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Active-electrode biosensor of SnO_2 nanowire for cyclodextrin detection from microbial enzyme

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Abstract

Cyclodextrin (CD) is a conical compound used in food and pharmaceutical industry to complexation of hydrophobic substances. It is a product of microbial enzymes which converts starch into CD during their activity. We aim to detect CD using active-electrode biosensor of SnO_2. They were grown on active electrode by the VLS method. The CD consists of several glucose units which have hydroxyl groups which tend to bind to interface states present in nanowires changing their conductivity. Experimental results of electrical conductivity at different CD concentrations are presented. A model that describes the influence of adsorbed glucose on nanowires and its electrical properties is also presented. Some general observations are performed on the applicability of the CD adsorption method by the nanowire-based biosensor.

Keywords: biosensor, cyclodextrin, enzyme, SnO_2, nanowire, electrical conductivity

(Some figures may appear in colour only in the online journal)

1. Introduction

CDs (cyclodextrins) are glucose units linked by1, 4-bonds in the form of a truncated cone [1]. The CDs ability to form inclusion complexes with many molecules ensuring that it is used into industry [2–4]. The CDs result from starch degradation by the action of the enzyme CD glycosyltransferase (CGTase) produced by microorganisms, mainly bacteria of genus *Bacillus*. Enzymatic activity of CGTase occurs when a starch source is hydrolyzed to produce CD by transglycosylation therefore it is measured by the formation of these product. Therefore, the goal of any enzymatic detection assay is to measure the conversion of substrate into product. An enzymatic reaction could be observed by the appearance of a colored compound using spectrophotometers which determine the absorbance wavelengths of these compounds. Enzyme activity can be performed by electrochemical methods which is used to determine pH changes however its

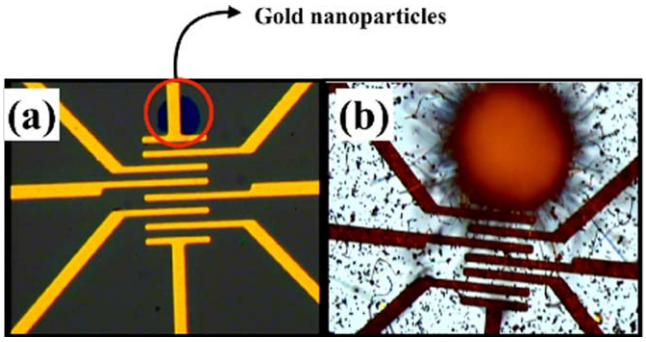

Figure 1. (a) An optical micrograph of the metal electrodes obtained by photolithography. The highlighted region depicts the place where a thin gold layer was deposited and it is responsible for the formation of the catalyst nanoparticles that act as the preferential site for the nanowires growth; (b) completed device after the nanowires growing on the gold nanoparticles region grown by the VLS method.

detection is often described with errors due to lack of accurate and reproducible data. CGTase can be applied to starch hydrolysis to produce CD by means of transglycosylation in industry. Enzymatic activity measures the amount of active enzyme present in a reaction medium which is accomplished by the formation of these by-products [5]. Although the descriptions of enzymatic assays can be found in detail in literature [6], procedures may be modified due to the special features of a single enzyme. Thus, alternative methods for enzymatic detection have great appeal, both academic and commercial. Conductive-type electrical sensors, i.e. those that alter the conductivity of the active layer, have shown a great advantage for detection of microbial metabolism. The metal oxide-based devices have been known since the 1950s when effects of the reaction of metal oxides with the surrounding atmosphere were observed [7–10].

Metal oxide semiconductors (MOS) have been extensively employed as gas sensors due to their advantages including high selectivity and sensitivity toward specific gases, low production cost, fast response, recovery time, ease of use and simple electronic interface [8, 11]. In general, metal oxides are classified in two types: n-type (the majority of carriers are electrons) and p-type (the majority is the 'holes'). Among the MOS, tin oxide (SnO_2) has been explored as a very sensitive material to different stimuli. SnO_2 is classified as a n-type semiconductor presenting a 3.6 eV wide band gap at 300 K that enables its application in a variety of technologies. The sensing principle of SnO_2 is based on changes in the electrical conductivity influenced by adsorbed oxygenated species on its surface [12, 13].

More recently, nanomaterials MOS—including SnO_2—in many different nanoscale devices have emerged enabling the development of novel chemical and biological sensors and also improving the analytical performance by particle size, surface area, functionality, adsorption capability and electron-transfer properties [14, 15]. The high performance of SnO_2 nanomaterials can be achieved through reducing the size and also the dimensionality of the material by modifications on its morphologies resulting in structures such as nanoparticles, nanowires, nanotubes, nanoneedles, and nanoflowers [16–19]. The use of pure or doped nano-MOS as biological and chemical sensors are very promising due to its distinguish features: high surface to volume ratio; modified-surface work function; high surface reactivity; high electron mobility and high effectivity in retaining biomolecules. They have been used in a wide range of scenarios, such as clinical, industrial, agricultural, pharmaceutical and military fields. Despite high-performance gas sensors are the most representative application of MOS, the effectiveness as biological sensing has been attracted attention due to their fast response, high sensitivity and selectivity. In this work SnO_2 nanowires were used as active electrodes for the CD detection and the comparison with the traditional biological method of enzymatic activity quantification.

2. Material and methods

2.1. Microorganism and CGTase production

The strain Bacillus lehensis was isolated from wastewater samples from a cassava flour mill in Brazil and 16 S rRNA sequence was deposited in the genetic sequence databank of the US National Center for Biotechnology Information (GenBank: HQ399547). Bacillus lehensis HQ399547 is maintained in the Industrial Microbiology Laboratory of the Biological Sciences Institute of Rio Claro (SP, Brazil) [20]. The Bacillus lehensis CGII was cultivated in 5 l stirring tank reactor (Bio-t-mini Zeta, Rapperswil, Switzerland) at 35 °C, 150 rpm, initial pH of 9.2, containing 2 l production medium [21].

2.2. CGTase Purification and cyclodextrin production

The cell-free supernatant of fermentation was purified using the method described by [22]. The same volume of purified enzyme (0.035 mg ml^{-1}) and potato starch (1% w/v) solution were incubated at 55 °C, pH 8.0 for obtention of CD in production times.

Beta CD was analyzed using a high-performance liquid chromatography (HPLC) system equipped with a UV detector in a 210-nm column (4.6 × 250 mm; Phenomenex, Torrance, USA) at 25 °C.

2.3. Device construction and synthesis of SnO_2 nanowires

The biosensors were constructed following the conventional photolithography procedure [23–26]. Initially, metal electrodes (separated by 5 μm) were defined on a Si/SiO_2 (500 nm thick oxide layer) substrate. Then a small region (10 μm in diameter) was selected (figure 1(a)) for the growth of SnO_2 nanowires by the vapor liquid solid (VLS) method and following the experimental procedure reported by Costa *et al* [27]. Then, a thin layer of gold (2 nm) was deposited under high vacuum (better than 10^{-6} mbar), responsible for the formation of the catalyst nanoparticles used as the preferred site for adsorption of the vapor precursor elements. The precursor tin metal powder (Aldrich, purity > 99.99%) was placed in an alumina crucible and placed in the center of a

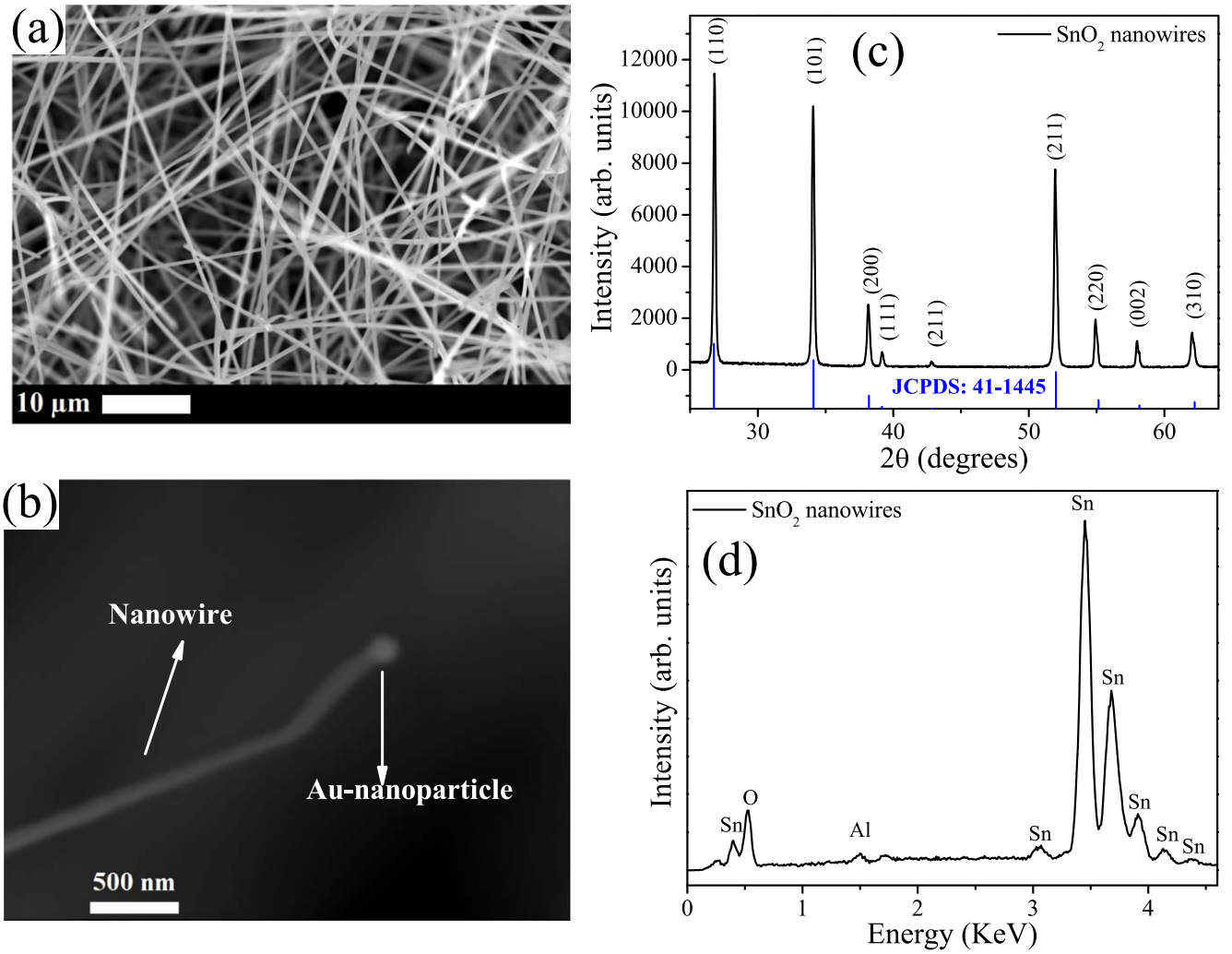

Figure 2. Structural data and chemical composition of the SnO_2 nanowires: (a) SEM image of as-grown nanowires exhibiting lengths of tens of micrometers and (b) SEM image of a nanowire's tip where the catalyst nanoparticle can be seen confirming the grown by VLS; (c) XRD pattern obtained from SnO_2 nanowires' sample, agreeing with JCPDS Card No. 41-1445; in (d) EDX analysis for the SnO_2 nanowires confirmed that the nanowires are composed only by Sn and O.

tubular reactor (Lindberg Blue-M). The substrates with the electrodes and a small region covered with gold (figure 1(a)) were placed 8 cm downstream from the precursor powder. The synthesis temperature was adjusted to 950 °C (heating rate 20 °C min^{-1}) and remained at this value for 90 min. The pressure inside the tube was controlled by a vacuum pump and a mixture of oxygen/argon with constant flow of 100 sccm was used to transport the precursor vapor to synthesis region. After this process, the oven was naturally cooled to room temperature (figure 1(b)).

Figure 2 shows the structural data and chemical composition of the as grown SnO_2 nanowires. For a first investigation of the samples SEM images were taken. Figure 2(a) reveals nanowires with a good uniformity both on the diameters and lengths (several tens of micrometers) and figure 2(b) highlights a single nanowire with a catalyst nanoparticle of Au on its tip confirming the grown by VLS mechanism. In order to identify the crystal structure of the sample x-ray diffraction measurements were performed. In figure 2(c), the XRD pattern indicates that the nanowires have a good crystalline quality and in close agreement to the JCPDS Card No. 41-1445 (represented by blue bars) indicating that the as-grown material presents a tetragonal SnO_2 structure (space group P42/mnm). Energy-dispersive x-ray spectroscopy (EDX) spectrum as depicted in figure 2(d), confirms the chemical composition of the nanowires, indicated the presence of Sn, O (Al signal was generated by SEM support).

2.4. Electrical setup

The experimental setup for characterization of the biosensor is shown in figure 3(a): it is constituted of three electrodes: (i) working electrode formed by a set of nanowires grown by VLS method; (ii) reference electrode and (iii) common electrode. The last two were made of a 100 nm layer of titanium. Electrical characterization was performed by a four-probe measurement, whose scheme is shown in figure 3(b). The electric current–voltage characterization was performed by applying an electric current (0–250 nA) between the working

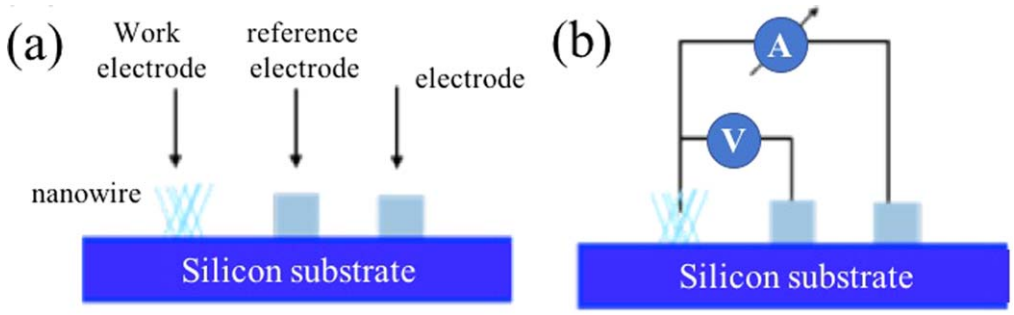

Figure 3. (a) Sketch of the used devices and the electrical connections and (b) diagram of the 4-probe setup which was performed to perform the electrical measurements.

electrodes and the common electrode and the voltage between the working electrode and the reference electrode was measured. Measurements with respect to solvent and cyclodextrin solution were performed by inserting 20 μl of solution on the device, closing the electrical circuit.

Electrical characterization was performed in two different devices: (i) all electrodes only made of titanium and (ii) one of electrodes consisting of nanowires, as seen in figure 3. In both configurations measurements were taken only with the solvent to eliminate its contribution. The configuration (i) is only intended to demonstrate the effectiveness of nanowires as CD detectors. The CD were tested in concentrations of 0.001 mmol to 0.1 mol. In addition, current–time characterization was performed, maintaining a fixed voltage of 5.0 V and increasing CD concentration from 0.001 mmol to 10 mmol.

3. Results and discussion

3.1. Cyclodextrin production

A microbial enzyme was produced by Bacillus lehensis CGII and purified by two-step method using ammonium sulfate precipitation and bispecific affinity chromatography. Enzymes are present in all living cells and therefore in all microorganisms. Each microorganism strain produces a large number of enzymes that hydrolyzing, oxidizing or reducing metabolites in nature. The quantity of microbial enzymes produced varies among species and strains. Microbial CGTases are extracellular enzymes that differing in the amount and type of CDs produced, most being α, β and γ-CDs. The optimization of the fermentation conditions for CGTase production has been studied to increase the rates for this.

Cassava starch was used as substrate to perform a chemical reaction for production a mixture of α-, β- and γ-CDs under the optimum conditions to form the enzyme substrate complex. The amylases enzymes are catalytic converters of biological processes specifically cleave the starch and related compounds by hydrolysis of the α-1, 4 and / or α-1, 6 glycosidic bonds. There are some amylases that have additional activities beyond the properties of amylolytic enzymes, such as the CGTase which is capable of converting starch into CDs and derivatives by performing the cyclization (conversion of α-1, 4-glucan starch to CDs by intramolecular transglycosylation reaction), coupling (by opening the rings of the CDs and transferring the linear maltooligosaccharides formed to the acceptors) and the disproportionation by means of the intermolecular transglycosylation reaction.

Table 1. Production of β- CD from Bacillus lehensis CGII.

Ciclodextrin production by ciclodextrin glycosiltransferase	
Concentration (mmol)	Enzimatic activity (U ml^{-1})
0.06	30
0.15	45
0.20	50
1.00	110
2.00	175
4.00	238
5.00	303

The conversion of starch into CDs by CGTase enzyme from Bacillus lehensis CGII HPLC was obtained in hours with production ratio of β- CD (g l^{-1}) was 6.33 (table 1).

The CDs can reversibly capture molecules of varying size to form inclusion complexes which is the result of the balance between the included substance and the CD molecule. Specific CDs (α-, β-, or γ-CD) will complex guest molecules of specific sizes. A, β and γ-CD have external diameters (nm) of 1.52, 1.66 and 1.77 and internal cavity diameter (nm) of 0.45, 0.60 and 0.75. Enzyme activity has been measured based on the discoloration of solutions as phenolphthalein during the formation of inclusion complexes. The rate of enzymatic activity (U ml^{-1}) in a sample can be determined from the rate of enzyme-catalyzed reaction. The enzymatic activity corresponds to the amount of enzyme that catalyzes a reaction with 1 micromole of product per minute.

3.2. Electrical characterization of biosensor

Initially, the electrical response towards the presence of CD was evaluated by comparing (i) the device composed only by metallic electrodes and (ii) device containing SnO_2 nanowires. The concentration of CD was ranged from 0.001 mmol to 10 mmol in order to verify the effect of a large range of CD concentration on the sensor's responsivity. Figure 4 shows the

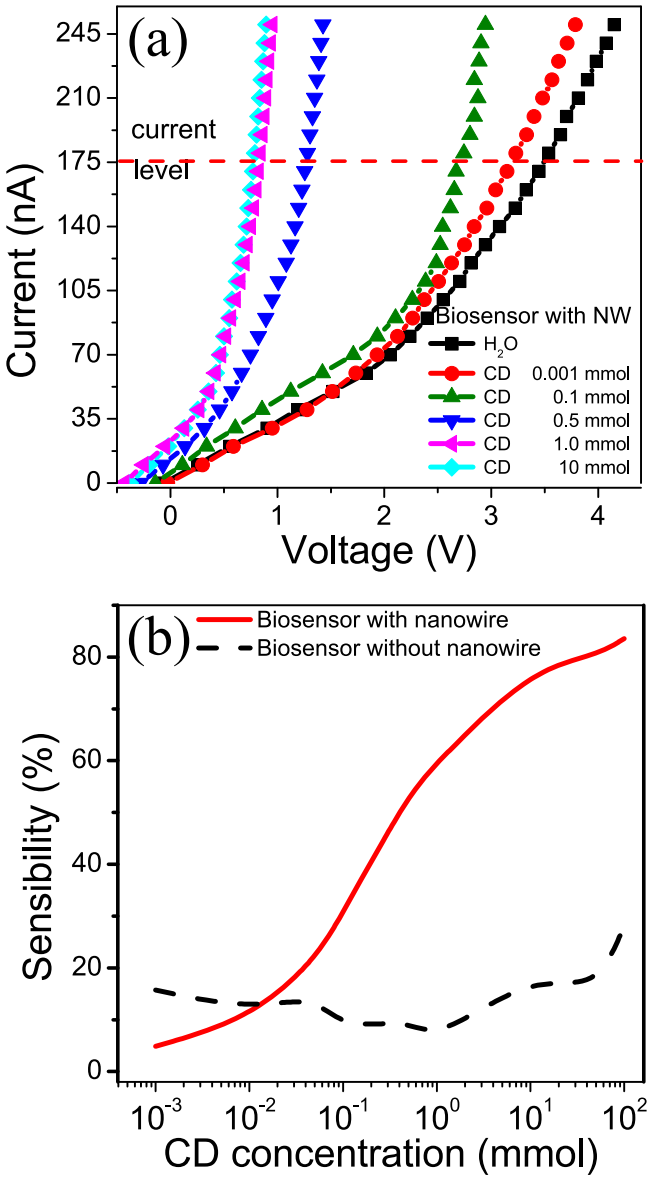

Figure 4. Experimental results concerning the biosensors electrical characterization: (a) current–voltage characterization for different cyclodextrin concentrations for the device using SnO_2 nanowires as working electrode; (b) sensitivity versus cyclodextrin concentration for the control sample, without SnO_2 nanowires and for those with SnO_2 nanowires on the electrodes.

biosensor electrical response regarding the presence of CD in the two device configurations discussed earlier.

Figure 4(a) shows the current–voltage curves for the device described in case (ii) with different CD concentrations. Initially, the sensor was measured in contact with the solvent (H_2O) used to dissolve the CD (black line-square symbol). In this figure it is easy to observe that there is a shift of the origin for lower concentrations of cyclodextrin, consequently it shows the control measurement (H_2O). This may be due to a potential drop caused by photovoltaic effects, charge polarization or space-charge effect. Also, we observe that with increasing concentration of CD the device needs a lower voltage to reach the current level of 175 nA (highest resolution range highlighted in the graph). At concentrations a

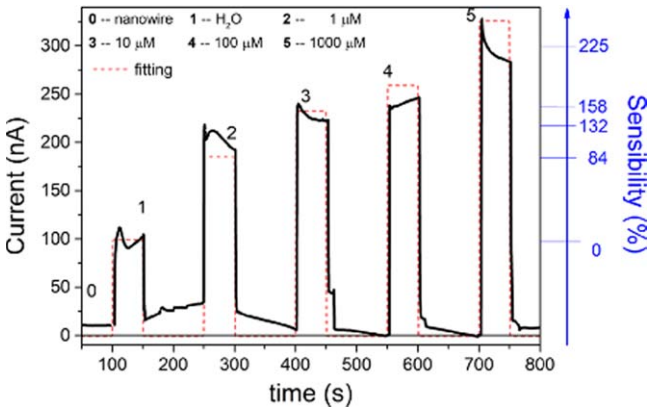

Figure 5. Current–time characterization for the nanowire biosensor containing SnO_2 nanowires as electrodes under various cyclodextrin concentrations.

higher than1mmol the device response tends to saturate and the response is almost superimposed at those concentrations. Data regarding the configuration without nanowires as electrodes were omitted because they were very noisy and inconclusive.

The biosensor sensibility S_{NW} can be defined in terms of conductivity (or electric current), and it is simply written as

$$S_{NW} = \frac{G_{NW} - G_{solv}}{G_{solv}} = \frac{I_{NW} - I_{sol}}{I_{solv}}, \quad (1)$$

where G_{sol} and G_{NW} are the electrical conductance of SnO_2 nanowire under only solvent (w.r.t. H_2O) and CD solution, and I_{sol} and I_{NW} are corresponding currents, respectively. Sensitivity zero was considered to be that obtained with respect to H_2O. Since higher S_{NW} was observed in the forward bias than the reverse bias, the analysis of the S_{NW} was performed in the forward bias conditions for highly sensitive detection. To calculate the S_{NW} of the SnO_2 NW biosensors, the currents were extracted at a given forward bias considering the constant electric current level of 175 nA; it was done using the *I–V* characteristics before and after exposure to CD solution. The calculated S_{NW} is shown in figure 4(b)

As can be seen in figure 4(b), the responsivity behavior as a function of the increase of CD concentration is considerably enhanced by the presence of SnO_2 nanowires, while for the electrode made only metal the response was poorly increased by CD. It was found an enhancement factor up to four times when the SnO_2 nanowires were present on the sensor. This can be explained by the adsorption of CD molecules on the surface of SnO_2 nanowires by oxygen interactions and then resulting in the increase of the electrical conductivity, which is not observed for the device without nanowire as electrode. Therefore, the presence of SnO_2 nanowires enhances the sensor's response when there is an increase of CD concentration. For the device formed only by metal electrodes there is not a clear response with increasing concentration.

The time-dependence of the current flowing through SnO_2 nanowire device under contact to different concentrations of CD (from 0.001 to 1 mmol) is presented in figure 5. It can be seen a fast and pronounced increasing of the current

Table 2. Comparison of analytical methodologies for cyclodextrin sensing.

Analytical technique	Concentration range	Remarks	References
Absorbance/photometry	0.17–2 mmol l^{-1}	Indirect method (derivatization with phenolphthalein)	[29]
Capillary electrophoresis—fluorescence detection	0.01–1 mmol l^{-1}	Indirect method (derivatization with 2-anilinonaphthalene-6-sulfonic acid)	[30]
Liquid chromatography/refractive index detection	0.01–4 mg ml^{-1}	Chromatographic running time of 20 min	[31]
Liquid chromatography/pulsed amperometric detection	20–1500 pmol	Chromatographic running time of 20 min	[32]
Hydrophilic interaction chromatography/light scattering detection	0.5–1.5 mg ml^{-1}	Runtime of 10 min	[33]
Nanowire electrode/conductivity	1–1000 μmol l^{-1}	Direct method response time of 50 s	This work

after contact with CD (down to 0.001 mmol). The sensor's response time can be estimate in 50 s, which is the time where the current remains unchanged, followed by the recovery time of 100 s—when the current values reach the baseline—before the measurement of the next concentration. For calibration purposes, the analytical signal was evaluated by measuring the electrical current of different concentrations of CD in triplicate. The linear relation between analytical signal and concentration was obtained within the range of 0.001 mmol to 1 mmol of CD, which is suitable for its use in quantification from enzymatic activities.

As seen previously, it is very clear that increasing the CD concentration, the electrical current arises accordingly. In this study, the SnO_2 nanowires which present a great area/volume ratio, allowed a dramatic increase of the surface area subjected to the aqueous solution containing CD. The variation of the electrical current depends itself on how much electron activity is affected by the amount of chemisorbed oxygen species from the carbohydrate [28]. In this way, the hydroxyl groups present in the CD interacts with the SnO_2 nanowires surface, causing notable changes in resistivity and electric current of the device. The results obtained in this study were compared to other methodologies for cyclodextrin sensing already published in literature which are summarized in table 2. Our results have showed advantages in terms of fast response (i.e. 50 s), wide concentration range of CD that can be probed, no need of derivatization steps and easiness to use.

4. Conclusion

In conclusion, we have built biosensors which use SnO_2 nanowires grown by VLS method as active electrodes. The sensory characteristics of biosensors have been carefully studied the high sensitivity and fast responses for the biosensor were observed at room temperature. These results demonstrate the potential of the SnO_2 nanowires to CD detection placing them as strong candidates for the *in situ* detection of enzymatic activity of CD glucosyltransferase.

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