International Journal of Pharmaceutical Chemistry

ISSN: 2249-734X (Online)
Journal DOI:10.7439/ijpc
CODEN:IJPCI4 (American Chemical Society)

Research Article

Cyclic Voltammetric Studies of Simvastatin at Glassy Carbon Electrode Modified with Poly(p-toluene sulfonic acid).

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Abstract

Glassy carbon electrode (GCE) is modified with electropolymerised film of p-toluene sulfonic acid (p-TSA). This polymer (p-TSA) modified electrode is used to study the electrochemical behavior of simvastatin (SMV), a lipid lowering drug in aqueous alcohol medium by Cyclic Voltammetry. It was found that the oxidation peak current of simvastatin 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 simvastatin were examined. The proposed method was sensitive and simple. It was successfully employed to determine simvastatin in pharmaceutical samples.

Keywords: Simvastatin; Cyclic voltammetry; p-toluene sulfonic acid; glassy carbon electrode.

1. Introduction

Simvastatin (SMV), chemically known as (1*S*, 2*S*, 8*S*, 8a*R*)-1,2,6,7,8,8a-hexahydro-1-(2-((2*R*,4*R*)-terahyro-4-hydroxy-6-oxo-2H-pyran-2-yl)-2,6-dimethylnapthalen-8-yl 2,2-dimethyl butanoate (Figure 1), belongs to the group of cholesterol lowering lactones known as statins, which in 2007, have been identified as being the most widely prescribed drugs in the world[1]. Statins are potent and effective inhibitors of cholesterol biosynthesis that are widely used to treat hypercholesterolemia. SMV, a lipid lowering agent that is a synthetic derivate of a fermentation product of *Aspergillus terreus* has been found to lessen both normal and elevated low density lipoprotein (LDL) concentrations[1]. The drug is officially listed in the 2004 United States Pharmacopocia and the offical method of its determination is high-performance liquid chromatography[2]. Beyond this well-defined mode of action for statins, several clinical trials such as 4S [3], WOSCOPS[4], CARE[5], and HPS[6] have demonstrated that this class of drugs can protect against cardiovascular disease (CVD) through an additional mechanism that is independent of cholesterol lowering[7]. Since its introduction, there has been a large debate surrounding the price for lipid-lowering treatment and its benefits on atherosclerosis. Although this has affected the other statins as well, SMV was the first statin drug to be used extensively in clinical practice. A number of large epidemiological studies were conducted to discover which patients would benefit most from SMV as the study drug. The most influential studies were the Scandinavian Simvastatin Survival Study (4S) and the Heart protection study (HPS). It has now become apparent that patients with one or more risk factors for cardiovascular disease (such as diabetes mellitus, hypertension or a positive family history) can benefit from statins—even if they do not have substantially elevated

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cholesterol levels. 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[8]. A recent meta-analysis of 14 randomized trials demonstrated benefits of statin therapy to reduce the vascular mortality in diabetic patients[9]. Consequently, millions of diabetic people are receiving statins [10] despite the fact that their local effects on certain tissues like the retina remain largely unknown. After conversion of this lactone prodrug to its active hydroxyl acid form, the compound is a potent competitive inhibitor of HMG-CoA reductase, the rate limiting enzyme in cholesterol biosynthesis[11].

Different analytical methods have been reported for the determination of simvastatin. Apostolou et al[12] developed a fully automated high-throughput liquid chromatography/tandem mass spectrometry method for the simultaneous quantification of simvastatin and simvastatin acid in human plasma. Basavaiah et al[13] developed two simple and sensitive spectrophotometric methods for the determination of simvastatin in pure form and in tablets using in situ generated bromine, and p-phenylenediamine or o-dianisidine as reagents in bulk drugs and in tablets. Nigovi et al[14] developed a cathodic square-wave stripping voltammetry method for determination of simvastatin at trace levels. Arayne et al[15] developed a simple UV spectrophotometric method for the determination of simvastatin in methanol and compared this with the existing pharmacopoeial HPLC method. Jitender et al[16] developed and validated a sensitive HPLC assay for simvastatin and its corresponding simvastatin hydroxyl acid for their simultaneous estimation in solutions of various studies. Coruh et al[17] studied the electrochemical behavior and determination of simvastatin in aqueous alcohol medium at a stationary glassy carbon electrode.

Abu-Nameh et al[18] proposed a simple and rapid HPLC method for the determination of simvastatin using a C18 column and acetonitrile-phosphate buffer methanol (5: 3: 1, v/v/v) as a mobile phase with detection at 230 nm. Barrett et al[19] presented a validated, highly sensitive and selective isocratic HPLC method for the quantitative determination of simvastatin and its metabolite simvastatin hydroxy acid. Godoy et al[20] developed a simple HPLC method for the determination of simvastatin in tablet dosage forms. Malenovic et al[21] used a novel and unique approach for retention modeling in the separation of simvastatin and six impurities by liquid chromatography using a micro emulsion as mobile phase. Srinivasu et al[22] developed a micellar electrokinetic chromatographic method for the quantification of lovastatin and simvastatin. Tan et al[23] developed and validated a simple and sensitive reversed-phase liquid chromatographic method for the analysis of simvastatin in human plasma. Wang et al[24] developed a second derivative UV spectroscopic method for the determination of simvastatin in the tablet dosage form. Ochiai et al[25] developed a highly sensitive and selective high performance liquid chromatographic method for the determination of simvastatin (I) and its active hydrolyzed metabolite (II) in human plasma. Carlucci et al[26] developed and validated a fast, simple and accurate method for determining simvastatin and simvastatin acid concentrations in human plasma. However, some of these methods HPLC, HPLC-MS/MS, derivative spectrophotometry and voltammetric techniques 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.

The concept of chemically modified electrodes (CME's) is one of the exciting developments in the field of electroanalytical chemistry[27]. The electropolymerisation generally results in polymer which is uniform and strongly adhere to the electrode surface. Polymer-modified electrodes have many advantages in the detection of analytes because of their selectivity and homogeneity in electrochemical deposition, physical and chemical stability [28,29]. Glassy carbon electrode (GCE) has been very popular because of its excellent electrical and mechanical properties, wide potential range, extreme chemical inertness and relatively reproducible performance[30-35].

In this present work, a poly (p-TSA) film was fabricated on the surface of a GCE in 0.1M NaCl solution by Cyclic Voltammetry(CV). The polymer was found to be electrocatalytically active for the oxidation of SMV. With its good sensitivity, selectivity and stability, the polymer coated GCE has been used for the determination of SMV.

2. Experimental

2.1 Reagents

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 H_2SO_4 and Britton Robinson buffer solution,

2.2 Apparatus

Electrochemical measurements were carried out with a model EA-201 electroanalyser (chemlink systems) a three electrode system was employed. The poly (p-TSA) 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. Each polishing step is 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 0.1 M H_2SO_4 solution. The electrode was subsequently placed in a solution containing 0.10 M NaCl and 1 mM p-TSA and cyclic potential sweep was applied in the range of -2000 to +2500 mV at a scan rate of 100 mV/s for 15 times. The poly (p-TSA) fabricated modified GCE after polymerisation washed with distilled water.

3. Results and Discussion

3.1 The electropolymerisation of p-TSA at the glassy carbon electrode surface

Electrooxidation of alkyl substituted aromatic compounds is believed to occur by the removal of an electron from the alkyl side chain or the aromatic ring and by the formation of a radical cation. The reaction mechanism of the electropolymerization of p-TSA has been reported[36]. Fig. 2 shows the cyclic voltammograms of 1.0 mM p-TSA in 0.1M NaCl solution at glassy carbon electrode. In the first cycle, with the potential scanning from -2000 to 2500 mV, three strong reduction peaks were observed at 309 mV (peak A), -581 mV (peak B) and -1706 mV (peak C), which might be due to reduction of the monomer. From the third cycle onwards, one obvious oxidation peak appeared with potential at 1524 mV (peak D). In the second and subsequent cycles the larger peaks were observed upon continuous scanning, which was reflecting the continuous growth of the film. This indicated p-TSA was gradually deposited on the surface of GCE by electropolymeriszation. A uniform adherent blue colored polymer was formed on the GCE. After modification (15 cycles), the poly (p-TSA) modified electrode was carefully rinsed with distilled water and was used for the determination of SMV.

3.2 Electrochemical behavior of simvastatin at poly (p-TSA) modified GCE

Cyclic voltammetry was utilized to investigate the electrochemical behavior of simvastatin at the p-TSA polymer film GCE (Figure 3c), a bare GCE (Figure 3b) and cyclic voltammogram of bare GCE in blank solution containing 0.1M H_2SO_4-10% ethanol solution (Figure 3a). It showed that only one oxidation peak at +1151 mV and a peak current of 32 µA at bare GCE, whereas an oxidation peak at 1052 mV and a peak current of 80.4 µA at the poly (p-TSA) modified GCE, in the potential range -50 to +1500 mV. No reduction peak was observed in the reverse scan, suggesting that the electrochemical reaction is a totally irreversible process. From the figure 3b, the oxidation peak at the bare GCE is broad due to slow electron transfer, while the response was considerably improved at the poly (p-TSA) film electrode and the peak potentials shifted in negative direction.

3.3 Effect of the poly (p-TSA) film thickness on the electrochemical response of simvastatin

The thickness of poly (p-TSA) film could be controlled by the cyclic number of voltammetric scans during the electrochemical modification. The effect of the thickness of poly (p-TSA) film on the electrode surface on the electrochemical response of simvastatin was investigated by cyclic voltammetry. The current (I_{pa}) response of poly (p-TSA) films increase gradually as the number of cycles increases during film formation from 5 to 15 cycles. Afterwards I_{pa} starts to decrease by increasing the number of cycles which was examined upto 25 cycles (Fig.4) In order to obtain better oxidation peak and higher sensitivity of current for the electrochemical response of SMV, 15 scans were chosen to control the thickness of the poly (p-TSA) film.

3.4 Effect of supporting electrolyte concentration

It was not obvious from the literature the particular choice of supporting electrolyte or its concentration for. The electrochemical oxidation of simvastatin was studied in various supporting electrolytes such as CH_3COOH-CH_3COONa, 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 with respect to single enhancement accompanied by sharper response were

obtained with H_2SO_4. The effect of the concentration of H_2SO_4 was tested over the 0.02 – 0.1 M range. The cyclic voltammograms of 0.1 M SMV with the varying concentrations of supporting electrolyte, H_2SO_4 on the poly (p-TSA) modified GCE was shown in Fig. 5a And the variation of oxidation peak current of SMV with the variation of concentration of H_2SO_4 on poly (p-TSA) modified GCE was shown in Fig. 5b.

3.5 Effect of Ethanol

Owing to the insolubility of simvastatin in aqueous solution, ethanol was used as solvent. The effect of % of ethanol on the oxidation peak current of SMV on poly (p-TSA) modified GCE was therefore examined in the range of 10-30% (Fig. 6) 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.

3.6 Effect of scan rate

The effect of scan rates on the electrochemical response of 0.1mM SMV at poly (p-TSA) modified GCE was studied at different scan rates including 25, 50, 75, 100 and 125 mV/s by CV. From Figure 7a, it was found that the oxidation peak current increases linearly with the increase in scan rate with a correlation coefficient of 0.9922 and slope of 0.8856, which revealed that an adsorption controlled process occurring at the poly (p-TSA) modified GC in the range of 25-125 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 0.9771 in Fig 7b. In the experiment, the relationship between the oxidation peak potentials and the scan rates can be described as following (Figure 7c) :E_{pa}= 0.0664 log υ +0.9132; r=0.9921. According to Laviron's theory[37], the slope was equal to 2.303RT/αn_αF which lends to value of αn_α calculated as 0.985. As for a totally irreversible electrode reaction process, α was assumed as 0.5 which lends to the n_α calculated to be 1.97 indicating that two electrons were involved in the oxidation process of SMV at the poly (p-TSA) 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 (p-TSA) film modified GCE can therefore be summarized as in scheme I.

3.7 Effect of simvastatin concentration

The variation of concentration of SMV was studied at poly (p-TSA) film modified GCE at a scan rate of 100 mV/s. Fig. 8a shows the cyclic voltammograms of SMV at poly (p-TSA) film modified 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 $0.4X10^{-3}$ M with a correlation co-efficient of 0.9979 in Fig 8b.

3.8 Effect of pH

The influence of solution pH on the oxidation of 0.1mM SMV at the poly (p-TSA) modified 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 protons take part in these electrode reactions. Fig. 9 shows the linear relationship between the anodic peak current and pH of the solution with a negative slope of 7.3714 mV and beyond pH 2, a great decrease of the oxidation peak current could be observed, then it decreased gradually with the further increasing the pH of solution.

4. Conclusions

In the present study, a novel method of use of poly (p-TSA) modified GCE for the determination of simvastatin was developed. The electrochemical behavior of simvastatin on the modified electrode was investigated by cyclic voltammetry. The poly (p-TSA) film showed electrocatalytic action for the oxidation of simvastatin, 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 oxidation mechanism of SMV is also proposed. The oxidation peak current was linear in the range of 0.1X10-^{3} M to $0.4X10^{-3}$ M with a detection limit of 1.5 $X10^{-7}$. Together with low cost and ease of preparation, this film modified electrode seems to be of good utility for further sensor development

Acknowledgements

Deepa M.B. thanks the Department of Science and Technology (DST), New Delhi, for the award of INSPIRE Fellowship (No.DST/INSPIRE Fellowship/2010/[73] Dated: 21st December, 2010)

Figure 1. Chemical structure of simvastatin.

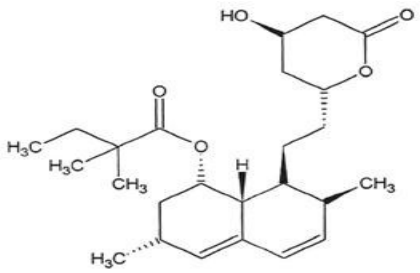


Figure 2. Cyclic voltammograms for the electropolymerisation of 1mM p-tolune sulfonic acid in 0.1M NaCl solution on a GCE. Initial potential -2000 mV, Terminal potential 2500 mV. Scan rate: 100mVs^{-1}.

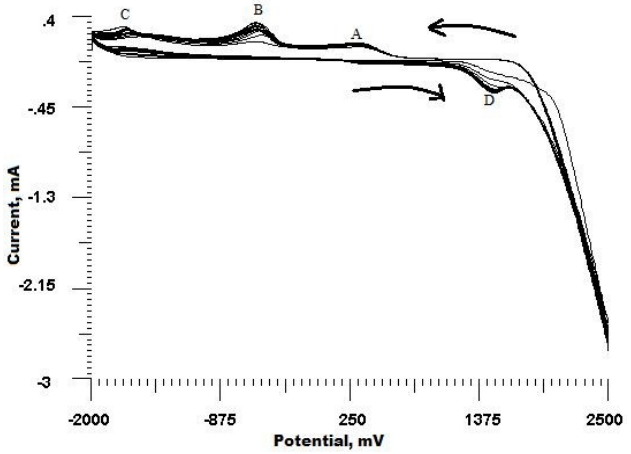


Figure 3. Typical Cyclic Voltammograms of 0.1 X 10^{-3} M simvastatin at the poly(pTSA) modified GCE (c), a bare GCE (b), and without simvastatin (a) in 0.1M H_2SO_4-10% ethanol, scan rate: 100mVs^{-1}.

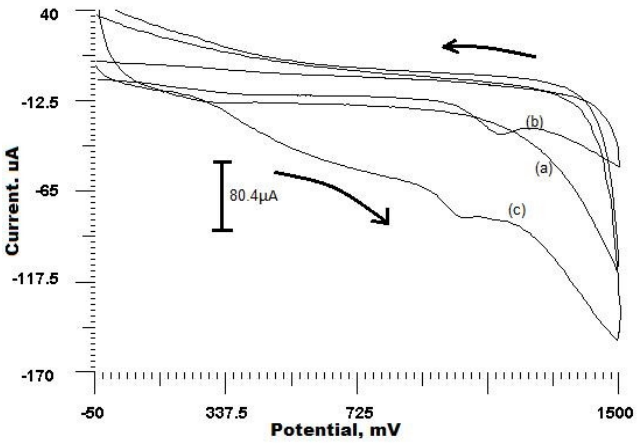


Figure 4. The plot of Oxidation peak current versus number of cycles.

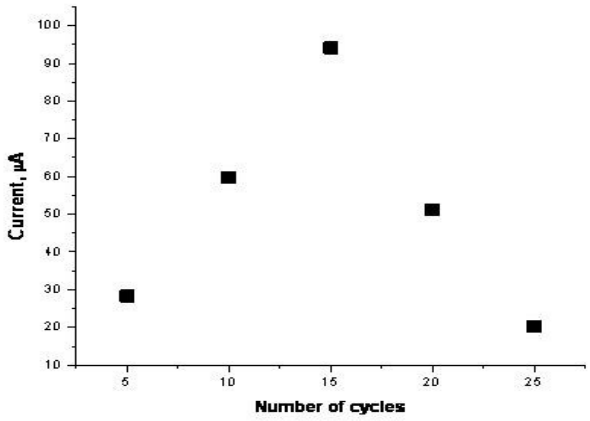


Figure 5a. Cyclic voltammograms of 0.1X10^{-3} M SMV at the poly(pTSA) GCE with the ariation of concentration of H_2SO_4, 0.02 M, 0.04 M, 0.06 M, 0.08 M and 0.1 M.

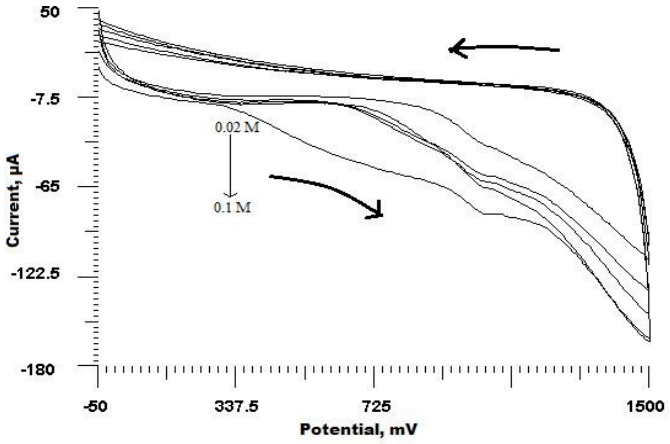


Figure 5b. The plot of Oxidation peak current versus concentration of H_2SO_4.

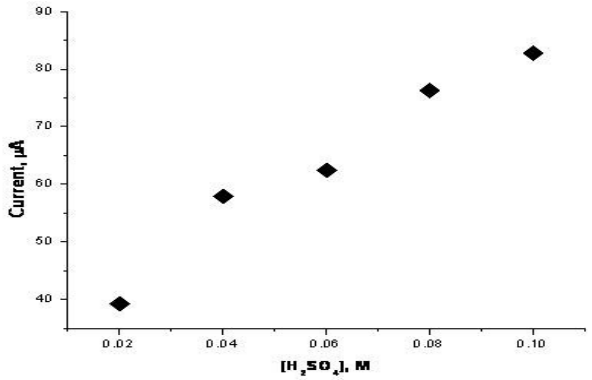


Figure 6. Dependance of the oxidation peak current on the solution % of ethanol, 10, 15, 20, 25 and 30%.

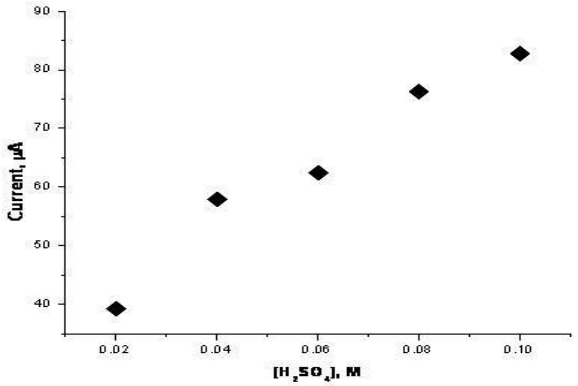


Figure 7a. The plot of Oxidation peak current versus Scan rates.

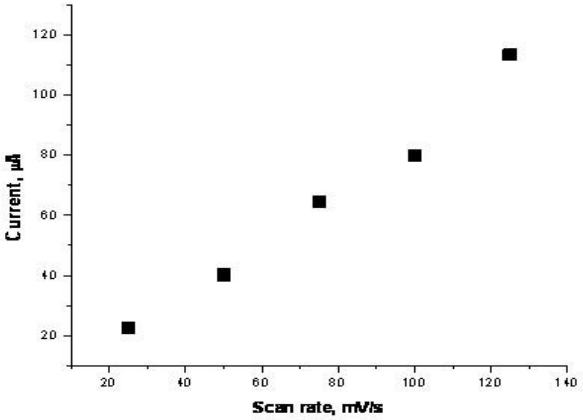


Figure 7b. The plot of oxidation peak current and the square root of Scan rates.

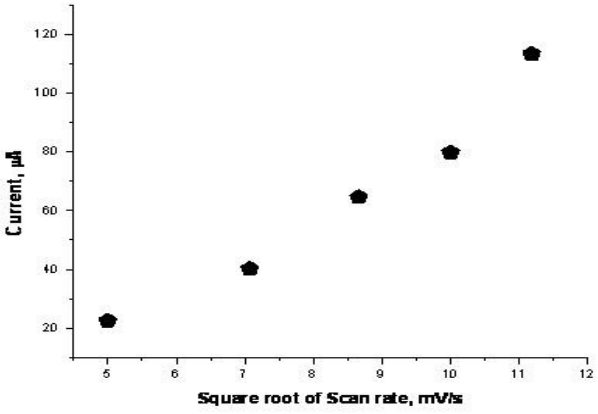


Figure 7c. The plot of oxidation peak potential, E_{pa} versus logarithm of Scan rates.

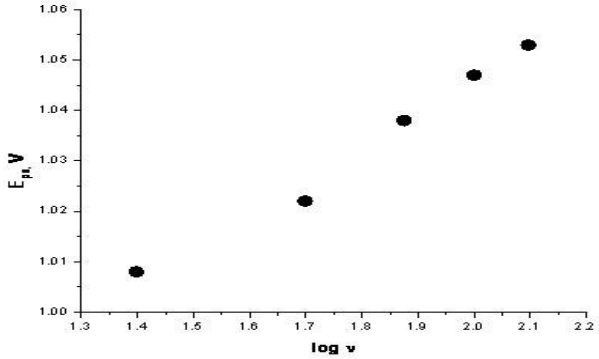


Figure 8a. Cyclic voltammogram of variation of concentration of simvastatin from 0.1mM to 0.4mM at the poly(pTSA) GCE.

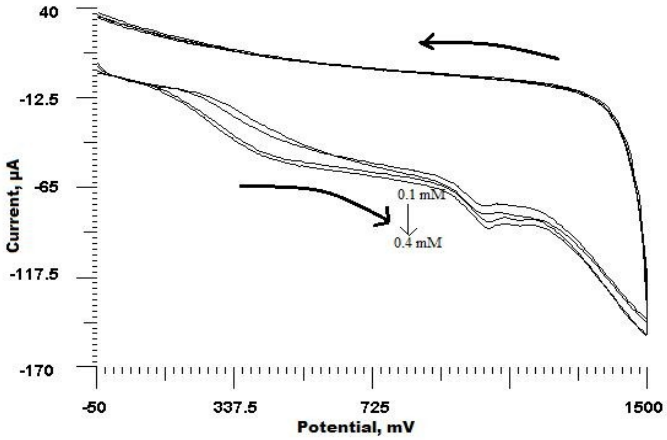


Figure 8b. Effect of variation of concentration of simvastatin on the anodic peak current at the poly(pTSA) GCE.

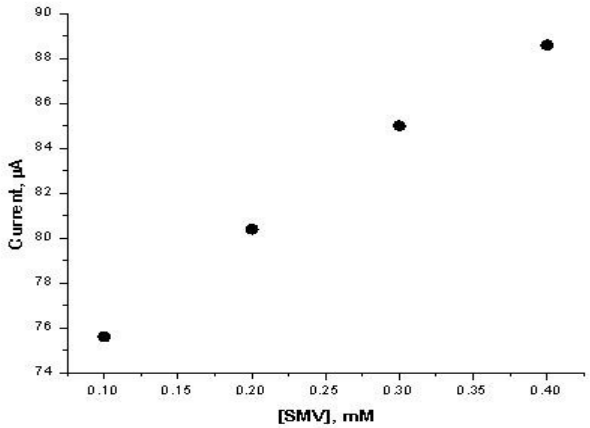
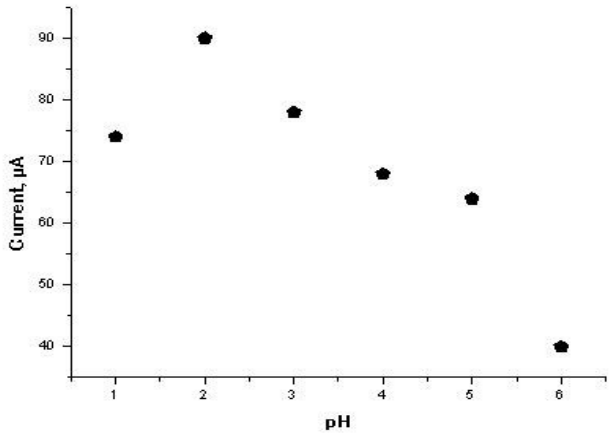
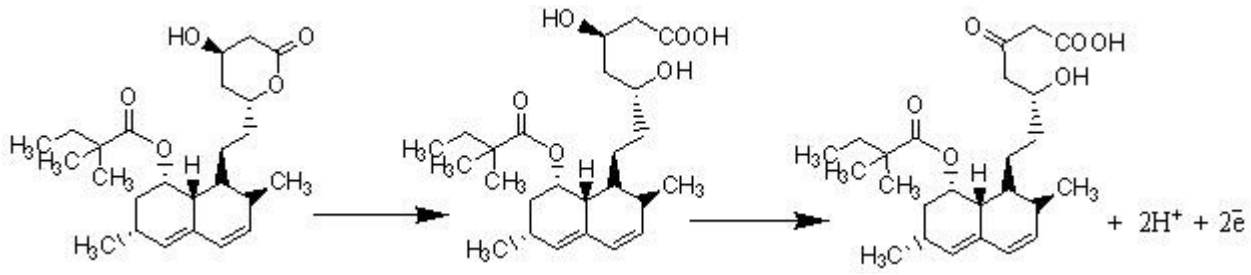


Figure 9. The plot of oxidation peak current on the solution pH.



Scheme I. Probable reaction mechanism for the oxidation of SMV.



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