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Electrochemical Determination of Phenazopyridine Hydrocloride using Poly(*p*-Aminobenzene Sulfonic Acid) Film Modified Glassy Carbon Electrode

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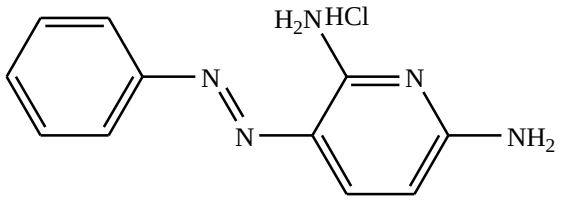
Phenazopyridine hydrochloride is an antiviral effective drug active compound. A glassy carbon electrode (GCE) was modified with an electropolymerized film of *p*-aminobenzene sulfonic acid (*p*-ABSA) in phosphate buffer solution (PBS). The electrochemical properties of the polymer film were studied by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques. The polymer film-modified electrode was used to electrochemically detect phenazopyridine hydrochloride. Polymer film showed excellent electrocatalytic activity for the reduction of phenazopyridine hydrochloride. The catodic peak potential value of the phenazopyridine hydrochloride at the poly(*p*-ABSA) modified glassy carbon electrode was -334 mV obtained by DPV. A linear calibration curve for DPV analysis was constructed in the phenazopyridine hydrochloride concentration range $6x10^{-6}$-$1x10^{-5}$ mol L^{-1}. Limit of detection (LOD) and limit of quantification (LOQ) were obtained as $2.1x10^{-7}$ and $7x10^{-7}$ mol L^{-1} respectively. The proposed method exhibits good recovery and reproducibility. The mechanisms of electropolymerization of *p*-ABSA are also suggested**.**

Keywords: *p*-Aminobenzene sulfonic acid; Modified glassy carbon electrode; Electropolymerization; Electrocatalytic reduction; Determination of phenazopyridine hydrochloride; Voltammetry.

1. INTRODUCTION

Phenazopyridine hydrochloride (PAP) [Scheme 1] is a heterocyclic aromatic azo compound that exists at room temperature as brick-red microcrystals with a slight violet luster [1]. PAP (2,6-Pyridinediamine-3-phenylazo monohydrochloride), urinary tract infections (prostatitis, urethritis,

cystitis etc.), combustion caused by surgery and endoscopic procedures burning, itching, used to reduce symptoms such as frequent and less urine out [2,3].



Scheme 1. Chemical structure of phenazopyridine hydrochloride (PAP).

PAP has been determined in various pharmaceutical preparations, blood plasma and urine by zero-crossing first derivative spectrophotometry [4], imprinted polymer-electrospray ionization ion mobility spectrometry [5], highly selective potentiometric sensor [6], high performance liquid chromatograph [7 - 9], gas chromatography – mass spectrometry [10].

A few electrochemical studies have been conducted on the electrochemical behavior and determination of PAP in pharmaceuticals and biological samples by voltammetric and polarographic methods. The electrochemical behavior of PAP was studied using different electrodes such as glassy carbon electrode [11, 12], hanging mercury drop electrode [13], mercury drop electrode [14] and $MgCrO_4$ nanoparticles decorated multi-walled carbon nano tubes - modified glassy carbon electrode [15].

In recent years modification of electrode surfaces has been an important research area in electrochemistry. Compared with other electrode concepts in electrochemistry, the distinguishing feature of a chemically modified electrode is that generally a thin film of a selected chemical is bonded or coated onto the electrode surface to endow the electrode with the chemical, electrochemical, optical, electrical, transport, and other desirable properties of the film in a rational, chemically designed manner [16]. One of the methods used for the modification of electrode surfaces is electropolymerization. Electropolymerization can accelerate transmission of electrons onto the surface of the electrode, it has high selectivity and sensitivity due to the film homogeneity in electrochemical deposition, and it has strong adherence to the electron surface and large surface area [17, 18]. Researchers have employed polymeric film-modified electrodes to detect organic and inorganic molecules in recent years.

Although the reduction reactions of PAP were studied, there have not been sufficient studies on the determination and electrocatalytic-reduction behavior at poly(p-ABSA)-modified glassy carbon electrodes. In this study, we prepared the poly(p-ABSA) modified GCE [19, 20] and studied the electrochemical properties of PAP on modified glassy carbon electrode. Quantitative analysis was performed on the drug active substance in tablet dosage form at modified GCE electrode. It showed excellent electrocatalytic activity for reduction of PAP in 0.1 mol L^{-1} PBS (pH 7.0).

2. EXPERIMENTAL

2.1. Reagents and Materials

PAP and Azosilin® were kindly supplied by Faco Inc., (Istanbul, Turkey). Their stock solutions (0.01 mol L^{-1}) were prepared with water. *p*-aminobenzene sulfonic acid (Sigma Aldrich) solution was prepared with 0.1 mol L^{-1} PBS and used without any further purification. Phosphate buffer solutions were prepared with 0.1 mol L^{-1} NaH_2PO_4-Na_2HPO_4 and by adjusting the pH with 0.1 mol L^{-1} H_3PO_4 and 0.1 mol L^{-1} NaOH. All aqueous solutions were prepared in twice-distilled de-ionized water and used analytical grade chemicals. To remove oxygen in experimental solutions argon gas (99% purity) was used.

2.2. Apparatus

A Model Metrohm 757 VA Trace Analyzer (Herisau, Switzerland) and Metrohm Autolab PGSTAT 101 (Netherlands) were used for the voltammetric measurements, with a three-electrode system consisting of glassy carbon working electrode (GCE) [surface area (ϕ) 7 mm; disc diameter (R) 2 mm, Metrohm], a platinum wire auxiliary electrode and Ag/AgCl (KCl 3 M, Metrohm) reference electrode. The bare GCE was polished successively with 0.3, 0.1 diamond suspension and 0.05 μm Al_2O_3 slurry on a polishing cloth before electrochemical modification. Firstly, the deoxygenating process of the supporting electrolyte solutions was carried out with argon gas for 5 min before all experiments. Then, the argon gas was also passed through the solutions for 60 s after the addition of each sample solution in the experiments. Monomer solutions were purged with argon gas for about 30 min before polymerization and the solution was blanketed with the same gas during electropolymerization. In each new experiment, a new bare electrode surface was used. All pH measurements were made with EZDO 5011 A model pH meter. All measurements were carried out at ambient temperature of the laboratory (15-20 °C). Wise Clean model sonicator was used to clean the surface of electrodes. The 0.055 μS/cm ultra pure water (UPW) was used throughout the experiments.

For analytical application, the following parameters were employed: pulse amplitude (pulse amplitude of the voltage pulse superimposed on the direct voltage) 50 mV; pulse time (time interval during which a voltage pulse is superimposed on the direct voltage) 0.04 s, voltage step (voltage step for direct voltage ramp) 0.009 V, voltage step time (time interval after which the voltage in the sweep is increased or decreased by the amount of the voltage step) 0.04 , potential step 10 mV (DPV); and scan rate (display of the ramp slope calculated as voltage step/voltage step time) in the range 20-100 mV s^{-1} (CV).

2.3. Preparation of poly(p-Aminobenzene Sulfonic Acid)-modified GCE

Prior to electrochemical modification, the bare GCE was polished successively with 1 μm diamond paste, 3 μm diamond paste and 0.05 μm Al_2O_3 slurry on a polishing cloth. Then it was rinsed with double-distilled water, and sonicated in 1:1 nitric acid, acetone and double-distilled water for 10

min, respectively. After being cleaned, the electrode was immersed in 2.0×10^{-3} mol L^{-1} *p*-ABSA solution and was conditioned by cyclic sweeping between −1.5 to +2.4 V at 200 mV s^{-1} for 10 scans (Fig. 1). In order to get a stable response prior to measurements, the resultant modified electrode was also continuously cycled from 0.5 to 1.5 V in pH 7.0 PBS for another few scans. Finally, the modified electrode was carefully rinsed with distilled water, and used for analysis of PAP and stored in air for later use [20, 21]. We studied the reduction of PAP at bare and modified-GCE (0.1 M of pH 5-8 PBS). Best results were obtained at 0.1 mol L^{-1} PBS (pH 7.0). Therefore PBS (pH 7.0) was selected for detection of PAP by modified GCE.

3. RESULTS AND DISCUSSION

3.1. Electropolymerization of p-aminobenzene sulfonic acid at the GCE surface

The electropolymerization of *p*-ABSA on GC electrode surface was performed by repetitive cyclic voltammetry (Fig. 1).

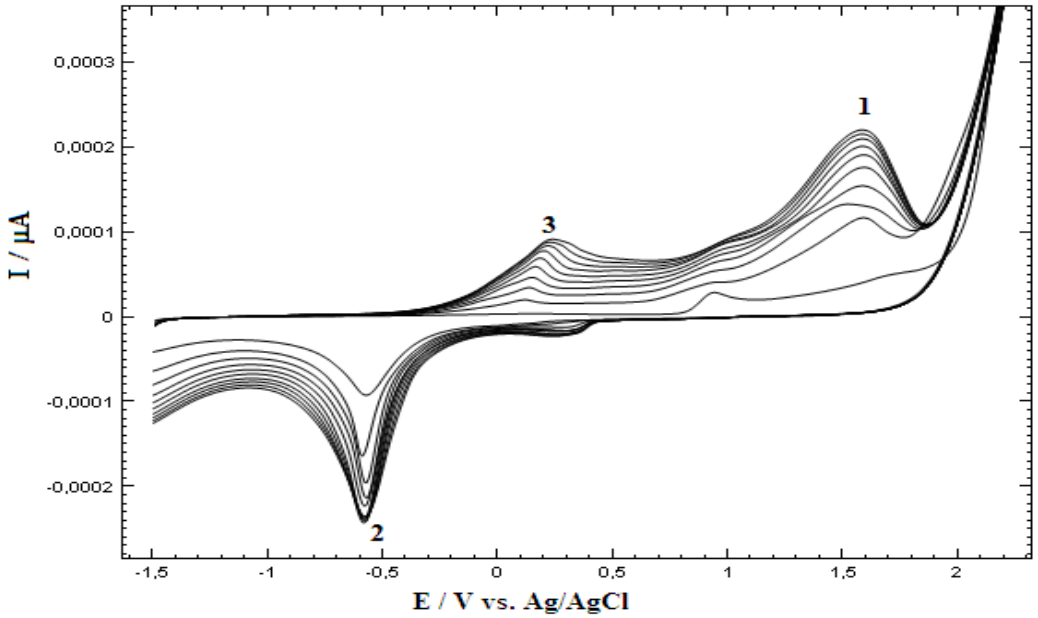
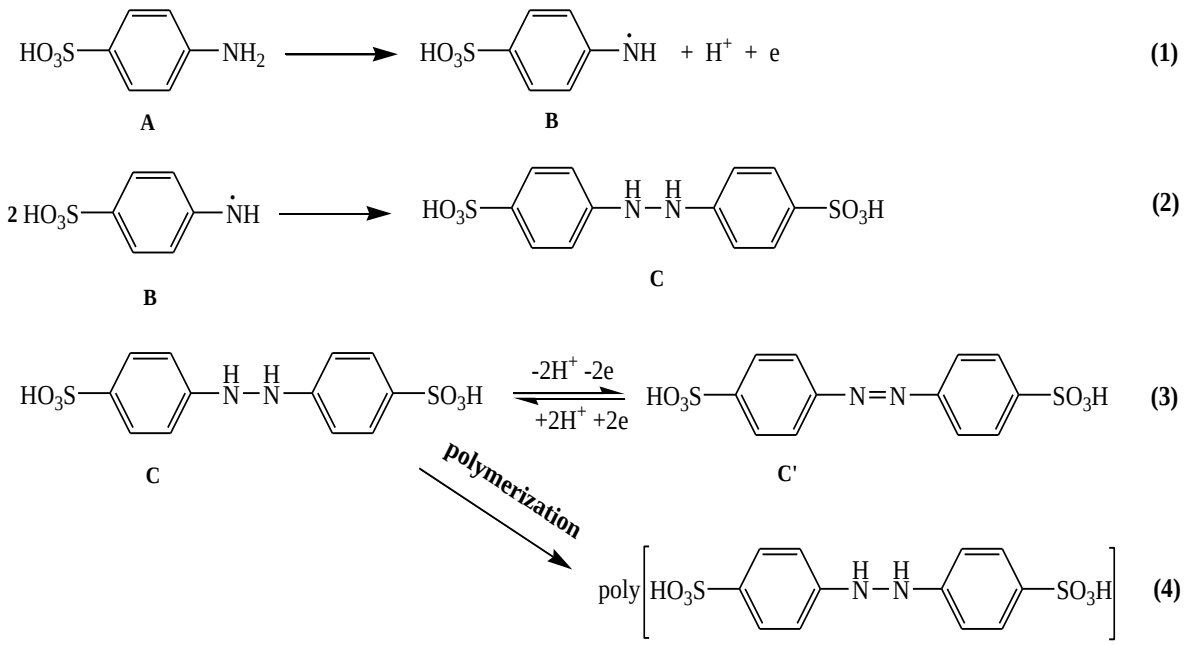


Figure 1. Repetitive cyclic voltamograms of 2.0×10^{-3} mol L^{-1} *p*-ABSA at bare GCE. Initial potential: -1.5 V; terminal potential : +2.4 V. Scan rate: 200 mVs^{-1}; supporting electrolyte: 0.1 mol L^{-1} PBS (pH 7.0).

As can be seen in Fig. 1, in the first scan, anodic peak 1 and cathodic peak 2 were observed with peak potential value at about +1.5 V and −0.65 V, respectively. From the second cycle on, anodic peak 3 appeared with potential at about +0.15 V. Then larger peaks were observed upon continuous scanning, reflecting the continuous growth of the film. These facts indicated *p*-ABSA was deposited on the surface of GCE by electropolymerization. A uniform adherent blue polymer film was formed on

the GCE surface. After modification, the poly(*p*-ABSA) film electrode was carefully rinsed with doubly distilled water, then stored in pH 7.0 PBS and for later use.

The electropolymerization behavior of *p*-ABSA at GCE was similar to the references reported [20, 22]. The reaction mechanism may be similar to that proposed in Scheme 2.



Scheme 2. Suggested electrochemical polymerization of *p*-ABSA at GCE.

As can be seen from Scheme 2, *p*-ABSA (A) was first oxidized to free radical (B) (peak 1); the free radical (B) combined together rapidly to form hydrazobenzene sulfonic acid (C); then hydrazobenzene sulfonic acid (C) was oxidized to azobenzene sulfonic acid (C′) (peak 3), and azobenzene sulfonic acid (C′) was reduced to hydrazobenzene sulfonic acid (C) (peak 2) and finally the electrode surface was covered by the formed polymer (D).

3.2. Electrochemical response of PAP at poly(p-ABSA)-modified GCE

Fig. 2 shows CV curves of 5.0×10^{-5} mol L^{-1} PAP at bare GCE and poly(*p*-ABSA)-modified GCE in 0.1 mol L^{-1} PBS (pH 7.0), respectively. As can be observed from Fig. 2, PAP katodic peak showed about -0.639 V at bare GCE and -0.603 V at the modified-GCE. Reduction peak potential of PAP was shifted 36 mV positively and peak current increased about 10 times at the modified-GCE. The electrostatic interaction between modified electrode and PAP contributed to the enhancement of sensitivity and electroactivity.

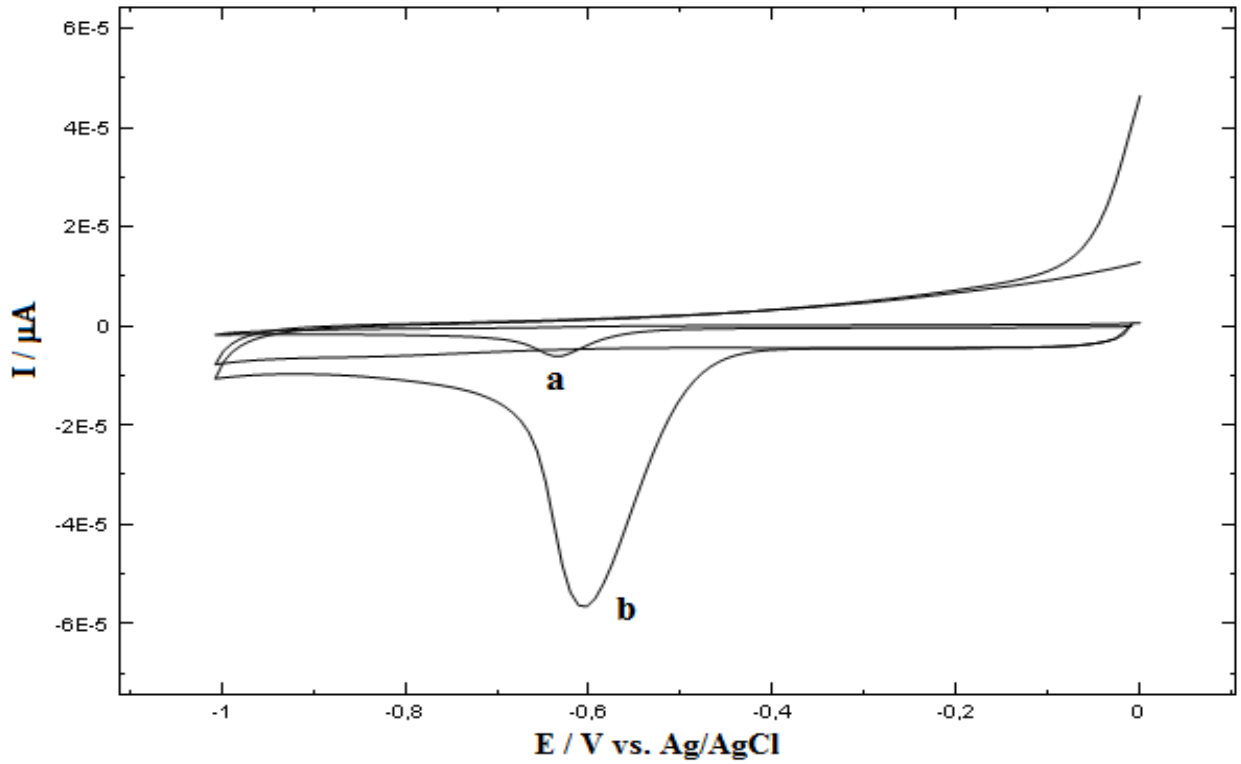


Figure 2. CV voltamograms of $5.0x10^{-5}$ mol L^{-1} PAP a) at bare GCE b) at poly(*p*-ABSA)-modified GCE. Scan rate: 100 mVs^{-1}; supporting electrolyte: 0.1 mol L^{-1} PBS (pH 7.0).

3.3. Effect of scan rate and pH on reduction of PAP

In order to obtain the optimum experimental conditions, some variables affecting the peak current and peak potential, which are pH and the species of supporting electrolyte, were studied for PAP solution of $8.0x10^{-6}$ mol L^{-1} on modified-GC electrode by the proposed voltammetric techniques. When the effect of pH on the peak current was studied, the highest peak current was observed at pH 7.0 PBS Therefore, pH 7.0 PBS was preferred for further work.

The effect of scan rate on the reduction peak current of $8.0x10^{-6}$ mol L^{-1} PAP was studied. With increasing scan rate, the katodic peak current increased. A good linearity between the square root of scan rate and peak current was obtained between the range of 20-100 mV s^{-1} (Fig. 3). The linear regression equation was $I_p(\mu A)= 0.8213\upsilon^{1/2}-1.5384$ with correlation coefficient (r=0.99). A linear relationship was observed between peak current and square root of scan rate with a correlation coefficient of r=0.99. 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 0.5019 which is almost close to the theoretical value of 0.5. The equation was $\log I_p(\mu A)=0.5019\log\upsilon-0.2044$ (r=0.93) on modified electrode. These indicate a diffusion controlled electron process of PAP reduction at poly(*p*-ABSA)-modified GCE.

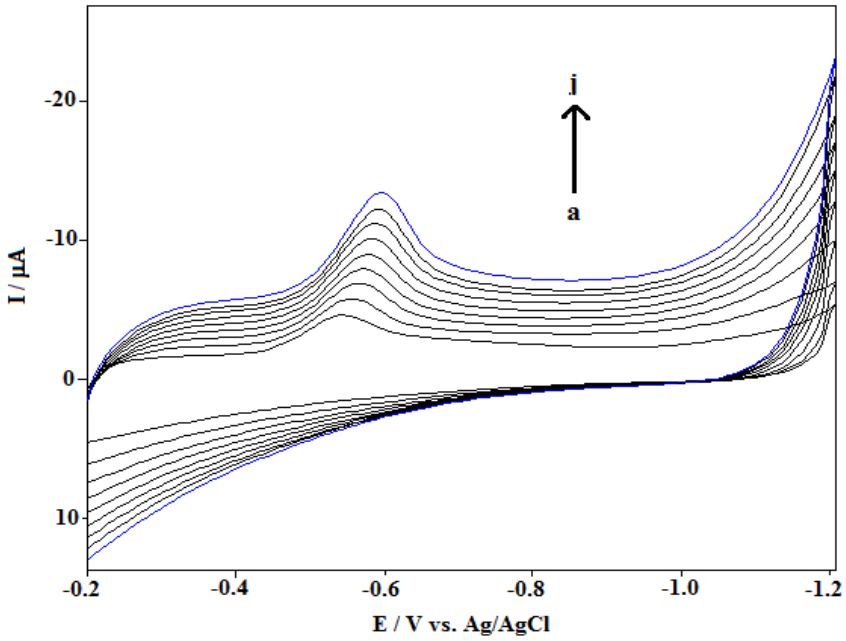


Figure 3. CV voltamograms of $8.0x10^{-6}$ mol L^{-1} PAP at poly(*p*-ABSA)-modified GCE; Scan rates: a) 20, b) 30, c) 40, d) 50, e) 60, f) 70, g) 80, h) 90 and i) 100 mVs^{-1}; supporting electrolyte: 0.1 mol L^{-1} PBS (pH 7.0).

3.5. Determination of PAP

The determination of PAP concentration at poly(*p*-ABSA)-modified *GC* electrode was performed with differential pulse voltammetry (DPV). Under the optimum analytical conditions, the determination of PAP at different concentrations was performed. A linear calibration curve (Fig**. 4**) was obtained for PAP in the range $6x10^{-6}$-$1x10^{-5}$ mol L^{-1} for 0.1 M PBS supporting electrolyte **(**Fig**. 5)**.

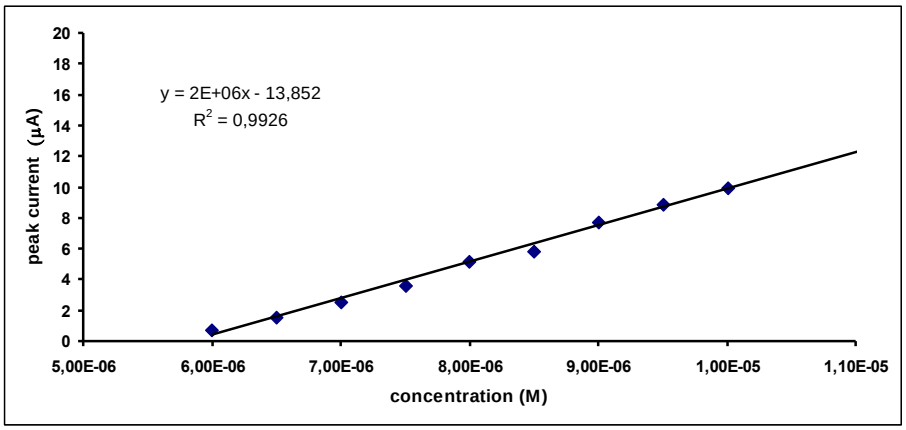


Figure 4. Plot of concentration versus current for PAP.

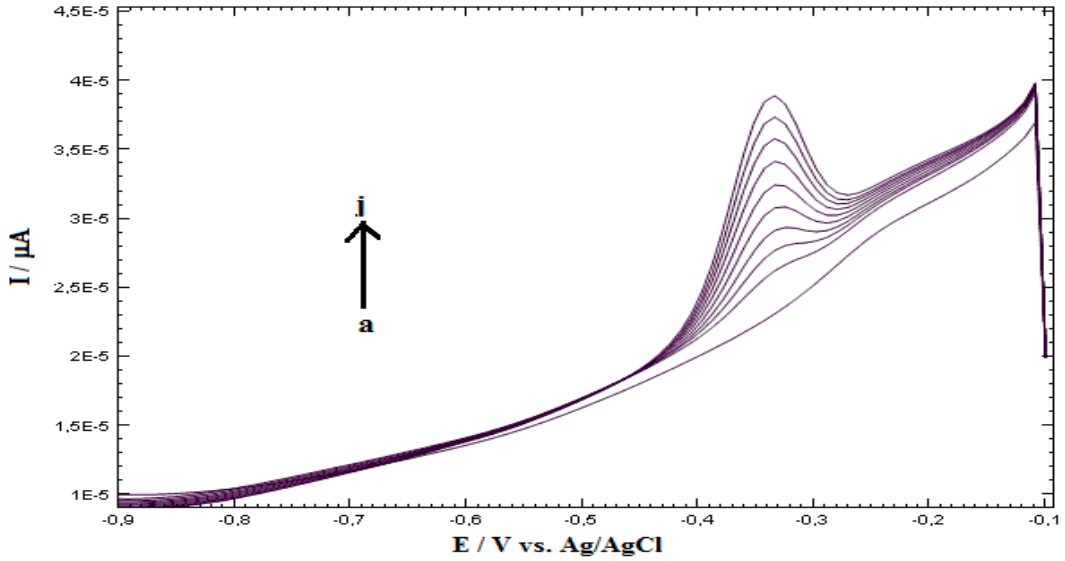


Figure 5. DPV voltamograms of PAP at poly(*p*-ABSA)-modified GC electrode; a) blank; b) $6.0x10^{-6}$; c) $6.5x10^{-6}$; d) $7.0x10^{-6}$; e) $7.5x10^{-6}$; f) $8.0x10^{-6}$; g) $8.5x10^{-6}$; h) $9.0x10^{-6}$ i) $9.5x10^{-6}$; j)$1.0x10^{-5}$ mol L^{-1}.

LOD and LOQ were calculated for the electro-reduction peak current using the following equations. LOD = 3 s/m LOQ = 10 s/m; where, s is the standard deviation of the peak currents (for five runs) and m is the slope of the calibration curve. The achieved LOD and LOQ were $2.10x10^{-7}$ and $7.00x10^{-7}$ mol L^{-1} for poly(p-ABSA)-modified GCE, respectively.

Validation of the procedure for the quantitative determination of PAP was investigated via evaluation of the limit of detection (LOD), limit of quantification (LOQ) and recovery studies by DPV technique (Table 1).

Table 1. Detection of PAP in commercial tablets and mean recoveries at poly(*p*-ABSA)-modified GCE by DPV

Parameters	Results
Labeled PAP (mg)	50
Amount of PAP found (mg)	49.84
RSD %	0.38
Bias (%)	0.32
Spiked PAP (mg)	5.00
Found (mg)	4.95
Recovery %	98.90
RSD % of recovery	0.28
Bias %	1.09

$7.0x10^{-6}$ mol L^{-1} PAP was investigated repeatedly at an identical surface of poly(*p*-ABSA)-modified GCE for 20 successive times. Also, the stability of the modified electrode was investigated. Modified electrode was stored in PBS (pH 7.0) at 4 °C in a refrigerator. The peak current retained

98.3% of its initial response after storage in air for 15 days. This indicates that the modified electrode has good stability.

3.6. Analytical Applications

Ten tablets of Azosilin® Yavuz İlaç Ecza Deposu Medikal Ürünler San. ve Tic. A.Ş, Istanbul), containing 50 mg PAP per tablet, were accurately weighed and ground to a fine powder. An adequate amount of this powder, corresponding to a stock solution of concentration $1x10^{-3}$ mol L^{-1}, was weighed and transferred into a 10 mL calibration flask and the volume was adjusted with distilled water. The contents of the flask were centrifuged for 15 min at 4000 rpm to affect complete dissolution. The non-dissolved excipients were settled on the bottom. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquor and diluting with deionized water. Each solution was transferred into the voltammetric cell. The amount of PAP in Azosilin commercial tablets was calculated by reference to the appropriate calibration plots. For this reason, the proposed techniques were checked by performing recovery tests. The results obtained are given in Table 1. The proposed techniques could be successfully applied to PAP assay in tablets without any interference.

4. CONCLUSIONS

A poly(*p*-aminobenzene sulfonic acid)-modified GCE was fabricated by electropolymerization techniques in PBS using cyclic voltammetry method. The modified GC electrode showed good electrocatalytic activity for the reduction of PAP. The modified electrode provides greater sensitivity and selectivity in the determination of PAP. Moreover, the modified electrode showed easy regeneration, good reproducibility and stability. Proposed methods can be applied to the detection of PAP in practical drug samples.

ACKNOWLEDGEMENTS
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