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Short communication

Characterization of different diamond-like carbon electrodes for biosensor design

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Abstract

Diamond-like carbon (DLC) films are gaining big interest in electrochemistry research area. DLC electrodes made with different ratio of sp^3/sp^2 carbon hybridization or doped with different percentages of nickel were characterized electrochemically by cyclic voltammetry and by amperometric measurements towards hydrogen peroxide. SiCAr1 and SiCNi5% were chosen as sensitive transducers for the elaboration of amperometric glucose biosensors. Immobilization of glucose oxidase was carried out by cross-linking with glutaraldehyde. Measurements were made at a fixed potential + 1.0 V in 40 mM phosphate buffer pH 7.4. SiCAr1 seems to be more sensitive for glucose, 0.6875 μA/mM, than SiCNi5%, 0.3654 μA/mM. Detections limits were 20 μM and 30 μM, respectively. Apparent Michaelis-Menten constants were found around 3 mM. Forty-eight percent and 79% of the original response for 0.5 mM glucose remained after 10 days for both biosensors, respectively.

Keywords: Diamond-like carbon; Biosensors; Glucose oxidase; Amperometric measurements

1. Introduction

Biosensors, combining a selective biological recognition element and a sensitive transducer, are of increasing importance in many areas such as medicine, food quality and safety control, and environmental pollution monitoring. Amperometric enzyme electrodes hold a leading position among biosensor systems presently available and have already found a large commercial market. The most common enzymes used in monoenzymatic systems are oxidases; especially glucose oxidase due to its low price and high stability. Such devices combine the specificity of the enzyme for recognizing a given target analyte, whilst their sensitivity is greatly influenced by the transducer [1–3]. The most employed electrochemical transducers are platinum, gold and carbonaceous materials [4,5]. Carbon electrodes exist in different allotropes and they are widely used in the field of biosensors. Glassy carbon (GC) electrodes are often used as transducers [6–8] by employing a modified GC electrode or by changing the methods of enzyme immobilisation in the aim to get sensitive, stable and reproducible biosensor. Graphite, porous carbon [9] and carbon films [10,11] electrodes are also used as transducers in the field of biosensors.

Diamond-like carbon films are gaining now big interest. These films are chemically stable, optically transparent and achieve good mechanical properties, low coefficient of friction, and strong wear resistance. Due to these properties, DLC films found application in variety of areas such as electronic, optical, mechanical and biomedical applications. Pulsed laser deposition of diamond-like carbon leads to high purity films with a predominance of sp^3 hybridization (diamond) at low deposition temperature. Since the last ten years, femtosecond lasers have been used in pulsed laser deposition. In this case, the kinetic energy of the ejected particles can be increased up to a few keV, which is much higher than in nanosecond regime. Original properties of these coatings, including lower stress and good adherence, may be related to such a

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high value of the kinetic energy. These advantages allow the development of DLC coatings without any adhesion underlayer [12].

Diamond-like-carbon films constitute a new research area in electrochemistry. They have been used as electrodes in electrochemical microgravimetry on quartz crystal electrodes [13], as nitrogenated DLC films (N:DLC) for metal tracing analysis [14] and as coatings for polycarbonate membranes used as permselective barriers in glucose oxidase biosensors [15,16]. We have tested amorphous DLC electrodes in a preliminary work for the development of a glucose amperometric biosensor. It can be seen that DLC films can be used as transducers in the field of biosensors nevertheless they are less sensitive then glucose oxidase/glassy carbon electrodes [17]. Properties of DLC films can be adjusted depending on their application. Thus, the sp^3/sp^2 carbon hybridization ratio may be adjusted and controlled according to the deposition process and conditions. Their conductivity also can be controlled by doping elements such as metals, Si and N.

The present study deals with characterization and the comparison of different diamond-like carbon films as matrices for biosensor design. Some of these films are elaborated with different ratio of sp^3/sp^2 carbon hybridization and others are doped with Ni. Cyclic voltammetry and amperometric measurements aim to see whether properties of DLC films affect their sensitivities towards hydrogen peroxide. The behavior of the most sensitive electrodes for H_2O_2 will be studied for biosensor elaboration by using a conventional glucose oxidase assay system.

2. Experimental

2.1. Materials

Glucose Oxidase (GOD, EC 1.1.3.4, 130 U/mg) was kindly given by the Laboratory of Biomolecular Electronics, IMBG, Kiev, Ukraine. Bovine Serum Albumin (BSA, Fraction V) and α-D(+)-glucose were obtained from Sigma. Glutaraldehyde, 24-wt.% solution in water, was purchased from Acros Organics. Hydrogen peroxide 30% and chemicals used for preparing buffer solution, sodium hydroxide and potassium dihydrogen phosphate were obtained, respectively, from Fluka, Sigma and Prolabo. Glucose standard solutions were elaborated by diluting a 1 M α-D(+)-glucose stock solution prepared 24 h before use to establish the anomeric equilibrium between α and β forms of D-glucose. Ultra-pure water (resistivity > 18.2 MΩ cm, Elga System) was used for the preparation of all solutions.

2.2. Measurements and apparatus

Voltammetric and amperometric experiments were carried out using a Voltalab 10 (PGZ100 & VoltaMaster 4). The electrochemical cell consisted of a three-electrode system with a platinum plate (0.54 cm^2) and a saturated calomel electrode (SCE) as counter and reference electrode, respectively. Diamond-like carbon electrodes with an effective surface of 0.15 cm^2 were used as working electrodes. A magnetic stirred and a stirring bar provide the convective transport. All potentials were reported versus SCE. The background current was allowed to decay to a steady value before aliquots of substrate solution were added. The biosensor response was measured as the difference between total and residual current.

2.3. Diamond-like carbon films deposition

These films were prepared by the Laboratory of Signal Processing and Instrumentation, Jean Monnet-University, France. Nickel containing DLC films have been deposited by femtosecond pulsed laser deposition by ablating alternatively graphite (purity 99.997%) and nickel (purity 99.9%) targets under vacuum conditions at room temperature onto p-silicon substrates at a target to substrate distance of 36 mm. The femtosecond laser (Concerto, BMI/TCL, Ti-Saphir, $\lambda = 800$ nm, pulse duration 300–350 fs, repetition rate 1 kHz, energy per pulse 1 mJ, energy density 2.6 J/m^2) has been alternatively focused on the targets with an incident angle of 45°, by using a shutter rotating at 32 rpm.

Two percentages of nickel have been introduced in diamond-like carbon films according to the conditions mentioned below. For the first sample, six sequences of ablation on each target have been performed alternatively during 89 s for graphite and 1 s for nickel. A similar procedure has been carried out for the second sample, with 16 sequences of ablation on each target during 32 s for graphite and 1 s for the nickel. Taking into account the deposition rate of pure carbon (22 nm/min) and pure nickel (35 nm/min), nickel incorporated DLC film thickness is estimated in the range of 200 nm for both samples. Percentages of nickel in diamond-like carbon films have been determined by X-ray photoelectron spectroscopy (XPS) and Rutherford backscattering spectroscopy (RBS). They showed 2 at.% for the first sample (SiCNi2%) and 5 at.% for the second one (SiCNi5%) [14].

Three other types of DLC films have been elaborated by femtosecond pulsed laser ablation onto p-silicon substrates under argon pressure at room temperature. Normally DLC films made under vacuum show a predominance of sp^3 hybridized carbon [15]. Thus, working under argon pressure and modifying this pressure leads to DLC films with different ratio of sp^3/sp^2 carbon hybridization. $P_{Ar} = 2 \times 10^{-2}$ mbar (SiCAr1), $P_{Ar} = 5 \times 10^{-1}$ mbar (SiCAr2) and $P_{Ar} = 5 \times 10^{-3}$ mbar (SiCAr3) were used, respectively, for the deposition of DLC films by ablation graphite target during 10 min.

All diamond-like-carbon electrodes cited below were cleaned with ultra-pure water and with optical paper before use.

2.4. Enzyme immobilization

Immobilization was carried out by cross-linking with glutaraldehyde. Thus, a mixture of 5% glucose oxidase, 5% BSA and 10% glycerol in phosphate buffer 20 mM pH 7.4 was prepared. The glycerol was used as a plasticizer in order to avoid cracks appearing in the biolayer. DLC electrodes were coated with a thin layer of this mixture and kept 20 min in

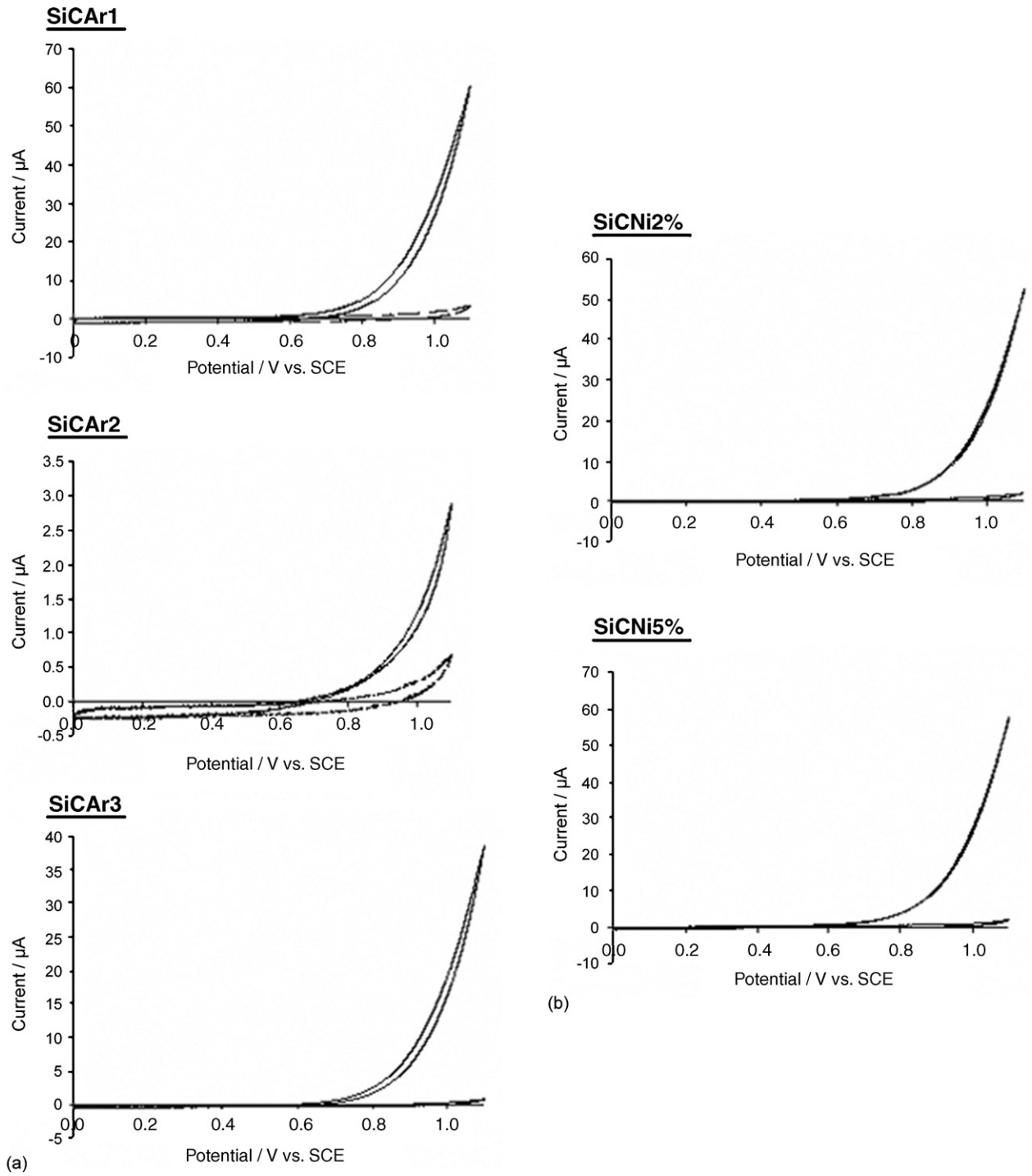

Fig. 1. (a) Cyclic Voltammograms of DLCs films with different ratio of sp^3/sp^2 carbon hybridization in 40 mM PB solution, pH 7.4 with and without 5 mM H_2O_2. (b) Cyclic Voltammograms of Ni-containing DLCs films in 40 mM PB solution, pH 7.4 with and without 5 mM H_2O_2.

glutaraldehyde vapor at room temperature. This bifunctional compound (OHC–$(CH_2)_3$–CHO) links covalently from each side to the amine groups of GOD and BSA, respectively, creating a stable biopolymer. The resulting enzyme electrodes were allowed to dry in air and were thoroughly washed and stored in 40 mM phosphate buffer (PB) solution, pH 7.4, at 4 °C when not in use.

3. Results and discussions

3.1. Hydrogen peroxide detection

Glucose biosensors are based on the fact that glucose oxidase, a flavoenzyme, catalyses the oxidation of glucose to gluconic acid in the presence of oxygen. Since hydrogen peroxide is the

Table 1
Hydrogen peroxide sensitivity of different diamond-like carbon electrodes in 40 mM PB solution, pH 7.4

Electrodes	SiCArl	SiCAr2	SiCAr3	SiCNi2%	SiCNi5%
Sensitivity (μA/mM)	4.4463	1.1872	2.0397	3.4659	4.6401

co-product of this reaction; its electroactivity can be used to obtain a measurable current signal.

$$\beta\text{-d-Glucose} + O_2 \leftrightarrow \text{GluconicAcid} + H_2O_2$$

$$H_2O_2 \leftrightarrow O_2 + 2H^+ + 2e^-$$

DLC electrodes were initially characterized electrochemically by recording their cyclic voltammograms between 0.0 V and 1.1 V in 40 mM phosphate buffer solution, pH 7.4 with and without 5 mM H_2O_2. The catalytic voltammograms obtained in the presence of hydrogen peroxide exhibit a greater sensitivity for SiCAr1 and SiCNi5% electrodes; no observable plateau was distinguished (Fig. 1a and b). After analysis of these responses, a potential of +1.0 V was chosen for H_2O_2 amperometric detection.

Amperometric measurements were carried out in PB solution at +1.0 V by injecting H_2O_2 aliquots; each addition result in a 0.02 mM increment in concentration. The comparison of DLCs electrodes according to H_2O_2 sensitivity is shown in Fig. 2. Their corresponding sensitivities are presented in Table 1. Since SiCAr1 and SiCNi5% electrodes enable a more satisfactory determination of hydrogen peroxide, 4.4463 μA/mM and 4.6401 μA/mM, respectively, their behavior will be studied for biosensor elaboration by using a glucose amperometric assay system.

3.2. Enzyme measurements

To evaluate the possible application and the behavior of the above-mentioned DLCs electrodes in biosensor construction, glucose biosensors were fabricated following the procedure previously described. Kinetics studies of the immobilized enzyme

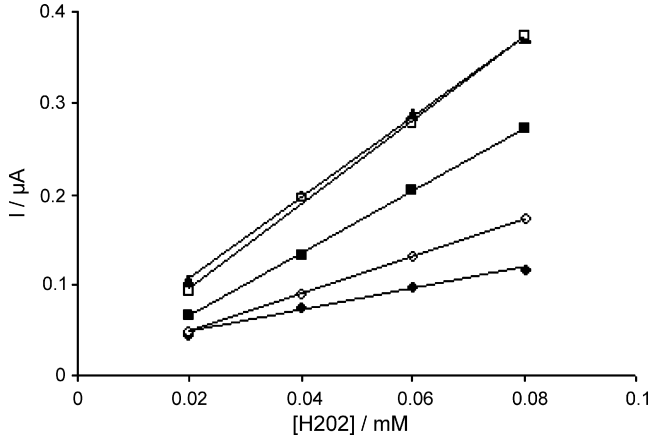

Fig. 2. Hydrogen peroxide calibrations curves for SiCAr1 (Δ), SiCAr2 (♦), SiCAr3 (◊), SiCNi2% (■) and SiCNi5% (□) electrodes for successive additions of 0.02 mM H_2O_2 in 40 mM PB, pH 7.4.

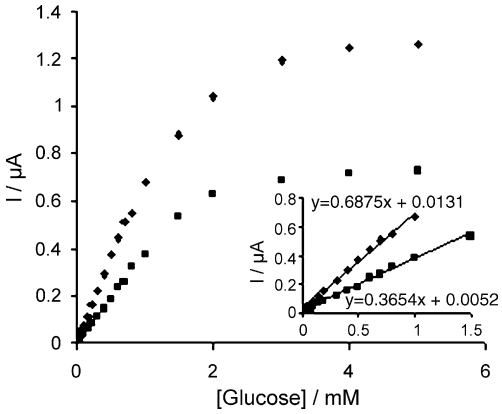

Fig. 3. Glucose Calibration curves for SiCAr 1 (♦) and SiCNi 5% (■) biosensors for successive additions of glucose in 40 mM PB, pH 7.4.

were carried out by plotting the electrochemical response to increasing concentrations of glucose. Fig. 3 shows the curves of DLCs electrodes, SiCAr1 and SiCNi5%, in 40 mM PB, pH 7.4 at a measurement potential of +1.0 V for successive additions of glucose.

The SiCAr1 glucose biosensor displays a linearity range up to 1 mM and until 1.5 mM for SiCNi5%. Corresponding detections limits were 20 μM and 30 μM, respectively. Even if these two electrodes have exhibited the same sensitivity for hydrogen peroxide, SiCAr1 biosensor shows higher sensitivity for glucose, 0.6875 μA/mM with $R = 0.9984$, than SiCNi5% biosensor, 0.3654 μA/mM with $R = 0.9982$. This fact can be explained by the use of two different topographical and constitutional structure electrodes leading to different and various interaction phenomenons between the immobilized biopolymer and the electrode surface. From the Lineweaver-Burk plots, apparent Michaelis-Menten constants of 3.38 mM and 3.09 mM were obtained, respectively, for SiCAr1 and SiCNi5% biosensors. The apparent K_m which depends on enzyme and not on the substrate is almost the same for both electrodes.

3.3. Operational and storage stability

The stability of enzyme sensors was usually limited by the deactivation and loss of enzyme. The deactivation of enzyme was mainly caused by unsuitable temperature. So, enzyme electrode needs to be kept at 4 °C. The loss of enzyme is highly related to the way the enzyme is fixed to the electrode (adsorption to the electrode, entrapment into a polymer, cross-linking with a bifunctional compound . . .).

The operational stability during 10 h for SiCAr1 and SiCNi5% glucose biosensors and their storage stability were tested in 40 mM phosphate buffer pH 7.4 containing 0.5 mM glucose.

The operational stability of both biosensors during ten working hours is presented in Fig. 4. The current response for 0.5 mM glucose seems to be very stable for SiCAr1 while it increases very slowly for SiCNi5%. It may be explained by the swollen of the biolayer with time allowing easily access of glucose to the electrode surface leading therefore to increasing quantity of

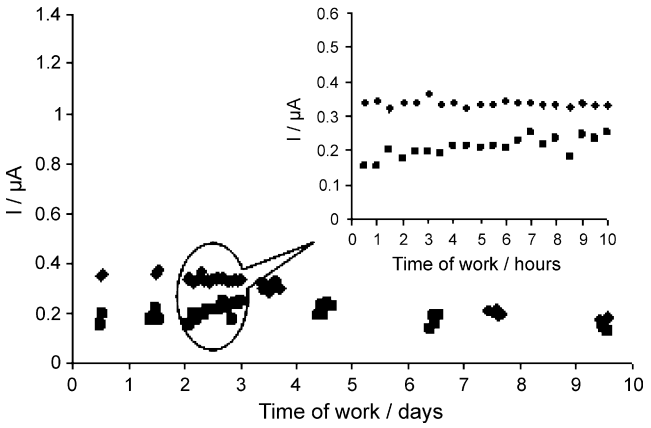

Fig. 4. Operational and storage stability of SiCAr1 (♦) and SiCNi5% (■) biosensors for 0.5 mM glucose concentration.

H_2O_2. It is illustrated in Fig. 4 by the slow increasing of the slope after 10 h of work.

The storage stability of SiCAr1 and SiCNi5% has been examined by checking periodically their activities. The current response of these two biosensors for 0.5 mM glucose remained almost unchanged during the three first days, and ∼48% and ∼79% of the original response remained after 10 days, respectively (Fig. 4). In fact, it can be deduced from X-ray photoelectron spectroscopy (XPS), near edge X-ray absorption fine structure (NEXAFS) and transmission electron microscope (TEM) that nickel is predominantly present in the metallic form in DLC films. They have the shape of nodules which are dispersed in the carbonaceous matrix [18]. In addition, AFM measurements provide an average roughness (Ra) of 2 nm for non-doped diamond-like carbon electrodes and 32 nm for nickel doped DLC electrodes. Thus, the higher roughness of SiCNi5% electrodes explains the better biolayer immobilization leading therefore to a more stable biosensor.

4. Conclusion

This work has been concerned with the characterization and the comparison of different diamond-like carbon electrodes for biosensor construction. DLC films appear to be very interesting in the electrochemical biosensor field. Evaluation of these electrodes was carried out by comparing their sensitivities towards hydrogen peroxide. The most sensitive electrodes were explored and studied for the development of a conventional glucose biosensor. SiCAr1 glucose oxidase based biosensor seems to be more sensitive than SiCNi5% but its main drawback is the stability that may be increased by fixing the enzyme differently on the electrode surface.

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