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Utilization of electrochemical methods in determination of trace elements in beverages

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Abstract: Electrochemical methods have many inherent advantages such as simplicity, low cost and amenability to miniaturization. A new, sensitive and selective electrochemical method for the caffeine determination using boron doped diamond electrode was developed. It was found by cyclic voltammetry that caffeine provided highly reproducible and well-defined irreversible oxidation peak, at very positive potential of +1.55 V vs. Ag/AgCl electrode. The effect of pH and scan rate on the voltammetric response of caffeine oxidation were studied to select the optimum experimental conditions. Linear response of peak current on the concentration in the range from 4×10^{-7} to 2.5×10^{-5} mol L^{-1}, good repeatability (RSD of 2.1 %) and the detection limit of 1.5×10^{-7} mol L^{-1} without any chemical modifications and electrochemical surface pretreatment were observed by differential pulse voltammetry in 0.4 mol L^{-1} perchloric acid. The effect of possible interfering compounds appeared to be negligible which evidently proved very good selectivity. The proposed method was successfully applied for the caffeine determination in commercially available beverage samples, with results in a close statistical agreement to these declared by manufacturer.

Keywords: caffeine, 1,3,7-trimethylxantine, boron-doped diamond electrode, voltammetry

Introduction

Caffeine (3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione) or 1-3-7-trimethylxantine is a natural alkaloid belonging to *N*-methyl derivatives of xanthine. It is found in various kinds of beverages and food such as coffee, coca-cola, tea, cocoa beans and chocolate. Because of high popularity of coffee and other caffeine containing beverages including soft and energy drinks, caffeine is the most commonly used psychoactive substance in daily human life. For humans, caffeine has many important physiological effects, such as stimulation of the central nervous system, diuresis and gastric acid secretion (Rostagno et al., 2011). However, high amounts of caffeine can cause trembling, nausea, nervousness and seizures and mutation effects such as inhibition of DNA (Chen et al., 2008). A fatal dose of caffeine has been evaluated to be more than 10 g (about 170 mg kg^{-1} of body weight). Due to the above mentioned facts detection and quantification of caffeine is important and does not have only clinical significance, but it can also give beneficial advice to people's health and life.

Numerous studies aimed towards the development of analytical methods for the caffeine determination in different matrix (environmental, biological, plants, food, etc.) has been published. From the optical techniques UV (Fernandez-Maestre, Hill, 2009), FT-infrared (Ito et al., 2008), FT-Raman (Koleva et al., 2008) and NMR (Armenta et al., 2005) spectrometry were usually employed for caffeine determination. The separation methods such as capillary electrophoresis (Zhao, Lunte, 1997), thin-layer chromatography (Sullivan, Sherma, 2005), gas chromatography (Jafari et al., 2011) and liquid chromatography (Tzanavaras, Themelis, 2007) were used for the analysis of mixtures containing caffeine and other drugs or metabolites. However, these techniques are mostly very expensive and long time is required for some procedures as derivatization, extraction and purification (Goyal et al., 2011) therefore, the development of reliable, low-cost, rapid, simple and accurate method for caffeine determination in various foodstuffs, pharmaceutical formulations and biological fluids is needed.

This opens the opportunities for the electrochemical methods employment, however only a few papers dealing with an electroanalysis of caffeine on more common electrode materials had appeared. This is because the oxidation of caffeine occurs at a very high positive potential, and may overlap with electrochemical reactions limiting potential window from the anodic side. This often gives low reproducible analysis. Boron-doped diamond (BDD) is a modern electrode material which opens new possibilities of electrochemical investigations due to its excellent features, such as the wide potential window in aqueous solutions, low background current, long-term stability of response, low sensitivity to dissolved oxygen and a good resistance to surface fouling due to weak adsorption (Pecková et al., 2009; Pleskov, 2002; Lawrence et al., 2006).

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Based on the above mentioned facts this paper demonstrates the application of BDD electrode as very sensitive electrochemical sensor for the voltammetric determination of caffeine without any chemical modifications and/or electrochemical pretreatment of electrode. This simple and practical analytical approach is illustrated on several commercial beverages as real samples.

Materials and Methods

The voltammetric measurements were carried out using Autolab PGSTAT-302N (Metrohm, The Netherlands) potentiostat controlled with the NOVA 1.7 software. All the electrochemical experiments were conducted in a three electrode single compartment glass cell. An Ag/AgCl (3.0 mol L^{-1} KCl) electrode was used as reference and the counter electrode was a Pt wire. The working electrode was BDD (Windsor Scientific Ltd., United Kingdom) with inner diameter of disc of 3 mm. All pH values were measured with pH meter Model 215 (Denver Instrument, USA). Caffeine was obtained from Zentiva (Hlohovec, Slovak Republic) and used as received. All reagents were of analytical grade purity. The stock solution of caffeine (1.0×10^{-3} mol L^{-1}) was prepared using double-distilled deionized water. The caffeine solutions of lower concentrations were freshly prepared by diluting with supporting electrolyte. Perchloric acid was used as supporting electrolyte (Lachema Brno, Czech Republic). The caffeine containing beverages was purchased from the local store.

Cyclic voltammetry and differential pulse voltammetry (DPV) were employed without deaeration, since dissolved oxygen did not interfere in anodic potential window of BDD electrode. After optimization of instrumental parameters DPV voltammograms were recorded and then calibration curve was constructed from the average of six consecutive measurements for each addition of standard. Calibration curve was analyzed by linear least-square regression in OriginPro 7.5 (OriginLab Corporation, USA) and the relevant results (slope and intercept) were reported with 95 % confidence level. The detection limit was calculated using the 3σ criterion. In order to fit into linear range of calibration curve, samples of coca-cola, pepsi-cola and energy drink were diluted by a factor 1:200 (v/v) with the supporting electrolyte after sonical elimination of gas. Generally, the diluting process can actually help to reduce the matrix effects (Zhao et al., 2011). The standard addition method was used for analysis of coca-cola sample spiked with aliquots amounts of caffeine.

Results and Discussion

First, cyclic voltammetry was applied to elucidate the electrochemical behavior of caffeine on BDD electrode (Fig. 1). It shows the anodic peak at the potential of about +1.55 V *vs.* Ag/AgCl and no presence of any cathodic peak on the reverse scan, indicating irreversible oxidation. Further, as it is apparent in the absence of caffeine no oxidation peak is observed and background current is very low.

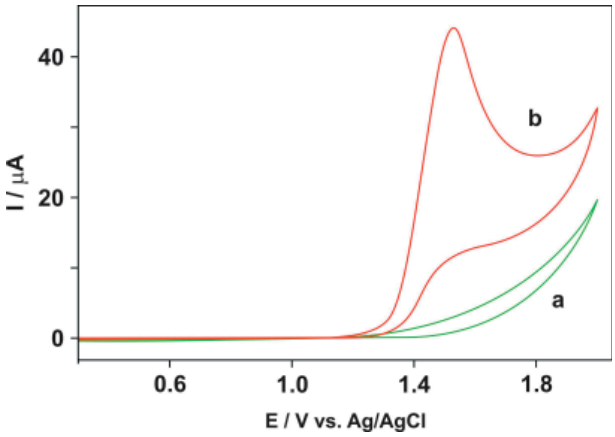

Fig. 1. Cyclic voltammograms of (a) 0 mol L^{-1} and (b) 1.0×10^{-5} mol L^{-1} caffeine in 0.4 mol L^{-1} $HClO_4$ on bare BDD electrode, scan rate 0.05 V s^{-1}.

It was previously observed that low pH has a significant influence on the oxidation of caffeine (Schrenk *et al.*, 2002). We decided to choose and test perchloric acid in the pH range of 0.5—3 (achieved by diluting of 0.4 mol L^{-1}) with 1.0×10^{-5} mol L^{-1} caffeine concentration. Apparently the magnitude of peak current was found to be highest in pH equal to 0.5. Based on this fact 0.4 mol L^{-1} $HClO_4$ was chosen and used in further experiments. As shown in Fig. 2, the peak potential is slightly shifted towards more negative potentials and peak current decreases as the pH increases in the range from 0.5 to 3.0.

Next, we performed further experiments to study the effect of the scan rate on the voltammetric response of caffeine oxidation at bare BDD electrode and characterize the transport in a diffusion layer. Fig. 3 shows the cyclic voltammograms in the presence of 1.0×10^{-5} mol L^{-1} caffeine in 0.4 mol L^{-1} $HClO_4$ recorded at various scan rates. The slight shift of peak potential towards more positive potential was observed as the scan rate increased. From the inset of Fig. 3 it can also be seen that peak current is linearly proportional ($R^2 = 0.998$) to the square root of the scan rate within the range of 0.01—0.3 V s^{-1} indicating that the electrode reaction is controlled by diffusion thus rate-limiting adsorption and/or

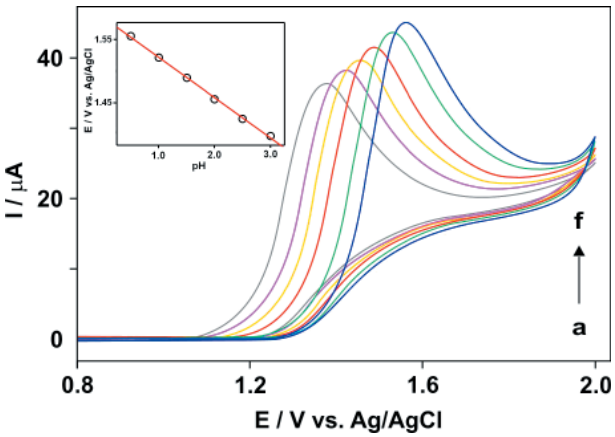

Fig. 2. Cyclic voltammograms of 1.0×10^{-5} mol L^{-1} caffeine in $HClO_4$ on bare BDD electrode at different pH: (a) 3.0, (b) 2.5, (c) 2.0, (d) 1.5, (e) 1.0 and (f) 0.5 with scan rate of 0.05 V s^{-1}. The dependence between peak potential (V) and pH appears in the inset.

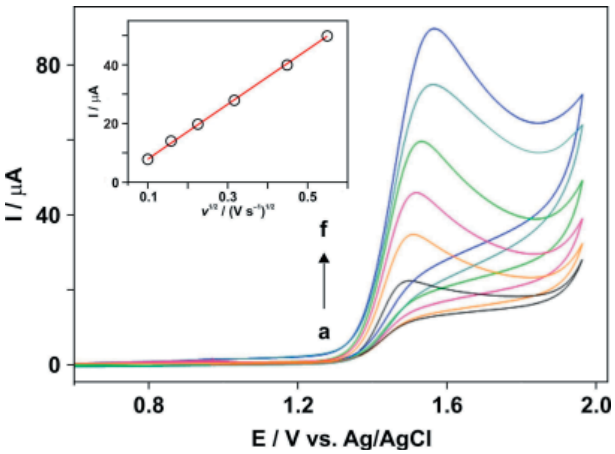

Fig. 3. Cyclic voltammograms of 1.0×10^{-5} mol L^{-1} caffeine in 0.4 mol L^{-1} $HClO_4$ on BDD electrode for scan rates (v): (a) 0.01, (b) 0.025, (c) 0.05, (d) 0.1, (e) 0.2 and (f) 0.3 V s^{-1}. The dependence between peak current (µA) and square root of the scan rate appears in the inset.

specific interactions on bare BDD electrode surface are negligible.

Differential pulse voltammetry (DPV) was chosen as more sensitive voltammetric technique in comparison with cyclic voltammetry to investigate the dependence between peak currents and caffeine concentrations. This procedure involves an optimization of the experimental parameters affecting the peak current such as modulation amplitude, modulation time and scan rate. During optimization each parameter was changed while the others were kept constant. It was found that the peak current increased with the increasing of modulation amplitude accompanied by the widening peak width at the same time. When the modulation amplitude is higher than 0.05 V, the peak becomes much wider. As for the modulation time, peak currents decreased with an increasing of modulation time and the most stable peak current was observed at about 0.02 s. Table 1 shows studied range of DPV parameters and their optimum values which were used for DPV calibration curve construction.

Tab. 1. Optimized DPV parameters for the caffeine concentration of 1.0×10^{-5} mol L^{-1}.

Parameters	Studied range	Optimum value
Modulation amplitude (V)	0.02–0.1	0.05
Modulation time (s)	0.01–0.05	0.02
Scan rate (V s^{-1})	0.01–0.1	0.05

The calibration curve was constructed by measuring of peak current with optimized DPV parameters. Fig. 4 displays DPV voltammograms at various concentrations of caffeine in 0.4 mol L^{-1} $HClO_4$. An average of six consecutive measurements was used for calibration curve construction.

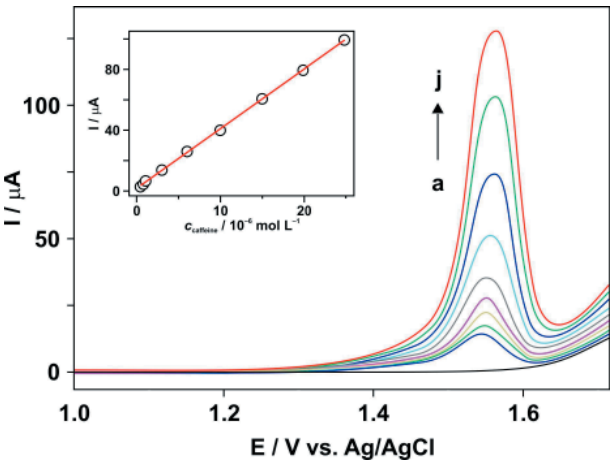

Fig. 4. DPV voltammograms of caffeine solutions with various concentrations: (a) 0 , (b) 4×10^{-7}, (c) 8×10^{-7}, (d) 1×10^{-6}, (e) 3×10^{-6}, (f) 6×10^{-6}, (g) 1×10^{-5}, (h) 1.5×10^{-5}, (i) 2×10^{-5} and (j) 2.5×10^{-5} mol L^{-1} (supporting electrolyte 0.4 mol L^{-1} $HClO_4$) on bare BDD electrode at optimized DPV parameters: modulation amplitude of 0.05 V, modulation time 0.02 s and scan rate 0.05 V s^{-1}. The dependence between peak current (µA) and caffeine concentrations (mol L^{-1}) appears in the inset.

The dependence of peak current on caffeine concentration shows a good linearity in the concentration range from 4×10^{-7} to 2.5×10^{-5} mol L^{-1} as depicted in the inset of Fig. 4. and is expressed by

the equation (1):

$$I_p = A + B \times c \quad (1)$$

where the intercept A = 2.4 μA (SD_A = 0.1 μA) and slope B = 3.2×10^6 μA mol^{-1} L ($SD_B = 1.5 \times 10^4$ μA mol^{-1} L), R^2 = 0.999. The detection limit was calculated according to 3σ criterion and found to be 1.5×10^{-7} mol L^{-1}. The repeatability was evaluated by six successive measurements of 1.0×10^{-5} mol L^{-1} caffeine solution under the same operating conditions over the short time interval. The relative standard deviation of the peak current of 2.1 % was obtained revealing good repeatability of the proposed method. The potential interferences influencing the caffeine determination was investigated by addition of possible interferent to a solution containing fixed amount of 1.0×10^{-5} mol L^{-1} caffeine. The various species such as glucose, fructose, sucrose and ascorbic acid can be present in coca-cola, pepsi cola and energy drink so they were tested under the same experimental conditions. The results showed that more than 100-fold their excess had no influence on the peak current of caffeine.

In order to estimate the accuracy of the proposed analytical technique, the standard additions method was used for beverage sample analysis spiked with aliquots amount of caffeine standard. The average results for six replicate measurements with standard deviations and confidence interval for 95 % probability are summarized in Table 2. To investigate matrix effects the caffeine standard was added to the diluted coca-cola sample and the recoveries were calculated. Their values (range from 97.4 to 102.2 %) reveal good accuracy of the presented method.

The oxidation peak current of caffeine is sensitive to each standard addition, however in the case of coca-cola sample it occurs at slightly more positive potential. This shift is probably the consequence of presence of the residual content of carbon dioxide in the coca-cola sample.

In order to evaluate the validity and practical applicability of the proposed method, three commercially available caffeine containing beverages were directly analyzed. Real samples analysis results of caffeine content in beverage samples are summarized in Table 3. The determined value of caffeine content in coca-cola is in good agreement with a content declared by manufacturer.

Conclusions

Proposed analytical technique is simple and rapid in comparison with other analytical methods used for the caffeine determination. The low detection limit (1.5×10^{-7} mol L^{-1}) was obtained as a consequence of very high S/N ratio without any chemical modification of the BDD surface and also no electrochemical pretreatment is involved. Method is highly selective because species present in beverages as real samples like glucose or ascorbic acid do not interfere even in a high excess. When tested the accuracy of the method recoveries from 97.4 to 102.2 % were achieved. Based on these facts, the

Tab. 2. Caffeine spiked coca-cola samples analysis on BDD electrode in DPV mode (n = 6).

Added 10^4 mol L^{-1}	Expected 10^4 mol L^{-1}	Found* 10^4 mol L^{-1}	SD 10^4 mol L^{-1}	Confidence interval for 95 % probability** 10^4 mol L^{-1}	Recovery %
0	–	2.47	0.13	(2.47 ± 0.11)	
0.5	2.97	2.92	0.17	(2.92 ± 0.14)	98.3
1.0	3.47	3.38	0.21	(3.38 ± 0.17)	97.4
1.5	3.97	4.01	0.23	(4.01 ± 0.19)	101.0
2.0	4.47	4.57	0.29	(4.57 ± 0.24)	102.2

*Average for six replicate measurements (n = 6): $\bar{x}$

**Calculated according ($\bar{x} \pm t_{n-1,\alpha} \times SD/\sqrt{n}$); from tables $t_{5;\,0.05}$ = 2.0150

Tab. 3. Real caffeine samples analysis (n = 6).

Beverage samples	Caffeine content (mg L^{-1})		
	Proposed method bare BDD (DPV)	SD	Declared by manufacturer
Coca-cola	98	8	100
Pepsi-cola	117	13	120
Energy drink	202	16	195

presented method offers green and sensitive possibility for quality control analysis of food products or pharmaceutical formulations containing caffeine.

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