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Determination of indacaterol from inhaler capsules by square-wave voltammetry at the surface of the boron-doped diamond electrode

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Abstract: This research paper presents an electroanalytical investigation using the voltammetric method to quantify indacaterol maleate (IND) employing an unmodified boron-doped diamond (BDD) electrode. IND exhibited a distinct, irreversible oxidation peak at approximately 1.06 V (*vs*. Ag/AgCl) in a 0.1 mol L^{-1} phosphate buffer solution (PBS) with a pH of 2.5, as demonstrated by cyclic voltammetry (CV). A hypothetical mechanism for the electro-oxidation of IND, based on data gathered from CV investigations, was suggested. The square wave-adsorptive stripping voltammetric technique achieved acceptable linearity in PBS (pH 2.5) at approximately 0.90 V. The methodology demonstrated linearity within the concentration range of 1.0 to 30.0 μg mL^{-1} (equivalent to 1.97×10^{-6}–5.89×10^{-5} mol L^{-1}) and yielded a limit of detection (*LOD*) of 0.22 μg mL^{-1} (equivalent to 4.33×10^{-7} mol L^{-1}). The proposed method's applicability was assessed through the sensing of IND in drug formulations.

Keywords: indacaterol maleate; square-wave voltammetry; boron-doped diamond electrode; inhaler capsules.

INTRODUCTION

In the realm of medical research, it is widely acknowledged that inhaled bronchodilators hold a position of utmost importance as the fundamental symptomatic maintenance therapy for the effective management of chronic obstructive pulmonary disease (COPD). This particular ailment, which afflicts a significant portion of the population, is recognized for its characteristic slow and progressive development of airflow limitation.[1] COPD is characterized by its non-

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-reversible nature and usually corresponds with an aberrant inflammatory reaction within the lungs triggered by exposure to harmful particles and gases.[2,3] Therapeutic interventions aimed at relaxing bronchial smooth muscle and improving lung function in patients with COPD have demonstrated rapid, productive, and durable therapeutic results.[4,5]

Indacaterol maleate (IND), 5-[2-[(5,6-diethyl-2,3-dihydro-1*H*-inden-2-yl)-amino]-1-hydroxyethyl]-8-hydroxyquinolin-2(1*H*)-one, $C_{24}H_{28}N_2O_3 \cdot C_4H_4O_4$, molecular weight 508.6 as a maleate salt (392.49 as a base). The compound exhibits a water solubility of approximately 7.9×10^{-3} g L^{-1}, a log *P* value of 4.0 and pK_a values of 9.8 for the essential group and 8.5 for the acidic group. Functioning as a long-acting β2-agonist (LABA), it stimulates *β*2-receptors, increasing intracellular cyclic adenosine monophosphate (cAMP) levels.[6] Recently approved in the European Union, this novel medication is a pre-metered single-unit dose capsule-based ultra-long-acting *β*2-agonist for once-daily oral inhalation. It has gained approval from the United States Food and Drug Administration (FDA) for COPD management,[7,8] leading to improved bronchial muscle tissue smoothness. This once-daily regimen, containing an FDA-approved LABA dose, enhances health status, reduces dyspnea and lowers exacerbations in COPD patients.[9–11]

The deposition of inhaled medications in the pulmonary system is influenced by factors such as particle dimensions, inhalation apparatus structure, patient maneuver proficiency and airflow dynamics.[12,13] Various pharmacokinetic techniques assess lung deposition and therapeutic equivalency of inhaled treatments, using human plasma or urine as experimental samples.[14,15] Another method evaluates the effects of inhalation strategies and flow rates. IND, a recently developed long-acting *β*2-agonist, exhibits prolonged action due to its strong binding affinity to lipid raft domains in the airway membrane, impacting comparative lung deposition and systemic exposure upon inhalation.[16]

IND, administered at a daily dosage of 150–300 μg, demonstrates a slow dissociation rate from receptors.[17] Swift absorption upon oral administration results in extensive dispersion throughout the body, with an apparent volume of distribution of 10±4 L kg^{-1}. Plasma protein binding percentages range from 94.1 to 95.3 % and 95.1 to 96.2 %.[17] Extensive metabolic processes lead to the formation of the phenolic *O*-glucuronide. Primary adverse effects, especially in overdose cases, include nasopharyngitis, tremor, cough, upper respiratory tract infection, headache, tachycardia, palpitations, nausea, hypokalemia, vomiting, sleepiness, acidosis, cardiac arrhythmias, metabolic issues and hyperglycemia. IND's notable safety contributes to enhanced patient compliance and therapeutic effectiveness. Recent studies indicate potential advantages of IND in COPD management.[18,19]

Drug combinations, such as IND and glycopyrronium, are employed for the symptomatic management of chronic obstructive pulmonary disorder in adults. It

is crucial to emphasize consulting local prescribing information to obtain specific details on contraindications, special cautions, and precautions related to these medications. In this context, it is imperative to employ analytical methodologies that effectively substantiate the pharmacological attributes, therapeutic effectiveness and tolerability investigations concerning IND.[20] A limited range of physicochemical approaches has been documented for the quantification of IND in biological fluids and pharmacological formulations. Some involve high-performance liquid chromatography (HPLC) with various detectors,[21–27] ultra-performance liquid chromatography-diode array detector (UPLC-DAD),[28] ion-pairing chromatographic (IPC),[29] spectrophotometry,[30–32] capillary electrophoresis (CE),[33,34] potentiometry[35] and voltammetry.[36] However, only one study has shown that IND examination can be conducted using voltammetric approaches.[36]

In analytical chemistry, a critical application involves the analysis of pharmaceutical and clinical specimens.[37] Determining chemicals in various matrices is vital in pharmaceutical and medical sciences. For reliability and sensitivity, analytical techniques for such analyses should be applicable across a wide range of concentrations, quick, simple (suitable for non-experts) and cost-effective.[38] Electrochemical techniques, particularly voltammetric approaches, have gained prominence in drug and clinical analyses due to their benefits, including simplification, cost-effectiveness, rapid evaluation and high sensitivity. Understanding the oxidation and reduction mechanisms of pharmacologically relevant chemicals is equally crucial.[39]

A boron-doped diamond (BDD) electrode is a type of carbonaceous electrode material with unique properties, including a wide operational range in both cathodic and anodic directions. The research focuses on key aspects, such as investigating low background current, analyzing low signal-to-noise ratio, developing a process suitable for corrosive conditions, and establishing a repeatable response for electrochemical evaluations, all commonly observed in BDD. The wide operational range of the electrode material allows the analysis of electroactive compounds often inaccessible using conventional carbon and metal electrodes.[40] In contrast to many electrode materials like glassy carbon, BDD electrodes have limited capacity for modifications using modifying chemicals. This limitation stems from the restricted adsorption of both the sample and the modifying agent on the surfaces of BDD electrode materials.[41]

According to available data, a singular electroanalytical technique for detecting IND has been documented. The primary objective of this study is to establish a viable approach for IND analysis using a voltammetric modality that is cost-effective, expeditious and easy to apply. The effectiveness of the methodology was demonstrated through its application in a pharmaceutical formulation specimen under ideal conditions.

EXPERIMENTAL

Chemicals

The standard reference of IND maleate (ReagentPlus®, 99.81 %), purchased from ChemScene LLC (USA), was obtained. Due to its limited solubility in water, IND stock solutions were prepared by dissolving in ethanol to a concentration of 1.0 mg mL^{-1} and refrigerating until intended use. IND stock solutions were produced in the selected electrolytes at a reduced concentration. Ultra-pure water from a Milli-Q water purifying system (Millipore, resistivity ≥ 18.2 MΩ cm) was used for preparation. Analytical-grade materials, including 0.1 mol L^{-1} acetate buffer solution (ABS) at pH 4.7, 0.1 mol L^{-1} phosphate buffer solution (PBS) at pH 2.5 and 7.4, and 0.04 mol L^{-1} Britton–Robinson (BR) buffer among pH 2.0–8.0, were utilized. Voltammetric investigations conducted in aqueous buffer solutions were found to be independent of the presence of ethanol. The concentration of ethanol in the voltammetric cell was consistently kept below 10 % of the total volume.

Apparatus and analytical procedure

Using an electrochemical analyzer, specifically the μAutolab type III manufactured by Metrohm Autolab B.V. in the Netherlands, electrochemical measurements were conducted. The obtained data were collected and processed using GPES software, version 4.9. Baseline correction and smoothing of the peaks observed in the square wave voltammograms were performed using the Savicky–Golay method with a peak width of 0.01 V. For the electrochemical measurements, a three-electrode glass cell system with a 10 mL volume, maintained at ambient temperature, was employed. The cell configuration included a boron-doped diamond (BDD) working electrode, provided by Windsor Scientific Ltd., UK, with a boron content of 1000 ppm and a diameter of 3 mm. A platinum wire served as the auxiliary electrode, and an Ag/AgCl electrode immersed in a 3 mol dm^{-3} NaCl solution, specifically the RE-1 model from BAS, USA, functioned as the reference electrode. All electrode potentials in the paper are referred to Ag/AgCl scale. The impact of employing BDD electrode pretreatment methodologies on IND signals was examined. At the beginning of each experimental day, the BDD electrode underwent treatment, subjecting it to an anodic potential of 1.8 V for 180 s, followed by exposure to a cathodic potential of –1.8 V for an equivalent duration. The activation procedure began with anodic polarization to cleanse the fouled BDD surface, restoring its reactivity for subsequent use. Subsequently, cathodic polarization was applied to regenerate the primarily hydrogen-terminated electrode surface, inducing its hydrophobic characteristics. To mitigate potential detriment to the electrode surface, a rudimentary manual cleansing procedure was executed before each electrochemical assessment. The BDD electrode was gently abraded using a moistened, sleek polishing cloth (BAS velvet polishing pad) for less than 60 s, followed by a thorough water rinse.[44] This implemented methodology ensures the attainment of a pristine electrode surface, preventing any potential adsorption of IND or its oxidation byproducts.

The CV approach in the anodic direction (from 0.2 to 1.4 V) was employed to assess the electrochemical behavior and reaction mechanism of IND on the surface of a boron-doped diamond (BDD) electrode in the selected supporting electrolyte. Subsequently, square wave voltammetry (SWV) was used within a potential range of 0.25 to 1.30 V to identify the optimal conditions, including the supporting electrolyte (at various pH levels), and to compare differential pulse voltammetry (DPV), accumulation variables and SWV parameters, all aimed at enhancing the sensitivity for IND detection. Additionally, the analytical capability of the

developed methodology, the influence of interfering substances, and the practical applicability of the technique were evaluated.

To perform SW-AdSV sensing of IND, a three-electrode configuration was immersed in a voltammetric cell containing IND solutions and phosphate buffer solution (PBS) at pH 2.5. The voltammetric cell, under open-circuit conditions with a deposition time of 30 s, received a voltage application and was stirred at 500 rpm. After 10 s of equilibration, anodic scanning was recorded within the potential range of 0.25 to 1.3 V. The electrochemical analyses were conducted in triplicate, and the detection of IND in actual samples was carried out using the standard addition methodology.

Preparation of samples

The IND sample was obtained from a local pharmacy in the form of inhaler capsules (Inbroxa®, Deva Co., Türkiye), each containing 150 μg (195 μg IND maleate). The mass of ten capsules was precisely measured. After carefully extracting the outer shell of the capsules, the mass of the empty capsules was determined. The net weight per capsule was calculated by subtracting the weight of the empty capsules from the overall net weight. An appropriate amount of powder, equivalent to 1.0 mg of IND maleate, was quantified and placed in a 1-mL test tube, then saturated with the requisite volume of ethanol. Continuous agitation for approximately 15 min ensured complete dissolution. An appropriate volume of the solution (50 μL) was transferred to an electrochemical cell containing 10 mL of phosphate buffer solution (PBS) at pH 2.5 and was examined on the same day of preparation using the developed method. The content of IND in the sample was determined using the standard addition method, by adding IND at concentrations of 1.0, 2.5, 5.0, 7.5, 10 and 15 μg mL^{-1} to the product. Consequently, SW-AdS voltammograms were recorded after each addition.

RESULTS AND DISCUSSION

Investigation of the electrochemical behavior of IND on the boron-doped diamond electrode

Applying the CV method to the BDD electrode allowed us to evaluate the electrochemical properties of IND. Using a voltage scanning rate of 100 mV s^{-1}, three sequential cyclic voltammograms (CVs) for 100 μg mL^{-1} (1.97×10^{-4} mol L^{-1}) of IND were recorded between 0.2 and 1.40 V in 0.1 mol L^{-1} PBS at pH 2.5. The IND compound exhibited a distinct anodic peak (I_p) at a potential of approximately 1.06 V, following the completion of the initial cycle in the anodic direction (depicted in Fig. 1A). If the reversed scan does not exhibit a reduction peak, it indicates that the IND electrode reactions at the BDD electrode surface are irreversible, distinguishing them from other surface reactions. During the acquisition of sequential CVs, a decrease in the oxidation signal was observed, and potentially attributed to the adsorption of IND and/or its oxidation products on the surface of the BDD electrode, leading to deactivation, possibly through fouling. The investigation focused on exploring the influence of potential scan rate (v) on the anodic peak current of 100 μg mL^{-1} IND, employing CV in a 0.1 mol L^{-1} PBS solution (pH 2.5) to ensure that the anodic peak current could be clearly observed and measured with sufficient sensitivity and accuracy. The volt-

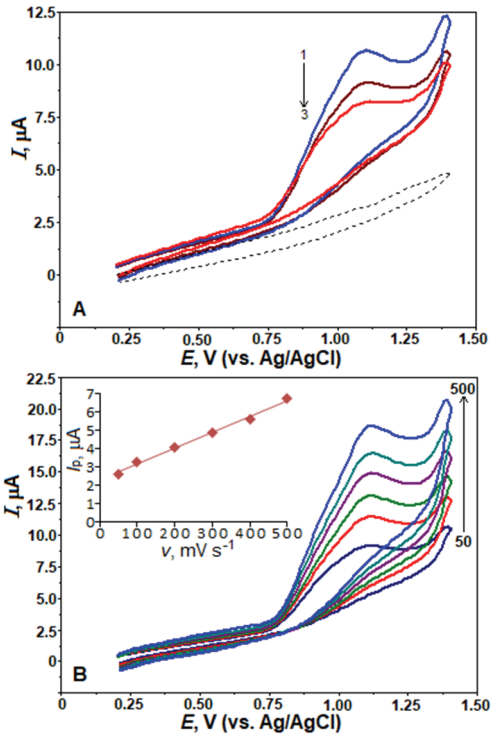
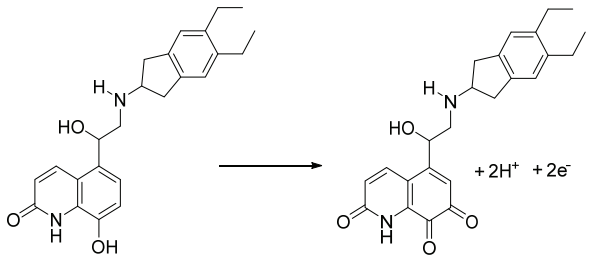


Fig. 1. A) The first three cycles (1–3) of cyclic voltammograms of 100 µg mL^{-1} IND, scan rate 100 mV s^{-1} (the dashed lines indicate the background current); B) cyclic voltammograms of 100 µg mL^{-1} IND recorded at 50, 100, 200, 300, 400 and 500 mV s^{-1} (the inset shows the linear relationship between I_p *versus* v).

age scan rate varied from 50 to 500 mV s^{-1}, as shown in Fig. 1B. The peak current responses increased as v was raised, indicating the irreversibility of the electrode process, which is evident from the voltammograms where the oxidation peak potentials of IND shifted to slightly more positive values with increased scan rates. The relationship between the peak currents (I_p) and the v was linear, as depicted by the equation I_p (µA) = 0.008v (mV s^{-1}) + 1.623, r = 0.993 (n = 6). This suggests that the electrode process is controlled by adsorption. Furthermore, a linear relationship was also evident when I_p was plotted against the square root of the scan rate ($v^{1/2}$), following the equation I_p (µA) = 0.290$v^{1/2}$ (mV s^{-1}) – – 0.693, r = 0.992, indicating the involvement of a diffusion mechanism alongside adsorption. The plot of log I_p versus log v, which is represented by the equation log I_p (µA) = 0.581log v (mV s^{-1}) – 0.806, r = 0.996, further confirms this behaviour. The slope of approximately 0.59 suggests that the oxidation of IND is governed by both diffusion and adsorption processes, a dual mechanism corroborated by data from our group and others, not only for boron-doped diamond electrodes,[40] but also for various carbon-based electrode materials.[42,43] To find the quantity of electrons (n) engaged in the IND oxidation phenomenon at the BDD electrode, the n value was computed utilizing the equation αn = 47.7/ /(E_p – $E_{p/2}$), where E_p – $E_{p/2}$ was determined to be 52 mV. In an entirely irrev-

ersible electrode process, the α (charge transfer coefficient) is conventionally regarded as 0.5. Upon analysis, it was determined that the n value, denoting the stoichiometric coefficient, amounted to 1.83, which approximates to 2. This finding aligns with the observations reported in a prior investigation pertaining to the oxidation mechanism of IND.[36] Although the primary objective of this investigation does not encompass a comprehensive examination of the mechanism underlying the electrochemical oxidation of IND, an assessment will be conducted utilizing the CVs obtained at the BDD electrode. Furthermore, by examining the voltammetric behaviour of IND at a titanium(IV) oxide nanoparticle (TiO_2-NPs) and the ionic liquid (IL) *n*-hexyl-3-methylimidazolium hexafluorophosphate modified carbon paste electrode (referred to as TiO_2-NPs–––IL-MCPE), it is possible to propose a preliminary oxidation reaction scheme for IND at a BDD electrode (as shown in Scheme 1).



Scheme 1. Proposed oxidation reaction scheme for IND on the BDD electrode.

The SW mode stripping waveform was employed in the subsequent stage of the study due to its improved sensitivity, lower consumption of electroactive molecules and quicker examination rate. Preliminary examinations revealed that the untreated BDD electrode was ineffective in countering passivation challenges, especially when confronted with high IND concentrations. The repeatable and sensitive response of the BDD surface could be compromised by the electrochemical oxidation of this compound, leading to potential passivation without pretreatment. SW-AdSV was employed to examine the efficacy of three distinct methodologies for a concentration of 30 μg mL^{-1} (5.89×10^{-5} mol L^{-1}) of IND under open-circuit conditions, with an accumulation duration of 30 s. This period was chosen because it was sufficient for effective accumulation. The concentration was selected because SWV is a very sensitive technique compared to CV, enabling effective manipulation and examination of the reaction on the BDD electrode surface in PBS at a pH of 2.5. Initially, anodic pretreatment (APT) was implemented on the BDD electrode surface, involving subjecting the electrode to a potential of 1.8 V for 180 s in a solution of 0.5 mol L^{-1} H_2SO_4. Secondly, the evaluation assessed the effects of cathodic pretreatment (CPT) on the BDD electrode surface, involving applying a potential of –1.8 V for 180 s in a 0.5 mol L^{-1}

H_2SO_4 solution. In due course, the BDD electrode surface underwent anodic pretreatment followed by cathodic pretreatment. During the preceding pretreatment protocol, the BDD electrode surface underwent necessary preparations. Notably, the most discerning outcomes were achieved through this procedure for the analysis of IND, as depicted in Fig. 2. Henceforth, this protocol was perpetuated in the subsequent segments of the investigation.

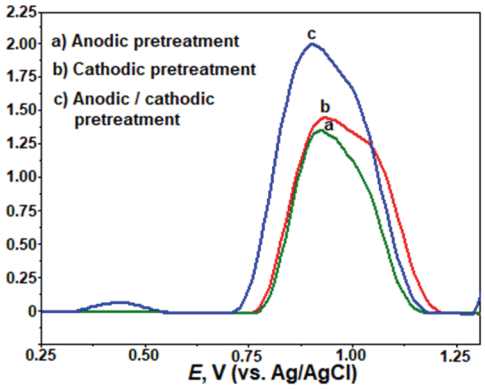


Fig. 2. The electrochemical pretreatments were employed to acquire the SW voltammograms at a concentration of 30.0 μg mL^{-1} of IND in a 0.1 mol L^{-1} PBS solution at a pH of 2.5. The BDD electrode was used for this purpose. During the open-circuit condition, the duration of accumulation is 30 s. The parameters for SWV are as follows: ΔE_s = 10 mV; ΔE_{sw} = 40 mV; f = 50 Hz.

To determine the optimal medium, the SW-AdSV technique was applied to investigate how various pH levels influence the oxidation peak current responses of IND at the BDD electrode interface. SW-AdSVs were recorded within a potential range of 0.25 to 1.30 V, utilizing a BR buffer with a pH spanning from 2.0 to 8.0. These measurements were conducted for a 30.0 μg mL^{-1} solution of IND, as depicted in Fig. 3A. As observed in Fig. 3A, the peak current densities exhibited a decline upon reaching a pH of 5.0, followed by an increment until reaching a pH of 7.0, and subsequently experienced a decline once again. Upon examining the anodic peak potentials within the pH range of 2.0–8.0, a discernible shift towards more negative values was observed with an increase in pH. The given equation illustrates the linear relationship between the pH values within the range of 2.0 to 8.0 and the anodic peak potentials of IND. It can be expressed as E_p (V) = –0.067pH + 1.083, r = 0.992.

The equation mentioned above demonstrates the pH-dependent nature of the IND oxidation on the BDD electrode. Based on experimental results, it can be inferred that the electrode reaction involved an equimolar exchange of protons and electrons. This is supported by the observed slope of 0.067 V/pH, which closely aligns with the expected value of 0.059 V. The observed anodic peak of IND in this investigation is likely attributed to the oxidation of the hydroxyl moiety within the molecular structure. A plausible mechanism outlining the electrooxidation of IND was presented in the Scheme 1. Fig. 3B illustrates the SW-AdSV signals obtained in different electrolyte solutions. Oxidation peak

potentials of 0.89, 0.77 and 0.57 V were observed, along with anodic peak currents of 2.09, 1.62 and 1.82 μA in 0.1 mol L^{-1} PBS pH 2.5, ABS pH 4.7 and PBS pH 7.4, respectively. The obtained outcomes are consistent with those achieved in the BR buffer. As can be seen from Fig. 3A and B, the most distinctive SW--AdSV signal of 30 μg mL^{-1} IND on the BDD electrode with the maximum peak current and the best shaped peak was obtained at PBS pH 2.5. Hence, this buffer was used for further studies.

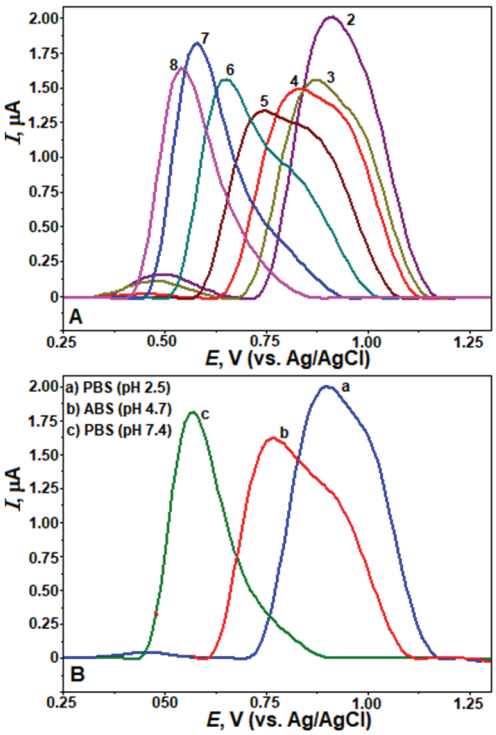


Fig. 3. SW-AdS voltammograms of 30 μg mL^{-1} IND in BR buffer pH 2.0–8.0 (A), and in various supporting electrolytes at different pH values (B) on BDD electrode. Other operating conditions were as indicated in Fig. 2.

A comparative analysis was performed to assess the relative sensitivity of two pulse approaches, namely DPV and SWV, in detecting the anodic peak currents of IND. The experimental findings revealed that the anodic peak currents of IND measured by SWV were approximately 5.58 times greater than those obtained by DPV (Fig. 4). Henceforth, further investigation shall be conducted employing the SWV methodology.

Considering the adsorptive properties of IND on the BDD electrode, we investigated the effect of accumulation time (t_{acc}) and deposition potential (E_{acc}) on 20.0 μg mL^{-1} IND. This concentration was chosen to ensure that the adsorptive properties of IND were clearly observable, facilitating a thorough investigation of the effects of t_{acc} and d E_{acc} on the electrochemical behavior of IND, while

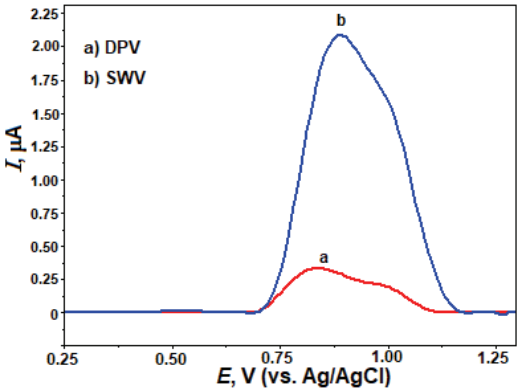


Fig. 4. DPAdS (a) and SW-AdS (b) voltammograms of 30 µg mL^{-1} IND in 0.1 mol L^{-1} PBS at pH 2.5 on the BDD electrode. DPV parameters: modulation amplitude, 50 mV; step potential, 8 mV and modulation time 0.05 s. Other operating conditions were as indicated in Fig. 2.

adhering to the ideal experimental conditions. Utilizing the open-circuit condition, an investigation was conducted to examine the impact of t_{acc} on the signal related to the anodic peak within the time frame of 0–150 s. It was observed that the peak currents increased up to 30 s. After reaching this point, they remained approximately constant, indicating that the electrode surface might have reached saturation. Therefore, t_{acc} of 30 s was selected for all the SW-AdSV experiments. Subsequently, the peak currents showed a state of near-constant stability, indicating that the IND had achieved saturation on the BDD electrode (Fig. 5A).

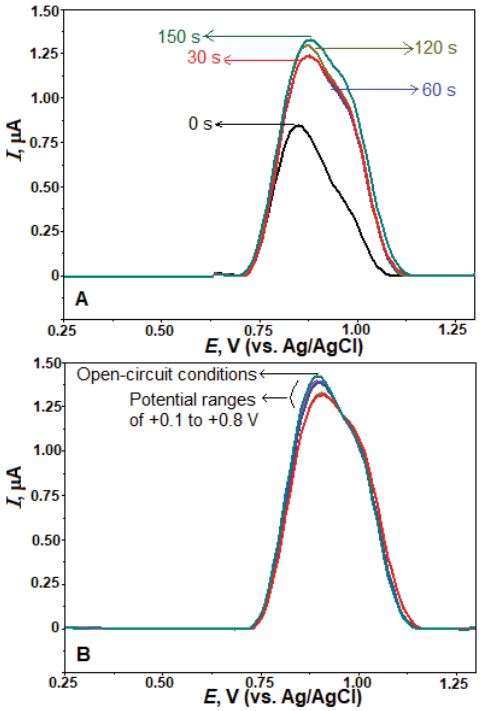


Fig. 5. SW-AdS voltammograms for 20 µg mL^{-1} IND recorded in PBS (pH 2.5) after different accumulation period, t_{acc} in the range 0–150 s at open-circuit condition (A) and after different accumulation potentials, E_{acc} at open-circuit condition or over the potential range 0.1 to 0.8 V using t_{acc} at 30 s (B). The other operating conditions as indicated in Fig. 2.

Conversely, the anodic peak current was evaluated at the specified E_{acc} by subjecting the system to voltage values ranging from 0.1 to 0.8 V or by maintaining open-circuit conditions for a duration of 30 s. The current associated with the IND peak remained relatively constant throughout its operating range, indicating that this parameter had a negligible effect on the determination of the compound (Fig. 5B). Accordingly, the oxidation peak currents were ascertained while the system was in an open-circuit state. Consequently, endeavors were undertaken to enhance the impact of pulse parameters under the given conditions (frequency, f, 25–125 Hz; step potential, ΔE_s, 8–18 mV; square-wave amplitude, ΔE_{sw}, 30–80 mV). The optimization was conducted through the manipulation of a singular parameter while keeping all other variables in a state of constancy. Maximum sensitivity and best peak shape were obtained via the following parameters: f = = 75 Hz; ΔE_s = 12 mV and ΔE_{sw} = 50 mV (Fig. 6A–C).

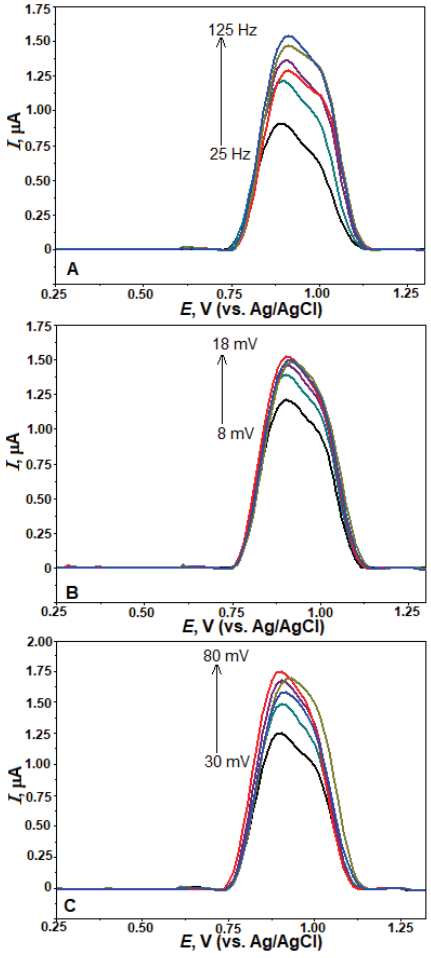


Fig. 6. SW-AdS voltammograms for 20 μg mL^{-1} IND were recorded in PBS (pH 2.5) using different frequencies (f, between 25-125 Hz) (A), step potentials, ΔE_s, 8–18 mV (B) and pulse amplitudes ΔE_{sw}, 30–80 mV (C) on the BDD electrode. The accumulation duration under open-circuit conditions was 30 s.

Analytical applications

The results presented thus far were utilized to assess the analytical performance by observing the concentration oxidation peaks of IND. Fig. 7 displays the voltammetric responses, while Table I elucidates the corresponding analytic parameters. The limits of detection (*LOD*) and quantification (*LOQ*) were determined using analytical curve data in the following manner: the *LOD* was calculated by multiplying three times the standard deviation of the peak currents (from ten runs) for the lowest concentration within the linear range and then dividing it by the slope of each calibration curve.

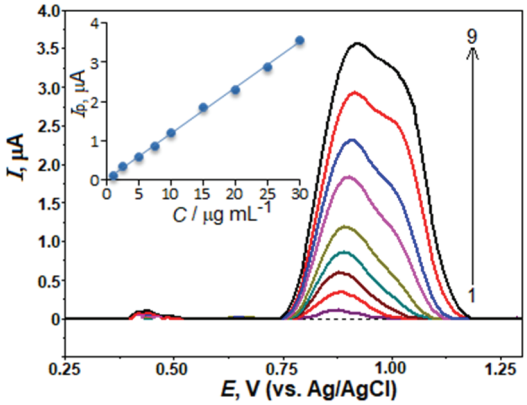


Fig. 7. SW-AdSW voltammograms for the IND concentrations of 1.0, 2.5, 5.0, 7.5, 10.0, 15.0, 20.0, 25.0 and 30.0 μg mL^{-1} (1–9) for the oxidation peaks in 0.1 mol L^{-1} PBS at pH 2.5 on the BDD electrode. The depiction of background current is represented by the delineation of dotted lines. The IND quantification calibration charts are visually presented in the inset. During the open-circuit condition, the duration of accumulation is 30 s, and the SWV parameters: ΔE_s = = 12 mV; ΔE_{sw} = 50 mV; f = 75 Hz.

Repeatability (intra-day precision) was investigated by conducting six tests on the same day, while intermediate precision (inter-day precision) was determined by conducting three assays on five separate days for 1.0 μg mL^{-1} IND under ideal experimental conditions (Table I). The positive outcomes demonstrate that the BDD electrode is an effective electrochemical sensor for accurately determining the amounts of IND in samples taken from the wild.

TABLE I. Analytical variables determined for the oxidation peak of IND by utilizing SW-AdSV on the BDD electrode; E_p = peak potential; LWR = linear working range; LRE = linear regression equation; r = correlation coefficient; *LOQ* = limit of quantification; *LOD* = limit of detection

Analytical parameter	Value
E_p	+0.90 V
LWR	1.0–30.0 μg mL^{-1} (1.97×10^{-6}–5.89×10^{-5} mol L^{-1})
LRE	I_p (μA) = 0.118C (μg mL^{-1}) + 0.011
r	0.999
LOQ	0.73 μg mL^{-1} (1.44×10^{-6} mol L^{-1})
LOD	0.22 μg mL^{-1} (4.33×10^{-7} mol L^{-1})
Intra-day repeatability (*RSD* / %, n = 10)	4.83
Inter-day repeatability (*RSD* / %, n = 5)	5.58

To the best of our knowledge, previous studies have not employed bare electrodes for determining IND. Our analysis, utilizing non-modified BDD electrodes, produced results showing lower sensitivity in terms of the limit of detection compared to prior findings[36] (see Table II). However, the suggested methodology exhibits enhanced practicality, cost-effectiveness, and efficiency in measuring IND compared to earlier research endeavors. Additionally, the use of non-modified BDD electrodes eliminates the need for complex electrode modifications, reducing both the time and costs associated with electrode preparation.

TABLE II. A contrast of the IND's linear range and detection limit with the previously reported work; TiO_2-NP–IL-MCPE: titanium (IV) oxide nanoparticles ionic liquid carbon paste electrode, BDDE: boron-doped diamond electrode

Electrode	Linear range (mol L^{-1})	LOD / mol L^{-1}	Ref.
TiO_2-NP–IL-MCPE	2×10^{-9}–2×10^{-5}	5×10^{-10}	36
BDDE	1.97×10^{-6}–5.89×10^{-5}	4.33×10^{-7}	This work

It is essential to highlight that the presence of electroactive chemicals has the potential to disrupt the peak of the analyte in both drug formulations and biological samples. The evaluation of IND determination selectivity on the BDD electrode involved investigating the impact of various molecules and ions commonly found in pharmaceutical formulations or urine specimens. This was accomplished by observing the alterations in the IND signal when these substances were introduced to a solution containing 1.0 μg mL^{-1} IND at concentration ratios of 1:1, 1:10 and 1:25 (IND:interfering compound) under ideal conditions. The determination of the tolerance limit was assessed through the quantification of the concentration at which an average deviation of ±5 % was observed in the oxidation signal of IND. The experimental findings revealed that the presence of a 25-fold surplus of anions and cations (chloride, nitrate, sodium, potassium, magnesium, calcium, copper, iron and aluminum) did not exert a noteworthy influence on the anodic response of IND. Furthermore, it was noted that the impacts of substances commonly present in pharmaceutical compositions, such as microcrystalline cellulose, gelatine, and lactose monohydrate, on the anodic currents response of IND were inconsequential when a 25-fold surplus was employed. The investigation focused on the effects of ascorbic acid (AA), dopamine (DOP) and uric acid (UA), which are commonly found in urine samples. The experimental findings indicate that the oxidation peak currents of IND were influenced by the separate solutions of DOP, AA, as well as UA while equimolar concentration was employed (Fig. 8). As a result, the direct use of the developed method for IND determination may be limited when analyzing real biological samples, such as plasma or urine. If needed, this issue can be addressed before the voltammetric measurement of IND by employing appropriate sep-

aration techniques. However, this limitation does not apply when analyzing pharmaceutical samples.

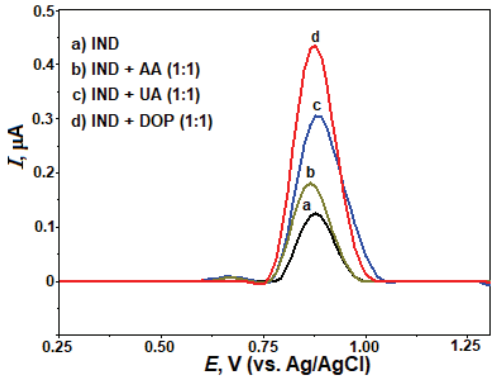


Fig. 8. SW-AdS voltammograms of 1.0 µg mL^{-1} IND (a) in the presence of equimolar concentration ascorbic acid (b), uric acid (c) and dopamine (d) in 0.1 mol L^{-1} PBS at pH 2.5. Other operating conditions as indicated in Fig. 7.

In consideration of these findings, a range of standard addition techniques were employed to assess the concentration of the IND compound in the sample. The samples were prepared as described in the Experimental (*Preparation of samples*). In Fig. 9, we present a graphical representation of the standard addition technique applied to evaluate the oxidation signal of IND. Additionally, we provide a set of sample SW voltammograms for reference.

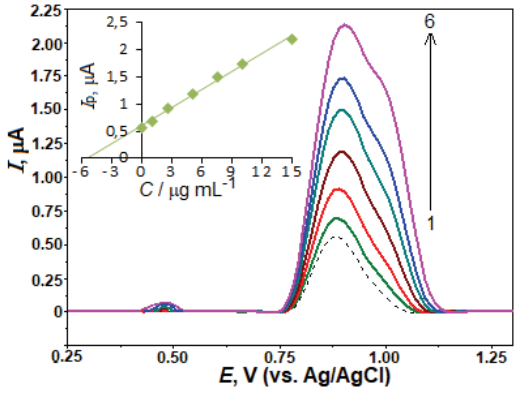


Fig. 9. SW-AdSV of the diluted drug sample (dashed lines) and evaluated by using standard additions of 1.0 and 15.0 µg mL^{-1} IND (1–6) in 0.1 mol L^{-1} PBS at pH 2.5. Inset depicts the result of analysis by standard addition method. Other operating conditions as indicated in Fig. 7.

Standard solutions of IND (ranging from 1.0 to 15.0 µg mL^{-1}) were prepared in an appropriate electrolyte solution and introduced into a voltammetric cell containing 10 mL of the sample solution. The voltammetric responses were then recorded to confirm the reliability of the established methodology for practical use. To quantify the recovered IND, we compared the concentrations of the spiked and unadulterated compounds. After carefully diluting the samples, we determined that the inhaler capsules contained 203.58 µg of IND maleate (with a relative standard deviation of 2.9 %). This value is 4.4 % higher than the manu-

facturer's stated label value of 195.0 μg, indicating that the measured amount is very close to the labeled value. The proximity of the measured value to the labeled value is considered close, as it falls within a 5 % range, which is generally acceptable in quality control assessments. The determined values for IND recovery are presented in Table III. As a result, the proposed methodology ensures the accuracy of voltammetric determination of IND in the commercial pharmaceutical formulation sample.

TABLE III. The inhaler capsule samples were subjected to analysis using SWV on the BDD electrode after treatment with standard solutions of the active pharmaceutical ingredient (IND)

Added[a], μg mL^{-1}	Expected[b], μg mL^{-1}	Found[c], μg mL^{-1}	Recovery ± *RSD*, %
0	-----	5.22	-
1.0	6.22	6.24	100.3 ± 4.7
2.5	7.72	7.94	102.9 ± 3.8
5.0	10.22	10.66	104.3 ± 3.6

[a]IND concentration in the electrochemical cell containing 10 mL of PBS; [b]the value to be obtained after adding IND to the electrochemical cell containing 10 mL of PB; [c]the average of three replicate measurements

CONCLUSIONS

Developing a novel and alternative electroanalytical approach for IND evaluation required the use of the SW-AdSV methodology, paired with the BDD electrode. The anti-fouling features of the BDD electrode, achievable through a quick and easy cleaning approach, are crucial to highlight in this context. These characteristics allow the electrode to be utilized for an extended amount of time while maintaining consistent responses. The BDD electrode provided an electrochemical method for IND measurement that did not require any modifications and effectively replaced the need for modified electrodes. The results revealed an anodic peak corresponding to IND when using CV at a positive potential of approximately 1.06 V in a pH 2.5 PBS as the supporting electrolyte. Additionally, the evaluated analytical parameters demonstrated good sensitivity, with a *LOD* of 4.33×10^{-7} and satisfactory repeatability (*RSD* = 4.83 %). The developed technique was tested for the evaluation of IND in a commercially available pharmaceutical composition and demonstrated excellent recoveries. Furthermore, the provided method has the potential for immediate use without the need for waste formation, complex sample extraction, increased chemical consumption, or expensive instrumentation. Evidently, the BDD electrode offers a straightforward, speedy, and cost-effective method for the routine pharmaceutical examination of IND.

И З В О Д

ОДРЕЂИВАЊЕ ИНДАКАТЕРОЛА НА ПОВРШИНИ БОРОМ ДОПИРАНЕ ДИЈАМАНТСКЕ ЕЛЕКТРОДЕ, У КАПСУЛАМА ЗА ИНХАЛАЦИЈУ, КОРИШЋЕЊЕМ ВОЛТАМЕТРИЈЕ ПРАВОУГАОНИХ ТАЛАСА

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У овом раду је приказано електроаналитичко испитивање помоћу волтаметријске методе за квантификацију индакатерол малеата (IND) употребом немодификоване дијамантске електроде допиране бором (BDD). Применом цикличне волтамерије IND је показао јасан, иреверзибилан пик оксидације на приближно 1,06 V (у односу на Ag/AgCl) у 0,1 mol L^{-1} раствору фосфатног пуфера (PBS), pH 2,5. Предложен је хипотетички механизам електрооксидације IND на основу података добијених цикличном волтаметријом. Применом адсорпционе стрипинг волтаметрије правоугаоних таласа постигнута је прихватљива линеарност у PBS раствору на pH 2,5 на приближно 0,90 V. Добијена је линеарност у опсегу концентрација од 1,0 to 30,0 μg mL^{-1} (еквивалентно $1{,}97\times10^{-6}$–$5{,}89\times10^{-5}$ mol L^{-1}) и граница детекције од 0,22 μg mL^{-1} (еквивалентно 4.33×10^{-7} mol L^{-1}). Применљивост предложене методе је процењена одређивањем IND у формулацијама лекова.

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