ABSTRACT:
A polymerized film of gabapentin was prepared on the surface of a glassy carbon electrode (GCE) in phosphate buffer solution by electropolymerisation method using cyclic voltammetric technique. The poly (gabapentin) film modified glassy carbon electrode was calibrated with standard potassium ferrocyanide solution in 1 M KCl as a supporting electrolyte. This modified electrode used to study the electrochemical behavior of gemfibrozil, a lipid lowering drug in aqueous alcohol medium by cyclic voltammetric technique. It was found that the oxidation peak current of gemfibrozil at the modified GCE was greatly improved compared with that at the bare GCE. The effects of scan rate, pH, supporting electrolyte concentration, % of solvent and concentration of gemfibrozil were examined. With high sensitivity and selectivity, micro molar detection limit, high reproducibility together with ease of preparation and regeneration of the electrode surface make the electrode very stable for the determination of gemfibrozil in pharmaceutical and clinical preparations.

Keyword: Cyclic Voltammetry, Gemfibrozil, Gabapentin, Electropolymerisation, Modified glassy carbon electrode

1. Introduction
Gemfibrozil (Figure 1), (2, 2-dimethyl-5-(2,5-xylyloxy) valeric acid,) is an acidic drug, which has been demonstrated to be a safe and effective hypolipemic agent and to lower the incidence of coronary heart disease in humans. Gemfibrozil is a potent lipid regulating drug whose major effects are to increase plasma high density lipoproteins (HDL) and to decrease plasma triglycerides (TG) in a wide variety of primary and secondary dyslipoproteinemias. Gemfibrozil reduces plasma triglyceride (very low-density lipoprotein [VLDL]) concentrations and increases high-density lipoprotein (HDL) concentrations. Although gemfibrozil may slightly reduce total and low-density lipoprotein (LDL) cholesterol concentrations, use of gemfibrozil in patients with elevated triglycerides associated with type IV hyperlipidemia often results in significant increases in LDL; LDL concentrations are not significantly affected by gemfibrozil in patients with Type IIb hyperlipidemia (although HDL is significantly increased). The mechanism of this action is not completely understood but may involve inhibition of peripheral lipolysis; reduced hepatic extraction of free fatty acids, which reduces hepatic triglyceride production; inhibition of synthesis and increased clearance of VLDL carrier, apolipoprotein B, which also reduces VLDL production and according to animal studies, reduced incorporation of long-chain fatty acids into newly formed triglycerides, accelerated turnover and removal of cholesterol from the liver (stimulates incorporation of cholesterol precursors into precursors into liver sterols), and increased excretion of cholesterol in the feces.

Gemfibrozil is determined by high performance liquid chromatography (HPLC) liquid chromatography (LC) gas chromatography (GC) gas chromatography-mass spectrometry.
The above methods however, require relatively expensive instrumentations and take relatively long time for determination of gemfibrozil. Voltammetric methods have been used to determine organic drugs in pharmaceutical dosage forms and related metabolites in biological fluids. The advance in experimental voltammetric techniques in the field of analysis of drugs is due to their simplicity, high sensitivity, low cost and relatively short analysis time as compared with other ones. Electropolymerisation is a good approach to immobilize polymers to prepare polymer modified electrodes (PMEs) as adjusting the electrochemical parameters can control film thickness, permeation and charge transport characteristics. Polymer-modified electrodes have many advantages in the detection of analytes because of its selectivity, sensitivity and homogeneity in electrochemical deposition, strong adherence to electrode surface and chemical stability of the film. Selectivity of PMEs as a sensor can be attained by different mechanisms such as size exclusion, ion exchange, hydrophobicity interaction, and electrostatic interaction. To our best knowledge, there are no reports for quantitative determination of gemfibrozil by using poly (gabapentin) film modified glassy carbon electrode.

In the present work, the modification was carried out by preparing poly-(gabapentin) film modified glassy carbon electrode for the electrochemical investigation of gemfibrozil by using cyclic voltammetry. Gabapentin, (1-(aminomethyl) cyclohexane-acetic acid) (Figure 2) is extensively used for the treatment of convulsive-type cerebral disorders, such as epilepsy, hyperkinesias, and cranial trachoma. It is sometimes prescribed for the treatment of neuropathic pain.

2. EXPERIMENTAL
2.1 Reagents: Gemfibrozil was purchased from Medrich Company, Bangalore and used without further purification. Gabapentin was purchased from Fluka. The stock solution of the gemfibrozil (25mM) was prepared by dissolving it in absolute ethanol and kept in the dark under refrigeration to avoid any degradation of the drug. Freshly prepared solutions were used in each experiment. All chemicals were of analytical grade quality and were used without further purification. Other dilute standard solutions were prepared by appropriate dilution of stock solution in 0.1M Acetate buffer solution-5% ethanol.

2.2 Apparatus: Electrochemical measurements were carried out with a model EA-201 electroanalyser (chemlink systems) a three electrode system was employed. The poly (gabapentin) modified glassy carbon electrode is used as working electrode with a saturated calomel electrode as reference electrode (SCE) and the platinum electrode as auxiliary electrode for all experiment.

2.3 Modification procedure: Before the modification, the glassy carbon electrode surface was polished with a fine emery sheet and then rinsed with distilled water. After each polishing step followed by electrochemical pretreatment of the GCE by cycling the potential between -1200 mV and +1000 mV at a scan rate of 100 mV/s for 10 times in 0.1 M H2SO4 solution. The 1 mM gabapentin was placed in the electrochemical cell along with 0.2 M phosphate buffer solution at pH 10 to maintain basic condition to oxidize the monomer (gabapentin). The GCE was scanned 15 multiple cycles between the potential ranges from −700 to 1800 V at 100 mV/s scan rate. After this process, the modified electrode was electroactivated by cyclic Voltammetry (CV) from -700 to 1800 mV at 100 mV/s in acetate buffer solution (ABS) pH 5.5.

3. RESULTS AND DISCUSSION
3.1 Electropolymerisation of gabapentin on a glassy carbon electrode: Glassy carbon electrode (GCE) is modified with electropolymerised film of gabapentin. A solution of monomer, gabapentin is oxidized to an activated form that polymerizes to form a polymer film directly on the electrode surface. This procedure results in few pinholes since polymerization would be accentuated at exposed (pinholes) sites at the electrode surface. Electro catalysis at a modified electrode is usually an electron transfer reaction between the electrode and solution substrate which, when mediated by a immobilized redox couple (i.e., the mediator), proceeds at a lower Over potential than would otherwise occur at the bare electrode and enhances the peak current. We have tried other
compounds for the modification of GCE. For example Eriochrome black-T 28 and Patton and Reeder's indicator 29. Electropolymerisation of gabapentin was fabricated in 0.2 M phosphate buffer solution containing 1mM gabapentin on GCE. The film was grown on GCE by cyclic voltammetric scans between -400 to +1800 mV. The optimized scan number under the experimental conditions was determined as 15 for reaching the steady response. As shown in Figure 3, in the first cycle, with the potential scanning from -400 to +1800 mV the anodic peak was observed at 1552 mV corresponding to the oxidation of gabapentin monomer. The peak descended gradually with the increase in cyclic time; such decrease indicates the poly (gabapentin) membrane forming and depositing on the surface of the GCE by electropolymerisation. Gabapentin was oxidized to free radical at the surface of GCE rapidly resulting in the possible structure of electropolymnerised poly (gabapentin) (Scheme 1). After polymerisation the poly (gabapentin) modified GCE was carefully rinsed with distilled water to remove the physically adsorbed material. Then the film electrode was transferred to an electrochemical cell and cyclic voltammetric sweeps were carried out to obtain electrochemical steady state. In order to confirm the formation of poly (gabapentin) on GCE, the cyclic voltammetric sweep was carried out in 0.1 M acetate buffer (pH 5.5) in the range of -700 to 1800 mV at 100 mV/s. A broad cyclic voltammogram compared to blank was obtained which confirms the deposition of polymer film on the electrode surface.

3.2 Effect of the poly (gabapentin) film thickness on the electrochemical response of gemfibrozil: The thickness of poly (gabapentin) film could be controlled by the cyclic number of voltammetric scans during the electrochemical modification. The effect of the thickness of poly (gabapentin) film on the electrochemical response of gemfibrozil was investigated in 0.1 M acetate buffer solution of pH 5.5 at poly (gabapentin) film modified GCE using cyclic voltammetric technique. Figure 6 shows cyclic voltammograms of 0.2mM gemfibrozil at bare GCE (curve b) and at poly (gabapentin) film modified GCE (curve d) along with bare GCE in blank solution (curve a) containing 0.1 M acetate buffer solution at pH 5.5 and poly(gabapentin) film modified GCE in blank solution (curve c), a broad cyclic voltammograms compared to bare GCE in blank was obtained which confirms the deposition of polymer film on the electrode surface. Above studies showed that only one oxidation peak at +1391 mV and a peak current of 5.8 µA at bare GCE, whereas an oxidation peak at 1321 mV and a peak current of 16.2 µA at the poly(gabapentin) film modified GCE, in the potential range -700 to +1800 mV. No reduction peak was observed in the reverse scan, suggesting that the electrochemical reaction is a totally irreversible process and the oxidation peak at the bare GCE is broad due to slow electron transfer, while the response was considerably improved at the poly (gabapentin) film electrode and the peak potentials shifted to negative direction, the shape of the peak turns...
the peak current and square root of scan rate in coefficient of R=0.9978 was obtained between explained by plotting the scan rate vs. oxidation peak potential and scan rate can be Figure 9c. The relationship between the correlation coefficient of 0.9944 shown in linearity was also obtained for the plot of scan rate vs. the oxidation peak current with a poly (gabapentin) modified GCE. However, at higher content of ethanol, the peak current decreased and the peak potential shifted in negative direction. From Figure 7 it was found that the peak current was maximum in 5% ethanol and gradually decreased in the range of 10-30%. Therefore the ethanol content in the supporting electrolyte was controlled at the 5% levels for subsequent experiments.

3.6 Effect of pH: The electro oxidation of gemfibrozil was studied at 0.2 mM stock solution over pH range from 3.5 to 8.5 using 0.1 M ABS at a scan rate of 100 mV/s on poly (gabapentin) modified GCE using cyclic voltammetry. The oxidation peak current increases with increase of pH from 3.5 to 5.5 and becomes maximum and peak potential shifted negatively. While pH beyond 5.5, a great decrease of the oxidation peak current could be observed, then it decreased gradually with the further increasing the pH of solution. Figure. 8 shows the relationship between the anodic peak current and pH of the solution.

3.7 Effect of scan rate: Useful information involving electrochemical mechanism usually can be acquired from the relationship between peak current and scan rate. Therefore, the effect of scan rates on the electrochemical response of 0.2 mM gemfibrozil at poly (gabapentin) modified GCE was studied at different scan rates including 50, 100, 150, 200, 250, 300, 350 and 400 mV/s by CV and the cyclic voltammograms were shown in Figure 9a. As shown in Figure 9b, a linear relationship with a correlation coefficient of R=0.9978 was obtained between the peak current and square root of scan rate in the range of 50-400 mV/s, which revealed that a diffusion controlled process occurring at the poly (gabapentin) modified GCE. However linearity was also obtained for the plot of scan rate vs. the oxidation peak current with a correlation coefficient of 0.9944 shown in Figure 9c. The relationship between the oxidation peak potential and scan rate can be explained by plotting the scan rate vs. oxidation peak potentials (Figure 9d) by considering the relation: $E_{pa}= 0.0565 \ln \mu + 1.4625$; $r=0.9904$.

According to Laviron’s theory $^{30}$, the slope is equal to RT/αnF. Then the value of $\alpha n$ calculated as 0.4542. As for a totally irreversible electrode reaction process, $\alpha$ was assumed as 0.5. On the basis of the above discussion, the $n_\alpha$ was calculated as 0.91 which indicated that one electron was involved in the oxidation process of gemfibrozil at the poly (gabapentin) film modified electrode. Since the equal number of electron and proton took part in the oxidation of gemfibrozil, therefore one electron and one proton transfer were involved in the electrode reaction process. The electrochemical reaction process for gemfibrozil at poly (gabapentin) film modified GCE can therefore be summarized as in scheme I. From the deduced mechanism of gemfibrozil, an intermediate of a free radical was formed. It may be just the free radical polymerizes and comes into being as insoluble products that deposit on the electrode surface, which agrees with the phenomena of voltammograms recorded from multi-cycles.

3.8 Calibration of gemfibrozil concentration: A series of gemfibrozil solutions were prepared to investigate the relationship between the oxidation peak current ($I_{pa}$) and the gemfibrozil concentration at poly (gabapentin) modified GCE at a scan rate of 100 mV/s by CV. As shown in Figure. 10, a linear concentration range was found to occur from 1.0 X 10^{-5} to 2.0 X 10^{-4} M and can be described by a linear regression equation: $I_{pa} (\mu A) = 0.4192 C (10^{-5} M) +9.8184$ (r= 0.9995). The limit of detection (LOD) and limit of quantification (LOQ) were 4.34 µM and 14.48 µM, respectively. The LOD and LOQ were calculated on the peak current using the following equation:

$$\text{LOD} = 3s/m , \quad \text{LOQ} = 10s/m$$

Where s is standard deviation and m is the slope of calibration plot.

3.9 Determination of gemfibrozil in pharmaceutical dosages: The applicability of the proposed voltammetric method for the assay of gemfibrozil was examined by analyzing the commercially available Lopid tablets (declared content is 300 mg of gemfibrozil in one tablet). The powder collected from 10 capsules was weighed accurately and transferred into 25 ml volumetric flask and dissolved in ethanol. The mixture was sonicated for 30 minutes and it was then filtered. A suitable aliquot of the clear filtrate was quantitatively diluted with 0.1M ABS-5% ethanol. A typical cyclic voltammograms for the determination of
gemfibrozil in the commercial Lopid tablets at bare glassy carbon electrode and poly (gabapentin) modified glassy carbon electrode were shown in Figure, 11a and 11b Figure respectively. Gemfibrozil in commercial Lopid tablets obtained from cyclic voltammetric determination are given in Table 1. The mean recovery (%) value of gemfibrozil is 99.22 in Table 1 is showing better value compared with the mean recovery (%) values of gemfibrozil, 90.15±6.9% from high-performance liquid chromatographic method 31. The results were satisfactory, showing that the proposed method could be efficiently used for the determination of gemfibrozil in pharmaceutical preparations.

CONCLUSIONS
In the present study, a chemically modified electrode based on the electropolymerisation of gabapentin has been prepared for the electrochemical determination of gemfibrozil. Results showed that the oxidation peak current of gemfibrozil was improved at poly (gabapentin) film modified GCE. The electrochemical response is diffusion controlled and irreversible in nature. A linear concentration range was found to occur from 1.0 X 10^−5 to 2.0 X 10^−4 M with the limit of detection (LOD) and limit of quantification (LOQ) were 4.34 µM and 14.48 µM, respectively. The probable reaction mechanism involved in the oxidation of gemfibrozil was also proposed. The applicability of the proposed voltammetric method for the assay of gemfibrozil was examined by analyzing the commercially available Lopid tablets (declared content is 300 mg of gemfibrozil in one tablet) and successfully applied for the determination of gemfibrozil in pharmaceutical dosages.

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Step -1

![Scheme I. Proposed mechanism of the electrochemical oxidation of gemfibrozil at the poly (Gabapentin) modified GCE.](image)

**Scheme I. Proposed mechanism of the electrochemical oxidation of gemfibrozil at the poly (Gabapentin) modified GCE.**

**Figure. 1 Chemical structure of Gemfibrozil.**
Figure 2 Chemical structure of Gabapentin

Figure 3 Cyclic voltammograms for the electropolymerisation of 1mM Gabapentin in 0.2M phosphate buffer solution on a GCE. Initial potential: -400 mV, Terminal potential: 1800 mV. Scan rate: 100mVs⁻¹.

Figure 4 The plot of Oxidation peak current versus number of cycles.
Figure. 5 Cyclic voltammogram of 1mM potassium ferrocyanide in 1M KCl at bare GCE (solid line) and modified GCE (dashed line). Scan rate: 50mVs⁻¹.

Figure. 6 Cyclic voltammograms of 0.2mM gemfibrozil at bare GCE (curve b) and at poly (gabapentinate) film modified GCE (curve d) along with bare GCE in blank solution (curve a) containing 0.1 M acetate buffer solution at pH 5.5 and poly (gabapentinate) film modified GCE in blank solution (curve c).

Figure. 7 The plot of Oxidation peak current with the variation of % of ethanol, 5%, 10%, 15%, 20% and 25%.
Figure 8 The plot of oxidation peak current on the solution pH, 3.5, 4.5, 5.5, 6.5, 7.5 and 8.5.

Figure 9a Cyclic voltammograms of 0.2 mM gemfibrozil at the poly (gabapentin) modified GCE with different scan rates were 50, 100, 150, 200, 250, 300, 350 and 400 mV/s.

Figure 9b The plot of oxidation peak currents vs. scan rates at poly (gabapentin) modified GCE.
Figure 9c The plot of oxidation peak currents vs. square root scan rates at poly (gabapentin) modified GCE.

Figure 9d The plot of oxidation peak potential vs. natural logarithm of the scan rate at poly (gabapentin) modified GCE.

Figure 10 Effect of variation of concentration of gemfibrozil, 1.0 X 10^{-5}, 2.5 X 10^{-5}, 5.0 X 10^{-5}, 7.5 X 10^{-5}, 10 X 10^{-5}, 15 X 10^{-5} and 20 X 10^{-5} M on the anodic peak current at poly (gabapentin) modified GCE; \( \nu = 100 \text{mV/s} \).
Figure. 11 Typical cyclic voltammograms for the determination of gemfibrozil in a commercial tablet sample at bare GCE (curve a) and poly (gabapentin) modified GCE (curve b) with a scan rate of 100 mV/s.

Table 1. Determination results of gemfibrozil in the commercial lopid capsules

| Sample no. | Specified (mg/tab) | Detected (mg/tab) | Recovery (%) | RSD % (n=3) |
|------------|--------------------|-------------------|--------------|-------------|
| 1          | 300                | 293.02            | 97.67        |             |
| 2          | 300                | 302.31            | 100.77       | 1.55        |
| 3          | 300                | 297.67            | 99.22        |             |