CHAPTER 5

Functionalized Graphene Oxide as an Electrochemical Sensing Platform for Detection of Bisphenol A
Bisphenol A, chemically known as (2,2-bis(4-hydroxyphenyl)propane) is an important industrial chemical owing to its popular uses in widespread fields such as an additive in the manufacture of polycarbonate plastics, epoxy resins, as dental sealants, water bottles and baby feeding bottles etc. to name a few. This molecule has recently raised much concern among the scientific community which is ascribable to its potential toxic implications in human animal and aquatic life and also in issues pertaining to the environment. Due to the extensive use of this chemical in consumer products, high levels of BPA are regularly being leached into the environment which is commonly caused by incomplete reaction on exposure to heat or acidic and basic conditions accelerating the hydrolysis of the ester bonds linking BPA monomers from plastic food packaging into water or food, with greatest concentrations expected in surface and industrial waste water. BPA is also being listed as an Endocrine Disrupting Chemical (EDC) as it is known to imitate the biological activity of naturally occurring hormones like estrogen with higher concentrations in the blood stream causing reproductive dysfunction, endometrial hyperplasia, recurrent miscarriages, abnormal karyotypes, and polycystic ovarian syndrome, etc. in both man and animals along with increasing the risk of cancer. Further, leaching of Bisphenol A from baby feeding bottles have led to negative health effects in infants. Owing to the negative effects of Bisphenol A, it has posed recent concern among the scientific community to design and develop easy, clean, cost-effective, selective and sensitive materials and methods for easy detection of this hazardous pollutant.
Conventional methods for detection of Bisphenol A involve chromatographic techniques such as Gas Chromatography or HPLC coupled with Mass Spectroscopy or capillary electrophoresis, solid-phase microextraction, etc. These analytical methods suffer from the drawbacks of being expensive and needs complicated sample pretreatment in addition to not being suitable for on-site operation. Therefore, search for easy and effective ways which are fast and allow easy detection of BPA at a lower cost with high sensitivity is in great demand. In this regard electrochemical techniques can prove to be a useful alternative to the existing traditional methods. It has a host of advantages including rapid response, low energy consumption, on-site applicability, and as a clean and eco-friendly method. The detection of BPA via electrochemical methods using different modifications of the glassy carbon electrode has already been reported in the literature. For instance, Apodaca et al. investigated electrochemical sensing of BPA via electrochemical impedance spectroscopy (EIS) measurements using an electropolymerized molecularly imprinted polymer film composed of varying ratios of BPA to terthiophene and carbazole monomer complex deposited onto indium tin oxide (ITO) substrates via anodic electropolymerization using cyclic voltammetry (CV). Li et al. reported carboxylated multi-walled nanotube modified Glassy Carbon Electrode (GCE) for BPA detection. Ndlovu et al. demonstrated a cost-effective bare exfoliated graphite electrode for electrochemical detection of BPA.

5.1 The Motivation

It has been observed that a number of reports are available for detection of BPA in solution using various analytical techniques ranging from electrochemical to
spectroscopic and employing a variety of materials like CNT, various polymers employing different metals and metal oxides like Pt, Au, SiO₂, Al₂O₃ etc. Contemporarily, it is quite evident that the reported systems very often make use of certain toxic organic and inorganic compounds such as azobenzene and its derivatives, thiophenes, carbazoles, silanes etc which have a negative impact on the environment. Therefore, clean and effective analytical techniques employing materials that are biocompatible but with the same or improved sensitivity towards detection of BPA has been in demand lately. Graphene, a zero band-gap semiconductor material comprising of a two-dimensional hexagonal honeycomb network of sp² hybridised carbon atoms and often termed as a ‘wonder material’ has been in limelight recently on account of its interesting properties like high conductivity, good biocompatibility, high adsorptivity, electronic property, and potential applications in electrochemical sensors. Furthermore, due to high electron transport mobility graphene can act as electron transfer bridges making them suitable for electrocatalytic applications. On the other hand graphene functionalized with different surface functional moieties, make them useful non-toxic materials with a large surface area thereby facilitating adsorption of biomolecules which is favourable for designing sensors with improved sensitivity. Furthermore, graphene is free from catalytic impurities such as Fe, Ni etc. present in carbon nanotubes thereby limiting the probable interference caused by these metals and hence improving the reproducibility of results.

In the present work, we demonstrate the electrochemical detection of Bisphenol A in solution by three different types of functionalized graphene viz. graphene oxide (GO), ester functionalized graphene oxide (GO-ES) and amine functionalized
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graphene oxide (GO-en) modified glassy carbon electrode (GCE) using a very simple drop casting method without the use of any toxic organic compounds or polymeric binders, based purely on cast and dry strategy under ambient conditions. The system developed showed detection of BPA via a reduction in the oxidation peak current value associated with a significant shift in the peak potential value. Further, the electrochemical sensing materials developed showed good sensitivity compared to already reported systems and furthermore high selectivity in presence of other structurally similar kinds of molecules in solution thereby demonstrating the practical applicability of the material and the technique developed. However, the detection mechanism is not proposed to be based on the oxidation of BPA at the electrode which is contrary to earlier reported systems for electrochemical detection of BPA. A plausible mechanism to justify the detection process is also being discussed.

5.2 Materials

Commercially available graphene platelet nanopowder (GPN Type 1, thickness 6–8 nm) (Sisco Research Laboratories Pvt. Ltd.), ethylene diamine (≥98%) (Merck), concentrated sulfuric acid H₂SO₄ (98%) (Merck), concentrated nitric acid HNO₃ (70%) (Merck), 4-nitrotoluene (Merck), m-nitrophenol (Merck), 1-amino-2-naphthol-4-sulphonic acid (Merck), potassium ferricyanide K₃[Fe(CN)₆] (Merck), potassium chloride KCl (Merck), poly(ethylene glycol) (Sigma Aldrich) and 2,2’-dithiobenzoic acid(Sigma Aldrich) were used as received without further purification. The water used throughout the experiments was from a Milli-Q water purification system.
5.3 Experimental

5.3.1 Synthesis of graphene with different surface functionalities

Functionalized graphene samples were prepared using different chemical reactions to incorporate various surface functionalities on to the graphene surface. The pictorial representation of the reaction processes is demonstrated in Scheme 5.1.

**Scheme 5.1** Schematic representation of the synthesis of functionalized graphene samples using various chemical reactions.

(a) Synthesis of graphene oxide (GO) with carboxylic acid (-COOH), hydroxyl (-OH) and epoxy (-O-) surface functional groups

Graphene platelet nanopowder was exfoliated and oxidized in a one-step process employing an acid mixture oxidant i.e., a 3:1 mixture of concentrated H$_2$SO$_4$/HNO$_3$ using a simple bath sonication and centrifugation technique at room temperature.\textsuperscript{77} The reactive species in the process responsible for the oxidation was nitronium ion (NO$_2^+$).\textsuperscript{212}

\[
H_2SO_4 + HNO_3 = HSO_4^- + NO_2^+ + H_2O
\]

Briefly, a 50 mL dispersion of graphene platelet nanopowder was prepared by dispersing 0.01 g of graphene platelet nanopowder in a 3:1 acid mixture of
concentrated $\text{H}_2\text{SO}_4$ (98 wt%):$\text{HNO}_3$ (70%). This dispersion was then subjected to bath sonication for a time period of 24 hours in a water bath. After sonication the dispersion was diluted by adding 100 mL of deionized water and centrifuged at 8000 rpm for 30 minutes to separate the exfoliated graphene from the acid mixture. The supernatant solution containing the acid mixture was then separated from the heavier particles and this process of centrifugation and washing was repeated until all the acid content was washed away. Finally the exfoliated graphene part was redispersed in deionized water and this dispersion was termed as the graphene oxide (GO) solution.

(b) *Synthesis of ester functionalized graphene oxide (GO-ES) with ester (-COOR) and hydroxyl (-OH) surface functional groups*

Graphene oxide (GO) prepared from the above step was then subsequently functionalized via incorporation of ester (-COOR) functional groups using covalent bonding interactions according to a method previously reported by our laboratory. In a typical procedure, the aqueous dispersion of GO was mixed with poly(ethylene glycol) (PEG) in a 1:2 ratio (5 mL GO dispersion + 10 mL PEG), and to this mixture, approximately 40 μL of concentrated sulphuric acid was added as the catalyst. The solution was subsequently heated at 120°C under reflux for 90 minutes with continuous stirring. The resultant dispersion obtained was the esterified GO solution, termed as GO-ES. In this case, the fruity smell of the product was used as a preliminary indication of the formation of ester-functionalized GO.

(c) *Synthesis of amine/amide functionalized graphene oxide (GO-en) with amine (-NHR), amide (-CONHR) and hydroxyl (-OH) surface functional groups*
Graphene oxide sample prepared was reduced and functionalized in a single step via incorporation of amine and amide functionalities on the surface using ethylene diamine (en) in accordance with our previous reports.\textsuperscript{79} For the reduction and functionalization, typically, 10 mL of the as-prepared GO dispersion was taken in a round bottom flask and to it 15 mL of 33\% ethylene diamine solution was added dropwise with continuous stirring. The pH of the reaction mixture was adjusted at around 10 and the dispersion was refluxed for 8 hours at 80\degree C. The mechanism of reduction and functionalization of GO by ethylene diamine via ring opening of epoxy groups had already been reported earlier by us and other groups.\textsuperscript{79,213} The proposed mechanism for the reduction of GO by ethylene diamine is known to occur via reaction of diamine with epoxy groups by a ring opening addition reaction; the driving force for the reaction being the strain on the three-membered epoxy ring which always has a tendency to open up. Further, some of the amine groups would also react with the carboxylic acid groups on the GO surface to form covalent amide linkages thereby leading to covalent functionalization. The GO dispersion reduced and functionalized with ethylene diamine was termed as GO-en.

5.3.2 Preparation of functionalized graphene-modified Glassy Carbon Electrode (GCE)

The functionalized graphene modified GCE was prepared by a simple drop casting method. Firstly, the surface of GCE was mechanically polished using alumina powder of diameter 0.05 \( \mu \)m and rinsed ultrasonically with Milli-Q water and ethanol respectively. The electrode was then completely dried at room temperature prior to use. Approximately 1 mg of graphene oxide, ester functionalized graphene oxide and
amine functionalized graphene oxide were dispersed in 1 mL of distilled water and ultrasonicated for 30 minutes for proper dispersibility. About 5 μL of the respective functionalized graphene dispersions was then cast on the surface of GCE and dried at 60°C in a hot air oven to form a uniform film on the surface.

5.3.3 Electrochemical detection of Bisphenol A (BPA) by the functionalized graphene modified GCE

Cyclic voltammetry (CV) was performed to demonstrate the electrochemical sensing of BPA by the functionalized graphene modified GCE. All measurements were done using 10 mL of a 1:1 mixture of 2 mM potassium ferricyanide K$_3$[Fe(CN)$_6$] and 0.1 M potassium chloride KCl as the supporting electrolyte. The operating potential ranges were found to be different for different functionalized graphene-modified electrodes. In all cases the cyclic voltammograms were recorded at a scan rate of 0.05 mV/s. The three-electrode system viz. the working electrode (the functionalized graphene modified GCE), the reference electrode (Ag/AgCl) and the counter electrode (Pt wire) were dipped in the electrolyte solution and all connections were made accordingly. A specific amount of the analyte i.e. Bisphenol A solution in ethanol was added to the electrolyte mixture at regular intervals and the corresponding CV graph was recorded.

5.4 Characterization

UV-visible absorption spectra of the prepared samples were collected on a Shimadzu UV-VIS Spectrophotometer, UV-2600; zeta potential measurements were carried out on a Malvern Zetasizer Nanoseries, Nano-ZS90. Scanning Electron Microscope (SEM) images were collected on a Carl Zeiss Sigma VP instrument. Powder X-ray diffraction (XRD) spectrum was collected on a Bruker D8 Advance diffractometer.
The presence of different functionalities on the prepared samples was confirmed using Fourier Transform Infrared Spectra collected on a Nicolet-6700 FT-IR Spectrophotometer. Cyclic Voltammetry (CV) experiments for electrochemical detection of Bisphenol A were performed on a CH instrument Electrochemical Workstation using the conventional three-electrode system viz. a platinum wire auxiliary electrode, an Ag/AgCl electrode as the reference electrode and the bare or functionalized graphene modified glassy carbon electrode (GCE).

5.5 Results and Discussion

5.5.1 Physical characterization of the functionalized graphene samples prepared

The functionalized graphene samples prepared were characterized in a stepwise and systematic manner using UV-Visible absorption spectroscopy, zeta potential measurements, Scanning Electron Microscopy, Powder XRD and Fourier Transform Infrared (FT-IR) Spectroscopy.

(a) UV-Visible Spectroscopy

Graphene oxide (GO), ester functionalized graphene oxide (GO-ES) and amine/amide functionalized graphene oxide (GO-en) samples were characterized using UV-Visible spectroscopy. The corresponding stacked UV-Visible absorption spectra of the samples are shown in Figure 5.1.
The UV-Visible absorption spectrum of Graphene oxide (GO) showed two distinct absorption peaks viz. at 201 nm and 280 nm which could be ascribed respectively to π–π* transition of the C=C bond and to n–π* transition of the –COO− groups arising due to oxidation of graphene. The presence of these characteristic absorption peaks indicated the successful oxidation of graphene nanoplatelets to graphene oxide. On the other hand, the ester functionalized graphene (GO-ES) showed an absorption peak at 272 nm corresponding to n–π* transition of the ester (–COOR) group. This indicated the successful incorporation of ester functional groups into the graphene surface. Further, after reduction cum functionalization of graphene oxide with ethylene diamine, the absorption peak of GO corresponding to n–π* transition of the –COO− groups arising due to oxidation of graphene was found to disappear indicating a successful reduction of –COOH groups with a steep increase in absorption around 200 nm. This increase might however be ascribed to n–σ* transitions of the –OH and –NH₂ groups on the surface of GO-en. Therefore, UV-Visible spectroscopy provided convincing evidence supporting the successful incorporation of different functional groups on to the graphene surface.

(b) Scanning Electron Microscopy and Zeta Potential Measurements
Evidence for successful synthesis of the functionalized graphene samples could be drawn from Scanning Electron Microscopy and Zeta Potential Measurements. Figure 5.2 (A)-(C) shows the SEM micrographs of GO, GO-ES and GO-en respectively. All the images showed the planar sheet-like structure of the exfoliated graphene functionalized with different surface functional groups. Figure 5.2 (D)-(F) shows the zeta potential distribution graphs of the samples GO, GO-ES and GO-en respectively. GO was found to exhibit a zeta potential value of -33.5 mV thereby showing the negative potential on the surface of GO. After esterification of GO to GO-ES, the value of zeta potential was found to be reduced to -27.8 mV indicating a positive change in the surface charge from more negative to less negative upon esterification. This observation was quite justifiable based on the fact that the –COOH group bearing GO became less negative after esterification to form –COOR (R= alkyl chain). On the other hand, reduction cum amination of GO using ethylene diamine caused an increase in the zeta potential value of GO from -33.5 mV to -45.3 mV. This observation could be ascribed to the increased electron density upon formation of –OH groups via ring opening of epoxy groups and formation of –NHCH₂–CH₂–NH₂ linkages. Further, upon reduction with en, the C=C structure was somewhat restored leading to an increase in the overall negative charge. Therefore, zeta potential measurements provided strong and convincing evidence supporting the successful functionalization of graphene samples bearing different surface functionalities.

(c) FT-IR and Powder XRD Analysis
All the three samples of functionalized graphene prepared were characterized by FTIR spectroscopy for inquiring the successful incorporation of the respective functional groups. Figure 5.3 (A) shows the stacked FTIR spectra of GO, GO-ES and GO-en. The stretching frequencies observed for GO appeared at 3483(-O-H stretch), 2961(-C-H stretch), 1708(C=O carboxylic acids), 1384, 1159(C-O stretch alcohols), 1081(=C-H bend) and 883(O-H bend carboxylic acid). GO-ES showed the characteristic peaks at 3401(–O-H stretch), 2881(C-H stretch alkanes), 1642(C==O ester), 1458(C-H bend alkanes), 1352(C-O stretch alcohols) and 1118 cm\(^{-1}\)(=C-H bend). The stretching frequencies for GO-en were found to appear at 3400(–O-H stretch), 2984(-C-H stretch), 1587(N-H bend \(^1\)\(^\circ\) amines), 1385(C-O stretch alcohols), 1050(C-O stretch alcohols) and 811 cm\(^{-1}\) (C-H rock). Thus from FTIR spectroscopy it could be very well established that the graphene samples had been successfully functionalized by different functional moieties.

**Figure 5.3** Stacked (A) FT-IR spectra and (B) Powder XRD spectra of functionalized graphene samples prepared viz. GO, GO-ES and GO-en.
The functionalized graphene samples prepared were also subjected to powder X-ray diffraction analysis and the corresponding stacked diffractograms are shown in Figure 5.3 (B). It was very clear from the graph that the diffractograms for all the functionalized graphene samples viz. GO, GO-ES and GO-en showed a very sharp and well-defined diffraction peak at $2\theta \approx 25^\circ$ (002) corresponding to the disorderely stacked graphene sheets. Further, the sharpness of the peaks observed clearly indicated the crystalline nature of the graphene samples.

5.5.2 Electrochemical Characterization of Functionalized Graphene Modified Glassy Carbon Electrode

The electrochemical behavior of functionalized graphene modified glassy carbon electrode was investigated using potassium ferricyanide as the redox marker via cyclic voltammetry (CV) measurements. All measurements were carried out using 10 mL of a 1:1 mixture of 2 mM potassium ferricyanide $K_3[Fe(CN)_6]$ and 0.1 M potassium chloride $KCl$ as the supporting electrolyte. The operating potential ranges were found to be different for different functionalized graphene-modified electrodes. In all cases, the cyclic voltammograms were recorded at a scan rate of 0.05 mV/s. Figure 5.4 shows the stacked cyclic voltammograms of bare and different functionalized graphene modified GCE. From the plot it was quite clear that all of the functionalized graphene samples
exhibited a well-defined oxidation/reduction peak when compared to the bare GCE in the potential window of -0.2-0.6 V. The CV graph of GO showed a well-defined oxidation peak at about 0.28 V. From the plot it was very clear that the electron transfer process was an irreversible one. On the other hand, the voltammogram of esterified graphene i.e., GO-ES showed a well-defined reduction peak at a potential value of 0.109 V. In this case too, the electron transfer was found to be an irreversible one although in the reverse order as compared with that of GO. For ethylene diamine reduced and functionalized graphene, i.e., GO-en, two distinct oxidation-reduction peaks were found to appear at 0.24 V and 0.15 V respectively giving rise to an approximately symmetrical CV graph thereby indicating a reversible electron transfer process. It was to be noted that in all the cases the oxidation/reduction peak currents were found to be higher than that of the bare GCE indicating that the prepared graphene samples were successfully deposited on the surface of GCE and thereby exhibited higher conductivity as compared to the bare GCE.

5.5.3 Electrochemical Detection of Bisphenol A by the Functionalized Graphene Modified Glassy Carbon Electrode

After careful investigation of the electrochemical response of different functionalized graphene modified GCE, we subsequently carried out the CV experiments in a similar manner in presence of the environmentally hazardous analyte Bisphenol A. All measurements were carried out as before using 10 mL of a 1:1 mixture of 2 mM potassium ferricyanide $K_3[Fe(CN)_6]$ and 0.1 M potassium chloride KCl as the supporting electrolyte. Different concentrations of the analyte i.e. Bisphenol A solution in ethanol was added to the electrolyte mixture at regular intervals and the corresponding CV graph was recorded at a scan rate of 0.05 mV/s. Figure 5.5 shows
the electrochemical response of various functionalized graphene modified GCE both in the presence and absence of Bisphenol A in solution.

Figure 5.5 Stacked CV graphs showing the electrochemical response towards the presence of Bisphenol A for (A) GO, (B) GO-ES and (C) GO-en modified GCE. Calibration curves (Current vs Concentration of BPA) showing the linear response of (D) GO, (E) GO-ES and (F) GO-en modified GCE towards the presence of Bisphenol A in solution.

Figure 5.5 (A) shows the stacked cyclic voltammograms of GO-modified GCE in both the presence and absence of BPA. The CV graph of GO showed a well-defined oxidation peak at about 0.28 V. From the plot it was very clear that the electron transfer process for GO-GCE was an irreversible one. However, in presence of BPA, the oxidation peak current was found to be reduced as evident from the CV graph of GO-GCE in presence of BPA. Figure 5.5 (D) shows the calibration curve of the oxidation peak current against various concentrations of BPA added. From the plot it was evident that the reduction in the oxidation current showed a good linear response with a linear regression constant value of $R^2 = 0.8202$. Figure 5.5 (B) shows the stacked cyclic voltammograms of GO-modified GCE both in the presence and absence of BPA. The voltammogram of esterified graphene modified GCE i.e., GO-
ES-GCE showed a well-defined reduction peak at a potential value of 0.109 V. In this case the reduction peak current was found to appear at a potential value of -0.044 V in the presence of BPA with an increase in the value of reduction peak current and a marked shift in the peak maxima of 0.065 V. Figure 5.5 (E) shows the calibration curve of the oxidation peak current against various concentrations of BPA added for GO-ES-GCE. From the plot it was evident that the reduction in the oxidation current showed a good linear response with a linear regression constant value of $R^2 = 0.8427$. Similarly, for ethylene diamine reduced and functionalized graphene modified GCE, i.e., GO-en-GCE, two distinct oxidation-reduction peaks were found to appear at 0.24 V and 0.15 V respectively. In this case too there was an observed decrease in the oxidation/reduction peak currents in the presence of BPA as the analyte as evident from Figure 5.5(C). Figure 5.5 (F) shows the calibration curve of the oxidation peak current against various concentrations of BPA added for GO-en-GCE. From the plot it was evident that the decrease in the oxidation/reduction current values showed a good linear response with a linear regression constant value of $R^2 = 0.8035$. Thus from the plots it was quite evident that in all of the functionalized graphene modified GCEs there was an observed change in the redox behavior of the native material in presence of Bisphenol A.

**Figure 5.6.** The complete cyclic voltammogram profile showing the electrochemical response of (A) GO, (B) GO-ES and (C) GO-en towards detection of Bisphenol A in solution.
The complete profile showing the electrochemical response of GO, GO-ES and GO-en towards Bisphenol A in the concentration range $1.99 \times 10^{-7} - 11.15 \times 10^{-8}$ M is shown in Figure 5.6 (A), (B) and (C) respectively. From the plot 5.6 (A) it was quite evident that there was a reduction in the oxidation peak current of GO-GCE in the presence of BPA in the concentration range $2 \times 10^{-7} - 9.09 \times 10^{-8}$ M along with a significant shift in the oxidation potential towards a lesser value. Similarly, the complete profile showing the electrochemical response of GO-ES towards Bisphenol A in the concentration range $2 \times 10^{-7} - 11.16 \times 10^{-8}$ M is shown in Figure 5.6 (B). From the plot 5.6 (B) it was quite evident that there was a reduction in the oxidation peak current of GO-ES-GCE in the presence of BPA in the concentration range $2 \times 10^{-7} - 11.16 \times 10^{-8}$ M along with a significant shift in the oxidation potential towards a lesser value. Further, the complete profile showing the electrochemical response of GO-en towards Bisphenol A in the concentration range $2 \times 10^{-7} - 11.16 \times 10^{-8}$ M is shown in Figure 5.6 (C). From the plot 5.6 (C) it was quite evident that there was a marked reduction in the oxidation peak current of GO-en-GCE in the presence of BPA in the concentration range $2 \times 10^{-7} - 11.16 \times 10^{-8}$ M along with a significant shift in the oxidation potential towards a lesser positive value.

5.5.4 Mechanistic Insight

As evident from the cyclic voltammograms of different functionalized graphene modified GCEs in presence of BPA, there was an observed decrease in the oxidation peak current of the native graphene materials along with a significant shift in the oxidation peak potential. Although most of the earlier reports on electrochemical detection of BPA by modified GCEs were based primarily on the oxidation of BPA, the system we report herein do not claim the oxidation of BPA at the
electrode. Rather it could probably be demonstrated as a simple detection of BPA in solution with the detection process being driven by the $\pi$-$\pi$ attraction between the functionalized graphene modified surface of the GCE and the BPA molecules in solution as demonstrated in Scheme 5.2.

**Scheme 5.2** Schematic representation of the electrochemical sensing of BPA in solution as a result of $\pi$-$\pi$ stacking of BPA molecules on the functionalized graphene modified GCEs.

Further, it was to be noted that the $\pi$-stacking of BPA molecules on the graphene modified GCE did not lead to an increase in the oxidation current and rather showed a decrease which is contrary to available literature reports.\textsuperscript{217} This contrasting observation could be attributed to the fact that the functionalized graphene material used viz. GO, GO-ES or GO-en was composed primarily of a sp$^3$ C-framework with small sp$^2$ domains in the sheet structure. $\Pi$-stacking of BPA molecules occurred specifically at those domains and these sp$^2$ regions being discrete, the resulting current increase was not observed. However, they served as the driving force to draw the BPA molecules to the electrode surface as was evident from the reduced oxidation peak current of the graphene samples along with a marked shift in the oxidation
potential from more to less negative side thereby leading to electrochemical detection of BPA in solution by the functionalized graphene modified GCEs.

5.5.5 Selectivity of the functionalized graphene based electrode material developed towards electrochemical detection of Bisphenol A

For a material to be applicable as a sensor it must exhibit good selectivity towards the detection of the specific analyte concerned. Therefore, keeping this point in view, we carried out the electrochemical sensing of a few other analytes structurally similar to BPA viz. 2,2’-dithiobenzoic acid (DTBA), 4-nitrotoluene (4-NT), m-nitrophenol (m-NP) and 1-amino-2-naphthol-4-sulphonic acid (ANSA) using GO-modified GCE. The stacked cyclic voltammograms of GO modified GCE both in the presence and absence of different analytes is shown in Figure 5.7 (A-D).

![Figure 5.7](image.png)

**Figure 5.7** The stacked cyclic voltammograms of GO modified GCE both in the presence and absence of (A) 2,2’-dithiobenzoic acid (DTBA), (B) 4-nitrotoluene (4-NT), (C) m-nitrophenol (m-NP) and (D) 1-amino-2-naphthol-4-sulphonic acid (ANSA) respectively.
From all the plots it was very well evident that in all the four cases there was no significant change in the CV graph of native GO-GCE in the presence of similar kinds of analytes in solution. The concentration of the analyte added was $1.99 \times 10^{-7}$ M and since the same amount of BPA was found to cause a significant reduction in the oxidation peak current of GO-GCE, therefore the electrode material developed could be said to be highly selective for electrochemical detection of Bisphenol A in solution. Figure 5.8 shows the histogram plot of the change in oxidation peak current of GO-modified GCE in the presence of various types of analytes bearing a variety of functional groups. All experiments were performed under exactly same conditions as was done for BPA. The concentration of the analytes used was $1.99 \times 10^{-7}$ M similar to the lowest detectable amount of BPA by the functionalized graphene modified GCE. It was quite clear from the plot that the change in current was highest in presence of BPA as compared to all other analytes in solution. Therefore, we can say that GO-modified GCE can be potentially used for the selective detection of Bisphenol-A in solution in presence of other similar types of molecules.

5.5.6 Comparative Analysis of the sensitivity of the electrodes developed towards detection of Bisphenol A: A review of literature
For comparative analysis of the materials we developed viz. GO, GO-ES and GO-en for preparation of functionalized graphene-modified GCE for electrochemical detection of Bisphenol A, we present herein a summary of different systems already reported for detection of BPA using various analytical techniques.

**Table 5.1.** Comparative analysis of the system developed (Functionalized Graphene) with already reported systems available for detection of environmentally hazardous Bisphenol A.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Material used</th>
<th>Method of detection</th>
<th>Lower detection limit (M)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>An electropolymerized molecularly imprinted polymer (E-MIP) film composed of varying ratios of BPA-terthiophene and carbazole monomer complex deposited onto indium tin oxide (ITO) substrates</td>
<td>Electrochemical Impedance Spectroscopy (EIS)</td>
<td>$4.2 \times 10^{-2}$</td>
<td>194</td>
</tr>
<tr>
<td>2</td>
<td>Graphene-graphene oxide</td>
<td>Cyclic voltammetry &amp; Electrochemical Impedance Spectroscopy (EIS)</td>
<td>$4.69 \times 10^{-8}$</td>
<td>206</td>
</tr>
<tr>
<td>3</td>
<td>A composite material based on a metal organic framework, nanowhisker of Al$_2$O$_3$ silanized with (3-aminopropyl)trimethoxysilane and gold nanoparticles.</td>
<td>Differential Pulse Voltammetry (DPV)</td>
<td>$3.78 \times 10^{-7}$</td>
<td>216</td>
</tr>
<tr>
<td>4</td>
<td>Graphene-CNTs nanocomposite with a sandwich lamination Structure loaded with Pt nanoparticles</td>
<td>Differential Pulse Voltammetry (DPV)</td>
<td>$4.2 \times 10^{-6}$</td>
<td>218</td>
</tr>
<tr>
<td>5</td>
<td>Surface molecular imprinting polymer microspheres synthesized on silica Microspheres using a water-soluble acenophene-containing 4-[(4-methacryloyloxy)phenylazo]benzenesulfonic acid as the functional monomer</td>
<td>UV-Visible spectroscopy</td>
<td>$2.19 \times 10^{-5}$</td>
<td>219</td>
</tr>
<tr>
<td>6</td>
<td>Our system</td>
<td>Cyclic voltammetry</td>
<td>$2 \times 10^{-7}$</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.1 lists the different types of materials and analytical techniques already reported for detection of Bisphenol A along with the corresponding detection limits based on literature review. From the table it is quite clear that there had been already a number of reports available for detection of BPA in solution using various analytical techniques ranging from electrochemical to spectroscopic and so on. On the other hand, the materials reported range from graphene, CNT, to various polymers employing different metals and metal oxides like Pt, Au, SiO$_2$, Al$_2$O$_3$ etc.
At the same time it was quite evident that the reported systems made use of certain toxic organic and inorganic compounds such as azobenzene and its derivatives, thiophenes, carbazoles, silanes etc. On the other hand, the system we report herein is based purely on graphene derivatized with different surface functional groups. Moreover, we carried out the electrode modification without the use of any polymeric binder, based purely on cast and dry strategy under ambient conditions. Further, the limits of detection reported for the various systems were found to be quite comparable with that obtained with the system we developed in addition to the fact that we did not make use of any toxic organic chemicals or metals in the development of the graphene-based electrode material and that only simple chemical reactions were carried out to incorporate the desired surface functionalities.

5.6 Conclusion
In the present study we demonstrated the successful electrochemical detection of the environmentally hazardous endocrine disruptor Bisphenol A using three different chemically functionalized graphene samples viz. GO, GO-ES (ester-functionalized) and GO-en (amine reduced and functionalized) graphene. All the functionalized graphene samples were subjected to systematic and stepwise characterization using various instrumental techniques. Subsequently, the graphene samples prepared were used to modify the surface of the GCE using a simple drop casting technique under ambient conditions and the corresponding electrode was used for electrochemical sensing of BPA in solution using cyclic voltammetry as a tool. However, the detection mechanism was attributed to simple π-stacking interactions between the sp² domains on the graphene layer structure and the Bisphenol A molecules which were in stark contrast to earlier reported sensors which were primarily based on oxidation of
Bisphenol A. Therefore, we successfully designed and developed a simple, cost-effective, environmentally benign electrode material based on functionalized graphene for selective and sensitive detection of Bisphenol A in solution with a lower detection limit of $2 \times 10^{-7}$ M. The significance of using electrochemical sensing technique over other optical methods of detection was that comparatively higher sensitivity of detection could be achieved with greater ease and effectiveness.