

Review paper

# Graphene: A Comprehensive Review on Its Utilization in Carbon Paste Electrodes for Improved Sensor Performances

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Nowadays, Graphene (Gr), a 2-dimensional  $sp^2$ -hybridized carbon sheet has been considered a hot material. It has received tremendous amount of attraction owing to some extraordinary features such as superb electrical and thermal conductivities and high mechanical strength. Hence, it has been used widely in electrochemistry in order to achieved desired sensitivities. Very recently, it has been utilized for the development of various paste types electrodes due to their simple preparation methods, cost effectiveness and easy recycling. Several reports appeared on the utilization of pure graphene paste electrode (GPE), modified GPE and modifications of traditionally prepared carbon paste electrode (CPE) with Gr for sensitive determination of various analytes. The goal of this review is to focus on all the applications of Gr in paste type of electrodes which have been appeared in literature. This review has been written in summary style and encompasses the fabrication schemes, electrochemical determination methods, characterization techniques, and analytical performances from all the contributions made on this topic.

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**Keywords:** Graphene, Graphene paste electrode, carbon paste electrode

**Abbreviations:** Gr, Graphene; GPE, Graphene paste electrode; CPE, Carbon paste electrode; RGO, Reduced graphene oxide; IL, Ionic liquid; CTS, Chitosan; Nf, Nafion; SEM, Scanning electron micrograph; TEM, Transmission electron micrograph; EIS, Electrochemical impedance spectroscopy; AFM, Atomic force microscopy; CILE, Carbon ionic liquid electrode

## 1. INTRODUCTION

Among several types of carbon electrodes, the carbon paste electrode (CPE) was first introduced by Ralph Norman Adams almost five decades ago [1, 2]. Later, with the pioneering work of Kuwana followed by Baldwin research, it became possible to obtain the modern type of CPE where carbon (graphite) powder was mixed with solid binder (pasting liquid) in order to achieve homogenous

paste. It became extremely popular and commenced the vast research activities in the field of electrochemistry owing to some superb features such as simple preparation, economic, easy renewability of electrode surface just by polishing, negligible background current and wide potential window [2].

In spite of its appealing nature, the presence of no or less conducting binder in the CPE leads to sluggish electron transfer kinetics, thus providing decreased sensitivity of the detection system. Hence, various new materials have been introduced during the preparation of conventional CPE in order to improve the electrochemical performance of the sensor electrodes.

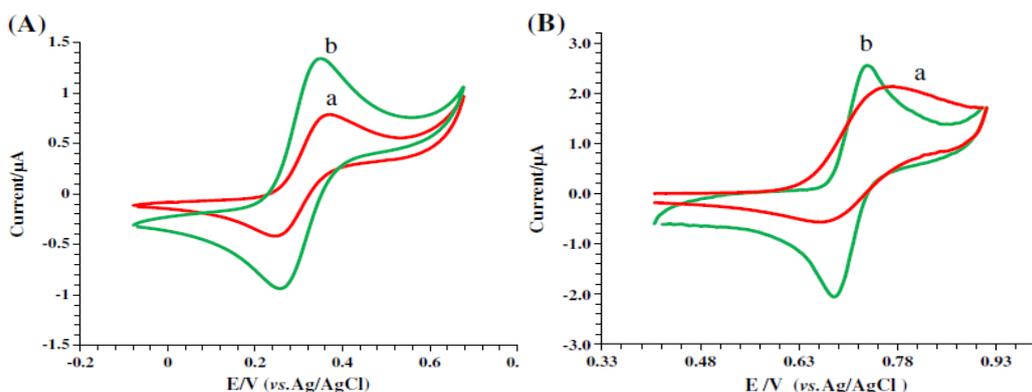
One of the most promising strategies used to improve the sensitivity of a sensor is to utilize a material with great surface area and conductivity as transducer. Among various Carbon nanomaterials used as transducer, the application of Gr has progressed rapidly.

In recent years, graphene and graphene related materials including graphene oxides have witnessed tremendous interest among scientific community. It is a 2-dimensional  $sp^2$ -hybridized carbon sheet possessing some supreme physicochemical properties such as extremely high surface area, high thermal and electrical conductivity and robust mechanical strength due to the long range  $\pi$ -conjugation. These extraordinary properties make it suitable for electrochemistry applications particularly in sensing and energy storage. It has also been suggested that Gr has the potential to replace the some existing materials used for the fabrication of chemical and biosensors [3-5].

Therefore, recently, several reports have been appeared including either as pure Gr paste electrode (GPE), doped GPE or Gr doped CPE for the fabrication of various types of sensors. This review intends to focus on the utilization of Gr in several ways. For the convenience, the review has been categorized based on the type of utilization of Gr as discussed in the following sections.

## 2. PURE GPE

The utilization of GPE was first reported by Parvin et al. in 2011 for the determination of chlorpromazine (CPZ). In this report, firstly, Gr was modified by Hummers method [6, 7] followed by mixing of as-synthesized Gr with an appropriate amount of mineral oil to achieve a homogeneous paste. Finally, the GPE was filled into a piston-driven Gr electrode holder. For comparison purpose, piston-driven carbon electrode holder was also prepared just by substituting Gr with carbon. Figure 1(A) and (B) compare the cyclic voltammograms (CVs) for 0.1 mM  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  and 0.1 mM CPZ recorded at the CPE and GPE. It can be observed that GPE provided dramatically enhanced redox peaks for chlorpromazine with a small peak potential separation ( $\Delta E$ ) of 0.63 mV. On the other hand, CPE shows a pair of poor redox peaks providing high peak potential separation of  $\sim 120$  mV. The poor performance of CPE was attributed to its low electrical conductivity and surface area.



**Figure 1.** (A) CVs for 0.1 mM  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  using CPE (a), and GPE (b), and (B) CVs for 0.1 mM CPZ using CPE (a), and GPE (b). Electrolyte: 0.5 M phosphate solution with pH 4.0. Scan rate:  $60 \text{ mV s}^{-1}$  (reproduced with permission from Reference [8]).

The Differential pulse voltammetry (DPV) was used to get the calibration curve. This method exhibited limits of detection (LOD) and the limits of quantitation (LOQ) values of 6.0 and 20.0 nM, respectively, with a relative standard deviation (RSD) of 3.6% ( $n=10$ ), while RSD was 4.4% for five electrodes [8].

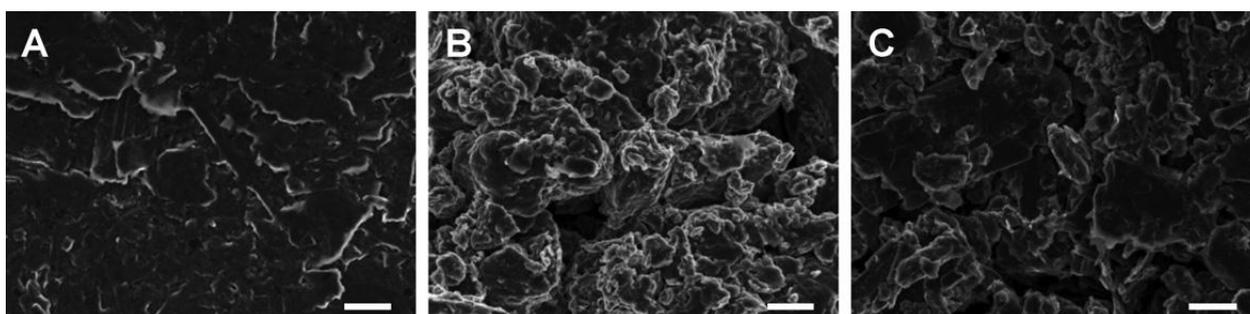
The same author used the similar strategy for electrochemical determination of Acetazolamide (ACZ), N-(5-sulfamoyl-1,3,4-thiadiazol-2-yl) acetamide, is a carbonic anhydrase inhibitor (CAI) used clinically in the management of glaucoma. The piston-driven GPE and CPE were prepared using reduced Gr oxide (RGO) and graphite with mineral oil, respectively.

The reported method established linear relationship from 0.007–3.0  $\mu\text{M}$  and the values of LOD and LOQ values were calculated to be 2.2 and 7.3 nM, respectively. This method also showed excellent tolerance limit which was defined as the concentration ratio of additive of ACZ causing less than  $\pm 5.0\%$  relative error. The tolerance limit of additives to 60.0  $\mu\text{M}$  ACZ was 700 times for  $\text{Mg}^{+2}$ ,  $\text{Fe}^{+2}$ ,  $\text{Co}^{+2}$ ,  $\text{Ca}^{+2}$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{-2}$ ,  $\text{Br}^-$ , and glycine; 250 times for glucose, sucrose, fructose, valine, 150 times for aspartic acid, urea, 50 times for cysteine and 37 times for ascorbic acid and saturated starch solution. Furthermore, the analytical applicability of the method was investigated by analyzing real samples of human urine and serum. The excellent recovery values of ACZ between 96.0% and 99.2% were achieved using voltammetric method comparable with those obtained from HPLC method [9].

In a study done by Hu et al. reduced Graphene nano-sheets (GN) paste electrodes (GNPE) were prepared for phenol sensing in the presence of surfactant in buffer solution. The electrodes were obtained by mixing pre-synthesized reduced GN by using modified Hummer's method with 180 mg of paraffin oil thoroughly and finally filled in a Teflon tube of 3mm id. For comparison study, CPE was also prepared by using graphite powder instead of reduced GN. The study showed that GNPE provided large current response compared to CPE due to the fast electron transfer property of GN. To study the effect of a surfactant on electrochemical response of phenol, sodium dodecyl sulfate (SDS) was dissolved in buffer. It was observed that current response increased by increasing the concentration of SDS. However, when the concentration of SDS was 200  $\mu\text{mol/L}$ , the response was leveled off. Hence, this concentration was used for further optimization of pH value of buffer. This method exhibited

linear current response from  $8.0 \times 10^{-8}$  to  $8.0 \times 10^{-5}$  mol/L ( $R = 0.99926$ ,  $n=9$ ) with LOD value of  $5.0 \times 10^{-8}$  mol/L. The reported method showed good electrocatalytic selectivity for 5  $\mu\text{mol/L}$  phenol. The 5  $\mu\text{mol/L}$   $\alpha$ -naphthol, 5  $\mu\text{mol/L}$  p-chlorophenol, 10  $\mu\text{mol/L}$  catechol and 50  $\mu\text{mol/L}$  hydroquinone showed some interference signals, however, 100  $\mu\text{mol/L}$  p-nitrophenol and 100  $\mu\text{mol/L}$  2-aminophenol did not show interferences. Furthermore, three polluted soil samples were analyzed and outstanding recoveries of phenol upto 99.4% (mean value) were achieved and results were found to be in close agreement with the results obtained by ultraviolet-visible absorption spectrophotometry [10].

In an interesting study, Gasnier et al. used GPE for direct quantification of nicotinamide adenine dinucleotide (NADH). Before preparation of various GPE, the Gr oxide was obtained using the Hummers method followed by its reduction (Gr) with Ascorbic acid (AA). Finally, several electrodes were prepared by mixing various amounts of Gr, graphite and mineral ratio in w/w%. The Gr oxide, Gr and graphite were characterized in details by using FT-IR and Raman spectroscopy.



**Figure 2.** SEM images for CPE (A), Gr<sub>50</sub>PE (B) and Gr<sub>30</sub>PE (C) at a 10.00K $\times$  magnification. Scale bar: 10  $\mu\text{m}$  (reproduced with permission from reference [11]).

Figure 2 depicts the SEM characterization of different composites such as with no Gr (CPE), 50% (Gr<sub>50</sub>PE) and 30% Gr (Gr<sub>30</sub>PE). It can be easily deduced from Fig 3A, B and C that that varying the amount of Gr generates quite different surface morphology.

It is worth mentioning that the Gr content of 30% (w/w) provided the excellent electrocatalytic response. Increasing the Gr content beyond 30% did not enhance the current response significantly. The Gr<sub>30</sub>PE demonstrated robust and reproducible amperometric response of NADH without any significant passivation. The same electrode exhibited excellent quantification of NADH in the presence of AA because both bioanalytes were oxidized at very different peak potentials. The calibration plots was obtained at Gr<sub>30</sub>PE for NADH using DPV in the presence of  $1.0 \times 10^{-5}$  and  $5.0 \times 10^{-5}$  M AA with average sensitivities. The average sensitivities were obtained using 3 different electrodes from three calibration plots and estimated to be  $(6.7 \pm 0.5)$ ,  $(6.6 \pm 0.5)$ , and  $(6.9 \pm 0.2)$   $\mu\text{A/mM}$  [11].

Very recently, Parvin et al. used the same similar strategy as has been used by them in other reports for the determination of Medetomidine, an analgesic sedative for animals. The authors utilized the reduced GPE modified by Hummers method for the preparation of piston driven GPE and CPE. In order to exploit the maximum potential, some experimental parameters including pH, supporting electrolyte and scan rate were optimized. It was found that the used method exhibited high tolerance

limits against several inorganic and organic interfering analytes commonly existing in pharmaceuticals and biological samples. The resulting GPE electrode was characterized by using DPV techniques and yielded excellent linear range from 0.009 to 2.5 mM. The LOD and limit of quantitation (LOQ) values for the determination of medetomidine were observed to be 2.8 and 9.2 nM, respectively. This fabricated GPE was used to detect medetomidine in Rabbit serum and urine by spiking method with excellent precision and sensitivity [12].

### 3. MODIFICATIONS OF GPE

In this section, reports based on the utilization of Gr as a constituent of paste electrode and its modification by incorporation of some other dopants will be described.

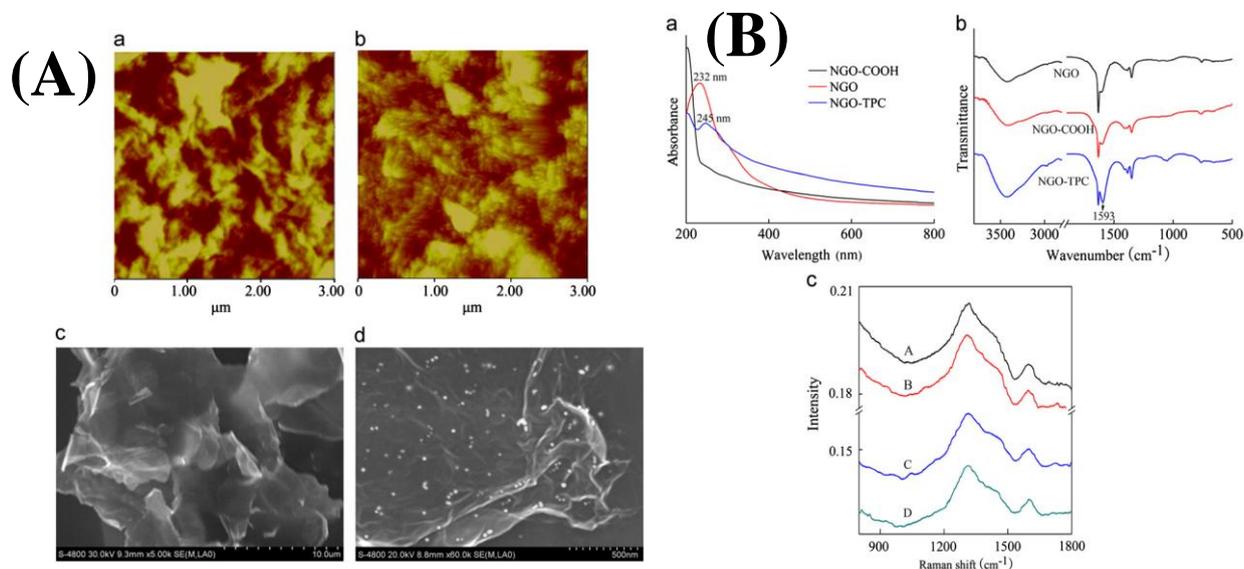
In 2011, Bo et al. fabricated a DNA biosensor based on GPE modified with electrodeposition Prussian blue (PB) followed by coating with chitosan. Firstly, GPE was prepared by mixing different ratio of pre-synthesized Gr with Nujol oil, filled into Teflon tube and electrical contact was made by a Cu wire. Then, electrodeposition of PB was carried out in an aqueous solution consisting of 2 mM  $\text{FeCl}_3$ , 2 mM  $[\text{Fe}(\text{CN})_6]^{3-/4-}$ , 0.1 M KCl, 0.1 M HCl and a constant potential of +0.4 V (vs. SCE) was applied for 120 s in order to enhance the electrocatalytic feature of the DNA biosensor. However, due to low stability of PB modified electrodes, a protective layer of chitosan was formed over PB by simple casting method and air dried. Finally, an ssDNA probe was immobilized through oligonucleotide and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) chemistry in order to achieve ssDNA/Chit/PB/GPE sensor.

Some experimental variables such as paste compositions, DNA hybridization time and effect of EDC were studied thoroughly. The effects of different sort of DNA hybridization with complimentary DNA (cDNA, Target DNA), three base mis-matched containing sequence (mcDNA) and non-complementary DNA (ncDNA) were investigated by comparing the DPV responses current of ssDNA/Chit/PB/GPE. It was observed that response current of cDNA was greatly enhanced compared to mcDNA and ncDNA which exhibited poor responses. The LOD of reported DNA biosensor was estimated to be  $1.58 \times 10^{-11}$  M and it showed excellent repeatability with RSD of 2.83% (n=4) and stability of over 1 week [13].

In an approach by Hunag et al., a GPE functionalized with gold nanoparticles (AuNPs) and Nafion (Nf)-Cysteine conjugate was applied to make disposable immunosensor against Hepatitis B surface antigen (HBsAg). The surface morphology of Gr was analyzed by SEM and TEM, respectively. The SEM image confirmed the typical wrinkled surface of Gr. The TEM result was in agreement with SEM image. The author also studied the interfacial properties of each surface modification with electrochemical impedance spectroscopy (EIS). The electrochemical characterization of immunosensor was carried out by using CV. Some experimental conditions including incubation time, temperature and pH of the system were also optimized to achieve maximum current response. The reported immunosensor provided linear current relationship to concentrations of HBsAg from 0.5 to 800 ng/mL and the LOD was estimated to be 0.1 ng/mL (S/N =3). The specific selectivity of immunosensor was assessed by using carcinoembryonic antigen (CEA),  $\alpha$ -Fetoprotein

(AFP) prostate-specific antigen (PSA), and human immunoglobulin (HIgG). The result shows that all the interfering species exhibited negligible responses. The reproducibility results of disposable immunosensor showed that it maintained excellent reproducibility for over 6 months with only 10% decrease in current response. Furthermore, the analytical effectiveness of immunosensor was investigated for the determination of HBsAg in six human serum samples obtained from local hospital and the results were found to be in close agreement of the results of enzyme-linked immunosorbent assays (ELISA) method [14].

Yang et al. proposed a highly selective Silver (Ag)-ion electrode based on conjugate of 2-thiophenecarboxylic acid (ionophore, TPC), AuNPs and nanoscale Gr oxide (NGO). Initially, to obtain the conjugated material, NGO was synthesized. The NGO-TPC conjugation was carried out through hydroxysuccinimide/1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (NHS/EDC) chemistry. Later, colloidal AuNPs were fabricated and decorated over NGO-TPC to obtain AuNPs-NGO-TPC conjugate. The GPE was prepared by thorough hand mixing of AuNPs-NGO-TPC conjugate, graphite powder and paraffin oil. Finally, electrode was made by filling the homogenous paste into disposable polyethylene syringe and Cu wire was inserted from opposite side to make electrical contact.



**Figure 3.** (A) AFM images of the (a) NGO and (b) NGO–AuNP–TPC nanosheets, SEM images of the (c) NGO and (d) NGO–AuNP–TPC nanosheets; (B) (a) UV/vis spectra of the NGO, NGO–COOH and NGO–AuNP–TPC in aqueous solution, (b) FT-IR spectra of the NGO, NGO–COOH and NGO–TPC measured in KBr pellets, (c) Raman spectra of NGO (A), NGO–COOH (B), NGO–TPC (C) and NGO–AuNP–TPC (D) (reproduced with permission from reference [15]).

The as-fabricated electrode was characterized by collecting data for morphological information using SEM and AFM images as shown by Figure 3(A) and (B), respectively. The AFM images shown in Figure 3 (A) a and b supported and corresponded the SEM images data in Figure 3 (B) c and d that particle or sphere-like AuNPs were successfully loaded over crumbled and wrinkled surface of Gr. In

addition, UV-Vis, FT-IR and Raman measurements provided the exact information on AuNPs-NGO-TPC conjugation process as illustrated in Figure 7 (B) a, b, and c, respectively.

The potentiometric responses confirmed that the AuNPs-NGO-TPC based GPE was highly selective towards Ag-ion over other various cations and exhibited an excellent Nernstian slope of 59.3 mVdec<sup>-1</sup> ranging from  $8.4 \times 10^{-7}$  to  $1.0 \times 10^{-1}$  M with a LOD of  $6.3 \times 10^{-7}$  M. The high sensitivity of the system was also supported by EIS results. Furthermore, the fast dynamic time of 10 s, stability of over 3 months, high reproducibility and no ligand leaching of this method indicated its promising utility in detection of Ag-ion over other Ag-ion selective electrodes in literature [15].

Ojani et al. developed a platinum nanoparticles (PtNPs) modified GPE system for the efficient determination for sodium borohydride electrooxidation used in direct borohydride fuel cells (DBFC). The Gr was prepared by Hummers method while the Pt colloidal solution was synthesized with Xiao and Bard method by using borohydride as reducing agent [16]. Finally, PtNPs-GPE was prepared by hand mixing of an appropriate amount of Gr, PtNPs and paraffin and the resulting paste was filled into glass tube followed by electrical connection implemented by Cu wire. For comparison purpose, CPE and GPE were also prepared by using graphite powder and Gr with paraffin, respectively.

The electrochemical characterizations of as-prepared electrodes were carried out through CV and EIS. The results suggested that highest responses were obtained with PtNPs-GPE due to the high conductivity of Gr and PtNPs. The high response behavior of PtNPs-GPE was also confirmed by LSV technique which provided a linear dependence slope of peak current density versus borohydride concentration for PtNPs /GPE 1.34 times sharper than PtNPs/CPE. The difference between these two slopes can be due to higher electrocatalytic activity of PtNPs/GPE [17].

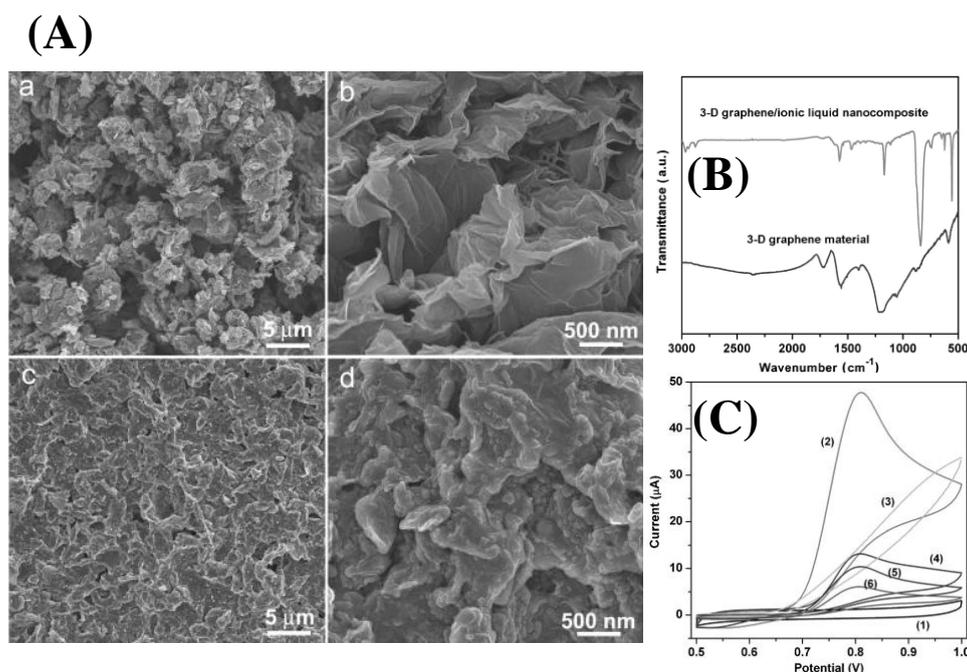
In one of the novel report by Saber-Tehrani et al. GPE was modified with an azo dye 2-(4-((4-acetylphenyl) diazenyl)phenylamino) ethanol (ADPE) for the effective electrochemical determination of a herbicide, Oxadiargyl a broad-spectrum of broadleaf weeds, grass and annual sedges.

The Gr was synthesized according to Stankovich method [18] and reduced chemically. The ADPE/GPE was prepared by blending appropriate amount of ADPE in diethyl ether, graphite powder and Gr with a mortar and pestle. After solvent evaporation, a 70:30 (w/w) mixture of ADPE-Gr powder and paraffin oil was mixed and packed into the end of a Teflon tube. Electrical contact was made from opposite end of the tube. For comparison studies, unmodified GPE was prepared without adding ADPE while ADPE/CPE was prepared without Gr in the paste. The characterization of Gr was performed with AFM and FT-IR. The CV was used to investigate the electrocatalytic activities of ADPE/GPE, ADPE/CPE and bare CPE toward oxadiargyl. It was observed that except ADPE/GPE, no other electrodes exhibited considerable reduction current for oxadiargyl. Therefore, a calibration curve was plotted by obtaining current responses of ADPE/GPE toward reduction of oxadiargyl by DPV technique at different concentrations in PBS buffer. A linear relationship was achieved in the concentration of oxadiargyl from 0.03 to 1.4 mgL<sup>-1</sup> while the LOD was calculated to be 1.3 mgL<sup>-1</sup>. Furthermore, the specificity of present system was ascertained among several coexisted electroactive pesticides including diazinon, parathion, paraquat and diuron and cationic interferents such as Pb<sup>2+</sup>, Cu<sup>2+</sup>, and Cr<sup>3+</sup> found to be acceptable with high stability for over 2 weeks. The method applicability was performed in various real samples (water, soil, rice and rough rice) by the standard addition method for prevention of any matrix effect and excellent recoveries of oxadiargyl were achieved [19].

#### 4. IONIC LIQUID MODIFIED GPE AND CPE

Ionic Liquids (ILs), composed of organic cations and various anions, have been explored widely in electrochemical sensors owing to some unique features such as wide electrochemical windows, negligible vapor pressure, high ionic conductivity and electrochemical stability and good solubility. Hence, to improve the sensitivity and to expand the diversity of research in electrochemical sensors, the incorporation of ILs has become a trend. Recently, due to the thirst in Gr chemistry, the incorporation of ILs and Gr has provided a new dimension to the electrochemical sensors.

In 2010, Ng et al. proposed an interesting approach for the detection of NO based on the Gr-IL nanocomposite gel. Initially, 3-dimensional (3-D) Gr was prepared by modified Hummers method.



**Figure 4.** (A) SEM images of 3-D Gr material with low (a) and high (b) magnification and of 3-D Gr/IL nanocomposite with low (c) and high (d) magnification; (B) FTIR spectra of 3-D Gr material and 3-D Gr/IL nanocomposite; (C) CVs of 0 mM of NO in PBS using 3-D Gr/IL electrode (curve 1), and 0.2 mM of NO in PBS using 3-D Gr/IL (curve 2), 3-D Gr/mineral oil (curve 3), MWCNTs/IL (curve 4), graphite/IL (curve 5) and GCE (curve 6). Electrode surface area of 0.071 cm. Scan rate: 0.05 Vs<sup>-1</sup> (reproduced with permission from Reference [20]).

For the fabrication of desired electrode (Gr-IL), the as-prepared 3-D Gr (or MWCNTs and graphite as a comparison) was blended thoroughly with BMIMPF<sub>6</sub> (IL), forming a homogenous black paste. For the 3-D Gr/mineral oil nanocomposite, all the same procedures were carried out with the exception of using mineral oil as the binder. The resulting mixture was dispersed in acetonitrile and casted over glassy carbon electrode (GCE) followed by the immobilization of Nafion (Nf) to avoid the interferences from nitrite and ascorbic acid.

The morphology and composition of resulting electrode were characterized by SEM and FT-IR, respectively as shown in Figure 4 (A) and (B). The SEM images confirmed the formation of uniform and porous structure of the electrode via the interaction of Gr with IL through cation- $\pi$  and/or  $\pi$ - $\pi$  interactions formed. The Figure 4 (B) showed the FT-IR result which also supported the proper composition of fabricated electrode. For comparison, two different type of electrodes namely, MWCNTs-IL and graphite-IL were prepared by using the same method except inclusion of Gr. The Figure 4 (C) illustrated the electrocatalytic behaviors of various modified electrodes toward NO in PBS buffer using CV. It was observed that Gr-IL electrode outperformed the other two types of electrodes. The amperometric response of Gr-IL offered LOD of 16 nM in just less than 4 s and an excellent sensitivity of  $11.2 \text{ mAcm}^{-2}$  was obtained [20].

In another report, the detection of Bisphenol A (BPA) was carried out by utilizing the chitosan-Gr-carbon ionic liquid electrode (CTS-Gr/CILE) modified electrode as shown by Wang et al. For the fabrication of CILE, the IL, 1-ethyl-3-methylimidazolium tetrafluoroborate (EMIMBF<sub>4</sub>) was hand mixed into graphite and paraffin oil to get a homogenous carbon paste. This paste was filled into glass tubes and electrical contact was established with Cu wire. Then, equal volume of Gr and CTS was mixed together and the resulting mixture was casted over CILE and dried. For comparison, CTS/CILE was fabricated with the similar procedure. The electrochemical characteristics of electrodes were analyzed with EIS and CV. In EIS, the CTS-Gr/CILE exhibited lower electrode transfer resistance compared to CTS/CILE and bare CILE that may be due to the excellent conductivity of Gr. The EIS results were found to be in close agreement with CV data. To achieve highest current response, the buffer pH, scan rate and optimum accumulation conditions were optimized. The Calibration curve was plotted for various concentrations of BPA with function of current response using DPV technique offering two LDR from 0.1  $\mu\text{mol/L}$  to 100.0  $\mu\text{mol/L}$  and 100.0  $\mu\text{mol/L}$  to 800.0  $\mu\text{mol/L}$  and a LOD of  $2.64 \times 10^{-8} \text{ mol/L}$  was estimated for BPA. Furthermore, the CTS-Gr/CILE was found to be highly selective toward BPA compared to 50 fold concentrations of organic compounds such as phenol, p-nitrophenol, 2,4-dinitrophenol, hydroquinone, resorcinol, ascorbic acid, uric acid and 200 fold concentration of inorganic ions such as  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cl}^-$ ,  $\text{I}^-$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{PO}_4^{3-}$  etc with less than 5% deviation. To validate the method, the prepared electrode was used to assess the BPA in 3 spiked samples of PVC food package film, rubber and dropper latex head and excellent recoveries from 94.36% to 101.88% with RSD in the range of 0.50%~2.95% were obtained [21].

Very recently, Ma et al. performed the simultaneous detection of Hydroquinone (HQ), catechol (CC) and resorcinol (RS) based on Gr-CILE successfully. Firstly, the Gr-CILE paste was prepared by inclusion of the commercially available Gr with an IL, octylpyridinium hexafluorophosphate (OPFP) and graphite by simple hand mixing in mortar. The resulting paste was packed into glass tubes firmly and electrical contact was implemented. Another electrode without inclusion of Gr into CILE was also prepared for comparison. In order to study the prepared electrodes response toward simultaneous determination of HQ, CC and RS, CV experiments were carried out in acetate buffer. It was observed that the Bare CILE exhibited only two unobvious and broad anodic peaks denoted to HQ and CC, while no peak was observed for RS. On the other hand, three well defined and sensitive anodic peaks were appeared for Gr-CILE that may be attributed to the high surface area, excellent catalytic activity, and good conductivity of Gr. The proposed method exhibited linear concentrations range from  $1.0 \times$

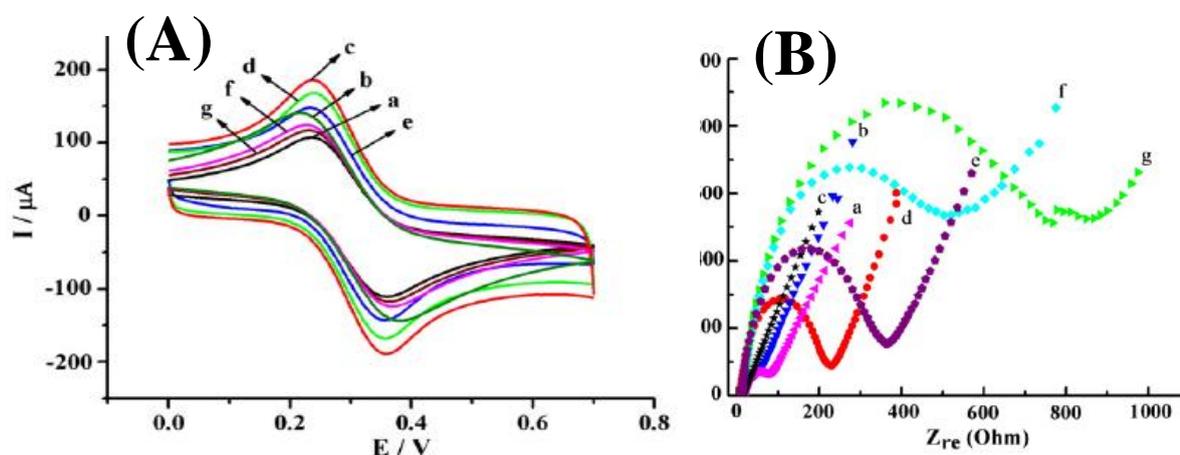
$10^{-5}$  M to  $4.0 \times 10^{-4}$  M for HQ, from  $1.0 \times 10^{-5}$  M to  $3.0 \times 10^{-4}$  for CC and  $1.0 \times 10^{-6}$  M to  $1.7 \times 10^{-4}$  for RS, respectively. The LOD was estimated to be  $1.8 \times 10^{-6}$  M for HQ,  $7.4 \times 10^{-7}$  M for CC, and  $3.6 \times 10^{-7}$  M for RS, respectively. To investigate the analytical applicability of proposed electrodes, local tap water and lake samples were spiked with 10  $\mu$ L of different concentrations of dihydroxybenzenes and excellent recoveries in the range of 98.0% to 106.0% were calculated. Moreover, the as-prepared electrodes were reproducible and remained stable for 15 days [22].

Sun et al. prepared Gr modified CILE for the detection of adenine. The authors fabricated the CILE by hand mixing 1-Butylpyridinium hexafluorophosphate (BPPF<sub>6</sub>) together with graphite and paraffin oil to achieve a homogenous paste and then it was packed into glass tube. The surface of the resulting electrode was modified with Gr followed by CTS to obtain final electrode referred as CTS/GR/CILE. Other modified electrodes such as CTS/CILE or CTS/CNT/CILE were prepared by similar procedure except the utilization of Gr for comparison study. The morphological characterization was carried out by SEM and TEM analysis. The electrochemical characteristics of all the fabricated electrodes were studied via CV and EIS. The oxidation peak current for adenine with CTS/GR/CILE was found to be ~ 5.3 times higher than to the bare CILE which clearly points out that the presence of highly conductive GR on the electrode surface enhances the electrocatalytic activity toward the oxidation of adenine. The EIS results were found to be in agreement of CV results. On the other hand, the oxidation peak of adenine appeared at slightly lower potential with CTS/CNT/CILE compared to CTS/GR/CILEH. However, CTS/CNT/CILE showed poor current response compared to CTS/GR/CILE. The experimental conditions such as buffer pH, scan rate and accumulation conditions were optimized to achieve highest current response. Under optimum conditions, the proposed method exhibited LDR between 10.0 to 70.0  $\mu$ mol L<sup>-1</sup> with LOD value of 0.286 nmol L<sup>-1</sup>. The excellent recoveries from 98.7% to 101.5% of adenine from spiked milk samples were achieved to validate the method applicability successfully [23].

Du et al. published an interesting research for the simultaneous determination of guanine (G) and adenine (A) based on IL-Gr/CPE and electrochemical activities of three kinds of modified carbon electrodes including CILE, Gr/carbon paste electrode (Gr/CPE), and ionic liquid-functionalized Gr (IL-Gr)/CPE, were compared in detail. In order to fabricate the desired electrodes, Hummers method was used to synthesize Gr and followed by Gr functionalization with 1-butyl-3-methylimidazolium hexafluorophosphate (BMIMPF<sub>6</sub>) via an epoxide ring-opening reaction of 3-bromopropylamine hydrobromide. The traditional CPE was prepared by using graphite and paraffin and the resulting homogenous mixture was packed into glass tube. The same procedure was applied to prepare CILE except the inclusion of BMIMPF<sub>6</sub> instead of paraffin. Finally, Gr/CPE and IL-Gr/CPE were prepared by dropping the appropriate amount of Gr and IL functionalized Gr over CPE, respectively. The SEM image and Raman spectrum provided the evidence of successful incorporation of Gr on the electrode surface. Out of all three kinds of electrodes, the electrochemical response of IL-Gr/CPE was highest with dopamine as redox probe. The CVs of simultaneous oxidation of G and A presented higher electrocatalytic behavior of IL-Gr/CPE which may be ascribed to the accelerated electron transfer rate of Gr and enhanced affinity of Gr to G and A. The IL-Gr/CPE exhibited linear concentration range for G from  $2.0 \times 10^{-7}$  to  $1.0 \times 10^{-6}$  M and LOD was calculated to be  $6.5 \times 10^{-8}$  M while linear concentration range for A was from  $2.0 \times 10^{-7}$  to  $1.0 \times 10^{-6}$  M with LOD value of  $3.2 \times 10^{-8}$  M via DPV analysis.

Furthermore, the authors claimed the successful analytical application of IL-Gr/CPE with various mismatched sequences of DNA with outstanding reproducibility and stability of the electrode [24].

Recently, Yu et al. used a complex strategy for AuNPs and RGO functionalized CILE for the fabrication of a novel electrochemical carcinoembryonic antigen (CEA) immunosensor. Firstly, RGO and AuNPs were synthesized and then CILE electrode was prepared by combining graphite powder and 1-Butylpyridinium hexafluorophosphate (BPPF<sub>6</sub>) thoroughly. The resultant homogenous carbon paste was filled into glass tube and Cu wire was used for electrical contact. Later, the RGO was dropped over CILE and dried. It was followed by electropolymerization of L-Arg to obtain a poly (L-Arg) film over it. The poly (L-Arg)/RGO/CILE was immersed into AuNPs solution and AuNPs/poly (L-Arg)/RGO/CILE was obtained. Then, the modified electrode was immersed in the anti-CEA solution for some time and then it was again incubated into BSA solution for blocking the remaining active sites and to avoid the non-specific adsorption. The final modified electrode was referred as anti-CEA/AuNPs/Arg/RGO/CILE.



**Figure 5.** CV of different modified electrodes (A) (a) CILE; (b) RGO/CILE; (c) Arg/RGO/CILE; (d) AuNPs/Arg/RGO/CILE; (e) anti-CEA/AuNPs/Arg/RGO/CILE; in pH 7.0 PBS at 50 mV s<sup>-1</sup>; (B) EIS of different electrodes in 5 mM [Fe(CN)<sub>6</sub>]<sup>3-/4-</sup> solution containing 0.1 M KCl: (a) CILE; (b) RGO/CILE; (c) Arg/RGO/CILE; (d) AuNPs/Arg/RGO/CILE; (e) anti-CEA/AuNPs/Arg/RGO/CILE; (f) BSA/anti-CEA/AuNPs/Arg/RGO/CILE; (g) CEA/BSA/anti-CEA/AuNPs/Arg/RGO/CILE (reproduced with permission from Reference [25]).

The SEM image of the resultant electrode shows the typical wrinkled structure of Gr. The stepwise modifications of electrode were characterized CV and EIS shown in Figure 5 (a) and (b). It can be deduced from the figure that both data are well coincided.

The experimental variables such as influence of incubation time and temperature were optimized. In order to obtain calibration plot, the DPV analysis was carried out for various concentrations of CEA. The response of CEA decreased with the increased concentrations of CEA. The proposed electrochemical immunosensor offered linear oxidation current response in the concentration range from 0.5 to 200 ng mL<sup>-1</sup>. The immunosensor showed selective determination of

CEA when it was incubated in some potential co-existed species with CEA, such as PSA, AFP, HIgG, LDL and BSA, ascorbic acid, dopamine, L-glucose, tryptophan, and tyrosine. The authors reported the successful practical application of the proposed method on six human serum samples and results were compared with ELISA. The proposed method and ELISA showed acceptable RSD from -7.7 % to 6.0 % [25].

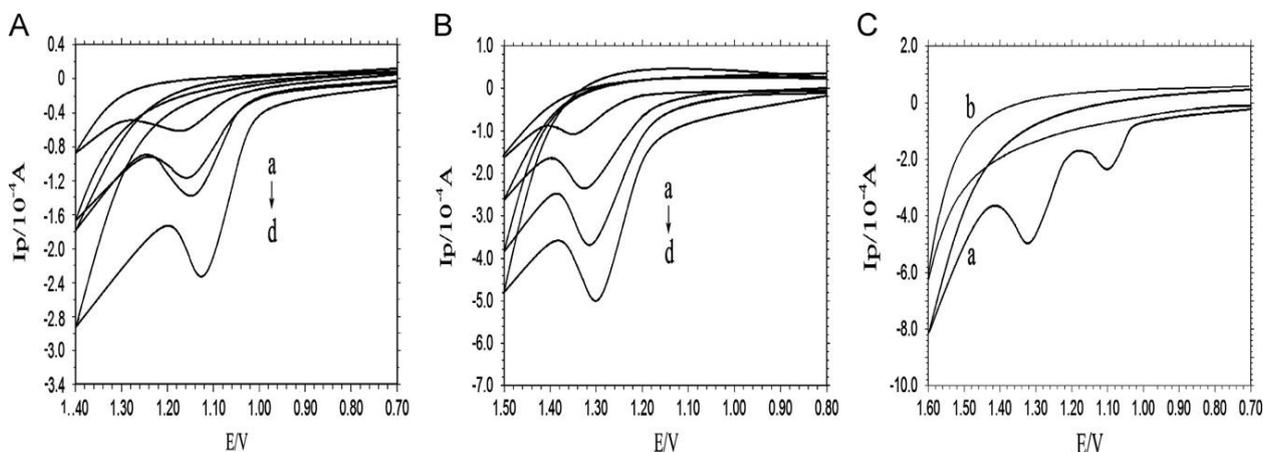
The group of Sun et al., described the fabrication of electrochemically reduced Gr modified CILE for detection of rutin. The CPE was prepared in traditional way while the CILE was prepared by thorough mixing of IL (1-Butylpyridinium hexafluorophosphate, BPPF<sub>6</sub>), graphite powder and liquid paraffin. For the preparation of Gr modified CILE (Gr/CILE), the CILE was dispersed in GO solution followed by application of constant potential -1.3V for 300 s for a stable electrochemical reduction of Gr.

The SEM images were obtained for different electrodes. The SEM images supported the characteristics surface distinction related to each type of electrode. The electrochemical behaviors of all the electrodes were monitored by CV and EIS. It was suggested from the CV results that Gr/CILE offered highest redox peak current response toward rutin. The EIS Nyquist plots also supported the CV results and indicated the lower electron resistance value for Gr/CILE compared to CPE and CILE. The authors noted that the proposed Gr/CILE sensor worked well in two linear concentrations range of rutin from 0.070 to 10.0 μmol/L and 10.0 to 100.0 μmol/L with LOD of 24.0 nmol/L. Besides its excellent reproducibility and stability, the proposed sensor was found to be highly selective against 100 fold concentrations of some common cations, anions, 50 fold concentrations few biologically valuable compounds i.e. glucose, glycine, L-tryptophan L-tyrosine, citric acid, folic acid; and 10-fold of ascorbic acid, sodium dodecylsulfate, human serum albumin, quercetin, morin. Furthermore, the utility of the rutin sensor was demonstrated in human serum and urine samples by standard addition method and acceptable recovery of 96.4% to 98.0% was achieved [26].

The Gao and coworkers explored the electrochemical DNA biosensor for the Detection of Transgenic Soybean Gene Sequence of MON89788 based on Gr, TiO<sub>2</sub> nanorods, and chitosan (CTS) nanocomposite modified CILE.

Initially, CILE was prepared by mixing 1-Butylpyridinium hexafluorophosphate (BPPF<sub>6</sub>) and graphite powder. The resultant paste was filled into glass tubes and Cu wire was inserted from other end for establishing electrical contact. Then, nanocomposite of CTS-Gr-TiO<sub>2</sub> was prepared and casted over CILE to achieve CTS-Gr-TiO<sub>2</sub>/CILE. Similarly other electrodes namely CTS/CILE, CTS-TiO<sub>2</sub>/CILE and CTS-Gr/CILE were also prepared for comparison. The immobilization of probe ssDNA was carried out through electrostatic attraction between probe ssDNA and CTS film while the methylene blue (MB) was used as an indicator during hybridization assay when target ss DNA approached to probe ssDNA. In order to explore the maximum utility of fabricated DNA biosensor, one-base mismatched and three-base mismatched DNA were also detected. The electrochemical behaviors of various prepared electrodes were investigated by using CV and EIS with [Fe(CN)<sub>6</sub>]<sup>3-/4-</sup> as an redox probe. It was found from CV and EIS results that CTS-Gr-TiO<sub>2</sub>/CILE exhibited highest response and lower electron transfer resistance, respectively. The indicator MB also showed increasing current response with increasing nanomaterial modification stages on the electrode. The compositions of nanomaterial, the concentration and accumulation time of MB were tuned to achieve best efficiency

of the sensor. The selectivity of the ssDNA/CTS-Gr-TiO<sub>2</sub>/CILE was performed by using target ss DNA in DPE technique. The target ssDNA sequence could be detected in the range from  $1.0 \times 10^{-12}$  to  $1.0 \times 10^{-6}$  mol/L with a detection limit of  $7.21 \times 10^{-13}$  mol/L. While the probe ssDNA on the electrode surface was hybridized with three-base mismatched sequence and one-base mismatched sequence, the decrease of the reductive peak current was much smaller than that obtained from the complementary ssDNA sequence. The author utilized as-fabricated electrochemical DNA biosensor to the polymerase chain reaction (PCR) product of transgenic soybeans with satisfactory results [27].



**Figure 6.** (A) CVs of  $100 \mu\text{mol L}^{-1}$  guanosine on different electrodes in pH 6.0 B–R buffer solution; (B) CVs of  $200 \mu\text{mol L}^{-1}$  adenosine on different electrodes in pH 5.5 Britton–Robinson buffer solution (electrode from (a) to (d): CILE, CTS-ZrO<sub>2</sub>/CILE, CTS-Gr/CILE, CTS-Gr-ZrO<sub>2</sub>/CILE); (C) CVs of a mixture solution of  $100 \mu\text{mol L}^{-1}$  guanosine and  $200 \mu\text{mol L}^{-1}$  adenosine on CTS-Gr-ZrO<sub>2</sub>/CILE in pH 5.7 Britton–Robinson buffer solution (a) and that of the buffer solution (b) (reproduced with permission from Reference [28]).

Sun et al., reported on the development of Gr-ZrO<sub>2</sub>-CTS modified CILE for the simultaneous determination of guanosine and adenosine. The authors synthesized the reduced Gr and ZrO<sub>2</sub> nanoparticles as reported elsewhere. For the preparation of CILE, appropriate amount of 1-pyridinium hexafluorophosphate (HPPF<sub>6</sub>) and graphite powder were mixed. The resulting paste was filled firmly into glass tube and electrical contact was established. After polishing the surface of CILE, certain amount of pre-dispersed composite solution of Gr-ZrO<sub>2</sub>-CTS was dropped over CILE and dried in the air to achieve Gr-ZrO<sub>2</sub>-CTS/CILE. The SEM images recorded for Gr and Gr-ZrO<sub>2</sub> phases proved the successful synthesis of both the products. The EIS plot was further used to confirm the stepwise modifications of Gr-ZrO<sub>2</sub>-CTS/CILE. To investigate the electrochemical behavior of prepared sensor, firstly, guanosine and adenosine were measured individually followed by simultaneous determination of both the nucleosides successfully. It could be concluded from CV results that Gr-ZnO<sub>2</sub>-CTS/CILE exhibited distinguished ability and sensitive simultaneous determination of guanosine and adenosine compared to CILE, CTS-ZrO<sub>2</sub>/CILE and CTS-GR/CILE as illustrated in Figure 6.

The sensor exhibited LOD value of  $0.117 \mu\text{mol L}^{-1}$  and  $0.144 \mu\text{mol L}^{-1}$  for guanosine and adenosine, respectively. Furthermore, the sensor was reproducible, stable and highly selective against

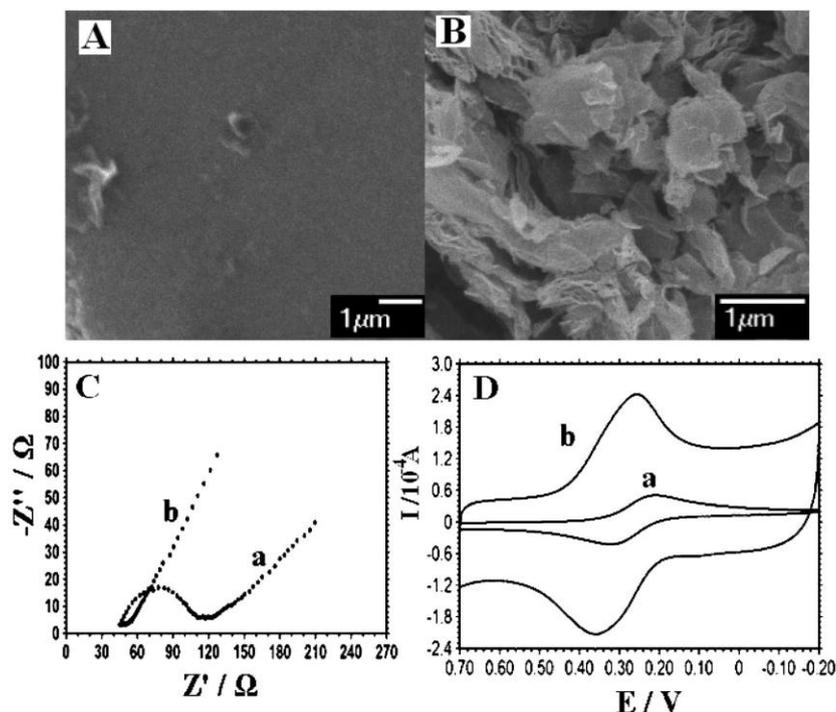
some common existing interferences in biological fluid i.e. adrenaline, dopamine, uric acid and ascorbic acid. The analytical method was validated by spiking the two human urine samples with known concentrations of both nucleosides. The sensor offered good recoveries in the range of 95.56% - 102.91% for guanosine and 96.38% - 105.40% for adenosine [28].

## 5. GRAPHENE MODIFIED CPE

Li and coworkers used a simple method by Gr doped CPE for an excellent electrochemical detection of AA. The unmodified CPE was prepared by hand mixing graphite and paraffin oil in mortar. The resulting mixture was packed into glass tube and Cu wire was used for electrical contact. The Gr-CPE was obtained by same procedure except the inclusion of various amounts of Gr into CPE. The morphology of Gr was studied by using SEM and TEM, while the CV and EIS data were obtained for electrochemical behavior of prepared sensor toward  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  as redox probe. It was found that bare CPE and Gr-CPE showed electrocatalytic activity for AA, however, Gr-CPE demonstrated an improved electrochemical response compared to CPE. The proposed sensor exhibited acceptable reproducibility with RSD of 2.8% and retained its 96.3% response after 28 days of storage. The method was found to be in the linear range of AA concentrations from  $1.0 \times 10^{-7}$  to  $1.06 \times 10^{-4}$  M with a LOD of  $7.0 \times 10^{-8}$  M estimated at  $S/N=3$ . This method showed lower LOD and oxidation overvoltage compared to some other reported sensor for AA. In addition, the proposed sensor was capable to simultaneously discriminate the AA, UA and DA, thus, selective toward AA. The real samples analysis was done by spiking the three known amount of AA into vitamin C injection and excellent recoveries in the range of 95.3% -102.6 % were achieved [29].

Recently, molecular wires (MW) have attracted much attention owing to few potential features such chemical and thermal stability, high conductivity and wide electrochemical windows. Hence, in 2011, Ruan et al. reported on the fabrication of CTS-Gr modified MW-CPE for electrochemical detection for Adenosine-5'-triphosphate (ATP). The authors prepared MW-CPE by simply blending certain amount of graphite and diphenylacetylene (DPA), a smallest conjugated oligomer in mortar. The resulting paste was introduced into glass tube from one end and electrical contact was maintained from other end. After polishing the surface, few drops of CTS-Gr suspension was spread over it and dried at ambient temperature.

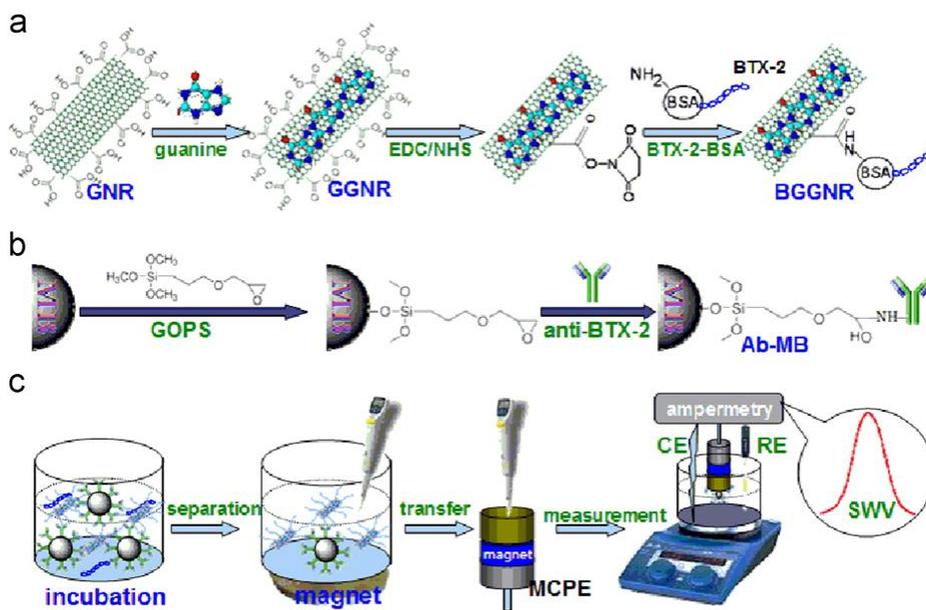
The resulting sensor was referred as CTS-Gr/MW-CPE. The SEM image of MW-CTS in Figure 7 (A) and (B) depicted a smooth surface while CTS-Gr/MW-CPE exhibited non-smooth, crimped and typical sheet like structure of Gr. The EIS and CV were used to investigate the electrochemical characteristics of MW-CPE and CTS-Gr/MW-CPE as shown in Figure 7 (C) and (D), respectively.



**Figure 7.** SEM images of (A) MW-CPE and (B) CTS-Gr/MW-CPE. (C) EIS for (a) MW-CPE and (b) CTS-Gr/MW-CPE in a solution of 10.0 mM  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  and 0.1 M KCl with the frequencies swept from  $10^5$  to  $10^{-1}$  Hz. (D) CVs of (a) MW-CPE and (b) CTS-Gr/MW-CPE in a solution of 1.0 mM  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  and 0.5 M KCl at a scan rate of  $100 \text{ mVs}^{-1}$  (reproduced with permission from Reference [30]).

The CV result clearly indicated that the peak current of the  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  redox probe was much higher at CTS-GR/MW-CPE than that of MW-CPE. This observation was also supported by EIS. It could be deduced from these data that the combined effect of MW and Gr enhanced the electron transfer for CTS-GR/MW-CPE. On the other hand, EIS provided evidence on successful immobilization of Gr over MW-CPE as it showed lower electron transfer resistance. The CV results offered better current response with CTS-GR/MW-CPE compared to MW-CPE for ATP. It was found that increasing the pH buffer decreased the overpotential. Furthermore, scan rate, effect of accumulation potential and time for ATP were also tuned to get maximum efficiency of the sensor. The proposed sensor worked well in two linear concentrations range from 1.0 nM to 700.0 mM and from 200.0 mM to 700.0 mM. The LOD was estimated to be 0.342 nM. The author noted the good reproducibility, stability and high selectivity of the sensor. Finally, two real injection samples were assessed for the determination of ATP with acceptable recoveries [30].

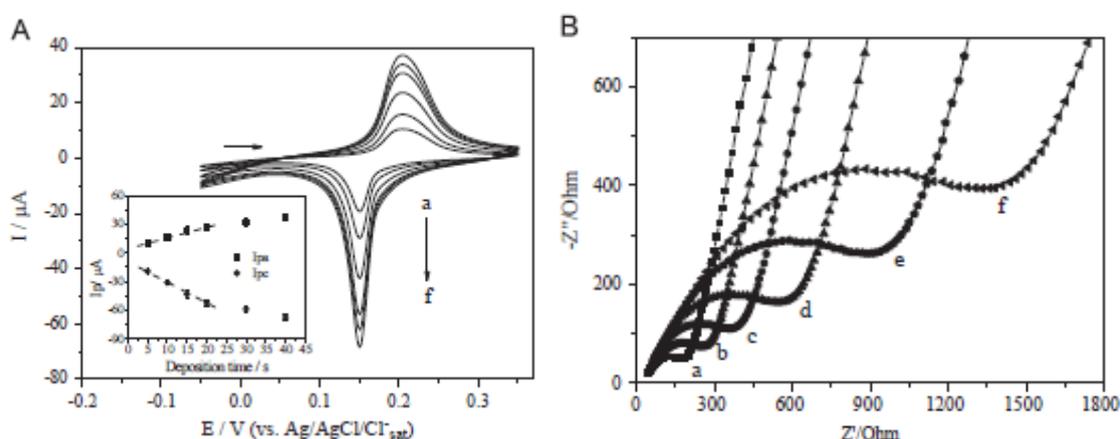
The Tang and coworkers proposed a facile magneto-controlled electrochemical immunoassay for the detection of brevetoxin B (BTX-2) in seafood based on guanine-functionalized Gr nanoribbons (GGNRs). The schematic diagram of sensor fabrication has been shown in Figure 8. The successful preparation of GGNRs was confirmed by TEM and UV-vis absorption. After optimization of various experimental parameters, the proposed electrochemical immunosensor showed dynamic concentration range spanned from  $1.0 \text{ pgmL}^{-1}$  to  $10.0 \text{ ngmL}^{-1}$  with a LOD of  $1.0 \text{ pgmL}^{-1}$  BTX-2 [31].



**Figure 8.** Fabrication scheme of electrode (a) guanine functionalized Gr nanoribbons conjugated with BTX-2-BSA and (b) anti-BTX-2 antibody functionalized magnetic beads, and (c) competitive-type immunoassay protocol and measurement method; RE: reference electrode, CE: auxiliary electrode (reproduced with permission from Reference [31]).

Wonsawat and coworkers described the development of in-situ Bismuth (Bi)-modified Gr-CPE (Bi-Gr-CPE) for the determination of  $\text{Cd}^{+2}$  and  $\text{Pb}^{+2}$  ions in flow based system. For desired sensor, initially, commercially available Gr powder was well dispersed in isopropanol. Then, graphite powder was added into well dispersed Gr powder and dried to achieve Gr-graphite powder. Gr-CPE was prepared by mixing the Graphite powder with varying proportions of Gr-graphite powder. The resulting homogenous paste was packed firmly in electrode-holder and a Cu wire was inserted for electrical contact. The CPE was made with same procedure but except Gr. Finally, Bi-Gr-CPEs and Bi-CPEs were obtained by in-situ plating of a Bi film from a  $\text{Bi}^{3+}$  plating solution. CV and square wave anodic stripping voltammetry (SWASV) coupled with sequential injection analysis (SIA) were employed to study the electrochemical behavior of the electrodes. Various experimental parameters such as Gr and graphite mass ratio,  $\text{Bi}^{3+}$  concentration in the plating solution, HCl concentration in the electrolyte and SW parameters (i.e. deposition potential, SW frequency, and step potential) were optimized to investigate the influence on electrode. The electrochemical detection of  $\text{Cd}^{+2}$  and  $\text{Pb}^{+2}$  ions were carried out using CV. The anodic peak currents of both metal ions exhibited distinct peaks, with the highest current response being observed with the Bi-Gr-CPE followed by Bi-CPE, Gr-CPE and CPE. It was suggested that the high electron transfer rate between analytes and electrode surface due to the presence of Gr could enhance the sensitivity. Furthermore, the formation of Bi layer over Gr-CPE appeared to be more sensitive. The proposed sensor showed linear concentrations range between  $0.1\text{-}50.0 \mu\text{g L}^{-1}$  for both metal ions with LOD of  $0.07$  and  $0.04 \mu\text{g L}^{-1}$  for  $\text{Cd}^{+2}$  and  $\text{Pb}^{+2}$ . The Bi-Gr-CPE sensor was also explored for real sample analysis in a non-complicated (tap water) or complicated (sea bass fish and undulated surf clam tissues) matrix and recoveries were ranged from  $70.4\%$  to  $120\%$  for  $\text{Cd}^{+2}$  and  $65.8\%$  to  $113.5\%$  for  $\text{Pb}^{+2}$  [32].

Very recently, the electrochemical detection of captopril (CPT) and Hydrochlorothiazide (HCT) was performed on a Gr/Ferrocene (Fc) Composite CPE (Gr/Fc/CPE) by Gholivand et al. For the preparation of Gr/Fc/CPE sensor, graphite, Gr and Fc were mixed thoroughly in ethanol followed by addition of paraffin oil to obtain a homogenous paste. The resulting paste was filled into Teflon tube and electrical contact was made from opposite end with Cu wire. For comparison purpose, two other electrodes i.e. Gr/CPE and Fc/CPE were also prepared with same procedure but without adding Fc and Gr, respectively. The electrochemical behaviors of CPT and HCT were individually and simultaneously investigated at the surface of the Gr/Fc/CPE, Gr/CPE and Fc/CPE. The author noted that the all three electrodes provided current responses, however, the highest peak current values were achieved with Gr/Fc/CPE for both analytes suggesting the enhanced kinetics of the electron transfer process and sensitivity due to the presence Gr and electron mediator (Fc) in CV.



**Figure 9.** (A) CVs of electrodeposited Prussian blue films on GPE using an electrodeposition potential of 0.4V during (a) 5, (b) 10, (c) 15, (d) 20, (e) 30 and (f) 40 s in 0.1 mol L<sup>-1</sup> KCl and 0.1 mol L<sup>-1</sup> HCl at 40 mVs<sup>-1</sup>. (B) Nyquist plots of electrodeposited Prussian blue films on GPE during (a) 5, (b) 10, (c) 15, (d) 20, (e) 30 and (f) 40s, for a frequency range of 10<sup>5</sup>–10<sup>-1</sup> Hz. Experimental conditions: 0.04 mol L<sup>-1</sup> Britton–Robinson buffer (pH5) 4.75×10<sup>-5</sup> mol L<sup>-1</sup> 4-AMP as redox mediator, and 0.2 V of conditioning potential (reproduced with permission from Reference [34]).

The DPV was applied for the simultaneous determination of analytes and analytical plots were found to be linear in the concentration ranges of 1.0–430 μM and 0.5–390 μM for CPT and HCT, respectively. The LOD of CPT and HCT were found to be 0.87 and 0.38 μM, respectively. The proposed method was validated for the determination of CPT and HCT in pharmaceutical preparations (CAPOZIDE) and human urine samples with acceptable recoveries (n=5) between 94%–97% and 92%–96% for CPT and HCT, respectively, with RSD less than 7% [33].

Recently, Oliveira and coworkers reported on a novel method of an enzymatic biosensor based on Laccase on Gr-CPE functionalized with Prussian blue film (LACC/PB/Gr-CPE) for carbamate pesticides quantification. Initially, for the preparation of desired biosensors, CPE was made in traditional way followed by addition of Gr in varying amounts. Then, PB film was electrodeposited

under certain conditions and finally LACC was dropped over its surface and dried at room temperature to obtain LACC/PB/Gr-CPE.

The compositions of electrode were optimized by using full-factorial central composite design (CCD) and RSM. The Gr was characterized by TEM. Figure 9 showed the electrochemical behavior of proposed biosensor using CV and corresponding EIS technique results. It can be seen that highest response was achieved with LACC/PB/Gr-CPE that may be attributed to the synergic effect of LACC, PB and Gr. The proposed biosensor was utilized for quantification of five carbamates widely applied on tomato and potato crops and acceptable LDR with less dispersion of data at low concentrations. This method offered excellent reproducibility in the range of 4.1- 6.3% RSD, intra- and inter-day repeatability from 1.8 to 3.8% RSD and accuracy and stability for approximately 20 days [34].

## 6. CONCLUSION

This review provided detailed account on the utilization of Gr in various types of paste electrodes. It has been shown that pure GPE offered higher sensitivities toward analytes. On the other hand, the modification of paste electrode with nanocomposite of graphene with various types of IL and CTS exhibited even higher LOD and LDR. Few reports have been appeared on the development of graphene modified traditional CPE. The resulting electrodes performed better than to the bare CPE. Therefore, it can be concluded that graphene has made its niche and shown potential in the field of electrochemical methods. It can be predicted that being an outstanding material, graphene will play important role in various aspects of catalysis, chemical and biosensors and drug delivery applications. Furthermore, there is still room for better understanding for the scientific research and application development of graphene-based theory, materials, and devices.

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