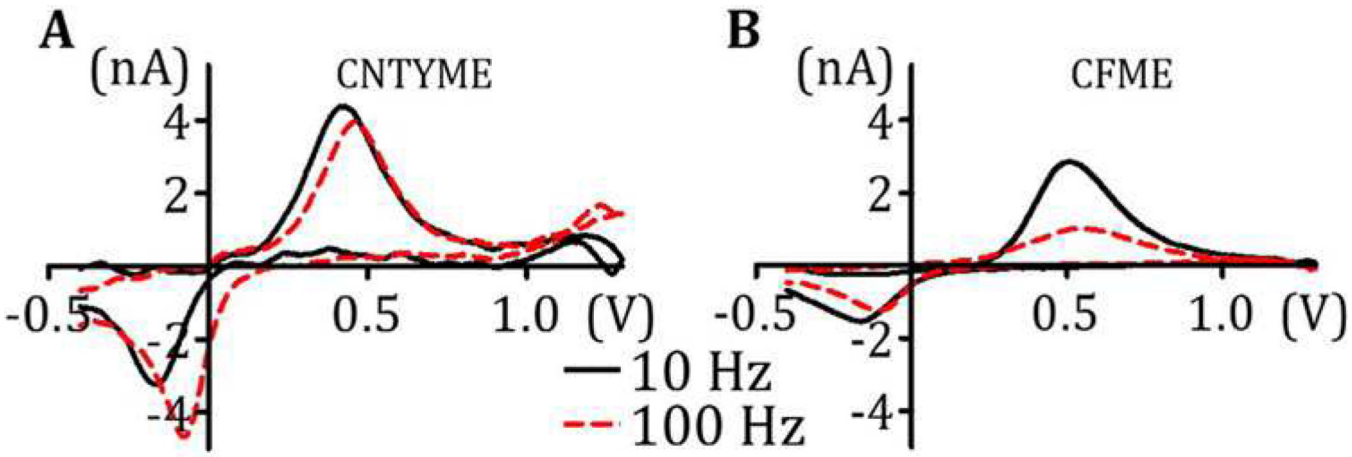

Figure 1.

Comparison of the effect of FSCV scan repetition rate at (A) a carbon nanotube yarn disk microelectrode (CNTYME) and (B) a carbon fiber disk microelectrode (CFME). 1 μM dopamine is detected using a scan rate of 400 V/s. The repetition rate is either 10 Hz (solid black traces) or 100 Hz (dashed red traces). Carbon nanotube yarn electrodes are not dependent on scan repetition frequency. Adapted with permission from reference [47].

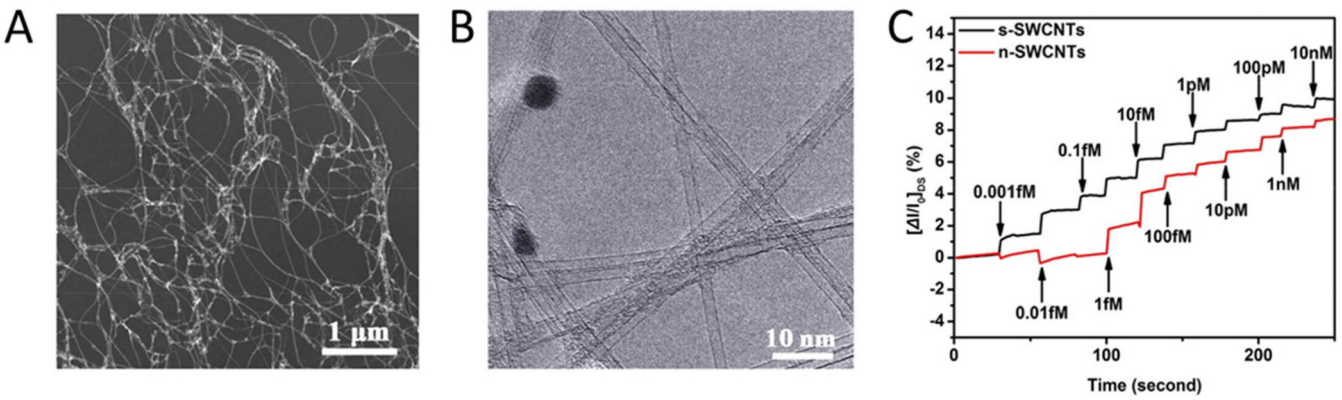

Figure 2.

FET-based sensor for detection of dopamine. (A)SEM and (B) TEM images of the semiconducting SWCNTs (s-SWCNTs). (C) Typical real time current ($\Delta I/I_0$) changes with dopamine concentration in a PBS solution for FET sensor with s-SWCNT and normal SWCNTS (n-SWCNTs). Adapted with permission from reference [51].

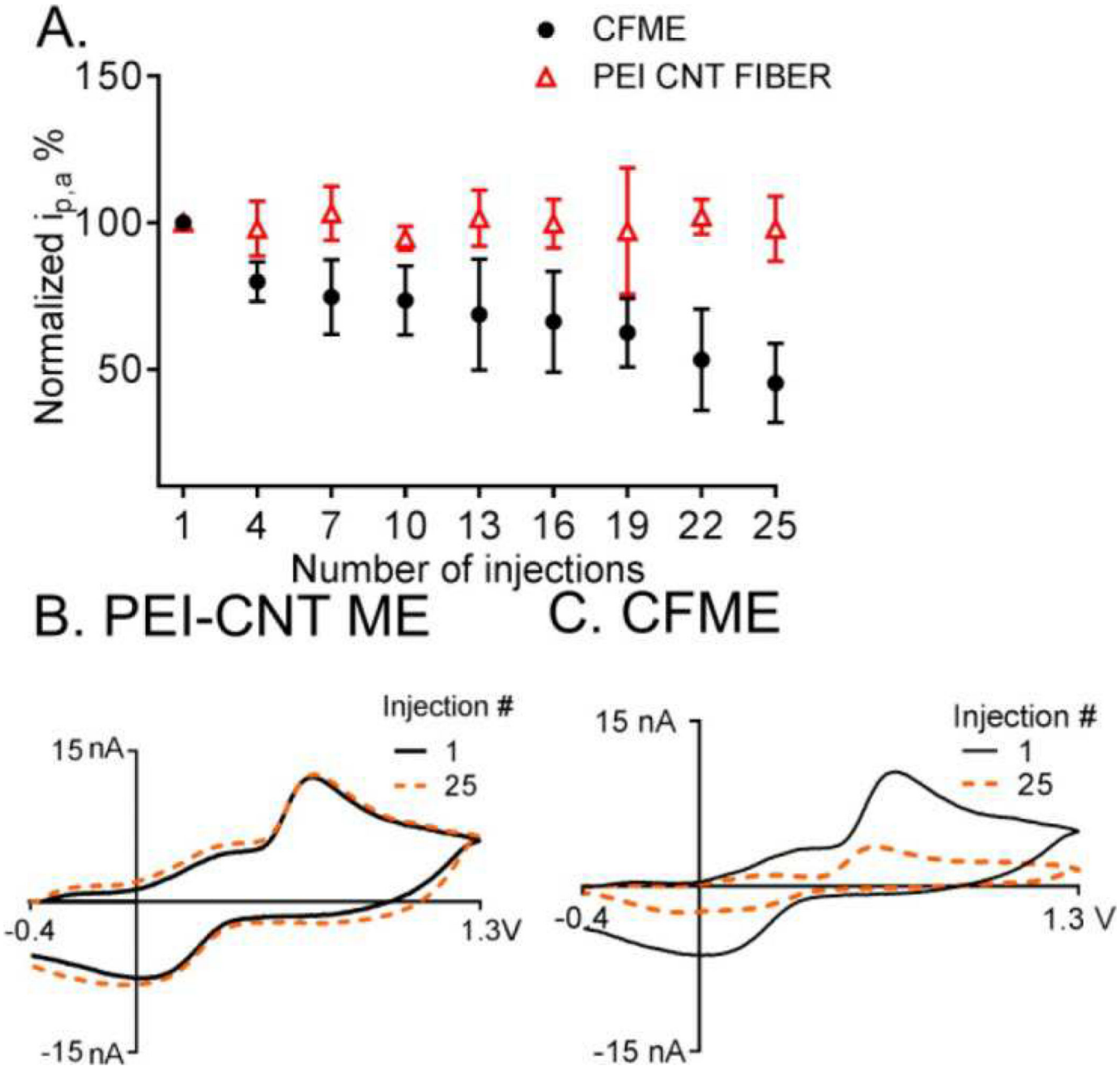

Figure 3.

Serotonin fouling at a PEI-CNT fiber microelectrode. (A) Repeated injections of serotonin (1 μM) every 15 s for 25 injections lead to no decrease in current for serotonin at PEI-CNT fiber electrodes (red) as opposed to the 50% decrease for carbon-fiber microelectrodes (CFMEs) (black). (B) Example cyclic voltammograms of 1 μM serotonin for a PEI-CNT fiber microelectrode for the 1st (solid black) and 25th injection (dashed red), approximately 6.25 min apart. The CVs are similar. (C) Example cyclic voltammograms of 1 μM serotonin at CFMEs for the 1st and 25th injection, indicating serotonin fouling does occur at the surface of the CFME. Adapted with permission from reference [68].

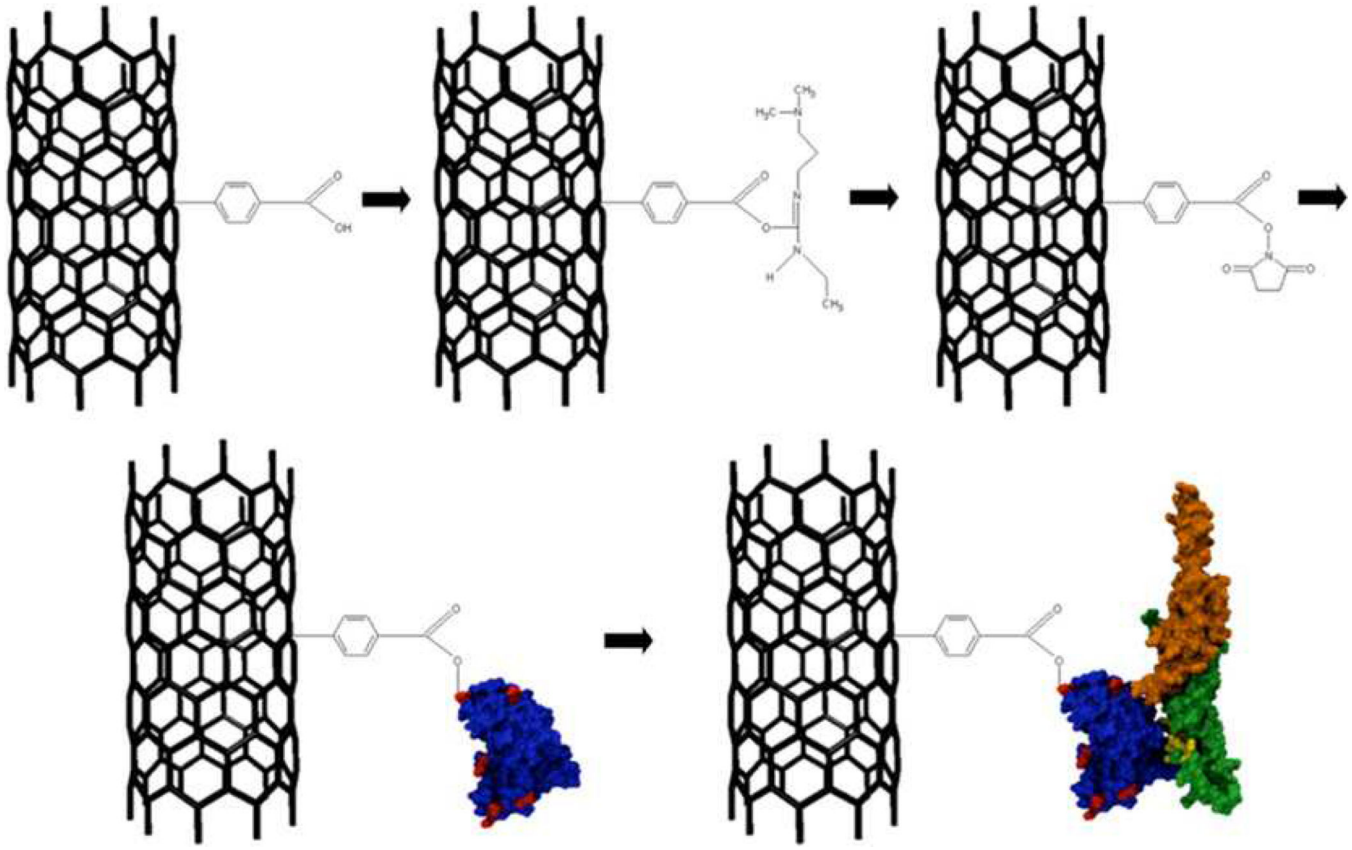

Figure 4.

CNTs for direct detection of the cancer biomarker OPN. Functionalization scheme for OPN attachment: first, carboxylic acid sites are created on the nanotube sidewall by incubation in a diazonium salt solution. The carboxylic acid group is then activated by EDC and stabilized with NHS. ScFv antibody displaces the NHS and forms an amide bond (surface amine-rich lysine residues responsible for this bond are depicted in red), and OPN binds preferentially to the scFv in the detection step. The OPN epitope is shown in yellow, and the C- and N-termini are in orange and green, respectively. Reprinted (adapted) with permission from reference [160].

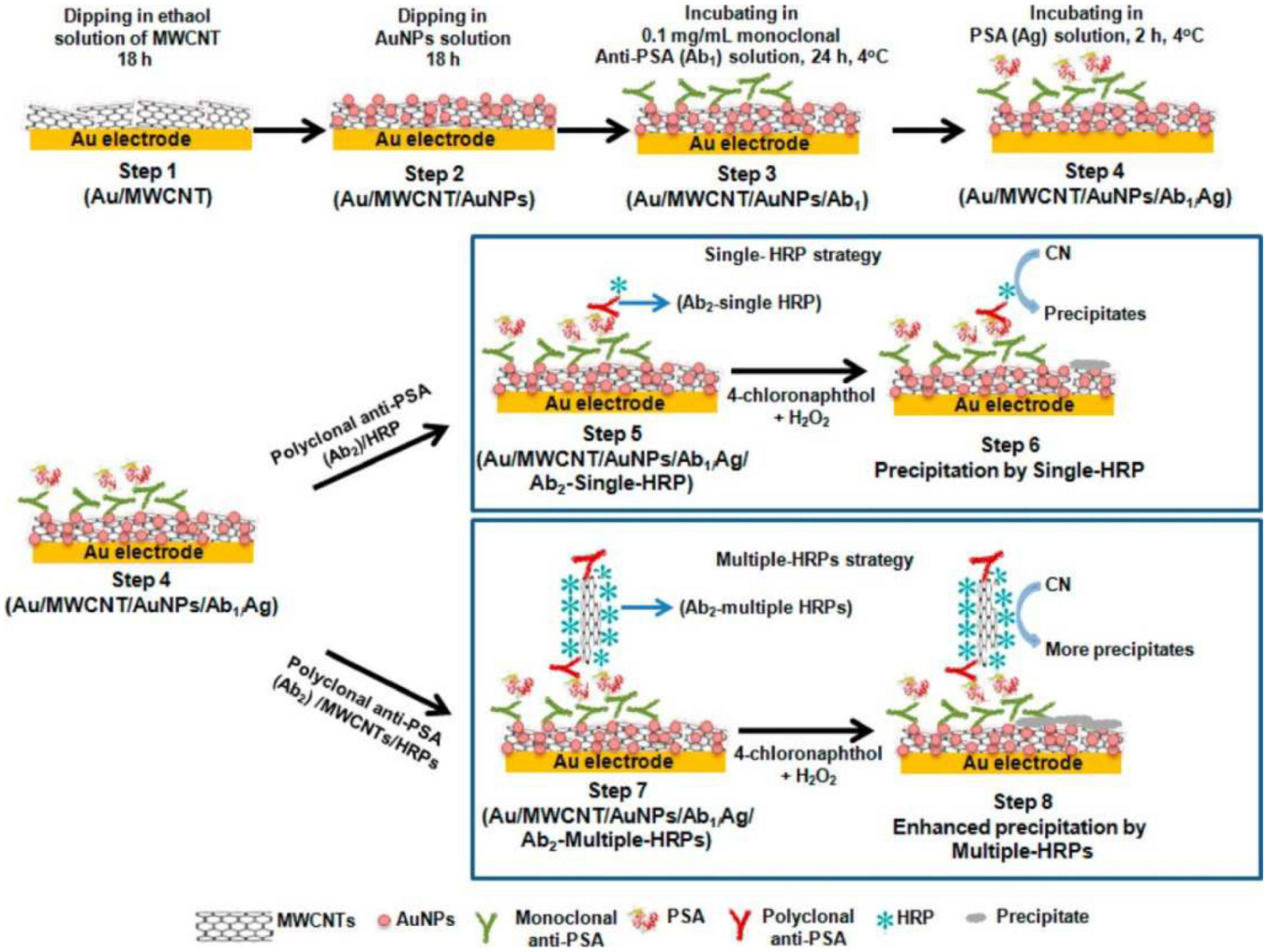

Figure 5.

Schematic illustrations detailing the fabrication of both a single-HRP and multiple-HRP strategy-based PSA immunosensor. For the single-HRP strategy, the HRP is bound to the antibody. In the multiple HRP strategy, multiple copies of both HRP and the antibody are bound on the MWCNTs, so that a larger quantity of precipitate is formed from the interaction of the labeled antibody and the sensor surface. Adapted with permission from reference [114].

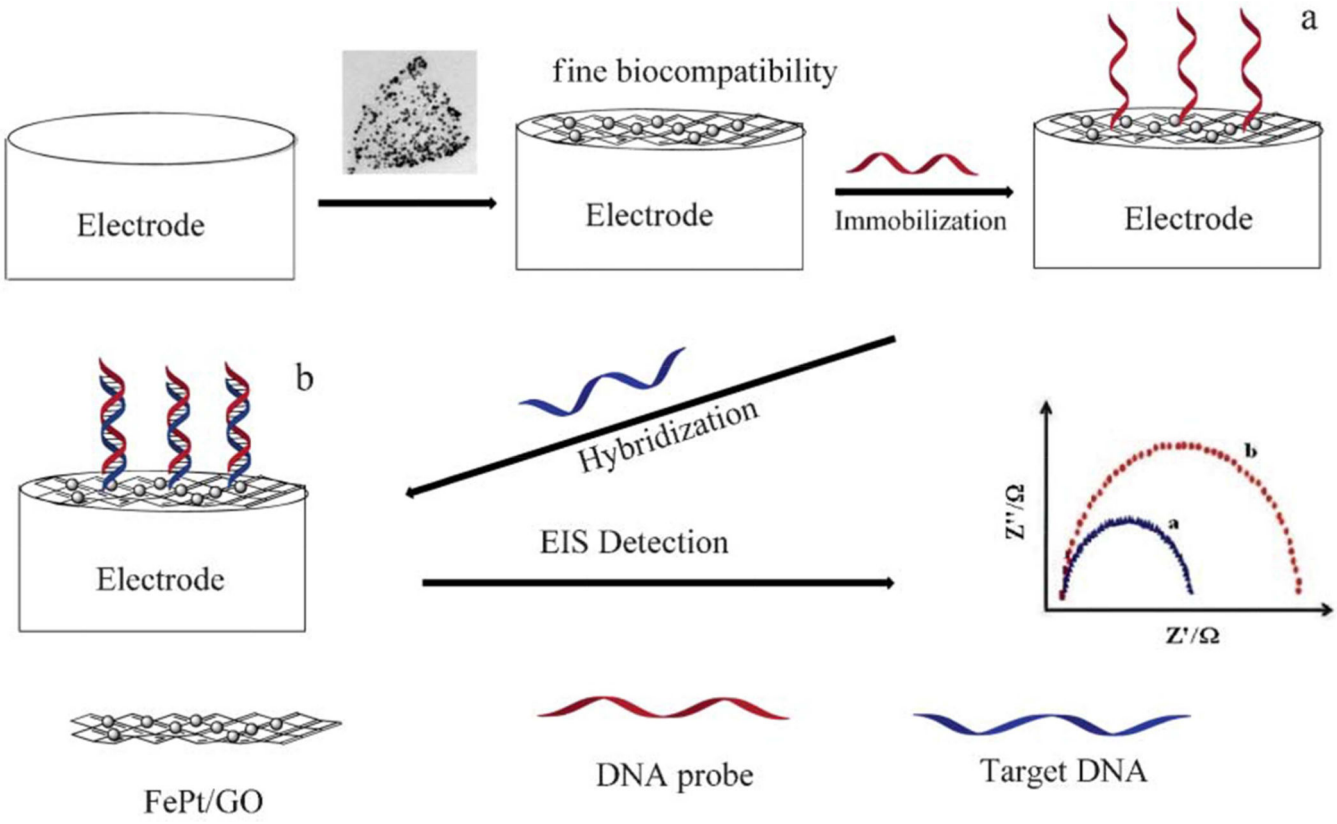

Figure 6.

Schematic representation of biosensing of DNA hybridization with EIS. A GCE electrode is modified with FePt–GO. Probe DNA is immobilized by dipping in ssDNA probe solution for 2 h, followed by washing and rinsing steps. Upon hybridization with the target gene, the EIS signal of $[Fe(CN)_6]^{3-/4-}$ increases (from a to b). Reprinted (adapted) with permission from reference [141]

Table 1

Carbon nanomaterial-based electrochemical sensors for detection of dopamine, ascorbic acid, and uric acid

Sensor	Method	Analyte	LOD	Ref.
Carbon Nanotube-Based Sensors				
$CONH_2$-CNT/CFME	FSCV	DA	0.13 μM	[3]
COOH-CNT/CFME	FSCV	DA	0.18 μM	[3]
SWCNT forest/CFMEs	FSCV	DA	0.017 μM	[33]
Helical CNTs GCE	DPV	DA	0.8 μM	[44]
		AA	0.92 μM	
		UA	1.5 μM	
PDDA/Helical CNT/GCE	DPV	DA	0.08 μM	[45]
		AA	0.12 μM	
		UA	0.22 μM	
CNT yarn disk electrode	FSCV	DA	0.021 μM	[48]
CNTYMEs	FSCV	DA	0.01 μM	[47]
CNF/GCE	DPV	DA	0.05 μM	[50]
s-SWCNT/PET	FET	DA	10^{-12}μM	[51]
Graphene Based Sensors				
Graphite oxide bulk/CPE	DPV	DA	0.015 μM	[55]
		UA	2.7 μM	
Graphene flower/CFE	DPV	DA	0.5 μM	[13]
		AA	24.7 μM	
		UA	2 μM	
3D graphene foam electrode	Amperometry	DA	0.025 μM	[14]
SWCNH/GCE	LSV	DA	0.06 μM	[9]
		AA	5 μM	
		UA	0.02 μM	
Whole Graphene solution-gated graphene transistor	SGGT	DA	0.001 μM	[61]
		AA	0.01 μM	
		UA	0.03 μM	
N-doped Carbon Based Sensors				
N-doped graphene/SPCE	CV	DA	0.93 μM	[62]
N-CNRs-Nafion/GCE	DPV	DA	0.0089 μM	[63]
N-PCNPs/GCE	DPV	DA	0.011 μM	[64]
		AA	0.74 μM	
		UA	0.021 μM	
Polymer Coatings				
PEDOT/RGO/GCE	Amperometry	DA	0.039 μM	[67]
PEDOT/CNT/CPE	DPV	DA	0.020 μM	[66]

Sensor	Method	Analyte	LOD	Ref.
PEI/CNT/CFME	FSCV	DA	0.005 μM	[68]
Gr/(PDDA–[PSS-MWCNTs])$_5$ graphite electrode, LBL	Amperometry	DA	0.15 μM	[70]
CNP/functionalized silicate particles/ITO, LBL	DPV	DA	0.125 μM	[71]
PANANA–MIPs/GCE	DPV	DA	0.0033 μM	[74]
MIPs-Graphene/GCE	DPV	DA	10^{-5} μM	[75]
PPy/CNTs-MIPs/GCE	DPV	DA	10^{-5} μM	[76]
Indirect Detection using Enzymes and DNA				
Cysteamin/MWCNT–tyrosine–Nafion/Au electrode biosensor	Amperometry	DA	0.003 μM	[79]
	DPV	DA	0.05 μM	
Uricase/Chitosan/CNT nanofiber/AgNP/Au electrode biosensor	Amperometry	UA	1 μM	[80]

* No detection limit was reported so the value is the lowest concentration detected experimentally.

Table 2

Carbon nanomaterial-based electrochemical sensors for detection of serotonin, epinephrine, and norepinephrine

Sensor	Method	Analyte	LOD	Ref.
Serotonin				
rGO/GCE	Amperometry	Serotonin	0.005 μM	[15]
$CONH_2$-CNT/CFME	FSCV	Serotonin	0.09 μM	[3]
COOH-CNT/CFME	FSCV	Serotonin	0.07 μM	[3]
CNF/GCE	DPV	Serotonin	0.25 μM	[50]
rGO/PANI–MIPs/AuNPs/GCE	DPV	Serotonin	0.0117 μM	[81]
IL/7-(1,3-dithiolan-2-yl)-9,10-dihydroxy-6H-benzofuro [3,2–c]chromen-6-one/f-CNT/GCE	DPV	Serotonin	2 μM	[82]
		Norepinephrine	0.049 μM	
Benzofuran derivative/CNT/TiO_2NPs/IL/GCE	DPV	Isoproterenol	0.028 μM	[83]
		Serotonin	0.154 μM	
Nafion/$Co(OH)_2$– MWCNTs/CILE	DPV	Serotonin	0.023 μM	[84]
		L-dopa	0.12 μM	
	Amperometry	Serotonin	0.36 μM	
		L-dopa	0.47 μM	
Epinephrine and Norepinephrine				
Oxidized SWCNH/SPE	DPV	Epinephrine	0.1 μM	[85]
SDS/pristine MWCNT/CPE	Amperometry	Epinephrine	0.045 μM	[87]
CNT-mer dispersed MIP-modified PGE	DPASV	Epinephrine	0.001 μM*	[88]
Graphene/AuNPs/GCE	CV	Epinephrine	0.007 μM	[89]
MWCNTs-$Ni(OH)_2$NPs/GCE	DPV	Epinephrine	0.29 μM	[90]
		Piroxicam	0.11 μM	
	Amperometry	Epinephrine	0.47 μM	
		Piroxicam	0.61 μM	

*No detection limit was reported so the value is the lowest concentration detected experimentally.

Table 3

Carbon nanomaterial-based non-enzymatic and enzyme sensors for hydrogen peroxide

Sensor	Method	Analyte	LOD	Linear Range	Ref.
polyXa/FAD/MWCNTs	Amperometry	Hydrogen peroxide	100 μM	100 – 2900 μM	[91]
CNT/PB/CNT paste	Amperometry	Hydrogen peroxide	4.74×10^{-3}μM	0.05 – 5.0 μM	[92]
Nafion-PB-MWCNTs/SPCE-IL	Amperometry	Hydrogen peroxide	0.35 μM	5 – 1645 μM	[93]
EDTMP/Fe^{III}-DETPA/PAH-MWCNT/Au	Amperometry	Hydrogen peroxide	6.3×10^{-3}μM	0.0125 – 4750 μM	[94]
Co_3O_4/MWCNTs/gelatin/HRP/Nafion/GCE	Amperometry	Hydrogen peroxide	0.74 μM	0.74 – 19 μM	[95]
L-cysteine-HRP-SWNTs-Au film	Amperometry	Hydrogen peroxide	2.1×10^{-7}μM	1.0×10^{-6}– 1.0×10^{-7}μM	[96]
GC/MWCNTs/[bmim][PF6]/CAT	EIS	Hydrogen peroxide	2.5×10^{-5}μM	5.0×10^{-3}– 1.7 μM	[97]

Table 4

Carbon nanomaterial-based enzyme sensors for enzymatic substrates.

Sensor	Method	Analyte	LOD	Linear range	Ref.
CNT fiber/GOx/GAD	Amperometry	Glucose	25 μM	25 – 2000 μM; 2000 –30,000 μM	[100]
GOx/NH2-MWCN Ts/GCE	Amperometry	Glucose	9 μM	17 – 650 μM	[101]
GN-Py-GOx	Amperometry	Glucose	50 μM	50 – 2200 μM	[102]
ACE(C0.15/OF73)/GOx	Amperometry	Glucose	100 μM	5000 – 15,000 μM	[103]
mSWNT-GOx/PPF/Au	Amperometry	Glucose	7.1 μM	250 – 1400 μM	[104]
sSWNT-GDH/PPF/Au	Amperometry	Glucose	20 μM	250 – 2500 μM	
CS-FC/SWNTs/GOD/3D	Amperometry	Glucose	1.2 μM	5.0 – 19,800 μM	[99]
GCE-MWCNT-Nf-HRP-SG/Chit-AOx-PEI	Amperometry	Ethanol	5 μM	8 – 42 μM	[105]
GCE-MWCNT-Nf-HRP-SG/Chit-FcA Ox-PEI	Amperometry	Ethanol	2 μM	5 – 3000 μM	[106]
Chox-ALP/CNT–CS/GCE	DPV	Cholesterol	10 μM	50 – 2000 μM	[110]
Chox/PANI/ZnO/JLCNT-FET	FET	Cholesterol	250 μM	500 – 16,600 μM	[111]
XOD/BSA/CNT/CF	Amperometry	Hypoxanthine	0.75 μM	0.75 – 135 μM	[112]
XOD–AuNPs–SWCNH/AU	Amperometry	Hypoxanthine	0.61 μM	1.5 – 35 μM	[10]

Table 5

Carbon nanomaterial based sensors for direct detection of proteins, biomarkers, and bacteria.

Sensor	Method	Analyte	LOD	Linear range	Ref
Multi-HRP/MWCNTs/AuNP-b ased PSA immunosensor	SWV	PSA	0.40 pg mL^{-1}	1.0 – 1.0 $\times 10^4$ pg mL^{-1}	[114]
Bienzyme/Ab2/SWCNHs bioconjugates	EIS	AFP	0.33 pg mL^{-1}	1.0 – 6.0$\times 10^4$ pg mL^{-1}	[116]
rGO/Thi/AuNP nanocomposite	DPV	CEA	0.650 pg mL^{-1}	10 – 3.0$\times 10^5$ pg mL^{-1}	[117]
		AFP	0.885 pg mL^{-1}	10 – 3.0$\times 10^5$ pg mL^{-1}	
Anti-OPN/NT-FET	FET	OPN	1 pg mL^{-1}	1 – 1.0$\times 10^6$ pg mL^{-1}	[113]
SPCE/Anti-AFP+Ab_2/nanoAu/CNH	DPV	AFP	0.07 pg mL^{-1}	0.1 – 1.0$\times 10^3$ pg mL^{-1}	[115]
VACNF/Linker/anti-CRP	EIS	CRP	11000 pg mL^{-1}	N/A	[118]
Anti-CRP/SWCNT/FET	FET	CRP	100 pg mL^{-1}	100 –1.0$\times 10^8$ pg mL^{-1}	[119]
BSA-sip/MNPs/CS-MWNT/GCE	DPV	BSA	28 pg mL^{-1}	100 –1.0$\times 10^8$ pg mL^{-1}	[120]
Anti-*GAS*/SWCNT/IDE/SPQC	EIS	Streptoco ccus	12 cfu/mL	3 $\times$ 10^2–10^6 cfu/ml	[121]

N/A: not available.

Table 6

Carbon nanomaterial-based DNA sensors

Sensor	Method	Analyte	LOD	Ref
PtNPs/CNT bioconjugate and DNA/Au electrode sandwich scheme	Chronoamp	Test DNA	6×10^{-10} μM	[124]
DNA/CNT-GOx modified GCE	DPV and LSV	Test DNA	600 μg/L	[125]
AuNPs/Polydopamine/MWCNTs-COOH on GCE	Chronocoulo metry	Test DNA	3.5×10^{-9} μM	[126]
AuNP/molybdenum disulfide/MWCNT modified GCE	CV	Test DNA	7.9×10^{-10} μM	[128]
AuNPs/vertically aligned SWCNT arrays/SiO_2/Si substrate	EIS	Test DNA	1.0×10^{-15} μM	[129]
CNF/CHIT modified GCE	DPV	Test DNA	8.8×10^{-5} μM	[131]
RGO with peptide nucleic acid probe	FET	Test DNA	1.0×10^{-7} μM	[133]
Graphene FET array on SiO_2/Si substrate	FET	Test DNA	1.0×10^{-7} μM	[134]
Vertical GNFs on Si wafers	DPV	Fish sperm, calf thymus DNA	1 μg/mL	[135]
Graphene nanowall (RGNW) modified graphite electrode	DPV	Test DNA	9.4×10^{-15} μM	[136]
Graphene nanosheets (RGNS) modified graphite electrode	DPV	Test DNA	5.4×10^{-9} μM	[136]
Poly(indole-6-carboxylic acid)/COOH-MWCNTs/GCE	CV	HBV DNA	2.0×10^{-9} μM	[137]
DNA/AuNP/SWCNT on SiO_2/Si	EIS	HBV and HPV DNA	1.0×10^{-12} μM	[138]
DNA-wrapped CNTs	DPV	HPV DNA	9.0×10^{-7} μM	[139]
GO-CHIT nanocomposite modified ITO	DPV	*Salmonella typhi* specific DNA	1.0×10^{-8} μM	[140]
FePt-CNT/GCE	EIS	PML-RARA fusion gene	2.1×10^{-7} μM	[141]
FePt-graphene oxide(GO)/GCE	EIS	PML-RARA fusion gene	3.5×10^{-8} μM	[141]
PABSA−RGO/CPE	EIS	PML-RARA fusion gene	3.7×10^{-11} μM	[142]
Ruthenium-GO	ECL	*rpoB gene*	4.0×10^{-5} μM	[143]
Nanoporous carbon-modified disposable electrical printed electrodes	EIS	Synthetic oligonucleotides (Alzheimer's disease)	0.1 μM*	[144]
DNA-Glu-polyaniline(PANI)-CNT/ITO	DPV	*N. gonorrhoeae* DNA	1.2×10^{-11} μM	[146]

*No detection limit was reported so the value is the lowest concentration detected experimentally