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Anodic voltammetric behavior and determination of cefixime in pharmaceutical dosage forms and biological fluids

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Abstract

The voltammetric behavior of cefixime was studied using cyclic, linear sweep, differential pulse and square wave voltammetric techniques. The oxidation of cefixime was irreversible and exhibited diffusion controlled process depending on pH. The oxidation mechanism was proposed and discussed. Different parameters were tested to optimize the conditions for the determination of cefixime. The dependence of current intensities and potentials on pH, concentration, scan rate, nature of the buffer was investigated. According to the linear relationship between the peak current and the concentration, differential pulse (DPV) and square wave (SWV) voltammetric methods for cefixime assay in pharmaceutical dosage forms and biological fluids were developed. For the determination of cefixime were proposed in acetate buffer at pH 4.5, which allows quantitation over the 6×10^{-6}–2×10^{-4} M range in supporting electrolyte and spiked serum sample; 8×10^{-6}–2×10^{-4} M range in urine sample; 6×10^{-6}–1×10^{-4} M range in breast milk samples for both techniques. The repeatability, reproducibility, precision and accuracy of the methods in all media were investigated. No electroactive interferences from the excipients and endogenous substances were found in the pharmaceutical dosage forms and in the biological samples, respectively.

Keywords: Cefixime; Voltammetry; Pharmaceuticals; Serum samples; Urine samples; Breast milk samples; Oxidation

1. Introduction

Cefixime (CEF) is a semi synthetic and generally classified as a third-generation cephalosporin antibiotic for oral administration. It is used for the treatment of susceptible infections, including gonorrhea, otitis media, pharyngitis, lower respiratory-tract infections such as bronchitis and urinary-tract infections. CEF is available as the trihydrate [1–3] (Scheme 1).

Forty to 50% of the oral dose of CEF is absorbed from the gastrointestinal tract. CEF is better absorbed from oral suspension than from tablet dosage forms. The plasma half-life is usually about 3–4 h and may be prolonged when there is renal impairment. About 65% of CEF in the circulation is bound to plasma proteins [1]. Primarily the kidney excretes CEF. About 20% of an oral dose (or 50% of an absorbed dose) is excreted unchanged in the urine within 24 h. CEF is crosses the placenta. There is no evidence of metabolism of CEF in vivo [1].

CEF has been studied and determined by relatively few procedures such as spectrophotometric [4–7], fluorimetric [5,8], high performance liquid chromatographic [9] and high performance thin layer chromatographic [10] methods. The widespread use of this compound and the need for clinical and pharmacological study require fast and sensitive analytical techniques to assay the presence of the drug in pharmaceutical dosage forms and biological samples. Already published methods show that there are no methods for the determination of CEF in biological fluids except spiked urine sample application [11].

Electroanalytical methods have proved to be useful for development of very sensitive and selective methods for the determination of organic molecules, including drugs and

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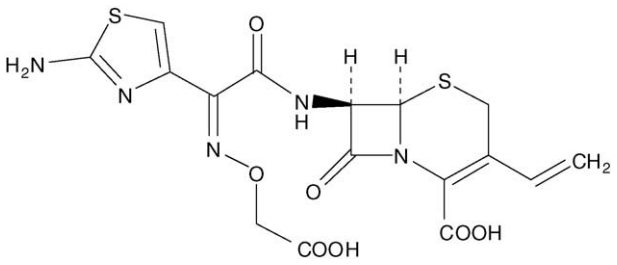

Scheme 1. The chemical structure of Cefixime.

related molecules such as cephalosporins [12–14] in dosage forms and biological fluids [15–18]. Additional application of electrochemistry includes the determination of electrode mechanisms. Redox properties of organic molecules can give insights into their metabolic fate or their in vivo redox processes or pharmacological activity [19].

Surveying the literature revealed that no electrochemical methods for determination of CEF was reported except one cathodic study related to Reddy et al. [11]. This literature is related to the electroreduction of CEF and its determination on HMDE in pharmaceuticals and human urine. However, nothing has been published concerning electrochemical oxidation mechanism and anodic voltammetric determination of CEF at solid electrode neither from pharmaceuticals nor in biological samples.

The goal of this study was to development of new, fully validated, rapid and selective voltammetric methods for the simple and direct determination of cefixime in raw materials, drug dosage forms, spiked human serum, urine and breast milk samples without any time-consuming extraction or separation steps prior to drug assay. Our aim of this study was also to establish the experimental conditions, to investigate the voltammetric behavior and oxidation mechanism of CEF using cyclic, linear sweep, differential pulse and square wave voltammetric techniques. The proposed methods might be alternatives to the HPLC techniques in therapeutic drug monitoring or the experimental data might be used for the development HPLC-EC method.

2. Experimental

2.1. Apparatus

All voltammetric measurement at a glassy carbon electrode was performed using a BAS 100 W (Bioanalytical System, USA) electrochemical analyzer. A glassy carbon working electrode (BAS; Φ: 3 mm diameter), an Ag/AgCl reference electrode (BAS; 3 M KCl) and platinum wire counter electrode and a standard one-compartment three-electrode cell of 10 mL capacity were used in all experiments. The glassy carbon electrode was polished manually with aqueous slurry of alumina powder (Φ: 0.01 μm) on a damp smooth polishing cloth (BAS velvet polishing pad), before each measurement. All measurements were realized at room temperature.

The pH was measured using a pH meter Model 538 (WTW, Austria) using a combined electrode (glass electrode-reference electrode) with an accuracy of ±0.05 pH.

DPV conditions were: pulse amplitude, 50 mV; pulse width, 50 ms; scan rate, 20 mVs^{-1} and SWV conditions were: pulse amplitude, 25 mV; frequency, 15 Hz; potential step, 4 mV.

For the comparison, the UV-spectrophotometric method was realized using Shimadzu 160A double beam spectrophotometer with a slit width 2 nm. The absorbance values were measured at 288 nm using 1 cm quartz cells.

2.2. Reagents

Cefixime and its two different type of dosage forms (Suprax® tablets and oral suspensions) were kindly provided by Eczacıbaşı Pharm. Comp. (Istanbul, Turkey). Model compound Cefepime and 2-Aminothiazole were kindly supplied by Bristol Myers Squibb (Istanbul-Turkey) and Merck, respectively. All chemicals for preparation of buffers and supporting electrolytes such as H_2SO_4, H_3PO_4, NaH_2PO_4, Na_2HPO_4, H_3BO_3, CH_3COOH, NaOH were reagent grade (Merck or Sigma).

Stock solutions of CEF (1×10^{-3} M) were prepared in methanol and kept in the dark in refrigerator. Four different type of supporting electrolytes, were used in this study. Working solutions under voltammetric investigation were prepared by dilution of the stock solution and contained 20% methanol. 0.1 M H_2SO_4, 0.5 M H_2SO_4, 0.2 M Phosphate buffer at pH 2.0–9.0, 0.04 M Britton-Robinson buffer at pH 2.32–12.0 and 0.2 M Acetate buffer at pH 3.5–5.5 were used for supporting electrolyte. Standard solutions were prepared by dilution of the stock solution with selected supporting electrolyte to give solutions containing CEF in the concentration range of 6×10^{-6} to 2×10^{-4} M. The calibration equation for DPV and SWV was constructed by plotting the peak current against CEF concentration.

Standard solutions were prepared by dilution of the stock solution with methanol to give solutions containing CEF in the concentration range of 1×10^{-5}–5×10^{-5} M for UV-spectrophotometric method. The calibration plot was constructed by plotting the absorbance against the compound concentration. All validation parameters were also calculated for the comparison study.

2.3. Validation of the methods

The ruggedness and precision were checked in the same day ($n = 5$) and three different days ($n = 5$) over a week period. Precision and ruggedness were expressed as relative standard deviation (R.S.D.%).

The precision and accuracy of analytical methods are described in a quantitative fashion by the use of relative errors (Bias%). One example of relative error is the

accuracy, which describes the deviation from the expected results.

All solutions were kept in the dark and were used within 24 h to avoid decomposition. However, voltammograms of the sample solutions recorded a week after preparation did not show any appreciable change in assay values.

2.4. Pharmaceutical dosage forms assay procedure

Ten tablets of Suprax® (each tablet contains 400 mg CEF) were accurately weighed and finely powdered by pestle in a mortar. An adequate amount of this powder, corresponding to a stock solution of concentration 1×10^{-3} M, was weighed, transferred into a 50 mL-calibrated flask and completed to the volume with methanol. The content of the flask were sonicated for 10 min to effect complete dissolution. The sample from the clear supernatant liquor was withdrawn and quantitatively diluted with the selected supporting electrolyte and solution contained constant amount methanol as 20%. This solution was then transferred to a voltammetric cell and DP and SW voltammograms were recorded. The drug content in per tablet was determined referring to the related regression equations. Inactive ingredients contained in the tablets are: dibasic calcium phosphate, hydroxypropyl methylcellulose 2910, light mineral oil, magnesium stearate, microcrystalline cellulose, pregelatinized starch, sodium lauryl sulfate and titanium dioxide.

In the case of oral suspension, first of all the suspension was prepared and well shaked after addition necessary amount of water. The required volume (accurately pipetted) of the suspension sample, equivalent to a stock solution of concentration ca 1×10^{-3} M, was transferred to a 50 mL-calibrated flask and complete to the volume with methanol. The contents were ultrasonicated for 10 min to effect complete dissolution. Aliquots from the upper clear layer were taken and diluted with the selected supporting electrolyte and contained 20% methanol. Oral suspension samples were then transferred to a voltammetric cell and voltammograms were recorded. The drug content in per 5 mL oral suspension was determined referring to the related regression equations.

Inactive ingredients contained in the oral suspensions are: strawberry flavor, sodium benzoate, sucrose and xanthan gum.

2.5. Recovery studies

Because other components of the matrix of tablet or oral suspension dosage forms may interfere with the analysis or accurate quantitation of the analyte, potential effects from matrix components must be investigated. If the proposed method is used to measure an analyte in a complex sample matrix (e.g., a pharmaceutical formulation), a standard addition recovery method can be used. Recovery experiments are performed in the presence of the matrix [20,21]. To study the accuracy, reproducibility and to check the interference from the excipients used in the formulations of these techniques, recovery experiments were carried out using the standard addition method. In order to know whether the excipients show any interference with the analysis, known amounts of the pure CEF were added to the pre-analyzed tablet and oral suspension dosage forms. The mixtures were analyzed by the both proposed techniques. The recovery results obtained after five repeated experiments for both techniques.

2.6. Analysis of biological samples

The serum, urine or breast milk samples was fortified with CEF dissolved in methanol to achieve final concentration of 1×10^{-3} M and treated with 1 mL acetonitrile as endogenous substance precipitating agent, and then volume was completed to 2.5 mL with the same biological samples. These samples were vortexed for 10 min and then centrifuged for 5 min at $5000 \times g$ for getting rid of residues. The supernatant was taken carefully and used as described below subsections.

The amount of CEF in spiked serum, urine and breast milk samples for the recovery studies was calculated from the related regression equations. Necessary validation parameters were also calculated for spiked serum, urine and breast milk samples.

2.6.1. Analysis of spiked serum samples

Serum samples of healthy individuals (after having obtained their written consent) were stored frozen until assay. After gentle, thawing, an aliquot volume of serum sample was spiked with CEF. After above procedure, the supernatant was taken carefully. The concentration of CEF was varied in the range of 6×10^{-6}–2×10^{-4} M in human serum samples. These solutions were analyzed in the voltammetric cell containing acetate buffer at pH 4.5 and constant amount of methanol (20%).

2.6.2. Analysis of spiked urine samples

The urine samples were taken from healthy individuals immediately before the experiments. Aliquot volumes of urine sample were placed into the calibrated flask. It was fortified with CEF. After above procedure, the supernatant was taken carefully. The concentration of CEF was varied in the range of 8×10^{-6}–2×10^{-4} M in human urine samples. These solutions were analyzed in the voltammetric cell containing acetate buffer at pH 4.5 and constant amount of methanol (20%).

2.6.3. Analysis of spiked breast milk samples

The drug free breast milk samples were taken from one healthy female subject immediately before the experiments. An aliquot volume of breast milk sample was spiked with CEF. After above procedure, the supernatant was taken carefully. The concentration of CEF was varied in the range of 6×10^{-6}–1×10^{-4} M in breast milk samples. These serial dilutions were analyzed in the voltammetric cell containing acetate buffer at pH 4.5 and constant amount of methanol (20%).

2.6.4. Validation parameters of the biological fluids

The ruggedness and precision were checked in the same day ($n = 5$) and three different days ($n = 5$) over a week period. Precision and ruggedness were expressed as relative standard deviation (R.S.D.%).

The precision and accuracy of analytical methods are described in a quantitative fashion by the use of relative errors (Bias%). One example of relative error is the accuracy, which describes the deviation from the expected results.

All solutions were used within a short time period to avoid decomposition.

3. Results and discussion

CEF appears to be an electroactive drug for both directions. Specifically, the drug is capable to be both, oxidable and reducible. The cathodic behavior of CEF has been studied using HMDE electrode by Reddy et al. [11]. However, no previous electrooxidation data were available concerning the solid electrode behavior of CEF. To demonstrate the usefulness of a solid electrode for determination of CEF, which may offer advantages for the use of such electrodes as sensors, the electrochemical behavior of CEF on a glassy carbon electrode was investigated in this research. As a first step CEF was subjected to a voltammetric study in the differential pulse and square wave modes, and to a cyclic and linear sweep voltammetric studies with the aim of characterizing its electrochemical oxidation behavior. Therefore, several measurements with different electrochemical techniques were performed using various supporting electrolytes and buffers in order to obtain such information.

Cyclic and linear sweep voltammograms of CEF exhibited one distinct and well defined anodic peak and one ill-defined anodic wave at different potential values until pH 7.0. (Fig. 1a–c). After pH 7, the sharp anodic peak becomes broader (Fig. 1d). Cyclic voltammetric measurements showed an irreversible nature of the oxidation process. The

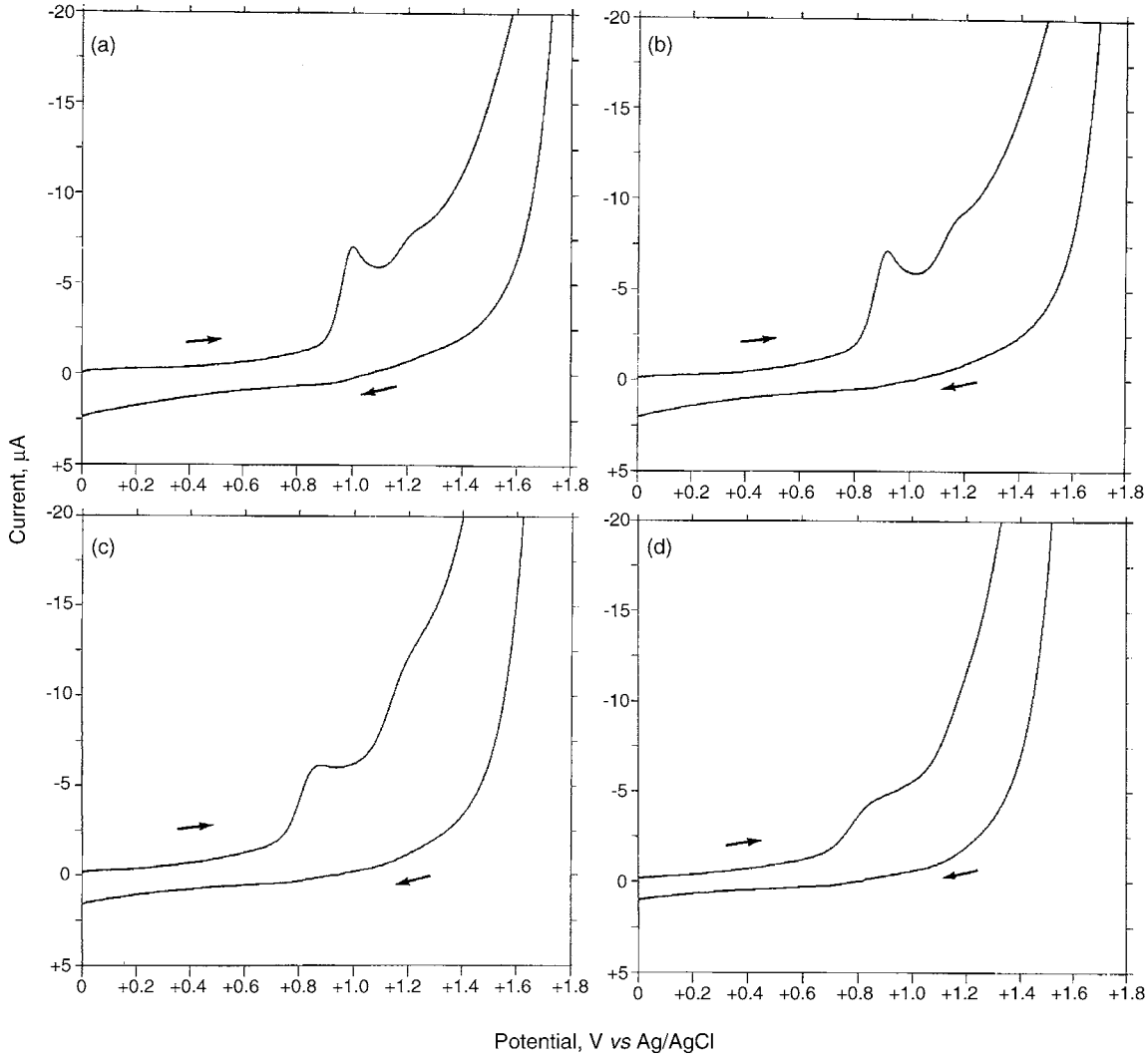

Fig. 1. Cyclic voltammograms of 2×10^{-4} M CEF in Britton-Robinson buffer (methanol 20%) at pH 2.32 (a) at pH 4.02 (b); at pH 6.00 (c); at pH 8.00 (d). Scan rate 100 mVs^{-1}.

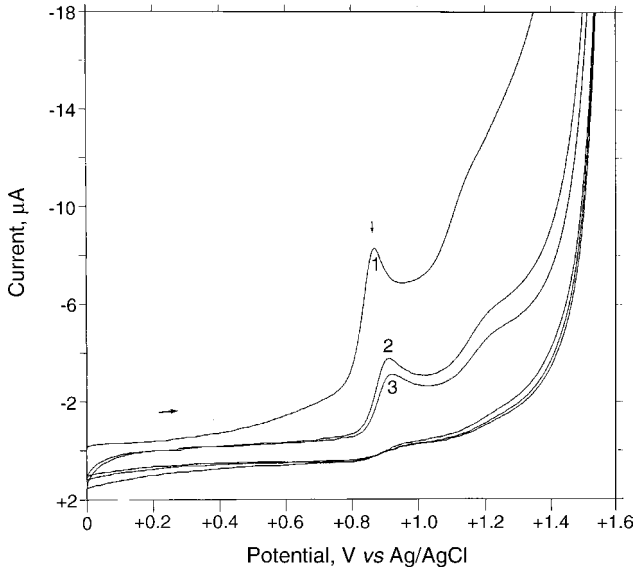

Fig. 2. Multisweep cyclic voltammograms of 2×10^{-4} M CEF solutions in acetate buffer (methanol 20%) at pH 4.50. Scan rate, 100 mVs^{-1}. The numbers indicate the number of scans.

scanning was started at −0.25 or 0.0 V in the positive direction at pH 4.5 acetate buffer, the anodic oxidation of CEF did not occur until about +0.85 V. By reversing at +1.80 V no reduction signal corresponding to the anodic response was observed on the cathodic branch. The CEF wave decreased to the second or higher cycles. (Fig. 2). This phenomenon may be partly attributed to the consumption of adsorbed CEF on the electrode surface.

Due to the poorly resolved signal obtained by cyclic voltammetry with a decrease in pH above 8.0, the effect of pH on peak intensity and peak potential were studied also using DPV and SWV techniques. The peak potential of the anodic process moved to less positive potential values and oxidation peak ill defined by raising the pH (Fig. 1). Both obtained graphs from DPV and SWV were found similar. For this reason, only DPV graph for the main oxidation step was given as Fig. 3. According to the Fig. 3a, the plot of the first peak potential versus pH showed one straight line between 1.5 and 5.7, which can be expressed by the following equations in all studied buffer systems:

$$E_p(\text{mV}) = 1150.7 - 59.3\,\text{pH} \quad r: 0.975\,(n = 12)$$

for cyclic voltammetry

$$E_p(\text{mV}) = 1085.7 - 55.90\,\text{pH} \quad r: 0.989\,(n = 12)\,\text{for DPV}$$

$$E_p(\text{mV}) = 1108.6 - 54.6\,\text{pH} \quad r: 0.990\,(n = 12)\,\text{for SWV}.$$

Then the peak potential looks like pH independent above pH 5.7. The linearity was observed in the pH range 1.5–5.7, giving a negative slope of approximately 55 mV per pH unit for all using techniques. The intersection observed in the plot between pH 1.5 and 5.7 can be explained by changes in protonation of the acid-base functions in the molecule. The intersection point of the curve is close to the pK_a value of 2-aminothiazole molecule present in CEF molecule which is pK_a 5.5 [22]. As it can be seen in Fig. 3a, the peak potential nearly becomes pH independent after pH 5.7. The study of the influence of pH on peak currents (Fig. 3b) was also carried out to determine the pH value for the maximum signal. The height of the peak reaches a maximum and the shape of the curves is better in acetate buffer at pH 4.5 than other buffers. This supporting electrolyte was chosen with respect to sharp response and better peak shape for the calibration equation for pharmaceutical dosage forms and all biological samples. 0.2 M concentration of the buffer was selected to obtain an adequate buffering capacity.

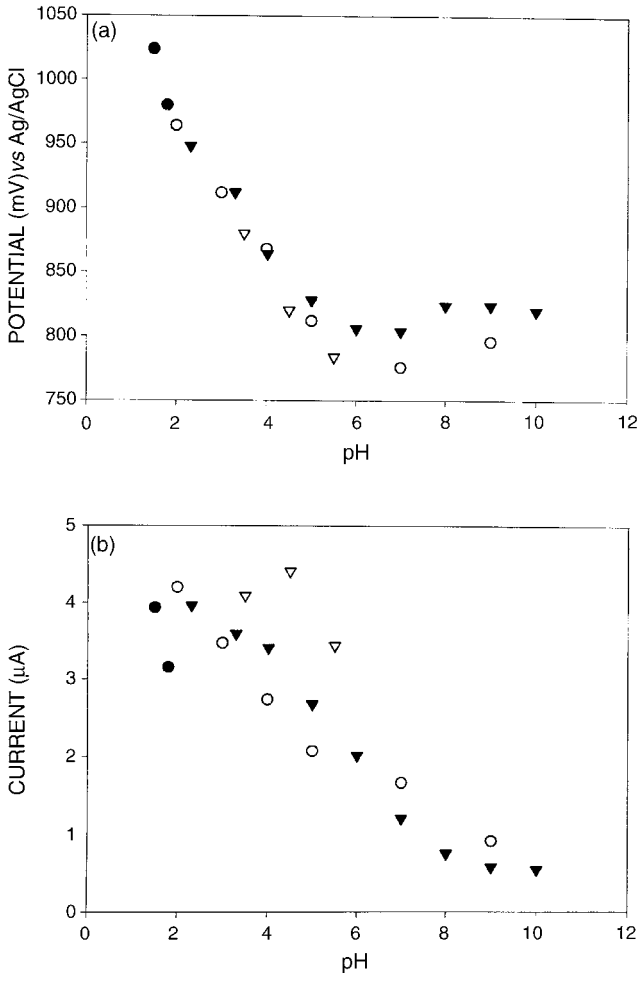

Fig. 3. Effects of pH on CEF anodic peak potential (a) and peak current (b); CEF concentration 2×10^{-4} M. 0.1 and 0.5 M H_2SO_4 (●); 0.04 M Britton-Robinson (▼); 0.2 M Acetate (▽) and 0.2 M Phosphate (◯) buffers.

The effects of potential scan rates (v) between 5 and 1000 mVs^{-1} on the peak potential and peak current of CEF was also evaluated. Scan rate studies were then carried out to assess whether the processes on glassy carbon electrode were under diffusion or adsorption control. A linear relationship on the oxidation peak current with the square root of the scan rate showed the diffusion control process. The equation

is noted below in acetate buffer at pH 4.5:

$$I_p(\mu A) = 0.36v^{1/2}(mVs^{-1}) + 0.27 \quad r = 0.990\,(n = 10)$$

The effect of scan rate on peak current was also examined under the above conditions with a plot of logarithm of peak current (log i) versus logarithm of scan rate (log v), giving a straight line within the same scan rate range. This linear relationship was obtained as follow:

$$\log i_p(\mu A) = 0.51 \log\, v\,(mVs^{-1}) - 0.44$$

$$r = 0.991\,(n = 10)$$

The slope (0.51) of the relationship is close to the theoretically expected (0.5) for an ideal reaction of solution species [23], so in this case the process had a diffusive component. A 61 mV positive shift in the peak potential confirmed the irreversibility of the oxidation process.

The Tafel plots (log i versus E) was obtained with a scan rate of $5\,mVs^{-1}$ beginning from a steady-state potential in pH 4.5 acetate buffer and from the slope of the linear part α_n was found to be 0.42. The exchange current density (i_o) is $3.16 \times 10^{-6}\,A\,cm^{-2}$ for this system. These values together with the absence of cathodic response and a 61 mV positive shift of the peak potential in cyclic voltammetry (Figs. 1 and 2) indicated the irreversibility of the anodic reaction.

To identify the grouping responsible for the oxidation process, the oxidation of CEF was compared to that of cephalexin, cefotaxime, cefadroxil and cefepime to exclude the possibility of the oxidation of the cephalosporin structure. According to the obtained results, cephalexin was oxidized at glassy carbon electrode at more higher potential than CEF. Furthermore, the pH dependence of the peak currents and potentials of the anodic process of cephalexin follows a completely different pattern from that observed for CEF. The oxidation of cephalexin probably involves the primary amino group in the side chain at C-7 [12] and this compound mechanism discussion is out of scope in this study. Considering the obtained results from CEF, and bearing in mind the electrochemical behavior of 2-aminothiazole, the side chain of cephalosporin structure of CEF at glassy carbon electrode, we may assume that the oxidation process is located on the 2-aminothiazole moiety in the molecule. It is thus possible to exclude the oxidation of the cephalosporin structure as responsible for the anodic waves of CEF. For cefadroxil, it seems most probable that the oxidation may occur because of the phenolate anion on the side chain of the cephalosporine structure [24]. Cefepime and cefotaxime results were also confirmed that the oxidation involves the 2-aminothiazole ring in the side chain at C-7 [12].

The anodic behavior of CEF was also compared to the 2-aminothiazole oxidation, which was performed by cyclic voltammetry at the glassy carbon electrode, as a function of pH in order to identify the oxidation process of CEF. Our results revealed a good agreement with the oxidation mechanism postulated for similar compounds in the literature [12,25] and our obtained results from 2-aminothiazole molecule and similar cephalosporine compounds. According to the experimental results, 2-amino group showed similar oxidative behavior when compared the oxidation of CEF. The oxidation of 2-aminothiazole occurs as a single and sharp peak, which resembles the anodic peak of CEF. The oxidation potentials of CEF when compared with similar compounds and 2-aminothiazole occur about 0.10 V less positive. As the oxidation of the thiazole ring occurs only at very high positive potentials [26]. The oxidation of 2-aminothiazole and CEF were observed very less positive potentials than thiazole potentials [26]. This must involve a conversion of the 2-amino group on the thiazole ring. Moreover, the oxidation of CEF also shows the oxidation wave at potentials more positive than main peak potentials, which is obtained also for the oxidation of 2-aminothiazole. It is thus possible to conclude that the two electron oxidation of CEF corresponds to an oxidation of the 2-aminothiazole moieties in the side chain on C-7. This molecule is extensively metabolized in vivo [1,3], mainly through oxidative processes. As a result of we may assume that the oxidation occurred first on the 2-aminothiazole moiety of the molecule, which is electroactive in both acidic and basic media.

3.1. Analytical applications and methods validation

Differential pulse (DPV) and square wave (SWV) voltammetry are effective and rapid electroanalytical techniques with well-established advantages, including good discrimination against background currents and low detection limits [19,27]. The advantages of SWV are greater speed of analysis, lower consumption of electroactive species in relation to the other electroanalytical techniques and reduced problems with blocking of the electrode surface. SWV showed similar results with other techniques. In this study DPV were used as an alternative technique. Various electrolytes, such as sulphuric acid, Britton-Robinson, phosphate and acetate buffer were examined as a supporting electrolyte. Based on the above study, the best condition for analytical applications proved to be an acetate buffer of pH 4.5. This supporting electrolyte with a constant amount of methanol (as 20%) was chosen for the subsequent experiments.

The quantitative evaluation is based on the dependence of the peak current on CEF concentration. Peak currents increased linearly with increasing amounts of CEF by DPV and SWV techniques. The effects of methanol on peak current potential were also studied. As expected, the peak current decreased owing to a lowering of the diffusion coefficient with changing ionic strength and viscosity of the medium. For example, peak current in solutions containing 40% (v/v) methanol decreased by ca 20% of that obtained in aqueous solution. Besides, a very slight decrement in peak potential was observed. For analytical purposes, best response (with regard to peak current sensitivity, morphology, reproducibility and solubility) was obtained by working with 20% constant amount of methanol in selected supporting electrolyte.

Two calibration equations from the standard solution of CEF according to the procedures described above were constructed by using DPV and SWV techniques. Using the selected conditions, a linear relation in the concentration range between 6×10^{-6} and 2×10^{-4} M was found, indicating that the response was diffusion controlled in this range. Above this concentration a loss of linearity was probably due to the adsorption of CEF on the electrode surface. The characteristics and related validation parameters of the calibration equations are summarized in Table 1. The limits of detection (LOD) and limits of quantification (LOQ) were also shown in Table 1. Several approaches are given in the ICH guideline to determine the LOD and LOQ values. In this study, LOD and LOQ were based, on the standard deviation of response and the slope of the corresponding curve using following equations [20,21];

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

The low values of SE of slope and intercept and greater than 0.999 correlation coefficient nearly in all media established the precision of the proposed method.

The stability of the reference substance and sample solutions was checked by analyzing prepared standard solution of CEF in supporting electrolyte aged at +4 °C in the dark against sample freshly prepared. The results demonstrated that the working reference solutions were stable at least for up to 2 weeks. The CEF response for the assay reference solutions over 2 weeks did not considerable change.

The developed methods were validated according to the standard procedures [20,21] and the results obtained are tabulated also in Table 1. Accuracy, precision and reproducibility of the proposed method were assessed by performing replicate analysis of the standard solutions in supporting electrolyte and biological media within calibration curves, the selected concentrations were prepared in all media and assayed with related calibration curves to determine within day (repeatability) and between day (reproducibility) variability. The within day and between day precision, accuracy and reproducibility were determined as the R.S.D.% and the results were shown in Table 1. Precision, accuracy and reproducibility results shown in Table 1 demonstrate good precision, accuracy and reproducibility.

3.2. Determination of CEF in pharmaceutical dosage forms

When working on standard solutions and according to the obtained validation parameters, results encourage the use of the proposed method described for the assay of CEF in pharmaceutical dosage forms and spiked biological samples. The CEF was commercially provided by Eczacıbaşı Pharm. Ind. Products in presentation named Suprax® tablets and oral suspensions containing 400 mg and 100 mg/5 mL of CEF, respectively. On the basis of above results, both DPV and SWV techniques were applied to the direct determination of CEF in pharmaceutical dosage forms, using the related calibra-

Table 1
Regression data of the calibration lines for quantitative determination of CEF by DPV and SWV in supporting electrolyte, serum, urine, breast milk and spectrophotometric method

	DPV				SWV				Spectrophotometric method
	Supporting electrolyte	Serum	Urine	Breast milk	Supporting electrolyte	Serum	Urine	Breast milk	
Measured potential (V)/absorbance (nm)	+0.85	+0.89	+0.85	+0.84	+0.90	+0.88	+0.87	+0.87	288
Linearity range (M)	6×10^{-6}–2×10^{-4}	6×10^{-6}–2×10^{-4}	8×10^{-6}–2×10^{-4}	6×10^{-6}–1×10^{-4}	6×10^{-6}–2×10^{-4}	6×10^{-6}–2×10^{-4}	8×10^{-6}–2×10^{-4}	6×10^{-6}–1×10^{-4}	1×10^{-5}–5×10^{-5}
Slope (μAM^{-1})	1.33×10^{4}	1.42×10^{4}	2.61×10^{4}	1.85×10^{4}	1.36×10^{4}	1.84×10^{4}	2.64×10^{4}	2.77×10^{4}	1.93×10^{4}
Intercept (μA)	2.52×10^{-2}	8.79×10^{-3}	7.48×10^{-2}	-6.37×10^{-2}	-3.61×10^{-2}	-2.16×10^{-4}	7.05×10^{-2}	−0.173	-2×10^{-3}
Correlation coefficient	0.999	0.999	0.999	0.998	0.999	0.999	0.998	0.999	0.999
SE of slope	1.30×10^{2}	1.299×10^{2}	1.18×10^{2}	3.09×10^{2}	1.59×10^{2}	2.67×10^{2}	4.41×10^{2}	4.39×10^{2}	2.54×10^{2}
SE of intercept	1.08×10^{-2}	1.08×10^{-2}	1.04×10^{-2}	1.63×10^{-2}	1.32×10^{-2}	2.22×10^{-2}	3.88×10^{-2}	2.31×10^{-2}	7.82×10^{-3}
LOD (M)	6.42×10^{-7}	4.28×10^{-7}	5.31×10^{-8}	1.77×10^{-7}	2.72×10^{-7}	5.41×10^{-7}	2.45×10^{-7}	1.86×10^{-7}	2.70×10^{-6}
LOQ (M)	2.14×10^{-6}	1.43×10^{-6}	1.77×10^{-7}	5.92×10^{-7}	9.05×10^{-7}	1.80×10^{-6}	8.17×10^{-7}	6.19×10^{-7}	9.02×10^{-6}
Repeatability of peak current/absorbance (R.S.D.%)	0.65	0.27	1.20	0.47	0.83	0.63	1.88	0.92	0.86
Repeatability of peak potential/wavelength (R.S.D.%)	0.25	0.20	0.21	0.42	0.76	0.25	0.35	0.26	0.32
Reproducibility of peak current/absorbance (R.S.D.%)	1.95	1.06	2.25	1.10	1.31	1.02	2.32	1.25	1.06
Reproducibility of peak potential/wavelength (R.S.D.%)	0.75	0.21	0.22	0.51	0.95	0.41	0.21	0.25	0.47

Table 2
Comparative and recovery studies for CEF studies from pharmaceutical dosage forms

	Tablet (mg)			Oral Suspension (mg/5 mL)		
	DPV	SWV	Spectrophotometric method	DPV	SWV	Spectrophotometric method
Labeled claim	400.0	400.0	400.0	100.0	100.0	100.0
Amount found[a]	397.65	397.77	395.55	100.05	99.70	100.27
R.S.D.%	0.53	0.64	0.33	0.83	0.59	0.68
Bias%	0.588	0.558	1.113	−0.05	0.3	−0.27
T_{value}	t_{calc}: 0.047	t_{calc}: 0.060	$t_{theoretical}$: 2.31	t_{calc}: 0.33	t_{calc}: 0.099	$t_{theoretical}$: 2.31
F_{value}	F_{calc}: 0.37	F_{calc}: 0.22	$F_{theoretical}$: 2.60	F_{calc}: 0.71	F_{calc}: 0.78	$F_{theoretical}$: 2.60
Added (mg)	20.00	20.00	20.00	10.00	10.00	10.00
Found (mg)[a]	19.98	19.97	20.08	9.99	10.01	10.01
Recovery %	99.87	99.84	100.38	99.97	100.08	100.1
R.S.D.% of recovery	0.78	0.56	0.52	0.28	0.56	0.28
Bias%	0.10	0.15	−0.40	0.03	−0.1	−0.1

[a] Each value is the mean of five experiments.

tion straight lines without any sample extraction, evaporation or filtration and after adequate dilutions. The results show that the proposed techniques were successfully applied for the assay of CEF in its dosage forms (Table 2). The accuracy of the methods was determined by its recovery during spiked experiments. Recovery studies were carried out after addition of known amounts of the pure drug to various pre-analyzed formulations of CEF. Recovery experiments using the developed assay procedure further indicated the absence of interference from commonly encountered pharmaceutical excipients used in the selected formulations (Table 2).

CEF pharmaceutical dosage forms were also determined with the UV-spectrophotometric method, which is proposed for the comparison with the DPV and SWV techniques. Also the proposed UV-spectrophotometric method was fully validated. All validation parameters were found for the UV-spectrophotometric method (Table 1). The results obtained for the pharmaceutical dosage forms are also listed in Table 2. Recovery experiments were also realized for the UV-spectrophotometric method (Table 2).

Table 2 compares the results of the analysis of CEF between the electroanalytical and UV-spectrophotometric methods. All methods showed similar accuracy and precision. According to the student's t- and F test, the calculated t and F values did not exceed the theoretical value for a significance level of 0.05 statistical analysis of the results showed no significant difference between the performance of the compare UV-spectrophotometric and proposed methods as regards to simplicity. On the other hand, the voltammetric assays are very rapid, used without any filtration steps and have better accuracy, precision, linearity range and determination limits than UV-spectrophotometric assay.

3.3. Determination of CEF in spiked biological samples

In order to check the applicability of the proposed techniques to the human serum, urine and breast milk samples, the calibration equation were obtained in spiked biological samples. Acetonitrile and methanol were tried as a biological sample precipitating agents. The best response was received with acetonitrile. Also different amount of acetonitrile were examined. The best results were obtained using 1 mL acetonitrile. The measurements of CEF in biological samples were performed as described in Section 2. Calibration equation parameters and related validation data were shown in Table 1. The results for the analysis of spiked serum, urine and breast milk are abridged in Table 3. Analysis of drugs from serum or urine or especially breast milk samples usually requires extensive time-consuming sample preparation, use of expensive organic solvents and other chemicals. In this study, the proteins and endogenous substances in serum, urine and breast milk samples are precipitated by the addition of acetonitrile, which is centrifuged at $5000 \times g$, and the supernatant was taken and diluted with the supporting electrolyte and directly analyzed.

Table 3
Application of the DPV and SWV methods to the determination of Cefixime in spiked human serum, urine and breast milk samples

Technique	Medium	Cefixime added (M)	n	Cefixime found (M)	Average recovery (%)	R.S.D.%	Bias%
DPV	Serum	6×10^{-5}	5	5.97×10^{-5}	99.42	1.87	0.5
DPV	Urine	6×10^{-5}	5	5.87×10^{-5}	97.76	2.31	2.16
DPV	Breast milk	6×10^{-5}	5	5.83×10^{-5}	97.13	3.08	2.83
SWV	Serum	6×10^{-5}	5	5.91×10^{-5}	98.52	2.83	1.50
SWV	Urine	6×10^{-5}	5	6.17×10^{-5}	102.86	2.22	−2.83
SWV	Breast milk	6×10^{-5}	5	6.07×10^{-5}	101.25	2.05	−1.17

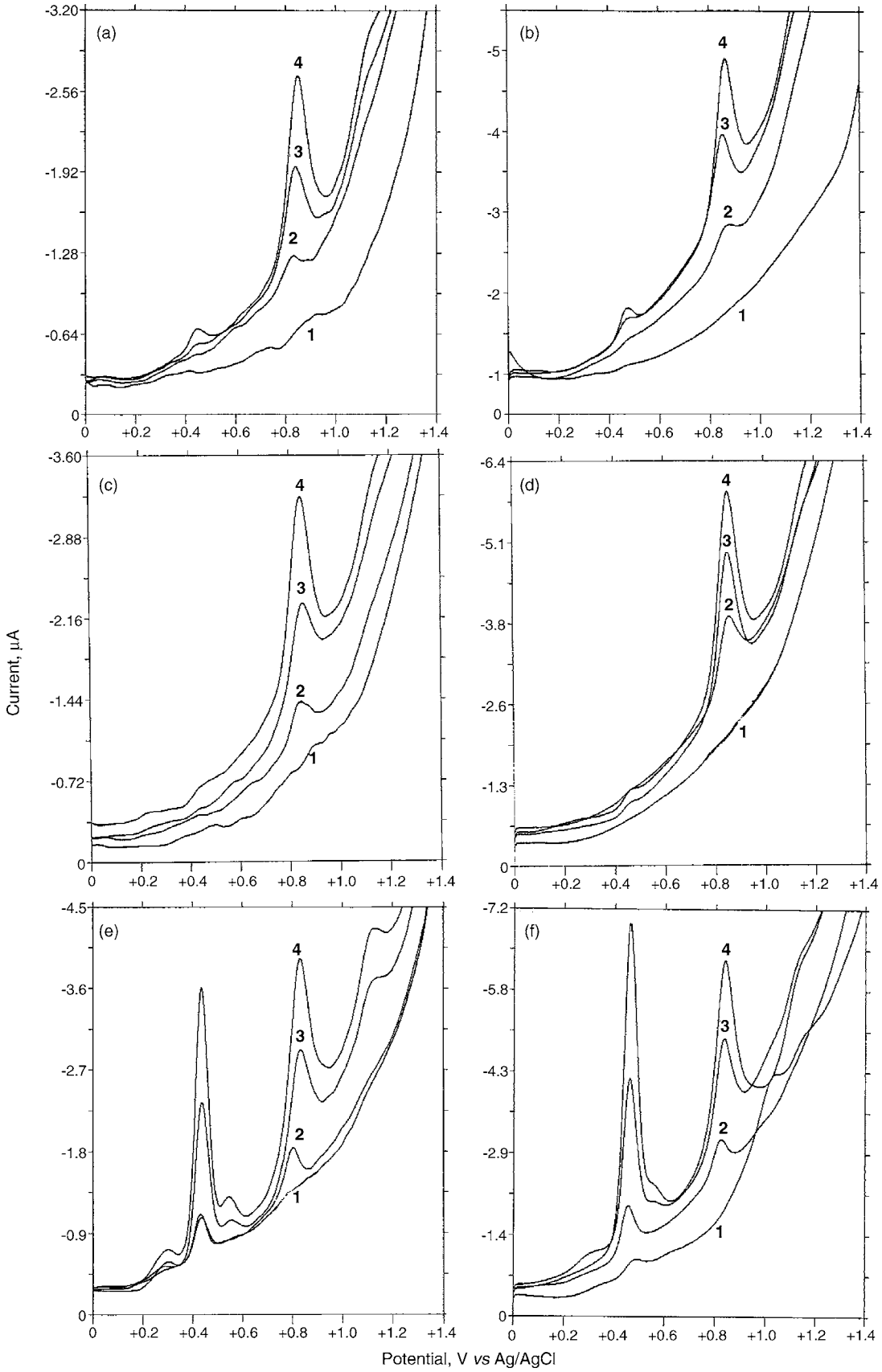

Fig. 4. Differential pulse (a, c, e) and square wave (b, d, f) voltammograms obtained for the determination in spiked serum (a, b); breast milk (c, d) and urine (e, f) samples (1) blank; (2) 1×10^{-5} M; (3) 4×10^{-5} M; (4) 6×10^{-5} M CEF extract in pH 4.50 acetate buffer.

Typical DPV and SWV curves of CEF examined in serum samples are shown Fig. 4a and b; in breast milk samples are shown in Fig. 4c and d; in urine samples are shown in Fig. 4e and f. Using both voltammetric techniques, no sample preparation was required, other than precipitation and dilution steps. The recovery results of CEF (Table 3) in biological samples were calculated from the related linear regression equations, which are given in Table 1.

As it can be seen in Fig. 4, no oxidation compounds and no extra noise peaks present in biological material peak occurred in the potential range where the analytical peak appeared. Stability of biological samples kept in refrigerator (+4 °C) was tested by making consecutive analyses of the sample over a period of approximately 4h. There were no significant changes in the peak currents and potentials between the first and last measurements.

4. Conclusions

The electrochemical behavior of CEF on glassy carbon electrode was established and studied for the first time. CEF is irreversibly oxidized at high positive potentials. The electroreduction method which is found in literature [11] related to the of CEF determination on HMDE in pharmaceuticals and human urine. This literature method includes extraction and evaporation step for the determination of CEF from the urine samples. The proposed DPV and SWV techniques for the determination of CEF in pharmaceutical dosage forms and biological samples was found to be more simple, selective, rapid, cheaper, fully validated and as sensitive as the reported method [11]. The analytical results obtained from pharmaceutical dosage forms by DPV and SWV are in good agreement with those obtained by the UV-spectrophotometric method. Although both, voltammetric and UV spectrophotometric methods showed similar simplicity, the principal advantage of the proposed voltammetric methods over the spectrophotometric one is that the absence of influence of matrix effects, higher selectivity and sensitivity because of the possibility of higher sample dilution. The proposed voltammetric methods can be applied directly to the analysis of pharmaceutical dosage forms and biological samples without the need for separation or complex sample preparation, since there was no interference from the excipients and endogenous substances. These methods are very rapid, requiring less than 5 min to run sample. Consequently, the above-presented techniques are a good analytical alternative for determining CEF in pharmaceutical dosage forms and spiked serum, urine and breast milk samples. The proposed methods might be alternatives to the HPLC techniques in therapeutic drug monitoring or the experimental data might be used for the development HPLC-EC method.

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