Investigation of the Voltammetric Behavior of Methyldopa at Poly (*p*-Aminobenzene Sulfonic Acid) Modified Sensor

Poli (*p*-Aminobenzen Sülfonik Asit) Modifiye Sensör ile Metildopanın Voltametrik Davranışının İncelenmesi

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ABSTRACT

Objectives: Modification of carbon electrodes with poly (*p*-aminobenzene sulfonic acid) and use as a sensor for sensitive and reliable detection of methyldopa (MD) and ascorbic acid.

Materials and Methods: Electropolymerization was performed by cyclic voltammetry (CV) in 0.1 M KCl solution. The modified sensor has high electrocatalytic effect for oxidation of methyldopa, which appeared at pH range of 2-11 by differential pulse voltammetry (DPV) techniques.

Results: For the voltammetric determination of MD, the best results were acquired by DPV technique in phosphate buffer solution (PBS) (pH 3). The calibration plot of the proposed sensor is linear in two concentration ranges of 1.0-30 and 30.0–300.0 µM. The calibration equation over theses ranges are: I_{pa} (µA) = 1.21 × C (µM) +30.81, R^2 = 0.994 and I_{pa} (µA) = 0.53 x C (µM) + 53.30, R^2 = 0.9975 respectively. In sensitivity studies, the limit of quantification (LOQ) and the limit of detection (LOD) were 10.6 nM and 5.0 nM, respectively. This modified electrode was used as a sensor for determination of MD in pharmaceutical and serum samples with satisfactory results.

Conclusion: The obtained results revealed that prepared modified electrode and the proposed method have good sensitivity, repeatability, reproducibility and stability.

Key Words: Methyldopa, voltammetry, poly (*p*-aminobenzene sulfonic acid), ascorbic acid, glassy carbon electrode.

ÖZET

Amaç: Karbon elektrotların poli (*p*-aminobenzen sülfonik asit) ile modifikasyonu ve metildopa (MD) ve askorbik asitin hassas ve güvenilir bir şekilde tayini için bir sensör olarak kullanılması.

Gereç ve Yöntemler: Elektropolimerizasyon, 0.1 M KCl çözeltisi içerisinde dönüşümlü voltametri (CV) ile gerçekleştirildi. Modifiye edilmiş sensör metildopanın oksidasyonu için yüksek elektrokatalitik etkiye sahiptir, bu da 2-12 pH aralığında diferansiyel puls voltametri tekniği ile gözlenmiştir.

Bulgular: MD'nın voltametrik tayini için, en iyi sonuçlar fosfat tampon çözeltisinde (PBS) (pH 3) DPV tekniği ile elde edildi. MD için konsantrasyon aralığı, DPV ile 3.2 x 10^{-8} ile 4.7 x 10^{-7} M arasındaydı. Duyarlılık çalışmalarında, tayin alt sınırı (LOQ) ve tayin sınırı (LOD) sırasıyla 10.6 nM ve 5.0 nM idi. Modifiye edilmiş sensör, MD ile askorbik asit (AA) gibi girişim yapan maddelerin gerçek örneklerde eş zamanlı tayini için kullanıldı.

Sonuç: Elde edilen sonuçlar, modifiye edilmiş elektrot ve önerilen yöntemin iyi duyarlılık, tekrarlanabilirlik, tekrar üretilebilirlik ve kararlılığa sahip olduğunu ortaya çıkardı.

Anahtar kelimeler: Metildopa, voltametri, poli (*p*-amino benzen sülfonik asit), askorbik asit, camsı karbon elektrot

INTRODUCTION

Methyldopa, is a catecholamine which is known by its chemical name 2-Methyl-3-(3,4-dihydroxyphenyl)-DL-alanine (Figure 1), and it is widely used to lower blood pressure. MD is a centrally acting adrenal-receptor that reduces high blood pressure and sympathetic tone.[1] In adrenergic nerve terminals, it is converted to α-methyl noradrenaline, and its antihypertensive effect seems to be owing to this agent stimulate the central adrenoreceptors.[2]

Various methods like high-performance liquid chromatography with UV detection[3], polarographic[4], potentiometry[5], ultra violet visible spectrophotometry[6] and flow injection techniques[7,8] were reported previously for the determination of MD. However, many of these techniques require expensive equipment and time consuming. In addition, since these catecholamines are electrochemically active, it is also possible to determine the nature of the molecules that provide neurotransmission by electrochemical methods. Therefore, it is important to detect of MD in the presence of AA by a reliable method, which has good selectivity and sensitivity.

Ascorbic acid (vitamin C) is a biologically and industrially important substance[9]. The coexistence of ascorbic acid, MD and other catecholamines with very close oxidation potentials leads to the response obtained by electrochemical techniques. For this reason, the increased sensitivity and selectivity of the new sensors produced to the MD has long been the subject of researchers working on. Using the polymer modified electrodes solves this problem. Electrochemical behavior of MD was studied at various polymer electrodes.[10-17]

However, some disadvantages exist in the previously reported modified electrodes. AA exists as an anion in physiological pH (7.4), whereas MD exists as a cation. There are high electron density sulfo groups and electron-rich N atoms in the structure of *p*-(ABSA). For this reason, a negatively charged polymer film is required to eliminate the interference of AA in the determination of MD. The *p*-aminobenzene

sulfonic acid molecule has a high electron density sulfo groups, and *p*-(ABSA) films are negatively charged. Due to the electrostatic repulsion between the negatively charged sulfo groups and the ascorbic acid anions in the modified sensor, the ascorbic acid shifts to a more negative potential and the dopaminic acid can be easily separated from ascorbic acid. The *p*-(ABSA) modified sensor can show high selectivity against MD.[18]

In this study, electroanalytical methods were developed to detect methyldopa in drug samples rapidly, reliably and sensitively using the electrode modified with poly (p-ABSA). It has been determined that the modified sensor can be utilized to the MD determination even in the presence of ascorbic acid at the same time. Another significant advantage of these techniques over other techniques is that they can be applied directly to the analysis of pharmaceutical dosage forms and biological samples, without the need for extensive sample preparation, as there is no interaction between the adjuvants and the endogenous substances.

The analytical determination parameters such as the limit of detection (LOD), the limit of quantification (LOQ) and the concentration range were determined, and the amount of MD in the drug tablets and blood serum was found. To test the accuracy of the applied voltammetric method, MD recovery studies were performed in real samples.

MATERIALS AND METHODS

Materials

Alfamet tablet containing 250 mg MD was kindly supplied by I.E. Ulagay (Turkey). All chemicals were of analytical purity and were procured from Merck (Darmstadt, Germany) or Sigma Chemical Company. Prior to the polymerization, the solutions of monomer were held in the nitrogen gas atmosphere for about 10 minutes, and during the electropolymerization, the electrochemical cell was covered with nitrogen gas. Voltammetric experiments were carried out in the phosphate buffer solution (pH 3.0). Methyldopa and AA solutions were freshly prepared before the experiments. All solutions were prepared in with ultra-pure water.

Instrumentation

In voltammetry experiments, BAS (Bioanalytical Systems, Inc.) 100BW electrochemical analyzer was used. This analyzer is connected to a personal computer and the device is controlled, data stored and processed by means of software loaded and running under MS-Windows. The electrode system consisting of an Ag/AgCl reference electrode (CHI), a glassy carbon disc working electrode (geometric area: 6.85 mm^2, CHI) and a Pt wire coil as auxiliary electrode (CHI) was used.

Modification of Poly (p-ABSA) Sensor

Before modification, the working glassy carbon electrode (GCE) was cleaned using 0.3 and 0.05 μm Al_2O_3 slurry on polishing materials. Then polished GCE was sonicated in 1:1 nitric acid solution for 10 min and washed with ultra-pure water. Afterward, GCE was electrochemically cleaned by 20-times cycling in the potential range of (−0.7) to (1.7) V with a scan rate of 100 mV/s in 0.5 M H_2SO_4. After that, the electrode was plunged into 0.1 M KCl solution containing 5.0 mM *p*-ABSA and modification procedure was performed by cyclic sweeping from (-1.5) to (2.5) V for 14 cycles at 50 mV/s. Then, the modified sensor was conditioned by cyclic voltammetry in the potential range of (-0.5) - (0.5) V with 100 mV/s in pH 3.0 PBS and was stored in PBS (pH 3.0).

Preparation of real samples

Tablet of MD with the commercial name of Alfamet were prepared. Each tablet contains 250 mg methyldopa. Five MD tablets were finely powdered using a mortar and pestle and then, appropriate amount of this sample containing a known amount of the active material was weighed and dissolve with double distilled water. The prepared mixture was filtered using a filter paper and diluted to appropriate amounts with double distilled water. The serum samples were collected from research hospital and were sonicated (15 minutes with 5000 rpm) and then diluted 10 times with doubly

distilled water without any additional pretreatment. Before voltammetric determination, appropriate amount of the prepared real samples were added to 10 mL of phosphate buffer solution with optimum pH (pH 3.0) and then was transferred to the electrochemical cell for electrochemical measurements. The standard addition method was used to determine MD in the real and spiked samples.

RESULTS AND DISCUSSION

Electropolymerization of p-Aminobenzene Sulfonic Acid

Figure 2 shows the electropolymerization of p-ABSA at the GCE surface. The electropolymerization was performed in 0.1 M KCl solution containing 5.0 mM p-ABSA at a GCE by cyclic voltammetry technique in the potential range of (-1.5)-(2.5) V. In the first cycle, two reduction peaks were obtained at 0.452 V (peak A) and 0.449 V (peak B), which might be related to the reduction of *p*-ABSA. Again, in the first cycle, an oxidation peak was observed at 0.824V (peak C). In the next and subsequent cycles, following the continuous scan, broader peaks were monitored providing that the polymer film was constantly growing. It could be observed that the film growth was faster for the first five cycles than for the other cycles and also, the next cycles are no longer exist. From these, it could be said that *p*-ABSA was coated on the GCE surface by electrodeposition. A brown polymer was formed that was properly bonded on the GCE surface.

The Effect of Film Thickness on MD Response

The film thickness, which is determined with the number of cycles of electropolymerization, is one of the most important factors determining the polymer film selectivity property. By altering the amount of charge consumed during electropolymerization, it is possible to obtain poly (*p*-ABSA) films at desired thicknesses. Different film thicknesses were obtained by varying the cycles of the cyclic voltammetry. The selectivity of poly (*p*-ABSA) sensors prepared in the range of 8–18 cycles to MD and AA were systematically examined. From the DPV results of MD, it was observed that the regular and repetitive responses could be obtained at 14 cycles film thickness. It can be seen from the Figure 3. Furthermore, the effect of

the number of cycles on the electropolymerization was calculated as 64.42%. The value was calculated from the ratio of the highest peak current to the peak current of the first polymer film.

Electrochemical behavior of MD at poly (p-ABSA) modified sensor

The voltammograms achieved by the cyclic voltammetry technique of MD show a reduction wave at a potential of nearly 200 mV potential and an oxidation peak of nearly 220 mV (Figure 4). The electrochemical oxidation of MD was studied by cyclic voltammetry at the surface of the bare and poly (*p*-ABSA) modified GCE. The oxidation of MD shows a weak peak on the bare GC at nearly 0.590 V but the experimental results for modified GCE show well-defined anodic peak at the peak potential of 0.220 V, respect to Ag/AgCl reference electrode. The peak current and peak potential values recorded at the GCE electrode were 0.61 μA and 0.590 mV, respectively. However, at the poly (*p*-ABSA) electrode these values were observed to be 30.48 μA and 0.220 mV, respectively (Figure 4). Consequently, in comparison with the data recorded from the bare GCE electrode, an increase in peak current and a decrease in overpotential of MD were obtained at modified GC electrode. Therefore, it was assessed as an electrocatalytic effect for the oxidation of MD on the modified surface. It could be observed that the oxidation peak current for modified electrode significantly increased and it was almost 50 times higher than that unmodified electrode. This behavior is due to adsorption of MD on the surface of *p*-ABSA by interaction of MD functional groups such as NH_2, COOH and OH with carboxyl groups of activated *p*-ABSA on the surface of electrode. Thus sensitivity significantly enhanced due to preconcentration of MD on the active surface of *p*-ABSA. Also, as shown in Fig. 4B, the onset potential for MD oxidation at poly (*p*-ABSA) electrode is lower than its oxidation at a bare GCE (Fig. 4B) because of catalytic behavior of modified electrode. However, the potential peak at the bare GCE (0.59 V) is higher than the potential peak at the modified GCE (0.220 V). The effect of scan rate on the oxidation peak current of 0.01 mM MD was studied. With the scan rate increasing, the anodic peak current increased. A good linearity between the square root of scan rate and peak current was obtained between the range of 10–

250 mV s^{-1}. The linear regression equation was $I_p(\mu A)= 0.502v^{1/2}–0.899$ with correlation coefficient $R^2 = 0.998$. Correlation coefficient is very close to 1.0 showing that the oxidation process is diffusion controlled. Also, the plot of logarithm of peak current versus logarithm scan rate has a slope of 0.63 which is almost the theoretical value of 0.56. The equation was $\log I_p(\mu A)=0.63\log v–0.7041$ ($R^2 = 0.998$) on modified electrode. This indicates diffusion controlled electron process of MD oxidation at poly(*p*-ABSA) modified GCE.

The electrostatic interaction between the modified GCE electrode and MD contributed to the enhancement of sensitivity and electroactivity. The oxidation peak of MD in the pH of 3.0 is irreversible and thus with increase in the peak height, the peak potential shifts to lower potential. But onset potential that show the kinetic of the reaction, decreased for the modified GCE compare to the bare GCE and thus sensitivity and selectivity increased because of these effects.

Electrolyte Type Effect on Voltammetric Behavior of Methyldopa

By selecting an appropriate supporting electrolyte solution, it creates a conductive environment between the submerged electrodes.

The choice of supporting electrolyte depends on MD's resolution, dissociation degree and nucleophilic character. For this purpose, voltammograms of MD in Na_2SO_4, PBS (pH 7.0), $NaNO_3$, $NaClO_4$, NaCl and KCl supporting electrolytes (electrolytes concentration, 0.1 M) were taken (Figure 5). While taking a voltammogram at pH 7.0 for PBS, voltammograms were taken at the native pH of the other electrolyte species.

Effect of pH on The Peak Potential and Peak Current of MD

The peak current and potential are dependent on pH of solution. To find the optimum pH, the influence of pH over the range of 2.0–11.0 on the performance of the sensor was investigated. Experimental results for MD are shown in Figure 6. It was found that the anodic peak current of MD increased with increment of acidity, and reached to maximum value at pH (3.0). Therefore, pH 3.0 was selected as the optimum pH for the determination of MD. Increasing the peak current with the

increase of the acidity showed that the mechanism for oxidation of MD was a proton dependent reaction.

It was observed that as the pH of solution was increased, the oxidation peak potential shifted to negative potential values. The negative shift and the peak potential, showed a linear relationship with the slope of −52.4 mV/ pH in the pH range of (2.0)–(5.0). This slope approximately revealed that the number of proton in the process was equal with the number of electron transfer in the oxidation reaction of MD.

Determination of MD in the presence of Ascorbic Acid

Determination of MD in poly (*p*-ABSA) was done with differential pulse voltammetry. Differential pulse voltamograms of different concentrations of MD on poly (*p*-ABSA) modified glassy carbon electrode are shown in Figure 7. The data of the obtained calibration charts were shown in Table 1. The calibration plot of the proposed sensor is linear in two concentration ranges of 1.0 – 30.0 and 30.0 – 300.0 µM.

The calibration equation over theses ranges are: I_{pa} (µA) = 1.21 × C (µM) + 30.81, R^2 = 0.9944 and I_{pa} (µA) = 0.53 C (µM) + 53.30, R^2 = 0.9975, respectively. LOD and LOQ were calculated as 5.0 nM and 10.6 nM *(S/N=3),* respectively. The relative standard deviation (RSD) for MD and 5 repeated measurements was less than 3%.

It is readily seen from the Figure 8 that peak currents increase linearly with increasing MD concentration even in the presence of ascorbic acid. Morover, the MD peak current was unaffected with the increasing ascorbic acid concentration (Figure 9). Moreover, from the successive runs of the modified electrode in the binary mixture, it was observed that the voltammetric responses were almost invariable. The relative standard deviation (RSD) for MD and 5 repeated measurements was less than 3%.. This behavior reflects that the stability of the modified electrode was satisfactory.

Analytical applications

Five Alfamet tablets containing 250 mg MD in each tablet were directly weighed and powdered in the mortar. The calculated amount of MD corresponding to the 100 mM

concentration stock solution was weighed and transferred to a 10 mL volumetric flask and the volume was supplemented with ultra-pure water. The contents of the flask were subjected to centrifugation at 5000 rpm for 15 minutes to effect complete dissolution. The prepared mixture was filtered using paper filter and diluted to appropriate amounts double distilled water. The serum samples were collected from research hospital and were centrifuged (15 min with 5000 rpm) and then diluted 10 times with doubly distilled water without any additional pretreatment. Before voltammetric determination, appropriate amount of prepared real samples were added to 10 mL of phosphate buffer solution with optimum pH (pH 3.0) and then was transferred to the electrochemical cell for electrochemical measurements. The standard addition method was used to determine methyldopa in the real and spiked samples.

The quantity of MD in the tablets was computed from the suitable calibration graphs. Furthermore, the accuracy of the proposed techniques was checked by carrying out recovery studies. Recovery results obtained from the calibration graph can be seen in Tables 2. The proposed method was successfully performed to real samples in the presence of interferences.

CONCLUSION

Poly (*p*-ABSA) modified electrode was applied for electrocatalytic assay of MD. The modified GCE indicated high electrocatalytic activity for MD. The modified GCE provides much sensitivity and selectivity in the assay of MD. Besides, the modified electrode showed easy regeneration, good repeatability and stability. The modified GCE can be used under the selected conditions (in PBS, pH 3) for the determination of MD. The results show that the proposed method can be easily used in the determination of MD in drug samples and clinical analyzes. It has been observed that this method can be used to identify MD in the blood serum.

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Electrode Modified with Graphene Oxide Nanosheets and 3-(4′-Amino-3′-hydroxy-biphenyl-4-yl)-acrylic Acid, Electroanalysis, 2015, 27, 2421-2430.

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Tables

Tablo 1. The data of calibration charts

	Linear concentration range (μM)	
Parameters	1.0 - 30.0	30.0 - 300.0
Correlation coefficient	0.9944	0.9975
Standard Error of Slope	0.0263	0.0075
Standard Error of Intercept	0.3538	1.167

Table 2. Detection of MD in commercial tablets

Parameters	Labeled, mM	Found, mM	*RSD, %	Bias, %	Recovery, %	RSD* of recovery, %	Bias of recovery, %
Proposed method	1	0.979	0.14	0.84	97.9	0.77	1.98
Blood Serum	1	0.764	0.22	0.79	76.4	0.82	2.00

* RSD: relative standard deviation.

Each value is an average of five determinations..

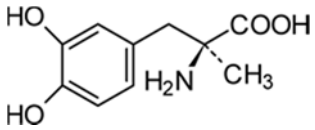

Figure 1. Molecular structure of methyldopa.

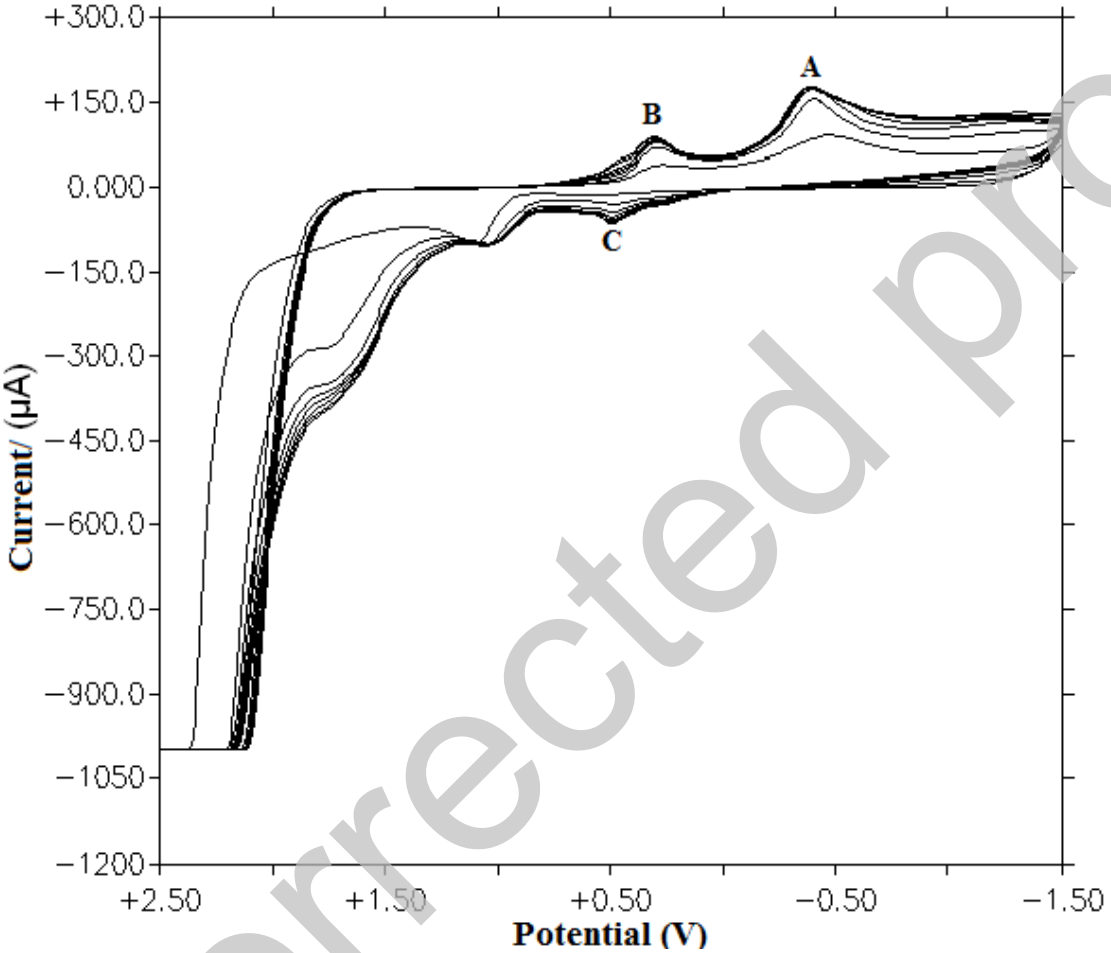

Figure 2. CVs of 5 mM *p*-aminobenzene sulfonic acid in 0.1 M KCl at GCE, Scan rate: 50 mV/s, 14 cycle.

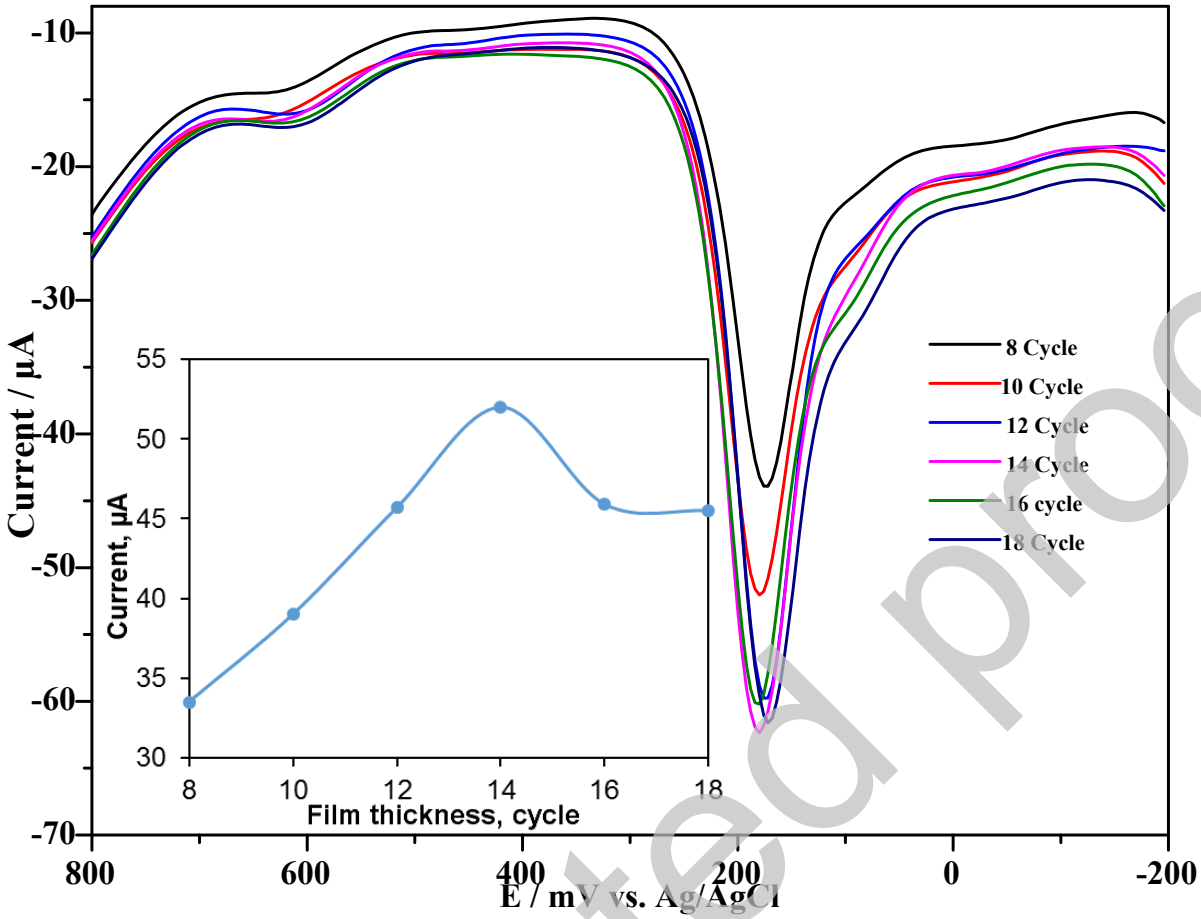

Figure 3. DPVs of increasing film thicknesses of 0.01 mM MD in 0.1 M PBS (pH 3.0) at poly (*p*-ABSA) modified sensor. The relationship between film thickness and the peak current of MD (inset).

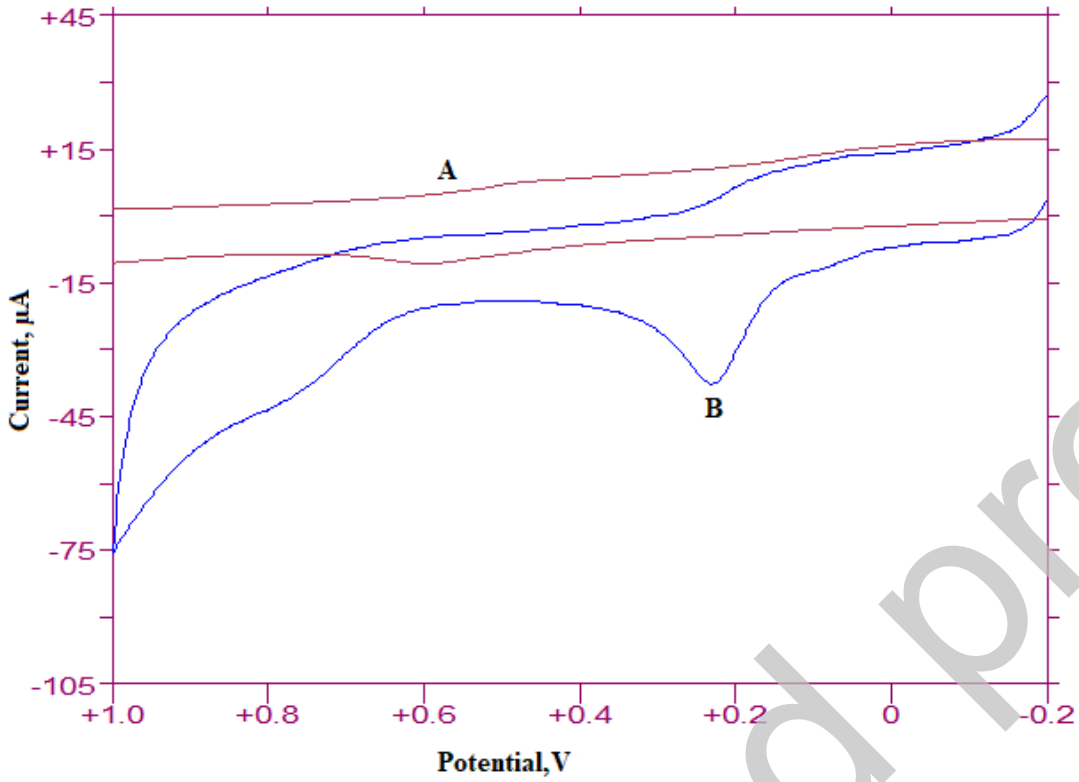

Figure 4. CVs of 0.01 mM MD in 0.1 mM PBS (pH 3.0) (A) GCE (B) at poly (*p*-ABSA) modified sensor. Scan rate: 50 mV/s.

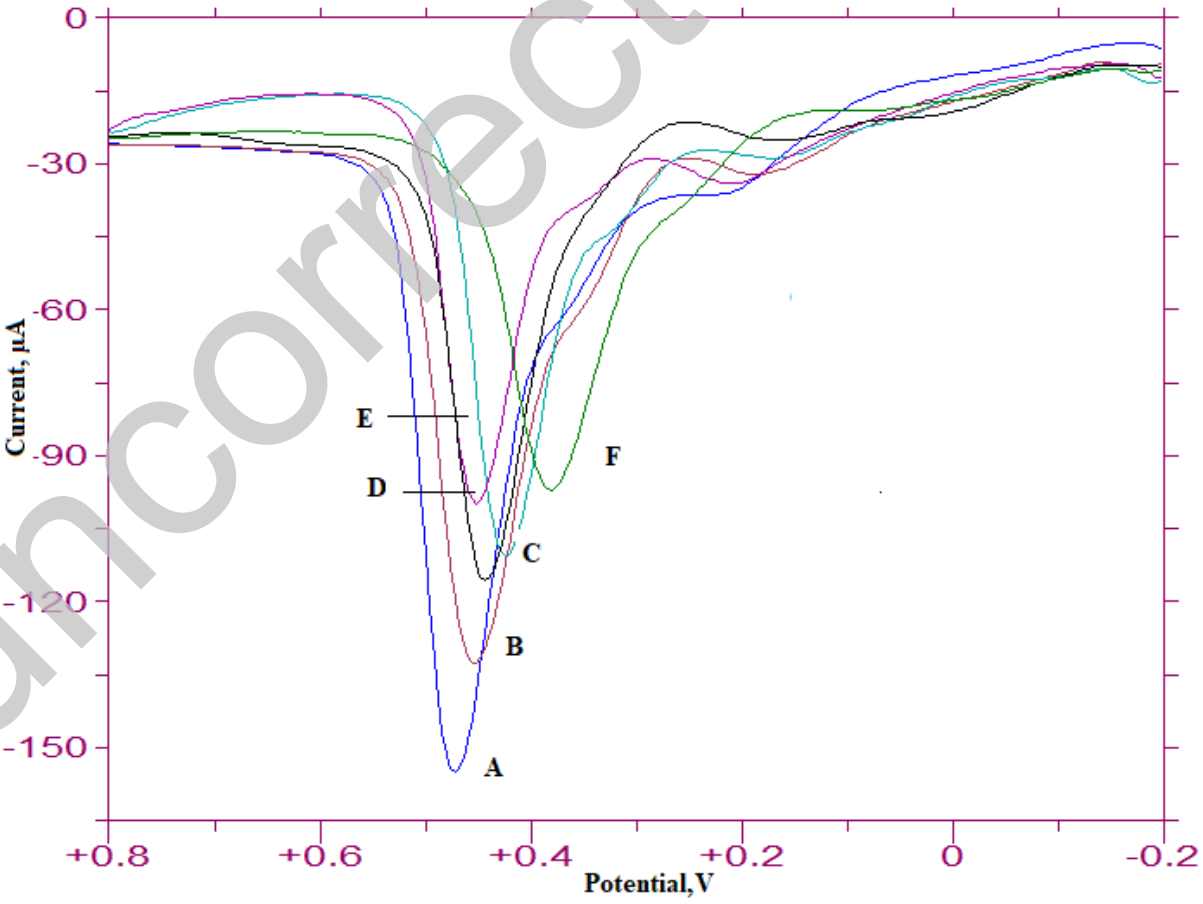

Figure 5. Electrolyte effect on voltammetric analysis of 0.01 mM Methyldopa at poly (*p*-ABSA) sensor. A) PBS (pH 7.0), B) $NaClO_4$ C) KCl, D) NaCl, E) $NaNO_3$ and F) Na_2SO_4. Electrolyte concentration was 0.1 M.

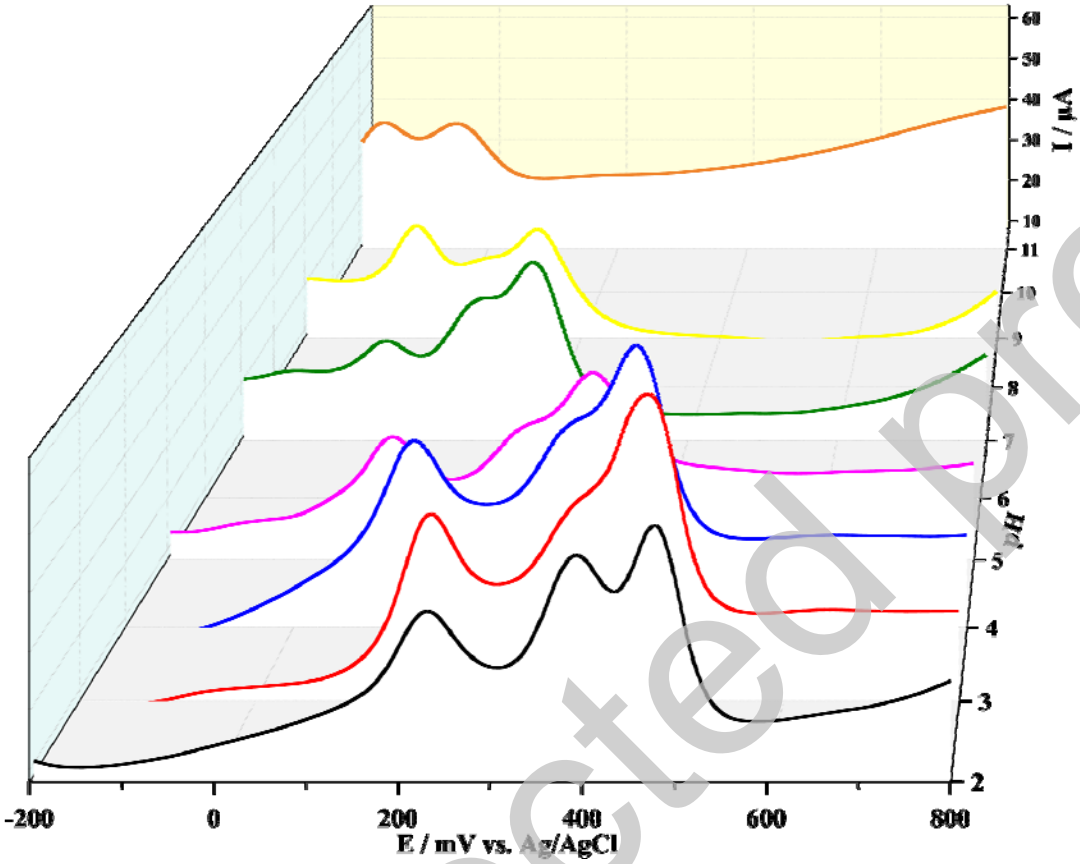

Figure 6 DPV responses of 0.01 mM MD and 1.0 mM AA at modified sensor in PBS medium at different pHs; 2.0, 3.0, 4.0, 5.0, 7.0, 9.0, 11.0. Scan rate, 50 mV/s.

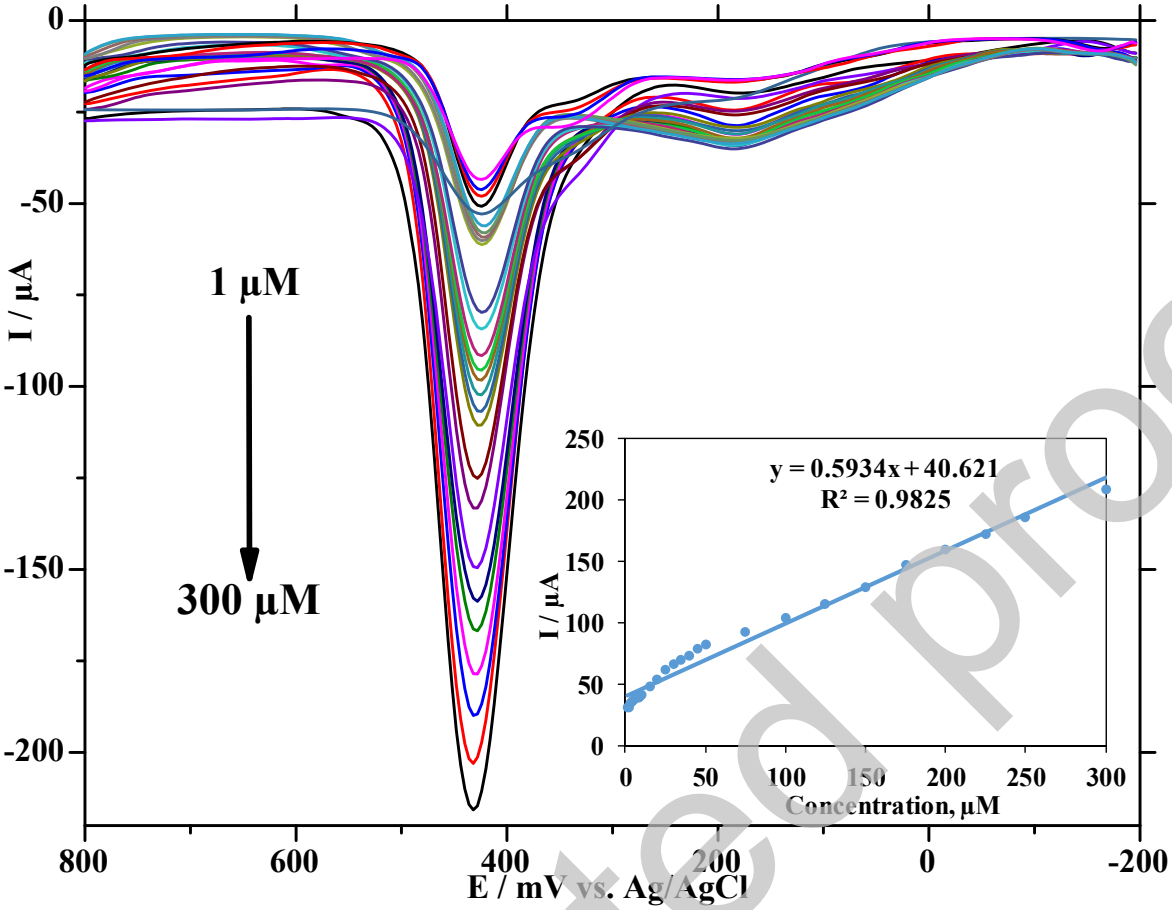

Figure 7. Differential pulse voltammograms and calibration graphs in increasing concentration of MD in 0.1 M PBS (pH 3.0) at poly (*p*-ABSA) modified sensor. The calibration chart of 1.0-300.0 µM MD (inset).

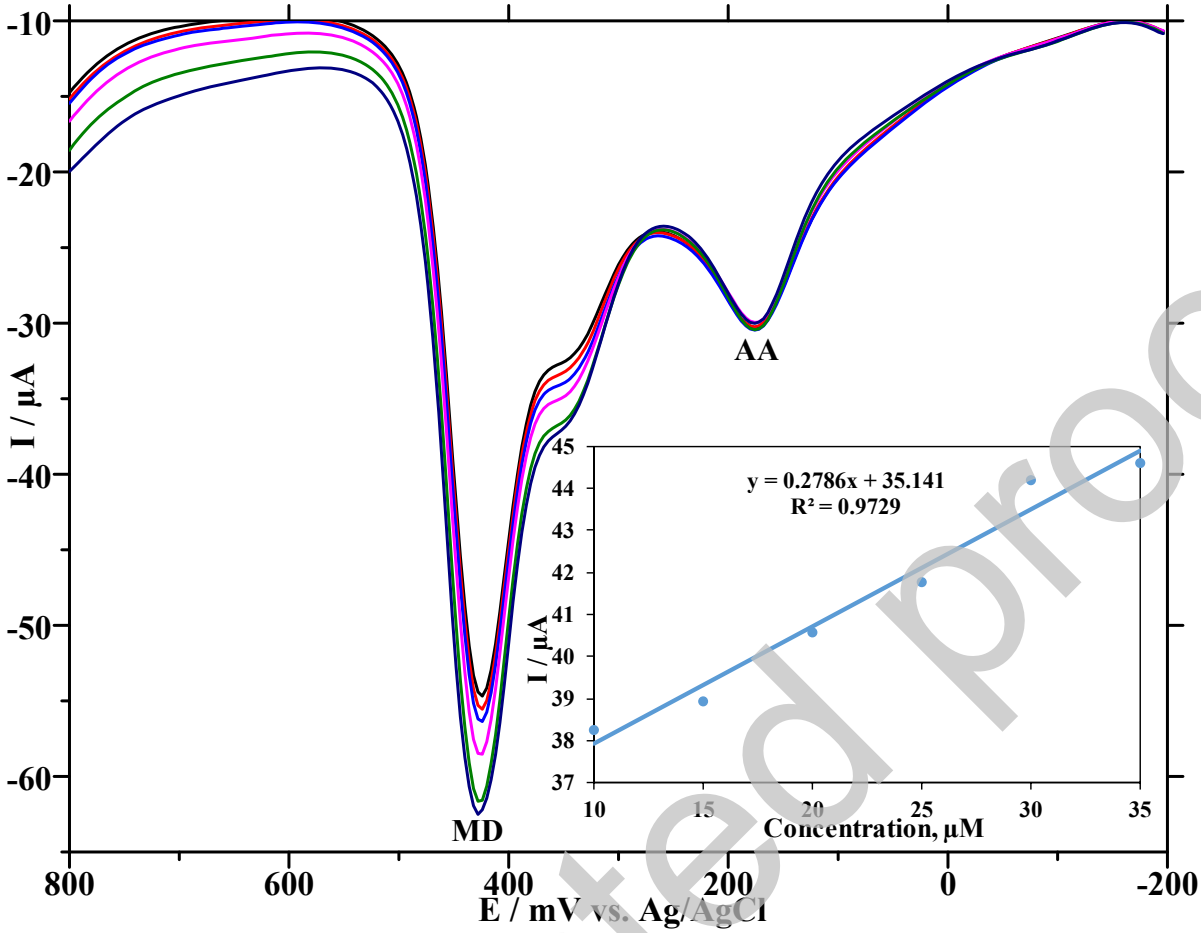

Figure 8. The increasing concentration of MD (0.01, 0.015, 0.020, 0.025, 0.030, 0.035 mM) with 0.5 mM ascorbic acid at poly (*p*-ABSA) modified sensor in 0.1 M PBS (pH 3.0).

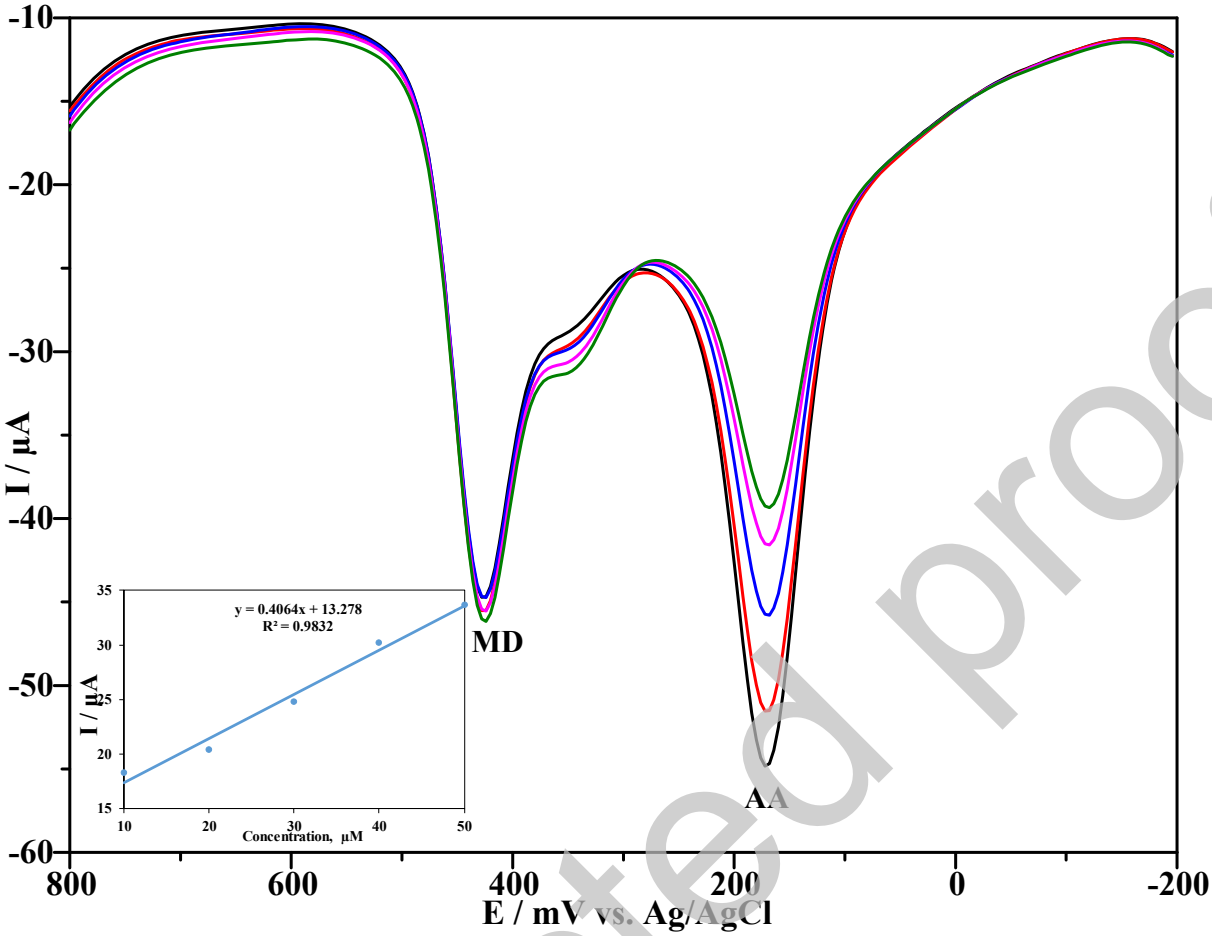

Figure 9. The increasing concentration of ascorbic acid (0.1, 0.2, 0.3, 0.4, 0.5 mM) in the presence of 0.01 mM methyldopa at poly (*p*-ABSA) in 0.1 M PBS (pH 3.0).