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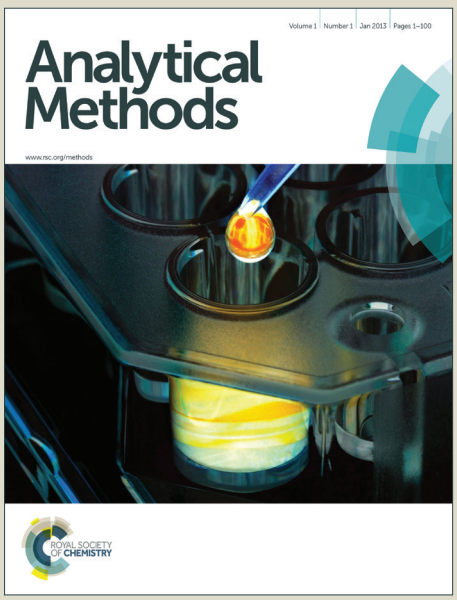

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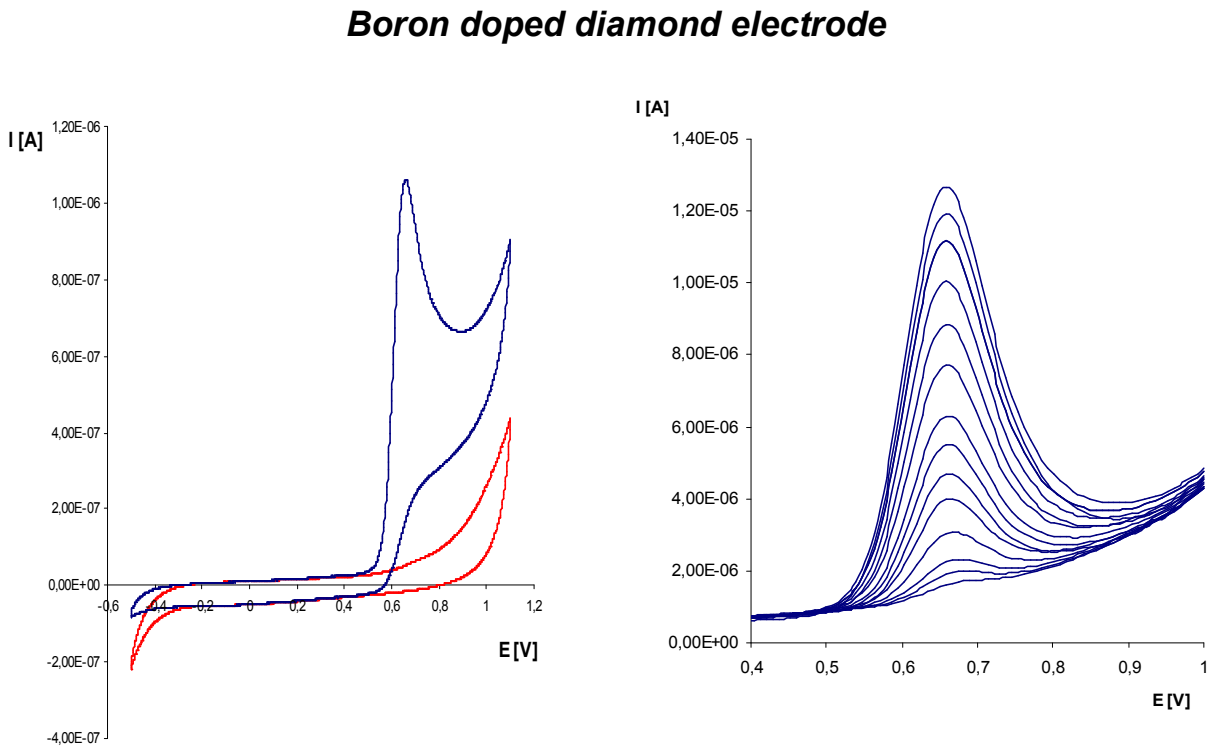
The following paper presents attractive methods for the determination of two antifungal agents, ketoconazole and ciclopirox olamine. The recommended procedures are based on oxidation of the said compounds on a new electrode material, boron-doped diamond electrode. The properties of the BDD electrode and the usage of the sensitive SWV technique facilitated the development of simple and sensitive and accuracy procedures intended to determine the studied compounds in various pharmaceutical cosmetics forms.



Voltammetric Determination of Antifungal Agents in Pharmaceuticals and Cosmetics Using a Boron-Doped Diamond Electrodes

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Abstract

Ketoconazole and ciclopirox olamine remain two most popular antifungal agents. Determinations of these substances were carried out by means of square-wave voltammetry method (SWV) and a boron-doped diamond electrode (BDD). The electrochemical oxidation of ketoconazole and ciclopirox olamine demonstrated irreversible peaks of oxidation for both the substances studied at 0.59 V for the first and at 0.66 V for the latter substance. Examining the influence of scan rate has allowed to demonstrate that the currents registered remain diffusion-controlled process. The following paper has established the optimal oxidation conditions for both compounds studied. pH value together with the nature of the supporting electrolyte and the SWV characteristic parameters were analysed. Under optimized conditions for ketoconazole and ciclopirox olamine oxidation, the influence of concentration over the value of the current registered was examined. A linear dependence was achieved in the range between $2.87 \cdot 10^{-7}$ mol/L and $3.13 \cdot 10^{-6}$ mol/L for ketoconazole and from $2.53 \cdot 10^{-5}$ mol/L to $4.19 \cdot 10^{-4}$ mol/L for ciclopirox olamine. The calibration curves achieved demonstrated high correlation outcomes. Obtained limit of detection reached $8.29 \cdot 10^{-8}$ mol/L for ketoconazole and $6.66 \cdot 10^{-6}$ mol/L for ciclopirox olamine. The recovery, precision and accuracy of the recommended methods were examined as well. Measurement precision has not exceeded

0.95% for ketoconazole and 2.87% for ciclopirox olamine. The aforementioned methods were applied successfully with regards to the ketoconazole and ciclopirox olamine analyses in several pharmaceutical dosage forms, e.g. tablets, creams and anti-dandruff shampoos. The elaborated methods remain accurate, which is acknowledged by the recovery values obtained ranging from 97.67% to 99.99%. The proposed procedures were compared with pharmacopoeial reference methods. Statistical t-student and F-Snedecor tests were conducted as well in order for the developed methods to be contrasted with pharmacopoeial reference methods.

Keywords:, boron-doped diamond electrode, ketoconazole, ciclopirox olamine, voltammetry, determination, pharmaceuticals, cosmetics

1 Introduction

Ketoconazole and ciclopirox olamine are synthetic, highly effective broad-spectrum antifungal agents. Their structures are shown in Fig.1a and 1b respectively.

Ketoconazole is an imidazole derivative, currently applied for the treatment of systemic infections. It has been found to demonstrate strong activeness against many fungal and some gram-positive microorganisms when applied orally [1]. The mechanism of its action is based on damaging fungal cytoplasmatic membrane as well as impairment of mitochondrial and microsomal enzymes of the fungi. Ketoconazole has been introduced into therapeutic use as an active ingredient of commercial antifungal formulations, such as tablets, creams and anti-dandruff shampoos. Many methods of ketoconazole quantitative determination in pharmaceutical preparations and biological fluids, such as HPLC [2-7], spectrofluorimetric [8], spectrophotometric [9,10] and electrophoretic capillary [11] ones, have been reported in

literature on the subject. On the other hand, there exist voltammetric methods characterized by lower cost, shorter analysis time and diminished requirements for sample pretreatment compared to chromatographic methods. The ketoconazole electrochemical properties were studied with the application of polarographic [12,13] and voltammetric [14-19] procedures. The electrochemical procedures described in the literature were carried out with the use of mercury, platinum, carbon paste and glassy carbon electrodes. The sensitivity of the said methods allows for them to be applied while determining ketoconazole in blood, urine, pharmaceutical preparations and cosmetics.

Ciclopirox olamine is a substituted antimycotic pyridone, widely used as an antifungal substance in pharmaceutical preparations as well as an antiseborrheic agent in cosmetics [20]. Its mechanism of action is anticipated to involve chelating with polyvalent metal ions such as Fe^{3+} and Al^{3+} and inhibiting the metal-dependent enzymes within fungal cells [21]. The recommended method for ciclopirox olamine determination is described in pharmacopeia. This methods involes spectrophotometric measurements using ferrous sulphate with the maximum absorption at 440 nm [22].The analytical method for ciclopirox olamine quantitative determination, which is referred to in literature, is essentially based on gas chromatography [23], spectrophotometry [24] spectrofluorimetry [25] electrophoretic capillary [26] and HPLC [27-29]. Ciclopirox cannot be directly quantified by HPLC or reverse phase chromatography, because of strong complexation of its N-hydroxylpyridone group with metal cations on the stationary phases of HPLC columns. Therefore, it is necessary to use pre-column derivatization methods to eliminate the chelating effect by ciclopirox N-hydroxyl group methylation or by using EDTA as a chelating agent in the sample preparation [21, 29].

The voltammetric method facilitates sensitive estimation of ciclopirox olamine. Its structure is characterized by the occurrence of two electroactive groups. The review of

literature has revealed that only two polarographic methods have been described for the ciclopirox olamine determination: one of those being based on the reduction of the carbonyl group [30] while the other one on oxidation of N-OH group [31] on the dropping mercury electrode.

In this work, the recommended procedures described the use of a novel carbon material – boron-doped diamond electrode (BDD) – for the determination of ciclopirox olamine and ketoconazole. Increasing use of BDD electrode for electroanalytical measurement of biological and pharmaceutical significance has been reported in recent years [32-41]. BDD electrode is a new electrode material that has received great attention recently. The reason for it is the material's extremely wide electrochemical potential window in aqueous and non-aqueous electrolytes, low and stable capacitive background current, high response reproducibility and long-term response stability as well as an inert surface with low adsorption properties, morphological and microstructural stability at extreme anodic and cathodic potentials etc. Owing to these properties, BDD electrode is an ideal electrode substrate for an extensive range of electrochemical applications [42-49].

There are no reports to be found in literature concerning the ketoconazole and ciclopirox olamine determination on BDD electrode. This paper describes electrochemical behaviour of ketoconazole and ciclopirox olamine on BDD electrode and their square-wave voltammetric determination. The proposed methods were successfully applied for ketoconazole and ciclopirox olamine determination in tablets, creams and anti-dandruff shampoos.

2 Experimental

2.1 Apparatus

The voltammetric experiments were performed using P101 potentiostat - galvanostat manufactured by from Metrohm, Autolab. B.V. In all the measurements, a three-electrode cell of 25 mL capacity and a BDD electrode (Windsor Scientific LTD, A=0.07cm^2, diamond was doped with boron, around 0.1%) or a glassy carbon electrode (BAS; A=0.07cm^2) were used as a working electrode, while saturated calomel electrode - as a reference electrode and a platinum wire counter electrode as an auxiliary electrode one.

Prior to each measurement, the working electrode was cleaned. The BDD electrode was cleaned by holding it in ethanol or methanol, respectively for ciclopirox olamine and ketoconazole, for 10 minutes. The glassy carbon electrode was polished manually with aluminum oxide on a BAS velvet polishing cloth (BAS, polishing kit, PK-4), then rinsed thoroughly with deionized water. Additionally, the diamond or glassy carbon electrode was cleaned electrochemically. The electrodes were transferred into an optimal blank supporting electrolyte solution and the cyclic voltammetry experiments were conducted. The potential was scanned in the range from -0.5 V to 1.2 V at 100mV/s for GC electrode. BDD was cathodic conditioned by cycling in within the potential ranges from -2.9 V to 0.3 V with the scan rate of 500 mV/s.

The cyclic voltammograms were also recorded to selected the best electrolyte pH value for electroanalytical purposes. The initial and the final potentials in this experiment were variable, depending on the supporting electrolyte pH and dependent on the substances studied. Values of pH were analyzed using a pH meter (InoLab, WTW, Austria) with a glass combination electrode.

As far as the SWV method is concerned, the potential was scanned from 0.0 V to 1.0 V. Experimental parameters of this method, such as frequency, pulse amplitude and step potential, were selected. Optimal values of those were applied for electrochemical

determination of ketoconazole and ciclopirox olamine. All the measurements were carried out at ambient temperature.

For the purposes of a comparison study, HPLC experiments were carried out using Thermo separation products (USA) with a diode array detection system (SCM 1000) and a symmetry shield C-18 column. The studied compounds were separated using mobile phases composed of methanol with diisopropylamine: ammonium acetate (7:3, v/v). The effluent was recorded at 225 nm for ketoconazole and at a flow rate of 1.2 mL/min [50]. The results were also compared with the spectrophotometric procedure for ciclopirox olamine recommended by the United States Pharmacopoeia 2006 [22]. That procedure is based on the specific reaction of ciclopirox olamine with iron ions (II). The reaction produces an orange-coloured complex with an absorption maximum of 440 nm.

2.2 Chemicals

Ketoconazole and ciclopirox olamine were obtained from Sigma-Aldrich Chemicals (USA). All aqueous solutions were made using ultrapure water obtained with Milli-Q equipment (Millipore, MA, USA). All other chemicals and reagents used were of analytical grade. A stock solution of ketoconazole was prepared by dissolving the substance in 0.2 mol/L HCl. The stock solution of ciclopirox olamine was prepared by dissolving the substance in methanol. Ketoconazole and ciclopirox olamine stock solutions were kept in the dark inside a refrigerator. Standard solutions were prepared by appropriate dilution of the stock solutions with water.

In the aforementioned investigations, the following were used as the supporting electrolytes: 0.04 mol/L Britton-Robinson buffer with pH 1.8 – 11.2, 0.06 mol/L phosphate buffer with pH 2 – 11, 0.2 mol/L ammonium buffer with pH 8.64 – 10.52, 0.2 mol/L acetate buffer with pH 3.6 – 5.6, Clark and Lubs buffer with pH 7 and McIlvaine's buffer with pH 7.

All the solutions were prepared using reagent grade chemicals and water purified by Milli-Q water.

Ketokonazol® tablets containing 200 mg of ketoconazole were supplied by Anpharm, Warsaw, Poland; Nizoral® cream containing 20 mg/g of the ketoconazole and Nizoral® shampoo (20 mg/g) were supplied by Janssen Pharmaceutica, Belgium. Pursuant to the information on the label Stieprox® shampoo (Stiefel, a GSK company) contains 15 mg of ciclopirox olamine per 1 mL of the shampoo.

2.3 Preparation of Tablets, Creams and Shampoo Samples

Ten ketoconazole tablets, containing 200 mg of ketoconazole each, were accurately weighed and powdered. An adequate amount of ketoconazole tablet (about 0.2 g) was weighed, transferred into a 100 mL calibrated flask and dissolved in 50 mL of 0.2 mol/L HCl. The solution was shaken for 30 min and complemented to the volume with 0.2 mol/L HCl. The excipients were separated by filtration. The resultant solution was subsequently transferred to a voltammetric cell and the measurements were recorded.

An accurately weighed amount of ketoconazole cream sample (about 1 g) was transferred into a 100 mL calibrated flask and dissolved in 50 mL of 0.2 mol/L HCl. The content of the flask was sonicated for 10 min and complemented to the volume with 0.2 mol/L HCl. Afterwards, the solution was filtered through Celite® 545 filter agent so as to obtain a clear filtrate. The solution was analyzed in the voltammetric cell in optimally chosen supporting electrolyte. The ketoconazole content in the studied sample was calculated on the basis of current values of the oxidation peak of the studied substance and equation of calibration curves.

Appropriate volumes (1g) of ketoconazole or ciclopirox olamine containing shampoos were accurately weighed, transferred to a 100 mL flask and completed to the final volume

with 0.2 mol/L HCl for ketoconazole and with methanol for ciclopirox olamine, respectively. These solutions were stirred for 30 min. Then, the solutions were electrochemically tested in optimally chosen measurement conditions.

3 Results and Discussion

3.1. Cyclic Voltammetric Behaviour of Ketoconazole on BDD Electrode

The electrochemical behaviour of ketoconazole on BDD electrode was studied. The process of electrooxidation of ketoconazole was studied in several supporting electrolytes. The measurement was studied over a wide pH range: from 1.8 to 11.2. Cyclic voltammograms of ketoconazole were recorded in ammonium, phosphate, acetate and Britton-Robinson buffers. Voltammograms for the studied substance were recorded in the range from -0.25 V to 1.0 V at the scan rate of 100 mV/s. Fig. 2 shows selected ketoconazole cyclic voltammograms in studied buffers. The voltammetric plots indicated that ketoconazole in pH range 1.8 – 6.5 achieves the oxidation peak as well as the related reduction peak in the potential window of 0.45 - 0.75 V. However, alongside the increase in solution pH value, the decrease in the reduction peak current value occurs. For the pH value over 6.5, an irreversible ketoconazole oxidation peak was observed. Cyclic voltammograms for the electrolyte solutions demonstrated that the BDD electrode background is not susceptible to the nature and pH value of the electrolyte applied (Fig.2).

In acidic solutions, ketoconazole occurs in two protonated forms. At pH below 2, the (KCH_2^{2+}) form of the analyte prevails, whereas in the pH range of 4-7, the (KCH^+) form prevails, which is shown in the following equations [9]:

$$KCH_2^{2+} \rightleftharpoons KCH^+ + H^+ \quad pKa_1 = 2.97$$

$$KCH^+ \rightleftharpoons KC + H^+ \qquad pKa_2 = 6.51$$

The experiments performed acknowledge the reversible properties of the aforementioned reactions. Hence, the oxidation and reduction peaks have been observed on CV curves. (Fig.2). Detailed analysis has shown that, this process was not completely reversible. The difference between the potentials was about 83 mV, higher than the expected value of 59/n mV for a theoretical reversible process, so the process is quasi-reversible.

In an alkaline solutions with pH above 8, the (KC) form of ketoconazole is dominant, which undergoes irreversible oxidation reaction. The studies showed that alkaline solutions is optimal for carrying out electrochemical qualitative and quantitative analyses of ketoconazole, because the recorded oxidation currents of the compound display peak values (Fig.3b). The potential of the peak of ketoconazole is shifted to less positive values with increasing pH (Fig. 3a). The diagram clearly demonstrates three sections related to pKa_1 and pKa_2 ketoconazole values.

The highest peak of ketoconazole oxidation was found in NH_3- NH_4Cl buffer at pH 9.42. This buffer was selected for further tests for ketoconazole on BDD electrode. In this buffers, ketoconazole is irreversibly oxidized in a single step, giving a well-defined peak at the potential at 0.59 V (SCE). Studies of the character of the recorded current were carried out at different scan rate. In the range 5-500 mV/s, the current function values $Ip^a \sim v^{1/2}$ were found increased linear with $v^{1/2}$ ($R^2 = 0.9989$). The current function values $\log Ip^a \sim \log v$ increased linear with slope 0.5. This value is identical with the theoretical value expressed with regards to a reaction for a diffusion-controlled electrode process (Fig.4).

The survey of the literature in the field reveals that ketoconazole examinations on BDD electrode have not been performed so far. It was advisable, though, to compare the voltammograms obtained for BDD electrode with the ones found for any other carbon

material commonly described in the literature. The cyclic curves for ketoconazole and the background on BDD electrode and glassy carbon electrode (GC) were performed. (Fig.5) The ketoconazole cyclic voltammogram yielded one well-defined irreversible oxidation peak of the investigated substance on both electrodes. The BDD electrode has a lower background current than GC electrode. The measurements recorded on the BDD electrode have higher sensitivity of determinations than on the GC electrode. In the case of ketoconazole, it was observed that the signal-to-background ratio (S/B) is 11.9 for BDD electrode and 4.4 for glassy carbon electrode.

3.2. Cyclic Voltammetric Behaviour of Ciclopirox olamine on BDD Electrode

The cyclic voltammetric behaviour of ciclopirox olamine on BDD electrode is shown in Fig. 6. Ciclopirox olamine is irreversible oxidized, producing one anodic peak at ~ 0.66 V.

On a par with ketoconazole, comparable curves using CV voltammetry for a compound studied were registered on GC electrode. It registered higher values of the background current and lower current values for the substances in comparison to BDD electrode. The BDD electrode showed higher sensitivity than the GC electrode for the detection of ciclopirox olamine because of its low and stable background current. It was observed that the signal-to-background ratio (S/B) is 20.64 for BDD and 3.49 for GC, respectively.

Then the process of electrooxidation of ciclopirox olamine on BDD electrode was studied in several supporting electrolytes. The cyclic voltammograms ciclopirox olamine were recorded in ammonium, phosphate, acetate, Britton-Robinson, Clark and Lubs and McIlvaine's buffers. Voltammograms were recorded in the range from -0.5 to 1.2 V at the scan rate of 100 mV/s. The voltammetric responses showed that, for pH values below 7, no

well-defined peak of ciclopirox olamine was observed (Fig.7). Alongside the increase in electrolyte solution pH value, higher current values have been achieved. Best shaped voltammograms were obtained for the solutions with pH value around 7. The pK_a value, most commonly referred to in the literature for ciclopirox olamine, equals 7.2 [21]. The peak oxidation of the ciclopirox olamine is related to the oxidation of N-OH group [31]. The highest peak on oxidation of ciclopirox olamine was found in McIlvaine's buffer, so this buffer was selected for further investigations of this substance.

The effect of the scan rate on peak current (Ip) was studied in the range 10-500 mV/s. The plot of Ip versus the square root ($v^{1/2}$) of the scan rate and a plot of the logarithm of the peak current versus the logarithm of the scan rate was examined. It was found that the current response was linear with the square root of the scan rate (correlation coefficient 0.9939). Plots of the logarithm of peak current of ciclopirox olamine versus the logarithm of scan rate gave a straight line with a slope of 0.434, indicating that the currents are diffusion-controlled for studied substance. (Fig.4)

CV voltammograms for both studied substances were carried out first in absence of oxygen and then in presence of oxygen. It can be concluded that the presence of oxygen in studied solutions does not affect the shape of the curves recorded on the BDD electrode (not shown).

3.3. Analytical Application and Validation of the Proposed Procedures

The following paper has taken advantage of the square-wave voltammetry (SWV) technique for quantitive determinaiton of ketoconazole and ciclopirox olamine. The parametres that characterise the SWV method and which influence the value of the current measures are: frequency *f*; amplitude E_{SW} and step potential ΔE. In order to select the best

parametres for ketoconazole and ciclopirox olamine electrooxidation by means of the SWV method, the measurements to optimise such parametres were performed. The research was conducted by amending the value of a parametre studied and maintaining other values as constant. While selecting the optimal conditions, the height and shape of the registered oxidation peaks were taken into account.

Studies over ketoconazole demonstrated that alongside the increase in amplitude within the range from 5 to 100 mV, the increase in registered currents occurred. The increase remains linear until 25 mV while for higher values the said dependance is not linear anymore. Hence, the value 25 mV has been selected for further research. Frequency measurements were performed within the range between 8 and 250 mV. The value of 100 mV was chosen as the optimal one. The values obtained for frequencies exceeding 100 Hz are characterised by noticeable dispersion and the lack of linear increase tendency. The measurements referring to the step potential were performed for the range 2-10 mV. The highest current peak value was reached at 4mV, hence the value was used for further research.

Examinations of ciclopirox olamine showed the increase in the values of the currents registered together with the increase in amplitude. The analysis of the dependence $I = f(E_{sw})$ allowed to state that within the range 5 - 25 mV the points form a linear correlation, while for higher values the linear dependence is not observed. Therefore, the value of 25 mV was regarded as the optimal one for further examinations. The impact of frequency was analysed within the range 8-250 mV. Studies on the dependency of the analyte oxidation peak current as a function of the square root of frequency showed the linear current increase within the aforementioned range. However, in conjunction with the frequency growth the registered curves were becoming less ans less symmetrical. That was the reason why the frequency of 150 Hz was nominated the optimal one. The step size was analysed within the range 2-10 mV. It has been observed that the ciclopirox olamine oxidation peak current increases

alongside the increase in the optimalised value. 8 mV was selected for further research due to high and well-shaped curves.

When the best operating parameters were selected, two calibration graphs were recorded from standard substances of ketoconazole and ciclopirox olamine. While making the calibration curves, BDD electrode background was controlled prior to the measurements and during the analysis. The obtained values of the background curves demonstrated that the background does not change during the measurement series, therefore it is not necessary to perform background correction when using SWV for the construction of calibration curve.

A linear relation of the ketoconazole peak current versus its concentration was observed between $2.87 \cdot 10^{-7}$ mol/L and $3.13 \cdot 10^{-6}$ mol/L, with the limit of detection at $8.29 \cdot 10^{-8}$ mol/L. The results are shown in Fig.8. A linear relation within the concentration range between $2.53 \cdot 10^{-5}$ mol/L and $4.19 \cdot 10^{-4}$ mol/L, with the limit of detection at $6.66 \cdot 10^{-6}$ mol/L was found for ciclopirox olamine. The results are shown in Fig.9. The characteristics of these calibration plots are reported in Table 1. The limit of detection (LOD) was obtained using equation LOD = 3s/m, where standard deviation (s) is obtained in five runs and (m) is the slope of the dependence of the measured current on concentration. The limits of quantification (LOQ) used the equation LOQ = 10s/m, respectively [51].

Repeatability, reproducibility and precision of the ketoconazole and ciclopirox olamine were calculated by repeating five experiments for the same solutions within the same day and five experiments on different solutions for five days. The concentrations used for the experiments amounted to $1.48 \cdot 10^{-4}$ mol/L for ciclopirox olamine and $1.42 \cdot 10^{-6}$ mol/L for ketoconazole. The precision, repeatability and reproducibility determined as RSD% demonstrated good results with regards to these parameters (Table 1).

3.4. Assay of Ketoconazole and Ciclopirox olamine in Commercial Formulations

The present procedures can be applied for direct determination of ketoconazole and ciclopirox olamine in pharmaceutical preparations and cosmetics. The preparation procedure of the studied samples was described in the Experimental part. The measurements were carried out under optimally selected conditions in ammonium buffer at pH 9.42 for ketoconazole and in McIlvaine's buffer at pH 7 for ciclopirox olamine.

The developed method of voltammetric ketoconazole determination was used to determine this substance in tablets, cream and shampoo. The established determined contents of ketoconazole in preparations subject to examination are close to the values declared by the manufacturers. The approximation error of ketoconazole determination in *Ketokonazol* tablets, *Nizoral* cream and *Nizoral* shampoo was, respectively, -0.04%, +0.05% and -1.0%. Voltammetric plots recorded for ketoconazole in tablets, creams and shampoo are shown in Fig. 10.

The voltammetric method of ciclopirox olamine determination was used to determine this analyte in 1.5% *Stieprox* shampoo. The experiment conducted by means of the SWV method allowed to determine ciclopirox olamine content at 14.97 mg (with the declared value being 15 mg). The relative determination error (0.2%) proves high accuracy of the proposed procedure.

The pharmacopoeial method of ketoconazole determination recommended in Polish Pharmacopoeia [50] was applied as a comparative method. The comparative method regarding ciclopirox olamine determination describe the spectrophotometric method with UV-VIS detection, in line with the recommendations from The United States Pharmacopeia of 2006 [22].

It was found that the ketoconazole content determined by means of the SWV method the reference HPLC method remained in conformity. The approximation error of ketoconazole determination with regards to the pharmacopoeial HPLC method is -0.96%, -0.75% and

2.55% for the samples of tablets, cream and shampoo respectively. Example curves obtained for commercial ketoconazole preparations (tablets, cream and shampoo) with the use of the comparative method are presented in Figure 11. It was also discovered that the results of ciclopirox olamine contents determinations in the studied shampoo obtained through the developed voltammetric method and through the spectrophotometric pharmacopoeial method are similar. The low approximation error of determination equals -1.44%, which proves the accuracy of the method developed.

The determination results obtained for ketoconazole and ciclopirox olamine were subjected to statistical analysis using the t-student test and the F-test. The values obtained by way of the SWV method were compared to the data acquired through the reference methods for both studied substances. At 95% confidence level, the calculated t and F values were lower than their theoretical counterparts. It means that the results obtained with the use of the proposed procedures as well as those acquired through the methods recommended in literature are burdened with a comparable statistical error. The values of t-student test prove that the differences between the results obtained by means of the compared methods are not statistically significant and result solely from incidental mistakes. The relevant outcomes are shown in Table 2. The proposed procedures might constitute a good alternative for the ketoconazole and ciclopirox olamine determinations in cosmetic and pharmaceutical preparations as well as a substitute for the HPLC technique.

In addition, the selectivity of the proposed procedures was checked. It was examined in the presence of some typical excipients (e.g., lactose, glucose, glycerine, starch) used in cosmetic and pharmaceutical preparations as well as in the presence of common electroactive substances in the tested range of potentials. The concentration produced an error ±5% that was adopted as the tolerance limit. The study showed that the excipients in the ketoconazole formulation did not interfere with the assay, except for glycerine, sodium benzoate and SO_3^{2-}.

Among the studied electroactive substances, ascorbic acid and ions such as Cu^{2+} can demonstrate some interference. Peaks of these substances were similar to the peak of ketoconazole oxidation. While performing the ciclopirox olamine determination lactose, ascorbic acid, starch and SO_3^{2-} can interfere. Precipitate formed in the presence of ions such as Cu^{2+}, Ca^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+} precipitate formed.

4 Conclusions

The following paper presents attractive methods for the determination of two antifungal drugs, ketoconazole and ciclopirox olamine. The recommended procedures are based on oxidation of the said compounds on a new electrode material, BDD. The properties of the BDD electrode and the usage of the sensitive SWV technique facilitated the development of simple and quick procedures intended to determine the studied compounds in various pharmaceutical and cosmetics forms. These methods marked with high sensitivity, precisions and accuracy. With regards to ketoconazole, the determination ranges for the calibration plots received as well as the LOD and LQD values remain lower compared to the works recommended in the literature [14-16,18]. Electroanalytical methods for ciclopirox olamine determination suggested in literature by Ibrahim [30,31] use the measurements on the mercury electrode, which is nowadays frequently replaced by other electrode materials.

The benefits resulting from the newly developed methods encompass quick measurement process in the solutions that do not require oxygen elimination. Additionally, they secure comfortable work with an electrode that does not necessary demand constant cleaning provides high signal-to-background ratio. Research conducted on the GC electrode acknowledged the advantages of the BDD electrode for they demonstrated noticeably higher oxidation peak values and low and stable background current. By referring the developed methods to other procedures described in literature, e.g. chromatographic ones, one must

underline that they provide direct measurement in verified matrices without them having to be separated, they do not require any preliminary derivation nor complicated instruments to be applied or expensive and environmentally-harmful agents to be used. Examinations on the accuracy of the methods, comparable analyses with the pharmacopeial procedures as well as the outcomes obtained from t-student and F-Snedecor tests prove that the recommended procedures may well be applied for routine analyses to be performed in research laboratories.

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Legend:

Fig. 1. Structure of ketoconazole (A) and ciclopirox olamine (B)

Fig. 2. Cyclic voltammograms of $1 \cdot 10^{-4}$ mol/L ketoconazole in: (1)B-R buffer pH=1.8; (2) acetate buffer pH=3.6; (3) phosphate buffer pH=5.9; (4) phosphate buffer pH=6.98; (5) B-R buffer pH=8.95; (6) B-R buffer pH=11.2, v=50 mV/s; at the window (CV voltammograms for the blank solutions).

Fig. 3. Effect of pH on 1×10^{-5} mol/L ketoconazole; (A) anodic peak current, (B) anodic peak potential.

Fig. 4. (A) Plot of the peak current versus the square root of the scan rate (B) Plot of the logarithm of peak current versus logarithm of scan rate for ciclopirox olamine and ketoconazole

Fig. 5. Cyclic voltammograms of ketoconazole: (A) on the BDD electrode, (B) on the GC electrode, (1) blank solution, (2) $1 \cdot 10^{-5}$ mol/L ketoconazole in NH_3-NH_4Cl buffer pH 9.42, v=100 mV/s

Fig. 6. Cyclic voltammograms of ciclopirox olamine in McIlvaine'a buffer pH 7, (A) on the BDD electrode, (B) on the GC electrode, (1) blank solution; (2) $1.58 \cdot 10^{-4}$ mol/L ciclopirox olamine; v=100 mV/s

Fig. 7. Cyclic voltammograms of $1.58 \cdot 10^{-4}$ mol/L ciclopirox olamine in phosphate buffer at different value of pH: (1) 2; (2) 5.91; (3) 6.98; (4) 8.04; (5) 10.97; v= 50 mV/s

Fig. 8. SWV voltammograms for different concentrations of ketoconazole in NH_3-NH_4Cl buffer pH 9.42, (range of concentration $2.87 \cdot 10^{-7} - 3.13 \cdot 10^{-6}$ mol /L)

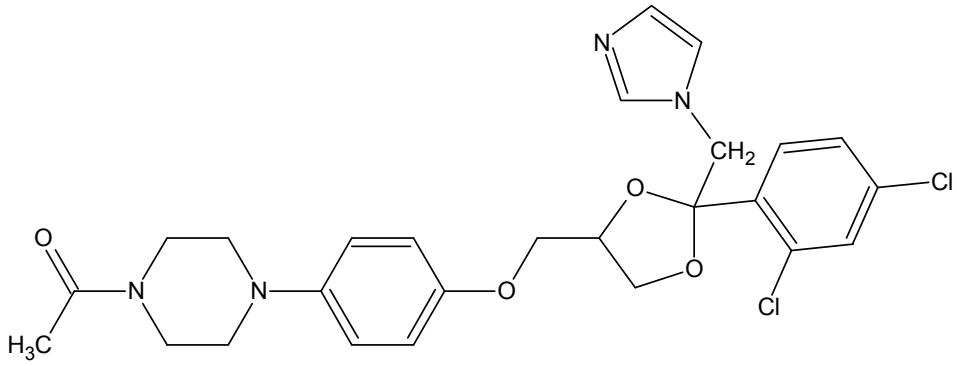
Fig. 9. SWV voltammograms for different concentrations of ciclopirox olamine in McIlvaine'a buffer pH 7, (range of concentration $2.53 \cdot 10^{-5} - 4.19 \cdot 10^{-4}$ mol /L)

Fig. 10. SWV voltammograms of ketoconazole in: (A) *Ketoconazol* tablets, (B) *Nizoral* cream, (C) *Nizoral* shampoo.

Fig. 11. Chromatogram of ketoconazole in: (A) *Ketokonazol* tablets, (B) *Nizoral* cream, (C) *Nizoral* shampoo.

Fig.1

A



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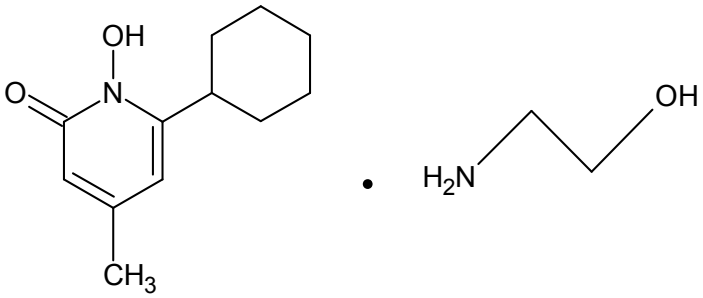
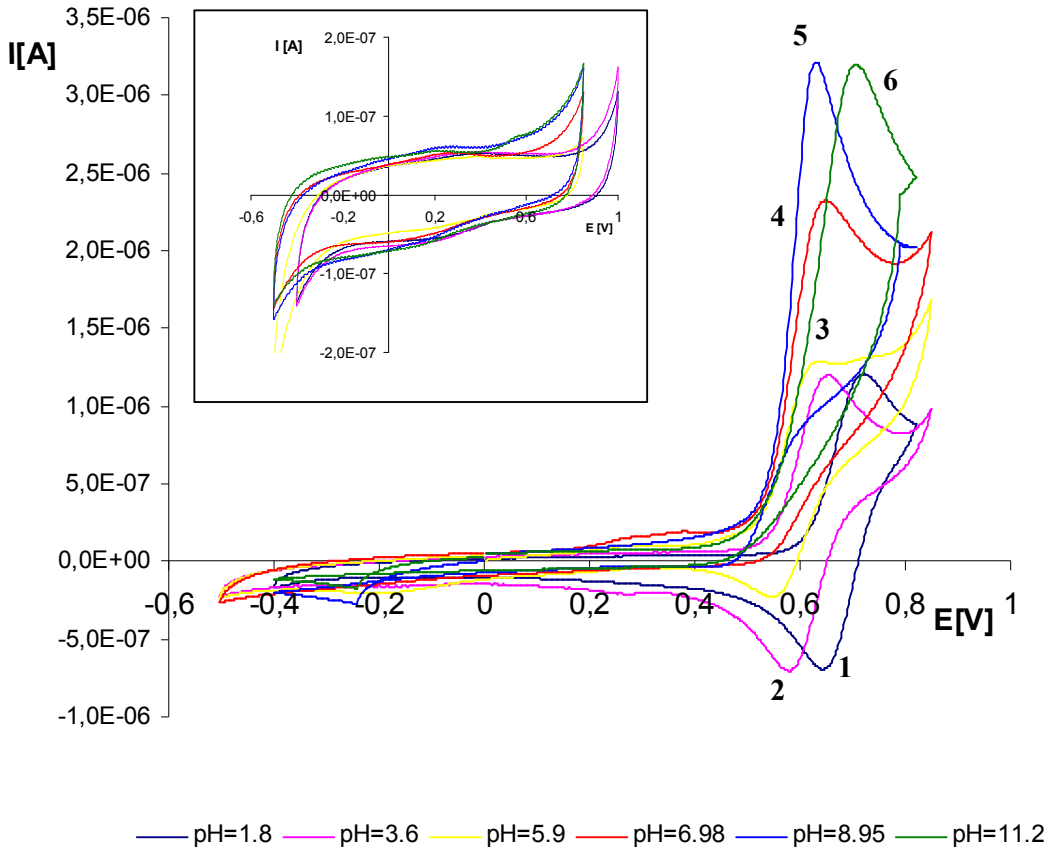


Fig.2.



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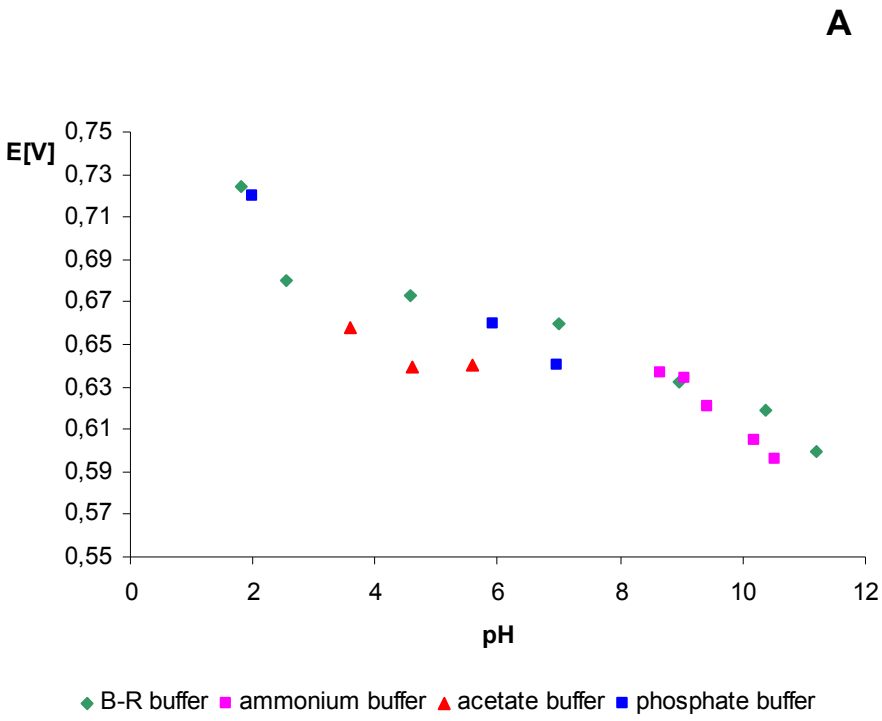


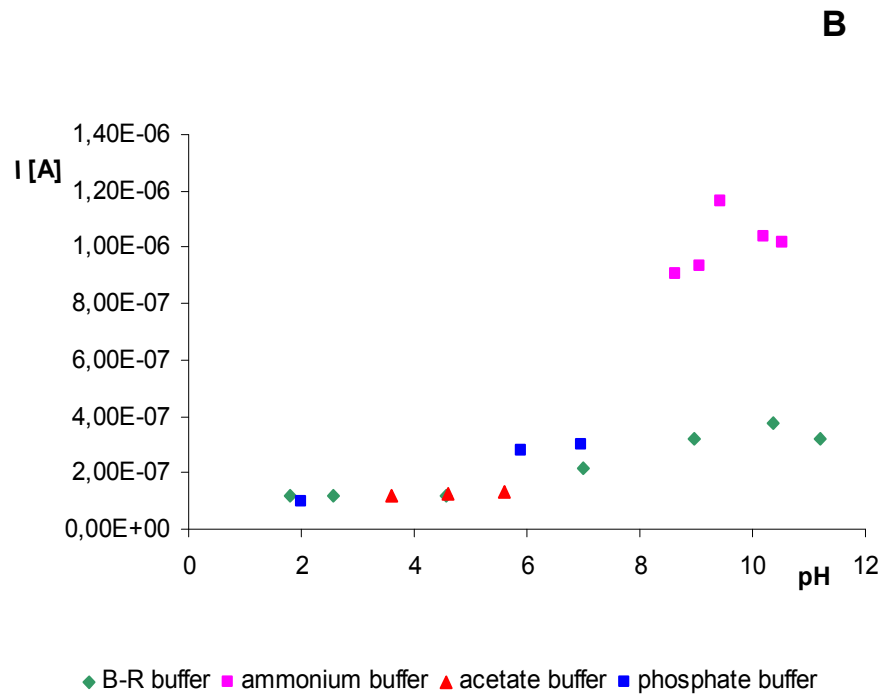
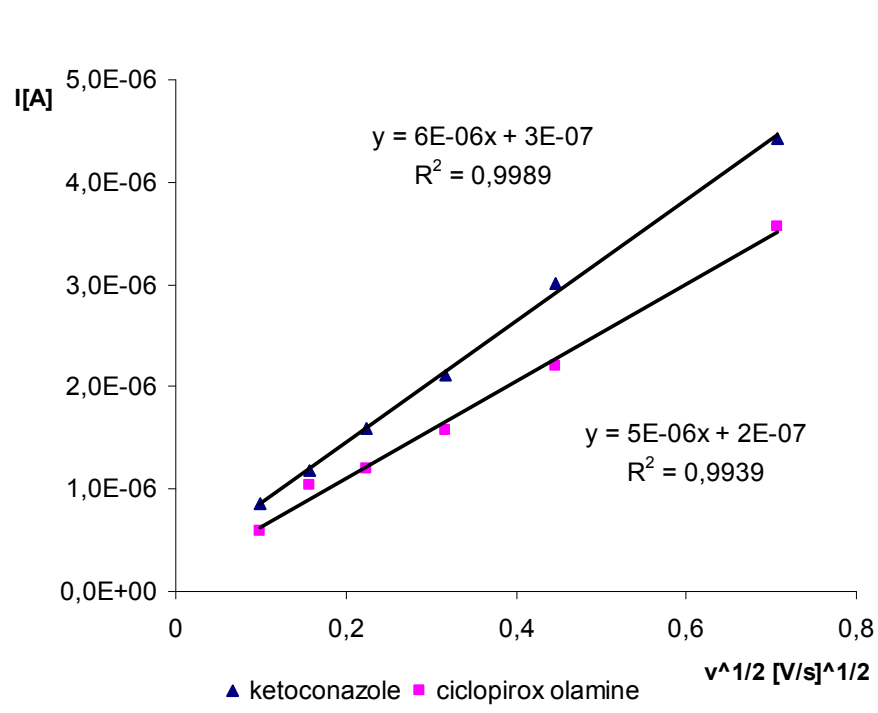
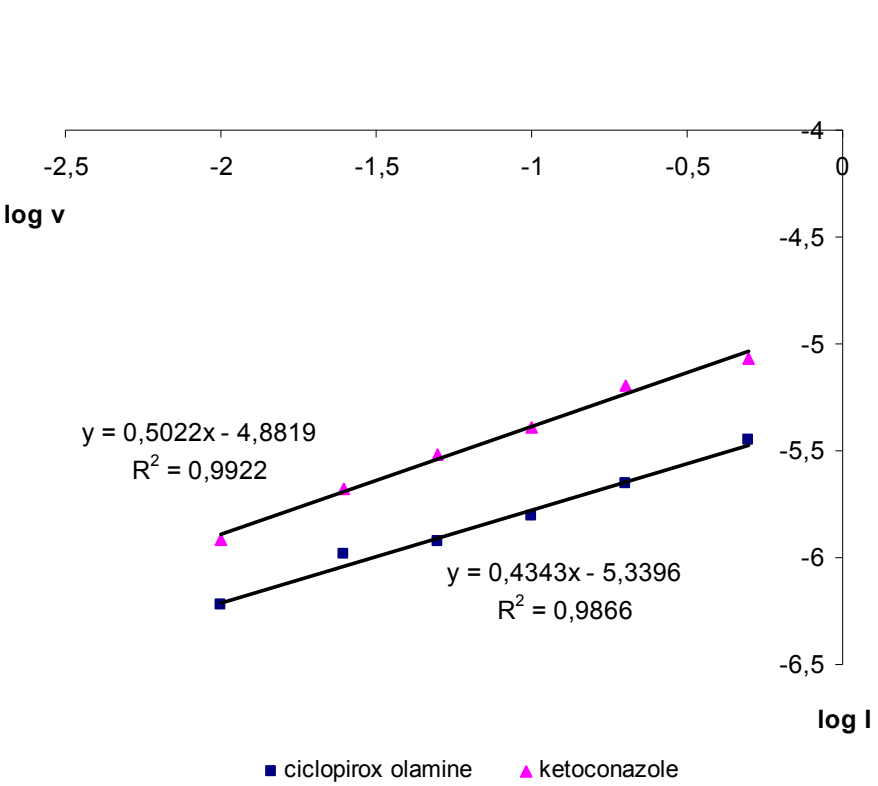


Fig.4.





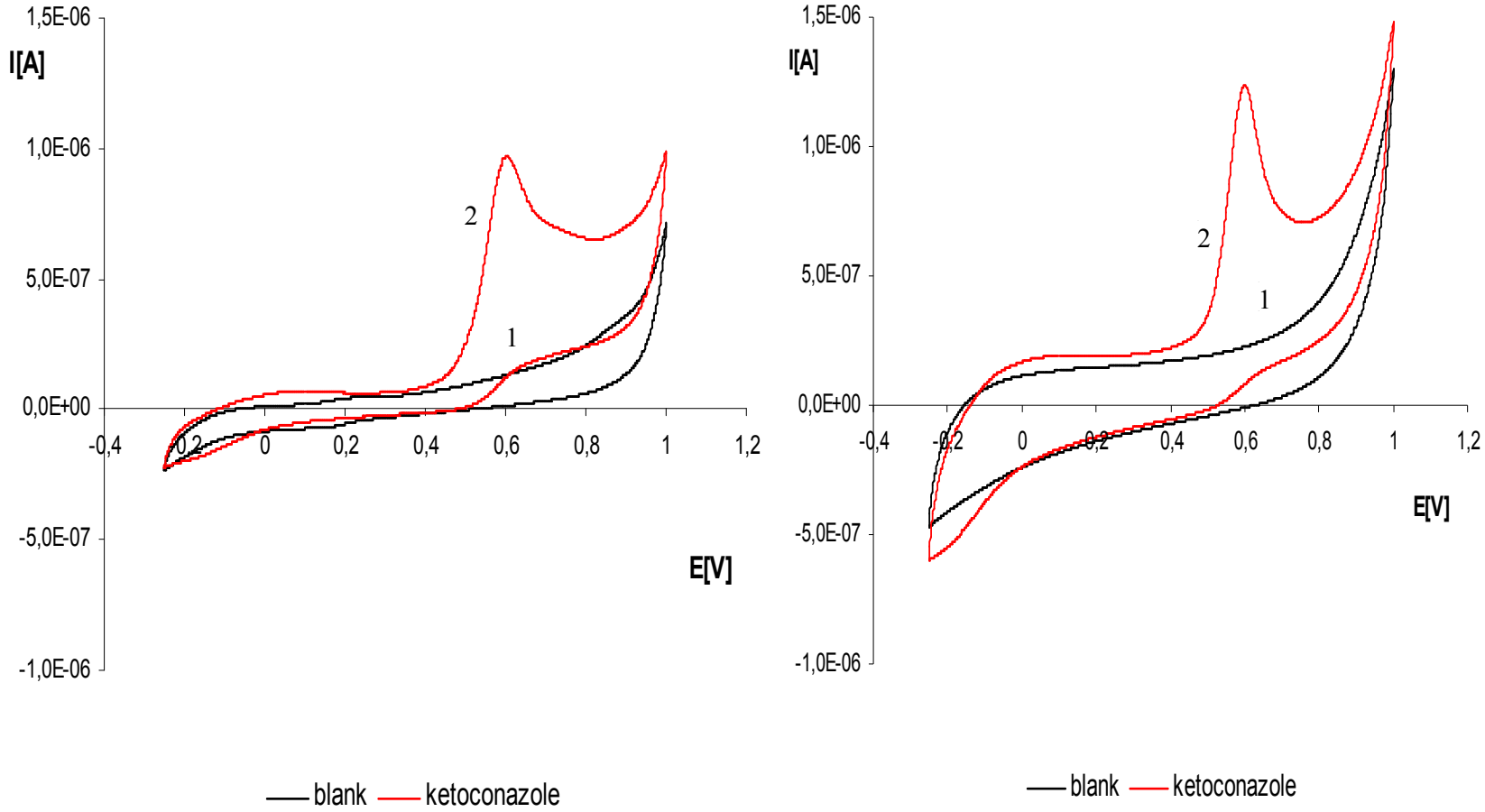
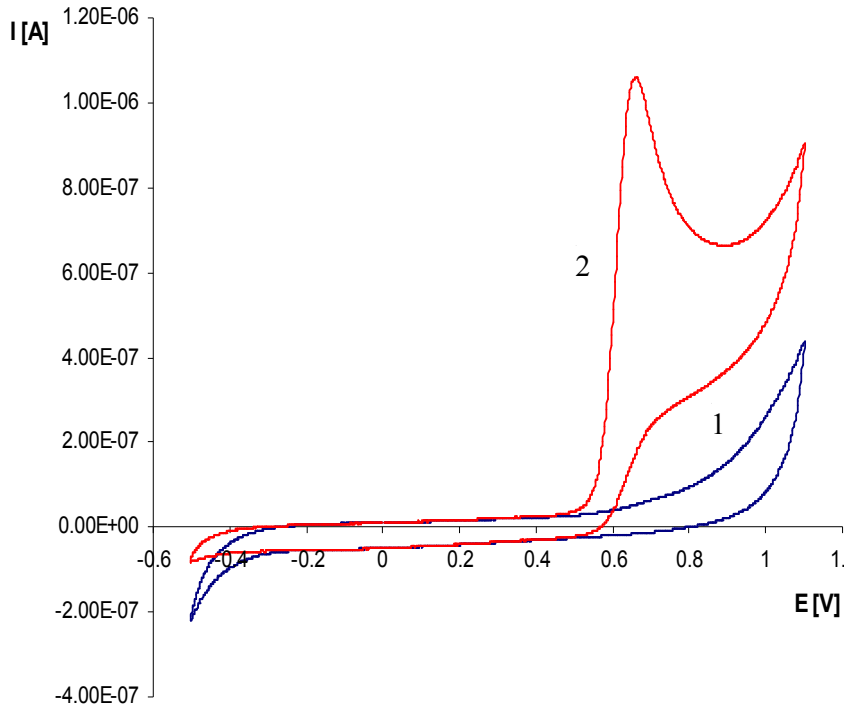


Fig.5.

Fig.6.

A



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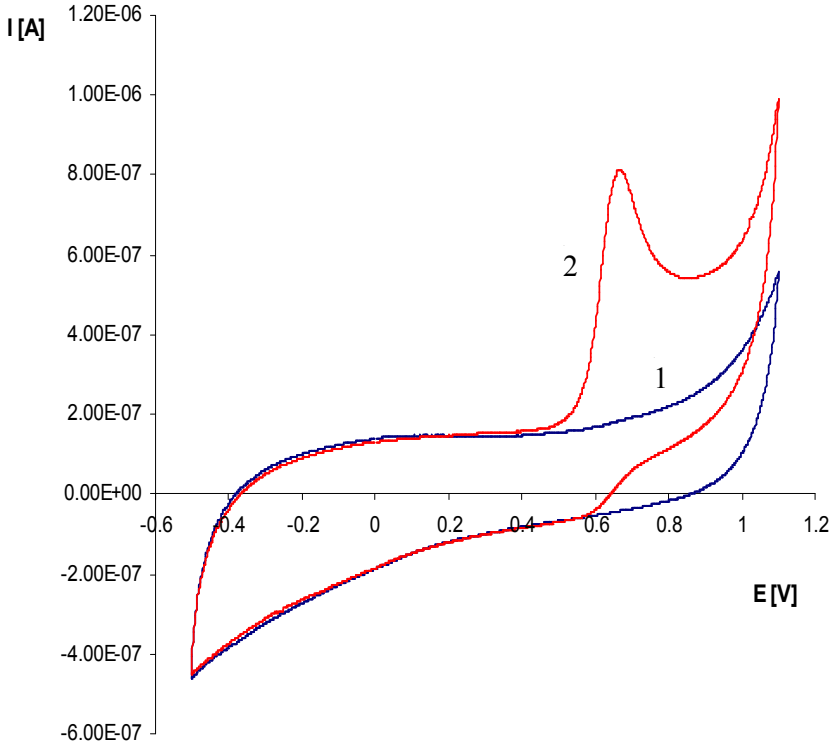

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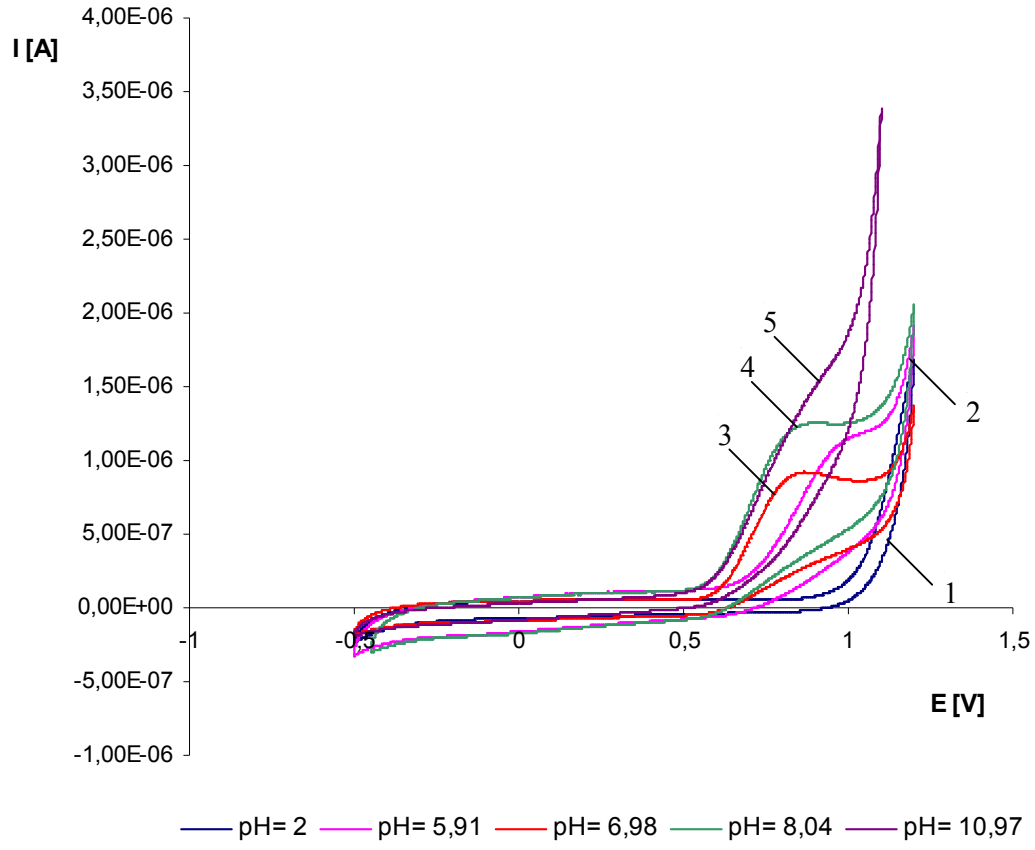
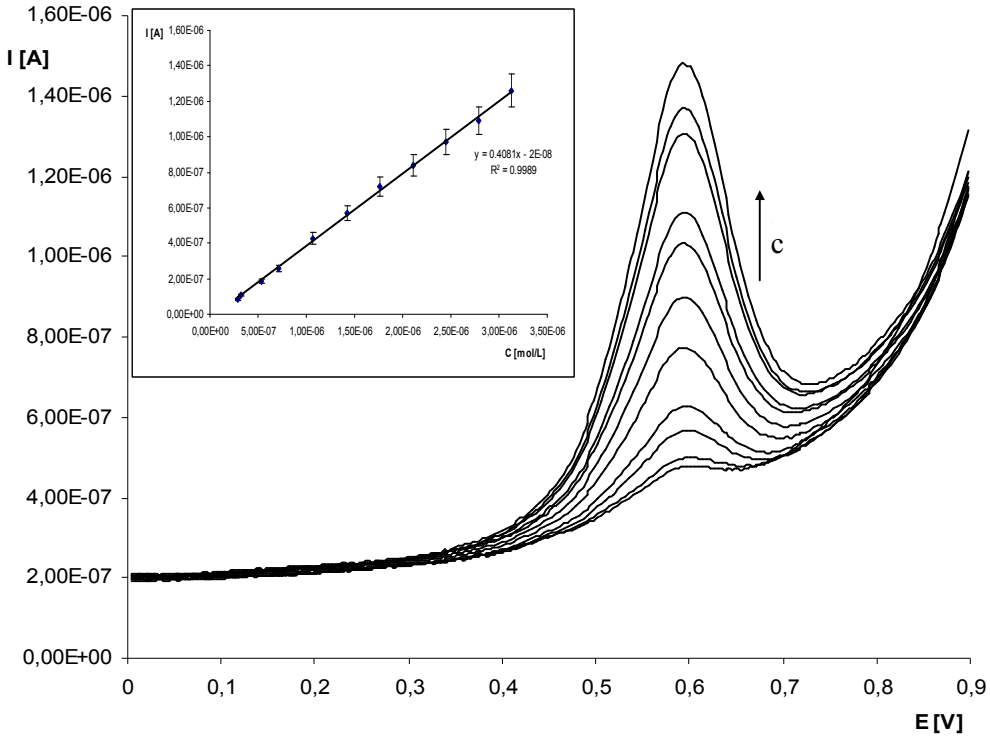


Fig.8



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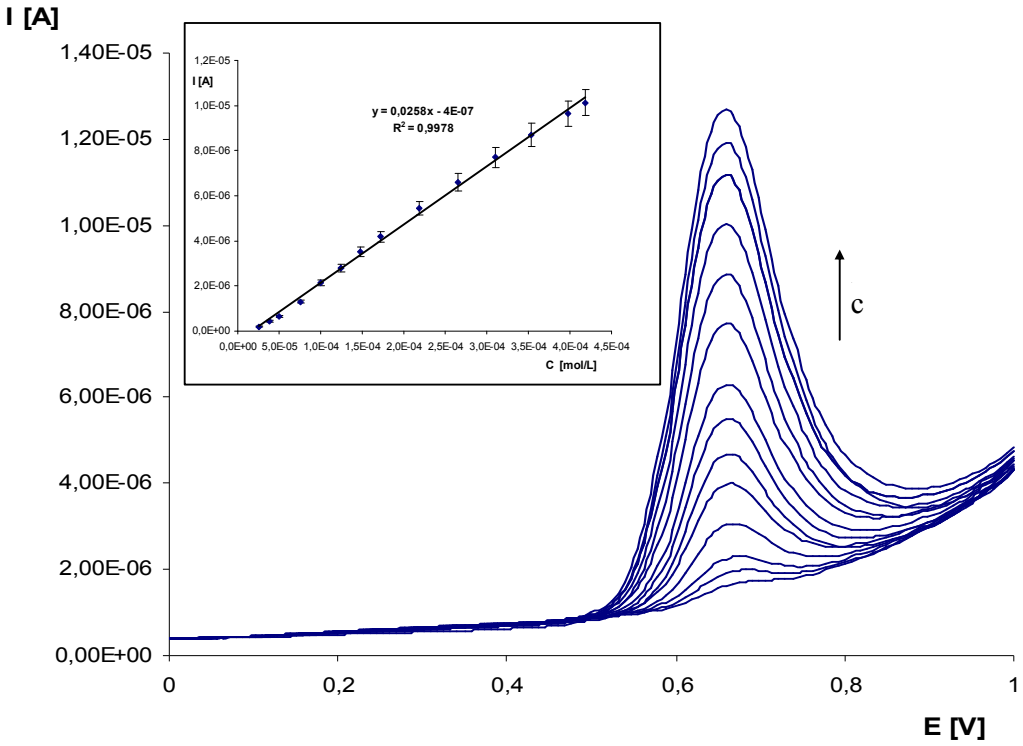
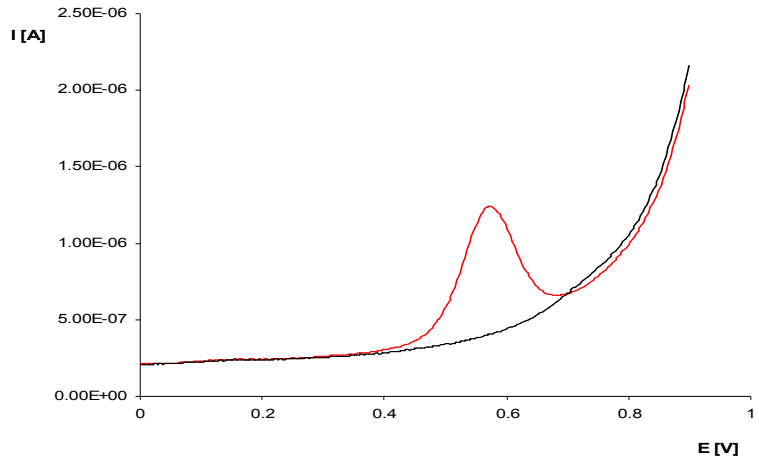


Fig.10.

A



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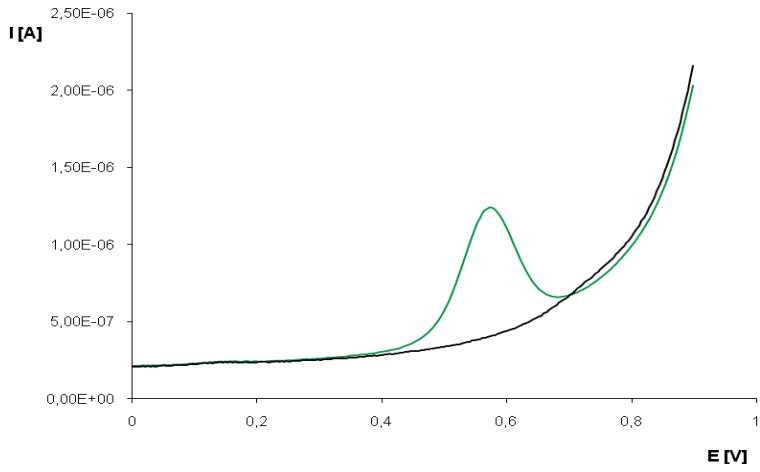

C

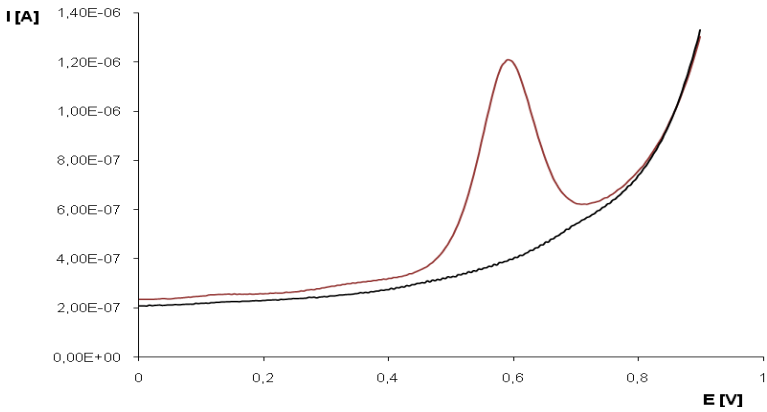

Fig.11

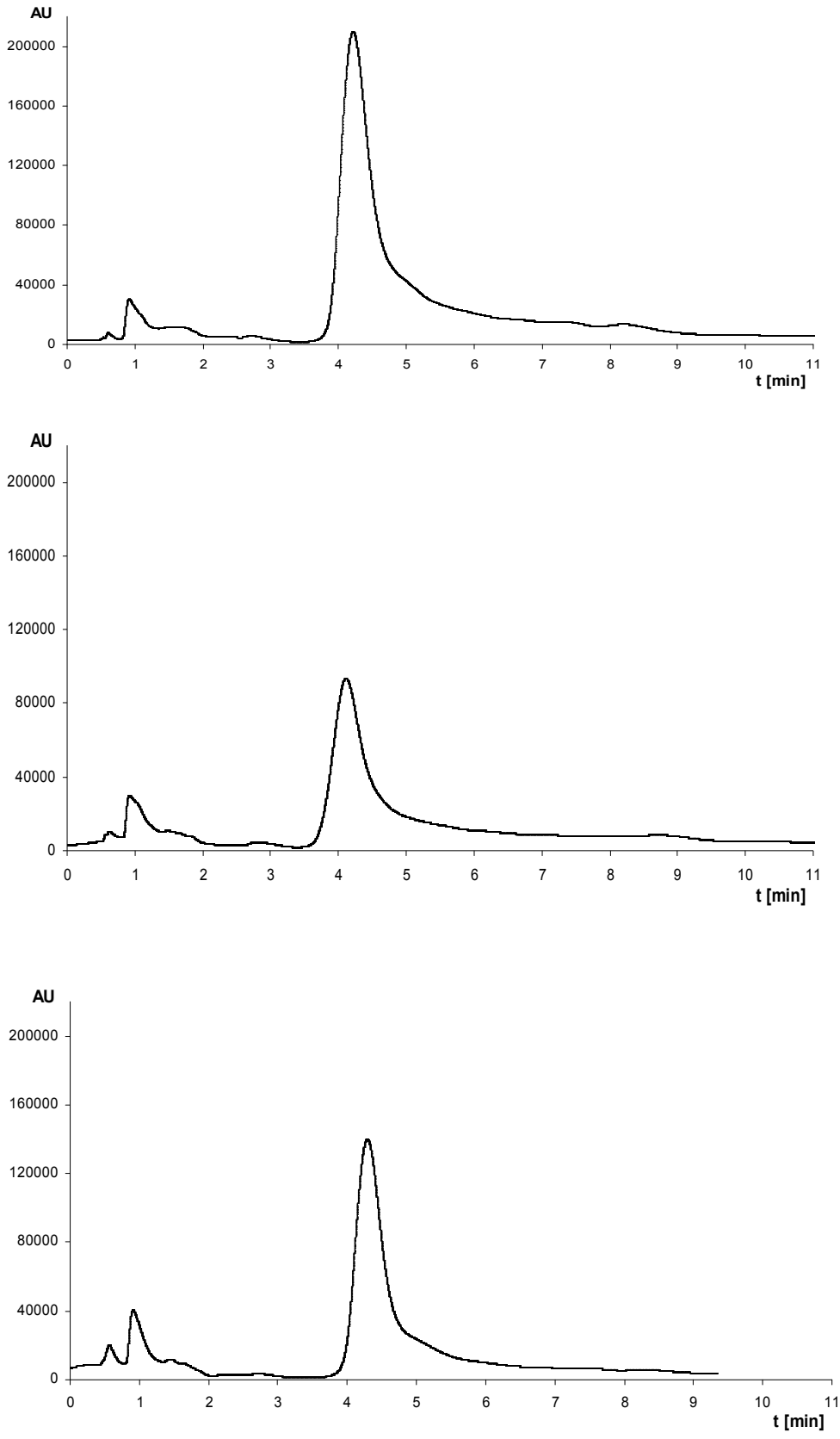

Table 1 Quantitative determination of ketoconazole and ciclopirox olamine by SWV method

	Ketoconazole	Ciclopirox olamine
Working electrode potential (V) (vs. SCE)	0.59	0.66
Studied range (mol/L)	$2.87 \cdot 10^{-7}$ to $1.2 \cdot 10^{-5}$	$1.77 \cdot 10^{-5}$ to $6.43 \cdot 10^{-4}$
Linearity range (mol/L)	$2.87 \cdot 10^{-7}$ to $3.13 \cdot 10^{-6}$	$2.53 \cdot 10^{-5}$ to $4.19 \cdot 10^{-4}$
Slope (μA×L/mol)	$4.08 \cdot 10^{5}$	$2.58 \cdot 10^{4}$
Intercept (μA)	0.02	0.40
Correlation coefficient (r)	0.9989	0.9978
LOD (mol/L)	$8.29 \cdot 10^{-8}$	$6.66 \cdot 10^{-6}$
LOQ (mol/L)	$2.51 \cdot 10^{-7}$	$2.02 \cdot 10^{-5}$
Repeatability of peak current (R.S.D.%)	0.33	0.85
Repeatability of peak potential (R.S.D.%)	0.37	0.66
Reproducibility of peak current (R.S.D.%)	0.95	2.87
Reproducibility of peak potential (R.S.D.%)	0.48	0.79

Table 2 Results obtained from the SWV determination and recovery experiments of ketoconazole and ciclopirox olamine in pharmaceuticals

	Ketoconazole						Ciclopirox olamine	
	SWV in tablets	official method [50]	SWV in cream	official method [50]	SWV in shampoo	official method [50]	SWV in shampoo	official method [22]
Labeled claim (mg)	200	200	20	20	20	20	15	15
Amount found (mg)[a]	200.09	198.18	19.99	19.84	20.20	20.73	14.97	14.76
S.D.	0.969	1.601	0.123	0.132	0.404	0.468	0.136	0.181
R.S.D. %	0.484	0.810	0.616	0.666	2.003	2.260	0.910	1.230
Error %	-0.04	0.91	0.05	0.80	-1.00	-3.65	0.20	1.60
t- student test (2.306)[b]	2.276		1.880		1.906		2.086	
F-test (6.39)[b]	0.364		1.151		1.340		1.777	
Added (mg)	0.043	0.0204	0.043	0.043	0.043	0.043	0.27	0.27
Found (mg)[a]	0.0421	0.0199	0.042	0.0427	0.043	0.0425	0.269	0.268
Recovered %	97.91	97.79	97.67	98.12	99.99	98.71	99.72	99.24
R.S.D. % of recovery	2.91	1.44	3.03	1.63	3.10	2.61	2.51	2.75
Error %	2.09	2.45	2.32	0.69	0	1.16	0.37	0.74

[a] Each value is the mean from five experiments

[b] The values in parenthesis are corresponding to the theoretical values of t and F at (P=0.05)

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The following paper presents attractive methods for the determination of two antifungal agents, ketoconazole and ciclopirox olamine. The recommended procedures are based on oxidation of the said compounds on a new electrode material, boron-doped diamond electrode. The properties of the BDD electrode and the usage of the sensitive SWV technique facilitated the development of simple and sensitive and accuracy procedures intended to determine the studied compounds in various pharmaceutical cosmetics forms.