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FULL PAPER

Development of an Electrochemical Methodology for the Quantification of β-estradiol in Pharmaceutical Formulation

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Abstract:

This paper describes a simple, inexpensive, highly sensitive, and efficient electrochemical method to determine Estradiol in pharmaceutical formulations. The oxidation reaction on the electrode surface was electrochemically characterized by cyclic voltammetry (CV) and square wave voltammetry (SWV). The investigation of Estradiol at carbon paste electrode revealed a non-reversible oxidation peak at +640 mV *vs.* Ag/AgCl, which was used for electrochemical detection of Estradiol. The operating parameters (pH, frequency, step potential, and amplitude) were optimized in relation to the peak current intensity, and a calibration curve was set up in a concentration range of 0.059 - 2.997 mg L^{-1}, with a detection limit of 21.85 µg L^{-1}. After calibration curve was plotted, the developed procedure was applied to determine Estradiol in parmacheutical formulation. These results show that the proposed method can be used for Estradiol quantification in pharmaceutical formulations with high sensitivity, specificity, stability, and reproducibility, and can be applied in analytical routines in laboratories of quality control analysis in drugs.

Keywords: voltammetry; carbon paste electrode; hormones; medications; determination

1. Introduction

Estrogens are biologically active hormones that are derived from cholesterol and released by glands - the adrenal cortex, testes and ovary - and placenta during pregnancy in humans and animals. Steroid estrogens can be classified as natural or synthetic hormones and can act as endocrine disrupting chemicals (EDCs) [1]. EDCs may interfere with function of the body's endocrine system in wildlife and humans, causing adverse effects in an intact organism or its progeny, by blocking or mimicking the normal effect of hormones, affecting their synthesis or metabolism, and altering hormone receptor levels [2,3]. EDCs also constitute a group of organic pollutants with increasing importance due to their impact in the environment and human health [3]. The substances exhibiting endocrine disrupting properties include a wide range of chemical groups, among which are natural or synthetic steroid estrogens. Particularly the natural hormone 17-β-estradiol (E2) and the synthetic estrogen 17-α-ethinylestradiol (EE2) are described as the EDCs with higher disrupting potency [3].

Natural steroidal estrogens, also known as the C18 steroidal group, have the same tetracyclic molecular framework comprising four rings: one phenolic group, two cyclohexane and one cyclo-pentane ring. Structural differences within the C18 group occur in the configuration of the D-ring at C16 and C17 positions. For example, estrone (E1) has a carbonyl group on C17, whilst 17β-estradiol (E2) has a hydroxyl group on C17 [1].

Estradiol (E2), (17β)-estra-1,3,5(10)-triene-3,17-diol, is the major estrogen in vertebrates, being associated with the female reproductive system and maintenance of female sexual

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characteristics [3,4]. E2 has been manufactured in large quantities to be used as oral hormonal contraceptives and for hormone replacement therapy, and illegal animal growth promotion [4].

Due to E2 presence as active ingredient in pharmaceutical formulations and in the environment as organic pollutant as well as interest in its quantification in biological samples (tissues and biofluids), the development of simple, sensitive, reliable, and cost-effective analytical methods for the determination of E2 levels in different types of samples has received considerable attention [4].

Based on a review considering 114 papers [3], gas or liquid chromatography coupled to mass spectrometry (GC–MS or LC–MS, respectively) are increasingly becoming the methods of choice to determine estrogens in biological samples [5,6]. Numerous analytical methods have been developed and subsequently optimized for E2 and EE2 determination in environmental samples, and most of them are also chromatography based methodologies. Although chromatography techniques are highly reliable and sensitive, these methods involve long analysis times, complex sample preparation procedures, and high costs [4]. Other analytical approaches such as electrochemical methods involving oxidation of estrogenic compounds on electrode surface have attracted some interest due important advantages including low cost, simple operation, portability, ease of miniaturization, high sensitivity, good selectivity and fast response [3,4].

Some electrochemical methods are proposed for E2 determination in pharmaceutical formulations [7-12]. However, from our best knowledge there no electrochemical methods for estradiol (E2] determination in pharmaceutical formulations using carbon paste electrode (CPE) without chemical modification and taking its advantages of simple and low-cost preparation.

This paper describes a simple, inexpensive, highly sensitive, and efficient electrochemical method to determine Estradiol (E2) in pharmaceutical formulations using carbon paste electrode (CPE) and square wave voltammetry (SWV). The oxidation reaction on the CPE surface was electrochemically characterized by cyclic voltammetry (CV).

2. Results and Discussion

2.1. Electrochemical behavior of E2 at CPE

Cyclic voltammograms (CVs) obtained using a 0.2 mol L^{-1} BR buffer solution at pH 5.00 in the absence and presence of 5.79 mg L^{-1} of E2 are shown in Fig. 1. The voltammogram in black shown in Fig. 1 indicates an absence of peaks, suggesting that there are no oxidation/reduction processes occurring on the surface of working electrode (CPE), thus indicating that potential range can be adopted to investigate the redox process on the surface of the working electrode. The voltammogram (blue), Fig. 1, obtained when E2 is present in the electrochemical cell, has an oxidation peak for the working electrode surface. The values obtained for Ep (mV)/Ip (µA) were: 648.86/0.84, respectively. The oxidation peak presented in the voltammogram (Fig. 1) can be attributed to the irreversible oxidation of the hydroxyl groups in the molecular structure of E2, leading to the formation of quinones [13,14].

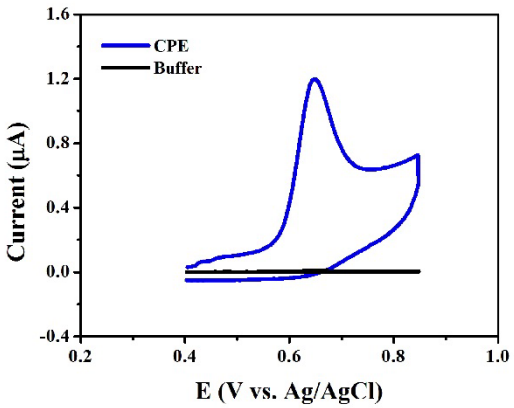

Figure 1. Cyclic voltammograms obtained in the absence (Black) and presence (Blue) of 5.79 mg L^{-1} of E2. Experimental conditions: Buffer [BR] = 0.2 mol L^{-1}, pH = 5.00. *v* = 100 mV s^{-1}.

2.2. Influence of pH

The effect of pH on the electrochemical behavior of E2 was investigated using 0.2 mol L^{-1} BR buffer in the pH range of 2.00 to 8.00 and a concentration of 5.79 mg L^{-1}. CVs using CPE as the working electrode were recorded in the potential range of 300 to 1,100 mV at a scan rate of 100 mV s^{-1} (Fig. 3A). As pH increased, Ep values shifted linearly to more negative values (Fig. 3B), indicating the intervention of protons in the electrochemical process of E2 on CPE surface. The values obtained were adjusted using

the least squares method, yielding the following equations: Ep (mV) = 964.76±12.18 – 64.43 ΔpH, r = 0.991. The values obtained for the angular coefficient of the curves Ep *vs.* pH were 64.43 mV/pH for, suggesting identical numbers of protons and electrons involved in the electrochemical oxidation of E2 on the surface of working electrode [15]. Fig. 3B show the variation in the peak currents with pH, and an increase of Ip is observed when the pH increases. A maximum Ip value was recorded at pH 5.0, which was selected for the electrochemical study of E2. It was also used for the development of an electrochemical method for determination of E2 in the pharmaceutical formulations.

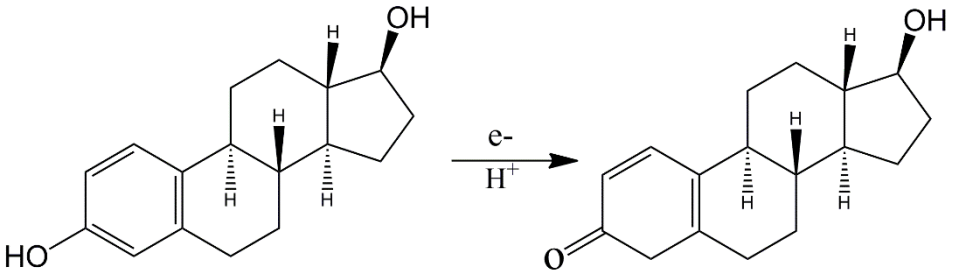

Figure 2. Possible oxidation reaction of E2 on the CPE surface [14].

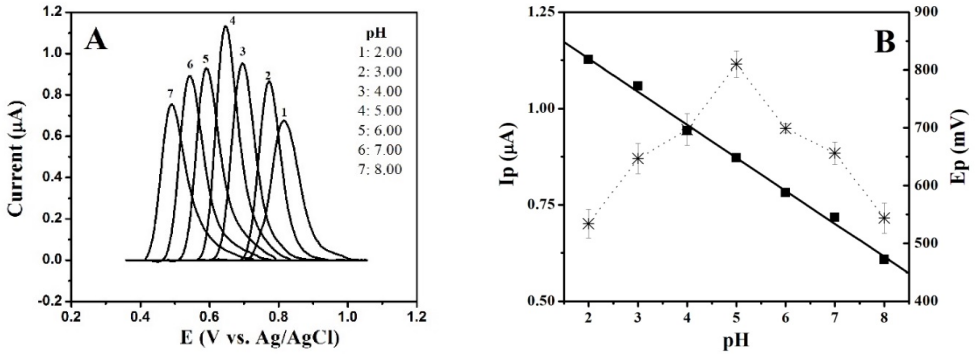

Figure 3. A) Anodic region of CVs. B) *Ip vs*. pH and *Ep vs*. pH curves. Experimental conditions: v = 100 mV s^{-1}. BR buffer: 0.2 mol L^{-1}. [E2] = 5.79 mg L^{-1}. Working electrode: CPE.

2.3. Scan rate effect

The influence of the scan rate on the Ip and Ep of E2 was evaluated in a range of 5 to 400 mV s^{-1} in CV. The obtained voltammograms are shown in Fig. 4A. An increase of Ip is observed when *v* increases and, consequently, there is a displacement of Ep to more positive values. The variation of Ip x ν (Fig. 4B) presents a linear behavior according to the following equation: I_p (μA) = -0.021 + 0.013 ν (mV s) (r = 0.998). This result indicates a adsorption controlled process with adsorption at the working electrode surface [15]. The correlation between log I_p *vs.* log v (Fig. 4C) showed a linear behavior according to the following equation: log I_p = -2.142 + 1.102 log *v* (r = 0.998). The obtained angular coefficient is close to 1, suggesting an irreversible, adsorption controlled electron transfer process [15].

2.4. Analytical curve, limit of detection and limit of quantification.

The method to be used with CPE was developed through the optimization of the following instrumental and experimental parameters in relation to the current at oxidation peak: frequency, amplitude, step potential, and pH (Table 1). Due its high sensitivity and lower LDs, SWV was the electrochemical method selected for E2 quantification under optimized conditions. The analytical curve (Fig. 5) exhibited good linearity for I_p *vs*. [E2] concentration curve in the range of 0.059 – 2.997 mg L^{-1}. The linear

equation obtained was I_p *(μA) = -0.280 ±0.060 (μA) + 8.237± 0.084 (mg L^{-1}) [E2]*, with *r* = 0.999. The obtained values of *LD* and *LQ*, determined from the analytical curve using the methodology described in item 3.4.2 (Materials and Methods), were 21.85 and 72.84 μg L^{-1}, respectively. The detection performance of CPE was compared to those of other sensors (Table 2). It is observed from the values presented in the Table 2 that the analytical performance obtained with the methodology proposed using the unmodified sensor is better when the comparison is made in terms of LDs values. Another relevant aspect to be considered is the ease of preparation of the proposed sensor when compared with the other sensors presented in said table, since it is obtained by simply mixing its constituents.

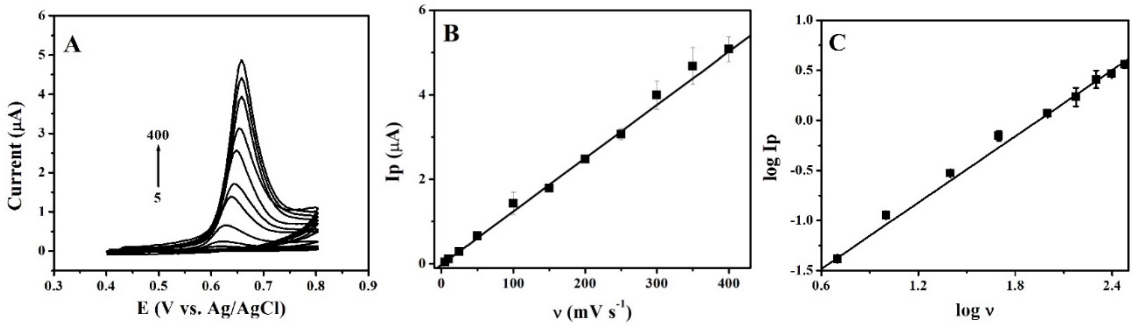

Figure 4. (A) Cyclic voltammograms reading obtained using CPE. (B) Ip *vs.* ν. (C) log Ip *vs.* log *v*. Experimental conditions: [E2] = 3.82 mg L^{-1}, BR buffer: 0.2 mol L^{-1}, pH = 5.00. ν = 5; 10; 25; 50; 100, 150, 200, 250, 300, 350 and 400 mV s^{-1}. (n = 3).

Table 1. Optimal parameters in SWV. [BR] = 0.2 mol L^{-1}.

Parameters	Range	Optimized value
pH	2.00 - 8.00	5.00
Amplitude (mV)	10 - 80	40
Step potential (mV)	1 - 20	12.5
Frequency (Hz)	5 - 80	60

2.5. Precision, reptibility and stability

Studies of precision, repeatability and stability of the method were investigated to evaluate the analytical performance of the proposed method. This study was based on the values of Ip and Ep, which obtained using previously optimized parameters and a solution containing 1.91 mg L^{-1} of E2 in the electrochemical cell. The intra-day precision of the working electrode was evaluated using three different electrodes of the same composition, the results obtained showed a relative standard deviation (RSD) of less than 3%. The repeatability was evaluated by measuring the values of Ip and Ep in 30 voltammograms on the same day, those obtained from RSD were less than 3%. The stability of the CPE was investigated using three different electrodes prepared in the same composition and the values of Ip and Ep were measured in 30 voltammograms for 6 alternate days, the results obtained showed a RSD of less than 5%. The results for precision, repeatability and stability are shown in Fig. 6. The low RSD values demonstrate that CPE has high accuracy, repeatability and stability, and can be used in analytical applications for BPA quantification in real samples.

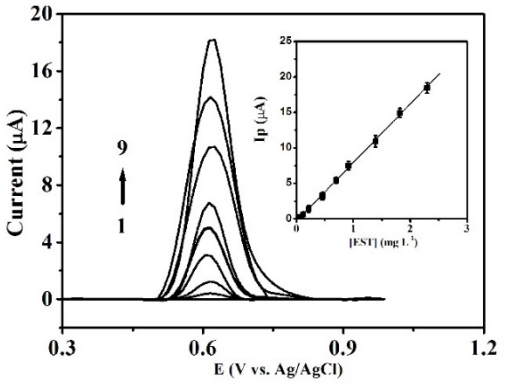

Figure 5. SWVs readings and analytical curves for E2, using CPE as the working electrode. Experimental conditions: BR buffer at 0.2 mol L^{-1} in pH 5.00. [E2]: 1) 0.059; 2) 0.114; 3) 0.213; 4) 0.456; 5) 0.694; 6) 0.913; 7) 1.389; 8) 1.816; 9) 2.297 mg L^{-1}.

Table 2. Studies reporting E2 quantification using electrochemical methods.

Electrodes	Methods	Linear range (g L^{-1})	LD (μg L^{-1})	Samples analyzed	Ref.
GCE	DPV	10.93 x 10^{-3} – 273.39 x 10^{-3}	3,295.90	Serum, pharmaceutical formulation	[9]
GCE/GQDs/PSSA	DPV	2.7 x 10^{-7} – 1.63 x 10^{-3}	0.06	Serum, pharmaceutical formulation	[10]
CPE/OA	LSV	4.08 x 10^{-4} – 2.72 x 10^{-3}	109.00	pharmaceutical formulation	[8]
CCE/FER	DPV	5.44 x 10^{-6} - 8.17 x 10^{-3}	13.60	pharmaceutical formulation	[11]
CPE/CuO	SWV	16.34 x 10^{-6} – 217.91 x 10^{-6}	5.72	Urine, Buttermilk	[4]
CPE/GNR-FS-Au-CA	DPV	27.24 x 10^{-6} – 1.36 x 10^{-3}	2.01	milk and pharmaceutical samples	[16]
CPE	SWV	0.059 x 10^{-3} – 2.997 x 10^{-3}	21.85	pharmaceutical formulation	This study

GCE: glassy carbon electrode. GQDs: graphene quantum dots. PSSA: doped poly-sulfosalicylic acid. DPV: Differential pulse voltammetry. CPE: carbon paste electrode. OA: oleic acid. LSV: linear scan voltammetry. CCE: carbon ceramic electrode. FER: ferrierite. CuO: Copper Oxide. GNR-FS-Au-CA: Cysteamine self-assembled gold nanoparticle modified fumed silica decorated graphene nanoribbon nanocomposite.

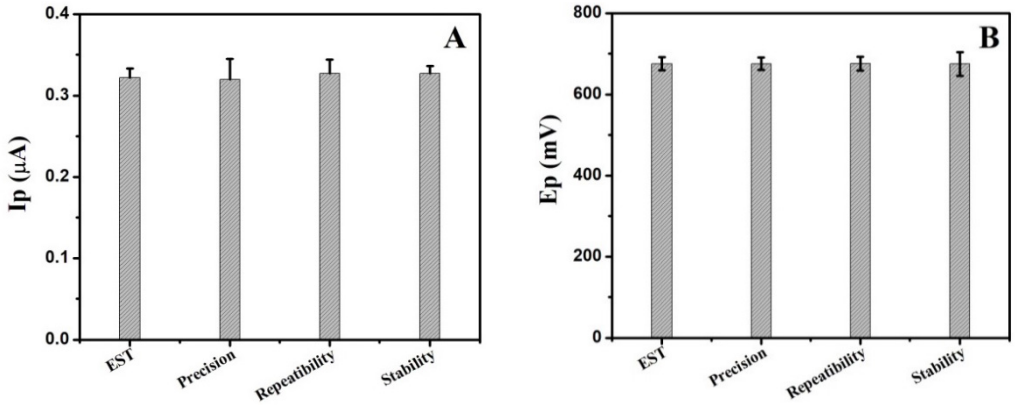

Figure 6. Precision, repeatability and stability. Ip (A). Ep (B). Experimental Conditions: as shown in Fig. 5. [E2] = 1.91 mg L^{-1}. (n = 3).

2.7. Application of proposed method in real samples

To verify the feasibility and validity of the present method for E2 analysis in actual samples (pharmaceutical samples). For this purpose the developed methodology was applied in the determination of estradiol in two pharmaceutical formulations, Fornulation A (Estrell) and B (Natifa) were purchased from local drugstores, with quantity declared on its label of 1 mg/tablet. The preparation of the formulations is described in item 3.5 (Materials and Methods), and the determination of amounts of E2 in each formulation was performed using the standard addition method. The results obtained in the E2 determination in the formulations under study are presented in Table 3. The results presented show a high agreement between the values found with the values declared in label of the each formulation.

Table 3. Found quandities of E2 and relative standard deviation in each sample analyzed. (n = 5).

Samples	Quantity declared (mg)	Quantity found (mg)	RSD (%)
A	1	0.99	2.02
B	1	1.02	2.94

3. Material and Methods

3.1. Reagents and solutions

A Britton-Robinson (BR) buffer solution was

prepared by mixing equal amounts of H_3BO_3 (VETEC), H_3PO_4 (SYNTH), and CH_3COOH (NUCLEAR) solutions, all at a concentration of 0.2 mol L^{-1}. The pH was adjusted by adding a NaOH solution at the same concentration used for the used acids. The stock solution of E2 was prepared in ethanol from an analytical standard (Sigma-Aldrich 99%, w/w). The working solution was obtained from the dilution of the stock solution in phosphate buffer BR at a concentration of 0.20 mol L^{-1} at the desired pH in each day of experiments. Water purified in a Milli-Q system (Waters) was used as a solvent, being also used to prepare the solutions for this study.

3.2. Apparatus

All electrochemical measurements were performed on an Autolab PGSTAT12 system (Eco Chemie, Utrecht, The Netherlands). The experiments were carried out in a three electrode glass cell at room temperature (25±1 ^oC), using a platinum wire as the counter electrode, Ag/AgCl in KCl (3 mol L^{-1}), as the reference electrode, and carbon paste electrode (CPE) as the working electrode. The volume used in all the electrochemical measurements was 5 mL. The cell was placed in a Faraday cage in to minimize background noise. Square wave voltammetry (SWV) and cyclic voltammetry (CV) were used to investigate the electrochemical behavior of E2. A pH meter (Hanna Instruments HI 3221), equipped with a combined glass electrode, was used for adjusting pH values.

3.3. Preparation CPE

Carbon paste was prepared by mixing spectroscopic-grade graphite powder (Sigma-Aldrich, as-received, <20 µm) with mineral oil (Sigma-Aldrich) at 75:25 (w: w) proportion. The mixture was homogenized in a mortar for 40 min and inserted into a 1.0 mL plastic syringe, with a geometric area of 0.054 cm^2. Electrical contact was established via a copper wire to the paste. The surface of the working electrode (CPE) was smoothed against weighing paper and carefully rinsed with distilled water.

3.4. Electrochemical Measures

3.4.1. Cyclic Voltammetry (CV)

In the evaluation of the electrochemical behavior of the E2, cyclic voltammetry (CV) was used. The study of the influence of the scan rate and the pH were carried out with this electrochemical method.

3.4.2. Square wave voltammetry (SWV)

The square wave voltammetry (SWV) due to its high sensitivity was used to optimize instrumental parameters (frequency, amplitude and potential step), optimization was performed by evaluating the values of Ip and Ep. Using the optimized instrumental and experimental parameters, an analytical curve was constructed in the E2 concentration range of 0.059 - 2.997 mg L^{-1} in the electrochemical cell. The analytical parameters, limit of determination (LD) and quantification (LQ) were determined according to the IUPAC recommendations [17], using the parameters of the analytical curve, where LD = 3SD s^{-1} and LQ = 10SD s^{-1}, where SD is the standard deviation of the intercept and s is the slope, both of the analytic curve obtained.

3.4.3. Precision, Repeatabilityand Stability

Sensor precision, repeatability, and stability were estimated by analyzing the Ip e Ep values for E2 (1.91 mg L^{-1}), making measurements at different times at the same day (precision), as well as performing 30 consecutive measurements (repeatability) and using three different electrodes of identical composition (stability). This study was performed using the same experimental and instrumental parameters to obtain the analytical curve.

3.5. Real Samples: Pharmaceutical Formulation

The methodology developed was applied in the determination of estradiol in pharmaceutical formulation, acquired in drugstores in the local market. A stock solution of each formulation at 0.122 mg L^{-1} concentration of E2 was prepared using the same procedure used in the preparation of the analytical standard, from the stock solution, working solutions were prepared using the BR buffer as concentration diluent of 0.2 mol L^{-1} at pH

5.00. To minimize the interfering effect of the matrix, the standard addition method was used for the sample in all determinations.

4. Conclusion

The present study was developed and an electrochemical method applied to quantify the Estradiol in pharmaceutical formulation. The best analytical conditions were reached by using a CPE as working electrode. The detection limit was determined to be 21.85 μg L^{-1}, thus proving that this method can be useful for detecting Estradiol in pharmaceutical formulation with high sensitivity, precision, repeatabiliy, repeatability and stability. The results obtained are in agrement with the declared (label) ones and the found values provided a relative standard deviation of less than 3%, indicating that the method can be applied in the determination of Estradiol in drugs with high sensitivity, precision.

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References and Notes

[1] Adeel, M.; Song, X.; Francis, D.; Yang, Y. *Environ. Int.* **2017**, *99*, 107. [Crossref]

[2] Bókony, V.; Üveges, B.; Ujhegyi, N.; Verebélyi, V.; Nemesházi, E.; Csíkvári, O.; Hettyey, A. *Sci. Total Environ* **2018**, *634*, 1335. [Crossref]

[3] Barreiros, L.; Queiroz, J. F.; Magalhães, L. M.; Silvia, A. M. T.; Segundo, M. A. *Microchem. J.* **2016**, *126*, 243. [Crossref]

[4] Antoniazzi, C.; Lima, C.; Marangoni, R.; Spinelli, A.; Castro, E.G. *J. Solid State Electrochem.* **2018**, *22*, 1373 [Crossref].

[5] Feng, H.; Ning, L.; Xiao-Li, L. *J. Northeast Agric. Univ.* **2016**, *2*, 44. [Crossref]

[6] Shahbazi, Y.; Malekinejad, H.; Tajik, H. *J. Food Drug Anal.* **2016**, *24*, 457. [Crossref]

[7] Hu, S.; He, Q.; Zhao, Z. *Analyst* **1992**, *117*, 181. [Crossref]

[8] Hu, S.; Guo, M.; Hu, G.; Jiang, M. *Anal. Lett.* **1995**, *28*, 1993. [Crossref]

[9] Salcı, B.; Biryol, I. *J. Pharm. Biomed. Anal.* **2002**, *28*, 753. [Crossref]

[10] Arvand, M.; Hemmati, S. *Talanta* **2017**,*174*, 243. [Crossref]

[11] Robak, J.; Burnat, B.; Kisielewska, A.; Fendrych, K.; Skrzypek, S. *J. Electrochem. Soc.* **2017**, *164*, B574. [Crossref]

[12] Smajdor, J.; Piech, R.; Ławrywianiec, M., Paczosa-Bator, B. *Anal. Biochem.* **2018,** *544*, 7. [Crossref]

[13] Moraes, F. C.; Rossi, B.; Donatoni, M. C.; Oliveira, K. T.; Pereira, E. C. *Anal. Chim. Acta* **2015**, *881*, 37. [Crossref]

[14] Ngundi, M. M.; Sadik, O. A.; Yamaguchi, T.; Suye, S. *Electrochem. Commun.* **2003**, *5*, 61. [Crossref]

[15] Pletcher, D.; Greff, R.; Peat, R.; Peter, L. M.; Robinson, J. Potential sweep techniques and cyclic voltammetry. In Instrumental Methods in Electrochemistry; 2nd ed. Woodhead Publishing Limited: New Delhi, India 2011, 178-228. [Crossref]

[16] Ozcan, A.; Topçuogullari, D. *Sens. Actuators, B* **2017**, *250*, 85. [CrossR\ref]

[17] Analytical Methods Committee, Recommendations for the definition, estimation and use of the detection limit, Analyst. 1997, 112, 199. [Crossref]