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Voltammetric determination of mixtures of caffeine and chlorogenic acid in beverage samples using a boron-doped diamond electrode

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ABSTRACT

Herein, a boron-doped diamond (BDD) electrode that is anodically pretreated was used for the simultaneous determination of caffeine (CAF) and chlorogenic acid (CGA) by cyclic and adsorptive stripping voltammetry. The dependence of peak current and potential on pH, scan rate, accumulation parameters and other experimental variables were studied. By using square-wave stripping mode after 60 s accumulation under open-circuit voltage, the BDD electrode was able to separate the oxidation peak potentials of CAF and CGA present in binary mixtures by about 0.4 V in Britton–Robinson buffer at pH 1.0. The limits of detection were 0.107 μg mL^{-1} (5.51×10^{-7} M) for CAF, and 0.448 μg mL^{-1} (1.26×10^{-6} M) for CGA. The practical applicability of this methodology was tested in commercially available beverage samples.

1. Introduction

Caffeine (CAF), 3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione, also known as 1,3,7-trimethylxanthine (Fig. 1 A), is an naturally occurring alkaloid widely distributed in natural products, commonly used as a flavoring agent in a variety of beverages. CAF may be also considered as the most widely used drug in the world. It is consumed daily in coffee, tea, cocoa, chocolate, some energy or soft drinks, as well as in drug formulations for the treatment of asthma, nasal congestion, headache or to improve athletic endurance and facilitate weight loss. CAF is also representing a mild stimulant for central nervous system, muscle, heart and circular systems of the human body [1]. It is generally associated with improvements in alertness, learning capacity and exercise performance when moderately consumed. However, drinking large amounts of CAF or taking sufficiently high doses may cause many undesired symptoms and even potentially adverse effects on health, especially for infants and children, such as agitation, chills, irritability, loss of appetite, weakness, insomnia, hypertension, gastrointestinal problem, fever, delusions, tachycardia and even death [2,3]. It has been also reported coma and death in cases of CAF overdose (> 200 mg/day) [4]. CAF is also known to aggregate with polyphenolic compounds. This association can also alter the mouth feel of beverages.

Polyphenolic compounds are widely found in the plant kingdom, and they play an important role in plant resistance and growth regulation [5]. The main polyphenol dietary sources are fruit and beverages and, to a lesser extent, vegetables, dry legumes, and cereals. In food and beverages, they contribute to the oxidative stability and organoleptic characteristics (e.g., bitterness, flavor, color, odor and astringency). Moreover, they have been shown to have multiple biological and pharmacological properties including anti-inflammatory, antitumor, antiviral and antibacterial effects [6–8]. These properties are attributed mainly to their powerful antioxidant and antiradical activity, which is related to the redox properties of polyphenols [9]. Main groups of these bioactive compounds are phenolic acids and flavonoids. Phenolic acids include benzoic acid and cinnamic acid and their derivatives. Chlorogenic acid (CGA), (1*S*,3*R*,4*R*,5*R*)-3-{[(*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy}-1,4,5-trihydroxycyclohexane-1 carboxylic acid (Fig. 1B), a member of hydroxycinnamic acid class, is an ester of quinic acid and caffeic acid. CGA is a prominent polyphenol compound found in considerably amounts in coffee beans and varying forms of coffee as well as in many other fruits and vegetables of human diet, such as apple, pear, peach, plum, apricot, cherry, blueberry, strawberry, eggplant, tomato and potato [10]. This compound has also been found to inhibit the release of glucose into the blood and appears to help people lose weight [11].

With respect to the above mentioned facts, the determination of CAF and CGA is important not only for the quality (taste and health benefit of the product) control of final product in different industries

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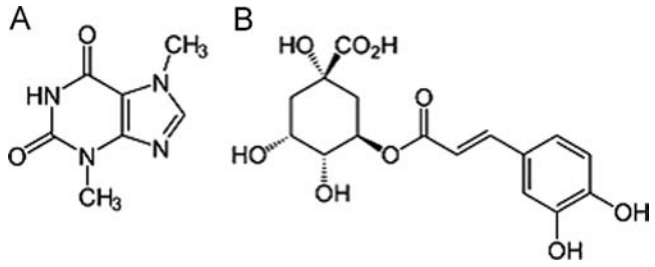

Fig. 1. Chemical structures of the CAF (A), and CGA (B).

(e.g., food, agriculture, drug and cosmetic), but also in order to study their biological effects on the human body. To date, a number of studies have been published for the individual or multicomponent analysis of CAF and/or CGA in various matrices (natural sources, marketed food products, drugs, biological fluids, etc.) covering a broad spectrum of instrumental techniques. Many recent publications include applications of high-performance liquid chromatography with different detectors and column types [12–18], micellar electrokinetic chromatography and capillary electrophoresis [19–22], gas chromatography [23], thin-layer chromatography and high-performance thin-layer chromatography [24–26], UV–vis spectrophotometry [27,28], infrared spectroscopy with Fourier transformation [29,30], and NMR spectroscopy [31]. Chemiluminescence technique has frequently been employed for analysis of CGA using a batch experimental set-up (no convection) or under flow conditions, namely flow injection, HPLC and CE [32–35].

Of the aforementioned methods, the most popular and widely used are separation techniques especially high-performance liquid chromatography and capillary electrophoresis. Generally, some of these methods are considered as being highly sensitive (limits of detection up to 10^{-11} M) and selective, but they are long lasting, expensive and often too laborious, when some procedures such as derivatization, extraction and purification are included. Besides, they require skilled personnel manipulating sophisticated instrumentation. On the other hand, despite the easy availability of photomers, colorimeters or single beam spectrophotometers, spectral methods suffer from disadvantages such as complicated and time-consuming sample preparation and as well as oftentimes lower sensitivity of analysis without preconcentration step.

Electroanalytical methods, particularly the voltammetric ones, satisfy many of the requirements for such tasks, particularly owing to their simplicity, fast response, low cost, satisfactory sensitivity and more selectivity to matrix effects in comparison with separation and spectral methods. The electroanalysis of CAF has been discussed in more detail by Švorc in a review appeared in the literature recently [36]. Although, there are few studies available for the individual or simultaneous voltammetric determination of CAF when using conventional bare electrodes [37,38], a large number of papers for this purpose involve the use of modified electrodes [39–44]. The majority of the studies carried out using voltammetric techniques have been based on the oxidation of CGA in order to determine the antioxidant capacity of this compound, like other polyphenolic compounds, by using the oxidation peaks, at bare [45–47] or chemically modified electrodes [45,48] in several kinds of matrices (foodstuff, beverages, processed food and biological samples). Some other voltammetric investigations have been conducted in order to detect or estimate the individual content of CGA [45,49] or total content of polyphenols [50]. Alternatively, biologically modified electrodes (enzyme-based and DNA-based sensors) have been proposed for the above purposes [51–56].

Despite the fact that CAF and CGA are oxidizable organic compounds, however, bare electrode materials have rarely been used for their analysis. The main reason is the problem of high oxidation potential of CAF where oxygen evolution current interferes, and electrode surface fouling and regeneration for CGA since the oxidation of phenolic compounds produces phenoxy radicals which couple to form a passivating polymeric film on the electrode surface. Various chemically modified electrodes have been developed to solve these problems. However, the disadvantage of these types of electrodes is in their preparation. In most cases, the processes of modifying bare electrodes are often complicated, time-consuming and inconvenient, and the prices of modifying substances are usually high. Furthermore, the surface stability and reproducibility of these electrodes are not always good. The boron-doped diamond (BDD) is a novel carbon-based material which has received much attention in last twenties years for electroanalysis [57,58], thanks to its commercial availability and advantageous electrochemical and mechanical properties. For voltammetric techniques a low and stable background current, a wide working potential window in aqueous media, and high resistance to deactivation by fouling are the most important ones. However, the analytical performance of BDD electrodes greatly depends on their surface termination (e.g. hydrogen or oxygen terminated) [59]. Recently, the use of BDD electrode has been reported for CAF quantification individually in beverage samples [60–62] as well as simultaneously in combination with some compounds commonly found in analgesic and antipyretic formulations [63–65]. Concerning CGA, however, no literature data were found on its electrochemical behavior at BDD electrode, except in a recent work of our group dealing with the estimation of its antioxidant capacity in coffee samples [66]. It is important to remark that, in the revised bibliography, it has not been found any voltammetric method in which CAF and CGA were determined simultaneously. On the other hand, voltammetric determination of caffeine simultaneously in combination with other compounds in beverage samples has not yet been reported.

Keeping the above knowledge in mind, and in continuation of our earlier reports on the voltammetric methodology established by means of BDD electrode for the determination of naturally occurring bioactive compounds, such as indole-3-acetic [67], capsaicin [68], vanillin [69] and rutin [70] in plants and foodstuffs, the present study is intended to demonstrate the possibility of using BDD electrode without any chemical modifications for the determination of CAF and CGA separately as well as simultaneously, with an eye to possible practical applications in several commercial beverages.

2. Experimental

2.1. Chemicals

All reagents were of analytical grade; specifically, CAF and CGA from Sigma (USA). Stock solutions of 2.0 mg mL^{-1} CAF and 1.0 mg mL^{-1} CGA were prepared by dissolving in water. On the day of the experiment working solutions were prepared by diluting with a selected supporting electrolyte. Three different supporting electrolytes, namely acetate (0.1 M, pH 4.8), phosphate (0.1 M, pH 2.5) and Britton–Robinson (0.1 M, pH 1–6) buffer solutions were used. The Britton-Robinson buffer solution was prepared from a mixture of phosphoric acid, acetic acid and boric acid, with all components at concentration of 0.1 M and adjusting to the required pH value with hydrochloric acid (0.2 M) or sodium hydroxide (0.2 M). The prepared stock solutions were preserved at 4 °C when not in use and protected from daylight during use in the laboratory. Aqueous solutions were prepared with deionized water further purified via a Milli-Q unit (Millipore).

2.2. Apparatus

All voltammetric measurements were carried out using a μAutolab type III (EcoChemie, The Netherlands) potentiostat/galvanostat

controlled with the GPES 4.9 software. For a better and clearer identification of the original peaks, the experimental square-wave (SW) voltammograms presented were baseline-corrected using the Savicky and Golay filter (level 2) and a moving average application (peak width=0.01 V) of the GPES software. Cyclic voltammetry (CV) was employed for preliminary studies on the electrochemical behavior of CAF and CGA. Square-wave adsorptive stripping voltammetry (SW-AdSV) was used for the development of electroanalytical methodology.

A classical three-electrode cell of volume 10 mL was used with a BDD working electrode (Windsor Scientific Ltd.; Ø: 3 mm, diameter), a platinum wire auxiliary electrode and an Ag/AgCl (3 M NaCl) reference electrode (Model RE-1, BAS, USA) to which all electrode potentials hereinafter are referred. BDD electrode was activated at the beginning of each working day in 0.5 M H_2SO_4 by applying a potential of +2.0 V for 180 s. Between individual measurements an activation program was used for 30 s under the same experimental conditions. The pretreatment procedure was carried out in an independent electrochemical cell.

All pH measurements were made on a digital pH meter a WTW inoLab pH 720 pH-meter with a combined electrode (glass-reference electrodes).

2.3. Analytical procedure

The general procedure adopted for obtaining adsorptive stripping voltammograms was as follows: The required aliquots of the CAF and/or CGA working solutions were placed into the voltammetric cell containing selected supporting electrolyte (Britton–Robinson buffer) at a desired pH (pH 1.0). The previously treated electrode was placed in the cell, and a selected accumulation potential (open-circuit) was then applied for a selected preconcentration period (60 s) at a stirring speed of 400 rpm. After an equilibration time of 10 s, the voltammogram was recorded, while the potential was scanned from +0.2 to +1.8 V using SW modulation with 10 mV scan increment, 40 mV pulse amplitude, and a frequency of 100 Hz.

Successive measurements were carried out by repeating the above assay protocol on the working electrode. All measurements were performed in triplicate. Quantifications for samples were performed by means of the calibration curve method from the related regression equation. The described method was validated for the parameters such as linearity, limits of detection (LOD) and quantification (LOQ), precision and accuracy. The LOD and LOQ values were calculated on using following equations: LOD=3 s/m; LOQ=10 s/m where s is the standard deviation of the response (three runs) of the lowest concentration of the linearity range and m is the slope of the related calibration curve. To determine the precision, standard solution mixture with 2.0 μg mL^{-1} CAF and 20.0 μg mL^{-1} CGA (medium concentrations of their linearity ranges) were analyzed ten times within the same day (intra-day variation) and on four different days (inter-day variation). Data were reported as the mean values, standard deviations (SD) and relative standard deviations (RSD %). All data were obtained at room temperature (~25 °C).

2.4. Treatment of commercial beverage sample

Samples of commercial instant coffee, cola soft and energy drinks were purchased from the local supermarket, and briefly stored at room temperature until submitted to the sample preparation procedure. All samples examined were analyzed shortly after opening. Coffee solutions were prepared by dissolving 1 g of coffee powder in 100 mL of boiling water. The sample solution was placed in an ultrasonic bath for 30 min to complete dissolution. In order to fit into the linear range, the sample was diluted with the Britton–Robinson buffer (pH 1.0). The dilution process can actually help in reducing the matrix effect of real sample. An aliquot volume of 250 μL of the final solution was transferred to a voltammetric cell already containing 10 mL of the above mentioned supporting electrolyte and analyzed in the day of preparation. In the case of the samples of cola and energy drink beverages, the content of three bottles was mixed thoroughly. After sonical elimination of gas, 150 μL aliquots of the samples (without any previous dilution or extraction) were transferred into the voltammetric cell containing 10 mL of Britton–Robinson buffer (pH 1.0).

3. Results and discussion

3.1. Electrochemical behavior of CAF and CGA on BDD electrode

The oxidation behavior of these compounds was first studied by CV without an accumulation step (t_{acc}=0 s) obtained with anodically pretreated BDD electrode. Fig. 2 shows the CV curves of single component of 350 μg mL^{-1} CGA (curve a) and 60 μg mL^{-1} CAF (curve b) in Britton–Robinson buffer pH 1.0 solution recorded within the potential window from +0.2 and +1.8 V at a scan rate of 100 mV s^{-1}. As can be seen, both compounds were oxidized on anodically pretreated BDD electrode yielding single oxidation process at +0.83 V and +1.51 V, respectively, and the obtained voltammograms presented an irreversible behavior. The oxidation mechanism of CAF and CGA has already been elucidated. Briefly, the overall process involves four electrons and four protons for CAF [60,62] whereas CGA is oxidized to corresponding *o*-quinone by a one step, two electron redox reaction including protonation [45,52,66].

The electrochemical response of BDD electrode is strongly affected by the type of pretreatment applied to the surface before experiments. Thus, this effect is big importance in the case of electroanalytical studies. Although BDD electrodes are known to be resistant to fouling, a preliminary conclusion indicated that slight fouling occurred at BDD electrode without pretreatment during especially CGA oxidation, and thus a way to restore the initial activity of the BDD electrode surface was necessary. Three different cleaning procedures were considered. First, the electrode was treated by mechanical cleaning (polishing manually with alumina, 0.01 μm/water slurries on felt pads). A second procedure consisted in a cathodic cleaning (−2.0 V for 180 s). Finally, an anodic cleaning program (+2.0 V for 180 s) was applied. In order to decrease the background current, the acidic media of 0.5 M H_2SO_4 was used for both electrochemical cleanings. Cathodically pretreated BDD electrode presented more positive oxidation peak potential and lower current intensity for both compounds than those obtained on the other procedures. By applying mechanical

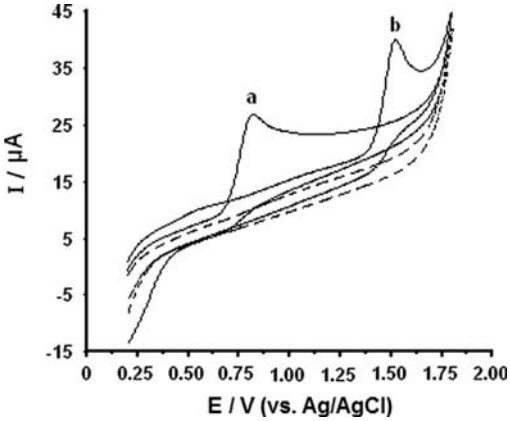

Fig. 2. Cyclic voltammograms of 350 μg mL^{-1} CGA (curve a) and 60 μg mL^{-1} CAF (curve b). Electrode, BDD; supporting electrolyte, Britton-Robinson buffer pH 1.0; scan rate, 100 mV s^{-1}. Dashed lines represent background current.

polishing, anodic peak potential was displaced to much lower positive values and the magnitude of the peak current reached a maximum. However, this procedure is not usual for BDD electrode because microcrystals of diamond can be damaged or removed from the electrode surface. On the other hand, anodically pretreated BDD presented a best peak definition and more intense current signal than that on cathodically pretreated one. Additionally, highest reproducibility of the measurements was obtained with the anodic activation procedure. Consequently, the anodic pretreatment was chosen. This pretreatment, which was repeated daily before starting the voltammetric measurements, was always preceded by a 30 s anodic pretreatment, which cleansed the electrode surface by oxidizing any adsorbed contaminant.

Next, the effect of scan rate on the voltammetric response of CAF and CGA oxidation was investigated under the above experimental conditions. The oxidation peak currents increased with a positive shift in the potential when the scan rate increased, a typical characteristic of irreversible electrochemical reactions. The current response (i_p) was linearly proportional to the square root of scan rate ($v^{1/2}$) within the range 50–500 mV s^{-1} according to the relationships:

$$i_p(\mu A) = 1.26v^{1/2}(mVs^{-1}) + 1.03, r = 0.999 \text{ (for CAF)}$$

$$i_p(\mu A) = 1.02v^{1/2}(mVs^{-1}) + 1.53, r = 0.996 \text{ (for CGA)}$$

In addition, the plots of log i_p of the peak current versus log υ were straight lines with slopes of 0.48 (r=0.999) for CAF and 0.43 (r=0.996) for CGA, which are close to the theoretical value of 0.5 that is expected for ideal reaction of solution species. All these facts pointed toward the diffusion-controlled nature of the electrode process for both compounds.

However, the plot of i_p versus v was also linear expressed by the equations:

$$i_p(\mu A) = 0.042\nu(mVs^{-1}) + 9.25 \ (n = 6, r = 0.989) \text{ (for CAF)}$$

$$i_p(\mu A) = 0.034\nu(mVs^{-1}) + 8.12 \ (n = 6, r = 0.994) \text{ (for CGA)}$$

This demonstrates that in spite of there being a diffusive process, there is also adsorption component (more effective for CGA) in the electrochemical process. This phenomenon may be attributed to the partial involvement of the diffusive compound molecules in the electrode reaction of the adsorbed ones. Thus, the electrochemical process has a mixed control for both compounds.

After this previous study, to identify the adsorptive character of the compounds on BDD electrode, the effect of accumulation potential (E_{acc}) and time (t_{acc}) was investigated individually for 5 μg mL^{-1} CAF or 25 μg mL^{-1} CGA in Britton–Robinson buffer (pH 1.0) within the potential range +0.2 to +1.8 V (data not presented). For this purpose, AdSV response was examined using the SW excitation waveform due to intense sensitivity with high speed, and reducing problems with blocking of the electrode surface. The dependence of the stripping peak current on the accumulation potential was evaluated either at open-circuit condition or over the potential range from +0.2 to +0.8 V for CGA and from +0.2 to +1.1 V for CAF in stirred solution with an accumulation time of 60 s. The maximum values for the stripping current were obtained at +0.2 and +0.3 V which were nearly equal to the value obtained at open-circuit voltage. Hence, an accumulation potential of open-circuit condition was used throughout the present study. After fixing the accumulation potential at this value, the accumulation time was varied between 0 and 120 s. The peak current of CAF increased with time up to 60 s and then leveled off. In the case of CGA, the peak current increased at a fast rate up to about 60 s then rather slowly between 60 s and 120 s. However, considering the speed of the measurement, 60 s was deemed to be the optimum accumulation time for preconcentration prior to stripping.

The attention was then turned to the influence of changing the pH upon the AdSV performance for both compounds. It is worth noting that CGA is not stable to high pH whereas it is stable to acid pH [71]. On the other hand, reported studies have demonstrated that low pH has a significant influence on the oxidation of CAF [60,63–65]. Keeping the above knowledge in mind, various supporting electrolytes at pH values from the range 1.0–6.0 including acetate, phosphate and Britton-Robinson buffer solutions were tested by carrying out stripping measurements on 10 μg mL^{-1} CAF and 50 μg mL^{-1} CGA separately, with an open-circuit mode at 60 s. For both CAF and CGA oxidation, increasing the pH of the supporting electrolyte, the magnitude of this current was decreased (data not shown). The voltammetric responses for both compounds were characterized by well-defined oxidation peaks and highest analytical signals in the case of Britton−Robinson buffer equal to pH 1.0. Then, this supporting electrolyte was used for the subsequent development of an electroanalytical procedure.

Before recording analytical curve for CAF and CGA determination, the experimental SWV parameters such as frequency (f), scan increment (ΔE_s) and pulse amplitude (ΔE_{sw}) that affect stripping response were optimized. The ranges studied were: f=25–150 Hz,

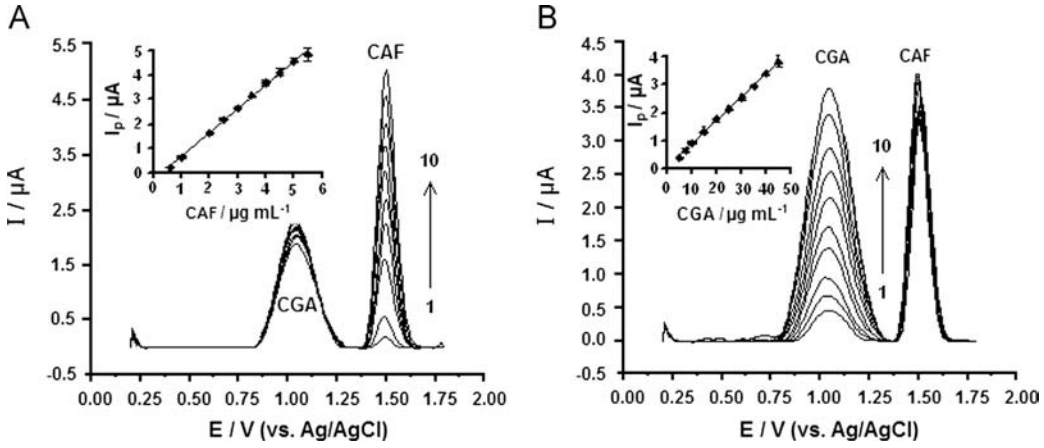

Fig. 3. SW stripping voltammograms for various concentrations of CAF (A) and CGA (B) at fixed concentrations of 20.0 μg mL^{-1} CGA and 5.0 μg mL^{-1} CAF, respectively. CAF concentrations (1–10): 0.60–5.5 μg mL^{-1}. CGA concentrations (1-10): 5.0–45.0 μg mL^{-1}. Insets: the respective analytical curves for CAF (A) and CGA (B) (error bars are constructed for three measurements). Electrode, BDD; supporting electrolyte, Britton-Robinson buffer pH 1.0; Preconcentration period, 60 s at open circuit condition; SWV parameters: frequency, 100 Hz; step potential, 10 mV; pulse amplitude, 40 mV.

ΔE_s=4–14 mV and ΔE_{sw}=10–60 mV. For entire analysis the optimized values were: *f*, 100 Hz; ΔE_s, 10 mV; and ΔE_{sw}, 40 mV.

3.2. Analytical applications

Having characterized the response of both CAF and CGA on the anodically pretreated BDD electrode, well-defined peaks presented a good peak-potential separation (more than 0.4 V), using Britton–Robinson buffer at pH 1.0, and optimized accumulation time of 60 s at open-circuit voltage. To further investigate the voltammetric response when CAF and CGA co-exist, SW-AdSV behavior of CAF (or CGA) was tested in the presence of a large excess of CGA (or CAF).

Initially, for the separate determination of CAF, its concentration was varied in the range 0.6–5.5 μg mL^{-1} in solutions containing CGA at the fixed concentration of 20 μg mL^{-1} (Fig. 3A). Similarly, keeping the concentration of CAF was constant at 5 μg mL^{-1}, the determination of CGA was accomplished in the concentration range

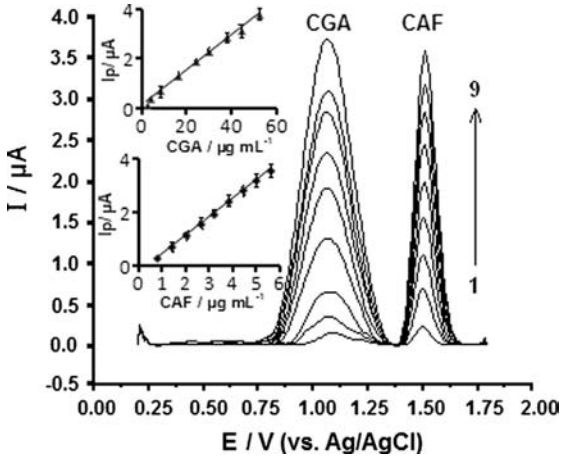

Fig. 4. SW stripping voltammograms of CAF and CGA. The concentrations of both compounds were changed simultaneously (1–9). CAF concentrations: 0.8–5.6 μg mL^{-1}. CGA concentrations: 2.0–52.0 μg mL^{-1}. Insets: the respective analytical curves for CAF and CGA (error bars are constructed for three measurements). Other operating conditions as indicated in Fig. 3.

5.0–45.0 μg mL^{-1} (Fig. 3B). As seen from Fig. 3A and B, stripping current for CAF and CGA increased regularly as their corresponding concentration was increased, respectively, while the one of the compound kept at constant concentration did not change. The analytical curves shown in the insets of Fig. 3 depict linear responses for both compounds according to the regression equations:

$$i_p(\mu A) = 0.988C\ (\mu g\ mL^{-1}) - 0.315\ (r = 0.998, n = 9)\ (\text{forCAF})$$

$$i_p(\mu A) = 0.084\ C\ (\mu g\ mL^{-1}) + 0.032\ (r = 0.999, n = 9)\ (\text{for CGA})$$

where i_p is the adsorptive stripping peak current, C concentration of compounds. From the data obtained by the analytical curves, LOD was achieved as 0.067 μg mL^{-1} (4.49×10^{-7} M) for CAF, and 0.630 μg mL^{-1} (1.79×10^{-6} M) for CGA. The obtained results showed that neither CAF nor CGA interfere with the oxidation signals of each other, indicating that their responses are independent.

After this initial study, CAF and CGA were determined by simultaneously changing their concentrations (Fig. 4). By analyzing the insets in Fig. 4, one can concluded that the corresponding analytic curves presented good linear responses in the concentration 0.8–5.6 μg mL^{-1} (4.12×10^{-6} M – 2.88×10^{-5} M), and 2–52 μg mL^{-1} (5.64×10^{-6} M – 1.47×10^{-4} M) for CAF and CGA, respectively. Using the stripping peak currents at potentials of +1.50 and +1.08 V, the respective calibration equations are

$$i_p(\mu A) = 0.695\ C(\mu g\ mL^{-1}) - 0.266(r = 0.999, n = 9)\ (\text{for CAF})$$

$$i_p(\mu A) = 0.071C(\mu g\ mL^{-1}) + 0.097(r = 0.998, n = 9)\ (\text{for CGA})$$

From these plots, LOD and LOQ were calculated as 0.107 μg mL^{-1} (5.51×10^{-7} M) and 0.357 μg mL^{-1} (1.84×10^{-6} M) for CAF while they were found to be 0.448 μg mL^{-1} (1.26×10^{-6} M) and 1.49 μg mL^{-1} (4.20×10^{-6} M) for CGA, respectively. These regression equations show that the sensitivity of anodically pretreated BDD electrode towards CAF is nearly 3 times higher than that towards CGA. Some examples of the voltammetric applications published previously for determination of these compounds are presented in Table 1. Comparison of the results shows that the sensitivity in terms of LOD for CAF and CGA obtained on BDD

Table 1
Comparison of the efficiency of the BDD electrode with literature electrodes for voltammetric determination of CAF and CGA.

Analyte	Electrode	Modifier	Technique	LOD (μM)	Sample	Reference
Caffeine						
CAF+some other alkaloids	EA-GCE	n.a.	DPV	0.02	Drugs, Urine	[37]
CAF+ASA	EPPGE	n.a.	SWV	0.008	Coffee, Drugs, Urine	[38]
CAF	GCE	Nafion	DPV	0.798	Cola	[39]
CAF	GCE	Nafion/MWCNTs	DP-AdSV	0.513	Cola, Drugs	[40]
CAF	GCE	CTAB/GR	DPV	0.091	Soft drink	[41]
CAF+PAR	GCE	Cu^{II}TAPcSAM	DPV	0.03	Cola, Drugs, Serum	[42]
CAF	CPE	MIP	DPV	0.015	Tea	[43]
CAF	CCE	SWCNT	DPV	0.12	Beverages	[44]
CAF+CGA	BDD	n.a.	SW-AdSV	0.551	Beverages	This work
Chlorogenic acid						
CGA (total antioxidant activity)	GCE	MWNT	DPV	0.21	Coffee	[48]
CGA	Au	MIP	DPV	0.148	Coffee, Tea	[49]
CGA	CPE	Ir- BMI.PF_6-PPO	SWV	0.918	Coffee	[52]
CGA	CPE	$[Cu_2(\mu\text{-OH})(bpbpmp\text{-}NO_2)]_2[ClO_4]_2$	SWV	0.8	Coffee	[56]
CGA+CAF	BDD	n.a.	SW-AdSV	1.26	Beverages	This work

Compound: CAF, caffeine; ASA, acetyl salicylic acid; PAR, paracetamol; CGA, chlorogenic acid. Electrode: GCE, glassy carbon; EA-GCE; electrochemically activated glassy carbon; EPPGE, edge plane pyrolytic graphite; CPE, carbon paste; CCE, carbon ceramic; BDD, boron-doped diamond; Au, gold. Modifier: MWCNT, multi-walled carbon nanotube; CTAB, cetyltrimethylammonium bromide; GR, graphene; CuIITAPcSAM, self-assembled monolayer of non-peripheral amine substituted copper(II) phthalocyanine;; MIP, molecular imprinted polymer; SWCNT, single-walled carbon nanotube; Ir- BMI.PF6-PPO, ionic liquid containing iridium nanoparticles and polyphenol oxidase; H2bpbpmp-NO2, the ligand 2-[N-bis-(2-pyridylmethyl)aminomethyl]-4-methyl-6-[N′-(2-pyridylmethyl)(2-hydroxy-5-nitrobenzyl)aminomethyl]phenol; Technique: DPV, differential pulse voltammetry; SWV, square-wave voltammetry; AdSV, adsorptive stripping voltammetry. Other: LOD, limit of detection for caffeine or chlorogenic acid; n.a. not applicable.

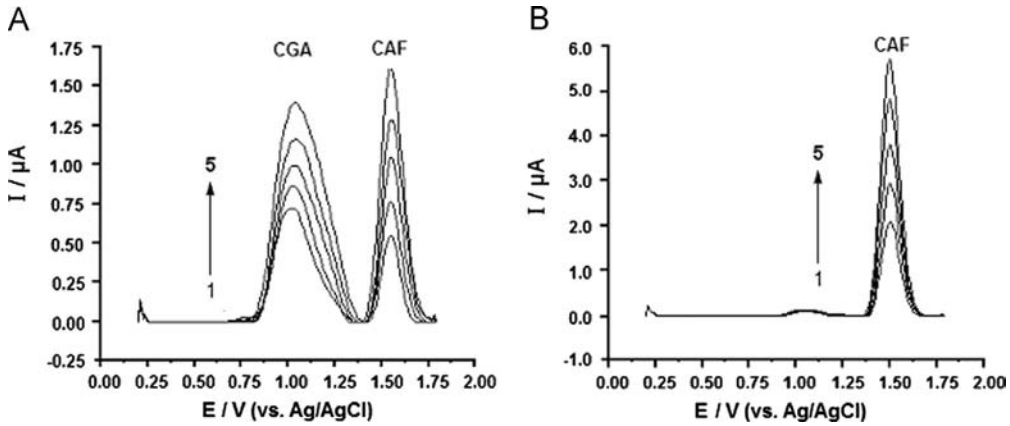

Fig. 5. (A) SW stripping voltammograms of diluted samples of coffee extract before (curve 1) and after standard additions of 0.8–2.6 and 2.0–8.0 μg mL^{-1} CAF and CGA, respectively, (curves 2–5). (B) SW stripping voltammograms of energy drink sample before (curve 1) and after standard additions of 1.0–4.0 μg mL^{-1} CAF (curves 2–5). Other operating conditions as indicated in Fig. 3.

electrode without any series of chemical modification steps are slightly lower or quite similar with respect to those reported earlier for some modified electrodes.

For intra-day and inter-day precision, the RSD values were calculated to be 3.09% and 5.92% for CAF, and 3.98 and 6.74% for CGA, respectively, which are acceptable for practical applications.

It is noteworthy to underline once again that CGA is unstable in alkaline solutions [71]. All working solutions (in strongly acidic medium) used for the validation experiments were freshly prepared, protected from light and used within 10 h.

In order to evaluate the selectivity of the proposed method, increasing concentrations of the possible interfering agents such as some ions and phytochemicals which are usually present in both fluids and edible plants samples were added to a solution mixture with 2.0 μg mL^{-1} CAF and 20.0 μg mL^{-1} CGA, and the corresponding voltammograms were recorded. The tolerance limit was defined as the maximum concentration of potential interfering substance that causes a change in the signal of ±5% for the determination of both compounds. The results showed that a 100-fold of Na^+, Cl^-, NO_3^-, Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, Fe^{3+}, and 50-fold of glucose, fructose, and sucrose had almost no influences on the peak currents and potentials of CAF and CGA. However, ascorbic acid and vanillic acid did significantly interfere with their current response. Nevertheless, interferent-to-analyte concentration ratio is much less in studied examples. On the other hand, the detection of CGA in the presence of other hydroxycinnamic acids (caffeic acid, *p*-coumaric acid, ferrulic acid) and hydroxybenzoic acids (gallic acid) was complicated even when an interferent-to-analyte ratio of 1:1 by the fact that all compounds have similar redox centers that can undergo electrochemical oxidation on the surface. Therefore, the experimental results confirmed that voltammetric signals observed in real samples have been assigned not only to the individual oxidation of CGA but also to the combined effects of other naturally occurring phenolic compounds.

In order to confirm the performance of the proposed methodology in real samples, its applicability was tested in three different samples of commercial beverages (instant coffee, cola and energy drink) frequently consumed in Turkey. Following the sample preparation, that is quick and easy, the voltammetric procedure under the experimental conditions was carried out. Representative voltammograms of coffee and energy drink samples are shown in Fig. 5. A well-defined oxidation peak at about +1.55 V, and two at about +1.10 and +1.55 V were observed after transference of the samples of energy drink and coffee, respectively, with peak heights proportional to the sample addition. The peaks appeared at +1.55 V (Fig. 5 A and B) can be assigned to the oxidation of CAF, since multiple standard additions of CAF exhibited a concomitant increase in the peak currents without any distortion of the peak potential. Although instant coffee contains relatively high amount of CGA, during processing of this type coffee, it is observed a partial decay of CGA with formation of caffeic acid [14]. Thus, it should be mentioned at this point that the intensity of the oxidation peak at about +1.10 V is associated with the synergistic or additive effect of other hydroxycinnamic acids (especially caffeic acid) present in this coffee sample (i.e. as the sum of their peak intensities). As can be seen from Fig. 5 A, this peak height also increases by adding standard solutions of CGA which indicates that total polyphenolic content can be quantified from the standard addition of CGA, without the specific determination of this compound. On the other hand, in neither energy drink nor cola any peak could be observed corresponding to the oxidation of polyphenolics (Fig. 5B).

Table 2
CAF and CGA recovery data in the samples of coffee extract and energy drink based on SW-AdSV assay using BDD electrode.

CAF			CGA_		
Added (μg mL^{-1})	**Found[a] (μg mL^{-1})**	**Recovered (%) ± RSD (%)**	**Added (μg mL^{-1})**	**Found[a] (μg mL^{-1})**	**Recovered (%) ± RSD (%)**
Coffee			*Coffee*		
0.0	1.27		0.0	7.34	
0.8	2.03	95.0 ± 2.7	2.0	9.21	93.5 ± 3.9
1.4	2.85	112.0 ± 3.3	4.0	12.02	117.0 ± 2.2
2.0	3.40	106.5 ± 3.2	6.0	12.76	90.3 ± 1.9
2.6	3.77	96.2 ± 1.8	8.0	15.99	108.1 ± 1.1
Energy drink			*Energy drink*		
0.0	2.53		0.0	n.d.[b]	
1.0	3.71	117.5 ± 1.2	–		
2.0	4.54	100.5 ± 1.3	–		
3.0	5.48	98.3 ± 1.7	–		
4.0	6.40	96.7 ± 1.0	–		

[a] Average of three measurements.
[b] n.d. = not detected.

To check the validity of the proposed method, the spike/recovery experiments were performed. To do so, the appropriate amount of standard CAF and CGA was added to the matrices of commercial samples and the voltammetric responses were evaluated. In Table 2, the results of the analysis of spiked samples of coffee and energy drink are shown, which indicates that there were no important matrix interferences for the samples analyzed by the proposed voltammetric method. As shown in Table 3, the coffee sample contained both compounds whereas CGA was not detected in cola

Table 3
Results of the analysis of CAF and estimated polyphenol content in beverage samples based on SW-AdSV assay using BDD electrode.

Sample	CAF	Total phenolics (as CGA equivalent)
Coffee[a,b]	25.5 ± 1.82	146.8 ± 3.35
Energy drink[a,c]	160.1 ± 5.54	n.d.[d]
Cola[a,c]	119.1 ± 1.92	n.d.[d]

[a] Average of three measurements.
[b] mg g^{-1} ± SD.
[c] mg L^{-1} ± SD.
[d] n.d. = not detected.

and energy drink beverages. Total polyphenolics determined in coffee sample were expressed in CGA equivalents. The results of CAF obtained with coffee and cola samples were found to be in accordance with the legal limits of Turkish Food Codex [72] and with the amount reported in the label by the manufacturer (for instant coffee, 25–54 mg g^{-1}; for beverages, ≤ 150 mg L^{-1}). In the case of energy drink, caffeine content is slightly higher than the Turkish standard. However, it should be important to say that it is difficult to compare the content of these compounds from various type of coffee, cola and energy drinks because it may vary depending on several factors such as the type of the brand, roasting process (coffee), packing and storage conditions as a canned or bottled beverage, environmental variables namely climate (temperature, light and water), etc.

4. Conclusions

It should be mentioned once again that this paper, to our knowledge, is the first report on the determination of mixtures of CAF and CGA by a voltammetric technique. The proposed methodology is based on the use of anodically pretreated BDD electrode in combination with the SW-AdSV technique. Compared to those obtained with the modified electrodes for analysis of these substances individually or simultaneously in combination with other compounds, comparable or slightly less analytical sensitivity of the unmodified BDD electrode (except electrochemical pretreatment) makes this electrode very promising for simultaneous determination of caffeine and the estimated polyphenol content in chlorogenic acid equivalents in beverage samples, which may find application in industry sector.

Voltammetric procedure has the disadvantage of individual determination of CGA in case of analyzing natural sources (vegetables and plants) or food products. However, taking into account that in many cases, it is more important to measure the total content of polyphenolic compounds than to determine each of them individually, electrochemical measurements using less complicated apparatus and inexpensive material compared with chromatographic techniques, could be suitable as a quick control test for global amount of polyphenolics and their antioxidant activities in real samples. Besides, CGA oxidation at a BDD electrode could be used for the more sensitive and individual detection of this compound after chromatographic separation.

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