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## Carbon nanotube array-based biosensor

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**Abstract** Aligned multi-wall carbon nanotubes (MWNT) grown on platinum substrate are used for the development of an amperometric biosensor. The opening and functionalization by oxidation of the nanotube array allows for the efficient immobilization of the model enzyme, glucose oxidase. The carboxylated open-ends of nanotubes are used for the immobilization of the enzymes, while the platinum substrate provides the direct transduction platform for signal monitoring. It is also shown that carbon nanotubes can play a dual role, both as immobilization matrices and as mediators, allowing for the development of a third generation of biosensor systems, with good overall analytical characteristics.

**Keywords** Carbon nanotube · Biosensor · Array · Glucose oxidase

### Introduction

Carbon nanotubes are of special interest due to their unique electronic, metallic, and structural characteristics [1]. These nano-materials are very promising for the development of novel technological applications, such as batteries [2], tips for scanning probe microscopy [3], electrochemical actuators [4], sensors [5] etc. Among the anticipated applications of carbon nanotubes is their use as components in biological devices. Until now, most of the research undertaken in this direction has been focused on attaching biological molecules onto these nano-materials. It has been shown that small proteins can be entrapped into the inner channel of opened nanotubes by simple adsorption [6, 7, 8]. Attachment of small proteins on the outer surface of carbon nanotubes has also been achieved,

either by hydrophobic [9] and electrostatic interactions [10], via covalent bonding [11] or by functionalization of the nanotube sides by polymer coating [12]. However, application of these practices has been limited to the development of microelectrodes to promote bio-electrochemical reactions [13, 14]. Until today there has been no report describing the use of these materials for the design and development of biological sensors.

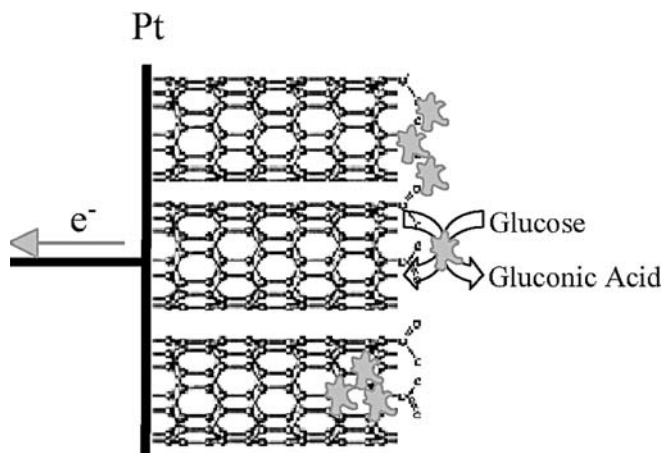
One of the key issues in biosensor design is the establishment of a fast electron-transfer between the enzyme active site and the electrochemical transducer. Additional restrictions apply when miniaturization of the system is attempted. Small surface area leads to constraints concerning the minimum enzyme loading, that can be repeatedly immobilized for analytical applications [15]. The structure-dependent metallic character of carbon nanotubes should allow them to promote electron-transfer reactions at low overpotentials. This characteristic, along with their high surface area, provides the ground for unique biochemical sensing systems. The latest advances in production of well-controlled aligned carbon nanotube arrays [16] have shown the way for incorporation of nanotechnology in biosensor technology.

### Materials and methods

The nanotube array used was a multi-wall carbon nanotube array, with closed nanotubes 15–20 microns long and 150 nm internal diameter. Multi-wall nanotube arrays, grown by the CVD method [17] on platinum substrate, were purchased from NanoLab Inc. (Watertown, MA, USA). Cyclic voltammetry was used to monitor the electrochemical characteristics of the nanotubes, proving the metallic character of the array and the chemical stability upon treatment at high oxidative potentials.

The original nanotube array was opened using two previously reported etching procedures [18, 19], namely acid oxidation (sensor 1) and air oxidation (sensor 2) with small modifications. In short, the chemical etching was achieved using a mixture of concentrated  $\text{H}_2\text{SO}_4$  and  $\text{HNO}_3$  acids (3:1, 98% and 65% respectively) for 8 h at 40 °C. Alternatively, air oxidation was achieved at 600 °C for 5 min under air flow. Upon completion of the etching procedure the array was washed with deionized water and dried at 100 °C overnight. Scanning electron microscopy was used to determine the effect of these oxidation procedures on the arrays. SEM images

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**Fig. 1** Schematic diagram of the carbon nanotube array biosensor. The enzyme immobilization allows for the direct electron transfer from the enzyme to platinum transducer

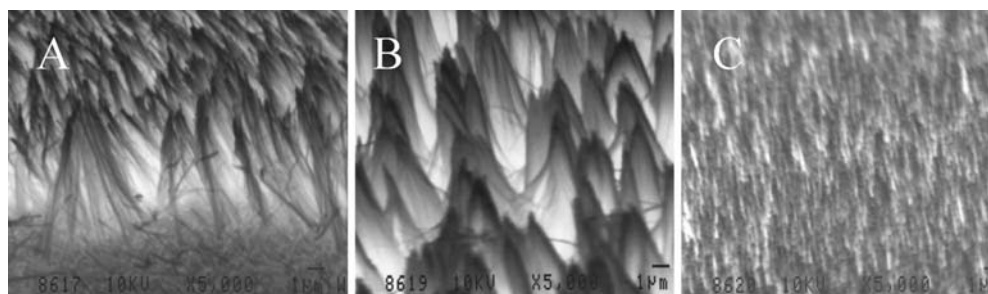
were obtained with a JEOL-JSM 840 scanning microscope at 10 KV. Subsequently, enzyme immobilization was achieved by incubation for 3 h of both freshly oxidized sensors in an enzymatic solution (1500 to 2500 U/mL).

## Results and discussion

Figure 1 shows the schematic diagram of the carbon nanotube array biosensor presented in this work. The immobilization of the enzyme into the nanotubes would allow for the mediated direct electron transfer to the platinum transducer.

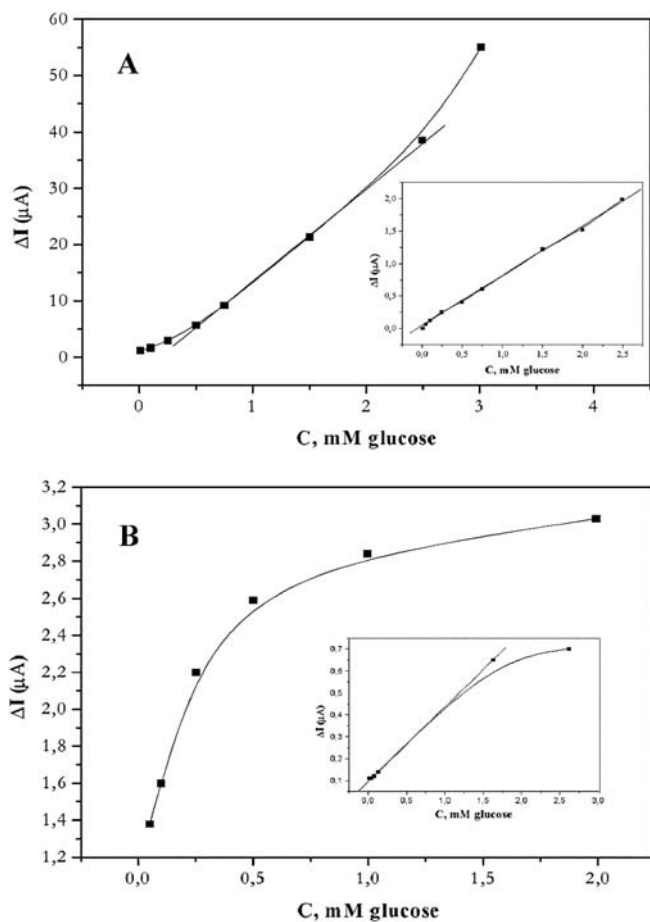
The two array systems used in this work were either acid treated, or air treated. Acid treatment purified the array by removing any amorphous carbon material or other impurities that occurred during the production procedure. It also reduced the length of the nanotubes by approximately 50% (Fig. 2B), compared with the original array (Fig. 2A). On the contrary, air-oxidation caused the peeling of the outer graphitic layers from the nanotubes producing thinner nanotubes (Fig. 2C).

**Fig. 2A–C** SEM images of the Pt-aligned carbon nanotube array **A** in original state, **B** after being chemically etched with a mixture of concentrated  $\text{H}_2\text{SO}_4$  and  $\text{HNO}_3$  acids (3:1, 98% and 65% respectively) for 8 h at 40 °C, washed with deionized water and dried at 100 °C overnight, and **C** after air oxidation at 600 °C for 5 min under air flow. SEM images were obtained with a JEOL-JSM 840 scanning microscope at 10KV. Bar is 1  $\mu\text{m}$



After immobilization of the enzyme, the calibration curves to glucose were recorded. Sensor 1, which was constructed using the acid oxidized array (Fig. 3A), had a linear range of response from 0.25 to 2.5 mM glucose ( $r=0.9986$ ) and sensitivity of  $93.9 \pm 0.4 \mu\text{A mM}^{-1} \text{cm}^{-2}$ . The limit of detection, based on a signal-to-noise ratio of 3, was 0.19 mM. These values are among the best reported for glucose biosensors and make the application of the system in micro-analysis feasible. One very important feature was that even after incubation of the sensor in buffer solution for 24 h (at 4 °C) there still was a significant remaining sensitivity ( $4.53 \pm 0.01 \mu\text{A mM}^{-1} \text{cm}^{-2}$ ) (Fig. 3A, inset). On the other hand, sensor 2, which was based on the air oxidized array (Fig. 3B), showed completely different analytical characteristics. The linear range of response of the air-treated sensor was from 0.05 to 0.5 mM glucose ( $r=0.9707$ ) and the sensitivity was  $15.6 \pm 0.5 \mu\text{A mM}^{-1} \text{cm}^{-2}$ , a value much lower than the one obtained from the acid-treated sensor 1, but yet high if compared to traditional biosensor electrodes. It is important to note that sensor 2 lost all its activity after the first day of operation, suggesting a weaker enzyme immobilization, or lower enzyme stabilization. In order to exclude any possible adsorption of the enzyme on the platinum substrate, a blank experiment was undertaken. A platinum foil was immersed in an enzyme solution for 20 h and the possible response to the substrate was examined. No signal was recorded, thus the enzyme must have been indeed immobilized on the nanotubes.

Based on the above-mentioned results, the procedure of chemical etching is more efficient in opening carbon nanotubes and allowing the entrance of the enzyme at the inner channel. What seems most probable is that upon oxidation of the array, the introduced carboxylic groups at the open-ends, provide a stabilizing hydrophilic environment that allows for the adsorption and insertion of the enzyme into the cavity of the nanotubes, while preserving its functionality. Even if blocking of the entry-ports could occur by the introduced functional groups [20], this is not significant in this case due to the large internal diameter of the nanotubes (150 nm) compared to the enzyme's hydrated diameter (approximately 7 nm). The possible electrostatic interaction of the enzyme with the outer wall of the nanotubes is weak [12], and cannot account for the observed extended lifetime of the sensor. The remaining sensitivity of sensor 1 on the second day shows that a significant amount of the adsorbed enzyme could be in the inner walls of the nanotubes.



**Fig. 3A,B** Initial calibration curves in phosphate buffer (10 mM, pH 7.5) at +800 mV to glucose of: **A** sensor 1 on the first and the second day (*inset*) of oxygen, and **B** sensor 2 in the presence and absence (*inset*) of oxygen

Since carbon nanoparticles have been successfully used as mediators in enzyme biosensors [21], sensor 2 was additionally evaluated under anaerobic conditions (argon flow) to examine the mediation capabilities of the nano-material (Fig. 3B, inset). It has been reported that air oxidation does not lead to significantly increased work functions of carbon nanotubes, as opposed to acid oxidation [22]. Higher work functions would impede reversible electron transfer between the co-factor of the enzyme and the believed mediator. For this reason, only the mediation capabilities of the air oxidized nanotubes (sensor 2) were evaluated. In this case, the observed sensitivity to glucose was  $2.003 \pm 0.001 \mu A \text{ mM}^{-1} \text{ cm}^{-2}$ , indicating that the absence of oxygen did not obstruct the enzyme's catalytic reaction or the operation of the sensor. This observation

further enhances the unique character and usefulness of these nano-materials.

In conclusion, we report here for the first time the use of carbon nanotubes as immobilization matrix for the development of an amperometric biosensor. The opening and functionalization of large nanotube arrays allowed for the efficient immobilization of the enzyme, while the platinum substrate served as the direct transduction platform for signal monitoring. The carbon nanotube-based biosensor showed good overall analytical characteristics. It was also shown that carbon nanotubes can play a dual role, both as immobilization matrices and as mediators, allowing for the development of a third generation of biosensor systems.

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