Determination of Amoxicillin in Co-Amoxiclav and Urine Samples by Differential Pulse Adsorptive Stripping Voltammetry

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Abstract

A novel selective and sensitive method is developed for determination of amoxicillin by adsorptive stripping voltammetry. Amoxicillin gave well resolved diffusion-controlled cathodic peaks at -0.514 V, respectively (versus Ag/AgCl) in phosphate buffer at pH 10.2. Optimal conditions were obtained at pH 10.2, accumulation potential of 0.15 V (vs. Ag/AgCl), accumulation time of 60 s, and scan rate of 120 mV/s. Under the optimized conditions, a linear calibration curve was established for the concentration of amoxicillin in the range of 0.07-5 µM with a detection limit of 0.0098 µM. The procedure was successfully applied to the determination of amoxicillin in various medicine and biological samples. The relative standard deviation of the method at 0.09 and0.4 µM amoxicillin was 2.65%, 2.01%, for 10 runs, respectively.

Keywords: amoxicillin; Adsorptive stripping voltammetry; medicine and biological samples.

Introduction

Amoxicillin, D-α-amino-*p*-hydroxybenzylpenicillin trihydrate, (Figure. 1) is one of the most frequently used β-lactam antibiotics in the world and it is employed to treat humans and animals [1-3]. Amoxicillin is antibiotic that is used to treat certain infections caused by bacteria, such as pneumonia, bronchitis, venereal disease, and ear, lung, nose, urinary tract and skin infections. It is also used before some surgery [4-6]. Amoxicillin trihydrate is a white odorless crystalline powder [7]. As others β-lactam antibiotics, AMX, presents a structure based on a β -lactam ring responsible for the antibacterial activity and variable side chains that account for the major differences in their chemical and pharmacological properties. After a single oral dose of 500mg, 60–86% of the drug excreted is unchanged in the urine during the first 6 hours [8,9]. co-amoxiclav is an antibiotic useful for the treatment of a number of bacterial infections. It is a combination antibiotic consisting of amoxicillin trihydrate, a β-lactam antibiotic, and potassium clavulanate, a β-lactamase inhibitor. This combination results in an antibiotic with an increased spectrum of action and restored efficacy against amoxicillin-resistant bacteria that produce β-lactamase.

Several methods were used for the determination of AMX such as: chromatography (HPLC) [10-13], spectrophotometic [14-16], LC/MS/MS [17] and electrochemical methods [18-20]. It is necessary to develop a simple, sensitive and selective method for determination amoxicillin.
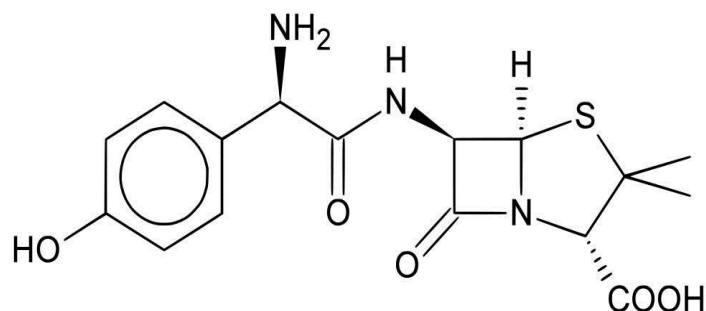
Figure 1: Structure of amoxicillin.

In this paper we report a Differential Pulse Adsorptive Stripping Voltammetry (DPAdSV) procedure for determination of amoxicillin. The method is applied to the determination of amoxicillin in medicine and biological samples with satisfactory results.

Experimental

Apparatus

DPAdsv measurement were made using a 746 VA-Trace Analyzer, (Metrohm, Switzerland) connected to an electrode stand, 747 VA-Stand,(Metrohm, Switzerland). The three-electrode configuration was used comprising a Metrohm multimode electrode(MME) in hanging mercury drop electrode (HMDE) state as working electrode, a double junction Ag/AgCl (3M KCl, saturated AgCl, and 3M KCl in the bridge) reference electrode and a Pt wire auxiliary electrode. All potential quoted are relative to the Ag/AgCl reference electrode. A rotating Teflon rod stirred solutions in the voltammetric cell. The mercury was triple-distilled quality, and medium drop size of the HMDE was selected. All experiments were done at the room temperature. pH measurement were made with a Metrohm pH meter model 827 (Switzerland). Eppendorf reference variable micropipettes (10-100 and 100-100 µl) were used to pipette micro liter volume of solution. All glass ware and storage bottle were soaked in 10 % nitric acid overnight and thoroughly rinsed with deionized water prior to use.

Reagents and solutions

All chemical reagents were of analytical grade and were purchased from Merck (Germany). All solutions were prepared with doubly distilled water. The stock solutions of 1.0×10^{-3} M amoxicillin was prepared with The 0.0182 g of pure amoxicillin accurately weighed and transferred to a 50 mLvolumetric flask and then dissolved in ethanol-water (1:1) solution.

Preparation and determination of real samples

Determination of amoxicillin in co-amoxiclav and Capsule samples

Ten tablets of co-amoxiclav were completely ground and homogenized. Then, 37 mg of the powders was accurately weighed and dissolved with ultrasonication in 100 mL of ethanol-water (1:1) solution. This solution was diluted 10-times, and then 1mL portion of solution was diluted in a voltammetric cell to 10 mL of 0.1 mol L^{-1} phosphate buffer (pH =10.2) for the analysis of the sample (Figure.2). Capsule samples were also prepared with the same procedure [8]. The concentration of amoxicillin in the working solution was determined under the optimum conditions by DPAdSV method. The results for the determination are listed in Table1.
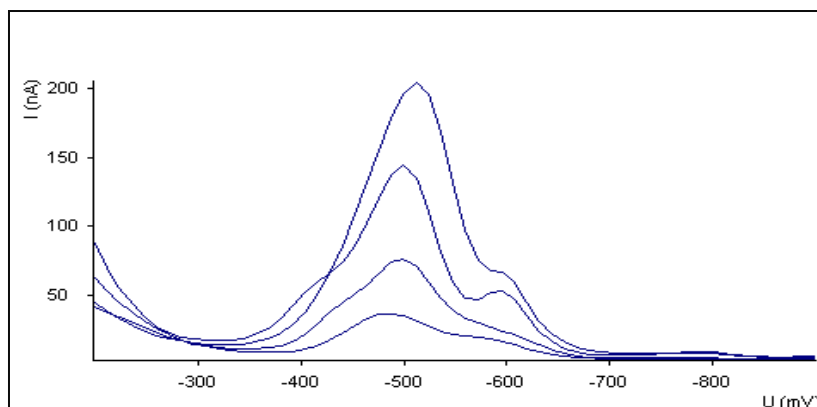
Figure 2: Typical voltammograms for determination of amoxicillin in real sample (co-amoxiclav) under optimum conditions.

Determination of amoxicillin in urine

The fresh urine sample was taken. Deproteinization of the sample was achieved by adding 2 ml of 10% trichloroacetic acid and centrifuged the mixture at 4500 rmp for 20 min. Then 5.0 ml aliquot of the supernatant fluid was taken into a 100 ml calibrated flask for determination amoxicillin. The accuracy was tested by standard addition method. The results for the determination of urine are listed in Table 1 [21].

Samples	Added (µM)	Found(µM)	Recovery %
Capsule	0.0	0.01±0.291	-
	0.2	0.06±0.515	104
	2.00	0.11±2.288	99.88
co-amoxiclav	0.0	0.009±0.272	-
	0.2	0.01±0.475	100.63
	2.00	0.18±2.211	97.43
urine	0.0	0.002±0.104	-
	0.2	0.05±0.295	97.14
	2.00	0.29±2.049	97.68

Table 1: Determination of amoxicillin in Capsule, co-amoxiclav and urine samples.

Results and Discussion

Amoxicillin electrochemical behavior was studied. The results of the initial testing of the drug in phosphate buffer solution at pH =10.2 indicated amoxicillin characteristics is absorbed surface hanging mercury drop electrode. Amoxicillin have a one peak with appropriate current in -0.514 v potential, that Probably related to Absorption and then oxidation OH Phenol groups to Carbonyl group on electrode surface (Figure 3). This peak current increased with increasing concentration of amoxicillin.
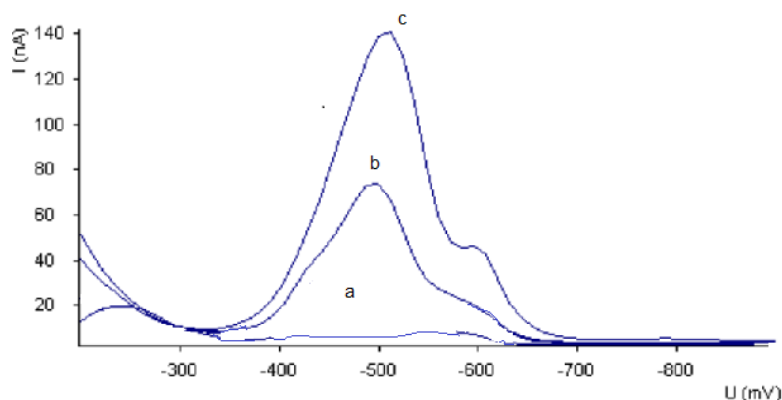
Figure 3: Differential pulse voltammograms of phosphate buffer (pH 10.2) containing: a): 0.00 ml, b): 0.01 ml and c): 0.04ml of amoxicillin 0.001M. Conditions: accumulation potential: +0.1 V, accumulation time: 50 s and scan rate: 60 mV/s.

Effects of variables

To obtain the best sensitivity in the determination of amoxicillin the influence of different parameters such as pH, accumulation potential, and accumulation time and scan rate were investigated.

Influence of supporting electrolyte and pH: Preliminary experiments were carried out with different types of buffer. The result showed that the peak shape for amoxicillin was improved in the presence of phosphate buffer solution. Therefore, phosphate buffer was used for optimization of pH. The electrochemical behavior of amoxicillin is dependent on pH value of the aqueous solution [18] , The influence of pH on the cathodic stripping peak currents of amoxicillin was studied in the pH range of 8.0-11.4 of phosphate buffer (t_{acc}=50 s and E_{acc}= 0.1 V). The results are shown in Figure 4. The results show that the peak currents of amoxicillin increasing the pH to about 10.2.
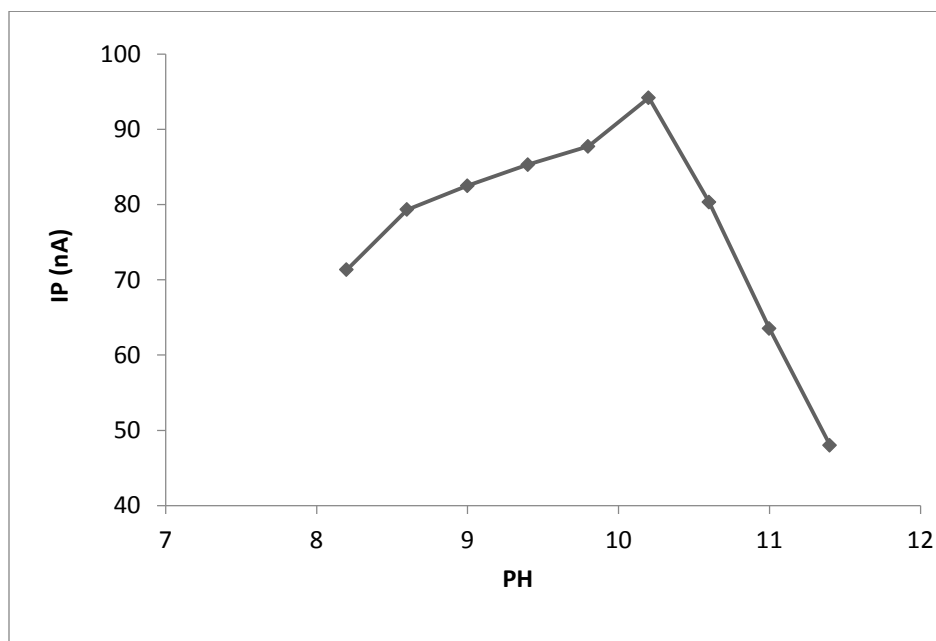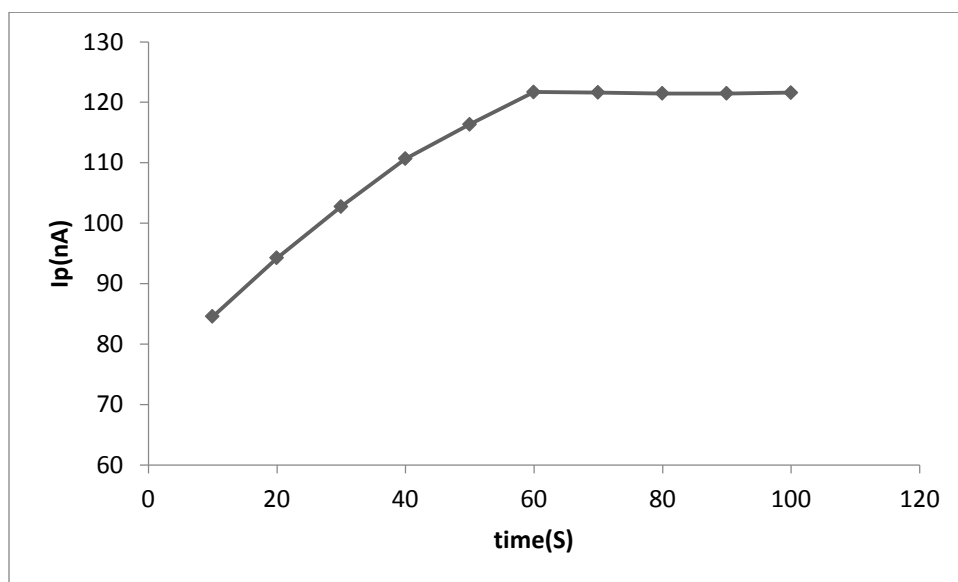

Figure 4: Effect of pH on the peak current of 0.04 ml of amoxicillin 0.001M. Conditions: accumulation potential: +0.1 V, accumulation time: 50 s and scan rate: 60 mV/s.

Influence of accumulation potential: The effect of the accumulation potential on the peak of amoxicillin was studied in the range of 0.3 to -0.15 (t_{acc}=50 s). As shown in Figure 5. 0.15 v pre-concentration potential of the reference electrode Ag /AgCl was chosen as the appropriate pre-concentration potential.
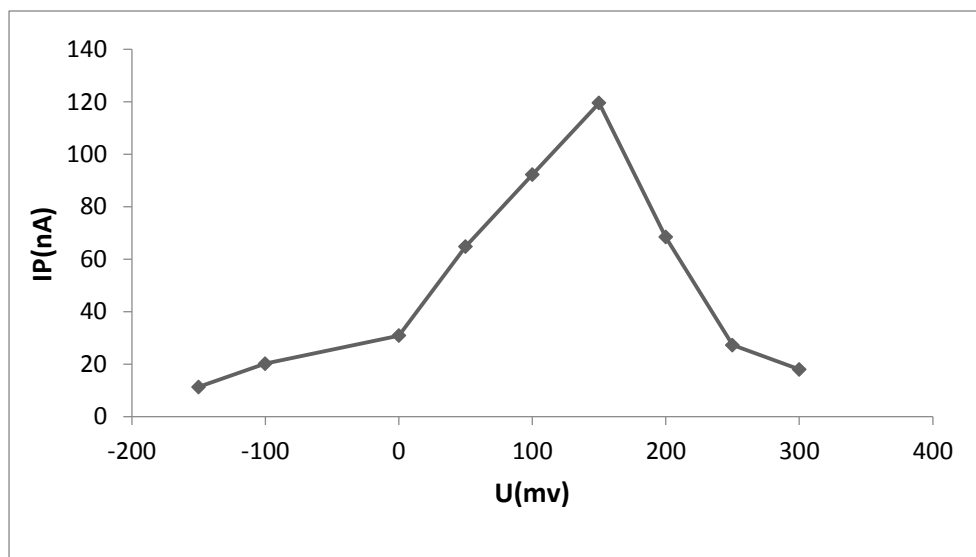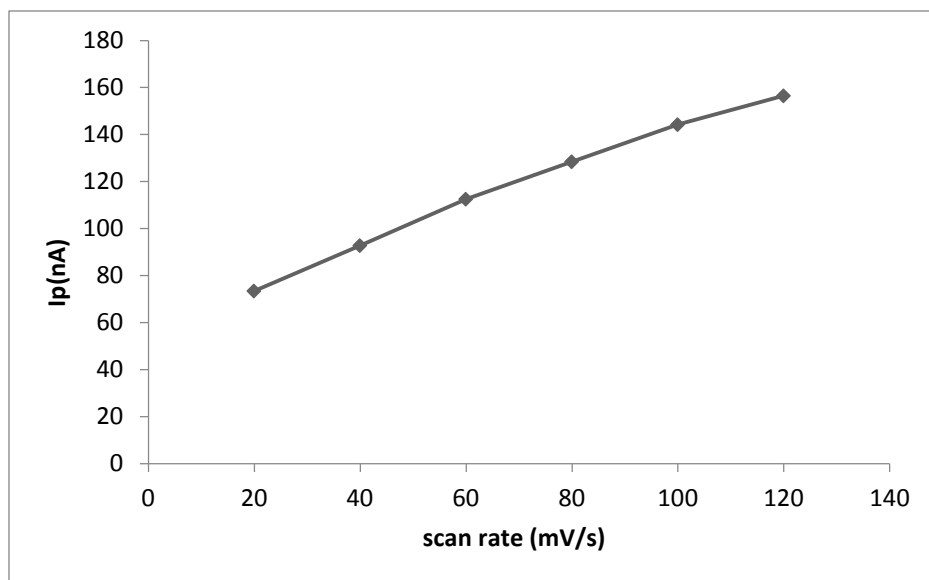

Figure 5: Effect of accumulation potential on the peak currents of amoxicillin. Conditions: phosphate buffer (pH 10.2), accumulation time: 50 s and scan rate: 60 mV/s.

Influence of accumulation time: The effect of the accumulation time on the stripping peak currents of fenoprofen was studied in the range of 10-100 s (E_{acc}=0.15 V). As shown in Figure 6, the peak currents increased initially with increasing pre-concentration time. After a specific period of accumulation time, the peak currents tend to level off slowly as the equilibrium surface concentration of the adsorbed amoxicillin was approached. Therefore, an accumulation time 60 s was selected for further investigations.

Figure 6: Effect of accumulation time on the peak currents of amoxicillin. Conditions: phosphate buffer (pH 10.2), accumulation potential, +0.15 V and scan rate: 60 mV/s.

Influence of scan rate: To obtain good potential scan rate under optimal conditions, potential scan rate in the range of 20 to120 mV/s was investigated. The results show that the peak for amoxicillin increase from 20 to 120 mV/s. therefore, the scan rate 120 mV/s was selected. The results are shown in Figure 7.

Figure 7: Effect of scan rate on the peak currents of amoxicillin. Conditions: phosphate buffer (pH 10.2), accumulation potential, +0.15 V and accumulation time, 60 s.

Linear range, detection limit and precision

To investigate the linear relationship between peak currents and concentration of amoxicillin under optimum conditions (pH 10.2, accumulation potential 0.15 V, accumulation time 60 s and scan rate 120 mV/s) a calibration graph was plotted. The results are shown in figure 8 and calibration equation, obtained by least-squares method (Figure 9). The relative standard deviation for 10 replicate analyses of solution containing 0.09 and 0.4 µM amoxicillin was 2.65% and 2.01%, respectively. A detection limit of 0.0098 µM of amoxicillin was estimated from 10 replicate determinations of blank solution under optimum conditions.
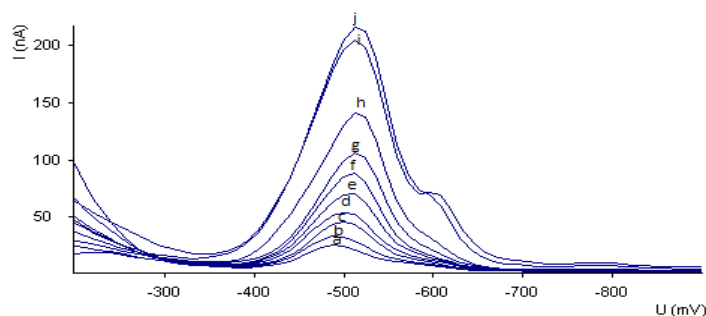
Figure 8: Typical voltammograms for determination of amoxicillin under optimum conditions a)0.07 µM , b)0.09 µM , c) 0.2 µM , d) 0.3 µM , e) 0.6 µM , f) 0.8 µM , g) 2 µg/ml , h) 3 µM , i) 4 µM and j) 5 µM of amoxicillin**.**
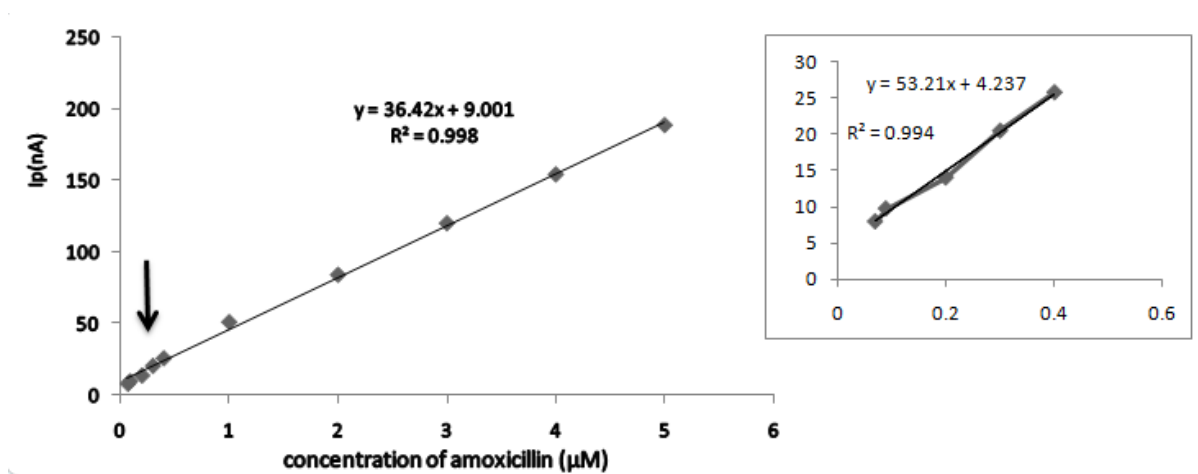
Figure 9: Calibration graph obtained using the optimized conditions.

Interference study

The amount of possible interference in the presence of other species in real samples was analyzed by addition of the interfering species to a solution containing 0.04 ml of amoxicillin 0.001M under the optimized conditions. The maximum tolerable concentrations of foreign species are shown in Table.2, where the tolerance limit was defined as the concentration of foreign species that produces a change in height of peak current of less than 5%. According to the results, the method is highly selective and has been successfully applied to trace determinations of amoxicillin in real sample.

Species	Tolerance limit $S_{species}/W_{fenoprofen})$
K^+, Na^+, CO_3^{-2}, NH+, Cl^-, ، glucose ,saccharose	1000
Mg^{+2}, Ca^{+2}, SO_4^{-2},NO_3^-	500
Al^{+3} ،Cu^{+2}	100
Mn^{+2},Fe^{+2},CN^-	5

Table 2: Interference study for amoxicillin determination.

Method	Linear range (μM)	LOD (μM)	Reference
LC-MS/MS	0.0136-54.7	0.0136	[8]
Chromatography	0.041-1.36	0.041	[22]
SWV	2-25	1.06	[23]
Cyclicvoltammetry	10-200	0.812	[24]
SWV	18.9-91.9	8.49	[25]
Cyclicvoltammetry	0.6-8 and 10-80	0.2	[18]
DPAdSV	0.07-5	0.0098	This work

Table 3: Some critical points in present work compared with some previous works applied for determination of amoxicillin.

Conclusion

A novel method is developed for determination of trace amount of amoxicillin by DPAdSV. This method is sensitive, precise, selective and simple for determination of amoxicillin.Table.3 show some critical properties of present work compared with previous studies. Comparison of the present work with the results in this table shows a good detection limit or linear calibration range compared to other studies.

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