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Adsorption of Glucose Oxidase to 3-D Scaffolds of Carbon Nanotubes: Analytical Applications

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Abstract

This study is the first to focus on the potential use of carbon nanotube (CNT) scaffolds as enzyme immobilization substrates for analytical purposes. Besides all the well-known advantages of CNT, three-dimensional scaffolds can significantly increase the amount of enzymes adsorbed per unit area, preserve the catalytic activity of the adsorbed molecules, and allow effective exposure to substrates present in the adjacent medium. Additionally, our results indicate that the sensitivity of analytical probes based on enzyme-loaded CNT scaffolds is proportional to the thickness of the scaffold providing 3-fold sensitivity improvements with respect to the control surfaces.

Keywords

Carbon nanotube; Nanostructures; Enzyme adsorption; Ellipsometry; Glucose oxidase; Biosensors

1. INTRODUCTION

Since the first report of the observation of carbon nanotubes (CNT) [1], many papers have described significant advancements regarding their applications in different fields of chemical sciences and biotechnology [2-7]. In many cases, CNT are functionalized to allow coupling the unique properties of CNT with those of other materials of interest [8-11]. Among them, immobilization of macromolecules to CNT is often achieved by means a combination of covalent and non-covalent interactions. Due to its strong affinity and spontaneity the latter typically dominates the process over the much slower covalent reactions yielding to irreversible immobilization of proteins to CNT [12].

It has been also shown that CNT can be attached to conventional electrodes and act as wires, connecting the underlying electrode to the corresponding biorecognition elements [13-16]. In addition, CNT have been also used as immobilization substrates for various enzymes in analytical devices and biosensors [17-21]. CNT have the potential to promote the attachment of biorecognition elements [22], retention of catalytic activity [23], and enhancement of the electron transfer rate, in some cases even with a direct [24, 25] and quasi-reversible redox process [22, 23, 26-28]. Moreover, CNT have been incorporated into composite matrices of

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6. SUPPORTING INFORMATION AVAILABLE. Information provided as Supporting Information include a SEM micrograph of a selected substrate and the comparison of enzyme adsorbed amounts (from ellipsometric observations, Γ_{MAX} , and calculated from activity, Γ_{activity}) as a function of the thickness of the CNT scaffold.

biosensors to enhance the sensitivity and selectivity towards certain analytes [4, 29-31]. One additional advantage of coating the electrode surface with CNT is that they can significantly increase the effective area of the sensor [32], enabling the adsorption of higher amounts of enzyme and leading to significant increases in the sensitivity.

It is evident, however, that there is a limit for enhancing the amount of enzyme adsorbed to the surface of a flat substrate. CNT scaffolds (three-dimensional structures of carbon nanotubes) can potentially overcome this difficulty by not only accommodating a larger amount of enzyme but also keeping the enzyme active and accessible for substrates. Although the production of CNT films have been realized by different methods, [33-35], no study has been reported on their potential as three-dimensional scaffolds for the development of biosensors. Aiming to address that gap in knowledge, this paper demonstrates that glucose oxidase (GOx, a model enzyme for bioanalytical applications [36-39]) can penetrate a three-dimensional CNT scaffold, attach to the nanotubes present in the inner layers of the scaffold, and retain most of its catalytic activity. Thus, this study provides evidence about the advantages of using CNT scaffolds to improve the sensitivity of biosensors. To achieve these goals, the adsorption and desorption process of GOx to CNT scaffolds was investigated by variable angle spectroscopic ellipsometry. Then, the catalytic activity of GOx adsorbed to the CNT scaffold was evaluated by spectrophotometry. Finally, the GOx-modified CNT scaffolds of different thicknesses were integrated in a flow injection analysis system and used as electrochemical biosensors. Atomic force microscopy images were used to characterize the topography of the scaffolds and to visualize the adsorbed enzymes.

2. EXPERIMENTAL

2.1 Preparation and optical characterization of the scaffolds

The CNT scaffolds used herein were prepared by Unidym Inc. (Sunnyvale, CA, USA) using <111> silicon wafers (Si/SiO₂; Sumco, Phoenix, AZ, USA) as substrates and a proprietary ink composed of multi-wall CNT suspended in a mixture of alcohols (see Supplementary Information). According to the provider, the Si/SiO₂ wafers were first immersed in a 1 % w/v solution of APTES (3-aminopropyl-triethoxysilane) for 30 min and then thoroughly rinsed with DI water. This procedure renders a -NH₂ terminated surface that promotes adhesion of the CNT, among other materials [40]. The APTES-treated wafers were subsequently coated with the CNT ink (produced by chemical vapor deposition and purified with standard methods [41]) using a Mayer rod [42]. The thickness of the CNT scaffold is determined by the concentration of CNT in the ink and the conditions used during the preparation. The substrates were then dried, annealed at 300 °C overnight, and finally rinsed with DI water. The formed coating is essentially a layer of pure CNT and contains no residual organic additives or polymeric constituents. In all cases, the CNT scaffolds were rinsed thoroughly with ultrapure water (18 MΩ-cm, NANOpure Diamond, Barnstead; Dubuque, Iowa, USA) before use.

The optical characterization of the substrates and adsorption experiments were conducted in a modified version of a commercially available cell designed for spectroscopic ellipsometry (J.A. Woollam Co; Lincoln, NE, USA). Details of the selected cell, which allows monitoring of the adsorption under well-controlled hydrodynamic conditions, have been reported elsewhere [43]. All experiments were performed at room temperature by mounting the sample on a micrometer-position-controlled translation stage with the gradient direction perpendicular to the plane of incidence. In all cases, changes in the reflectance and phase difference between the parallel (R_p) and perpendicular (R_s) components of a polarized light beam upon reflection from a surface were translated to the ellipsometric angles (Ψ , amplitude and Δ , phase difference) using Equation 1.

$$\tan(\Psi) e^{i\Delta} = \frac{R_p}{R_s} \quad \text{Equation 1}$$

Prior to each experiment the cell and tubing were filled with buffer and the substrate was aligned with respect to the incident beam and the detector. Substrates were initially characterized in buffer, at the incident angle of 70° (with respect to the substrate normal, limited by the geometry of the flow cell), and using wavelengths between 600 nm and 900 nm (10 nm steps). For all adsorption experiments, the variations of Ψ and Δ at 700 nm as a function of time were recorded at an angle of incidence of 70°. The experiments were initiated by pumping the buffer through the cell for ~5 min to set the baseline. Subsequently, enzyme solution was introduced to the cell and the adsorption process started. After reaching a steady state, the pump was stopped and a more accurate spectroscopic scan (in the 600 nm to 900 nm range) was performed. The collected data were then modeled using the WVASE software package (J.A. Woollam Co; Lincoln, NE, USA). The mean square error (MSE, performed with a built-in function in WVASE) was used to quantify the difference between the experimental- and model-generated data.

Unless otherwise stated, enzyme solutions (final concentration of 0.1 mg·mL⁻¹) were prepared by dissolving glucose oxidase (GOx, 21200 U·g⁻¹, Sigma, USA) in a 10 mM buffer composed of acetic acid and sodium acetate (pH=4.2). This buffer was selected because it provides high buffer capacity around the isoelectric point of glucose oxidase [44, 45].

2.2 Optical model

The optical model used to represent the substrate consisted of three uniaxial layers with optical axes parallel to the substrate surface. The bottom layer was silicon ($d = 1$ mm) with a native layer of SiO₂ (2.5 ± 0.5 nm). A two-media Bruggeman effective medium approximation (EMA) consisting of arc-evaporated carbon [46] and void space was used to represent the optical properties of the third layer (CNT scaffold). In all cases, the ambient was defined using the optical properties of water. The thickness of the CNT scaffold and percentage of arc-evaporated carbon in the EMA layer were initially considered as the model variables. After investigating the carbon percentage for different substrates (average 40 ± 9 %), a 40% value was assumed for all samples and the thickness values were recalculated. In order to verify CNT density used in the described optical model, atomic force microscopy (AFM) was utilized. Tapping mode AFM was carried out with a Nanoscope V AFM (multimode, VEECO Instruments, USA) and using phosphorus-doped silicon probes. Figure 1A shows a typical image of the substrates in which the interconnected network of CNT forming the scaffold is shown. It is worth mentioning that although this optical model has been successfully applied to represent the optical properties of CNT films prepared by different providers [47-49], these results may not represent the properties of CNT films prepared by other methods [50-54].

To calculate the amount of enzyme adsorbed to the CNT films, an additional component (representing the adsorbed enzyme) was included in the EMA layer of the optical model. To represent the adsorption process, the model was allowed to dynamically replace void space (filled with water) with the selected enzyme (GOx). The resulting optical model is schematically shown in Figure 1B. In agreement with previous reports describing the adsorption of transparent polymers [55-57], the optical properties of the enzyme were described using a Cauchy model ($A=1.45$, $B=0.1$, $C=0$). This volumetric interpretation of the adsorption process is also coherent with previous reports [58, 59].

To account for slight variations in the thickness of the different CNT substrates, the percentage of volume of GOx (calculated as the % volume of Cauchy in the EMA layer) was multiplied by the thickness of the CNT layer to obtain an “effective thickness” of GOx inside the CNT scaffold. This calculation, which enabled the comparison of experiments performed with different substrates, was verified by adsorbing GOx under identical experimental conditions (concentration, pH, buffer, and flow rate), but using different substrates. In all cases, variations of less than 5% were obtained. Consequently, this optical model was used to analyze the results of the adsorption experiments presented in this manuscript.

The amount of enzyme adsorbed (Γ , expressed in $\text{mg}\cdot\text{m}^{-2}$) was then calculated using Equation 2,

$$\Gamma = \frac{d(n - n_0)}{(dn/dc)} \quad \text{Equation 2}$$

where d is the effective thickness of the adsorbed layer and n and n_0 (1.465 and 1.33) are the refractive indices of the adsorbed layer and the ambient (H_2O), respectively. In accordance with previous reports, [47, 48, 57] a constant refractive index increment ($dn/dc = 0.180 \text{ cm}^3\text{g}^{-1}$) was assumed for the adsorbed layer of enzyme.

In all cases, a good agreement was obtained between the experimental and the model-generated data ($\text{MSE} < 11$), indicating that the system can be described with the proposed model. Although outside the scope of the present study, it is worth mentioning that other models considering the possibility of having the enzyme either adsorbed on top of the surface of the scaffold [48, 60] or distributed with a gradient of concentrations within the scaffold [61, 62] were evaluated. However, none of those models render reasonable results and were not further considered.

2.3 Measurement of the catalytic activity of adsorbed GOx

The activity of GOx adsorbed to the CNT scaffolds was evaluated by two different methods: spectrophotometrically and electrochemically. The former method is based on the reaction of β -D-glucose (Sigma, USA) with O_2 and H_2O in the presence of GOx to produce β -D-glucono- δ -lactone and H_2O_2 . The H_2O_2 was then used to oxidize α -dianisidine in the presence of horseradish peroxidase (HRP, 188000 purpurogallin Ug^{-1} , Sigma, USA) to commence a color change that is monitored spectrophotometrically at 500 nm [37]. In this study, CNT scaffolds (with known geometric area and different thicknesses) were immersed in a solution containing $0.1 \text{ mg}\cdot\text{mL}^{-1}$ GOx (in acetate buffer $\text{pH}=4.2$) for 2 h. Subsequently, the GOx-modified CNT scaffolds were rinsed thoroughly with buffer (to remove loosely bound GOx) and placed in a quartz cuvette filled with a mixture of glucose, α -dianisidine, and HRP in acetate buffer at pH 5.1 (as indicated in the assay protocol). In order to constantly homogenize the solution, a magnetic bar was placed inside the cuvette and was stirred using a magnetic stirrer (Spinette, Sterna; Atascadero, CA, USA). To calculate the enzymatic activity, the change in absorbance at 500 nm was monitored for 45 min, using a spectrophotometer (Genesys 10 UV, Thermo Scientific, USA).

In order to evaluate the analytical performance of CNT scaffolds as suitable platforms for the development of electrochemical biosensors, the catalytic activity of GOx adsorbed to CNT scaffolds was also investigated by amperometry. For those experiments, the GOx-modified scaffolds were integrated to a flow injection analysis (FIA) system. The system consists of a peristaltic pump (Minipuls 3, Gilson, France), a multichannel injection valve (Model 9725, Rheodyne, USA), a thin-layer flow cell (MW-5051, Bioanalytical Systems Inc., USA), a 0.001" teflon gasket (MF-1044, Bioanalytical Systems Inc., USA), and an

electrochemical detector (model 810B, CH Instruments; Austin, TX, USA). GOx was adsorbed to CNT scaffolds (with different thicknesses of CNT) following the procedure previously described. Next, the CNT/GOx composites were rinsed with buffer and placed in the cell, with the CNT scaffold facing the flow. A drop of high-conductivity silver paint and a copper cable were used to provide electrical contact between the CNT scaffold and the electrochemical detector. In all cases, the cell block (stainless steel) served as the auxiliary electrode and a Ag/AgCl/KCl_{3M} electrode was used as reference electrode. Flow injection determinations were performed in amperometric mode, by following the anodic peak current originated by the electrochemical oxidation of the H₂O₂, released upon the re-oxidation of GOx by molecular oxygen [63, 64]. A 10 mM acetate buffer (pH= 5.1, flow rate = 0.2 ml min⁻¹) was selected as the background electrolyte. The detection potential (+0.7 V vs Ag/AgCl 3M) was selected by performing the corresponding hydrodynamic voltammograms (data not shown) and is in good agreement with other biosensors previously published [65].

3. RESULTS AND DISCUSSION

In the following sections, the results related to the interaction of GOx with CNT scaffolds are presented. Experiments, performed by spectroscopic ellipsometry and complemented by measurements of the catalytic activity of the adsorbed protein, were designed to evaluate the analytical implications the adsorption of the selected enzyme to 3D scaffolds.

3.1 Adsorption/Desorption of GOx to CNT scaffolds

Initially, the dynamic adsorption of GOx to two CNT scaffolds of different thickness and to a bare Si/SiO₂ wafer (used as a control substrate) was investigated. As can be observed in Figure 2, GOx was able to interact with the selected substrates (Si/SiO₂, 27.5 nm scaffold, and 49.1 nm scaffold) displaying three distinct phases within each of the adsorption curves. Initially, the adsorbed amount increased in a linear fashion, the slope of which determined the initial adsorption rate. This phase was followed by a transition period during which the adsorption rate gradually decreased until reaching a plateau (Γ_{SAT}), where the adsorbed amount remained constant over time (third part in the curve). As can be also observed, a significantly larger amount of GOx (up to 11.8 mg·m⁻²) was adsorbed to the 49.1 nm CNT film in comparison to the 27.5 nm film (up to 7.0 mg·m⁻²). As expected, a much lower amount of GOx (up to 2.0 mg·m⁻²) was adsorbed to the base Si/SiO₂ surface, supporting the hypothesis that most of the GOx is adsorbed to the CNT scaffold and not to the supporting substrate. Most importantly, Figure 2 shows that similar initial adsorption rates were obtained for the selected CNT scaffolds, regardless of their thickness, and that this adsorption rate was larger than that on a bare Si/SiO₂ wafer. This finding suggests that given the option, GOx will first populate the CNT surface rather than adsorbing to the SiO₂ substrate. Complementary adsorption experiments as a function of pH and ionic strength (data not shown) indicated that a combination of hydrophobic and (to a lower degree) electrostatic interactions between the CNT scaffold and GOx were the main driving forces for the interaction. In addition, both the initial adsorption rate and the saturation amount were strongly dependent on the bulk concentration of GOx. In all cases, the higher the protein concentration, the faster the Γ_{SAT} was reached, and the greater the Γ_{SAT} value. Consequently, our results support the hypothesis that high initial adsorption rates hinder the relaxation of the adsorbed proteins, rendering a much crowded surface [66]. The main advantage of such high adsorption rate is that by limiting the relaxation process, adsorbed enzymes have higher chances to preserve their tertiary structure and consequently, their catalytic activity.

One concern arising from the use of adsorption as immobilization technique is that enzymes may desorb from the substrate [67, 68] as there is no covalent bond that attaches them to the scaffold. In order to evaluate the stability of the GOx-CNT scaffolds, desorption

experiments were performed by rinsing the GOx-CNT scaffolds with buffer. These experiments were also performed in the liquid cell, immediately after the adsorbed amount (Γ) reached a stable value (marked as $\downarrow$ in Figure 2). As can be observed, no significant changes in adsorbed amount were observed upon switching from pumping a solution containing GOx to a solution containing only buffer. These results indicate a rather strong interaction between the GOx and the CNT scaffold. These observations are also in good agreement with previous studies reporting on the adsorption of other proteins to CNT [47, 69, 70] as well as the efficiency of different immobilization techniques [12].

As previously stated, one of the hypotheses of this work was that GOx would be able to penetrate into the CNT scaffold. To confirm this, the adsorption of GOx to scaffolds of different thickness was investigated. Figure 3 compares the saturation adsorbed amount of GOx (Γ_{SAT}) to a bare wafer and six CNT scaffolds with thicknesses ranging from 27 nm up to 61 nm (limited by the reflectance of the scaffolds).

As can be observed, the thicker the scaffolds, the higher the amount of GOx adsorbed. This finding clearly indicates that GOx is able to penetrate into the scaffold rather than adsorbing just on the outer surface of the film (which would have a constant area). Assuming a linear change in the adsorbed amount with respect to the thickness of the scaffolds, the collected results suggest that only $1.5 \text{ mg}\cdot\text{m}^{-2}$, would be adsorbed to a flat surface. This value is in close agreement with the amount of GOx adsorbed to the bare Si/SiO₂ substrate ($2.0 \pm 0.1 \text{ mg}\cdot\text{m}^{-2}$).

Although proteins in aqueous solutions readily adsorb to almost any available solid-liquid interface, the adsorption process often involves changes in protein conformation at the surface, and in some cases this can result in inactivation of the adsorbed protein.[71] For that reason, reconstructed 3D images of the scaffolds before and after the adsorption of GOx were also analyzed to probe the location and conformation of the adsorbed GOx. In the case of the bare scaffold (Figure 1A), the smooth walls of the CNT (individual and bundles) were clearly observed. On the contrary, when GOx was adsorbed to the scaffold a less smooth topography was observed (Figure 4). In addition, it was observed that the size (average $\pm$ standard deviation, based on measurement of tube diameters at 20 different spots) of the features in the scaffold increased from $27 \pm 6 \text{ nm}$ (CNT) to $39 \pm 6 \text{ nm}$ (GOx-CNT).

The average increase in diameter (12 nm) observed upon adsorbing the enzyme suggests that a dense single layer of GOx ($7 \text{ nm} \times 5.5 \text{ nm} \times 8 \text{ nm}$ [72]) was adsorbed to the outside walls of the nanotubes, and that the adsorbed GOx preserved most of its native structure. Although images of proteins adsorbed onto single-wall CNT have been presented in the literature [17, 73], ours is the first observation of such high packing density of GOx. The present observation does not rule out the possibility of having unbound GOx molecules in parts of the scaffold that are not accessible to the AFM tip. Additionally, AFM images presented herein show a rather uniform layer of GOx adsorbed to the CNT at different depths of the scaffold the results and are in agreement with the optical model used to interpret the ellipsometric data.

3.2 Activity of GOx adsorbed to the CNT scaffolds

In order to investigate the effect of the thickness of the scaffold on the catalytic activity of the nanocomposites, the enzymatic activity of various substrates was evaluated spectrophotometrically. As shown in Figure 5 (activity per unit area as function of the thickness of the scaffold), the enzymatic activity of the GOx-loaded scaffold was found to be proportional to the thickness of the scaffold ($1.02 \text{ U m}^{-2}\cdot\text{nm}^{-1}$, $R^2=0.97$).

The importance of these results is twofold. First, they indicate that the catalytic activity of a nanocomposite can be significantly increased by using thicker CNT scaffolds. Specifically, an increase of 345% of the catalytic activity was obtained (with respect to the flat control surface, SiO₂) when a scaffold of 67 nm was used. Second and in agreement with the results collected by ellipsometry and AFM, these results further support the hypothesis that GOx adsorbed to the CNT scaffold is catalytically active and accessible to the enzyme's substrate (glucose).

It is worth mentioning that proteins are well-known to undergo spreading upon adsorption to solid surfaces [74]. The extent of this process determines the catalytic activity of the nanocomposites and is crucial to evaluate the suitability of CNT as a suitable platform for the development of biosensors. Therefore, to estimate the degree of denaturation of GOx adsorbed to the CNT scaffold, the maximum amount of adsorbed enzyme (calculated from Figure 2, Γ_{MAX}) was compared to the amount of enzyme derived from the activity measurements (from Figure 5, $\Gamma_{activity}$). A figure showing the comparison has been included in the Supplementary Information. It was observed that despite the continuous increase in the amount of enzyme adsorbed (both Γ_{MAX} and $\Gamma_{activity}$) as a function of the thickness of the scaffold (d), a much lower slope was obtained for the dataset derived from the activity measurements ($d\Gamma_{MAX}/dd = 0.210 \text{ mg}\cdot\text{nm}\cdot\text{m}^{-2}$; $d\Gamma_{activity}/dd = 0.089 \text{ mg}\cdot\text{nm}\cdot\text{m}^{-2}$). This difference suggests that although GOx was able to penetrate the scaffold and display enzymatic activity, a non-trivial fraction of the activity (~40%) was lost upon adsorption. Comparing these results with others stating that GOx adsorbed to CNT can retain up to 90 % of its native activity,[75] suggest that a much better characterization of the CNT and understanding of the attachment process is still required. Nevertheless, application of advanced methods for engineering the structure of CNT scaffolds can also lead to better control over the distribution and accessibility of adsorbed enzymes [33].

3.3 Detection of glucose using FIA and GOx adsorbed to CNT scaffolds

Coupling amperometric detection to flow injection analysis (FIA) has proven to be extremely useful for many analytical applications. FIA offer advantages such as improved detection limits, high sampling rate, low sample volume, inexpensive equipment, and accuracy of its results [76, 77]. Considering this, scaffolds of two different thicknesses were modified with GOx, integrated to a FIA system, and used to perform glucose measurements.

In all cases, the typical features (transients in current) were observed upon the application of the detection potential with a relative standard deviation (RSD) smaller than 5% for both scaffolds (n=60). Figure 5 summarizes the dependence between peak current (I_p) and glucose concentration obtained with both scaffolds. The data points and error bars in the figure represent the average and standard deviation, respectively, obtained for at least three consecutive injections. As it can be observed, although both scaffolds displayed linear responses up to ~50 mM glucose, the 65.5 nm scaffold provided significantly larger signals (I_p) than the 44.5 nm scaffold. Specifically, a sensitivity improvement of 390% was obtained ($38.5 \times 10^{-4} \mu\text{A mM}^{-1}$ for the 65.5 nm / $9.92 \times 10^{-4} \mu\text{A mM}^{-1}$ for the 44.5 nm scaffold). The limit of detection (calculated as 3 times the noise value of the baseline, $0.4 \pm 0.1 \text{ nA}$) for the electrode with thicker CNT scaffold was considerably lower (0.47 mM) than the one obtained with the thinner scaffold (8.4 mM).

These results indicate that the sensitivity of the GOx-loaded bioanalytical probe can be adjusted by varying the thickness of the CNT scaffold.

The obtained results (data not shown) also revealed that the probe was stable during the measurements and the response remained nearly constant for a single concentration of glucose after at least 40 injections.

4. CONCLUSIONS

According to our experiments, GOx was able to penetrate the CNT scaffold. Such arrangement enabled both adsorbing significantly higher amounts of enzyme (with respect to the flat control surface) and obtaining nanocomposites with higher catalytic activities. Hence, controlling the CNT scaffold thickness can be used as a simple and efficient way to tune the sensitivity of analytical probes based on enzyme-attached substrates. Our experiments show that GOx adsorbed to CNT scaffolds retains catalytic activity and can be used to facilitate electrochemical oxidation of glucose. AFM images suggest that a monolayer of GOx was adsorbed with high packing density over each CNT within the scaffold. Although only GOx was selected for the experiments discussed in this manuscript, the conclusions should be applicable to a wide range of enzymes. In principle, other enzymes, at least the ones with similar properties (size, charge distribution, glass transition temperature, etc) to GOx, should be able to penetrate and attach to inner layers of such CNT scaffolds. The optimal condition for adsorption that results in maximum activity of enzymes, however, should be separately characterized for each enzyme. Moreover, the adsorbed enzymes are stable on CNT while molecules of substrate can access the exposed enzymes in the 3D scaffold. The sensitivity of the GOx-loaded probes can be tuned by varying the thickness of the CNT scaffold. These findings provide a scientific ground for development of a new generation of analytical devices based on enzyme immobilization within 3D nanostructures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

A) AFM image of the bare CNT scaffold, B) Optical model used to represent the properties of the CNT scaffold and the adsorption of GOx

Figure 2. Dynamic adsorption data obtained for a bare Si/SiO₂ wafer and two CNT scaffolds of 27.5 nm and 49.1 nm.

Figure 3. Adsorbed amount of GOx to a bare wafer and six CNT scaffolds with different thicknesses. Error bars represent the errors associated with calculations of the adsorbed amounts using the presented optical model.

Figure 4.
AFM image of the CNT scaffold after adsorption of GOx

Figure 5. Enzymatic activity of GOx-modified scaffolds per unit area of the substrate as a function of the thickness of the scaffold

Figure 6.

Peak current versus concentration of glucose obtained with two GOx-modified CNT scaffolds. Error bars represent the standard deviation of three peaks obtained at three separate injections.