



Voltammetric Analysis of Cephalexin and Cefazolin in Pharmaceutical Formulations and Biological Samples

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Abstract: This study presents the use of disposable pencil graphite electrode to investigate the electrochemical behaviors and voltammetric determination of cephalexin and cefazolin in phosphate buffer at different pH values (4.5-6.0). Sample analysis was performed in phosphate buffer at the optimum medium determined for each sample. Validation parameters were studied to show the correctness, sensitivity, and consistency of the method developed for the cephalosporins. It was concluded that disposable pencil graphite electrode could be used effectively in the determination of cephalosporins. The limits of detection (LOD) (S/N=3) were found to be 0.117 mM and 0.293 mM, for cephalexin and cefazolin, respectively. The analysis of these compounds in pharmaceutical formulations and in human blood serum and urine samples was carried out at defined optimum conditions.

Keywords: Beta-lactam antibiotics, pencil graphite electrode (PGE), voltammetric methods, electrochemical biosensors.

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INTRODUCTION

Drugs are increasingly used worldwide to provide public health for a reason that many active ingredients are used to prevent / treat human health and animal diseases (1), but their development requires the implementation of a strict waste management system (2). Antibiotics are drugs that kill (bactericidal) or slow down (bacteriostatic) the growth of bacteria. Broad-spectrum antibiotics treat a wide range of infections, as narrow-spectrum antibiotics are effective against only a few types of bacteria. Despite the importance of antibiotics, their uses have received much criticism from the consumers, responsible stakeholders and policy makers (3). Antibiotics can be classified into several groups according to their chemical structure: beta-lactams, tetracyclines, macrolides, aminoglycosides, sulfonamides, quinolones, aminoglycosides, glycopeptides and

oxazolidinones. Beta-lactam antibiotics are the most frequently used antibiotic group because of their bactericidal properties and low side effects. Penicillins and cephalosporins are among the most consumed species of beta-lactam antibiotics. Cephalosporins are semisynthetic antibiotics and are classified into four major groups according to their antimicrobial activity spectrum. As a rule, first-generation compounds have better activity against gram (+) organisms while other compounds have improved activity against gram (-) aerobic organisms (4).

It is important to estimate and detect amount of antibiotics in pharmaceutical and clinical samples because of their numerous pathological procedures. This is necessary in the field of human health and in food and fermentation industry for checking illegal use of antibiotics in food reservation and processing (5). Chromatographic methods using various

detectors, due to simultaneous detection, accurate quantification, automation and the high selectivity based on the chemical structures of the analyte, have been the most commonly used for the detection of antibiotics (6-8). Furthermore, various techniques have been reported to determine these compounds including use of electrophoresis (9-11), diode array (12, 13) or enzyme linked immunosorbent assay (ELISA) (14, 15). However, there are some well-known disadvantages of these methods such as costly equipment and consumables, long analysis time, laborious sample preparation and the need for a well-trained technical personnel (16). On the other hand, electrochemical methods that use in sensor technology have proved to very useful in the analysis of antibiotics due to some advantages such as simplicity, high sensitivity, low cost, reliable, portable and relatively short analysis time (20-22). Among various types of electrodes used in electroanalytical applications, pencil graphite electrodes have some advantages such as high electrochemical reactivity, commercial availability, good mechanical rigidity, disposability, renewability, low costs, low technology, and easy of modification. Due to their useful and important functions, these electrodes have already been used in many electroanalytical applications (23-25).

In this work, the voltammetric behaviors of cephalexin and cefazolin were investigated by differential pulse voltammetry (DPV) using pencil graphite electrodes as a sensor in phosphate buffer at pH between 4.5 and 6.0. The sensor was applied to determine cephalosporins in commercial pharmaceutical and biological samples without physiological interferents.

MATERIALS AND METHODS

Chemicals and Apparatus

Cephalexin (>99.9%) and cefazolin (>99.9%) were obtained from Sigma-Aldrich (Germany). Sodium hydroxide ($\geq 98\%$, Merck, Germany), phosphoric acid (85%, Merck, Germany), are commercially available as analytical grade reagents. Ultra-pure deionized water with a resistivity of 18.2 M Ω cm, was used for all experiments. SEF-500 tablet (Mustafa Nevzat) and Sefazol I.M. (Mustafa Nevzat) were purchased from a local pharmacy store.

Experiments were conducted using an Autolab Potentiostat/Galvanostat PGSTAT-302N device controlled by Nova 2.1 program on a personal computer. Ag/AgCl in 3.0 M KCl electrode as reference electrode, Pt-wire electrode as counter electrode, and 0.7 mm HB Tombo carbon-based pencil graphite leads based on a three-electrode system were used to conduct the experiments. Mettler Toledo S220 Seven Compact pH/ion meter and Mettler Toledo InLab 416 Ag/AgCl combined electrode were used for pH adjustments of buffer solutions used in electrochemical studies.

Preparation of Solutions

0.20 M phosphate buffer stock solution was prepared by dissolving an amount of phosphoric acid in 25.0 mL of deionized water. From this stock solution, buffer solutions of pH 4.5-6.0 were prepared by adding 3.0 M NaOH and mixing. 25.0 mM stock solutions were prepared pure cephalexin and cefazolin dissolving it in 25.0 mL of deionized water. The necessary dilutions for DPV studies was taken from the stock solution. Pharmaceutical sample preparation; two pills of SEF-500 Tablet weighing 1.351 g, containing 1.0 g of cephalexin, were crushed and powdered. Then, 0.222 g was dissolved in 25.0 mL deionized water and the solution was diluted for 10 minutes in an ultrasonic bath. The concentration of cephalexin in solution was 25.0 mM. After the completion of precipitation process, certain volumes are used during the experiments when deemed necessary. 0.271 g of Sefazol I.M. containing 250 mg of cefazolin was measured. Then, 0.250 g was dissolved in 25.0 mL deionized water and the solution was diluted for 10 minutes in an ultrasonic mixer. The concentration of cefazolin in solution was 20.0 mM. After the completion of precipitation process, certain volumes are used during the experiments when deemed necessary.

Serum sample was obtained from healthy and non-treated people. A specific amount of serum was centrifuged at a rate of 5000 rpm for 10 minutes to precipitate the proteins. Then, 1.0 mL of filtered serum was added to 9.0 mL of buffer solution and put on the electrochemical cell. Urine samples were taken from healthy individuals who were not taking any medications. A certain amount of urine was centrifuged at 5000 rpm for 10 minutes to precipitate the proteins. The centrifuged urine was taken and filtered. For each study, 2.0 mL of urine was added to 8.0 mL of buffer solution and put on the electrochemical cell.

Analytical Procedure

Electrochemical behaviors of some beta-lactam antibiotics (cephalexin and cefazolin) were studied by DPV using pencil graphite electrodes. An 8.0 mL aliquot of phosphate buffer (supporting buffer) at desired pH was pipetted in a clean and dry voltammetric cell and the required standard solutions of cephalosporins were added. DPV was employed in the potential range of 0- (+2.25) V, with a repetition of $n=3$. Changes in peak currents with support electrolyte in various pH values were assessed. All measurements were performed at room temperature. Analyzes of cephalexin and cefazolin in pharmaceutical dosage, as well as human blood serum and urine samples have been carried out under determined optimal experimental conditions.

RESULTS AND DISCUSSION

Influence of pH

In general, the pH of the support electrolyte has an important role on the electrochemical behavior of biologically active molecules. The pH dependence of cephalosporins' peak currents were investigated by using DPV in phosphate buffer solution within the pH range of 4.5-6.0 (26, 27). For this purpose, voltammograms were taken 3 times with DPV between 0 V to +2.25 V of the compounds and the supporting electrolyte and pH at which the highest oxidation currents were determined. Oxidation peak curves of cefazolin versus pH for each buffer were plotted to determine which one provides the highest

electroactivity (Figure 1). The results showed that the peak currents of cephalosporins increased until reaches 5.0, and then decreased drastically. As a result of these observations, pH 5.0 was chosen as the optimal supporting electrolyte to obtain high sensitivity for cephalosporins and used in subsequent experiments. Previous studies showed that most electrocatalytic is observed in more acidic solutions, and in solutions where pH value is higher than 5.0, cephalosporins underwent deprotonization and the formation of carboxylate anions occurs.

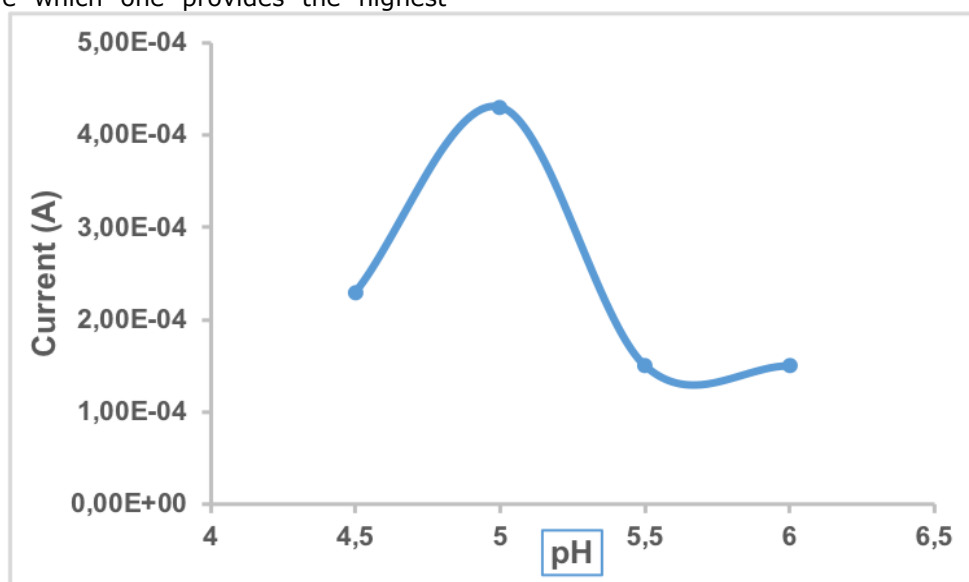


Figure 1. pH-current graph for 1.0 mM cefazolin.

Analytical Performance

Under optimum experimental conditions, the calibration graphics for cephalixin and cefazolin are established. As it can be seen in Figure 2, peak current linear with concentrations in the concentration ranges of 0.5-4.0 mM and 1.0-3.5 mM for cephalixin and cefazolin, respectively. The limit of detection (LOD) and limit of quantification (LOQ) of cephalosporins with

single-use disposable electrode were calculated using the equations are mentioned below.

$$\text{LOD} = 3 s/m; \text{LOQ} = 10 s/m$$

where s is the standard deviation for the peak current of the blank and m is the slope of the calibration graph. Given in Table 1 are the results of these calculations.

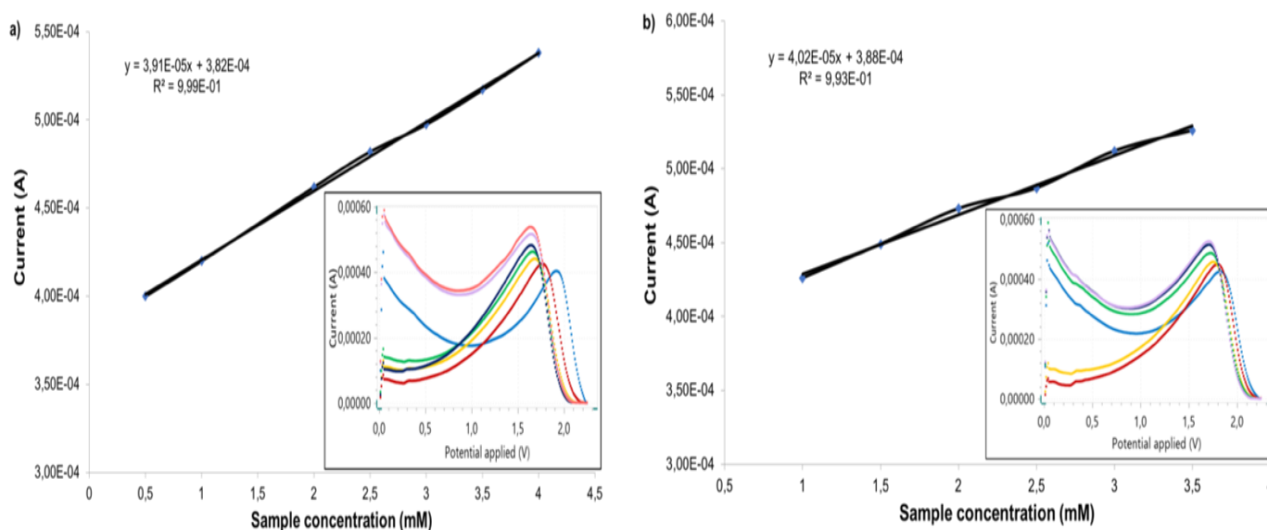


Figure 2. The calibration curves for cephalixin (a) and cefazolin (b) between peak current and concentration at PGE.

Table 1. Characteristics of calibration curves.

	Cephalexin	Cefazolin
Linearity range (mM)	0.5-4.0	1.0-3.5
Regression equation	$y = 3,91E-05x + 3,82E-04$	$y = 4,02E-05x + 3,88E-04$
SD of slope	5.79×10^{-7}	1.63×10^{-6}
SD of intercept	1.53×10^{-6}	3.93×10^{-6}
Correlation coefficient	9,99E-01	9,93E-01
LOD (mM)	0.117	0.293
LOQ (mM)	0.391	0,978

Analytical Application

Analysis and recovery studies of drug substances have been carried out to determine the amount of cephalexin and cefazolin in blood, urine and pharmaceutical samples and to check the accuracy of the method developed. To this end, the samples were prepared as described in the experimental section. Differential pulse voltammograms were recorded under exactly the same conditions used to record the voltammograms to plot the calibration plot. In order to clarify the applicability of the sensor in some commercially available pharmaceutical formulations, analysis of the cephalexin and cefazolin was carried out. Voltammograms of the prepared pharmaceutical sample (SEF-500 and Sefazol I.M.) were taken with pencil graphite electrode and the amount of cephalexin and cefazolin in the sample were determined. Figure 3(a) and Figure 4(a) show the voltammograms

taken by DPV of the pharmaceutical samples. The results obtained are given in Table 2.

To investigate the performance of the sensors in the complex matrix, urine and blood samples were spiked with different concentrations of drugs and analyzed by the proposed method. Analysis of the blood and urine samples calibration line were performed by standard addition method at the concentration interval. Figure 3(b-c) and Figure 4 (b-c) demonstrate the DPV voltammograms of the cephalosporins containing blood and urine samples, respectively. Peak formation was observed in all samples and the recovery data are given in Table 2 below. The obtained results confirm that the disposable pencil graphite electrode can be employed for determination of cephalexin and cefazoline in real samples with different matrices.

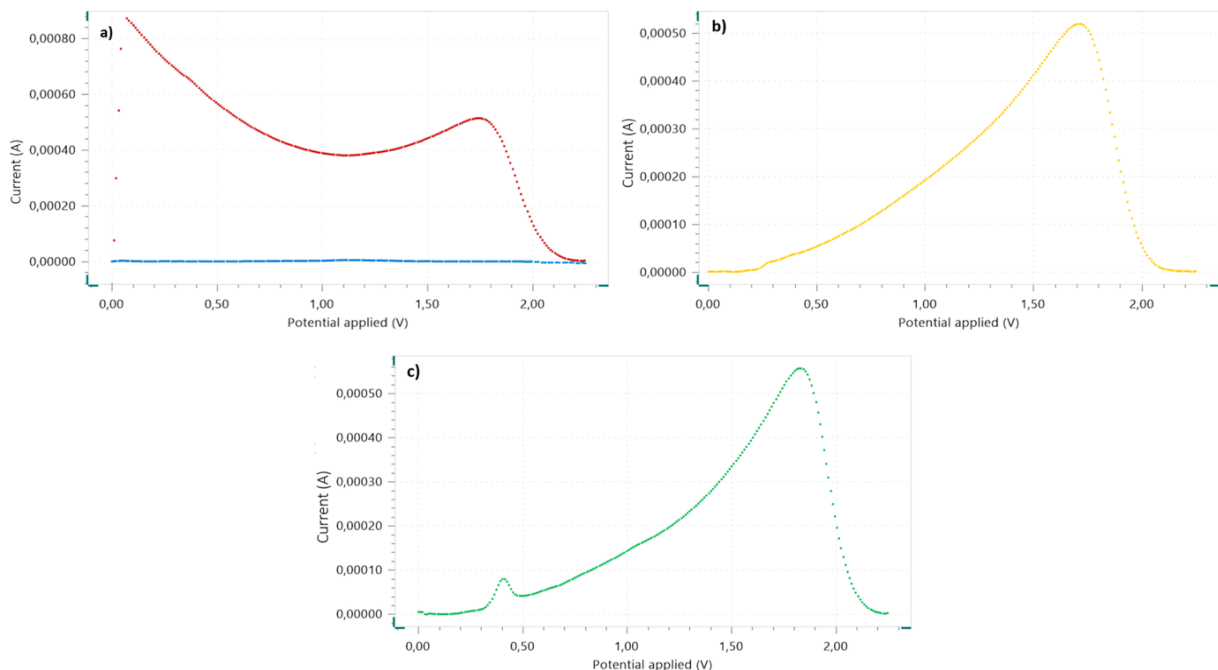


Figure 3. Voltammograms of cephalexin in a) pharmaceutical, b) blood and c) urine samples.

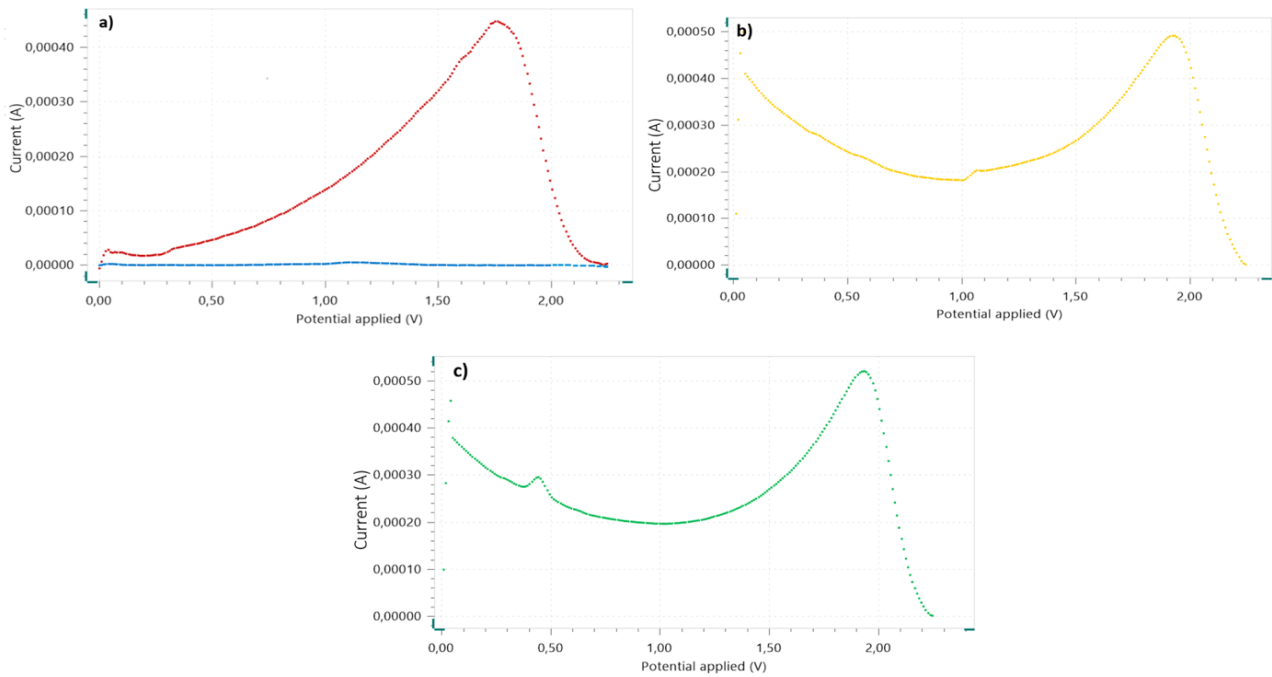


Figure 4. Voltammograms of cefazolin in a) pharmaceutical, b) blood and c) urine samples.

CONCLUSIONS

The analysis of various beta-lactam antibiotics without any pretreatment, with a small amount of analytical work, is an advantage observed during the experiments and demonstrates the simplicity of the method. In addition, it has been determined that the method applied during the studies is a fast, distinctive and economical method. The proposed method could find application in pharmaceutical products and in clinical samples.

In this study, disposable pencil graphite electrode for detection of beta-lactam antibiotics and its

applicability in electrochemical biosensor studies has been provided. The cephalixin electrochemical biosensor response was linear with the variation of the analyte concentrations, with the following parameters: analytical linear range from 0.50 mM to 4.00 mM, LOD and LOQ of 0.117 mM and 0.391 mM, respectively. A linear relationship between the concentration of cefazolin and current response was obtained with excellent reproducibility of the current and a low LOD (0.293 mM) and LOQ (0.978 mM). Considering the advantages of reproducibility, low LOD, low cost and disposable use, this study is considered to be a significant contribution to the literature.

Table 2. The obtained results from detection of cephalexin and cefazolin in pharmaceutical and biological samples.

	CEPHALEXIN					CEFAZOLIN				
	Added content (mM)	Average detected content (mM) \pm SD	RSD %	Recovery %	Recovery RSD%	Added content (mM)	Average detected content (mM) \pm SD	RSD %	Recovery %	Recovery RSD%
Blood Serum	3.25	3.50 \pm 0.05	1.43	107.70	1.41	1.25	1.25 \pm 0.07	5.60	100.00	5.65
Urine	3.25	4.25 \pm 0.05	1.17	130.77	1.19	3.25	3.32 \pm 0.09	2.71	102.15	2.79
Pharmaceutical Sample	3.25	3.24 \pm 0.04	1.23	99.69	1.50	3.25	2.16 \pm 0.03	1.39	66.66	1.33

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