**Determination of tramadol in pharmaceutical forms and urine samples using a boron-doped diamond electrode**

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*Abstract:* The present work describes the electroanalytical investigation and a novel voltammetric method for a cheap, fast and simple quantification of tramadol (TRH) using a boron-doped diamond (BDD) electrode. TRH displayed one well-defined, irreversible and adsorption-controlled oxidation peak at about +1.58 V (vs. Ag/AgCl) in Britton-Robinson buffer (BR, 0.1 mol L-1, pH 3.0) using cyclic voltammetry (CV) technique. The voltammetric responses of the oxidation peaks are dependent on pH and its sensitivity was significantly enhanced in the existence of surfactant media (sodium dodecyl sulfate, SDS). In optimized experiment conditions, employing square-wave stripping mode, it was found that there was an excellent correlation between oxidation peak current and TRH concentration in the range of 0.25 to 50.0 μg mL-1 (8.34×10-7-1.67×10-4 mol L-1), with a detection limit of 0.072 μg mL-1 (2.40×10-7 mol L-1) in 0.1 mol L-1 BR buffer (pH 3.0) solution comprising 8×10-4 mol L-1 SDS at +1.52 V (after 30 s accumulation at open-circuit condition). The developed approach can be used for the quantification of TRH in the pharmaceutical formulations and the spiked human urine samples with acceptable recoveries.

*Keywords:* tramadol; boron-doped diamond electrode; pharmaceutical formulation; urine samples; sodium dodecyl sulfate.

RUNNING TITLE: DETERMINATION OF TRAMADOL AT BDD ELECTRODE

INTRODUCTION

The main goal of pain management is to reduce trauma and to ameliorate the quality of life of the patient. Opioid class narcotics are of the class of drugs commonly used for increasing patient comfort. Tramadol, (IUPAC name: (1R,2R)-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl) cyclohexan-1-ol hydrochloride, here abbreviated as TRH (Fig.1), is one of the atypical opioid class drugs that serving for this purpose1. TRH is also a synthetic analogue of opioid class codeine that especially applied in the medication of chronic and acute pains as centrally acting analgesic2. Although the pain relieving dose varies depending on the response of the patient and the intensity of the pain, the therapeutic concentration of TRH was reported to be 100-300 ng L-1 level in the plasma3. It relieves pain with a double-acting action mechanism both by acting as an opioid receptor and by inhibiting the reuptake of serotonin and norepinephrine4. This opioid can also be used in combination with other analgesics and antipyretics in the treatment of advanced cancer. TRH is immediately absorbed following oral administration and reaches a bioavailability level of 65-70% after the first pass metabolism5. Approximately 30% of the parent drug is excreted through the urine without any change in the body and the rest 70% through the kidney converted into metabolites6. Therefore, its determination in biological samples such as blood, urine, and serum are great importance because the results of analyses provide information about long-term abuse and can also be used for forensic purposes2–6.



**Figure 1.** Molecular structure of tramadol

To date, a number of studies have been performed including high performance liquid chromatography7,8, spectrophotometry9, electrochemiluminescence10, gas chromatography11 and electrochemical methods12–14 for quantitative analysis of TRH from drugs, biological fluids, environmental water samples. Electrochemical methods, one of these methods, provide not only benefits such as speed, precision, selectivity and cost compliance for chemical analysis, but also provide useful information about the oxido-reduction mechanism of the corresponding electroactive species. Therefore, it is one of the most frequently used methods in the analysis of electroactive compounds in drugs, environmental samples and foods15.

Electrochemical studies developed for TRH analysis from different samples are presented in Table 1. Most of these studies were carried out either attaching specific chemicals the glassy carbon electrode or by filling different composite materials to the carbon paste electrode. After these processes, some modified electrodes reached very satisfactory detection limit levels.

Boron doped diamond (BDD) electrodes are the prestigious carbon material that gains a new horizon to electrochemical analysis and it enables the analysis of many electroactive species that can not be performed by traditional solid electrodes. The electrode material to be used in electrochemical analyzes is expected to produce a stable electrode response, have a large potential window, enable to work in even the most aggressive environments, respond quickly, have low baseline current, and be economical. BDD is one of the outstanding electrode materials that fulfill all of these expectations16. As a result of these properties, the BDD electrode has increased both the extent and quantity of electrochemical analyses17.

The surfactants increase the solubility of the electroactive species by forming a micelle structure in solution and allow the electroactive species to adsorb to the electrode surface more easily. As a result, the quantitative analysis of the related compound can be achieved with better sensitivity18. To the best of our knowledge, any study on the electrochemical behavior and quantitative analysis of TRH using unmodified BDD electrodes has not been found so far except for the two papers which perform simultaneous electroanalytical determination of acetaminophen and TRH using a BDD electrode19,20. The objective of this work was to examine the electrochemical properties of TRH on unmodified BDD substrate in the existence of sodium dodecyl sulfate (anionic surfactant, SDS) and then was to develop a fast, simple and environment-friendly voltammetric technique for the quantification of TRH by unmodified BDD in connection with square-wave voltammetric (SWV) technique. Based on the results obtained, the practical applicability of the developed technique was demonstrated in commercial pharmaceuticals containing TRH and the spiked urine samples.

EXPERIMENTAL

*Chemicals and solutions*

The reference standard of TRH (Reagent Plus®, ≥99%) was purchased from Sigma-Aldrich and used without further purification. The purified water from a Millipore Milli-Q system (Millipore, resistivity ≥ 18.2 MΩ cm) and analytical-grade reagents were used for the preparation of Britton-Robinson buffer (BR, 0.1 mol L-1, pH 2-8), phosphate buffer (0.1 mol L-1, pH 2.5 and 7.4), acetate buffer (0.1 mol L-1, pH 4.7), HClO4 (0.1 mol L-1) and HNO3 (0.1 mol L-1) solutions. Stock solution (1.0 mg mL-1) of TRH was prepared in water. It was stored in a refrigerator at +4 oC when not in use and protected from exposure to direct daylight during use in the laboratory. The solutions of TRH used in calibration studies and sample analysis were prepared by diluting the stock solution to the appropriate volume with the supporting electrolyte.

*Apparatus and measurements*

The electrochemical analysis was fulfilled with a µAutolab type III potentiostat/galvanostat (Metrohm Autolab B.V., the Netherlands), which was managed by GPES 4.9 software. The raw signals of square wave voltammograms generated by the electrochemical instrument were recorded after the correction processing by moving average method (0.01 V peak widths) and the smoothing processing by Savicky and Golay algorithm in this software. All voltammetric experiments were conducted in three electrodes system in a glass electrochemical cell (volume of 10 mL) maintained at ambient temperature. A platinum wire and an Ag/AgCl (3 mol L-1 NaCl, Model RE-1, BAS, USA) were used as the counter and reference electrodes, respectively. The BDD electrode was obtained from Windsor Scientific Ltd., (UK). The BDD film electrode (boron content 1000 ppm), with poly-crystalline structure deposited on a polyether ether ketone tube with a 0.5 mm thickness and diameter of 3 mm declared by the provider was employed as working electrode. At the beginning of every experiment day, an anodic potential of +1.8 V for 180 s followed by a cathodic potential of -1.8 V for 180 s was applied to the BDD electrode in order to form oxygen- and hydrogen-terminated on its surface in 0.5 M H2SO4. Before each voltammetric experiment, electrode was softly rubbed with a polishing pad (which takes less than 1 min) and then rinsed with deionized water21. A pH meter model WTW inoLab720 equipped with a combined glass electrode was used to measure all pH values.

Cyclic voltammetry (CV) method was firstly used in order to enlighten the electrochemical behavior of TRH on bare BDD electrode for preliminary studies followed by square wave adsorptive stripping voltammetry (SW-AdSV) method for testing analytical performance and practicability of the method.

The employed procedure for SW-AdSV analysis of TRH was as follows: The formerly treated BDD electrode was immersed in a stirred (at 500 rpm) sample solution for a certain time period, at a chosen accumulation potential in order to accomplish TRH pre-concentration. After a rest period of 5 s, anodic scans were implemented in the range of +0.4 to +1.8 V using the SW waveform to settle the solution and decrease the background current.

Prior to analytical applications, the best device signals were obtained at 50 Hz frequency, 50 mV pulse amplitude, and 14 mV step potential values among SWV variables. Consecutive measurements were performed by applying the above procedure to the working electrode recursively. All measurements were performed at room temperature (25 ± 50C) and in triplicate.

*Preparation of samples*

TRH injection solution (Tradolex®, Mentapharma Co., Turkey) containing 50 mg TRH per mL-1 was used for drug sample analysis. 2 mL of this injection solution was transferred to a calibrated amber glass flask and the volume was made up to 1 L with deionized water. The known amounts of this solution were smoothly added to a 10 mL volume electrochemical cell containing 8x10-4 mol L-1 SDS and 0.1 mol L-1 BR buffer solutions (pH 3.0). Then, the unknown sample analysis was calculated by the corresponding regression equation in the calibration graph obtained for the standard TRH solutions.

 Drug-free human urine samples were obtained from a healthy 19-year-old male donor before the day of the experiment. After adding 9 mL of urine sample to 1 mL of TRH stock solution (1 mg mL-1) in a test tube, the resulting mixture was vortexed for one minute. The appropriate volume of this mixture was then transferred into the voltammetric cell containing the selected supporting electrolyte.

RESULTS AND DISCUSSION

*Cyclic voltammetric behavior of TRH on the BDD electrode*

The electrochemical behavior of the TRH was firstly tried CV technique in the 0.1 mol L-1 BR buffer solutions (pH 3.0) within the potential range from +0.8 V to 1.8 V at scan rate of 100 mV s-1 without accumulation step. In addition, the CV of the solution containing only the supporting electrolyte (without TRH) was recorded for comparison purposes (Figure 2A, dashed line). As can be seen from Figure 2A, a well-shaped oxidation peak was observed at approximately +1.58 V in the first cycle, whereas no reduction peak was viewed in the reverse scan. Consequently, it can be concluded that the oxidation reaction of TRH on the BDD electrode is irreversible22. Decrease in anodic peak current in the 2nd and 3rd scans of consecutive CVs can be interpreted as the adsorption of TRH on the BDD electrode and/or the oxidation products occur on the BDD electrode. To clarify this situation, the effect of scan rate on the current response of 100 μg mL-1TRH was explored by CV in BR buffer pH 3.0 using the BDD electrode (Fig. 2B). There was a slight shift of the oxidation peak potentials of TRH towards more positive values as the scan rate was increased. The linear relation between the oxidation peak current (*Ip*) and scan rate (ν) was obtained in the range of 50-500 mV s-1 (n=7). The equation is noted below;



In addition the linearities of plots of log *i*p versus log *v* are expressed as follows:



 These results strongly indicated that the TRH oxidation reaction is under adsorption control process at the BDD electrode.



**Figure 2.** The repetitive cyclic voltammograms at scan rate of 100 mV s-1 (A), and the cyclic voltammograms at different scan rates (50, 100, 200, 300, 400 and 500 mV s-1) (B) of 100 μg mL-1 TRH in BR buffer solution (pH 3.0). A; Dashed lines represent background current. B; Inset depicts the plot of peak current vs. scan rate (*ν*).

*Effect of supporting electrolyte and pH*

 The influence of the pH on the oxidation peak current response of TRH was investigated by SW-AdSV on the BDD electrode using the different supporting electrolyte at various pH values in order to obtain the best voltammetric response for analytical purposes. In Fig. 3A, the baseline corrected SW-AdSV voltammograms are depicted within the pH range 2.0-8.0 in BR buffer by performing SW-AdSV measurement on 20 μg mL-1 TRH solution, with an open-circuit accumulation at 30 s, with the potential window from +0.4 V to +1.8 V. As can be seen in this figure, SW-AdS voltammograms recorded at the BDD exhibit one oxidation peak in the working potential range except of pH 8.0. At pH 8.0, a small additional anodic peak was noticed at about +1.71 V. The shift of the oxidation peak potential of TRH to low values while increasing from pH 2.0 to 8.0 is a clear indication that the oxidation process of TRH on the BDD electrode is accompanied by a protonation reaction. The dependences of peak potential, *Ep*, of TRH on pH were investigated in the ranges pH 2.0-8.0. It was found to be linear in the pH range of 2.0-8.0 and can be described by the below equation:



The slope was found to be 0.0215 V per pH unit which indicated that the numbers of electron and proton taking part in the electrode reaction are unequal23.

 The SW-AdS voltammograms of TRH in various supporting electrolytes are shown in Figure 3B. Using 0.1 mol L-1 HClO4, HNO3, PBS pH 2.5, ABS pH 4.7 and PBS pH 7.4, oxidation peak potentials of +1.57 (5.41 µA), 1.57 (4.47 µA), 1.57 (4.19 µA), 1.52 (4.77 µA) and 1.45 V (3.38 µA) were obtained, respectively. Meanwhile, in case of phosphate buffer at pH 7.4 one more oxidation peak potential was detected at +1.69 V (0.98 μA).



**Figure 3.** The stripping voltammograms of 20 μg mL-1 TRH in BR buffer solution pH 2.0-8.0 (A), and in various supporting electrolytes (B). Inset of (A) depicts the plot of *Ep* vs. pH. Electrode, BDD; accumulation time 30 s at open-circuit condition. SWV parameters: frequency, 50 Hz; step potential, 8 mV; pulse amplitude, 30 mV.

 As can be seen from Figures 3A and B, the maximum and best-shaped signal of 20 μg mL-1 TRH on BDD electrode was obtained at BR buffer pH 3.0. Thus, 0.1 mol L-1 BR buffer (pH 3.0) was used for further studies.

*Effect of accumulation time and accumulation potential*

 Considering the apparent adsorptive character of TRH on the BDD electrode, it can be predicted that the accumulation time and potential have an effect on the oxidation signal of TRH. To verify this prediction, the effects of accumulation time (*tacc*) and accumulation potential (*Eacc*) effects (data not shown) were investigated under optimized experimental circumstance for 10 μg mL-1 TRH. The effect of *tacc* on the oxidation signal of TRH was examined in the range of 0-240 s by applying open circuit potential to the electrochemical cell. No significant increase at oxidation peak was observed in accumulation times applied for more than 30 s. For this reason, it was chosen as the shortest time for optimum *tacc* and doubtlessly practical use of the electrode. Although the usage of accumulation slightly increased oxidation peak currents (*tacc*= 30 s, *Ip*=2.18 µA), compared to SW voltammograms obtained without accumulation (*tacc*= 0 s, *Ip*=1.37 µA), its use contributed to increased sensitivity and regeneration of the diffusion layer between measurements. The dependence of the stripping peak current on the *Eacc*, was evaluated either at open-circuit condition or over the potential range +0.1 to +1.2 V. No important effects were determined under the studied potential values. Thus, *tacc* of 30 s and *Eacc* of open-circuit accumulation were found reasonable, respectively, for the rest of present analytical investigation.

*Effect of SWV parameters*

 The parameters individually analyzed in SWV were frequency (*f*), pulse amplitude (*ΔEsw*) and step potential (*ΔEs*) in order to obtain the highest value of the oxidation peak current, the maximum selectivity and the improved reproducibility. While one parameter was varied all others were kept fixed. The f value was evaluated in a range from 25 to 125 Hz (with the *ΔEs* and *ΔEsw* fixed at 8 mV and 30 mV, respectively). The higher the *f* value, the higher the oxidation peak current was obtained. However, for values larger than 50 Hz a considerable widening in the peak width was obtained. Evaluation of this phenomenon in SW voltammetric responses represents a loss in analytical selectivity. Thus, *f*= 50 Hz was chosen for all subsequent experiments. The influence of the *ΔEsw* (remaining parameters: *ΔEs*= 8 mV, *f* = 50 Hz) on the oxidation peak current intensity was also examined in the range from 30 to 70 mV. A linear increase was observed between the oxidation peak current values and the *ΔEsw* in the investigated range. However, at higher values of 50 mV, an increase in *ΔEsw* resulted in a considerable widening in the SW voltammograms were seen. The *ΔEs* was varied between 6-16 mV with a fixed parameters of *f* = 50 Hz and *ΔEsw*= 50 mV. The recorded voltammetric signal increased gradually until the value of 14 mV, after which it slightly increased. This effect was also accompanied by peak broadening. Thus, *ΔEs*=14 mV was chosen. For further SW-AdS voltammetric measurements the optimal values were: *f*, 50 Hz; *ΔEsw*, 50 mV and *ΔEs*, 14 mV.

*Effect of anionic surfactant*

 Finally, to improve the sensitivity of the electrochemical process, the influence of anionic (negatively charged) surfactant, SDS was also evaluated on the oxidation signals of TRH. This effect was examined by keeping the TRH concentration constant at 7.5 µg mL−1 in the electrochemical cell containing BR buffer (pH 3.0) and changing of SDS concentrations in the range from 1×10-4 mol L-1 to 1×10-3 mol L-1. When compared the voltammetric behavior of TRH in the absence and presence of SDS (Figure 4), a slight negatively shift was observed in the peak potentials of the electrolyte solution containing SDS, but the increase in SDS concentration did not change the position of it. On the other hand, an important signal enhancement was observed in the cooperation of SDS. The stripping peak currents increased with SDS concentration up to 8×10-4 mol L-1. Above this concentration, a very small change was remarked (Fig. 4 inset). Therefore, the concentration of SDS at 8×10-4 mol L-1 was chosen for the rest of present analytical investigation. In this case, TRH signals increased approximately 2.5 times higher compared to the solution without surfactant.

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**Figure 4.**The stripping voltammograms of 7.5 µg mL-1 TRH in 0.1 mol L-1 BR buffer solution (pH 3.0) in the presence of different SDS concentrations (1.0×10−4–1.0×10−3 mol L-1). Dashed line represents the voltammogram without SDS. Inset: plot of *Ip* vs. CSDS. Electrode, BDD; accumulation time 30 s at open-circuit condition. SWV parameters: frequency, 50 Hz; step potential, 14 mV; pulse amplitude, 50 mV.

*Analytical performance evaluation*

After optimization of working conditions (instrumental parameters and chemical conditions), the analytical performance was evaluated by examining the oxidation peak current as a function of concentrations of TRH. Construction of the analytical curve was obtained for TRH on the BDD electrode. In this context, known amounts of TRH stock solution were added sequentially to the voltammetric cell and the current responses obtained from SW-AdS for each addition were evaluated. The SW-AdS voltammograms were recorded by additions of TRH over the 0.25 to 50.0 μg mL-1 (8.34×10-7 mol L-1 - 1.67×10-4 mol L-1) concentration range and the respective analytical curve is shown in Fig. 5, inset.



**Figure 5.** The stripping voltammograms for TRH levels of (1) 0.25, (2) 0.50, (3) 1.0, (4) 2.5, (5) 5.0, (6) 7.5, (7) 10, (8) 20, (9) 30, (10) 40 and (11) 50 µg mL-1 in 0.1 mol L-1 BR buffer solution (pH 3.0) in the presence of 8×10-4 mol L-1 SDS. Inset depicts a corresponding calibration plot for the quantitation of TRH. Other operating conditions as indicated in Fig. 4.

 A highly linear calibration graph was obtained by plotting oxidation peak currents against TRH concentrations in the specified range expressed by the equation 4;

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In the equation, *Ip* represents the oxidation peak current, *C* TRH concentration, *r* correlation coefficient and *n* the number of experiments. From the obtained value by the analytical curve, limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.072 μg mL-1 (2.40×10-7 mol L-1) and 0.24 μg mL-1 (8.01×10-7 mol L-1), respectively. LOD and LOQ were calculated as three and ten times the standard deviation of the lowest concentration (in the linearity range) divided by the slope of the calibration curve, respectively.

 Table 1 depicts a comparison of BDD electrode analytical performance created in this study with the other electroanalytical studies in earlier published report. As understood from this table, BDD electrode presented a more sensitive electroanalytical response than modified carbon paste electrode (M-CPE)12, coated wire electrode (CWE)24, carbon nanoparticles glassy carbon electrode (CNPs/GCE)25 and multiwalled carbon nanotube glassy carbon (MWCNT/GCE)26 electrodes. However, some electroanalytical studies (1,3,13,14,27–30) reported in the literature shown higher sensitivity than the developed method. But, it should be pointed out that in all reported works use chemically modified electrodes (based on glassy carbon and carbon paste electrodes). Despite the higher sensitivity of chemically modified electrodes have some drawbacks, such as their poor reproducibility, high costs and longtime preparation. In this work, using of the BDD electrode without any modiﬁcation presented good performance including suﬃcient sensitivity, simplicity, rapidity and low cost for TRH quantification in pharmaceutical form and urine samples.

 The precision of the developed method was assessed in terms of the intra- and inter-day repeatability under the optimum experimental conditions. The intra-day repeatability of the magnitude of oxidation peak current was determined by ten times repeated measurements of 0.25 µg mL-1 for TRH solution. The results depicted that a relative standard deviation (RSD) of 4.51% demonstrating that the results are repeatable. Then, inter-day repeatability was assessed by measuring the magnitude of oxidation peak current response of the BDD electrode for five consecutive working days for the same level of TRH concentration and the RSD was calculated to be 5.33%. Bearing in the mind the obtained precision values, it suggests that the BDD electrode has proven to be suitable electrochemical sensor for the repeatable quantification of TRH in the pharmaceutical formulation.

**Table 1.**Comparison of the efficiency of the BDD electrode with literature electrodes for TRH determination

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Analyte** | **Electrode** | **Linearity Range** **(mol L-1)** | **LOD****(mol L-1)** | **Sample** | **Ref.** |
| TRH | PdNPs/GCE | 5.0×10-8 - 2.0×10-4 | 1.5×10-8 | Drug, plasma, urine | 1 |
| TRH + ACP | La+3CuO/MWCNT/GCE | 5.0×10-7 - 9.0×10-4 | 1.4×10-8 | Drug, urine | 3 |
| TRH | Modified CPE | 9.2×10-6 - 1.0×10-1 | 6.2×10-6 | Drug, urine, milk | 12 |
| TRH | MIP/MWCNT/CPE | 1.0×10-8 - 2.0×10-5 | 4.0×10-9 | Drug, urine | 13 |
| TRH+ACP+CAF | PNB/GCE | 2.0×10-7 - 1.6×10-5 | 8.0×10-8 | Drug | 14 |
| TRH | CWE | 1.0×10-5 - 1.0×10-1 | 1.2×10-6 | Drug | 24 |
| TRH+ACP | CNPs/GCE | 1.0×10-5 - 1.0×10-3 | 1.0×10-6 | Drug, plasma | 25 |
| TRH +ACP | MWCNT/GCE | 2.0×10-6 -3.0×10-4 | 3.6×10-7 | Drug, urine, serum | 26 |
| TRH+ACP | D50wx2/GNP/GCE | 3.3×10-8 - 4.2×10-5 | 1.1×10-8 | Drug, blood serum | 27 |
| TRH+ACP | NiFe2O4NPs/GR/CPE | 1.0×10-8 - 9.0×10-6 | 3.6×10-9 | Drug, blood serum | 28 |
| TRH | P@SG/MIP/fMWCNT/GCE | 2.0×10-10 -2.0×10-9 | 3.0×10-11 | Drug, urine | 29 |
| TRH | Sb2O3NPs/MWCNTs/GCE | 4.0×10-8 -3.0×10-5 | 9.5×10-9 | Drug, blood serum | 30 |
| TRH | BDD | 8.3×10-7 - 1.7×10-4 | 2.4×10-7 | This work |  |

**Analyte:** TRH, tramadol hydrochloride; ACP, acetaminophen; CAF,caffeine **Electrode:** CVE, coated wire electrode, La+3CuO/MWCNT/GCE, lanthanum doped CuO multiwalled carbon nanotube glassy carbon electrode ; D50wx2/GNP/GCE , dowex50wx2 gold nanoparticle glassy carbon electrode; NiFe2O4NPs/GR/CPE , NiFe2O4 nanoparticles graphen carbon paste electrode; PdNPs/GCE Pd nanoparticles glassy carbon electrode; CNPs/GCE carbon nanoparticles glassy carbon electrode; Nafion®/CTAB-Au/GCE ,nafion cetyl trimethylammonium bromide Au nanoparticles glassy carbon electrode; PNB/GCE , poly(nile blue) glassy carbon electrode; MIP/MWCNT/CPE, molecularly imprinted polymer multiwalled carbon nanotube carbon paste electrode; P@SG/MIP/fMWCNT/GCE polypyrol sol-gel molecularly imprinted polymer carboxylic acid functionalized multiwalled carbon nanotube glassy carbon electrode; MWCNT/GCE multiwalled carbon nanotube glassy carbon electrode; Sb2O3NPs/MWCNTs/GCE, antimony oxide nanoparticles/ multiwalled carbon nanotubes/ glassy carbon electrode.

*Effect of interfering compounds*

 Prior the analyses of the real samples, the influence of potentially interfering compounds, mostly present in the pharmaceutical formulation or biological samples were examined by SW-AdSV for 2.5 µg mL-1 TRH under the same experimental conditions. The tolerance limit was defined as the maximum concentration of the selected interfering compounds, which caused an approximately ±10% relative error for the oxidation peak current of TRH. It was found that inorganic ions such as sodium (Na+), potassium (K+), calcium (Ca2+), magnesium (Mg2+), zinc (Zn2+), iron (Fe3+), titanium (Ti4+), nitrate (NO3-), chloride (Cl-) and sulfate (SO42-) do not affect the oxidation signal even at 100 times the concentration of TRH. With regards to carbohydrate compounds such as glucose, fructose, sucrose and lactose insignificant effect on TRH oxidation peaks were recorded in their 100-fold excess. The agents present in the pharmaceutical formulations, such as cornstarch, magnesium stearate, microcrystalline cellulose on the oxidation current responses of TRH was found to be also negligible. The effect of ascorbic acid (AA), dopamine (DOP) (Fig. 6A) and uric acid (UA) (Fig. 6B), which could be present in biological fluids, were evaluated in a molar concentration at the ratio (TRH solution: interfering agent) of 1:1, 1:10 and 1:50.



**Figure 6.** The stripping voltammograms of TRH (2.5 µg mL-1) mixture in the presence of (A) equimolar concentration, 10-fold and 50 fold excess DOP and (B) equimolar concentration, 10-fold and 50 fold excess UA. Other operating conditions as indicated in Fig. 4.

Obviously, the oxidation peak current of TRH not affected the oxidation peak currents of individual solutions of AA, DOP and UA in the working concentrations. Also paracetamol (100-fold excess), coexisting of TRH in some pharmaceutical formulations, did not affected the oxidation peak current of TRH. This results show that the developed approach can be successfully applied to the real samples.

 *Analytical application*

 Based on obtained results, in the finally step the pharmaceutical and urine samples were used for the quantification of TRH as the examples of the applicability of the developed method. Firstly, the practical applicability of the BDD electrode for SW-AdSV quantification of TRH was verified by analysis in the pharmaceutical formulation (injectable solution). The injectable solution was diluted with water to obtain the required concentration for assay. The analysis of the sample was undertaken using the calibration curve method from the related regression equation. The TRH content of the injectable solution which was declared by the producer, was 50 mg mL-1, the TRH content was found to be 48.80 mg mL-1 (RSD of 3.86%) by the developed method. The validity of the developed approach was also assessed by applying the recovery experiments. Recovery studies were carried out adding standard TRH solutions (0.25, 1.0 and 2.5 µg mL-1) prepared in the supporting electrolyte to 10 mL of sample solution in voltammetric cell and the SW-AdSV responses were assessed. The acceptable recoveries were obtained in the ranged from 97.15 to 104.62%, demonstrating that the BDD electrode response is not influenced by the sample matrix. Secondly, the satisfactory sensitivity and good selectivity of the proposed approach was also used for the quantification of TRH in the human urine samples with more complex matrices in comparison with pharmaceutical forms. The preparation of the samples is described in detail in Section 2.3. The sample does not contain TRH, so it was artificially spiked. Quantification was performed by means of the standard addition method for urine sample spiked with TRH (2.5 µg mL-1). Obtained results are summarized in Table 2. So, from these obtained results and recovery values, it can be concluded that developed approach is suitable for quantification of TRH in the urine samples. The related voltammograms of the urine sample by standard addition method are depicted in Fig. 7A. It can be concluded that an appeared oxidation peak at about +1.55 V is due to the TRH oxidation since its peak current increased after each TRH standard addition. In the absence of TRH, there were no detectable oxidation peaks in the working potential range where the analytical peak observed (Fig. 7B). On the other hand, an unknown oxidation peak at about +1.00 V was observed in blank urine samples, which could be due to the oxidation of uric acid (UA)31,32. After several standard additions of UA, it was observed an increasing on this oxidation peak. Since its peak potential was well differentiated of TRH, it did not interfere with its quantification.

**Table 2.** Measurement results for addition and recovery of TRH from urine sample using proposed method.

|  |  |  |
| --- | --- | --- |
| Added (μg mL-1) | Found[a] (μg mL-1) | Recovery (%) ± RSD (%) |
| 2.50 | 2.44 | 97.60 ±2.57 |

[a] Calculated by the use of standard addition method. Values reported are the average of three independent analysis of the same sample.



**Figure 7.** The stripping voltammograms of the urine sample (diluted with supporting electrolyte in the ratio of 5:100, v/v): (..) in the absence of TRH (dashed line), (a) in the presence of 2.5 µg mL-1 TRH, (b–g) after standard additions of 0.5, 2.5, 5.0, 10, 20, 30 µg mL-1 TRH in 0.1 mol L-1 BR buffer solution (pH 3.0) in the presence of 8×10-4 mol L-1 SDS. Inset depicts the result of analysis by standard addition method (A). The stripping voltammograms of the diluted urine sample under the experimental condition (B). Other operating conditions as indicated in Fig. 4.

CONCLUSION

Up till now antecedently published electroanalytical works dealing with the TRH determination are generally based on modification of the carbon electrodes. In this study, the BDD electrode was used for the first time to develop a simple, alternative and novel voltammetric approach for electroanalytical quantification of TRH. The electrochemical behavior of irreversibly oxidized TRH on the BDD electrode was examined by CV and SW-AdSV technique. The obtained results showed that the oxidation peak currents of TRH can be affected by the anionic surfactant SDS. Using the optimized experimental conditions the developed voltammetric method exhibited the limit of detection in the 2.40 x 10-7 mol L−1 concentration level in 0.1 mol L-1 BR buffer (pH 3.0) solution with 8x10-4 mol L-1 SDS. The methods were sufficiently selective with the negligible effect of possible interfering. This method was validated on model and spiked samples. Pharmaceutical formulations of TRH and the urine as an example of biological fluid served as real samples.

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