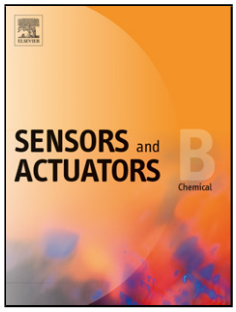

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Mercury-free and modification-free electroanalytical approach towards bromazepam and alprazolam sensing: A facile and efficient assay for their quantification in pharmaceuticals using boron-doped diamond electrodes

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Highlights

- For the first time, BDD electrodes were used in benzodiazepines sensing
- Facile and efficient approach for determination of benzodiazepines is presented
- Low LODs of 10^{-7} mol/L were achieved using bare BDD electrodes
- Green and effective alternative to mercury-based and chemically modified electrodes
- BDD platforms are promising tools for pharmaceutical applications

Abstract

The boron-doped diamond electrodes (BDDEs) with different dopant levels were applied as advanced, mercury-free and modification-free electroanalytical platforms for simple and reliable quantification of benzodiazepines, bromazepam (BZ) and alprazolam (ALZ). Cyclic voltammetric measurements revealed that the electrode reactions of both analytes were manifested by one irreversible and diffusion-controlled reduction peak at −1.10 V in Britton-Robinson buffer of pH 11 for BZ using lab-made BDDE with 1000 ppm B/C ratio and −0.84 V in BR buffer of pH 5 for ALZ using commercial BDDE with 1000 ppm B/C ratio. Differential pulse voltammetry was used for construction of calibration curves for BZ and ALZ, respectively, with the analytical parameters as follows: the linear dynamic ranges of 1×10^{-6} – 1×10^{-4} and 8×10^{-7} – 1×10^{-4} mol/L, the detection limit of 3.1×10^{-7} and 6.4×10^{-7} mol/L and the excellent repeatability with the relative standard deviation below 3% for both drugs. The developed methods were applied to analysis of the pharmaceutical tablets with the recoveries from 97.33 to 100.85% for BZ and from 94.14 to 101.32% for ALZ. The usage of BDDEs as advanced electrochemical sensors in drug analysis represents a facile and effective analytical approach which may replace mercury-based sensors and chemically modified electrodes in previous benzodiazepines sensing.

Keywords: benzodiazepines, boron-doped diamond electrode, electrochemical sensor, differential pulse voltammetry, pharmaceutical tablet

1. Introduction

Benzodiazepines (BDZs) have become one of the most widely used drugs, since the chlordiazepoxide and diazepam were launched on global market in 1960s. These drugs were found to exhibit anticonvulsant, hypnotic, sedative and muscle-relaxant effects [1]. For this reason, BDZs have been indicated in medical use for the treatment of anxiety, insomnia, depression, psychiatric disorders and alcohol withdrawal syndromes [2, 3]. Nowadays, these drugs are mostly abused due to their toxic effects and widespread availability [4]. Based on the pharmaceutical relevance and medicinal objectives of BDZs, advanced and perspective analytical approaches of high efficiency for the rigid control of these important substances in pharmaceutical formulations and different biological fluids are still required.

Many analytical methods have been recently developed for determination of BDZs, in particular, high performance liquid chromatography (HPLC) coupled with diode array detector (DAD) [5, 6] and tandem mass spectrometry (MS/MS) [7, 8], gas chromatography (GC) in combination with MS [9], electrophoresis [10] and spectrofluorimetry [11]. These remarkable analytical methods are high sensitive and selective requiring low sample consumption and enabling simultaneous determination of plenty of miscellaneous analytes. On the other hand, they suffer from expensive instrumentation, time consuming and very costly analysis as well as need for the sample derivatization is required prior to analysis.

Electroanalytical methods represent financially unassuming, temporally efficient and sensitive tool for detection and determination of various structurally and biologically interesting substances [12, 13]. The literature survey states that hanging mercury drop

electrodes (HMDE) [14-16] and silver solid amalgam electrode (AgSAE) [17] have been applied to sensitive determination of several BDZs derivatives based on their electrochemical reduction. Besides, various chemically modified carbon-based electrodes such as glassy carbon electrode (GCE) modified with multi-walled carbon nanotubes (MWCNT) [18] and 3-methylthiophene [19] as well as carbon paste electrode (CPE) modified with MWCNT [20] and bentonite [21] have been exploited as mercury-free electrochemical platforms for quantifying BDZs. In order to achieve low detection limit (LOD), a modification of carbon-based electrode substrates appeared to be favorable for supporting electron transfer in redox reaction, for decreasing peak potential and principally for increasing sensitivity. Nevertheless, bare (unmodified) conventional carbon-based electrode materials are known for their higher background with limited usable potential window in negative area due to hydrogen evolution reaction. For electroanalytical determination of BDZs, differential pulse voltammetry (DPV) and square-wave voltammetry (SWV) coupled with stripping steps such as square-wave adsorptive cathodic stripping voltammetry (SWAdCSV), square-wave cathodic stripping voltammetry (SWCSV), differential pulse stripping voltammetry (DPSV), differential pulse adsorptive cathodic stripping voltammetry (DPAdCSV) and linear sweep adsorptive cathodic stripping voltammetry (LSAdCSV) have been commonly used. In this respect, stripping voltammetry is an efficient technique routinely capable of achieving LODs lower than 10^{-8} mol/L due to preconcentration of analyte on particular working electrode [22]. In spite of convenient application of HMDEs and modified carbonaceous electrodes in BDZs sensing, researchers are still forced to search the advanced and perspective electrode materials to be applied as sensitive electrochemical sensors, even without performing any surface modification and using toxic mercury electrodes. It is worth noting that the authors of the present work have recently demonstrated the use of meniscus modified silver solid amalgam

electrode (m-AgSAE) as prospective electrode material for the quantification of selected BDZs with LODs of 10^{-7} mol/L [17].

Boron-doped diamond (BDD) is a perspective and "green" carbon-based electrode material exhibiting many advantages when compared to conventional materials such as CPE, GCE or HMDE. BDD electrode (BDDE) renders high chemical and electrochemical stability owing to sp^3 hybridization of carbon in diamond structure (chemically inert character), wide potential window in the aqueous and non-aqueous media as well as low and stable background current [23, 24]. Apart from these properties, it yields excellent biocompatibility, mechanical robustness and stability in alkaline and acidic media [25, 26]. BDD electrode surface can be treated electrochemically by applying very negative and positive potentials (cathodic and anodic pretreatment) to obtain predominantly hydrogen and oxygen terminated surface (hydrophobic and hydrophilic nature), respectively, which may change overall chemical properties of this working electrode [13]. Recently, interesting reviews on practical guide to usage of BDDE in electrochemical research [27] as well as possibilities of chemical modification of this material for biosensors and biosensing [28] have also been published. In addition, BDDE has seemed to be an efficient electrochemical sensor for detection and determination of significant biologically active compounds used in protection of human health [29], environment [30] and food analysis [31].

This paper reports the novel application of BDDEs as advanced, mercury-free and modification-free electrochemical platforms suitable for the individual determination of the selected electrochemically reducible BDZs (Fig. S1), namely *bromazepam* (7-Bromo-5-(2-pyridyl)-3*H*-1,4-benzodiaxepin-2(1*H*)-one, BZ) and *alprazolam* (8-Chloro-1-methyl-6-phenyl-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine, ALZ). The feasibility of the developed assays was demonstrated by the analysis of commercial pharmaceuticals. Moreover, as far as we know, these procedures could be considered as the first usage of BDD as advanced

carbon-based electrode material for the reliable quantification of BDZs in pharmaceutical samples.

Here Fig. S1

2. Experimental

2.1. Chemicals

BZ (CAS No. 1812-30-2, purity ≥ 98%, in all figures presented as **A**) and ALZ (CAS No. 28981-97-7, purity ≥ 98%, in all figures presented as **B**) were purchased from Sigma Aldrich (Czech Republic). 1×10^{-3} mol/L stock solutions were prepared by dissolution of appropriate amount of BZ and ALZ in 25 mL methanol (p.a., Lachema, Czech Republic) without any further purification. These solutions were stored in the fridge for following use. Britton-Robinson buffer (BR) was prepared by mixing of 0.04 mol/L H_3BO_3, H_3PO_4, CH_3COOH adjusted with 0.2 mol/L NaOH (p.a., Lachema, Czech Republic). Prior to the analysis, 1 mol/L H_2SO_4 (p.a., Lachema, Czech Republic) was used as transitional medium for applying the highly positive and negative potentials on the BDDE surface to be anodically and cathodically pretreated. The individual working and calibration solutions of BDZs were prepared by suitable dilution of the stock solution of BZ and ALZ with supporting electrolyte. All aqueous solutions were prepared in deionized water with resistivity above 18 MΩ cm.

2.2. Equipments

All voltammetric measurements were performed using potentiostat PSTAT 910 mini (Metrohm Ltd., Switzerland) driven by software PSTAT 1.0. The three electrode configuration consisted of platinum wire as counter electrode, Ag/AgCl/3 mol/L KCl reference electrode and working electrodes as follows: either commercial BDDE (boron

concentration expressed as B/C ratio in gaseous phase of 1000 ppm, BDD film with diameter of 3 mm, Windsor Scientific Ltd., UK) or lab-made BDDEs with the different boron doping levels (B/C ratio of 1000 ppm, 2000 ppm, 4000 ppm and 8000 ppm, BDD film with diameter of 740 μm) fabricated by the co-workers at the Faculty of Electrical Engineering and Information Technology, Slovak University of Technology in Bratislava. pH-meter Model 25 (Denver Instrument, USA) with combined glass electrode was applied for the pH measurements.

2.3. Working electrode surface pretreatment

At the beginning of every work day, the particular BDDE was firstly rinsed with deionized water and very softly rubbed with a piece of damp silk cloth until a mirror-like appearance of surface was obtained. After this procedure, an anodic pretreatment was carried out in 1 mol/L H_2SO_4 by applying +2.0 V for 60 s to get rid of any impurities on the BDDE surface. Subsequently, a cathodic pretreatment using −2.0 V for 30 s in the same medium was undertaken to obtain predominantly H-terminated electrode surface. Afterwards, thus pretreated BDDE surface was directly utilized for the voltammetric measurements with repeatable signals.

2.4. Measurement procedure

10 mL of particular 1×10^{-4} mol/L BDZ solution was placed into the electrochemical cell. Nitrogen gas (Linde, Slovakia) was used for bubbling each analyzed solution for 5 min in order to remove oxygen from solution and to maintain the inert atmosphere in the cell. Since the BDDEs with the different active surface areas were applied in this work, instead of the peak currents, the particular peak current densities (current density J is defined as a ratio of current signal to electrode area) for electrochemical reduction of BZ (J_{BZ}) and ALZ (J_{ALZ})

were always evaluated. Cyclic voltammetry (CV) was used for investigation of the effect of supporting electrolyte (pH) and scan rate. Calibration curves were assessed from the differential pulse (DP) voltammograms with the optimized operating parameters such as modulation amplitude and modulation time. The peak current densities (J_{BZ} and J_{ALZ}) were estimated from the straight lines connecting the minima before and after the voltammetric peak maximum, without using any background correction. Analysis of the pharmaceuticals was accomplished by standard addition method with three consecutive additions of 50 µL of the particular BDZ stock solution into the electrochemical cell. LODs were calculated as standard deviation of intercept divided by slope of the particular calibration curve. The calibration curves were statistically analyzed by OriginPro 8.0 (OriginLab, USA) and the relevant results (slope and intercept) were evaluated with a 95% confidence interval. All voltammetric measurements were performed in triplicate at room temperature.

2.5. Preparation of pharmaceutical samples

The commercial pharmaceutical tablets of Lexaurin® (declared amount of BZ according to the leaflet was 3 mg per tablet) and Xanax® (declared amount of ALZ was 2 mg per tablet) were purchased from Krka (Slovenia). The procedure for preparing and treating these samples was as follow: ten tablets of each brand of pharmaceuticals were weighed and powdered using mortar and pestle. 300 mg of particular powder of pharmaceuticals was dissolved in 25 mL methanol and filtered through the filter paper (the pore size of 20 µm). The clear filtrates of both pharmaceuticals were filled up with methanol to the total volume of 50 mL volumetric flask and stored in the fridge.

2.6. Fabrication of the BDDEs

Apart from the commercial BDDEs used in this work, the lab-made BDDEs with the different boron doping levels were prepared and applied. The polycrystalline BDD films were grown by bias enhanced hot filament chemical vapor deposition (HF CVD) technique. As a substrate, highly conductive (0.008-0.024 Ω cm) N (100) type silicon substrate with 2 μm thick SiO_2 layer (CVD, Oxford PlasmaLab 80) was used. The deposition process was divided into three steps: (*i*) 40 min ultrasonic seeding of diamond nanoparticles (CAS No. 7782-40-3, Sigma-Aldrich) diluted in deionized water, (*ii*) 2 h growth of the BDD thin film with 2 % concentration of CH_4 in H_2 and trimethylboron (TMB) to obtain the 1000 ppm, 2000 ppm, 4000 ppm and 8000 ppm boron to carbon ratio (B/C) within the gas mixture. The total pressure in the reactor during deposition was kept at 3 kPa and temperature was set up to 650±20 ˚C, (*iii*) a hydrogen termination of the as-grown BDD layer within the one vacuum cycle (10 min, H_2, 3 kPa, 650±20 ˚C). Resistivity of the 1000 ppm, 2000 ppm, 4000 ppm and 8000 ppm films was 150.21, 1.58, 8.25 and 3.65×10^{-3} Ω cm and B concentration was 0.24, 0.56, 4.92 and 2.65×10^{21}, respectively, as measured by 4-point Hall method. The active surface of working electrode (0.43 mm^2) was created in 400 nm SiO_2 (CVD, Oxford PlasmaLab 80) using a standard optical lithography (SUSS, MA6) and wet etching in the buffered oxide etch (BOE) solution (6:1 volume ratio of 40% NH_4F in water to 49% HF in water). Subsequently, the electrode chip (10×3 mm^2) was electrically connected by Ag polymer paste (CB115, DuPont) to a printed circuit board support and completely passivated by non-conducting paste (548X, DuPont).

3. Results and discussion

3.1. Electrochemical behavior study of BZ and ALZ on BDDEs

3.1.1. Effect of pH and reversibility study

The choice of the suitable pH of supporting electrolyte is an important stage in the electroanalytical studies because it affects the properties of the solution and the electrode-solution interface, thus modifying the thermodynamics and kinetics of the particular charge transfer process. The effect of pH on the peak current density (J_{BZ} and J_{ALZ}) for the individual BDZ solutions of 1×10^{-4} mol/L was investigated using differential pulse voltammetry (DPV) in BR buffers (pH 2 – 12). The results accomplished for the commercial and lab-made BDDEs with various boron doping levels are listed in Table S1. It is apparent from these data that the maximum peak current densities for BZ and ALZ varied from the particular working electrode and pH used. The optimal medium for the electrochemical reduction of BZ with the highest J_{BZ} (–0.329 μA/mm^2) was shown at the peak potential of –1.10 V in the BR buffer of pH 11 using the L-M BDDE with 1000 ppm B/C ratio. As for the electrochemical reduction of ALZ, the highest J_{ALZ} (–0.686 μA/mm^2) was observed at –0.84 V when the BR buffer of pH 5 and the commercial BDDE with 1000 ppm B/C ratio were used. The recorded DP voltammograms characterizing these redox processes in relation to the pH of BR buffer on the selected BDDEs are demonstrated in Fig. 1 (A for BZ and B for ALZ). The insets of Fig. 1 depict the dependences between the particular peak current density (J_p) and pH suggesting the pH 11 and 5 as the most suitable ones for BZ and ALZ, respectively. Besides, these insets also reflect E_p versus pH dependences with a good linearity achieved over the particular pH range with the following equations (Eqs. 1 and 2):

E_{BZ} (V) = −0.826 – 0.022×pH R = −0.992 pH studied range of 4-12 (1)

E_{ALZ} (V) = −0.615 – 0.045×pH R = −0.998 pH studied range of 2-7 (2)

Overall, the L-M BDDE with the BR buffer of pH 11 and the commercial BDDE with the BR buffer of pH 5 were applied for the further voltammetric measurements of BZ and ALZ, respectively. Concerning the electrode reactions of both analytes, Fig. 2 demonstrates the CV records for the respective 1×10^{-4} mol/L BZ and ALZ solutions using the optimal supporting

electrolytes in the potential range from +1.0 to –1.5 V. In both cases, one reduction peak at –1.10 V and –0.84 V at forward scan with the absence of any oxidation peak at reverse scan was observed for BZ and ALZ, respectively, obviously confirming the totally irreversible electrode reactions on BDDEs. This behavior is mostly consistent with those reported for mercury [17, 32-34] and chemically modified carbon-based electrodes [18-21] in BDZs sensing. Besides, it is also apparent from Fig. 2 that the background current appeared to be relatively low at the more negative potentials (less than –1.0 V) using both BDDEs, thus approving excellent properties of this electrode material for detection of highly reducible analytes. Additionally, to corroborate this phenomenon, we also noticed higher hydrogen overpotential when the bubbles of the electrogenerated hydrogen did not occur even at the potentials of more negative than −1.5 V. Therefore, the hydrogen evolution on BDDEs did not significantly complicate the voltammetric behavior of BZ and ALZ.

Here Table S1

Here Fig. 1

Here Fig. 2

3.1.2. Mechanism of electrochemical reduction of BDZs

The electrode reaction mechanism of some BDZs using various working electrodes such as m-AgSAE [17], CPE [34] and GCE [35] is well described in literature, in particular, suggesting the azomethine functional group to be reduced with the participation of $2H^+/2e^-$ transfer. However, as to BDDE, the mechanism of the electrode reaction of BDZs on this advanced carbon material has not been well known and described yet. Although the clarification of the detailed mechanism of the electrochemical reduction of BDZs has not been the subject matter of this work, to give some closer information for this aspect, the slope values of dependences (E_p versus pH of supporting electrolyte as shown in the insets of Fig.

1) for BZ and ALZ were recognized to be 0.022 V/pH and 0.045 V/pH, respectively (Eqs. 1 and 2). This fact indicates that the reduction of the reactive imine functional group of BZ as a part of the azomethine part could occur by $1H^+$ and $2e^-$ exchange to most likely form the radical anion [17]. In regard to ALZ, the slope of this dependence (Eq. 2) notifies that the electrochemical mechanism probably involves the transfer of $2H^+/2e^-$ process due to the reduction of the imine groups to the amine functionalities [32]. Furthermore, it is noticeable from these results that the proposed reduction mechanism of studied BDZs is in good agreement with the literature data when using other working electrode materials [17, 32, 35]. On the other hand, for a detailed insight into the reaction mechanism of the studied BDZs, some additional measurements should be carried out e.g. using controlled potential coulometry with subsequent spectral analysis (NMR, MS) for identification of corresponding reduction products.

3.1.3. Effect of scan rate

The influence of scan rate (v) on the peak current density (J_p) was investigated by CV using 1×10^{-4} mol/L BZ and ALZ solution on the particular BDDE in the potential window from +1.0 to –1.5 V. Fig. S2 depicts the CV records of BZ and ALZ exhibiting the reduction peaks for the range of scan rate from 25 to 200 mV/s. From the insets of Fig. S2 it is evident that the respective linear relationships between J_p and the square root of the scan rate ($v^{1/2}$) for both BDZs clearly reflect a diffusion-driven mechanism of the electrode reactions. The particular equations (Eqs. 3 and 4) with the significant linearity can be summarized as follows:

$$J_{BZ}\ (\mu A/mm^2) = -0.025\times v^{1/2}\ (mV/s)^{1/2} - 0.047 \qquad R = -0.995 \qquad (3)$$

$$J_{ALZ}\ (\mu A/mm^2) = -0.011\times v^{1/2}\ (mV/s)^{1/2} - 0.030 \qquad R = -0.999 \qquad (4)$$

Here Fig. S2

The linear relationships were also obtained for the dependences of the logarithm of the absolute values of J_{BZ} and J_{ALZ} against the logarithm of scan rate with the slopes of 0.42 and 0.38 for BZ and ALZ, respectively (data not shown). These values are close to the theoretical one (0.50) indicating that the electrochemical reduction of BZ and ALZ on BDDEs is largely a diffusion-controlled process. Therefore, it may be concluded that the effect of adsorption as a rate-determining step during the electrode reactions of the studied analytes seemed to be negligible. Another proof of the minor effect of adsorption resulted from the intercept values given in Eqs. 3 and 4 which were found to be satisfactory low. Additionally, as can be seen in Fig. S2, the small shift of the peak potentials (E_{BZ} and E_{ALZ}) towards negative direction was noticed for both BDZs as the scan rate gradually increased. This behavior points out the irreversible nature of the electrode process of BZ and ALZ on BDDE which is in good accordance with those previously observed on other electrode materials [18].

3.2. Individual analytical determination of BZ and ALZ

3.2.1. Optimization of differential pulse voltammetric parameters

DPV was applied as a sensitive voltammetric technique for the determination of BDZs with good significant resolution against the background current [13]. The optimization of the DPV operating parameters was performed to obtain the highest current densities for BZ and ALZ, thus ensuring the distinguished analytical sensitivity for their individual determination on BDDEs. The optimization was carried out using 1×10^{-4} mol/L BDZs in the particular medium with the fixed scan rate value of 10 mV/s. The operating parameters of DPV such as modulation amplitude (pulse height) and modulation time (pulse time) were investigated for both analytes. During the optimization one parameter was changed while the other was kept constant. As shown in DP voltammograms in Fig. 3, a variation of modulation amplitude in

the range of 10 – 150 mV (with the modulation time fixed at 50 ms) showed that the current responses of BZ and ALZ increased, however, at the same time the background currents gradually increased as well. The optimal modulation amplitude with the best compromise between the faradaic and background current was set on 50 mV for BZ and 100 mV for ALZ. Furthermore, the modulation time was explored in the range from 10 to 150 ms. The insets of Fig. 3 illustrate the decrease of J_{BZ} and J_{ALZ} as the modulation time increased. The optimal modulation time of 25 ms and 50 ms were chosen as suitable values for the subsequent DPV determination of BZ and ALZ, respectively. The optimal operating parameters of DPV for the determination of BDZs are also summarized in Table S2.

Here Fig. 3

Here Table S2

3.2.2. Quantification of BDZs

The applicability of DPV for the individual determination of BZ and ALZ was investigated under the optimized experimental conditions by plotting of J_{BZ} and J_{ALZ} against the particular concentration. The DP voltammograms, involving the calibration curves with the linear dynamic ranges of 1×10^{-6} – 1×10^{-4} mol/L and 8×10^{-7} – 1×10^{-4} mol/L for BZ and ALZ, respectively, are gathered in Fig. 4. The analytical parameters of this electroanalytical approach are summarized in Table 1. LODs for BZ and ALZ were calculated to be 3.1×10^{-7} and 6.4×10^{-7} mol/L, respectively. The repeatability of the proposed methods was tested by four replicate DPV measurements for 1×10^{-5} mol/L of each BDZ using the same conditions with the achieved relative standard deviations (RSDs) of 2.93 % for BZ and 1.98 % for ALZ. The low RSD values reflect good precision of the proposed method and confirm the negligible adsorption effect for this advanced electrode material. It may be concluded from

the attained results that the developed methods provide considerably sensitive and precise quantification of both studied BDZs.

Here Fig. 4

Here Table 1

3.2.3. Interference studies

In order to evaluate the selectivity of the proposed electrochemical protocols, the influence of some common species (usually present in pharmaceuticals) on the reduction signal of 1×10^{-5} mol/L BZ and ALZ was examined under the optimum experimental conditions. The tolerance limit was taken as the maximum concentration of the foreign substances, which caused an approximately ±5% relative error in the determination of both drugs. The 200-fold excess of inorganic ions such as Ca^{2+}, Mg^{2+}, Na^{+} and K^{+} and sugars (glucose, lactose, starch and sucrose) exhibited no significant changes of BZ and ALZ signals. It was found that the individual determination of BZ and ALZ on BDDEs was not affected significantly by the common interfering species.

3.2.4. Analysis of pharmaceutical tablets

In order to investigate the accuracy and validity of the developed methods, standard addition method was applied to the analysis of two different commercially available pharmaceuticals Lexaurin® (BZ) and Xanax® (ALZ). The preparation of the pharmaceutical samples is discussed in detail in section 2.5. An aliquot volume of 1×10^{-3} mol/L BDZ standard solution was added to the electrochemical cell containing 9 mL of supporting electrolyte and 1 mL of the particular pharmaceutical sample. Each experiment was performed in triplicate. The respective DP voltammograms of analysis of the commercial pharmaceutical tablets with the graphical evaluation of standard addition method are illustrated in Fig. 5. The recovery values for both pharmaceutical samples are summarized in

Table 2 and Table 3. The recoveries were ranged from 97.33 to 100.85% and from 94.14 to 101.32% for the individual BZ and ALZ determination, respectively. Obviously, these results indicate that the methods did not suffer from any significant matrix interferences. Therefore, the BZ and ALZ amount can be quantitatively recovered by the proposed methods, being thus a guarantee of the accuracy of the voltammetric determination of these BDZs in routine pharmaceutical analysis.

Here Fig. 5

Here Table 2

Here Table 3

3.2.5. Comparison with mercury and chemically modified working electrodes

A comparison between the analytical parameters of the proposed advanced approach and some methods previously reported for the determination of BZ, ALZ and other related BDZs is summarized in Table 4. From these data, it can be emphasized that the electroanalytical methods with the use of mercury electrodes in combination with the pulse voltammetric techniques with stripping steps have been considered to be the highly sensitive platforms with the low LODs for the quantifying BDZ, mostly below 10^{-8} mol/L [14-16, 32]. In addition, the linear range for studied BDZs using mercury electrodes was at the concentration level of three orders and the particular peak potentials varied from –0.5 to –1.2 according to the supporting electrolyte used (BR buffers of pH 5-11). The excellent analytical performance for BDZs sensing mostly arises from high hydrogen overpotential and self-renewing atomically smooth surface of this electrode material. However, mercury has been considered as toxic substance and the problems with mechanical stability could occur for electrochemical measurements in flowing systems and for field measurements, therefore an adequate care should be taken in its

handling. Likewise, the current trends in the field of modern electroanalytical chemistry often classify mercury as obsolete electrode material [36].

Hence, several electrochemists have focused on the usage of the perspective and environmentally acceptable electrode materials as sensitive electrochemical sensors capable of the trace quantification of the particular BDZ. In this respect, the application of silver solid amalgam electrodes has seemed to be efficient in the BDZ sensing combining advantages of mercury and solid working electrodes. In contrast to mercury electrodes, they are mechanically stable and thus compatible with measurement in combination with flowing systems. The use of m-AgSAE as alternative electrode material for the individual determination of four BDZs (diazepam, nordiazepam, BZ, ALZ) by DPV with LODs of about 10^{-7} mol/L has been recently demonstrated [17]. For BZ and ALZ, the best environment appeared to be alkaline with the peak potentials of –0.35 and –1.06 V using BR buffer of pH 2 and phosphate buffer (PB) of pH 11, respectively. Otherwise, so far the most sensitive electroanalytical procedure with LOD of 2×10^{-10} mol/L has been rendered for lorazepam determination at +0.19 V in neutral media (PB of pH 6.8) using a GCE coated with MWCNT and Au nanoparticles [18]. This chemically modified electrode enhanced the transport of electrons that led to substantial improvement of sensitivity when compared with bare GCE.

Concerning the analytical performance of BDZ sensing, it should be pointed out that the LODs accomplished by applying BDDEs in the proposed paper are slightly lower with those obtained with m-AgSAE [17] and 3-methylthiophene modified GCE electrodes [19] in acid medium. The usage of other chemically modified electrodes such as MWCNT modified CPE [20] and bentonite modified CPE [21] has resulted in LODs of 10^{-8} mol/L using SWV for both cases. Owing to above mentioned facts, it is worth stating that the application of amalgam electrodes is usually accompanied by extending the total procedure time because of a requirement of the preparation of nontoxic amalgam. Then, the preparation of chemically

modified electrodes mostly requests demanding surface modification procedure, which on the one hand improves the sensitivity and selectivity, but on the other hand oftentimes gives the low reproducible signals. Furthermore, the analytical parameters achieved by modified electrodes may suffer from the variableness due to the singularity in the actual status of the modified electrode surface which can restrict their reliable usage in routine analysis. Accordingly, herein presented BDDEs in connection with the DPV technique meet all required characteristics for the contemporary electrochemical sensors (rapidity, simplicity, sufficient sensitivity, good repeatability and low cost) to be successfully used as mercury-free (nontoxic) and modification-free alternative platforms for determination of BDZs traces in pharmaceutical samples.

Here Table 4

4. Conclusion

Recent analytical methods dedicated to BDZs quantification are relatively of high cost, generate a high amount of toxic organic solvent with time consuming analysis and need for sample derivatization. In this work, the commercial and lab-made BDDEs with various boron-doping levels were exploited for the first time in connection with DPV technique to elaborate the novel and advanced electrochemical protocols for simple analytical determination of the selected BDZs. CV experiments revealed that both BZ and ALZ provided one irreversible and diffusion-controlled reduction peak at –1.10 and –0.84 V, respectively. Under the optimized experimental conditions, the analytical parameters were determined as follows: LOD = 3.1×10^{-7} and 6.4×10^{-7} mol/L, the linear dynamic ranges of 1×10^{-6} – 1×10^{-4} mol/L and 8×10^{-7} – 1×10^{-4} mol/L as well as the significant repeatability (RSD = 2.93 and 1.98%) for BZ and ALZ, respectively. The methods were sufficiently selective with the negligible effect of

possible interfering agents. The developed procedures were applied to the analysis of the pharmaceutical samples without a complex sample pretreatment with the recoveries in the range of 97.33 – 100.85% and 94.14 – 101.32% for BZ and ALZ, respectively. The innovative aspect of here reported work consists in the application of the bare BDDEs as advanced, mercury-free and modification-free electrochemical platforms replacing toxic and obsolete mercury electrodes as well as chemically modified carbon electrodes in BDZs sensing. Clearly, the coupling of BDDE with DPV is a low cost and adequate electrochemical tool for routine pharmaceutical analysis.

Acknowledgements

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Captions for figures

Fig. 1.

(A) Effect of pH on the peak potential (■) and the peak current density (■) of 1×10^{-4} mol/L BZ in BR buffer of pH 11 on L-M BDDE of 1000 ppm B/C using DPV. The used DPV parameters: modulation amplitude of 50 mV, modulation time of 80 ms and scan rate of 25 mV/s.

(B) Effect of pH on the peak potential (■) and the peak current density (■) of 1×10^{-4} mol/L ALZ in BR buffer of pH 5 on commercial BDDE of 1000 ppm B/C using DPV. The used DPV parameters: modulation amplitude of 50 mV, modulation time of 80 ms and scan rate of 25 mV/s.

Fig. 2.

(A) CV records of 0 (blank) and 1×10^{-4} mol/L BZ in BR buffer of pH 11 on L-M BDDE of 1000 ppm B/C in the potential range from +1.0 to –1.5 V with the scan rate of 50 mV/s.

(B) CV records of 0 (blank) and 1×10^{-4} mol/L ALZ in BR buffer of pH 5 on commercial BDDE of 1000 ppm B/C in the potential range from +1.0 to –1.5 V with the scan rate of 50 mV/s.

Fig. 3.

(A) DP voltammograms of 1×10^{-4} mol/L BZ in BR buffer of pH 11 on L-M BDDE of 1000 ppm B/C for various modulation amplitudes: (a) 10, (b) 25, (c) 50, (d) 100 and (e) 150 mV. The optimization of modulation time: (a) 10, (b) 25, (c) 50, (d) 100 and (e) 150 ms appears in the inset.

(B) DP voltammograms of 1×10^{-4} mol/L ALZ in BR buffer of pH 5 on commercial BDDE of 1000 ppm B/C for various modulation amplitudes: (a) 10, (b) 25, (c) 50, (d) 100 and (e) 150 mV. The optimization of modulation time: (a) 10, (b) 25, (c) 50, (d) 100 and (e) 150 ms appears in the inset.

Fig. 4.

(A) DP voltammograms of supporting electrolyte (BR buffer of pH 11, curve a) and BZ for the concentration range with two (I and II) linear segments: **(I)** (b) 1×10^{-6}, (c) 2×10^{-6}, (d) 4×10^{-6}, (e) 6×10^{-6}, (f) 8×10^{-6} and (g) 1×10^{-5} mol/L, **(II)** (h) 2×10^{-5}, (i) 4×10^{-5}, (j) 6×10^{-5}, (k) 8×10^{-5} and (l) 1×10^{-4} mol/L in BR buffer of pH 11 on L-M BDDE of 1000 ppm B/C. The optimized DPV parameters: modulation amplitude of 50 mV, modulation time of 25 ms and scan rate of 10 mV/s. The respective calibration curves $J_{BZ} = f(c_{BZ})$ with corresponding error bars appear in the inset.

(B) DP voltammograms of supporting electrolyte (BR buffer of pH 5, curve a) and ALZ for the concentration range with two (I and II) linear segments: **(I)** (b) 8×10^{-7}, (c) 1×10^{-6}, (d) 2×10^{-6}, (e) 4×10^{-6}, (f) 6×10^{-6} and (g) 8×10^{-6} mol/L, **(II)** (h) 1×10^{-5}, (i) 2×10^{-5}, (j) 4×10^{-5}, (k) 6×10^{-5}, (l) 8×10^{-5} and (m) 1×10^{-4} mol/L in BR buffer of pH 5 on commercial BDDE of 1000 ppm B/C. The optimized DPV parameters: modulation amplitude of 100 mV, modulation time of 50 ms and scan rate of 10 mV/s. The respective calibration curves $J_{ALZ} = f(c_{ALZ})$ with corresponding error bars appear in the inset.

Fig. 5.

(A) DP voltammograms of analysis of the pharmaceuticals tablets Lexaurin® (curve b) with declared content of 3 mg BZ using standard addition method in BR buffer of pH 11 (curve a) on L-M BDDE of 1000 ppm B/C. The respective standard additions: (c) 50, (d) 100 and (e) 150 µL ($c_{BZ} = 1\times10^{-3}$ mol/L). The optimized DPV parameters: modulation amplitude of 50 mV, modulation time of 25 ms and scan rate of 10 mV/s. The determination of BZ by standard addition method is depicted in the inset.

(B) DP voltammograms of analysis of the pharmaceuticals tablets Xanax® (curve b) with declared content of 2 mg ALZ using standard addition method in BR buffer of pH 5 (curve a) on commercial BDDE of 1000 ppm B/C. The respective standard additions: (c) 50, (d) 100 and (e) 150 µL ($c_{ALZ} = 1\times10^{-3}$ mol/L). The optimized DPV parameters: modulation amplitude of 100 mV, modulation time of 50 ms and scan rate of 10 mV/s. The determination of ALZ by standard addition method is depicted in the inset.

Fig. 1

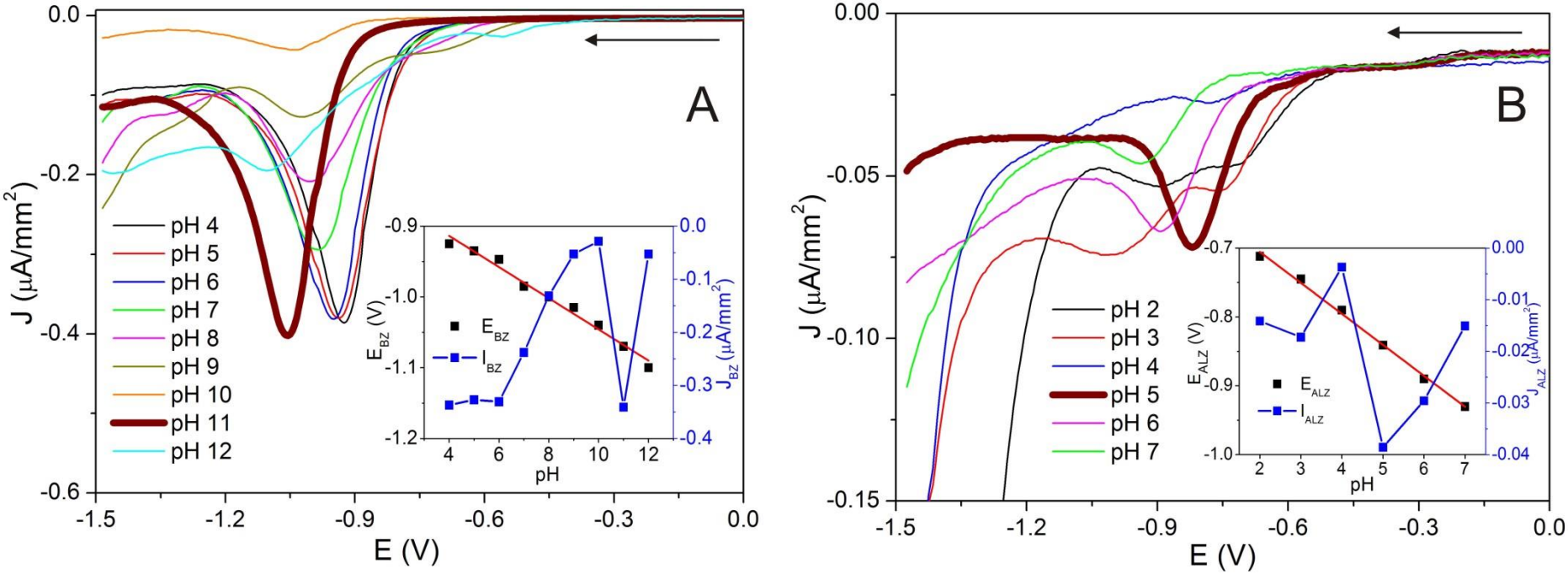

Fig. 2

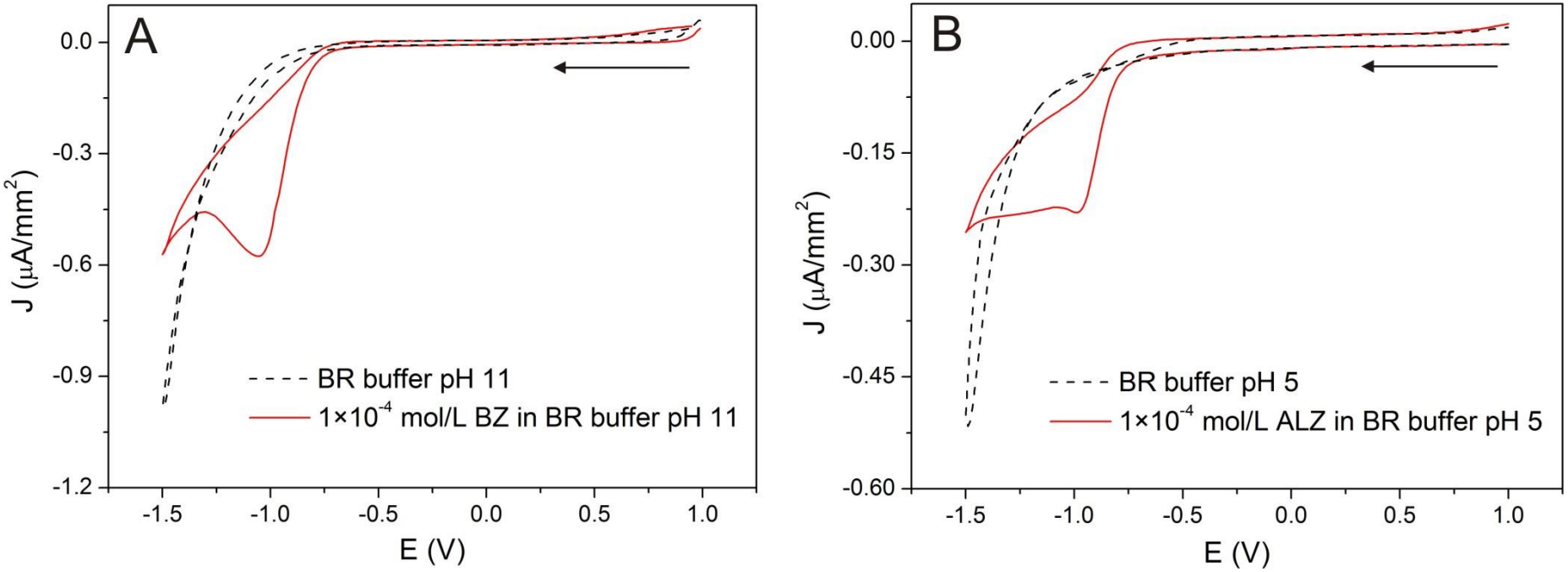

Fig. 3

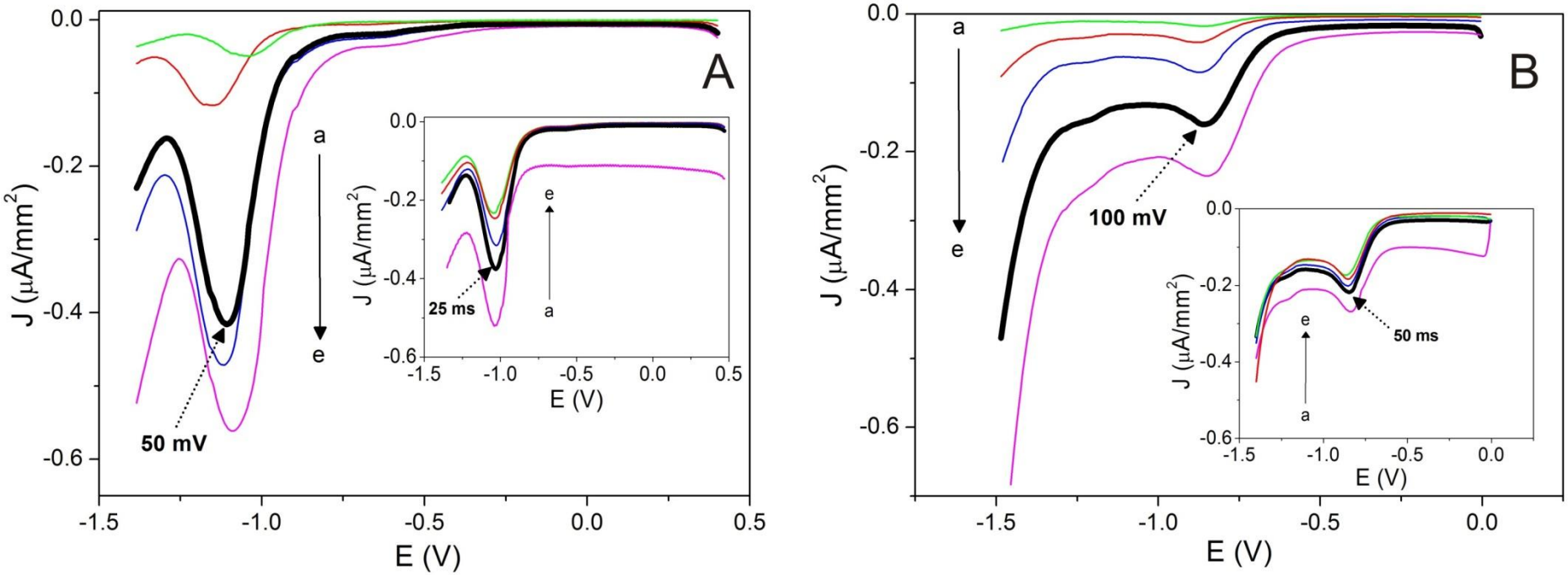

Fig. 4

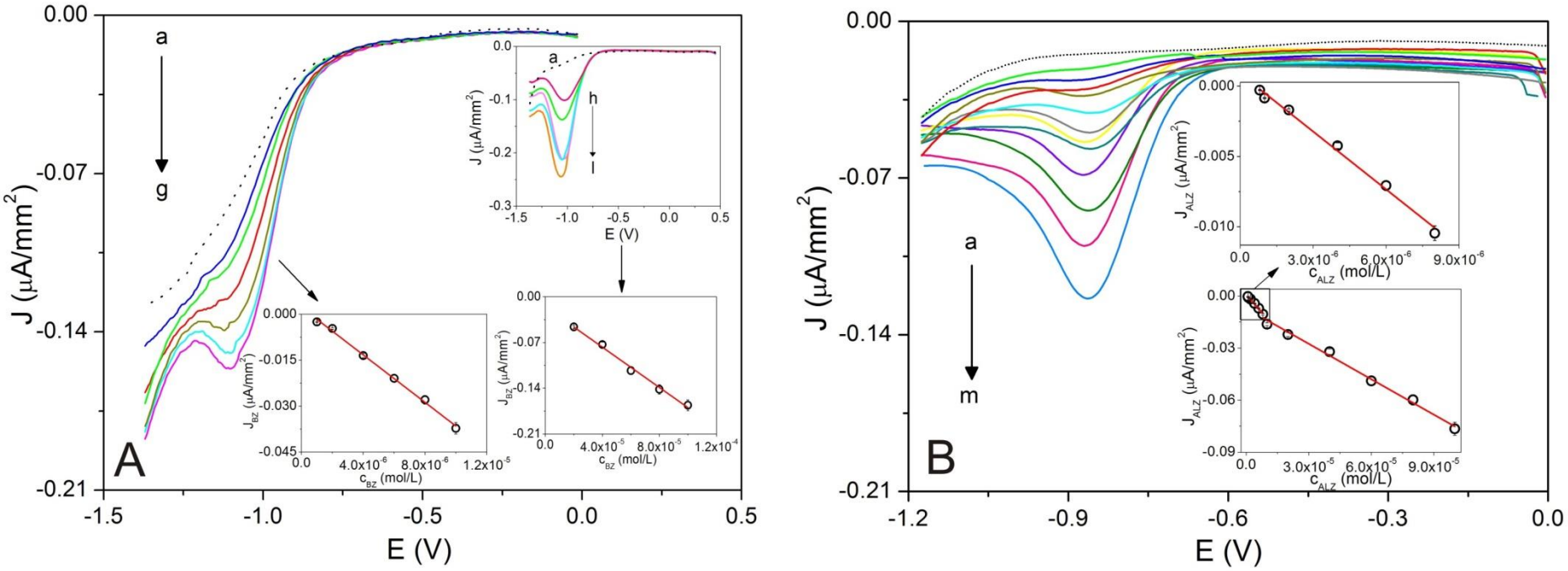

Fig. 5

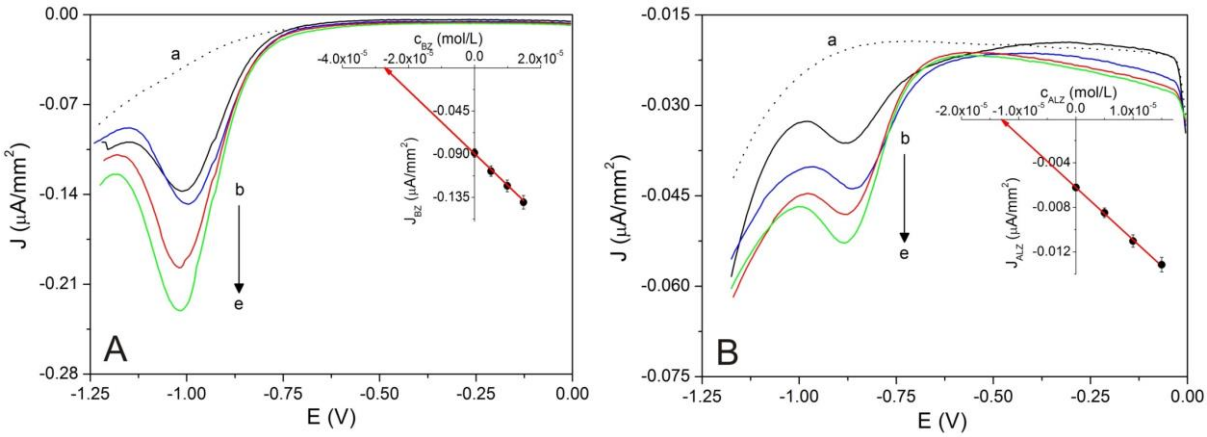

Table 1

The basic characteristics of the developed methods for the individual determination of BZ and ALZ on BDDEs using the DPV technique with the optimized parameters (n = 3).

Characteristics of the developed methods	**Studied analyte**	
	BZ	**ALZ**
Type of BDDE	Lab-made	Commercial
BDDE active surface area (mm^2)	0.43	7.1
B/C ratio in BDDE (ppm)	1000	1000
Supporting electrolyte	BR buffer, pH 11	BR buffer, pH 5
Peak potential (V vs. Ag/AgCl/3 mol/L KCl)	–1.10	–0.84
Linear dynamic range (mol/L)	1×10^{-6} – 1×10^{-4} (with 2 linear segments: 1×10^{-6} – 1×10^{-5}, 2×10^{-5} – 1×10^{-4})	8×10^{-7} – 1×10^{-4} (with 2 linear segments: (8×10^{-7} – 8×10^{-6}, 8×10^{-6} – 1×10^{-4})
Slope ($\mu A\times L/mm^2\times mol$) for 1st linear segment	–3870 ± 107	–1411 ± 54
Intercept ($\mu A/mm^2$) for 1st linear segment	0.0022 ± 0.0004	–0.0009 ± 0.0003
Correlation coefficient	–0.998	–0.996
Detection limit (mol/L)	3.1×10^{-7}	6.4×10^{-7}
Repeatability (%, for 1×10^{-5} mol/L, n = 4)	2.93	1.98

Table 2

The analysis of the pharmaceutical tablets (Lexaurin®) containing BZ using the proposed method (n = 3).

Added (µL)	**Expected (mg)**	**Found*** **(mg)**	**Standard deviation (mg)**	**Recovery (%)**
0	3.000	2.920 ± 0.083	0.049	97.33
50	3.016	2.945 ± 0.089	0.053	97.64
100	3.032	2.982 ± 0.061	0.036	98.35
150	3.047	3.073 ± 0.054	0.032	100.85

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

Table 3

The analysis of the pharmaceutical tablets (Xanax®) containing ALZ using the proposed method (n = 3).

Added (µL)	**Expected (mg)**	**Found*** **(mg)**	**Standard deviation (mg)**	**Recovery (%)**
0	2.000	1.917 ± 0.086	0.051	95.85
50	2.015	1.897 ± 0.125	0.074	94.14
100	2.031	2.043 ± 0.083	0.049	100.59
150	2.046	2.073 ± 0.089	0.053	101.32

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

Table 4

The overview of voltammetric methods for the determination of BDZs on various mercury-like and chemically modified working electrodes.

Studied BDZ	Working electrode	Supporting electrolyte	E_p (V)	Techniques	Linear range (mol/L)	LOD (mol/L)	LOQ (mol/L)	Ref.
Diazepam	m-AgSAE	0.1 mol/L NaOH	−1.21	DPV	$4\times10^{-7}-1\times10^{-4}$	9.0×10^{-8}	–	17
	3-methylthiophene/GCE	0.1 mol/L H_2SO_4	−0.85	DPSV	$7\times10^{-7}-3.8\times10^{-6}$	3.5×10^{-7}	–	19
	MWCNT/CPE	BR pH 7	−0.98	SWV	$7\times10^{-8}-2.7\times10^{-6}$	1.4×10^{-8}	–	20
	Bentonite/CPE	BR pH 10	–	SWAdCSV	$9.8\times10^{-8}-9\times10^{-7}$	1.4×10^{-8}	–	21
Nordiazepam	m-AgSAE	BR pH 10.2	−1.09	DPV	$6\times10^{-7}-1\times10^{-4}$	1.1×10^{-7}	–	17
Bromazepam	m-AgSAE	BR pH 2	−0.35	DPV	$6\times10^{-7}-1\times10^{-4}$	3.7×10^{-7}	–	17
	HMDE	BR pH 5	−0.55	DPAdCSV	$1\times10^{-8}-8\times10^{-8}$	3.5×10^{-9}	–	32
	HMDE	0.1 mol/L KNO_3/BR pH 6	–	SWCSV	$1\times10^{-7}-1\times10^{-5}$	1.2×10^{-8}	–	33
Alprazolam	m-AgSAE	PB pH 11	−1.06	DPV	$6\times10^{-7}-1\times10^{-4}$	3.5×10^{-7}	–	17
	HMDE	Ringer buffer pH 10	−1.05	AdCSV	$1\times10^{-6}-3\times10^{-5}$	–	–	36
Chlordiazepoxide	Bentonite/CPE	0.05 mol/L FA/NaOH	–	SWAdCSV	$1.1\times10^{-7}-1\times10^{-6}$	1.6×10^{-8}	–	21
	HMDE	BR pH 8	−1.22	SWAdCSV	$5\times10^{-9}-2\times10^{-7}$	–	1.5×10^{-9}	14
Lorazepam	Au/MWCNT/GCE	PB pH 6.8	0.19	SWV	$5\times10^{-10}-1\times10^{-8}$	2.0×10^{-10}	–	18
	HMDE	BR pH 2	−0.65	DPAdCSV	$1.6\times10^{-7}-3.6\times10^{-6}$	5.9×10^{-8}	–	15
Tetrazepam	HMDE	BR pH 11	−0.97	DPP	$5\times10^{-6}-1.1\times10^{-4}$	1.5×10^{-6}	–	16
	HMDE	BR pH 11	−0.97	DPAdCSV	$3\times10^{-7}-1\times10^{-5}$	–	3×10^{-7}	16
	HMDE	BR pH 11	−0.97	LSAdCSV	$1\times10^{-8}-1\times10^{-6}$	–	1×10^{-8}	16
	HMDE	BR pH 11	−0.97	SWAdCSV	$3\times10^{-9}-6\times10^{-7}$	–	3×10^{-9}	16

Abbreviations: AdCSV – adsorptive cathodic stripping voltammetry, BR – Britton-Robinson buffer, CPE – carbon paste electrode, DPAdCSV – differential pulse adsorptive cathodic stripping voltammetry, DPP – differential pulse polarography, DPSV – differential pulse stripping voltammetry, DPV – differential pulse voltammetry, E_p – peak potential, FA – formic acid, GCE – glassy carbon electrode, HMDE – hanging mercury drop electrode, LOD – detection limit, LOQ – quantification limit, LSAdCSV – linear sweep adsorptive cathodic stripping voltammetry, m-AgSAE – meniscus modified silver solid amalgam electrode, MWCNT – multi-walled carbon nanotube, PB – phosphate buffer, SWAdCSV – square-wave adsorptive cathodic stripping voltammetry, SWCSV – square-wave cathodic stripping voltammetry, SWV – square-wave voltammetr