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Cathodic Stripping Voltammetric Determination of Cefadroxil in Pharmaceutical Preparations and in Blood Serum

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Abstract

An analytical method has been developed using hanging mercury drop electrode (HMDE) for the quantitative determination of antibacterial drug cefadroxil (CFL) from pharmaceutical preparations and blood serum. Cathodic adsorptive stripping voltammetry was carried out in hydrochloric acid (0.1M): methanol (80: 20 v/v) and potassium chloride (0.1M) as supporting electrolyte. The reduction wave was obtained within -700 to -800 mV. Linear calibration curve was within 1-50µg/mL with detection limit of 0.1µg/mL of cefadroxil. Relative standard deviation for inter and intra day analysis of CFL was within 1-2%. The number of additives present in pharmaceutical preparations did not interfere the determination of cefadroxil. The analysis of pharmaceutical preparations and blood serum after chemotherapy with cefadroxil indicated relative standard deviation (RSD) within 0.8-1.2% and 2.6-3.8% respectively. The satisfactory results were obtained for quality control of cefadroxil in pharmaceutical preparations and in blood serum.

Keywords: Cathodic Stripping Voltammetry, Phenolic Beta Lactam, Cefadroxil, Pharamaceuticals, HMDE.

Introduction

The phenolic β-lactam antibiotic cefadroxil (CFL) is a broad spectrum first generation cephalosporin and is used for the treatment of urinary tract infections. It has significant activity against both Gram-positive and Gram-negative bacteria [1]. It contains a β-lactam ring fused with a six membered dihydrothiazine ring bearing substitutes R^1 and R^2 in the side chain at C_3 and C_7. Various analytical procedures are described for the determination of CFL mainly, spectrophotometry [2-3], derivative spectrophotometry [4-5], spectrofluorometry [6-7], liquid chromatography [8-11], atomic absorption [12], capillary zone electrophoresis [13], chemiluminescence [14-15] and electroanalytical technique [16-18]. The British pharmacopeias, 1998, describes a liquid chromatographic method for the analysis of bulk drug [19].

Electroanalytical methods are sensitive, selective and suitable for biological active compounds. They involve less expensive equipment. Ivaska et al [16] reported a differential pulse polarographic method for CFL at pH 1-2 as a distinct peak at -1.25 to 1.30 V (vs Ag/AgCl) and a linear scan voltammetric method at glassy carbon electrode as anodic oxidation wave at +0.8V (vs Ag/AgCl) at pH 7.3. Ozkan et al [17] reported electro oxidation of CFL using glassy carbon electrode by cyclic voltammetry and differential pulse voltammetry. The method was applied for determination from pharmaceutical preparations. Gaber et al [18] examined differential pulse polarography and cyclic voltammetry to investigate the coordination of CFL with Cd(II), Pd(II) and Zn(II). However these methods have not been applied for the determination of CFL from biological fluids.

The procedure which could be used for the analysis of CFL from biological fluids, without extensive sample preparations or derivatization in the presence of excipients could be of analytical interest. Hanging mercury drop electrode (HMDE) can be examined, because glassy carbon electrode requires surface polishing during each run and may be time consuming. The precision and accuracy may also be affected. The present work examines simple and sensitive method for the determination of CFL from pharmaceutical preparation and blood serum using differential pulse cathodic stripping voltammetry at HMDE. The determination of CFL from pharmaceutical preparations could be of interest for quality control and analytical procedure used for the analysis of CFL in biological fluids may be of value in evaluation of bioavailability and pharmacokinetic properties.

Experimental

Chemicals and Reagents

GR grade methanol, hydrochloric acid (37%), potassium chloride, potassium nitrate, lithium chloride, sodium acetate, potassium dihydrogen phosphate (E. Merck, Germany) and cefadroxil (CFL) (Sigma, Switzerland) were used. Freshly prepared doubly distilled deionized water was used throughout the study. A stock solution of CFL (1mg/mL) was prepared by dissolving 100 mg of CFL in methanol (20 mL) and adjusting the volume with hydrochloric acid (0.1N) to 100mL. This solution (5mL) was further diluted to 100mL with methanol: HCl (0.1M) (20:80v/v) on each working day. Potassium chloride (0.1M) as base electrolyte prepared in deionized water was used. Buffer solutions within pH 1-10 at unit interval were prepared from the following:

Hydrochloric acid (0.1M), potassium chloride (0.1M), acetic acid (0.1M), sodium acetate (0.1M), ammonium acetate (0.1M) sodium bicarbonate (0.1M), sodium carbonate (0.1M) ammonium chloride (0.1M) and ammonia (0.1M).

Instrumentation

The pH measurements were made with pH meter (WTW – Inolab, Germany) with glass electrode and internal reference electrode. Voltammetric measurements were performed with Metrohm automatic 746-VA Trace analyzer equipped with 747-VA stand (Metrohm, Switzerland). The 747 stand includes a three electrode system, an Ag/AgCl (3M KCl), reference electrode, a platinum wire as auxiliary electrode and hanging mercury drop electrode as working electrode. A printer Epson LX-300 was used for printing purposes. PTFE coated string bar, rotated by a magnetic stirrer was used during preconcentration step.

Analytical Procedure

Determination of Cefadroxil

Solution of cefadroxil (in methanol: (0.1M) HCl 1:4 v/v) (10mL) containing 10-500 µg and 1mL of potassium chloride (0.1M) was transferred into polarographic vessel. The pH was adjusted to 4 by adding 1mL of (0.1M HCl:KCl) buffer. The sample was purged for 200 seconds with oxygen-free nitrogen (British Oxygen Company, BOC, Karachi). The preconcentration potential (-700 mV) measured against Ag/AgCl reference electrode was applied to the fresh mercury drop for 60 seconds (t_{acc}= 60 sec) while the solution was stirred. The stirring was then stopped for a period of 10 seconds (equilibration time=10 sec). The voltammogram was then recorded by applying a cathodic differential pulse scan with pulse amplitude of -50 mV. The voltammograms were recorded in triplicate for each run automatically by the instrument. The average peak heights (n=3) were assessed on the basis of the difference between peak height of the analyte and that of base electrolyte alone recorded under the same conditions. The quantitation was carried out by calibration curve and standard addition technique.

Determination of Cefadroxil in Pharmaceutical Preparations

Eight tablets of each Helicef (Helix Pharma (Pvt.) Ltd. Karachi, Pakistan), Neucef (Sami Pharmaceutical (Pvt.) Ltd. Karachi, Pakistan), Evacef (Highnoon laboratories, Lahore, Pakistan) were separately ground to fine powder. A quantity equivalent to one tablet (500mg of cefadroxil) of each was weighed, dissolved in 20mL methanol, transferred to a 100 mL volumetric flask and diluted to the mark with

(0.1M) HCl. This solution was slightly turbid but no further treatment was made. Further dilution was made with 20% methanol in HCl (0.1M). 10 mL of this solution and 1 mL potassium chloride (0.1M) were transferred to polarographic cell and analysis was carried out by analytical procedure 2.3.1.

Determination of Cefadroxil in Blood Serum

The blood sample (5mL) of volunteers was collected after 2hrs of taking a tablet Helicef containing 500 mg of cefadroxil. The blood sample was collected by vein puncture in a clean screw capped vial and centrifuged at 3000 rpm for 15 minutes. Supernatant was separated and added acetonitrile (1mL) and contains were mixed for two minutes. The sample was centrifuged for 15minutes at 4000 rpm and supernatant was collected and organic solvent was evaporated at 45°C under nitrogen stream. Subsequently the sample was transferred to volumetric flask, volume was adjusted to 10mL with methanol: HCl (0.1M) (1:4 v/v) and was analyzed using analytical procedure 2.3.1. A blank determination was carried out following the same procedure with blood sample (5mL) from the volunteer who had not taken any medicine at least for one week.

Results and Discussion

The electrochemical reduction of CFL by differential pulse cathodic stripping voltammetry (CSV) was examined by hanging mercury drop electrode (HMDE). The effect of pH and supporting electrolyte was examined on electrochemical reduction of CFL.

The influence of pH was examined within pH 2–10. It was observed that peak potential shifted linearly to more negative side with an increase in pH indicating that the mechanism of electrode reaction is pH dependent. The highest peak current was obtained at pH 4 at -0.75 V (Figure 1) and was selected. The effects of different electrolyte on peak potential (mV) and peak current (nA) were examined. The solution (1mL) of potassium chloride, lithium chloride, sodium oxalate, potassium citrate, sodium carbonate, sodium acetate of 0.1M concentration were added and determination were carried out at pH 4. The peak potential was measured at -0.75V. Average response of at least (n=3) determination was calculated. The maximum peak current was obtained when potassium chloride was used. At optimized conditions the peak current was observed to be concentration dependent and linear calibration curve was obtained by recording average peak current (n=6) against concentration of CFL and was obtained with 1-50 μg/mL (Figure 2).

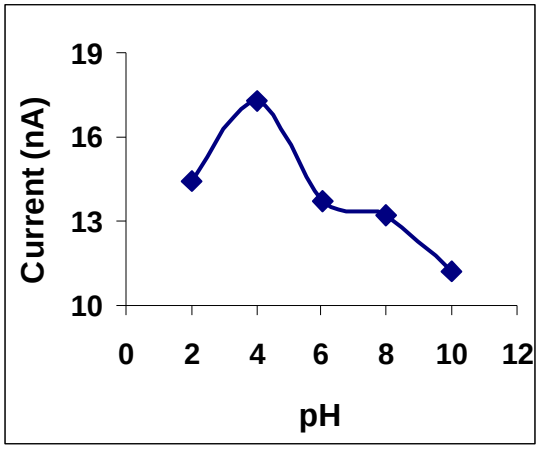

Fig. 1. Effect of pH on current (nA) for cefadroxil in 20% methanol plus 80% 0.1M HCl plus 0.1 M KCl.

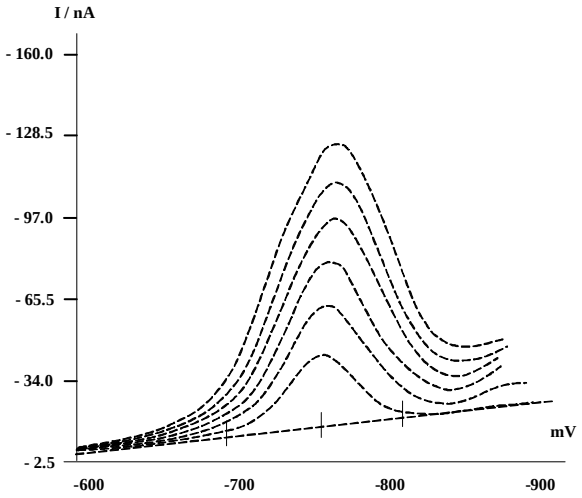

Fig. 2. Cathodic stripping voltammogram for CFL indicating potential (mV) verses current (nA) in 20% methanol plus 80% 0.1M HCl plus 0.1 M KCl, pH 4.0, concentrations 1, 10, 20, 30, 40, 50 μg/ mL, peak potential -0.75 V.

The coefficient of determination, R^2 for a six points calibration was obtained 0.9822 with y= 4.8542x (Figure 3). The analysis of test solutions

of CFL (n=4) was carried out within the calibration range and relative % error for the analysis was obtained within ± 0.5%. The reproducibility of the measurements in terms of peak potential and peak current for CFL with 10μg/mL (n=5) was calculated and relative standard deviations (RSD) were obtained with 0.5% and 0.6% respectively. The detection limit measured as signal to noise ratio (3:1) as compared to the blank was obtained 0.1μg/mL. The inter and intra day variation in the response for 10μg/mL was examined for five days and each day analysis was carried out in triplicate (n=3). The RSD for pooled data was obtained 1.2%. For the determination of CFL in pharmaceutical preparations, the interfering effects of common additives glucose, lactose, sorbitol, gum arabic were examined at the concentration five times the CFL (10μg/mL). The average variation (n=3) in the response was observed less than 5% and did not interfere the determinations. The results obtained were compared with a spectrophotometric method [20] by measuring the absorbance at 455nm and a good agreement with observed results with CSV was obtained (shown in Table 1).

The method was applied for the determination of CFL in the pharmaceutical preparations Helicef, Neucef and Evacef.

The results of analysis are summarized in Table 1 and the results agreed with the labeled values with relative % deviation within 1.2 to 5.2%. The RSD for replicate analysis (n=3) was obtained within 0.8 – 1.2%.

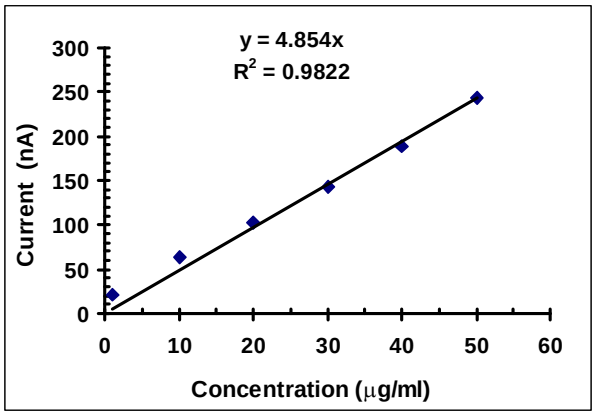

Fig. 3. Calibration plot for CFL from Figure 2.

The method was examined for the analysis of CFL from human serum. The blood samples of healthy volunteers were collected after 2hrs from a single dose of 500mg of cefadroxil. The serum after de-proteinization with acetonitrile was analyzed. The results of analysis are summarized in Table 2 and are within 15 to 20 μg/mL with RSD 2.6-3.8%. The peak potential was however observed at slightly shifted position towards negative but stable signals were observed including after spiking the sample with 100 μg of CFL.

Table 1. Results of analysis for cefadroxil in pharmaceutical preparations

S. No.	Name of Drug	Labeled Amount (mg/ Tablet)	Amount Found (mg/ Tablet) ± C.L at 95%	*Amount Found (mg/ Tablet) ± C.L at 95%	RSD % (n=3)
1	Helicef	500	497 ± 4	492±2	0.8
2	Neucef	500	479 ± 6	472±5	1.2
3	Evacef	500	493 ± 4	488±4	0.8

***Amount found by spectrophotometric method [20]**

Table 2. Results of analysis for cefadroxil in blood serum

Blood Samples No.	Amount taken Helicef (mg)	Amount found (μg/mL) ± C.L at 95%	% RSD
1	500	15.2±0.5	3.8
2	500	20.4±0.5	2.7
3	500	17.6±0.4	2.6

Conclusion

Simple cathodic stripping voltammetric method has been developed with HMDE for quantitative determination of cefadroxil at pH-4. The CSV methodology is simple, sensitive and rapid for the determination of the drug in pharmaceuticals and in blood serum. The sample preparation procedure is simple without need to eliminate the excipients. The satisfactory results obtained with RSD 0.6% and allow recommending the procedure for quality control of cefadroxil in concentration range 1-50 μg/mL in pharmaceutical preparations and blood serum.

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