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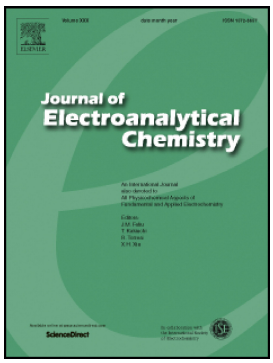

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PII: S1572-6657(18)30378-3
DOI: doi:10.1016/j.jelechem.2018.05.026
Reference: JEAC 4082

To appear in: *Journal of Electroanalytical Chemistry*

Received date: 23 March 2018
Revised date: 29 April 2018
Accepted date: 17 May 2018

An advanced approach for electrochemical sensing of ibuprofen in pharmaceuticals and human urine samples using a bare boron-doped diamond electrode

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Abstract

Herein, an advanced electroanalytical approach for the determination of ibuprofen based on the use of a bare and electrochemically untreated boron-doped diamond electrode is presented. Cyclic voltammetric study revealed that the electrode reaction of the analyte was manifested by the presence of well-shaped irreversible and diffusion-driven oxidation peak at very high potential (+1.75 V vs. Ag/AgCl/3 mol L^{-1} KCl reference electrode) in 1 mol L^{-1} perchloric acid. After optimization of experimental conditions, the peak current of ibuprofen was proportionally linear from 9.49×10^{-7} to 6.69×10^{-5} mol L^{-1} providing both differential pulse (DPV) and square-wave voltammetric (SWV) techniques, respectively. The elaborated electroanalytical protocol rendered low detection limits of 4.1×10^{-7} and 9.3×10^{-7} mol L^{-1} in association with favourable intra-day repeatability (relative standard deviation of 3.6 and 4.6%) using DPV and SWV procedures, respectively. The effect of interfering compounds such as ascorbic acid, dopamine, caffeine, uric acid and glucose on the current response of ibuprofen was explored in details. The usefulness of the proposed approach was verified in the analysis of a variety of commercial brands of pharmaceuticals and spiked human urine samples with the significant range of recovery percentages (for pharmaceuticals: 99.8 – 107.5% and 99.8 – 105.0% by DPV and SWV, for urine: 95 – 107% and 97 – 103% by DPV and SWV). Taking these features into account, the developed protocol may be exploited as a novel, simple and efficient tool in drug quality control analysis and analysis of biological samples. In addition, a bare and electrochemically untreated boron-doped diamond electrode may be applied as a progressive electrochemical sensor and helpful alternative to previously utilized electrochemical platforms in this field.

Keywords: ibuprofen, boron-doped diamond electrode, differential pulse voltammetry, square-wave voltammetry, pharmaceutical dosage

1. Introduction

Ibuprofen (IUPAC name: (RS)-2-(4-(2-methylpropyl)phenyl)propanoic acid; here abbreviated as IBP) is a non-steroidal anti-inflammatory substance representing a broad group of drugs that are greatly used as painkillers and anti-pyretic agents [1]. This drug blocks the enzyme cyclooxygenase, thus inhibiting prostaglandin biosynthesis [2]. It is metabolized mostly in the liver (90%) to hydroxy and carboxy metabolites of IBP, with less than 10% excreted unchanged in urine and bile [3]. On the other hand, IBP is an over-the-counter and highest-selling drug worldwide, consequently making it the first choice for various short-term non-specific pain indications. Its most common use is as a reliever for fever symptoms, menstrual cramps, headaches, arthritis and many other common pains [4,5]. High consumption of IBP is predominantly affected by its relatively weak impacts and low toxicity in humans in comparison with other analgesic and anti-inflammatory drugs. This leads to high doses in commercial pharmaceutical products (the recommended daily dose of IBP according to WHO is 1200 mg [6]) which are sold under a wide variety of brand names across the world. Therefore, the popularity and availability make IBP one of the most frequently detected and quantified analyte in pharmaceutical analysis. Besides, the effectiveness, safety and tolerability of IBP are in accordance with the benefit and risk evaluation in clinical practice. Based on the pharmaceutical relevance and medicinal objectives of IBP, novel and progressive analytical approaches of high efficiency for the rigid control of this non-steroidal anti-inflammatory substance in pharmaceutical dosages and different biological fluids are still desired.

In past few years, many attempts have been made by researchers for the development of novel analytical methods, method procedures and analytical protocols for the detection and quantification of IBP in miscellaneous kinds of samples. In particular, high performance liquid chromatography (HPLC) [7,8], gas chromatography [9], capillary electrophoresis [10]

and spectrophotometry [11,12] have been among the most recently developed. In spite of doubtless accuracy, precision and sensitivity, all these methods have some practical limitations that come into consideration in case of experiment design including expensive instrument; sophisticated but tedious and time-consuming sample preparation/derivatization; usage of toxic organic solvents generating high amounts of waste and/or very costly analysis [13]. Therefore, there is an immediate need for novel, relative simple and rapid analytical methods and procedures with sufficient sensitivity, reliability and low costing for the determination of IBP in pharmaceutical dosages and biological samples.

Nowadays, electrochemical methods represent financially unassuming, time-efficient and comfortable sensitive tool for quantifying various structurally and biologically interesting drugs [14]. Up to now, the literature survey has reflected several reports on detection and quantification of IBP. In this respect, the simple, reliable and low cost conductometric method has been recently developed, based on the inclusion complexation of IBP in β-cyclodextrin [15]. With regards to potentiometric sensing, various membrane sensors providing detection limit (LOD) for IBP in the range of 10^{-4} – 10^{-5} mol L^{-1} have been explored, based on glassy carbon electrode (GCE) incorporated with MWCNT/β-cyclodextrin [16], drug-ferroin ion-association complexes as electroactive material [17], different plasticizers [18] and commercial cyclodextrins [19] as well as various quaternary ammonium salts as ion exchangers [20] and mercury(I) ibuprofenate indicator [21]. Amperometric determinations of IBP utilizing various bare and/or modified carbon-based working electrodes have been introduced by Montes et al. [22] and Chaves et al. [23] with LOD of 1.9×10^{-6} and 1.3×10^{-7} mol L^{-1}, respectively. As to voltammetric techniques with bare (chemically unmodified) and conventional working electrodes, screen printed graphite electrode (SPGE) has shown rapid, cost-effective and reproducible performance for in-field IBP determination with LOD of 6.3×10^{-6} mol L^{-1} accomplished by square-wave voltammetric mode (SWV) [24]. Concerning

modified working electrodes, carbon paste electrode (CPE) has been applied as promising electrode substrate in analysis of river water and commercial tablets, with montmorillonite (Mt) as significant modifier able to catalyze the oxidation of IBP (LOD = 6.8×10^{-8} mol L^{-1}) [25]. In other study, silver-modified zeolite-MWCNT-epoxy composite electrode has been employed as novel electrochemical sensor for SWV determination of IBP with achieved LOD of 1.5×10^{-6} mol L^{-1} [26]. Similarly, silver-decorated CNF-epoxy and silver-modified natural zeolite-CNF-epoxy composite electrodes have appeared to be efficient sensors in IBP sensing, with LODs of 4.8×10^{-8} and 3.9×10^{-8} mol L^{-1} [27], respectively. Despite the useful utilization of conventional bare and chemically modified electrodes, electrochemists are constantly pushed to explore the novel and perspective material platforms as fool-proof electrochemical sensors for detection and determination of IBP and related drugs. Besides, the improvement of electrochemical sensors and biosensors in order to ensure their practical and safe use for IBP sensing is still of great value for the safety of professionals working with this drug, patients and the environment which we know to be at risk of contamination.

In last two decades, boron-doped diamond (BDD) has been established as an advanced and environmental-friendly electrode material supplying distinguished properties unlike conventional and chemically modified electrodes. This electrode material renders significant chemical stability (owing to sp^3 hybridization of carbon atoms in diamond lattice), low capacitive current, good mechanical stability and notable biocompatibility as well as the widest potential window in both aqueous and non-aqueous environment [28,29]. Recently, interesting papers dealing with progressive electrochemical sensors based on BDD electrodes (BDDE) have been introduced by our working group, focusing on simple, rapid and reliable determination of miscellaneous drugs in pharmaceuticals and human urine samples [30-32]. In relation to IBP sensing, the working group of prof. Santos firstly reported short communication on the usage of cathodically and anodically pretreated highly doped BDDE

(boron content of 8000 ppm) for differential pulse voltammetric (DPV) determination of IBP solely in pharmaceuticals [33]. The obtained LOD was found to be 5×10^{-6} mol L^{-1}. Moreover, they recently also developed the DPV procedure for simultaneous determination of paracetamol and IBP in pharmaceuticals on this working electrode (LOD of 7.1×10^{-6} mol L^{-1} for IBP) [34].

As evidenced above, the literature survey has indicated a few published papers predominantly dealing with the use of chemically modified electrodes for electrochemical determination of IBP. Herein, an advanced electroanalytical approach for simple, rapid and reliable determination of IBP is proposed, based on the use of a bare and electrochemically untreated commercial low doped BDDE using DPV and SWV procedures. The methodology involves the selection of suitable experimental conditions for IBP oxidation on BDDE, characterization of electrode reaction, analytical performance and interference study. Moreover, a variety of commercial pharmaceutical samples as well as model human urine samples are considered to be simply and reliably analyzed. In view of this fact, the elaborated protocol could be considered as a sensitive, rapid and cost-effective alternative for IBP sensing to previously reported methods and procedures based mostly on chemically modified electrodes.

2. Experimental

2.1. Reagents and solutions

Ibuprofen (CAS No. 15687-27-1, purity ≥ 98%) was purchased from Sigma Aldrich (Slovakia). A suitable amount of its powder was dissolved in 50 mL methanol (p.a., Lachema, Czech Republic) to get stock solution of 1×10^{-3} mol L^{-1}. This solution was constantly stored in refrigerator (when not used), without any consistency changes during a few weeks. $HClO_4$, HNO_3 and H_2SO_4 of different concentrations as well as Britton-Robinson (BR) buffers were

used as potential supporting electrolytes for purposes of this work. BR buffers were prepared by mixing of 0.04 mol L^{-1} H_3BO_3, H_3PO_4, CH_3COOH adjusted with 0.2 mol L^{-1} NaOH (p.a., Lachema, Czech Republic). The working and calibration solutions of different concentrations of IBP were always made directly in electrochemical cell by diluting the appropriate volume of stock solution in supporting electrolyte. All solutions were prepared in deionized water with resistivity above 18 MΩ cm.

2.2. Instrumentation

The voltammetric measurements were undertaken with an Autolab PGSTAT 101 (Metrohm Autolab B.V., The Netherlands) using a conventional three-electrode cell with a bare and electrochemically untreated commercial low doped BDDE (Windsor Scientific Ltd., UK declares a film diameter of 3 mm, resistivity of 0.075 Ω cm and B/C ratio in gaseous phase during the deposition step of 1000 ppm), a platinum wire as auxiliary electrode and an Ag/AgCl/3 mol L^{-1} KCl electrode as reference electrode. All pH values were measured using pHenomenal® pH 1100L meter (VWR, Slovakia) with a combined glass-reference electrode.

2.3. Measurement procedures

Cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square-wave voltammetry (SWV) were used for exploring the electrochemical behaviour of IBP on the BDDE and for assessing its analytical performance under the optimal experimental conditions. The BDDE surface was treated as follows: at the beginning of every work day, it was firstly rinsed with deionized water and then very gently smoothed with a piece of wet silk material to reach a mirror-like appearance of the electrode surface. Afterward, it was again washed down with deionized water and the BDDE was ready for voltammetric measurements of IBP with repeatable signals (no cathodic and/or anodic pretreatment of BDDE was

necessary to perform as no considerable changes of current response of IBP from the viewpoint of selectivity and sensitivity were registered after application of –2.5 V (cathodic) and/or +2.5 V in 1 mol L^{-1} H_2SO_4 for 40 s). All voltammograms were recorded after addition of particular volumes of IBP stock and/or working solutions to the electrochemical cell (already containing supporting electrolyte). The calibration curves were evaluated from the peak currents of analyte achieved by DPV and SWV procedures with the optimized instrumental parameters (modulation amplitude, modulation time and frequency). Subsequently, they were statistically analyzed by OriginPro 8.0 (OriginLab, USA) and the relevant results (slope and intercept) were assessed in a 95% confidence interval. LODs were calculated as standard deviation of intercept divided by slope of the particular calibration curve [30,31]. All voltammetric measurements (except for repeatability evaluation) were carried out in triplicate at ambient temperature.

2.4. Preparation of pharmaceutical samples and their analysis

The five different brands of pharmaceutical dosages with the declared IBP content such as *Brufen*, *Ibalgin*, *Ibuprofen Rapid*, *Brufedol* and *Ibalgin Junior* were purchased without prescription in local drugstore in Bratislava (Slovakia). The procedure for preparation of the respective samples (except liquid pharmaceutical product *Ibalgin Junior*) was as follows: ten tablets of each brand were always weighed and powdered using mortar and pestle. The particular amount of powder was dissolved in 25 mL methanol and filtered through the filter paper (the pore size of 20 μm). The transparent filtrate was filled up with methanol to 100 mL volumetric flask to get stock solution of pharmaceutical sample. 100 μL of this solution was added to the electrochemical cell to 20 mL of supporting electrolyte and analyzed by DPV and SWV procedures. Subsequently, the multiple standard addition method was utilized while three consecutive additions of 500 μL of 1×10^{-3} mol L^{-1} IBP working solution were applied

prior to next application of DPV and SWV procedures. The recovery percentages were identified graphically, with the abscissa referring to the IBP concentration in the electrochemical cell. Furthermore, the extrapolation of the curve along this axis yielded the sample concentration, allowing for the calculation of the recovery values. Concerning *Ibalgin Junior* brand, 5 mL of this liquid was mixed with 25 mL methanol to get limpid solution. Subsequently, it was filled up quantitatively with methanol to 100 mL volumetric flask to get stock sample solution. The procedure for analysis of this pharmaceutical drug was similar as aforementioned.

2.5. Preparation of model human urine samples and their analysis

Human urine samples were collected from four healthy and non-smoking volunteers (V1: female, 24 years; V2: male, 35 years; V3: female, 54 years, V4: male, 24 years) immediately prior to launching the experiments. At the time of experiments and shortly before, these volunteers did not undergo any treatments with multivitamin formulations and other drug dosages. It should also be taken into account that the experiments were undertaken in compliance with respective law (Parliamentary Act no. 40/1964 Coll. Civil Code as amended) with the informed consent obtained from the volunteers prior to the experiments. In regards to the preparation of the human urine samples, the particular volume (1 mL) of fresh urine was directly transferred to 19 mL of supporting electrolyte in the electrochemical cell. The solution in cell was subsequently fortified with aliquot of IBP working solution to form model (spiked) human urine sample with the total IBP concentration of 1×10^{-5} mol L^{-1}. Afterwards, the multiple standard addition method with the corresponding total volumes of 200, 400 and 600 µL of 1×10^{-3} mol L^{-1} IBP working solution was applied and analysis by DPV and SWV was undertaken.

3. Results and discussion

3.1. Electrochemical behaviour of IBP on bare BDDE

At first step, CV technique was applied to examine the electrochemical behavior of 1×10^{-4} mol L^{-1} IBP on the bare BDDE. As electrochemical pretreatment of working electrode surface (neither anodic nor cathodic, the details are given in section 2.3.) did not cause any significant improvement of the current response of IBP, an electrochemically untreated BDDE was utilized in all experiments. This approach differs from previous ones applying also cathodic and anodic pretreatment of BDDE for improvement of IBP signal [23,33,34] as well as other protocols for drug sensing developed in our laboratory [35-37]. All required factors which may affect the current response of IBP were thoroughly explored to accomplish the experimental conditions with most favourable analytical performance. In this respect, the various supporting electrolytes with different pH values such as Britton-Robinson (BR) buffers (pH 2 – 12) and strong acids (sulphuric acid, nitric acid and perchloric acid) were studied. The results showed that the use of BR buffers from pH 6 to 12 was not convenient owing to any distinct current responses of IBP on the BDDE. The more favourable voltammograms, with the peak potentials of IBP higher than +1.5 V, however, with higher background current, were obtained in the pH range of 2 – 5 indicating the fact that acidic medium could be suitable for the electrochemical activity of IBP on the BDDE (the results not shown). In general, in a more acidic environment, the background current appears to be low, most likely due to forming of hydroxyl radicals as products of water oxidation, thus corroborating the respective electrochemical oxidation of analyte [38]. Accordingly, we explored several strong acids as supporting electrolytes including sulphuric acid, nitric acid and perchloric acid (all in the concentration range from 0.1 to 2 M). In regards to nitric and sulphuric acid, the peak current of IBP was found to be comparable mutually, however, with twice higher magnitude and better repeatability than that obtained in BR buffers.

Nevertheless, the most distinctive voltammetric profile of 1×10^{-4} mol L^{-1} IBP on the bare BDDE with the highest magnitude and the lowest background current was noticed in the presence of 1 mol L^{-1} perchloric acid. Thus, bearing in mind the well-defined IBP oxidation peak compared to other acids and BR buffers, perchloric acid was chosen as the supporting electrolyte for subsequent electrochemical studies.

IBP is an electrochemically active compound which may transfer electrons at highly positive potentials on carbonaceous electrodes [23,24,33]. In order to assess the preferences of the use of BDDE in IBP sensing with respect to traditional carbonaceous electrode materials, the electrochemical behavior of IBP on bare working electrodes were subjected to CV technique applying GCE and BDDE. Fig. 1 points to the comparison of CV records of 0 (1 mol L^{-1} $HClO_4$ as supporting electrolyte) and 1×10^{-4} mol L^{-1} IBP in 1 mol L^{-1} $HClO_4$ on the GCE and BDDE in the potential range from +0.5 to +2.0 V. In the case of GCE, the peak current of IBP (red line) appeared to be very poor as evidenced by the presence of small and oval "hump" at +1.75 V. The low peak current of analyte is a consequence of its interference with remarkably higher background current (caused by oxygen evolution and/or decomposition of supporting electrolyte) typical for GCE at very positive potential. Obviously, the observed phenomenon considerably restricts the utilization of this traditional carbonaceous electrode material in reliable sensing of highly oxidizing analytes including IBP. On the other hand, BDDE known for its wide potential range and low background current provided sufficiently sensitive current response of IBP at +1.75 V (green line in Fig. 1) in contrast to the GCE. By reversing at +2.0 V, no voltammetric signals corresponding to the reduction of IBP were noticed on the cathodic branch for both working electrodes indicating that electrode reaction of IBP is totally irreversible. The respective electrochemical behavior of IBP well agrees with those reported for chemically modified electrodes [25,27]. Apparently, the background current on the BDDE was found to be approximately 10-times

lower when compared that obtained on GCE (e.g. at potential of +1.8 V the current responses gained 4×10^{-6} and 5×10^{-5} A for BDDE and GCE, respectively). This fact proves the benefits and legitimacy of the use of BDDE for IBP determination. Additional information from Fig. 1 is concerned with the peak potential values of IBP, which stay the unchanged on the BDDE and GCE indicating the fact that analogous mechanisms of electrode reaction were attained.

Here Fig. 1

3.2. Effect of scan rate

The influence of scan rate (v) was explored in the range from 5 to 500 mV s^{-1} by CV technique on the bare BDDE using 1 mol L^{-1} $HClO_4$ containing 1×10^{-4} mol L^{-1} IBP. As evidenced in Fig. 2, the scan rate variation yields the obvious increase in the peak currents (I_p) of IBP. At the same time, its peak potential (E_p) shifts towards more positive potentials confirming the irreversible nature of the electrode reaction of IBP on the BDDE. In the light of these facts, the plot of peak current (I_p) *vs.* square root of scan rate ($v^{1/2}$) revealed the dependence (first inset in Fig. 2, Eq. 1):

$$I_p\,(\mathrm{A}) = (2.42 \pm 0.27)\times10^{-7} + (9.97 \pm 0.22)\times10^{-8}\times v^{1/2}\ (\mathrm{mV\ s^{-1}})^{1/2} \quad (1)$$

with significant linearity (R^2 = 0.996), indicating the occurrence of diffusion-driven oxidation process of IBP. To confirm if the diffusion is absolutely the rate limiting step of the electrode reaction of analyte, linear fitting of log I_p *vs.* log v was also done (second inset in Fig. 2), as expressed by Eq. 2:

$$\log I_p = (0.43 \pm 0.01)\times\log v - (6.63 \pm 0.03) \quad (2)$$

This plot exhibited the considerable linear dependence (R^2 = 0.990) with the slope value of 0.43 close to the theoretical value of 0.50 [31] for the diffusion-controlled mechanism of the electrode reaction of IBP on the BDDE. Additionally, as mentioned above and evidenced from Fig. 2, the moderate shift of E_p towards positive direction was recorded as v gradually increased. This tendency was described by the linear relationship of E_p *vs*. log v (third inset in Fig. 2, R^2 = 0.988, Eq. 3):

$$E_p\,(\mathrm{V}) = (0.06 \pm 0.01)\times\log v + (1.65 \pm 0.08) \qquad (3)$$

From the slope of this dependence ($2.3RT/\alpha nF$), αn value for IBP was considered to be 0.98. In view of irreversible system with α value usually equal to 0.5 [31], the number of electrons (n) participating in the electrode reaction of IBP on the bare BDDE was calculated to be approximately 2.

Here Fig. 2

3.3. Mechanism of electrochemical oxidation of IBP

Based on the Naproxen as structurally related derivative to IBP [39], the mechanism of electrochemical oxidation of (racemic) IBP could be postulated as follows (Scheme 1): the first single-electron oxidation provides after deprotonation the benzyl radical **I** and carboxyl radical **II**, respectively. By elimination of CO_2 from both radicals a common benzyl radical **III** is generated. Its subsequent (second) single-electron oxidation results in a benzyl cation **IV** which is the substrate for the formation of the final products: reaction of **IV** with water followed by deprotonation to generate alcohol **VI**, whereby the *in situ* formation of the alcohol **VI** to the carbocythine **IV** gives symmetrical ether **VIII**. In parallel, the formation of

the labile hydroperoxide **V**, which disproportionately produces a mixture of alcohol **VI** and ketone **VII**, is formed by the addition of oxygen to the benzyl radical **III**.

Here Scheme 1

3.4. Analytical performance evaluation

3.4.1. Optimization of DPV and SWV instrumental parameters

With the aim to achieve the current response of IBP with the highest magnitude and best shape, the optimum instrumental parameters were selected studying the variation of the modulation amplitude and modulation time for DPV and frequency and amplitude for SWV (Fig. 3). In regards to the variations in the modulation amplitude values for DPV from 10 to 200 mV (with the modulation time fixed at 25 ms, Fig. 3A), the results indicated that its increase promoted a displacement of the peak potential toward less positive values with a moderate widening in the half-peak width and increasing in the background current. This phenomenon resulted in a loss in analytical selectivity and sensitivity, thus as a compromise modulation amplitude of 100 mV was chosen. Considering modulation time value in the interval from 10 to 200 ms (with the modulation amplitude fixed at 100 mV, inset of Fig. 3A), its increase stimulated a decrease in the peak current of IBP accompanied by a shift of peak potential toward less positive values. The optimum value was considered to be the lowest one (10 ms). As to the SWV, the appropriate values of amplitude (studied range of 10-150 mV with the frequency fixed at 25 Hz, Fig. 3B) and frequency (studied range of 10-125 Hz with the amplitude fixed at 50 mV, inset of Fig. 3B) in the viewpoint of analytical selectivity and sensitivity aspect were found to be 50 mV and 50 Hz, respectively. It is worth mentioning that in first reported short communication on IBP sensing using BDDE [33], the oxidation peak of IBP accomplished by SWV was not as well-defined (RSD above 10%) as using DPV thus restricting this fast scanning voltammetric technique for IBP determination. In our approach,

the use of SWV is reliably feasible for sensitive quantification of IBP on BDDE with oxidation peak of analyte to be distinctively recorded.

Here Fig. 3

3.4.2. Figures of merit

After optimization of experimental conditions, the following step consisted in the construction of calibration curves for IBP using both DPV and SWV procedures and the evaluation of whole analytical performance. The DP and SW voltammograms recorded at different IBP concentrations on the bare BDDE and the corresponding calibration curves are shown in Fig. 4A-B. The analytical performance evaluation towards IBP sensing is listed in Table 1. Obviously, DPV technique provided almost 4-times higher sensitivity to IBP quantification (0.0536 A L mol^{-1}) than SWV (0.0135 A L mol^{-1}). This fact also resulted in lower LOD value for DPV (4.1×10^{-7} mol L^{-1}) when compared to SWV (9.3×10^{-7} mol L^{-1}). The linear concentration range of $(9.49 – 669)\times10^{-7}$ mol L^{-1} was found to be similar for both pulse voltammetric techniques. Bearing in the mind the intra-day repeatability, it was explored by 6 replicates of DPV and SWV measurements for 1×10^{-5} mol L^{-1} IBP. Low RSD values (both below 5%) indicated good precision and confirmed minimal adsorption effect of analyte and its oxidation products during the electrode reaction on the bare BDDE. Therefore, the BDDE has proven to be appropriate electrochemical sensor for the sensitive and precise determination of IBP under the selected experimental conditions. Compared with other reported electrochemical works, the use of bare and electrochemically untreated BDDE in this work provided the LOD value of approximately comparable and/or 1 order lower than those obtained by other voltammetric procedures and protocols using SPGE [24] and silver-modified zeolite MWCNT-epoxy composite electrode [26]. In comparison to amperometric sensing of IBP, the proposed protocol yields 5-times lower LOD than that attained by Montes

et al. [22] using modified carbonaceous working electrode. Concerning the other electrochemical works using BDDE, the developed protocol although exhibits the narrower linear concentration range for IBP sensing, however, with the comparable repeatability (RSD below 5%) and the determined LOD value of 1 order lower in comparison with previous voltammetric ones on anodically pretreated highly doped BDDE [33,34]. Taking flow injection determination of IBP on BDDE into consideration, Chaves et al. also achieved distinguished analytical parameter values such as LOD of 1.3×10^{-7} mol L^{-1}, 150 injections h^{-1} and repeatability with RSD below 1%, moreover, in multiple-pulse amperometric mode ensuring selective quantification of IBP in the presence of paracetamol and caffeine [23]. The attained LOD in this work was also found to be 2-3 concentration orders lower than those obtained by potentiometric sensing of IBP [16-21]. With regards to recently published separation methods for IBP sensing, HPLC coupled with diode array detector although provided the LOD of 2 orders lower than in our work, however, with tedious procedure based on the application of molecularly imprinted polymer as selective sorbent in the solid-phase extraction of analyte [8]. On the other hand, spectrophotometric methods having advantages of simplicity and analysis time yielded the LOD of 1-2 orders higher [11,12] than that described in this protocol.

Here Fig. 4

Here Table 1

3.5. Selectivity study

In next step, the selectivity of the proposed electroanalytical protocol was estimated by introducing some possible interfering agents in the solution containing IBP and applying DPV procedure under the optimum experimental conditions. The following substances, typically present as excipients in pharmaceutical formulations, were studied: magnesium stearate,

starch, cellulose, benzoic acid and sodium benzoate. As to other components usually found in biological samples (human urine), glucose (GLU), ascorbic acid (AA), uric acid (UA), dopamine (DOP) and caffeine (CAF) were subjected to the detailed interference study [31]. Likewise, it should be emphasized that the effect of aforementioned urinary compounds on IBP signal has not been explored in previous works using anodically pretreated highly doped BDDE [23,33,34]. For such purposes, a working solution of 1×10^{-5} mol L^{-1} IBP in 1 mol L^{-1} $HClO_4$ was spiked with each interferent at the following concentration ratios (IBP : interferent): 1:1, 1:10 and 1:100. Subsequently, the respective current responses of IBP were compared with those for the working solution in the absence of any interfering agent. The results indicated that excipients typically present in pharmaceuticals had no significant effect on the current response of IBP (the particular voltammograms not shown). This is related to the fact that they are not electrochemically active on the bare BDDE. In regards to GLU, no remarkable changes for the IBP signal were noticed, even in 100-fold excess to IBP. The impact of the presence of electrochemically active substances (AA, UA, DOP and CAF) is illustratively depicted in Fig. 5A-D. As can be seen from this figure, the variations in the current responses of IBP caused by the presence of these substances are different. In the case of AA, its 10-fold excess rendered the slight decrease of IBP signal by approximately 20%. Moreover, in 100-fold excess of AA, IBP signal was not able to reliably evaluate owing to the strong overlapping of its signal with AA response (Fig. 5A). Concerning the effect of UA, its 100-fold excess gave rise to the moderate decrease of the current response of IBP (about 20%), while 20-fold excess yielded no substantial IBP signal changes (Fig. 5B). Furthermore, no distinguished effect on IBP was observed in the presence of DOP (Fig. 5C). With regard to CAF, its presence in 1:1 concentration ratio caused no remarkable impact on the oxidation peak of IBP. However, in the higher excess of CAF (1:10 and 1:100), the selectivity was not satisfactory since there were overlapping of the oxidation peaks of IBP and CAF noticed in

DP voltammograms (Fig. 5D). This also results from the proximity of their peak potentials. Taking the above mentioned results into account, the selectivity of the proposed protocol for the determination of IBP could be considered as reasonable.

Here Fig. 5

3.6. Sample analysis

3.6.1. Analysis of pharmaceutical samples

In order to verify the accuracy and validity of the proposed electroanalytical protocol, the multiple standard addition method was applied to the analysis of several commercial pharmaceutical dosages with IBP content available in drugstores in Slovakia. The sample preparation and specifics of the multiple standard addition method is discussed in section 2.4. As an illustrative example, the particular DP voltammograms of analysis of the pharmaceutical dosage *Ibalgin* (with the declared content of 200 mg IBP) on the bare BDDE with the graphical evaluation of the multiple standard addition method are given in Fig. 6. The recovery percentages determined for all brands by both pulse techniques are summarized in Table 2. Apparently, the obtained values were generally in a good agreement with the declared quantities of producers and ranged from 99.8 to 107.5% and from 99.8 to 105.0% by DPV and SWV procedures, respectively. These values revealed the fact that proposed protocol did not suffer from any considerable matrix effect despite the absence of sample treatment for the removal of interfering possible thus corroborating the suitability of the bare BDDE for this purpose. Therefore, the IBP amount can be quantitatively recovered in pharmaceutical dosages by the proposed protocol, being thus a liability of the accuracy of the voltammetric determination in routine pharmaceutical analysis.

Here Fig. 6

Here Table 2

3.6.2. Analysis of spiked human urine samples

Analysis of real and/or model biological samples has not been previously investigated on BDDE in relation to the presence of IBP traces as evidenced by data in last scientific papers [23,33,34]. Therefore, the viability of the developed protocol for analysis of samples with more complicated matrix with IBP content was verified on the set of the spiked human urine samples taken from four volunteers (V1 – V4). The preparation of these samples and specifics of the multiple standard addition method are given in section 2.5. It is well known that human urine is essentially composed of water, salts, uric acid and other biomolecules which may restrain the reliable trace determination of required analyte. Herein, as evidenced by the results in Table 3, the adequate recovery percentages close to 100% (99.8 – 107.5% and 99.8 – 106.0% for DPV and SWV, respectively) were attained proving the minor impact of potential matrix interferences throughout the analysis. Likewise, the highly positive oxidation potential of IBP on the bare BDDE in comparison with common urinary compounds (the peak potentials of AA, UA and DOP generally occurred up to +1 V on bare BDDE [40-42]) allows it to be reliably quantified in human urine by the presented protocol. Fig. 7 displays an illustrative example of the DP voltammograms for the analysis of spiked human urine sample of volunteer V2 on the bare BDDE using the multiple standard addition method (graphical evaluation appears in the inset). It is evident that the voltammetric peak at about +1.6 V can be assigned to the oxidation of IBP, since it increases proportionally after each standard addition. Finally, the proposed electroanalytical protocol yielded the reasonable accuracy for the IBP determination in spiked human urine samples. However, for deeper enlightenment of analysis of real human urine samples with IBP traces (e.g. after single and/or multiple taking of 400 mg oral dose of IBP by patient, while less than 10% of IBP is excreted unchanged in urine [3]), determination of hydroxy and carboxy metabolites of IBP on BDDE would be also necessary to be carried out and studied in details.

Here Table 3

Here Fig. 7

4. Conclusion

Up to now, the electrochemical data from scientific literature on IBP sensing registered predominantly the papers focused on potentiometry, amperometry and voltammetry mostly based on the application of chemically modified working electrodes. Herein, an advanced approach is introduced and elaboration of novel electroanalytical protocol for the reliable determination of IBP based on the use of a bare and electrochemically untreated BDDE is presented. One irreversible and diffusion-driven oxidation peak was noticed by CV at +1.75 V in 1 mol L^{-1} $HClO_4$. Utilizing the optimized instrumental parameters, the analytical performance of the proposed protocol was assessed for both pulse techniques with the validation parameters such as LOD at submicromolar concentration levels, linear concentration range of $(9.49 – 669)\times10^{-7}$ mol L^{-1} and intra-day repeatability with RSD values lower than 5%. The results of the impact of possible interfering compounds such as excipients derived from pharmaceuticals and selected agents usually present in urine (glucose, ascorbic acid, uric acid, dopamine and caffeine) revealed the reasonable selectivity for IBP sensing in commercial pharmaceutical dosages and human urine samples under the optimized experimental conditions. The analysis of the various commercially available pharmaceutical dosages (without a demanding sample pre-treatment and electrochemical pretreatment of working electrode surface) proved the meaningful accuracy of the elaborated methodology as the recovery percentages varied from 99.8 to 107.5% and 99.8 – 105.0% by DPV and SWV procedure, respectively. The tolerable recovery values (95 – 107%) were also accomplished in analysis of the human urine samples fortified with IBP. Apparently, the coupling of the bare and electrochemically untreated BDDE with sensitive pulse voltammetric techniques may

constitute a regular, low-cost and profitable electrochemical tool for routine pharmaceutical analysis, especially in drug quality control and assurance. Finally, this work proposes the novel approach for IBP sensing with enhanced analytical performance and adequate LOD, using a simpler, rapid and cost-effective approach than other previously proposed electrochemical platforms. Likewise, the BDD-based sensors could be utilized within the extraordinary analytical protocols capable of simple, fast and proper biomedical usage, thus providing invaluable services not only to analysts and clinicians in diagnosis of certain diseases but also to nanotechnologists and material engineers in the design of miniaturized sensors.

Acknowledgements

This work has been supported by the Grant Agency of the Slovak Republic (grant No. 1/0489/16), the Ministry of Education, Science and Technology, the Republic of Serbia (Project No. OI 172030) and MagBioVin project (FP7-ERAChairs-Pilot Call-2013, Grant agreement: 621375).

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Table 1

The analytical parameters obtained for IBP determination using DPV and SWV on the BDDE.

Analytical parameter	Pulse voltammetric technique	
	DPV	**SWV**
Intercept (A)	$(1.86 \pm 0.08)\times10^{-7}$	$(3.68 \pm 0.42)\times10^{-8}$
Slope (A L mol^{-1})	(0.0536 ± 0.0009)	(0.0135 ± 0.0003)
Linear range (mol L^{-1})	$(9.49 – 669)\times10^{-7}$	$(9.49 – 669)\times10^{-7}$
R^2	0.997	0.993
Detection limit* (mol L^{-1})	4.1×10^{-7}	9.3×10^{-7}
Intra-day repeatability** (%)	3.6	4.6

* Calculated as 3×standard deviation of intercept/slope of calibration curve

** RSD calculated for 6 replicate DPV or SWV measurements at 1×10^{-5} mol L^{-1} IBP

Table 2

The analysis of the various commercially available pharmaceutical dosages with IBP content using the proposed protocol (n = 3).

Pharmaceutical dosage	Declared content (mg)	Pulse voltammetric technique			
		DPV		SWV	
		Determined content* (mg)	Recovery percentage (%)	Determined content* (mg)	Recovery percentage (%)
Brufen	400	(417 ± 18)	104.3	(403 ± 13)	100.8
Ibalgin	200	(202 ± 6)	101.0	(201 ± 10)	100.5
Ibuprofen Rapid	400	(401 ± 19)	100.3	(399 ± 11)	99.8
Brufedol	400	(399 ± 14)	99.8	(402 ± 15)	100.5
Ibalgin Junior	40**	(43 ± 5)	107.5	(42 ± 3)	105.0

*Confidence interval for 95% probability calculated as $[\bar{X} \pm t_{n-1,\alpha}\, SD/n^{1/2}]$; $t_{2;\,0.05} = 2.92$

**mg mL^{-1}

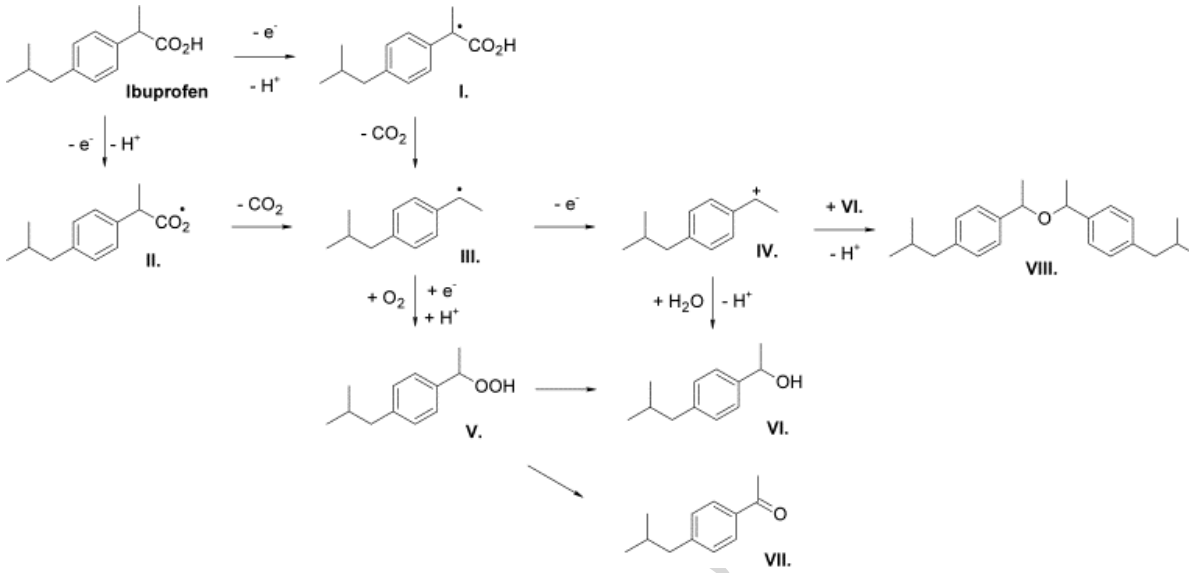
Table 3

The analysis of the spiked human urine samples of volunteers (V1 – V4) using the proposed method (n = 3).

Human urine sample	Expected concentration (mol L^{-1})	Pulse voltammetric technique			
		DPV		SWV	
		Determined concentration* (mol L^{-1})	Recovery percentage (%)	Determined concentration* (mol L^{-1})	Recovery percentage (%)
V1	1×10^{-5}	$(1.07 \pm 0.16)\times10^{-5}$	107	$(0.98 \pm 0.15)\times10^{-5}$	98
V2	1×10^{-5}	$(1.04 \pm 0.11)\times10^{-5}$	104	$(0.99 \pm 0.06)\times10^{-5}$	99
V3	1×10^{-5}	$(0.96 \pm 0.18)\times10^{-5}$	96	$(0.97 \pm 0.13)\times10^{-5}$	97
V4	1×10^{-5}	$(0.95 \pm 0.13)\times10^{-5}$	95	$(1.03 \pm 0.16)\times10^{-5}$	103

*Confidence interval for 95% probability calculated as $[\bar{\chi} \pm t_{n-1,\alpha}\, SD/n^{1/2}]$; $t_{2;\,0.05} = 2.92$

Scheme 1



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E-mail address: lubomir.svorc@stuba.sk (Dr. Ľubomír Švorc)

Fig. 1

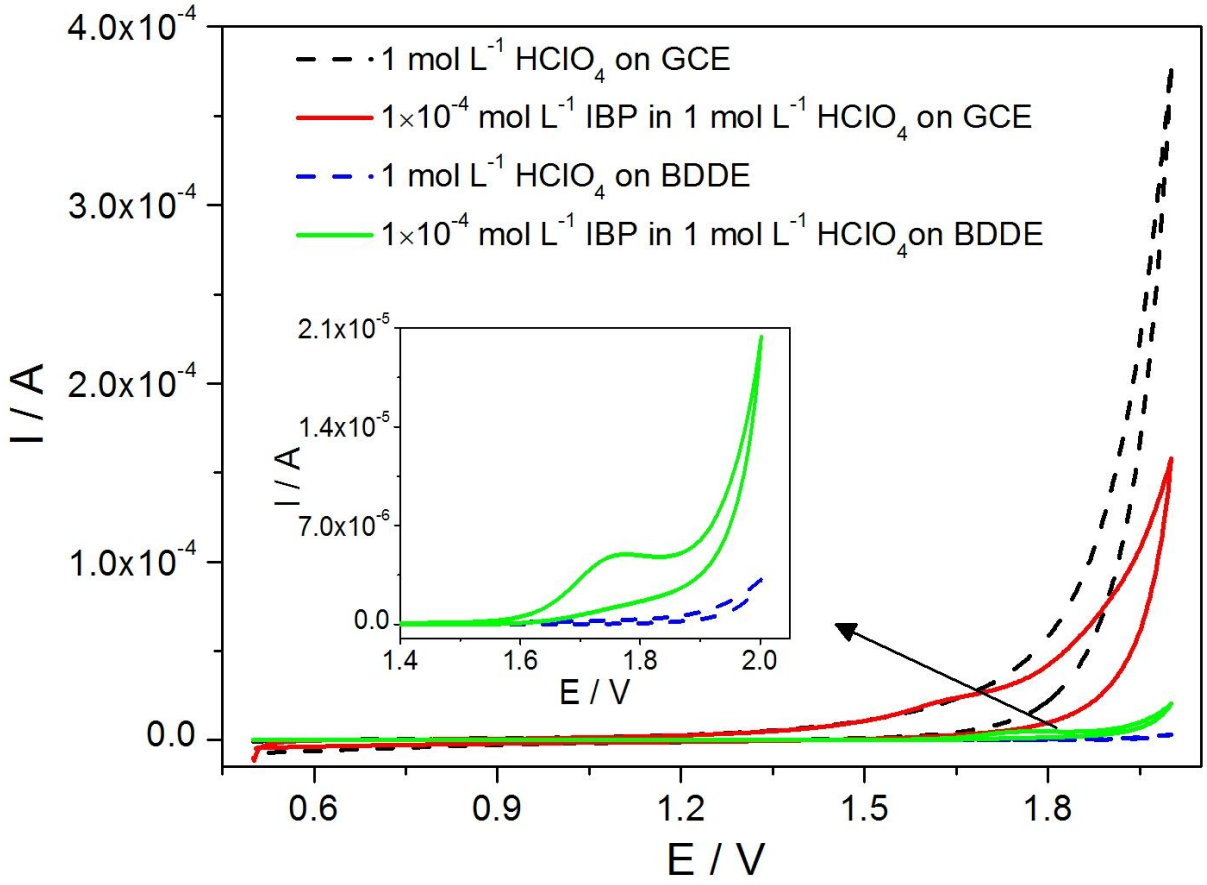

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Fig. 2

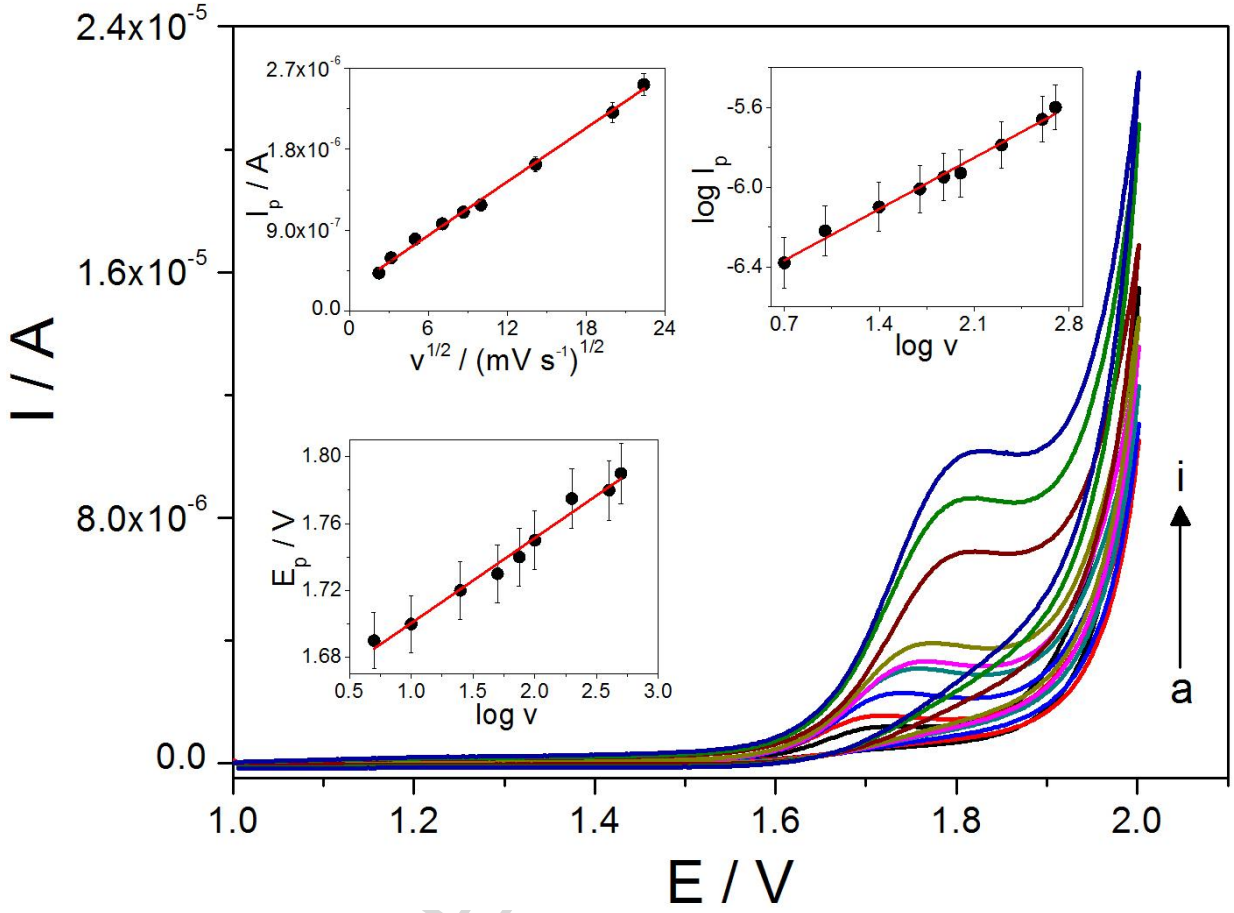

Fig. 3

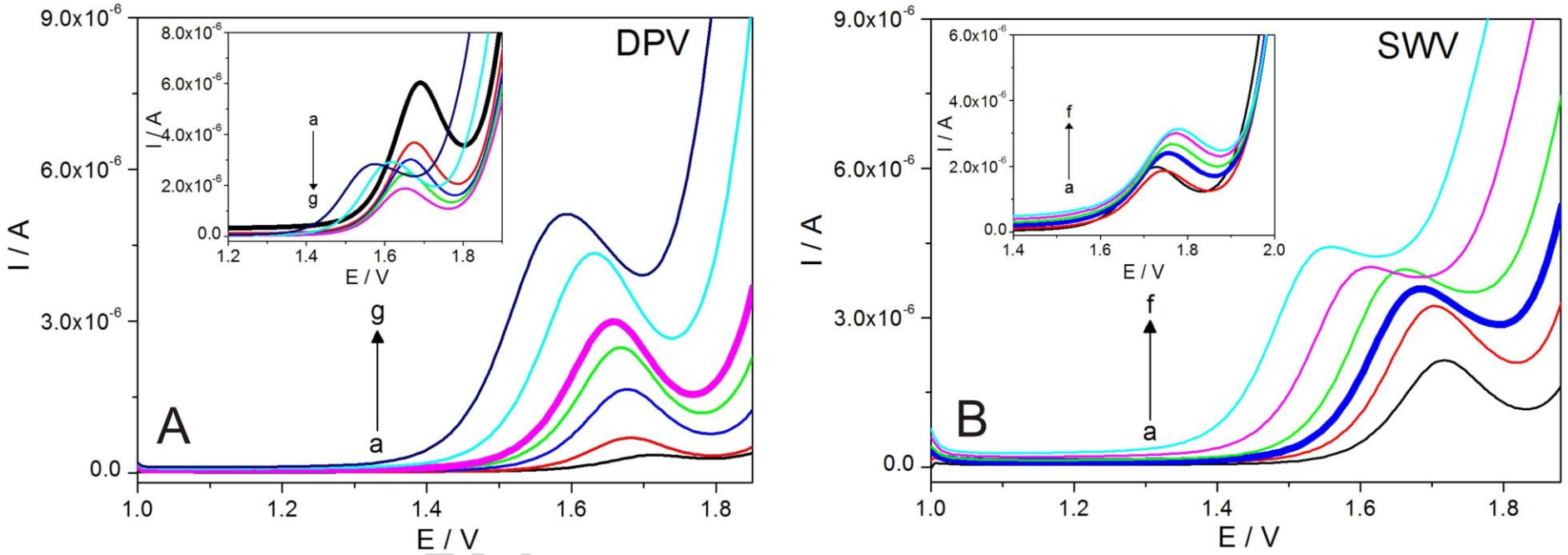

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Fig. 4

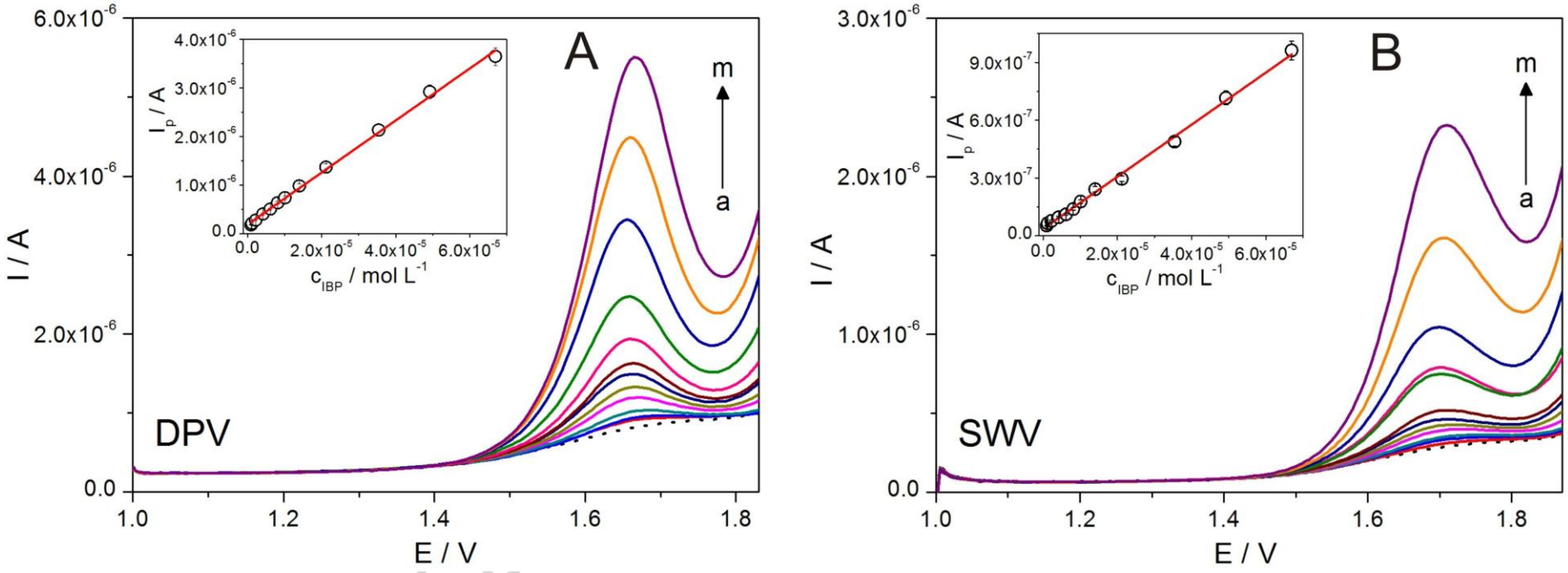
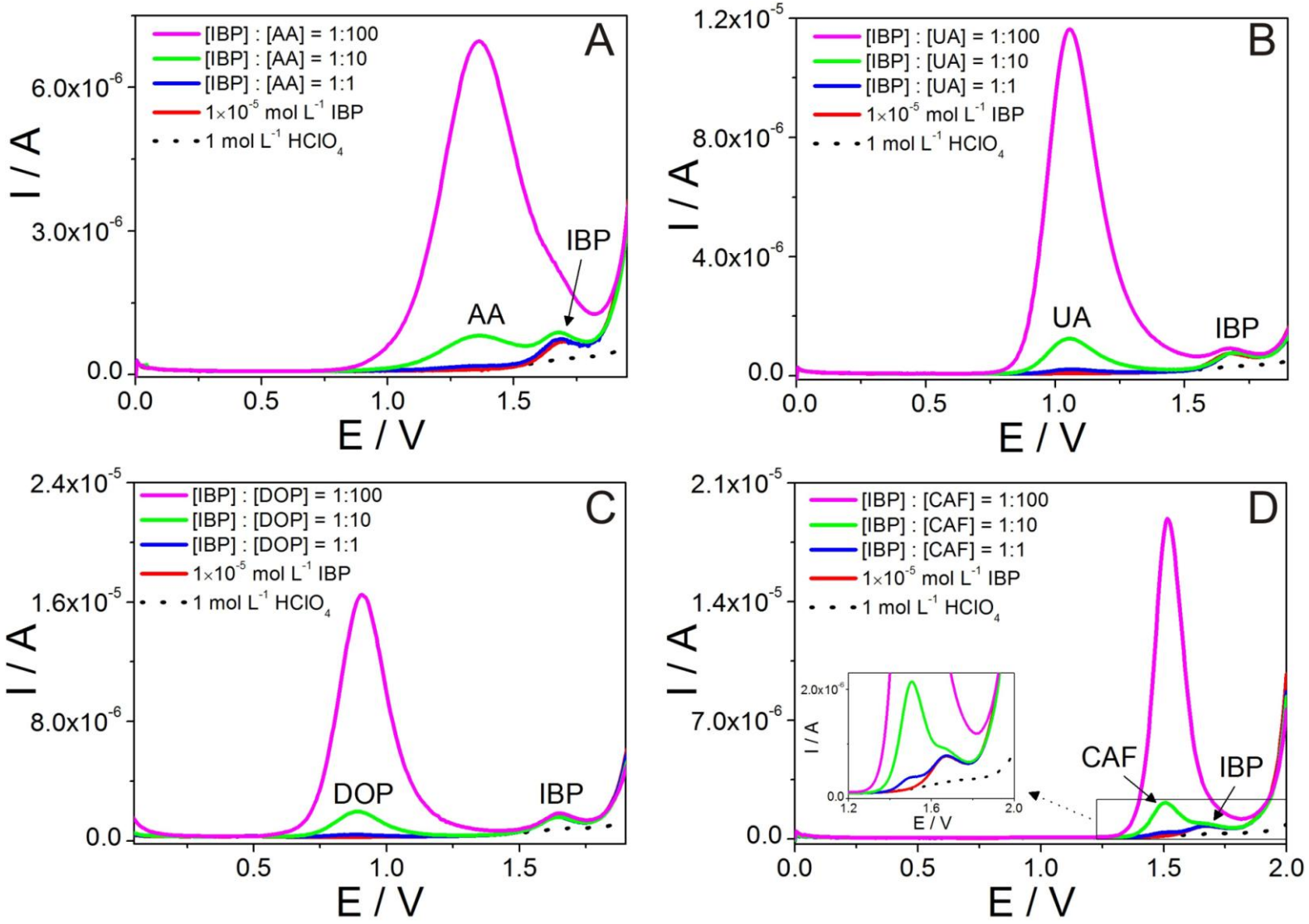


Fig. 5


A
[IBP] : [AA] = 1:100
[IBP] : [AA] = 1:10
[IBP] : [AA] = 1:1
1×10-5 mol L-1 IBP
1 mol L-1 HClO4
AA
IBP
I / A
E / V
B
[IBP] : [UA] = 1:100
[IBP] : [UA] = 1:10
[IBP] : [UA] = 1:1
1×10-5 mol L-1 IBP
1 mol L-1 HClO4
UA
IBP
C
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1 mol L-1 HClO4
DOP
IBP
D
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1 mol L-1 HClO4
CAF
IBP

Fig. 6

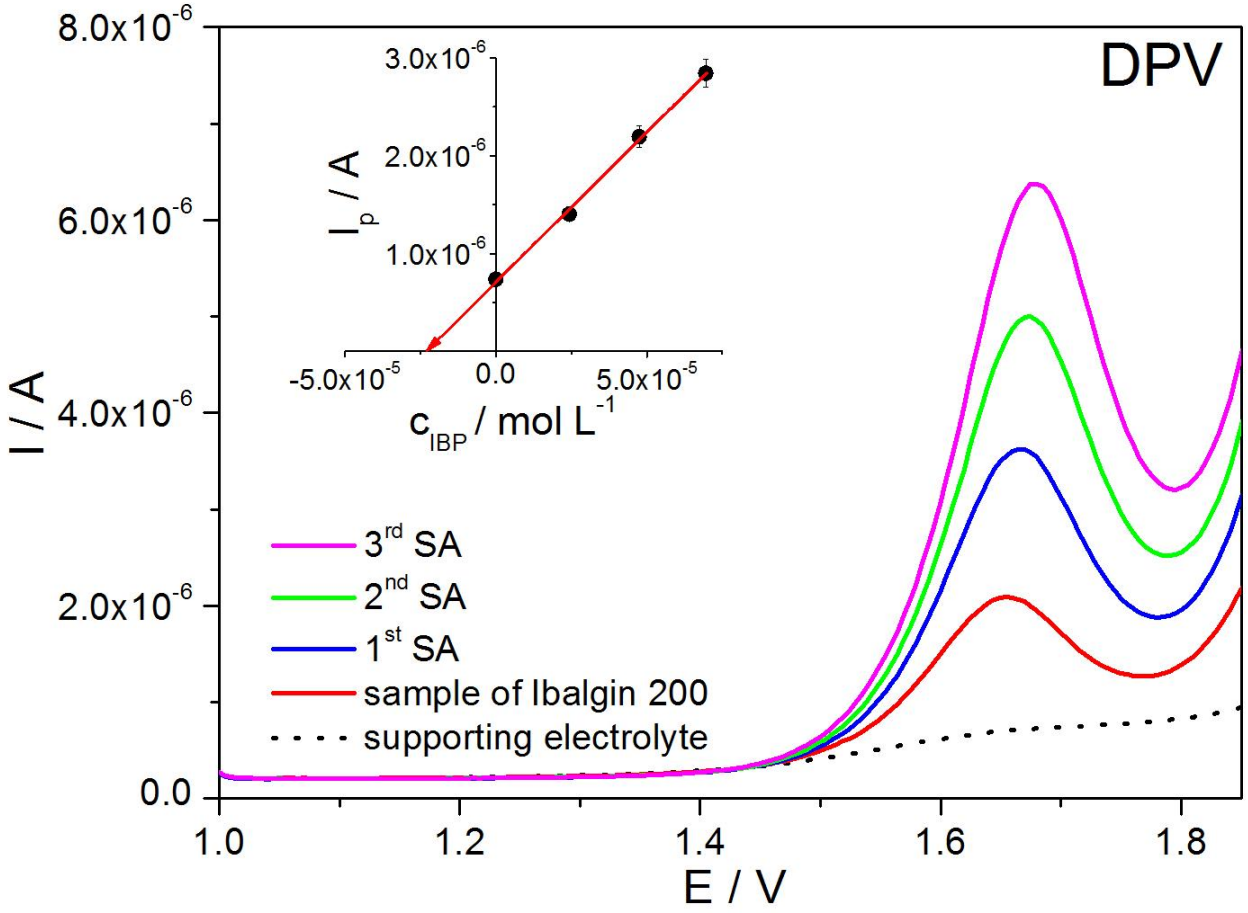

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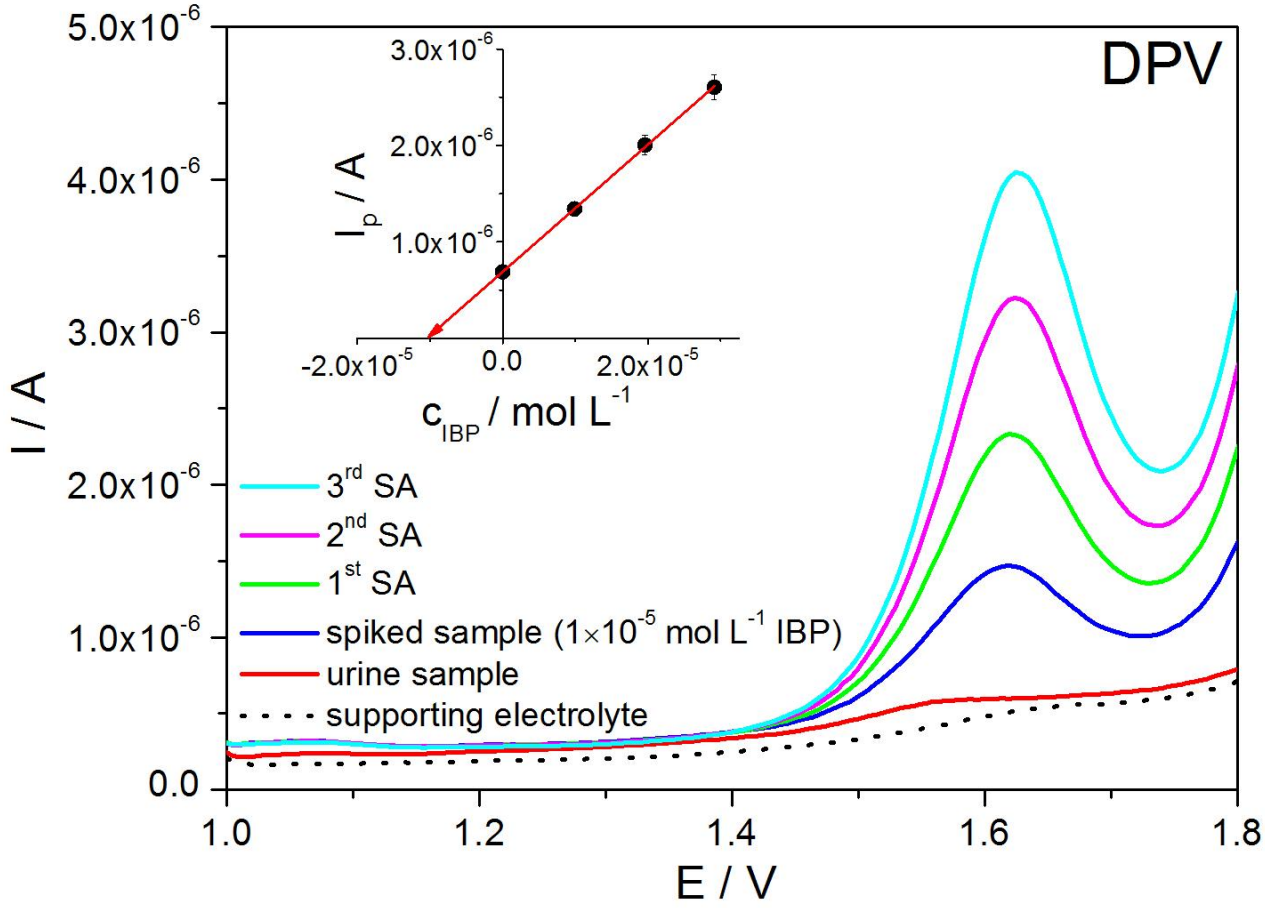
Fig. 7



Captions for figures

Scheme 1 The proposed mechanism of electrochemical oxidation of IBP.

Fig. 1 CV records of 0 (1 mol L^{-1} $HClO_4$ as supporting electrolyte) and 1×10^{-4} mol L^{-1} IBP in 1 mol L^{-1} $HClO_4$ on the GCE and BDDE in potential range from +0.5 to +2 V with scan rate of 100 mV s^{-1}.

Fig. 2 CV records of 1×10^{-4} mol L^{-1} IBP for various scan rates: (a) 5, (b) 10, (c) 25, (d) 50, (e) 75, (f) 100, (g) 200, (h) 400 and (i) 500 mV s^{-1} in 1 mol L^{-1} $HClO_4$ on the bare BDDE in potential range from +1 to +2 V. The corresponding calibration curves are displayed in the insets.

Fig. 3 (A) DP voltammograms of 1×10^{-4} mol L^{-1} IBP in 1 mol L^{-1} $HClO_4$ on the bare BDDE for various modulation amplitudes: (a) 10, (b) 25, (c) 50, (d) 75, (e) 100, (f) 150 and (g) 200 mV (modulation time fixed at 25 ms). The optimization of modulation time: (a) 10, (b) 25, (c) 50, (d) 75, (e) 100, (f) 150 and (g) 200 ms is appended in the inset (modulation amplitude fixed at 100 mV).
(B) SW voltammograms of 1×10^{-4} mol L^{-1} IBP in 1 mol L^{-1} $HClO_4$ on the bare BDDE for various amplitudes: (a) 10, (b) 25, (c) 50, (d) 75, (e) 100 and (f) 150 mV (frequency fixed at 25 Hz). The optimization of frequency: (a) 10, (b) 25, (c) 50, (d) 75, (e) 100 and (f) 125 Hz is appended in the inset (amplitude fixed at 50 mV).

Fig. 4 (A) DP and (B) SW voltammograms for various IBP concentrations: (a) 0, (b) 9.49×10^{-7}, (c) 1.20×10^{-6}, (d) 2.20×10^{-6}, (e) 4.18×10^{-6}, (f) 6.16×10^{-6}, (g) 8.13×10^{-6}, (h) 1.01×10^{-5}, (i) 1.40×10^{-5}, (j) 2.12×10^{-5}, (k) 3.54×10^{-5}, (l) 4.92×10^{-5} and (m) 6.69×10^{-5} mol L^{-1} in 1 mol L^{-1} $HClO_4$ on the bare BDDE. The optimized DPV parameters: modulation amplitude of 100 mV, modulation time of 10 ms and scan rate of 10 mV s^{-1}. The optimized SWV parameters: amplitude of 50 mV, frequency of 50 Hz and scan rate of 250 mV s^{-1}. The corresponding calibration curves are displayed in the insets.

Fig. 5 DP voltammograms manifesting the impact of the presence of selected interfering compounds on the current response of 1×10^{-5} mol L^{-1} IBP in 1 mol L^{-1} $HClO_4$ on the bare

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BDDE. The studied interfering agents were: ascorbic acid (AA), uric acid (UA), dopamine (DOP) and caffeine (CAF) in various concentration ratios (IBP : interferent = 1:1, 1:10 and 1:100). The optimized DPV parameters: modulation amplitude of 100 mV, modulation time of 10 ms.

Fig. 6 DP voltammograms of analysis of the pharmaceuticals dosages *Ibalgin* with the declared content of 200 mg IBP using the multiple standard addition method in 1 mol L^{-1} $HClO_4$ on the bare BDDE. The corresponding standard additions (SA): 500, 1000 and 1500 µL (c_{IBP} = 1×10^{-3} mol L^{-1}). The optimized DPV parameters: modulation amplitude of 100 mV, modulation time of 10 ms. The quantification of IBP by the multiple standard addition method is appended in the inset.

Fig. 7 DP voltammograms of analysis of the model human urine sample of the volunteer 2 (V2) spiked with 1×10^{-5} mol L^{-1} IBP and assessed by application of the multiple standard addition method in 1 mol L^{-1} $HClO_4$ on the bare BDDE. The corresponding standard additions: 200, 400 and 600 µL (c_{IBP} = 1×10^{-3} mol L^{-1}). The optimized DPV parameters: modulation amplitude of 100 mV, modulation time of 10 ms. The quantification of IBP by the multiple standard addition method is depicted in the inset.

Highlights

- Simple and advanced electroanalytical protocol for ibuprofen sensing is presented
- Ibuprofen provided one irreversible oxidation peak at very positive potentials
- LOD values at submicromolar levels were achieved by DPV and SWV procedures
- BDDE constitutes a regular and profitable electrochemical tool in drug analysis