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Electro-oxidation and Determination of Deferiprone on a Glassy Carbon Electrode

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The electrochemical oxidation and determination of the anti-thalassemia and anti-HIV replication drug, deferiprone, was investigated at a glassy carbon electrode. In a phosphate buffer solution, pH = 7.40, four anodic peaks for deferiprone appeared. Cyclic voltammetric study indicated that the oxidation process is irreversible and diffusion-controlled. The diffusion and the electron transfer coefficients of deferiprone were found to be 3.21×10^{-6} cm^2 s^{-1} and 0.48, respectively, for the first step of the oxidation process. A sensitive, simple and time-saving differential pulse voltammetric procedure was developed for determination of this drug. Using our proposed method, deferiprone can be determined with a detection limit of 5.42×10^{-7} mol L^{-1}. The applicability of the method to direct assays of spiked human serum and urine is described.

Elektrochemiczne utlenianie deferipronu, leku przeciw talasemii oraz hamującego replikację HIV, badano na elektrodzie z węgla szklistego. Stwierdzono, że w buforze fosforanowym o pH 7,40, występują cztery piki leku. Badanie metodą cyklicznej woltamperometrii wykazało, że proces utleniania jest nieodwracalny i kontrolowany dyfuzyjnie. Współczynniki dyfuzji i przeniesienia elektronu wynoszą odpowiednio $3{,}21 \times 10^{-6}$ cm^2 s^{-1} i 0,48. Do oznaczani deferipronu opracowano metodę z zastosowaniem pulsowej woltamperometrii. Opracowana metoda jest czuła, szybka i prosta. Granica wykrywalności wynosi $5{,}42 \times 10^{-7}$ mol L^{-1}. Opisano zastosowanie opracowanej procedury do oznaczania leku w surowicy krwi i moczu.

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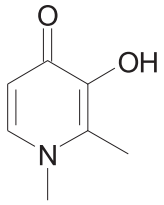
β-thalassemia syndromes are a heterogeneous group of Mendelian disorders characterized by a lack or decreased synthesis of either the α- or β-globin chain of hemoglobin. This deficiency causes ineffective erythropoiesis as well as lysis of mature red cells in the spleen [1]. Patients with these syndromes require regular blood transfusions to maintain hemoglobin level of around 1.2 g mL^{-1}, which leads to transfusional iron overload.

Heart failure due to iron overload can develop either as a result of excess dietary absorption (hereditary hemochromatosis) or from repeated blood transfusions. The most striking model of cardiac iron overload is seen in thalassemia major, in which heart failure remains the major cause of death (60%), greatly exceeding deaths from infection (13%) and liver disease (6%) [2].

Deferiprone (1,2-dimethyl-3-hydroxypyrid-4-one, Scheme 1) is the first oral iron chelator to be used clinically, mainly in thalassemia patients [3, 4]. Deferiprone belongs to the family of the α-ketohydroxypyridines, a relatively new class of chelating agents, some of which are naturally occurring. These chelators have a high affinity for binding iron, and are able to remove it from proteins that are transporting and storing it in the body, largely sparing other biologically important metals. They are stable in conditions that exist in the human digestive system and are readily absorbed. Deferiprone can remove excess iron from various parts of the body of iron-loaded patients, including the liver and particularly, the heart [3]. This drug is also used worldwide to treat cancer, leukemia, hemodialysis and other patients. It is worth noting that the drug deferiprone may be used in the detoxification of other metals, such as aluminum in hemodialysis patients, plutonium used in the nuclear industry and uranium used by the military [5–7].



Scheme 1

Iron is also involved in replication of the human immunodeficiency virus type 1 (HIV-1) [8]. Iron chelators such as deferiprone inhibit the replication of HIV-1 through several routes. Deferiprone can inhibit nuclear factor-κB activation and the subsequent replication of human immunodeficiency virus type 1 [9]. Deferiprone can also render iron-dependent ribonucleotide reductase inactive, thereby inhibiting DNA synthesis and therefore HIV replication [10].

Drug analysis has an extensive impact on public health. Polarography and voltammetry can be considered as convenient alternatives to routinely employed analytical methods, in that they present the great advantage of permitting a direct, simple and rapid determination requiring a minimum volume of sample. Electrochemical techniques have also been used for the determination of a wide range of drug compounds. They have the advantage of not requiring, in most instances, derivatization, and they are less sensitive to matrix effects than other analytical techniques. Additionally, electrochemical techniques include the determination of the drug's electrode mechanism. Redox properties of drugs can provide insight into their metabolic fate, their *in vivo* redox processes and their pharmacological activity [11–17].

Voltammetric oxidation and electrochemical determination of deferiprone on a glassy carbon (GC) surface have not, to the best of our knowledge, been reported in the literature. In the present work, the electrochemical behavior of deferiprone at a GC electrode was studied, with the aim of developing an electroanalytical procedure for quantification of deferiprone in both bulk form and human biological fluids.

EXPERIMENTAL

All chemicals used in this work were of analytical reagent grade from Merck. Deferiprone was obtained as a gift from Arasto Pharmaceutical Chemicals Inc., Tehran, Iran.

The standard solution of authentic deferiprone was prepared by dissolving an accurate mass of the bulk drug in an appropriate volume of 100 mmol L^{-1} phosphate buffer solution, pH 7.40 (PBS), which was also used as supporting electrolyte, and then stored in the dark at 4°C. Additional dilute solutions were prepared daily by accurate dilution just before use. Deferiprone solutions were stable and their concentrations did not change with time.

Drug-free serum samples were obtained from healthy male volunteers and stored frozen until the assay. The serum samples were diluted (1:7) with the supporting electrolyte and filtered through a 30 kDalton filter to produce protein-free human serum. Various portions of stock deferiprone solution were transferred into 10 mL volumetric flasks containing 3.3 mL of the serum sample. These solutions were then diluted to the mark with the supporting electrolyte for preparation of spiked samples (final dilution of 1:20 with the supporting electrolyte). The protein-free spiked serum solutions were directly analyzed by the calibration method, according to our proposed procedure.

Urine samples taken from a healthy person were diluted (1:10) with PBS after adding an appropriate amount of deferiprone standard solution. The resulting solution was directly analyzed, according to our proposed procedure, without any pretreatment or extraction steps.

Electrochemical measurements were carried out in a conventional three-electrode cell (from Metrohm) powered by an electrochemical system comprising the AUTOLAB system with PGSTAT30 (Eco Chemie, Utrecht, The Netherlands). The system was run on a PC using the GPES 4.9 software. An Ag/AgCl-Sat'd KCl (from Metrohm) and a platinum disk (from Azar Electrode Co., Iran) were used as reference and counter electrodes, respectively. The working electrode was a glassy carbon electrode (from Azar Electrode Co., Iran), exposing a geometric surface area of 0.0314 cm^2. For differential pulse voltammetry (DPV) measurements, a pulse width of 25 mV, a pulse time of 50 ms, and a scan rate of 10 mV s^{-1} were employed. Before

each measurement, the GC electrode was polished with 0.05 μm alumina suspension on a polishing microcloth, followed by sonication for 3 min in an ultrasonic bath. The electrode was then transferred to the supporting electrolyte. Potential in the range of –1000 to 1700 mV in a regime of cyclic voltammetry was applied until a stable voltammogram was achieved. The solution pH was adjusted using a Metrohm 744 pH meter. All studies were carried out at room temperature.

RESULTS AND DISCUSSION

Figure 1 shows a typical cyclic voltammogram of PBS containing 5.0 mmol L^{-1} deferiprone. For comparison, the cyclic voltammogram of the blank electrolyte is also shown. Deferiprone exhibits four oxidation peaks, located at 524, 703, 1230 and 1480 mV (indicated as a'1, a1, a'2 and a2, respectively), at a potential sweep rate of 50 mV s^{-1}. In the reverse sweep, however, no peaks appeared, indicating an irreversible heterogeneous electron transfer process for the oxidation of deferiprone at the GC surface. From cyclic voltammograms recorded at different potential sweep rates using the GC electrode (vide infra), it can be inspected that both peak currents a'1 and a'2 depend linearly on the potential sweep rate (Fig. 1, insets A'1 and A'2). Therefore, both peaks a'1 and a'2 corresponded to surface-confined processes and can be related to the adsorption of reaction products produced in peaks a1 and a2, *i.e.* peaks a'1 and a'2 are two pre-peaks for peaks a1 and a2. It is well known that the adsorption of reaction product on the electrode surface represents a pre-peak [18].

Controlled-potential coulometry was performed in PBS containing 2.09 mmol L^{-1} deferiprone, at 703 and 1480 mV. The electrolysis progress was monitored by cyclic voltammetry. The extrapolated charge consumption for total electrolysis of the solution after corrections for background/charging currents was derived, with the number of exchanged electrons for peaks a1 and a2 obtained as 2.1 and 2.0, respectively. Hence, deferiprone is oxidized via two two-electron steps on the GC electrode surface.

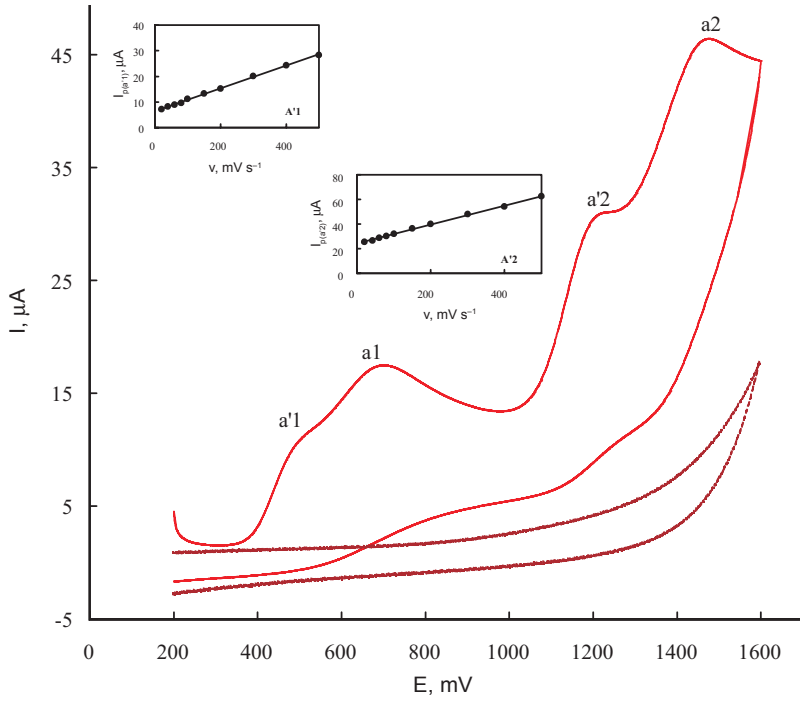


Figure 1. Cyclic voltammograms of PBS containing 5.0 mmol L^{-1} deferiprone. Potential sweep rate was 50 mV s^{-1}. The base voltammograms in the absence of deferiprone are also depicted. Insets A'1 and A'2 – dependency of peaks a'1 and a'2 currents on the corresponding potential sweep rate. Data were collected from cyclic voltammograms recorded at different potential sweep rates using the GC electrode

Figure 2 represents a steady-state current-potential curve recorded for the first step of oxidation of deferiprone (peak a'1 in Fig. 1). As the Figure indicates, a typical S-shape plot was obtained. The slope of the E *vs* log I plot was found to be 123.59 mV dec^{-1} for deferiprone (Fig. 2, inset). From the kinetic theory of electrode reaction, this Tafel slope indicates that a unit reaction involving the first electron transfer is a rate-limiting step. Also, the electron transfer coefficient (a) was determined to be 0.48.

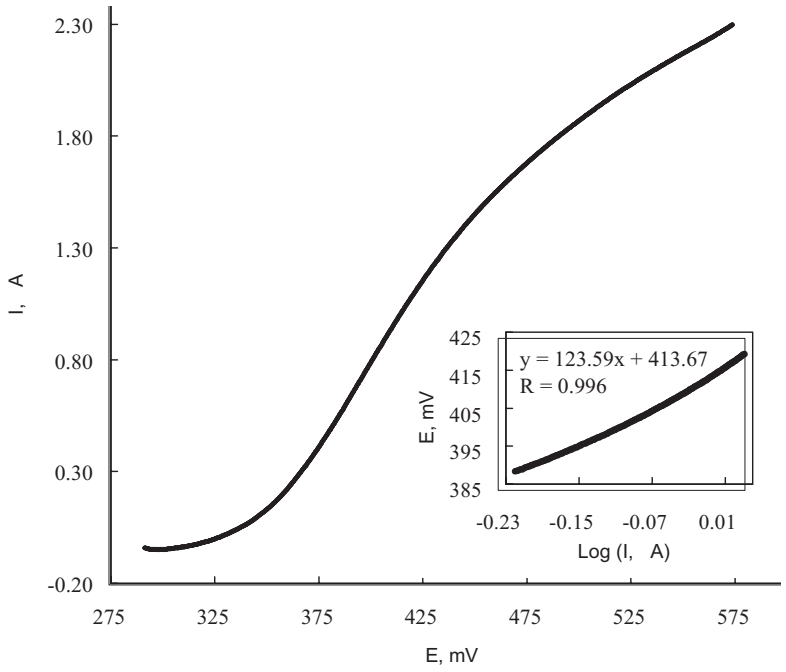


Figure 2. Main panel – potentiodynamic polarization curve of deferiprone in PBS, recorded using a potential sweep rate of 0.5 mV s^{-1}. Inset – the corresponding Tafel plot

The effect of potential sweep rate was studied in the range of 2 to 900 mV s^{-1}. As the potential sweep rate increased, both peak currents increased and the peak potentials shifted to more positive values (Fig. 3), confirming the irreversible nature of the reaction processes. Also, the a1 peak current depends linearly on the corresponding square root of potential sweep rate (Fig. 3, inset). This relationship indicates that mass transport phenomena have occurred in the oxidation process *via* diffusion. From the slope of the linear dependency of a1 peak current on the square root of potential sweep rate, and using the Randles-Sevcik equation for totally irreversible electron transfer processes [19], we can calculate the diffusion coefficient of deferiprone as follows:

$$i_p = (2.99 \times 10^5)\, n\, \alpha^{0.5}\, A\, C^*\, D^{0.5}\, \nu^{0.5} \qquad (1)$$

where α is the electron transfer coefficient, n is the number of exchanged electrons, A is the surface area of the working electrode, C* and D are the bulk concentration and diffusion coefficients of the electroreactant species, respectively, and ν is the potential sweep rate. The diffusion coefficient of deferiprone was found to be 3.21×10^{-6} cm^2 s^{-1}.

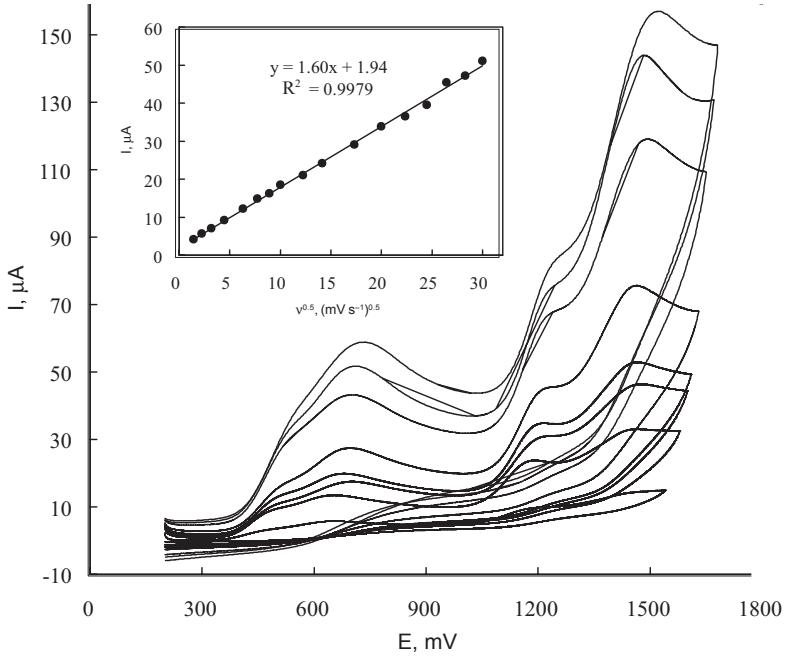
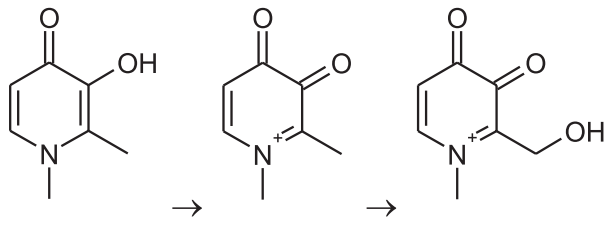


Figure 3. Main panel – cyclic voltammograms of 2.2 mmol L^{-1} deferiprone in PBS recorded at different potential sweep rates. Potential sweep rates from inner to outer are: 5, 40, 80, 100, 200, 500, 700 and 900 mV s^{-1}. Inset – the dependency of anodic peak current on the square root of potential sweep rate in a wide range of 2–900 mV s^{-1}

On the basis of our results (and assuming one can be proposed), we have depicted a mechanism for the oxidation of deferiprone in Scheme 2. In the first step, deferiprone is oxidized to corresponding dione. Then the dione undergoes a probable anodic hydroxylation of the methyl side chain [20].



Scheme 2

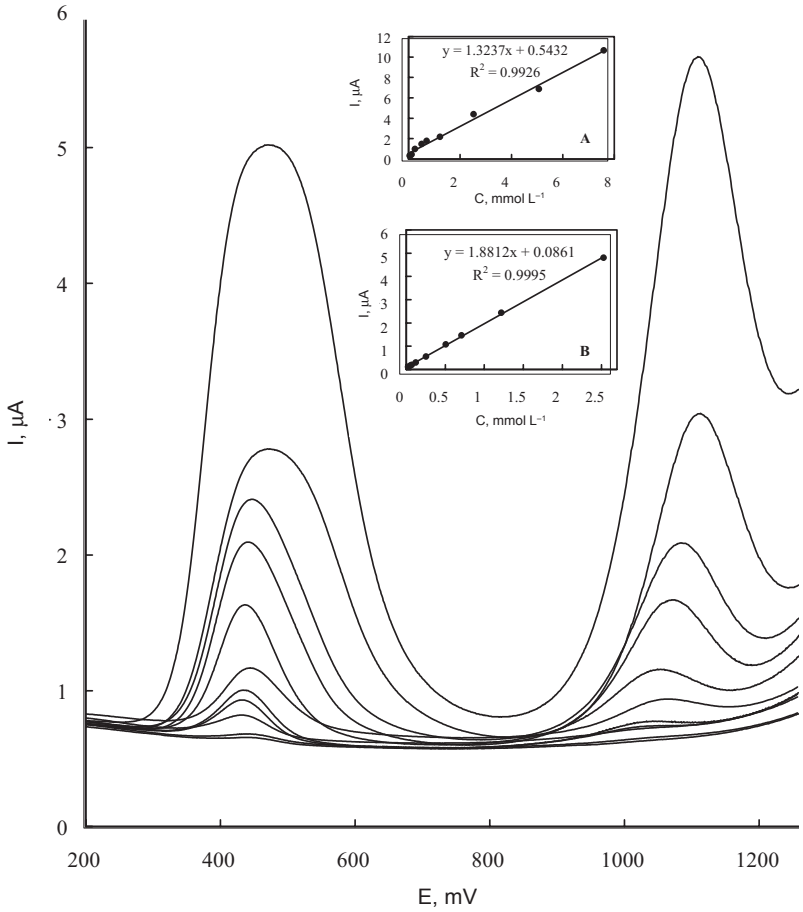


Figure 4. Main panel – differential pulse voltammograms obtained for determination of deferiprone in PBS; deferiprone concentrations from inner to outer are: 0.025, 0.051, 0.071, 0.122, 0.253, 0.507, 0.709, 1.22, 2.53, 5.07 and 7.59 mmol L^{-1}. Insets – A: The related calibration graph for peak a1; B: The related calibration graph for peak a2

The calibration curve for deferiprone in PBS was obtained by differential pulse voltammetry. Figure 4 shows typical DPV curves for different concentrations of deferiprone in buffer solution. The dependency between peak current and drug concentration was rectilinear for peak a1 within the range of 0.051 to 7.60 mmol L^{-1} (Fig. 4, inset A), and for peak a2 within the range of 0.025 to 2.53 mmol L^{-1} (Fig. 4, inset B). The limits of detection (LOD) and quantitation (LOQ) of the procedure were calculated according to the 3 S.D./m and 10 S.D./m criteria, respectively, where S.D. is the standard deviation of the intercept and m is the slope of the calibration curves [21]. The limits of detection and quantitation were found to be 5.42×10^{-7} mol L^{-1} and 1.77×10^{-6} mol L^{-1} for peak a1, and 8.73×10^{-7} mol L^{-1} and 2.76×10^{-6} mol L^{-1} for

peak a2, respectively. Precision and accuracy were assessed by performing replicate analyses of deferiprone samples. The precision of the method was calculated as the relative standard deviation (RSD). The procedure was repeated on the same day on the same spiked solutions at concentrations in the range of the standard series. The intra-assay RSDs of the proposed method, determined on the basis of peak current for 10 replications, were 1.38% and 0.82% for peaks a1 and a2, respectively, and showed good reproducibility.

The accuracy of the proposed method was determined by spiking serum and urine samples with different concentrations of deferiprone. Good recoveries were obtained from both samples (Tab. 1).

Table 1. Results obtained for deferiprone analysis from spiked human serum and urine samples

Medium	Serum		Urine
	Peak a1	Peak a2	Peak a1
Linearity range, mol L^{-1}	3.53×10^{-6}–2.12×10^{-3}	1.76×10^{-6}–2.12×10^{-3}	5.00×10^{-5}–2.00×10^{-3}
Slope, μA mol L^{-1}	2.90×10^{3}	1.79×10^{3}	1.61×10^{3}
Correlation coefficient, r	0.997	0.999	0.997
RSD, %[a]	1.61	0.29	3.29
LOD, mol L^{-1}	1.02×10^{-7}	1.14×10^{-7}	2.34×10^{-6}
LOQ, mol L^{-1}	3.26×10^{-7}	3.34×10^{-7}	7.80×10^{-6}
Recovery, %[b]	98.98	99.90	97.63

[a] Each value is obtained from ten experiments.

[b] Recovery value is the mean of ten experiments.

The applicability of the proposed method for the determination of deferiprone in biological fluids was examined by measuring the peak a1 and a2 currents as a function of the bulk concentration of the drug in urine and serum samples. The urine and serum samples were diluted 10 and 20 times with PBS prior to taking the measurements, to prevent the matrix effect of real samples. The generally poor selectivity of voltammetric techniques can pose problems in the analysis of biological samples, which contain oxidizable substances. However, no current due to oxidation of the compounds in either the serum or the urine samples appeared. The results obtained from our voltammetric technique for determining deferiprone in serum and urine samples are listed in Table 1. The recovery of deferiprone was determined by comparing the peak currents of a known drug concentration in both media with their

equivalents in calibration curves; these results are also summarized in Table 1. Good recoveries of deferiprone were achieved from these matrices, meaning that application of our proposed voltammetric method to the analysis of deferiprone in biological fluids could be easily assessed.

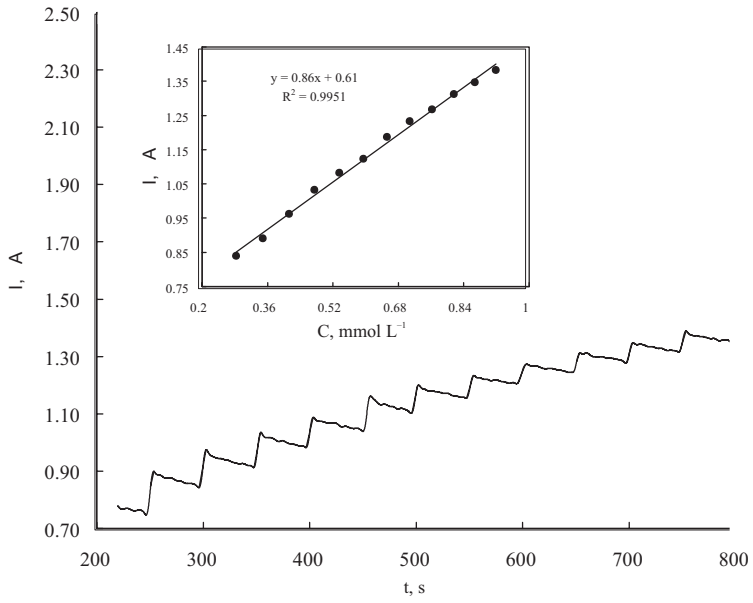


Figure 5. Amperograms obtained for a GC electrode during the successive addition of 0.3 mL solution of 5.0 mmol L^{-1} deferiprone into a 20 mL stirred solution of PBS at an applied potential of 750 mV. Inset – variations of measured net current after addition of each increment of deferiprone as a function of its concentration in solution

In order to more sensitive determination of deferiprone, the amperometric response of GC electrode on successive step changes of applied potential of 750 mV was determined (Fig. 5). It is clear that the steady-state currents rise with the addition of deferiprone, and quickly reach a stable value (in less than 5 s).

CONCLUSION

The electrochemical behaviour of deferiprone was studied in phosphate buffer solution, pH 7.40, on a glassy carbon electrode surface using cyclic voltammetry. The kinetic parameters such as the electron transfer coefficient for oxidation and the diffusion coefficient of deferiprone, were determined using our proposed method. A differential pulse voltammetry procedure was optimized and successfully applied

for quantification of deferiprone in bulk form and human biological fluids. The simplicity, sensitivity, selectivity and short time of analysis are the main advantages of these procedures, making them useful for routine analysis.

Acknowledgment

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