Int. J. Electrochem. Sci., 11 **(2016)** 1676 - 1690

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Determination of Ketoconazole on a Gold Disk Microelectrode

O. Gładysz[1,*], *P. Łoś*[2], *B. Karolewicz*[3], *A. Górniak*[4]

[1] Department of Inorganic Chemistry, Wroclaw Medical University, Borowska 211a, 50-556 Wroclaw, Poland
[2] Industrial Chemistry Research Institute, Rydygiera 8, 01-793 Warsaw, Poland
[3] Department of Drug Form Technology, Wroclaw Medical University, Borowska 211a, 50-556 Wroclaw, Poland
[4] Laboratory of Elemental Analysis and Structural Research, Wroclaw Medical University, Borowska 211a, 50-556 Wroclaw, Poland
[*]E-mail: olimpia.gladysz@umed.wroc.pl; gladysz.olimpia@gmail.com

Received: 3 November 2015 / *Accepted:* 4 December 2015 / *Published:* 1 January 2016

The oxidation of ketoconazole (an antifungal agent and strong CYP3A inhibitor) was investigated using a gold disk microelectrode of 25μm in diameter. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were applied as electrochemical techniques. The stability of the gold microelectrode was tested by CV and A.C. impedance methods. Also, diffusion coefficients were determined. The influence of pH on the electroanalytical response was studied. It was found that voltamperometric responses are selective for ketoconazole and the linear equations of the peak current versus ketoconazole concentration were obtained for pharmaceutical preparations: tablets, shampoo and cream. The advantages of the use of microelectrodes include the possibility of miniaturization, very low currents in the range of 10^{-9}A and simplicity of sample preparation which may help to develop an analytical method. To our knowledge, the data presented here represent the first systematic studies of ketoconazole oxidation using microelectrodes.

Keywords: ketoconazole oxidation; gold disk microelectrode; diffusion coefficients

1. INTRODUCTION

The antifungal agent known as ketoconazole (cis-1-acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl] methoxy] phenyl] piperazine) acts as an inhibitor of ergosterol biosynthesis. The structure of ketoconazole is presented on Figure 1.

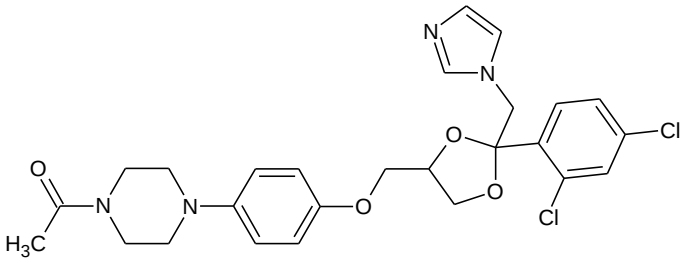

Figure 1. The structure of ketoconazole.

Ketoconazole-associated hepatoxicity is a common side effect of oral treatment [1-4]. Ketoconazole is one of the strongest inhibitors of CYP3A4 in clinical use. Cytochrome P450 3A (CYP3A) catalyzes numerous biotransformations of drugs and ketoconazole may inhibit metabolic pathways causing disadvantageous drug-drug interactions [5]. The Summary of Product Characteristics concerning ketoconazole warns against hepatotoxicity and recommends the monitoring of hepatic function. The risk of serious hepatic toxicity increases with a longer duration of treatment. When a liver dysfunction occurs, it may be necessary to reduce the dose. In order to support the safety of ketoconazole usage, continuous control of ketoconazole concentration during drug administration is recommended. It is known that ketoconazole is absorbed at acidic pH from the digestive tract, so bioavailability (ketoconazole concentration in human plasma) might be studied over the long term. The main hepatic metabolic pathways are the oxidation and degradation of the imidazole and piperazine rings, oxidative O-dealkylation and aromatic hydroxylation. As many as sixteen metabolites have been proposed from rat and human microsomal metabolisms [6] and these can be followed by subsequent metabolic oxidation. Metabolites are mainly excreted through bile and to a limited extent in urine. The level of an unmetabolized drug is a few percentages in urine. Apart from pharmacokinetic studies, the use of microelectrodes may be also beneficial for quality control of pharmaceutical formulations.

According to the European Pharmacopeia, potentiometric titration is recommended as the method of quantitative analysis of ketoconazole [7]. There are numerous papers reporting various methods for determination of ketoconazole in biological and pharmaceutical samples, such as thermogravimetry [8], spectrofluorimetry [9], high-performance thin layer chromatography [10], column liquid chromatography [11] and high-performance liquid chromatography [12,13], together with spectrophotometry [14,15] and capillary electrophoresis [16]. The disadvantage of these methods is their relatively long time of analysis that requires sample pre-treatment, and this makes their on-line application not advisable.

Direct, selective and sensitive methods are based on electrochemical properties. Potentiometry has been employed to improve ketoconazole determination and selective potentiometric electrodes have been developed to analyze pharmaceutical preparations [17,18]. Moreover, polarographic and voltamperometric techniques are frequently applied to investigate the electroactivity and dependence of ketoconazole concentrations. Ketoconazole electroreduction has been studied by the polarographic method using a dropping mercury electrode [19,20], hanging mercury drop electrode [21] and silver solid amalgam electrode [22]. Nowadays, the risk of mercury poisoning and its accumulation in the environment limit the widespread application of mercury. Thus the development of mercury-free

methods is very popular. Instead of a mercury electrode, other solid electrodes may be used. Because the polarization range is related to positive potentials, ketoconazole electro-oxidation studies are recommended. There are papers reporting ketoconazole electro-oxidation by voltammetric methods, in which boron-doped diamond [23], glassy carbon [20,24,27,28], multi-walled carbon nanotubes-modified glassy carbon [29], carbon paste [30], platinum [20,24,26] and gold [24,26] electrodes have been applied in different media, buffers and pH conditions. A common feature of the above-mentioned electrodes was their conventional size, for example the diameter in the range of a few millimeters.

A great opportunity for innovation might be to minimize the diameter of the electrode to micrometers. When the dimension of the electrode is within a μm range, the size of the diffusion layer formed during voltammetric measurements is larger than the characteristic small dimension of a microelectrode [31]. This critical dimension is defined in aqueous solutions differently, as smaller than 50 μm [32], 25 μm [33] or 20 μm [31]. The term "ultramicroelectrodes" [34-38] or "microelectrodes" [39-41] is applied when the characteristic dimension of an electrode is smaller than the thickness of the diffusion layer [33] and a steady state is achieved after a sufficient time dependent on the surface area of the electrode [41]. In comparison to conventional-size electrodes, the diffusion layer at the micro-disk of a microelectrode is not flat for long periods. For a micro-disk it becomes hemispherical (convergent) after sufficiently long periods. The mass transport to the electrode is intensified and new features are demonstrated: much smaller currents in the range of nA, increased current density, lower ohmic drops, higher ratio of faradaic current to capacitance current and improvement in signal to noise ratio [42]. The novel application of microelectrodes refers to *in vivo* [43-47] measurements due to small currents, the possibility of device miniaturization and very short measurement time. Alternative *in vitro* methods, based on microelectrodes, have been developed in neurochemistry and neurotoxicology [48-54]. A small sample volume is not a limitation any more. Another application might be voltammetry in resistive media with no supporting electrolyte [55,56]. Also, large concentrations or even pure substances [57,58] are possible can be examined. Microelectrodes integrated in an array have been used in testosterone [59], oxygen [60], dopamine [61-63] and heavy metal detection [32,64]. The above mentioned microelectrode advantages brought possibility to apply gold microelectrodes for drug determination, for example bisacodyl [65], sodium valproate [66], nortriptyline [67], lidocaine [68], ketotifen [69], erythromycin [70], metformin [71], diphenhydramine [72] and tamoxifen [73].

This paper presents a study on testing of ketoconazole oxidation using a gold disk microelectrode at different pH and measurement parameters. The ketoconazole concentration dependence was analyzed for pharmaceutical products such as tablets, cream and shampoo. Voltammetric methods such as cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were applied.

2. EXPERIMENTAL

2.1. Electrodes

Almost all experiments were conducted using a three-electrode configuration at ambient temperature (23°C). A gold disk microelectrode of a 25μm diameter was employed as the working

electrode. The reference electrode was an Ag/AgCl electrode and a platinum plate with a surface of about 0.3cm^2, acting as an auxiliary electrode. For the two-electrode configuration, the platinum plate acted as both the auxiliary and the reference electrode. Because the plate has a large surface in comparison to the disk-shaped microelectrode ($4.9 \cdot 10^{-6}$ cm^2), it can be assumed that such an electrode did not undergo polarization. The two-electrode electrochemical cell was tested because simplicity and easy-of-use might be an important factor in any prospective application. The application of a two-electrode device may cause fewer problems in terms of its operation by untrained workers in the future commercial applications. A glassy carbon electrode (the diameter of 3mm) was used to compare the repeatability of CV measurements.

2.2. Apparatus

Voltamperometric measurements were carried out using the AUTOLAB GSTST30 system (EcoChemie), and General Purpose Electrochemical System version 4.5 was the program used for data acquisition. Adjustment of pH values was carried out using a pH-meter (Beckman ϕ720) equipped with a glass combined electrode. A.C. impedances were recorded using the frequency response analyzer (FRA) which was a module of the AUTOLAB GSTST30 system (Eco Chemie). Frequency Response Analyzer version 2.4 was the program used for data acquisition and analysis.

2.3. Reagents and solutions

Reagent grade ketoconazole was donated by the Hasco Lek pharmaceutical company (Poland) as a gift sample. The stock solution of ketoconazole was prepared via dissolving in 99.8% ethanol and was stored at 4°C to avoid degradation. A Britton-Robinson buffer (mix of 0.04M H_3BO_3, H_3PO_4, CH_3COOH) was used as the supporting electrolyte and the desired pH value was adjusted by adding 1M NaOH. Ethanol was used to improve ketoconazole solubility. During the first measurements at pH=2, the Britton-Robinson buffer was sufficient to dissolve ketoconazole, and ethanol was not necessary.

Ketoconazole in tablets (Ketokonazol 200mg, Polfarmex S.A., Poland), shampoo and cream (Nizoral 20mg/g, McNeil Products Ltd., Great Britain) was purchased as commercial pharmaceutical formulations.

Suitable amounts of powdered tablets were dissolve in ethanol, vigorously stirred and shaken to quantitatively extract the drug into solution. This was filtered afterwards. The stock solution of ketoconazole (10^{-2} mol dm^{-3}) was diluted using the Britton-Robinson buffer and ethanol. As a result ethanol-buffer solutions (1:4) were prepared. The same procedure was followed in the case of cream.

2.4. Procedure

The microelectrode was mechanically polished with wet alumina powder of a grain diameter of 0.3 μm and 0.05 μm (Escil, France) and washed with water. For the purposes of this paper,

measurements were carried out three times during the quantitative analysis and the average currents were used for the calculations. The scan rate during cyclic voltammetry (CV) was 0.1V s^{-1}. During differential pulse voltammetry (DPV), optimum parameters were chosen: a modulation amplitude of 0.05 V and a scan rate of 0.02 V s^{-1}. Impedances were recorded from 3 kHz to 22Hz and a sinusoidal wave with the amplitude of 10mV was applied.

3. RESULTS AND DISCUSSION

3.1. Cyclic voltammetry

Ketoconazole oxidation in the Britton-Robinson buffer (pH=2) was analyzed by cyclic voltammetry (CV) for the positive potential range of 0.4-1.7V (Fig.2). During the anodic cycle, a well-formed oxidation wave was observed. Above 1.4V, a weak wave is linked to the subsequent oxidation process. A further potential sweep towards a positive potential is not recommended because current values increase rapidly due to possible (surface) gold dissolution and possible subsequent reactions of such oxidation products with ketoconazole. On the cathodic reverse curve at potential 0.8 V, a reduction peak of oxidation products is observed. Forward and backward scans do not retrace. Both the oxidation wave and reduction peaks are concentration dependent in a 10^{-3}-10^{-4} mol dm^{-3} range.

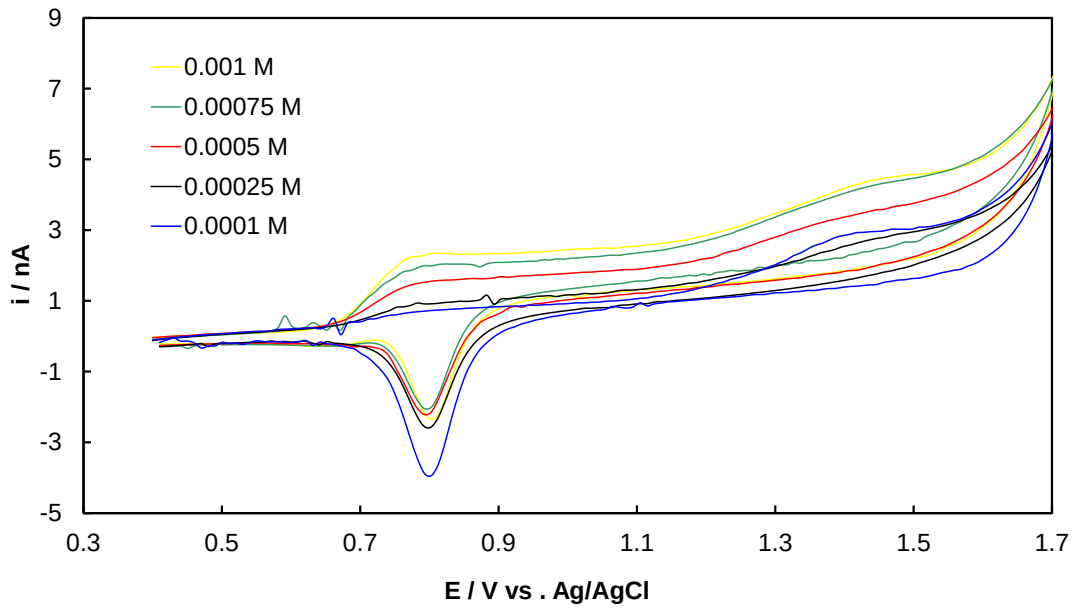

Figure 2. Cyclic voltammograms of varying ketoconazole concentrations (10^{-3}-10^{-4} mol dm^{-3}). Au electrode (ϕ 25μm), buffer (pH 2), scan rate 0.1V s^{-1}.

Tomes criterion was used to check the reversibility of the ketoconazole electro-oxidation wave:

$$\left|E_{3/4} - E_{1/4}\right| = 56.4n \qquad (1)$$

where $E_{3/4}$ and $E_{1/2}$ are potentials for i/i_{dif} =0.75 and i/i_{dif} =0.25 respectively, i_{dif} is a diffusional current, n is the number of electrons [33].

In the case of the voltammogram for 0.001 mol dm^{-3} ketoconazole (Fig. 2), a graphically determined half-wave potential $E_{1/2}$ equals 712mV and a calculated value of $|E_{3/4} - E_{1/4}|$ equals 60.4mV. This may indicate a slight kinetic limitation to maintain electrochemical reversibility. In the case of reversibility the slope should equal 59.1 mV and the intercept should be a half-wave potential $E_{1/2.}$ Estimated values 59.8 and 705 mV are slightly higher than the reversibility criterion. Ketoconazole oxidation under those experimental conditions seems to be very close to a reversible (Nernstian) system. According to the literature, the mechanism of ketoconazole oxidation on a solid conventional electrode in chloroform [24] or acetonitrile[26] is reversible and diffusion-controlled. One electron is exchanged and a radical cation is formed:

$$KC \xleftrightarrow{\pm e} KC^{\cdot +} \quad (2)$$

The radical cation follows chemical reactions to form stable products:

$$KC^{\cdot +} \longrightarrow P_1 \quad (3)$$

Ketoconazole can undergo further irreversible oxidation:

$$KC^{\cdot +} \xrightarrow{-e} P_2 \quad (4)$$

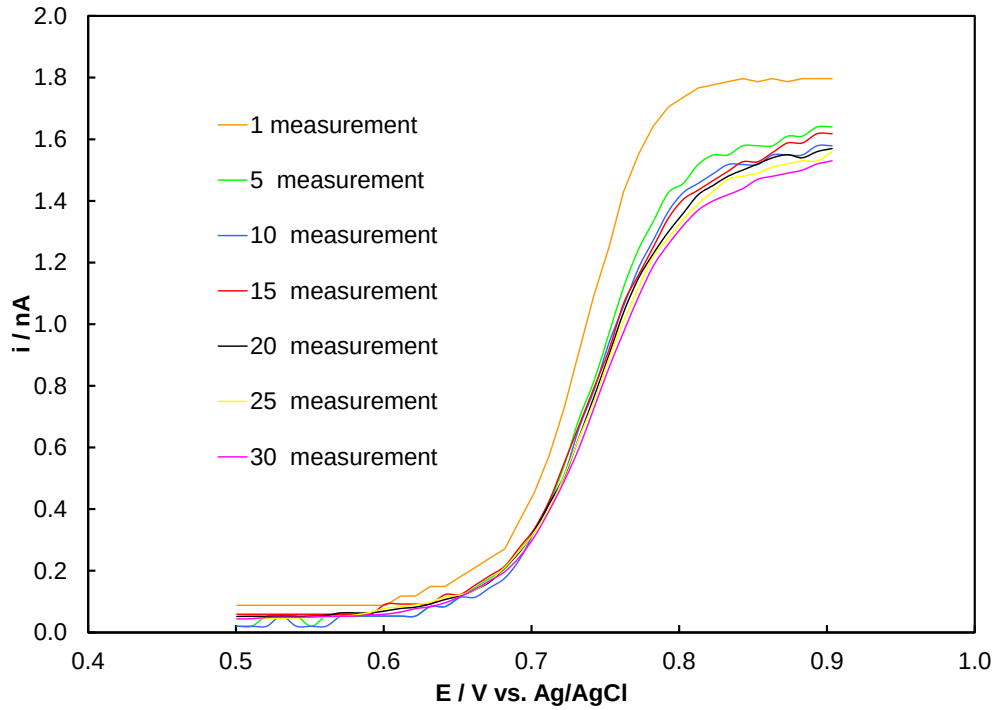

Figure 3. The repeatability of CV curves without the polishing the Au electrode (ϕ 25μm) between measurements. C_{KC}=7·10^{-4} mol dm^{-3}, buffer (pH 2), scan rate 0.1V s^{-1}.

Possible products of Eq. (3) and (4) might be similar in structure to natural ketoconazole metabolites [24] because an *in vivo* ketoconazole biotransformation is oxidation [74]. On the other hand, according to the study of ketoconazole oxidation on solid, glassy carbon electrode in phosphate buffer in 20% ethanol [20] or ammonia buffer in water [27], the process is irreversible. The

electroactivity is probably caused by the presence of methoxyl residues [20,27]. When a glassy carbon electrode was used, the strong adsorptive properties were observed in ammonia buffer in water-ethanol (1:1) [25] and in phosphate buffer in 20% ethanol [20].

Our results of cyclic voltammetry (CV) indicate that there is no adsorption of ketoconazole oxidation products on a gold microelectrode. Similarly, there was no adsorption indicated on conventional gold electrodes [24,26]. The best method to confirm such an observation is an A.C. impedance investigation. Electrochemical Impedance Spectroscopy (EIS) enables an *in situ* study of the electrode-electrolyte interface during CV tests. The stability of a gold microdisk electrode are presented on Figure 3. The electrode was polished only before the first measurements.

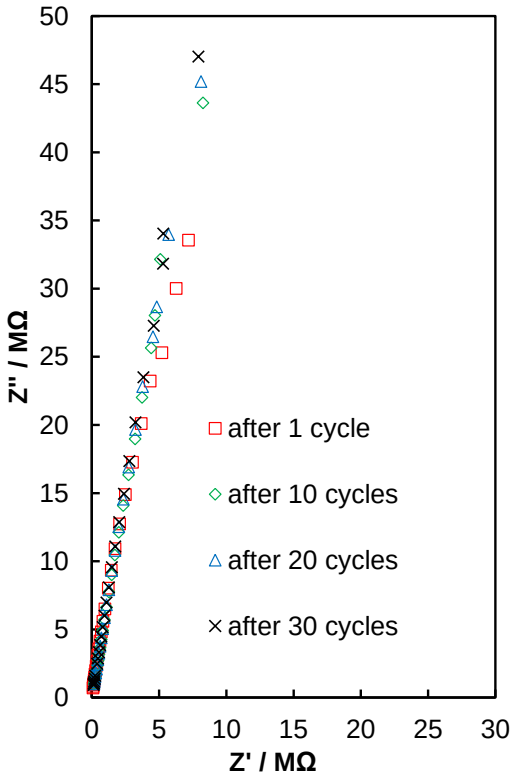

Figure 4. The Nyquist graphs registered after 1, 10, 20, 30 CV cycles of ketoconazole oxidation. Au electrode (ϕ 25µm), $C_{KC}=7\cdot10^{-4}$ mol dm^{-3}, buffer (pH 2), 3kHz-22Hz, amplitude 10mV.

The Nyquist graphs registered after the first, tenth, twentieth and thirtieth of the oxidation cycles (without removing the microelectrode from the solution) are depicted on Figure 4. It is very clear that the A.C. impedance results (Fig.4) are in accordance with the CV results (Fig.3), i.e. the A.C. impedance graphs are practically the same after the tenth, twentieth and thirtieth CV measurements. The only minor difference is observed between the curve registered after the first measurement and the rest. However, such behavior is typical of most solid electrodes immersed in electrolytes. A.C. impedance curves (Fig.4) do not present features typical of adsorption. In fact, they represent an equivalent circuit of simple ohmic resistance (R) and a constant phase element (CPE) connected in series. A detailed description of such A.C. impedance systems may be found in many papers, e.g. [75].

The approximation of the obtained results by an R-CPE equivalent circuit shows that CPE capacitive parameters change for all curves from $1.3 \cdot 10^{-10}$ to $1.6 \cdot 10^{-10}$ F cm^{-2} s CPA^{-1}. A constant phase angle (CPA) equals 0.90 for the A.C. impedance curves registered after 10, 20 and 30 CV cycles, and 0.92 for the A.C. impedance results registered after the first CV cycles. Double layer capacitances of approximately 10 µF cm^{-2} were estimated. The stability of the double layer capacitance during the repeated CV measurements suggests a lack of accumulation of adsorption species during ketoconazole oxidation on the gold microelectrode.

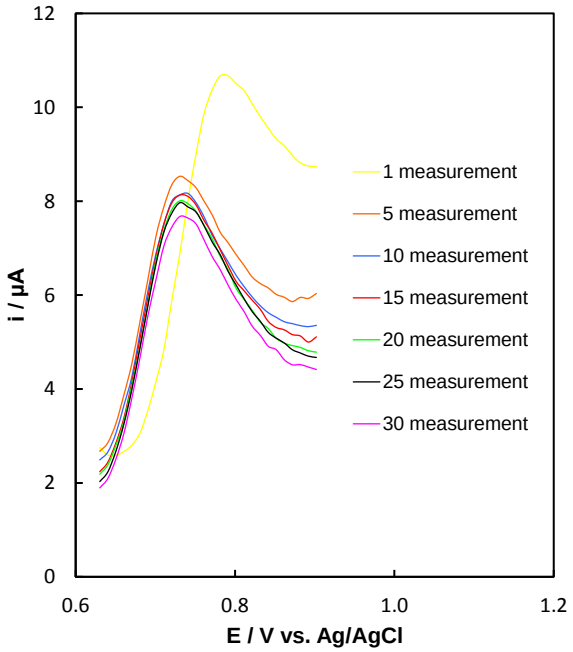

Figure 5. The repeatability of CV curves without the polishin the glassy carbon electrode (ϕ 3mm) between measurements. $C_{KC}=7 \cdot 10^{-4}$ mol dm^{-3}, buffer (pH 2), scan rate 0.1V s^{-1}.

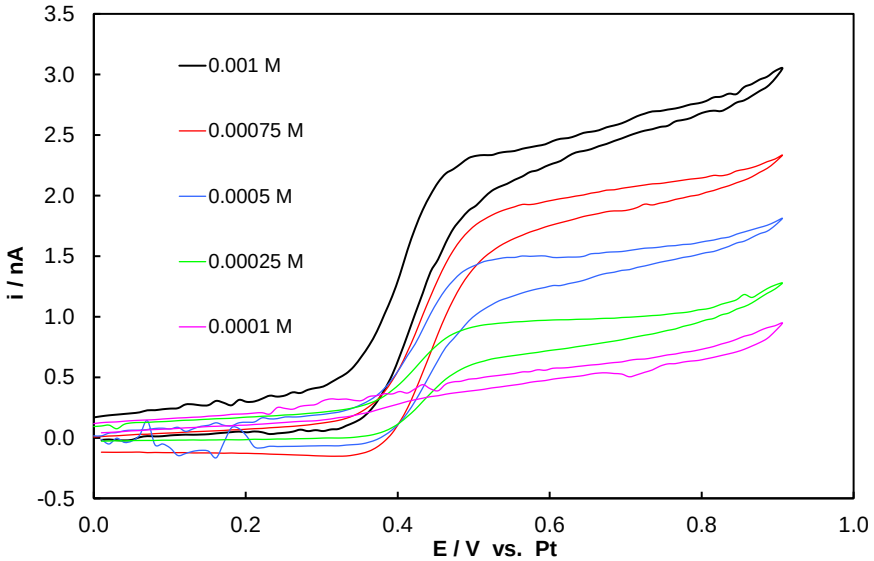

Figure 6. Cyclic voltammograms of varying ketoconazole concentrations(10^{-3}-10^{-4} mol dm^{-3}) . Au electrode (ϕ 25µm), buffer (pH 2), scan rate 0.1V s^{-1}.

The relative standard deviation (RSD) for CV measurements (Fig.3) equals 3.7%. The RSD was calculated for the 29 currents at 0.9V. The first measurement was not considered. Figure 5 presents the repeatability of CV measurements when a glassy carbon electrode is used. The RSD calculated for 29 peak currents equals 6%. Gold might be considered as the electrode material of choice. In order to check the two-electrode system, cyclic voltammetry measurements were carried out (Fig. 6) in a shorter potential range.

The current-potential curve is sigmoidal in shape and oxidation current values are concentration dependent in the studied range. This is predicted by the theory of steady-state voltammetry [33], where the maximum diffusion-limited current in the case of a disk microelectrode is described by Eq. (5):

$$i_{dif} = 4nFDcr \quad (5)$$

where *n* is the number of exchanged electrons, *F* is the Faraday constant, *D* is the diffusion coefficient, *c* is the electroactive species concentration, *r* is the disk radius. The calculated diffusion coefficients of ketoconazole in Britton-Robinson buffer (pH=2) are $4.9 \cdot 10^{-6}$ cm^2 s^{-1} for $1 \cdot 10^{-3}$mol dm^{-3} ketoconazole, $5.3 \cdot 10^{-6}$ cm^2 s^{-1} for $7.5 \cdot 10^{-4}$ mol dm^{-3}, $6.2 \cdot 10^{-6}$ cm^2 s^{-1} for $5 \cdot 10^{-4}$ mol dm^{-3}, $7.9 \cdot 10^{-6}$ cm^2 s^{-1} for $2.5 \cdot 10^{-4}$ mol $dm^{-3}$and the average value $6.1 \cdot 10^{-6}$ $cm^2 s^{-1}$. The currents were measured at 0.55 V vs. Pt (Fig.6), because the current increases for higher potentials. For lower ketoconazole concentrations, steady-state diffusion-limited currents are difficult to indicate due to a lack of a clear current plateau.

The analysis of ketoconazole reduction by cyclic voltammetry using an electrode made of gold was limited by hydrogen ion reduction at negative potentials which started below -1V under the experimental conditions used in this study. Solid metal electrodes are not recommended for studies at negative potential, especially in a strong acidic solution.

3.2. Differential pulse voltammetry

In the case of a microelectrode, cyclic voltammetric curves are waves but steady-state currents are difficult to define (see Fig. 6). Differential pulse voltammetry (DPV) supports the suppression of the charging currents and DPV measurements give a peaked response instead of a wave. Such a shape is additionally more convenient to interpret quantitatively. For this reason, differential pulse voltammetry (DPV) is more suitable. DPV curves for ketoconazole solutions in a three-electrode system are presented on Figure 7.

In comparison to Figure 2, regular-shaped peaks exhibit at 0.7V. The relationship between ketoconazole concentration and peak current is not linear (the best approximation results are obtained for a quadratic equation). The curves were recorded in Britton-Robinson buffer at pH=2. In order to study the influence of higher pH, ketoconazole solubility in water had to be improved. Therefore, solutions of ethanol-buffer solutions (1:4) were used. The ketoconazole concentration of $2.5 \cdot 10^{-4}$ mol dm^{-3} was tested in a wide range of pH by differential pulse voltammetry (DPV).

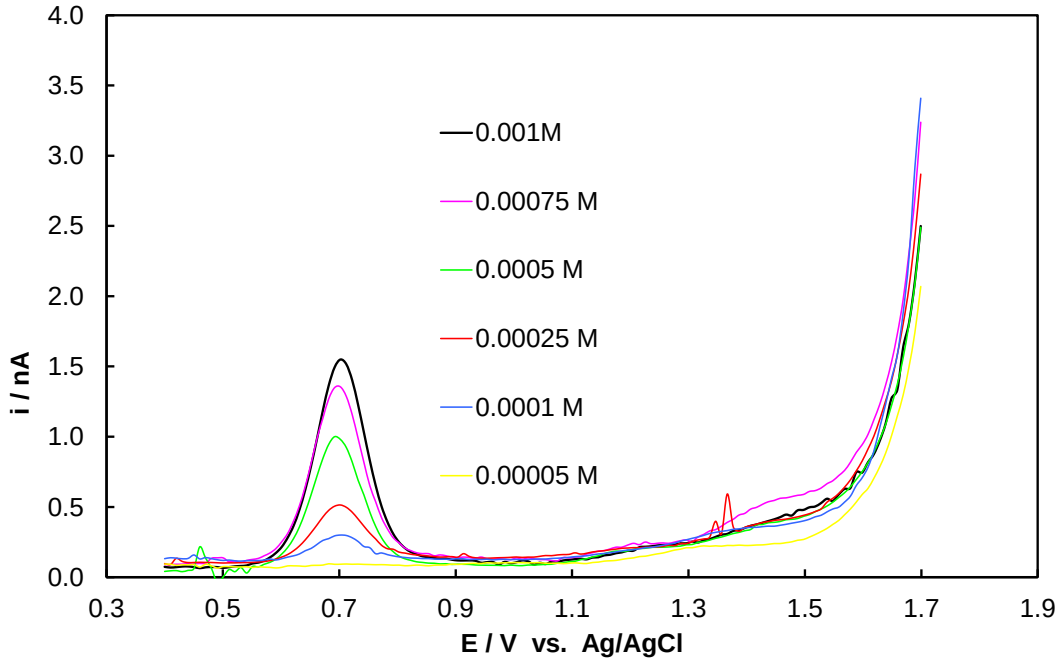

Figure 7. Differential pulse voltammograms of varying ketoconazole concentrations(10^{-3}-$5{\cdot}10^{-5}$ mol dm^{-3}). Au electrode (ϕ 25μm), buffer (pH 2), scan rate 0.02V s^{-1}.

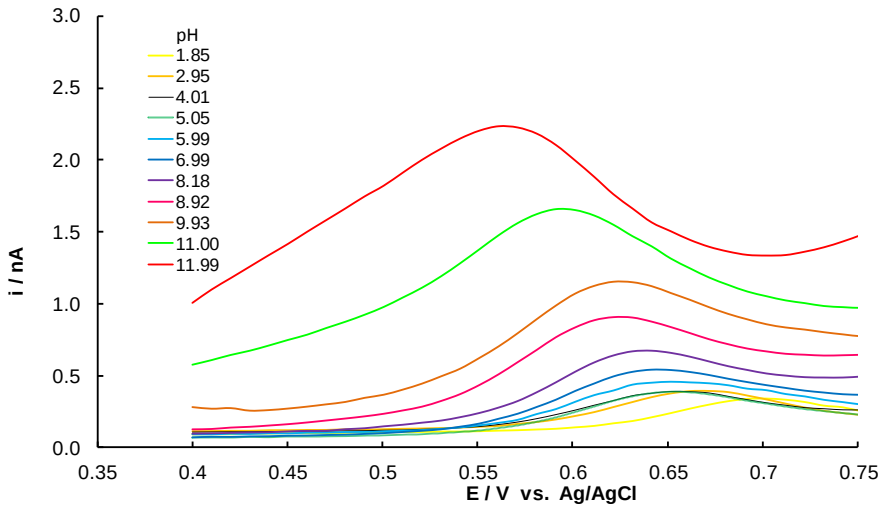

Figure 8. Differential pulse voltammograms of ketoconazole ($2.5{\cdot}10^{-4}$ mol dm^{-3}) for varying pH. Au electrode (ϕ 25μm), ethanol-buffer (1:4), scan rate 0.02V s^{-1}.

As seen in Figure 8, when pH is more basic the maximum of the current increases. Together with increasing pH values, the peak potential is shifted from 0.7V to 0.56 V.

In Figure 9 voltammograms of ethanol – buffer solutions (1:4) at pH=9 are presented. Peak current is directly proportional to ketoconazole concentration in the range of 10^{-3}-$5{\cdot}10^{-5}$ mol dm^{-3}. The calibration curve equation is in Table 1. Although increasing pH caused an increased maximum of

current, when pH was raised to 9, the limit of quantification ($5 \cdot 10^{-5}$ mol dm^{-3} ketoconazole) did not change in comparison to pH=2.

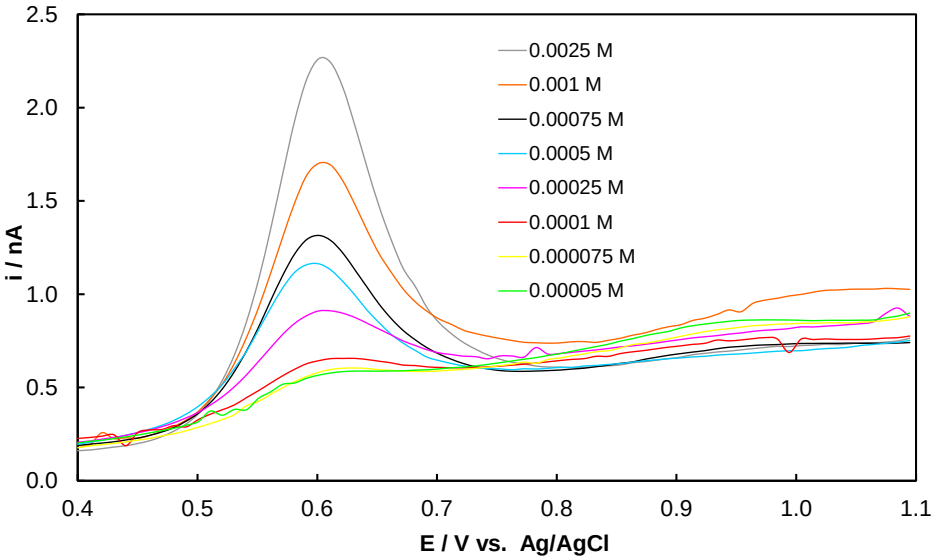

Figure 9. Differential pulse voltammograms of varying ketoconazole concentrations ($2.5 \cdot 10^{-3}$-$5 \cdot 10^{-5}$mol dm^{-3}). Au electrode (ϕ 25µm), ethanol-buffer (1:4), pH 9, scan rate 0.02V s^{-1}.

At pH=9, it is difficult to obtain a clear solution when the declared ketoconazole concentration is more than 10^{-3} mol dm^{-3}. Precipitation is not negligible and for $2.5 \cdot 10^{-3}$ mol dm^{-3} the current value is smaller than it would be predicted by linear approximation.

3.3. Analysis of pharmaceutical formulations

The ketoconazole solutions of shampoo were analyzed by differential pulse voltammetry (DPV). Other ingredients of shampoo are: sodium lauryl ether sulfate (SLES), disodium monolauryl ether sulfosuccinate, coconut fatty acid diethanolamide, laurdimonium - hydrolyzed animal collagen, macrogol 120 methyl glucose dioleate, sodium chloride, hydrochloric acid, imidurea, sodium hydroxide, and erythrosine sodium. Due to the addition of an anionic detergent and surfactant (SLES), ketoconazole in shampoo is easily soluble in the Britton-Robinson buffer. The amount of 0.01g, 0.1g, 0.5g, 1g, 2g, 3g, & 4g of shampoo was dissolved to obtain ketoconazole concentrations of $1.5 \cdot 10^{-5}$, $7.5 \cdot 10^{-4}$, $1.5 \cdot 10^{-4}$, $3 \cdot 10^{-3}$, $4.5 \cdot 10^{-3}$, & $6 \cdot 10^{-3}$ mol dm^{-3}. The shampoo composition is complex, but the peak at about 0.68-0.7 V is selective for ketoconazole, in the studied concentration range. The shapes of voltamperograms are similar to those registered in solutions. For such shampoo solutions the relationship between the peak current and ketoconazole concentration is not linear. In order to avoid possible influences of various excipient concentrations, the solutions of a fixed amount of shampoo and extra ketoconazole additions were prepared in the ethanol-buffer solutions. The relationship between the peak current and ketoconazole concentration is linear (Table 1).

Table 1. Calibration curve equations for ketoconazole in its formulations. The RSD of peak currents below 2%.

Method: DPV	Calibration equation I(nA) C(mol·dm^{-3})	R^2	Linearity range (mol·dm^{-3})	E_{peak} (V) vs. Ag/AgCl
solutions	i=1116.1C+0.5514	0.9924	$5\cdot10^{-5}$-$1\cdot10^{-3}$	0.601-0.632
shampoo	i=986.29C+0.2357	0.9928	$1\cdot10^{-4}$-$1\cdot10^{-3}$	0.692-0.693
	i=963.95C+0.2461	0.9989	$1\cdot10^{-4}$-$2.5\cdot10^{-3}$	0.692-0.823
tablets	i=822.08C+0.1521	0.9829	$5\cdot10^{-5}$-$1\cdot10^{-3}$	0.712-0.722
	i=861.78C+0.1389	0.9968	$5\cdot10^{-5}$-$2.5\cdot10^{-3}$	0.712-0.722
cream	i=676.46C+0.1781	0.9734	$5\cdot10^{-5}$-$1\cdot10^{-3}$	0.702-0.732
	i=795.44C+0.1385	0.9918	$5\cdot10^{-5}$-$2.5\cdot10^{-3}$	0.702-0.732

When the concentration of ketoconazole is above 10^{-3} mol dm^{-3}, the solution is observed to become slightly cloudy and less foaming. The linear regression for a wider range of ketoconazole concentration seems to differ insignificantly in comparison to a smaller concentration range where solutions are entirely clear.

In the case of tablets, other ingredients are: lactose monohydrate, corn starch, povidone, microcrystalline cellulose, colloidal anhydrous silica and magnesium stearate. For ketoconazole in ethanol-buffer solutions (1:4), regular-shaped peaks are obtained and the relationship between the peak current and ketoconazole concentration is linear (Table 1). When the ketoconazole concentration is above 10^{-3} mol dm^{-3}, opalescence is observed. However, linear regression for a wider range of ketoconazole concentration differs insignificantly from the smaller concentrations range, where solutions are clear.

Cream containing ketoconazole also consists of: propylene glycol, stearyl alcohol, cetyl alcohol, sorbitan stearate, polysorbate 60, polysorbate 80, isopropyl myristate and sodium sulfite. For ketoconazole in ethanol-buffer solutions (1:4), the relationship between the peak current and ketoconazole concentration is linear (Table 1).When the ketoconazole concentration equals $2.5\cdot10^{-3}$ mol dm^{-3}, the consistency of gel is revealed. There are visible differences between the two trend equations of $5\cdot10^{-5}$-$2.5\cdot10^{-3}$ and $5\cdot10^{-5}$-10^{-3} mol dm^{-3} ketoconazole. The gel formation implies a change of diffusion coefficients and viscosity, which may influence the peak current of ketoconazole oxidation.

In comparison to other papers, although a conventional-size gold electrode was tested for ketoconazole oxidation in papers [24,26] it was not used for quantitative determination of ketoconazole. The CV method and a glassy carbon electrode was used for ketoconazole determination in the range of 10^{-5}-$5\cdot10^{-4}$ mol·dm^{-3} in 20% ethanol [20]. Adsorptive stripping voltammetry and a glassy carbon electrode was used in the range of 10^{-6}-10^{-10} mol·dm^{-3} in ethanol-water solution (1:1) [25]. Square wave voltammetry and a glassy carbon electrode was used in the range of $3.2\cdot10^{-7}$-$9.58\cdot10^{-6}$ mol·dm^{-3} in ammonia buffer [27]. DPV and linear sweep voltammetry using carbon paste

electrode were methods for determination in the range $2.4 \cdot 10^{-8}$-$4.8 \cdot 10^{-7}$ mol·dm^{-3} and $9.1 \cdot 10^{-7}$- 10^{-5} mol·dm^{-3} respectively, in buffer solutions [30].

There are lower detection limits and concentration ranges in papers [20,24,25,27,30] and preconcentration steps are required for methods in papers [25,30]. The calibration curves in the present paper are the first where a gold electrode is used for quantitative analysis of ketoconazole.

4. CONCLUSIONS

Ketoconazole electrooxidation described by Eq.(2) on a gold disk microelectrode during a differential pulse voltammetric measurement results in a well-formed peak of the current. Ketoconazole electro-oxidation on a gold microelectrode is concentration dependent.

An analysis of the relationship between the peak current and ketoconazole concentration revealed DPV as the appropriate method for ketoconazole determination in pharmaceutical preparations (shampoo, tablets, cream) and in ethanol-buffer solutions (1:4) at pH 9. Studies of pharmaceutical preparations showed that a complex composition of excipients does not interfere with the voltammetric determination. Further investigation of a lower detection limit, measurement optimization, electrolyte composition and solubility improvement might be beneficial. However, the equations of current-concentration relationships concern a relevant concentration range for the quality control of these commercial drugs. As regards ketoconazole in human plasma, its concentration has been reported as 94 μmol dm^{-3} after oral administration [76]. In the case of ketoconazole in ethanol-buffer solutions at pH=9, the linear range is $2.5 \cdot 10^{-3}$-$5 \cdot 10^{-5}$ mol dm^{-3} (Fig.9). The preliminary linear range includes this ketoconazole concentration after absorption into blood.

In conclusion, to our knowledge the present study is the first investigation of ketoconazole oxidation using microelectrodes. Voltamperometric responses are selective for ketoconazole under the present experimental conditions. Ketoconazole determination by DPV is selective in complex pharmaceutical formulations. The combination of all the advantages of microelectrodes and voltammetry such as the possibility of miniaturization due to the disk radius in μm, very small currents in the range of 10^{-9}A, non-toxic materials, non-time-consuming measurements and very simple sample preparation, may create a promising tool for an *in vitro* and *in vivo* analysis.

References

1. Y.J. Ying, N.X. Lu and T.Q. Mei, *Biomed. Environ. Sci.*, 26 (2013) 605
2. A. K. Gupta and Lyons D. C. A., *J Cutan. Med. Surg*., 19 (2015) 352
3. A. K. Gupta, D. Daigle and K. A. Foley, *Expert Opin. Drug. Saf*., 14 (2015) 325
4. H. K. Greenblatt and D. J. Greenblatt, *J Clin. Pharmacol*., 54 (2014) 1321
5. S.V. Mandlekar, A.V. Rose, G. Cornelius, B. Sleczka, C. Caporuscio, J. Wang, and P.H. Marathe, *Xenobiot.*, 37 (2007) 923
6. W. L. Fitch, T. Tran, M. Young, L. Liu and Y. Chen, *Drug Metab. Lett.*, 3 (2009) 191
7. European Pharmacop. Online 8.0, 8th Edition (2014)

8. B. Gomes, A.P. Correia, L. Pinto, S. Simoes and M. Oliveira, *J. Therm. Anal. Calorim.*, 91 (2008) 317
9. F. Jalali, A. Afshoon and M .Shamsipur, *Chemia Analityczna*, 52 (2007) 115
10. S. Suwanna, L. Boonsom and L. Saisunee, *J. Cosmet. Sci.*, 61 (2010) 367
11. P. Nirali, V.V. Kumar, S. Ramakrishna, *Chromatogr.*, 71 (2010) 941
12. P.M.A. Lima, N.D. Prado, M.L.M. Silva, R.D.L. Diniz, K.M. Queiroz, I.D. Cesar, G.A. Pianetti and D.A. Santos, *J. AOAC Int.*, 92 (2009) 1076
13. D.A Hamdy and D.R. Brocks, *J. Pharm. Biomed. Anal.*, 53 (2010) 617
14. E.R.M. Kedorhackmann, M.M.F. Nery and M. I.R.M. Santoro, *Anal. Lett.*, 27 (1994) 363
15. S. M. Fraihat and K. M. Bahgat, *Trop. J. Pharm. Res*., 13 (2014) 1511
16. M. Castro-Puyana, C. Garcia-Ruiz and A. Cifuentes, *J. Chromatogr. A*, 1114 (2006) 170
17. K.K. Girish, A. Pearl and J. Sareena, *Anal. Lett.*, 41 (2008) 1144
18. M. Shamsipur and F.Jalali, *Anal. Sci.*, 16 (2000) 549
19. Z. Fijałek, J. Chodkowski and M. Warowna, *Acta Poloniae Pharm.*, 49 (1992) 1
20. K. Sarna and Z. Fijałek, *Acta Poloniae Pharm. - Drug Res.*, 53 (1996) 163
21. P. Arranz, A. Arranz, J.M. Moreda, A. Cid and J. Arranz, *J. Pharm. Biomed. Anal*., 33 (2003) 589
22. A.N. de Sousa Dantasa, D. de Souzaa, J.S. de Limab, P. de Lima-Netoa and A.N. Correia, *Electrochim. Acta*, 55 (2010) 9083
23. K. Mielech-Lukasiewicz and K. Roginska, *Anal. Metod*, 6 (2014) 7912
24. M. Shamsipur and K. Farhadi, *Electroanal.*, 12 (2000) 429
25. T. Peng, Q. Cheng and C.F. Yang, *Fresenius J. Anal. Chem*., 370 (2001) 1082
26. M. Shamsipur and K. Farhadi, *Chem. Anal.*, 46 (2001) 387
27. K. Mielech-Łukasiewicz, H. Puzanowska-Tarasiewicz and A. Niedzielko, *Anal. Lett*., 44 (2011) 955
28. H. Knoth, G. K. E Scriba and B. Buettner, *Pharm*., 70 (2015) 374
29. J. Borowiec, L. Wei and L. Zhuet, *Anal. Met*., 4 (2012) 444
30. M. Shamsipur and K. Farhadi, *Analyst*, 125 (2000) 1639
31. J. Heinze, *Angew. Chem. Int. Ed. Engl*., 30 (1991) 170
32. G. Herzog and V. Beni, *Anal. Chim. Acta*, 769 (2013) 10
33. C.G. Zoski, *Electroanal.*, 14 (2002) 1041
34. R.M. Wightman, *Science*, 240 (1988) 415
35. R.M. Wightman and D.O. Wipf, Voltammetry at Ultramicroelectrodes, in: A.J. Bard (Ed.), *Electroanalytical Chemistry*, Vol.15, Marcel Dekker, New York (1988)
36. M. Fleishmann, S. Pons, D.R. Rolison and P.P Schmidt, *Ultramicroelectrodes*, Datatech Systems Inc, Morganton (1987)
37. J. Heinze, *Angew. Chem Int Ed Engl*, 32 (1993) 1268
38. L. Danis, D. Polcari and A. Kwan, *Anal. Chem*., 87 (2015) 2565
39. M.I. Montenegro, M.A. Queiros and J.L. Daschbach (Eds.), *Microelectrodes: Theory and Applications*, NATO ASI Series, Series E: Applied Sciences, Vol. 197, Kluwer Academic Publishers, Dordrecht (1991)
40. B.R. Scharifker, *Microelectrode Techniques in Electrochemistry*, in: J.O'M. Bockris (Ed.), No.22, Plenum, New York (1992)
41. K. Stulik, Ch. Amatore, K. Holub, V. Marecek and W. Kutner, *Pure Appl. Chem*., 72 (2000) 1483
42. Z. Stojek, *Mikrochim. Acta II* (1991) 353
43. J. Suzuki, N. Ozawa, Y.L. Murashima, T. Shinba and M. Yoshii, *Brain Res.* 1460 (2012) 63
44. M.A. Makos,Y.Ch. Kim, K.An. Han, M.L. Heien and A.G. Ewing, *Anal. Chem*., 81 (2009) 1848
45. T. Mishima, S. Sakatani, H. Hirase, *J. Neurosci. Method.*, 166 (2007) 32
46. B.M. Dixon, J.P. Lowry and R.D. O'Neill, *J. Neurosci. Method.*, 119 (2002) 135
47. A. Suzuki; A.T. Ivandini, K.Yoshimi, A. Fujishima, G. Oyama,T. Nakazato; N. Hattori; S. Kitazawa and Y. Einaga, *Anal. Chem*., 79 (2007) 8608

48. H.T. Hogberg, T. Sobanski, A. Novellino, M. Whelan, D.G. Weiss and A.K. Bal-Price, *NeuroToxicol.*, 32 (2011) 158
49. J.J. Burmeister and G.A Gerhardt, Neurochemical arrays, in: C.A Grimes, E.C. Dickey, M.V Pishko (Eds.), *Encyclopedia of Sensors*, vol.6 (2006)
50. A. Stett, U. Egert, E. Guenther, F. Hofmann, T. Meyer, W. Nisch and H. Haemmerle, *Anal. Bioanal. Chem*., 377 (2003) 486
51. J.T. Cox, Ch.G. Gunderson and B. Zhang, *Electroanal.,* 25 (2013) 2151
52. N. Vasylieva; S. Marinesco and D. Barbier, *Biosens. Bioelectron*., 72 (2015) 148
53. Z. Fekete, *Sens. Actuators B – Chem*., 215 (2015) 300
54. M. E. J. Obien, K. Deligkaris and T. Bullmann, *Front. Neurosci*., 8 (2015) 423
55. A. M. Bond, *Analyst*, 119 (1994) R1
56. L. Kiss, G. Nagy and B. Kovacs, *Electroanal*., 24 (2012) 2117
57. M.A. May, K.V. Gupta and K. Hounsokou, *Rev. Sci. Inst*., 71 (2000) 516
58. M. Ciszkowska, Z. Stojek and J. Osteryoung, *Anal. Chem.*, 62 (1990) 349
59. O. Laczka , F.J. del Campo , F.X. Muñoz-Pascual and E. Baldrich, *Anal. Chem*., 83 (2011) 4037
60. A. Moya, X. Guimera and F. J.del Campo, *Microchim. Acta*, 182 (2015) 985
61. A. M. H. Ng, L. Kenry and T. Chwee, *Biosens. Bioelectr*., 65 (2015) 265
62. C. Lete, S. Lupu and B. Lakard, *J. Electroanal. Chem*., 744 (2015) 53
63. S. Lupu, C. Lete and F. Javier del Campo, *Electranal*., 27 (2015) 1649
64. O. Ordeig, J. del Campo, F. Xavier Munoz, C.E. Banks and R.G. Compton, *Electroanal.* 19 (2007) 1973
65. P. Daneshgar, P. Norouzi and M.R. Ganjali, *Sens. Actuators B* 136 (2009) 66
66. P. Norouzi , S. Shirvani-Arani, P. Daneshgar and M.R. Ganjali, *Biosens. Bioelectron*. 22 (2007) 1068
67. P. Norouzi, R. Ganjali, S. Shirvani-Arani and A. Mohammadi, *J. Pharm. Sci.* 96 (2007) 893
68. P. Norouzi, M.R. Ganjali , P. Daneshgar , R. Dinarvand , A.A. Moosavi-Movahedi, A.A. Saboury, *Anal. Chim. Acta*, 590 (2007) 74
69. P. Daneshgar, P.Norouzi and M.R. Ganjali, *Chem. Pharm. Bull*., 57 (2009) 117
70. P. Norouzi , P. Daneshgar and M.R. Ganjali, *Mater. Sci. Eng*., C 29 (2009) 1281
71. M.R. Ganjali, P. Norouzi, M. Zare, *Russ. J. Electrochem*., 44 (2008) 1135
72. P. Norouzi, M.R. Ganjali, E. Nouryousefi, S.J. Shahtaheri and R. Dinarvand, *Croatica Chem. Acta*, 83 (2010) 135
73. P. Daneshgar, P. Norouzi, M.R. Ganjali and H.A.Zamani, Talanta 77 (2009) 1075
74. L.W. Whitehouse, A. Menzies, B. Dawson, T.D. Cyr, A.W. By, D.B. Black and J. Zamecnik, *J. Pharm. Biomed. Anal*., 12 (1994) 1425
75. P. Los, A. Rami. A. Lasia, *J. Appl. Electrochem*., 23 (1993) 135
76. R.J. Rodriguez and D. Acosta, *Drug Metab. Dispos*., 25 (1997) 772