



A novel nanogold–single wall carbon nanotube modified sensor for the electrochemical determination of 8-hydroxyguanine, a diabetes risk biomarker



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ABSTRACT

An electrochemical study of the oxidation of 8-hydroxyguanine (8-OH-Gua) at gold nanoparticles attached to single walled carbon nanotube modified edge plane pyrolytic graphite electrode (AuNP-SWCNT/EPPGE) has been carried out to develop a method for the self diagnosis of diabetes. The level of 8-OH-Gua, an important biomarker of oxidative DNA damage, is higher in urine of diabetic patients than control subjects. A detailed comparison has been made between the square wave voltammetric (SWV) response of SWCNT/EPPGE and AuNP-SWCNT/EPPGE towards the oxidation of 8-OH-Gua in respect of several essential analytical parameters viz. sensitivity, detection limit, peak current and peak potential. The AuNP-SWCNT/EPPGE exhibited a well defined anodic peak at potential of ~221 mV for the oxidation of 8-OH-Gua as compared to ~312 mV using SWCNT/EPPGE at pH = 7.2. Under optimized conditions linear calibration curve for 8-OH-Gua is obtained over a concentration range of 0.01–10.0 nM in phosphate buffer solution (PBS) of pH = 7.2 with detection limit and sensitivity of 5.0 (± 0.1) pM and 4.9 (± 0.1) $\mu\text{A nM}^{-1}$, respectively. The oxidation of 8-OH-Gua occurred in a pH dependent process and the electrode reaction followed adsorption controlled pathway. The electrode exhibited an efficient catalytic response with good reproducibility and stability. The method has been found selective and successfully implemented for the determination of 8-OH-Gua in urine samples of diabetic patients.

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1. Introduction

Diabetes, termed ‘diabetes mellitus’ in clinical terminology, is a serious and growing health care problem worldwide and is associated with acute and chronic complications [1]. The excessive production of reactive oxygen species (ROS) due to aerobic cellular metabolism can accelerate oxidative damage to macromolecules including lipids, proteins as well as nucleic acids [2]. Reactive oxygen species are known to cause cleavage of strands and base modifications in nuclear and mitochondrial DNA which in turn results in the cellular dysfunction and apoptosis that are critical to the pathogenesis of many diseases including diabetes and its complications [3]. Substantial evidences have been demonstrated to suggest the increased production of reactive oxygen species in diabetic patients [4,5]. Electrochemical oxidation of DNA can occur at any of the four bases out of which guanine can undergo the easiest oxidative damage [6]. The major product of guanine oxidation is 8-hydroxyguanine (8-OH-Gua) which is widely accepted as a biomarker of oxidative DNA damage [7]. 8-OH-Gua is excreted

into urine from cellular DNA without further metabolism [8]. Thus, its urinary excretion reflects oxidative DNA damage and the “whole body” repair of DNA. Therefore, an assay that is able to determine the level of 8-OH-Gua in biological fluids can better reflect the extent of oxidative damage of cellular DNA. Since urine concentration of metabolites symbolizing DNA damage is very low, and urine as such is a complex matrix, measurement of these lesions in urine is an analytical challenge [9]. Hence, the quantification of 8-OH-Gua in biological fluids requires sensitive approach. Moreover, method should be selective, as guanine and other major urinary metabolites viz. uric acid, and ascorbic acid may also interfere the 8-OH-Gua determination. In addition common-diabetic patients are subjected to glucose test in blood serum and are required to undergo painful blood suction.

Literature survey reveals that various analytical methods including capillary electrophoresis with precolumn derivatization [10], high performance liquid chromatography (HPLC) equipped with an electrochemical detector, [11] flow injection biosensor with an online microdialysis sampling method [12] and gas chromatography–mass spectrometry (GC/MS) [13] have been discovered for 8-OH-Gua determination with low detection limit. These methods are complicated, time consuming, need expensive instrumentation and cannot be used for self-diagnosis. Therefore, these methods are not widely used, diabetic patients want to

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utilize methods that offer ease of detection, do not require blood suction, have numerically sensitive quantification and allow self-diagnosis anywhere. Hence, still there is a vital need to develop a specific method that is fast, sensitive, easy to use, and cost effective having lower detection limit for the determination of oxidative DNA damage and its effects.

In the present method a new approach is proposed using edge plane pyrolytic graphite electrode (EPPGE) after surface modification with gold nanoparticles attached to single walled carbon nanotubes. The modified electrode was used for the typical analysis of 8-OH-Gua in biological fluids for detecting any oxidative DNA damage which further clues to the diabetic diagnosis. Square wave voltammetry is a versatile technique for electroanalytical determination as it has higher sensitivity and effectively suppresses background current. A detailed comparison has been made to examine electrochemical response of single walled carbon nanotube modified edge plane pyrolytic graphite electrode (SWCNT/EPPGE) and gold nanoparticles attached to single walled carbon nanotube modified edge plane pyrolytic graphite electrode (AuNP-SWCNT/EPPGE) towards 8-OH-Gua oxidation. The comparative study shows that the catalytic effect of nanotubes gets enhanced significantly on attachment with gold nanoparticles. Improved sensitivity, selectivity, reproducibility and stability of AuNP-SWCNT/EPPGE are observed in comparison to SWCNT/EPPGE, which make it attractive for further developments in the field of electrochemical sensors. In this paper, the concentration of 8-OH-Gua in urine samples of diabetic patients is determined and compared with non-diabetic (control) samples using the proposed sensor.

2. Experimental

2.1. Materials

Pyrolytic graphite pieces ($2 \times 2 \times 10 \text{ mm}^3$) were obtained from Pfizer Inc. New York, U.S.A. Guanine and 8-OH-Gua were obtained from Fluka and Adams Chem. Co. Illinois, USA respectively and used without further purification. Ascorbic acid and uric acid were purchased from Wako Pure Chemicals Industries Ltd., Japan and Sigma-Aldrich, respectively. All solutions were prepared in double distilled water. The first urine samples of the day were collected from three departmental personnel and used as control (male: 53 years, 69 kg, male: 50 years, 76 kg, male: 55 years, 75 kg) whereas, three urine samples of diabetic patients (male: 52 years, 61 kg, male: 57 years, 72 kg, male: 54 years, 84 kg) were obtained from the hospital of Indian Institute of Technology, Roorkee after clearance from Ethics Committee of I.I.T. Roorkee. The urine samples collected were stored in a refrigerator until used. Prior to recording voltammograms, urine samples were diluted 60 times with phosphate buffer solution of pH = 7.2 to minimize matrix complexity. Single walled carbon nanotubes (SWCNT) of purity >98% were purchased from Bucky, USA. HAuCl_4 was purchased from Aldrich (USA). The stock solution of 8-OH-Gua was prepared in 2 M Na_2CO_3 and the stock solutions of guanine, uric acid and ascorbic acid were prepared

in double distilled water and then diluted with phosphate buffer solution (PBS) of pH = 7.2 to achieve the desired concentration. Other solvents and chemicals used were of analytical grade obtained from Merck. Phosphate buffer solutions of pH range of 2.4–10.0 and ionic strength $\mu = 1.0 \text{ M}$ were prepared according to the method of Christian and Purdy by mixing standard solutions of Na_2HPO_4 and NaH_2PO_4 [14].

2.2. Apparatus and experimental procedure

All voltammetric measurements were carried out using BAS (Bioanalytical Systems, West Lafayette, USA) CV-50W voltammetric analyzer. The voltammetric experiments were performed using three electrode single compartment cells equipped with a SWCNT/EPPGE or AuNP-SWCNT/EPPGE as working, platinum wire as counter and Ag/AgCl (3 M NaCl) as reference electrode (Model MF-2052 RB-5B). The surface morphology of the modified electrodes was characterized using Quanta 200-F (FEI Company) FE-SEM instrument. All potentials are referred to the Ag/AgCl reference electrode at an ambient temperature of $27 \pm 2^\circ \text{C}$. The pH of the buffer solutions was measured using Eutech Instruments pH 510, pH meter after standardization with 0.05 M potassium hydrogen phthalate (pH 4.0 at 25°C) and 0.01 M borax (pH 9.2 at 25°C). The voltammetric experiments were performed in 1.0 M phosphate buffer solution of different pH containing 8-OH-Gua at AuNP-SWCNT/EPPGE in a suitable potential range. The modified electrodes gave reproducible results for three consecutive runs in the same solution, however, before the next sample a potential of -100 mV for 60 s was applied to overcome the problem of adsorption of analyte at the electrode surface. The optimized square wave voltammetric parameters used were: square wave amplitude (E_{sw}): 25 mV; potential step (E): 4 mV; and square wave frequency (f): 15 Hz.

2.3. Preparation of electrode

A Pyrex glass tube of appropriate length and diameter was cleaned thoroughly and dried. One end of the glass tube is filled with epoxy resin (Araldite, Ciba Geigy) up to a height of about 2 cm, with the help of a thin glass rod. Pyrolytic graphite piece was then slid in the glass tube carefully from the other open end of the tube so that its edge plane side reaches at the bottom of the tube. The graphite piece was pushed with a thin glass rod till 3/4th portion of it got covered with epoxy resin to avoid any air pocketing between the tube and the graphite piece. The electrode was then allowed to stand for 24 h until resin gets solidified. The glass tube was rubbed on an emery paper till the edge plane side of graphite appeared at the resin end. Finally, the electrode was washed several times with double distilled water in order to remove the fine powder adhered to the electrode surface of pyrolytic graphite. Mercury was filled into the glass tube and a copper wire was inserted to make proper contact of electrode to the outer circuit.

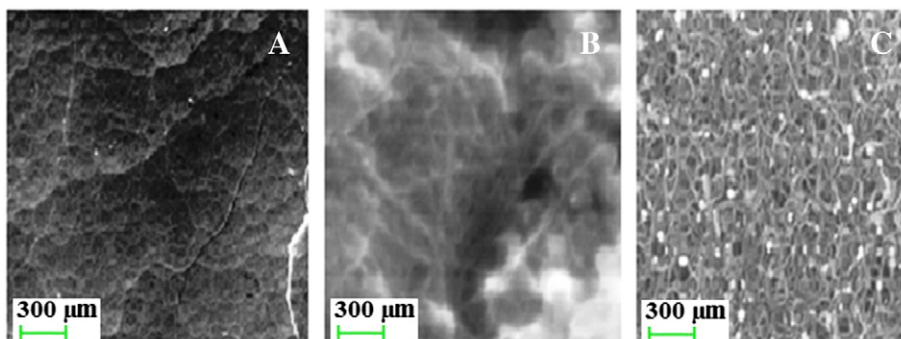


Fig. 1. Comparison of typical FE-SEM images of (A) EPPGE, (B) SWCNT/EPPGE and (C) AuNP-SWCNT/EPPGE.

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