Electrochemical Determination of Propranolol using Reduced Graphene Oxide Modified Carbon Paste Electrode

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Abstract- In present work, a cardiovascular drug, propranolol (PROP) has been investigated using reduced graphene oxide modified carbon paste electrode (rGO-CPE). A simple, selective and sensitive method has been developed using cyclic voltammetry (CV), differential pulse voltammetry (DPV), square wave voltammetry (SWV) and electrochemical impedance spectroscopy (EIS). At pH-7.0 in 0.1 M phosphate buffer solution (PBS) PROP shows excellent electrooxidation process on rGO-CPE. The EIS data provides charge transfer resistance behaviour at carbon paste electrode (CPE, 96.6 kΩ) and rGO-CPE (46.7 kΩ), which shows the increased electron transfer process at rGO-CPE. The linear calibration curves were obtained in the concentration range of 0.1–2.5 µM (for DPV) and 0.1–5.0 µM (for SWV), respectively. The lower limits of detection values were found to be 0.4×10⁻⁸ M and 0.2×10⁻⁹ M, respectively. The effect of pH, scan rate, electrode kinetics, electroactive surface area, sensitivity and selectivity on the determination of PROP has been studied. The modified electrode rGO-CPE has been successfully employed for the determination of PROP present in pharmaceutical sample.

Keywords- Cyclic voltammetry, Carbon paste electrode, Electrochemical impedance spectroscopy, Propranolol, Square wave voltammetry
1. INTRODUCTION

Propranolol hydrochloride (PROP, 1-(isopropylamino)-3-(1-naphthyloxy)-2-propanol), is a non-selective β-blocker of bronchial and cardiac adrenergic receptors. It is widely used to treat coronary atherosclerosis which reduces exertion-induced angina. In addition to angina, β-blockers reduces force of contraction of muscles and also the heart-rate, hence these drugs are helpful in treating high blood pressure and cardiac arrhythmias. But PROP in combination with calcium channel blockers and organic nitrates enhance its anti-anginal efficacy [1]. During the initial stage of certain cardiac arrhythmias, PROP blocks the role of the sympathetic nervous system. The electrophysiological property of PROP lowers the adrenergically increased calcium influx through β-receptor blockade. At normal therapeutic doses PROP decreases neurologically induced automaticity. But at higher doses it shows some anesthetic effects which leads to lower conduction velocity, lower excitability and also prolonged effective refractory period. The β-blocking effects are more important during normal therapeutic situations than any local anesthetic effects [2]. Therefore the sensitive and selective investigation of PROP using electrochemical method in pharmaceutical formulations is more promising.

Different analytical methods were employed for the investigation of PROP such as chemiluminescence [3,4], spectrophotometry [5,6], spectrofluorimetry [7-10], potentiometry [11-13], flow injection analysis [3], colorimetry [14] and atomic absorption spectrometry [15-17]. However these methods may be selective and accurate but have some drawbacks such as long duration of analysis, complex handling, expensive instruments and extensive pretreatment of sample [18]. Thus the development of convenient method for the selective and sensitive determination of PROP is needed. Electroanalytical method is one such type in which a simple, rapid, selective and sensitive electrochemical sensor can be used for the investigation of electroactive species [19-24]. Different types of electrodes were used for the determination of PROP such as boron-doped diamond electrode [25], MWCNT with graphite and silicon rubber [26], glassy carbon electrode (GCE) [27], nafion coated GCE [28], carbon paste electrode (CPE) [29], gold electrode [30] and graphite-silicon rubber electrode [31].

Electroanalytical methods based on the chemically modified electrodes with reduced graphene oxide (rGO) shows high applicability, selectivity and sensitivity towards the investigation of electroactive species [32-34]. Various reducing agents were used for the synthesis of rGO such as sodium borohydride, hydrazine, hydroquinone, dimethylhydrazine etc. Since the formation of irreversible agglomerates through π-π stacking and van-der Waals force of attractions of rGO leads to an increase in the electrocatalytic activity [35]. Thus rGO modified electrodes are found to be the promising electrochemical sensors for the investigation of electroactive molecules. In present work rGO modified carbon paste electrode has been used for the quantification of PROP.
2. EXPERIMENTAL

2.1. Chemicals and apparatus

All the chemicals used were of analytical grade and used as received without any further purifications. Propranolol (PROP) was purchased from Sigma-Aldrich, Bangalore, India. Potassium dihydrogen phosphate (KH₂PO₄), dipotassium hydrogen phosphate (K₂HPO₄) and potassium chloride (KCl) were purchased from Merck, Mumbai, India. Potassium ferricyanide (K₃[Fe(CN)₆]) was procured from HiMedia Laboratories Pvt. Ltd. Reduced graphene oxide (rGO) procured from United Nanotech Innovations Pvt. Ltd., Bangalore, India.

CV, DPV and SWV measurements were carried out using electrochemical workstation CHI660D (CH Instruments, USA) and pH measurements were performed by Equiptronics pH meter (EQ-611). Artificial urine and serum samples were prepared according to B.C. Lourencaio et al. [36]. From local pharmaceutical shop PROP tablet samples (Inderal-10) were procured for the real sample analysis. Doubly distilled water was used to prepare the solutions. A typical three electrode system which consists reference electrode (Silver/Silver chloride), auxiliary electrode (platinum wire) and working electrode (carbon paste electrode) has been used for the electrochemical experiments.

2.2. Preparation of modified electrode

The carbon paste electrode (CPE) was prepared by mixing the graphite powder and mineral oil in the ratio of 70:30 (w/w) in an agate mortar. The resulting homogeneous paste was filled firmly into a glass tube of 6 cm length and 3 mm inner diameter. The obtained CPE surface was renewed by polishing on the butter sheet until the shiny surface was formed. An electrical contact has been established by inserting the copper wire on the other end of the tube consisting carbon paste. Similarly modified carbon paste electrode was developed by mixing various amount of rGO (2–6%) into the graphite powder and mineral oil. The 3% rGO composite CPE showed very good results compared to other compositions of the modifier (Fig. 3). Based on the results the optimum composition of rGO-CPE has been found to be 77:20:3 (w/w) with respect to graphite powder: mineral oil: rGO.
3. RESULTS AND DISCUSSION

3.1. Electrochemical characterization of modified electrodes

The modified electrode has been characterized by using cyclic voltammetry (CV). The redox behaviour of the 1 mM K₃[Fe(CN)₆] exhibits the electron transfer kinetics at the unmodified (CPE) and modified electrodes (rGO-CPE). Fig. 1 shows the reversible peaks of [Fe(CN)₆]³⁻⁻/⁴⁻ redox couple on CPE (curve a) with a peak to peak separation (ΔEₚ) of 130 mV and for rGO-CPE (curve b) ΔEₚ was found to be 80 mV. The rGO-CPE showed good peak current enhancement because of the good electron transfer activity at the surface of the modified electrode. Randles-Sevcik equation has been used to calculate the electroactive surface area [37]:

\[ I_p = 2.69 \times 10^5 n^2 A D_0^{1/2} C_0^* \nu^{1/2} \]

where \( I_p \) refers to the anodic peak current (µA), \( n \) is the number of electrons transferred, \( A \) is surface area of the electrode (cm²), \( D_0 \) refers to the diffusion coefficient (cm²s⁻¹), \( C_0^* \) is the concentration of K₃[Fe(CN)₆] (mol cm⁻³) and \( \nu \) is the scan rate (Vs⁻¹). For K₃[Fe(CN)₆], \( n=1, D_0=7.6\times10^{-6} \text{ cm}^2\text{s}^{-1} \) and electroactive surface area of the electrodes can be calculated using the slope of \( I_p \) verses \( \nu^{1/2} \). By substituting the values, the electroactive surface area of CPE and rGO-CPE have been calculated and found to be 0.103 and 0.256 cm², respectively. This clearly indicates that the rGO modified CPE has got higher surface area and showed good electron transfer than CPE.

![Cyclic voltammogram for 1 mM K₃[Fe(CN)₆] in 0.1 M KCl solution on (a) CPE and (b) rGO-CPE at pH-7, \( \nu=100 \text{ mV s}^{-1} \)](image)

Furthermore, the electrochemical impedance spectroscopy (EIS) has been used to study the charge transfer capacity of the modified (rGO-CPE) and unmodified electrodes (CPE).
Nyquist plots were obtained in the frequency range of 0.1 to $10^5$ Hz on [Fe(CN)$_6$]$_{3/4}$- redox couple (Fig. 2). The plots consists two parts, a semicircle part (at higher frequency) and linear plot (at lower frequency). The semicircle part shows the charge transfer resistance ($R_{ct}$) with electron transfer limited process and linear part indicates the diffusion limiting step [38,39]. In Fig. 2, the inset shows the Randle’s equivalent circuit in which $C_{dl}$ refers to electrical double-layer capacitance, $R_s$ is the solution resistance, $W$ is the Warburg diffusion element and $R_{ct}$ refers to the charge transfer resistance. Larger semicircle with linear tail indicates the increase in $R_{ct}$ (CPE=96.6 kΩ) and smaller semicircle with linear tail shows the decrease in $R_{ct}$ (rGO-CPE=46.7 kΩ). Thus the rate of electron transfer is more at rGO-CPE compared to the CPE.

The apparent electron transfer rate constant ($k_{app}$) has been calculated by using the equation [40]:

$$k_{app} = \frac{RT}{F^2R_{ct}C}$$

where $R$ = universal gas constant (8.3142 JK$^{-1}$mol$^{-1}$), $T$=absolute temperature (298 K), $F$=Faraday’s constant (96,487 Cmol$^{-1}$), $R_{ct}$=charge transfer resistance in kΩ and $C$=concentration of K$_3$[Fe(CN)$_6$] in mM.

The $k_{app}$ values were found to be 2.7551×10$^{-9}$ and 5.6990×10$^{-9}$ cm s$^{-1}$ for CPE and rGO-CPE, respectively. This clearly indicates that the increase in $k_{app}$ value and low charge transfer resistance ($R_{ct}$) promotes the electron transfer process on the rGO-CPE. Hence the proposed rGO-CPE acts as better sensor for the investigation of the analyte of interest.

![Electrochemical impedance spectra of CPE and rGO-CPE](image-url)

**Fig. 2.** Electrochemical impedance spectra of CPE and rGO-CPE
3.2. Influence of modifier

Modification of carbon paste electrode results in good sensitivity and selectivity for detection of electroactive substances [41]. The rGO is one such modifier which increases the surface activity and also the conductivity of the carbon paste electrode. The modifier mixed with carbon paste in various ratio (wt%) was used for voltammetric determination of 1 mM PROP in 0.1 M phosphate buffer solution (pH -7.0). Fig. 3 shows the effect of rGO concentration (2.5, 5.0, 7.5, 10.0 and 12.5 mg in wt%) on peak current of PROP. The composition of 3-wt% of rGO-CPE showed better current enhancement compared to other compositions of modifier. Decrease in peak intensity was observed above 3-wt% of rGO which may be attributed to the increased resistivity of the electrode surface [42]. Hence, the composition of rGO in the modified electrode rGO-CPE was fixed at 3-wt% has optimum for the determination of PROP.

![Fig. 3. (a) Effect of modifier (rGO) composition on peak current at pH-7.0; (b) Variation of peak current with the composition of modifier (wt%)](image)

3.3. Influence of pH

The electrochemical reactions in aqueous solution are pH dependent which involves the addition or removal of electrons from the electroactive substance and results in the gain or loss of a proton [43]. The effect of pH on the oxidation of PROP at rGO-CPE was studied at various pH ranging from 2.0 to 9.0 (Fig. 4a). Anodic peak current increases with increase in pH up to 7.0 and it was found to be decreased above 7.0 (Fig. 4b). Thus pH-7.0 was selected as optimum for the analysis of PROP. The electro-oxidation of PROP indicates that it involves two protons and two electrons transfer processes [44]. The probable oxidation mechanism of PROP has given in Scheme 2.
3.4. Electrocatalytic activity of PROP on rGO-CPE

Cyclic voltammetric method showed good electrocatalytic activity of PROP in 0.1 M phosphate buffer solution (PBS) with scan rate of 100 mVs\(^{-1}\) at CPE and rGO-CPE (Fig. 5). The potential was applied in between 0.0 to 1.6 V and the anodic peak was observed at 1.0733 V at rGO-CPE and 1.1196 V at CPE. The higher peak current was observed on rGO-CPE (382.4 µA) than that of CPE (87.57 µA). The anodic peak may be due to the oxidation of secondary alcoholic group in PROP [45]. This clearly indicates that the rGO-CPE shows better electron transfer kinetics towards the oxidation of PROP. The increased electroactive surface area and excellent electron transfer ability of rGO makes the rGO-CPE as a better sensor for the investigation of PROP. The irreversible anodic peak indicates the formation of carbonyl group which involves 2e\(^{-}\) and 2H\(^{+}\) transfer process [46]. Thus rGO-CPE exhibits good electrocatalytic performance than CPE towards the determination of PROP.
3.5. Scan rate studies

The diffusion or adsorption nature of PROP on rGO-CPE was examined by applying different scan rates using cyclic voltammetry. Effect of scan rate (v) on peak current (I_{pa}) was studied by recording cyclic voltammograms on rGO-CPE in 1 mM PROP (0.1 M PBS, pH 7.0) at different scan rates varying from 25 to 200 mVs^{-1}. Fig. 6 clearly indicated that the anodic peak current has been increased with increase in scan rate according to Randles-Sevcik equation. Also, a shift in peak potential towards more positive side was observed and it may be due to the double layer capacitance (C_d), uncompensated resistance (R_u) and adsorption of small amount of electroactive species or faradaic changes at the electrode surface [47]. It also indicates the irreversible electrochemical reaction of PROP. The electron transfer process is more rapid compared to the diffusion rate of the analyte from bulk solution to the electrode surface, hence the process is diffusion controlled [48]. The linear regression equation for scan rate (v) and peak current (I_{pa}) is given by: I_{pa} (\mu A)=13.7+3.7266 v (Vs^{-1}); R^2=0.9906. Similarly, the linear regression equation for peak current (I_{pa}) verses square root of scan rate (v^{1/2}) is given by: I_{pa} (\mu A)=210.27+60.8328 v^{1/2} (Vs^{-1}); R^2=0.9984. The linear relationship between log I_{pa} and log v is given by: log I_{pa} (\mu A)=0.9549+0.6803 log v (Vs^{-1}); R^2=0.9947. The obtained slope value of 0.6 is nearly equal to the theoretical value of 0.5, hence the process is diffusion controlled [38].

The standard rate constant value has been calculated by using Laviron equation [34] for an irreversible reaction:

\[ E_p = E^0 + \left( \frac{2.303RT}{anF} \right) \log \left( \frac{RTk^0}{anF} \right) + \left( \frac{2.303RT}{anF} \right) \log v \]
where, $R$=universal gas constant (8.314 J mol$^{-1}$K$^{-1}$), $T$=absolute temperature (298 K), $k_0$=standard rate constant in s$^{-1}$, $\alpha$=electron transfer co-efficient, $F$=Faraday’s constant (96,487 C mol$^{-1}$).

The linear relationship between $E_p$ and log $v$ is given by the equation: $E_p (V)=0.5655+0.2782 \log v$ (V s$^{-1}$); $R^2=0.9903$. By substituting the slope value of 0.2782 in the above equation the standard rate constant ($k^0$) has been calculated. For irreversible electrode reaction the value of $\alpha$ is equal to 0.5 and the number of electrons transferred in the electrochemical oxidation process of PROP was found to be 2 [48]. Hence, the standard rate constant ($k^0$) is found to be 16.30 µs$^{-1}$.

**Fig. 6.** (a) Effect of scan rate on electrooxidation of PROP in 0.1 M PBS at rGO-CPE. Linear plots of (b) $I_{pa}$ and $v^{1/2}$, (c) log $I_{pa}$ and log $v$ and (d) $E_p$ and log $v$
Fig. 7. (a) SWV for the oxidation of PROP at different concentrations (0.1 to 5.0 µM) in 0.1 M PBS of pH-7.0; (b) Linear plot of concentration and Ipa

Table 1. Comparison between the previously reported analytical parameters at different modified electrodes

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Analytical Technique</th>
<th>Linear Range (µM L⁻¹)</th>
<th>LODᵃ (µM L⁻¹)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MWCNTs/GCEᵇ</td>
<td>DPVⁱ</td>
<td>0.09-7.0</td>
<td>0.05</td>
<td>[27]</td>
</tr>
<tr>
<td>AgNPs-IL-GO-NF/GCEᶜ</td>
<td>SWVʲ</td>
<td>0.079-2.96</td>
<td>0.013</td>
<td>[38]</td>
</tr>
<tr>
<td>SPEᵈ</td>
<td>DPV</td>
<td>0.033-3.22</td>
<td>0.0056</td>
<td>[43]</td>
</tr>
<tr>
<td>MWCNTs-PAH/GCEᵉ</td>
<td>DPAdASVᵏ</td>
<td>0.074-0.63</td>
<td>0.026</td>
<td>[45]</td>
</tr>
<tr>
<td>CuNPs-PEDOT:PSS/GCEᶠ</td>
<td>SWV</td>
<td>0.5-2.9</td>
<td>0.18</td>
<td>[49]</td>
</tr>
<tr>
<td>rGO-CPEᵍ</td>
<td>SWV</td>
<td>0.1-5.0</td>
<td>0.002</td>
<td>Present work</td>
</tr>
</tbody>
</table>

ᵃLimit of detection
ᵇMulti-walled carbon nanotube glassy carbon electrode
ᶜSilver nanoparticles, ionic liquids, graphene oxide, nafion glassy carbon electrode
ᵈScreen printed electrode
ᵉMulti-walled carbon nanotube-poly(allylamine hydrochloride) modified glassy carbon electrode
ᶠCopper nanoparticles-graphene oxide-carbon black-poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) modified glassy carbon electrode
ᵍNitrogen-Containing Tetrahedral Amorphous Carbon Electrode
ʰReduced graphene oxide modified carbon paste electrode
ⁱDifferential pulse voltammetry
ʲSquare wave voltammetry
ᵏDiferential-pulse adsorptive anodic stripping voltammetry
3.6. Analytical application of PROP

3.6.1. Square wave voltammetry (SWV)

In comparison with CV and DPV, SWV possesses good electrochemical response with increased electron transfer kinetics. The parameters such as frequency ($f$), amplitude ($a$), accumulation potential ($E_p$) and quiet time ($t$) were optimized. The optimum values obtained for these parameters in 1 mM PROP were as follows: $f=60 \text{ Hz (10–100 Hz)}$, $a=20 \text{ mV (10–60 mV)}$, $E_p=4 \text{ mV (1–6 mV)}$ and $t=3 \text{ s (1–5 s)}$. The SWV curves were obtained in the linear concentration range of 0.1 to 5.0 µM (Fig. 7). The linear regression equation obtained for anodic peak current verses concentration is given by: $I_{pa} (\mu A)=2.1822\times10^{-6} M+1.73248\times10^{-4}$; $R^2=0.9955$. The limit of detection (LOD) was calculated using the formula $3\times(\sigma/m)$, where $\sigma$ is the standard deviation of blank and $m$ is the slope of calibration graph. The LOD value was found to be 0.2 nM. Thus the proposed rGO-CPE shows excellent electrocatalytic activity towards the determination of PROP. The previously reported experimental data of PROP has been shown in Table 1.

3.7. Interference study

For successful investigation of PROP on rGO-CPE, it is necessary to evaluate interfering species which affects the sensitivity and reliability of the electrode. Various ionic species (sodium chloride, potassium chloride, calcium carbonate and magnesium sulphate) and biological samples (ascorbic acid, glucose, glycine, uric acids, dopamine and epinephrine) were added to the test solution in order to check the interference in the oxidation of PROP. The species Na$^+$, K$^+$, Ca$^{2+}$, Mg$^{2+}$ and ascorbic acid, glucose, glycine, uric acid, dopamine and epinephrine didn’t show any interferences in the electrooxidation of PROP in 0.1 M PBS at pH-7.0. Thus the electrocatalytic performance of rGO-CPE towards the oxidation of PROP is independent of interference substances.

3.8. Real sample analysis

The proposed sensor successfully employed for the investigation of PROP present in the pharmaceutical samples using SWV. The Aquazide-25 tablet samples were used for the analysis. The tablets were powdered in a mortar and dissolved in 0.1 M phosphate buffer solution. The obtained solution was filtered and diluted to get the required experimental concentration. Finally the square wave voltammograms were recorded for two concentrations (Fig. 8). Table 2 shows the percent recoveries of PROP and were found to be 100.34% and 100%, respectively. Hence the proposed sensor exhibited the good analytical applicability, reliability and high sensitivity towards the investigation of PROP present in the pharmaceutical sample.
Fig. 8. Square Wave Voltammograms of PROP present in tablet sample (Aquazide-25) using standard addition method: (a) 1 µM (tab) (b) 2 µM (tab) (c) 2 µM (tab) + 1 µM (std) and (d) 2 µM (tab)+2 µM (std) (e) 2 µM (tab)+3 µM (std) (f) 2 µM (tab)+4 µM (std) at pH-7 in 0.1 M PBS

Table 2. Detection of PROP present in pharmaceutical samples

<table>
<thead>
<tr>
<th>Technique</th>
<th>Tablet Sample</th>
<th>Added (µM)</th>
<th>Found (µM)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWV*</td>
<td>1</td>
<td>1</td>
<td>1±0.5</td>
<td>100.34</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>2±0.1</td>
<td>100</td>
</tr>
</tbody>
</table>

*Square Wave Voltammetry

3.9. Analysis of propranolol in human serum and urine samples

The presence of PROP in human urine and serum samples were determined by SWV using rGO-CPE.

Table 3. Results obtained from detection of PROP present in an artificial urine and serum samples by SWV on rGO-CPE

<table>
<thead>
<tr>
<th>Technique</th>
<th>Analyte</th>
<th>Added (µM L⁻¹)</th>
<th>Found (µM L⁻¹)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWV*</td>
<td>Urine</td>
<td>1.0</td>
<td>1.0±0.1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>2.0</td>
<td>2.2±0.2</td>
<td>102</td>
</tr>
</tbody>
</table>

*Square wave voltammetry

The percentage recovery for the detection of PROP in spiked urine and serum samples were tabulated (Table 3). The percentage recovery values have been calculated from the analytical curves obtained from the standard sample concentration to the actual spiked concentration. Thus SWV shows good analytical response even for smaller amount of PROP.
present in urine and serum sample. Hence rGO-CPE exhibits better sensitivity, stability and reproducibility for the determination of trace amount of PROP present in biological samples.

4. CONCLUSION

In the present work, the reduced graphene oxide modified carbon paste electrode (rGO-CPE) showed enhanced electron transfer activity and increased charge transfer efficiency towards the electrooxidation of propranolol (PROP). The electrochemical impedance spectra (EIS) shows the charge transfer resistance was high at the unmodified electrode compared to the modified electrode. The rGO-CPE shows better selectivity, sensitivity and reliability towards the determination of PROP. The electrooxidation of PROP on rGO-CPE follows diffusion-controlled process which involves two electron and two proton transfer reaction. The good linearity concentration range shows the low limit of detection. The analytical utility of the proposed sensor for real sample analysis has been evaluated by investigating the PROP present in the pharmaceutical sample.

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