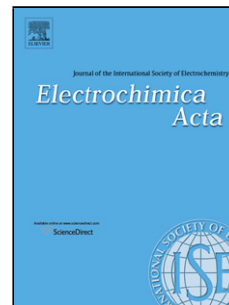


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Boron-doped diamond electrodes explored for the electroanalytical detection of 7-methylguanine and applied for its sensing within urine samples

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Highlights

- Facile determination of N7-methylguanine (7-mG) by electrochemical methods, which may represent a tool for diagnosis, prevention and the monitoring of diseases.
- SWV provides good resolution of the anodic peak for 7-mG and the main nucleic bases interferences, such as adenine and guanine.
- It is plausible the simultaneous determination of 7-mG and another of the most common modified nucleobases, 8-oxoguanine (8-oxoG).
- 7-mG electrochemical determination within a human urine sample is feasible with linear regression between peak current intensity versus 7-mG concentration.

Abstract

Epigenetic modifications have been associated by many studies with several types of diseases and metabolic dysfunctions. Specifically, N7-methyl modification of guanine (7-mG) is well established to be used as a biomarker for the detection and determination of DNA methylation. The use of an electrochemical sensor has the potential to provide a simpler and more economic sensing methodology for the determination of 7-mG compared to traditionally utilised laboratory based approaches. In this paper we demonstrate the feasibility of an electrochemical sensor which could potentially be easily applied towards the determination of 7-mG within biological samples, such as human urine. A practical electrochemical configuration was employed consisting of a boron-doped diamond electrode (BDD) as the working electrode and a screen-printed graphite electrode (SPE) providing the counter electrode and the reference electrode. With this new protocol, the electrochemical behaviour of 7-mG has been investigated *via* cyclic voltammetry (CV) and square wave voltammetry (SWV) using a BDD electrode with a simplified electrochemical set-up. The electrochemical behaviour of 7-mG within acetate buffer solutions at a BDD electrode has been compared and contrasted to a glassy carbon electrode with the following parameters studied: voltammetric scan rate, solution pH, 7-mG concentration and electrode surface pretreatment. The oxidative mechanism elucidation has been performed at controlled potential and such results have provided the dimer formation as the major product. The simultaneous electroanalytical identification of 7-mG together with the presence of guanine, adenine and 8-oxoguanine has been investigated under the optimum experimental conditions. Furthermore, the feasibility of using a BDD electrode for the detection of 7-mG is explored in a human urine sample.

Keywords: 7-methylguanine; epigenetic modification; urine; electrochemical sensor; Boron Doped Diamond electrode.

1. Introduction

Studies focused upon DNA and the genetic fields have become considerably attractive since they both play an important role within living organisms. DNA and RNA consist of nucleic acids, which are polymers of high amounts of monomers called nucleotides which are linked by phosphodiester bonds forming long linear chains. Its sequence within DNA determines the genetic information and is responsible for both body function and the hereditary transmission. In contrast, nucleotide sequences in RNA determines the expression of proteins, *e.g.* regulatory enzymes [1].

Mutagenic changes in DNA sequences and epigenetic modifications can cause an incorrect DNA replication and transcription, and this is well known to be linked with several types of diseases and metabolic disorders [2–4]. Epigenetic modifications are defined as covalent chemical changes in DNA nucleic bases with no sequence modifications. The most important and common events in epigenetic modifications are oxidation and alkylation [5]. The former can be produced by oxidizing agents, free radicals or ionizing radiation, while the latter is produced by alkylating agents such as alkyl sulfonates, methyl halides, N-nitroso compounds and tobacco-specific nitrosamines [6,7]. There exists some well-known modified nucleobases, called DNA adducts, which its elevated presence in DNA, RNA and biological fluids has been related to certain diseases. Modified nucleobases which are shown in scheme ESI-1 may be the main and most important examples [4] Most of these are commonly generated in humans as a result of unhealthy habits, bad or inappropriate diet and exposure to methylating agents [8]. Consequently, modified nucleobases identification and quantification represents a useful way to find biomarkers for cancer diseases, DNA damage, premature cell ageing, exposure to methylating and toxic agents, oxidative stress, among many others [9–11].

Specifically, 7-methylguanine (N7-methylguanine, 7-mG, 7-MeG, m⁷-Gua) is a methylated form of guanine, which has been relatively less studied than other modified forms. It has always been considered to be innocuous and non-mutagenic due to its chemical instability within the DNA structure and as it is able to participate in Watson Crick base pairing [4,12]. In addition, 7-mG is presented inherently in messenger and transfer RNA where it plays a significant role within gene regulation, providing stability to these molecules [10,11]. However, several studies present a close relationship between high levels (abnormal patterns) of 7-methylguanine (within the DNA, RNA

and biological samples), and the presence of other mutagenic adducts cause the development of pathologies such as carcinogenesis, neurodegeneration, male infertility and problems during assisted reproduction [13]. The advantage of determining 7-mG as a biomarker consists of it is generated in higher amounts than other promutagenic nucleobases and thus this makes simpler its determination [8,12–14].

7-mG (see Scheme ESI-1) is the most common DNA adduct because the N7 atom of guanine moiety is the most reactive to be attacked by alkylating electrophiles as it has the highest negative electrostatic potential of all atoms within DNA nucleobases [4]. Moreover, 7-mG is frequently presented close to 5-methylcytosine in CpG islands (CGI). CpG islands are a Cytosine, Guanine rich region located within the promoter region of many genes. Normal pattern of methylation within CpG islands has an important function in gene regulation. Nonetheless, high amounts (abnormal patterns) of 7-mG levels in CpG islands (Hypermethylation) has been related to the diseases discussed previously. For those reasons, detection of aberrant methylation levels may represent a marker of disease activity [10,15–18].

For the determination of 7-mG, and other modified nucleotides, a variety of analytical techniques have been described in the literature, as follows: immunoassays [19,20], ^{32}P -postlabeling methods [7,20], capillary electrophoresis coupled with electrospray ionization mass spectrometry (CE-ESI-MS) [9], liquid chromatography-tandem mass spectrometry (LC-MS/MS) [12,21–23] and high-performance liquid chromatography coupled with UV-Vis detection [2,3,24,25] and electrochemical detection [11,14,26]. Regarding the electrochemical detection in the case of 7-mG, glassy carbon electrode is normally used, being presented the 7-mG response about 1 V versus standard Ag/AgCl reference electrode. Nevertheless, although these methods are sensitive and accurate, they are tedious and not cost-effective for routine analysis. Furthermore they involve a laborious sample preparation as well as the *in situ* analysis is not possible. As an interesting alternative, the preparation and use of an electrochemical sensor may provide a more economic and cost-effective method for 7-mG determination. Such devices also present the advantage of having a small size, low limit of detection (LOD) and possibility for *in-situ* analysis. Nonetheless, it should be noted that reproducibility and repeatability of these methods is not as high as the relative to the combination between chromatographic, spectroscopic and spectrometric methods. In the case of using an electrochemical approach, our group has recently investigated the voltammetric behavior of 7-mG at screen-printed graphite electrodes

(SPGE) [27] in phosphate buffer solutions. The above pioneering work provided a reasonable sensitivity and a good reproducibility for the electroanalytical detection of 7-mG in buffer solutions. However, the reusability and the validation of the electrochemical sensor based on the SPGE platform using real samples is still lacking, as well as the electro-oxidative mechanism of 7-mG is uncertain.

In this present paper, the electrochemical behavior of 7-mG at boron doped diamond electrode (BDD) and glassy carbon (GC) electrodes has been reported. BDD electrode was considered for this work because of its useful properties which are principally: high thermal conductivity, electrical conductivity, considerable hardness, chemical inertness in harsh conditions, surface with low adsorption properties, low background current and high chemical and electrochemical stability [28]. Furthermore, BDD presents a large voltammetric potential window between oxygen and hydrogen evolution, both in aqueous and non-aqueous media [29–33]. In this case, the electrochemical response of 7-mG has been investigated by cyclic voltammetry (CV) and square wave voltammetry (SWV). Square wave voltammetry is one of the most sensitive and powerful electrochemical techniques applied, whose limits of detection are generally similar to the obtained with the chromatographic and spectroscopic techniques [32,34]. For electroanalytical applications, Scheme 2 depicts the electrochemical configuration used with working solution dropped onto a screen-printed graphite electrode (SPGE) which provides the counter and reference electrode. The electrochemical behavior of 7-mG has been carried out upon pH, scan rate, 7-mG concentration, pretreatment of the electrode surface and the presence of other nucleobases as interfering substances. Preparative oxidative electrolysis of 7-mG at controlled potential using a BDD electrode has been performed for the elucidation of the electro-oxidation of the methylated guanine compared to the unmodified one. Finally, the electrochemical response of 7-mG has been examined within complex fluid such as in real human urine to assess the feasibility of 7-mG determination in a biological complex sample. As mentioned above, the interest lies in the possibility of employing this modified nucleotide as biomarker for early diagnosis of cancer and other diseases by the use of an electrochemical sensor as a complementary tool for other diagnostic methods.

2. Experimental

2.1. Reagents and chemicals.

Free DNA bases (Adenine and Guanine) as well as the modified ones (7-methylguanine and 8-oxoguanine) were purchased from Sigma Aldrich at the highest analytical grade available and were used without further purification. Solutions were prepared using doubly distilled water with a resistivity not less than 18.2 M Ω ·cm. Guanine solutions were prepared in 0.1 M acetate buffer solution of pH 5.0 (Sodium Acetate, Scharlau Chemie S.A., reagent grade) up to saturated conditions and were filtered with a 45 μ m pore filter (Millipore MILLEX-HN) in order to remove excess insoluble guanine. Final guanine concentration was determined by UV-visible spectrophotometry (UV-2401PC, Shimadzu) with an extinction coefficient of 10,700 cm⁻¹ M⁻¹ at 243.0 nm [35]. Unless stated elsewhere, Adenine, 8-oxoG and 7-methylguanine tested in this work were dissolved in 0.1 M acetate buffer solution at pH 5.0. Concentration of these solutions was also determined by UV-visible spectrophotometry using an extinction coefficient of 7,300 cm⁻¹ M⁻¹ at 283.0 nm for 7-mG [3], an extinction coefficient of 13,400 cm⁻¹ M⁻¹ at 261.0 nm for Adenine [36] and 7,762 cm⁻¹ M⁻¹ at 285 nm for 8-oxoguanine [37]. Otherwise, acetate buffer solutions were made up setting pH values with glacial acetic acid (J. T. Backer, 99-100 %) and concentrated NaOH solution (Scharlau Chemie S.A., reagent grade). The pH measurements were carried out with a Crison Micro pH 2000 pH-meter.

2.2. Electrodes and Electrochemical Configuration.

Two different electrochemical cell configurations based on a three-electrode standard electrochemical cell were employed. On one hand, an electrochemical glass cell with a 10 mL volume was used. A polycrystalline boron doped diamond (BDD) film with 3 mm diameter and mounted in polyether ether ketone [PEEK] doped with a 0.1 % of boron (Windsor Scientific Company, UK) was used as the working electrode. For a comparative study, a cylindrical bar of glassy carbon electrode (GC) with a 3 mm diameter (\geq 99.95% purity, Goodfellow) was used as working electrode. A gold wire was used as counterelectrode spirally wound, and the reference electrode (RE) was an Ag/AgCl (3 M KCl) standard electrode set in a separate compartment and connected to the electrochemical cell by a Luggin capillary. On the other hand, for electroanalytical applications, Scheme 1 depicts the electrochemical configuration used with working

solution drop on a screen-printed graphite electrode (SPGE) of about 50 μL . A 3.0 mm diameter graphitic working surface of the SPGE platform was used as the counter electrode and the Ag/AgCl pseudo reference acts as reference electrode. Again, a 3 mm diameter GC or BDD electrodes were the working electrodes. Sample solutions -50 microliters- were dropped onto the SPE platform (scheme 2) before the BDD was introduced into the solution with meniscus. The distance between the BDD working electrode and SPE counter electrode was set at 0.5 cm for all the experiments with the help of a tweezer regulated and fixed to be always at the same distance. Every experiment, BDD electrode was washed with deionized water, while SPE platform was thoroughly washed with 0.1 M acetate buffer solution and then equilibrated with the sample solution before measurements. Electrochemical configuration from Scheme 1 was employed because of its simplicity, and faster experiments (desirable properties in analytical tests) and the handling of low biological sample volumes.

The edge-plane like screen-printed graphite electrodes (SPGE) were fabricated at Manchester Metropolitan University utilising appropriate stencil designs with a microDEK 1760RS screen-printing machine (DEK, Weymouth, UK). For each of the screen-printed sensors a carbon-graphite ink formulation (Product Code: product code: C2000802P2; Gwent Electronic Materials Ltd, UK) was first screen-printed onto a polyester flexible film (Autostat, 250 μm thickness) [38]. This layer was cured in a fan oven at 60 degrees Celsius for 30 min. Next a silver/silver chloride (40:60) reference electrode was applied by screen-printing Ag/AgCl paste (Product Code: C2040308P2; Gwent Electronic Materials Ltd, UK) onto the plastic substrate. This layer was once more cured in a fan oven at 60 degrees Celsius for 30 min. Last a dielectric paste ink (Product Code: D2070423P5; Gwent Electronic Materials Ltd, UK) was printed to cover the connections and define the 3 mm diameter graphite working electrode. After curing at 60 degrees Celsius for 30 min the screen-printed electrode is ready to use. An edge-connector was used to ensure the reproducibility of the electrochemical connections throughout the studies [39]. Similar SPGE platforms have been electrochemically characterized in a previous contribution [40,41].

2.2. *Electrode pretreatments.*

Unless stated otherwise, different working electrodes were polished prior to the electrochemical measurements by using an alumina slurry suspension (Buehler, 0.3 μm

particle size), with deionized water as lubricant, for 5 minutes. BDD electrode was polished only before starting the electrochemical measurements. Then, the electrodes were thoroughly washed with deionized water and immersed into an ultrasonic cleaning bath for 2 minutes removing any alumina residues adsorbed onto the carbonaceous surface. In the case of using the SPE electrochemical configuration, BDD and SPE electrodes were electrochemically conditioned by performing 5 successive cyclic voltammograms within 0.1 M acetate buffer solution pH 5.0 over the range of 0 V and +1.0 V versus pseudo Ag/AgCl. In the case of the SPE configuration, BDD electrochemical conditioning was performed in 0.1 M acetate buffer solution pH 5.0 in order to clean both SPE and BDD electrodes.

Anodic and cathodic electrochemical pretreatments of the BDD electrode were performed to investigate the electrochemical behavior of 7-mG in 0.1 M acetate buffer solution pH 5.0. Both electrochemical pretreatment consisted of cycling the BDD electrode in 1.0 M HNO₃ solution with a 0.1 V/s of scan rate under vigorous stirring conditions. In the case of cathodic pretreatment cycles were recorded between 0 and -2 V and, in the case of anodic pretreatment cycles were recorded between 0 and +2 V, versus Ag/AgCl. After pretreatment, BDD electrode was washed thoroughly with water and finally dried under nitrogen atmosphere. All electrochemical pretreatments were carried out in the conventional three electrodes electrochemical setup using a graphite rod as a counter electrode.

2.3. Experimental Procedure.

CV and SWV measurements were carried out using an Autolab PGSTAT 30 (Eco Chemie, The Netherlands) potentiostat/galvanostat and controlled by Autolab GPES software version 4.9 for Windows XP. All electrochemical experiments were carried out under atmospheric conditions and at room temperature, being the laboratory temperature between 22 and 25°C. SWV parameters used in all experiments were the following: modulation amplitude, 50 mV; modulation frequency, 8 Hz; modulation step, 5 mV. SWV parameters were optimized regarding the current intensity for the electro-oxidation of 7-mG. Different frequencies were examined between 8 and 25 Hz, and even though the higher frequencies resulted to be beneficial, 8 Hz was the optimum frequency allowing a better resolution under the presence of other nucleic bases such as A and G. All potentials are referred to an Ag/AgCl/(3 M KCl) reference electrode in the case of using a three electrodes conventional electrochemical cell and versus Ag/AgCl

pseudo-reference electrode in the case of the configuration with a SPE, as displayed within Scheme 1.

Confidence intervals of the measurements were obtained using the statistical value “t student” (for N-2 freedom degrees, where N is the number of standard solutions used) for a confidence level of 95 %.

2.4. Electro-oxidation of 7-mG. Preparative electrolysis.

Preparative electro-oxidation of 7-mG was performed using a two compartment H-shaped electrochemical cell separated by a Nafion 117 cationic exchange membrane. A bipolar polycrystalline BDD electrode supported on silicon (from Adamant Technologies Switzerland of 2.5 x 4.0 cm) and doped with 700-800 ppm of boron was the anode and the cathode, respectively. The anodic compartment was filled with 50 mL of a 200 μ M 7-mG solution ($2 \cdot 10^{-5}$ moles) in 0.1 M acetate buffer solution pH 5.0. In the same way, the cathodic compartment was filled with 50 mL of a 0.1 M acetate buffer solution pH 5.0. Electrolysis was carried out at controlled potential at 1.2 V versus Ag/AgCl using a Potentiostat/Galvanostat (AMEL Instruments Model 2049 General-Purpose Potentiometer) at room temperature under stirring and aerated conditions. Charge passed was 108 % of the theoretical charge passed assuming an electron transfer of 2 electrons per mole of 7-mG. Solutions exposed to different charges were analysed using a liquid chromatography-mass spectrometry system (LC/MS; Trap SL Agilent 1100). The mobile phase was a mixture of two components [A – water + 0.1% formic acid (JT Baker), and B – acetonitrile (HPLC grade Sharlau) + 0.1% acid formic (JT Baker)]. A C18 commercial column of 250 mm \times 4 mm with 5 μ m particle size (Hysil ODS) was used. An injection volume of 100 μ L was always used for the samples of the electrolyzed solution.

3. Results and discussion.

3.1. Cyclic voltammetric (CV) and SWV behavior of 7-MeG: comparison between BDD and GC electrodes.

First, the comparative study of the electrochemical behaviour of 7-mG between BDD and GC electrodes using a standard three electrode electrochemical cell as described in the experimental section were performed. Both electrodes have been widely employed due to their attractive properties for the study of the electrochemical

behavior of biological compounds [42]. Fig. 1A shows the CV profiles of 50 μM 7-mG in 0.1 M acetate buffer solution pH 5.0 at BDD and GC electrodes. The anodic peak potential is observed at +1.20 V in the case of BDD and at +1.08 V for GC versus Ag/AgCl. For the SWV response of the same concentration of 7-mG, depicted in Fig.1, the anodic peak potential appears at +1.04 V using the BDD and +1.06 V for the GC

As shown within Fig.1A the current intensity obtained for GC is slightly higher than that obtained for BDD, but the BDD capacitive current intensity is lower. In addition, the oxidative peak obtained at a BDD electrode is better defined than for GC because it is not so close to the solvent oxidation potential. SWV, in Fig.1B current intensities obtained for the electro-oxidation of 7-mG are similar for both electrodes but, again, the oxidative wave at BDD electrode exhibits a more defined peak, for the same reasons stated above. SWV is a very interesting technique for this determination, as can be checked putting our attention on the successive scans obtained with this technique, as depicted in Fig. 2, for BDD and GC electrodes. A decrease in current intensity was remarkably obtained when using the GC electrode, denoting adsorption, fouling or blocking of the active sites of the electrode surface by 7-mG or its oxidative products, as observed for guanine using GC electrodes [43], ascribed mainly to a higher sp^2 character in GC. Nevertheless, differences in peak current intensity are not significant between the two electrodes. In conclusion, we have considered the use of the BDD as most suitable electrode for the determination of 7-mG in this work.

3.2. Electrochemical response for 7-mG through the SPE configuration.

The use of the SPE electrochemical cell configuration exhibits a wide number of advantages in terms of small sample volume required, miniaturization of the electrochemical system, fast measurements, and low cost, among other things. With the aim of contributing in this regard, we explored whether the SPE configuration, as shown in Scheme 1, was adequate to perform the electro-oxidative response and the electroanalytical determination of 7-mG. Fig. 3 displays the CV and SWV voltammograms for 50 μM 7-mG solution with an anodic peak potential of 7-mG for CV at + 0.82 V versus pseudo Ag/AgCl and +0.77 V in the case of SWV. There is a potential difference of ~ 0.28 V between the potential obtained versus the pseudo and the standard Ag/AgCl. It is worth noting that the same SPE was used for all consecutive measurements shown in Fig. 3, maintaining the oxidative response stable and

reproducible, as depicted in Fig. ESI-1. Furthermore, no electrode surface blocking is observed and both CV and SWV show well-defined peaks and peak potential remains constant with consecutive measurements, confirming the pseudo-reference electrode stability over time and with use. A small slope in CV voltammograms is recorded, which can be attributed to an electric resistance due to the relatively low electric conductivity by 0.1 M acetate buffer solution at pH 5.0, according to the following data: the resistivity value of 0.1 M acetate buffer solution at pH 5.0 is $0.234 \Omega\cdot\text{m}$ and for BDD film electrode is $\sim 7.5 \cdot 10^{-4} \Omega\cdot\text{m}$.

To further explore the electrochemical response of 7-mG at BDD electrode, potential and current intensity dependence with the scan rate was analyzed by CV technique. Fig.4A depicts the CV response of 7-mG in 0.1 M acetate buffer pH 5.0 with scan rates. Subsequently, peak current intensity (I_p) is plotted versus scan rate (see Fig. ESI-2A) and versus square root of the scan rate (see Fig ESI-2B), and peak potential values (E_p) is plotted versus scan rate (see Fig. ESI-2C).

Moreover, the dependence of peak potential logarithm ($\text{Log } E_p$) and peak current logarithm ($\text{Log } I_p$) with the scan rate logarithm ($\text{Log } v$) is represented, as can be seen in Fig. 4B and ESI-4D. After analyzing all the plots of Fig. 4 and ESI-2, it is concluded that there is a linear dependence of I_p with $v^{1/2}$ ($R^2 = 0.994$; slope = $8.045 \mu\text{A} [\text{V}\cdot\text{s}^{-1}]^{-1/2}$) and a linear dependence of $\log_{10} I_p$ with $\log_{10} v$ ($R^2 = 0.997$; slope = 0.558) too. Linear plot of $\text{Log } I_p$ versus $\text{Log } v$ with a slope close to the theoretical value of 0.5 indicates that the process is diffusion controlled. Linear dependence of I_p with $v^{1/2}$ also supports this view [44]. Furthermore, the dependence of peak potential with scan rate is characteristic of an irreversible process. This kind of performance observed for 7-mG on BDD at this work is similar to the obtained for G at carbonaceous electrodes by Li et al [44].

We next investigated the effect of the pretreatment of the BDD electrode on the electrochemical response of 7-mG in 0.1 M acetate buffer solution pH 5.0. These electrochemical pretreatments were performed in order to investigate the electrochemical performance of 7-mG. Surface modification of BDD electrode was performed by anodic or cathodic pretreatments to assess the electrochemical response of 7-mG electro-oxidation. Fig. ESI-3 depicts the CV and SWV responses as a function of the pretreatment. There are not significant differences in current intensity obtained by the different pretreatments. However, pretreatments can have a major influence on the electrochemical response as they can functionalize the carbon surface, remove

contaminants and ensure a more homogeneous surface; associated with greater reproducibility of results [45]. In this regard, an improved response may be likely defined in terms of a better peak definition, greater reproducibility, lower overpotential and/or increased current intensity. However, Fig. ESI-3 (Left) shows CV responses with a higher capacitive current intensity and an electrode resistance associated with the cathodic and anodic pretreatments compared to the mechanical polishing one.

3.3. Effect of pH on the electrochemical 7-mG SWV response at BDD: pH dependence on peak potential and peak current.

The effect of pH of the solution on the electrochemical oxidation of 7-mG has been studied. The use of acetate buffer solutions instead phosphate buffer solutions as described by Brotons et al. [27] was based on the evaluation of pH on the electrochemical behaviour of 7-mG for the optimum electroanalytical determination in terms of sensitivity. In literature, several researchers have established that Guanine peak potential (precursor of 7-mG) and other modifications (such as 8-oxoguanine) shifts linearly with pH, presenting slopes close to the Nernstian theoretical value of 59 mV at 25° C, which is due to a mechanism involving the same number of protons that electrons transferred [46–48]. However, less is known about the effect of pH solution upon the electro-oxidation of 7-mG and, consequently, a pH-dependence study over the range of 2.5 – 8.5 has been performed. As it can be seen in Fig. 5, SWV response of 50 μ M 7-mG solution shows a peak potential shifting to lower positive potentials with pH and a linear pH-dependence of the anodic peak potential with a slope of 56 mV (Inset Fig. 5), close to the theoretical Nernstian value of 59 mV. This indicates that an equal amount of protons and electrons is involved in electrode reaction as well. However, it is worth noting that at pH 4.19, SWV response deviates from the linear trend attributed to the pKa1 of the substance near 4.4 [49]. By contrast, Brotons et al. [27] reported a value of pH dependence for the electro-oxidation of 7-mG at screen printed graphite electrodes (SPGE) of 27 mV pH⁻¹ within a pH range of 3 and 9 in phosphate buffer solutions. The relationship of anodic peak current as a function of pH does not present any clear tendency (Fig. ESI-4). Moreover, the electrochemical response of the 7-mG with buffer solutions with pH lower than 3.76 or higher than 5.76 has been checked, providing similar peak potentials for the 7-mG electro-oxidation and identical pH dependence. However, although pH 8.5 presents a SWV response with better electrocatalysis than the rest of pH, we chose pH 5.0 as the optimum pH for analytical measurements

because at this pH, SWV voltammetric response presents high and satisfactory peak current intensity and acetate/acetic acid solution remains its pH buffering capacity.

3.4. Calibration curves of current intensity versus 7-MeG concentration obtained by SWV.

Electroanalytical quantification of 7-mG was accomplished in 0.1 M acetate buffer solution pH 5.0 using SWV (Fig. 6) in the range of 10 μ M to 230 μ M. Linearity range was found to be from 10 to 200 μ M, since 230 μ M standard solutions did not follow the linear trend. From the SWV of all standard solutions, a straight-line calibration plot was obtained by linear regression by the method of least squares (inset of Fig. 6 shows the calibration curve, peak current intensity as a function of 7-mG concentration). The equation of the line is as follows: $I_p/\mu\text{A} = (0.0332 \pm 0.0012) [7\text{-mG}/\mu\text{M}] + (0.30 \pm 0.13)$ ($r^2 = 0.997$, $n = 3$) for 7-mG. Repeatability and reproducibility were evaluated obtaining coefficients of variations of 2 and 6 %, respectively ($n = 3$ in both cases, as shown in Fig. ESI-5). The limit of detection was 1.2 μ M and the limit of quantification was 39 μ M, based on three and ten times the noise level, respectively.

3.5. Simultaneous detection of 7-mG in the presence of other nucleobases.

We have considered the presence of other nucleobases which can mostly interfere with 7-mG electro-oxidation at BDD electrode. The main interfering compounds must be G and A, since their oxidation potential remains close to the 7-mG one [10]. First, it is interesting to study the electrochemical response of 7-mG + G and 7-mG + A mixtures to better analyse the effect of each nucleobase on the 7-mG behaviour and to exam if their simultaneous detection is viable. In the case of 7-mG + G mixtures, as shown in Fig. 7A, 7-mG peak potential is located about at 0.79 V versus pseudo Ag/AgCl while G peak potential is found at 0.67 V. Potentials remain fairly constant with the variation of the relative proportion between 7-mG and G. This result indicates that there is not any cooperative effect upon the electrochemical responses of each nucleobase. There is a difference of 120 mV between peak potentials between G and 7-mG, sufficient to enable their simultaneous detection. However, the peaks are slightly overlapped; such a fact may affect the determination of peak current and peak potential for each compound. Moreover, as reported in Fig. 7B, 7-mG peak potential occurs at 0.80 V versus pseudo Ag/AgCl while the A peak potential occurs at about 1.1

V. Peak potentials do not vary significantly with the relative ratio of 7-mG and A. Apparently; there is not a cooperative effect between both components. Therefore, A will not be an interfering compound for the quantification of 7-mG as we can distinguish a peak separation of 300 mV between 7-mG and A.

We turn next to explore the electroanalytical behaviour of 7-mG in the presence of the three nucleobases together (G + 7-mG + A) (Fig. ESI-6) and the mixture of the three nucleobases with 8-oxoG (see Fig. 8). The mixture with the presence of 8-oxoG results very interesting because 8-oxoG is another of the most common modified nucleobases in DNA [46]. Consequently, simultaneous analysis of 7-mG and 8-oxoG, in biological samples, might be quite useful from an electroanalytical point of view. Accordingly, the electro-oxidation of G overlaps that one for the 7-mG with just 100 mV of difference between their peak potentials, while the peak potential differences still remains unaltered between 7-mG and A nucleobases. The presence of 8-oxoG shifts G and A peak potentials circa 20-30 mV to more positive values. This could be attributable to 8-oxoG competing with G and A by the adsorption sites of the electrode surface. Also, the introduction of 8-oxoG appears generally to decrease peak current intensities, so that processes involving electrode surface blocking dominate the electrochemical response of every nucleobases. This fact is also corroborated by the fouling of electrode with consecutive SWV experiments. Hence, the simultaneous analysis of 7-mG and 8-oxoG is entirely possible as there is a difference of 380 mV between their peak potentials. Finally, repeatability of all measurements was also studied by calculating coefficient of variation for four different experiments Table 1, polishing the electrode surface every electrochemical measurement.

3.6. Mechanism of the 7-mG electro-oxidation.

To propose a scheme for the mechanism for the electro-oxidation of 7-mG at BDD electrode, we have performed the preparative electrolysis of 7-mG at a controlled potential of 1.20 V versus Ag/AgCl, as described previously in the experimental section. Fig. 9A depicts the SWV monitoring the electrochemical response of the anolyte solution with charge passed. After 1.95 C and 4.18 C of charge passed (the latter corresponds to the 108 % of the theoretical charge assuming a 2 electrons process involved in the electro-oxidation of 7-mG), the SWV shows a new peak at about 0.97 V versus pseudo Ag/AgCl. The presence of the new anodic peak was ascribed previously to the formation of the dimer species from the electro-oxidation of G [44] at glassy

carbon electrodes or the electro-oxidation of 7-mG at screen printed graphite electrodes [27] using SWV. Nevertheless, to shed light on the final products from the electro-oxidation of 7-mG in 0.1 M acetate buffer solutions pH 5.0, HPLC coupled to mass spectrometry experiments of the anolyte solutions exhibits the major formation of dimer species coming from the dimerization process of two 7-mG radical species, as shown in Fig. 9B within the pathway A with a m/z of 166 which corresponded to $[7\text{-mG} + \text{H}^+]$. The formation of oxygenated derivatives species like 7-methy-8-oxoguanine; as shown in Fig. 9B within the pathway B, was not found experimentally. Neither the starting product 7-mG nor the dimer species were observed in the catholyte solution.

On the other hand, G electro-oxidation mechanism at carbonaceous electrodes is well-known and described in literature [44]. In the case of the 7-mG, it is expected that its electro-oxidation mechanism retains parallelisms with the mechanism for G. However, it must be taken into account that the introduction of the methyl group at the N7 position can modify it. Firstly, from the pH-dependence versus peak potential study, it was concluded that the same number of protons and electrons are involved in the mechanism. Secondly, from the dependence of peak potential with the logarithm of the scan rate (see Fig. 4B); the kinetic parameter $(\alpha \cdot n_a)$ was calculated. This requires the employing of Equation 1, which defines such dependency [50]:

$$E_p = cte. + \frac{1}{2} \cdot \frac{RT}{(\alpha \cdot n_a) \cdot F} \cdot 2.3 \cdot \log(v) \quad (1)$$

where n_a is the number of electrons exchanged in the rate-determining step, α is the electronic transfer coefficient, F is the Faraday constant (96,485 C / mole e^-) and R is the molar gas constant (8.31 J·K⁻¹·mol⁻¹). Therefore, being aware of the slope value of the plot E_p versus $\log v$, which is 0.0314 V (see Fig. ESI-2D), we can estimate that the value of $(\alpha \cdot n_a)$ is equal to 0.94. Thirdly, and knowing the value of $(\alpha \cdot n_a)$, we proceeded to estimate the number of electrons exchanged in the process from the plot peak current versus the square root of the scan rate from the CV experiments (see Fig. ESI-2B). For an irreversible process, that dependence follows the equation 2 [50].

$$I_p = (2,99 \cdot 10^5) \cdot (n_a \cdot \alpha)^{\frac{1}{2}} \cdot n \cdot D^{1/2} \cdot A \cdot C \cdot v^{\frac{1}{2}} \quad (2)$$

where n is the number of electrons involved in the mechanism, A is the electrode area (projected area of BDD = 0.071 cm^2), D is diffusion coefficient of 7-mG (assumed to be equal to the guanine one: $7.3 \cdot 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ [44]) and C is the concentration of the electroactive species (7-mG, $50 \cdot 10^{-9} \text{ mole} \cdot \text{cm}^{-3}$). Slope obtained was $8.05 \cdot 10^{-6} \text{ A} \cdot [\text{V} \cdot \text{s}^{-1}]^{-1/2}$ (see Fig. ESI-2B). Thereafter, the number of electrons involved in the electro-oxidation mechanism was equal to 2. Taking into account the above data together with the same number of electrons and protons exchanged during the electro-oxidation of 7-mG, the electro-oxidative mechanism should follow the plausible route described in Figure 9B, leading to the formation of 7-methyl-8-oxoguanine. Furthermore, the proposed mechanism in Fig. 9B involves that the oxidation of 7-methyl-8-oxoguanine either is precluded or is conducted at higher potentials, such a fact that can be justified by the presence of the methyl group in N7 position, thereby impeding the formation of imino bond ($\text{N} = \text{C}$) between C5 and N7 positions of the G moiety, unlike what happens to guanine which bond is easily formed. However, according to the analysis of distinct analytes during the electro-oxidation of 7-mG by mass spectrometry, the only product observed was the dimer species, involving one proton and one electron for the whole mechanism, so the scenario is entirely different from an experimental point of view.

3.7. Interference study in a complex matrix

The feasibility of the electrochemical sensing of 7-mG has been investigated in a real urine matrix with high amount of components which could interfere the 7-mG analysis. Human urine sample from a healthy volunteer was collected the same day of the experiments, and before use, the urine sample was filtered through $0.45 \mu\text{m}$ nylon filters and diluted two times with 0.1 M acetate buffer solution and final pH was set at pH 5.0 using acetic acid glacial. This was made to reduce the electrochemical signal, in order to the concentrated urine sample presents too high current intensities. Nonetheless, the SWV of the diluted urine sample at pH 5.0 still depicts two wide oxidative peaks at about 0.5 and 0.9 V, respectively, as can be seen in Fig. ESI-7 and Fig. ESI-8.. Urea is one of the major organic compound present in urine samples [51-52], but urea resulted to be electrochemically stable within the potential range used (see Fig. ESI-8). In addition, Fig. ESI-8 also depicts the electrochemical response of uric acid, revealing an anodic peak at 0.2 V versus pseudo Ag/AgCl. Moreover, dopamine and ascorbic acid solutions were evaluated as likely interference compounds; showing

anodic peak potentials at 0.45 and 0.3 V, respectively (see Fig. ESI-8). Hence, the presence of urea, uric acid, dopamine and ascorbic acid exhibit no interference with the electrochemical oxidation of 7-mG, but there exists still a wide number of metabolites, e.g., creatinine or other unidentified metabolites, present in human urine which can be responsible for the anodic main peak shown in Fig. 10 (or Fig ESI-7 and ESI-8).

7-mG standard stock solutions with concentrations between 25 and 200 μM were prepared with this solution as a matrix and their SWV response was recorded whose results are presented in Fig. 10. As can be checked in Fig. 10, 7-mG determination in urine sample is feasible as anodic peak of 7-mG appears at 1.05 V versus pseudo Ag/AgCl where interferences by urine components are quite slight on that potential region. Interestingly, the anodic peak associated to the electro-oxidation of 7-mG shifted near 200 mV to more positive potentials. In addition, peak current intensity shifts linear with the concentration (inset Fig in Fig.10).

3.8. Comparison between different analytical methods for 7-mG determination.

In the process of validation of an analytical method, it is important to compare its properties with other methods with the same purpose (see Table 2, obtained from reference [27]). Therefore, here we provide a brief comparison between the method of this study and others widely employed techniques for 7-mG determination. In the case of the electrochemical method for 7-mG determination with screen-printed graphite electrodes in PBS buffer solution at pH 4.0 presented by Brotons et al. [27], our work showed a higher linear range and a lower LOD. Nevertheless, our electrochemical method exhibits a LOD higher than the other conventional methods. This is since mass spectrometry is a very sensitive technique and, besides, these techniques carry out the separation of all components of the sample by chromatography before determination. Moreover, it is important to bear in mind that range of concentrations studied in this work is higher than that values presented by convectional techniques. Most importantly is the fact that the reproducibility obtained is similar to the other techniques.

4. Conclusions

The feasibility of a sensor for the electroanalytical determination of 7-mG using a BDD electrode has been evaluated. According to the results, we can conclude that the method is suitable for the detection of 7-mG as its electro-oxidation in acetic acid /acetate 0.1 M aqueous buffer solutions pH 5.0 (optimum determined pH) can give rise

well-defined anodic peaks by CV and SWV techniques. It has been determined that the electro-oxidation reaction is diffusion controlled and it is estimated that the mechanism involves two protons and two electrons. However, preparative electrolysis at controlled potential of 7-mG reveals that the electro-oxidation process of 7-mG at controlled potential takes place throughout the participation of one electron and one proton leading to the formation of the dimer species.

We can assess that this electroanalytical method is also suitable for quantifying 7-mG, since the electrochemical sensor has been validated in terms of linearity, sensitivity, LOD, LOQ, repeatability and reproducibility, obtaining a calibration curve in the range of linearity of 10 - 200 μM , with suitable regression ($R^2 = 0.997$) and a sensitivity of $0.0332 \mu\text{A} \cdot \mu\text{M}^{-1}$. Furthermore, the use of the BDD electrode for the electrochemical sensing of 7-mG has revealed proper repeatability and reproducibility. The simultaneous determination of 7-mG with its possible interference compounds present in samples with DNA has also been satisfactorily fulfilled. Finally, this work has demonstrated the feasibility of an electrochemical sensor for determining the 7-mG in a complex matrix like urine samples and lays the groundwork for their development. Future work would be needed to developing electrochemical sensors for 7-mG detection in biological samples (DNA by extraction or other biological fluids, such as urine), which can serve as a biomarker for the detection of abnormal methylation patterns and both as a method for the early diagnosis and monitoring of pathologies derived from such methylation.

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References

- [1] R.R. Sinden, DNA Structure and Function, Academic Press, 1994.
- [2] S. Loft, P. Svoboda, H. Kasai, A. Tjønneland, P. Møller, M. Sørensen, K. Overvad, H. Autrup, O. Raaschou-Nielsen, Prospective study of urinary excretion of 7-methylguanine and the risk of lung cancer: Effect modification by mu class glutathione-S-transferases., *Int. J. Cancer*. 121 (2007) 1579–1584.
- [3] P. Svoboda, H. Kasai, Simultaneous HPLC analysis of 8-hydroxydeoxyguanosine and 7-methylguanine in urine from humans and rodents., *Anal. Biochem*. 334 (2004) 239–250.
- [4] N. Shrivastav, D. Li, J.M. Essigmann, Chemical biology of mutagenesis and DNA repair: cellular responses to DNA alkylation., *Carcinogenesis*. 31 (2010) 59–70.
- [5] B. Alberts, *Molecular Biology of the Cell: Reference Edition*, Taylor & Francis, 2008.
- [6] P.A.J. Crosbie, K. Harrison, R. Shah, A.J. Watson, R. Agius, P.V. Barber, G. Margison, A. Povey, Topographical study of O(6)-alkylguanine DNA alkyltransferase repair activity and N7-methylguanine levels in resected lung tissue., *Chem. Biol. Interact*. 204 (2013) 98–104.
- [7] A. a Saad, P.J. O'Connor, M.H. Mostafa, N.E. Metwalli, D.P. Cooper, G.P. Margison, A. Povey, Bladder tumor contains higher N7-methylguanine levels in DNA than adjacent normal bladder epithelium., *Cancer Epidemiol. Biomarkers Prev*. 15 (2006) 740–743.
- [8] K. Tamae, K. Kawai, S. Yamasaki, K. Kawanami, M. Ikeda, K. Takahashi, T. Miyamoto, N. Kato, H. Kasai, Effect of age, smoking and other lifestyle factors on urinary 7-methylguanine and 8-hydroxydeoxyguanosine, *Cancer Sci*. 100 (2009) 715–721.
- [9] E. Rodríguez-Gonzalo, R. Hernández-Prieto, D. García-Gómez, R. Carabias-Martínez, Capillary electrophoresis-mass spectrometry for direct determination of

- urinary modified nucleosides. Evaluation of synthetic urine as a surrogate matrix for quantitative analysis., *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.* 942-943 (2013) 21–30.
- [10] A. Brotons, L.A. Mas, J.P. Metters, C.E. Banks, J. Iniesta, Voltammetric behaviour of free DNA bases, methylcytosine and oligonucleotides at disposable screen printed graphite electrode platforms., *Analyst.* 138 (2013) 5239–5249.
- [11] B. Thomas, S. Matson, V. Chopra, L. Sun, S. Sharma, S. Hersch, H.D. Rosas, C. Scherzer, R Ferrante, W. Matson, A novel method for detecting 7-methyl guanine reveals aberrant methylation levels in Huntington disease., *Anal. Biochem.* 436 (2013) 112–120.
- [12] H.-J.C. Chen, Y.-F. Liu, Simultaneous quantitative analysis of N3-ethyladenine and N7-ethylguanine in human leukocyte deoxyribonucleic acid by stable isotope dilution capillary liquid chromatography-nanospray ionization tandem mass spectrometry., *J. Chromatogr. A.* 1271 (2013) 86–94.
- [13] S.J. Stocks, R.M. Agius, N. Cooley, K.L. Harrison, D.R. Brison, G. Horne, A. Gibbs., A.C. Povey, Alkylation of sperm DNA is associated with male factor infertility and a reduction in the proportion of oocytes fertilised during assisted reproduction., *Mutat. Res.* 698 (2010) 18–23.
- [14] J.W. Park, B.N. Ames, 7-Methylguanine adducts in DNA are normally present at high levels and increase on aging: analysis by HPLC with electrochemical detection., *Proc. Natl. Acad. Sci.* 85 (1988) 7467–7470.
- [15] H. Kroeger, J. Jelinek, M.R.H. Estécio, R. He, K. Kondo, W. Chung, L. Zhang, L. Shen, H.M. Kantarjian, C.E. Bueso-Ramos, J. Issa, Aberrant CpG island methylation in acute myeloid leukemia is accentuated at relapse., *Blood.* 112 (2008) 1366–1373.
- [16] K. Wallace, M.V. Grau, A.J. Levine, L. Shen, R. Hamdan, X. Chen, J. Gui, R.W. Haile, E.L. Barry, D. Ahnen, G. McKeown-Eyssen, J.A. Baron, J.P.J. Issa, Association between folate levels and CpG Island hypermethylation in normal colorectal mucosa., *Cancer Prev. Res. (Phila).* 3 (2010) 1552–1564.

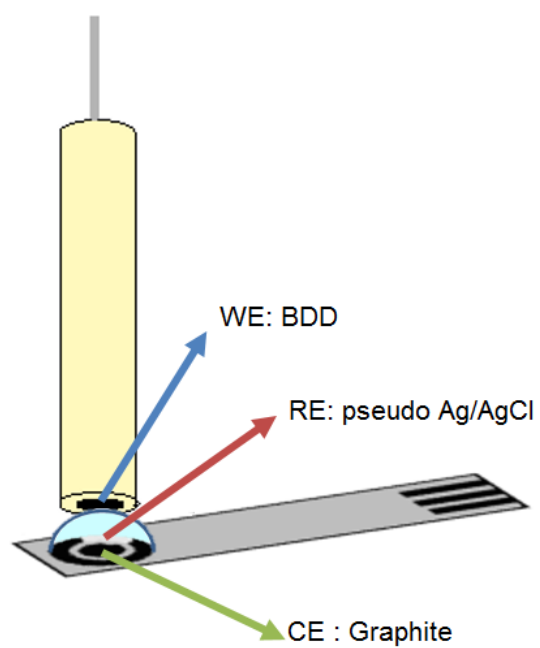
- [17] S. Watanabe, T. Ichimura, N. Fujita, S. Tsuruzoe, I. Ohki, M. Shirakawa, M. Kawasuji, M. Najao, Methylated DNA-binding domain 1 and methylpurine-DNA glycosylase link transcriptional repression and DNA repair in chromatin., *Proc. Natl. Acad. Sci. U. S. A.* 100 (2003) 12859–12864.
- [18] W. Chung, J. Bondaruk, J. Jelinek, Y. Lotan, S. Liang, B. Czerniak, J. Issa, Detection of bladder cancer using novel DNA methylation biomarkers in urine sediments., *Cancer Epidemiol. Biomarkers Prev.* 20 (2011) 1483–1491.
- [19] A.J. Sasco, F. Rey, C. Reynaud, J.-Y. Bobin, M. Clavel, A. Niveleau, Breast cancer prognostic significance of some modified urinary nucleosides, *Cancer Lett.* 108 (1996) 157–162.
- [20] K.L. Harrison, M. Wood, N.P. Lees, C.N. Hall, G.P. Margison, A.C. Povey, Development and Application of a Sensitive and Rapid Immunoassay for the Quantitation of N7-Methyldeoxyguanosine in DNA Samples, *Chem. Res. Toxicol.* 14 (2001) 295–301.
- [21] C.-W. Hu, H. Lee, J.-L. Chen, Y.-J. Li, M.-R. Chao, Optimization of global DNA methylation measurement by LC-MS/MS and its application in lung cancer patients., *Anal. Bioanal. Chem.* 405 (2013) 8859–8869.
- [22] H.-L. Lee, Y.-M. Hsueh, C.-J. Chung, Y.-S. Pu, L.W. Chang, D.P.H. Hsieh, S.-H. Liou, P. Lin, Correlation between the urine profile of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone metabolites and N7-methylguanine in urothelial carcinoma patients., *Cancer Epidemiol. Biomarkers Prev.* 17 (2008) 3390–3395.
- [23] F. Zhang, M.J. Bartels, L.H. Pottenger, B.B. Gollapudi, M.R. Schisler, Simultaneous quantitation of 7-methyl- and O6-methylguanine adducts in DNA by liquid chromatography-positive electrospray tandem mass spectrometry., *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.* 833 (2006) 141–148.
- [24] J. Chadt, D. Sykora, R. Nilsson, P. Vodicka, Monitoring of dimethyl sulphate-induced N3-methyladenine, N7-methylguanine and O(6)-methylguanine DNA adducts using reversed-phase high performance liquid chromatography and mass

- spectrometry., *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.* 867 (2008) 43–48.
- [25] G. Xu, H.R. Schmid, X. Lu, H.M. Liebich, P. Lu, Excretion pattern investigation of urinary normal and modified nucleosides of breast cancer patients by RP-HPLC and factor analysis method, *Biomed. Chromatogr.* 14 (2000) 459–463.
- [26] H. Kaur, B. Halliwell, Measurement of oxidized and methylated DNA bases by HPLC with electrochemical detection, *Biochem. J.* 318 (1996) 21–23.
- [27] A. Brotons, I. Sanjuán, C.E. Banks, F.J. Vidal-Iglesias, J. Solla-Gullon, J. Iniesta, Voltammetric behaviour of 7-methylguanine using screen-printed graphite electrodes: towards a guanine methylation electrochemical sensor, *Electroanalysis.* 27 (2015) 1–8.
- [28] Ľ. Švorc, K. Kalcher, Modification-free electrochemical approach for sensitive monitoring of purine DNA bases: Simultaneous determination of guanine and adenine in biological samples using boron-doped diamond electrode, *Sensors Actuators, B Chem.* 194 (2014) 332–342.
- [29] E.A McGaw, G.M. Swain, A comparison of boron-doped diamond thin-film and Hg-coated glassy carbon electrodes for anodic stripping voltammetric determination of heavy metal ions in aqueous media., *Anal. Chim. Acta.* 575 (2006) 180–189.
- [30] A. Manivannan, M.S. Seehra, D.A. Tryk, A. Fujishima, Electrochemical Detection of Ionic Mercury At Boron-Doped Diamond Electrodes, *Anal. Lett.* 35 (2002) 355–368.
- [31] A. Manivannan, R. Kawasaki, D. Tryk, A. Fujishima, Interaction of Pb and Cd during anodic stripping voltammetric analysis at boron-doped diamond electrodes, *Electrochim. Acta.* 49 (2004) 3313–3318.
- [32] T.M. Arantes, A. Sardinha, M.R. Baldan, F.H. Cristovan, N.G. Ferreira, Lead detection using micro/nanocrystalline boron-doped diamond by square-wave anodic stripping voltammetry, *Talanta.* 128 (2014) 132–40.

- [33] R. Daghrir, P. Drogui, J. Tshibangu, Efficient treatment of domestic wastewater by electrochemical oxidation process using bored doped diamond anode, *Sep. Purif. Technol.* 131 (2014) 79–83.
- [34] L. Codognoto, S.A.S. Machado, L.A. Avaca, Square wave voltammetry on boron-doped diamond electrodes for analytical determinations, *Diam. Relat. Mater.* 11 (2002) 1670–1675.
- [35] G.D. Fasman, C.R. Company, *Handbook of Biochemistry and Molecular Biology*, CRC Press, 1977.
- [36] M.S. Swaraga, M. Adinarayana, Kinetics and mechanism of protection of adenine from sulphate radical anion by caffeic acid under anoxic conditions.
- [37] D.A. Boateng, *Kinetics of Formation and Oxidation of 8-oxo-7,8-dihydroguanine (8oxoG)*, East Tennessee State University, 2014.
- [38] C.W. Foster, J. Metters, D.K. Kampouris, C.E. Banks, Ultraflexible Screen-Printed Graphitic Electroanalytical Sensing Platforms, *Electroanalysis*. 26 (2014) 262–274.
- [39] F.E. Galdino, C.W. Foster, J.A. Bonacin, C.E. Banks, Exploring the electrical wiring of screen-printed configurations utilised in electroanalysis, *Anal. Methods*. 7 (2015) 1208–1214.
- [40] R.O. Kadara, N. Jenkinson, C.E. Banks, Characterisation of commercially available electrochemical sensing platforms, *Sensors Actuators B Chem.* 138 (2009) 556–562.
- [41] E.P. Randviir, D.A.C. Brownson, J.P. Metters, R.O. Kadara, C.E. Banks, The fabrication, characterisation and electrochemical investigation of screen-printed graphene electrodes., *Phys. Chem. Chem. Phys.* 16 (2014) 4598–4611.
- [42] E.R. Sartori, A.B. Trench, R.C. Rocha-Filho, O. Fatibello-Filho, Determination of Propylthiouracile in Pharmaceuticals by Differential Pulse Voltammetry using

- a cathodically pretreated Boron-Doped Diamond Electrode, *J. Braz. Chem. Soc.* 24 (2013) 1504–1511.
- [43] A.M.O. Brett, F. Matysik, Sonoelectrochemical studies of guanine and guanosine, *Bioelectrochemistry Bioenerg.* 42 (1997) 111–116.
- [44] Q. Li, C. Batchelor-mcauley, R.G. Compton, Electrochemical Oxidation of Guanine: Electrode Reaction Mechanism and Tailoring Carbon Electrode Surfaces To Switch between Adsorptive and Diffusional Responses, *J. Phys. Chem.* 114 (2010) 7423–7428.
- [45] L. Švorc, D. Jambrec, M. Vojs, S. Barwe, J. Clausmeyer, P. Michniak, et al., Doping level of boron-doped diamond electrodes controls the grafting density of functional groups for DNA assays, *ACS Appl. Mater. Interfaces.* 7 (2015) 18949–18956.
- [46] A.M.O. Brett, J.A.P. Piedade, S.H.P. Serrano, Electrochemical Oxidation of 8-Oxoguanine, *Electroanalysis.* 12 (2000) 969–973.
- [47] C. Deng, Y. Xia, C. Xiao, Z. Nie, M. Yang, S. Si, Electrochemical oxidation of purine and pyrimidine bases based on the boron-doped nanotubes modified electrode., *Biosens. Bioelectron.* 31 (2012) 469–474.
- [48] P. Wang, H. Wu, Z. Dai, X. Zou, Simultaneous detection of guanine, adenine, thymine and cytosine at choline monolayer supported multiwalled carbon nanotubes film., *Biosens. Bioelectron.* 26 (2011) 3339–3345.
- [49] S.-G. Hwang, Y.-H. Jang, H. Cho, Y.-S. Kim, Study of 7-Methylguanine on pK_a Values by Using Density Functional Theoretical Method, *Bull. Korean Chem. Soc.* 31 (2010) 168–170.
- [50] A.J. Bard, L.R. Faulkner, *ELECTROCHEMICAL METHODS: Fundamentals and Applications.*, John Wiley & Sons, Inc., 2001.

- [51] C. Rose, A. Parker, B. Jefferson, E. Cartmell, The Characterization of Feces and Urine: A Review of the Literature to Inform Advanced Treatment Technology, *Crit Rev Env. Sci Technol.* 45 (2015) 1827–1879.
- [52] S. Bouatra, F. Aziat, R. Mandal, A.C. Guo, M. R. Wilson, C. Knox, T.C. Bjorndahl, R. Krishnamurthy, F. Saleem, P. Liu, Z.T. Dame, J. Poelzer, J. Huynh, F.S. Yallou, N. Psychogios, E. Dong, R. Bogumi, C. Roehring, D.S. Wishart, The Human Urine Metabolome, *PLOS one* 8 (2013) 1-28.
- [53] C.W. Hu, C.M. Chen, H.H. Ho, M.R. Chao, Simultaneous quantification of methylated purines in DNA by isotope dilution LC-MS/MS coupled with automated solid-phase extraction, *Anal. Bioanal. Chem.* 402 (2012) 1199–1208.
- [54] Y. Ohba, M. Kai, K. Zaitso, Simultaneous determination of 7-methylguanine and O⁶-methylguanine in DNA by liquid chromatography with postcolumn fluorescence derivatization using 3,4-dimethoxyphenylglyoxal, *Anal. Chim. Acta.* 365 (1998) 233–239.

Figure Captions

Scheme 1: Electrochemical cell configuration used for the electroanalytical detection of 7-mG.

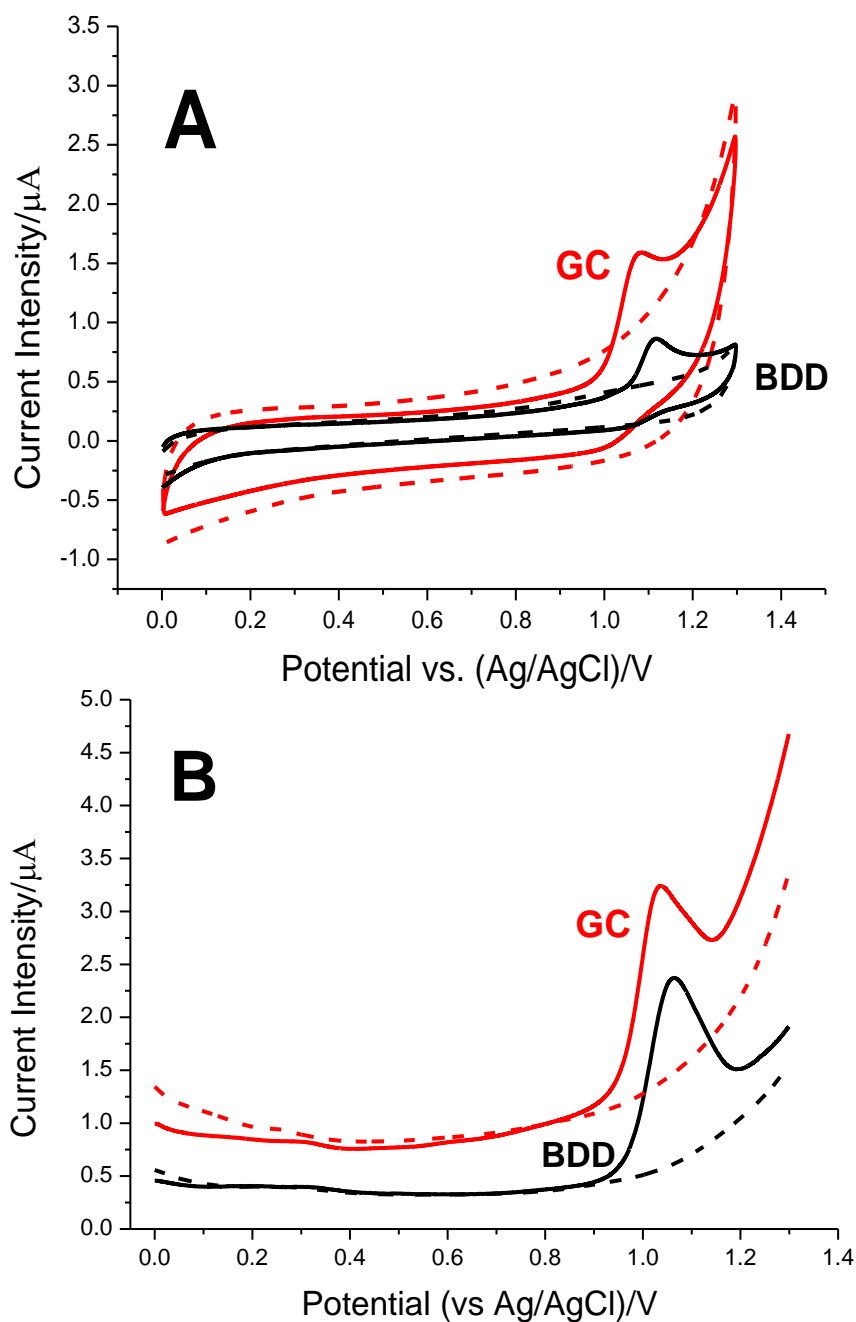


Fig.1. (A) Cyclic voltammetry response of 50 μM 7-mG in 0.1 M acetate buffer solution pH 5.0, at BDD (black line) and GC (red line). Background was recorded (n a dashed line. 50 mV/s. (B) Square wave voltammetry response of 50 μM 7-mG, in 0.1 M acetate buffer solution pH 5.0, at BDD (Black line) and GC (Red line). SWV parameters: modulation amplitude, 50 mV; modulation frequency, 8 Hz; modulation step, 5 mV. Experiments were carried out at a standard three electrodes electrochemical cell. The electrochemical behaviour of 7-mG is irrespective of the presence of nitrogen in the electrochemical cell.

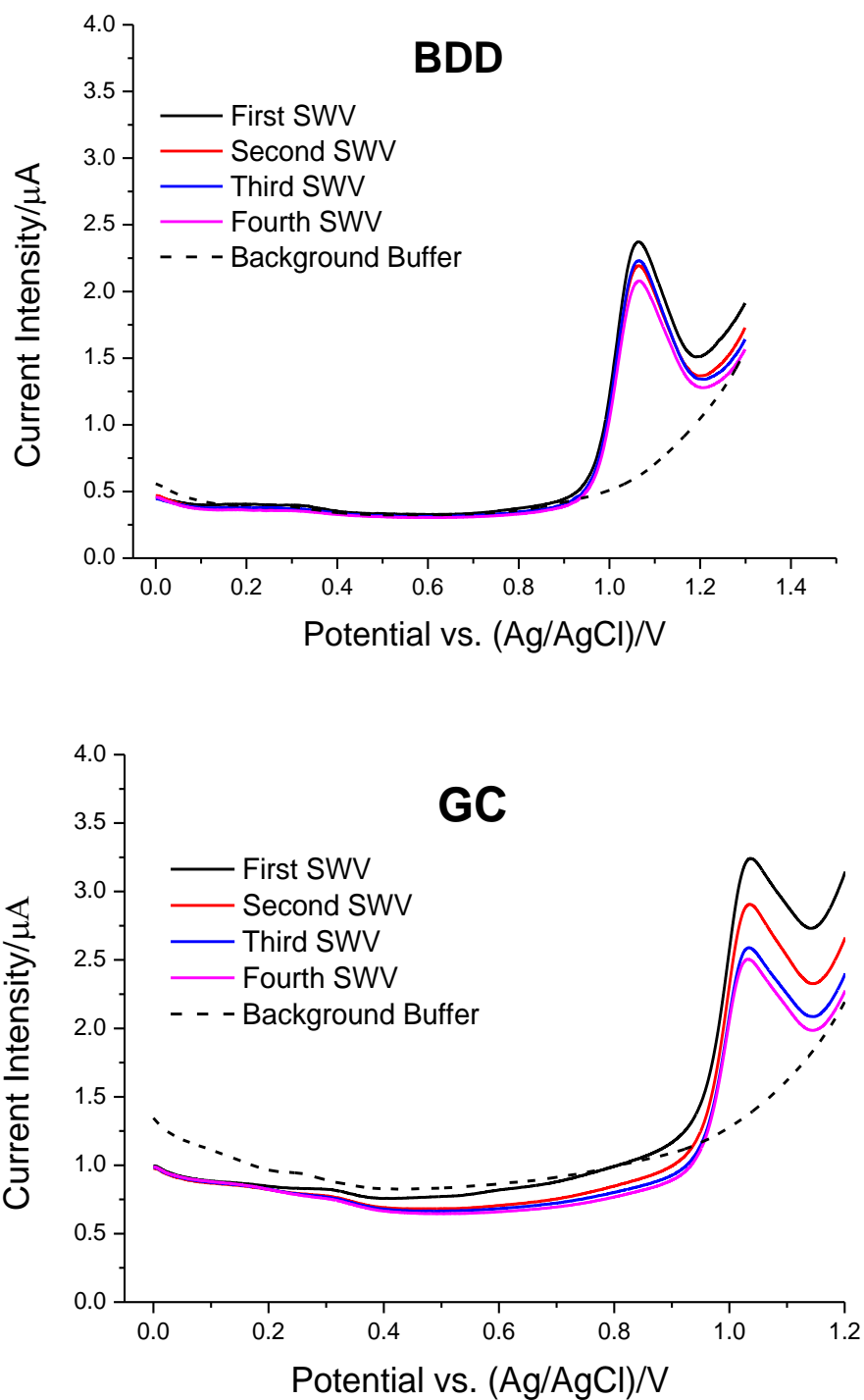


Fig. 2. Five consecutive SWV measurements of 50 μM 7-mG in 0.1 M acetate buffer solution pH 5.0; at BDD and GC electrodes. In the legend, the order of voltammograms recorded is highlighted in colors. SWV parameters: modulation amplitude, 50 mV; modulation frequency, 8 Hz; modulation step, 5 mV.

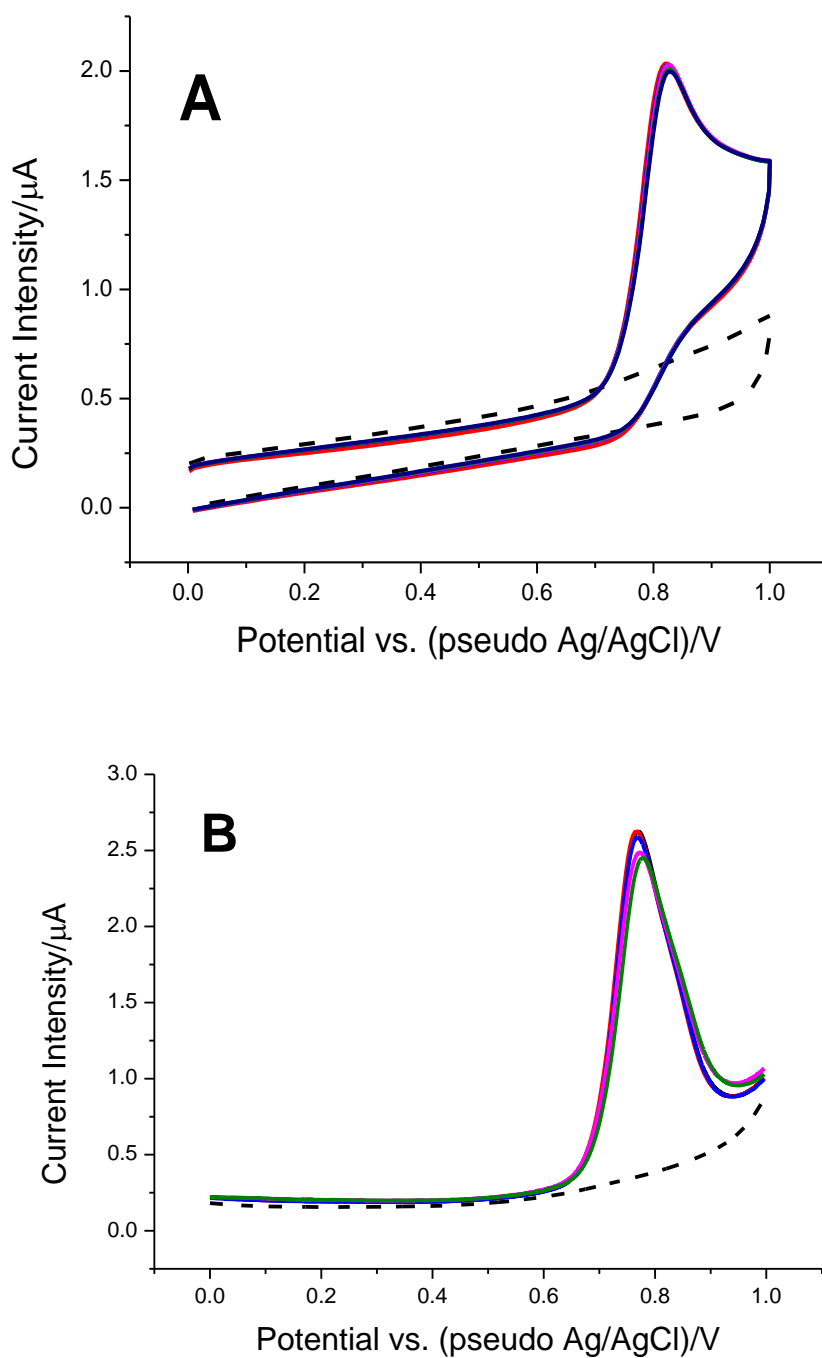


Fig. 3. Consecutive voltammograms A) CV and B) SWV in BDD electrode for 50 μM 7-mG in 0.1 M acetate buffer solution pH 5.0 (solid lines) and background of acetate buffer solution without 7-mG (dashed line). CV parameters: scan rate: 50 mV/s, step potential: 5 mV. SWV parameters: modulation amplitude, 50 mV; modulation frequency, 8 Hz; modulation step, 5 mV.

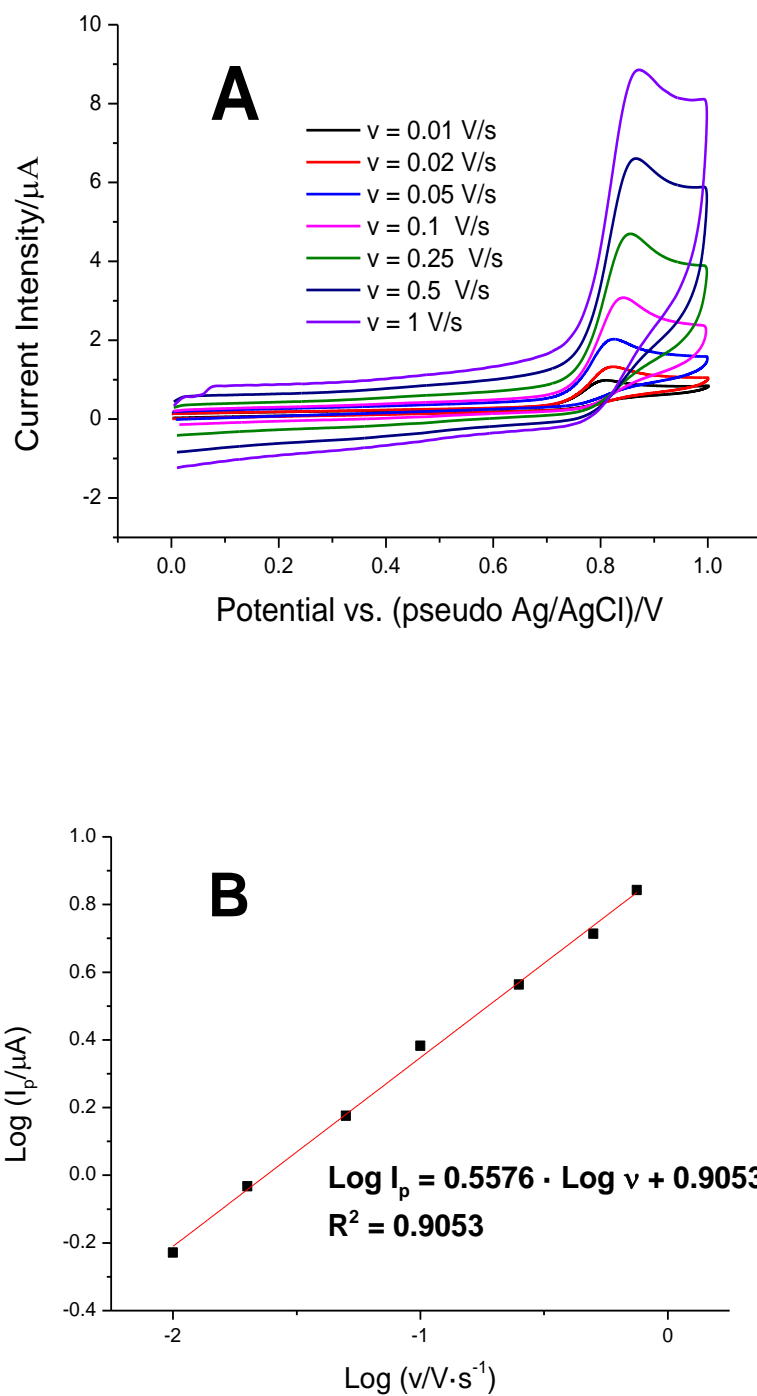


Fig. 4. (A) Cyclic voltammetry response of 50 μM 7-mG in 0.1 M acetate buffer solution pH 5.0 as a function of scan rate. Scan rates values are highlighted in colors in legend. (B) Plot of $\text{Log}_{10} I_p$ versus $\text{Log } v$. First scan recorded.

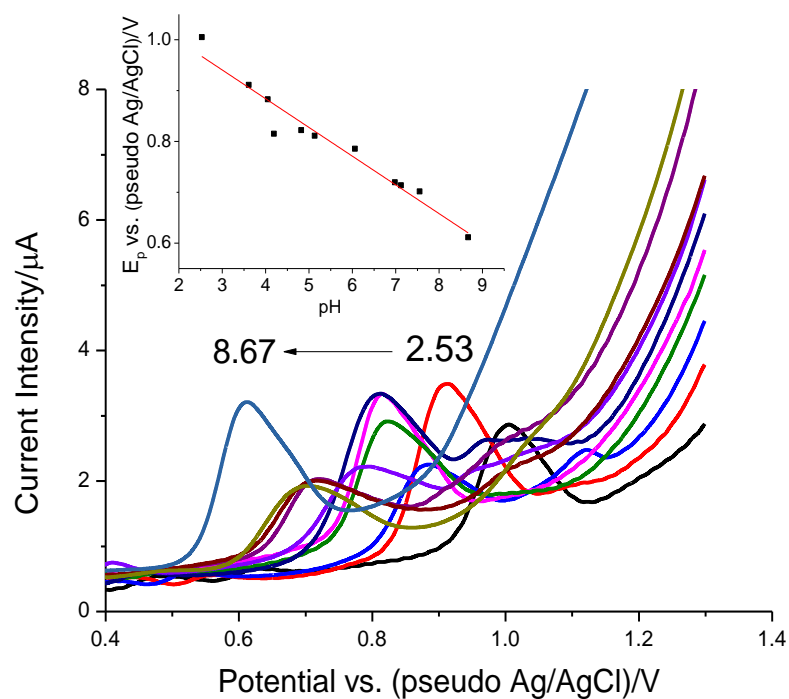


Fig. 5. SWV response of voltammograms 50 μ M 7-mG as a function of pH. 0.1 M acetate buffer solution adjusted at pH values between 2.53 and 8.67. Figure inset shows the plot E_p versus pH. SWV parameters: modulation amplitude, 50 mV; modulation frequency, 8 Hz; modulation step, 5 mV. First scan recorded.

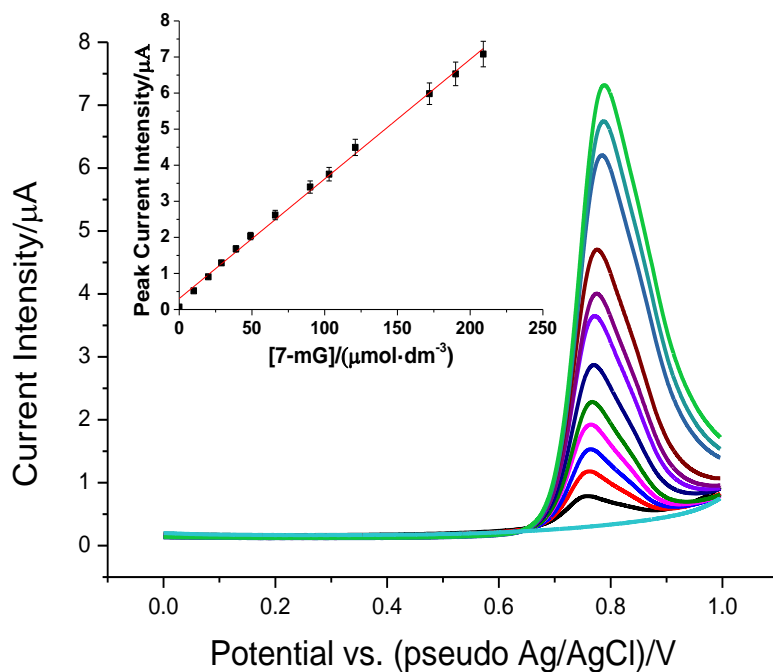


Fig. 6. SWV response of standard solutions with 7-mG (10, 20, 29, 39, 49, 66, 90, 103, 121, 172, 190, 209 μM) in 0.1 M acetate buffer solution pH 5.0. Figure inset shows the calibration plot of 7-mG standard solutions with concentration within 10 – 200 μM . SWV parameters: modulation amplitude, 50 mV; modulation frequency, 8 Hz; modulation step, 5 mV. First scan recorded.

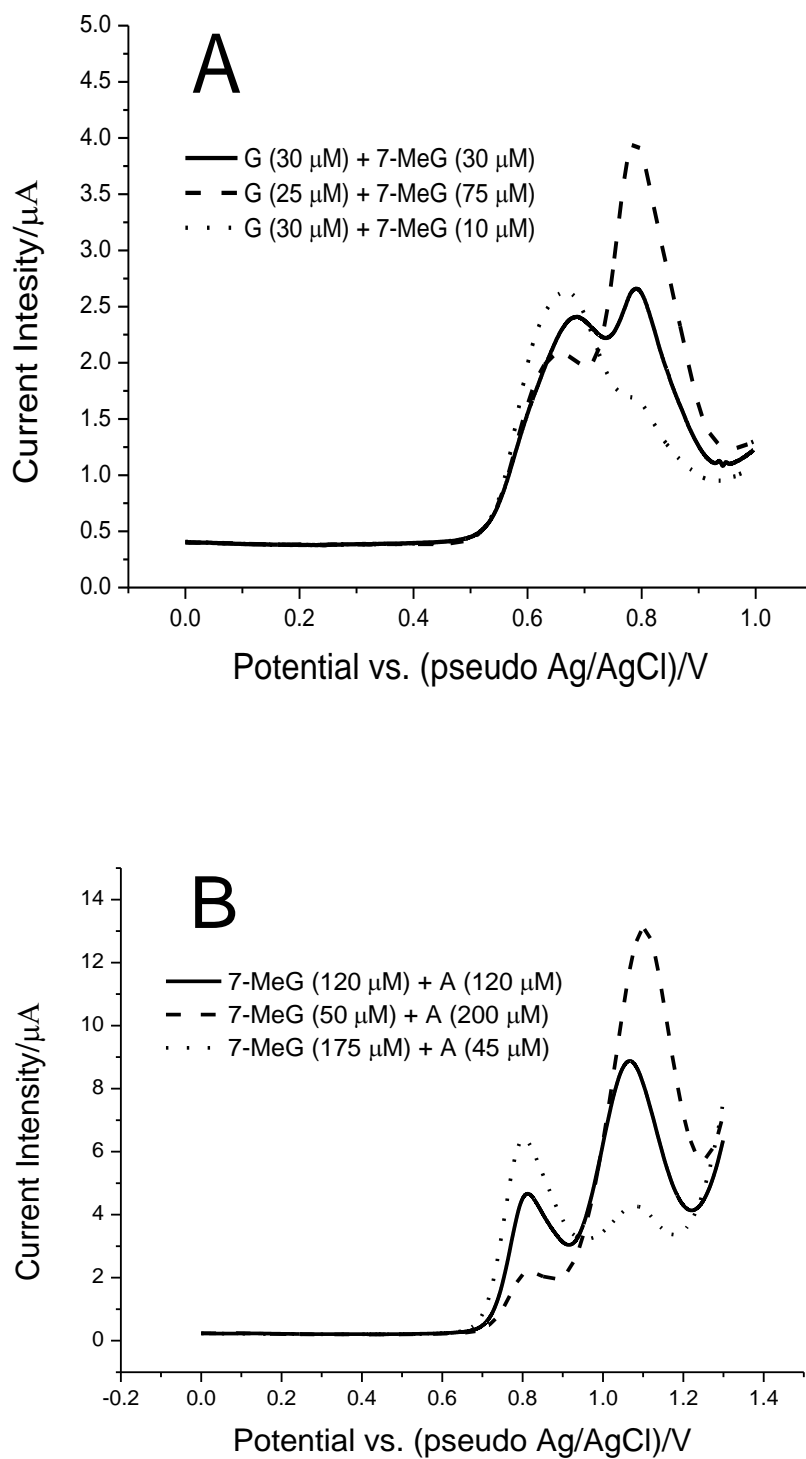


Fig. 7. (A) SWV response of 7-mG + G mixtures at different 7-mG to G ratios. (B) SWV response of A + 7-mG mixtures at different A to 7-mG ratios. SWV parameters: modulation amplitude, 50 mV; modulation frequency, 8 Hz; modulation step, 5 mV. First scan recorded.

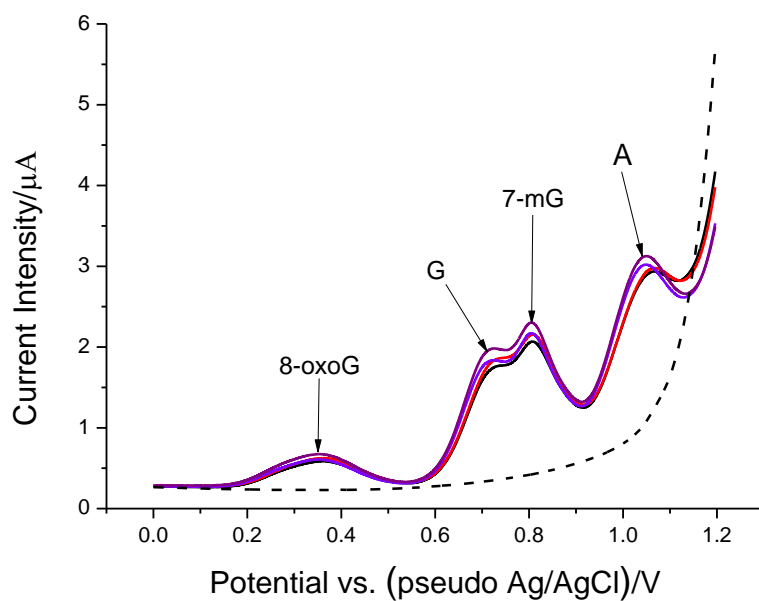


Fig. 8. SWV response of a mixture of 8-oxoG (25 μM), G (30 μM), 7-mg (45 μM) and A (30 μM) in 0.1 M acetate buffer solution pH 5.0. Dashed line corresponds to the background 0.1 M acetate buffer solution pH 5.0. SWV parameters: modulation amplitude, 50 mV; modulation frequency, 8 Hz; modulation step, 5 mV. First scan recorded.

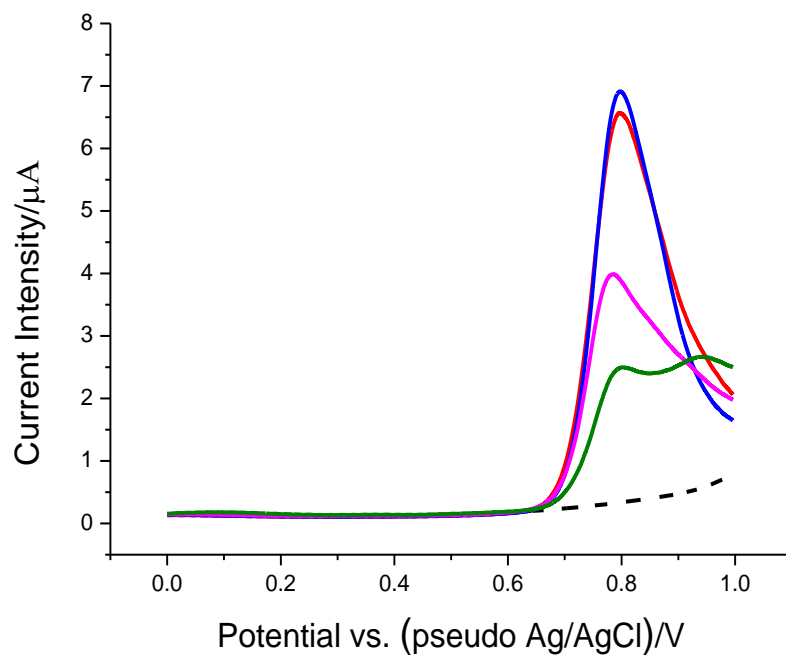
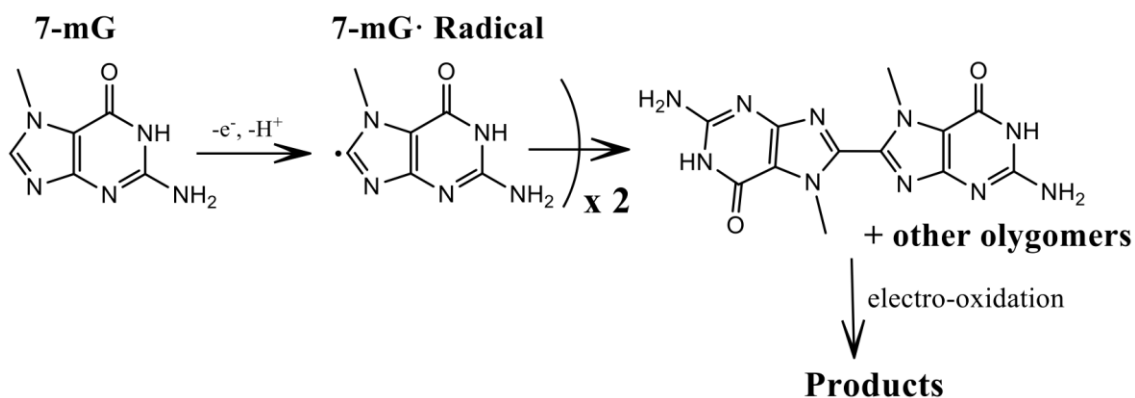


Figure 9A. SWV monitoring the electrochemical response of the analyte solution (Initially 200 μM 7-mG in 0.1 M acetate buffer solution pH 5.0) with a certain charge passed during the electrolysis: 0 C (Red line), 1.95 C (Pink line) and 4.18 C (Green line). Blue line corresponds to the response of initial 200 μM 7-mG solution in 0.1 M acetate buffer pH 5.0 before being introduced in the analyte compartment. Black dashed line corresponds to the background response. SWV parameters: modulation amplitude, 50 mV; modulation frequency, 8 Hz; modulation step, 5 mV. First scan recorded. Starting 7-mG concentration: 200 μM 0.1 M acetate buffer solution pH 5.0

Pathway A:



Pathway B:

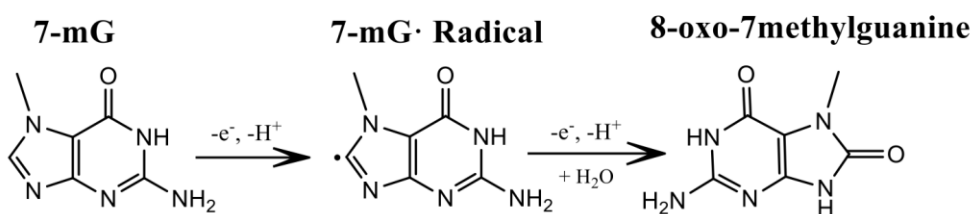


Figure 9B. Mechanism proposed for the electro-oxidation of 7-mG at BDD electrode.

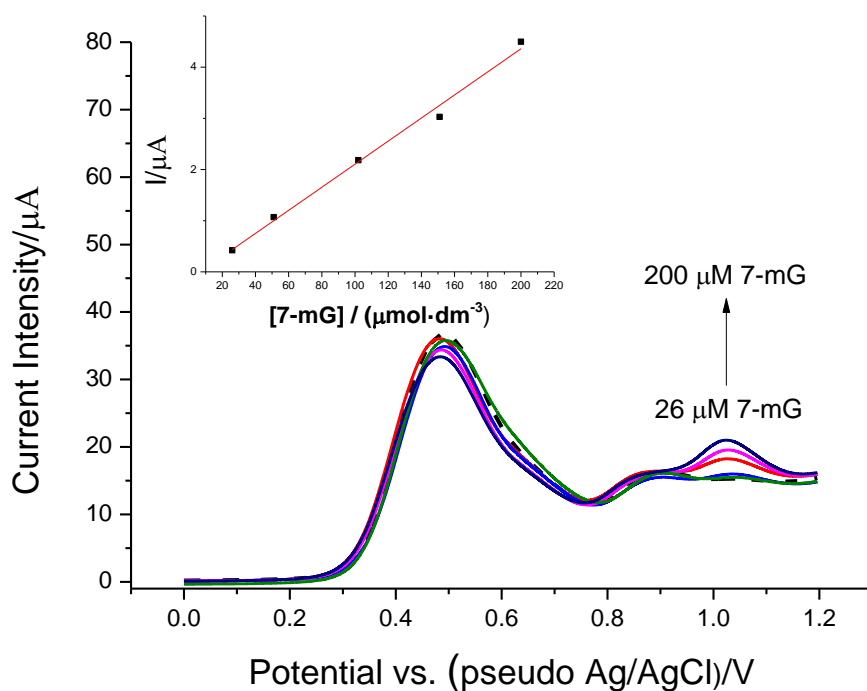


Fig. 10. SWV response of urine sample from a healthy human previously diluted to half by using 0.1 M acetate buffer solution pH 5.0 as a function of 7-mG concentration (26, 51, 102, 151, 200 μM). Dashed line corresponds to the background. Square wave voltammetry parameters: modulation amplitude, 50 mV; modulation frequency, 8 Hz; modulation step, 5 mV. First scan recorded. Figure inset depicts the calibration curve with a slope of $0.022 \mu\text{A} \cdot \mu\text{M}^{-1}$ and an intercept of $-0.15 \mu\text{A}$. LOD of $21 \mu\text{M}$ 7-mG.

Tables

Table 1. Peak potential (E_p) and peak current intensity (I_p) values obtained from the SWV response depicted in Figure 8. Coefficients of variations (CoV) are shown for each nucleic base in both mixtures.

Nucleobase (Concentration)	G + 7-mG + A mixture			8-oxoG + G + 7-mG + A mixture			
	G (30 μ M)	7-mG (30 μ M)	A (30 μ M)	G (30 μ M)	7-mG (45 μ M)	A (30 μ M)	8-oxoG (25 μ M)
E_p/V vs pseudo Ag/AgCl (N = 4)	0.696	0.803	1.034	0.730	0.806	1.055	0.357
CoV (%) (N = 4)	0.5	0.06	0.4	1.0	0.14	0.9	1.9
I_p/μ A (N = 4)	1.,7	0.90	1.5	1.52	0.96	1.76	0.36
CoV (%) (N = 4)	6	7	8	6	10	4	11

Table 2. Comparison between different analytical methods for the determination of 7-mG in terms of LOD, reproducibility and linearity.

Analytical method	LOD	Reproducibility	Linear range	Ref.
Square Wave Voltammetry (BDD electrode)	2 µg/mL	R.S.D. = 5,7%	1.65-33 µg/mL	This work
Square Wave Voltammetry (Screen printed graphite electrode)	2.4 µg/mL	R.S.D. = 5.1%	1.0-6.6 µg/mL	[27]
Reversed-phase high performance liquid chromatography with mass spectrometry.	0.2 ng/mL	R.S.D. = 4.8%	0.5–50 ng/mL	[24]
Liquid chromatography–positive electrospray tandem mass spectrometry	0.5 ng/mL	R.S.D. ≤ 11.0%	1 – 2000 ng/mL	[23]
Isotope dilution LC-MS/MS coupled with automated solid-phase extraction	0.02 ng/mL	R.S.D ≤ 8.0%	0.03 – 0.968 ng /mL	[53]
Liquid chromatography with postcolumn fluorescence derivatization	55 ng/mL	R.S.D. = 5.2 %	0 – 8275 ng/mL	[54]