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Short communication

Voltammetric studies on the antibiotic drug cefoperazone Quantification and pharmacokinetic studies

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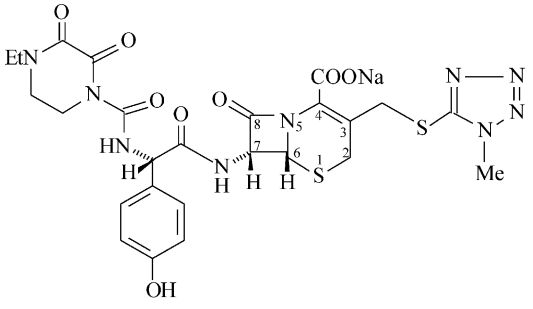
Abstract

A fully validated simple, sensitive and selective square-wave stripping voltammetry procedure was described for the trace quantification of cefoperazone in bulk form, formulations and human serum/plasma. The procedure was based on reduction of the adsorbed drug onto a hanging mercury drop electrode. The procedural conditions were optimized as: frequency = 60 Hz, scan increment = 8 mV, pulse amplitude = 25 mV, preconcentration potential = −0.3 V (versus Ag/Ag/KCl_s), preconcentration duration = 60–150 s and an acetate buffer of pH 4.2 as a supporting electrolyte. A limit of detection of 4.5×10^{-10} M and a limit of quantitation of 1.5×10^{-9} M bulk cefoperazone were achieved following preconcentration of the drug onto the hanging mercury drop electrode for 150 s. The proposed square-wave adsorptive cathodic stripping voltammetric procedure was successfully applied for trace quantification of cefoperazone in human serum and plasma. The achieved limits of detection and quantitation of the drug in human serum were 6×10^{-10} M (0.375 ng ml^{-1}) and 2×10^{-9} M (1.250 ng ml^{-1}), respectively. The pharmacokinetic parameters of cefoperazone in plasma of hospitalized volunteers were successfully estimated.

Keywords: Cefoperazone; Cefazone®; Cefobid®; Human serum; Stripping voltammetry; Quantification; Pharmacokinetics

1. Introduction

Cefoperazone, is one of the third-generation semi-synthetic cephalosporins antibiotic active against a wide range of Gram-positive and Gram-negative bacteria, including β-lactamases produced by Enterobacteriaceae and *Pseudomonas* spp. [1]



(Structure of cefoperazone)

Cefoperazone is widely distributed into most body fluids and tissues reaching concentrations higher than the minimum inhibitory concentrations of susceptible bacteria [1]. Peak-plasma concentration is achieved within 1–2 h after i.m. and 5 min of i.v. administration of the drug. About 90% of the drug is bound to plasma proteins. Plasma half-life time is about 2 h [1].

Several analytical techniques have been used for quantification of cefoperazone in pharmaceutical formulations and human biological fluids. These include spectrophotometry [2–6], spectrofluorimetry [7,8], colorimetry [9], capillary zone electrophoresis [10], high-performance liquid chromatography [11–15], and voltammetry [16–18].

This work aimed to describe a fully validated, simple, and sensitive adsorptive stripping voltammetric procedure for trace quantification of cefoperazone in pharmaceutical formulations and human blood and to estimate its pharmacokinetic parameters in plasma of volunteers each administrated a single dose by i.m. injection.

2. Experimental

2.1. Apparatus

The Electrochemical Analyzers Models 263A-PAR and 394A-PAR (Princeton Applied Research, Oak Ridge, TN,

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USA) were used for voltammetry measurements. The electrode assembly (Model 303A-PAR), incorporated with a micro-electrochemical cell of three electrode system comprising of a hanging mercury drop electrode (HMDE) as a working electrode (area: 0.026 cm^2), an Ag/AgCl (saturated KCl) reference electrode and a platinum wire auxiliary electrode, was used. A magnetic stirrer (305-PAR) and a stirring bar were used to provide the convective transport during the preconcentration step. All the measurements were automated and controlled through the programming capacity of the apparatus.

A Shimadzu UV–vis recording Spectrophotometer (160A) was used for a comparative assay of the drug in its pharmaceutical formulations by means of a reported spectrophotometric method [3].

2.2. Reagents and solutions

(i) A standard stock solution (1×10^{-3} M) of bulk cefoperazone was prepared in de-ionized water and stored at 4 °C. More dilute solutions (10^{-6} to 10^{-4} M) were prepared daily by accurate dilution with de-ionized water just before use.

(ii) The powder content of two vials of each of Cefazone® (500 mg cefoperazone/vial, Pharmaco Pharmaceuticals, Alexandria, Egypt) and Cefobid® (500 mg cefoperazone/vial, Pfizer, Cairo, Egypt) were weighed and the average mass per vial was determined. A quantity of the powder of each of these samples was weighed accurately then transferred into a 50 ml-volume calibrated flask, completed to volume with de-ionized water then sonicated for about 15 min. The desired concentrations (1×10^{-6} to 1×10^{-4} M) of each of the two formulations were obtained by accurate dilution with de-ionized water.

(iii) Aliquots of serum of healthy volunteer were introduced into seven centrifugation tubes, then successively fortified with various concentrations of cefoperazone and completed to a 1.0 ml volume with methanol (as a precipitating agent for proteins). After vortexing for 30 s, the precipitated proteins were separated out by centrifugation for 3 min at 14,000 rpm. The clear supernatant layer was filtrated through a 0.45 μm milli-pore filter to produce a protein-free human serum spiked with various concentrations of cefoperazone.

(iv) Series of a Britton–Robinson (B–R) universal (pH 2–11) and an acetate (pH 3.6–5.5) buffers as supporting electrolytes were prepared.

2.3. Pharmacokinetic studies

A pharmacokinetic study was performed on human plasma of two healthy volunteers. The two volunteers gave their written informed consent prior to participating in the study (at Ramadan Specialized Hospital, Tanta City, Egypt). Both volunteers fasted overnight for 8 h before injection. A venous blood sample was taken immediately before drug injection to serve as a blank and sampling was continued for a period of up to 12 h following an i.m. injection of a single vial (Cefazone®, 500 mg cefoperazone). The blood samples were centrifuged immediately at 2000 rpm for 10 min and the plasma fractions were rapidly separated and stored in coded polypropylene tubes at −20 °C until assayed. Analysis of the human plasma samples was carried out according to the general analytical procedure.

2.4. General analytical procedure

A known volume of the solution containing cefoperazone (proteins free-solution in case of human serum or plasma samples) was transferred into a 10 ml volume calibrated flask and then made up to the volume with the buffer solution. Then the solution was introduced into the electrolysis cell and deoxygenated with pure nitrogen gas for about 10 min in the first cycle and for 30 s in each successive cycle, while the nitrogen gas was kept over the solution during the measurements. Preconcentration of cefoperazone onto the surface of the HMDE was performed at −0.3 V (versus Ag/AgCl/KCl_s) for a selected duration (60–150 s) while stirring the solution at 400 rpm. After an equilibrium time of 5 s, the voltammograms were recorded by scanning the potential towards the negative direction using the square-wave potential waveform.

3. Results and discussions

3.1. Cyclic voltammetry studies

Cyclic voltammograms of 1×10^{-6} M cefoperazone exhibited a single well-defined irreversible cathodic peak in the B-R universal buffer (pH 2–8) and acetate buffer (pH 3.6–5.5). A much developed peak current intensity was achieved in acetate buffer of pH 4.2. The observed irreversible cathodic peak of cefoperazone may be attributed to the reduction of an electro-active group in the side-chain on C-7.

Fig. 1 shows the cyclic voltammograms for 1×10^{-6} M cefoperazone in an acetate buffer (pH 4.2) following: its precon-

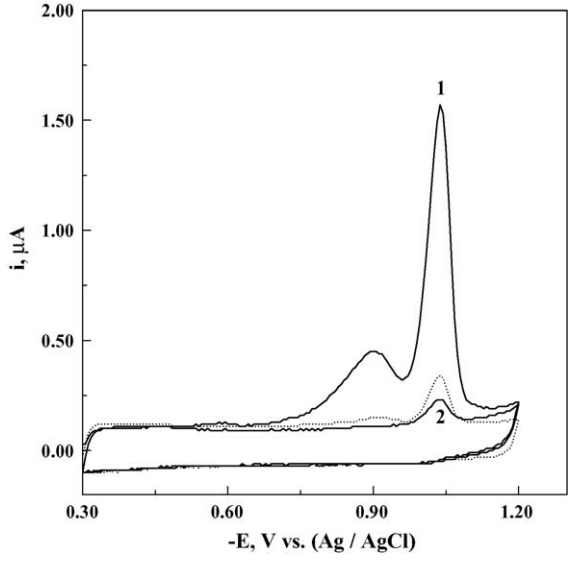

Fig. 1. Cyclic voltammograms for 1×10^{-6} M bulk cefoperazone in an acetate buffer of pH 4.2 following preconcentration onto the HMDE: at open circuit (dashed scan) and −0.3 V for 30 s (scan 1); replicate scan at the same mercury drop (scan 2); scan rate = 200 mV s^{-1}.

centration onto the HMDE at open circuit (dashed curve) and at −0.3 V for 30 s (curve 1). A much developed peak current intensity was achieved following preconcentration of cefoperazone onto the HMDE surface (curve 1), whereas the second cycle at the same mercury drop (curve 2) exhibited a much lower peak current intensity. This behavior indicated the adsorptive accumulation of cefoperazone onto the HMDE (curve 1) then its desorption from the electrode surface (curve 2).

Cefoperazone surface coverage (Γ_0) was evaluated using the relation [19]: $\Gamma_0 = Q/nF$, where Q is the charge consumed by the surface process as calculated by the integration of the area under the peak of reactant, corrected for residual current, n is the number of electrons transferred in the electrode reaction ($n = 4$) and A is the area (0.026 cm^2) of the HMDE. A monolayer surface coverage of 1.22×10^{-10} mol cm^{-2} was obtained. Each adsorbed molecule therefore occupied an area of 1.36 nm^2.

3.2. Square-wave stripping voltammetry studies

3.2.1. Optimization of the procedural conditions

The influence of pH on the square-wave voltammetric response for 5×10^{-7} M cefoperazone was examined in the acetate buffer (pH 3.6–5.5) following preconcentration at −0.1 V for 30 s. The voltammograms showed a single well-defined irreversible cathodic peak over the pH range. The peak current intensity was much developed at pH 4.2.

The optimum instrumental conditions of the proposed procedure were identified from a study of the peak current intensity for 2×10^{-7} M cefoperazone in an acetate buffer of pH 4.2, following its preconcentration onto the HMDE for 30 s, on changing the frequency (f), scan increment (ΔE_s) and pulse-amplitude (a), within the ranges, 10–100 Hz, 2–8 mV and 10–50 mV, respectively. Although the peak current intensity was almost directly proportional to each of f, ΔE_s and a, however a much developed peak current intensity was achieved under the following instrumental conditions: $f = 60$ Hz, $\Delta E_s = 8$ mV and $a = 25$ mV.

Effect of varying the preconcentration potential (E_{acc}) from 0.0 to −0.8 V on the peak current intensity for 2×10^{-7} M cefoperazone in an acetate buffer of pH 4.2 following preconcentration for 30 s was also studied. A much developed peak current intensity was achieved over the potential range of −0.2 to −0.4 V. Hence, a preconcentration potential of −0.3 V was chosen throughout the present analytical study.

The square-wave adsorptive cathodic stripping voltammograms for 1×10^{-8}, 5×10^{-8} and 1×10^{-7} M cefoperazone, were recorded following different preconcentration durations. Linear relationships of peak current (i_p) versus preconcentration duration t_{acc} (60–150 s) were obtained. This means that the optimal preconcentration duration should be chosen according to the concentration level of cefoperazone in the investigated solution.

3.2.2. Method validation

Calibration curves within various concentrations of bulk cefoperazone were attempted following different preconcentration durations (60–150 s). The linear range (1.5×10^{-9} to 7×10^{-8} M) and the estimated limit of detection (4.5×10^{-10} M) and limit of quantitation (1.5×10^{-9} M) indicated the high sensitivity of the proposed square-wave adsorptive cathodic voltammetric procedure for assay of bulk cefoperazone.

Table 1
Repeatability, precision, robustness and intermediate precision of the results for 1×10^{-8} M cefoperazone, using the proposed square-wave adsorptive stripping voltammetric procedure

Variables	Procedural conditions	%R ± S.D. ($n = 3$)
Repeatability and precision[a]		
Day 1	pH 4.2, $E_{acc} = -0.3$ V, $t_{acc} = 150$ s	101.0 ± 1.15
Day 2		102.0 ± 0.50
Day 3		102.5 ± 0.37
Robustness[a]		
pH of the medium		
4.0	$E_{acc} = -0.3$ V, $t_{acc} = 150$ s	98.8 ± 1.15
4.2		101.0 ± 1.15
4.4		100.8 ± 0.46
Accumulation potential (E_{acc})		
−0.25	pH 4.2, $t_{acc} = 150$ s	99.8 ± 0.37
−0.30		101.0 ± 1.15
−0.35		97.2 ± 0.7
Intermediate precision		
Potentiostat 263A-PAR	pH 4.2, $E_{acc} = -0.3$ V, $t_{acc} = 150$ s	101.0 ± 1.15
Potentiostat 273A-PAR		98.5 ± 2.17

[a] Potentiostat 263A-PAR.

Repeatability of the results was examined by performing five replicate measurements for 1×10^{-8} M bulk cefoperazone using the proposed square-wave adsorptive cathodic stripping voltammetric procedure for 1 day (intra-day precision) using the same standard solution of cefoperazone and for 5 days (inter-day precision) over a 1 week using different standard solutions of cefoperazone. The recoveries obtained (Table 1) confirmed both the high precision of the proposed procedure and stability of cefoperazone solutions.

The robustness [20] of the proposed procedure was examined by studying the effect of small variation of pH (4 – 4.4) and preconcentration potential E_{acc} (−0.25 to −0.35 V) on recovery of the drug. Recovery results (Table 1) were not significantly affected and consequently the described procedure was reliable for the assay of cefoperazone and it could be considered robust. The intermediate precision [20] was examined by applying the proposed procedure to assay of cefoperazone under the optimal procedural conditions using two potentiostats, 263A-PAR (Lab 1) and 273A-PAR (Lab 2). The results obtained due to Lab 1 to Lab 2 were found reproducible (Table 1).

3.2.3. Applications

3.2.3.1. Analysis of Cefazone® and Cefobid® vials. The described procedure was successfully applied for the assay of cefoperazone in the pharmaceutical formulations Cefazone® and Cefobid® vials (500 mg cefoperazone/vial). Recoveries of cefoperazone in both formulations, based on the average of five replicate measurements are reported in Table 2. The results were statistically compared with those obtained by a reported spectrophotometric method [3]. Values of F-calculated, F-theoretical, t-calculated and t-theoretical are also included in

Table 2
Assay of cefoperazone in pharmaceutical formulations (vials) by the proposed square-wave adsorptive cathodic stripping voltammetric procedure and a reported spectrophotometric method [3]

Formulation	Claimed value (mg/vial)	Proposed procedure (% R ± S.D.)		Reported method [3] (% R ± S.D.) ($n = 5$)
		Calibration curve	Standard addition	
Cefazone®	500	102.1 ± 1.14, $F = 1.13$ (6.39), $t = 1.12$ (2.30)	102.7 ± 0.82	101.3 ± 1.21
Cefobid®	500	100.4 ± 0.39, $F = 2.14$ (6.39), $t = 2.27$ (2.30)	100.9 ± 0.23	99.7 ± 0.57

Table 2. Since the calculated F-value did not exceed the theoretical one, there was no significant difference between the proposed and reported methods with respect to reproducibility [21]. Also, no significant difference was noticed between the two methods regarding accuracy and precision as revealed by t-value [21]. The accuracy of the proposed procedure was also judged by applying the standard addition method [22].

3.2.3.2. Analysis of human serum. From the data illustrated in Fig. 2 linear calibration graph over the concentration range of 2×10^{-9} to 5×10^{-8} M cefoperazone spiked in human serum was obtained. Limits of detection (LOD) and quantitation (LOQ) of 6×10^{-10} M (0.393 ng ml^{-1}) and 2×10^{-9} M (1.250 ng ml^{-1}) were achieved, respectively.

Accordingly the proposed square-wave adsorptive cathodic stripping voltammetric procedure was successfully applied to estimate the pharmacokinetic parameters of cefoperazone in human plasma following an i.m. injection of a single vial of Cefazone®—500 mg to each of two healthy male volunteers. A venous blood sample was taken immediately before drug injection to serve as a blank and sampling was continued for a period of half-hour up to 12 h following medication. Fig. 3 shows the plasma concentration–time profiles of two volunteers obtained by the proposed procedure at specified intervals. These profiles were re-plotted semi-logarithmically

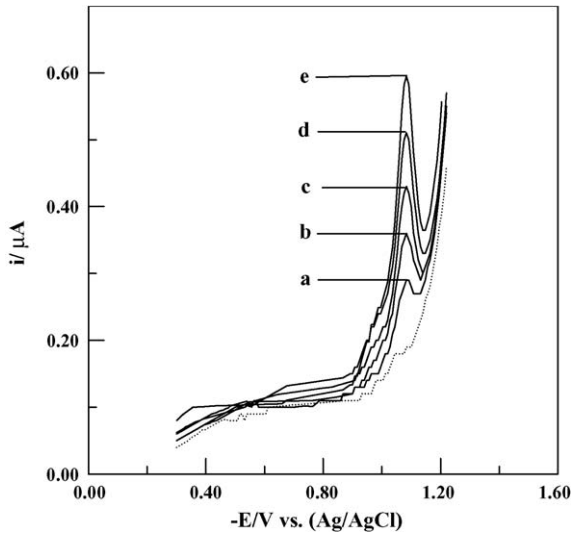

Fig. 2. SWCAdS voltammograms for various concentrations of cefoperazone in human serum samples recorded following preconcentration at −0.3 V for 150 s: (a) 1×10^{-8}; (b) 2×10^{-8}; (c) 3×10^{-8}; (d) 4×10^{-8}; (e) 5×10^{-8}. The dotted line represents the background; $f = 60$ Hz, $\Delta s = 8$ mV and $E_{sw} = 25$ mV.

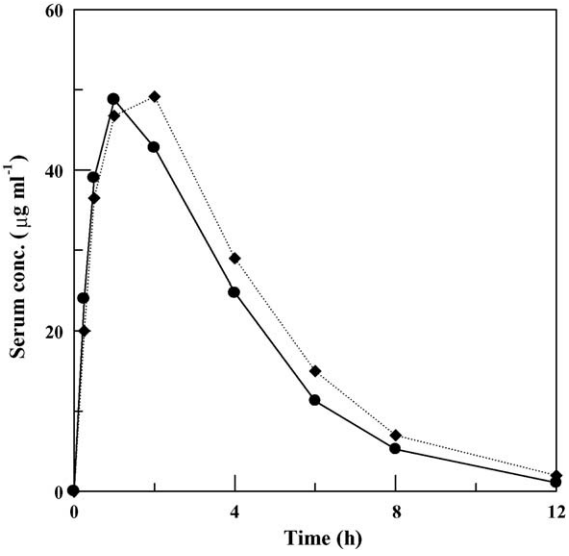

Fig. 3. Plasma concentration–time profiles for two volunteers: 1 (—) and 2 (···) following an i.m. injection of a single vial of Cefazone® (500 mg).

and the pharmacokinetic parameters were estimated (peak plasma concentration $C_{max} = 46.8$–48.7 µg ml^{-1}, peak plasma time $t_{max} = 1$–1.9 h, area under the plasma concentration–time curve AUC = 214–246 µg h ml^{-1}, elimination rate constant $k_c = 0.31$–0.40 1/h and elimination half-life time $t_{1/2} = 1.8$–2.1 h) and found to agree with the reported values [1].

4. Conclusion

A fully validated highly sensitive, rapid, selective and precise square-wave adsorptive cathodic stripping voltammetric procedure was described for quantification of cefoperazone in bulk form, pharmaceutical formulations and human serum. Pharmacokinetic parameters of the drug in human plasma were also estimated using the described procedure. The procedure showed clear advantages such as a short period of real time of drug analysis and no samples pretreatment and/or time-consuming extraction steps were required prior to analysis of the drug. The procedure could be applied in clinical laboratories.

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