Dhanauri International Journal of Multidisciplinary Research (DIJMR)
Volume 2 Issue 1, July (2026)
ISSN: 3107-9563 (online)
doi.org/10.5281/zenodo.21407170

Role of Graphene-Based Nanostructures in Biosensing and Biomedicine

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ABSTRACT

In 2004, the separation of single layer from highly oriented pyrolytic graphite via mechanical exfoliation method to obtained two-dimensional graphene nanosheets by A. K. Geim and K. S. Novoselov was a Nobel winning research work, revealed the extraordinary material properties and capabilities of atomic-level thin graphene nanosheets. The large active surface area, high mechanical durability, and superior thermal and electrical conductivities of graphene sheets make its suitable to interact with foreign nanomaterials that open exciting research avenues in the biosensing and biomedical application field, including mapping, precise drug delivery, and gene therapy. Over the past few decades, application based research in the field of two-dimensional nanosheets and its nanocomposites have grown tremendously and opens new possible effective path in the field of biosensing and biomedicine. In this review, a comprehensive study of the role of graphene based nanocomposites in the field of electrochemical biosensing and biomedicine. Furthermore, we focus on the superior properties and advancement in graphene-based biosensors in respect of available alternatives, and we discuss future prospects for the possible advancement of graphene-based electrochemical sensors in the biomedical field.

KEYWORDS: Nanomaterials, Graphene, Nanocomposites, Electrochemical Sensor, Biomedicine.

1. INTRODUCTION: Science and technology deals with the minimization of size of bulk materials in nanometer range (< 100 nm) is known as Nanoscience and nanotechnology. As the bulk material size reduces, its material properties such as electrical, mechanical, and optical properties etc. also modified. In past few decades, fabrication and controlled modification of size of bulk materials in nanometer range to form nanomaterials have considerable attention due to various potential and efficient applications across various technological fields [Rao, C. N. R., & Cheetham, A. K. 2001, Roduner, E. 2006, Buot, F. A. 1993, Hutchison, J. E. 2008, Guisbiers, G. 2010, Patra, S. et al, 2017]. The physical and chemical properties of nanomaterials are highly influenced by two factors: (i) ratio of surface area with volume enclosed by it (S/V) and (ii) the confinement effect of object in quantum regime [Pan, H., &

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Published online-17 July 2026

Feng, Y. P. 2008, Hua, Z. J. et al. 2003]. The electronic energy states of the material directly depend on the degree of spatial confinement. The materials can be grouped into planer-structure (2D), rod like (1D), or quantum dots (0D), depends on the material has one degree of spatial confinement, two degree of spatial confinement or three degree of spatial confinement respectively (Figure-1) [Van Zeghbroeck, B. J. 2011].

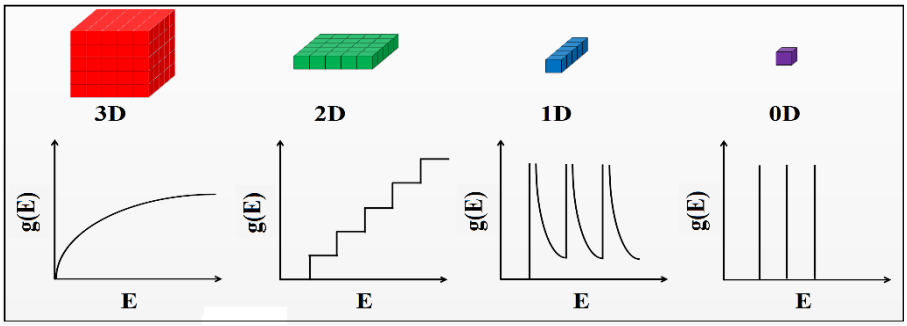

Figure 1: Block diagram and density of state of bulk (3D), planer (2D), 1 D and 0 D structures.

The two main approaches first one is top-down method and second is bottom-up method to synthesize various nanomaterials. In top down approach involves cutting down the bulky size of materials up to the nanometer regime in a controlled manner via physical or chemical process. Some standard and precision lithographic techniques with source setup such as FIB (focused ion beam), X-ray, electron, and photolithography are also used in top-down approach category [Hu, J. et al. 1999, Li, J. et al. 2003]. **(ii) Bottom-Up Approach:** Alternatively, bottom up approach involves targeted self-assembly/ nucleation of individual atoms or molecules to construct complex structure having size in nanometer regime via physical or chemical process. In nature, the formation of DNA is perfect example of bottom up approach and it's a cornerstone of advanced molecular engineering [So, H. M. et al. 2005, Yang, N. et al. 2016]. In the past several decades, a huge number of nanomaterials including metallic and non-metallic nanomaterials are synthesized by using various methods by scientific community. In the era of rapid expansion of nanomaterials field, carbon-based nanostructures such as most notably buckminsterfullerene (C_{60}), carbon nanotubes (CNTs), carbon nanofibre (CNFs) and graphene nanosheets has extraordinary prominence in application field [Kroto, H. W. et al. 1985, Iijima, S. 1991]. The unmatchable versatile performance of carbon based nanostructures has a wide range of applications in both nanotechnology and modern carbon chemistry [Hu, J. et al. 1999, Li, J. et al. 2003, So, H. M. et al. 2005, Yang, N. et al. 2016, Kroto, H. W. et al. 1985, Iijima, S. 1991]. An extraordinary turning point in carbon based nanomaterials science occurred, when A. K. Geim and his team member successful exfoliate graphene sheet; a single, two-dimensional (2D) sheet from bulk graphite. In now days, graphene sheets is universally recognized as the fundamental building structural for all other carbon based nanostructures such as fullerenes, nanoribbons, quantum dots, carbon nanotubes, and complex 3D carbon foams are ultimately derived, Figure 2 [Geim, A. K., & Novoselov, K. S. 2007, Novoselov, K. S. et al. 2005]. The atomically thin two dimensional transparent Graphene sheet of hexagonally arranged carbon atoms showing zero-band gap. Graphene sheets have theoretical very large surface area (~2600 m^2/g), possesses very high mechanical durability, and superior thermal and electrical conductivities [Ferrari, A. C. et al. 2006, Ferrari, A. C., & Robertson, J. 2000, Rao,

C. N. R. et al. 2009]. The unique charge carrier functions as Dirac fermions in graphene, enabling it to show the quantum Hall effect at room temperature. The extraordinary materialistic properties of graphene sheet make it an right candidate for various cutting-edge application in the field of nanoelectronics (e.g., Cellular integration systems and field-effect transistors), energy storage, biomedicine (e.g., Next-generation biomolecular devices, bio-imaging, and biosensors) [Dreyer, D. R. et al. 2010, Hummers, W. S., & Offeman, R. E. 1958, Park, S., & Ruoff, R. S. 2009, Dreyer, D. R. et al. 2010, Joshi, R. K. et al. 2010, Shao, Y. et al. 2010, Wang, L. et al. 2017, Valappil, M. O. et al. 2015. Bollella, P. et al. 2017, Ali, Md. A. et al. 2016]. In current era, scientist and researchers are actively searching the possibilities of structural variations of nanomaterials (Example: graphene, such as quantum dots, nanosheets, nanorods, and nanotubes) to optimize its performance [Yang, X. et al. 2008, Nakagawa, M. V. et al. 2016, Qiu, H. J. et al. 2017, Valles, C. et al. 2008]. In case of graphene sheets, rich edge activity, high electron mobility, and tunable charge carrier concentration by chemical doping, molecular adsorption, surface coating, and ion implantation. In case of pristine graphene, only edges play as active sites, while the richness of oxygen functional groups (such as carboxyl and hydroxyl groups) in graphene oxide (GO) sheets, imparts hydrophilic and biocompatibility, makes it a highly valuable derivative of graphene. Graphene oxide (GO) sheets are alternative, cost-effective and efficient filler for composite materials, and a prime component for super-capacitors and energy-conversion systems [Steenackers, M. et al. 2011, Zhan, B. et al. 2014, Nair, A. K. et al. 2016, Liao, L. et al. 2014, Alwarappan, S. et al. 2012, Alwarappan, S. et al. 2012, Wu, J. et al. 2011, Jahan, M. et. al. 2012].

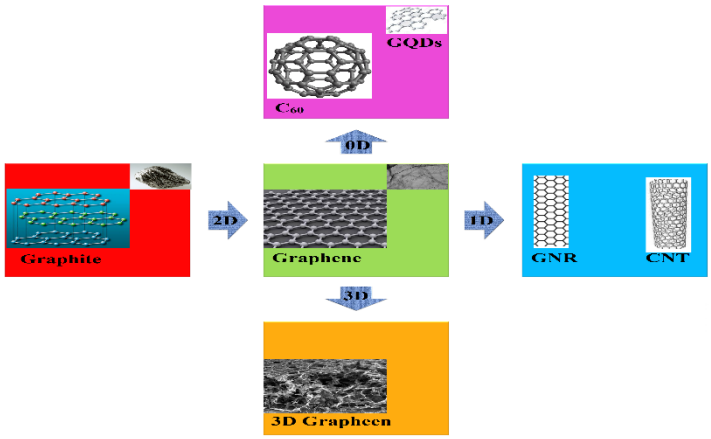

Figure 2: Schematic representation showing to different forms of carbon based nanomaterials.

In general, graphene sheets can be prepared by using various methods such as **(i) Mechanical Exfoliation Method:** In 2004, Geim and co-workers mechanically peel off layers of graphene from highly oriented pyrolytic graphite (HOPG) with the help of scotch tape. This method is used for the production of highly crystalline graphene flakes but is small scale production [Geim, A. K., & Novoselov, K. S. 2007, Novoselov, K. S. et al. 2005]. **(ii) Scalable Manufacturing:** Other methods such as CVD (chemical vapor deposition), epitaxial growth (thermal sublimation of SiC wafers), and chemical exfoliation methods are preferred for large scale synthesis of graphene sheets. Among the above listed methods, chemical exfoliation

method stands out as the most economically for industrial scaling [Dreyer, D. R. et al. 2010, Hummers, W. S., & Offeman, R. E. 1958].

(a) Graphene-Based Biosensors: The performance of conventional biosensor depends on two basic components: (i) Biological recognition element, which binds selectively with a target such as glucose, urea, or specific drugs and (ii) Transducer, that enhanced and translates interaction of biological recognizer and target in the form of measurable electronic signal. Preferred biological recognizers are enzymes, nucleic acids, antibodies, or whole microorganisms etc. The performance of electrochemical biosensors depends on several factors such as biocompatibility, conductivity, surface area, and catalytic capabilities of working electrode. Carbon-based electrodes are superior as compared to conventional metallic electrodes due to its expansive working potential windows in aqueous medium as well as in non-aqueous media, rich surface activity, chemical inertness, and affordability etc. fullerenes' and carbon nanotubes (CNTs) have proven useful electrochemical biosensors; graphene stands out due to its tunable band gap, combined with unparalleled charge carrier mobility and conductivity [Yang, X. et al. 2008, Nakagawa, M. V. et al. 2016, Qiu, H. J. et al. 2017, Valles, C. et al. 2008, Steenackers, M. et al. 2011, Zhan, B. et al. 2014, Nair, A. K. et al. 2016, Liao, L. et al. 2014, Alwarappan, S. et al. 2012, Alwarappan, S. et al. 2012, Wu, J. et al. 2011, Jahan, M. et. al. 2012]. Additionally, the electrochemical activity of synthesized carbon based nanosheets can be precisely tailored by introducing specific hetero-atom doping such as B, N, S, P, Ag, Fe, Ni, Co, Mn, Pd, Pt, or Rh etc. during the fabrication process [Su, C., & Loh, K. P. 2013, Su, D. S. et al. 2013, Dai, L. et al. 2015]. Both graphene sheets and carbon nanofibers (CNFs) shows high conductive, while cylindrically stacked graphitic layers are frequently utilized in modern sensing paradigms [Stavyiannoudaki, V. et al. 2009]. The remarkable research work in this field include: **(i) Non-Enzymatic Platforms:** Rathod et al. (2010), demonstrated the research work based on physiological glucose sensor operating at pH 7 by using CNF electrode modified with platinum nanoparticles and coated in insulating Nafion. The larger active surface area of CNFs boost the oxidation of glucose, allowing it to out-perform similar platinum-modified CNT designs. In another research work performed by Liu et al. (2009) to fabricate renewable nickel nanoparticles modified CNF electrode modified to achieve broad linear detection range and enhanced sensitivity. **(ii) Enzymatic Platforms:** Specific enzymes based modified graphene sheets shows excellent direct electrochemistry toward various targets such as hydrogen peroxide (H_2O_2) and glucose [Shan, C. S. et al. 2009, Wang, Z. J. et al. 2009, Zhou, M. et al. 2009, Kang, X. H. et al. 2009, Wu, H. et al. 2009, Lu, J. et al. 2007]. The research work based on graphene/polyethylenimine-based glucose biosensor was performed by Shan et al. (2009) shows high reproducibility, prolonged stability, and a linear response range of 2 to 14 mM. In separate research work, Zhou et al. (2009) demonstrated that chemically reduced graphene oxide (rGO) strongly amplifies the amperometric signal during glucose detection. While, the extensive research work based on two dimensional nanomaterials (e.g., Graphene, MoS_2, and WS_2) point out its exceptional stability, conductivity, and biocompatibility, but a critical demand for ultrasensitive, highly selective biosensors capable of analyzing complex bio-molecules is always in demand [Shah, P. K. et al. 2015, Narayanan, T. N. et al. 2014, Wang, L. et al. 2014, Yu, X. et al. 2017, Zhang,

H. et al. 2017, Loh, K. P. et al. 2016]. This review chapter will provides a compiled details about the synthesis, characterization, and possible uses of graphene based biosensors.

2. SYNTHESIS AND CHARACTERIZATIONS

2.1 Preparation of Graphene sheets

Graphene sheets can be obtained by extracting single atomic layers from bulk graphite such as mechanical exfoliation method and chemical exfoliation method, (top-down technique) or synthesize the two-dimensional material from scratch using molecular or atomic carbon precursors such as epitaxial growth and chemical vapor deposition (bottom-up technique) [Geim, A. K., & Novoselov, K. S. 2007, Novoselov, K. S. et al. 2005, Dreyer, D. R. et al. 2010, Hummers, W. S., & Offeman, R. E. 1958, Park, S., & Ruoff, R. S. 2009, Dreyer, D. R. et al. 2010]. The useful methods for graphene synthesis are detailed below:

(a) Mechanical Exfoliation Method: Originally pioneered by Geim and his research team, using top-down approach with the help of adhesive/scotch tape to repeatedly cleave highly ordered pyrolytic graphite (HOPG) until a freestanding, single atomic monolayer of graphene is achieved. Mechanical exfoliation method produces pristine, highly crystalline graphene (measuring up to 100 µm) that are perfect for establishing fundamental material characterization, its inherently low yield [Geim, A. K., & Novoselov, K. S. 2007, Novoselov, K. S. et al. 2005].

(b) Epitaxial Growth Method: In the epitaxial growth method; a bottom-up approach silicon carbide (SiC) wafer is used as an embedded carbon rich source. This SiC wafer is subjected to high thermal sublimation under vacuum or inert conditions, causing the silicon atoms to preferentially vaporize. As the SiC wafer cools, the residual carbon atoms left on the SiC wafer surface naturally self-assemble into the signature 2D hexagonal lattice and form graphene sheet.

(c) Chemical Vapor Deposition Technique: During chemical vapor deposition (CVD) process, methane (CH_4) is used as the carbon source, introduced into a high-temperature, inert environment, the hydrocarbon gas undergoes catalytic dissociation, breaking down the bonds and isolated carbon atoms. During a subsequent cooling process, these isolated carbon atoms nucleate and crystallize in hexagonal lattice forming a high-quality graphene sheet.

(d) Chemical Exfoliation Method: Chemical exfoliation method is based on Hummers' method is regarded as the most scalable top-down approach. In the Hummers' method, Bulk graphite is subjected to oxidation process by using strong oxidizing agents (such as potassium permanganate) combined with sulfuric acid at ice temperature. The oxygen-rich functional groups introduced during oxidation process of graphite, intercalate between the graphite layers and causes expansion in interlayer spacing, drastically weakening the van der Waals interactions and allowing the graphite to separate into individual layers. These state of individual layers of graphite is called graphene oxide (GO). Subsequent GO treatment with reducing agents to remove oxygen functional groups, yielding conductive, reduced graphene oxide (rGO) [Dreyer, D. R. et al. 2010, Hummers, W. S., & Offeman, R. E. 1958]. The degree of oxidation can be tuned in controlled way by modulating the concentration and introduction rate of the oxidizing agents.

2.2 Characterizations of Graphene Nanosheets

The quality of synthesized graphene sheets are characterized by using transmission electron microscopy (TEM) and Raman spectroscopy. In Figure 3 (a-b), TEM imaging of Graphene sheets clearly reveals the characteristic sheet-like morphology of the prepared graphene sample.

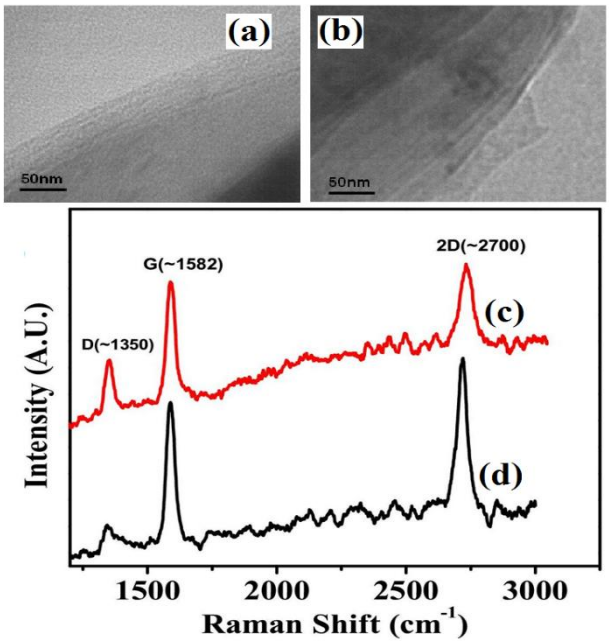

Figure 3: (a-b) TEM image and (c-d) Raman spectra of lab prepared and commercially prepared graphene sheets respectively (J. Nanosci. Nanotechnol. 13, 4040, 2013).

Furthermore, Raman spectroscopy is used as an essential tool for evaluating the quality and determining the number of layers present in the graphene nanosheets [Ferrari, A. C. et al. 2006, Dreyer, D. R. et al. 2010, Venezuela, P. et al. 2011, You, Y. et al. 2008, Mishra, M. et al. 2013, Zabel, J. et al. 2012, Mishra, M. et al. 2015]. The Raman spectrum of graphene sheets (Figure 3 (c-d)) is characterized by three signature peaks, each corresponding to specific vibration phenomena within the hexagonal carbon lattice:

(i) D band: In Raman spectra of graphene sheet, D-peak at ~1350 cm^{-1} is associated with the breathing mode of the hexagonal carbon rings present in graphene. It becomes active due to translational symmetry breaking, serving as a primary indicator of structural defects and edge effects within the graphene sheets.

(ii) G band: In Raman spectra of graphene sheet, G-peak at ~1582 cm^{-1} originates from the first-order, in-plane vibrational stretching of the sp^2 hybridized carbon-carbon (C–C) bonds.

(iii) 2D band: In Raman spectra of graphene sheet, 2D-peak at ~2700 cm^{-1} results from a second-order, two-phonon resonant scattering process near the Brillouin zone boundary. 2D-peak is used to identify the number of sheets present in the graphene.

3. ELECTROCHEMISTRY OF GRAPHENE NANOSHEETS

In electrochemical analysis, the efficiency of working electrode (such as gold, silver, copper, platinum, palladium, nickel, and glassy carbon (GC)) is governed by the characteristics of target analyte's redox and the background current generated across the applied potential range. The cost-efficiency and expansive electrochemical window of glassy carbon electrode (GCE)

remains the industry choice. The selectivity and sensitivity of working electrode are strongly influenced by its active surface area, electrical conductivity, biocompatibility, charge carrier mobility, and the presence of localized defect sites [Shao, Y. et al. 2010]. Various research work based on the functionalized GCEs with graphene, demonstrates significantly elevates electrochemical capabilities compared to other carbon-based modifiers. Graphene shows distinct advantages over carbon nanotubes (CNTs): it is highly cost-effective, devoid of toxic metallic impurities, and easier to modify. The exceptional properties of graphene such as its high charge carrier mobility, tunable band gap, superior conductivity, and large active surface area makes it an unparalleled electrode modifier [Yang, N. et al. 2016, Ali, Md. A. 2016, Loh, K. P. et al. 2016, Zhu, C. et al. 2017, Zhu, C. et al. 2015, Chen, F. et al. 2009, Bo, X. et al. 2017, Bharath, G. et al. 2015, Celik, N. et al. 2015, Tian, F. et al. 2017, Wang, L. et al. 2016]. Graphene/reduced graphene oxide (rGO) synthesized via Hummers' method are ideal for electrochemical applications, offering enriched functional groups, abundant active edge defects, and highly conductive networks [Celik, N. et al. 2015, Ohno, Y. et al. 2010, Cinti, S., & Arduini, F. 2017, Alwarappan, S., & Li, C. Z. 2010, Palaniselvam, T. et al. 2014, Claussen, J. C. et al. 2012, Shown, I., & Ganguly, A. 2016]. Typically, the cyclic voltammetry (CV) technique is used for electrochemical capability of graphene-modified platforms with the help of standard redox probes (e.g., $[Fe(CN)_6]^{3-/4-}$, $[Ru(NH_3)_6]^{3+/2+}$, and $Fe^{3+/2+}$) to extract qualitative data regarding surface reaction kinetics [Tang, L. H. 2009].

3.1 Working Principle of Cyclic Voltammetry

Cyclic voltammetry (CV) is a standard three-electrode cell setup, in which disc-shaped glassy carbon electrode (GCE) as a **working electrode,** Ag|AgCl in 3.0 M KCl is used as a **reference electrode**, platinum wire as **auxiliary electrode,** here glassy carbon electrode is a 3 mm diameter disc-shaped electrode [Mabbott, G. A. 1983]. The resulting data is plotted as a cyclic voltammogram (current versus applied potential), mapping the electrode's specific electrochemical behavior. The stability and biosensing potential of such graphene networks are evaluated using $[Fe(CN)_6]^{3-/4-}$ and $[Ru(NH_3)_6]^{3+/2+}$ redox couples. CV technique characterizes the electrochemical reactions at the working electrode by measuring the resulting current as the applied potential is linearly swept back and forth between two defined limits. The electrochemical cell's potential (E), which fluctuates based on the reaction quotient (Q), is mathematically described by the Nernst equation:

$$E = E^0 - \frac{2.303RT}{nF}\log Q \qquad (1)$$

Cyclic voltammetry (CV) analysis provides critical quantitative and qualitative data of reaction mechanisms and charge transfer dynamics. Graphene-modified working electrode can be prepared by drop casting of graphene (~ 10 µL in de-ionized water) onto the polished surface of glassy carbon electrode and dry at room temperature. Before electrochemical testing, graphene-modified GCE is gently rinsed with de-ionized water to remove any impurities.

3.2 Characteristic Parameters of Electrochemistry

$[Fe(CN)_6]^{3-/4-}$, $[Ru(NH_3)_6]^{3+/2+}$, and $Fe^{3+/2+}$ are used as standard redox couples in cyclic voltammetry (CV) measurement, which shows well-defined redox peaks with graphene-modified GEC electrodes. In the 0.1 mM phosphate buffer solution (pH 7), the Graphene-treated GCE exhibit a wide electrochemical potential window [Zhou, M. et al. 2009]. The linear correlation between the square root of the scan rate and the peak currents was observed

in case of diffusion-controlled redox process [Lin, W. J. et al. 2009]. The peak-to-peak potential separation (ΔE_p) for a single-electron transfer, in case of graphene falls in the range 61.5-73 mV (at 10 mV/s) for $[Fe(CN)_6]^{3-/4-}$, and 60-65 mV (at 100 mV/s) for $[Ru(NH_3)_6]^{3+/2+}$ [Tang, L. H. et al. 2009, Yang, S. L. et al. 2009, Shang, N. G. et al. 2008, Wang, J. F. et al. 2009]. These data closely matches with the theoretical ideal of 59 mV and significantly lower than those of GCEs [McCreery, R. L. 2008]. The result of Lower ΔE_p-values correspond to higher electron transfer coefficients, these confirm that graphene enhanced accelerates electron transfer kinetics. (i) $[Ru(NH_3)_6]^{3+/2+}$ acts as an outer-sphere probe, insensitive to surface defects, making it ideal for baseline comparisons between carbon electrodes, (ii) $[Fe(CN)_6]^{3-/4-}$ is highly sensitive to surface characteristics but not to oxides, and (iii) $Fe^{3+/2+}$ is highly sensitive to both surface structure and the presence of oxides. Kinetic profiling via CV reveals that the electron transfer rate constants for graphene-modified GC electrodes are ~ 0.18 cm/s for $[Ru(NH_3)_6]^{3+/2+}$ and 0.49 cm/s for $[Fe(CN)_6]^{3-/4-}$. This vastly out-performs bare GCEs, which sluggish rates of just 0.055 cm/s and 0.029 cm/s for the respective probes. Such extent of improvement makes graphene sheets as a highly efficient electro-catalytic surface [Tang, L. H. et al. 2009]. A broad electrochemical potential window ~ 2.5 V for Graphene-modified working electrodes plays a crucial for biosensing, as it allows for the reliable detection of various biomolecules - such as specific nucleic acids - that require higher potentials for successful oxidation and reduction. Furthermore, the physical orientation of the graphene sheets (perpendicular versus parallel to the substrate) highly affects the sensitivity of sensor. Graphene serves as an optimal scaffold for immobilizing enzymes, peptides, and nucleic acids, ensuring highly reproducible and selective biological detection over extended life-cycles.

4. GRAPHENE BASED ELECTROCHEMICAL SENSORS

The ultra-sensitivity, super selectivity, and high accuracy of electrochemical biosensors make it widely useful for the reliable detection of various biological and chemical analytes, making it highly adaptable for diverse practical applications [Ronkainen, N. J. et al. 2010, Chikkaveeraiah, B. V. et al. 2012, Qureshi, A. et al. 2012, Xu, X. et al. 2009, Wang, J. 2006]. In modern electrochemical analysis, glassy carbon (GC) has largely replaced conventional metallic electrodes, because of its cost-efficiency, wide electrochemical potential window, distinct chemical inertness, and highly stable baseline activity. Modifying the surface of GC electrode with carbon-based nanomaterials strongly elevates its sensitivity, biocompatible, operational stability, and signal reproducibility. As a result, modified GC electrodes serve as foundational platforms for DNA, protein, and enzymatic sensors. Modified GC electrodes used to monitor a wide array of targets, ranging from dissolved gases and waterborne heavy metals to environmental disease biomarkers and neurotransmitters [Yang, N. et al. 2016]. Various other two dimensional nanostructure such as hexagonal boron nitride (h-BN), and transition metal dichalcogenides (TMDs) modified GCE are used as modern electrochemical and biochemical sensors (Figure 4). Furthermore, the intrinsic electrochemical performance of carbon electrode can be substantially enhanced by doping with transition metals or heteroatom’s. The main motivation behind the enzymatic biosensors is to demonstrate the direct transfer of charge carriers between the active electrode surface and the active site of enzyme. The demonstration of "direct electrochemistry" with unmodified electrodes is always challenging, as the redox-active centers of most enzymes are deeply shielded within

hydrophobic molecular pockets [Shan, C. S. et al. 2009, Zhang, W. J., & Li, G. X. 2004, Ghindilis, A. L. et al. 1997, Gan, T., & Hu, S. 2011]. To overcome this fundamental hydrophobic shielding, researchers modify GC electrodes with highly conductive nanomaterials, such as graphene sheets, metallic nanoparticles, or carbon nanotubes etc., which act as efficient mediator to boost electron transfer. For example, when a graphene-modified electrode is functionalized with glucose oxidase (GOx), the system yields clearly defined redox peaks upon interacting with glucose. This distinct electrochemical signaling verifies that a successful, reversible electron transfer has occurred directly at the enzyme's active core. Based on their reliance on and integration of these biological catalysts, electrochemical sensing platforms are fundamentally divided into two major classifications: (i) enzymatic sensors and (ii) non-enzymatic sensors.

4.1 Enzyme-Functionalized Graphene Electrodes

The exceptional electro-catalytic performance of Graphene sheets due to its large active surface area make it suitable for direct electrochemistry of enzymes [Shan, C. S. et al. 2009, Wang, Z. J. et al. 2009, Zhou, M. et al. 2009, Kang, X. H. et al. 2009].

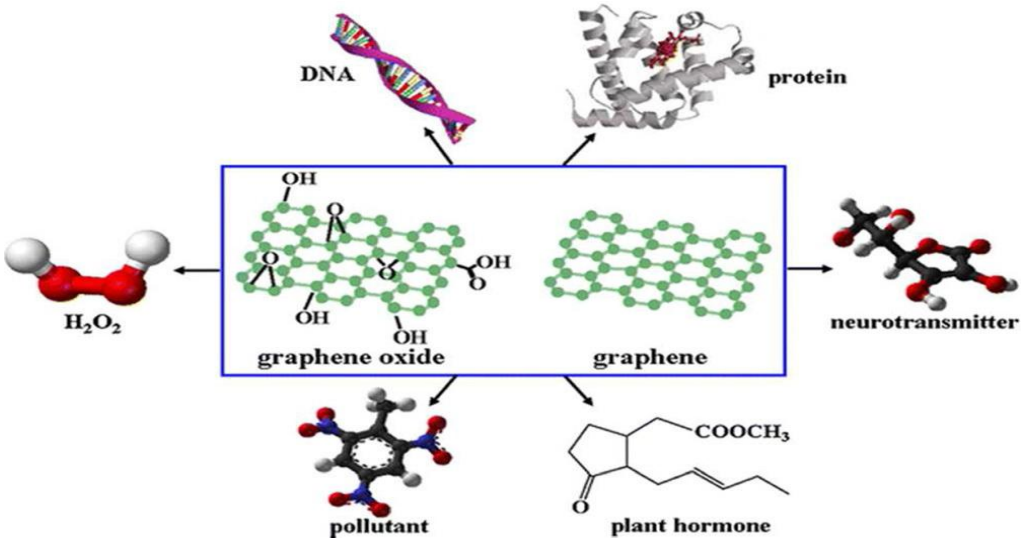

Figure 4: Graphene based electrochemical and biochemical sensors (Microchimica Acta, 175, 1-19, 2011).

Since, the biologically function of enzymes is highly specialized and it required a precise spatial conformation to target specific substrates (e.g., proteases for proteins, lipases for lipids, lactase for lactose, and amylase for carbohydrates). Imparting graphene networks into them, shows remarkably potential towards specific target sensing platforms. Zhou et al. perform the demonstration of modified graphene-alcohol dehydrogenase (ADH) electrode selectively for ethanol detection that shows an exceptionally quick response, an expanded linear detection range, at lower detection limit. That support the inherent biocompatibility and superior ability of graphene sheets to mediate charge transfer between electrode surface and enzymatic reaction products [Zhou, M. et al. 2009].

(a) Glucose and Carbohydrate Sensors

As millions of individuals are globally affected by diabetes and millions more are remaining undiagnosed, an urgent requirement to develop a rapid, and highly accurate glucose monitoring systems on priority basis in healthcare sector. Electrochemical glucose biosensors can address

such urgent need, by isolating the specific oxidation of target analytes at the electrode interface. On the basis of detailed analysis of various experimental data it was observed that the incorporation of graphene sheets significantly amplifies sensitivity without sacrificing selectivity [Celik, N. et al. 2015, Ohno, Y. et al. 2010, Cinti, S., & Arduini, F. 2017, Alwarappan, S., & Li, C. Z. 2010, Palaniselvam, T. et al. 2014, Claussen, J. C. et al. 2012, Shown, I., & Ganguly, A. 2016]. In a standard graphene-enhanced glucose sensor (Figure 5), glucose oxidase (GOx) serves as the primary biological recognition element. The glucose concentrations quantification can be done by measuring the hydrogen peroxide (H_2O_2) generated as a byproduct of glucose oxidation (detection limits below 0.5 mM in physiological fluids). The main huddle in GOx biosensing process is that the redox-active core of enzymes is encased within a bulky
glycoprotein shell, which obstructs direct electron transfer to conventional electrodes. Graphene modified with metal NPs effectively acts as a conductive bridge across this spatial gap. Subbiah et al. (2010) used a polypyrrole/graphene/GOx composite to maintain enzyme bioactivity and achieved a rapid heterogeneous electron transfer. Zeng et al. (2010) modified a dual-enzyme biosensor incorporating both GOx and glucoamylase onto a graphene scaffold to detect maltose, during the process glucoamylase first hydrolyzes maltose into glucose, and then subsequently oxidized by GOx to produce measurable H_2O_2. Similar electrochemical mechanisms have been successfully used with graphene-Cu/CuO architectures on indium tin oxide (ITO) substrates for fructose detection. The large surface area of graphene sheets permits high loading capacity of enzyme (reaching approximately 1.12×10^{-9} mol/cm^2) translates into ultra-sensitive analyte responses and accelerated electron-transfer rate constants (k_s = 0.18 to 2.83 s^{-1}) that overcome, the achievable range with carbon nanotubes [Kang, X. H. et al. 2009, Guiseppi-Elie, A. et al. 2002, Deng, C. Y. et al. 2008, Cai, C. X., & Chen, J. 2004].

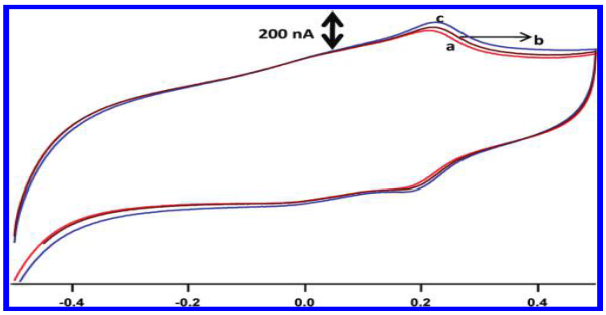

Figure 5: CV plot of 10 mM glucose in (a) SLG-GOD, (b) FLG-GOD, and (c) MLG-GOD (J. Phys. Chem. C, 116, 6556-6559, 2012).

(b) Nucleic Acid Sensors

The molecular-level analysis of nucleic acids is basic need for detecting genetic mutations that provide the early diagnosis and management of chronic neurological conditions such as Alzheimer's disease and Tourette's syndrome. The well resolved electrochemical oxidation potentials for adenine (A), guanine (G), cytosine (C), and thymine (T) of DNA, makes it possible to individually targeted and quantified (Figure 6) recorded in 0.1 M phosphate-buffered saline (PBS, pH 7.0) using graphene-modified glassy carbon (graphene/GC, green), graphite-modified glassy carbon (graphite/GC, red), and unmodified glassy carbon (bare GC, black) electrodes. The concentration of G, A, T, C, ssDNA, and dsDNA was maintained at 10 mg mL^{-1} [Zhou, M. et al. 2009]. Biosensors based on Graphene-modified GC offer a cost

effective, highly selective, and ultra-sensitive method for detecting specific DNA sequences and identifying genetic abnormalities [Zhou, M. et al. 2009, Sassolas, A. et al. 2008, Drummond, T. G. et al. 2003]. Bo et al. (2017) utilize modified graphene/ polyaniline nanowires electrode for surface-controlled electrochemical DNA sensor, showing a direct, linear connection between the peak current and applied scan rates varying from 25-150 mV/s, Huang K J et al. (2011) utilized strongly -COOH functionalized reduced graphene oxide (rGO) to detect guanine at limits of 50 nM and adenine at limit ~ 25 nM.

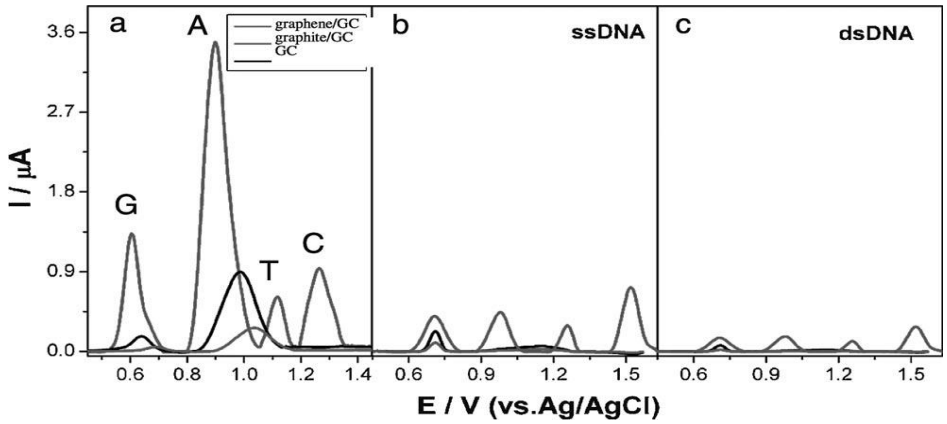

Figure 6: Differential pulse voltammograms (DPVs) of (a) mixed DNA nucleobases (G, A, T, and C), (b) single-stranded DNA (ssDNA), and (c) double-stranded DNA (dsDNA)

The enhancement in the detection limit due to hydrogen bonding, π-π stacking interactions between the target nucleobases and the modified carbon lattice [Huang K J et al. 2011]. In his research work Pumera M (2010) demonstrated that in case of stacked nanofabric graphene the DNA sensing sensitivity increased by four fold as compared with carbon nanotubes (CNTs) due to its highly improved directly attributed to a high density of exposed, electroactive edge planes. Research works performed by Goh, M. S., & Pumera, M. (2010) confirm that multi-layered graphene architectures inherently possess superior sensing capabilities compared to standard bilayer variants. rGO modified electrode perform significantly faster electron transfer kinetics as compared to unmodified glassy carbon electrodes enables the detection of all four nucleobases at same time for both ssDNA and dsDNA configurations. Consequently, these platforms can successfully identify single nucleotide polymorphisms (SNPs) directly, completely bypassing the need for cumbersome labeling or complex hybridization procedures. Interestingly, the presence of active sites present on structural defects is essential for functionality. Highly pristine, perfectly crystalline graphene lacks the requisite edge-plane active sites, rendering it largely ineffective for the electrochemical detection of adenine and guanine bases [Lim, C. X. et al. 2010].

(c) Small Molecule Detectors: Hydrogen Peroxide (H_2O_2)

The accumulation of hydrogen peroxide (H_2O_2) over time may cause extensive biological toxicity, because it serves as a ubiquitous by product of oxidizes enzymes and a primary substrate for peroxides. The precise quantification of H_2O_2 is utmost requirement across the clinical diagnostics, pharmaceutical research, and environmental monitoring [Borgmann, S. 2009]. The motivation behind the electrochemical sensing of hydrogen peroxide (H_2O_2) to achieve reliable, highly sensitive detection with low operational over potentials. Graphene-

based platforms have proven highly suitable in achieving this goal. Shan C. et al (2010) integrated graphene, gold nanoparticles (Au NPs), and chitosan to engineer electrode to delivered outstanding electro-catalytic efficiency toward both H_2O_2 and O_2. Zhou, M. et al. (2009) established that graphene matrices highly enhanced electron transfer rates during H_2O_2 detection, vastly outperforming conventional carbon-based electrodes. Analytical data derived from cyclic voltammograms (Figure 7) underscores these kinetic benefits, revealing highly favorable onset potentials. Furthermore, when operating at an applied potential of 0.2 V, graphene-based sensors exhibit a significantly broader linear detection range than platforms utilizing standard carbon nanotubes (CNTs).

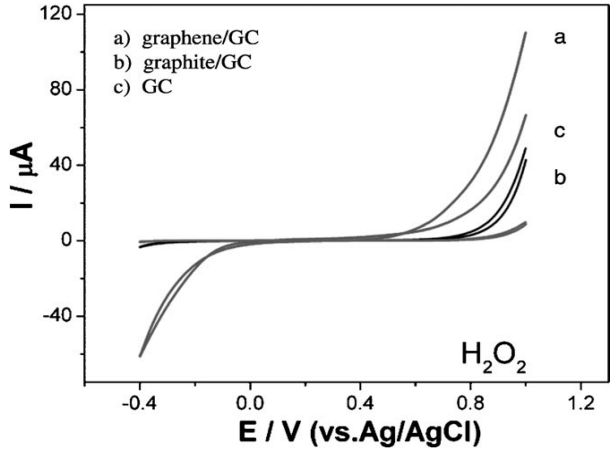

Figure 7: CV Plot on (a) graphene/GC, (b) graphite/GC and (c) GC electrodes (scan rate 50 mV/s, 4 mM H_2O_2 0.1 M PBS (pH 7.0) (Anal. Chem., 81, 5603, 2009).

(d) Reduced β-Nicotinamide Adenine Dinucleotide (NADH) Sensors

Enzymes that depend on the NAD^+/NADH redox couple (dehydrogenases) are basic for the advanced amperometric biosensors. The anodic oxidation of NADH to regenerate NAD^+, is main concept behind the operation for unstoppable detection of metabolic substrates like lactate, alcohol, and glucose. The electrochemical sensing of NADH using traditional electrodes has been severely limitation of rapid electrode surface fouling and the necessity for massive anodic over potentials to drive the reaction. The integration of chemically reduced graphene oxide (CR-GO) effectively neutralizes and drastically accelerates heterogeneous electron transfer dynamics to facilitating biological electron transfer. In Figure 8, the kinetic efficiency successfully depresses the required oxidation peak potential from approximately 0.70 V (the standard threshold for glassy carbon and bare graphite) down to a highly manageable 0.40 V [Ronkainen, N. J. et al. 2010]. Liu, H. et al. (2009) shows that modifying the graphene architecture with methylene green (MG) can drive the required oxidation potential down even further, achieving successful NADH detection at an exceptionally low ~0.14 V. Research conducted by Lin, W. J. et al. (2009) established that graphene substrates fundamentally out-perform traditional edge-plane pyrolytic graphite electrodes (EPPGEs) in facilitating NADH oxidation. These studies underscore a critical structural prerequisite: a high density of edge-plane defects on the carbon lattice provides the indispensable active sites required for the rapid, stable, and highly efficient electro-catalysis of small bio-molecules.

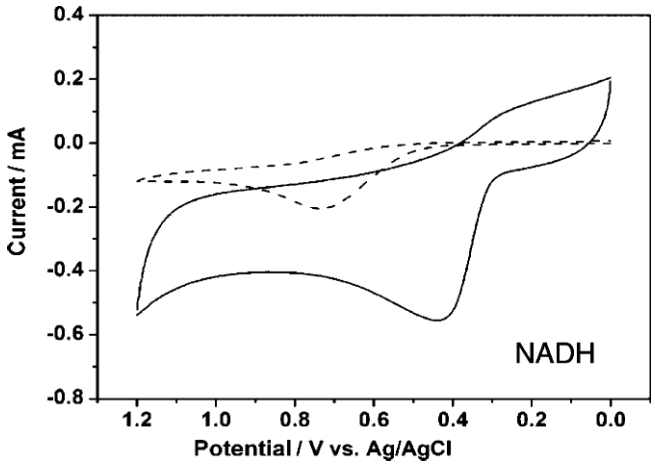

Figure 8: CV plot at scan rate 100 mV/s, in 0.1 M pH 6.8 PBS/1 mM NADH at bare GC (dashed line) and graphene/GC electrodes (solid line) (Adv. Funct. Mater., 19, 2782, 2009).

(e) Detection of Neurotransmitters and Metabolites

If the essential catecholamine neurotransmitter dopamine (DA) within the central nervous system depleted, it causes progression of Parkinson's disease. Analytically, quantification of DA at standard physiological concentrations (ranging from 0.1 μM to 1 mM) is main challenge; such type of huddles arises because the electrochemical oxidation potential of dopamine severely overlaps with those of uric acid (UA) and ascorbic acid (AA) [Shang, N. G. et al. 2008, Wang, Y. et al. 2009, Alwarappan, S. et al. 2009]. To overcome such overlapping, graphene-based electrodes can be precisely engineered that allow them to successfully differentiate these overlapping signals. Hou, S. F. et al. (2010) effectively neutralized signal interference by engineering a graphene electrode functionalized with Nafion and EDTA, resulting in a highly selective sensing platform specifically tailored for dopamine. Tan, L. et al. (2010) achieved ultra-sensitive DA detection by using unique cyclodextrin-graphene structural pairing allowed the platform to accurately detect dopamine as low as 5 nM. Expanding the application to coexisting metabolites, Hong, W. et al. (2010) fabricated a robust sensor by decorating pyrene butyrate-functionalized reduced graphene oxide (rGO) sheets with gold nanoparticles. In Figure 9, this specialized architecture yielded an improved detection limit of 0.2 μM for uric acid.

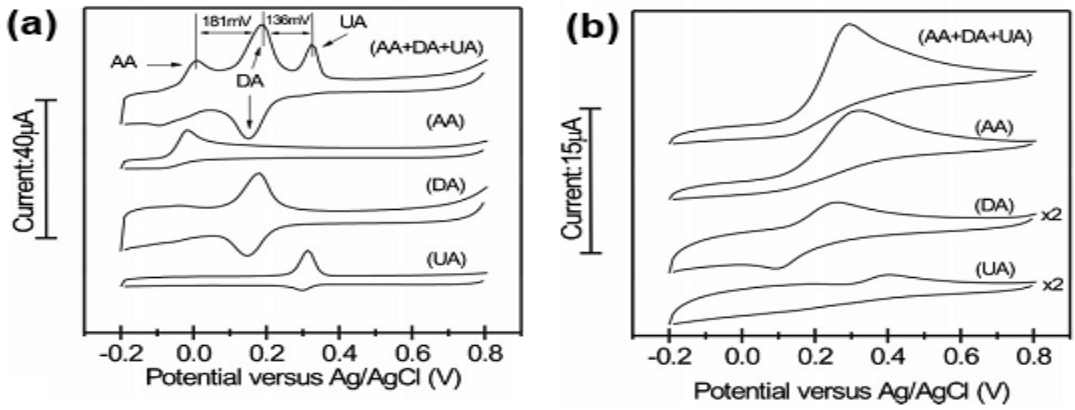

Figure 9: CV plot for (a) graphene and (b) bare GC electrodes, in the solution 50 mM, pH 7.0 PBS/1mM AA, 0.1mM DA/0.1mM UA (Adv. Funct. Mater. 2008, 18, 3506).

(f) Cellular Detection Platforms

The early stage detection of malignant and pathogenic cells are utmost important in modern oncology for disease management. The superior property of easily functionalized of Graphene surface to recognize specific biological targets make it suitable scaffold for cytological biosensors. Feng, L. et al. (2011) engineered reduced graphene oxide (rGO) capped with 4,9,10-perylene tetracarboxylic acid (PTCA) on GCE and treated with target-specific oligonucleotide aptamers to achieve a highly selective detection capability towards human cervical carcinoma and breast cancer cells (detection limit ~ 1,000 cells/mL). Xia, Y. et al. (2012) covalently attached double-stranded DNA (dsDNA) with anti-HER2 antibodies on GO-modified electrode for amperometric sensor targeting the HER2 biomarker on SKOV-3 cancer cells. Daunorubicin acts as an intercalating agent to generate a distinct electrochemical signal, such amperometric sensor achieved an ultra-low detection limit of 5.2 cells/mL. Guo, C. X. et al. (2010) indirectly quantified live cell populations by continuously measuring the real-time, in situ release of extracellular hydrogen peroxide (H_2O_2).

(g) Protein Biomarker Analysis

Protein biomarkers are main quantifying entity in several pathological, pharmacological, or physiological conditions. Graphene matrices are used to highly improve the electrochemical signals necessary to detect proteins traces at clinical diagnostics. Du, D. et al. (2010) designed a novel immune-sensor by using horseradish peroxidase (HRP)/ carbon nanospheres/graphene to detect α-fetoprotein (AFP) by dual-signal amplification mechanism that achieved an ultrahigh-sensitivity ~ 20 pg/mL. A single-step, in situ process adopted by Tuteja, S. K. et al. (2014) to prepare 2-aminobenzylamine/graphene electrode to detect Cardiac Troponin I (cTnI) a critical indicator of myocardial infarction below to 0.01 ng/mL. Su, B. et al. (2010) efficiently quantified AFP in human serum by immobilizing HRP-anti-AFP conjugates on a biomimetic nanogold/graphene electrode. Later, a sandwich-type electrochemical immunosensor was designed by Han, J. et al. (2012) to quantify both total Prostate Specific Antigen (PSA) and free PSA (fPSA) at ultra-low detection limits ~3.4 pg/mL and ~ 6.7 pg/mL respectively.

4.2 Non-Enzymatic Graphene-Modified Electrodes

The enzymatic detectors are target selective, sensitive and their real- world implementation is oppressively restricted because of complex immobilisation procedures, high manufacturing costs, environmental vulnerability, and fast biocatalyst degradation. The limited use of enzyme-based detectors, experimenters are exploring the alternate possibilities of robust, enzyme-free (non-enzymatic) electro-catalytic sensors generally made from pure metals, metal oxides, alloys, or semiconductors. The catalytic activity at atomic level rely on the direct interaction of the target analyte with d-orbital electrons of transition metal and catalytic efficiency of such exchange is estimated by the system's Gibbs free energy of system and intermediate bond strength that promotes sufficient reactant adsorption without preventing the subsequent release of products [Pletcher, D. 1984]. The inherent electro-catalytic efficiency of carbon nanostructures can be highly improved by doping with metal nanoparticles, such as Pt or Au NPs [Yoo, E. et al. 2009, Zhou, K. et al. 2010, Shan, C. et al. 2010, Baby, T. T. et al. 2010, Liu, Y. et al. 2010]. Likewise, the sp^2-hybridized lattice of graphene requires a low applied potential to eventuality to induce largely reactive hydroxyl radicals at its active spots, a critical step for the electrochemical oxidation of several organic molecules [Koppang, D. et al. 1999]. Graphene sheets decorated with carbon nanofibers, metal and metal oxide NPs, leads

modern non-enzyme based sensors [Jin, C., & Taniguchi, I. 2007, Schechner, P. et al. 2007, Appleby, A. J., & Van Drunen, C. 1971, Prilutsky, S. et al. 2010, Davis, F., & Higson, S. P. J. 2007, Kerzenmacher, S. et al. 2008]. Liu, M., Ruan, R., & Chen, W. (2013) fabricated a graphene-modified GCE decorated with Cu_2O nanotubes, yielding a well resolved glucose oxidation peak and high sensitivity ~ 0.285 mA cm^{-2} mM^{-1}. Graphene treated with Mn_3O_4 or Co_3O_4 nanoparticles has proven exceptionally able of low-limit glucose sensing. Gao, L. et al. (2012) prepared a hybrid hemin-graphene sheets for dual-detection platform, when activated with phosphate buffer, it possess robust, intrinsic peroxidase-like activity, driving both the oxidation of TMB (3,3,5,5-tetramethylbenzidine) and the reduction of H_2O_2. As compared with bare graphene, this hybrid generated higher catalytic currents and an earlier onset potential for H_2O_2 reduction, yielding an expansive linear range (0.5 μM to 0.4 mM) with sensitivity limit ~ 0.3 μM for glucose and ~ 0.2 μM for H_2O_2. Song, H. et al. (2013) further advanced this composite by incorporating gold nanoparticles to form an ultra-sensitive Au-hemin-graphene platform. The groups of researchers Shan, C. S. et al. (2009) and Kang, X. H. et al. (2009) demonstrated the direct electrochemistry of glucose oxidase (GOx) using thermally and chemically reduced graphene oxides. The transition to metal-oxide/graphene composites remains the most effective strategy for prostrating the inherent structural fragility of biological enzymes. Beyond clinical applications, graphene-modified GC are highly valuable tools for environmental monitoring, specifically in detecting poisonous heavy metal ions like cadmium (Cd^{2+}) and lead (Pb^{2+}). Li et al. developed a Nafion-graphene based electrode that displayed extensively enhanced sensitivity toward these metallic pollutants, although the commerce between the Nafion polymer and the graphene sheets can sometimes induce minor signal interference (Figure 10). When analysed using differential pulse anodic stripping voltammetry (DPASV), these nano-modified electrodes produce sharply defined, easily distinguishable current peaks. They induce significantly large stripping currents and superior sensitivity limits as compared to conventional materials, consistently delivering: (i) A broad linear detection range for Cd^{2+} (1.5 to 30 mg/L) and Pb^{2+} (0.5 to 50 mg/L), (ii) Exceptional detection limits of 0.02 mg/L for both targets. Such level of analytical performance nearly parallel with Nafion/CNT-coated bismuth electrodes but comprehensively outpaces standard bismuth-modified GCEs or mesoporous carbon platforms [Li, J. et al. 2009, Li, J. et al. 2009, Xu, H. et al. 2008, Kefala, G. et al. 2004, Zhu, L. D. et al. 2008]. The enrichment electrochemical sensing nature of Graphene sheets due to its immense active planer surface morphology, and high electrical conductivity, that maximize the localized accumulation of target ions, elevate overall detection sensitivity, and effectively prevent the sensor fouling typically induced by environmental surfactants [Li, J. et al. 2009, Li, J. et al. 2009].

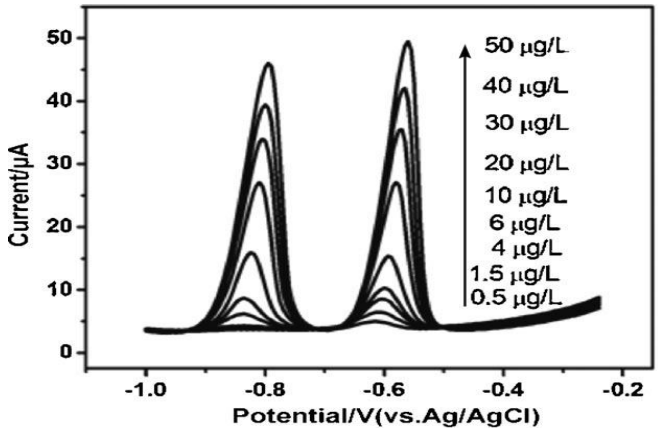

Figure 10: Striping voltammograms on in situ plated Nafion-G-BFE (bismuth film electrode) for the Cd^{2+} and Pb^{2+} in 0.4 mg L^{-1} Bi^{3+} solution (Anal. Chim. Acta 2009, 649, 196).

5 GRAPHENE: IN THE FIELD OF BIOMEDICAL APPLICATIONS

To revolutionize the patient care, the basic need to development of highly specific, ultra-sensitive, and user-accessible diagnostic sensors. The real time precise measurement of targeted biomarkers such as proteins, nucleic acids, and small molecules is vital for the timely identification of bacterial and viral infections, neurodegenerative diseases, and malignancies. In present era, electrochemical biosensors become essential diagnostic and prognostic instruments in this field. To maximize analytical performance across these categories, modern research heavily leverages functionalized nanomaterials, with two-dimensional nanosheets driving unprecedented diagnostic breakthroughs.

Cancer Biomarker Detection

(a) α-Fetoprotein (AFP) Quantification: The utmost necessity of precise detection and measurement of α-fetoprotein (AFP) at clinical diagnostics. Scientists have developed several high level sophisticated graphene/ immune-sensors to confined and quantify specific antigen. TiO_2/Graphene/Chitosan/AuNP platform was synthesized by incubating a modified electrode in an anti-AFP solution under 4 °C for 12 hours, followed by BSA (bovine serum albumin) treatment to stop the non-specific adsorption of serum proteins. Experimental verification of the integrated gold nanoparticles (Au NPs) functioned as highly effective electron shuttles in a 0.1 M PBS buffer (pH 7) containing a $[Fe(CN)_6]^{3-/4-}$ redox probe and achieved a remarkable limit of detection (LOD) of 0.03 ng/mL across two distinct linear ranges (0.1 to 10 ng/mL and 10 to 300 ng/mL). Furthermore, it exhibited excellent retaining tendency (~ 95% of its initial signal after 30 days) [Huang, J. et al. 2013, Huang, K. et al. 2013]. Wu, H. et al. 2009 designed an electrochemical sensor by using glucose to reduce Ag-ions directly by reduced graphene oxide (rGO) nanosheets. The cyclic voltammetry (CV) analysis demonstrated the logarithmic correlation between target concentration and anodic peak intensity with a linear detection range of 0.1-100 ng/mL and at sensitivity of 39.86 mA/log(ng/mL). When actual human serum cross-validated with the commercial ELISA kits, the results matched perfectly with sensor operational stability of 86.9 % over 20 days [Wu, S. et al. 2013]. Zhao, L. et al. (2013) extended analytical domain by reduction of $HAuCl_4$ and K_2PdCl_4 at same time to design bimetallic nanocrystals. By covalently attaching primary antibodies via amide linkages, this architecture synergized the immense catalytic activity of the bimetallic crystals with the exceptional electrical conductivity of N-doped graphene.

(b) Human Epidermal Growth Factor Receptor 2 (HER2) Analysis

When HER2 gene encodes a 185 kDa trans-membrane glycoprotein over-expressed, it acts as an active tyrosine kinase receptor that drastically accelerates metastasis in breast cancer. It is also intrinsically linked to gastric, salivary, bladder, lung, and ovarian carcinomas. Ali, Md. A. et al. (2015) successfully synthesize a label-free, highly sensitive micro-fluidic immune-sensor utilizing a 3D porous graphene foam doped with $nTiO_2$/carbon and functionalized with anti-ErbB2 receptors to estimate breast cancer markers via specific antigen-antibody binding events through EIS and DPV. The common diagnostic imaging techniques such as ultrasound, MRI, computed tomography, and X-ray mammography provides limited, localized diagnostic information. On the other side, advanced analytical platforms like nano-transistors, semiconductor quantum dots, photonic crystals, micro-cantilevers, and surface plasmon resonance (SPR) deliver highly precise, quantitative molecular data [Yu, X. et al. 2006, Krishnan, S. et al. 2011, Chan, L. L. et al. 2007, Wee, K. W. et al. 2005, Wu, X. et al. 2002].

(c) Multiplexed Platforms and Alternative Biomarkers

The advancement in analytical platforms for clinical diagnostic led to the development of sensors capable to target multiple disease markers: Ho and colleagues designed an immune-magnetic system capable of simultaneously quantifying multiple oncology proteins, including prostate-specific antigen (PSA), carcinoembryonic antigen (CEA), and AFP [Ho, S. L. et al. 2016]. While high-performance field effect transistor (FET) immune-sensors uses graphene-encapsulated nanoparticles to detect breast cancer biomarkers with an extraordinary LOD of 1.0 pM. For CEA detection, Gao, X. et al. (2011) achieved an impedimetric LOD of 0.06 ng/mL (linear range: 0.1 to 160 ng/mL) by immobilizing anti-CEA onto a CNT/AuNP matrix. Wang, J., Xu, D., & Polsky, R. (2002) successfully developed a magnetically induced solid-state sensor to identify breast cancer markers via DNA hybridization (sensing limit ~ 200 ng/mL). Table 1 summarizes the core performance metrics of advanced two dimensional nanomaterials based sensing platforms.

Table 1: Summarized List of Electrochemical Biosensors based on carbon nanotubes, graphene and other nanomaterials

Authors	Type of Sensor
H. M. So et al. 2005	SWNT-FET DNA biosensor as an alternative to protein-based sensing
N. Yang et al. 2016	SWNTs electrode as molecular wires for direct protein electrochemistry
R. K. Joshi et al. 2010	Graphene Ribbons electrode for O_2, CO and NO_2 Sensing under practical conditions
Y. Shao et al. 2010 Shan C. S. et al. 2009	Graphene/polyethylenimine functionalized ionic liquid for glucose (wide linear detection range 2 to 14 mM) with exceptional reproducibility. It exhibits high stability, retains 95.1% of its initial current response after one week.

Shan C et al. 2010	Graphene/AuNPs/chitosan based H_2O_2 and O_2 sensor.
Wu H. et al. 2009	GOD/graphene/Pt NPs/chitosan composite as a Enhanced glucose detector (Limit of 0.6 mM).
Zhou M. et al. 2009	CR-GO treated electrode for H_2O_2 Electrochemical behavior, ADH-graphene GC electrode for Ethanol sensors, and CRGO GC electrode for DNA sensors
Kang X H et al. 2009	In Glucose Oxidase/graphene/chitosan modified electrode, chitosan acts as an excellent dispersion agent, yielding a highly sensitive glucose response (**37.93 mA mM^{-1} cm^{-2}**) and high stability in direct electrochemistry.
Tang L H et al. 2009	CRGO GCE shows improved electron transfer rate with respect to graphite/GC and GC electrodes towards NADH.
Liu H et al. 2009	Graphene/methylene green (MG) based electrode shows enhanced performance toward the oxidation of NADH. Oxidation of NADH on MG-graphene electrode takes place ~ 0.14 V, while for pristine graphene electrode this value ~ 0.40 V
Lin W et al. 2009	EPPGE possess high density edge defective sites for electron transfer towards biological species that improve oxidation/reduction activity of small bio-molecules
Pumera M. 2010	Molecular dynamics and HRXPS confirm that oxygen-containing groups of graphene are key factor to enhanced activity towards NAD^+ molecules
Shang N G et al. 2008	Multilayer graphene nano-flakes (MGNFs) based electrode used to detect DA at limit ~ 0.17 mM (resolved discrimination of AA, DA, and UA)
Alwarappan, S et al. 2009	Graphene GCE shows better sensitivity toward DA than SWCNTs and capable to effectively distinguishes AA, DA, and ST.
Wang Y. et al. 2009	Graphene electrode shows high selectivity towards DA (linear range 5 mM - 200 mM).
J Li et al 2009	Nafion/Graphene/bismuth electrode for Pb^{2+}, Cd^{2+} ions, shows Linear range

L Wang et al. 2017	For cancer diagnosis
Rathod D et al . 2010	CNF/Nafion/platinum NPs electrode as glucose sensor
Liu Y et al. 2009	CNF/Ni NPs GC electrode used for Glucose sensor shows wide linear range and high sensitivity
Alwarappan, S et al. 2010	Graphene/polypyrrole electrode as a glucose detector shows fast heterogeneous electron transfer rate, better biocompatibility and performance
Zeng G et al. 2010	Bienzymatic graphene-glucose oxidase- glucoamylase electrode for maltose detection Graphene-Cu/CuO at ITO electrode for fructose sensors
Hong W et al. 2010	Graphene oxide GC electrode enrich with –COOH used for G and A detection shows high sensitivity at detection limits 50 nM and 25 nM
Pumera M 2010	Stacked graphene nanofibers GC electrode used for detection of components of DNA, shows four-fold higher sensitive than CNT based electrodes
Lim C X et al. 2010	Epitaxial grown graphene on SiC used for both ssDNA and dsDNA detection
Lin W J et al. 2009	Graphene modified electrodes exhibits superior sensing capability towards toward NADH oxidation as compared with bare EPPGE.
Hou S F et al. 2010	Nafion/N- (trimethoxysilylpropyl)/ (EDTA) modified graphene electrode used for DA detection.
Tan L et al. 2010	Cyclodextrin/graphene GC electrode for DA detection
Hong W et al. 2010	Au NPs/pyrene butyrate RGO (PFG) for detection of UA with detection limit 0.2 μM
Feng L et al. 2011	Breast cancer cells and human cervical carcinoma cells detection: Graphene/4,9,10-perylene tetracarboxylic acid (PTCA)/ oligonucleotides GCE based electrochemical biosensor.
Xia Y et al. 2012	graphene -amperometric sensors for HER2 on SKOV-3 cancer cells identification

Du D et al. 2010	Graphene/ CNSs/ (HRPAb2) sensor for sensitive cancer biomarker α-fetoprotein (AFP)
Tuteja S K et al. 2014	2-ABA/graphene for immune sensing of cTnI with sensitivity ~ 0.01 ng/mL.
Su B et al. 2010	HRP-anti-AFP immobilized graphene / Au-functionalized biomimetic interfaces to detect α-fetoprotein (AFP) inhuman serum
Han J. et al. 2012	Sandwich-type Au @ PB NPs/OGS and Au @ Ni NPs/OGS for electrochemical immune sensor detection of PSA
Gao L et al. 2012	Non-enzymatic hemin-Graphene hybrid nanomaterials towards H_2O_2 and glucose detection.
Liu M et al. 2013	Cu_2O nanotubes/graphene GC electrode for glucose detection.
Song H et al. 2013	Au-hemin-graphene electrode for improve performance of Non-enzyme based H_2O_2 sensors
K. Huang et al. 2013	TiO_2/Graphene/chitosan/AuNPs used to quantify the AFP cancer biomarkers.
Ho S et al. 2016	Immune magnetic platform: Multiplex quantification of various cancer-associated proteins
Yu X et al. 2006	SWCNT/multi-label secondary antibodies combined immune-sensors for ultra sensitive detection of cancer biomarkers.
Gao X et al. 2011	Immobilizing anticarcinoembryonic antigen by CNT/Au NPs electrode for impedimetric detection of cancerous molecules
Wang J et al. 2002	Magnetically induced solid-state electrochemical DNA hybridization for breast cancer biomarkers detection.

6. CURRENT CHALLENGES AND FUTURE PERSPECTIVES IN GRAPHENE BIOSENSING

Graphene sheet is widely recognized as a preferred nanosheet in the electrochemical analysis particularly for glucose monitoring due to its ultra-sensitivity and strict target selectivity. The main concerns include its potential cytotoxicity, long-term operational stability, and high susceptibility to surface fouling, which is largely driven by the hydrophobicity of the material. Overcoming such type of obstacles is essential for the successful commercialization of a new generation of ultra-sensitive, interference-free diagnostic platforms. While the research work in this area during the past decade has yielded tremendous progress in graphene-enhanced electrochemical analysis, continued innovation is critical. To optimize the capabilities of graphene, the scientific community must address several technical hurdles: (i) Engineering

method for highly reproducible for the exact fabrication of graphene sheets, controlled heteroatom doping, and targeted nanostructure, (ii) Extended theoretical framework to specific microstructural defects, the density of states, and varied surface functional groups etc. (iii) Designing and maximizing surface morphologies such as nanocages, ordered arrays, nanowires, gap channels, and highly porous matrices to maximize overall catalytic efficiency. This foundational optimization will not only revolutionize biosensing but will also drive critical advancements in targeted drug delivery, advance medical imaging, and broad-scale electro-catalysis. Ultimately, graphene-based nanostructures have proven to be an exceptional platform for biosensors; it continues to present a vast frontier for fundamental theoretical exploration, material refinement, and next-generation technological deployment in field of biosensors and biomedicine.

7. CONCLUSIONS

The superior material properties such as extremely large surface-to-volume ratio, zero band gap, tunable electronic behavior, and enhanced physical and chemical activity of Graphene sheets makes it a remarkably ultra-sensitive electrochemical biosensor. Electrochemical analysis with GC electrode modified with graphene is uniquely equipped to direct electrochemistry of biological enzymes and detect a broad spectrum of biomolecules across various fields, including biomedical diagnostics, environmental surveillance, and bio-analysis. To optimize the analytical capabilities, the modification of targeted chemical by two-dimensional nanosheets remains a primary focus of modern nanomaterials research. The tuning of electronic behavior of graphene sheet by integrating non-metallic heteroatoms (e.g., P, S, N, and B atoms or transition metals including Rh, Pt, Pd, Mn, Co, Ni, Fe, and Ag) consistently provides substantial enhancements in overall electrocatalytic performance. Furthermore, graphene functions as a highly resilient foundational scaffold for supporting secondary nanomaterials by anchoring metallic nanocrystals (NCs), transition metal dichalcogenides (such as MoS_2), or gold and silver nanoparticles (Au/Ag NPs), researchers can forge highly conductive, efficient interfaces between the underlying electrode and the biological targets, laying the groundwork for next-generation sensing platforms. The superior structural and electronic advantages of graphene sheets translate into performance upgrades across diversified applications in H_2O_2 detection, in which Graphene exhibits dual utility in hydrogen peroxide sensing. For enzymatic systems, graphene nanocomposites function simultaneously as a stable immobilization matrix and as conductive "molecular nanowires" that bridge the gap to deeply buried peroxide. In non-enzymatic systems, unmodified graphene or doped graphene provides an ideal electro-catalytic surface that inherently maximizes reaction efficiency. The large active surface area, pristine biocompatibility, and superior electrical conductivity of graphene facilitate immense signal amplification. This structural synergy drastically elevates the ultimate analytical sensitivity of immune-based diagnostic assays. The analytical success of graphene has sparked a broader revolution within materials science. It has directly inspired the development and application of analogous inorganic 2D materials such as metallic chalcogenides in novel electrochemical sensor architectures. The ultimate scientific challenge now lies in bridging the gap between profound academic discoveries and viable, scalable clinical technologies. The rapid acceleration of biosensor research is fueled by critical, real-world imperatives: the need for real-time medical diagnostics, rigorous food safety monitoring,

and advanced homeland security measures. Fortunately, with the recent advent of scalable, high-volume production techniques for graphene, the widespread commercialization and global deployment of these transformative analytical platforms are rapidly becoming a tangible reality.

ACKNOWLEDGEMENT

The author Dr. Mukesh Mishra (MM) would like to thank Shri R. K. Parikh Arts and Science College and CSIR-CECRI for providing the necessary facilities and support. The author M.M. expresses sincere gratitude to the mentor, Dr. Subbiah Alwarappan, for his continuous guidance and acknowledging the financial support received by DST-SERB (PDF/2015/563).

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