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Electrochemical studies of Simvastatin at glassy carbon electrode and immobilized by Sodium dodecyl sulfate surfactant

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ABSTRACT

Simvastatin, a lipid lowering drug whose electrochemical behavior was studied in aqueous alcohol medium at glassy carbon electrode by Cyclic Voltammetry. Cyclic voltammetric studies of simvastatin showed one well defined oxidation peak in Britton Robinson buffer. The effects of scan rate, pH, supporting electrolyte concentration, % of solvent and concentration of simvastatin were examined. The adsorption behavior of sodium dodecyl sulphate (SDS) surfactant on a glassy carbon electrode was investigated. The detection limit of the SDS modified glassy carbon electrode is 2.5X10^{-7}M. The probable reaction mechanism involved in the oxidation of simvastatin was also proposed. The proposed method was sensitive and simple. It was successfully employed to determine simvastatin in pharmaceutical samples.

Keywords: Simvastatin, Cyclic Voltammetry, Sodium dodecyl sulfate, Glassy carbon electrode.

INTRODUCTION

Simvastatin (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-dimethyl butanoate. It is used for the treatment of hypercholesterolemia [1]. Statins are potent and effective inhibitors of cholesterol biosynthesis that are widely used to treat hypercholesterolemia. Beyond this well-defined mode of action for statins, several clinical trials such as 4S [2], WOSCOPS [3], CARE, [4] and HPS [5] have demonstrated that this class of drugs can protect against cardiovascular disease (CVD) through an additional mechanism that is independent of cholesterol lowering [6]. Guidelines from the UK National Institute for Health and Clinical Excellence (NICE) recommend statin therapy for primary prevention of CVD in adults who have a 20% or greater 10-year risk [7]. A recent meta-analysis of 14 randomised trials demonstrated benefits of statin therapy to reduce vascular mortality in diabetic patients [8]. Consequently, millions of diabetic people are receiving statins [9] despite the fact that their local effects on certain tissues like the retina remain largely unknown. Following conversion of this lactone prodrug to its hydroxyl acid form, the compound is a potent competitive inhibitor of HMG-CoA reductase, the rate limiting enzyme in cholesterol biosynthesis [10].

Different analytical methods have been reported for the determination of simvastatin, which include HPLC [11-14], HPLC-MS/MS [15], derivative spectrophotometry [16] and Voltammetric techniques [17]. However, some of these methods require expensive equipment and are time-consuming. In some cases, the methods entail an extraction and

derivatization procedures due to their relatively low sensitivities. Hence, a more rapid and simpler method for identification and determination of simvastatin at tracelevels is highly desirable.

Drug analysis has an extensive impact on public health. Electrochemical techniques have been used for the determination of the drug's electrode mechanism. The redox properties of drugs can provide insight into their metabolic fate, their invivo redox processes and their pharmacological activity [18]. The chemical modifications of bare electrodes with redox active thinfilms offer significant advantages in the design and development of electrochemical sensors. In operation, electrode surface modification has been tried as a means to reduce the overvoltage and to overcome the slow kinetics of many electrode processes. A further advantage of the chemically modified electrode is their being less prone to surface fouling compared to bare electrodes [19]. Glassy carbon electrode has been very popular because of its excellent electrical and mechanical properties, wide potential range, extreme chemical inertness and relatively reproducible performance [20-27].

Surfactants are a kind of amphiphilic molecule with a polar head on one side and a long hydrophobic tail on the other. The applications of surfactants in electrochemistry and electro analytical chemistry have been widely reported [28]. Many of the studies of modified electrodes were undertake simply because electrochemists were curious about new species attached to electrode surface behavior compared to these species in solution [29]. Some less soluble surfactants were employed in the immobilization of macro molecules or other functional materials, Wu et al [30] developed a stable multi-wall carbon nanotube (MWNT) modified electrode based on the immobilization of MWNT in the film of insoluble dihexadecyl phosphate (DHP) on a glassy carbon electrode. This electrode exhibited an electro catalytic activity towards biomolecules and has been used as a sensor for the determination of these species [31, 32].

In this paper, the electrochemical behavior of simvastatin was determined on the surface of bare GCE and sodium dodecyl benzene sulfonate (SDS) immobilized GCE by cyclic voltammetry (CV), the resulting electrode exhibited good performance on the electrochemical oxidation of SMV. With its good sensitivity, selectivity and stability, the surfactant modified GCE has been used for the determination of simvastatin.

EXPERIMENTAL SECTION

2.1 Reagents

Simvastatin was purchased from Medrich Company, Bangalore and used without further purification. SDS was purchased from Fluka. 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 H_2SO_4-10% ethanol and Britton Robinson buffer solution- 10% ethanol.

2.2 Apparatus

Electrochemical measurements were carried out with a model EA-201 electroanalyser (chemlink systems) a three electrode system was employed. The SDS 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 100mV/s for 10 times in 0.10 M H_2SO_4 solution. The surfactant immobilized glassy carbon electrode was prepared by drying the known quantity of SDS surfactant on the bare glassy carbon electrode.

RESULTS AND DISCUSSION

3.1 Electrochemical behavior of simvastatin at GCE

Cyclic voltammetric technique was utilized to investigate the electrochemical behavior of simvastatin on GCE [Figure.2(a)] and in blank solution containing 0.1M H_2SO_4-10%ethanol solution [Figure.2(b)]. It showed that only one oxidation peak at +1261 mV and a peak current of 6.5 µA. No reduction peak was observed in the reverse scan, suggesting that the electrochemical reaction is a totally irreversible process.

3.2 Effect of supporting electrolyte concentration

It was not obvious from the literature as to the particular choice of supporting electrolyte or its concentration. The electrochemical oxidation of simvastatin was studied in various supporting electrolytes such as HAc-NaAc, H_2SO_4, H_3PO_4 -Na_2HPO_4, Britton Robinson buffer. Simvastatin yielded a single oxidation peak in all the above supporting electrolyte. However, the best results were obtained with H_2SO_4. The effect of the concentration of H_2SO_4 was tested over the 20, 40, 60, 80 and 100 mM. The cyclic voltammograms of 0.1mM SMV with the varying concentrations of supporting electrolyte, H_2SO_4 on the surface of GCE was shown in Fig. 3a and the plot of oxidation peak current of SMV with the variation of concentration of H_2SO_4 on GCE was shown in Fig. 3b.

3.3 Effect of Ethanol

Owing to the insolubility of simvastatin in water, ethanol was used as solvent. Figure. 4a shows the cyclic voltammograms of 0.1mM SMV with the variation of percentage of ethanol at GCE. The effect of % of ethanol on the oxidation peak current of SMV on the surface of GCE was examined in 10%, 15%, 20%, 25% and 30% (Figure. 4b) and the results showed that the content of ethanol should be higher than 10% (v/v) to avoid precipitation of SMV. According to the above studies, the optimal supporting electrolyte was 0.1M H_2SO_4 – 10% ethanol.

3.4 Effect of scan rate

The effect of scan rates on the electrochemical response of 0.1mM SMV at GCE was studied between the range 25 to 150 mV/s and the cyclic voltammograms were shown in Fig. 5a. From figure 5b, it was found that the oxidation peak current increases linearly with the increase in scan rate with a correlation coefficient of 0.9980 and slope of 0.0610, which indicates an adsorption controlled process occuring at the GCE. However linearity was also obtained for the plot of square root of scan rate vs. the oxidation peak current with a correlation coefficient of 0.9908 in Fig 5c.

3.5 Effect of simvastatin concentration

The variation of concentration of SMV was studied at GCE at a scan rate of 100 mV/s. Fig. 6a shows the cyclic voltammograms of SMV at GCE. The plot of i_{pa} versus concentration of SMV showed the linear relationship between the anodic peak current i_{pa} and the SMV concentration in the range of $0.1X10^{-3}$ M to $0.5X10^{-3}$ M with a correlation co-efficient of 0.9766 in Fig 6b.

3.6 Effect of pH

The influence of pH on the oxidation of 0.1mM SMV at the GCE using Britton Robinson buffer of pH 1 to 6 were investigated by CV. It shows that, by increasing the pH of the Britton Robinson buffer , a negative shift was observed in the oxidation peak potentials, showing that the involvement of protons in these electrode reactions. Fig. 7 shows the linear relationship between the anodic peak current and pH of the solution with a negative slope of 7.3714 mV and when pH value beyond 2, a great decrease of the oxidation peak current could be observed, then it decreased gradually with the further increasing the pH of solution.

3.7 Effect of SDS surfactant

The electrochemical responses of SMV at glassy carbon electrode were shown in Figure.8a with 0.1M H_2SO_4 - 10%ethanol as supporting electrolyte and a scan rate of 100 mv/s. Owing to the complex properties and the roughness of the bare glassy carbon electrode surface, the cyclic voltammogram of SMV is low signal [Figure.8(a)]. However the voltammetric response is apparently improved in the presence of 25mM of 5µL SDS, reflected by the enlargement of anodic peak current and the oxidation peak shifted to lower potential of +1240 mV from +1261 mV [Figure.8(b)]. The peak current enhancement was undoubtedly attributed to the interaction of SDS with SMV and GCE. It is well known that surfactants can be adsorbed on hydrophobic surface to form surfactant film, which may alter the overvoltage of the electrode and influence the rate of electron transfer [33, 34]. The probable mechanism is the SDS surfactant molecule diffuses into the glassy carbon electrode along with the SMV results increase in the signal.

3.8 Effect of SDS surfactant concentration on electrochemical response of simvastatin.

The effect of SDS surfactant concentration on oxidation of SMV was studied from 0 µL to 20 µL. The peak current increases with the concentration of surfactant upto 5 µL and then decreases with increase in concentration of SDS above 5 µL (Figure. 9). The oxidation found to be occur at lower potential.

3.9 Effect of scan rate in 5 µL SDS immobilization of GCE

The cyclic voltammograms of 2.0×10^{-4} M SMV at different scan rates from 50 to 400 mV/s at SDS immobilized GCE is shown in Figure. 10a. A linear plot of i_p vs. $v^{1/2}$ should be obtained when the electrode process is diffusion-controlled, whereas the adsorption-controlled process should result in a linear plot of i_p versus v [35]. When the potential was scanned at increasing rates from 50 to 400 mV/s, under the same experimental conditions, a linear

relationship was observed between the peak intensity i_p and scan rate v (Fig. 10b), with a correlation coefficient of 0.9976, suggesting the adsorption of SMV on the SDS immobilized electrode surface and also the linearity was obtained between the peak current, i_p and $v^{1/2}$ with a correlation coefficient of 0.9923 shown in Figure. 10c.

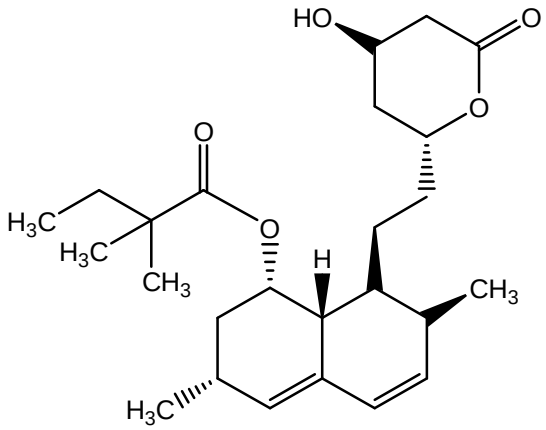


Fig 1. Chemical structure of simvastatin.

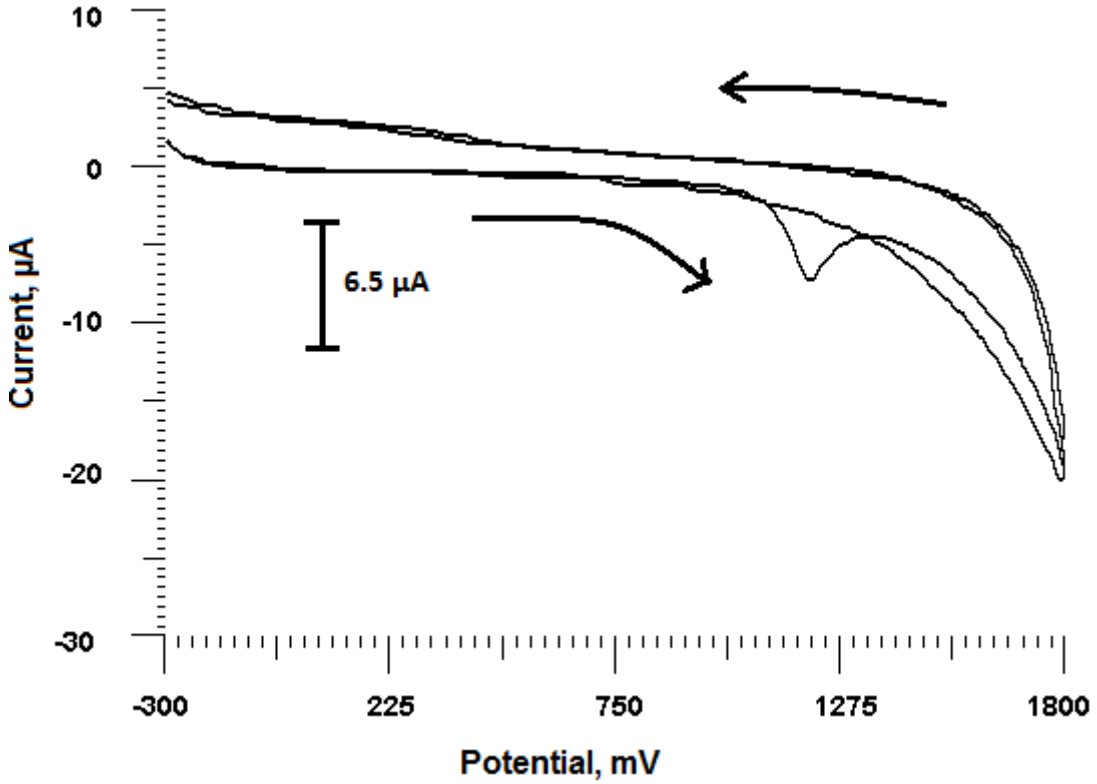


Fig 2. Cyclic voltammogram obtained for 0.1mM simvastatin on GCE in 0.1M H_2SO_4-10% ethanol: (a) simvastatin and (b) blank at scan rate: 100mV/s

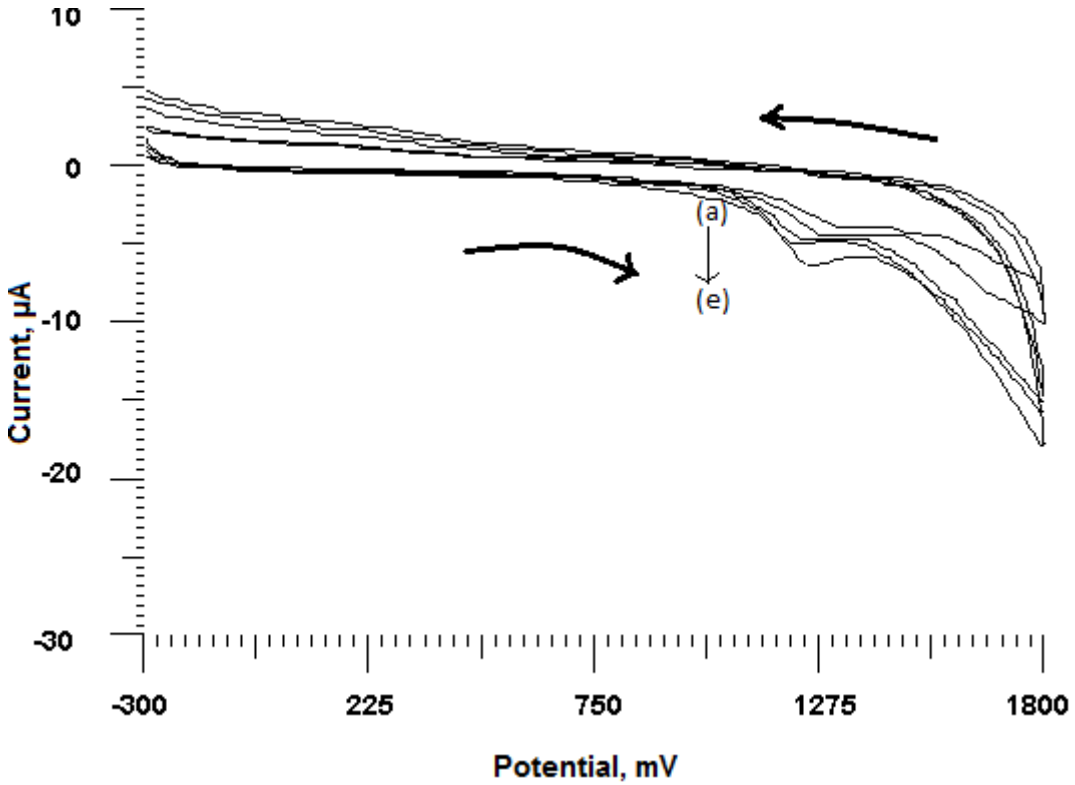


Fig 3a.Cyclic voltammograms of 0.1 mM SMV with the variation of concentration of H_2SO_4 from (a) 20 mM (b) 40 mM (c) 60 mM (d) 80 mM and (e) 100 mM

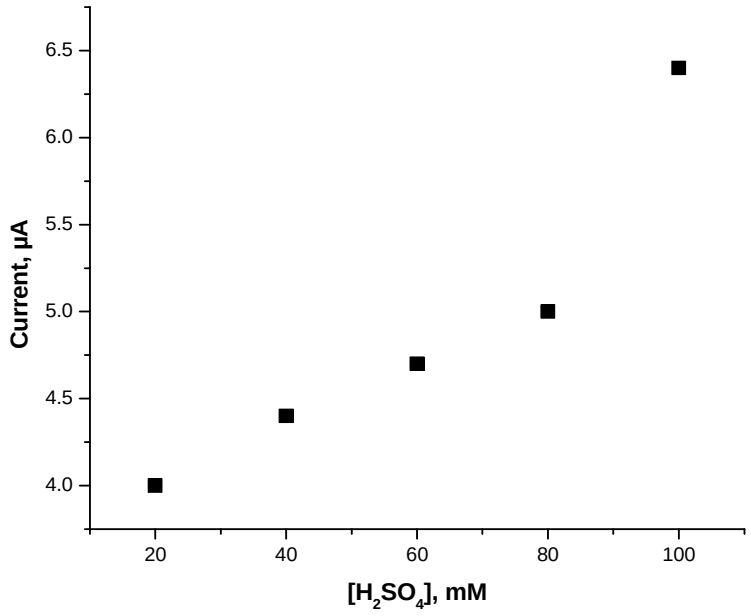


Fig 3b.The plot of Oxidation peak current versus concentration of H_2SO_4

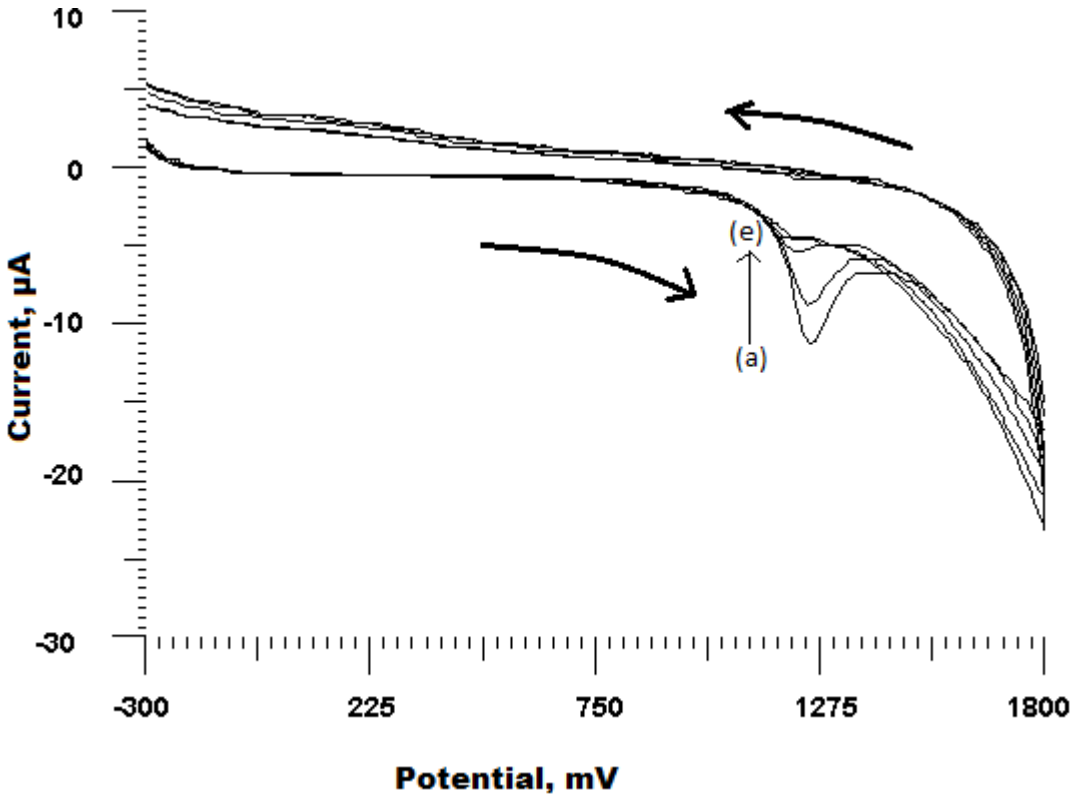


Fig 4a Cyclic voltammograms of 0.1mM SMV with the variation of % of ethanol, (a)10%, (b)15%, (c) 20%, (d)25% and (e) 30%.

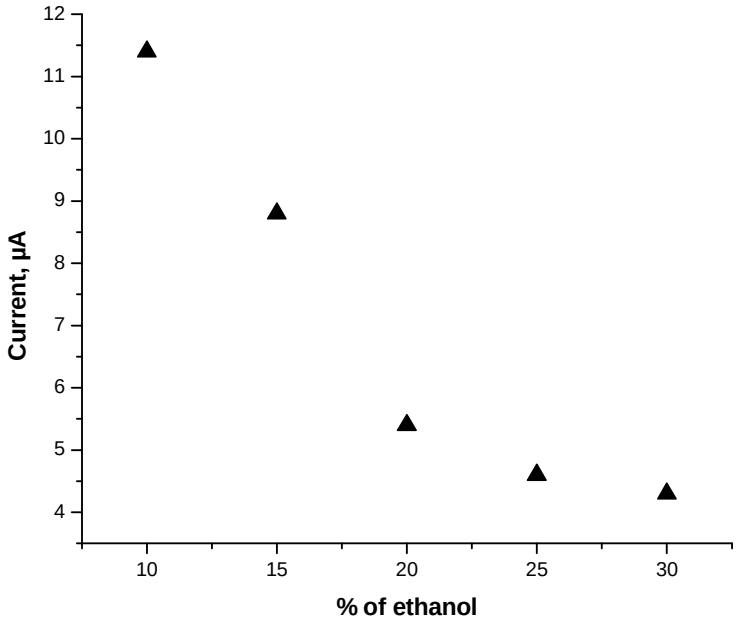


Fig 4b. The plot of the oxidation peak current on the solution % of ethanol,

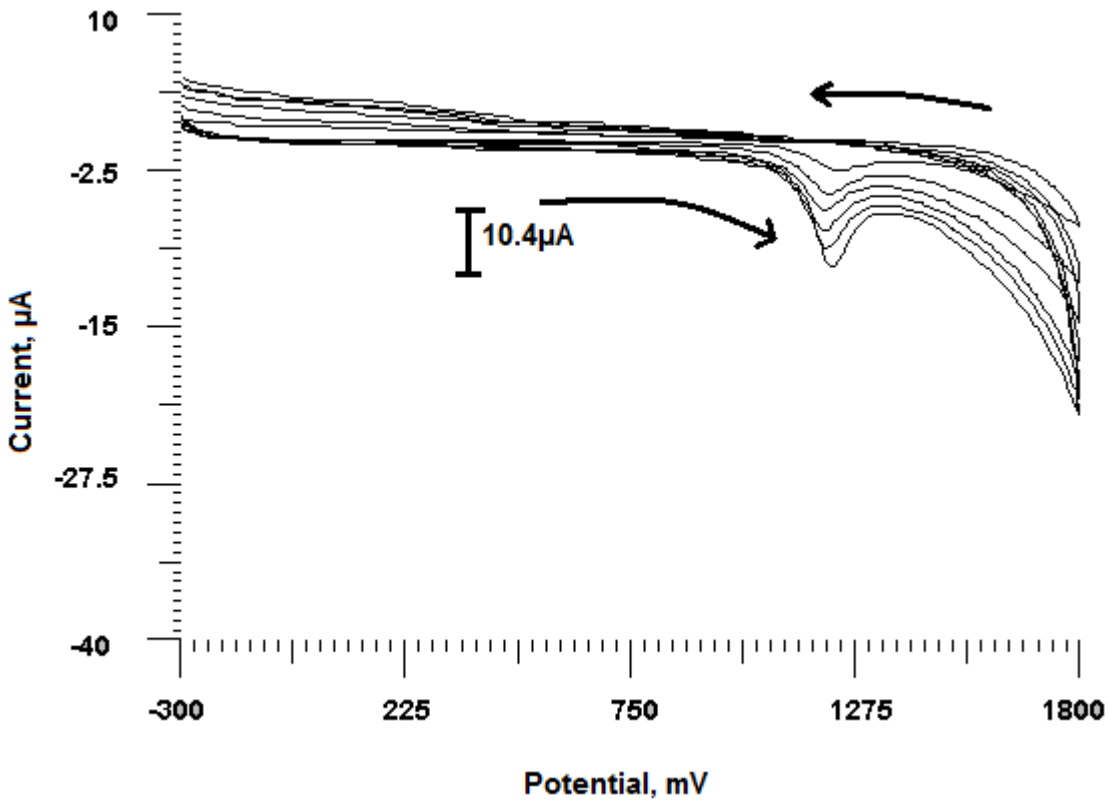


Fig 5a. Cyclic voltammograms of 0.1 mM SMV at the GCE in 0.1M H_2SO_4-10% ethanol with scan rates 25, 50, 75, 100, 125 and 150mV/s.

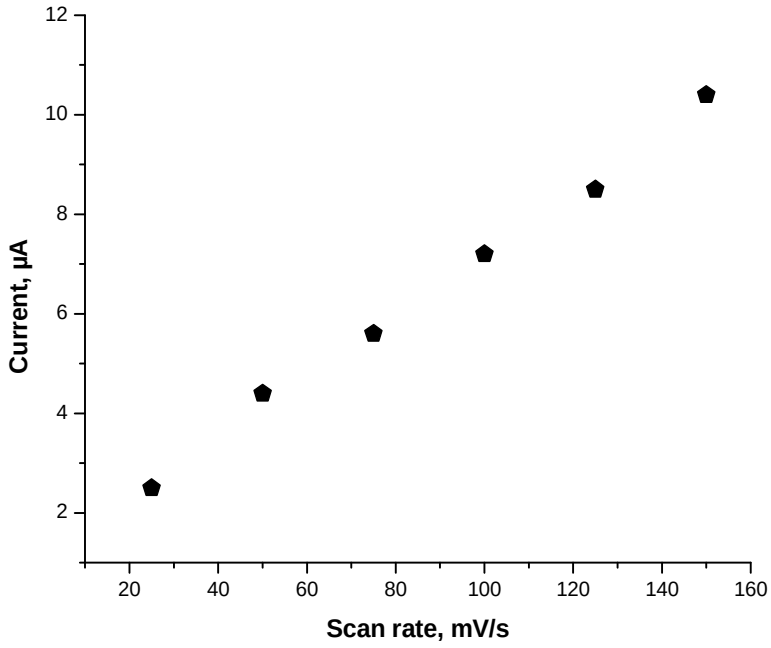


Fig 5b. The plot of oxidation peak currents vs. scan rates. (r= 0.9980)

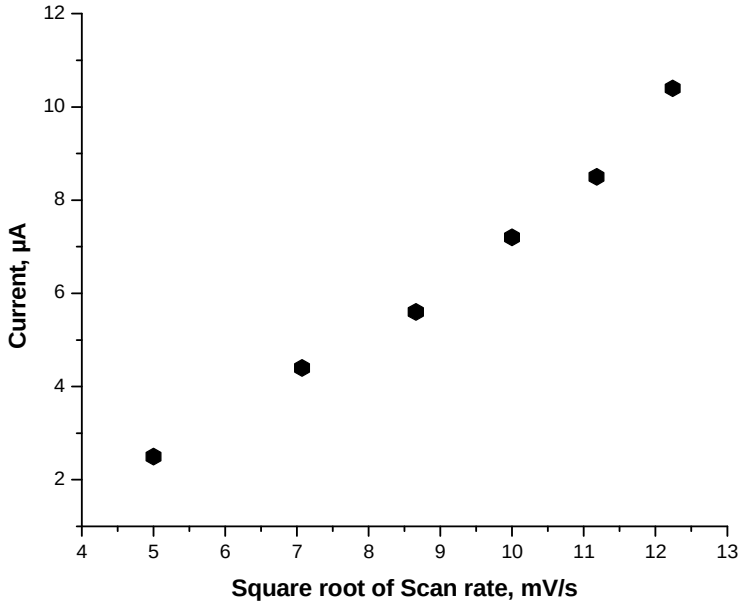


Fig 5c. The plot of peak currents vs. the square root of scan rates. (r= 0.9908)

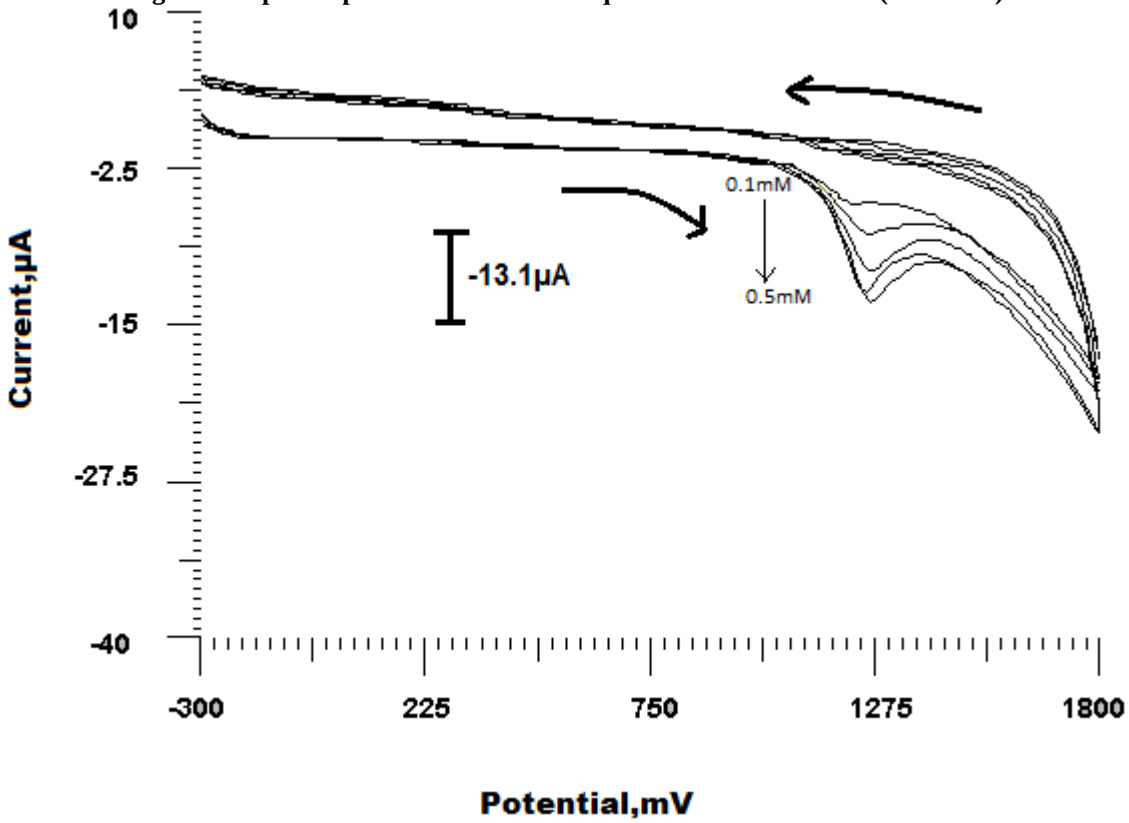


Fig.6a. Cyclic voltammogram of variation of concentration of SMV, 0.1mM, 0.2mM, 0.3mM, 0.4mM and 0.5mM in 0.1M H_2SO_4 -10%ethanol at GCE ; υ=100mV/s.

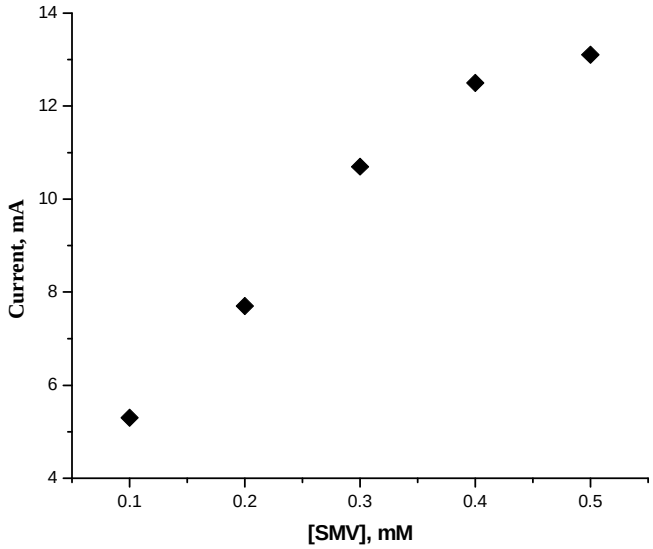


Fig 6b. Effect of variation of concentration of simvastatin on the anodic peak current 5mM in 0.1M H_2SO_4 - 10%ethanol at GCE ; υ=100mV/s.

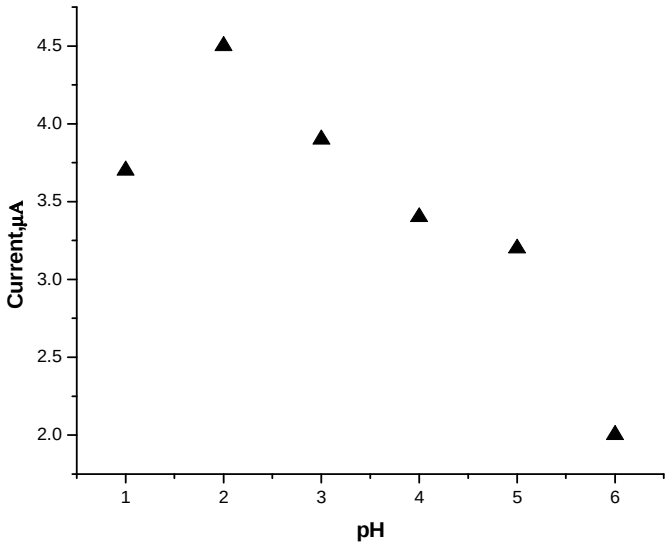


Fig 7. The plot of the oxidation peak current on the solution pH

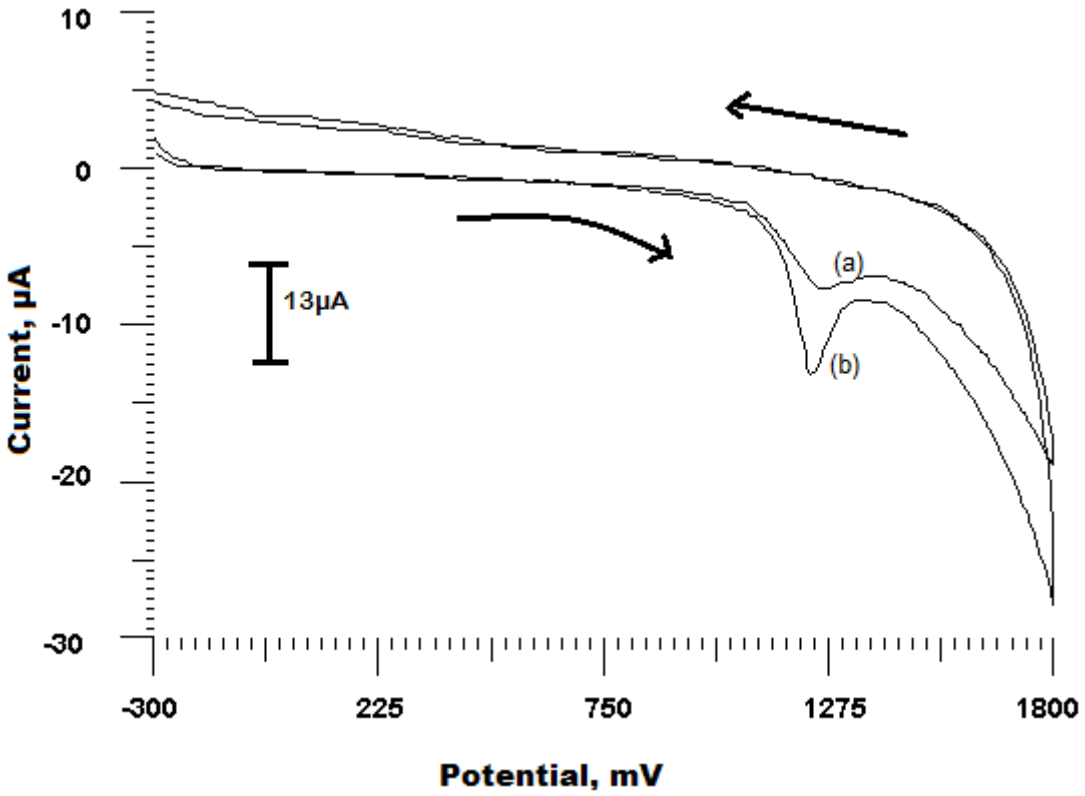


Fig 8. Cyclic voltammogram of 0.1 mM SMV for the comparision of bare GCE (a) and SDS surfactant immobilised GCEs (b).

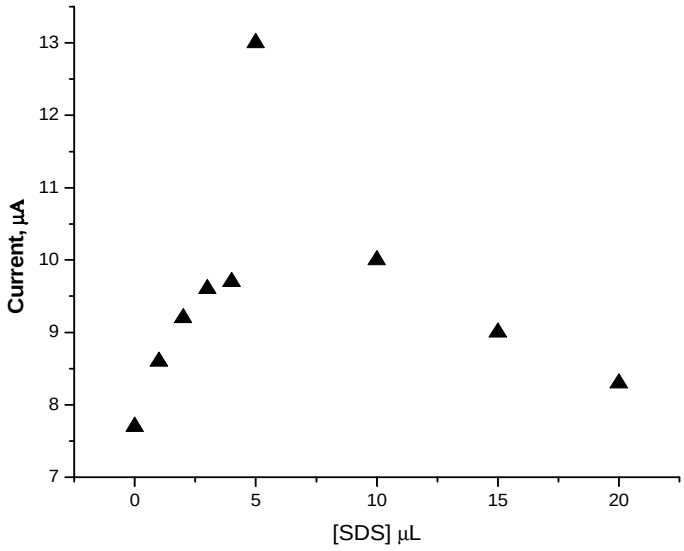


Fig. 9 Effect of SDS surfactant concentration on SMV oxidation peak at (bare GCE, 1 µL, 2 µL, 3 µL, 4 µL, 5 µL, 10 µL, 15 µL, 20 µL) immobilized GCE

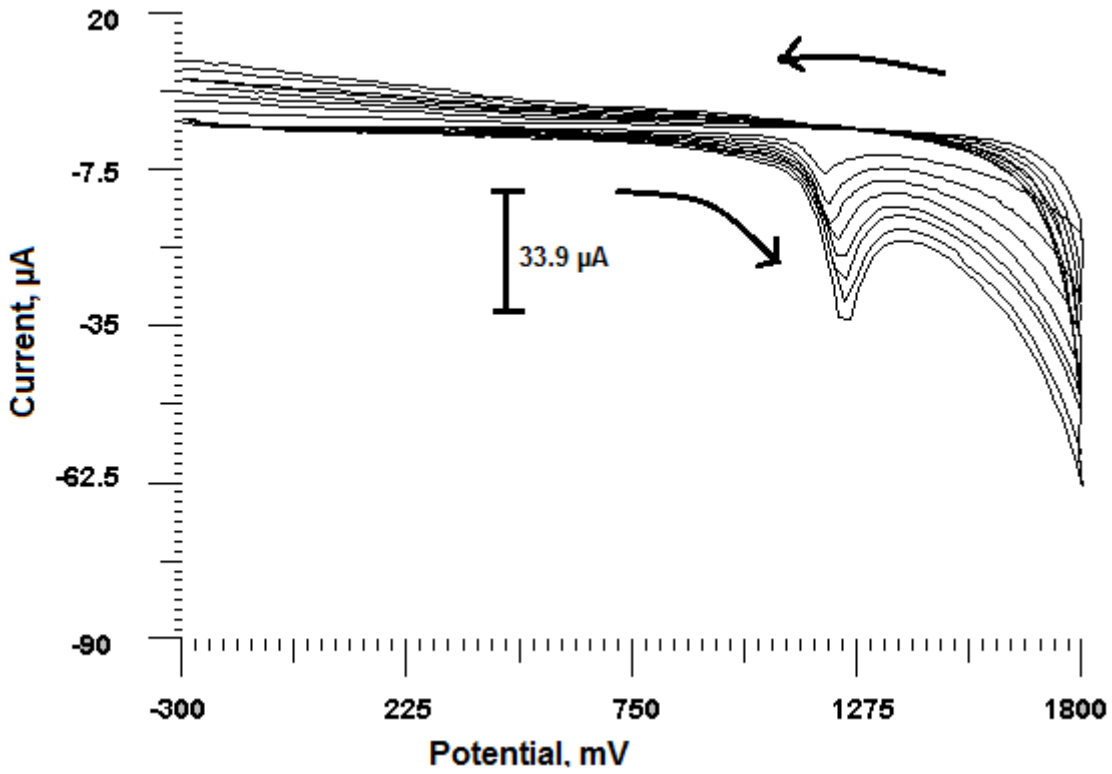


Fig. 10a. Cyclic voltammograms of 0.2 mM SMV at the SDS immobilized GCE in 0.1M H_2SO_4-10%ethanol with scan rates 50, 100, 150, 200, 250, 300, 350 and 4000mV/s.

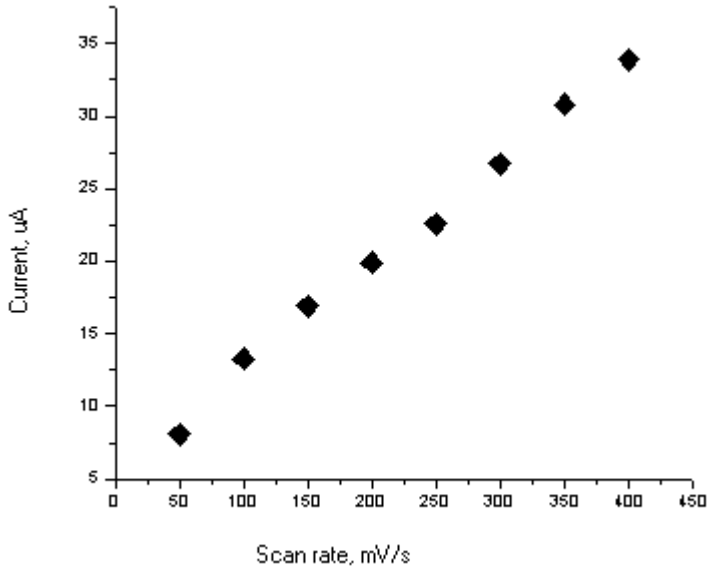


Fig. 10b. The plot of oxidation peak currents vs. scan rates at SDS immobilized GCE (r= 0.9976)

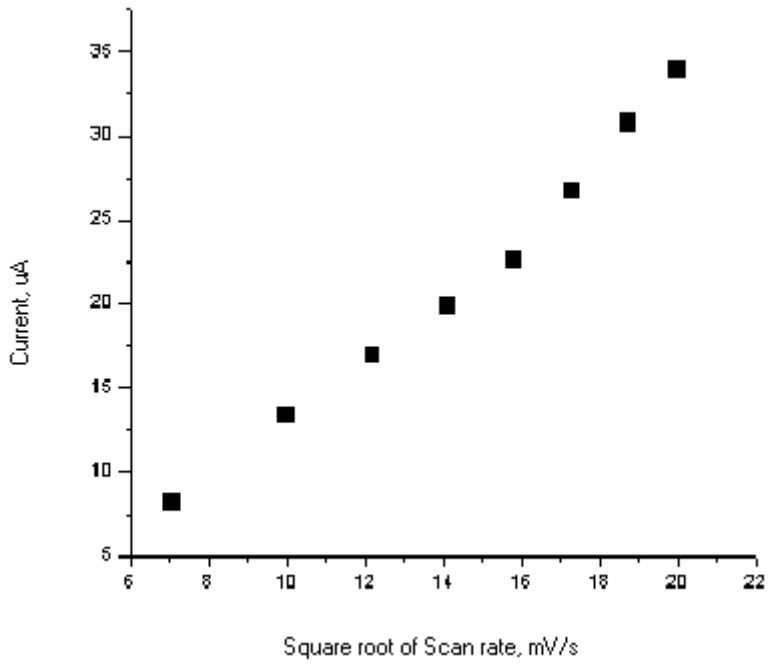


Fig. 10c. The plot of oxidation peak currents vs. square root scan rates at SDS immobilized GCE (r= 0.9923)

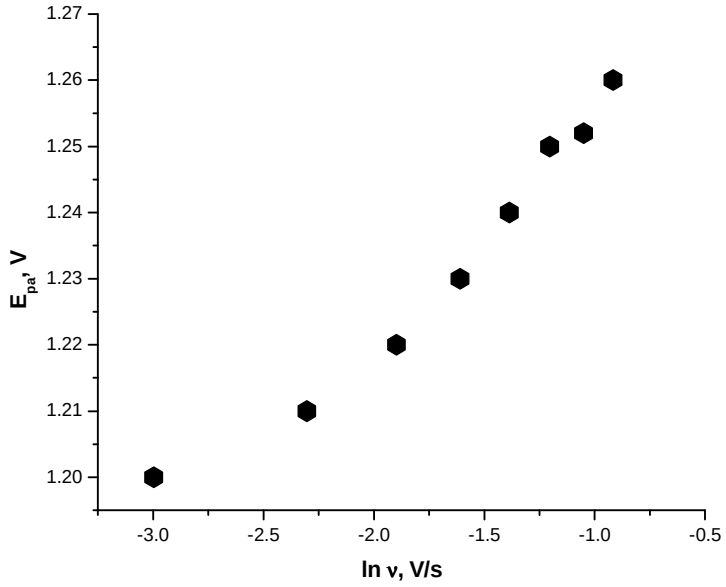
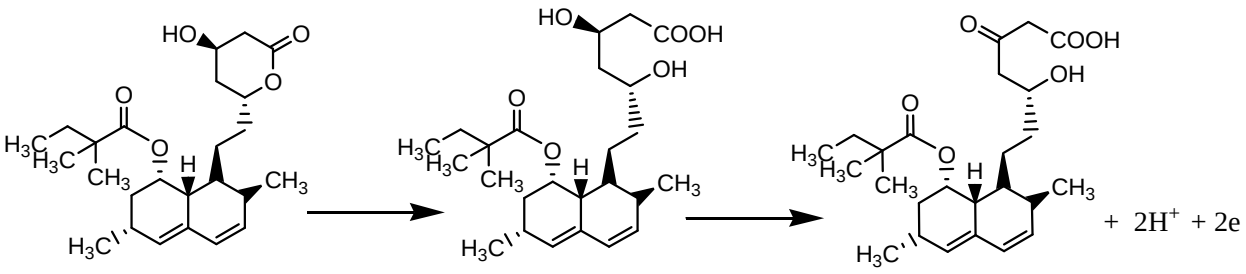


Fig. 10d. The plot of oxidation peak potential vs. natural logarithm of the scan rate at SDS immobilized GCE



A **B** **C**

Figure 11.Probable reaction mechanism for the oxidation of SMV.

Determinations of *α*, n and k_s

The cyclic voltammograms of 0.2 mM SMV at different scan rates from 50 to 400 mV/s at SDS immobilized GCE shows that both the anodic peak potential (E_p), and peak current (i_p), are affected by scan rate, *v*. According to Laviron's theory [36] , for an irreversible anodic reaction, the relationship between *E*p and *v* is described as follows:

$$Ep = E^{o} - \frac{RT}{\alpha nF} \ln \frac{RTks}{\alpha nF} + \frac{RT}{\alpha nF} lnv$$

where E^0 is formal standard potential, α the charge transfer coefficient, n the number of the electrons transferred involved in the oxidation of SMV, *F* the Faraday constant (96485 C/mol) and k_s the standard heterogeneous reaction rate constant. *R* and *T* have their usual meaning. As shown in Fig. 10d, the plot of E_p versus ln*v* is a linear relationship in the potential scan rate ranging from 50 to 400 mV/s, following the linear equation E_p = 1.2825 + 0.0297 ln*v* (R = 0.9758), which confirms that the electrochemical oxidation of SMV in our experimental conditions is totally irreversible. The value of α can be calculated using the equation shown below [37].

$$\Delta E_p = E_p - E_{p/2} = (47.7) / \alpha$$

Where E_p is the peak potential and $E_{p/2}$ is the half wave potential. The values of n and α were found to be 2 and 0.5129 respectively. The value of heterogeneous rate constant, k_s can be calculated using the equation shown below [38].

$$k_s = \frac{i_p}{0.227nFAC \exp\{-\alpha nF(E_p - E^f)\}}$$

Where, i_p is oxidation peak current, n is number of electrons involved, F is Faraday constant, A is the area of the electrode used, E_p is oxidation peak potential and E^f is formal electrode potential. The value of k_s found to be 3.58 X 10^{-2} cms^{-1}. Since the equal numbers 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 SDS immobilized GCE can therefore be summerised as in Figure 11. The chemical structure of SMV contains a β-hydroxy-lactone (A in figure 11). The physiologically active form of the drug is the β-hydroxy acid (B in figure 11), which is formed by a ring opening reaction of the lactone ring. This undergoes oxidation to form the product C.

CONCLUSION

In the present study, the electrochemical behavior of simvastatin on the glassy carbon electrode was studied by cyclic voltammetry and SDS, surfactant used as a modifier to study the electrochemical response of simvastatin on the GCE. The SDS modified GCE showed electrocatalytic action for the oxidation of simvastatin, characterizing by the enhancement of the peak current and the reduction of peak potential. The anodic peak current increases linearly with the scan rate reveals the adsorption controlled reaction. The detection limit of the SDS modified glassy carbon electrode is 2.5X 10^{-7}. The probable reaction mechanism involved in the oxidation of simvastatin was also proposed. The surface of working electrode can be prepared and renewed easily by simple mechanical polishing. Together with low cost and ease preparation, this SDS modified glassy carbon electrode seems to be of good utility for further sensor development. The proposed method was sensitive and simple. It was successfully employed to determine SMV in pharmaceutical samples.

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