



# Adsorptive stripping voltammetric method for the determination of caffeine at integrated three-electrode screen-printed sensor with carbon/carbon nanofibers working electrode

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## Abstract

A commercially available screen-printed carbon electrode coated with carbon nanofibers (SPCE/CNFs) and differential-pulse adsorptive stripping voltammetry (DPAdSV) were used to determination of caffeine. The process of electrochemical oxidation of caffeine in 0.1 mol L<sup>-1</sup> sulfuric acid at the SPCE/CNFs surface was investigated by cyclic voltammetry (CV). The effect of the supporting electrolyte type, pH and concentration, time and potential of accumulation, amplitude and scan rate were studied to select the optimum experimental conditions. Under the optimized conditions, the well-defined caffeine peak was observed at 1.25 V (vs. screen-printed silver reference electrode) in 0.1 mol L<sup>-1</sup> sulfuric acid. The caffeine was accumulated at -0.8 V for 60 s. The oxidation peak current was proportional to concentration of caffeine from 2.0 × 10<sup>-7</sup> to 1.0 × 10<sup>-6</sup> mol L<sup>-1</sup> with detection limit of 5.6 × 10<sup>-8</sup> mol L<sup>-1</sup>. The presented method has been successfully applied for the determination of caffeine in beverage samples.

**Keywords** Integrated three-electrode screen-printed sensor with carbon/carbon nanofibers working electrode · Adsorption-controlled process · Diffusion-controlled process · Voltammetric determination of caffeine · Beverage samples

## 1 Introduction

Caffeine (1,3,7-trimethylxanthine or 3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione, Fig. 1) is a naturally occurring alkaloid belonging to N-methyl derivatives of xanthine (Yardımcı et al. 2013; Švorc et al. 2012). It is found in many plant products such as coffee and cacao beans, tea leaves, yerba mate, cola nuts or guarana berries (Gupta et al. 2013). Depending on its origin it is also known as theine, mateine or guaranine (Martínez-Huitle et al. 2010). It is consumed in various forms, most often in coffee and tea, as well as an ingredient of soft and energy drinks or over-the-counter medicines for the treatment of headache, asthma, nasal congestion and even to facilitate weight loss and improve

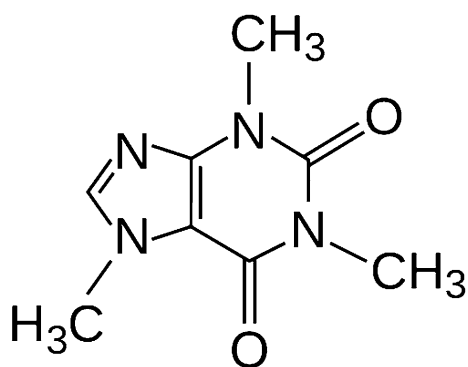
athletic endurance (Torres et al. 2014; Yardımcı et al. 2013). Owing to the high popularity of beverages containing caffeine, it is the most commonly used psychoactive substance in modern society (Švorc et al. 2012).

Caffeine has no nutritional value, but it has a specific physiological effect on the human body. It has the strongest effect on the cerebral cortex and the central nervous system. In moderate doses, caffeine has a positive effect on concentration, physical and mental performance and additionally reduces sleepiness and fatigue. It contributes to increased attention, perceptiveness and memory and helps to restore consciousness in states of fainting. It can also increase vigilance, visual and auditory reactions and improve mood, cognitive and psychomotor functions, as well as contribute to the facilitation of mental processes and general improvement of the body's coordination. In the circulatory system, caffeine additionally acts as a vasoconstrictor, increases blood pressure and improves blood supply to the heart. In addition, it reduces inflammatory reactions in the body, increases diuresis and gastric acid secretion and improves glucose metabolism (Torres et al. 2014; Jeevagan and John 2012; Yardımcı et al. 2013). Moderate intake of caffeine by healthy adults, in an amount of about 200–500 mg per day, has a positive

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**Fig. 1** The chemical structural formula of caffeine

effect on the human body, and does not addict or cause adverse effects (Jeevagan and John 2012). However, high amounts of caffeine can have an adverse effect on health, especially for infants and children, or pregnant women. It may cause restlessness, insomnia, nervousness, hyperactivity, nausea, seizures and headaches. It can mobilize cellular calcium, leading to bone mass loss and also cause emesis and dehydration, loss of appetite, gastrointestinal problems or kidney and liver damage. Caffeine is considered a risk factor for cardiovascular diseases. Overdose of it may result in hypertension, cardiac arrest or tachycardia and even death (Torres et al. 2014; Gupta et al. 2013; Jeevagan and John 2012; Chitravathi and Munichandraiah 2016; Yardım et al. 2013). It is estimated that the lethal dose of caffeine exceeds 10 g (about 170 mg kg<sup>-1</sup> body weight) (Švorc et al. 2012). In the light of its adverse effects it is very important to develop a fast, simple and accurate method for the determination of caffeine in real samples.

Many different procedures have been proposed for assay a caffeine in biological and pharmaceutical samples including gas chromatography/mass spectrometry (GC/MS) (Jeon et al. 2017), liquid chromatography/mass spectrometry (LC–MS) (Li et al. 2015), liquid chromatography using fluorimetric and UV detection (Ibrahim and Wahba 2014), high-performance liquid chromatography-tandem mass spectrometry (HPLC–MS/MS) (Rybak et al. 2014), ultra-high-performance liquid chromatography–tandem mass spectrometry method (UHPLC-MS/MS) (Krpó et al. 2018; Liu et al. 2013) and also solid surface fluorescence using membrane filters modified with MWCNTs (Talio et al. 2013) or multivariate calibration-prediction techniques, principal component regression (PCR), partial least squares (PLS) and artificial neural networks (ANN) were applied to the spectrometric multicomponent analysis (Aktaş and Kitiş 2014). However, these methods are generally expensive and often require a tedious, time-consuming sample pretreatment, which makes them unsuitable for the routine analysis of large number of samples. They also require combination with various detection methods.

Caffeine is electroactive and it can be detected by electrochemical methods. Accordingly, interest in electrochemical methods has increased due to their low-cost instrumentation, simplicity, speed, high sensitivity and good stability. Many different electrochemical methods can be found in the subject literature, that apply working electrodes with surfaces that have been subjected to various modification processes to improve their analytical properties. These include using glassy carbon electrodes modified with: Nafion (Torres et al. 2014), Nafion and graphene oxide (Zhao et al. 2011), lead and Nafion films (Tyszczyk-Rotko and Bęczkowska 2015), multiwall carbon nanotubes (Gupta et al. 2013), a composite film of poly(4-vinylpyridine) and multiwall carbon nanotubes (Ghadimi et al. 2016), a DNA-functionalized single walled carbon nanotube and Nafion composite film (Wang et al. 2014), novel N-doped carbon nanotubes functionalized with MnFe<sub>2</sub>O<sub>4</sub> nanoparticles (Fernandes et al. 2015). Also electrode modified through the electrodeposition of luteolin on a functionalized multi-wall carbon nanotube immobilized on the surface of a glassy carbon electrode (Amiri-Aref et al. 2014) or a glassy carbon disk electrodes after its modification with poly(Alizarin Violet 3B), multiwalled carbon nanotubes and graphene (Wang et al. 2016) were used for the determination of caffeine. Recently, the boron-doped diamond (BDD) variant has gained great popularity. To determine caffeine levels both bare boron-doped diamond electrode (BDDE) (Švorc et al. 2012), cathodically-pretreated BDDE and anodically-pretreated BDDE (Lourencão et al. 2009), Nafion-modified BDDE or a non-toxic bismuth particles Nafion covered boron-doped diamond electrode (Sadok et al. 2016) were applied.

Currently, nanocarbon materials attract growing interest in analytical chemistry due to their unique properties and structures (Fernandes et al. 2015). Nanomaterials have been widely used as chemical sensor and biosensor materials (Zhao et al. 2011). They are usually used to increase the surface immobilizing the biomolecules, which generally causes an increase in the number of binding sites available to detect a specific chemical analyte and also leads to signal amplification in electrochemical sensors (Zhao et al. 2011; Amiri-Aref et al. 2014). An excellent example here are the carbon nanofibers (CNFs), which are characterized by their extremely large specific surface area, high chemical and mechanical stability, excellent electrical and thermal conductivities and a possibility of their mass production (Zhang et al. 2016; Oularbi et al. 2017; Feng et al. 2014). The quest for miniaturization led to increasing popularity of the screen-printed electrodes (SPEs), for which production nanomaterials can be used. SPEs usually include a counter electrode, a reference electrode and also a working electrode (the surface of which can be modified) printed on the same strip. Because of their low production costs and commercial availability, they can be used

as highly-reproducible disposable electrodes. Moreover they have a lot of advantages such as appropriate linearity, repeatability, selectivity and sensitivity, ease of surface modification and portability (Puy-Llovera et al. 2017; Pérez-Ràfols et al. 2016). Therefore, the main purpose of this article was to develop and optimize a new, simple, fast and accurate voltammetric caffeine determination method using a commercially available screen-printed sensor.

## 2 Experimental

### 2.1 Apparatus

Electrochemical measurements were carried out using the Eco Chemie  $\mu$ Autolab analyzer (The Netherlands). A conventional quartz cell with a volume of 10 mL was used. On the surface of the commercially available electrochemical sensor (SPCE/CNFs) there is a three-electrode system consisting of a working electrode (screen printed carbon electrode covered with carbon nanofibers), auxiliary electrode (screen printed carbon electrode) and an reference electrode (screen printed silver electrode) (DropSens, Ref. 110CNF).

### 2.2 Reagents and beverage samples

The following substances were used as supporting electrolyte: 0.05–0.3 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> (Merck), 0.2 mol L<sup>-1</sup> HCl, 0.2 mol L<sup>-1</sup> CH<sub>3</sub>COOH, 0.2 mol L<sup>-1</sup> PIPES (pH 7.0 ± 0.1, Sigma-Aldrich) and 0.2 mol L<sup>-1</sup> acetate buffer pH 4.5 ± 0.1 prepared from CH<sub>3</sub>COOH and NaOH (Merck). The influence of the following interferents present in real samples was checked: glucose (standard, Poland), fructose (POCH, Poland), sucrose (Merck) and vitamins B<sub>3</sub> (niacin) from Fluka, B<sub>5</sub> (pantothenic acid), C (ascorbic acid) from Sigma and B<sub>6</sub> (pyridoxine) from Supelco. The stock standard solution of caffeine (1.0 × 10<sup>-3</sup>, 1.0 × 10<sup>-4</sup> and 1.0 × 10<sup>-5</sup> mol L<sup>-1</sup>) was prepared by dissolving the reagent (Sigma-Aldrich) in deionized water purified via a Milli-Q unit (Millipore system). All prepared solutions were stored in the dark at 4 °C in a refrigerator.

Two beverages containing caffeine were selected for the study: an energy drink composed of water, sucrose, glucose, citric acid, carbon dioxide, taurine (0.4%), sodium carbonates, magnesium carbonates, caffeine (32 mg/100 mL), niacin, pantothenic acid, pyridoxine, flavours, caramel, riboflavin and cola drink composed of water, sugar, carbon dioxide, E150d caramel, phosphoric acid, caffeine (0.01 g/100 mL). The samples were stirred at 250 rpm for 1 h in order to eliminate CO<sub>2</sub> and added directly to the supporting electrolyte in the voltammetric cell.

### 2.3 Voltammetric measurements

0.1 mol L<sup>-1</sup> sulfuric acid was selected as the basic electrolyte. Caffeine was accumulated at -0.8 V for 60 s. During this step the solution was stirred. The differential-pulse adsorptive stripping voltammetric (DPAdSV) curves were recorded in the potential range from 0.25 to 1.8 V with an amplitude of 100 mV and a scan rate of 125 mV s<sup>-1</sup>. Then the blank curve was subtracted from each voltammograms and obtained curves were cut in the range from 1.0 to 1.5 V.

In cyclic voltammetry (CV), the effect of the scan rate was tested in the range from 0.005 to 0.3 V s<sup>-1</sup> in the potential range from 0.25 to 1.8 V.

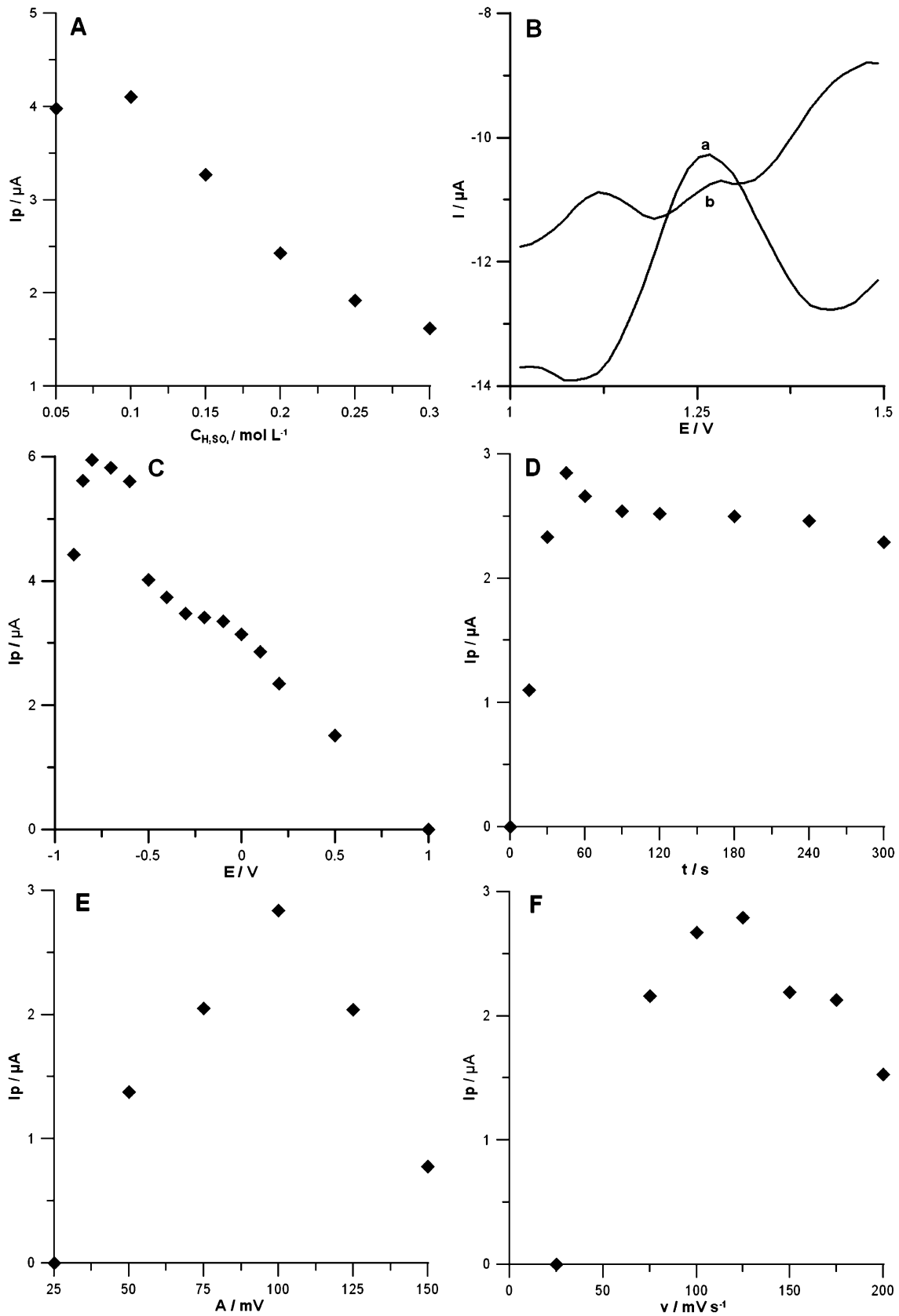
## 3 Results and discussion

In earlier studies our research team has developed a method for the determination of caffeine in river water samples using a screen-printed carbon electrode modified with bismuth film (BiF/SPCE) (Tyszczyk-Rotko and Szwagierek 2017). Preliminary studies have shown that the caffeine signal can also be obtained on commercially available integrated three-electrode screen-printed sensor with carbon/carbon nanofibers working electrode (SPCE/CNFs), so it was decided to use them for further measurements.

### 3.1 Composition of measurement solution

In the first stage of optimization of the caffeine determination method, the influence of the supporting electrolyte type on the obtained analytical signal of caffeine was investigated. For this purpose 0.2 mol L<sup>-1</sup> solutions of sulfuric acid, hydrogen chloride, acetic acid, acetate buffer of pH 4.5 ± 0.1, PIPES buffer of pH 7.0 ± 0.1 as well as 5.0 × 10<sup>-7</sup> and 1.0 × 10<sup>-6</sup> mol L<sup>-1</sup> caffeine were used. Based on the performed DPAdSV measurements, it was found that the highest caffeine peak current and the lowest background level were obtained in sulfuric acid. In the case of acetic acid, the signal was much lower and less well formed, while in other electrolytes the analytical signal of caffeine was not visible at all. Finally, sulfuric acid was selected as the supporting electrolyte for conducting the further part of the experiment.

After the selection of sulfuric acid, the influence of its concentration on the intensity of the caffeine peak current (5.0 × 10<sup>-7</sup> mol L<sup>-1</sup>) was also determined. For this purpose, a series of solutions of sulfuric acid in the electrochemical cell were prepared at concentrations of 0.05, 0.1, 0.15, 0.2, 0.25, 0.3 mol L<sup>-1</sup>. The dependence of the electrolyte concentration on the caffeine peak current is shown in Fig. 2a. The caffeine peak initially increases with an increase in the concentration of sulfuric acid from 0.05 to 0.1 mol L<sup>-1</sup>. A further increase in the concentration of sulfuric acid from



**Fig. 2 a** The dependence of peak current of caffeine ( $5.0 \times 10^{-7}$  mol L $^{-1}$ ) on the concentration of sulfuric acid. The caffeine was accumulated at  $-0.65$  V for 60 s. DPAdSV parameters: amplitude of 100 mV, scan rate of 150 mV s $^{-1}$ . **b** DPAdSV (a) and SWAdSV (b) curves obtained during determination of  $1.0 \times 10^{-6}$  mol L $^{-1}$  caffeine. The conditions are the same as in Fig. 2a. The dependence of the peak current of caffeine ( $1.0 \times 10^{-6}$  mol L $^{-1}$ ) on the potential (c) and time (d). The caffeine was accumulated at  $-0.8$  V (d) for 60 s (c). DPAdSV parameters: amplitude of 100 mV, scan rate of 150 mV s $^{-1}$ . The dependence of peak current of caffeine ( $1.0 \times 10^{-6}$  mol L $^{-1}$ ) on the amplitude (e) and scan rate (f). The caffeine was accumulated at  $-0.8$  V for 60 s. DPAdSV parameters: amplitude of 100 mV (f), scan rate of 150 mV s $^{-1}$  (e)

0.1 to 0.3 mol L $^{-1}$  causes gradual lowering of the analytical signal of caffeine. Based on the obtained results, it was found that the 0.1 mol L $^{-1}$  H $_2$ SO $_4$  solution should be selected for further measurements, as it was characterized by the highest obtained caffeine peak current.

### 3.2 Parameters of voltammetric procedure

The comparison of differential-pulse adsorptive stripping voltammetry (DPAdSV) and square-wave adsorptive stripping voltammetry (SWAdSV) for the determination of  $1.0 \times 10^{-6}$  mol L $^{-1}$  caffeine shows much higher signal in the case of DPAdSV (Fig. 2b). The next step was to optimize the DPAdSV parameters of the measurement, such as: time and potential of accumulation, amplitude and scan rate. Oxidation peak of caffeine molecules on the surface of the SPCE/CNFs depends on the potential applied to the electrode. In order to determine optimal accumulation potential, 0.1 mol L $^{-1}$  H $_2$ SO $_4$  solution containing  $1.0 \times 10^{-6}$  mol L $^{-1}$  of caffeine was prepared. The potential was changed in the range from  $-0.9$  to 1.0 V. The dependence of the caffeine peak current on the accumulation potential is shown in Fig. 2c. It is noted that the caffeine signal rises in the potential range from  $-0.9$  to  $-0.8$  V, then decreases in the potential range from  $-0.8$  to 1.0 V. At the potential of 1.0 V the caffeine signal is not visible. For the rest of the experiment the potential of  $-0.8$  V was selected, as optimal for the determination of caffeine.

Then the accumulation time of caffeine on SPCE was determined. For this purpose, a solution containing 0.1 mol L $^{-1}$  of sulfuric acid and  $1.0 \times 10^{-6}$  mol L $^{-1}$  of caffeine was prepared. A potential of  $-0.8$  V was applied to the electrode for a time ranging from 0 to 300 s. The dependence of the caffeine peak current from the accumulation time is shown in Fig. 2d. It was observed that the peak current of caffeine increases with increasing accumulation time and the highest values were recorded for 45 s. The current response reaching almost a steady state when caffeine was accumulated for the duration of 60–300 s. This behaviour suggested a saturation of accessible adsorption of caffeine at the (SPCE/CNFs). Compared voltammograms obtained for 45 and 60 s

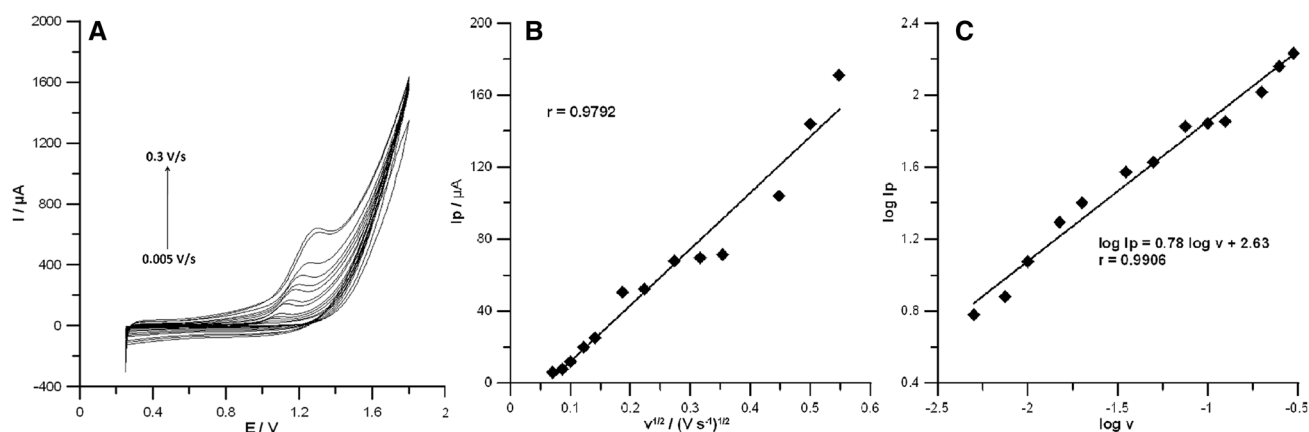
demonstrated that the accumulation over 45 s gives a slightly higher caffeine signal, however, during the 60 s accumulation a much lower background signal level was obtained, which is why 60 s was adopted as the most optimal accumulation time.

After optimization of the potential and time of accumulation, the effect of the amplitude (Fig. 2e) and scan rate (Fig. 2f) on the caffeine signal were investigated. A solution containing 0.1 mol L $^{-1}$  of sulfuric acid and  $1.0 \times 10^{-6}$  mol L $^{-1}$  of caffeine was prepared. The amplitude value was changed in the range of 25–150 mV, while the scanning rate was 25–200 mV s $^{-1}$ . It was observed that the caffeine peak current increases with the amplitude increase to 100 mV, further increase of the amplitude contributed to lowering of caffeine peak current. In the case of optimization of the scan rate, it was found that the analytical signal rises from 25 to 125 mV s $^{-1}$ , and then decreases in the range from 125 to 200 mV s $^{-1}$ . On this basis, an amplitude of 100 mV and a scanning rate of 125 mV s $^{-1}$  were selected as optimal values.

### 3.3 Scan rate study

In order to determine the type of process occurring on the surface of the electrode, as a result of which caffeine is deposited on its surface, tests were carried out using CV in 0.1 mol L $^{-1}$  sulfuric acid solution containing  $5.0 \times 10^{-5}$  mol L $^{-1}$  of caffeine. The CV curves were recorded in the potential window from 0.25 to 1.8 V. During subsequent measurements, the scan rate was varied in the range from 5 to 300 mV s $^{-1}$ . As it can be seen in Fig. 3a, only oxidation peak of caffeine is visible. The obtained results suggest, that charge transfer during caffeine oxidation is electrochemically irreversible. In addition, when the scan rate was increased, the oxidation peak potential shifted toward more positive values, such behaviour confirms that the electrode process is irreversible. It should be mentioned that oxidation mechanism of caffeine in acidic medium was already elucidated (Švorc et al. 2012). The oxidation peak of caffeine corresponds to an overall  $4e^-$ ,  $4H^+$  process. Reaction has two steps. The first one is  $2e^-$ ,  $2H^+$  oxidation of the C-8 to N-9 bonds and gives the 1,3,7-trimethyluric acid, followed by fast  $2e^-$ ,  $2H^+$  oxidation to 4,5-dihydroxy-1,3,7-trimethyltetrahydro-1H-purine-2,6,8-trione and 4,5-dihydroxy-1,7,9-trimethyltetrahydro-1H-purine-2,6,8-trione (both of them are 4,5-diol analogues of 1,3,7-trimethyluric acid).

On the basis of the obtained CV measurements, the relationship between the caffeine peak current ( $I_p$ ) and the square root of the scan rate ( $v^{1/2}$ ) was plotted. As shown in Fig. 3b, a linear correlation ( $r = 0.9792$ ) suggests that the process is diffusion-controlled. In order to confirm this assumption, the relationship between the logarithm of the caffeine peak current ( $\log I_p$ ) and the logarithm of the scan rate ( $\log v$ ) was plotted (Fig. 3c). The value of the



**Fig. 3** a Cyclic voltammograms obtained in 0.1 mol L<sup>-1</sup> sulphuric acid solution containing 5.0 × 10<sup>-5</sup> mol L<sup>-1</sup> caffeine at SPCE/CNFs. Scan rate was changed from 0.005 to 0.3 V s<sup>-1</sup>. Relationship

between: **b** peak currents of caffeine ( $I_p$ ) and the square root of the scan rates ( $v^{1/2}$ ); **c** logarithm of the peak current ( $\log I_p$ ) and the logarithm of the scan rate ( $\log v$ )

**Table 1** Influence of various potential interferents on the peak current of 1.0 × 10<sup>-6</sup> mol L<sup>-1</sup> caffeine

Interferents	Concentration (mol L <sup>-1</sup> )	Relative signal <sup>a</sup> (% n = 3)
Glucose	1.0 × 10 <sup>-7</sup>	100.1
	1.0 × 10 <sup>-6</sup>	98.7
	1.0 × 10 <sup>-5</sup>	93.9
Fructose	1.0 × 10 <sup>-7</sup>	96.6
	1.0 × 10 <sup>-6</sup>	100.7
	1.0 × 10 <sup>-5</sup>	104.1
Sucrose	1.0 × 10 <sup>-7</sup>	104.8
	1.0 × 10 <sup>-6</sup>	101.4
	1.0 × 10 <sup>-5</sup>	97.3
Ascorbic acid	1.0 × 10 <sup>-7</sup>	103.7
	1.0 × 10 <sup>-6</sup>	99.7
	1.0 × 10 <sup>-5</sup>	86.6
Niacin	1.0 × 10 <sup>-7</sup>	103.5
	1.0 × 10 <sup>-6</sup>	98.5
	1.0 × 10 <sup>-5</sup>	98.0
Pantothenic acid	1.0 × 10 <sup>-7</sup>	100.3
	1.0 × 10 <sup>-6</sup>	100.8
	1.0 × 10 <sup>-5</sup>	102.8
Pyridoxine	1.0 × 10 <sup>-7</sup>	98.8
	1.0 × 10 <sup>-6</sup>	96.8
	1.0 × 10 <sup>-5</sup>	94.0

<sup>a</sup>Relative signal (%) = 100 × (the caffeine peak current after addition of interferent/the caffeine peak current)

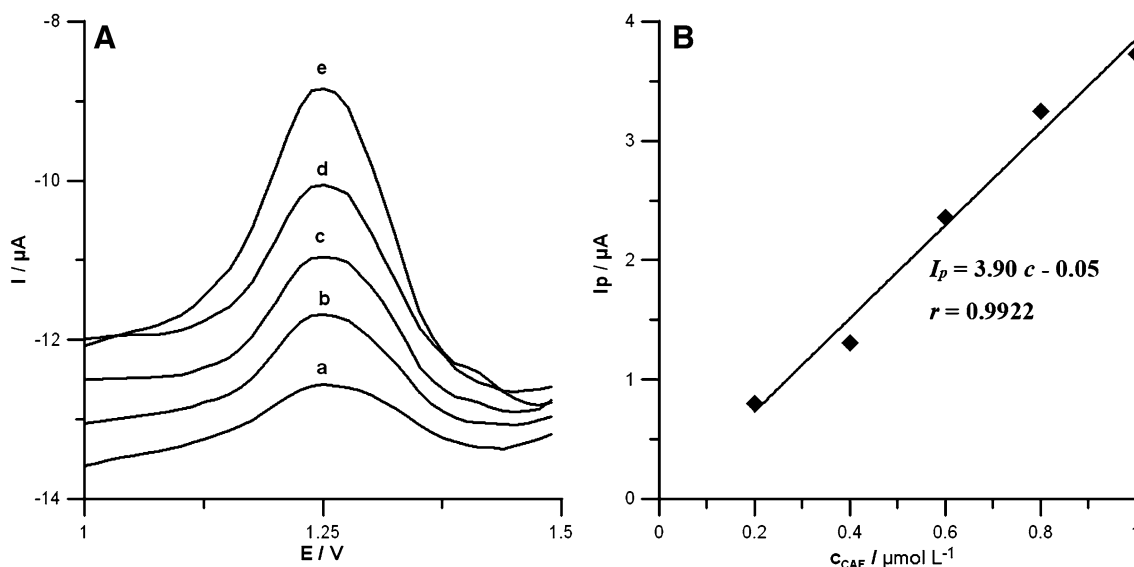
coefficient slope of 0.78 indicates that the electrochemical oxidation process of caffeine at the SPCE/CNFs surface is not purely diffusion- or -adsorption controlled.

### 3.4 Interference studies

During the tests, we investigated the effect of potential interferents, as present in real samples, on the analytical signal of caffeine. The composition of two drinks: cola and energy drink was analysed and the effect of each component on the caffeine peak current was analysed. 0.1 mol L<sup>-1</sup> sulfuric acid solution containing 1.0 × 10<sup>-6</sup> mol L<sup>-1</sup> caffeine and a series of interferent solutions with concentrations that were 10 times lower, equal to, and 10 times higher than the caffeine concentration, i.e.: 1.0 × 10<sup>-7</sup>, 1.0 × 10<sup>-6</sup> and 1.0 × 10<sup>-5</sup> mol L<sup>-1</sup>. As can be seen from Table 1, the all studied compounds have negligible effect on the signal of caffeine.

### 3.5 Calibration curve

After optimizing the composition of the basic electrolyte and the voltammetric procedure, DPAdSV measurements for calibration curve were made. The recorded voltammograms are shown in Fig. 4a. It was found that the caffeine calibration curve is linear over the range of concentrations of 2.0 × 10<sup>-7</sup> to 1.0 × 10<sup>-6</sup> mol L<sup>-1</sup> (Fig. 4b). The equation of the calibration curve is shown as follows:  $I_p = 3.9 c - 0.05$  [where:  $I_p$ —caffeine peak current (μA),  $c$ —caffeine concentration (μmol L<sup>-1</sup>)]. The correlation coefficient ( $r$ ) was 0.9922. The limit of detection (LOD) and quantification (LOQ) were calculated as 3 and 10-times of the standard deviation for the intercept ( $SD_a$ ,  $n = 3$ ) divided by the calibration curve slope ( $LOD = 3SD_a/b$ ;  $LOQ = 10SD_a/b$ ) and they equal 5.6 × 10<sup>-8</sup> and 1.9 × 10<sup>-7</sup> mol L<sup>-1</sup>, respectively. The comparison of the voltammetric methods for the determination of caffeine are presented in Table 2. As it can be seen, the detection limit for voltammetric methods is in the range 10<sup>-9</sup> – 10<sup>-6</sup> mol L<sup>-1</sup>. The detection limit obtained at the commercially available SPCE/CNFs (5.6 × 10<sup>-8</sup> mol L<sup>-1</sup>) is entered in the



**Fig. 4 a** DPAdSV curves obtained in 0.1 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> for various concentrations of caffeine: (a) 2.0 × 10<sup>-7</sup>, (b) 4.0 × 10<sup>-7</sup>, (c) 6.0 × 10<sup>-7</sup>, (d) 8.0 × 10<sup>-7</sup>, (e) 1.0 × 10<sup>-6</sup> mol L<sup>-1</sup>. **b** Linear range of the caffeine calibration curve: 2.0 × 10<sup>-7</sup> – 1.0 × 10<sup>-6</sup> mol L<sup>-1</sup>. The

obtained average values are shown with the standard deviation for n=3. DPAdSV parameters: accumulation potential of -0.8 V, accumulation time of 60 s, amplitude of 100 mV, scan rate of 125 mV s<sup>-1</sup>

**Table 2** The comparison of the voltammetric methods for the determination of caffeine

Electrode	Method	Linear range [mol L <sup>-1</sup> ]	Limit of detection [mol L <sup>-1</sup> ]	References
GCE/Nafion	DPV	1.0 × 10 <sup>-4</sup> – 7.0 × 10 <sup>-3</sup>	3.9 × 10 <sup>-8</sup>	Torres et al. 2014
GCE/Nafion/GO	DPV	4.0 × 10 <sup>-7</sup> – 8.0 × 10 <sup>-5</sup>	2.0 × 10 <sup>-7</sup>	Zhao et al. 2011
GCE/Nafion/PbFE	DPV	5.0 × 10 <sup>-8</sup> – 5.0 × 10 <sup>-6</sup> (peak 1) 5.0 × 10 <sup>-7</sup> – 1.0 × 10 <sup>-5</sup> (peak 2)	1.7 × 10 <sup>-8</sup> (peak 1) 2.2 × 10 <sup>-7</sup> (peak 2)	Tyszczyk-Rotko and Bęczkowska 2015
GCE/MWCNT	SWV	1.0 × 10 <sup>-5</sup> – 5.0 × 10 <sup>-4</sup>	3.52 × 10 <sup>-9</sup>	Gupta et al. 2013
GCE/P4VP-MWCNT	DPV	2.0 × 10 <sup>-6</sup> – 2.0 × 10 <sup>-4</sup>	1.19 × 10 <sup>-9</sup>	Ghadimi et al. 2016
GCE/DNA-WCNT/Nafion	SWASV	5.0 × 10 <sup>-7</sup> – 1.0 × 10 <sup>-4</sup>	1.0 × 10 <sup>-7</sup>	Wang et al. 2014
GCE/MnFe <sub>2</sub> O <sub>4</sub> @CNT-N	SWV	1.0 × 10 <sup>-6</sup> – 1.1 × 10 <sup>-3</sup>	0.83 × 10 <sup>-6</sup>	Fernandes et al. 2015
Lt/fMWCNT/MGCE	DPV	1.0 × 10 <sup>-5</sup> – 1.1 × 10 <sup>-4</sup>	3.54 × 10 <sup>-6</sup>	Amiri-Aref et al. 2014
poly(AV3B)/MWCNT-GR/GCE	DPV	1.0 × 10 <sup>-6</sup> – 1.2 × 10 <sup>-4</sup>	1.0 × 10 <sup>-7</sup>	Wang et al. 2016
BDDE	DPV	4.0 × 10 <sup>-7</sup> – 2.5 × 10 <sup>-5</sup>	1.5 × 10 <sup>-7</sup>	Švorc et al. 2012
BDDE	DPV	5.0 × 10 <sup>-7</sup> – 8.3 × 10 <sup>-5</sup>	3.5 × 10 <sup>-8</sup>	Lourencão et al. 2009
Bi/Nafion/BDDE	DPV	1.0 × 10 <sup>-8</sup> – 2.0 × 10 <sup>-5</sup>	2.8 × 10 <sup>-9</sup>	Sadok et al. 2016
BiF/SPCE	DPV	1.0 × 10 <sup>-7</sup> – 2.0 × 10 <sup>-5</sup>	2.7 × 10 <sup>-8</sup>	Tyszczyk-Rotko and Szwagierek 2017
SPCE/CNFs	DPAdSV	2.0 × 10 <sup>-7</sup> – 1.0 × 10 <sup>-6</sup>	5.6 × 10 <sup>-8</sup>	This work

GCE glassy carbon electrode, GO graphene oxide, PbFE lead film electrode, MWCNT multiwall carbon nanotubes, P4VP poly(4-vinylpyridine), DNA-SWCNT DNA-functionalized single walled carbon nanotube, MnFe<sub>2</sub>O<sub>4</sub>@CNT-N N-doped carbon nanotubes functionalized with MnFe<sub>2</sub>O<sub>4</sub> nanoparticles, Lt/fMWCNT functionalized multi-walled carbon nanotubes and luteolin, poly(AV3B)/MWCNT-GR poly(Alizarin Violet 3B)/multi-walled carbon nanotubes and graphene, BDDE boron doped diamond electrode, BiF/SPCE screen-printed carbon electrode modified with bis-muth film, SPCE/CNFs screen-printed carbon electrode coated with carbon nanofibers, DPV differential-pulse voltammetry, SWV square wave voltammetry, SWASV square wave anodic stripping voltammetry, DPAdSV differential-pulse adsorptive stripping voltammetry

middle of this range. However, the presented in this paper method does not require complicated modifications of the working electrode.

The repeatability of the voltammetric measurements was checked by three repeated scans at each caffeine concentration from the linear range of calibration curve. The relative

**Table 3** Determination of caffeine in beverage samples at the SPCE/CNFs

Beverage sample	Claimed [mmol L <sup>-1</sup> ]	Found ± SD [mmol L <sup>-1</sup> ]	Relative error [%]
Energy drink	1.65	1.66 ± 0.0047	0.6
Cola drink	0.52	0.52 ± 0.0031	0

SD standard deviation for n = 3

standard deviation (RSD) was changed from 1.6 to 3.0%, which confirms a good precision of the proposed method.

### 3.6 Analytical applications

In order to evaluate the possibility of using the proposed voltammetric procedure for caffeine determination on SPCE with the use of differential-pulse adsorptive stripping voltammetry in real samples, an analysis of popular beverages was carried out. The caffeine content in the tested beverages was determined using the standard addition method. The results are presented in Table 3. The results recorded with use of the proposed voltammetric method and SPCE/CNFs confirm the possibility of using it for the determination of caffeine levels in beverages containing caffeine in the presence of interferents.

## 4 Conclusions

This paper presents method of caffeine determination using differential-pulse adsorptive stripping voltammetry at a new integrated three-electrode screen-printed sensor with carbon/carbon nanofibers working electrode. This method is simple, fast, sensitive and does not require a long-lasting sample preparation and complicated modifications of the working electrode. The presented method has been successfully applied to analyse the caffeine content in beverages. It should also be mentioned that the use of commercially available modern miniature voltammetric sensors allows the analysis of biologically active substances without the need to transport the sample to a laboratory.

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