Simple Real-time Voltammetric Method for Captopril Determination in Pharmaceutical Formulation

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A novel voltammetric assay for captopril (CAP) determination by using an electrochemically pretreated pencil graphite electrode (PGE) is presented. The electrochemical oxidation reaction of CAP was investigated with PGE* by using cyclic voltammetry and linear sweep voltammetry techniques. CAP was electrochemically inactive at the non-pretreated pencil graphite electrode surface, while a sharp anodic wave with an anodic peak potential at around 200 mV resulted by using the PGE*. According to kinetic studies upon the electrode behavior, a new reaction mechanism for electrochemical oxidation of captopril is proposed. The sensor was examined as a selective, simple and precise new electrochemical disposable electrode for the determination of CAP in pharmaceutical samples in complex medical cases associated with sleep apnea, with good results.*

Keywords: disposable sensor, dopamine, voltammetry, pharmaceutical samples, sleep apnea

Captopril (CAP), 1-(3-mercapto-2-D-methyl-1-oxopropyl) proline, is used as a drug being an orally active inhibitor of the angiotensin-converting enzyme (ACE); by this action it causes artery walls to relax, lowering the blood pressure, improving so the pumping efficiency and cardiac output in patients with heart failure. Accordingly, CAP has been widely used for the treatment of hypertension, congestive heart failure, and left ventricular dysfunction after myocardial infarction [1]. This ACE inhibitor present a thiol group and can take up free radicals in living systems and exhibits antioxidant properties [2-4]. CAP is also sometimes prescribed for decreasing symptoms of cystinuria, reducing rheumatoid arthritis symptoms and treating Raynaud's phenomena [5].

Administering CAP for therapeutic purposes leads to undesirable side effects like a dry, persistent cough [6]. Toxicity from CAP includes bone marrow suppression and proteinuria [7]. In some instances, liver dysfunction and skin yellowing have been reported with captopril administration [5,8].

Like other thiols, CAP undergoes rapid oxidation to disulphide metabolites both in vitro and in vivo where it is eliminated together with unchanged captopril (40–60%) in urine [9]. In solution, CAP undergoes an oxygen facilitated, first order and free radical oxidation at its thiol to yield captopril disulfide. The reaction rate depends on *p*H (oxidation is delayed using lower pH) and oxygen concentration. Its oxidative dimerization to a disulphide is a significant pharmaceutical problem.

The determination of CAP is important in pharmacology and medicine for drug quality control purposes and to monitor patients with cardiovascular diseases (hypertension) or sleep disorders.

The association between sleep apnea syndrome and high blood pressure is well documented, especially with respect to night-time hypertension. Normally, at night, the blood pressure drops, a phenomenon defined as *dipping*. Sleep apnea syndrome (SAS) appears to be responsible for a large number of cases of high blood pressure or no decrease during night time.

Several analytical methods have already been applied for the determination of CAP in pharmaceutical formulations and clinical samples, including gas chromatography [10], high-performance liquid chromatography [3,4,11], colorimetry [2], spectrophotometry [12], chemiluminescence [13], and capillary electrophoresis [14].

Electrochemical techniques represent an alternative to the spectrophotometric and chromatographic methods due to their well-known advantages like simplicity, speed and low cost [15].

Captopril with its thiol group can oxidize at the surface of various electrodes or chemically modified ones and was determinate by different electrochemical methods [5,7,8,11] of which we mention those coupled with linear sweep voltammetric technique [16-19]. Most of the mentioned methods are applied to the determination of captopril in tablet.

Among many other sensors, pencil graphite electrode (PGE) was successfully used in multiple electroanalytical applications due to its unique and extremely useful properties [20].

A survey of the literature reveals that there is no report regarding the linear sweep voltammetric (LSV) determination of CAP using electrochemically pre-treated (activated) pencil graphite electrode (PGE*). Electrodes reported in the literature and coupled with this technique were chemically modified ones. They present some well-known disadvantages of which we mention the possible electrode surface passivation which blocks the electron transfer, and so a cleaning step or renewal of the electrode surface is required before each measurement [20]. By using disposable sensors this necessary step is avoided [21]. A facile LSV method based on a PGE* for CAP determination with a detection limit of 2.34×10^{-5} M is reported. The proposed method applied for CAP determination in real pharmaceutical samples is fast, selective, sensitive, and environmentally friendly.

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Experimental part

Reagents and solutions

Captopril was provided by Sigma-Aldrich. Britton-Robinson buffer (BRB) solution of different *p*H values (1.81 to 6.80) was used as supporting electrolyte. A 1.0 × 10^{-2} M standard stock solution of CAP in bi-distillated water was prepared and further diluted with BRB solution (pH 2.21) to the desired concentrations just before use. Pharmaceutical formulations containing CAP (tablets of 25 mg from Labormed, Romania) were used in order to evaluate the LSV procedure performance.

Apparatus

Cyclic voltammetric (CV) and linear sweep voltammetric (LSV) measurements were carried out with an Autolab PGSTAT 128N (Ecochemie B.V., Netherlands, www.metrohm-autolab.com) linked with a computer using Nova 1.8 software. A typical electrochemical cell configuration of 10 mL volume was used. Pencil graphite (PG) and an electrochemically pre-treated pencil graphite (PG*) were used as working electrodes together with a saturated Ag|AgCl (3.0 M KCl) as the reference and platinum (3 mm disk) as the auxiliary electrodes. PG electrodes were Rotring pencil-leads of different types (B, 2B, HB, H and 2H) with a diameter of 0.5 mm and 6 cm length purchased from local bookstore. The PGEs were prepared and electrochemically pre-treated as described elsewhere [20]. Each voltammetric recording was carried out on a new graphite pencil lead.

Procedures

The potential range used in cyclic voltammetry studies was between -0.2 V to +0.6 V. Under optimized conditions linear sweep voltammograms were recorded in the same potential window for different concentrations of CAP solutions prepared in BRB solution *p*H 2.21. The oxidation peak potential at +0.20 V resulted in LSV method was used for CAP quantitative determination in pharmaceutical formulation.

Preparation and analysis of the pharmaceutical samples

To demonstrate the analytical usefulness of the proposed activated PGE* pharmaceutical samples were analyzed. Five tablets containing CAP were weighted and then pestle in a mortar. For the analysis of pharmaceutical formulations, an accurate amount from this fine powder was transferred to a 25 mL volumetric flask and the volume was completed with distillated water. The solution was filtered through a quantitative Whatman type filter paper. From this solution 1 mL was taken and introduced in a 10 mL volumetric flask and adjusted to the final volume with BRB solution of *p*H 2.21 and analyzed by LSV. No additional treatment of the sample was required. The LSV peak currents of CAP were recorded on the PGE* for diluted samples before and after three consecutive standard additions of 0.1 mL from a 1 × 10^{-2} M CAP stock solution.

Results and discussions

Electrochemical studies

During experiments, PGEs of different types were electrochemically pre-treated by cycling the potential between -0.2 V and +3.0 V in BRB solution of *p*H 2.21, for different number of cycles (10 to 50) at a scan rate of 500 mV·s^{-1}. Typical cyclic voltammograms for the activated electrodes in the presence of a CAP showed an increase of the anodic peak current function of the cycle numbers. By inspecting the CVs presented in figure 1, one can observe that there is no further significative increase in current after a number of 40 cycles.

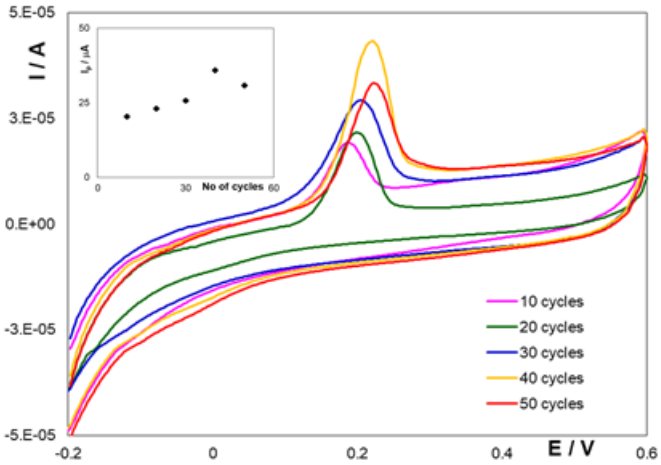

Fig. 1. Cyclic voltammograms of 1 × 10^{-3} M CAP in BRB solution *p*H 2.21 at Rotring HB model PGE* for different number of potential scans; scan rate of 100 mV·s^{-1}. Inset the graph of peak currents vs. number of cycles.

Moreover, as shown in figure 2, the best response was recorded for Rotring HB type.

The Rotring HB type of PGE activated under the mentioned conditions and using 40 cycles of potential scans in BRB solution pH 2.21 were inspected by CV and used in all further electrochemical studies.

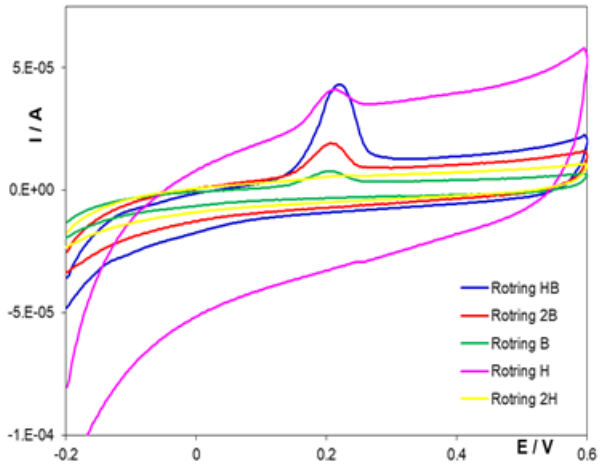

Fig. 2. Cyclic voltammograms for 1 × 10^{-3} M CAP in BRB solution *p*H 2.21 at different types of PGE* after 40 cycles; scan rate of 100 mV·s^{-1}

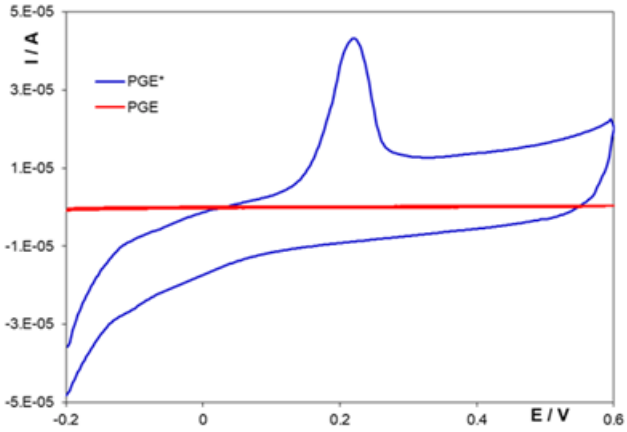

Fig. 3. Cyclic voltammograms of 1 × 10^{-3} M CAP in BRB solution pH 2.21 at PGE and PGE*; scan rate of 100 mV·s^{-1}

Cyclic voltammograms of 1 × 10^{-3} M CAP in BRB solution *p*H 2.21 at PGE and PGE* are shown in figure 3. The CV for non-activated PGE has no electrochemical significance, while in case of PGE* only an oxidation peak potential at

around +0.2 V was observed and no reverse peak; accordingly, CAP electrochemical behaviour at this electrode correspond to an irreversible process. Obviously, the electrochemical activation of the PGE surface (discussed in detail somewhere else) [20] facilitate the redox reaction of CAP at PGE*. A decrease in the oxidation potential for captopril is achieved with PGE* compared with the modified electrodes [5,8,22-25].

Method optimization

The electrochemical oxidation of thiolic compounds involves protons transfer to form a disulphide and thus, the electrochemical behaviour of these compounds can be affected by the solution *p*H. Figure 4 shows that the *p*H value of the supporting electrolyte influenced voltammetric response of CAP at PGE*. The anodic wave associated with CAP oxidation occurred at positive potentials at all used pH values ($1.81 < pH < 6.80$), but shifted to more positive potentials with decreasing pH, indicating the presence of protons in the electrode process. The anodic peak is mediated by the oxygen-containing functional groups of PGE* in the *p*H range of 1.81-6.80 for CAP oxidation. Captopril is a dibasic acid having dissociation constants pk_1 = 3.7 (carboxyl group) and pk_2 = 9.8 (thiol group) [5,26], and its maximum stability was found in acidic solutions below *p*H 4 [8,27]. The anodic peak potential (E_{pa}) increases semi-linearly with the decrease of the solution *p*H. Therefore, in solutions with pH less than 3.7 an inflection point can be observed for variation of E_{pa} function of *p*H (fig. 4) as shown by others also [5,8]. The peak current reaches a maximum in a solution with *p*H 2.21, which is selected as the optimized *p*H value for determination of captopril.

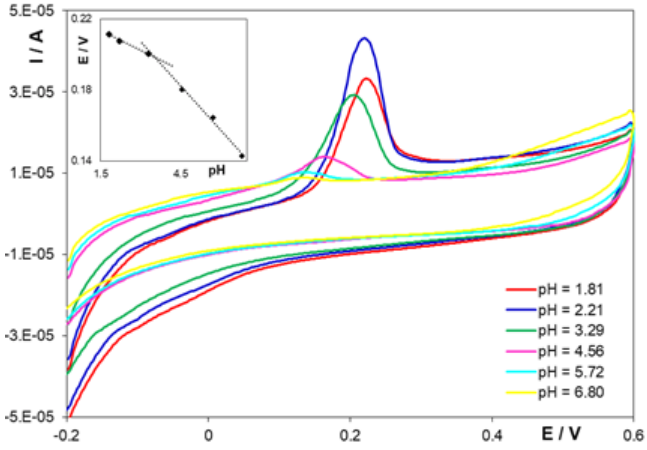

Fig. 4. Cyclic voltammograms for 1·x 10^{-3} M CAP in BRB solution of different *p*H values at PGE*; scan rate of 100 mV·s^{-1}.

Kinetics of the electrode reaction in the presence of CAP was investigated by evaluating the effect of scan rate on the voltammetric profile of CAP. As can be seen from figure 5, by increasing the scan rate the oxidation peak potentials (E_{pa}) shifted positively confirming the kinetic limitation in the electrochemical reaction. The anodic peak current (I_{pa}) increased linearly with the square root of the scan rate (fig. 5), which indicates that the CAP oxidation on the PGE* follows a diffusion-controlled process. Additionally, a plot of log(I_{pa}) vs. log(v) resulted in a straight line with a slope of 0.5174, which is close to the theoretical value of 0.50 for a diffusion-controlled mechanism.

Moreover, the anodic peak potential (E_{pa}) showed a linear relationship with the natural logarithm of scan rate E_{pa} = 0.0400 ln(v) + 0.0459, for scan rates in between 10 and 500 mV·s^{-1}. The slope of this graph was used to calculate

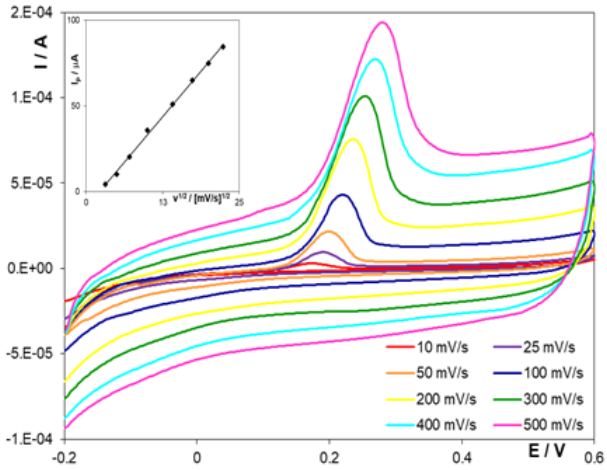

Fig. 5. Cyclic voltammograms of 1 × 10^{-3} M CAP in BRB solution *p*H 2.21 at PGE* for different scan rates. Inset, the graph of peak current vs. scan rate ($v^{1/2}$)

number of electrons involved in the electrode reaction rate-determining step (n) for the irreversible oxidation reaction of CAP according to the Laviron [28] theory, where the E_p is defined as:

$$E_p = E^{0\prime} + \frac{RT}{(1-\alpha)nF}\ln\frac{(1-\alpha)nF}{RTk_{et}} + \frac{RT}{(1-\alpha)nF}\ln v \qquad (1)$$

where k_{et} is the heterogeneous electron transfer rate constant, $E^{0\grave{E}}$ is the formal redox potential, n is the number of electrons involved in an electrode reaction, α is the charge transfer coefficient, v is the scan rate, R, T and F having their usual meaning. Thus, (1-α)n was 0.64. Considering α = 0.5 for an irreversible electrode process, n was estimated as 1, which suggest that one electron transfer process is involved in the rate-determining step of the mechanism for CAP oxidation at PGE*. The results are in good agreement with those presented in the literature [5,23,29]. By extrapolation of the plot of E_p vs. v to the vertical axis at v = 0, an $E^{0\grave{E}}$ value of 0.1458 was estimated. Further, using this $E^{0\prime}$ value, k_{et} was calculated according to eq. (1) from the intercept of the plot of E_{pa} vs. ln(v) and its value was 303.80 s^{-1}.

The overall electrochemical oxidation reaction of CAP at the electrode surface is a well-known two-electron transfer [23,30]:

$$2\,CAPSH \rightarrow CAPSSCAP + 2H^+ + 2e^- \qquad (2)$$

By investigation of the kinetic parameters of the electrochemical oxidation reaction of CAP at the PGE* surface in the pH range of 1.81-6.80, this reaction may occur by the following proposed mechanism:

$$CAPSH + H^+ \leftrightarrow CAPSH^{2+} \qquad (3)$$

$$CAPSH^{2+} \rightarrow CAPH^+S\bullet + H^+ + e^- \qquad (4)$$

$$2\,CAPH^+S\bullet \rightarrow CAPSSCAP + 2H^+ \qquad (5)$$

In this case mechanism (4) is the rate-determining step for CAPSH oxidation. The overall oxidation reaction mechanism is similar to other reports [8,23,30-32], with one electron and one proton responsible in dimerization of captopril to form disulfide.

LSV studies

After the optimization of the operational CV conditions, a LSV method was evaluated for quantification of CAP in real samples. Figure 6 shows typical LSVs for different CAP concentrations. The current values (at +0.20 V) presented

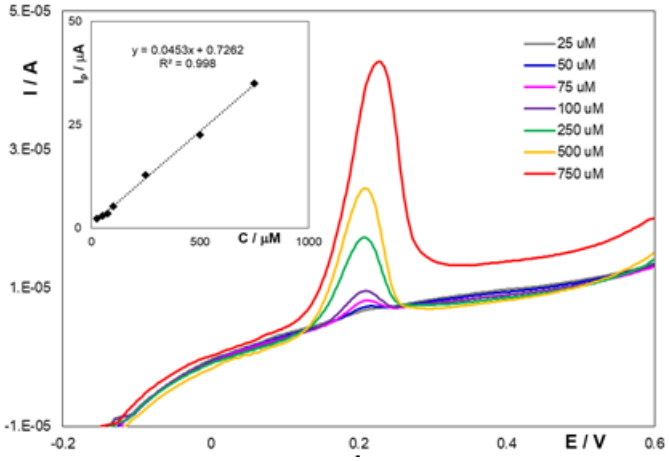

Fig. 6. Linear sweep voltammograms of 1·x 10^{-3} M CAP in BRB solution *p*H 2.21 at PGE* for different CAP concentrations. Inset, the calibration graph

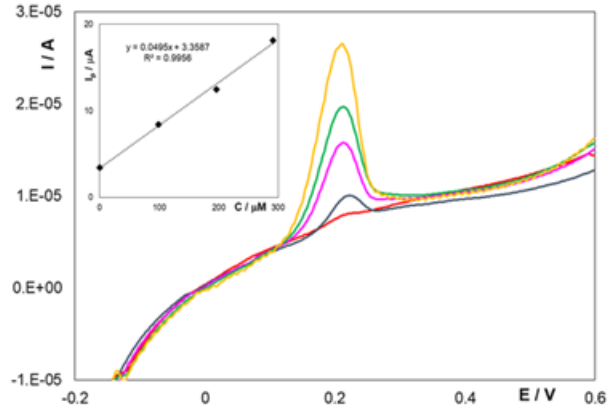

Fig. 7. Linear sweep voltammograms at PGE* for a CAP sample diluted in BRB solution *p*H 2.21 (red) before (blue) and after three additions of 0.1 mL from a 1 × 10^{-2} M CAP stock solution (pink, green and yellow). Inset, the calibration graph

Added CPT (µM)	Intra-day			Inter-day		
	CPT found ± SD (µM)	Precision (RSD,%)	Accuracy (R ± SD,%)	CPT found ± SD (µM)	Precision (RSD,%)	Accuracy (R ± SD,%)
25	25.56 ± 1.07	4.19	101.75 ± 5.00	25.58 ± 1.47	5.74	102.31 ± 5.88
100	100.09 ± 0.84	0.84	100.09 ± 0.84	99.18 ± 0.92	0.92	100.04 ± 0.92
750	750.39 ± 1.01	0.13	100.05 ± 0.14	750.52 ± 1.15	0.15	100.07 ± 0.15

Table 1
RESULTS FOR THE EVALUATION OF THE INTER-DAY PRECISION AND ACCURACY OF CPT DETERMINATION BY LSV

CPT declared weight mg	CPT weight recovered mg	R (%)	Weight of CPT ± SD mg; RSD (%); $R_{average}$ (%)
25	23.96	95.84	24.12 ± 0.22; 0.90%; 96.48%
	24.37	97.48	
	24.03	96.12	

Table 2
RESULTS FOR CAP DETERMINATION IN PHARMACEUTICAL SAMPLES

a linear relationship with captopril concentrations from 2.5 × 10^{-5} M – 7.5 × 10^{-4} M (fig. 3). The linear equation was I_p (µA) = 0.0453 C (µM) + 0.7262, where C is the captopril concentration, having a correlation coefficient of 0.9980 (n = 3).

The detection and quantification limits were calculated as $3.3s_a/b$ and $10s_a/b$, respectively, where s_a is the standard deviation of the intercept of the calibration curve and b is the slope of the calibration curve; the calculated values were 2.34 × 10^{-5} M and 7.08 × 10^{-5} M, respectively.

Analytical performance and applications

The effect of other components, usually present in the pharmaceutical formulations (starch, lactose, stearic acid, and cellulose) upon electrode response was investigated for a 1.0 × 10^{-4} M CAP solution. For concentrations of each mentioned compound that can be found in the commercial formulations there was no significant interference in the LSV procedure up to 10-fold excess for tested excipients.

The accuracy and precision of the LSV method were evaluated by analysis of CPT at three levels of concentration (2.5 × 10^{-5} M, 1.0 × 10^{-4} M and 7.5 × 10^{-4} M) by performing three experiments in the same day (intra-day) and for three successive days (inter-day). The data are presented in table 1.

Captopril contents were determined by the standard addition method. The peak currents of LSVs, recorded at PGE* before and after three consecutive standard additions of CAP (fig. 7) were measured and used to calculate CAP contents in the Labormed tablets.

The mean results in case of three determinations of CAP presented in table 2 are very close to the declared value of 25 mg. The found content was 24.12 mg with a standard deviation (SD) of 0.22 mg. Recoveries ranging from 95.84 to 97.48% of CAP were found using the LSV procedure.

On the basis of the average recoveries in the optimized conditions, it can be summarized that the voltammetric method for practical samples analysis is accurate and reliable and could be applied for the determination of CAP in pharmaceutical samples.

Conclusions

The electrochemical behaviour of PGE* as a new electrochemical sensor for CAP determination has been studied. The simple activation procedure used to make the modified electrode is an obvious advantage of the sensor. Acidic conditions were best suited for CAP determination with a detection limit of 2.34·10^{-5} M. No gas purging for dissolved oxygen removal from the sample solution [8] and/or preconcentration time as in stripping methods [33,34] are needed. The method is also faster than chromatographic ones. The proposed voltammetric method has some obvious advantages compare with others presented in the literature like selectivity (detection

of CAP without previous separation), extreme simplicity, rapid response, and low cost. Besides other interesting features, this facile and easy method of detection will be useful towards applications for CAP analysis and quality control of pharmaceutical samples in complex medical cases associated with sleep apnea (such as the lack of *dipping* under the conditions of appropriate anti-hypertensive therapy).

References

1. HEEL, R.C., BROGDEN, R.N., SPEIGHT, T.M., AVERY, G.S., Drugs, **20,** no. 3, 1980, p. 409.
2. PIMENTA, A.M., ARAUJO, A.N., MONTENEGRO, M.C.B.S.M., Anal. Chim. Acta, **438**, no. 1, 2001, p. 31.
3. MIRZA, T., TAN, H.S., J. Pharm. Biomed. Anal., **25,** no. 1, 2001, p. 39.
4. SALEM, I.I., SAIF, W.A., JMEIAN, Y., TAMIMI, J.I.A., J. Pharm. Biomed. Anal., **37**, no. 37, 2005, p. 1073.
5. SHAHROKHIAN, S., KARIMI, M., KHAJEHSHARIFI, H., Sens. Actuat. B Chem., **109**, no. 2, 2005, p. 278.
6. ROBERTSON, J.I.S., TILLMAN, D.M., J. Cardiovasc. Pharmacol., **10**, 1987, p. 43.
7. PARHAM, H., ZARGAR, B., Talanta, **65,** no. 3, 2005, p. 776.
8. REZAEI, B., DAMIRI, S., Sens. Actuat. B Chem., **134**, no. 1, 2008, p. 324.
9. DUCHIN, K.L., MCKINSTRY, D.N., COHEN, A.L., MIGDALOF, B.H., Clin. Pharmacokinet., **14**, no. 4, 1988, p. 241.
10. FRANKLIN, M.E., ADDISON, R.S., BAKER, P.V., HOOPER, W.D., J. Chromatogr. B Biomed. Sci. Appl., **705**, no. 1, 1998, p. 47.
11. WAKABAYASHI, H., YAMATO, S., NAKAJIMA, M., SHIMADA, K., J. Pharm. Biomed. Anal., **12**, no. 9, 1994, p. 1147.
12. Moldovan, Z., Rev. Roum. Chim., **57**, no. 7-8, 2012, p. 721.
13. PULGARIN, J.A.M., BERMEJO, L.F.G., LOPEZ, P.F., Anal. Chim. Acta, **546**, no. 1, 2005, p. 60.
14. PEREZ-RUIZ, T., MARTINEZ-LOZANO, C., GALERA, R., Electrophoresis, **27**, no. 12, 2006, p. 2310.
15. STEFANESCU, D.C., CIUCU, A.A., RABINCA, A.A., BULEANDRA, M., STOIAN, A.P., JECAN, R.C., HAINAROSIE, R., Rev. Chim. (Bucharest) **69**, no. 1, 2018, p. 277.
16. BAHRAMIPUR, H., JALALI, F., Int. J. Electrochem., 2011; Article ID 864358, 6 pages, doi:10.4061/2011/864358.
17. NIAZI, A., POURGHOBADI, Z., NEMATOLLAHI, D., BEIGINEJAD, H., J. Electrochem. Soc., **161**, no. 5, 2014, p. H284.
18. JALALI, F., RANJBAR, S., Russian J. Electrochem., **50**, no. 5, 2014, p. 482.
19. HABIBI, D., FARAJI, A.R., Gil, A., Sens. Actuat. B Chem., **182**, no. 1, 2013, p. 80.
20. BULEANDRA, M., RABINCA, A.A., BADEA, I.A., BALAN, A., STAMATIN, I., MIHAILCIUC, C., CIUCU, A.A., Microchim. Acta, **184**, no. 5, 2017, p. 1481.
21. BULEANDRA, M., RABINCA, A.A., TACHE, F., MOLDOVAN, Z,. STAMATIN, I., MIHAILCIUC, C., CIUCU, A.A., Sens. Actuat. B Chem., **241**, no. 3, 2017, p. 406.
22. KARIMI-MALEH, H., ENSAFI, A.A., ALLAFCHIAN, A.R., J. Solid State Electrochem., **14**, no. 1, 2010, p. 9.
23. RAOOF, J.B., OJANI, R., BAGHAYERI, M., Chin. J. Catal., 2011, **32**, no. 11-12, p. 1685.
24. Favaro, G., Fiorani, M., Anal. Chim. Acta, **332**, no. 2-3, 1996, p. 249.
25. PAIMARD, G., GHOLIVAND, M.B., SHAMSIPUR, M., GHOLIVAND, K., J. Electroanal. Chem., **738**, no. 2, 2015, p. 176.
26.. RIBERIO, P.R.DAS, SANTINI, A.O., PEZZA, H.R., PEZZA, L., Elect. Quim., 2003, **28**, no. 1, p. 39.
27. HILLAERT, S., VAN DEN BOSSCHE, W., J. Pharm. Biomed. Anal., **21**, no. 1, 1999, p. 65.
28. LAVIRON, E., J. Electroanal. Chem. Interface Electrochem., **101**, no. 1, 1979, p. 19.
29. MAZLOUM-ARDAKANI, M., SHEIKH-MOHSENI, M.A., MIRJALILI, B.F., ZAMANI, L., J. Electroanal. Chem., **686**, 2012, p. 12.
30. LIEDER, M., SCHLAPFER, C.W., J. Appl. Electrochem., **31**, no. 12, 2001, p. 1321.
31. RADOVAN, C., MANEA, F., Electroanalysis, **19**, no. 1, 2007, p. 91.
32. ZHOU, M.. DING, J., GUO, L., SHANG, Q., Anal. Chem., 2007, **79**, no. 14, p. 5328.
33. IOANNIDES, X., ECONOMOU, A., VOULGAROPOULOS, A., J. Pharm. Biomed. Anal., **33**, no. 2, 2003, p. 309.
34. ENSAFI, A.A., HAJIAN, R., J. Braz. Chem. Soc., **19**, no. 3, 2008, p. 405.

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