Novel Strategy for Electroanalytical Detection of Antipsychotic Drugs Chlorpromazine and Thioridazine; Possibilities for Simultaneous Determination

B. B. Petković¹,*, D. Kuzmanović², T. Dimitrijević², M. P. Krstić³, D. M. Stanković⁴,⁵

¹ Department of Chemistry, Faculty of Natural Sciences and Mathematics, University of Priština, Lole Ribara 29, 38220 Kosovska Mitrovica, Serbia
² Faculty of Chemistry, University of Belgrade, Studentski trg 12-16, 11000 Belgrade, Serbia
³ Faculty of Veterinary Medicine, University of Belgrade, Bulevar oslobođenja 18, 11000 Belgrade, Serbia
⁴ Innovation center of the Faculty of Chemistry, University of Belgrade, POB 51, 118, 11158 Belgrade, Serbia
⁵ The Vinča Institute of Nuclear Sciences, University of Belgrade, POB 522, 11001 Belgrade, Serbia
*E-mail: bedpet@orion.rs, branka.petkovic@pr.ac.rs

Received: 13 February 2017 / Accepted: 8 March 2017 / Published: 12 April 2017

A simple and fast method for determination of two phenothiazine drugs, chlorpromazine (CPZ) and thioridazine (TDZ), at the boron-doped diamond electrode (BDDE) was proposed. Oxidation peaks on potentials higher than 1 V were used for quantitation of investigated phenothiazines due to better selectivity over common interfering compounds in urine. Differential pulse voltammetry was applied for trace determination of CPZ and TDZ, in Britton-Robinson buffer solution at optimal pH. Under optimized DPV conditions a linear analytical curve was obtained from 1.0×10⁻⁷ to 4.0×10⁻⁵ M with a detection limit of 0.3×10⁻⁷ M (for CPZ) and in the concentration range of 2×10⁻⁷ M to 4.0×10⁻⁵ M, with a detection limit of 1.2×10⁻⁷ M (for TDZ). The applicability of the method was proved by determination of CPZ and TDZ by proposed procedures in spiked urine samples. The electrochemical behavior of those two important tranquilizers in different solvents was described, exploring the possibility of simultaneous determination.

Keywords: voltammetric method, chlorpromazine, thioridazine, boron-doped diamond electrode, human urine

1. INTRODUCTION

Phenothiazine is a prototypical pharmaceutical lead structure in modern medicinal chemistry. Its derivatives are firstly used for malaria, then allergy and predominantly for psychiatry treatment. More
recently, phenothiazine derivatives are under investigation as possible anti-infective drugs [1]. Chlorpromazine (CPZ), (2-chloro-10-(3-dimethylaminoprophyl) phenothiazine), is a phenothiazine derivative with an aliphatic side chain (Scheme 1, a) [2]. The field of its applications is the treatment of schizophrenia, control of excitation, nervousness and other psychomotor disturbances in schizophrenic patients and decrease of the manic phase of manic-depressive conditions. This compound is used as a sedative, to act as an anti-emetic [3]. CPZ is active in the treatment of cancer, viral and bacterial infections and neurodegenerative illnesses [4-6]. An overdose of this drug (>2g per day) commonly results in abnormal pigmentation of eyelids, interpalpebral conjunctiva, and cornea. It may cause tract disorders, accommodation interference, cataract and pigmenatory deposits in the lens and cornea [7-9]. Thioridazine (TDZ), 10-[2-(methyl-2-piper-Idyl)ethyl]-2-methylthiophenothiazine), a piperidine type of antipsychotic agent (Scheme 1, b) [2] is a phenothiazine neuroleptic drug used for the treatment of schizophrenia, other psychiatric disorders, and the short-term treatment of adults with major depression who have varying degrees of associated anxiety. It may be used in the psychiatric treatment of children [10]. A “new use” of TDZ is its capability to cure any form of drug-resistant tuberculosis [11, 12]. The serious side effect of TDZ is the potentially fatal narcoleptics malignant syndrome [13].

Bearing in mind the problems that can cause an overdose of those phenothiazine-based drugs, the different analytical techniques have been reported for their determination. These include flow injection potentiometry [14], spectrophotometry [15, 16], HPLC [17-20], gas chromatography [21, 22], chemiluminescence [23, 24], electrochemiluminescence [25] and spectroelectrochemical method [26]. Voltammetric techniques were also employed in this purpose, using ruthenium electrodes [27], platinum disc electrode [28], at carbon material electrodes: glassy carbon electrode (GCE) [29], graphene paste electrode (GPE) [30], carbon paste electrode (CPE) [31], and at modified electrodes with: nitrogen-doped carbon nanotubes/gold composites [32], multi-walled carbon nanotubes with immobilized cobalt nanoparticles [33] and nickel (II) incorporated aluminophosphate [34]. The most of these methods require several steps, highly evolved instruments, special training, or preparing special chemicals for electrode modification. Hence, it is important to find simple, fast, low cost, but equally sensitive and selective method for determination of CPZ and TDZ.

Boron-doped diamond (BDD), sp³ bonded carbon electrode material, has very useful electrochemical and mechanical properties and because of that, it has been used as an alternative to others electrodes [35]. This electrode has very useful features for its voltammetric use, such as a low and stable background current over the wide potential range (~3.2 V) in aqueous solutions, high stability and hardness and chemical stability [36-38]. BDD also has a good resistance to molecular adsorption and electrode fouling as the diamond surface is relatively non-polar when hydrogen terminated and contains no extended π-electron system [39]. The aim of this work was to find new, simple, selective, sensitive and fast analytical procedures for the determination of chlorpromazine and thioridazine in biological samples by differential pulse voltammetry at unmodified BDDE. Various parameters were optimized, and the influence of most common interferences was investigated. After optimization, proposed methods were successfully applied for the CPZ and TDZ content determination in spiked human urine samples. Additionally, the electrochemical behavior of CPZ and TDZ on BDDE was investigated in non-aqueous media to find the possibility of simultaneous determination of both phenothiazine derivates.
2. EXPERIMENTAL

2.1 Chemicals and reagents

Chlorpromazine hydrochloride (C_{17}H_{19}ClN_{2}S \cdot HCl) and thioridazine hydrochloride (C_{21}H_{26}N_{2}S_{2} \cdot HCl) were purchased from Sigma-Aldrich. The stock solutions (10^{-3} \text{ mol L}^{-1}) were prepared with deionized water (TDZ was dissolved in water-ethanol solution, 1:1 v/v) and stored at +4 \degree C in darkness. Working solutions were daily prepared by appropriate dilution of the stock solutions. Britton-Robinson buffer (BR buffer) as a universal buffer of pH 2–11 was used as a supporting electrolyte in the present study. Acetonitrile (AN), dimethylformamide (DMF) and dimethyl sulphoxide (DMSO), all of the highest purity from J.T.Baker, Netherlands, and high purity water (Millipore, 18 M\Omega cm resistivity) were used as solvents. Tetrabutylammonium hexa-fluorophosphate (for electrochemical analysis), produced by Sigma-Aldrich was used as an electrolyte in non-aqueous solutions. All the other chemicals were of analytical grade and used without further purification.

2.2 Apparatus and electrochemical measurements

Voltammetric measurements were performed using an electrochemical system CH Instruments (USA). A conventional three-electrode system was used with the boron-doped diamond electrode (inner diameter of 3 mm; Windsor Scientific Ltd., Slough, Berkshire, United Kingdom), an Ag/AgCl (saturated KCl) reference electrode and Pt counter electrode. BDDE was anodically pretreated as it is previously reported [40]. The cyclic and the differential pulse voltammograms of standard chlorpromazine and thioridazine solutions were recorded in the potential range from 0.0 to 1.5 V, using a scan rate from 10 to 300 mV/s (for CV) and 20 mV/s (for DPV), a modulation time of 40 ms, a pulse amplitude of 40 mV and pulse time of 0.2 s, for CPZ. For TDZ DPV voltammograms were recorder using pulse amplitude of 50 mV and all other parameters were same as for CPZ. All pH values were measured with pH meter model Jenco Instruments Model No. 6071 (Taiwan). All experiments were obtained at an ambient temperature.
2.3 Preparation of urine samples

The urine sample was acidified by adding 0.1 ml of 5 mol L\(^{-1}\) hydrochloric acid in 10 ml of urine, centrifuged at 4000 rpm for 10 min and the supernatant solutions were taken and stored in the refrigerator. Each 0.1 mL of the urine sample was taken and diluted to 10 mL with optimal Britton-Robinson buffer (at pH 4 for CPZ, and pH 6 for TDZ) and then directly analyzed. Prepared urine samples were spiked with the appropriate amount of standard CPZ/TDZ, and concentration of those phenothiazines and recovery were determined from the calibration curve.

3. RESULTS AND DISCUSSION

3.1 Electrochemical behavior of CPZ and TDZ at BDDE in aqueous buffer

Cyclic voltammetry was used to study electrochemical behavior of CPZ and TDZ at BBDE. BR buffer solution as used as supporting electrolyte, changing the pH values from 2 to 10. It can be seen from Fig.1 that one or two oxidation peaks for both investigated compounds were appeared in a range of 0.7 to 0.9 V and depended on the applied pH. They are originated from phenothiazine core and formation of cation/dication radicals [40-42]. The peak on potential higher than 1V was noticed by Takamura et al. [43] who studding the electrochemical behavior of CPZ at GCE. Amiri et al. suggested electrochemical oxidation mechanism of TDZ on modified CPE electrode which involves electrons and proton transfer [34]. The pH 4 for CPZ and pH 6 for TDZ were selected as optimal pH values for further investigations due to well defined first peak and especially peak on high potential value.

![Cyclic voltammetric profiles of 0.1 mM CPZ (a) and TDZ (b) in different pH BR buffer solutions at BDDE, scan rate of 20 mV/s](image)

**Figure 1.** Cyclic voltammetric profiles of 0.1 mM CPZ (a) and TDZ (b) in different pH BR buffer solutions at BDDE, scan rate of 20 mV/s

Cyclic voltammograms of 0.1 mM CPZ in BR buffer solution at pH 4 on BDDE were recorded at different potential sweep rates and this graph, and the graph of the linear dependence of peak current from the square root of potential scan rate with inserted graph of log I vs log v were shown in Fig. 2. A
linear relationship was observed between log I and log v and the slope of 0.51 was very close to the theoretically expected value of 0.5 for a purely diffusion controlled process [44]. On that way, it was confirmed that the mass transport in diffusion layer during oxidation reaction is controlled by diffusion and the adsorption and/or other specific interactions on self-assembled BDDE electrode surface are negligible. The same effect of scan rate is noticed for TDZ.

Figure 2.  a) Cyclic voltammetric profiles of 0.1 mM CPZ at BDDE immersed in BR buffer solution pH 4, at various scan rates; b) Dependence of the peak current versus function of $v^{1/2}$

3.1.1 Optimization of analytical method and calibration curves

Differential pulse voltammetry (DPV) was selected as a suitable electroanalytical technique for detection of trace amounts of CPZ and TDZ. This technique produced more distinct peaks about the square wave voltammetry. By varying one parameter while others were kept fixed, the optimal modulation time of 40 ms and pulse amplitude of 40 ms were chosen to take into account the width and height of the peaks of quantification (0.6 V and 1.3 V) for CPZ. In the case of TDZ the optimal modulation time and pulse amplitude were at the values of 40 ms and 50 mV, respectively. The peak on 0.6 V is usually used for quantization of phenothiazines [30, 34], while peaks at 1.3 V for CPZ and 1.1 V for TDZ are more selective over interferences due to high potential value.

Differential pulse voltammograms of various concentrations of CPZ from $1.0 \times 10^{-7}$ to $4.5 \times 10^{-5}$ M recorded on BDDE were showed in Fig.3 (a).

The calibration curve was constructed by plotting the current density of anodic peaks at 0.6 and 1.3 V vs. the concentration of CPZ. The resulting calibration plots for both oxidation peaks (Fig. 3, b and c)) are followed by equations: Ox$_{0.6}$ V: $I_p (A) = 2.45 \times 10^{-8} \times C_{CPZ} + 7.53 \times 10^{-8}$ and Ox$_{1.3}$ V: $I_p (A) = 1.99 \times 10^{-8} \times C_{CPZ} + 0.31 \times 10^{-8}$. Detection limit (LOD) of CPZ determination for oxidation peaks on 0.6 and 1.3 V are $0.6 \times 10^{-7}$ M and $0.3 \times 10^{-7}$ M, respectively, based on S/N = 3.

Fig. 4 (a) showed DPVs for different concentrations of TDZ in BR buffered solution at pH 6. A dynamic linear range in the concentration range of $2.0 \times 10^{-7}$ to $4.0 \times 10^{-5}$ M and detection limits for Ox$_{0.6}$V of $1.5 \times 10^{-7}$ M and for Ox$_{1.1}$V of $1.2 \times 10^{-7}$ M were obtained (Fig. 4. b) and c)) The linear
equations are \( I_p (A) = 4.51 \times 10^{-8} \times C_{TDZ} + 2.37 \times 10^{-7} \) (for \( O_{0.6} \)) and \( I_p (A) = 1.30 \times 10^{-8} \times C_{TDZ} + 3.04 \times 10^{-8} \) for \( O_{1.1} \).

![Graph](image)

**Figure 3.** DPV of CPZ added in concentration of \( 1.0 \times 10^{-7} - 4.5 \times 10^{-5} \) M, \( pH = 4 \) (a); calibration curves for \( O_{0.6} \) (b) and \( O_{1.3} \) (c)

The repeatability of proposed methods was evaluated by five replicate DPV measurements under the optimal operating conditions at the \( 15 \times 10^{-6} \) M CPZ and \( 20 \times 10^{-6} \) M TDZ concentration. The relative standard deviation for peak current for \( O_{0.6} \) and \( O_{1.3} \) were: for CPZ- 2.72 \% and 1.64 \%, and for TDZ-1.21\% and 1.07\%.

![Graph](image)

**Figure 4.** DPV of TDZ added in concentration of \( 2.0 \times 10^{-7} - 4.0 \times 10^{-5} \) M, \( pH = 6 \) (a); calibration curves for \( O_{0.6} \) (b) and \( O_{1.3} \) (c)
3.1.2 Interference study and analytical application

To investigate the concomitant effects of compounds usually present in urine samples, DP voltammograms at BDDE were carried out in a BR buffer solution, (pH 4 and 6), containing $5 \times 10^{-6}$ M of CPZ or TDZ in the presence of an equal, triple and fivefold concentration of uric acid (UA), dopamine (DOP) and ascorbic acid (AA). In the present study, no significant influence on the voltammetric response for the CPZ and TDZ was observed for the peaks on higher potential values (Fig. 5). This confirmed the possibility of selective and accurate determination of CPZ and TDZ in human urine samples.

![Figure 5](image)

**Figure 5.** The influence of common urinary compounds on current obtained for determination of CPZ and TDZ under optimized experimental conditions.

The applicability of the voltammetric procedure was tested by determination of low CPZ and TDZ concentrations in spiked urine samples. As can be seen from the Table 1, the recovery varied from 94.0 to 101.0 % for the lowest recorded concentrations of CPZ in the linear range, and from 95.5 to 106.5 % for concentrations of TDZ. By these results, it can be stated that the proposed voltammetric procedures using an unmodified BDDE represent a fast, accurate and reproducible tool for giving information about studied phenothiazines in human urine samples.

| Table 1. Determination results of chlorpromazine and thioridazine in human urine samples |
|---------------------------------|---------------------------------|----------------|----------------|
| **Spiked** $(\times 10^{-7}$ M CPZ) | **Found** $(\times 10^{-7}$ M CPZ) | **Recovery (%)** | **RSD (%)**, $n = 3$ |
| 1 | 0.94 | 94.0 | 7.2 |
| 2 | 2.02 | 101.0 | 5.8 |
| 3 | 2.95 | 98.3 | 5.6 |
| **Spiked** $(\times 10^{-7}$ M TDZ) | **Found** $(\times 10^{-7}$ M TDZ) | **Recovery (%)** | **RSD (%)** |
| 2 | 2.13 | 106.5 | 8.1 |
| 4 | 3.82 | 95.5 | 6.5 |
| 5 | 5.21 | 104.2 | 5.8 |
3.1.3 Comparison with other electrochemical methods

A comparison of the analytical performance of the proposed method with other reported voltammetric methods for determination of CPZ and TDZ is given in Table 2. As it can be seen, modified and unmodified electrodes were used in this purpose. Application of carbon paste electrode modified with cobalt nanoparticles led to the most sensitive determination of CPZ among all previously reported (LOD was 0.6 nM) [31]. Graphene paste electrode was found to be highly sensitive unmodified material for determination of CPZ and LOD for this method was 6 nM. Application of different modifiers on GCE did not lead to a significant decrease of the detection limit [32, 33, 34]. Bearing in mind the time-consuming preparation of modifiers for sensors, advantage of self-assembled BDDE is evident. Proposed method is quite comparable with other methods (modified GCE) in sensor characteristics and sometimes it is even better regarding concentration range or limit of detection [32, 34]. This simple, fast and inexpensive analytical procedure with unmodified BDDE could be a useful alternative to modified sensors and conventional analytical methods for determination of those prominent phenothiazine drugs.

Table 2. Comparison of proposed method with previously reported voltammetric methods for determination of CPZ and TDZ.

<table>
<thead>
<tr>
<th>Working electrode</th>
<th>Modifier</th>
<th>Analyte of interest for this work</th>
<th>Supporting electrolyte</th>
<th>Technique</th>
<th>LCR (μM)</th>
<th>LOD (μM)</th>
<th>Sample</th>
<th>Ref.</th>
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<td>GCE</td>
<td>-</td>
<td>CPZ</td>
<td>BR buffer pH 9</td>
<td>DPSV</td>
<td>50-1200</td>
<td>-</td>
<td>Synthetic mixtures and blood</td>
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<tr>
<td>GCE</td>
<td>-</td>
<td>CPZ</td>
<td>0.03 M HClO₄ in AN</td>
<td>DPV</td>
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<td>0.4</td>
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<tr>
<td>Ru</td>
<td>-</td>
<td>CPZ</td>
<td>H₂SO₄</td>
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<td>200-800</td>
<td>-</td>
<td>Tablets</td>
<td>29</td>
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<tr>
<td>GPE</td>
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<td>CPZ</td>
<td>Phosphate buffer, pH 4</td>
<td>DPV</td>
<td>0.01-9</td>
<td>0.006</td>
<td>Tablets</td>
<td>30</td>
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<tr>
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<td>Cobalt nanoparticles</td>
<td>CPZ</td>
<td>Phosphate buffer, pH 4</td>
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<td>31</td>
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<td>GCE</td>
<td>Nitrogen-doped carbon nanotubes/gold composites</td>
<td>TDZ</td>
<td>PBS buffer, pH 6</td>
<td>DPV</td>
<td>12-850</td>
<td>1.3</td>
<td>-</td>
<td>32</td>
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<tr>
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<td>DPV</td>
<td>50-100</td>
<td>0.05</td>
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<td>33</td>
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<tr>
<td>CPE</td>
<td>Nickel (II) incorporated alumino-phosphate</td>
<td>TDZ</td>
<td>Phosphate buffer, pH 6</td>
<td>DPV</td>
<td>0.1-10</td>
<td>0.09</td>
<td>Tablets and human serum</td>
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<td>CPZ TDZ</td>
<td>BR buffer pH 4</td>
<td>DPV</td>
<td>0.1-40</td>
<td>0.03</td>
<td>Urine</td>
<td>This work</td>
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3.2 Electrochemical simultaneous determination of CPZ and TDZ at BDDE

Because of the phenothiazine-based drugs frequent use and strong sedative effect, it is important to have a fast and reliable method to determine the amount of these drugs in body fluids, to determine the possible misuse and overuse. Phenothiazines are not usually used together in therapeutic purpose, but in the case of abuse and for forensic analysis, it is useful to have information about the possibility of their simultaneous electrochemical determination. It is noticed that electrochemical behavior of phenothiazines strongly depends on the used solvent.

As phenothiazine determination is usually done in aqueous solution, a significant difference between the peaks at higher potentials for TDZ and CPZ leaved open the possibility for their simultaneous determination. However, in the mixed CPZ and TDZ solution (10⁻⁵M) in BR buffer at selected pH values (4 and 6), reduction of peak current, displacement and overlapping of peaks was noticed (Fig.6). The aqueous medium was not suitable to electrochemically separate these two antipsychotic drugs.

Figure 6. DPV profiles of CPZ and TDZ mixture in concentrations of 10⁻⁵M in BR buffer solution at pH 4 and 6.

Electrochemical behavior of a mixture of CPZ and TDZ (prepared by adding 1×10⁻⁶M to 5×10⁻⁶M one compound to the other, and opposite) in non-aqueous solutions was examined in acetonitrile, dimethylformamide, and dimethyl sulphoxide. The differential pulse voltammograms on Fig. 7 showed that simultaneous determination of CPZ and TDZ at BDDE was possible in acetonitrile, similarly as Zimova at al. recorded on GCE [28], but it is pronounced in dimethyl sulphoxide, by measuring the peak current in the range of 0.6 to 0.8V. DMF was not a convenient solvent for separation of peaks. In AN, contrary to other used non-aqueous solvents, the peak of high potential was noticed. This peak can not be a quantification peak from the same reasons as in an aqueous medium. The forego peak for CPZ which occurred in aqueous media and DMF also could be used for determination of CPZ.
concentrations in DMSO. In DMSO there was a linear relationship between peak current (at 0.7 V) and concentration of TDZ (\(I_p (A) = 5.95 \times 10^{-2} \times C_{TDZ} + 8.79 \times 10^{-8}\), with correlation coefficient \(R = 0.981\)) and also for peak current (at 0.8 V) and concentration of CPZ (\(I_p (A) = 4.02 \times 10^{-2} \times C_{CPZ} + 7.52 \times 10^{-8}\), \(R = 0.983\)).

**Figure 7.** DPV profiles of CPZ and TDZ mixture (in concentrations \(1 \times 10^{-6} \text{M to } 5 \times 10^{-6} \text{M}\)) in acetonitrile, dimethylformamide and dimethyl sulphoxide.

### 4. CONCLUSION

The present study is related to the application of unmodified BDDE for determination of prominent phenothiazine derivates, chlorpromazine and thioridazine in human urine samples. The proposed sensor exhibited the advantage of simple preparation, good reproducibility, and electrochemical surface stability. As BDDE is, up to date, one of the best solid electrode materials, this investigation provides valuable information about the possibility of a selective determination of CPZ and TDZ over interfering compounds from biological samples. Moreover, the possibility of simultaneous determination of CPZ and TDZ in the non-aqueous solution was examined, giving the advantage to determining in DMSO. The developed procedures at BDDE have the potential for use in fast analyzing of biological fluids and pharmaceutical formulations.

### ACKNOWLEDGEMENT

Financial support for this study was granted by the Ministry of Science and Technological Development of the Republic of Serbia, Project Number III45022, OI172030 and Magbiovin project (FP7-ERAChairs-Pilot Call-2013, Grantagreement: 621375).
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