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# A simple and feasible determination of selective estrogen receptor modulator raloxifene in the pharmaceutical formulation using the pretreated boron-doped diamond electrode

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*Abstract:* This article reports on the development of an electroanalytical method for the quantitative determination of the selective estrogen receptor modulator raloxifene (RLX) using voltammetry at a pretreated boron-doped diamond (BDD) electrode. RLX exhibited irreversible cyclic voltammetric (CV) behavior in 0.04 mol $L^{-1}$ Britton-Robinson (BR) supporting electrolyte at pH 2, generating two anodic oxidation peaks at approximately +0.79 V($P_{A1}$) and +1.46 V ($P_{A2}$). Scan rate analysis revealed that both adsorption and diffusion mechanisms govern RLX transport to the electrode surface. Consequently, incorporating a preconcentration (deposition) step was hypothesized to enhance analytical sensitivity. Optimal deposition parameters, along with supporting electrolyte pH and square-wave voltammetry (SWV) modulation settings, were systematically optimized. Quantitative analysis was based on the first anodic peak ($P_{A1}$) in 0.04 mol $L^{-1}$ BR buffer at pH 2, exhibiting a linear dynamic range from 0.025 to 5.0 μg $mL^{-1}$ ($4.9\cdot10^{-8}$ to $9.9\cdot10^{-6}$ mol $L^{-1}$) and a detection limit of 0.0073 μg $mL^{-1}$ ($1.4\cdot10^{-8}$ mol $L^{-1}$). The method's applicability was validated by successfully quantifying RLX in pharmaceutical formulations.

*Keywords:* raloxifene; square-wave voltammetry; sensing; boron-doped diamond electrode; pharmaceutical formulation.

## INTRODUCTION

Osteoporosis and breast cancer are among the most serious health issues affecting women.[1,2] The main cause of both problems is the lack of estrogen hormone, which plays an important role in in bone remodelling in postmenopausal women. This condition is characterized by an increased risk of fragility fractures due to deterioration of bone microarchitecture.[3,4] The conventional approach to

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treating osteoporosis involves hormone replacement therapy (HRT), which has been proven effective through various studies.[5] However, reports indicate that HRT may be associated with adverse effects, including potential risks for cardiovascular diseases and increased risk of breast cancer[3]. Additionally, breast cancer is the second most common type of cancer among women, following lung cancer, with over one million women diagnosed annually worldwide.[6,7] Increasingly, the use of selective estrogen receptor modulators (SERMs) has gained acceptance as an alternative to HRT in the management of these related conditions. SERMs provide the benefits of estrogen on bone health while potentially minimizing the adverse effects of estrogen in breast and other tissues.[8]

Raloxifene hydrochloride (RLX) is one of the most significant second-generation SERMs frequently prescribed for prevention of postmenopausal osteoporosis, where it acts as an estrogen agonist, and for breast cancer treatment, where it acts as an estrogen antagonist.[9–11] Compared to other SERM drugs, RLX is associated with fewer side effects, making it a preferred choice among anti-cancer drugs.[12] Furthermore, the neuroprotective efficacy of RLX has also been demonstrated in animal models of age-related neurodegenerative disorders, such as Parkinson's disease.[13–15] There is an increasing need for robust, simple, rapid, and reliable quantitative analytical methods to reliably monitor adverse effects resulting from overdoses of this medication, particularly in patients undergoing cancer treatment.

Several articles have been published for the quantitative analysis of RLX based on different analytical methodologies, including spectrophotometric methods,[16–19] chromatographic techniques[20–22] and capillary electrophoresis.[23] While these techniques offer selective and sensitive results, they also present several drawbacks such as requiring costly reagents, expensive instrumentation and time-consuming sample preparation. Another notable method for RLX analysis, based on resonance Rayleigh scattering, is characterized by multiple processing steps and the use of costly reagents.[24]

Electrochemical methods have become important alternative to the above-mentioned analytical techniques due to their simplicity, speed, and cheap instrumentation.[25,26] Owing to the electroactive chemical structure of RLX [Scheme1, 6–hydroxy–2–(4–hydroxyphenyl)–benzothiophene–3–yl]–[4–[2–(1–piperidyl)ethoxy]phenyl]–methanone, there has been a growing number of quantitative analyses in recent years, particularly electrochemical ones. These studies, have achieved varying sensitivity levels by using bare electrodes such as graphite[27] and glassy carbon electrodes (GCE)[28] as well as GCEs modified with carbon nanoparticles,[29] reduced graphene oxide-carbon nanotubes (rGOCNT),[30] graphene nanocomposite[31] and carbon paste electrodes (CPE) modified with carbon nano powder,[32] nickel (II) oxide nanoparticle,[33] nanocomposite,[34] N–CQD/$Fe_3O_4$

nanoparticles.[35] Moreover, an electroanalytical study demonstrated quantitative analysis of RLX can be mentioned that performed the quantitative analysis of RLX at a sensitivity level of 3 nM on a PGE electrode modified with nanocomposite materials.[36]

Boron-doped diamond electrodes offer numerous advantages for electrochemical analysis. Among these, the most prominent advantage is their wide potential window, which enables the analysis of a broad range of species.[37] Furthermore, these electrodes exhibit high electrochemical reactivity, enhancing analytical sensitivity, and maintain low background currents that contribute to improved detection limits.[38] Their inert surface minimizes contamination, while their stability ensures consistent signals over extended periods. Additionally, robust durability prolongs their service life, and their compatibility with portable systems further elevates their status as a preferred choice for electrochemical applications.[39]

Despite numerous reports on chemically modified electrodes for RLX, there is currently no advanced electroanalytical study that uses simple electrochemically pretreated boron-doped diamond (BDD) electrodes in combination with square-wave (SW) modulation for analyzing this chemotherapeutic agent. This study involves the optimizing experimental conditions, elucidating the electrode reaction mechanism, and determining the supporting electrolyte pH following a simple pretreatment of the working electrode. Subsequently, the analytical performance and selectivity of the proposed method are evaluated. Overall, this study shows great potential to stimulate future investigations into rapid, reliable, sensitive, and cost-efficient electrochemical methods for detecting electroactive species, typically employing pretreated BDD electrodes.

## EXPERIMENTAL

### *Chemicals and solutions*

The raloxifen standard (raloxifen hydrochloride ReagentPlus®, 99.91%) was obtained from ChemScene LLC (USA). No additional purification step was performed for this standard prior to the experiments. A solution of raloxifen hydrochloride (hereinafter referred to as RLX) at a concentration of 1.0 μg mL$^{-1}$ was prepared in methanol. This solution was stored in a freezer at +4 °C when not in use, and exposure to direct light was avoided both during use and when it was not in use. Britton-Robinson buffer (BR, 0.04 mol L$^{-1}$, pH 2−11) solution used in the experiments was prepared by dissolving 2.47 g of $H_3BO_3$, and adding 2.76 mL of 85% $H_3PO_4$ and 2.29 mL of glacial acetic acid per liter of solution. The pH of the resulting mixture was adjusted to the desired values using 3 M NaOH and/or 3 M HCl. The preparation and dilution of the solutions were carried out using purified water obtained from a Millipore Milli-Q system (Millipore) with a resistivity of ≥ 18.2 MΩ·cm.

### *Apparatus and measurements*

Electrochemical measurements were conducted using an Autolab type III electrochemical analyzer (Metrohm Autolab B.V., located in Utrecht, The Netherlands). The data were managed using the general-purpose electrochemical software (GPES) that came with the device (Version 4.9). All square wave voltammograms were smoothed using a moving average filtering algorithm for baseline corrections, followed by the steps of the Savitzky and Golay algorithm (peak width: 0.01 V). The experiments were performed in a three-electrode electrochemical cell. It was equipped with an Ag/AgCl/ 3 mol $L^{-1}$ NaCl reference electrode (BAS, Model RE–1, USA), a commercially available BDD working electrode (with a 3 mm disc diameter and a claimed boron doping concentration of 1000 ppm, Windsor Scientific Ltd., UK), and a platinum wire auxiliary electrode (BAS, MW–4130, USA). pH measurements were recorded using an aWTWinoLab pH 720 m model pH meter (Xylem, New York, USA) equipped with a combined glass-reference electrode at 25 °C.

At the beginning of each experimental day, the BDD electrode was electrochemically pretreated in 0.5 mol $L^{-1}$ $H_2SO_4$. The pretreatment consisted of an anodic polarization step at +1.8 V for 180 s, followed by a cathodic polarization step at –1.8 V for 180 s, performed in a separate electrochemical cell. This procedure is known to produce oxygen-terminated and hydrogen-terminated surface characteristics, respectively. [40] After pretreatment, the electrode surface was gently polished using a polishing pad, rinsed with deionized water, and prepared for subsequent electrochemical measurements. During the day, only gentle polishing was applied between measurements, as no significant surface passivation was observed under the experimental conditions.

The electrochemical behavior of RLX in the supporting electrolyte used for the analyses, as well as the mechanism of driving forces such as diffusion and adsorption involved in the electrode reaction, were investigated using cyclic voltammetry (CV). Subsequently, the effects of supporting electrolyte pH, deposition parameters, and selected pulse technique (square wave) parameters on RLX signals were examined. Experimental and instrumental variables were optimized iteratively by selecting the conditions that produced the highest and best-shaped RLX responses; each parameter was optimized sequentially while holding the others constant. The analytical performance of the method, specifically the relationship between RLX concentration and its oxidation peak currents, was evaluated under the optimized conditions yielding the strongest signals. Finally, the practical applicability of the proposed method was demonstrated by analyzing RLX in tablet formulations.

Quantitative analyses of RLX were performed by immersing three electrodes in a voltammetric cell containing RLX solution prepared in 0.04 mol $L^{-1}$ BR buffer at pH 2 using the SW modulation technique. Following this, the potential was scanned from 0 V to +1.3 V employing the same pulse technique. The SWV method was utilized for the quantitative evaluation of RLX. All measurements were performed in triplicate, except for the intraday repeatability assessment, which was conducted with ten repetitions.

### *Sample preparation*

For real sample analyses, fixed dose combination tablets containing RLX HCl (Ralien®, Genveon Co., Türkiye) were obtained from a local pharmacy. The manufacturer declares that each tablet contains 60 mg RLX HCl. Ten tablets were first ground into powder using a mortar and pestle. An amount of the powder equivalent to 10 mg RLX hydrochloride was accurately weighed and transferred to a 250 mL volumetric flask, which was filled to volume with methanol. The contents of the flask were stirred for approximately 15 minutes to facilitate dissolution. Subsequently, a 100 µL aliquot of this solution was taken and added to a voltammetric cell containing 0.04 mol $L^{-1}$ BR buffer at pH 2 as supporting electrolyte. Analysis was performed on the same day sample preparation in accordance with the recommended procedures, applying calibration curve method based on the relevant regression equation.

## RESULTS AND DISCUSSION

### *Assessment of electrochemical behavior of RLX on pretreated BDD electrode*

The cyclic voltammetry (CV) technique is used to determine the potential values at which electroactive species are reduced/oxidized and to determine which force dominates the transport of the electroactive compound to the electrode surface. For this purpose, three consecutives cyclic voltammograms (CVs) of 100 μg mL$^{-1}$ RLX were recorded in the potential range of –0.7 V to +1.80 V in 0.04 mol L$^{-1}$ BR buffer solution at pH 2, which was established as the most suitable medium for analytical applications. The CV of the blank solution (without RLX) was also recorded for comparison. Figure 1A shows two distinct oxidation peaks observed at approximately +0.79 ($P_{A1}$, well-defined) and approximately +1.46 V ($P_{A2}$) in the first cycle of RLX and then the decrease in the intensity of these peaks in the following cycles.

This may be due to the deactivation of the working electrode after contamination or the adsorption of RLX oxidation products on the working electrode. On the other hand, the absence of any reduction signal in the subsequent reverse scan of the CV recordings can be considered as clear evidence that RLX on the BDD electrode is completely irreversible.

### *Effect of scan rate*

In cyclic voltammetry (CV), the variation in peak currents of electroactive species at different scan rates is examined. These data enable predictions regarding the reaction kinetics of the examined species at the working electrode. In this context, the effect of the scan rate on the peak current of RLX was investigated using BDD electrode in 0.04 mol L$^{-1}$ BR buffer solution at pH 2 with scan rates ranging from 50 to 700 mV s$^{-1}$ (n= 8 scans, Fig. 1B). As the first oxidation peak ($P_{A1}$) is more pronounced than the second ($P_{A2}$), the effect was investigated based on $P_{A1}$ of RLX. As a result, a linear relationship was observed between the scan rate ($v$) and the peak current ($i_p$) of $P_{A1}$, as described by Equation 1:

$$i_{pA1}\ (\mu A) = 0.010 \pm 0.0003\ v\ (mV\ s^{-1}) + 1.423 \pm 0.0498, \quad r = 0.998 \qquad (1)$$

A similar linear relationship was observed between the peak current ($i_p$) of the RLX and the square root of the scan rate ($v^{1/2}$), as described by the following equation 2:

$$i_{pA1}\ (\mu A) = 0.331 \pm 0.0106\ v^{1/2}\ (mV\ s^{-1}) - 0.925 \pm 0.0324, \quad r = 0.98 \qquad (2)$$

These results indicate that both adsorption and diffusion phenomena are effective for RLX oxidation on the BDD electrode. Additionally, the relationship between the logarithm of the peak current and the logarithm of the scan rate was investigated, and a linear fit to Eq.3 was obtained:

$$\log i_p\ (\mu A) = 0.563 \pm 0.0181 \log v\ (mV\ s^{-1}) - 0.721 \pm 0.0252, \quad r = 0.988 \quad (3)$$

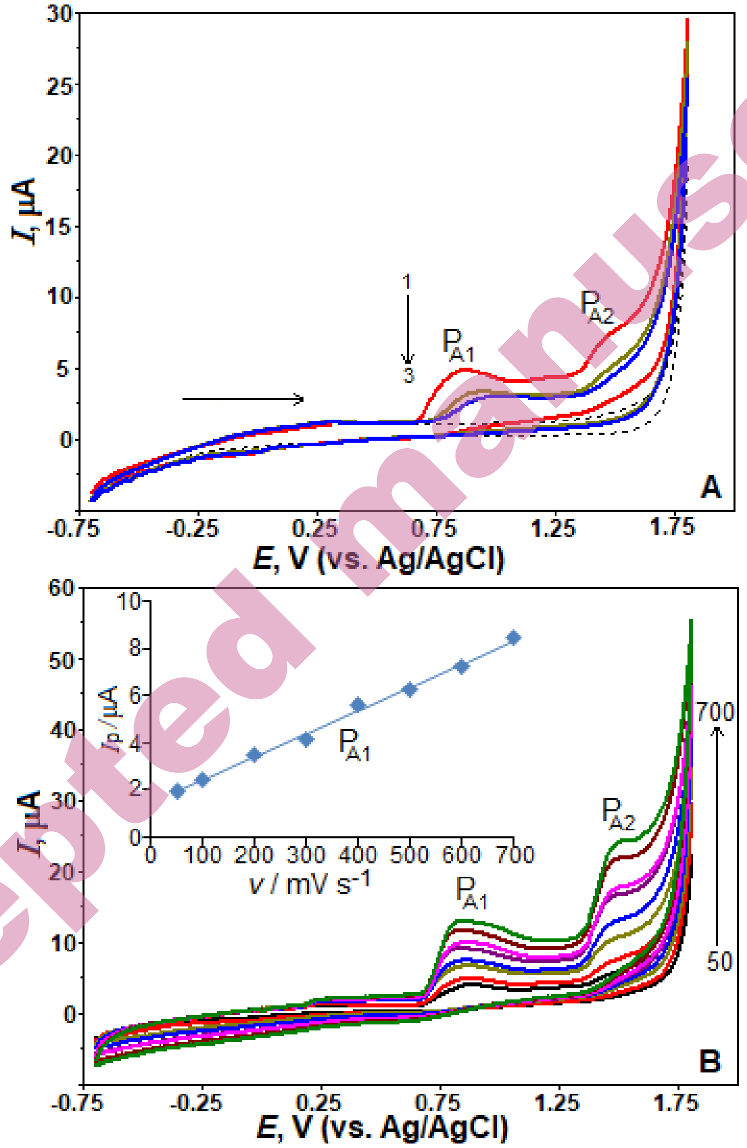


**Fig.1.** Triplicate CVs of 100 µg mL$^{-1}$ RLX obtained at a scan rate of 100 mV s$^{-1}$ (A), CV records of 100 µg mL$^{-1}$ at different scan rates (50, 100, 200, 300, 400, 500, 600, and 700 mV s$^{-1}$)

The slope of this line (~0.56), lying between the expected values of 1 and 0.5 for adsorption– and diffusion–controlled processes, respectively, confirms the dual mechanism of RLX oxidation. Similar results were reported in our research team's previously published work.[40] Scan rate studies indicate that RLX adsorbs onto the BDD electrode surface and suggest that improved sensitivity can be achieved by applying deposition processes in the quantitative analysis of RLX. Therefore, such an approach is expected to both mitigate electrode surface fouling and enhance sensitivity in investigating RLX oxidation processes in aqueous solutions. In the later stages of the study, adsorption-based modulation (square wave-adsorptive stripping voltammetry, SW–AdSV) was preferred due to its excellent sensitivity and lower consumption of electroactive molecules.

The electrochemically active surface area of the BDD electrode was evaluated by CV in a 0.1 M KCl electrolyte containing 1.0 mM $K_3[Fe(CN)_6]$. CV measurements were collected at scan rates between 25 and 400 mV $s^{-1}$. The electroactive surface area was obtained from the linear relationship between the peak current ($I_p$) and the square root of the scan rate ($v^{1/2}$), in accordance with the the Randles-Ševčík equation:

$$I_p = (2.69 \times 10^5)\, n^{3/2} A D^{1/2} C v^{1/2} \quad (4)$$

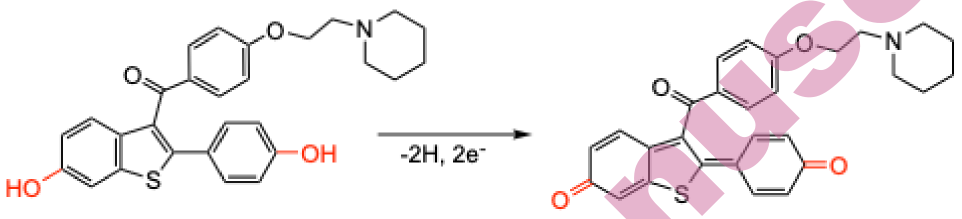
where $I_p$ denotes the anodic peak current (A), $n$ is the number of electrons involved in the redox process ($n = 1$), $A$ represents the electroactive surface area ($cm^2$), $D$ is the diffusion coefficient ($7.6 \times 10^{-6}$ $cm^2$ $s^{-1}$), $C$ corresponds to the concentration of the redox probe (M), and $v$ is the scan rate (V $s^{-1}$). Based on these parameters, the electrochemically active surface area of the BDD electrode was calculated to be 0.039 $cm^2$. This value is consistent with a previously published study.[41]

*Effect of supporting electrolyte pH and the nature of reaction mechanism*

It is a known fact that changing the pH of the supporting electrolyte also changes the response of the oxidation/reduction products of the electroactive species. This effect manifests both in the potential position of the peaks within the working potential window and in their current intensity. To investigate this influence more thoroughly, the effect of the BR supporting electrolyte on the RLX oxidation current at the BDD electrode was examined in the range of 2–11 (Fig.2). The study was carried out using SW–AdSV in the potential range of 0 to 1.30 V for a RLX concentration of 2.5 μg $mL^{-1}$ As clearly seen in Fig.3., the oxidation potentials of RLX shift toward more negative values with increasing pH. This observation provides evidence that RLX oxidation on the BDD electrode is pH dependent. Conversely, a general decreasing trend in the peak currents of RLX oxidation signals is also observed as the pH increases. Moreover, the variation in the peak potential positions (Ep) of RLX oxidation as a function of the working solution's pH exhibited a linear correlation, as described by Eq. (5) (inset of Fig. 2).

$$E_p\,(V) = -\,0.055\,pH + 0.862 \qquad r = 0.993 \quad (5)$$

The slope of this linear correlation is 55 mV which is close to the theoretical Nernstian value of 59 mV per pH unit. This can be interpreted as evidence that the number of protons and electrons involved in the RLX oxidation are equal.[42,43] Given the extant data and previously proposed mechanistic pathways, we posit that RLX undergoes oxidation to the products depicted in Scheme 1 at the pretreated BDD electrode.[27,29]



**Scheme 1.** Possible oxidation mechanism of RLX.

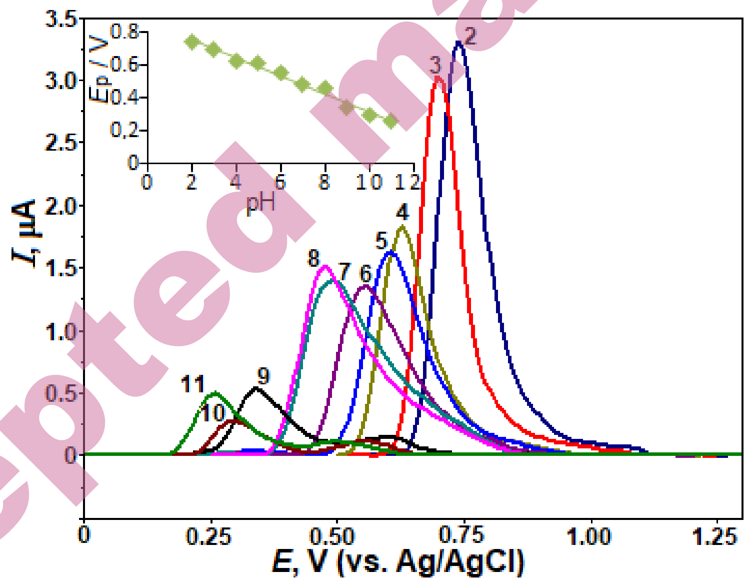


**Fig.2.** SW−AdS voltammograms for 2.5 μg $mL^{-1}$ RLX in BR buffer (pH 2−11) at the BDD electrode. At open circuit, the accumulation time is 30 s; the SWV parameters were just as follows: $f = 50$ Hz; $\Delta E_s = 8$ mV; $\Delta E_{sw} = 30$ mV.

*Effect of accumulation variables*

In processes where the adsorption significantly influences electrode kinetics, investigating the effects of accumulation parameters on the response of the electroactive species is an important step. Therefore, the effect of the accumulation time and accumulation potential on the electrochemical response of RLX were examined (data not shown). During these experiments, one parameter was kept constant while the other variable was varied. First, the accumulation potential was optimized under open circuit condition during the accumulation time ranging from

0 to 180 s for 2.5 µg mL$^{-1}$ RLX in BR supporting electrolyte at pH 2 supporting electrolyte using the BDD electrode. The results indicated that the maximum peak current for RLX was achieved after 30s of accumulation, with no significant increase observed at longer times. This suggest that the 30 s accumulation time was sufficient to adsorb the maximum amount of RLX onto the electrode surface. When longer deposition times were tested, RLX adsorption onto the electrode surface ceased, and the resulting current responses remained nearly unchanged.

The accumulation potential, the second accumulation parameter, was investigated over the range of +0.1 to +0.6 V as well as under open circuit conditions using the previously optimized accumulation time of 30 s. The results demonstrated that varying the accumulation potential, whether under open circuit conditions or applied potentials, did not significantly influence the oxidation peak currents of RLX. Therefore, subsequent experiments were conducted under open circuit conditions.

*Optimization of SWV instrumental parameters*

Optimizing the conditions that yield the highest peak current-considering chemical and accumulation variables, as well as SW modulation parameters such as frequency ($f$), step potential ($\Delta E_s$) and pulse amplitude ($\Delta E_{sw}$), is critical for enhancing the anodic peak current. In SW modulation optimization, two of the three variables are held constant while the third is varied to obtain the highest and best-shaped peak current. The study proceeded by sequentially fixing the optimized value of one parameter and optimizing the others to achieve the best instrumental response. All instrumental optimizations were conducted under open circuit with 30 s accumulation time in BR pH 2. First, the frequency was varied from 50 Hz to 125 Hz while step potential and pulse amplitude were held constant at 8 mV and 30 mV, respectively. As the frequency value increased, the anodic peak current of RLX also increased, but the peak broadening was observed beyond 100 Hz. Since the highest and the most well-defined peak was obtained at 100 Hz, this frequency was for subsequent experiments. Next, with the frequency fixed at 100 Hz and the step potential to 8 mV, and the pulse amplitude was varied between 30 and 70 mV. The anodic peak current increased linearly with pulse amplitude; however, a significant broadening of the peak shape was noted beyond 60 mV. Therefore, 60 mV was chosen as the optimal pulse amplitude. Finally, with the frequency and pulse amplitude fixed at their optimized values, the step potential was varied between 8 and 14 mV. The anodic peak current increased up to 12 mV and then decreased thereafter. Hence 12 mV was selected as the optimal step potential for further analytical studies. In conclusion, since the optimal instrumental parameters–100 Hz frequency, 60 mV pulse amplitude, and 12 mV step potential–yielded the best and highest peak currents, these values were adopted for subsequent stages of the study.

*Analytical performance of the method*

The functional applicability of the optimized experimental and instrumental parameters using SW–AdS voltammetry method was evaluated by monitoring the change in peak current with increasing RLX concentration in 0.04 mol $L^{-1}$ BR supporting electrolyte at pH 2. This was achieved by sequentially adding standard RLX solutions of varying concentrations to the voltammetric cell and recording the corresponding signals. Figure 3 shows the changes in SW-AdS voltammograms of the RLX oxidation peak currents at 0.74 V with increasing standard RLX concentration. The RLX concentration was varied from 0.025 (4.9 x $10^{-8}$ mol $L^{-1}$) to 5 µg $mL^{-1}$ (9.9 x $10^{-6}$ mol $L^{-1}$), with voltammograms recorded after each addition. The inset of Figure 4 presents the calibration curve demonstrating a linear relationship between the anodic peaks and RLX concentration. A linear correlation, as described by Equation 6, was obtained between the anodic peak signals and RLX concentration.

$$i_{\mathrm{p}}\,(\mu\mathrm{A}) = 2.576 \pm 0.0745\ c\ (\mu\mathrm{g\ mL^{-1}}) + 0.273 \pm 0.0085 \quad (r = 0.999,\ n = 8) \quad (6)$$

In the linear equation, $c$ indicates the RLX concentration, $i_{\mathrm{p}}$ indicates the anodic peak current, $n$ represents the number of measurements, and $r$ denotes the correlation coefficient.

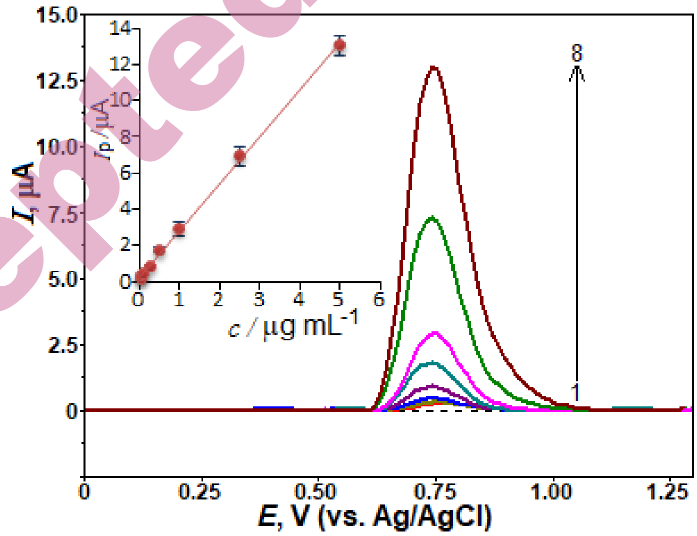


**Fig.3.** SW–AdS voltammograms with RLX concentrations of (1–8) 0.025, 0.05, 0.10, 0.25, 0.50, 1.0, 2.5, and 5.0 µg $mL^{-1}$ in BR buffer solution at pH 2 on the BDD electrode. The calibration graph for RLX measurement is shown inset. At open circuit, the accumulation time is 30 s, and the SWV parameters are all as shown: $f$ = 100 Hz; $\Delta E_s$ = 12 mV; $\Delta E_{sw}$ = 60 mV.

Using this analytical curve, the detection limit (LOD) and quantification limit (LOQ) were calculated as 0.0073 µg $mL^{-1}$ (1.4 x $10^{-8}$ mol $L^{-1}$), and 0.025 µg $mL^{-}$

$^{1}$ (4.9 x $10^{-8}$ mol L$^{-1}$), respectively. The 3*s/m* relation was used to calculate the detection limit. where *s* is the standard deviation of 10 measurements of the lowest concentration on the calibration line, and *m* is the slope of the corresponding calibration curve[44]. The analytical performance of the proposed method is compared with those of other previously published electrochemical analyses in Table I, which is arranged from the most sensitive to the least sensitive according to LOD values (except for our study, given in the last column). In electrochemical analyses, working electrodes are often functionalized with modifying agents to achieve better sensitivity,[28,32,34,35] however, some studies exhibit lower sensitivity compared to our results.[27,30,31,33] Achieving comparable sensitivity levels to modified electrode in RLX analyses with a sample pre-treatment step-without any electrode modification-represents the most original and prominent aspect of the method.

The precision of the method under the same conditions was investigated through intraday and inter-day repeatability experiments. The intraday repeatability of the anodic peak shapes was assessed by performing ten repetitions using 0.025 µg mL$^{-1}$ of the RLX solution. An RSD value of 5.22% shows that the method is repeatable. Additionally, the RSD value of 6.85% for inter-day repeatability, measured at the same concentration and under the same conditions over five consecutive days, also serves as a clear indicator of the method's repeatability of for analytical purposes.

TABLE I. Comparison of published electroanalytical methods for RLX detection.

| Working electrode | Supporting electrolyte | Technique | Linear range (µM) | LOD (nM) | Analyzed samples | Ref. |
|---|---|---|---|---|---|---|
| GO–CuO–PP/PGE | PBS pH 2.5 | SWV | 40–320 | 3 | Tablets, serum | 36 |
| CPE/NiO/SWCNTs/ 1B4MPTFB | PBS pH 7 | SWV | 0.03–520 | 7 | Tablets, serum | 33 |
| N–CQD/Fe$_3$O$_4$/N–B–3–MITFB/CPE | PBS pH 5 | DPV | 0.04–320 | 10 | Tablets, Urine | 35 |
| CNP/Mela/GCE | BR pH 3 | DPAdSV | 0.04–2 | 10 | Tablets | 29 |
| Nd$_2$O$_5$ NPs@GO | PBS pH 7 | Amperometry | 0.03–472.5 | 18.43 | Blood, urine | 31 |
| Carbon nanopowder/CPE | PBS pH 8 | AdLSSV | 0.001–40 | 19.5 | Tablets, serum | 32 |
| 1–M–3–OITFB/ZnO/CNTs/CPE | pH 8 | DPV | 0.08–400 | 40 | Tablets, Urine | 34 |
| GCE | PBS pH 3 | DPV | 0.2–50 | 75 | Tablets, plasma | 28 |
| BDD | BR pH 2 | SW-AdSV | 0.049–9.9 | 14 | Tablets | This work |

**Electrode:** GO-CuO-PP/PGE; graphene-CuO-polypyrrole pencil graphite electrode, CPE/NiO/SWCNTs/1B4MPTFB; carbon paste electrode nickel (II) oxide single-walled carbon nanotubes 1-butyl–4-methylpyridinium tetrafluoroborate, N-CQD/$Fe_3O_4$/N-B-3-MITFB/CPE: N-CQD/$Fe_3O_4$ nanoparticles and N-buty-3-methylimidazolium tetrafluoroborate, CNP/Mela/GCE; carbon nanoparticle melamine glassy carbon electrode, $Nd_2O_5$ NPs@GO; neodymium sesquioxide nanoparticles decorated graphene oxide nanocomposite, 1-M-3-OITFB/ZnO/CNTs/CPE; 1-methyl-3-octylimidazolium tetrafluoroborate and ZnO/CNTs nanocomposite, GCE; glassy carbon electrode, BDD; Boron doped diamond. **Technique:** SWV; square wave voltammetry, DPV; differential pulse voltammetry; DPAdSV; differential pulse adsorptive stripping voltammetry, AdLSSV; adsorptive linear sweep stripping voltammetry, SW-AdSV; square wave adsorptive stripping voltammetry

*Effect of interference*

Before testing the applicability of the method on real samples, the effect of potential interfering species in drug formulations was investigated using SW−AdSV under the same test conditions and for 0.1 µg $mL^{-1}$ RLX. The concentrations of interfering species causing ±5% error in RLX peak currents were adopted as tolerable limit values. It was observed that inorganic ionic species such as calcium ($Ca^{2+}$), magnesium ($Mg^{2+}$), zinc ($Zn^{2+}$), iron ($Fe^{3+}$), titanium ($Ti^{4+}$), potassium ($K^{+}$), sodium ($Na^{+}$), chloride ($Cl^{-}$), nitrate ($NO_3^{-}$) and sulphate ($SO_4{}^{2-}$) did not have a noticeable effect on RLX signals even in the presence of 50−fold excess concentrations of RLX. Similarly, the presence of sugars commonly used in drug formulations such as mannitol, glucose, sucrose and fructose at 50−fold excess concentration did not have a significant effect on RLX signals. Finally, drug additives such as microcrystalline cellulose, corn starch, and magnesium stearate, even at excessive concentrations (50−fold), did not alter RLX signals. These findings show that the proposed method has a high degree of selectivity and can be successfully applied to commercially available pharmaceutical formulations.

*Applicability of the method on real samples*

In the final stage, the practical applicability of the developed method was tested for the detection of RLX in commercially available drug formulations (Fig.4). Detailed procedure for drug sample preparation and subsequent evaluation are described in experimental section. After spiking the drug samples with standard RLX solutions, it was found that each tablet contained 56.9 mg RLX (3.3% RSD), which is very close to the 60 mg value declared by the manufacturer.

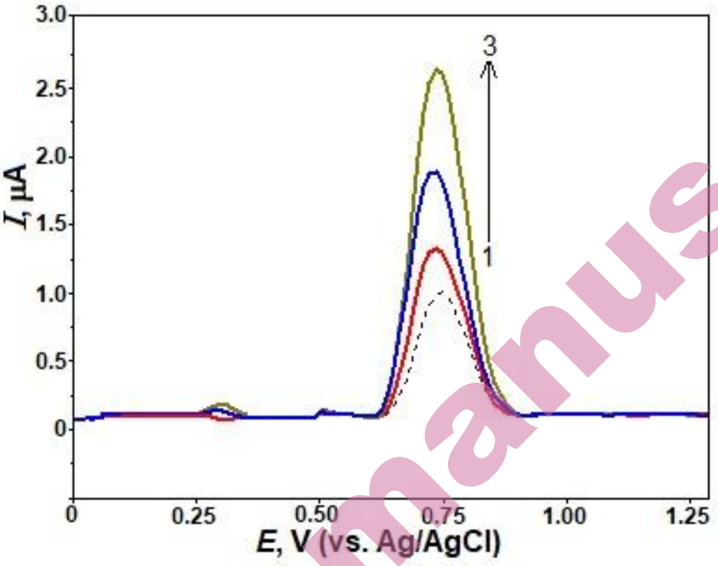


**Fig.4.** SW−AdS voltammograms of a such diluting pharmaceutical formulation (dashed lines) as well as standard additions to create the final concentration values of (1−3) 0.1, 0.25, and 0.50 μg mL$^{-1}$ RLX in BR buffer solution at pH 2. Other set of working circumstances is depicted in Fig. 4.

To verify the proposed method, a recovery study was conducted. This study involved spiking RLX standard solutions to the solution containing into the drug sample solution in the voltammetric cell at final concentrations of 0.1, 0.25 and 0.5 μg mL$^{-1}$. The RSD values obtained from three replicate measurements are presented in Table II. The satisfactory recovery results demonstrate that the proposed method can be effectively applied to the analysis of RLX in tablet formulations without significant matrix effect.

TABLE II. Recovery values of pharmaceutical formulations samples spiked with RLX standard solutions by using the proposed voltammetric method

| Added[a] (μg mL$^{-1}$) | Expected[a] (μg mL$^{-1}$) | Found[a,b] (μg mL$^{-1}$) | Recovery (%) ± RSD (%) |
|---|---|---|---|
| 0 | – | 0.237 | – ± 3.3 |
| 0.1 | 0.337 | 0.361 | 107.1 ± 3.1 |
| 0.25 | 0.487 | 0.493 | 101.2 ± 2.8 |
| 0.50 | 0.737 | 0.689 | 93.5 ± 2.5 |

[a] Concentration in the measured solution
[b] Average of three replicate measurements

## CONCLUSION

This study describes a procedure for applying a mechanical cleaning process to BDD electrode following anodic and cathodic pretreatment steps, respectively, before employing them for the quantitative analysis of RLX. The electrochemical behavior of RLX was elucidated using CV, while quantitative analysis was conducted based on SW modulation. Considering that adsorption, in addition to diffusion, influences the transport of RLX to the electrode surface, the effect of deposition parameters on the quantitative analysis were examined, and optimum conditions were identified. After a simple pretreatment applied of BDD electrode, a linear working range of 0.025 to 5 μg mL$^{-1}$ and a detection limit value of 0.0073 μg mL$^{-1}$ (1.4 x 10$^{-8}$ mol L$^{-1}$) were achieved under optimized experimental conditions including deposition step and the best SW modulation parameters. The method proposed herein offers a simpler and more rapid electrode preparation compared to electrochemical analysis techniques based on modified electrodes for RLX detection. It is also present advantages in terms of ease of use and cost-effectiveness avoiding the laborious electrode preparation processes commonly required. Compared to other analytical approach, this method provides a cost-efficient and straightforward alternative for RLX analysis with simpler instrumentation and procedure. Furthermore, the electrochemical protocol in this study demonstrates good sensitivity for analyzing other electroactive species such as active pharmaceutical ingredients and environmental contaminants, making it a promising candidate as an alternative method for rapid and economical future solutions.

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## ИЗВОД

### ЈЕДНОСТАВНО И ИЗВОДЉИВО ОДРЕЂИВАЊЕ СЕЛЕКТИВНОГ МОДУЛАТОРА ЕСТРОГЕНСКИХ РЕЦЕПТОРА РАЛОКСИФЕНА У ФАРМАЦЕУТСКОЈ ФОРМУЛАЦИЈИ КОРИШЋЕЊЕМ ПРЕТХОДНО ТРЕТИРАНЕ ДИЈАМАНТСКЕ ЕЛЕКТРОДЕ ДОПОВАНЕ БОРОМ

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У овом раду се приказује развој електроаналитичке методе за квантитативно одређивање селективног модулатора естрогенских рецептора ралоксифена (RLX) коришћењем волтаметрије на претходно третираној дијамантској електроди допованој бором (BDD). Циклична волтаметрија RLX је показала иреверзибилно понашање у Britton-Robinson (BR) основном електролиту концентрације 0,04 mol L$^{-1}$ и вредности pH 2, при чему су добијена два анодна оксидациона пика на приближно +0,79 V ($P_{A1}$) и +1,46 V ($P_{A2}$). Зависност волтамограма од брзине скенирања је показала да је транспорт RLX до површине електроде одређен и адсорпцијом и дифузијом. Сходно томе, претпостављено је да би се аналитичка осетљивост повећала укључивањем ступња претходне концентрације (таложења). Оптимални параметри таложења, заједно са pH вредношћу електролита и модулацијом параметара волтаметрије са правоугаоним сигналом (SWV) су систематски оптимизовани. Квантитативна анализа је заснована на анодном пику $P_{A1}$ у BR пуферу концентрације 0,04 mol L⁻¹ при pH 2, и показала је линеарни динамички опсег од 0,025 до 5,0 µg mL⁻¹ ($4,9\cdot10^{-8}$ до $9,9\cdot10^{-6}$ mo L⁻¹) и границу детекције од 0,0073 µg mL⁻¹ ($1,4\cdot10^{-8}$ L⁻¹). Применљивост методе је потврђена успешном квантификацијом RLX у фармацеутским формулацијама.

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## REFERENCES

1. Y. Wu, X. Jin, E. Ashrafzadeh Afshar, M. A. Taher, C. Xia, S. W. Joo, T. Mashifana, Y. Vasseghian, *Chemosphere* **305** (2022) 135392 (https://doi.org/10.1016/j.chemosphere.2022.135392)
2. I. Bozovic-Spasojevic, E. Azambuja, W. McCaskill-Stevens, P. Dinh, F. Cardoso, *Cancer Treat. Rev.* **38** (2012) 329 (https://doi.org/10.1016/j.ctrv.2011.07.005)
3. M. D. Ansari, I. Khan, P. Solanki, J. Pandit, R. N. Jahan, M. Aqil, Y. Sultana, *J. Drug Deliv. Sci. Technol.* **68** (2022) 103102 (https://doi.org/10.1016/j.jddst.2022.103102)
4. T. Sözen, L. Özışık, N. Çalık Başaran, *Eur. J. Rheumatol.* **4** (2017) 46 (https://pmc.ncbi.nlm.nih.gov/articles/PMC5335887/)
5. M. Gass, B. Dawson-Hughes, *American J. Med.* **119** (2006) S3 (https://doi.org/10.1016/j.amjmed.2005.12.017)
6. A. S. Abu Lila, M. H. Abdallah, S. U. D. Wani, H. V. Gangadharappa, K. M. Younes, E. S. Khafagy, T. M. Shehata, M. S. Soliman, *Colloids Surf. A Physicochem. Eng. Asp.* **625** (2021) 126971 (https://doi.org/10.1016/j.colsurfa.2021.126971)
7. A. Jemal, R. Siegel, E. Ward, Y. Hao, J. Xu, M. J. Thun, *CA Cancer. J. Clin.* **59** (2009) 225 (https://doi.org/10.3322/caac.20006)
8. B. H. Mitlak, F. J. Cohen, *Drugs* **57** (1999) 653 (https://doi.org/10.2165/00003495-199957050-00001)
9. G. Y. Stokes, J. C. Conboy, *J. Am. Chem. Soc.* **136** (2014) 1409 (https://doi.org/10.1021/ja409250y)
10. R. Abdelhamid, J. Luo, L. VandeVrede, I. Kundu, B. Michalsen, V. A. Litosh, I. T. Schiefer, T. Gherezghiher, P. Yao, Z. Qin, G. R. J. Thatcher, *ACS Chem. Neurosci.* **2** (2011) 256 (https://doi.org/10.1021/cn100106a)
11. H. D. Nelson, M E Beth Smith, J. C. Griffin, R. Fu, *Ann. Intern. Med.* **158** (2013) 604 (https://doi.org/10.7326/0003-4819-158-8-201304160-00005)
12. M. Y. Williams-Brown, S. M. Salih, X. Xu, T. D. Veenstra, M. Saeed, S. K. Theiler, C. R. Diaz-Arrastia, S. A. Salama, *J. Steroid Biochem. Mol. Biol.* **126** (2011) 78 (https://doi.org/10.1016/j.jsbmb.2011.05.001)
13. A. M. Baraka, A. A. Korish, G. A. Soliman, H. Kamal, *Life Sci.* **88** (2011) 879 (https://doi.org/10.1016/j.lfs.2011.03.010)
14. A. A. Poirier, M. Côté, M. Bourque, M. Morissette, T. Di Paolo, D. Soulet, *Neurobiol. Aging* **48** (2016) 61 (https://doi.org/10.1016/j.neurobiolaging.2016.08.004)
15. M. Bourque, M. Morissette, T. Di Paolo, *Neurobiol. Aging* **35** (2014) 2347 (https://doi.org/10.1016/j.neurobiolaging.2014.03.017)

16. Y. Wu, X. Jin, E. Ashrafzadeh Afshar, M. A. Taher, C. Xia, S. W. Joo, T. Mashifana, Y. Vasseghian, *Chemosphere* **305** (2022) 135392 (https://doi.org/10.1016/j.chemosphere.2022.135392)
17. F. Ibrahim, N. El-Enany, R. El-Shaheny, I. Mikhail, *J. Mol. Liq.* **252** (2018) 408 (https://doi.org/10.1016/j.molliq.2018.01.005)
18. K. Basavaiah, K. Tharpa, U. R. Anil Kumar, N. Rajedraprasad, S. G. Hiriyanna, K. B. Vinay, *Arch. Pharm. Res.* **32** (2009) 1271 (https://doi.org/10.1007/s12272-009-1913-7)
19. J. Dharuman, V. Ravichandran, N. Thirumoorthy, A. Dharamsi, *Pharmazie* **59** (2004) 720 (https://www.ingentaconnect.com/contentone/govi/pharmaz/2004/00000059/00000009/art00013)
20. K. Basavaiah, U. R. A. Kumar, K. Tharpa, *Acta Pharmaceutica* **58** (2008) 347 (https://doi.org/10.2478/v10007-008-0018-z)
21. J. Trontelj, T. Vovk, M. Bogataj, A. Mrhar, *Pharmacol. Res.* **52** (2005) 334 (https://doi.org/10.1016/j.phrs.2005.05.007)
22. J. Zweigenbaum, J. Henion, *Anal. Chem.* **72** (2000) 2446 (https://doi.org/10.1021/ac991413p)
23. T. Pérez-Ruiz, C. Martínez-Lozano, A. Sanz, E. Bravo, *J. Pharm. Biomed. Anal.* **34** (2004) 891 (https://doi.org/10.1016/j.jpba.2003.12.008)
24. S. P. Liu, Y. Q. He, Z. F. Liu, L. Kong, Q. M. Lu, *Anal. Chim. Acta* **598** (2007) 304 (https://doi.org/10.1016/j.aca.2007.06.054)
25. E. Keskin, A. S. Ertürk, *Ionics (Kiel)* **24** (2018) 4043 (https://doi.org/10.1007/s11581-018-2532-4)
26. M. Ghalkhani, N. K. Bakirhan, S. A. Ozkan, *Crit. Rev. Anal. Chem.* **50** (2020) 538 (https://doi.org/10.1080/10408347.2019.1664281)
27. X. Q. Li, J. B. He, L. Liu, T. Cui, *Electrochim. Acta* **96** (2013) 74 (https://doi.org/10.1016/j.electacta.2013.02.069)
28. A. Bagheri, H. Hosseini, *Bioelectrochemistry* **88** (2012) 164 (https://doi.org/10.1016/j.bioelechem.2012.03.007)
29. S. Shahrokhian, M. Ghalkhani, H. Balotf, R. Salimian, *J. Electroanal. Chem.* **780** (2016) 126 (https://doi.org/10.1016/j.jelechem.2016.09.012)
30. M. Ghalkhani, S. Shahrokhian, M. Navabi, *Mater. Chem. Phys.* **263** (2021) 124131 (https://doi.org/10.1016/j.matchemphys.2020.124131)
31. T. W. Chen, J. Princy Merlin, S. M. Chen, S. Anandaraj, M. S. Elshikh, T. W. Tseng, K. Wang, D. Qi, J. Jiang, *Ultrason. Sonochem.* **64** (2020) 104717 (https://doi.org/10.1016/j.ultsonch.2019.104717)
32. N. L. Teradal, R. D. Tandel, J. Seetharamappa, *Mater. Sci. Energy Technol.* **2** (2019) 337 (https://doi.org/10.1016/j.mset.2019.02.007)
33. S. Salmanpour, A. Sadrnia, F. Karimi, N. Majani, M. L. Yola, V. K. Gupta, *J. Mol. Liq.* **254** (2018) 255 (https://doi.org/10.1016/j.molliq.2018.01.105)
34. S. Cheraghi, M. A. Taher, H. Karimi-Maleh, *Appl. Surf. Sci.* **420** (2017) 882 (https://doi.org/10.1016/j.apsusc.2017.05.218)
35. F. Shalali, S. Cheraghi, M. A. Taher, *Mater. Chem. Phys.* **278** (2022) 125658 (https://doi.org/10.1016/j.matchemphys.2021.125658)
36. M. Fouladgar, H. Karimi-Maleh, F. Opoku, P. P. Govender, *J. Mol. Liq.* **311** (2020) 113314 (https://doi.org/10.1016/j.molliq.2020.113314)

37. R. R. Swara, A. I. M. Rahma, C. Y. Kharismasari, D. A. Wulandari, S. N. Zakiyyah, Y. Einaga, Y. W. Hartati, Irkham, *Clinica Chimica Acta* **574** (2025) 120285 (https://doi.org/10.1016/j.cca.2025.120285)
38. F. Zouaoui, G. Menassol, C. Ducros, P. Mailley, Y. Thomas, *Microchem. J.* **209** (2025) 112650 (https://doi.org/10.1016/j.microc.2025.112650)
39. T. Kondo, *Curr. Opin. Electrochem.* **32** (2022) 100891 (https://doi.org/10.1016/j.coelec.2021.100891)
40. H. İ. Özok, S. Allahverdiyeva, Y. Yardım, Z. Şentürk, *Electroanalysis* **34** (2022) 1487 (https://doi.org/10.1002/elan.202100654)
41. S. Allahverdiyeva, E. Keskin, P. T. Pınar, O. Yunusoğlu, Y. Yardım, Z. Şentürk, *Diam. Relat. Mater.* **108** (2020) 107962 (https://doi.org/10.1016/j.diamond.2020.107962)
42. N. Milhazes, P. Martins, E. Uriarte, J. Garrido, R. Calheiros, M. P. M. Marques, F. Borges, *Anal. Chim. Acta.* **596** (2007) 231 (https://doi.org/10.1016/j.aca.2007.06.027)
43. Ľ. Švorc, M. Vojs, P. Michniak, M. Marton, M. Rievaj, D. Bustin, *J. Electroanal. Chem.* **717–718** (2014) 34 (https://doi.org/10.1016/j.jelechem.2014.01.008)
44. H. B. Ammar, M. Ben Brahim, R. Abdelhédi, Y. Samet, *Materials Science and Engineering C* **59** (2016) 604 (https://doi.org/10.1016/j.msec.2015.10.025).