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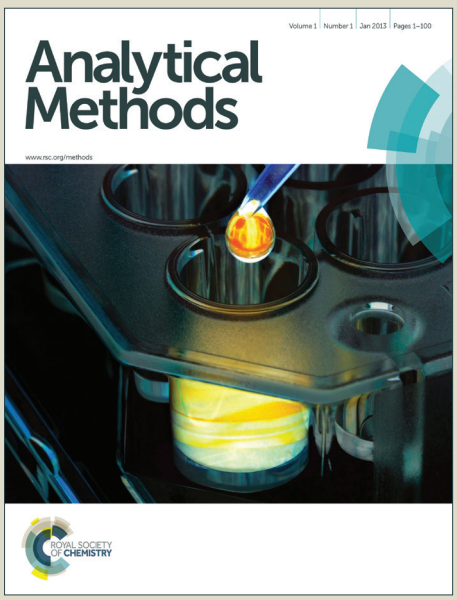

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ARTICLE

Electroanalytical application of a boron-doped diamond electrode for sensitive voltammetric determination of theophylline in pharmaceutical dosages and human urine

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In this paper, a novel voltammetric method for the determination of the 1,3-dimethylxanthine alkaloid theophylline was elaborated using differential pulse (DPV) and square-wave voltammetric (SWV) mode on a boron-doped diamond electrode. Direct oxidation of the analyte at very positive potentials was observed by cyclic voltammetry, as evidenced by the presence of well-shaped irreversible peak at +1.63 V (vs. Ag/AgCl electrode) in 1 mol L^{-1} sulphuric acid. After optimization of experimental conditions, the current response of theophylline was proportionally linear from 2 to 380 µmol L^{-1} using both pulse techniques. The developed electroanalytical method yielded low detection limits of 0.91 and 1.45 µmol L^{-1} associated with good intra-day repeatability (relative standard deviation of 3.2 and 2.5%) using DPV and SWV, respectively. The influence of some possible interferents was also evaluated. The practical feasibility of proposed methodology was tested in the analysis of pharmaceutical dosages and human urine samples and good recovery values were accomplished (93.2 – 102.5%). The results of analysis of pharmaceuticals were also in close agreement at a 95% confidence level with those obtained using titration (reference) method. Taking these attributes into consideration, the proposed sensor may be employed as a simple and effective analytical tool in drug control analysis and analysis of biological samples as well as useful alternative to previously utilized modified electrodes in this field.

1. Introduction

Theophylline (3,7-dihydro-1,3-dimethyl-1H-purine-2,6-dione, TP) is a type of 1,3-dimethylxanthine-based alkaloid that is present in some soft drinks, food stuffs as well as natural products. It may cause various physiological effects such as relaxation of bronchial muscle, gastric acid secretion and stimulation of the central nervous system[1,2]. TP is commonly prescribed for the treatment of neonatal apnea, cardiovascular diseases and as a respiratory stimulator in the case of acute and chronic obstructive pulmonary disease[3,4]. The effective TP concentration in adults is between 5 and 20 µg mL^{-1}. Levels below this range are non-therapeutic, however, higher levels can give rise to arythmia, fever, dehydratation, insomnia, anorexia, tachycardia and coma[5]. Because of the serious side effects, toxicity and even death of patients, the development of a simple, rapid and sensitive analytical method for the continuous monitoring of TP is of high importance.

Up to now, miscellaneous analytical methods including high-performance liquid chromatography[6], liquid chromatography coupled to mass spectrometry[7], gas chromatography[8], capillary gas chromatography[9], thin-layer chromatography[10], spectrophotometry[11] and electrophoresis[12] have been developed for the identification and quantification of TP. Possibilities of TP determination using immunoassay[13] and chemiluminiscence[14] were investigated as well. Some of these methods, such as separation techniques and spectrophotometry require preconcentration steps and tedious analytical processes prior to analysis, the use of organic solvents, thereby generating high amounts of waste, time-consuming derivatization steps and high implementation costs, thus justifying the need for reliable, low cost and simpler methods[15].

Electroanalytical methods are increasingly being used in the determination of a wide range of biologically active compounds. They may represent a suitable alternative to the above mentioned analytical techniques regarding the low cost of equipment, undemanding samples treatment, fast response, and satisfactory selectivity and sensitivity to matrix effects[16,17]. A survey of the literature shows many studies in recent years describing various electroanalytical methods for the

determination of TP and other related alkaloids. In general, glassy carbon electrodes (GCE) and carbon paste electrodes (CPE) are the most frequently used materials. The lowest detection limit (LOD = 0.4 nmol L^{-1}) was achieved by the utilization of gold nanoparticles (AuNPs)/L-cysteine (L-cys)/Graphene (Gr)/Nafion modified GCE[18]. This sensor showed excellent electrocatalytic activity towards the oxidation of TP in the presence of 0.1 mol L^{-1} sulphuric acid. Simultaneous determination of norepinephrine and TP using an electrochemically reduced graphene oxide modified GCE was demonstrated by Raj and John[19]. The LOD for TP was found to be 2.9 nmol L^{-1} and the proposed method was applied to the quantification of studied analytes in injections and tablets. Sun *et al.*[20] described a novel method for the determination of TP in pharmaceutical formulations based on the utilization of an aligned carbon nanotubes (ACNTs) film modified GCE with the LOD of 10 nmol L^{-1}. Voltammetric behavior of TP at multi-walled carbon nanotube (MWCNTs) modified CPE using DPV was studied by Malode *et al.*[21]. Recently, Güney *et al.*[22] developed a new electrochemical sensor based on an imprinted hybrid sol-gel film for selective and sensitive determination of TP in a drug sample. Ahn *et al.*[23] described a novel label-free electrochemical system utilizing silver ions captured within abasic site-incorporated duplex DNA on the gold electrode (AuE) surface. TP was reliably detected at a concentration of 3.2 µmol L^{-1}. Electrochemical oxidation of TP was investigating utilizing screen printed electrodes (SPE) by Wang *et al.*[24]. Sensor was applied with remarkable amount of success in aqueous solution at physiological pH and may be applied to the detection of TP at medicinally relevant concentrations. Comparison of basic characteristics and analytical parameters of herein proposed method with other previously reported electrochemical methods for direct determination of TP is summarized in Table 1.

In recent years, boron-doped diamond (BDD) electrodes have been applied to various research areas including pharmaceutical[25,26], food[27,28] and environmental[29,30] analysis. For voltammetric techniques, higher chemical stability, wider electrochemical potential window in aqueous solutions, lower capacitive current, excellent resistance to electrode fouling, good biocompatibility and stability of response are the most important ones[31]. The advantageous performance over conventional electrode materials lies also in the possibility of measurement at high anodic potentials and in extreme conditions such as strong acid media. However, the analytical performance of BDD electrodes greatly depends on their surface termination (e.g. hydrogen or oxygen terminated)[32]. As far as we know, there has been only one paper in scientific literature dealing with the development of a sensor for the determination of TP based on oxygen terminated boron-doped nanocrystalline diamond (B:NCD:O) modified with TP imprinted polypyrrole[33]. Hydrogen peroxide induced chemical formation of polypyrolle molecularly imprinted by TP was applied for the modification of conducting silicon substrate covered by B:NCD:O. Electrochemical impedance spectroscopy was applied to the evaluation of analyte-induced changes in electrochemical capacitance/resistance.

In this paper, a novel electroanalytical method for the determination of TP was established. An excellent analytical performance of this method was achieved using DPV and SWV on BDD electrode. Proposed method was applied to the determination of TP in pharmaceutical dosages and human urine samples. It may represent a sensitive, rapid and cost-effective alternative to methods based on complex analytical techniques such as HPLC. Moreover, the obtained results in the case of pharmaceuticals are compared with those obtained by titration (reference) method.

2. Experimental

2.1. Chemicals

Theophylline (TP, purity ≥ 99%) was purchased from Sigma-Aldrich (Slovakia) and used without any further purification. A stock solution of TP (10 mmol L^{-1}) was prepared by putting in the dissolved mass of its standard in 100 mL MeOH. On the day of the experiment, working solutions were prepared by appropriate dilution of the stock with a selected supporting electrolyte. Britton-Robinson buffer solutions (0.1 mol L^{-1}, pH 2-12), phosphate buffer solutions (0.1 mol L^{-1}, pH 2-11) and sulphuric acid (0.1-2 mol L^{-1}) were used as supporting electrolytes. Prepared stock solutions were preserved at 6 °C when not in use and protected from daylight during use in the laboratory. All other chemicals were of analytical reagent grade. Double-distilled deionized water with resistivity not less than 18 MΩ cm was used throughout the experiments.

2.2. Apparatus

Electrochemical measurements were performed with an AUTOLAB PGSTAT 302N (Metrohm Autolab B.V., The Netherlands) potentiostat/galvanostat controlled by NOVA 1.8 electrochemical software. A three-electrode cell system was used with a BDD working electrode (its fabrication and properties is mentioned in Section 2.6.), a platinum wire auxiliary electrode and an Ag/AgCl/3 mol L^{-1} KCl reference electrode to which all electrode potentials hereinafter are referred. All pH values were measured using a pH meter Model 215 (Denver Instrument, USA) with a combined electrode (glass-reference electrode). Raman spectroscopy (HORIBA JOBIN YVON LABRAM 300, He-Ne laser 632.8 nm) was used for structural characterization of the BDD electrode film.

2.3. Measurement procedures

At the beginning of every work day, the surface of BDD electrode was rinsed with deionized water. In order to get rid of any impurities and to activate the surface, 10 cyclic voltammetric (CV) scans in the potential range from -2.0 V to +2.0 V (100 mV s^{-1}) in 1 mol L^{-1} sulphuric acid were performed before first measurement. CV, SWV and DPV were employed to investigate the electrochemical behavior and the quantification of TP. With the utilization of optimal parameters, calibration curves were obtained by successive addition of

aliquots of TP stock solution (10 mmol L^{-1}) into the electrochemical cell already containing 25 mL of supporting electrolyte. Every point of the calibration curve represents the corresponding average of three successive measurements of standard solution of TP. The current peaks were evaluated without any background correction. The linear least-square regression (OriginPro 8.5, OriginLab Corporation, USA) was used for the assessment of calibration curves and the relevant results (slope and intercept) were reported with confidence interval for 95% probability. The detection (LOD) and quantification (LOQ) limit was calculated as three and ten times the standard deviation of intercept divided by slope, respectively. The analysis of real and model samples was performed by the standard addition method.

2.4. Sample preparation

Commercial pharmaceuticals Euphyllin® with declared TP content of 200 (sample A) and 300 mg (sample B) and Theoplus® with the content of 300 mg (sample C) were purchased in a local pharmacy. As for sample preparation, ten capsules of particular pharmaceutical dosage were weighed (average weight of one tablet was 265 mg for A, 395 mg for B and 518 mg for C, respectively) and 167 mg for A, 85.0 mg for B and 86.7 for C was dissolved in 5 mL MeOH with intensive stirring until the powder was completely dissolved. Subsequently, the mixture was filtered to obtain a clear filtrate and quantitatively transferred into 50 mL volumetric flask and completed with deionized water. An appropriate aliquot of this solution (0.5 mL) was added to the electrochemical cell already containing 25 mL of supporting electrolyte. The respective standard addition of 50, 100 and 150 µL of TP stock solution (10 mmol L^{-1}) was used to analyze the tablet samples.

Drug-free human urine samples were taken from three non-smoking volunteers (V1: female, 24 years; V2: female, 48 years; V3: male, 30 years) on the day of the experiment. These experiments were performed in compliance with named law (Parliamentary Act no. 40/1964 Coll. Civil Code as amended). Informed consent was obtained from the volunteers prior to the experiments. The urine samples were prepared as follows: 1 mL of particular fresh urine was placed to 100 mL volumetric flask and filled up by deionized water. An aliquot of this solution (0.1 mL) was added to electrochemical cell already containing 25 mL of supporting electrolyte. Subsequently, the solution was spiked with 0.6 mL of TP stock solution (10 mmol L^{-1}) to form model human urine sample. The analysis of TP in this kind of sample was undertaken using standard addition method with respective volumes of 0.9, 1.2 and 1.5 mL of stock solution.

2.5. Titration method

Besides the voltammetric techniques used in this work, the titration method was also handled to quantify TP content in pharmaceutical tablets. This procedure is considered to be a reference method for this application according to European Pharmacopoeia[34] and was carried out as follows: 0.160 g of tablet powder was dissolved in 5 mL MeOH and filled with deionized water into 100 mL volumetric flask. This solution was transferred in titration vessel. Subsequently, 20 mL of 0.1 mol L^{-1} silver nitrate and 1 mL of bromothymol blue was added in titration vessel and titrated with 0.1 mol L^{-1} sodium hydroxide. 1 mL of 0.1 mol L^{-1} sodium hydroxide was equivalent to 18.02 mg of TP in the sample.

2.6. Fabrication of BDD electrode

Heavily doped BDD film was grown by double bias enhanced hot filament chemical vapour deposition (HF CVD) technique previously described[35]. As a substrate, highly conductive (0.008-0.024 Ωcm) N (100) type silicon substrate with 2 µm thick SiO_2 layer (CVD, Oxford PlasmaLab 80) was used. A 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) 4 h growth of the BDD thin film with 1 % concentration of CH_4 in H_2 and trimethylboron (TMB) to obtain the 20,000 ppm boron to carbon ratio (B/C) within the gas mixture[36]. The total pressure in the reactor was kept at 3000 Pa 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, 3000 Pa, 650 ± 20 °C). The resistivity of the film was 2.1×10^{-3} Ωcm and B concentration 2.3×10^{21} cm^{-3} measured by 4-point hall measurement.

The working electrode active surface (0.43 mm^2) was created in 400 nm SiO_2 (CVD, Oxford PlasmaLab 80) by using a standard optical lithography (SUSS, MA6) and wet etching in 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 printed circuit board's support and completely passivated by non-conducting paste (548X, DuPont)[37].

3. Results and Discussion

3.1. Structural characterization of BDD electrode film

The Raman spectrum depicts characteristic bands typically observed for heavily doped BDD films (Fig. 1). Two broad bands (B) at approximately 470 and 1220 cm^{-1} dominate in this spectrum. The bands approximately agree with two maxima in the phonon density of states (PDOS). They may therefore be connected with a relaxation of the wavevector selection rules, and may well be associated with the actual boron incorporation in the lattice, rather than the hole concentration[38]. Moreover, the '470 cm^{-1}' band is proposed to originate from local vibrational modes of boron pairs. Following the study of Bernard *et al.*[39], we have fitted the 470 cm^{-1} band by one Lorentzian and one Gaussian line with resulting position of the Lorentzian maximum at 458.2 cm^{-1}, which corresponds to the boron dopation of 2.36×10^{21} cm^{-3}.

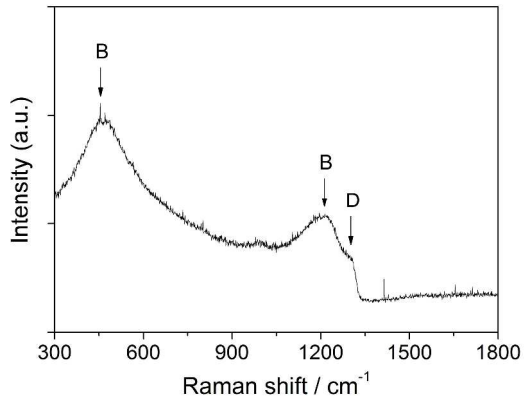

Fig. 1. Raman spectrum of the BDD film obtained using laser excitation at 632.8 nm.

3.2. Electrochemical behavior of TP on the BDD electrode

In order to obtain the most suitable conditions for the determination of TP, CV studies employing various supporting electrolytes with different pH values such as Britton-Robinson (pH 2.0-12.0) and phosphate (pH 2.0-11.0) buffers were performed. Sulphuric acid solutions (from 0.1 to 2 mol L^{-1}) were also tested. Nevertheless, the best results were obtained in strongly acidic media. The highest magnitude, lower background and well-defined voltammetric profile of the current response of 2 mmol L^{-1} TP was obtained in the presence of 1 mol L^{-1} sulphuric acid when compared to Britton-Robinson (pH 2.0) and phosphate (pH 2.0) buffers (see Fig. S1 in Electronic supplementary information (ESI)[†]). Hence, it was chosen as an optimal medium for further studies in this work. Typical CV voltammograms in the absence and presence of 1 mmol L^{-1} TP in 1 mol L^{-1} sulphuric acid are displayed in Fig. 2. As can be seen, TP was electrochemically oxidized with a well-defined signal at the potential of +1.63 V. In reverse scan, no reduction peak was observed indicating that the electrode reaction of TP on BDD electrode is totally irreversible. The electrochemical behavior of analyte on BDD electrode coincides with those explored on various electrode substrates[24,40,41].

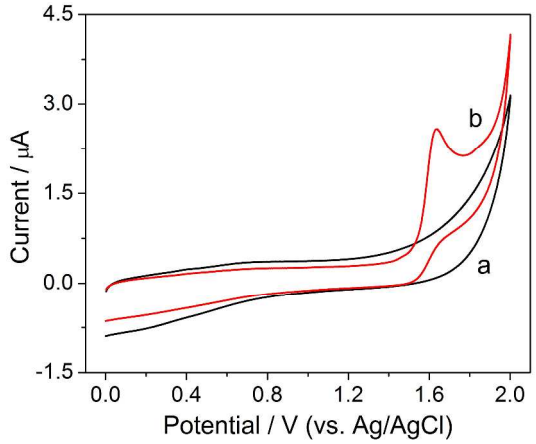

Fig. 2. CV voltammograms of (a) blank (1 mol L^{-1} H_2SO_4) and (b) 1 mmol L^{-1} TP in 1 mol L^{-1} H_2SO_4 on BDD electrode with the scan rate of 100 mV s^{-1}.

3.3. Effect of pH

The effect of pH on the peak potential (E_p) and current (I_p) of 1 mmol L^{-1} TP was investigated by CV (100 mV s^{-1}) in the presence of sulphuric acid in the pH range of 1.16-2.34 (these values corresponded with approximate concentration values from 2 to 0.1 mol L^{-1}. The peak potential (E_p) was slightly shifted towards more positive values (Fig. S2). This behavior confirms the participation of protons in the electrode reaction of TP on BDD electrode and the relationship between E_p vs. pH was found to be linear over the studied pH range. The following equation (Eq. 1) can be expressed as:

$$E_p(V) = 1.702(V) - 0.064 \times pH,\ R^2 = 0.984 \quad (1)$$

A slope of -0.064 (V pH^{-1}) suggested that the number of electron transfer is equal with that of hydrogen ions taking part in the particular electrode reaction. Despite the fact that the clarification of the oxidation mechanism of TP on BDD electrode has been beyond the scope of the study, the proposed mechanism is believed to occur via two electrons and two protons as in the case of the structurally similar guanine[24].

The effect of pH on the peak current (I_p) of TP was also investigated in the pH range of 1.16-2.34. The current response increased up to pH 1.47 (≈1 mol L^{-1}) and then sharply decreased. The highest peak current magnitude with lowest background was observed at this pH value, hence it was chosen as an optimal in all further experiments.

3.4. Effect of scan rate

The influence of scan rate (v) on the current response (I_p) of 1 mmol L^{-1} TP on BDD electrode was investigated by CV in the presence of 1 mol L^{-1} sulphuric acid at different scan rates from 10-200 mV s^{-1}. The slight potential shift to more positive values with increasing scan rate typical for irreversible systems[25] was noticed as depicted in Fig. 3. Further, it was found that the oxidation peak current varies linearly with the square root of scan rate which is an indication that the oxidation of TP is predominantly a diffusion controlled process[43]. The linear dependence (inset of Fig. 3) obeys the following equation (Eq. 2):

$$I_p(\mu A) = (0.513 \pm 0.011)(\mu A) + (0.008 \pm 0.001) \times v^{1/2}(mV\ s^{-1}),\ R^2 = 0.994 \quad (2)$$

In addition, there was a linear relationship between log I_p and log v, corresponding to the following equation (Eq. 3):

$$\log I_p = (0.55 \pm 0.04)\log v - (6.39 \pm 0.95),\ R^2 = 0.993 \quad (3)$$

The slope of 0.55 is close to the theoretically expected value of 0.5 for a purely diffusion controlled process[44].

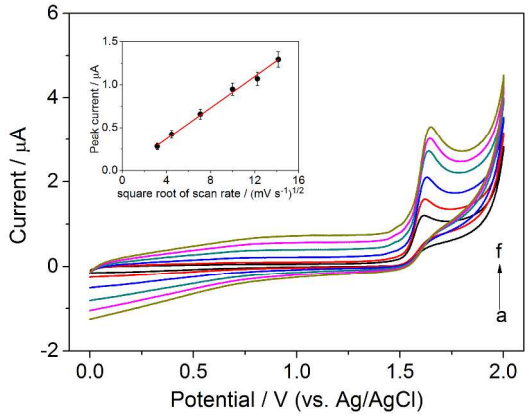

Fig. 3. Cyclic voltammograms of 1 mmol L^{-1} TP for various scan rates (v): (a) 10, (b) 20, (c) 50, (d) 100, (e) 150 and (f) 200 mV s^{-1} in 1 mol L^{-1} H_2SO_4 on BDD electrode. The peak current I_p as function of $v^{1/2}$ appears in the inset.

3.5. Analytical performance

In order to verify the feasibility of the method for the quantitative assessment of TP, the relationship between the oxidation peak current and the concentration of TP was studied using DPV and SWV with optimized experimental conditions (see ESI† and Fig S3 and S4). Respective DP and SW voltammograms in the concentration range from 2 to 380 μmol L^{-1} are displayed in Fig. 4 and in the inset of Fig. 4, respectively. The comparison of sensitivity of methods for determination of TP using both techniques is presented in Fig. 5. It is evident that DPV represents the more sensitive technique in this study and subsequently was selected for further experiments. The analytical parameters are summarized in Table 2.

According to the slope of calibration curves, DPV technique appeared to be more sensitive in comparison with SWV. The low LOD values were achieved as a consequence of high S/N ratio without any chemical modification of working electrode. In addition, in many cases they are comparable with LODs reported previously in the literature[54]. Intra-day repeatability was tested by 10 replicates of DPV and SWV measurements at 200 μmol L^{-1} TP. Low RSD values reflect good precision and confirm minimal adsorption of the analyte or its oxidation product on the BDD electrode surface. Thus, BDD has proven to be suitable electrochemical sensor for the precise determination of TP.

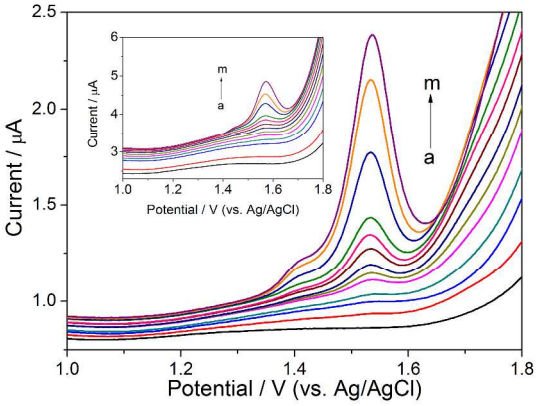

Fig. 4. DP and SW (inset) voltammograms for various concentrations of TP: (a) 0, (b) 2, (c) 4, (d) 8, (e) 20, (f) 28, (g) 40, (h) 60, (i) 80, (j) 99, (k) 200, (l) 290 and (m) 380 μmol L^{-1} in 1 mol L^{-1} H_2SO_4 on BDD electrode. DPV parameters: modulation amplitude of 50 mV, modulation time of 25 ms and scan rate of 10 mV s^{-1}. SWV parameters: amplitude of 25 mV, frequency of 50 Hz and scan rate of 250 mV s^{-1}.

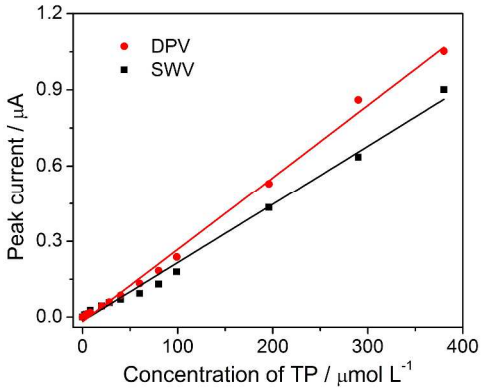

Fig. 5. Calibration curves for TP achieved by DPV and SWV.

Table 2 Analytical parameters for the determination of TP by DPV and SWV in 1 mol L^{-1} H_2SO_4 on BDD electrode (n = 3).

Analytical parameter	Technique	
	DPV	SWV
Intercept (nA)	-17.1 ± 0.8	-7.4 ± 1.1
Slope (nA L μmol^{-1})	2.90 ± 0.05	2.20 ± 0.05
Linear concentration range (μmol L^{-1})	2 - 380	2 - 380
Coefficient of determination (R^2)	0.996	0.993
Detection limit* (μmol L^{-1})	0.91	1.45
Intra-day repeatability** (%)	3.2	2.5

* Calculated as 3×standard deviation of intercept/slope of calibration curve
** RSD calculated for 10 replicate DPV or SWV measurements at 200 μmol L^{-1} TP

3.6. Interference study

Prior to analysis of samples (pharmaceutical dosages and human urine), the selectivity of proposed method was examined. The effect of possible interferents commonly present in human urine, such as ascorbic acid, uric acid, glucose, and dopamine as well as the effect other methylxanthines (caffeine, theobromine) on the oxidation peak current of 200 μmol L^{-1} TP in the concentration ratios from 1:0.1 to 1:100 was evaluated

(Fig. S5 – S10). The criterion was defined as the current response ratio of TP with its presence and absence. It was found that a 100-fold excess of glucose may be considered as minor without any substantial influence on the oxidation signal of TP (Fig. S5). The significant interferences were observed in a 5-fold excess of uric acid (Fig. S6) and 50-fold excess of dopamine (signal change of TP of 45%) (Fig. S7). According to those figures, a well-defined oxidation peak appeared at the potential of + 0.91 V and a second-one with a small magnitude at the potential of +0.76 V and +0.66 V for uric acid and dopamine, respectively. In the presence of ascorbic acid, the oxidation signal of TP declined and in 50-fold excess disappeared (Fig. S8). Furthermore, it was found that with 2-fold excess of theobromine the current response of TP decreased (signal value changed of about 90%) (Fig. S9). As depicted in Fig. S9, a second oxidation peak appeared with the increasing concentration of theobromine at the potential of +0.90 V. Caffeine underwent an electrochemical oxidation at the potential of +1.54 V vs. Ag/AgCl that is close to the potential of electrochemical oxidation of TP. The interference may occur if the concentration is 100 times higher than TP because its oxidation peak becomes enlarged and overlap with the oxidation peak of TP (Fig. S10). To sum up, the interference study revealed that the utilization of proposed method could be limited depending on the particular excess of some common urinary compounds and interferents with similar oxidation potentials when compared to TP.

3.7. Determination of TP in pharmaceutical dosages and human urine samples

Commercial pharmaceutical tablets containing TP were analyzed by standard addition method in order to evaluate the validity of the herein proposed method. The preparation of samples and measurement procedure is described in Section 2.4.). Corresponding DP voltammograms of pharmaceutical tablets (sample A) analysis is depicted in Fig. 6. The proportional increase of current response of TP was observed at the potential of +1.48 V vs. Ag/AgCl. Taking the molecular mass of TP (180.16 g mol^{-1}) into account, the volume in electrochemical cell (25.5 mL) and dilution factor (200), the content of TP is 130.5 mg (content in equivalent portion of 167 mg of powder used for the preparation of the stock sample solution). Regarding the average weight of one pharmaceutical tablet (0.265 for sample A), the average content of TP in original tablet is 0.208 g. Recovery experiment yielded sufficient values of 99.0 and 102.5% indicating that there were no important matrix interferences for the sample analyzed by the proposed method. The results obtained employing the proposed method as well as the reference titration method according to European Pharmacopoeia[34] are presented in Table 3. As can be seen, no significant differences were observed between the values found for the TP amounts using proposed and reference method. Moreover, the paired *t*-test was applied to the results obtained using both methods, since the calculated *t* value (0.743) is smaller than the critical value (2.919, α = 0.05), one may conclude that the results obtained with the proposed procedure is not statistically different from the reference titration method, at a 95% confidence level.

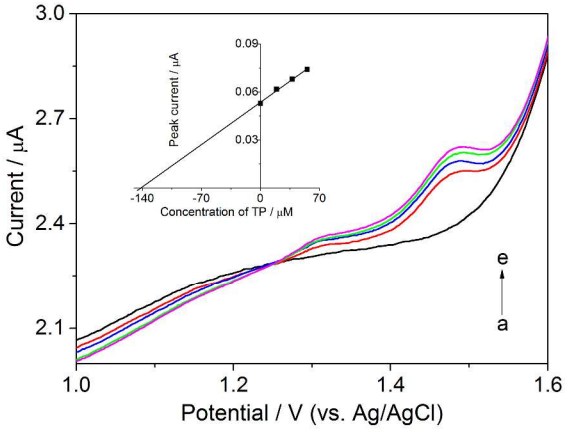

Fig. 6. DP voltammograms of analysis of pharmaceutical tablets Euphyllin® with declared content of 200 mg TP using the standard addition method in 1 mol L^{-1} H_2SO_4 on BDD electrode: (a) blank, (b) after addition of 0.5 mL of tablet sample and after spiking of (c) 50, (d) 100 and (e) 150 µL of solution of 10 mmol L^{-1} TP. The analysis by standard addition method is depicted in the inset. DPV parameters: modulation amplitude of 50 mV, modulation time of 25 ms and scan rate of 10 mV s^{-1}.

Table 3 Analysis of pharmaceuticals tablets with declared amount of TP using proposed and reference methods (n = 3).

Tablet sample	Declared (mg)	Proposed method		Reference method	
		Found* (mg)	Recovery (%)	Found* (mg)	Recovery (%)
A	200	205 ± 20	102.5	205 ± 6	98.0
B	300	298 ± 24	99.3	310 ± 14	103.3
C	300	297 ± 18	99.0	310 ± 12	103.3

* Confidence interval calculated according [mean ± $t_{n-1,\alpha}$ SD/sqrt(n)]; $t_{2;0.05}$ = 2.92

Subsequently, the proposed method was applied to determine TP in spiked human urine samples employing the standard addition method under the optimized experimental conditions. The preparation of this kind of sample is mentioned in Section 2.4. According to Table 4, sufficient recovery values were obtained (in the range from 93.2-102.2), since there was no significant interference by the matrix of human urine. Thus, the developed method provided good accuracy for the quantification of TP in both pharmaceutical dosages and human urine samples.

Table 4 Model human urine samples analysis using proposed method (n = 3).

Sample	Added (µmol L^{-1})	Found* (µmol L^{-1})	Recovery (µmol L^{-1})
V1	23	21.4 ± 1.7	93.2
V2	23	23.5 ± 5.3	102.2
V3	23	22.3 ± 1.6	97.0

* Confidence interval calculated according [mean ± $t_{n-1,\alpha}$ SD/sqrt(n)]; $t_{2;0.05}$ = 2.92

3.8. Comparison with other electroanalytical methods

Table 1 presents a comparison between the analytical characteristics of the proposed method with previously published voltammetric methods for the determination of TP. In general, various substrates such as GCE and CPE have been utilized in order to be chemically modified. There was only one paper dealing with unmodified AuE[23] and SPE[24]. As it was mentioned in introduction, the best results were achieved by a sensor based on GCE modified by AuNPs/L-cys/Gr/Nafion[18]. Comparable results were also obtained with the utilization of GCE modified by AuNPs/Chit-IL/Gr[47] and MIP/CNPs-SO_3H[22]. Significance of modification by MWCNTs obtained the best results in case of CPEs[21]. Voltammetric determination performed on CPE modified by BBFT/IL/GPE[55] and unmodified AuE[23] achieved relatively high LOD, thus considering them not so effective. Some of the papers (mostly including GCE modified electrodes) declare lower LOD when compared to our work. However, in pharmaceutical analysis, very low LOD are usually not required thus making the proposed method legitimately competitive in this field. In conclusion, sufficient LOD, wide linear concentration range and good repeatability were obtained by modification-free electrochemical sensor. In this sense, electrode modification steps may be tedious and the use of such electrode can be considered as an advantage over previous reports.

4. Conclusions

In this study, a BDD electrode in combination with DPV and SWV was applied to the electrochemical behavior study and determination of TP. A well-defined oxidation peak was observed by cyclic voltammetry at the potential of +1.63 V vs. Ag/AgCl in the presence of 1 mol L^{-1} sulphuric acid. The current response was found to be linearly dependent on the TP concentration from 2 to 380 µmol L^{-1} using both pulse techniques. Low detection limits of 0.91 and 1.45 µmol L^{-1} were obtained for DPV and SWV, respectively. These allow reducing matrix effects by working in diluted solutions at high anodic potentials. Practical applicability of the proposed method was demonstrated on the analysis of pharmaceutical dosages and human urine samples with satisfactory recoveries (varied from 93.2 to 102.5%). As a result, herein developed method is simple and rapid with utilization of modification-free electrochemical sensor providing a convenient technique for the TP quantification in pharmaceutical products. Moreover, the proposed method is cheaper when compared to commonly used chromatographic ones and also to other electroanalytical methods involving expensive modifiers. The possibility of a simultaneous determination of structurally related xanthines using BDD electrodes will be further investigated.

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