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Characterization of different DLC and DLN electrodes for biosensor design

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Diamond-Like Carbon and Carbon-Like Nanocomposite electrodes, novel materials in the field of biosensors, made with different ratio of sp^3/sp^2 carbon hybridization or doped with elements such as Ni, Si and W, were characterized electrochemically by cyclic voltammetry and by amperometric measurements towards hydrogen peroxide. SiCAr1 and SiCNi5% were chosen as sensitive transducers for 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 $\mu A/mM$) then SiCNi5% (0.3654 $\mu A/mM$). Detections limits were respectively 20 μM and 30 μM . Michaelis-Menten constants for the two electrodes were found around 3 mM. 48% and 79% of the original response for 0.5 mM glucose remained respectively for both electrodes after 10 days.

Keywords: Diamond-Like Carbon, Glucose oxidase, Amperometric measurements, Biosensor.

I. 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 Carbons electrodes exist in different allotropes and they are widely used in the field of biosensors. Glassy carbon electrodes are often used as transducers,⁶⁻⁸ either 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 carbon⁹ and carbon films^{10,11} electrodes are also used as transducers for elaboration of glucose biosensors.

Diamond-Like Carbon electrodes are gaining now big interest due to their chemical stability, mechanical properties and strong wear resistance. They have been tested in a preliminary work for development of glucose amperometric biosensor.¹² It can be seen that amorphous DLC films can be used as transducers in the field of biosensors. Properties of these films can be adjusted depending on their application. Thus, the Csp^3/sp^2 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 the characterization of different DLC and DLN films as matrices in biosensor design. Some of these films are elaborated with different ratio of sp^3/sp^2 carbon hybridization and the others are doped with elements such as Ni, Si and W. The aim of this work is to see whether changing properties of these films, we can change their sensitivities towards hydrogen peroxide and therefore elaborate a

glucose oxidase electrochemical enzyme biosensor with good sensitivity.

II. MATERIALS AND METHODS

II-A. 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 D(+)- glucose were obtained from Sigma. Glutaraldehyde, 24-wt% solution in water, was purchased from Acros Organics. Hydrogen peroxide 30% and chemical used for preparing buffer solutions, sodium hydroxide and potassium dihydrogen phosphate were obtained respectively from Fluka, Sigma and Prolabo. Glucose standard solutions were prepared by dilution of a 1 M D(+)-glucose stock solution. The stock solution was prepared 24 h before use to establish the anomeric equilibrium between α and β forms of D-glucose. Ultra-pure water (resistivity > 18.2 MO.cm, Elga System) was used throughout for the preparation of solutions.

II-B. Measurements and apparatus

Measurements were made in a one-compartment cell containing a platinum auxiliary electrode and a saturated calomel electrode (SCE) as reference. Voltammetric and amperometric experiments were carried out using a Voltalab 10 (PGZ100 & VoltaMaster 4). 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.

II-C. DLC and DLN films

II-C.a DLC 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. The films have been deposited 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) has been focused alternatively, by using a shutter, on each target, rotating at 32 rpm. The laser has been focused with an incident angle of 45° on the targets, with an energy density set to 2.6 J/m^2 .

Two percentages of nickel have been introduced in the DLC films according to the conditions mentioned below. For the first sample, six sequences of ablation on each target have been performed alternatively during 89s for graphite and 1 s for nickel. A similar procedure has been carried out for a 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 similar deposition rate of pure carbon (22nm/min) and pure nickel (35nm/min), the film thickness of the two kinds of samples is in the range of 200 nm. Percentages of nickel in DLC films have been determined by X-Ray photoelectron spectroscopy (XPS) and Rutherford background spectroscopy (RBS). They showed 2 at.% for the first sample (SiCNi 2%) while 5 at.% for the second one (SiCNi 5%).¹³

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.¹⁴ Thus working under argon pressure and modifying this pressure leads to DLC films with different ratio of sp^3/sp^2 carbon hybridization. $P_{\text{Ar}}=2.10^{-2}$ mbar (SiCAr1), $P_{\text{Ar}}=5.10^{-1}$ mbar (SiCAr2) and $P_{\text{Ar}}=5.10^{-3}$ mbar (SiCAr3) were used respectively for the deposition of DLC films by ablation graphite target during 10 minutes.

II-C.b DLN film deposition

These films were prepared by the Institute of Fluid Science, Tohoku University, Japan. Besides their numerous interesting properties, DLC films present two disadvantages: low adhesion strength and difficulty to be used in high temperature applications. Metal-containing diamond-like carbon-silicon nanocomposite films are thought to be one of the most perspective material for the wide temperature range applications, this class of materials being called diamond-like nanocomposites (DLN). In this work a W-DLN film was tested for electrochemical applications. W-DLN film was deposited onto polycrystalline substrate substrates with two processes: amorphous carbon-silicon matrix was fabricated by DC discharged of siloxane vapor and in the same time, the deposition DC magnetron sputtering was used for doping metals. Structural analysis was performed by raman spectroscopy with He-Ne laser. From Raman spectra, it appears that in these W-DLN films, the growth of sp^2 cluster size is observed starting from 22-25 at.% of

metal which correspond to the increase of conductivity of the film¹⁵.

All these electrodes were cleaned with ultra-pure water and with optical paper before use.

II-D. 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 used. DLC and DLN electrodes were coated with a thin layer of this mixture and kept 20 min in glutaraldehyde vapor at room temperature. This bifunctional compound ($\text{OHC}-(\text{CH}_2)_3\text{-CHO}$) links covalently from each side to the amine groups of GOD and BSA respectively creating a stable biolayer. The resulting enzyme electrodes were allowed to dry in air and were thoroughly washed and stored in 40 mM phosphate buffer solution, pH 7.4, at 4°C when not in use.

III. RESULTS AND DISCUSSIONS

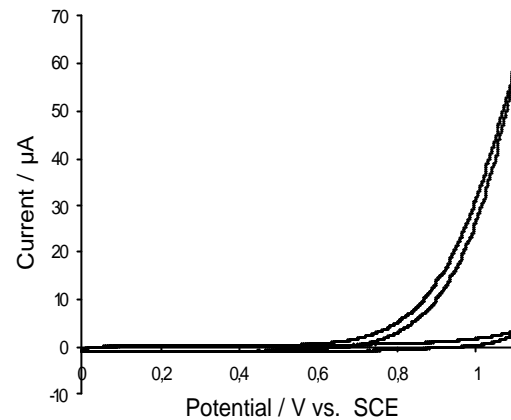
III-A. Hydrogen peroxide detection

Glucose biosensors are based on the fact that glucose oxidase, a flavoenzyme, catalyses the oxidation of glucose to gluconic acid in presence of oxygen. A co-product of this reaction is hydrogen peroxide and its electroactivity can be used to obtain a measurable current signal.

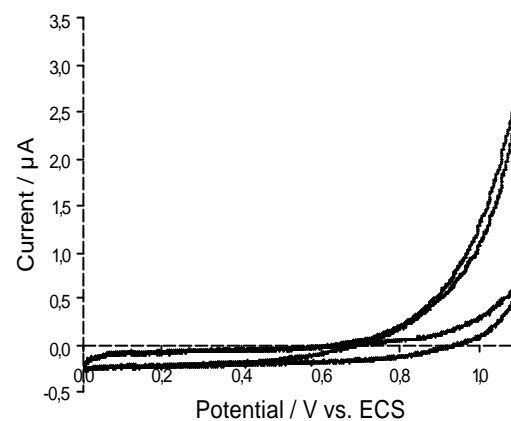
The DLC and DLN electrodes were initially characterized electrochemically by recording their cyclic voltammograms between 0.0 V and 1.1 V in 40 mM PB solution, pH 7.4 and with 5mM H_2O_2 in PB solution. Fig.1a and Fig.1b show the behavior of each electrode in PB and towards H_2O_2 . It can be seen that the response for hydrogen peroxide is higher for SiCar1, SiCNi5%

and W-containing DLN electrodes. After analysis of these responses, a potential + 1.0 V was chosen for H_2O_2 amperometric detection.

SiCar1



SiCar2



SiCar3

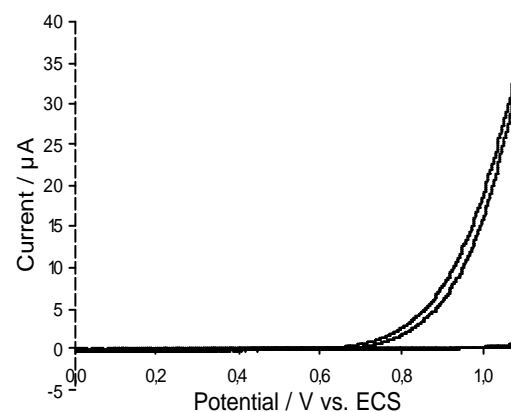
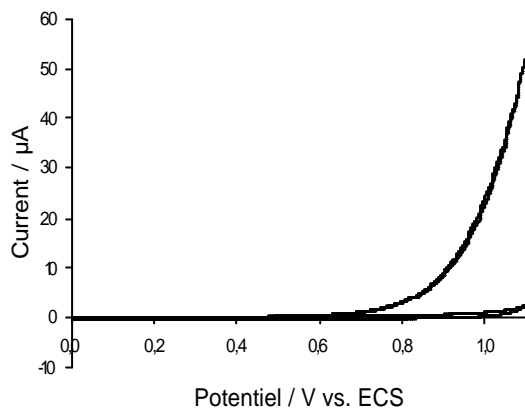
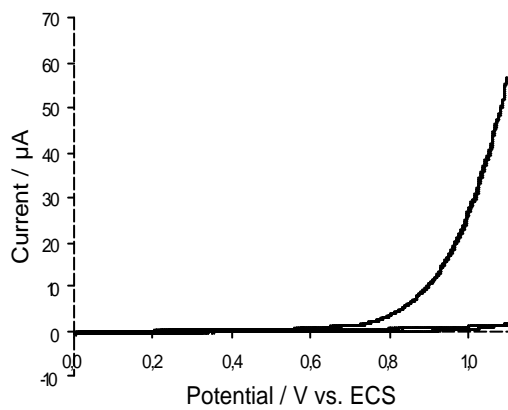


Fig.1a Cyclic Voltammograms of DLCs films with different ratio sp^3/sp^2 carbon hybridization in 40 mM PB solution, pH 7.4 and with 5 mM H_2O_2 in PB.

SiCNi2%



SiCNi5%



W-containing DLN

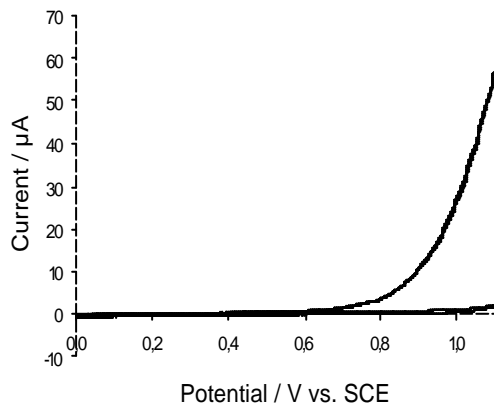


Fig.1b Cyclic Voltammograms of Ni-containing DLC and W-containing DLN electrodes in 40 mM PB solution, pH 7.4 and with 5 mM H_2O_2 in PB.

Amperometric measurements were carried out in PB solution at + 1.0 V by injecting aliquots of H_2O_2 to the cell, each addition resulted in a 0.02

mM increment in concentrations. The comparison of DLCs electrodes according to H_2O_2 sensitivity is shown in Fig. 2.

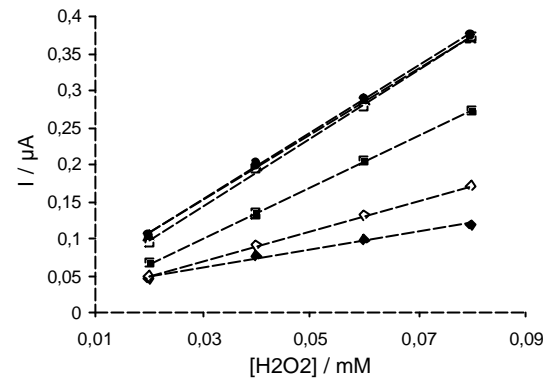


Fig.2 Hydrogen peroxide calibrations curves for SiCar1 (?), SiCar2 (?), SiCar3 (◊), SiCNi2% (◊), SiCNi5% (◻) and W-containing DLN (•) for successive additions of 0.02 mM H_2O_2 in 40 mM PB, pH 7.4.

SiCar1, SiCNi5% and W-containing DLN electrodes enables a more satisfactory determination of hydrogen peroxide. Their corresponding sensitivities are 4.4463 $\mu A/mM$ with $R = 0.9997$, 4.6401 $\mu A/mM$ with $R = 0.9993$ and 4.5119 $\mu A/mM$ with $R = 0.9995$, respectively.

Since these electrodes show the higher and approximately the same sensitivities towards hydrogen peroxide, they will be tested for elaboration of glucose amperometric biosensor.

III-B. Enzyme measurements

To evaluate the possible applications of the above-mentioned DLCs electrodes in biosensor construction, glucose biosensors were fabricated following the procedure previously described. Kinetics studies of the immobilized enzyme were carried out by plotting the electrochemical response to increasing concentrations of glucose. Fig. 3

shows the curves of DLCs electrodes, SiCar 1 and SiCNi 5%, in 40 mM PB, pH 7.4 at a measurement potential of +1.0 V for successive additions of glucose.

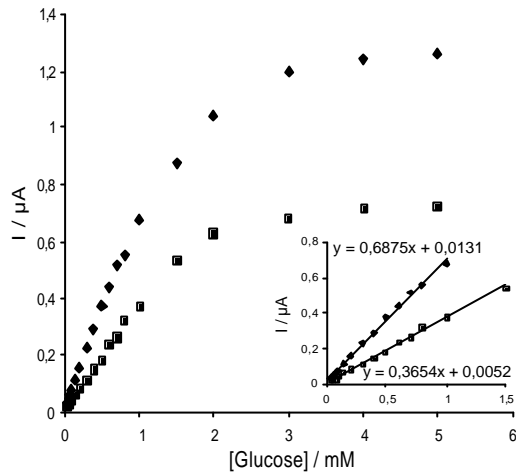


Fig. 3 Glucose Calibration curves for SiCar 1 (?) and SiCNi 5% (i) electrode for successive additions of glucose in 40 mM PB, pH 7.4.

Unfortunately, W-containing DLN electrode with immobilized glucose oxidase did not give any response for addition of glucose. It can be explained by the fact that after 2 months of storage, its sensitivity to hydrogen peroxide was decreased.

Glucose biosensor based on SiCar1 DLC electrode show a linearity range up to 1 mM and until 1.5 mM for SiCNi5% DLC electrode. Corresponding detections limits were 20 μ M and 30 μ M respectively. The sensitivity of SiCar1 electrode for glucose, 0.6875 μ A/mM with $R = 0.9984$, is higher then the one for SiCNi5%, 0.3654 μ A/mM with $R = 0.9982$. From the Lineweaver-Burk plots, an apparent Michaelis-Menten constant of 3.38 mM for SiCar1 and 3.09 mM for SiCNi5%, respectively, was obtained.

III-C. 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, crosslinking with a bifunctionnel compound...).

The operational stability during 10 hours for glucose biosensors, SiCar1 and SiCNi5%, and their storage stability in storage conditions (40mM PB, pH 7.4) were tested in 40 mM PB pH 7.4 containing 0.5 mM glucose.

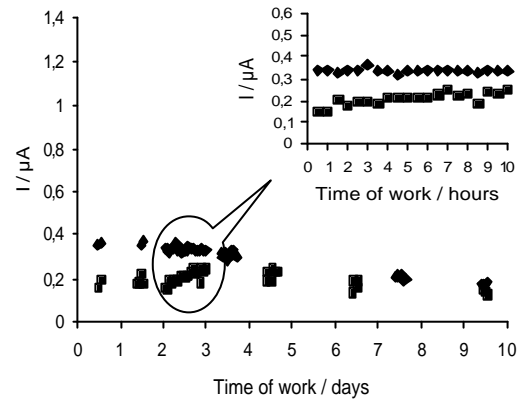


Fig.4 Operational and storage stability of SiCar1 (?) and SiCNi5% (i) biosensors for 0.5 mM glucose concentration.

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. At the tenth

day, the current response for 0.5 mM glucose is approximately the same for these two biosensors.

The operational stability of SiCAR1 and SiCNi5% during 10 hours is shown 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 and thus glucose can arrive more easily to the electrode.

IV. CONCLUSIONS

This work has been concerned with the development of electrochemical biosensors based on Diamond-Like Carbons electrodes. Evaluation of these electrodes was carried out using determination of glucose by glucose oxidase. Glucose oxidase biosensor based on SiCAR1 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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