CHAPTER IV

Results and Discussion
4. RESULTS AND DISCUSSION

4.1 Electrochemical Detection of Metronidazole and Chloramphenicol using GNF modified Glassy carbon Electrode

Carbon based solid materials have been used to immobilize redox catalyst which facilitates the electron transfer process. A graphene nanoflakes modified glassy carbon electrode has been utilized as an electron transfer catalyst for the determination of MNZ and CAP under optimized experimental conditions. Various analytical methods were used to characterize the synthesized GNF by exfoliation method. The electrochemical detection of MNZ and CAP using graphene flakes modified GCE and its study their electrochemical behavior of MNZ and CAP under various pH ranges, influence of potential scan rates and electron transfer kinetics. Enhanced peak current values were observed at the modified electrode with lower detection limits. Thus, the present method can be considered as an efficient for determination of MNZ and CAP in real samples like drug samples and human urine samples.

4.1.1 Characteristics of Graphite and GNF

The FT-IR spectra of a) graphite and b) GNF are shown in Fig. 4.1.1A. In graphite, there was no significant peak was obtained. In GNF, the absorption band at 3418 cm\(^{-1}\) and 1420 cm\(^{-1}\) corresponds to the stretching vibration of COOH/O-H and alcoholic O-H groups [1]. The peak at 2923 cm\(^{-1}\) is attributed to stretching vibration of the C-H group. The band at 1743 cm\(^{-1}\), 1658 cm\(^{-1}\) and 1035 cm\(^{-1}\) are due to the stretching vibration of C=O, C=C and alkoxy (C-O) groups. The oxygen-containing groups such as C-OH, C=O and O-H stretching vibration peaks are reduced as compared to GO [2]. Figure 4.1.1B represents the Raman spectra of a) graphite and b) GNF. The graphite exhibits two major peaks at 1570.60 cm\(^{-1}\) and 1336.14 cm\(^{-1}\) for G and D bands respectively. These bands are ascribed to first-order scattering of E\(_{2g}\) vibration mode and structural defects (symmetry A\(_{1g}\) mode). In GNF, the
intensity of G band is higher than D band corresponding to a low defect contents. A small D band at 1336 cm\(^{-1}\) is Raman inactive for pristine GNF where the symmetry is broken by edges or high density defects and the 2D band appears at 2688 cm\(^{-1}\), which is the overtone of the D band. In addition, the intensity ratio of G and 2D of graphite and GNF are 0.87 and 1.03, respectively [3]. The increase of intensity ratio indicates the decrease in the size of sp\(^2\) domains, possibly due to the extensive oxidation and the number of defects [4].

Figure 4.1.1C shows the comparative plot of a) graphite and b) GNF was characterized by using X-ray diffraction analysis. A sharp and high intensity peak at 20 value of graphite at 26.40\(^\circ\) corresponds to the plane of (002) with d spacing value of 3.36 Å. In contrast to graphite, the GNF exhibits an intense sharp peak at 26\(^\circ\) and a shallow less intense with a 20 value of 55\(^\circ\) are due to the formation of defect-free few layered graphene sheet. This results are matching with the previous reported results with a similar characteristic features [5]. A drastic decreasing of the peak intensity was noted in the case GNF when compared with the XRD pattern of graphite.

4.1.2 SEM and TEM images of Graphite and GNF

The surface morphology of graphene flake was confirmed by FE-SEM and TEM images. The flaky structure, smooth surface and a large proportion of flakes with various dimensions of graphene flakes are shown in Fig. 4.1.2A and B. From the analysis, flakes are low dissimilarity due to multi-layer stacked without agglomeration and size of the graphene flake can be calculated [6]. The average size of the graphene nanoflake was estimated to be 660 nm. Fig. 4.1.2C shows the TEM image of multi-layer GNF with an inter-planar spacing of ~ 0.55 nm was observed. The elemental composition of GNF was confirmed by energy dispersive X-ray analysis (EDAX) as shown in Fig.4.1.2D. The major constituents of flakes are carbon and oxygen.
Fig. 4.1.1 A) FT-IR spectra of a) graphite and b) GNF, B) Raman spectra of a) graphite and b) GNF and C) XRD pattern of a) graphite and b) GNF.
Fig. 4.1.2  *SEM images of A) and B) GNF, C) TEM image of GNF and D) EDAX spectrum of GNF.*

4.1.3 Electrochemical behavior of GNF/GCE

*Figure 4.1.3A* shows the CVs behavior of bare GCE and GNF/GCE in the presence of 10 mM [Fe(CN)$_6$]$^{3-/4-}$ containing 0.1 M KNO$_3$ as a redox probe at a scan rate of 50 mV/s. In bare GCE exhibits a reversible redox peaks of [Fe(CN)$_6$]$^{3-/4-}$ with a anodic and cathodic peak separation ($\Delta E_p$) of 73 mV was obtained (curve a). After GNF modified electrode, the peak current of CV was increased obviously (curve b), and the $\Delta E_p$ has 86 mV which close to Nernstian behavior (i.e., 59 mV). These result indicates that the surface charges of GNF repelled [Fe(CN)$_6$]$^{3-/4-}$ access to the electrode surface for electron communication [7].

The electrochemical behavior of the GNF/GCE was investigated by using 10 mM [Fe(CN)$_6$]$^{3-/4-}$ containing 0.1 M KNO$_3$ as redox probe at various scan rates of 5 - 200 mV/s (*Fig. 4.1.3B*). The difference between the anodic and cathodic peak potential were found to
be at + 0.118 V and + 0.032 V (vs. Ag/AgCl), respectively. The separation of anodic peak current is larger than that of cathodic peak current value and its potential peak separation ($\Delta E_p = E_{pa} - E_{pc}$) was found to be 86 mV at 50 mV/s and redox peak current ratio is equal close to one ($I_{pa}/I_{pc} \approx 1$) which indicates that the electrochemical reaction is reversible one. With increasing the potential scan rate of the GNF/GCE, the redox peak current values are also increasing and the anodic and cathodic peak potential ($E_{pa}$ and $E_{pc}$) separations does not change significantly from the scan rates of 5 - 200 mV/s. The observed peak current ($I_{pa}$ and $I_{pc}$) has a linear relationship with square root of scan rate and a linear regression equation of $I_{pa} (\mu A) = 0.1108 (\nu^{1/2}/mV^{1/2}/s^{1/2}) + 0.2036$ and $I_{pc} = (\mu A) = - 0.0885 (\nu^{1/2}/mV^{1/2}/s^{1/2}) - 0.3248$ with correlation coefficient ($R^2$) of 0.9923 and 0.9951 (Fig. 4.1.3C). These results indicate that the GNF/GCE is diffusion controlled electron transfer process.

The heterogeneous electron transfer rate constant value is calculated by using Laviron equation [8];

$$\log k_s = \alpha \log (1 - \alpha) + (1 - \alpha) \log \alpha - \log RT/nF - \alpha (1 - \alpha) n F\Delta E_p/2.3 RT \quad \cdots (4.1.1)$$

where $k_s$ is the standard heterogeneous reaction rate constant ($s^{-1}$), $n$ is the number of electrons involved in electrochemical reaction, $F$ is the Faraday constant (C/mol), $\nu$ is the scan rate (mV/s), $R$ is the universal gas constant (J/K/mol), $T$ is the absolute temperature (K), $\alpha$ is electron transfer coefficient. The electron transfer rate constant value of GNF/GCE was found to be 1.37 s$^{-1}$ using the above Equation.

Additionally, a straight line of log current versus log scan rate shows a linear plot and its linear regression equation of $\log I_p (\mu A) = 0.4650 \log \nu/mV/s - 0.8654$ with correlation coefficient ($R^2$) of 0.9978 (Fig. 4.1.3.D). This result confirms that the electroactive behavior of GNF/GCE interface is purely diffusion controlled electron transfer process.
Fig. 4.1.3  
A) Cyclic voltammograms of a) bare GCE and b) GNT/GCE in the presence of 0.1 M KNO$_3$ containing 10 mM [Fe(CN)$_6$]$_{3/4}$ at a scan rate of 50 mV/s. 
B) Cyclic voltammograms of GNF/GCE in the presence of 0.1 M KNO$_3$ containing 10 mM [Fe(CN)$_6$]$_{3/4}$ at various scan rates a) 5, b) 10, c) 20, d) 40, e) 60, f) 80, g) 100, h) 120, i) 140, j) 160, k) 180 and l) 200 mV/s. (C) Linear plot of anodic and cathodic peak current vs. square root of scan rate ($\nu^{1/2}$) from (B). D) Logarithmic plot of peak current vs. scan rate ($\nu$).
4.1.4 Electrochemical impedance spectroscopy of GNF/GCE

Electrochemical impedance spectroscopic (EIS) measurement was carried out to understand the electron transfer at electrode-electrolyte interface on the GNF modified electrode [9]. The curve of EIS includes a semicircular part and a linear part. The semicircular part at higher frequencies correspond to electron-transfer limited process and its diameter is equal to the electron transfer resistance ($R_{ct}$) which controls the electron transfer kinetics of redox probe at electrode interface. Meanwhile, the linear part at lower frequencies corresponds to diffusion process.

Fig. 4.1.4 Nyquist plots of a) bare GCE and b) GNF/GCE in the presence of 0.1 M KNO$_3$ containing 10 mM [Fe(CN)$_6^{3−/4−}$]. AC Amplitude: 5 mV; Frequency range: 0.01 Hz to 100 kHz. Inset is the Randles circuit.

The EIS spectra were carried out in 10 mM [Fe(CN)$_6^{3−/4−}$] containing 0.1 M KNO$_3$ as a redox probe with a frequency range from 0.01 Hz to $10^5$ Hz (Amplitude 5 mV) as shown in Figure 4.1.4. At bare GCE exhibited an almost straight line indicates a diffusion limited electrochemical process. For GNF/GCE shows a semicircular part (higher frequency) as well.
as linear portion, indicating a charge electron transfer and diffusion controlled electron transfer reaction. These results indicate that the GNF/GCE system has both diffusion and charge transfer limitation electrochemical process.

### 4.1.5 Electrochemical reduction of MNZ at GNF/GCE

The electrocatalytic reduction of MNZ using GNF/GCE was investigated by using cyclic voltammetric method. Cyclic voltammetry behavior of MNZ on bare GCE (absence and presence) and GNF/GCE in the presence of 0.1 M KCl containing PBS (pH 7.0) are shown in Fig. 4.1.5A. In bare GCE, MNZ shows a small reduction peak current and peak potential at - 0.44 V (vs. Ag/AgCl) however the GNF/GCE in the presence of MNZ exhibits a sharp, well-defined reduction peak current and peak potential at - 0.32 V (vs. Ag/AgCl) respectively. The electrocatalytic reduction of MNZ peak current value is three times higher than the bare GCE. Further, a linear peak current values are noted, while increasing the concentration of MNZ from 3.3 x 10^{-5} M to 23.1 x 10^{-5} M which exhibits a better electrocatalytic behavior of GNF/GCE as shown in Fig. 4.1.5B and C. Cyclic voltammogram was clearly depicted that the reduction peak current is increased linearly while increasing the potential scan rates from 10 to 120 mV/s (Fig. 4.1.5D). The observed reduction peak current value has linear relationship with square root of the scan rate and its linear regression equation of \( I_{pc} (\mu A) = -1.3487 (v^{1/2}/mV^{1/2}/s^{1/2}) + 2.8149 (R^2 = 0.9960) \) as shown in Fig. 4.1.5E. These results reveal that the electrocatalytic reduction of MNZ at modified electrode is a diffusion-controlled electron transfer process.

Moreover, the cathodic peak potential of MNZ increases linearly with natural logarithm of scan rate (\( \log v \)) and its linear regression equation of \( E_{pc} (V) = -0.0495 \log \nu/mV/s - 0.3028 (R^2 = 0.9987) \) (Fig. 4.1.5F). Since the reduction reaction of MNZ was irreversible in nature, a linear relationship between anodic peak potential (\( E_{pa} \)) and natural
Fig. 4.1.5 (A) CV of a) and b) bare GCE (absence and presence of MNZ) and c) GNF/GCE in the presence of 50 μL in 0.1 mM of MNZ at a scan rate 50 mV/s in 0.1 M KCl containing PBS (pH 7.0). (B) CV of GNF/GCE in different concentrations of MNZ (3.3 to 23.1 x 10^{-5} M) at a scan rate 50 mV/s. (C) Calibration plot of I_{pc} vs. MNZ concentration. (D) CV of MNZ at various scan rates (20 – 120 mV/s). (E) Plot of I_{pc} vs. \nu^{1/2}. (F) Plot of E_{pc} vs. log \nu.
logarithm of scan rate \((\log \nu)\). For the electrochemical reduction of MNZ, an irreversible process, the relationship between \(E_p\) and \(\log \nu\) obeyed the following equation [10];

\[
E_{pc} = E^0 - \frac{RT}{\alpha n_a F} \left[0.780 + \ln \left(\frac{D^{1/2}}{k^0}\right) + \ln \left(\alpha n_a F \nu RT\right)^{1/2}\right]
\] ...

\[(4.1.2)\]

\[
E_{pc} = k + \frac{RT}{2\alpha n_a F} (\log \nu)
\]

............... (4.1.3)

where \(E_{pc}\) is the formal redox potential, \(\alpha\) is the electron transfer coefficient, \(D\) is the diffusion coefficient \((\text{cm}^2/\text{s})\), \(k^0\) is the standard heterogeneous rate constant, \(R\) is the universal gas constant, \(T\) is the absolute temperature, and \(F\) is the Faraday constant \((\text{C/mol})\). The slope line is equal to \(RT/2\alpha n F\) and \(\alpha\) is found to be 0.7 in electrode reaction of MNZ due to irreversible electrode process. In the present study, number of protons and electrons involved during reduction process of MNZ is almost equal.

### 4.1.6 Electro catalytic reduction of CAP at GNF/GCE

Electrocatalytic behavior of GNF/GCE was used toward the cathodic peak current value of CAP. Figure 4.1.6A shows the cyclic voltammetric behavior of CAP on bare GCE (absence and presence) and GNF/GCE in presence of 0.1 M KCl containing PBS \((\text{pH } 7.0)\) at a scan rate of 50 mV/s. In bare GCE, the CAP exhibits one redox peak at - 0.207 V \((C_1)\) and - 0.122 V \((A_1)\) and the peak potential separation \((\Delta E_p = E_{pa} - E_{pc})\) was found to be 85 mV, indicates the reversible redox of the hydroxylamine \((-\text{NHOH})\). The cathodic peak at - 0.59 V \((C_2)\) corresponding to the irreversible reduction of nitro group \((-\text{NO}_2)\) to hydroxylamine group \((\text{NHOH})\). This process clearly indicates the development of \(C_2\) peak is responsible for the formation of \(A_1\) and \(C_1\) peaks respectively [11]. Whereas, a cathodic peak potential of GNF/GCE is shifted toward positive side and the observed peak current value is four times superior to bare GCE. The cathodic peak current increases with each addition of 0.1 mM CAP solution and its linear ranges from \(1.6 \times 10^{-5}\) to \(12.8 \times 10^{-5}\ M\) with the calibration plot as shown in Fig. 4.1.6B and C. Cyclic voltammogram was clearly depicted that the reduction
Fig. 4.1.6  (A) CV of a) and b) bare GCE (absence and presence of CAP) and c) GNF/GCE in the presence of 50 μl in 0.1 mM of CAP at a scan rate 50 mV/s in 0.1 M KCl containing PBS (pH 7.0). (B) CV of GNF/GCE in different concentrations of CAP (3.3 to 23.1 x 10^{-5} M) at a scan rate 50 mV/s. (C) Calibration plot of $I_p$ vs. CAP concentration. (D) CV of CAP at various scan rates (20 – 120 mV/s). (E) Plot of $I_p$ vs. $\nu^{1/2}$. (F) Plot of $E_p$ vs. log $\nu$. 
peak current is increased linearly while increasing the potential scan rates from 10 – 140 mV/s (Fig. 4.1.6D). The observed peak current has linear relationship with square root of the scan rate with linear regression equation of $I_{pc} \, (\mu A) = - 0.4066 \, (v^{1/2}/mV^{1/2}/s^{1/2}) - 1.5369 \, (R^2 = 0.9888)$ as shown in Fig. 4.1.6E. These results reveal that the electrocatalytic reduction of CAP at modified electrode is a diffusion-controlled electron transfer process.

Moreover, the cathodic peak potential of CAP increases linearly with natural logarithm of scan rate and its linear regression equation of $E_{pc} \, (V) = - 0.0291 \, \log v/mV/s - 0.4961 \, (R^2 = 0.9841)$ (Fig. 4.1.6F). Since the reduction reaction of CAP was irreversible in nature, a linear relationship between $E_{pa}$ and natural logarithm of scan rate ($\log v$) can be defined with the above Equation 4.1.2 and 4.1.3. The slope line is equal to $RT/2nF$ and $n$ is found to be 2.034. Assuming the electron transfer coefficient ($\alpha$) is approximately 0.5 for a totally irreversible electrode process, the value of $n$ is estimated to be 4.06, indicating that four electrons are involved in the electrochemical reduction of CAP.

4.1.7 Effect of pH on reduction of MNZ and CAP at GNF/GCE

The pH of solution is essential parameter which influences the reduction rate of MNZ and CAP [12]. The maximum peak current response of both MNZ and CAP was studied using 0.1 M KCl containing PBS with different pH ranges (pH 1.0 to 11.0) at GNF/GCE by using cyclic voltammetric method. A shift in reduction peak potential for both MNZ and CAP were observed, while increasing the pH of the medium, indicating the reduction behavior of MNZ and CAP at GNF/GCE are pH dependent reaction as shown in Fig. 4.1.7A and C. The observed cathodic peak current values are higher for both systems at pH 7.0. If pH values beyond 8, the reduction reaction is pH independent, and it is difficult to analyze the electrochemical reduction of MNZ and CAP. Figure 4.1.7B and D shows variation of $I_{pc}$ versus variation of pH and variation $E_{pc}$ versus variation of pH in MNZ and CAP, it can be
clearly observed that the cathodic peak potential and current were closely related to the pH value (pH 7.0) of supporting electrolyte. The linear regression equation of MNZ and CAP were $E_{pc} (V) = -0.1054 - 0.0487 \text{pH (R}^2 = 0.9968)$ and $E_{pc} (V) = -0.3487 - 0.0527 \text{pH (R}^2 = 0.9952)$ respectively. The slope value of 48.7 mV/pH unit and 52.7 mV/pH unit, which may close to the Nernstian equation for a proportion of electrons and protons involved in the reduction reaction as approximately 1:1 based on the slope of 59 mV/pH [13].

From the above observation, the reaction scheme would probably via the following mechanistic steps. Nitro group derivatives corresponds to the reduction of nitro group (R-NO) to hydroxylamine (RNHOH), involving 4 electrons and 4 protons followed by a two-electron reduction of hydroxylamine to amine [14]. The electrochemical reduction mechanism of MNZ and CAP is shown in Scheme 4.1.1.

![Scheme 4.1.1 Mechanism of the electrode reduction mechanism of MNZ and CAP using GNF/GCE.](image)
Fig. 4.1.7  Effect of pH on (A and B) CV curves of 0.1 mM of MNZ and CAP at GNF/GCE in the presence of 0.1 M KCl containing PBS with various pH ranges (pH 1, 3, 5, 7, 9 and 11) at a scan rate 50 mV/s. (C) Effect of pH on the peak potential ($E_{pc}$) and peak current ($I_{pc}$) of MNZ. (D) Effect of pH on the peak potential ($E_{pc}$) and peak current ($I_{pc}$) of CAP.
4.1.8 Individual/simultaneous determination of CAP and MNZ at GNF/GCE

The DPV technique was exploited for the selective and sensitive detection of MNZ and CAP at the GNF/GCE in the presence of 0.1 M KCl containing PBS (pH 7.0). The determination of MNZ and CAP mixtures were performed at GNF/GCE by DPV which eliminates the residual charge current values and the pure faraday current values where only measured. As can be seen from Fig. 4.1.8A, the sharp and well-defined reduction peak current increased linearly with increasing the concentration of MNZ. The reduction peak potential was observed at – 0.16 V (vs. Ag/AgCl) for MNZ. By varying the concentration of MNZ from 0.06 x 10^-7 M – 6.0 x 10^-6 M, the linear increase of peak current values were noted. The linear regression equation was expressed of I_{pc} (µA) = - 0.2038 C (10^-7 M) – 0.2493 with a correlation coefficient of 0.9980 as shown in Fig. 4.1.8B. The limit of detection (LOD) was found to be 0.9 x 10^-8 M for MNZ using 3σ/slope, where ‘σ’ is the standard deviation of the mean value for five independent voltammogram of blank solution. The comparison of our sensor with other previous reported literatures for MNZ determination was summarized in Table 4.1.1. This result implies that the reduction of MNZ at GNF/GCE has wider linear range, lower detection limit and good sensitivity.

Similarly, the electrochemical reduction of CAP was also investigated by varying the CAP concentration at GNF/GCE in presence of 0.1 M KCl containing PBS (pH 7.0). As can be seen from Fig. 4.1.8C, the reduction peak potential for CAP was found to be - 0.476 V (vs. Ag/AgCl) at the GNF/GCE. By varying the concentration of CAP from 0.25 x 10^-7 M – 32.5 x 10^-6 M, the linear increase of cathodic peak current values were noted. The linear regression equation was expressed as I_{pc} (µA) = - 1.4416 C (10^-7 M) + 0.0148 with a correlation coefficient of 0.9985 as shown in Fig. 4.1.8D. The limit of detection value (3σ/slope, where ‘σ’ is the standard deviation) was found to be 0.39 x 10^-8 M for CAP. The
Fig. 4.1.8  
(A) DPV of GNF/GCE in different concentrations of MNZ (0.06 - 6.0 x 10^{-7} M) at 0.1 M KCl containing PBS (pH 7.0). (B) Plot of $I_{pc}$ vs. conc. of MNZ. (C) DPV of GNF/GCE in different concentrations of CAP (0.25 - 3.25 x 10^{-7} M) at 0.1 M KCl containing PBS (pH 7.0). (D) Plot of $I_{pc}$ vs. conc. of CAP. (E) Simultaneous determination of MNZ and CAP using GNF/GCE in 0.1 M KCl containing PBS (pH 7.0). (F) Plot of $I_{pc}$ vs. conc. (MNZ – 0.1 to 1 x 10^{-7} M and CAP – 0.1 to 1 x 10^{-7} M). Scan rate: 20 mV/s, Pulse width: 20 mV, Pulse Amplitude: 25 mV.
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Graphene nanoflakes and nanoclay based ..........

detection limit of the proposed method is comparable with previously reported values as shown in Table 4.1.2.

Simultaneous determination of MNZ and CAP at GNF/GCE was studied by using DPV method. The concurrent study was carried out in the potential range from + 0.1 V to - 0.8 V. The concurrent determination of MNZ and CAP using GNF/GCE shows a well resolved separation between two cathodic peak potentials corresponding to their reduction peak current values. The peak separation between MNZ and CAP were found to be 280 mV as shown in Fig. 4.1.8E. The simultaneous determination of MNZ and CAP in a mixture was carried out at GNF/GCE when concentrations of two species were changed. From the Fig. 4.1.8F, the peak current of MNZ and CAP were linearly proportional to the same concentration of MNZ and CAP (0.1 × 10^-7 M to 100 × 10^-5 M). The linear relationship is obtained with the regression equation of $I_{pc} (\mu A) = -0.6380 \frac{C (\mu M)}{M} - 0.3380 (R^2 = 0.9929)$ and $I_{pc} (\mu A) = -0.2038 \frac{C (\mu M)}{M} - 0.2493 (R^2 = 0.9980)$ for MNZ and CAP respectively. The detection limit value ($3\sigma$/slope, where $\sigma$ is the standard deviation) was found to be 0.23 nM and 0.46 nM for MNZ and CAP respectively. These results strongly suggest that the individual and simultaneous determination of MNZ and CAP on GNF/GCE can be achieved with a high selectivity and sensitivity. The schematic diagram shows the simultaneous electrocatalytic reduction of MNZ and CAP corresponding to GNF/GCE (Scheme 4.1.2).

4.1.9 Amperometry detection of CAP and MNZ at GNF/GCE

Amperometry method can easily measure the current response for the each addition of MNZ and CAP with respective time under stirring condition. The typical steady-state catalytic current-time response of GNF/GCE under constant stirring for step-wise injection of 30 $\mu$L of 1$\mu$M MNZ (50 s) and 10 $\mu$L of 1 $\mu$M CAP (50 s) into 0.1 M KCl containing PBS (pH 7.0) at applied potential of - 0.16 V and - 0.476 V (vs. Ag/AgCl). Figure 4.1.9A and C
Table 4.1.1  Comparison table of MNZ determination in different electrodes

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Method</th>
<th>Linear range (mol/L)</th>
<th>$R^2$</th>
<th>LOD (mol/L)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au electrode</td>
<td>CV</td>
<td>$5 \times 10^{-7} - 1 \times 10^{-5}$</td>
<td>0.9975</td>
<td>$1.5 \times 10^{-7}$</td>
<td>[15]</td>
</tr>
<tr>
<td>HMDE</td>
<td>DPV</td>
<td>$2.3 \times 10^{-7} - 1.8 \times 10^{-6}$</td>
<td>0.9998</td>
<td>$3.6 \times 10^{-8}$</td>
<td>[16]</td>
</tr>
<tr>
<td>MIP-CP</td>
<td>DPV</td>
<td>$3.3 \times 10^{-10} - 1.5 \times 10^{-8}$</td>
<td>0.9970</td>
<td>$1.5 \times 10^{-10}$</td>
<td>[17]</td>
</tr>
<tr>
<td>Gr-IL/GCE</td>
<td>DPV</td>
<td>$1 \times 10^{-7} - 2.5 \times 10^{-5}$</td>
<td>0.9990</td>
<td>$4.7 \times 10^{-8}$</td>
<td>[18]</td>
</tr>
<tr>
<td>3D GNT/CPE</td>
<td>SWASV</td>
<td>$1 \times 10^{-9} - 2 \times 10^{-6}$</td>
<td>0.9969</td>
<td>$0.1 \times 10^{-9}$</td>
<td>[19]</td>
</tr>
<tr>
<td>Cysteic acid/</td>
<td>LSV</td>
<td>$1 \times 10^{-8} - 800 \times 10^{-6}$</td>
<td>0.9940</td>
<td>$2.3 \times 10^{-9}$</td>
<td>[20]</td>
</tr>
<tr>
<td>PDDA-GN/GCE</td>
<td>LSV</td>
<td>$0.05 \times 10^{-6} - 10 \times 10^{-6}$</td>
<td>0.9962</td>
<td>$28 \times 10^{-9}$</td>
<td>[23]</td>
</tr>
<tr>
<td>MMIP/MGCE</td>
<td>DPSV</td>
<td>$5 \times 10^{-8} - 1 \times 10^{-6}$</td>
<td>0.9946</td>
<td>$0.16 \times 10^{-7}$</td>
<td>[21]</td>
</tr>
<tr>
<td>Chit@CuTsPc</td>
<td>DPV</td>
<td>$0.8 \times 10^{-9} - 72 \times 10^{-7}$</td>
<td>0.9976</td>
<td>$4.1 \times 10^{-10}$</td>
<td>[22]</td>
</tr>
<tr>
<td>p-GR-Ag</td>
<td>LSV</td>
<td>$0.05 \times 10^{-6} - 10 \times 10^{-6}$</td>
<td>0.9962</td>
<td>$28 \times 10^{-9}$</td>
<td>[23]</td>
</tr>
<tr>
<td>MIP/NPAMR</td>
<td>AMP</td>
<td>$8 \times 10^{-14} - 1 \times 10^{-6}$</td>
<td>0.9946</td>
<td>$2.7 \times 10^{-14}$</td>
<td>[24]</td>
</tr>
<tr>
<td>MIP/GCE</td>
<td>DPV</td>
<td>$1 \times 10^{-9} - 1 \times 10^{-8}$</td>
<td>0.9934</td>
<td>$3.3 \times 10^{-10}$</td>
<td>[25]</td>
</tr>
<tr>
<td>MIP/AuNPs/GCE</td>
<td>DPV</td>
<td>$0.5 \times 10^{-6} - 1000 \times 10^{-6}$</td>
<td>0.9990</td>
<td>$0.12 \times 10^{-6}$</td>
<td>[26]</td>
</tr>
<tr>
<td>PDDA-GN/DNA/GCE</td>
<td>LSV</td>
<td>$0.05 \times 10^{-6} - 100 \times 10^{-6}$</td>
<td>0.9940</td>
<td>$24 \times 10^{-9}$</td>
<td>[27]</td>
</tr>
<tr>
<td>DMIP/CPE</td>
<td>DPV</td>
<td>$4.0 \times 10^{-7} - 2.0 \times 10^{-4}$</td>
<td>0.9938</td>
<td>$9.1 \times 10^{-8}$</td>
<td>[28]</td>
</tr>
<tr>
<td>BDD/GCE</td>
<td>SWV</td>
<td>$0.2 \times 10^{-6} - 4.2 \times 10^{-6}$</td>
<td>0.9968</td>
<td>$0.065 \times 10^{-6}$</td>
<td>[29]</td>
</tr>
<tr>
<td>GNF/GCE</td>
<td>Amp</td>
<td>$1.0 \times 10^{-8} - 110 \times 10^{-7}$</td>
<td>0.9983</td>
<td>$0.014 \times 10^{-9}$</td>
<td>This work</td>
</tr>
</tbody>
</table>

$GCE$ – Glassy carbon electrode  
HMDE – Hanging mercury drop electrode  
MIP – Molecularly imprinted polymer/carbon paste electrode  
SWASV – Square wave adsorptive stripping voltammetry  
GrIL – Graphene-ionic liquid/glassy carbon electrode  
DPSV – Differential pulse stripping voltammetry  
MMIP – Magnetic molecularly imprinted polymer  
LSV – Linear sweep voltammetry  
DMIP – duplex molecularly imprinted polymer  
p-GR-Ag – petal like graphene Ag composite
<table>
<thead>
<tr>
<th>Electrode</th>
<th>Method</th>
<th>Linear range (mol/L)</th>
<th>$R^2$</th>
<th>LOD (mol/L)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIP-CPE</td>
<td>DPV</td>
<td>$8.0 \times 10^{-9} - 1 \times 10^{-6}$</td>
<td>0.9948</td>
<td>$2.0 \times 10^{-9}$</td>
<td>[30]</td>
</tr>
<tr>
<td>Au/N-G/GCE</td>
<td>LSV</td>
<td>$2 \times 10^{-6} - 8 \times 10^{-5}$</td>
<td>0.9975</td>
<td>$5.9 \times 10^{-7}$</td>
<td>[31]</td>
</tr>
<tr>
<td>Aptamer/p-AHNSA/EPPG</td>
<td>DPV</td>
<td>$0.1 \times 10^{-9} - 2500 \times 10^{-9}$</td>
<td>0.9950</td>
<td>$0.02 \times 10^{-9}$</td>
<td>[32]</td>
</tr>
<tr>
<td>MWCNT@MIP/KM-3/P-r-GO/GCE</td>
<td>DPV</td>
<td>$5 \times 10^{-9} - 5 \times 10^{-7}$</td>
<td>0.9973</td>
<td>$1.0 \times 10^{-10}$</td>
<td>[33]</td>
</tr>
<tr>
<td>HEM/Apt/AuNPs/SBA-15@DABCO/SPE</td>
<td>DPV</td>
<td>$0.3 \times 10^{-6} - 0.15 \times 10^{-6}$</td>
<td>0.9895</td>
<td>$4 \times 10^{-9}$</td>
<td>[34]</td>
</tr>
<tr>
<td>MoS$_2$/PANI/CPE</td>
<td>DPV</td>
<td>$0.1 \times 10^{-6} - 10 \times 10^{-6}$</td>
<td>0.9925</td>
<td>$6.9 \times 10^{-8}$</td>
<td>[35]</td>
</tr>
<tr>
<td>GCE/Fe$_3$O$_4$-CMC@Au</td>
<td>SWV</td>
<td>$2.5 \times 10^{-6} - 25 \times 10^{-6}$</td>
<td>0.9935</td>
<td>$6.6 \times 10^{-8}$</td>
<td>[36]</td>
</tr>
<tr>
<td>TiN-rGO/GCE</td>
<td>DPV</td>
<td>$0.05 \times 10^{-6} - 100 \times 10^{-6}$</td>
<td>0.9950</td>
<td>$0.02 \times 10^{-8}$</td>
<td>[37]</td>
</tr>
<tr>
<td>AuNPs/GO/GCE</td>
<td>Amp</td>
<td>$1.5 \times 10^{-6} - 2.95 \times 10^{-6}$</td>
<td>0.9950</td>
<td>$0.25 \times 10^{-6}$</td>
<td>[38]</td>
</tr>
<tr>
<td>CSM@VSM/ITO electrode</td>
<td>DPV</td>
<td>$0.1 \times 10^{-12} - 3.6 \times 10^{-12}$</td>
<td>0.9944</td>
<td>$1.89 \times 10^{-6}$</td>
<td>[39]</td>
</tr>
<tr>
<td>GNF/GCE</td>
<td>Amp</td>
<td>$0.05 \times 10^{-8} - 55 \times 10^{-6}$</td>
<td>0.9980</td>
<td>$0.025 \times 10^{-9}$</td>
<td><em>This work</em></td>
</tr>
</tbody>
</table>

$^b$GCE – Glassy carbon electrode  
MIP-CPE – Molecularly imprinted polymer-carbon paste electrode  
Au/N-G – Nitrogen-doped graphene nanosheets decorated with gold nanoparticles  
CSM@VSM/ITO – Cylindrical surfactant micelles@vertical silica mesochannels/indium tin oxide  
AuNPs/GO – AuNPs decorated graphene oxide  
p-AHNSA – poly-(4-amino-3-hydroxynaphthalene sulfonic acid  
MWCNT@MIP – Multiwalled carbon nanotubes@molecularly imprinted polymer  
SBA-15@DABCO – 1, 4-diazabicyclo [2,2,2]octane supported mesoporous silica SBA-15  
SPE – Screen printed electrode  
SWV – Square wave voltammetry  
DPV – Differential pulse voltammetry  
Amp – Amperometry
clearly shows that the reduction peak current increases by increasing the concentration of MNZ and CAP. Amperometric response, increased linearly in ranges from $1.0 \times 10^{-8}$ M to $110 \times 10^{-7}$ M and $0.05 \times 10^{-8}$ M to $55 \times 10^{-6}$ M for MNZ and CAP, respectively. Linear calibration was obtained, with a coefficient of MNZ and CAP as 0.9983 and 0.9980 respectively, which demonstrates the better relationship between reduction peak current and concentration of MNZ and CAP. The limit of detection was calculated in a linear graph of MNZ and CAP were found to be 0.014 nM and 0.025 nM, based on the signal-to-noise ratio (S/N = 3) respectively (Fig. 4.1.9 B and D).

**Scheme 4.1.2** Schematic diagram represents the simultaneous electrocatalytic reduction of MNZ and CAP corresponding to GNF/GCE.

**4.1.10 Stability, reproducibility and Interference studies**

The long term stability of the prepared electrode (GNF/GCE) was tested and stored at RT. After two weeks, the CV response has retained about 99.2 % current response than the
(A) Amperometric response of GNF/GCE at an applied potential 160 mV to subsequent addition of different concentrations (1.0 \times 10^{-8} M - 110 \times 10^{-7} M) from 1 \mu M MNZ in 0.1 M KCl containing PBS (pH 7.0). (B) Calibration plot of cathodic peak current ($I_{pc}$) vs. concentration of MNZ. (C) Amperometric response of GNF/GCE at an applied potential 476 mV to subsequent addition of different concentrations (0.05 \times 10^{-8} M to 55 \times 10^{-6} M) from 0.1 \mu M CAP in the presence of 0.1 M KCl containing PBS (pH 7.0). (D) Calibration plot of cathodic peak current ($I_{pc}$) vs. concentration of CAP.
recently prepared one. After one month, the stability of the electrode was tested again 25 cycles recording by CV experiment in 0.1 M KCl containing PBS (pH 7.0) about 98.5 % current was retained and it indicates that the GNF/GCE shows a good stability and reproducibility. At different time intervals, the amperometry method were carried out for the determination of MNZ and CAP in 0.1 M KCl containing PBS (pH 7.0). The peak current was preserved the same with the relative standard deviation of 3.3 % for five determinations. This result suggests that the good stability and reproducibility of the GNF/GCE.

The influence of various substances as compounds potentially interfering with the determination of MNZ and CAP were studied under optimum conditions. The interfering substances were chosen from the pharmaceuticals and/or in biological fluids. The influence of interference species present in the reaction medium was also investigated at GNF/GCE along with MNZ and CAP by amperometric method. There is no interference was obtained for the determination of MNZ, the interference compounds such as orinidazole, chloramphenicol, cysteine, caffeine, glucose, caffeine, DA, oxalic acid, cellulose, NO$_3^-$, nitrophenol and AA as shown in Fig. 4.1.10A. And also to study the determination of CAP by using various interference species like chlorotetracycline, thiamphenicol, 4-nitrobenzoic acid, 4-nitroaniline, 4-nitrophenol, florfenicol, cysteine, glucose, AA, UA and streptomycin (Fig. 4.1.10B). In the determination of MNZ and CAP, a maximum concentration of the interfering substance was less than ±5 % error of the tolerance limit. The proposed GNF/GCE was applied for detection of MNZ and CAP in real samples using standard addition method.

4.1.11 Real sample analysis

4.1.11.1 Determination of MNZ in tablets

The proposed amperometry sensing method was scrutinized by analyzing the MNZ in five different tablets. The 200 mg and 400 mg of MNZ tablets were prepared by dissolving in 100 mL water by using ultrasonication. And then taken different volume of prepared solution
Fig. 4.1.10  A) Amperometric response of several interfering compounds at GNF/GCE in presence of 1 μM of MNZ in presence of 0.1 M KCl containing PBS (pH 7.0). B) Amperometric response of several interfering compounds at GNF/GCE in presence of 1 μM of CAP in presence of 0.1 M KCl containing PBS (pH 7.0).
Table 4.1.3  Determination of MNZ in tablet samples at pH 7

<table>
<thead>
<tr>
<th>S. No</th>
<th>Added (μM)</th>
<th>Found (μM)</th>
<th>R.S.D (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>98.2</td>
<td>2.6</td>
<td>98.2</td>
</tr>
<tr>
<td>2</td>
<td>150</td>
<td>151.1</td>
<td>1.2</td>
<td>100.7</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>191.3</td>
<td>0.83</td>
<td>95.65</td>
</tr>
<tr>
<td>4</td>
<td>250</td>
<td>255.3</td>
<td>1.65</td>
<td>102</td>
</tr>
<tr>
<td>5</td>
<td>500</td>
<td>507.8</td>
<td>2.97</td>
<td>101.5</td>
</tr>
</tbody>
</table>

Table 4.1.4  Determination of MNZ and CAP in human urine samples at pH 7

<table>
<thead>
<tr>
<th>Samples</th>
<th>Analyte</th>
<th>Detected (μM)</th>
<th>Added (μM)</th>
<th>Found (μM)</th>
<th>R.S.D (%)</th>
<th>^Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine 1</td>
<td>MNZ</td>
<td>-</td>
<td>10</td>
<td>10.57</td>
<td>3.3</td>
<td>105.7</td>
</tr>
<tr>
<td></td>
<td>CAP</td>
<td>9.72</td>
<td>10</td>
<td>19.96</td>
<td></td>
<td>101.2</td>
</tr>
<tr>
<td>Urine 2</td>
<td>MNZ</td>
<td>-</td>
<td>20</td>
<td>20.3</td>
<td>2.3</td>
<td>101.5</td>
</tr>
<tr>
<td></td>
<td>CAP</td>
<td>16.75</td>
<td>20</td>
<td>37.67</td>
<td></td>
<td>102.5</td>
</tr>
<tr>
<td>Urine 3</td>
<td>MNZ</td>
<td>-</td>
<td>30</td>
<td>29.91</td>
<td>2.9</td>
<td>99.7</td>
</tr>
<tr>
<td></td>
<td>CAP</td>
<td>10.03</td>
<td>30</td>
<td>38.77</td>
<td></td>
<td>96.8</td>
</tr>
</tbody>
</table>

^Recovery is calculated based on the clinical value
was transferred into 50 mL of PBS at pH 7.0. An aliquot of 10 mL of this solution was placed in the electrochemical cell for the determination of MNZ using above amperometry method. The analytical results are enumerated in Table 4.1.3. The recovery range from 95.65 % to 102 % and the R.S.D. (n = 5) was less than 3 %. These results were suitable and acceptable, showing that the proposed method could be efficiently used for the determination of MNZ in commercial sources.

4.1.11.2 Determination of MNZ and CAP in urine samples

In using amperometry method for the determination of real sample analysis of MNZ in human urine samples from patients were also investigated. Before the measurement, all urine samples were diluted 250 times with PBS (pH 7.0) in order to fit into the linear range of real samples. No other pretreatment process was performed. 1 mL of the urine sample was added to the electrochemical cell containing 15 mL of PBS medium and the certain amount of CAP was spiked using the standard addition method. The binary mixtures of MNZ and CAP were obtained between 96.8 % and 105.7 % as shown in Table 4.1.4. This recovery range indicates a good accuracy and repeatability of the proposed method in human urine samples.

In conclusion, a GNF was synthesized by surface mediated exfoliation method. The GNF was further characterized by FT-IR, Raman spectroscopy, XRD and SEM images. A sensitive electrochemical sensor with GNF/GCE was developed for the simultaneous determination of MNZ and CAP. The GNF/GCE shows an excellent electrocatalytic activity towards the electrochemical reduction of MNZ and CAP. The influence of many interfering substances were tested for peak current response and found that the present system is free from any momentous interference. Moreover, the sensor electrode shows a good sensitivity, selectivity, reproducibility and low detection limit in the electrochemical detection of MNZ and CAP at GNF/GCE. The developed sensor was successfully applied for electrochemical determination of MNZ and CAP in drugs and human urine samples.
4.2 Voltammetric Detection of Paracetamol and Pentoxifylline using GNF modified Glassy carbon Electrode

A novel electrochemical sensor for simultaneous detection of PAR and PTX were investigated based on GNF/GCE. The modified electrode exhibited a strong electrocatalytic activity towards the oxidation of PAR and PTX respectively. The electrochemical behavior of PAR and PTX were investigated using various pH ranges, influence of potential scan rates and electron transfer kinetics. An enhanced peak current value was observed at GNF/GCE with diffusion coefficient and lower detection limits. The interfering substances like biological and pharmaceutical samples were also investigated by using amperometry method. Thus, the present method can be considered as an efficient for the detection of PAR and PTX in drug formulations and urine samples.

4.2.1 Electrocatalytic oxidation of PAR at GNF/GCE

The electrocatalytic behavior of GNF/GCE was used towards the oxidation peak current value of PAR. Cyclic voltammetry behavior of 0.1 mM PAR on bare GCE (absence and presence) and GNF/GCE in the presence of 0.1 M KCl containing PBS (pH 7.0) are shown in Fig. 4.2.1A. In bare GCE, PAR exhibits a poor redox peak current and the anodic and cathodic peak potential ($E_{pa}$ and $E_{pc}$) at + 0.254 V and – 0.243 V (vs. Ag/AgCl) whereas, the GNF/GCE in the presence of PAR exhibits a sharp, well-defined anodic peak current ($E_{pa}$) at + 0.19 V and a small broad reduction peak ($E_{pc}$) at + 0.068 V, indicates the electrocatalytic behavior of PAR is a quasi-reversible one [40]. Even though, the oxidative peak current of PAR is four times higher than the bare GCE. Additionally, a linear peak current values are distinguished, while increasing the concentration of PAR from $0.5 \times 10^{-5}$ M to $5.5 \times 10^{-5}$ M which exhibits a better electrocatalytic behavior of GNF/GCE as shown in Fig. 4.2.1B and C. The effect of potential scan rate on the electrochemical oxidation of PAR at GNF/GCE was investigated by CV technique. With the increase of potential scan rate from
Fig. 4.2.1  (A) CV of a) and b) bare GCE (absence and presence of PAR) and c) GNF/GCE in the presence of 0.1 mM of PAR at a scan rate 50 mV/s in 0.1 M KCl containing PBS (pH 7.0). (B) CV of GNF/GCE in different concentrations (0.5 to 5.5 x 10^{-5} M) of PAR at a scan rate 50 mV/s. (C) Calibration plot of $I_{pa}$ vs. PAR concentration. (D) CV of PAR at various scan rates (5 – 180 mV/s). (E) Plot of $I_{pa}$ vs. $\nu^{1/2}$. (F) Plot of $E_{pa}$ vs. $\log \nu$. 
5 - 180 mV/s, the oxidation peak potential shifts to positive direction and the oxidation peak current increases gradually (Fig. 4.2.1D). A straight line can be attained for the oxidation peak current ($I_{pa}$) versus square root of potential scan rate ($\nu^{1/2}$), following a linear regression equation of $I_{pa} (\mu A) = 0.0433 \ \nu^{1/2} \ \text{mV}^{1/2}/\text{s}^{1/2} + 0.0733$ with correlation coefficient of ($R^2$) 0.9961 (Fig. 4.2.1E). This indicates that the electrochemical behavior of PAR is diffusion controlled electron transfer process [41].

Moreover the oxidative peak potential of PAR increases and the peak potential values shifted positively, designates that the kinetic limitation in the electrochemical reaction. The anodic peak potential have a linear relationship with natural logarithm of scan rate ($\log \nu$) and its linear regression equation of $E_{pc} (V) = -0.0445 \ \log \nu/\text{mV/s} + 0.1503 \ (R^2 = 0.9987)$ (Fig. 4.1.4F). According to the following equation [42]:

$$\log k_s = \alpha \log (1 - \alpha) + (1 - \alpha) \log \alpha - \log \frac{RT}{nFv} - \alpha (1 - \alpha) n F \Delta E_p / 2.3 RT \ \text{......(4.2.1)}$$

where $k_s$ is the standard heterogeneous reaction rate constant ($s^{-1}$), $n$ is the number of electrons involved in the electrochemical reaction of PAR, $F$ is the Faraday constant (C/mol), $\nu$ is the scan rate (mV/s), $R$ is the universal gas constant (J/K/mol), $T$ is the absolute temperature (K), $\alpha$ is the electron transfer coefficient. The electron transfer coefficient and number of electrons involved in the reaction were calculated to be 0.48 and 2.01. Using the above equation, the rate constant value was found to be 2.05 s$^{-1}$.

**4.2.2 Electrocatalytic oxidation of PTX at GNF/GCE**

The electrocatalytic behavior of GNF/GCE was used towards the oxidation peak current value of PTX. Cyclic voltammetry behavior of 0.1 mM PTX on bare GCE (absence and presence) and GNF/GCE in the presence of 0.1 M KCl containing PBS (pH 7.0) are shown in Fig. 4.2.2A. In bare GCE, PTX exhibits a poor anodic peak current and the anodic peak potential ($E_{pa}$) at +0.294 V (vs. Ag/AgCl) whereas, the GNF/GCE in the presence of
Fig. 4.2.2  

(A) CV of a) and b) bare GCE (absence and presence of PTX) and c) GNF/GCE in the presence of 0.1 mM of PTX at a scan rate 50 mV/s in 0.1 M KCl containing PBS (pH 7.0). (B) CV of GNF/GCE in different concentrations (0.02 to 1.4 x 10^{-4} M) of PTX at a scan rate 50 mV/s. (C) Calibration plot of \( I_{pa} \) vs. PTX concentration. (D) CV of PTX at various scan rates (20 – 120 mV/s). (E) Plot of \( I_{pa} \) vs. \( v^{1/2} \). (F) Logarithmic plot of \( I_{pa} \) vs. \( v \).
PTX exhibits a sharp, well-defined anodic peak current and peak potential ($E_{pa}$) at + 1.228 V (vs. Ag/AgCl). Although, the oxidative peak current of PTX is three times higher than the bare GCE. Additionally, a linear peak current values are distinguished, while increasing the concentration of PTX from $0.02 \times 10^{-4}$ M to $14 \times 10^{-3}$ M which reveals a better electrocatalytic behavior of GNF/GCE as shown in Fig. 4.2.2B and C [43]. The effect of potential sweep rate on the electrochemical oxidation of PTX at GNF/GCE was investigated by CV technique. With the increase of potential sweep rate from 20 - 120 mV/s, the oxidation peak current increases and also peak potential shifts positive direction was observed (Fig. 4.2.2D). A straight line can be attained for the oxidative peak current ($I_{pa}$) versus square root of potential sweep rate ($\nu^{1/2}$), resulting a linear regression equation of $I_{pa} (\mu A) = 0.2596 \nu^{1/2}$ (mV$^{1/2}$/s$^{1/2}$) + 1.0429 with correlation coefficient of ($R^2$) 0.9977 (Fig. 4.2.2E). This indicates that the electrochemical behavior of PTX is diffusion controlled electron transfer process.

Additionally, a straight line was observed between log current and log scan rate (Fig. 4.2.2F), corresponding to the following equation:

$$\log I_p / \mu A = 0.3166 \log \nu / \text{mV/s} - 0.0705; \ (R^2) = 0.9978 \ldots \ldots \ (4.2.2)$$

where $\nu$ is scan rate (mV/s). The slope of 0.31 is close to the theoretically expected value of 0.5. This result confirms that the electrochemical oxidation of PTX is purely diffusion controlled electron transfer process.

4.2.3 Effect of pH on oxidation of PAR and PTX at GNF/GCE

The pH of solution is essential parameter which influences the oxidation of PAR and PTX. The maximum peak current response of both PAR and PTX was studied using 0.1 M KCl containing PBS with different pH ranges (pH 1.0 to 11.0) at GNF/GCE by using cyclic voltammetric method. A shift in oxidation peak potential for both PAR and PTX were observed, while increasing the pH of medium, indicating the oxidative behavior of PAR and
PTX at GNF/GCE are pH dependent reaction as shown in Fig. 4.2.3A and B. The observed anodic peak current values are higher for both systems at pH 7.0. If pH values beyond 8, the oxidation reaction is pH independent, and it is difficult to analyze the electrochemical oxidation of PAR and PTX. Figure 4.2.3C and D shows variation of $I_{pa}$ versus variation of pH and variation $E_{pa}$ versus variation of pH in PAR and PTX can be clearly observed that the peak potential and current were closely related to pH value (pH 7.0) of supporting electrolyte. The linear regression equation of PAR and PTX were $E_{pa} (V) = -0.0596 \text{pH} + 0.6390 (R^2 = 0.9955)$ and $E_{pa} (V) = -0.0512 \text{pH} + 1.1899 (R^2 = 0.9829)$ respectively. The slope value of 59.6 mV/pH unit and 51.2 mV/pH unit, which may close to the Nernstian value for equal amount of electrons and protons involved in the oxidation reaction [44]. This indicates that the electrocatalytic oxidation of PAR and PTX at GNF/GCE implied as two protons and two electron redox process.

Scheme 4.2.1  Electrochemical oxidative mechanism of PAR and PTX at GNF/GCE.
Fig. 4.2.3  Effect of pH on (A and B) CV curves of 0.1 mM of PAR and PTX at GNF/GCE in the presence of 0.1 M KCl containing PBS with various pH ranges (pH 1, 3, 5, 7, 9 and 11) at a scan rate 50 mV/s. (C) Effect of pH on the anodic peak potential ($E_{pa}$) and peak current ($I_{pa}$) of PAR. (D) Effect of pH on the anodic peak potential ($E_{pa}$) and peak current ($I_{pa}$) of PTX.
From the above observation, the reaction scheme would probably via the following mechanistic steps [45]. The electrochemical oxidative mechanism of PAR and PTX are shown in *Scheme 4.2.1*.

### 4.2.4 Chronoamperometry method for PAR at GNF/GCE

The chronoamperometry method was employed to determine diffusion coefficient of PAR oxidation process at GNF/GCE. *Figure 4.2.4A* shows the current-time relationships of GNF/GCE obtained by setting the working electrode potentials of +0.2 V (vs. Ag/AgCl) for PAR at different concentration ranges in 0.1 M KCl containing PBS (pH 7.0).

![Graph showing current-time relationships](image)

*Fig. 4.2.4*  (A) CA obtained at GNF/GCE in the absence and presence of (0.05 to 0.2 mM) of PAR in 0.1 M KCl containing PBS (pH 7.0) at potential step of 20 mV. (B) Cottrell plots drawn using data obtained from CA b) to e) from (A).

In order to calculate the diffusion coefficient ($D$) of PAR, the experimental plot of peak current ($I_p$) versus $t^{-1/2}$ were drawn using comparison graphs of a) to d) that result in straight lines (*Fig. 4.2.4 B*). According to the Cottrell equation [46],

$$I = n F A C D^{1/2} \pi^{-1/2} t^{-1/2}$$  \hspace{1cm} .................................. (4.2.3)

where $n$ is the number of electrons, $F$ is the Faraday constant (C/mol), $A$ is the electrode area (cm$^2$), $C$ is the bulk concentration of an analytes (mol/cm$^3$) and $D$ is the
diffusion coefficient \((cm^2/s)\). The diffusion coefficient \((D)\) value can be obtained from the slopes of the linear plot \((I_p \text{ vs. } t^{-1/2})\) for PAR. The average value of PAR was found to be \(7.5 \times 10^{-6} \text{ cm}^2/\text{s}\), which agrees equitably with last previous literatures. Thus, this result shows that the better electrocatalytic oxidation of PAR occurs at GNF/GCE.

4.2.5 Simultaneous determination of PAR and PTX at GNF/GCE

DPV technique was exploited for the selective and sensitive simultaneous determination of PAR and PTX mixtures at the GNF/GCE in the presence of 0.1 M KCl containing PBS (pH 7.0). The simultaneous determination of PAR and PTX were performed at GNF/GCE by DPV which eliminates the residual charging current values and the pure faraday current values only measured. As can be seen from Fig. 4.2.5, the electrochemical determination of DPV shows the concentration of one species incessantly with other species kept constant. The sharp and well-defined oxidation peak current was increased linearly with increasing the concentration of PAR in the presence of 1 µM PTX as kept constant. On the other hand, oxidative peak current of PTX was increased linearly by the addition of PTX in the presence of 1 µM PAR as kept constant under optimized experimental condition.

The concentration of PAR and PTX was increased linearly, the peak potential of PAR and PTX at + 0.15 V and + 1.05 V (vs. Ag/AgCl) as remained constant (Fig. 4.2.5 A and C). A well-resolved peak potential window (900 mV) for the oxidation of PAR and PTX were observed at GNF/GCE. The calibration curves for PAR and PTX were obtained within the dynamic linear range from \(1 \times 10^{-8} \text{ M} – 13 \times 10^{-8} \text{ M}\) and \(0.5 \times 10^{-8} \text{ M} – 5.5 \times 10^{-8} \text{ M}\) were noted. The linear regression equation of PAR and PTX are \(I_{pa} (\mu A) = 2.2859 \cdot (10^{-8} \text{ M}) + 0.0157\) and \(I_{pa} (\mu A) = 0.8181 \cdot (10^{-8} \text{ M}) + 0.584\) with a correlation coefficient of 0.9987 and 0.9906 as shown in Fig. 4.2.5 B and D. The limit of detection (LOD) was found to be 1.2 nM and 4.5 nM for PAR and PTX using 3σ/slope, where ‘σ’ is the standard deviation of the
mean value for five independent voltammogram of blank solution. The PAR detection limit of the proposed method is comparable with previously reported values as shown in Table 4.2.1 and the PTX detection limit is lower than MWCNTPE [60] and GCE [61]. This result implies that the oxidation of both PAR and PTX at GNF/GCE has wider linear range, lower detection limit and good sensitivity. Schematic diagram illustrations the simultaneous electrocatalytic oxidation of PAR and PTX using GNF/GCE as shown in Scheme 4.2.2.

Scheme 4.2.2  Schematic diagram represents the simultaneous electrocatalytic oxidation of PAR and PTX corresponding to GNF/GCE.
Fig. 4.2.5  (A) DPV of PAR at GNF/GCE in 0.1 M KCl containing PBS (pH 7.0) in presence of 50 μM PTX (1 x 10^{-8} M – 13 x 10^{-8} M from a-m). (B) Plot of $I_{pa}$ vs. conc. of PAR. (C) DPV of PTX at GNF/GCE in 0.1 M KCl containing PBS (pH 7.0) in presence of 50 μM PTX (0.5 x 10^{-8} M – 5.5 x 10^{-8} M from a-j). (D) Plot of $I_{pa}$ vs. conc. of PTX. Scan rate: 20 mV/s, Pulse width: 20 mV, Pulse Amplitude: 25 mV.
Table 4.2.1 Comparison table of PAR determination in different electrodes

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Method</th>
<th>Linear range (µmol/L)</th>
<th>R²</th>
<th>LOD (mol/L)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAP/MWCNT/GCE</td>
<td>DPV</td>
<td>0.79 - 340</td>
<td>0.999</td>
<td>3.3 x 10⁻⁷</td>
<td>[47]</td>
</tr>
<tr>
<td>g-C₃N₄/CTS/GCE</td>
<td>DPV</td>
<td>1.7 - 280</td>
<td>0.9960</td>
<td>0.15 x 10⁶</td>
<td>[48]</td>
</tr>
<tr>
<td>rGO-PEDOT NT/GCE</td>
<td>DPV</td>
<td>1.0 - 35</td>
<td>0.9960</td>
<td>0.4 x 10⁶</td>
<td>[49]</td>
</tr>
<tr>
<td>Pt at CuNPs/C₆₀/GCE</td>
<td>SWV</td>
<td>0.004 - 0.4</td>
<td>0.9980</td>
<td>1.2 x 10⁶</td>
<td>[50]</td>
</tr>
<tr>
<td>MIP/GCE</td>
<td>DPV</td>
<td>1 - 4000</td>
<td>0.9940</td>
<td>0.33 x 10⁶</td>
<td>[51]</td>
</tr>
<tr>
<td>Nafion/TiO₂-graphene/GCE</td>
<td>DPV</td>
<td>1 - 100</td>
<td>0.9962</td>
<td>2.1 x 10⁻⁶</td>
<td>[52]</td>
</tr>
<tr>
<td>MIP/pABSA/ GCE</td>
<td>DPV</td>
<td>0.05 - 100</td>
<td>0.9982</td>
<td>4.3 x 10⁻⁸</td>
<td>[53]</td>
</tr>
<tr>
<td>Pd/GO/GCE</td>
<td>DPV</td>
<td>0.005 - 50</td>
<td>0.9946</td>
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<td>[54]</td>
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<tr>
<td>MIP/PB/GCE</td>
<td>DPV</td>
<td>0.001 - 1</td>
<td>-</td>
<td>5.3 x 10⁻¹⁰</td>
<td>[55]</td>
</tr>
<tr>
<td>SPGrE</td>
<td>CV</td>
<td>10 - 100</td>
<td>0.9879</td>
<td>20 x 10⁻⁹</td>
<td>[56]</td>
</tr>
<tr>
<td>Graphene/GCE</td>
<td>SWV</td>
<td>0 - 20</td>
<td>0.9984</td>
<td>3.2 x 10⁻⁸</td>
<td>[57]</td>
</tr>
<tr>
<td>ERG/GCE</td>
<td>DPV</td>
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<td>0.9960</td>
<td>1.2 x 10⁻⁶</td>
<td>[58]</td>
</tr>
<tr>
<td>P4VP/MWCNT GCE</td>
<td>DPV</td>
<td>0.02 - 450</td>
<td>0.9970</td>
<td>1.6 x 10⁻⁹</td>
<td>[59]</td>
</tr>
<tr>
<td>GNF/GCE</td>
<td>DPV</td>
<td>0.01 – 0.13</td>
<td>0.9987</td>
<td>1.2 x 10⁻⁹</td>
<td>This work</td>
</tr>
</tbody>
</table>

*P4VP/MWCNT – poly (4-vinylpyridine) and multi-walled carbon nanotube
ERG – Electrochemically reduced graphene
g-C₃N₄ – graphitic carbon nitride
Pd/GO – palladium nanoparticles anchored graphene oxide
pABSA – poly (p-aminobenzene sulfonic acid)
SPGrE – Screen printed graphene electrode
MIP – Molecularly imprinted polymer
GCE – Glassy carbon electrode
DPV – Differential pulse voltammetry
SWV – Square wave voltammetry
PB – Prussian blue
4.2.6  Amperometry detection of PAR and PTX at GNF/GCE

Amperometry method can be easily measure the peak current response for the every addition of PAR and PTX with respective time under stirring condition. The typical steady-state catalytic current-time response of GNF/GCE under constant stirring for step-wise injection of 50 μM PAR (50 s) and 50 μM PTX (50 s) into 0.1 M KCl containing PBS (pH 7.0) at applied potential of + 0.2 V and + 1.05 V (vs. Ag/AgCl). Figure 4.2.6 A and C clearly shows that the oxidation peak current increases by increasing the concentration of PAR and PTX. Amperometric response, increased linearly in ranges from 0.1 x 10⁻⁸ M to 1.5 x 10⁻⁸ M and 0.2 x 10⁻⁸ M to 3 x 10⁻⁸ M for PAR and PTX, respectively. Linear calibration was obtained, with a coefficient of PAR and PTX as 0.9982 and 0.9938 respectively, which demonstrates the better relationship between oxidation peak current and concentration of PAR and PTX. Limit of detection was calculated in the linear graph of PAR and PTX were found to be 0.43 nM and 0.75 nM based on signal-to-noise ratio (S/N = 3) respectively (Fig. 4.2.6 B and D). This result implies that the good performance of graphene flakes thus obtained towards the oxidation of PAR and PTX.

4.2.7  Stability, reproducibility and Interference studies

The long term stability of the prepared electrode (GNF/GCE) was tested and storing at RT. After two weeks, the CV response has retained about 99.3 % current response than the recently prepared one. After one month, the stability of the electrode was tested again 25 cycles recording by CV experiment in 0.1 M KCl containing PBS (pH 7.0) about 98.2 % current was retained and its indicates that the GNF/GCE shows a good stability and reproducibility. At different time intervals, the amperometry method were carried out for the determination of PAR and PTX in 0.1 M KCl containing PBS (pH 7.0). The peak current was preserved the same with the relative standard deviation of ± 3.3 % for five determinations.
Fig. 4.2.6  (A) Amperometric response of GNF/GCE at an applied potential + 0.2 V to subsequent addition of different concentrations (0.1 \times 10^{-8} \text{ M} \text{ to } 1.5 \times 10^{-8} \text{ M}) from 50 \, \mu\text{M} \text{ PAR in the presence of } 0.1 \text{ M KCl containing PBS (pH 7.0).} \text{ (B) Calibration plot of anodic peak current (I_{pa}) vs. concentration of PAR.} \text{ (C) Amperometric response of GNF/GCE at an applied potential + 1.05 V to subsequent addition of different concentrations (0.2 \times 10^{-8} \text{ M} \text{ to } 3 \times 10^{-8} \text{ M}) from 50 \, \mu\text{M PTX in the presence of } 0.1 \text{ M KCl containing PBS (pH 7.0).} \text{ (D) Calibration plot of anodic peak current (I_{pa}) vs. concentration of PTX.}
This result strongly suggests that the good reproducibility and stability of the GNF/GCE system.

![Graphene nanoflakes and nanoclay based system](image)

**Fig. 4.2.7** Amperometric response of several interfering compounds at GNF/GCE in 0.1 μM of PAR and PTX via 0.1 M KCl containing PBS (pH 7.0).

The influence of various substances as compounds potentially interfering with the determination of PAR and PTX were studied under optimum conditions. The interfering substances were chosen from the pharmaceuticals and/or in biological samples. The influence of interference species present in the reaction medium was also investigated at GNF/GCE along with PAR and PTX by amperometric method. There is no interference was obtained for determination of PAR and PTX, the interference compounds such as theophylline, guanine, adenine, aspirin, caffeine, folic acid, tyrosine, tryptophan, dopamine (DA), uric acid (UA), ascorbic acid (AA), Fe^{2+} and Na^{+} as shown in **Fig. 4.2.7**. These substances did not interfere with the concurrent determination of PAR and PTX peak current up to a minimum of 100
fold excess. In the determination of PAR and PTX, the maximum concentration of interfering substance was less than ± 5 % error of the tolerance limit. The proposed GNF/GCE was applied for the determination of PAR and PTX in real samples using standard addition method.

4.2.8 Real sample analysis

4.2.8.1 Determination of PAR and PTX in tablets

The proposed amperometric sensing method was examined by analyzing the PAR in four different tablets. The different amount of PAR was prepared by dissolving in 250 mL water by using ultrasonic bath. And then taken different volume of prepared solution was transferred into 50 mL of PBS at pH 7.0. An aliquot of 15 mL of this solution was placed in the electrochemical cell and the certain amount of PTX was spiked using standard addition method. The analytical results are enumerated in Table 4.2.2. The binary mixtures of PAR and PTX were obtained and the average recovery range of PAR and PTX are 99.66 % and 99.82 % were less than ±5% the R.S.D. (n = 5). These results were suitable and acceptable, showing that the proposed method could be efficiently used for the determination of PAR in drug formulations.

4.2.8.2 Determination of PTX in human urine samples

In using the amperometric method for determination of PTX in human urine samples were also investigated. Before measurement, all urine samples were diluted 200 times with PBS (pH 7.0) in order to fit into the linear range of real samples. 1 mL of the urine sample was added to the electrochemical cell containing 15 mL of PBS medium and the certain quantity of PTX was spiked using standard addition method. The PTX was obtained between 96.6 % and 104.8 % as shown in Table 4.2.3. These recovery ranges indicate a good accuracy, satisfactory and repeatability of the proposed method in urine samples.
### Table 4.2.2  Determination of PAR and PTX mixture in tablets (n = 5).

<table>
<thead>
<tr>
<th>Samples</th>
<th>PAR added (μM)</th>
<th>PTX added (μM)</th>
<th>PAR Found (μM)</th>
<th>PAR Recovery (%)</th>
<th>R. S. D. (%)</th>
<th>PTX Found (μM)</th>
<th>PTX Recovery (%)</th>
<th>R. S. D. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.95</td>
<td>95.0</td>
<td>3.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0.94</td>
<td>94.0</td>
<td>1.42</td>
<td>0.97</td>
<td>97.0</td>
<td>2.3</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
<td>0</td>
<td>1.53</td>
<td>102.0</td>
<td>2.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
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<td>4</td>
<td>1.5</td>
<td>1.5</td>
<td>1.45</td>
<td>96.7</td>
<td>3.3</td>
<td>1.56</td>
<td>104.0</td>
<td>3.12</td>
</tr>
<tr>
<td>5</td>
<td>2.0</td>
<td>0</td>
<td>2.15</td>
<td>107.5</td>
<td>3.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>2.0</td>
<td>2.0</td>
<td>2.11</td>
<td>105.5</td>
<td>1.90</td>
<td>2.03</td>
<td>101.5</td>
<td>1.87</td>
</tr>
<tr>
<td>7</td>
<td>2.5</td>
<td>0</td>
<td>2.42</td>
<td>96.8</td>
<td>2.43</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
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<td>9</td>
<td>3</td>
<td>0</td>
<td>2.91</td>
<td>97.0</td>
<td>3.12</td>
<td>-</td>
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<tr>
<td>10</td>
<td>3.0</td>
<td>3.0</td>
<td>2.94</td>
<td>98.0</td>
<td>2.72</td>
<td>2.98</td>
<td>99.4</td>
<td>3.0</td>
</tr>
</tbody>
</table>

### Table 4.2.3  Determination of PTX in urine samples (n = 5).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Added (μM)</th>
<th>Found (μM)</th>
<th>R.S.D (%)</th>
<th>bRecovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine 1</td>
<td>5</td>
<td>4.83</td>
<td>3.2</td>
<td>96.6</td>
</tr>
<tr>
<td>Urine 2</td>
<td>10</td>
<td>10.03</td>
<td>2.29</td>
<td>100.3</td>
</tr>
<tr>
<td>Urine 3</td>
<td>15</td>
<td>15.15</td>
<td>1.34</td>
<td>101</td>
</tr>
<tr>
<td>Urine 4</td>
<td>20</td>
<td>20.96</td>
<td>2.54</td>
<td>104.8</td>
</tr>
<tr>
<td>Urine 5</td>
<td>25</td>
<td>24.87</td>
<td>3.56</td>
<td>99.5</td>
</tr>
</tbody>
</table>

bRecovery is calculated based on the clinical value.
A novel electrochemical sensor for the simultaneous detection of PAR and PTX were investigated based on GNF/GCE. The modified electrode exhibited a strong electrocatalytic activity towards the oxidation of PAR and PTX respectively. The influence of many interfering substances were tested for the peak current response and found that the present system is free from any momentous interference due to presence of redox active molecules like AA and DA. Furthermore, the preparation of GNF/GCE reveals a simple and easier than all previously reported studies. Thus, the most important issues for the direct estimation of PAR and PTX in drug formulations and human urine samples have been proposed. Mechanisms are proposed to elucidate the variation in response owing to pH and reduction of signal due to interfering substances. GNF/GCE was found to be a potentially valuable tool for designing well-organized and tremendously selective electrochemical sensor for PAR and PTX in drug formulations and human urine samples.
4.3 Voltammetric Detection of L-Cysteine, N-Acetyl-L-Cysteine and Glutathione using CoPc@GNF modified Carbon Paste Electrode

Metal phthalocyanines (MPcs) are one of the important redox mediators for various oxidation reactions. The electrochemical oxidation behavior of some of the thiols has been investigated using CoPc@GNF/CPE as an excellent electron transfer mediator. The CoPc@GNF/CPE enhances the electrochemical oxidation peak current as well as to facilitate the redox reaction in the lower over potential ranges. The CoPc@GNF/CPE was utilized as an electron transfer catalyst for the determination of CySH, NAC and GSH under optimized experimental conditions. Due to the enhanced peak current values of CoPc@GNF/CPE with lower detection limits was observed. The present method can be used for detection of thiols in body fluids and human urine samples.

The surface modification of GNF with CoPc was accompanied by a \( \pi - \pi \) interaction as similar as reported in earlier studies. So the GNF was allowed to react with 0.1 M CoPc in ethanol under isolated by filtration.

4.3.1 FE-SEM and EDAX spectrum of CoPc@GNF

The FE-SEM image of cobalt phthalocyanine impregnated graphene flake showed the flaky structure of GNF attached to the surface of rod shaped CoPc. The average size of the CoPc@GNF was found to be 2.40 \( \mu \text{m} \) under the magnification of 10 \( \mu \text{m} \) (Fig.4.3.1a) [62].

![FE-SEM image of CoPc@GNF and EDAX spectrum of CoPc@GNF](image-url)
The elemental composition of CoPc@GNF was confirmed by an energy dispersive X-ray analysis (EDAX) as shown in Fig. 4.3.1b. The major constituents of CoPc@GNF are cobalt, nitrogen, carbon, oxygen and the corresponding composition as given in table.

### 4.3.2 Electrochemical behavior of CoPc@GNF/CPE

The electrochemical behavior of bare GCE, GNF/CPE and CoPc@GNF/CPE was investigated in both electrochemical impedance analysis as well as cyclic voltammetry method using 0.1 M KNO$_3$ containing 10 mM [Fe(CN)$_6$]$^{3-}/4^-$ redox probe. The electron transfer behavior of redox probe is shown in Fig. 4.3.2. A well-defined a single electron reversible transfer process was observed. In bare GCE, a reversible oxidation behavior with a peak potential separation ($\Delta E_p$) of 110 mV at a scan rate of 50 mV/s was observed. In the case of CoPc@GNF/CPE, the peak separation value was found to be 90 mV which is slightly higher than the GNF/CPE system ($\Delta E_p = 85$ mV). However, the peak current value of CoPc@GNF/CPE was higher than the peak current value of GNF/CPE which is due to diode characteristics of the redox reaction. Fig. 4.3.2 shows a peak potential at + 0.80 V (vs. Ag/AgCl) corresponds to single electron transfer process of CoPc i.e., (Co$^{3+}$/Co$^{2+}$) [63]. The enhanced electrocatalytic performance of CoPc@GNF/CPE system suggests that the CoPc modified GNF have a tendency to enhance redox probe.

### 4.3.3 EIS spectra of CoPc@GNF/CPE

Electrochemical impedance spectroscopic measurement (EIS) was carried out to understand the electron transfer at electrode-electrolyte interface on modified electrode surface. The EIS spectrum is consists of a semicircular part and a linear part. The semicircular part at higher frequencies correspond to electron-transfer-limited process and its diameter is equal to the electron transfer resistance ($R_{ct}$) which controls the electron transfer kinetics of redox probe at electrode interface. Meanwhile, the linear part at lower frequencies
Fig. 4.3.2  CV of a) bare GCE, b) GNF/CPE and c) GNF@CoPc/CPE in the presence of 0.1 M KNO$_3$ containing 10 mM [Fe(CN)$_6$]$^{3-/4-}$ at a scan rate of 50 mV/s. Inset shows the presence of cobalt peak in a) GO@CoPc/CPE and b) GNF@CoPc/CPE.

Fig. 4.3.3  Nyquist plots of a) bare GCE and b) GNF@CoPc/CPE in presence of 0.1 M KNO$_3$ containing 10 mM [Fe(CN)$_6$]$^{3-/4-}$. AC Amplitude: 5 mV; Frequency range: 0.01 Hz to 100 kHz. Inset is the Randles circuit.
corresponds to diffusion process [64]. **Figure 4.3.3** shows EIS was carried out in 0.1 M KNO₃ containing 10 mM [Fe(CN)₆]³⁻/⁴⁻ as a redox probe with a frequency range from 0.01 Hz to 10⁵ Hz (Amplitude 5 mV). Randles equivalent circuit model has been used to fit the experimental data where, \( R_s \) is electrolyte resistance, \( R_{ct} \) is charge transfer resistance, \( C_{dl} \) is double layer capacitance and \( Z_w \) is Warburg impedance (Inset: **Fig. 4.3.3**). At bare GCE exhibited a straight line indicates a diffusion limited electrochemical process. Whereas CoPc@GNF/CPE shows a very low resistance at the composite electrode due to the electrostatic interaction between negatively charged [Fe(CN)₆]³⁻/⁴⁻ and positive charged CoPc. This result attributed to the CoPc@GNF/CPE system has a good performance of synergistic effect of GNF and CoPc that could act as excellent electron transfer medium and enhance electron transfer process.

### 4.3.4 Electrocatalytic oxidation of CySH at CoPc@GNF/CPE

Electrocatalytic behavior of CoPc@GNF/CPE was investigated for the electrochemical oxidation of CySH. **Figure 4.3.4A** shows the cyclic voltammetric behavior of CySH at bare GCE (absence and presence), GNF/CPE and CoPc@GNF/CPE in presence of 1 mM of CySH in 0.1 M KCl containing PBS (pH 7.0) at a scan rate of 50 mV/s. In bare GCE, the CySH exhibits a poor oxidation peak current with a peak potential of +0.368 V (vs. Ag/AgCl) whereas, the oxidative peak potential of GNF/CPE is appeared at less positive side (less than 69 mV) and the observed peak current value is two times higher than that of bare GCE. In the case of CoPc@GNF/CPE, CySH exhibits a sharp, well-defined oxidation peak current with a peak potential of +0.36 V (vs. Ag/AgCl). The oxidative peak potential of CySH is shifted less negative (77 mV) and the peak current value is three times higher than the GNF/CPE, indicating that the combination of GNF and CoPc significantly enhanced the electrocatalytic activity towards the oxidation of CySH. Further, a linear increasing of peak current values was noted, while increasing the concentration of 1 mM CySH solution and its
linear ranges from $1.3 \times 10^{-4}$ to $10.4 \times 10^{-4}$ M as shown in Fig. 4.3.4B and C. Cyclic voltammogram were the influence of electrochemical oxidation of CySH using CoPc@GNF/CPE at different scan rates (10 - 100 mV/s) (Fig. 4.3.4D). The observed peak current values has linear relationship with square root of scan rate and its linear regression equation of $I_{pa} (\mu A) = 2.0994 \left( \nu^{1/2}/mV^{1/2}/s^{1/2} \right) - 0.7068 \ (R^2 = 0.9873)$ as shown in Fig. 4.3.4E. These results reveal that the electrocatalytic oxidation of CySH at CoPc@GNF/CPE is diffusion-controlled electron transfer process. According to Randles-Sevick equation [65],

$$I_p = 2.99 \times 10^5 \alpha^{1/2} n^{3/2} A C D^{1/2} X^{1/2} \quad \text{………… (4.3.1)}$$

where $n$ is the number of electrons involving in the electrochemical reaction, $D$ is the diffusion coefficient (cm$^2$/s), $C$ is the bulk concentration of the CySH (mol/cm$^3$), and $\nu$ is the scan rate (mVs$^{-1}$), $\alpha$ is the electron transfer coefficient and $A$ is the electrode surface area (cm$^2$). The diffusion coefficient was calculated to be $2.96 \times 10^{-5}$ cm$^2$/s. In addition, the electron transfer coefficient for the reaction can be obtained from the following equation;

$$E_p = b / 2 \log \nu + \text{constant} \quad \text{………………….. (4.3.2)}$$

The slope can be obtained from the anodic peak potential ($E_{pa}$) versus log scan rate ($\nu$). As shown in Fig. 4.3.4F, the Tafel slope value was found to be 41.3 mV; therefore $b = 82.6 \text{ mV}$, the slope line is equal to $RT/2anF$ and $\alpha$ is found to be 0.64 in the electrode reaction of CySH due to irreversible electrode process. In the present study, the number of protons and electrons involved during oxidation process of CySH is almost equal. This result implies that the electrochemical oxidation reaction of CySH described in the following equation [66]:

$$2\text{CySH} \rightarrow \text{CyS-SCy} + 2\text{H}^+ + 2\text{e}^- \quad \text{………… (4.3.3)}$$

4.3.5 Electrocatalytic oxidation of NAC at CoPc@GNF/CPE

Electrocatalytic behavior of CoPc@GNF/CPE was investigated for the electrochemical oxidation of NAC. Figure 4.3.5A shows the cyclic voltammetric behavior of
Fig. 4.3.4  (A) CV of a) and b) bare GCE (absence and presence of CySH), c) GNF/GCE and d) GNF@CoPc/CPE in the presence of 0.1 mM of CySH at a scan rate 50 mV/s in 0.1 M KCl containing PBS (pH 7.0). (B) CV of GNF@CoPc/CPE in different concentrations (1.3 to 10.4 x 10^{-4} M) of CySH at a scan rate 50 mV/s. (C) Calibration plot of I_{pa} vs. CySH concentration. (D) CV of CySH at various scan rates (10 – 100 mV/s). (E) Plot of I_{pa} vs. \nu^{1/2}. (F) Plot of E_{pa} vs. log \nu.
NAC at bare GCE (absence and presence), GNF/CPE and CoPc@GNF/CPE in presence of 1 mM of NAC in 0.1 M KCl containing PBS (pH 7.0) at a scan rate of 50 mV/s. In bare GCE, NAC exhibits a poor oxidation peak current with an oxidative peak potential of + 0.35 V (vs. Ag/AgCl) whereas, the oxidative peak potential of GNF/CPE is shifted less positive side (less than 53 mV) and the observed peak current value is three times higher than the bare GCE. The CoPc@GNF/CPE in the presence of NAC exhibits a sharp, well-defined oxidation peak current with a peak potential of + 0.36 V (vs. Ag/AgCl) respectively. The oxidative peak potential of NAC is shifted towards negative (63 mV) and the peak current value is four times higher than the GNF/CPE, indicating that the CoPc impregnated GNF enhanced the electrocatalytic activity towards the oxidation of NAC. Additionally, a linear peak current values are noted, while increasing the concentration of 1 mM NAC and its linear ranges from $1.3 \times 10^{-4}$ to $10.4 \times 10^{-4}$ M as shown in Fig. 4.3.5B and C. Cyclic voltammogram were recorded the influence of electrocatalytic oxidation of NAC using CoPc@GNF/CPE at different sweep rates (10 to 100 mV/s) as shown in Fig. 4.3.5D. The observed peak current value has linear relationship with square root of scan rate and its linear regression equation of $I_{pa} (\mu A) = 1.0234 \left( v^{1/2}/mV^{1/2}/s^{1/2} \right) + 2.963$ with correlation coefficient of ($R^2$) 0.9961 as shown in Fig. 4.3.5E. This result reveals that the electrochemical oxidation of NAC at CoPc@GNF/CPE is a diffusion-controlled electron transfer process. According to the Randles-Sevick Equation 4.3.1, the diffusion coefficient of NAC was calculated.

The diffusion coefficient was calculated to be $5.94 \times 10^{-5}$ cm$^2$/s. In addition, the electron transfer coefficient for the reaction can be obtained from the Equation 4.3.2. The slope can be obtained from the anodic peak potential ($E_{pa}$) versus log scan rate ($v$). As shown in the Fig. 4.3.5F, the Tafel slope value was found to be 111.0 mV, which indicates a one-electron transfer reaction is rate limiting step, assuming an electron transfer coefficient is
Fig. 4.3.5  
(A) CV of a) and b) bare GCE (absence and presence of NAC), c) GNF/GCE and 
d) GNF@CoPc/CPE in the presence of 0.1 mM of NAC at a scan rate 
50 mV/s in 0.1 M KCl containing PBS (pH 7.0).  
(B) CV of GNF@CoPc/CPE 
in different concentrations (1.3 to 10.4 x 10^{-4} M) of NAC at a scan rate 50 
M/s.  
(C) Calibration plot of $I_{pa}$ vs. NAC concentration.  
(D) CV of NAC at 
various scan rates (10 – 100 mV/s).  
(E) Plot of $I_{pa}$ vs. $\nu^{1/2}$.  
(F) Plot of $E_{pa}$ vs. log $\nu$.  

$y = 2.0718x - 0.5167$  
$R^2 = 0.9979$  

$y = 1.0234x + 2.963$  
$R^2 = 0.9961$  

$y = 0.0555x + 0.2735$  
$R^2 = 0.9902$
about 0.46 [67]. The Tafel plot depicts that the NAC is totally irreversible diffusion controlled process.

4.3.6 Electrocatalytic oxidation of GSH at CoPc@GNF/CPE

Electrocatalytic behavior of CoPc@GNF/CPE was investigated for the electrochemical oxidation of GSH. Figure 4.3.6A shows the cyclic voltammetric behavior of GSH on bare GCE (absence and presence), GNF/CPE and CoPc@GNF/CPE in presence of 1 mM of GSH in 0.1 M KCl containing PBS (pH 7.0) at a scan rate of 50 mV/s. In bare GCE, GSH exhibits a poor oxidation peak current with an oxidative peak potential of + 0.358 V (vs. Ag/AgCl) whereas, the oxidative peak potential of GNF/CPE is shifted less negative side (less than 38 mV) and the observed peak current value is two times higher than that of bare GCE. The CoPc@GNF/CPE in the presence of GSH exhibits a sharp, well-defined oxidation peak current with a peak potential of + 0.37 V (vs. Ag/AgCl) respectively. The oxidative peak potential of GSH is shifted towards less positive (50 mV) and the peak current value is two times superior than the GNF/CPE, indicating that the CoPc impregnated GNF enhanced and strong electrocatalytic activity towards the oxidation of GSH. Additionally, a linear peak current values are noted, while increasing the concentration of 1 mM GSH and its linear ranges from 0.1 x 10^{-4} to 7.0 x 10^{-4} M as shown in Fig. 4.3.6B and C. Cyclic voltammogram were recorded the influence of electrochemical oxidation of GSH using CoPc@GNF/CPE at different scan rates (10 to 100 mV/s) as shown in Fig. 4.3.6D. The observed peak current value has linear relationship with square root of scan rate and its linear regression equation of $I_{pa} (\mu A) = 0.3931 (v^{1/2}/mV^{1/2}/s^{1/2}) + 0.7608$ with correlation coefficient of $(R^2)$ 0.9923 as shown in Fig. 4.3.6E. This result reveals that the electrochemical oxidation of GSH at CoPc@GNF/CPE is a diffusion-controlled electron transfer process. According to the Randles-Sevick Equation 4.3.1, the diffusion coefficient of GSH was calculated.
Fig. 4.3.6  (A) CV of a) and b) bare GCE (absence and presence of GSH), c) GNF/GCE and d) GNF@CoPc/CPE in the presence of 0.1 mM of GSH at a scan rate 50 mV/s in 0.1 M KCl containing PBS (pH 7.0). (B) CV of GNF@CoPc/CPE in different concentrations (1.3 to 10.4 x 10^-4 M) of GSH at a scan rate 50 mV/s. (C) Calibration plot of $I_{pa}$ vs. GSH concentration. (D) CV of GSH at various scan rates (10 – 100 mV/s). (E) Plot of $I_{pa}$ vs. $\nu^{1/2}$. (F) Plot of $E_{pa}$ vs. log $\nu$. 
The diffusion coefficient was calculated to be $2.64 \times 10^{-5}$ cm$^2$/s. In addition, the electron transfer coefficient for the reaction can be obtained from the Equation 4.3.2. The slope can be obtained from the anodic peak potential ($E_{pa}$) versus log scan rate ($v$). As shown in the Fig. 4.3.6F, the Tafel slope value was found to be 125 mV, which indicates a one-electron transfer reaction is rate limiting step, assuming an electron transfer coefficient is about 0.52. The Tafel plot illustrates that the GSH is totally irreversible diffusion controlled process. In the present study, the number of protons and electrons involved during oxidation process of GSH is almost equal. This result suggests that the electrochemical oxidation reaction of GSH described in the following equation [68]:

$$2\text{GSH} \rightarrow \text{GS-SG} + 2\text{H}^+ + 2e^- \quad \text{(4.3.4)}$$

### 4.3.7 Influence of pH on the oxidation of CySH, NAC and GSH at CoPc@GNF/CPE

The pH of solution is an essential parameter which influences the oxidative peak current and peak potential of CySH, NAC and GSH. The influence of pH on oxidation peak current value was measured at different pH values containing 0.1 mM CySH, NAC and GSH in presence of KCl containing PBS (pH 1-11) at a scan rate of 50 mV/s (Fig. 4.3.7 A, C & E). In these analytes, the anodic peak potential shifted negatively as the increased pH values from 1 to 7, indicating the deprotonating involved in the oxidation process of –SH group and also the peak current was increased with the pH value from 1 to 7 [69]. In basic solution, the peak current and peak potential are disappeared and anodic peak potentials shifted positively as decreased in pH values from 7 to 11, depicts that distribution fraction of RS$^-$ as well as the corresponding peak currents decreased rapidly [70-72]. Therefore, in order to obtain high sensitivity under physiological environment, pH 7 was fixed at an optimum pH value for the determination of CySH, NAC and GSH in all the experiments. A plot of peak potential versus pH was found to be linear over the pH range 1 – 11 (Fig. 4.3.7 B, D & F) with a gradient value of 59 mV/pH consisting with a two proton/two electron transfer process.
Fig. 4.3.7 Effect of pH on CV curves of 0.1 mM of CySH, NAC and GSH at GNF@CoPc/CPE in the presence of 0.1 M KCl containing PBS with various pH ranges (pH 1, 3, 5, 7, 9 and 11) at a scan rate 50 mV/s (A, C and E). Effect of pH on the peak potential ($E_{pa}$) and peak current ($I_{pa}$) of CySH, NAC and GSH (B, D and F).
4.3.8 Chronoamperometric studies for CySH, NAC and GSH at CoPc@GNF/CPE

Chronoamperometry method was employed to determine diffusion coefficient and catalytic reaction rate constant of CySH, NAC and GSH oxidation process at CoPc@GNF/CPE. Figure 4.3.8A, D and G shows the current-time of CoPc@GNF/CPE obtained by setting the working electrode potentials at + 0.35 V, + 0.36 V and + 0.37 V vs. Ag/AgCl for various concentrations of CySH, NAC and GSH in 0.1 M KCl containing PBS (pH 7). In order to calculate the diffusion coefficient (D) of CySH, NAC and GSH, the experimental plots of \( I_p \) versus \( t^{1/2} \) were drawn using comparison graphs of a) to d) gave straight lines (Fig. 4.3.8B, E and H). According to the Cottrell equation [73],

\[
I = nFAD^{1/2}c^{1/2}t^{-1/2}
\]

where ‘n’ is number of electrons, ‘F’ is Faraday constant (C/mol), A is electrode area (cm\(^2\)), \( c \) is the bulk concentration of an analytes (mol/cm\(^3\)) and D is the diffusion coefficient (cm\(^2\)/s). The diffusion coefficient (D) value can be obtained from the slopes of linear plots of \( I \) vs. \( t^{1/2} \). The average value of CySH, NAC and GSH was determined as 2.93 x 10\(^{-5}\) cm\(^2\)/s, 5.9 x 10\(^{-5}\) cm\(^2\)/s and 2.87 x 10\(^{-5}\) cm\(^2\)/s respectively, which agrees equitably with last pervious literatures. Thus, this result shows that the better electrocatalytic oxidation of CySH, NAC and GSH occurred at CoPc@GNF/CPE and this value is in agreement with that obtained by cyclic voltammetry.

In addition, chronoamperometry is also employed to evaluate the catalytic rate constant of CySH, NAC and GSH at CoPc@GNF/CPE (Fig. 4.3.8C, F and I). The catalytic reaction rate constant was determined according to the method described below [74]

\[
\frac{I_C}{I_L} = \gamma^{1/2} [\pi^{1/2} \text{erf} (\gamma^{1/2}) + \exp (-\gamma)/\gamma^{1/2}]
\]

where \( I_C \) and \( I_L \) is the catalytic current and limiting current of CoPc@GNF/CPE in presence and absence of CySH, NAC and GSH and \( \gamma = k_bC_0\tau \) (\( C_0 \) is the bulk concentration of CySH, ...
Fig. 4.3.8  (A) Chronoamperograms obtained at GNF@CoPc/CPE in the absence and presence (0.05, 0.1 and 0.15 mM) of CySH in 0.1 M KCl containing PBS (pH 7.0) at potential step of 350 mV vs. Ag/AgCl/KCl electrode, respectively. (B) Cottrell plots drawn using data obtained from chronoamperograms b), c) and d) of A). (C) Plot of $I_c/I_L$ versus $t^{1/2}$. Data was obtained from the chronoamperograms of a) and d).
Fig. 4.3.8  (D) Chronoamperograms obtained at GNF@CoPc/CPE in the absence and presence (0.05, 0.1, 0.15 and 0.2 mM) of NAC in 0.1 M KCl containing PBS (pH 7.0) at potential step of 360 mV vs. Ag/AgCl/KCl electrode, respectively. (E) Cottrell plots drawn using data obtained from chronoamperograms b), c) d) and e) of D). (F) Plot of $I_c/I_L$ versus $t^{1/2}$. Data was obtained from the chronoamperograms of a) and e).
Fig. 4.3.8 (G) Chronoamperograms obtained at GNF@CoPc/CPE in the absence and presence (0.05, 0.1, 0.15 and 0.2 mM) of GSH in 0.1 M KCl containing PBS (pH 7.0) at potential step of 370 mV vs. Ag/AgCl/KCl electrode, respectively. (H) Cottrell plots drawn using data obtained from chronoamperograms b), c) d) and e) of G). (I) Plot of $I_c/I_L$ versus $t^{1/2}$. Data was obtained from the chronoamperograms of a) and e).
NAC and GSH (mol/cm³)) is the argument of error function. In all cases where γ exceeds 2, the error function is almost to 1 and the above equation can be written as:

\[ \frac{I_C}{I_L} = \gamma^{1/2} \pi^{1/2} = (\pi k_h C_b t)^{1/2} \]  \hspace{1cm} (4.3.7)

where \( k_h \) and \( t \) are the catalytic rate constant (cm³/mol/s) and time elapsed (s) respectively.

From the above equation 4 can be used to calculate the rate constant of catalytic process \( k_h \). The \( k_h \) value was found to be 6.9 x 10² cm³ mol⁻¹ s⁻¹, 4.6 x 10⁴ cm³ mol⁻¹ s⁻¹ and 2.1 x 10² cm³ mol⁻¹ s⁻¹ for CySH, NAC and GSH oxidation process. This value was derived from the slope of the plot of \( I_C/I_L \) vs. \( t^{1/2} \) for the oxidation of 2 mM CySH, NAC and GSH. The value of \( k_h \) illuminates the sharp feature of the catalytic peak observed for the catalytic oxidation of CySH, NAC and GSH at the surface of CoPc@GNF/CPE. The observed \( k_h \) value is nearly close to the previously reported results [75-77].

### 4.3.9 Individual determination of CySH, NAC and GSH at CoPc@GNF/CPE

The DPV method was used to determine the sensitive electrochemical oxidation of CySH, NAC and GSH using CoPc@GNF/CPE as shown in Fig. 4.3.9 A, C and E. The DPV experiments were carried out in the potential range of – 0.1 V to + 0.7 V in 0.1 M KCl containing PBS (pH 7.0) at various concentration of CySH, NAC and GSH. The sharp and well-defined oxidative peak current was increased linearly with increasing the concentration of PAR under optimized experimental condition. The calibration curves of CySH, NAC and GSH were obtained within the dynamic linear ranges from 0.1 x 10⁻⁶ M to 3.7 x 10⁻⁶ M, 0.01 x 10⁻⁶ M to 0.68 x 10⁻⁵ M and 0.01 x 10⁻⁶ M to 0.48 x 10⁻⁶ M respectively. The linear regression equation of CySH, NAC and GSH are \( I_{pa}(\mu A) = 0.3702(\mu M) + 0.0754 \), \( I_{pa}(\mu A) = 8.1480(\mu M) + 0.0129 \) and \( I_{pa}(\mu A) = 2.8219(\mu M) + 0.0651 \) with correlation coefficients of 0.9986, 0.9966 and 0.9902 respectively (Fig. 4.3.9 B, D and F). The limit of detection (3σ/s, σ is the standard deviation) value was found to be 8.1 x 10⁻⁸ M, 1.8 x
Fig. 4.3.9  (A) DPV of 0.1 mM CySH (0.1 μM – 3.7 μM) at GNF@CoPc/CPE in 0.1 M KCl containing PBS (pH 7.0). (B) Plot of $I_{pa}$ vs. conc. of CySH. (C) DPV of 0.1 mM NAC at GNF@CoPc/CPE in 0.1 M KCl containing PBS (pH 7.0). (D) Plot of $I_{pa}$ vs. conc. of NAC. (E) DPV of 0.1 mM GSH at GNF@CoPc/CPE in 0.1 M KCl containing PBS (pH 7.0). (F) Plot of $I_{pa}$ vs. conc. of GSH. Scan rate: 20 mV/s, Pulse width: 20 mV, Pulse Amplitude: 25 mV.
10^{-8} M and 5.3 \times 10^{-8} M for CoPc@GNF/CPE towards the oxidation of CySH, NAC and GSH. This result strongly suggests that the electrochemical oxidation of CySH, NAC and GSH at CoPc@GNF/CPE has lower detection limit, wider linear ranges and good selectivity.

**4.3.10 Amperometric detection of CySH, NAC and GSH at CoPc@GNF/CPE**

Amperometry experiment was utilized to estimate the electrochemical detection of CySH, NAC and GSH under hydrodynamic conditions to obtain the maximum sensitivity and low detection limit. The amperometric response was examined by successively increasing the step-wise injection of 20 \mu M CySH, 10 \mu M NAC and 50 \mu M GSH at every 50 s in presence of 0.1 M KCl containing PBS (pH 7.0). As shown in Fig. 4.3.10 A, C and E, a well-defined response was observed during the successive addition of CySH, NAC and GSH at CoPc@GNF/CPE. A linear relationship between anodic peak current and the concentration of CySH, NAC and GSH was observed and its linear dynamic ranges from 0.2 \times 10^{-7} M – 3.8 \times 10^{-7} M, 0.02 \times 10^{-7} M – 0.38 \times 10^{-7} M and 0.05 \times 10^{-7} M – 0.95 \times 10^{-7} M for CoPc@GNF/CPE respectively. The linear regression equation of CySH, NAC and GSH are

\begin{align*}
I_p (\mu A) &= 0.1592 \times (10^{-7} M) + 0.0428, \\
I_p (\mu A) &= 2.7710 \times (10^{-7} M) - 0.0715 \quad \text{and} \\
I_p (\mu A) &= 0.0085 \times (10^{-7} M) - 0.2060 
\end{align*}

with a correlation coefficient of 0.9972, 0.9983 and 0.9931 for CoPc@GNF/CPE (Fig. 4.3.10 B, D and F). The detection limit (S/N = 3) was calculated in the calibration plot of CySH, NAC and GSH were found to be 9.4 nM, 2.1 nM and 5.2 nM, respectively Thus, the CoPc@GNF/CPE can be used as an excellent electrocatalytic activity for sensitive amperometric detection of CySH, NAC and GSH. These detection limit, linear calibration range and sensitive determination for CySH, NAC and GSH at these modified electrodes are comparable with other modified electrodes as shown in Table 4.3.1, 4.3.2 and 4.3.3. Therefore, the proposed amperometry method for the detection of CySH, NAC and GSH are exhibits a good sensitivity, lower detection limits and thus it has promising
Fig. 4.3.10  Amperometric response of GNF@CoPc/CPE at an applied potential 320 mV, 270 mV and 350 mV to subsequent addition of different concentrations of 0.1 mM CySH, NAC and GSH in presence of 0.1 M KCl containing PBS (pH 7.0) (A, C and D). Calibration plot of peak current ($I_{pa}$) vs. conc. of CySH, NAC and GSH (B, D and F).
<table>
<thead>
<tr>
<th><strong>Electrode</strong></th>
<th><strong>Method</strong></th>
<th><strong>pH</strong></th>
<th><strong>Linear range (µmol/L)</strong></th>
<th><strong>R²</strong></th>
<th><strong>LOD (mol/L)</strong></th>
<th><strong>Ref.</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Au-SH-SiO₂@Cu-MOF/GCE</td>
<td>DPV</td>
<td>5.0</td>
<td>0.02 - 300</td>
<td>0.9940</td>
<td>0.027 x 10⁻⁶</td>
<td>[78]</td>
</tr>
<tr>
<td>AuNR/MWCNT/GCE</td>
<td>CA</td>
<td>7.0</td>
<td>5.0 - 200</td>
<td>-</td>
<td>0.008 x 10⁻⁶</td>
<td>[79]</td>
</tr>
<tr>
<td>MoS₂/PDDA-MC/GCE</td>
<td>Amp</td>
<td>6.0</td>
<td>0.45 - 155</td>
<td>0.9985</td>
<td>0.09 x 10⁻⁶</td>
<td>[80]</td>
</tr>
<tr>
<td>DMBQ/ZnO/NPs/CPE</td>
<td>SWV</td>
<td></td>
<td>0.09 – 340</td>
<td>0.9910</td>
<td>0.05 x 10⁻⁶</td>
<td>[81]</td>
</tr>
<tr>
<td>MAA/MIP/CPE</td>
<td>DPV</td>
<td>7.0</td>
<td>0.02 – 0.18</td>
<td>0.9974</td>
<td>9.6 x 10⁻⁹</td>
<td>[82]</td>
</tr>
<tr>
<td>PTh/TiO₂/FTO electrode</td>
<td>Amp</td>
<td>7.0</td>
<td>0.0006 - 0.005</td>
<td>0.9952</td>
<td>12.6 x 10⁻⁶</td>
<td>[83]</td>
</tr>
<tr>
<td>MoN/N-MWNTs-1/NF/GCE</td>
<td>Amp</td>
<td>7.0</td>
<td>0.0005 – 0.0079</td>
<td>0.9900</td>
<td>3.64 x 10⁻⁶</td>
<td>[84]</td>
</tr>
<tr>
<td>Y₂O₃-NPs/N-rGO/CPE</td>
<td>Amp</td>
<td>7.0</td>
<td>13 - 720</td>
<td>0.9951</td>
<td>0.8 x 10⁻⁶</td>
<td>[85]</td>
</tr>
<tr>
<td>Pt-Fe₃O₄/rGO/GCE</td>
<td>DPV</td>
<td>7.0</td>
<td>0.0001 - 0.001</td>
<td>0.9982</td>
<td>1.0 x 10⁻⁵</td>
<td>[86]</td>
</tr>
<tr>
<td>ZnBi₃₈O₅₈ nanorods/GCE</td>
<td>CV</td>
<td>7.0</td>
<td>0.0001 – 0.02</td>
<td>-</td>
<td>0.074 x 10⁻⁶</td>
<td>[87]</td>
</tr>
<tr>
<td>Carbon QDs/alizarin/GCE</td>
<td>Amp</td>
<td>7.0</td>
<td>0.3 – 3.6</td>
<td>0.9932</td>
<td>90 x 10⁻⁹</td>
<td>[88]</td>
</tr>
<tr>
<td>GNF@CoPc/GCE</td>
<td>Amp</td>
<td>7.0</td>
<td>0.02 – 0.38</td>
<td>0.9972</td>
<td>9.4 x 10⁻⁹</td>
<td><em>This work</em></td>
</tr>
</tbody>
</table>

*MOF – Metal organic framework  
AuNR – Gold nanorods  
MoS₂/PDDA-MC – Molybdenum-sulfur nanocube/poly(diallyldimethylammonium chloride)-mesoporous carbon  
MAA/MIP/CPE – Methacrylic acid based molecularly imprinted polymer modified carbon paste electrode  
PTh/TiO₂/FTO – Polythiophene layer sensitized anatased TiO₂ on F-doped tin oxide  
MoN/N-MWCNTs – Molybdenum nitride nanosheets/N-doped multi-walled carbon nanotubes  
N-rGO – Nitrogen doped reduced graphene oxide  
Pt-Fe₃O₄ – Platinum-magnetite  
ZnBi₃₈O₅₈ – Zinc bismuthate nanorods  
GCE – Glassy carbon electrode; DPV – Differential pulse voltammetry  
SWV – Square wave voltammetry; CA - Chronoamperometry
Table 4.3.2  Comparison table of NAC determination in various modified electrodes

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Method</th>
<th>pH</th>
<th>Linear range (µmol/L)</th>
<th>$R^2$</th>
<th>LOD (mol/L)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiCuNP/CPE</td>
<td>CV</td>
<td>7.0</td>
<td>99 - 890</td>
<td>0.9980</td>
<td>4.1 x 10^{-7}</td>
<td>[89]</td>
</tr>
<tr>
<td>BFT-CNT/GCE</td>
<td>DPV</td>
<td>8.0</td>
<td>10 - 600</td>
<td>0.9997</td>
<td>6.2 x 10^{-8}</td>
<td>[90]</td>
</tr>
<tr>
<td>DMBQ/Pt/CNTs/ CPE</td>
<td>SWV</td>
<td>7.0</td>
<td>0.1 - 600</td>
<td>0.9900</td>
<td>0.29 x 10^{-6}</td>
<td>[91]</td>
</tr>
<tr>
<td>2CBF-CNPE</td>
<td>SWV</td>
<td>7.0</td>
<td>0.05 - 400</td>
<td>0.9900</td>
<td>2.6 x 10^{-8}</td>
<td>[92]</td>
</tr>
<tr>
<td>CuO-CA/GCE</td>
<td>DPV</td>
<td>1.0</td>
<td>0.1 – 5.5</td>
<td>0.9990</td>
<td>1.0 x 10^{-8}</td>
<td>[93]</td>
</tr>
<tr>
<td>2,7-BFCNPE</td>
<td>DPV</td>
<td>7.0</td>
<td>0.07 - 300</td>
<td>0.9980</td>
<td>5.2 x 10^{-8}</td>
<td>[94]</td>
</tr>
<tr>
<td>N-DHPB-MWNT/CPE</td>
<td>DPV</td>
<td>7.0</td>
<td>0.5 - 200</td>
<td>0.9987</td>
<td>0.2 x 10^{-8}</td>
<td>[95]</td>
</tr>
<tr>
<td>CPE</td>
<td>DPV</td>
<td>6.0</td>
<td>30 - 2000</td>
<td>0.9930</td>
<td>10 x 10^{-6}</td>
<td>[96]</td>
</tr>
<tr>
<td>GNF@CoPc/GCE</td>
<td>Amp</td>
<td>7.0</td>
<td>0.2 – 3.8</td>
<td>0.9983</td>
<td>2.1 x 10^{-9}</td>
<td>This work</td>
</tr>
</tbody>
</table>

*SiCuNP – Copper nitroprusside adsorbed on 3-aminopropylsilica
BFT-CNT – 1-benzyl-4-ferrocenyl-1H-[1,2,3]-triazole/carbon nanotube
DMBQ – 8,9-dihydroxy-7-methyl-12H-benzothiazolo[2,3-b] quinazolin-12-one
2CBF – 2-chlorobenzoyl ferrocene
BF – 2,7-bis(ferrocenyl ethyl) fluoren-9-one
N-DHPB – N-(3,4-dihydroxyphenethyl)-3,5-dinitrobenzamide
GCE – Glassy carbon electrode
CPE – Carbon paste electrode
DPV – Differential pulse voltammetry
SWV – Square wave voltammetry
CA – Citric acid
Amp – Amperometry
Table 4.3.3  Comparison table of GSH determination in various modified electrodes

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Method</th>
<th>pH</th>
<th>Linear range (μmol/L)</th>
<th>$R^2$</th>
<th>LOD (mol/L)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA modified gold electrode</td>
<td>SWV</td>
<td>7.4</td>
<td>0.0005 - 5</td>
<td>0.9980</td>
<td>0.14 × 10⁻⁹</td>
<td>[97]</td>
</tr>
<tr>
<td>MB-MSN-DNA/SPE</td>
<td>DPV</td>
<td>7.0</td>
<td>0.001 - 1</td>
<td>0.9696</td>
<td>0.6 × 10⁻⁹</td>
<td>[98]</td>
</tr>
<tr>
<td>DNA-templated CuNPs/GE</td>
<td>DPV</td>
<td>7.5</td>
<td>0.001 - 1</td>
<td>0.9920</td>
<td>0.27 × 10⁻⁹</td>
<td>[99]</td>
</tr>
<tr>
<td>NiO/MWCNT-MEFPE</td>
<td>SWV</td>
<td>6.0</td>
<td>0.08 - 100</td>
<td>0.9951</td>
<td>0.6 × 10⁻⁸</td>
<td>[100]</td>
</tr>
<tr>
<td>pCAF-NC-GCE</td>
<td>CV</td>
<td>7.0</td>
<td>0.5 - 5000</td>
<td>-</td>
<td>-</td>
<td>[101]</td>
</tr>
<tr>
<td>NiHCF/CTAB/AuNPs/Pt UME</td>
<td>DPV</td>
<td>6.5</td>
<td>0.2 - 1.0</td>
<td>0.9987</td>
<td>0.08 × 10⁶</td>
<td>[102]</td>
</tr>
<tr>
<td>rGO/GCE</td>
<td>Amp</td>
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<td>5 - 875</td>
<td>-</td>
<td>5 × 10⁻⁶</td>
<td>[103]</td>
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<tr>
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<td>0.9989</td>
<td>4.0 × 10⁻¹³</td>
<td>[104]</td>
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<tr>
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<td>Amp</td>
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<td>5 - 915</td>
<td>0.9965</td>
<td>5 × 10⁻⁶</td>
<td>[105]</td>
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<tr>
<td>CNF-PDDA/PB/ITO</td>
<td>Amp</td>
<td>7.0</td>
<td>6 – 17.4</td>
<td>0.9956</td>
<td>2.07 × 10⁻⁹</td>
<td>[106]</td>
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<tr>
<td>GNF@CoPc/GCE</td>
<td>Amp</td>
<td>7.0</td>
<td>0.5 – 9.5</td>
<td>0.9931</td>
<td>5.2 × 10⁻⁹</td>
<td>This work</td>
</tr>
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*a* MSN – Mesoporous silica nanoparticles
SPE – Screen printed electrode
GE – Gold electrode
MEFPE – Modified ethynylferrocene carbon paste electrode
pCAF-NC-GCE – poly (caffeic acid) modified nano carbon glassy carbon electrode
rGO/GCE – reduced graphene oxide modified glassy carbon electrode
AuNP/GR/CILE – Gold nanoparticles and graphene and ionic liquid modified carbon paste electrode
Cu/Pt electrode – Copper nanoparticles modified platinum electrode
CNF-PDDA/PB – Carbon nanofibers-poly (diallyldimethylammonium chloride)/Prussian blue nanocomposite
UME – Pt ultramicro electrode
GCE – Glassy carbon electrode
DPV – Differential pulse voltammetry
SWV – Square wave voltammetry
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<td>[99]</td>
</tr>
<tr>
<td>NiO/MWCNT-MEFPE</td>
<td>SWV</td>
<td>6.0</td>
<td>0.08 - 100</td>
<td>0.9951</td>
<td>0.6 × 10^{-8}</td>
<td>[100]</td>
</tr>
<tr>
<td>pCAF-NC-GCE</td>
<td>CV</td>
<td>7.0</td>
<td>0.5 - 5000</td>
<td>-</td>
<td>-</td>
<td>[101]</td>
</tr>
<tr>
<td>NiHCF/CTAB/AuNPs/Pt UME</td>
<td>DPV</td>
<td>6.5</td>
<td>0.2 - 1.0</td>
<td>0.9987</td>
<td>0.08 × 10^{-6}</td>
<td>[102]</td>
</tr>
<tr>
<td>rGO/GCE</td>
<td>Amp</td>
<td>4.0</td>
<td>5 - 875</td>
<td>-</td>
<td>5 × 10^{-6}</td>
<td>[103]</td>
</tr>
<tr>
<td>AuNP/GR/CILE</td>
<td>CV</td>
<td>-</td>
<td>0.00001 - 0.1</td>
<td>0.9989</td>
<td>4.0 × 10^{-13}</td>
<td>[104]</td>
</tr>
<tr>
<td>Cu/Pt electrode</td>
<td>Amp</td>
<td>7.2</td>
<td>5 - 915</td>
<td>0.9965</td>
<td>5 × 10^{-6}</td>
<td>[105]</td>
</tr>
<tr>
<td>CNF-PDDA/PB/ITO</td>
<td>Amp</td>
<td>7.0</td>
<td>6 – 17.4</td>
<td>0.9956</td>
<td>2.07 × 10^{-9}</td>
<td>[106]</td>
</tr>
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<td>GNF@CoPc/GCE</td>
<td>Amp</td>
<td>7.0</td>
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<td>0.9931</td>
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<td>This work</td>
</tr>
</tbody>
</table>

*MSN – Mesoporous silica nanoparticles
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*CNF-PDDA/PB – Carbon nanofibers-poly (diallyldimethylammonium chloride)/Prussian blue nanocomposite
*UME – Pt ultramicro electrode
*GCE – Glassy carbon electrode
*DPV – Differential pulse voltammetry
*SWV – Square wave voltammetry
*ITO – Indium titanium oxide
Scheme 4.3.1 Schematic diagram represents the electrocatalytic oxidation of L-CySH corresponding to GNF@CoPc/CPE.

Scheme 4.3.2 Schematic diagram represents the electrocatalytic oxidation of NAC corresponding to GNF@CoPc/CPE.
Scheme 4.3.3 Schematic diagram represents the electrocatalytic oxidation of GSH corresponding to GNF@CoPc/CPE.

Table 4.3.4 Amperometric determination of CySH in human serum samples (n =5)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Added (µM)</th>
<th>Found (µM)</th>
<th>R.S.D (%)</th>
<th>Recovery (%)</th>
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<tr>
<td>1</td>
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<td>21.72</td>
<td>3.3</td>
<td>108.6</td>
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<td>19.51</td>
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<td>97.55</td>
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<td>101.1</td>
</tr>
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<td>21.02</td>
<td>1.2</td>
<td>105.1</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>19.88</td>
<td>3.9</td>
<td>99.4</td>
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</table>
application in real samples. The schematic diagram shows the electrocatalytic oxidation of CySH, NAC and GSH corresponding to CoPc@GNF/CPE (*Scheme 4.3.1, 4.3.2 and 4.3.3*).

### 4.3.11 Stability, reproducibility and Interference studies

The long term stability of the prepared electrode (CoPc@GNF/CPE) was tested and storing at room temperature. After two weeks, the CV response has retained about 99.4 % current response than the recently prepared one. After one month, the stability of the electrode was tested again 50 cycles recording by CV experiment in 0.1 M KCl containing PBS (pH 7.0) about 97.6 % current was retained and its indicates that the CoPc@GNF/CPE shows a better stability and reproducibility. At different time intervals, the amperometry method were carried out for the determination of CySH, NAC and GSH in 0.1 M KCl containing PBS (pH 7.0). The peak current was preserved the same with the relative standard deviation of 3.5 % for five determinations. This result suggests that the good reproducibility of the CoPc@GNF/CPE.

The influence of various substances as compounds potentially interfering with the determination of CySH, NAC and GSH were studied under optimized experimental conditions. The interfering substances were chosen from the pharmaceuticals and/or in biological fluids. The effect of typical interfering compounds like benzoic acid, tartaric acid, citric acid, oxalate, glucose, alanine, sodium citrate, glutamic acid, sucrose, fructose, lysine, ascorbic acid (AA), glycine, lactose and histidine are not potentially interfered with CySH as shown in *Fig. 4.3.11A*. And also we have investigated the interference of NAC by adding 100-fold excess of nictotinamide adenine dinucleotide (NADH), glutathione, L-cysteine, tryptophan, glycine, L-lysine, uric acid (UA), L-proline and dopamine (DA); 200-fold excess of $S^{3-}$ and $SO_4^{2-}$ as shown in *Fig. 4.3.11B*. The influence of various substances as potentially interfering compounds with the determination of GSH was studied under the optimum conditions with 0.1 μM GSH at pH 7.0. A 300-fold excess of glucose, sucrose, AA, UA,
glycine and alanine; 200-fold excess of methionine, thiourea, tryptophan and glutamic acid; 100-fold excess of NO$_3^-$, Na$^+$ and Mg$^{2+}$ are not affect the selectivity of GSH (Fig. 4.3.11C). These substances are not able to produce any current because of their inability for oxidation at this potential. In the determination of CySH, NAC and GSH, the maximum concentration of interfering substance was less than ± 5 % error of the tolerance limit. The proposed CoPc@GNF/CPE was applied for determination of CySH, NAC and GSH in real samples.

![Image](image_url)

**Fig. 4.3.11** (A) Amperometric response of several interfering compounds at GNF@CoPc/CPE in 0.1 μM of CySH at 0.1 M KCl containing PBS (pH 7.0). (B) Amperometric response of several interfering compounds at GNF@CoPc/CPE in 0.1 μM of NAC at 0.1 M KCl containing PBS (pH 7.0). (C) Amperometric response of several interfering compounds at GNF@CoPc/CPE in 0.1 μM of GSH at 0.1 M KCl containing PBS (pH 7.0).
4.3.12 Real sample analysis

4.3.12.1 Determination of CySH in human serum samples

The amperometric sensing method was examined to detect CySH in presence of human serum samples by using CoPc@GNF/CPE. To confirm the recovery of CySH by adding a certain amount of CySH in serum samples was investigated. An electrochemical cell containing 20 μM of CySH in presence of 10 mL of PBS at pH 7.0 and the certain amount of human serum samples was spiked using standard addition method. As shown in Table 4.3.4, the recovery results were found to be in the range of 97.55 % – 108.6 % and the R.S. D. (n = 5) value was less than 5 %. These recovery results imply that the CoPc@GNF/CPE is strongly used for the detection of CySH in human serum samples.

4.3.12.2 Determination of NAC in tablets and human urine samples

The proposed amperometry sensing method was scrutinized by analyzing the NAC in five different tablets by using CoPc@GNF/CPE. The 600 mg and 400 mg of NAC tablets were prepared by dissolving in 100 mL water by using ultra-sonication. And then taken different volume of prepared solution was transferred into 50 mL of PBS at pH 7.0. An aliquot of 10 mL of this solution was placed in the electrochemical cell for the determination of NAC using above amperometry method. The analytical results are enumerated in Table 4.3.5. The recovery range from 96.2 % to 102.55 % and the R.S.D. (n = 5) was less than 5%. These results were suitable and acceptable, showing that the proposed method could be efficiently used for the determination of NAC in commercial sources.

In using amperometry method for the determination of real sample analysis of NAC in human urine samples were also investigated. Before the measurement, all urine samples were diluted 200 times with PBS (pH 7.0) in order to fit into the linear range of real samples. No other pretreatment process was performed. The standard addition method was used for
### Table 4.3.5  Amperometric determination of NAC in tablets at (n =5)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Added (μM)</th>
<th>Found (μM)</th>
<th>R.S.D (%)</th>
<th>Recovery (%)</th>
</tr>
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<tr>
<td>1</td>
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<td>96.2</td>
<td>2.7</td>
<td>96.2</td>
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<td>200</td>
<td>205.1</td>
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<td>102.55</td>
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<td>100.64</td>
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<tr>
<td>5</td>
<td>600</td>
<td>598.8</td>
<td>2.9</td>
<td>99.8</td>
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### Table 4.3.6  Amperometric determination of NAC in human urine samples at (n =5)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Added (μM)</th>
<th>Found (μM)</th>
<th>R.S.D (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine 1</td>
<td>5</td>
<td>4.8</td>
<td>2.9</td>
<td>96</td>
</tr>
<tr>
<td>Urine 2</td>
<td>10</td>
<td>10.3</td>
<td>3.2</td>
<td>103</td>
</tr>
<tr>
<td>Urine 3</td>
<td>20</td>
<td>19.5</td>
<td>1.5</td>
<td>97.5</td>
</tr>
<tr>
<td>Urine 4</td>
<td>50</td>
<td>51.2</td>
<td>2.1</td>
<td>102.4</td>
</tr>
<tr>
<td>Urine 5</td>
<td>100</td>
<td>98.9</td>
<td>3.6</td>
<td>98.9</td>
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</table>

### Table 4.3.7  Amperometric determination of GSH in blood serum samples at (n =5)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Added (μM)</th>
<th>Found (μM)</th>
<th>R.S.D (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>0</td>
<td>2.72</td>
<td>2.1</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>7.53</td>
<td>1.4</td>
<td>101.4</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>12.32</td>
<td>3.4</td>
<td>98.5</td>
</tr>
<tr>
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<td>15</td>
<td>18.06</td>
<td>2.6</td>
<td>102.1</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>21.84</td>
<td>3.2</td>
<td>97.7</td>
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</tbody>
</table>
testing recovery. The recovery rates of the spiked samples ranged between 96 % and 103 %
(Table 4.3.6), indicating the suggested method is good accuracy, repeatability and free from
interferences of the urine sample matrix.

4.3.12.3 Determination of GSH in serum samples

A high selective and sensitive amperometry method was achieved for the
determination of GSH in blood serum samples by using CoPc@GNF/CPE. To confirm the
recovery of GSH by adding a certain amount of GSH was spiked in serum samples using
standard addition method was examined. These detection results are shown in Table 4.3.7.
The recovery results were found to be in the range of 97.7 % – 102.1 % and the R.S. D.
(n = 5) value was less than 5 %. These recovery results imply that the CoPc@GNF/CPE is
strongly used for the detection of GSH in blood serum samples.

In general, the electrochemical oxidation behavior of thiols has been investigated by
using CoPc@GNF/CPE as an excellent electron transfer mediator i.e., a CoPc impregnated
on GNF modified carbon paste electrode. It exhibits an enhanced oxidative peak current
towards L-CySH, NAC and GSH under optimized experimental condition. The
CoPc@GNF/CPE was used as an electron transfer mediator for the electrochemical oxidation
of thiols at a lower positive potential value ranges with greater sensitivity. The influence of
many interfering substances were tested for peak current response and found that the present
system is free from any momentous interference. Furthermore, the sensor electrode shows a
good sensitivity, selectivity, reproducibility and low detection limit in the individual
detection of L-CySH, NAC and GSH. The proposed method can be used for the
electrochemical detection of NAC in tablet and urine samples. Thus, the proposed method
can be considered as an efficient for detection of thiols in body fluids and urine samples.
4.4 Simultaneous Voltammetry Detection of Dopamine and Uric Acid using Fc/HNT Modified GCE

Ferrocene carboxylic acid have been widely used for numerous electrocatalytic oxidation studies because of its water solubility and used in a solution based electrocatalytic reactions. Ferrocene carboxylic acid forms a self-assembled monolayer on HNT by simple exposure of ferrocene carboxylic acid with in HNT surface. The self-assembled Fc/HNT was confirmed by FT-IR, TGA and cyclic voltammetry studies. In the present work, the development of immobilized redox active molecules like Fc within the HNT surface shows a facile electron transfer for electrochemical oxidation of bioactive molecules such as DA and UA. DPV method was exploited for the selective and sensitive detection of DA and UA at Fc/HNT/GCE as a result of its high current sensitivity. The amperometric method was applied for detection of DA and UA present in pharmaceutical products and urine samples.

4.4.1 FT-IR spectra of Fc/HNT nanocomposite

FT-IR spectra of Fc, HNT and Fc/HNT are shown in Fig. 4.4.1. From the spectrum of HNT, the absorption peak at 3712 cm$^{-1}$, 3616 cm$^{-1}$ and 3481 cm$^{-1}$ are attributed to the stretching of inner surface and inner hydroxyl groups [107]. The strong peaks at 1022 cm$^{-1}$, 787 cm$^{-1}$, 748 cm$^{-1}$ and 669 cm$^{-1}$; it is observed that the first two bands are assigned for symmetric stretching and in-plane vibration of Si-O groups; and last two bands are ascribed to the perpendicular stretching of Si-O groups (Fig. 4.4.1b). Deformation vibration of –OH inner hydroxyl groups (Al$_2$OH), Al-O-Si and Si-O-Si were exhibited at 914 cm$^{-1}$, 549 cm$^{-1}$ and 454 cm$^{-1}$ respectively. In Fig. 4.4.1a, a strong band at 1645 cm$^{-1}$ is attributed to stretching vibration of carbonyl group of Fc [108]. The modified Fc/HNT, a carboxylic acid (-COOH) group was shifted at 1660 cm$^{-1}$ (Fig. 4.4.1c), which confirms that the carbonyl group attaches to the HNT surface.
Fig. 4.4.1 FT-IR spectra of a) Fc, b) HNT and c) Fc/HNT.

Fig. 4.4.2 FE-SEM image of HNT and (B) EDAX spectrum of HNT.
4.4.2 FESEM and EDAX spectrum of HNT

The surface morphology of HNT nanoclay was investigated by FE-SEM image. As seen from Fig. 4.4.2A, the nanotubular structure of HNT with diameters in the range of 20 – 120 nm was found to be open-ended [109]. The average size of HNT nanoclay was estimated to be 20 nm. Elemental composition of HNT was confirmed by energy dispersive X-ray analysis (EDAX) as shown in Fig. 4.4.2B. The major elements for HNTs were Al, Si and O.

4.4.3 TGA of Fc/HNT nanocomposite

Thermal stability and surface modification of HNT and Fc/HNT were confirmed by TGA analysis as shown in Fig. 4.4.3. From HNT, a major weight loss attributed at 470 °C to 568 °C which corresponds to dehydroxylation of structural alumina group [110]. In Fc/HNT, the primary weight loss arise at 30 °C to 120 °C, which may correspond to the loss of adsorbed water molecules [111]; second weight loss arrive at 260 °C to 360 °C, may be due to structural decomposition [112] and third weight loss appear at 420 °C to 570 °C, correspond to dehydroxylation process of aluminosilicates [113].

![TGA curves of a) HNT and b) Fc/HNT.](image_url)
The final weight loss for Fc/HNT sample at 590 °C to 700 °C may be due to the loss of Fc nanoparticles on HNT that concludes the existence of Fc nanoparticles are immobilized on HNT surface.

4.4.4 Electrochemical behavior of Fc/HNT/GCE nanocomposite

Figure 4.4.4A shows the cyclic voltammogram of a) bare GCE, b) HNT and Fc/HNT modified GCE in the presence of 0.1 M KCl containing phosphate buffer solution (pH 7.0) at a scan rate of 50 mV/s. As can be seen, a bare GCE and HNT/GCE exhibits a flat and drab voltammetric response, whereas Fc/HNT/GCE shows a well resolved redox peaks and its anodic and cathodic peak potential were found to be at + 0.108 V and + 0.049 V (vs. Ag/AgCl), respectively. The observed anodic peak current is larger than that of cathodic peak current value and its potential peak separation \( \Delta E_p = E_{pa} - E_{pc} \) was found to be 59 mV at 50 mV/s and the peak current ratio is close to one \( (I_{pa} / I_{pc} \approx 1) \) which indicates that the electrochemical reaction is reversible one. Electrochemical stability and reversibility of Fc/HNT/GCE were done by a repetitive potential sweep at a scan rate of 50 mV/s.

Figure 4.4.4B shows that the peak current does not change during the continuous potential cycles which imply that the monolayer assembly of ferrocene carboxylic acid is stable enough for electrochemical studies.

With increase of potential scan rate of the surface modified Fc/HNT, the redox peak current values are also increasing and the anodic and cathodic peak potential \( (E_{pa} \text{ and } E_{pc}) \) separations does not change [114] significantly from the scan rates of 25-500 mV/s as shown in Fig. 4.4.4C. The observed anodic and cathodic peak current \( (I_{pa} \text{ and } I_{pc}) \) has a linear relationship with scan rate \( (v) \), which indicates that the modified GCE is adsorption controlled electron transfer process i.e. Fc molecules are strongly immobilized on the HNT surface (Fig. 4.4.3D).
Fig. 4.4.4  (A) CV of a) bare GCE, b) HNT/GCE and c) Fc/HNT/GCE in 0.1 M KCl containing PBS (pH 7.0). Scan rate: 50 mV/s. (B) CV of Fc/HNT/GCE in the presence of 0.1 M KCl containing PBS (pH 7.0) at continuous cycles (0-100 cycles). (C) CV of Fc/HNT/GCE in the presence of 0.1 M KCl containing PBS (pH 7.0) at various scan rates a) 25 b) 50 c) 100 d) 200 e) 300 f) 400 and g) 500 mV/s. (D) Linear plot of anodic and cathodic peak current (I_{pa} and I_{pc}) vs. scan rate (υ) from (C).
4.4.5 EIS spectra of Fe/HNT/GCE nanocomposite

The electron transfer behavior of surface-modified electrode process was further confirmed by electrochemical impedance spectroscopy (EIS) by employing electrochemical probe to study charge transfer across the electrode solution interface. The semicircular portion of Nyquist plot at higher frequencies corresponds to electron-transfer limited process and its diameter is equal to electron transfer resistance ($R_{ct}$), which reflects electron transfer kinetics of redox probe on electrode interface [115]. Meanwhile, the linear part of Nyquist plot at lower frequencies corresponds to diffusion process [116].

![Nyquist plots](image)

**Figure 4.4.5** Nyquist plots of a) bare, b) HNT and c) Fe/HNT/GCE in 0.5 mM $K_3[Fe(CN)_6]^{3+/4+}$ containing 0.1 M KCl as the supporting electrolyte. AC amplitude: 5 mV; frequency range: 0.01 Hz to 100 kHz. Inset is the Randles circuit.

**Figure 4.4.5** shows Nyquist plots for a) bare GCE, b) HNT and c) Fe/HNT modified GCE in the presence of 0.5 mM Fe(CN)$_6^{4+/3-}$ in 0.1 M KCl as a supporting electrolyte. The bare GCE exhibits a linearity that represents the characteristics of a diffusion limited process. Modified GCE contain semicircular portions, suggesting that the behavior is electron-transfer...
limited process. HNT/GCE shows a higher interfacial resistance (332Ω), indicating small interface impedance as compared to bare GCE. The interfacial resistance (494Ω) of Fc/HNT/GCE has higher than that of HNT/GCE, when a resistance is introduced into the electrode/solution system, leading to a lower rate of electron transfer of $K_3[Fe(CN)_6]^{3-/4-}$. This result implies that the Fc/HNT successfully immobilized on GCE surface. From the cyclic voltammetry and electrochemical impedance spectroscopy studies, the electrochemical behavior of Fc/HNT/GCE is purely surface confined redox process.

### 4.4.6 Electro-catalytic oxidation of DA at Fc/HNT/GCE

Electro-catalytic behavior of Fc/HNT/GCE was used towards the oxidation peak current value of DA [117]. Figure 4.4.6A shows the cyclic voltammetric behavior of DA on bare GCE (absence and presence) and Fc/HNT/GCE in presence of 0.1 M KCl containing PBS (pH 7.0) at a scan rate of 50 mV/s. In bare GCE, DA exhibits a poor anodic peak current with an oxidative peak potential of +0.025 V (vs. Ag/AgCl) whereas, the oxidative peak potential of Fc/HNT/GCE is shifted towards less positive side (less than 9 mV) and the observed peak current value is four times superior than bare GCE. The redox current increases with each addition of 0.1 M DA solution and its linear ranges from $3 \times 10^{-4}$ to $20 \times 10^{-4}$ M as shown in Fig. 4.4.6B and C. Cyclic voltammogram were recorded in DA at various scan rates in the ranges of 20 - 100 mV/s that increases redox peak current. The linearity of anodic peak current ($I_{pa} = 6.281x - 7.755$, $R^2 = 0.9972$) is possessed by diffusion controlled electron transfer process (Fig. 4.4.6D and E).

### 4.4.7 Electro-catalytic oxidation of UA at Fc/HNT/GCE

A similar trend was observed in case of electrochemical oxidation of UA [118] using Fc/HNT/GCE. Cyclic voltammetry behavior of UA on bare GCE (absence and presence) and Fc/HNT/GCE in the presence of 0.1 M KCl containing PBS (pH 7.0) are shown in
Fig. 4.4.6  (A) CV of a) and b) bare GCE (absence and presence of DA) and c) Fc/HNT/GCE in the presence of 50 μL in 0.1 M of DA at scan rate 50 mV/s in the presence of 0.1 M KCl containing PBS (pH 7.0). (B) CV of Fc/HNT/GCE in different concentrations (0.3 to 2.0 mM) of DA at a scan rate 50 mV/s. (C) Calibration plot of $I_{pa}$ vs. DA concentration. (D) CV of DA at various scan rates (20 – 100 mV/s). (E) Plot of $I_{pa}$ vs. $\sqrt{v}$. 
(A) CV of a) and b) bare GCE (absence and presence of UA) and c) Fc/HNT/GCE in the presence of 50 μL in 0.1 M of UA at scan rate 50 mV/s in the presence of 0.1 M KCl containing PBS (pH 7.0). (B) CV of Fc/HNT/GCE in different concentrations (0.33 to 2.64 mM) of UA at a scan rate 50 mV/s. (C) Calibration plot of $I_{pa}$ vs. UA concentration. (D) CV of UA at various scan rates (20 – 140 mV/s). (E) Plot of $I_{pa}$ vs. $\nu^{1/2}$. 
**Fig. 4.4.7A.** In bare GCE, UA shows a small oxidation peak current and peak potential at +0.27 V (vs. Ag/AgCl) however the modified Fc/HNT in the presence of UA exhibits a sharp, well-defined oxidation peak current and potential at +0.23 V (vs. Ag/AgCl). Further, linear peak current values are noted, while increasing the concentration of UA from $3 \times 10^{-4}$ M to $26 \times 10^{-4}$ M which exhibits the better electrocatalytic behavior of Fc/HNT/GCE as shown in Fig. **4.4.7B and C.** The influence of potential scan rate of electrochemical oxidation of UA was investigated at pH 7.0. The observed peak current values were found to be directly proportional to square root of sweep rate range of 20 - 100 mV/s, which implies that electrocatalytic oxidation of UA is diffusion controlled electron transfer process (**Fig. 4.4.7D and E**).

**4.4.8 Effect of pH on oxidation of DA and UA at Fc/HNT/GCE**

In general, the electrochemical activity of biologically important molecules redox behavior is dependent upon pH of the medium [119]. The electrochemical responses of both DA and UA was studied in 0.1 M KCl containing PBS with different pH range (pH 1.0 to 11.0) at Fc/HNT/GCE by cyclic voltammetric method (**Fig. 4.4.8A and B**). A shift in oxidation peak potential for both DA and UA was observed, while increasing the pH of medium, indicating that redox behavior of DA and UA at Fc/HNT/GCE are pH dependent reaction. The observed anodic peak current values are higher for both system at pH 7.0 and then decreases gradually with increasing the pH medium. **Figure 4.4.8C** shows variation of $I_{pa}$ versus variation of pH in DA and UA, it can be clearly observed that the peak potential and current were closely related to pH value (pH 7.0) of supporting electrolyte. The linear regression equation of DA and UA were $E_{pa}$ (V) = 0.7235 - 0.0526 pH ($R^2 = 0.9975$) and $E_{pa}$ (V) = 0.3561 - 0.0489 pH ($R^2 = 0.9977$) as shown in **Fig. 4.4.8D**. The slope value of 52.6 mV/pH unit and 48.9 mV/pH unit, which may close to the Nernstian value (0.059 V/pH) for a two protons/two electrons reaction. This indicates the electrocatalytic oxidation of both DA
Fig. 4.4.8  Effect of pH on (A and B) CV curves of 0.1 M of DA and UA at Fc/HNT/GCE in the presence of 0.1 M KCl containing PBS with various pH ranges (pH 1, 3, 5, 7, 9 and 11) at a scan rate 50 mV/s. (C) Variation of anodic peak current ($I_{pa}$) of DA (a) and UA (b) vs. pH and (D) Plot of peak potential of DA (a) and UA (b) (E/V) vs. pH.
Graphene nanoflakes and nanoclay based …

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and UA at Fc/HNT modified GCE implied as two protons and two electrons redox process [120, 121].

From the above observation, the reaction scheme would probably via the following mechanistic steps. In the first step, the zero valent ferrocene moiety can be oxidized into ferrocenium ion (Fc+) followed by an electron transfer reaction. Then, Fc+ catalysis the oxidation of DA or UA leads to form a dopaminequinone and allantoin. The electron settling between surfaces confined Fc and Fc+ enhanced peak current of DA and UA. Due to the reversible changes in electronic state of surface confined mediator, which facilitates the overall redox reactions [122] as shown in Scheme 4.4.1.

Scheme 4.4.1  Mechanism of the electrode reaction of DA and UA on Fc/HNT/GCE.

4.4.9  Chronoamperometry method for DA and UA Fc/HNT/GCE

The chronoamperometry method was employed to determine diffusion coefficient and catalytic reaction rate constant of DA and UA oxidation process at Fc/HNT modified GCE. Figure 4.4.9A and D shows the current-time relationships of Fc/HNT/GCE obtained by setting the working electrode potentials of + 0.08 V and + 0.22 V (vs. Ag/AgCl/KCl) for DA
and UA, respectively at different concentration ranges in 0.1 M KCl containing PBS (pH 7.0). In order to calculate the diffusion coefficient \((D)\) of DA and UA, the experimental plots of \(I\) versus \(t^{1/2}\) were drawn using comparison graphs of a) to d) that results in straight lines (Fig. 4.4.9B and E). According to the Cottrell equation [123],

\[
I = nFACD^{1/2} \pi^{-1/2} t^{-1/2}
\]

where \(n\) is the number of electrons, \(F\) is the Faraday constant (C/mol), \(A\) is the electrode area (cm\(^2\)), \(C\) is the bulk concentration of an analyte (mol/cm\(^3\)) and \(D\) is the diffusion coefficient (cm\(^2\)/s). The diffusion coefficient \((D)\) value can be obtained from the slopes of the linear plot \((I_p\ \text{vs.} \ t^{1/2})\) for DA and UA. The average value of DA and UA were found to be \(3.3 \times 10^{-6}\) cm\(^2\)/s and \(2.4 \times 10^{-6}\) cm\(^2\)/s respectively, which agrees equitably with last pervious literatures [124, 125]. Thus, this result shows that the better electrocatalytic oxidation of DA and UA occurs at surface modified Fc/HNT/GCE.

In addition, chronoamperometry is also employed to evaluate the catalytic rate constant of DA at Fc//HNT/GCE (Fig. 4.4.9C). The catalytic reaction rate constant was determined according to the method described in [126]:

\[
\frac{I_C}{I_L} = \gamma^{1/2} \left[ \frac{\pi^{1/2}}{2} \text{erf} \left( \frac{\gamma}{\sqrt{2}} \right) + \exp \left( -\gamma \right) \right] \frac{1}{\gamma^{1/2}}
\]

where \(I_C\) and \(I_L\) is the catalytic current and limiting current of Fc/HNT/GCE in the presence and absence of DA and \(\gamma = k_h C_b t\) (\(C_b\) is the bulk concentration of DA (mol/cm\(^3\))) is the argument of error function. In all cases where \(\gamma\) exceeds 2, the error function is almost to 1 and the above equation can be written as:

\[
\frac{I_C}{I_L} = \gamma^{1/2} \pi^{1/2} = (\pi^{1/2} k_h C_b t)^{1/2}
\]

where \(k_h\) and \(t\) are the catalytic rate constant (cm\(^3\)/mol/s) and time elapsed (s) respectively. Equation 4.4.3 can be used to calculate the rate constant of catalytic process \(k_h\). The \(k_h\) value was found to be \(1.9 \times 10^4\) cm\(^3\) mol\(^{-1}\) s\(^{-1}\) for oxidation of DA, which is close to
Fig. 4.4.9 (A) CA obtained at Fc/HNT/GCE in the absence and presence (0.05 – 0.25 mM) of DA in 0.1 M KCl containing PBS (pH 7.0) at potential step of 34 mV. (B) Cottrell plots drawn using data obtained from DA b) to e) of A). (C) Plot of \( I_{\text{C}}/I_{\text{L}} \) versus \( t^{1/2} \). Data was obtained from the CA of a) and d). (D) CA obtained at Fc/HNT/GCE in the absence and presence of (0.05 – 0.25 mM) of UA in 0.1 M KCl containing PBS (pH 7.0) at potential step of 220 mV. (E) Cottrell plots drawn using data obtained from UA b) to e) of D).
the reported value [127]. This value illuminates the sharp feature of the catalytic peak observed for the oxidation of DA at the surface of Fc/HNT/GCE. This value was derived from the slope of a plot of $I_c/I_L$ vs. $t^{1/2}$ for oxidation of 0.2 mM DA.

Further, the heterogeneous rate constant ($k_s$) for the oxidation of UA at Fc/HNT/GCE was calculated by Velasco equation [128]:

$$k_s = 1.11 \, D_o^{1/2} \,(E_p - E_{p/2})^{-1/2} \, \nu^{1/2}$$

where $D_o$ is apparent diffusion coefficient (cm$^2$/s), $E_p$ is oxidation peak potential, $E_{p/2}$ is half-wave oxidation peak potential and $\nu$ is scan rate (mV/s). The $D_o$ value was determined based on Cottrell slope obtained from chronoamperometry technique. The estimated $k_s$ value for oxidation of UA at Fc/HNT/GCE was found to be $1.47 \times 10^{-3}$ cm/s. The value of $k_s$ illuminates the sharp feature of the catalytic peak observed for the catalytic oxidation of UA at the surface of Fc/HNT/GCE. The observed $k_s$ value is nearly close to the previously reported results [129].

4.4.10 Simultaneous determination of DA and UA at Fc/HNT/GCE

The simultaneous determination of DA and UA mixtures was performed at Fc/HNT/GCE by DPV method, which eliminates the residual charging current and the pure Faraday current value only measured. DPV was employed for simultaneous determination of DA and UA at Fc/HNT/GCE in 0.1 M KCl containing PBS (pH 7.0). Electrochemical determination of DPV shows the concentration of one species incessantly with other species kept constant. Anodic peak current of DA increased linearly by addition of DA in the presence of 50 µL of 1mM UA kept constant. On the other hand, oxidative peak current of UA increased linearly by the addition of known amount UA in the presence of 20 µL of 1 mM DA kept constant under optimized pH value. The concentration of DA and UA increased simultaneously, the peak potential of UA or DA remained constant (Fig. 4.4.10A and C). The
oxidation peak current of DA and UA at surface of Fc/HNT was proportional to concentration of added substrates in linear ranges from $0.6 \times 10^{-6}$ M to $60 \times 10^{-4}$ M and $1.3 \times 10^{-6}$ M to $10 \times 10^{-4}$ M and correlation coefficient is 0.9959 and 0.9925 respectively (Fig. 4.4.10B and D). The detection limits ($3\sigma$/slope, $\sigma$ is standard deviation) of DA and UA were found to be $0.2 \times 10^{-7}$ M and $0.3 \times 10^{-7}$ M respectively. Comparison of Fc/HNT/GCE and other modified electrodes reported in previous literatures are shown in Table 4.4.1. The analytical parameter shows that Fc/HNT/GCE exhibits an electrocatalytic behavior for the independent determination of DA and UA. This result shows that the Fc and Fc$^+$ inside the clay nanotubes act as an electron transfer mediator, indicates the electrocatalytic oxidation of DA and UA.

The excellent electrocatalytic activity with well resolved peak separation provides a sensitive for simultaneous determination of DA and UA using Fc/HNT/GCE in PBS (pH 7.0) as shown in Fig. 4.3.10E. In the presence of both analytes, two well resolved independent anodic peaks were observed for DA and UA, peak separation between these two analytes was found to be 150 mV. The peak potential for an individual analyte does not affect in presence of other electroactive species, which is essential for the independent determination of each analytes. Simultaneous electrochemical response of DA and UA still increased linearly with increase in their concentrations and their linear range from $1.0 \times 10^{-6}$ M to $60 \times 10^{-5}$ M and $10 \times 10^{-5}$ M to $60 \times 10^{-5}$ M with the detection limit of 1.1 $\mu$M and 2.0 $\mu$M, respectively (Fig. 4.4.10F). These results demonstrate that the simultaneous determination of DA and UA at Fc/HNT/GCE achieved optimal electrocatalytic activity, lower detection limit, wider linear range, well-resolved peak potential window, higher selectivity and sensitivity. Schematic diagram illustrates the simultaneous electrocatalytic oxidation of DA and UA at Fc/HNT/GCE as shown in Scheme 4.4.2.
Fig. 4.4.10  (A) DPV of DA at Fc/HNT/GCE in 0.1 M KCl containing PBS (pH 7.0) in presence of 50 μM UA (0.66 – 6.0 μM). (B) Plot of $I_{pa}$ vs. conc. of DA. (C) DPV of UA at Fc/HNT/GCE in 0.1 M KCl containing PBS (pH 7.0) in presence of 20 μM DA (1.3 – 10.6 μM). (D) Plot of $I_{pa}$ vs. conc. of UA. (E) Simultaneous determination of DA and UA using Fc/HNT/GCE in 0.1 M KCl containing PBS (pH 7.0). (F) Plot of $I_{pa}$ vs. concentration (DA – 1 μM to 6000 mM and UA – 1 μM to 60 x 10^{-5} M). Scan rate: 20 mV/s, Pulse width: 20 mV, Pulse Amplitude: 25 mV.
<table>
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<th>Electrode</th>
<th>Method</th>
<th>Conc. range (µM)</th>
<th>pH</th>
<th>R²</th>
<th>Detection Limit (µM)</th>
<th>Ref.</th>
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<td>- -</td>
<td>0.02 0.10</td>
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<td>- -</td>
<td>3.0 20.0</td>
<td>[133]</td>
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<td>0.9959 0.9925</td>
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*GNP – Gold Nanoparticles
GCE – Glassy Carbon Electrode
PAA – Poly (Acrylic acid),
DPV – Differential Pulse Voltammetry
MWNTs – Multiwalled Nanotubes
CNPE – Carbon Nanotube Paste Electrode
MCMS – Magnetic Chitosan Microsphere
LSV – Linear Sweep Voltammetry
PPGE – Pretreated Pencil Graphite Electrode
CPE – Carbon Paste Electrode
PDOP@PtNP – Polydopamine @ Platinum Nanoparticles
PAM/rGO – Polyacrylamide/reduced Graphene oxide
Scheme 4.4.2 Schematic diagram represents the electrocatalytic oxidation of DA and UA corresponding to Fc/HNT/GCE.

4.4.11 Amperometry detection of DA and UA at Fc/HNT/GCE

Amperometry method can be easily measure the current response for the each addition of DA and UA with respective time under stirring condition. The typical steady-state catalytic current-time response of Fc/HNT/GCE under constant stirring for step-wise injection of 20 μM of 1mM DA (50 s) and 30 μM of 1 mM UA (30 s) into 0.1 M KCl containing PBS (pH 7.0) at applied potential of + 0.034 V and + 0.22 V (vs. Ag/AgCl). Figure 4.4.11 A and C clearly show that oxidation peak current increases by increasing the concentration of DA and UA. Amperometric response, increased linearly in range from 0.04 x 10^{-7} M to 4.4 x 10^{-6} M and 0.08 x 10^{-7} M to 6.4 x 10^{-6} M for DA and UA, respectively. Linear calibration plot was obtained, with a coefficient of DA and UA of 0.9977 and 0.9976
Fig. 4.4.11  (A) Amperometric response of Fc/HNT/GCE at an applied potential 34 mV to subsequent addition of different concentrations (4.0 x 10^{-7} M to 4.4 x 10^{-6} M) from 1 mM DA in 0.1 M KCl containing PBS (pH 7.0). (B) Calibration plot of anodic peak current ($I_{pa}$) vs. concentration of DA. (C) Amperometric response of Fc/HNT/GCE at an applied potential 220 mV to subsequent addition of different concentrations (8.0 x 10^{-7} M to 6.4 x 10^{-6} M) from 0.1 mM UA in the presence of 0.1 M KCl containing PBS (pH 7.0). (D) Calibration plot of anodic peak current ($I_{pa}$) vs. concentration of UA.
respectively, which demonstrates the better relationship between oxidation current and concentration. Limit of detection was calculated in the graph of DA and UA and were found to be 12 nM and 23 nM based on signal-to-noise ratio (S/N =3) respectively (Fig. 4.4.11 B and D).

4.4.12 Stability, reproducibility and interference studies

The reproducibility of the developed sensor was evaluated by using amperometric method. Five, different modified electrodes were constructed and their peak current response to 1 μM concentration of DA and UA were investigated. The relative standard deviation (RSD) was found to be 2.8 % and 2.3 % for DA and UA respectively (Fig. 4.4.12 A and B), confirming that the Fc/HNT/GCE was highly reproducible. The influence of interference species present in the reaction medium was also investigated at Fc/HNT/GCE along with DA and UA by amperometric method. Suppression of peak current values of DA and UA was investigated at Fc/HNT/GCE by various possible interfering substances like ascorbic acid (10 times), citric acid, cysteine, tyrosine, tartaric acid, caffeine, glucose, sucrose, NaCl, Ca^{2+}, Mg^{2+}, Zn^{2+}, aspartic acid, epinephrine (EP), and L- dopa as shown in Fig. 4.4.13. These substances did not interfere with the concurrent determination of DA and UA peak current up to a minimum of 100 fold excess.

4.4.13 Real sample analysis

4.4.13.1 Determination of DA in dopamine hydrochloride injection

The Fc/HNT modified electrode for analysis in practical samples was tested. Amperometric method was used in this experiment for the analyses of real sample of DA injection in pharmaceutical products. The injection solution (standard concentration of DA in 10 mg/mL, 1 mL per injection) was diluted in 50 mL standard flask with DD water and 100 μL was pipetted out into each series of 10 mL volumetric flasks and made up with 0.1 M PBS (pH 7.0). An aliquot of 15 mL of this solution was placed in the electrochemical cell for the
Fig. 4.4.12  (A) and (B) Linear current response of DA and UA (1 μM) at different freshly prepared Fc/HNT/GCE (n =5) in 0.1 M KCl containing PBS (pH 7.0).

Fig. 4.4.13 Amperometric response of several interfering compounds at Fc/HNT/GCE in presence of 0.1 μM of DA and UA via 0.1 M KCl containing PBS (pH 7.0).
Table 4.4.2  Determination of DA and UA mixture in dopamine injection (n = 5)

<table>
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<th>Samples</th>
<th>DA injection (μM)</th>
<th>UA Added (μM)</th>
<th>DA Found (μM)</th>
<th>Recovery (%)</th>
<th>R. S. D. (%)</th>
<th>UA Found (μM)</th>
<th>Recovery (%)</th>
<th>R. S. D. (%)</th>
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<td>1</td>
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Table 4.4.3  Determination of DA and UA in urine samples (n = 5)

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<thead>
<tr>
<th>Samples</th>
<th>Analyte</th>
<th>Detected (μM)</th>
<th>Added (μM)</th>
<th>Found (μM)</th>
<th>R.S.D (%)</th>
<th>bRecovery (%)</th>
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<td>2.3</td>
<td>91.4</td>
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<td></td>
<td>UA</td>
<td>10.68</td>
<td>5</td>
<td>16.02</td>
<td>102.1</td>
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<tr>
<td>Urine 2</td>
<td>DA</td>
<td>-</td>
<td>10</td>
<td>10.3</td>
<td>103</td>
<td></td>
</tr>
<tr>
<td></td>
<td>UA</td>
<td>15.87</td>
<td>10</td>
<td>25.67</td>
<td>99.2</td>
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<tr>
<td>Urine 3</td>
<td>DA</td>
<td>-</td>
<td>20</td>
<td>19.89</td>
<td>99.4</td>
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</tr>
<tr>
<td></td>
<td>UA</td>
<td>9.09</td>
<td>20</td>
<td>28.45</td>
<td>97.8</td>
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</table>

bRecovery is calculated based on the clinical value
determination of DA using above amperometric method. The analytical results are listed in Table 4.4.2. These results were satisfactory and acceptable, showing that the proposed method could be effectively used for the determination of DA in commercial sources.

**4.4.13.2 Determination of UA in human urine samples**

In using the amperometric method for determination of UA in human urine samples were also investigated. Before measurement, all urine samples were diluted 200 times with PBS (pH 7.0) in order to fit into the linear range of real samples. 1 mL of the urine sample was added to the electrochemical cell containing 15 mL of PBS medium and the certain amount of DA was spiked using the standard addition method. The binary mixtures of DA and UA were obtained between 91.4 % and 102.1 % as shown in Table 4.4.3. The recoveries indicate the accuracy and repeatability of the proposed method.

A novel electrochemical sensor for simultaneous detection of DA and UA were fabricated based on Fc/HNT modified GCE. Analysis by EIS revealed a lowering of charge transfer resistance by many fold due to the modification of the electrode. The modified electrode exhibited a strong electrocatalytic activity towards the oxidation of DA and UA, respectively. The influence of many interfering substances was tested for peak current response and found that the present system is free from any momentous interference due to presence of redox active molecules like AA, EP, aspartic acid, L-dopa or glucose. Moreover, modified electrode could be used for analysis in real samples. Furthermore, the preparation of modified electrode reveals a simple and easier than all previously reported studies. Thus, the most important issues for the direct estimation of DA and UA in pharmaceutical products and urine samples have been proposed. Mechanisms are proposed to elucidate the variation in response owing to pH, high response to AA and reduction of signal due to interfering substances. This Fc/HNT/GCE was found to be a potentially valuable tool for designing efficient and extremely selective electrochemical sensor design.
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