



# A comparison of electrochemically pre-treated and spark-platinized carbon fiber microelectrode. Measurement of 8-oxo-7,8-dihydro-2'-deoxyguanosine in human urine and plasma

Z. Bartosova<sup>a</sup>, D. Riman<sup>a</sup>, V. Halouzka<sup>a,b</sup>, J. Vostalova<sup>c</sup>, V. Simanek<sup>c</sup>, J. Hrbac<sup>d,\*\*</sup>, D. Jirovsky<sup>a,\*</sup>

<sup>a</sup> Department of Analytical Chemistry, Palacky University, Faculty of Science, 17.listopadu 12, CZ-771 46 Olomouc, Czech Republic

<sup>b</sup> Department of Physics and Materials Engineering, Faculty of Technology, Tomas Bata University in Zlin, nam. T.G. Masaryka 275, CZ-76001 Zlin, Czech Republic

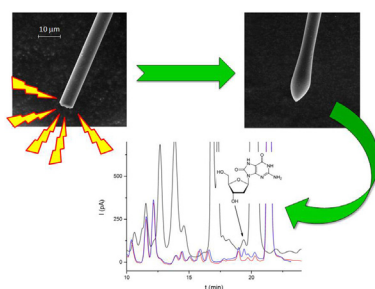
<sup>c</sup> Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacky University, Hnevotinska 3, CZ-775 15 Olomouc, Czech Republic

<sup>d</sup> Department of Chemistry, Faculty of Science, Masaryk University, Kamenice 5, CZ-625 00 Brno, Czech Republic

## HIGHLIGHTS

- Novel method of carbon fiber microelectrode activation and platinization using spark discharge.
- The activation procedure is efficient, fast and solvent-free.
- Modification of the surface and the shape of the carbon fiber microelectrode during the process.
- The spark-etched platinized carbon fiber sensors are highly sensitive.
- The sensor was successfully applied to HPLC analysis of 8-oxo-7,8-dihydro-2'-deoxyguanosine in plasma and urine.

## GRAPHICAL ABSTRACT



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## ABSTRACT

A novel method of carbon fiber microelectrode activation using spark discharge was demonstrated and compared to conventional electrochemical pretreatment by potential cycling. The spark discharge was performed at 800 V between the microelectrode connected to positive pole of the power supply and platinum counter electrode. Spark discharge led both to trimming of the fiber tip into conical shape and to the modification of carbon fiber microelectrode with platinum, as proven by scanning electron microscopy and electron dispersive X-ray spectroscopy. After the characterization of electrochemical properties using ferricyanide voltammetry, the activated electrodes were used for electrochemical analysis of 8-oxo-7,8-dihydro-2'-deoxyguanosine, an oxidative stress marker. Subnanomolar detection limits ( $0.55 \text{ nmol L}^{-1}$ ) in high-performance liquid chromatography were achieved for spark platinized electrodes incorporated into the flow detection cell.

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\* Corresponding author.

\*\* Corresponding author.

E-mail addresses: [jhrbac@atlas.cz](mailto:jhrbac@atlas.cz) (J. Hrbac), [david.jirovsky@upol.cz](mailto:david.jirovsky@upol.cz) (D. Jirovsky).

## 1. Introduction

Electrochemical detection employing carbon fiber microelectrodes (CFME) is a promising research direction in the field of separation and microfluidic techniques [1–4]. Due to their suitable dimensions (5–30  $\mu\text{m}$  in diameter, few millimeters in length), CFMEs can be directly inserted into flow paths such as outlets of HPLC columns or microfluidic channels. To achieve the best performance in sensing, CFMEs have to be activated or modified. The usual approach of CFME activation is electrochemical pretreatment based on potential cycling of CFME in solution using various protocols. In this regard, the CFME pretreatment is particularly important for dopamine sensing [5–7]. The application of a widely used protocol (0–3 V vs. Ag/AgCl, 70 Hz for 20 s in citrate-phosphate buffer, pH 7.4) increases the sensitivity towards dopamine (ca.  $4 \times$ ) and removes the interference from ascorbic acid by shifting its the anodic peak towards negative potential [8]. Studies addressing the effects of pretreatment are available also for analytes other than dopamine. In CV and DPV determination of folic acid the pretreatment of CFMEs by triangular waveform (0–2 V vs. SCE, 10 Hz, 1 min in Britton-Robinson's buffer, pH = 2) resulted in five-fold increase in sensitivity compared to bare CFME [9]. Similar sensitivity increase ( $4 \times$ ) was reported for CFME used for the stripping voltammetry of linurone herbicide after pretreatment (triangular waveform 0–1.5 V at 1 V  $\text{s}^{-1}$ , 20 cycles in  $\text{H}_3\text{PO}_4/\text{H}_2\text{PO}_4$ -mixture at pH = 2) [10]. The electrochemical pretreatment (triangular waveform, –1–2 V vs. Ag/AgCl, 20 V  $\text{s}^{-1}$ , 5 min, performed in 1M- $\text{H}_2\text{SO}_4$ ) resulted not only in sensitivity increase ( $15 \times$ ) towards methylene blue but also in improvement in reversibility of its voltammetric response [11].

In this work we suggest spark discharge as an alternative to CFME pretreatment, leading also to CFME modification by the material derived from the counter electrode used for sparking. Specifically, spark discharge between CFME and platinum counter electrode leads to platinization of CFME. To assess the advantage of a novel spark-platinized CFME-based sensor we have decided to test the analysis of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) in plasma and urine.

8-oxodG is a product of an oxidatively modified guanine base and can be determined in different biological samples, including blood, urine, saliva, cerebrospinal fluid, leukocytes and tissues [12]. It has been postulated that 8-oxodG is one of the sensitive biomarkers for oxidative damage of cellular DNA [13]. 8-oxodG is a useful marker for evaluating the cardiovascular risk factors and inflammatory status in hypertensive patients [14], for identifying persons at risk of developing cancer [15], for early prediction of lifestyle-related disease risk [16], and for assessing the environmental conditions that are expected to have a serious long-term impact on the health status in adults [17].

A variety of methods have been used to quantify 8-oxodG concentrations, such as enzyme-linked immunosorbent assay (ELISA), gas chromatography-mass spectrometry (GC-MS), liquid chromatography with tandem mass-spectrometry (LC-MS/MS) and high performance liquid chromatography with electrochemical detection (HPLC-ECD) [18].

Among instrumental analytical methods, the highly sensitive and specific electrochemical detection has been most widely used, with carbon electrodes being the most popular choice. Amperometric detection is a selective technique in 8-oxodG assays due to relatively low working potentials (around +500 mV vs. Ag/AgCl). Using commercial HPLC-ECD setup with glassy carbon electrode, sub-nanomolar LODs for 8-oxodG were reported in standard samples in the presence of excess uric acid, a strong interferent, while much higher LODs (in the order of 100 nM) were achieved in urine samples subjected to solid-phase extraction (SPE) [19].

In this work, we evaluated the performances of our previously described [20] carbon fiber-based detector in HPLC-ECD of 8-oxodG. For the first time, the CFME modification by sparking process was evaluated as an alternative to conventional electrode pretreatment. It has been found, that during the activation of the CFME by electric discharge against platinum counter electrode the effective modification of CFME with platinum occurs.

## 2. Materials and methods

### 2.1. Chemicals and reagents

The standards of potassium ferricyanide, potassium chloride and 8-oxo-7,8-dihydro-2'-deoxyguanosine were purchased from Sigma (Sigma, St. Louis, MO, USA). Stock solution of 8-oxo-7,8-dihydro-2'-deoxyguanosine (1.0 mg  $\text{mL}^{-1}$ ) was prepared and stored in refrigerator. The work solution was prepared freshly by diluting the stock solution by the mobile phase.

For the mobile phase preparation, disodium hydrogen phosphate and phosphoric acid (TraceSelect purity) purchased from Fluka (Fluka AG, Buchs, Switzerland) were used. Methanol (Merck, Darmstadt, Germany), was of gradient grade purity. The blood plasma and urine samples of patients were obtained from the University Hospital in Olomouc, Czech Republic.

### 2.2. CFME preparation, electrochemical pretreatment and spark treatment

A polyacrylonitrile-based carbon fiber (Sigrafil C30) was obtained from a local distributor (GRM-systems, Czech Republic). The procedure for microelectrode fabrication was as follows: carbon fiber was glued using conductive silver epoxy (Epotek H20E, Polytex, Germany) onto a copper wire, the junction was then hardened at 170  $^{\circ}\text{C}$  for 10 min. The fiber with copper contact attached was fitted into a glass capillary and about 5 mm of the fiber was left protruding from its tapered end. Both ends of the capillary were sealed using epoxy resin (L-200, Havel Composites Inc., Czech Republic). The fiber end of the electrode was briefly sonicated in dichloromethane in order to clean the fiber. Prior to use, the microelectrode was activated i. using the previously described electrochemical pretreatment procedure [7], ii. using spark discharge of positive or, iii. negative polarity. For electrochemical pretreatment the microelectrode was placed into electrochemical cell containing 1% (w/w) NaCl and cycled between 0 and 2.9 V vs. Ag/AgCl for 20 s, 50 Hz sinusoidal wave, followed by 5 s at constant potential –0.8 V and 5 s at 1.5 V [7]. For spark treatment, the CFME and a platinum foil electrode (99.95% Pt, Elektrochemie Detektor s.r.o., Czech Republic) were connected to an in-house built DC power supply, consisting of 10 kHz oscillator, transformer and a 5-stage Cockroft-Walton cascade, delivering the voltage of 800 V. The CFME was brought into close proximity of a platinum foil until a discharge occurred, manifested as a spark. For CFME connected to positive pole of the power supply the procedure is referred as (+) or positive sparking process, the opposite connection is referred to as negative or (–) sparking.

### 2.3. Cyclic voltammetry experiments

Cyclic voltammetry measurements and electrochemical pretreatment of CFMEs were performed using Nanoampere electrochemical workstation (L-Chem, Czech Republic). The electrochemical experiments were performed in three-electrode setup, employing RE-5b reference electrode (BASi, U.S.A.), platinum wire as auxiliary electrode and studied CFMEs as working electrodes. Voltammograms were processed using el-Chem Viewer software [21].

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