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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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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.

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

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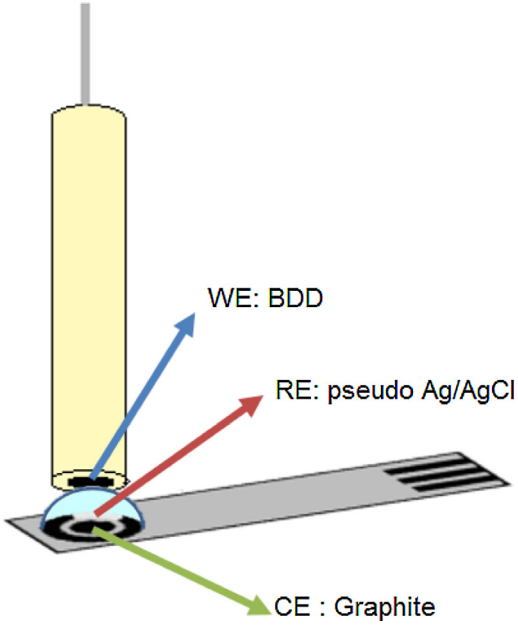
ionizing radiation, while the latter is produced by alkylating agents such as alkyl sulfonates, methyl halides, N-nitroso compounds and tobacco-specific nitrosamines [6,7]. There exists some well-known modified nucleobases, called DNA adducts, which its elevated presence in DNA, RNA and biological fluids 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, m^7-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].



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

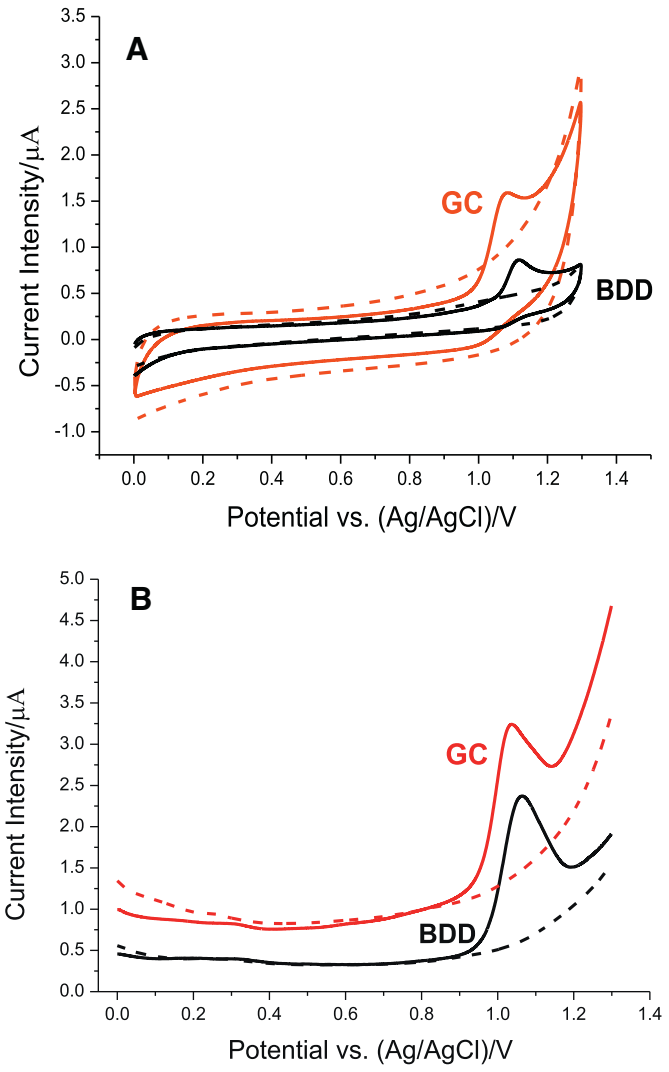


Fig. 1. (A) Cyclic voltammetry response of 50 μM 7-mG in 0.1 M acetate buffer solution pH 5.0, at BDD (black line) and GC (red line). Background was recorded in dashed line. 50 mV/s. **(B)** Square wave voltammetry response of 50 μM 7-mG, in 0.1 M acetate buffer solution pH 5.0, at BDD (Black line) and 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.

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

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