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Simultaneous Electrocatalytic determination of Simvastatin and Gemfibrozil at Poly (glycine) modified glassy carbon electrode

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ABSTRACT

Glassy carbon electrode (GCE) is modified with electropolymerised film of glycine. This polymer (glycine) modified electrode is used to study the simultaneous electrochemical detection of Simvastatin (SMV) and gemfibrozil (GBZ) and showed an excellent electrocatalytic effect on the oxidation of SMV and GBZ by cyclic voltammetry (CV) in 0.1 M acetate buffer solution (pH 5.5). The poly (glycine) modified electrode could separate the oxidation peak potentials of SMV and GBZ present in binary mixtures by about 276 mV though the bare electrode gave a single broad response. The detection limits for SMV and GBZ in binary mixtures at the poly (glycine) modified glassy carbon electrode were found to be 2.0×10^{-5} M and 1.26×10^{-5} M, respectively. The proposed method was sensitive and simple. It was successfully employed for the simultaneous detection of SMV and GBZ in pharmaceutical samples.

Key words: Cyclic Voltammetry, Simvastatin, Gemfibrozil, Electropolymerisation, Modified Glassy Carbon Electrode and Glycine.

1. INTRODUCTION

` Coadministration of statins and fibrates is beneficial in some patients by allowing simultaneous reduction of triglycerides and low-density lipoprotein cholesterol alongside elevation of high-density lipoprotein cholesterol. However, the potential for drug interactions must be taken into consideration. Combination of statins with fibrates can be used for combined dyslipidemia and it can decrease low density lipoprotein cholesterol more than 40 % [1]. Controlled trials have not only shown regression of atherosclerotic lesions with this combination, but have also demonstrated increased risk of myopathy [2-3].

Hyperlipidemia (HLP) is a group of lipid metabolism disorders with various pathogenesis. Its characteristic feature is an increase in cholesterol level, especially the low density lipoprotein fraction (LDL) level or triglyceride level in blood. An increase in total and LDL cholesterol level is related to the increased risk of ischemic heart disease as well as cerebral, coronary and peripheral circulation disorders. The medications against hyperlipidemia and cholesterol level reduction are 3-hydroxy-3-methylglutaryl- coenzyme A reductase inhibitors (HMGCoA) also known as statins and aryloxyalkylcarboxylic acid derivatives, also known as fibrates. Statins inhibit biosynthesis of endogenous cholesterol at the mevalonic acid synthesis level and reduce of total cholesterol, LDL fraction and triglycerides levels in plasma and increases HDL fraction level. The fibrates inhibit VLDL lipoprotein synthesis in liver and accelerate catabolism by increasing lipoprotein lipase activity. Also they improve HDL level and affect the return of cholesterol.

Simvastatin (SMV) (Fig. 1), a hypolipidemic drug belonging to the class of pharmaceuticals called statins is chemically designated as [(1S,3R,7R,8S,8aR)-8-[2-[(2R,4R)-4-hydroxy-6-oxo-oxan-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8ahexahydronaphthalen-1-yl] 2,2-dimethylbutanoate. This compound, acts as a highly potent and effective cholesterol-lowering agent, is being used in the control of hypercholesterolemia. It exhibits a very important hepatic first-pass metabolism, acting by blocking the 3-hydroxy-3- methylglutaryl coenzyme A reductase (HMG-CoA), and thereby reducing the low-density lipoproteins. Simvastatin is a potent

inhibitor of HMG-CoA reductase, which is a rate limiting enzyme in cholesterol bio-synthesis [4]. Several methods based on different techniques have been reported for the determination of Simvastatin in biological fluids, which include HPLC [5-7], HPLC-MS/MS [8], spectrophotometer [9]. Among them, HPLC methods have been described using expensive reagents or buffers in the mobile phase [10-14].

Fig-1: Chemical Structure of Simvastatin.

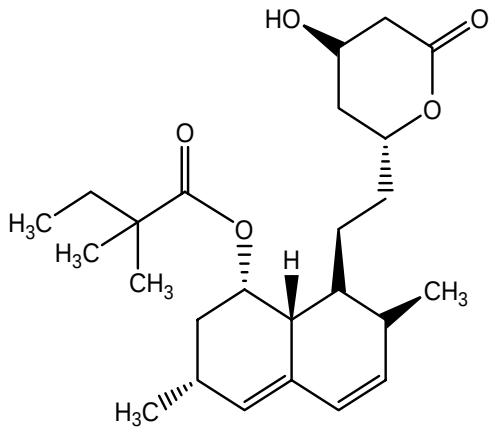
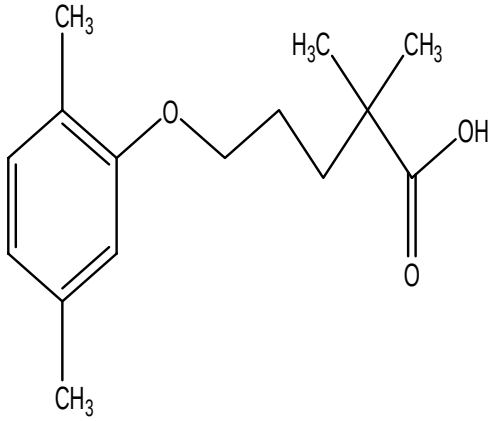


Fig -2: Chemical Structure of Gemfibrozil.



Gemfibrozil (GBZ), 5-(2,5-dimethylphenoxy)- 2,2-dimethylpentanoic acid (Figure 2), is a fibric acid derivative which is widely used as a hypolipidaemic agent. GBZ is extensively metabolized and excreted in urine as a glucuronide metabolite [15,16]. Accumulated evidences suggested that GBZ inhibited the glucuronidation of statins and this finding has been postulated as the mechanism of interaction between both drugs [17,18]. Gemfibrozil is determined by high performance liquid chromatography (HPLC) [19-22], liquid chromatography (LC)[23,24], gas chromatography (GC) [25], gas chromatography-mass spectrometry (GC-MS) [26]. These above methods, however, require relatively expensive instrumentations and take relatively long time for determination of gemfibrozil.

A simple, sensitive and validated HPLC method has been developed to determine gemfibrozil and simvastatin simultaneously in synthetic mixture form [27]. According to the information collected from literature there is no reported cyclic voltammetric method for simultaneous determination of gemfibrozil and simvastatin.

Electropolymerization 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 [28]. Selectivity of PMEs as a sensor can be attained by different mechanisms such as size exclusion [29], ion exchange [30], hydrophobicity interaction [31], and electrostatic interaction [32-33]. In the present work we are therefore focused on to the study of electrocatalytic response of both simvastatin and gemfibrozil on poly (glycine) modified glassy carbon electrode.

2. EXPERIMENTAL

2.1. Reagents

Gemfibrozil was purchased from Medrich Company, Bangalore and used without further purification. 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. Simvastatin was purchased from Medrich Company, Bangalore and used without further purification. The stock solution of the simvastatin (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 (glycine) modified glassy carbon electrode is used as working electrode with a saturated calomel electrode as reference

electrode (SCE) and the platinum electrode as auxillary electrode for all experiment.

2.3. Modification procedure

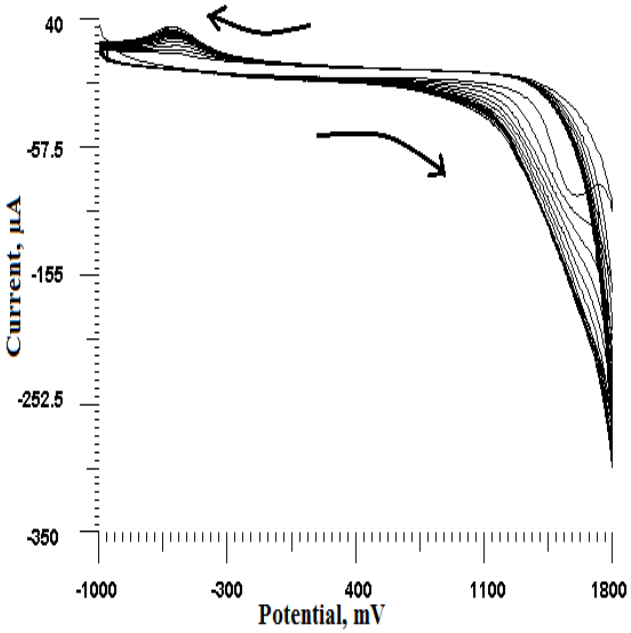
Before the modification, the glassy carbon electrode surface was polished with a fine emery sheet and then rinsed with distilled water. After each polishing step followed by electrochemical pretreatment of the GCE by cycling the potential between -1200 mV and +1000 mV at a scan rate of 100 mV/s for 10 times in 0.1 M H_2SO_4 solution. The 0.01M glycine was placed in the electrochemical cell along with 0.2 M phosphate buffer solution at pH 7. The GCE was scanned 15 multiple cycles between the potential ranges from −400 to 1800 V at 100 mV/s scan rate. After this process, the modified electrode was electroactivated by cyclic voltammetry from -700 to 1800 mV at 100 mV/s in pH 5.5 acetate buffer solution (ABS).

3. RESULTS AND DISCUSSION

3.1. Electropolymerisation of glycine on a glassy carbon electrode

Electropolymerisation of glycine was fabricated in 0.2 M phosphate buffer solution containing 0.01M glycine on GCE. The film was grown on GCE by cyclic voltammetric scans between -1000 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 -1000 to +1800 mV the anodic peak was observed at 1592 mV corresponding to the oxidation of glycine monomer and the cathodic peak was observed at -600 mV. The peak descended gradually with the increase in cyclic time; such decrease indicates the poly (glycine) membrane forming and depositing on the surface of the GCE by electropolymerisation. After polymerisation the poly (glycine) 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 (glycine) on GCE, the cyclic voltammetric sweep was carried out in 0.1 M acetate buffer (pH 5.5) in the range of -300 to 1600 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.

Fig-3: Cyclic voltammograms for the electropolymerisation of 0.01 **M** glycine in 0.2 **M** PBS, pH **7** on **a** GCE. Initial potential -1000 mV, Terminal potential 1800 mV. Scan rate-100mV/s



3.2. Electrocatalytic response of Simvastatin at the poly (glycine) modified electrode.

Fig. 4A shows the cyclic voltammograms (CVs) at bare GCE (Fig. 4A curve a) and poly (glycine) modified electrode (Fig. 4A curve c) in presence of 0.1 mM SMV in ABS pH 5.5 at a scan rate of 100 mV/s. It showed that only one oxidation peak at +1100 mV and a peak current of 5.12 µA at bare GCE, whereas an oxidation peak at 1012 mV and a peak current of 13.27 µA at the poly(glycine) modified GCE, in the potential range -300 to +1600 mV. No reduction peak was observed in the reverse scan, suggesting that the electrochemical reaction is a totally irreversible process. The peak current at poly (glycine) modified GCE is larger than the corresponding one at the bare GCE. These suggest that the poly (glycine) can act as a promoter to enhance the electrochemical reaction. Poly (glycine), itself, is electroinactive in the potential range from -300 to +1600 mV (Fig. 4A curve b). Due to the high porosity of the poly (glycine), the real surface area of the modified electrode is far greater than that of bare GCE. So the peak current increases evidently together with the background voltametric response at the poly (glycine)-coated GCE stronger than that at the bare surface.

Figure -4A: Cyclic voltammograms at bare GCE (curve a) and poly (glycine) modified electrode (curve c) in presence of 0.1 mM SMV and in the absence of 0.1 mM SMV (b) in ABS pH 5.5 at **a** scan rate of 100 mV/s.

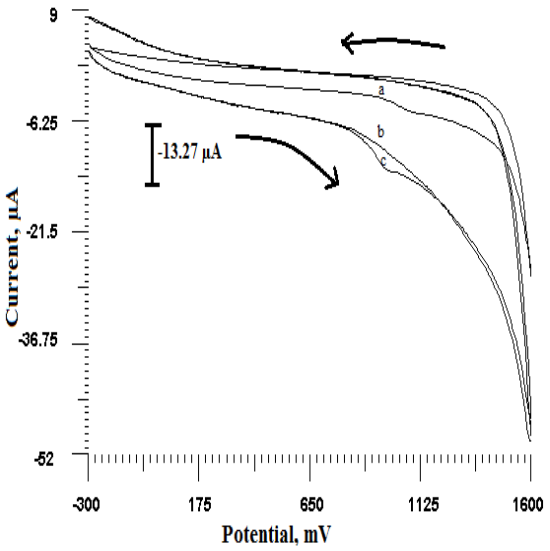


Fig. 4B shows the CVs of 0.1 mM SMV at the poly (glycine) modified GCE at different scan rates, 25, 50, 75, 100, 125 and 150 mV/s. From Fig. 4C, it was found that the oxidation peak current increases linearly with the increase in scan rate with a correlation coefficient of 0.9997 and slope of 0.1178, which revealed that an adsorption controlled process occuring at the poly (glycine) modified GC in the range of 25-150 mV/s. However linearity was also obtained for the plot of square root of scan rate vs. the oxidation peak current with a correlation coefficient of 0.9937 in Fig. 4D. The relationship between the oxidation peak potential and scan rate can be explained by plotting the scan rate vs. oxidation peak potentials. According to Laviron's theory [34] , the slope is equal to $RT/\alpha n_{\alpha}F$. Then the value of αn_{α} calculated as 0.985. As for a totally irreversible electrode reaction process, α was assumed as 0.5. On the basis of the above discussion, the n_{α} was calculated as 1.97 which indicated that two electrons were involved in the oxidation process of SMV at the poly (glycine) film modified electrode. Since the equal number of electron and proton took part in the oxidation of SMV, therefore two electrons and two protons transfer were involved in the electrode reaction process. The electrochemical reaction process for SMV at poly (glycine) film modified GCE can therefore be summarized as in scheme I.

Fig -4B: Cyclic voltammograms of 0.1 mM SMV at polyglycine modified electrode in 0.2 **M** ABS (pH 5.5) at different scan rates: (a) 25 mV/s; (b) 50 mV/s; (c) 75 mV/s; (d) 100 mV/s; (e) 125 mV/s; (f) 150 mV/s.

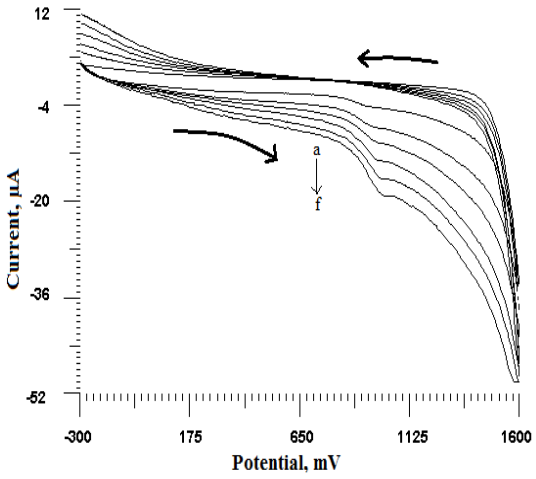
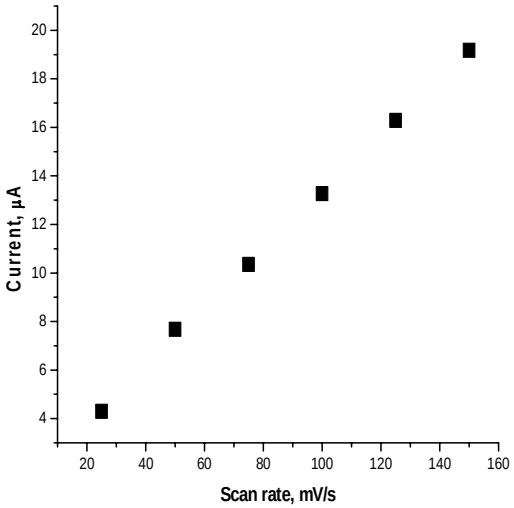


Figure -4C: The plot of Oxidation peak current versus Scan rates.



Scheme I. Probable reaction mechanism for the oxidation of SMV at poly (glycine) modified GCE.

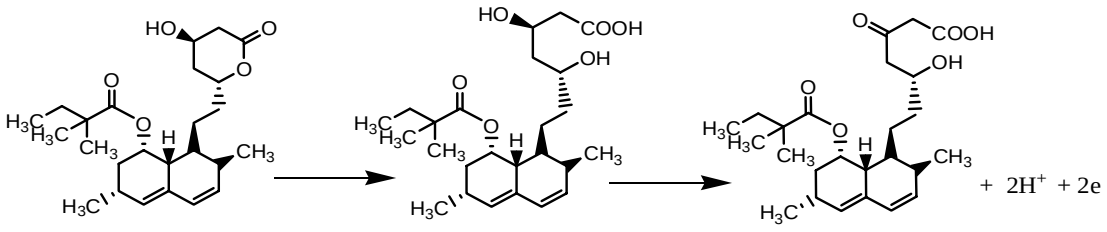
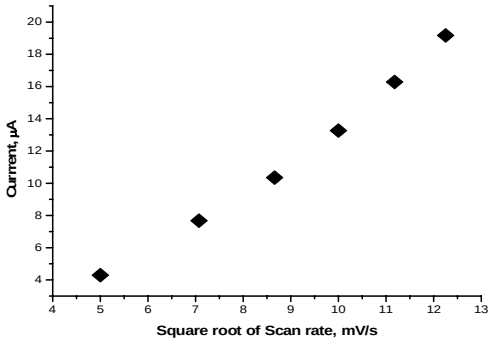


Figure -4D: The plot of Oxidation peak current and the square root of Scan rates.



3.3. Electrocatalytic response of Gemfibrozil at the poly (glycine) modified electrode.

The CVs of GBZ at poly (glycine) modified electrode is also compared with that at bare GCE. Fig. 5A shows the cyclic voltammograms (CVs) at bare GCE (Fig. 5A curve a) and poly (glycine) modified electrode (Fig. 5A curve c) in presence of 0.1 mM GBZ in ABS pH 5.5 at a scan rate of 100 mV/s. It showed that only one oxidation peak at +1328 mV and a peak current of 9.4 µA at bare GCE, whereas an oxidation peak at 1277 mV and a peak current of 30.4 µA at the poly (glycine) modified GCE, in the potential range -300 to +1600 mV. No reduction peak was observed in the reverse scan, suggesting that the electrochemical reaction is a totally irreversible process. The peak current at poly (glycine) modified GCE is larger than the corresponding one at the bare GCE. These suggest that the polyglycine can act as a promoter to enhance the electrochemical reaction. Polyglycine, itself, is electro inactive in the potential rage from -300 to +1600 mV (Fig. 5A curve b). Due to the high porosity of the polyglycine, the real surface area of the modified electrode is far greater than that of bare GCE. So the peak current increases evidently together with the background voltametric response at the polyglycine-coated GCE stronger than that at the bare surface.

Figure -5A: Cyclic voltammograms at bare GCE (curve a) and poly (glycine) modified electrode (curve c) in presence of 0.1 mM GBZ and in the absence of 0.1 mM GBZ (b) in ABS pH 5.5 at **a** scan rate of 100 mV/s.

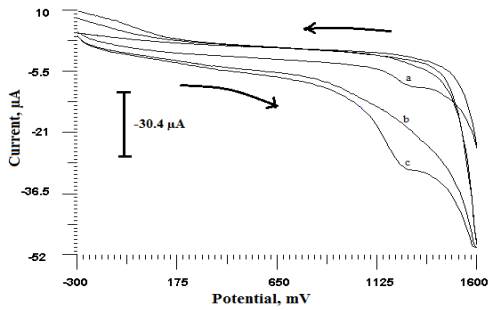


Figure -5B: Cyclic voltammograms of 0.1 mM GBZ at polyglycine modified electrode in 0.2 **M** ABS (pH 5.5) at different scan rates: (a) 25 mV/s; (b) 50 mV/s; (c) 75 mV/s; (d) 100 mV/s; (e) 125 mV/s; (f) 150 mV/s.

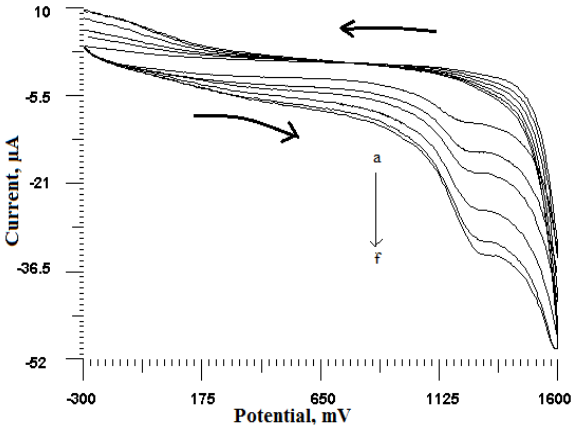


Figure -5C: The plot of Oxidation peak current versus Scan rates.

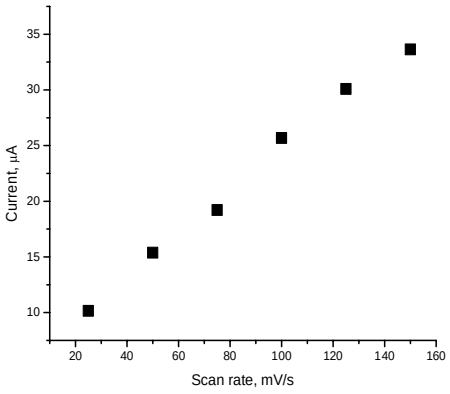


Figure -5D: The plot of Oxidation peak current and the square root of Scan rates.

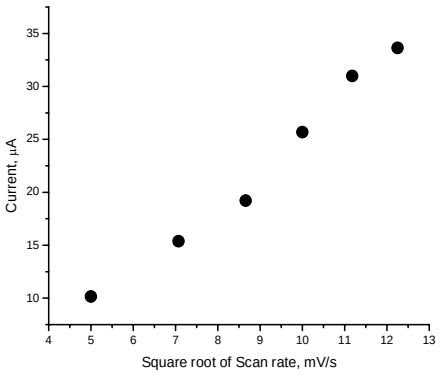
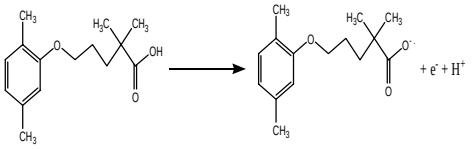


Fig. 5B shows the CVs of 0.1 mM GBZ at the poly (glycine) modified GCE at different scan rates, 25, 50, 75, 100, 125 and 150 mV/s. From Fig. 5C, it was found that the oxidation peak current increases linearly with the increase in

scan rate with a correlation coefficient of 0.9955 and slope of 0.1950, which revealed that an adsorption controlled process occuring at the poly (glycine) modified GC in the range of 25-150 mV/s. However linearity was also obtained for the plot of square root of scan rate vs. the oxidation peak current with a correlation coefficient of 0.9909 in Fig. 5D. The relationship between the oxidation peak potential and scan rate can be explained by plotting the scan rate vs. oxidation peak potentials. According to Laviron's theory [34], the slope is equal to $RT/\alpha n_{\alpha}F$. Then the value of αn_{α} calculated as 0.542. As for a totally irreversible electrode reaction process, α was assumed as 0.5. On the basis of the above discussion, the n_{α} was calculated as 1.084 which indicated that one electron was involved in the oxidation process of GBZ at the poly (glycine) film modified electrode. Since the equal number of electron and proton took part in the oxidation of GBZ, therefore one electron and one proton transfer were involved in the electrode reaction process. The electrochemical reaction process for GBZ at poly (glycine) film modified GCE can therefore be summarized as in scheme II.

Scheme II. Probable reaction mechanism for the oxidation of GBZ at poly (glycine) modified GCE.



3.4 Simultaneous detection SMV and GBZ

The electrochemical behavior of binary mixtures of 0.1 mM SMV and 0.1 mM GBZ at the poly (glycine) modified electrode was investigated using CV. For the binary mixtures, 0.1 M ABS was used to control the pH and the pH 5.5 was chosen, at this pH the oxidations of the two compounds have high electrochemical response. Fig. 6 shows the CVs obtained for SMV and GBZ coexisting at bare GCE (Fig. 6(curve.a) and modified electrode (Fig. 6 (curve.b). As shown in Fig. 6a, the bare GCE cannot separate the voltammetric signals of SMV and GBZ. Only one broad voltammetric signal was observed for both analytes. The fouling of the electrode surface by the oxidation products results in a single voltammetric peak for SMV and GBZ. Therefore it is impossible to use the bare electrode for the voltammetric detection of SMV in the presence of GBZ. Moreover, the polyglycine modified electrode resolved the mixed voltammetric signals into two well-defined voltammetric peaks at 981 and 1257 mV corresponding to the oxidation of SMV and GBZ, respectively. The poly (glycine) modified electrode shows good selectivity and excellent sensitivity in the simultaneous detection of SMV and GBZ. The separation between the voltammetric peaks of SMV and GBZ is large (276 mV) and thus the simultaneous detection of SMV and GBZ or the selective detection of GBZ in presence of SMV is feasible at the poly (glycine) modified electrode. The polyglycine modified electrode gave two peaks (Fig. 6, curve b). One peak was observed at 981 mV and the current response at this potential is approximately the same as that given by SMV in the absence of GBZ (Fig. 7, curve a). The another peak appeared with potential at 1257 mV and the current response at this potential is also approximately the same as that given by GBZ in the absence of SMV (Fig. 7, curve b). Thus, it can be confirmed that these two peaks are for SMV and GBZ, respectively.

Fig. 8A represents the CVs at different concentrations such as 10, 20, 40, 60 and 80 µM of GBZ where the concentration of SMV was kept constant. The oxidation peak current for GBZ was increased linearly with the increase in GBZ concentration (Fig. 8B) with the correlation coefficient of 0.9878 and the detection limit was 1.58×10^{-5} M based on the signal-to noise ratio of 3. Furthermore, while GBZ peak current increased with the increase in GBZ concentration, the peak current of SMV kept almost constant.

Overall facility of the poly (glycine) modified electrode for simultaneous determination of SMV and GBZ was demonstrated by simultaneously changing the concentration of SMV and GBZ. Fig. 8C illustrates the CV responses of the poly (glycine) modified electrode while simultaneously varying the concentrations of both SMV and GBZ. The calibration curves for SMV and GBZ were linear (Fig. 8D) for a wide range of concentrations (20, 40, 60, 80, 100, 120 and 140 µM for both of SMV and GBZ), with correlation coefficients 0.9897 and 0.9961, respectively. The detection limits for SMV and GBZ were found to be 2.0×10^{-5} M and 1.26×10^{-5} M, respectively.

To ascertain further the reproducibility of the results, three different GCE was modified with polyglycine and their responses towards the oxidation of of SMV and GBZ were tested. The separation between the voltammetric signals of SMV and GBZ and the sensitivities remained the same at all three modified electrodes, confirming that the results are reproducible. The stability of the polyglycine modified electrode was also investigated. Its electrocatalytic effect did not change after storage in air for at least one week.

Fig-6: Cyclic voltammograms at bare GCE (a) and polyglycine modified electrode (b) in

presence of 0.1 mM SMV and 0.1 mM GBZ in 0.2 **M** ABS (pH 5.5); scan rate-100 mV/s.

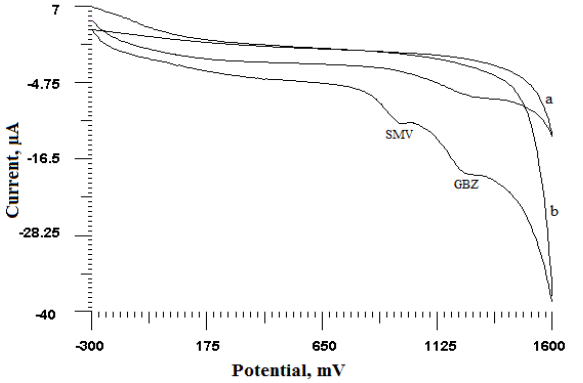


Fig-7: Cyclic Voltammograms for 0.1 mM SMV (a) and 0.1 mM GBZ (b) at poly (glycine) modified electrode in 0.2 **M** ABS (pH 5.0); scan rate 100 mV/s.

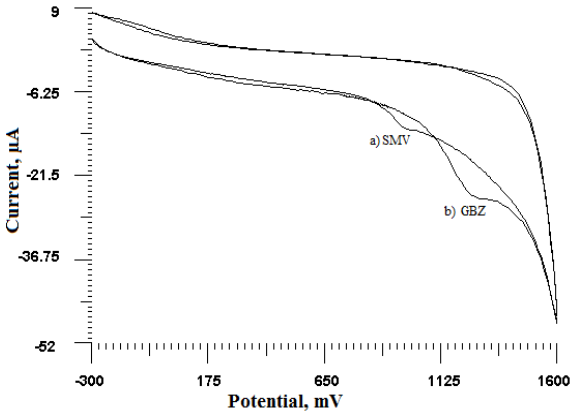


Fig-8A: Cyclic Voltammograms of SMV and GBZ at poly (glycine) modified electrode in 0.2 **M** ABS (pH 5.5),[SMV] was kept constant **&** [GBZ] was changed (i.e.,[SMV] = 0.1mM, [GBZ]: (a) 10, (b) 20, (c) 40, (d) 60 and (e) 80 µM .

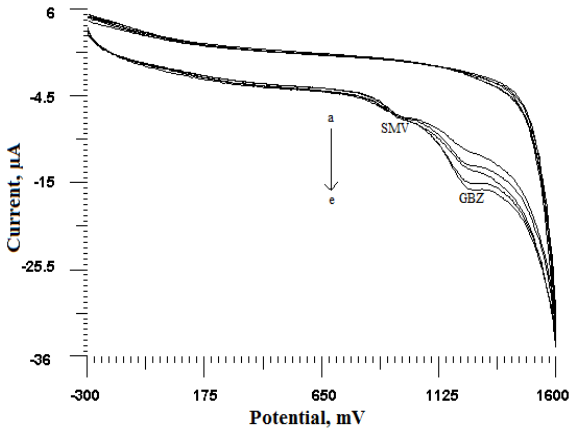


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Figure- 8B: The plot of variation of concentration of simvastatin on the anodic peak current at poly (glycine) modified electrode.

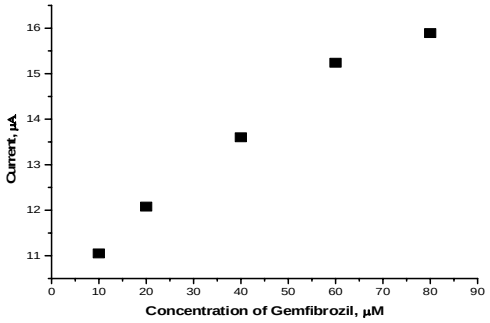


Figure 8C. Cyclic voltammograms for SMV and GBZ at poly (glycine) modified electrode in 0.2 **M** ABS (pH 5.5) while simultaneously changing their concentration (i.e., [SMV] = [GBZ]: (a) 20, (b) 40, (c) 60, (d) 80, (e) 100, (f) 120 and (g) 140 µM

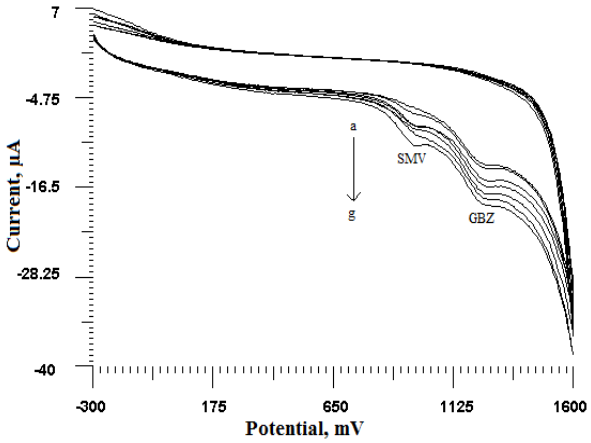
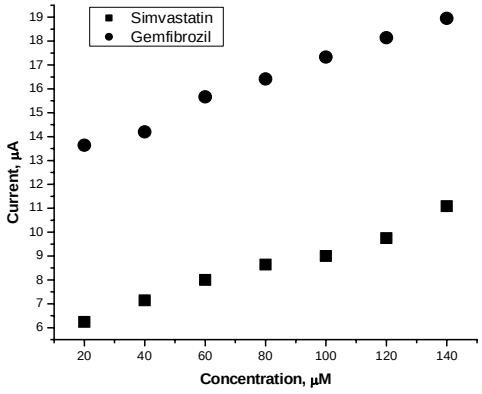


Figure 8D. Variation of oxidation peak current versus concentration.



3. Simvastatin and gemfibrozil in pharmaceutical formulations

Commercial pharmaceutical formulations of simvastatin and gemfibrozil were analyzed to evaluate the use of poly (glycine) modified GCE for

Table -1: Determination results of simvastatin in the simcard tablets and gemfibrozil in the commercial lopid capsules.

Tablet	Specified (mg/tab)	Detected (mg/tab)	Recovery (%)	RSD % (n=3)
	10	9.61	96.15	
SMV	10	10.09	100.96	2.43
	10	9.85	98.55	
	300	290.31	96.77	
GFZ	300	294.72	98.24	1.89
	300	301.29	100.43	

practical purposes. SIMCARD-10 tablets (Cipla LTD, India) and LOPID (300 mg) capsules were adopted for the tests. These tablets were accurately weighed, then transferred to a 100 ml standard flask, and finally dissolved in acetate buffer solution (pH 5.5). The prepared solution was examined using the poly (glycine) modified GCE using CV. The determined amounts of simvastatin in simcard tablets and gemfibrozil in commercial Lopid capsules obtained from cyclic voltammetric determination are 9.85 and 295.44 respectively, were similar with labeled claims. The recoveries of SMV and GBZ (Table 1), indicating that poly (glycine) modified GCE prepared in this study is accurate enough for practical applications.

4. CONCLUSIONS

The present study demonstrates an effective approach for the development poly (glycine) modified glassy carbon electrode, a novel voltammetric sensor for the electrochemical determination of simvastatin and gemfibrozil. The poly (glycine) film showed electrocatalytic action for the oxidation of simvastatin and gemfibrozil characterizing by the enhancement of the peak current and the reduction of peak potential. The electrochemical response is adsorption controlled and irreversible in nature. The poly (glycine) modified electrode showed excellent sensitivity, selectivity and antifouling properties and can separated oxidation peaks towards SMV and GBZ, which are indistinguishable at the bare electrode. The detection limit of both simvastatin and gemfibrozil were obtained. The poly (glycine) modified GCE exhibited very good performance for the determination of SMV and GBZ present in pharmaceutical tablets. Together with low cost and ease preparation, this film modified electrode seems to be of good utility for further sensor development.

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