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Simple electrochemical sensor for caffeine based on carbon and Nafion-modified carbon electrodes

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Abstract

A simple, economic, highly sensitive and highly selective method for the detection of caffeine has been developed at bare and Nafion-modified glassy carbon electrodes (GCE). The electrochemical behavior of caffeine was examined in electrolyte solutions of phosphate buffer saline, sodium perchlorate, and in choline chloride plus oxalic acid, using analytical determinations by fixed potential amperometry, phosphate buffer saline being the best. Modifications of the GCE surface with poly(3,4-ethylenedioxythiophene) (PEDOT), Nafion, and multi-walled carbon nanotubes were tested in order to evaluate possible sensor performance enhancements, Nafion giving the most satisfactory results. The effect of interfering compounds usually found in samples containing caffeine was examined at GCE without and with Nafion coating, to exclude interferences, and the sensors were successfully applied to determine the caffeine content in commercial beverages and drugs.

Keywords: caffeine; glassy carbon; Nafion coating; modified electrodes; food analysis

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1. Introduction

Caffeine (3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione or 1,3,7 -trimethylxanthine) is the active alkaloid component, together with other trace purines, of coffee, cola nuts, cocoa beans, tea leaves, yerbamate, guarana berries, amongst many varieties of plants, in which it acts as a natural pesticide (Clark, 1985). Caffeine is also the most pervasive drug in modern society, a constituent of coffee and tea and is added to many soft drinks. Even though some drugs containing caffeine together with other active substances have been discontinued, due to lack of evidence of the therapeutic utility of its association with other active components or because some associations have been found to have unwanted effects, caffeine is still used in the pharmacological preparation of analgesics (Derry C., Derry S., & Moore, 2012), diet aids (Westerterp-Plantenga, Lejeune, & Kovacs, 2005), and cold/flu remedies. Ingested caffeine undergoes extensive biotransformation in humans, and generates at least 17 detectable urinary metabolites, including theobromine (3,7-dimethylxanthine), paraxanthine (1,7-dimethylxanthine), theophylline (1,3-dimethylxanthine) and 1,3,7-trimethylurate (Nakajima, et al., 1994).

Caffeine is a stimulant of the central nervous system, affecting alertness and wakefulness (Nehling, Daval, & Debry, 1992). It also acts as a vasoconstrictor, increasing blood pressure (James, 2004), stimulating gastric secretion (Boekema, et al., 1999) and increasing respiration cycles, but may also cause emesis and dehydration, being a powerful diuretic (Maughan, & Griffin, 2003). It can mobilize calcium from cells leading to bone mass loss (Heaney, 2002) and is considered a risk factor for cardiovascular diseases (Nehling, Daval, & Debry, 1992).

The development of reliable methods for the evaluation and quantification of caffeine in real samples is thus an active field of research. Among the different methods that have been developed, the more advantageous are chromatographic (Srdjenovic, et al., 2008). However,

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63 they are generally expensive and require sample purification, so that simple, cheap and faster
64 methods are being investigated. Some recent electrochemical detection methods for caffeine
65 have been reported. These include using boron-doped diamond electrodes (BDD) (Švorc, et
66 al., 2012), Nafion-modified BDD (Martínez-Huitle, et al., 2010), cathodically-pretreated BDD
67 electrodes (Lourenção, et al., 2009), 1,4-benzoquinone or molecularly imprinted polymer
68 modified carbon paste electrodes (Aklilu, Tessema, & Redi-Abshiro, 2008; Alizadeh, et al.,
69 2010), Nafion/carbon nanotube (Yang, et al., 2010; Zhang et al., 2011) or Nafion/graphene
70 modified electrodes (Sun, et al., 2011, Zhao, et al., 2011), carbon fibre ultramicroelectrodes
71 (Nunes, & Cavalheiro, 2012), and polymer modified glassy carbon electrodes (GCE) (Amare,
72 & Admassie, 2012). One report appeared on caffeine detection at a Nafion-modified glassy
73 carbon electrode, the Nafion being used to both decrease the caffeine oxidation potential, so
74 as not to overlap with oxygen evolution, and increase electrode sensitivity (Brunetti,
75 Desimoni, & Casati, 2007). The benefits of using Nafion in electrode modification for more
76 sensitive caffeine detection when carried out in sulphuric acid solution have been attributed
77 mainly to pre-concentration in the Nafion polymer layer (Brunetti, Desimoni, & Casati, 2007;
78 Martínez-Huitle, et al., 2010).

79 This paper reports the use, for the first time, of bare GCE and Nafion-coated GCE for the
80 determination of caffeine, Nafion being used mainly to avoid the influence of negatively
81 charged interferents in real samples. The effect of pH on both peak current and peak potential
82 led to the proposal of a new oxidation mechanism and to choice of the optimal pH for sensor
83 operation. The usefulness of this fast, simple and practical analytical method is demonstrated
84 in caffeine detection in a number of commercial beverages and drugs.
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2. Experimental

2.1 Reagents and solutions

Caffeine was purchased from Sigma-Aldrich and fresh solutions of 0.10 M caffeine were prepared daily in water. The phosphate buffer saline solution (PBS) was constituted by di-sodium hydrogen phosphate (Na_2HPO_4), monobasic sodium phosphate (NaH_2PO_4), and sodium chloride (NaCl), purchased from Sigma-Aldrich. Nafion (5 % v/v) was from Aldrich. Choline chloride ($C_5H_{14}ClNO$) was purchased from Sigma and sodium perchlorate monohydrate ($NaClO_4$) was obtained from Merck. Buffer solutions employed had pH values from 3.0 up to 9.9. Buffer electrolyte solutions, 0.1 M, pH 3, 4, 5 were prepared by mixing HAcO + NaAcO, pH 6, 7, 8 from NaH_2PO_4 + Na_2HPO_4 and pH 9 and 9.9 from $NaHCO_3$+NaOH.

The monomer 2,3-dihydrothieno[3,4-b]-1,4-dioxin (EDOT) was from Aldrich. The solution used for the EDOT polymerisation contained 0.01 M of monomer dissolved in 0.1 M 4-styrenesulfonic acid sodium salt hydrate (NaPSS) (Aldrich).

Multi-walled carbon nanotubes (MWCNT) were purchased from NanoLab, USA.

Ascorbic acid, glucose, sucrose and fructose used in interference tests were purchased from Sigma, citric acid from Merck and sucrose from Panreac.

All chemicals were of analytical grade and used without further purification. Solutions were all prepared with Millipore Milli-Q nanopure water (resistivity $\geq$ 18 MΩ cm).

Experiments were performed at room temperature, 25 ± 1ºC.

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2.2 Electrochemical measurements and apparatus

A one-compartment 10 mL electrochemical cell contained a 2 mm diameter (geometric area 0.031 cm^2) glassy carbon electrode (GCE) as working electrode, a platinum wire auxiliary electrode and a saturated calomel electrode (SCE) as reference.

Electrochemical measurements were performed using a computer-controlled μ-Autolab Type II potentiostat/galvanostat (Metrohm-Autolab, Utrecht, Netherlands) running with GPES (General Purpose Electrochemical System) for Windows version 4.9 software.

The pH-measurements were done with a CRISON 2001 micro pH-meter.

2.3 Preparation of the modified GCE

The GCE surface was cleaned by polishing with diamond spray 1-μm particle size (Kemet International, UK) on a polishing cloth.

2.3.1 Electropolymerisation of EDOT

For electropolymerisation of EDOT, a 0.01M monomer solution was freshly prepared by dissolving the monomer in 0.10 M NaPSS, heating until complete monomer dissolution. EDOT was electropolymerised by potential cycling between −0.6 and +1.2 V vs. SCE for 10 cycles at a scan rate of 50 mV s^{-1}, a procedure optimised previously (Kahkhi, et al., 2012). PEDOT films were allowed to dry in air at room temperature, for at least 24 h, before use.

2.3.2. Modification with Nafion

A solution of 0.25% w/v Nafion was prepared by dissolving the required volume of Nafion®(5% w/v) in ethanol solution. A volume of 2 μL of this solution was dropped on top of the GCE and allowed to dry for at least 1 h. The modified electrode was then used directly,

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or a volume of 1 µL of 99.5% w/v dimethylformamide (DMF) was dropped on top of the Nafion/GCE. In this case, the electrode was then allowed to dry for a further1 h.

2.3.3. Modification with MWCNT

A mass of 60 mg of MWCNT was stirred in 10 mL of a 5 M nitric acid solution for 24 h. The solid product was collected, filtered and washed several times with pure water until the filtrate solution became pH 6.0. The activated MWCNTs obtained were then dried in an oven at 100 °C for 24 h.

For the dispersion of MWCNT, an aqueous solution of 1% (v/v) acetic acid was prepared in which chitosan was dispersed by agitation during 2 h to obtain a 1% (w/v) chitosan solution. The functionalised MWCNTs were dissolved in this chitosan solution, with a loading of 1% w/v of MWCNT. The dispersion was then immersed in an ultrasound bath for 2 h, to ensure a homogeneous mixture. The surface of the GCE was modified with the MWCNT dispersion, by drop-casting, and left to dry for 24 h before use.

2.4. Sample preparation

The samples used for the determination of caffeine, 3 different pharmaceutical preparations and 3 different types of beverages, were purchased locally.

Tablets of Ilvico®, Gurosan® and Dolviran®, as well as a sachet of Nescafé®, were diluted in water; the corresponding molar concentrations of these solutions were calculated and then a chosen volume of each directly added to the measurement cell. The beverages were used as purchased, without any other preparation, a chosen amount of each being added to the cell.

3. Results and discussion

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The voltammetric behaviour of caffeine was investigated at bare GCE, in three different electrolyte solutions, in order to choose the best medium. Following this, several sensor architectures involving surface modification with PEDOT, Nafion or MWCNT were tested in order to choose the electrode configuration with the best analytical properties. The reason for choosing these three modifiers will be given below in Section 3.2. The effect of interfering compounds on the sensor response to caffeine was assessed and measurements in commercial samples of beverages and drugs were carried out.

3.1. Evaluation of different media on sensor sensitivity

The voltammetric behaviour of caffeine at the bare GCE was first examined by cyclic voltammetry (CV). The CV scan presents an anodic peak at a high potential around +1.25 V vs SCE, and the absence of a cathodic peak on the reverse scan, indicating that the oxidation is irreversible, see Fig. 1.

The electrochemical behavior of caffeine may be influenced by the nature of the electrolyte solution. In order to evaluate the effect of different media on the sensor response, phosphate buffer saline, sodium perchlorate, and choline chloride solutions were tested and the analytical parameters in these media were obtained. Differential pulse voltammetry (DPV), a more sensitive analytical voltammetric technique than CV, was used to construct calibration curves, first in 0.1 M NaPBS pH 7.0 buffer solution since it mimics the medium of biological samples, such as serum. DPV scans for increasing concentrations of caffeine are presented in Fig. 2a with the corresponding calibration curve in inset. The optimum DPV conditions were found to be: 4 mV step potential, amplitude of 25 mV, scan rate 10 mV s^{-1} (data not shown), chosen to be applied in all further experiments. The sensitivity of the sensor was 170±7 µA cm^{-2} mM^{-1} (RSD 4.2%, n=6) and the detection limit 38.9±3.7 nM (RSD 9.5%, n=6).

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178 The second solution tested was sodium perchlorate, which has oxidizing properties and is
179 extremely soluble, even in organic solvents (Urbansky, 1998). Some previously reported
180 caffeine sensors operated in perchloric acid media (Alizadeh, et al., 2010; Švorc, et al., 2012).
181 DP voltammograms in 0.1 M $NaClO_4$, pH 5.9 and the corresponding calibration curve are
182 shown in Fig. 2b. The sensor sensitivity was 102±6 μA cm^{-2} mM^{-1} (RSD 5.6%, *n*=3), lower
183 than in 0.1 M NaPBS pH 7.0 and the detection limit was higher being 118±8 nM (RSD 6.8%,
184 *n*=3). Furthermore, no complexes are formed in solution, since the sensor exhibits a linear
185 increase in peak current with increasing concentration of caffeine.

186 Choline chloride (ChCl), a quaternary amine salt, which dissociates in water into the
187 corresponding positively charged quaternary hydroxyl alkylammonium ion and Cl^-, was also
188 tested. ChCl mixed together, in certain ratios, with organic acids, acting as proton donors,
189 such as oxalic, phthalic and formic acids are considered to be deep eutectic solvents, and have
190 been used in sensor applications, also being successfully applied in metal electrodeposition
191 e.g. (Golgovici, & Visan, 2012). A solution of 0.05 M ChCl + 0.05 M oxalic acid was
192 therefore chosen to evaluate sensor caffeine sensitivity. DP voltammograms for increasing
193 caffeine concentrations are shown in Fig. 2c, the sensor exhibiting a sensitivity of 151±7
194 (RSD 4.5%, *n*=3) μA cm^{-2} mM^{-1} which is higher than in perchlorate solution, but lower than
195 in 0.1 M NaPBS, and with a detection limit of 60.0±4.9 nM (RSD 8.2%, *n*=3).

196 In all media the DP calibration plot of caffeine is linear up to at least 7.0 mM caffeine, the
197 highest concentration tested. The highest sensitivity and lowest detection limit were found in
198 0.1 M NaPBS pH 7.0 solution, which was thus chosen for further studies.

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200 *3.2. The influence of different surface modifications on sensor performance*

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Different GCE surface modifications were done in order to assess possible enhancements of sensitivity of the caffeine sensor, namely PEDOT, Nafion and MWCNT, using DP voltammetry.

PEDOT conducting polymer is very attractive for use in sensors due to its high conductivity and good stability under ambient conditions (Crispin, et al., 2006). Electropolymerisation of EDOT was carried out by potential cycling from a solution containing 0.01 M EDOT dissolved in 0.1 M NaPSS, as in (Kahkhi, et al., 2012). The caffeine sensor sensitivity using PEDOT/GCE was almost three times lower, 57.6±3.4 μA cm^{-2} mM^{-1} (RSD 5.9%, *n*=3), than with bare GCE and the detection limit was higher, being 116±6 nM (RSD 4.9%, *n*=3). One of the possible justifications for the decrease in sensor sensitivity is the positive potentials needed that can cause over-oxidation and deterioration of the polymer.

Nafion is normally used to enhance sensor selectivity by electrostatic repulsion of unwanted species, especially anions, as well as minimising adsorption. Nafion/GCE as well as DMF/Nafion/GCE were prepared, DMF being used as a stabilizer of Nafion films (Gouveia-Caridade & Brett, 2005). DMF decreased the response to caffeine slightly so it was decided to use Nafion alone. The sensitivity, 176±8 (RSD 4.6%, *n*=3), was the same as at the bare GCE (170±7 μA cm^{-2} mM^{-1}), but the detection limit, of 128±6 nM (RSD 4.8%, *n*=3), was significantly higher. In this pH 7.0 phosphate buffer, there was no increase in the signal in the presence of Nafion as had been observed by (Brunetti, Desimoni, & Casati, 2007; Martínez-Huitle, et al., 2010) in sulphuric acid solution, which they attributed to pre-concentration of caffeine in the polymer layer. Nevertheless, coating with Nafion/GCE can be important to reduce interferences, for example in the measurement of caffeine in commercial samples in which the amounts are well above the detection limit, where the presence of ascorbate interferes in the detection of caffeine at the bare GCE (see Section 3.7).

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Experiments were also performed with MWCNT-modified GCE in order to evaluate possible sensitivity enhancement, but such a sensor could only measure caffeine in very acidic solutions of 0.1 M H_2SO_4 pH 1.1, as occurred in (Yang, et al., 2010) when the pH was 2.0, and displayed a non-linear response, the main reason probably being adsorption of caffeine inside the MWCNT structure.

As conclusion, the unmodified GCE exhibited the best analytical properties, the use of Nafion/GCE being advised when the sensor is used to detect caffeine in real samples containing ascorbate, in order to reduce its interference.

3.4. Influence of solution conditions on caffeine oxidation at GCE

The influence of pH on the oxidation peak potential and peak current of caffeine was investigated in buffer electrolyte solutions in the pH range from 3.0 to 9.9, all containing 0.5 mM caffeine.

Differential pulse voltammograms showed only a slight dependence on pH, as observed in previous work at carbon electrodes (Mersal, 2012) with a peak potential of around +1.30 V vs. Ag/AgCl. The value of the DPV half peak width, $\Delta E_{p/2}$, was found to be between 95 and 115 mV and values of E_p-$E_{p/2}$, extracted from cyclic voltammograms at different scan rates, shown in Fig.3a, were between 60 and 70 mV. Thus it can be deduced that 2 electrons are involved in the first step. The full oxidation mechanisms involves two oxidation steps, the second being a $2e^-$ 2H+ oxidation, involving overall 4 e^- and 4 H^+ (Nunes, & Cavalheiro, 2012; Mersal, 2012; Spataru, et al., 2002; Sun, et al., 2011).

The peak current value is slightly influenced by the pH value of the solution, increasing from pH 3.0 to 7.0, and then decreasing at higher values of pH. DP voltammograms, in solutions of pH higher than 8.0, have a broad oxidation wave, so accurate determination of caffeine was

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not possible. The results underline the advantage of this sensor that can be employed over a broad pH range, between 3 and 8.

Consequently, further measurements were performed in NaPBS pH 7.0, since at this pH the caffeine response was the highest.

3.5. Effect of scan rate

The influence of the scan rate in cyclic voltammetry on the oxidation peak current of caffeine was evaluated by recording CVs at different scan rates from 10 to 200 mV s^{-1} in 0.1 M NaPBS pH 7.0, containing 0.5 mM caffeine, see Fig. 3a. As seen in Fig. 3b, the anodic peak currents were linearly proportional to the square root of the scan rate following the linear regression equation $I_{pa} = 0.02+ 3.11\ v^{1/2}$ (I_{pa} in µA, v in V s^{-1}, R = 0.997), so it can be deduced that the electrochemical oxidation of caffeine at GCE is a diffusion-controlled process.

For scan rates higher than 50 mV s^{-1}, the anodic peak potential is slightly shifted towards more positive values with increase in scan rate, following the equation $E_{pa} = -5.2+ 1.7*\ln(v)$ (E_{pa} in V and v in Vs^{-1}), signifying a quasi-reversible process.

Square wave voltammetry was also performed in 0.1 M NaPBS containing 0.5 mM caffeine, by varying the frequency between 20 and 80 Hz, corresponding to scan rates between 51 to 204 mV s^{-1} (data not shown). The peak current increases linearly with square wave frequency, again characteristic of irreversible reactions.

3.6. Comparison of the sensor with the literature

Table 1 shows a comparison of caffeine sensors with similar surface modifications as those tested here. For example, a Nafion/MWCNT composite film-modified electrode had a much higher detection limit of 0.23 µM, a narrower linear range only up to $4.0x10^{-4}$ M, and the

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sensitivity was 125.2 μA cm^{-2} mM^{-1}, also lower than the values exhibited by the bare GCE caffeine sensor in this work (Yang, et al., 2010).

Caffeine sensors using the Nafion modified GCE had a much higher detection limit of 790 nM, compared with 38.9 nM here, the sensitivity of the sensor not being specified (Brunetti, Desimoni, & Casati, 2007; Zhang, et al., 2011). A GCE based on MWCNT covered with Nafion had a higher sensitivity and lower detection limit than the above, no response being recorded at bare GCE (Zhang, et al., 2011), and graphene oxide-Nafion had even higher sensitivity but a low linear range upper limit (Zhao et al., 2011) . A sensor based on 4-amino-3-hydroxynaphthalene sulfonic acid (AHNSA) (Amare, &Admassie, 2012) exhibited the highest sensitivity, but in very acidic media, 0.1 M HNO_3, the linear range being narrower, and the detection limit higher than that obtained in this work. A carbon paste electrode reported by was used at pH 2.7 for detection of caffeine at +1.5 V, with a LOD of 0.35 μM (Mersal, 2012).

The main advantages of the sensor developed in this work are the significantly lower detection limit, a very wide linear range and the fact that it exhibits good performance in solutions of pH between 3 and 8, the best being at pH 7.0. Other sensors mostly work in very acidic media: sulphuric acid (Brunetti, Desimoni, & Casati, 2007, Martínez-Huitle, et al., 2010; Sun, et al., 2011, Yang, et al., 2010), nitric acid (Amare, &Admassie, 2012) or perchloric acid (Alizadeh, et al, 2010; Švorc, et al., 2012). Few articles report the use of buffer solutions, for example pH 6.0 or 7.4 respectively (Aklilu, Tessema, & Redi-Abshiro, 2008; Nunes, & Cavalheiro 2012).

3.7. Interferences

An evaluation of possible interferences to the caffeine sensor operation was performed. The species tested were ascorbic acid, citric acid, fructose, glucose and sucrose, usually found in

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beverages and drugs together with caffeine. Two different interfering compound:caffeine concentration ratios, 1:1 and 1:2, were tested.

DPV curves were recorded in in 0.1 M NaPBS pH 7.0 containing 0.4 mM of caffeine, and again after the injection of the interfering compound (in a ratio of 1:1 and 1:2 of interfering compound:caffeine). For both ratios 1:1 and 1:2, ascorbate (AA) interfered with the caffeine response, increasing the overall oxidation peak current with 50 and 100 % respectively, see Table 2. However, using the Nafion/GCE, ascorbate is repelled by the Nafion film, and the caffeine response in the presence of AA is very close to 100%.

The consumption of caffeine is often associated with the addition of common sugars, such as sucrose, glucose and fructose. These sugars were tested as possible interferents. All sugars led to a slight decrease in the sensor response, Table 2, probably because the formation of a sugar-caffeine complex (Tavagnacco, et al., 2012).

3.8. Measurements in commercial samples

The amount of caffeine in six commercial beverages and drugs, described in *Section 2.4*, was measured at GCE and at Nafion/GCE in order to demonstrate the applicability of the proposed method. The Nafion/GCE electrode was mostly employed to reduce the interference from ascorbate, present in high concentration in the Guronsan® sample. It also has the effect of reducing the effects of blocking adsorption by other components of complex matrices.

The standard addition method was used in which an aliquot of the samples was injected into the buffer electrolyte followed by known amounts of caffeine. The results are presented in Table 3, and, as can be seen, they are in good agreement with the labelled values on the analysed products. As observed by comparing the caffeine concentration values at the GCE and Nafion/GCE, at Nafion/GCE the concentrations were lower than the labelled ones, the use of bare GCE being more accurate, except for the Guronsan® sample with a large amount of

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ascorbate. It is to be noted that Ilvico® contains paracetamol and ascorbate, Guronsan® ascorbate (six times the amount of caffeine) and glucuronamide, and Dolviran® acetylsalicylic acid and codeine. Thus, except from ascorbate, the other electroactive compounds, nor the other components of the beverages, do not interfere with the response at bare electrodes. The use of Nafion/GCE may only be needed for detection of caffeine in samples containing large amounts of ascorbate, unless there are large amounts of other adsorbable compounds present.

The results obtained demonstrated again the reliability of this simple, cheap, fast and easy method for caffeine detection.

4. Conclusions

A simple caffeine sensor based on differential pulse voltammetry at a bare GCE or Nafion-coated GCE when it is necessary to avoid interferents has been developed. Other surface modifications with PEDOT and or MWCNT did not lead to an increase in sensor performance. The best response of the sensor was achieved in 0.1 M NaPBS pH 7.0. Interference studies showed that ascorbate interfered with caffeine detection, the use of Nafion overcoming this problem. The sensor presents a very high sensitivity of 170±7 μA cm^{-2} mM^{-1}, a lower detection limit than other caffeine electrochemical sensors (38.9±3.7 nM) and the largest linear range, at least up to 7 mM. Drugs and beverages containing caffeine were analysed without any special pre-treatment and the results are in excellent agreement with the labelled values.

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438 **Tables**

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441 **Table 1.** Comparison of analytical parameters for caffeine determination with caffeine sensors in the literature

Electrode type	Solution pH	Linear range upper limit / mM	Sensitivity /μA cm^{-2} mM^{-1}	LOD /μM	*E* / V vs Ag/AgCl	Ref.
[1]BQMCPE	-	8.0	28.8	5.10	+1.45	Aklilu, Tessema, &Redi-Abshiro, 2008.
Nafion/MWCNT	2.0	0.40	125.2	0.23	+1.33	Yang, et al., 2010
MWCNT-Nafion/GCE	4.1	2.4	491.1	0.51	+1.34	Zhang, et al., 2011
[2]GO-Nafion/GCE	~2.0	0.080	2327	0.20	+1.45	Zhao, et al., 2011
Poly([3]AHNSA)/GCE	5.0	0.040	6384	0.14	+1.34	Amare, &Admassie, 2012
Nafion/GCE	≈1.0	0.011	-	0.79	+1.45	Brunetti, Desimoni, &Casati, 2007
Carbon paste electrode	2.7	1.0	255.8	0.35	+1.50	Mersal, 2012.
This work	7.0	7.0	169.7	0.04	+1.32	this work

442
443 [1] BQMCPE-1,4-benzoquinone modified carbon paste electrode; [2] GO – graphene oxide; [3]AHNSA- 4-Amino-3-hydroxynaphthalene sulfonic acid;
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447 **Table 2**. Interference effects of some compounds on caffeine sensor response.

Interferent species	Sensor response in the presence of interfering compound / %			
	1:1		**1:2**	
	GCE	**Nafion/GCE**	**GCE**	**Nafion/GCE**
Fructose	98.2	98.4	96.9	97.6
Sucrose	93.8	94.0	103.8	101.3
Glucose	95.6	95.8	97.4	98.5
Citric Acid	104.2	100.0	101.9	100.0
Ascorbic Acid	150.3	104.0	200.8	107.1

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450 **Table 3.** Determination of caffeine concentration in commercial samples

Sample	Labeled / µM	Obtained at bare GCE / µM	Obtained at Nafion/GCE / µM
Ilvico	166.7	156.4	150.1
Guronsan	128.1	323.5	125 3
Dolviran	173.8	166.8	159.3
Redbull	137.5	128.6	101.3
Coca-cola	113.8	109.2	103.9
Nescafé	233.2	229.5	220.3

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453 **Figure Captions**

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455 **Figure 1:** Cyclic Voltammograms (CV) recorded at bare GCE in 0.1 M NaPBS pH 7.0
456 containing 0.0, 0.5 and 1.0 mM caffeine.

457 **Figure 2:** DPVs at GCE for different concentrations of caffeine a) in 0.1 M NaPBS, b) 0.1 M
458 sodium perchlorate, and c) in 0.05 M ChCl + 0.05 M oxalic acid; in inset are the
459 corresponding calibration plots.

460 **Figure 3:** CVs recorded at GCE in 0.1 M NaPBS containing 0.5 mM caffeine at different
461 scan rates from 10 to 200 mV s^{-1} rate and b) the linear dependence of peak current vs. the
462 square root of scan rate.

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463 Figures

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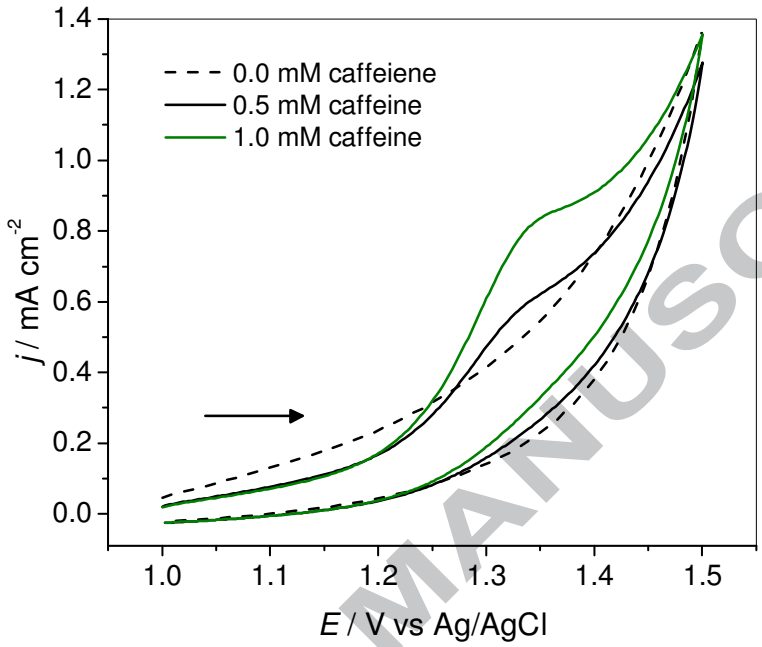

477 **Figure 1:** Cyclic Voltammograms (CV) recorded at bare GCE in 0.1 M NaPBS pH 7.0
478 containing 0.0, 0.5 and 1.0 mM caffeine.

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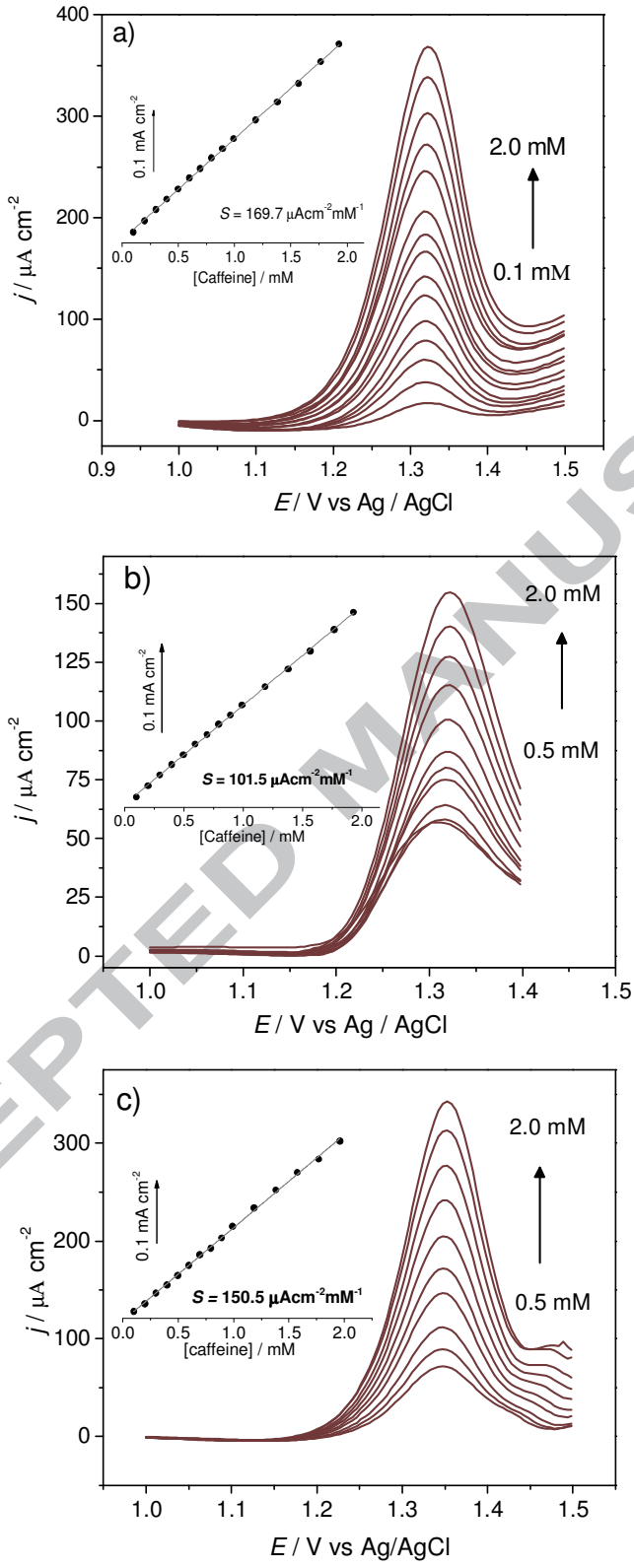

Figure 2: a) DPVs at GCE for different concentrations of caffeine a) in 0.1 M NaPBS, b) 0.1 M sodium perchlorate, and c) in 0.05 M ChCl + 0.05 M oxalic acid; in inset are the corresponding calibration plots.

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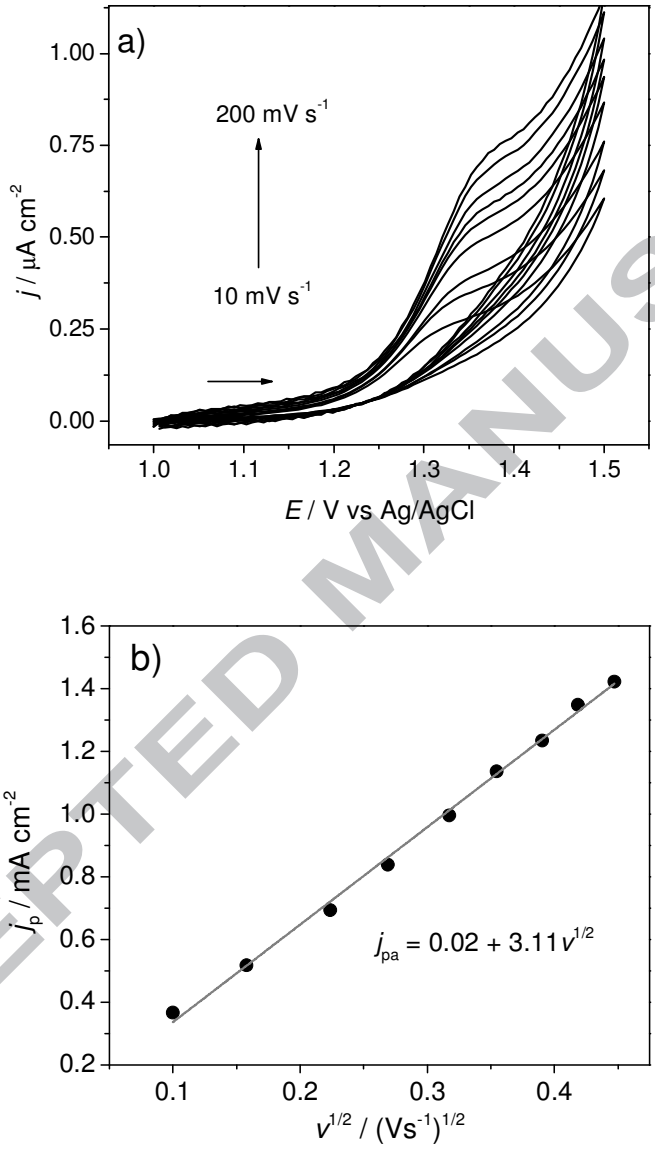

Figure 3: CVs recorded at GCE in 0.1 M NaPBS containing 0.5 mM caffeine at different scan rates from 10 to 200 mV s^{-1} rate and b) the linear dependence of peak current vs. the square root of scan rate.

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536 **HIGHLIGHTS**

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538 Simple electrochemical sensor for caffeine, superior to more complex sensor platforms

539 Electrode modification strategies and analysis medium optimised

540 Sensor based on bare or Nafion-modified glassy carbon gives best results

541 Successful interference-free application to beverages and drugs

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