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Electrochemical detection of hydrogen peroxide on platinum-containing tetrahedral amorphous carbon sensors and evaluation of their biofouling properties

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A B S T R A C T

Hydrogen peroxide is the product of various enzymatic reactions, and is thus typically utilized as the analyte in biosensors. However, its detection with conventional materials, such as noble metals or glassy carbon, is often hindered by slow kinetics and biofouling of the electrode. In this study electrochemical properties and suitability to peroxide detection as well as ability to resist biofouling of Pt-doped ta-C samples were evaluated. Pure ta-C and pure Pt were used as references. According to the results presented here it is proposed that combining ta-C with Pt results in good electrocatalytic activity towards H_2O_2 oxidation with better tolerance towards aqueous environment mimicking physiological conditions compared to pure Pt. In biofouling experiments, however, both the hybrid material and Pt were almost completely blocked after immersion in protein-containing solutions and did not produce any peaks for ferrocenemethanol oxidation or reduction. On the contrary, it was still possible to obtain clear peaks for H_2O_2 oxidation with them after similar treatment. Moreover, quartz crystal microbalance experiment showed less protein adsorption on the hybrid sample compared to Pt which is also supported by the electrochemical biofouling experiments for H_2O_2 detection.

1. Introduction

Hydrogen peroxide (H_2O_2) is a simple molecule that has widespread usage ranging from industry and food to clinical applications. It has high standard electrochemical potential [1] which makes it a powerful oxidizing agent. It can be either electrochemically oxidized to O_2 ($E^0 = 0.695$ V) or reduced to water ($E^0 = 1.763$ V) in diffusion controlled two-electron processes [2]. In industrial applications H_2O_2 and its redox reactions are closely linked to fuel cell technology as it has been proposed to be formed as reaction intermediate in the oxygen reduction reaction [2,3]. In living organisms cells produce H_2O_2 mitochondria to control growth and apoptosis. It has also been suggested that H_2O_2 has a key role in aging [4]. Furthermore, it is formed as a by-product in various enzymatic reactions, such as those catalyzed by glucose oxidase [5], glutamate oxidase [6], and cholesterol oxidase [7]. Enzymatic production makes H_2O_2 feasible for biosensor applications as it typically has lower oxidation potential compared to the enzyme substrate molecules.

Carbon-based materials are often considered as good candidates in sensor applications owing to their low cost, wide potential window, relative electrochemical inertness and adequate electrocatalytic activity in various redox reactions [8]. However, in detecting H_2O_2 carbon materials as such do not typically perform as well as noble metals, for instance Au and Pt [9]. It has been shown that pure glassy carbon does not catalyze redox reactions of H_2O_2 but by modifying the electrode surface for example with Ag [10–12], Pd [13,14], Pt [15] or carbon nanotubes [16] can result in measurable electrochemical reactions. In addition, Hrapovic et al. [15] demonstrated with their glassy carbon and carbon nanotube sensors that the detection limit for H_2O_2 with Pt-doped sensors strongly depends on the type of the carbon support.

Despite their good catalytic activity towards electrochemical oxidation and reduction of H_2O_2, the disadvantages of noble metals, especially Pt, include high cost and susceptibility to biofouling. Expenses can be lowered by using nanomaterials which also typically increases the surface area and can also increase the number of active sites [17]. Biofouling has been proposed as the main reason for biosensor failure in vivo [18]. It can be characterized as the accumulation of undesired biological material, such as proteins and cells, on sensor surface [18,19]. It is detrimental to the sensor function as it may prevent the necessary contact between the analyte and the surface.

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Moreover, the detection of H_2O_2 on conventional metal or carbon fiber electrodes is hindered by slow kinetics [10,20] and relatively high overpotential [21], which allows also the oxidation of interfering species, such as ascorbic acid and uric acid [10]. Thus, new materials that facilitate fast and interference-free detection of H_2O_2 as well as resist biofouling are in demand.

Tetrahedral amorphous carbon (ta-C) is hydrogen-free and consequently the hardest, strongest, and slickest form of diamond-like carbon (DLC). In addition, DLC has previously been shown to resist microbial and protein attachment [22] which indicates that ta-C also could possess similar antifouling properties. On the other hand, adsorption of bovine albumin serum (BSA) is suggested to be favored on ta-C when compared to other types of DLC with higher hydrogen content and lower sp^3 fraction [23]. With its large water window (3.7 V in H_2SO_4 and 3 V in PBS [24]) ta-C is a feasible material for electrochemical detection of various analytes in aqueous media [25]. However, in our experience pure ta-C surfaces typically perform poorly in detecting H_2O_2. In order to overcome this we have doped our ta-C samples with Pt in order to enhance the oxidation of H_2O_2. To our knowledge, no Pt-doped ta-C has been fabricated and used in electrochemical detection of H_2O_2 before. However, it has been shown that doping DLC with Pt affects its electrochemical properties [26–28]. Pleskov et al. [28] suggested that Pt acts as a catalyst for charge transfer in otherwise electrochemically inactive DLC. On the contrary, our ta-C is innately electrochemically active and adding Pt is aimed at oxidation of H_2O_2. Moreover, we have previously shown that functionalizing ta-C with NH_2 groups allows the immobilization of GluOx on ta-C [29] which makes the material a suitable candidate for biosensor applications. In addition, our ta-C electrodes with small exposed Pt areas were an order of magnitude more sensitive towards dopamine compared to Pt electrodes [30,31]. In this paper we inspect detection of H_2O_2 on hybrid Pt-doped ta-C in comparison with pure ta-C and Pt and evaluate biofouling properties of the samples by immersion in protein solutions.

2. Materials and methods

2.1. Sample fabrication

Studied samples consisted of 20 nm thick underlying titanium layer and 7 nm thick undoped or Pt-doped ta-C layer with Pt atomic concentration of 0 and 20%. Samples with 8 nm thick Pt layer only were fabricated as well. Substrates were either highly conductive p-type boron-doped (100) Si wafers with 0.001–0.002 Ω-cm resistivity (Ultrasil, USA) or silica-coated quartz crystals (Q-Sense, Biolin Scientific, Sweden) designed for quartz crystal microbalance with dissipation (QCM-D) experiments. Silicon wafers were cleaned by standard RCA-cleaning procedure before deposition.

Sample fabrication procedure consisted of direct current magnetron sputtering (DC-MS) for Ti layer and dual filtered cathodic vacuum arc (FCVA) deposition for carbon and platinum layers. Both deposition systems were installed in one chamber. DC-MS system was equipped with a circular and water-cooled magnetron sputtering source with 2 inch Ti target. Titanium underlying layers were deposited under the following deposition conditions: discharge power was fixed at 100 W, total pressure was 0.67 Pa, Ar gas flow rate was 28 sccm, deposition temperature was close to room temperature, and deposition time was 350 s. Cathodic arc deposition system (Lawrence Berkeley National Laboratory, USA) was equipped with a 60° bent magnetic filter for reduction of macroparticle contamination. In order to obtain Pt-doped ta-C, composite cathode made of 6.35 mm graphite rod and two embedded 1 mm Pt wires in the center was used. For deposition of ta-C or Pt layers only, separate graphite and platinum cathodes were utilized. The arc current pulses had amplitude of 0.7 kA and 0.6 ms pulse width. Each pulse was triggered at 1 Hz frequency. The 2.6 mF capacitor bank was charged to 400 V. The number of pulses was 360 for carbon and combined cathodes and 1440 for Pt. Total pressure during the deposition process was no less than $1.3 \cdot 10^{-4}$ Pa. The distance between the substrate holder and the filter was about 20 cm. Samples were placed in the rotating holder (rotational velocity used was 20 rpm).

After deposition wafers were cut into rectangular chips with an automated dicing saw. Samples were stored dry in room temperature. Before electrochemical characterization and biofouling experiments circular area (0.28 cm^2 or 0.50 cm^2) was defined from the sample with a PTFE tape (Irpola Oy, Finland) or with a custom made polycarbonate sample holder.

2.2. Physical characterization of samples

The sample morphology was studied with optical microscopy (Leica DMRX, Leica Microsystems, Germany), scanning electron microscopy (SEM, Hitachi-4700, Hitachi Ltd, Japan) and scanning probe microscopy (Ntegra Aura, NT-MDT Company, Russia). The latter, operated in ambient atmosphere with variable measuring facilities, was used for atomic force microscopy (AFM) and spreading resistance microscopy, i.e. conductive-AFM (c-AFM) and current–voltage spectroscopy. The measurements were carried out in 'scanning by sample' mode. For c-AFM, a diamond coated conductive probe (DCP10 or HA_HP_DCP, NT-MDT Company, Russia) was mounted on a special probe holder designed for the current measurements under the varied voltage applied to the probe. The measurements were performed in contact regime. The typical curvature radius of a tip was about 100 nm. The force constant values are nominally 11.5 N/m and 16 ± 3.2 N/m for DCP10 and HA_HP_DCP cantilevers, respectively. Bitmap images (topography and current maps) were processed with Gwyddion (version 2.34) software.

In order to define Pt atomic concentration of the hybrid sample, X-ray fluorescence (XRF) spectroscopy (Spectroscan MAX-GV, Spectron Ltd, Russia) was utilized. Quantitative analysis was made according to a calibration curve. Dependence of Pt atom number on intensity of Pt characteristic line (1312.6 Å for LiF200 detector) was determined for samples consisting of single platinum layers with different thicknesses deposited on Si wafer. Thickness was used as a measure of number of Pt atoms. The real thickness of Pt layers was measured with a Dektac XT profilometer (Bruker Corporation, USA) with 6 Å step height repeatability.

Samples containing ta-C were characterized with visible Raman spectroscopy (WITec alpha 300 spectrometer, WITec Wissenschaftliche Instrumente und Technologie GmbH, Germany). Spectra were acquired with 532 nm laser wave length and 0.5 s exposure time. All obtained spectra were processed by averaging of 50 spectra and a background subtraction. Non-linear spectrum background was caused by silicon signal masked partly by Ti and ta-C layers. In order to make accurate fitting of carbon peaks (Gaussian function), reduced Si spectra was subtracted. It is to be noted, that Raman spectrum of ta-C typically shows a D-band peak (~1360 cm^{-1}) and a G-band peak (~1560 cm^{-1}) [35,36]. Their ratio I(D)/I(G) is correlated to the sp^3/sp^2 fraction ratio, the band gap value, and film density.

2.3. Electrochemical measurements

Cyclic voltammetry was performed with a Gamry Reference 600 potentiostat and Gamry Framework software (Gamry Instruments, USA). Reference electrode was a skinny Ag/AgCl electrode (Sarissa Biomedical Ltd, UK) and counter electrode a glassy carbon rod.

In order to clean the samples they were cycled in nitrogen purged 0.15 M H_2SO_4 for at least 100 cycles between −0.4 V and 1.3 V vs. Ag/AgCl with 1 V s^{-1}. H_2O_2 solutions (0–100 mM) were freshly prepared from 30% H_2O_2 (Merck KGaA, Germany) by dilution in phosphate buffered saline (PBS, pH 7.4). The experiments were conducted by immersing the sample in nitrogen purged H_2O_2 solutions and cycling 3 times between −0.4 V and 1.3 V vs. Ag/AgCl with cycling rate of 50 mV s^{-1}.