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An enzymatic glucose detection sensor using ZnO nanostructure

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Abstract:

Glucose sensor based on ITO/ZnO NRs/GO_x/nafion is fabricated and tested under different glucose concentrations. Hydrothermal growth method along with sol-gel technique is used to grow high quality ZnO nanorods that have well-alignment and high density with an acceptable aspect ratio. The as-grown of ZnO nanorods are used to fabricate a working electrode that can be used for glucose detection in blood after a modification process with GO_x and nafion membrane. Annealing at 110 °C helped in improves the crystallinity of the seed layer and as a result, a high density and well alignment as-grown ZnO nanorods were obtained. High sensitivity and short response time were obtained from the fabricated device with an acceptable lower limit of detection.

1. Introduction:

Glucose electrochemical sensors based on nanostructure of materials have been investigated, but the demand of glucose detection devices pushes researchers to enhance glucose detection capabilities of devices with high accuracy, sensitivity, and selectivity [1-3]. Glucose is one of several chemical species in blood that must be detected accurately without being affected during detection process by the surrounding species [4]. This requires a material that can provide a fast electrochemical oxidation of glucose on its surface with lower applied potential to assure high level of selectivity and fast responded devices of glucose changes [5]. Electrochemical glucose sensors consist of three electrodes, working, counter, and reference electrode. However, some of which consist of only two electrodes. The role of the reference electrode is to control the applied voltage between the working and the reference electrodes, so the voltage is distributed equally between the two electrodes. This is an important issue in order to control the chemical reaction during the sensing mechanism, and as a result, the fabricated electrochemical sensor maintains high level of reliability and selectivity [6].

Zinc oxide ZnO is one of the semiconductor materials that attracted tremendous attention due to many exceptional properties that make this material is one of the best candidates in many devices and fabrication processes. Zinc oxide has a high binding energy, 60 meV that provides an enough time for the generated exciton to be collected [7]. This feature can be invested mainly in solar cells and other applications of energy harvesting devices. In addition, ZnO is a biocompatible material, which means it can be considered as a preferable semiconductor in biological and biomedicine devices [8]. Cost-effective and the easiness of growth are crucial factors in the regard of choosing ZnO nanostructure in electrochemical sensors since those kind of sensors require high quality materials that can sense glucose accurately. Zinc oxide nanostructure can be achieved in several growth methods, but the sol-gel and hydrothermal synthesis techniques are considered the best approaches since the growth time and growth temperature are under control. In addition, the growth of ZnO nanostructure can be done at low temperature around 85 °C and this is a low growth temperature to synthesize ZnO nanostructure comparing with other growth methods. Controlling the growth helps in producing ZnO nanorods in high aspect ratio and consequently, increasing the reaction active area of the working electrode [9-12]. The above mentioned parameters and features can be utilized to fabricate a reliable, selective, and highly sensitive electrochemical sensor that can detect glucose accurately and efficiently. It is an important for diabetic people to monitor their glucose level in blood regularly, and it is a significant point of the fabricated glucose sensors to reach the clinical level of glucose, so those sensors can be used for a real-time glucose monitoring. The regular glucose level in blood with a fasting stomach ranges from 3.9-5.5 mM/L, and this concentration might change with food or daily activities.

In this work, we report on fabrication and characterization of glucose detection sensor based ZnO nanorods. The growth and characterization of ZnO nanorods are investigated and sol-gel and hydrothermal method are used to synthesize high quality ZnO nanorods in terms of alignment, density, and aspect ratio. ZnO nanorods were chosen for several reasons. For instance, it is easy to control the alignment of the as-grown ZnO nanorods since that increases the electrochemical active area of the reaction during glucose detection process. Increasing the electrochemical area provides a fast oxidation process of H_2O_2, and thus no other oxidants rather than oxygen are needed. This helps in reducing noise of the detected output signal, which is an electric current in this sensing mechanism. Active area of electrochemical reaction = Area of nanorods *Density of nanorods*Area of substrate.

Active Area of electrochemical reaction =2πrh*No. of nanorods/Area*substrate Area.

Where r and h are radios and length of nanorods respectively. Zinc oxide nanorods lead to increase the active area of the reaction 5-6 times than bulk structure. Increasing the aspect ratio leads to increase the density of nanorods and this will lead to increase the total active area of the electrochemical reaction. Now, using Bulter-Volmer equation, $I = A_e q(K_f \rho_{H2O2} - K_b \rho_{O2})$, where A_e is the area of the working electrode, K_f is the rate constant for oxidation of H_2O_2, K_b is the rate constant for reduction of oxygen, ρ_{H2O2} is the concentration of hydrogen peroxide, and ρ_{O2} is the concentration of oxygen, one can see that the output detected current is proportional to the active area of the working electrode.

The second reason of choosing ZnO nanorods among other ZnO morphologies is the biocompatibility issue. Growth of ZnO nanorods can be done in a growth solution with pH 7, so no other base chemicals are needed to etch the nanorods to nanotubes. The fabricated and immobilized working electrode based on as-grown ZnO nanorods shows high sensitivity and short time response. The nanostructure of ZnO helps in enhancing the active electrochemical reaction area and detecting high output current. The sensor is fabricated based on the amperometric detection mechanism of glucose with two electrodes, working and reference electrodes. The working electrode is coated with GO_x and nafion membrane for better selectivity and sensitivity [13].

2. Experimental part:

2.1 Maerial growth and characterization

Zinc oxide nanorods are synthesized using hydrothermal growth method and sol-gel technique at low growth temperature. Zinc acetate dehydrate is dissolved in 10 mL of methoxyethanol and stirred in 300 rmp at 85 °C using a magnetic stirrer for seed solution synthesize. The precursor then is sonicated for 15 minutes and filtered to remove undissolved particles. The molarity of the prepared solution is 0.5 M and this molarity was chosen among many others because it provides seed layer with high density and as a result, high quality ZnO nanorods was obtained. The life time of the seed later is around one month and the distribution of the seed layer on the substrate plays a significant role in the uniformity of the ZnO nanorods and on the alignment as well.

The layers of the prepared seed solution are spin coated on the top of indium tin oxide ITO using a hot plate and the deposited layers annealed at 110 °C for one hour. The annealing process is very important since it provides a uniform distribution of the seed layer on top of the substrate. As it has been demonstrated previously, the uniform distribution of the seed layer is significant and it affects the density and alignment of the as-grown ZnO nanorods. To grow high quality ZnO nanorods, growth solution with a pH 7 should be synthesized carefully. The growth solution consists of zinc nitrate hexahydrate dissolved in 10 mL deionized water and hexamethylentetramine dissolved in 10 mL of deionized water as well in 0.025 M for both chemicals, and then they stirred at room temperature in 300 rpm for one hour. The two solutions mixed together and stirred for another hour and then 15 minutes sonication. Small filter is used to filter any undissolved particles. The growth solution must be used in the same day of preparation to avoid any accumulation.

The prepared samples are pasted into a glass slide substrate and placed upside down in the growth solution and located in a furnace at 85 °C for 6 hours growth time. The growth time is vary from 2 hours up to 24 hours and the growth temperature as well depending on the desired value of the aspect ratio, which is the ratio between the length of the nanorods to the diameter. After a certain growth time, the samples are rinsed by deionized water gently for three times to stop the effect of the growth solution on the samples. Drying the samples at 300 °C is important for around 15 minutes. The described method is used in this work to grow high quality ZnO nanorods that can be used for glucose detection in an electrochemical cell. A small part of the substrate is covered with a tape for contact during the characterization of the fabricated electrochemical setup. The effect of annealing is investigated, so scanning electron microscopy SEM is used to determine the effectiveness of the method and to calculate the length and diameters of the as-grown ZnO nanorods. SEM images were taken before and after annealing the seed layer at 110 °C as it can be shown in figure 1 (a and b). The annealing helped in enhancing the alignment of the as-grown ZnO nanorods since the seed layer distribution was enhanced by the annealing. This helps in increasing the active area of the working electrode and as a result, enhancing the sensing ability of the sensor. The detected current is proportional to the area of the working electrode, so the as-grown ZnO nanorods have high surface to bulk ratio. In addition, the density of the as-grown ZnO nanorods plays an important role regarding the output current enhancement. The annealing has a direct impact on that by forcing the seed layers to be crystalline and to be distributed uniformly. The detected current is proportional to the density of the ZnO nanorods since the density affects the area of the working electrode where the chemical reaction occurs. Annealing at 110 °C helps in synthesizing high quality ZnO nanorods in terms of alignment, density, and aspect ratio.

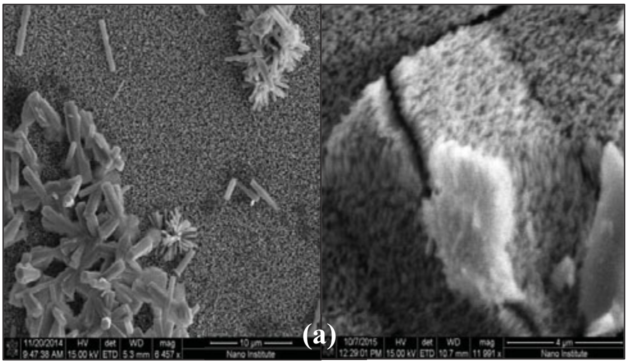

Figure 1 (a) SEM of the as-grown ZnO nanorods without annealing at 110 °C

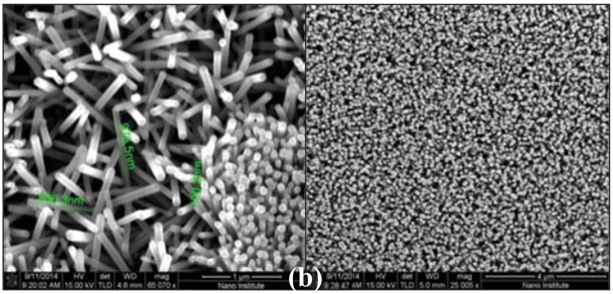

Figure 1 (b) SEM image of the as-grown ZnO nanorods after annealing at 110 °C

The well-aligned ZnO nanorods are shown in figure 1(b) with a small variety of the diameters and length. The aspect ratio of the as-grown ZnO nanorods can be enhanced by increasing the growth time and growth temperature. As shown from the SEM image, the aspect ratio is from 8.36 - 9.76, and this aspect ratio might be enhanced up to 10 times by manipulating growth time and growth temperature.

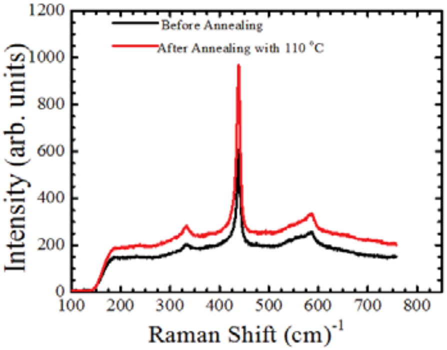

Figure 2. Raman shift of the as-grown ZnO nanorods before and after annealing.

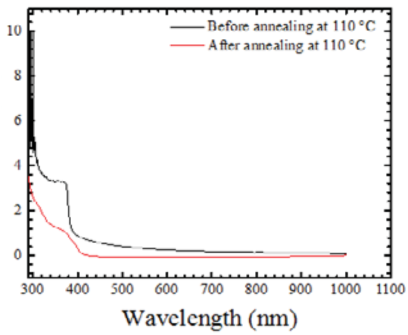

Figure 3. Absorbance spectrum of ZnO nanorods before and after annealing at 110 °C.

Annealing at 110 °C does not have a clear impact on Raman spectra as it can be clearly seen in figure 2. However, the intensity of the lattice and phonon vibration peak after annealing seems to be higher than before annealing. The position of the three peaks is still the same and the dominant peak is appeared at 438 cm^{-1} before and after annealing at 110 °C. Raman spectrum provides useful information regarding the lattice constant and phonon vibration modes. The other small peaks appeared at 330 cm^{-1} and 586 cm^{-1} and those peaks belong to different zinc to oxygen in the compound.

Absorbance spectra of the as-grown ZnO nanorods are shown in figure 3. The exciton peak before annealing at 110 °C is appeared at the wavelength 373 nm corresponding with the band gap of the ZnO, while it became a bit wider after annealing. As it can be seen from the graph, the as-grown ZnO nanorods have almost zero absorbance of the wavelengths that have energies lower than the band gap which is 3.37 eV for the bulk. This explains the importance of using ZnO in UV-applications. For energies that are higher than the band gap, the as-grown ZnO nanorods showed high absorbance spectra before and after annealing and the highest absorbance was observed at 300 nm. The band gap of materials can be calculated using the absorbance spectrum depending on the shoulder of the exciton peak. ZnO has a high binding energy, 60 meV which is the energy required to keep the electron-hole pair together before they recombine through the material. As it can be seen from the figure, the absorbance started at the wavelength 390 nm before annealing while the absorbance started at the wavelength 407 nm after annealing. This means the annealing might have an impact on the band gap of the as-grown ZnO nanorods.

2.2 Device fabrication and characterization:

The fabricated working electrode is based on ITO/ZnO NRs/GO_x/nafion membrane. Glucose oxidase GO_x is used as a mediator to increase the sensitivity and specificity or selectivity of the device. The working mechanism is as follows: An electrochemical reaction takes place between glucose and oxygen in the presence of GO_x on the surface of working electrode. The reaction product is gluconic acid and hydrogen peroxide. The second step of the electrochemical reaction is between the surface of the as-grown ZnO nanorods and hydrogen peroxide. Hydrogen peroxide is oxidized and gives two electrons, and oxygen is reduced, so the electrochemical reaction is called oxidation-reduction reaction. GOx has several chemical and physical properties that make it a desired enzyme for immobilization. Chemically, it is a stable enzyme in different pH solutions, and this helps in increasing the stability of the working electrode during the detection of glucose and during the storage time of the immobilized working electrode. In addition, it consists of two molecules of flavin adenine dinucleotide (FAD), and those molecules are bounded strongly, so GO_x cannot be analyzed easily especially in pH 7 solutions. Biologically, glucose oxidase is biocompatible enzyme that is highly recommended in biosensors or electrochemical sensor for glucose detections. The electrical current is promotional to the concentrations of hydrogen peroxide and hydrogen peroxide concentration is proportional to glucose concentrations in blood. The reaction between glucose and oxygen can occur only in the presence of the enzyme GO_x. Nafion membrane helps in enhancing the stability of the sensor and helps in increasing ion exchange during the oxidation reduction process. The sensor was characterized using sourceMeter keithley model 2410 to measure the output current with a fix value of the applied voltage, 0.8 volt. The applied voltage is to oxidize glucose or in other words, to make the reaction between oxygen and glucose happens and produce hydrogen peroxide. Figure 4 shows the time response of the fabricated working electrode. The sensor showed a fast time response around 3 second with different concentrations of glucose. The current was repeated each 20 seconds and the average of those data point was taken for each glucose concentrations. This is a useful study to examine the sensitivity of the device to any changes in glucose concentrations. Furthermore, the device did not show decay in current while moving from a glucose concentration to another one. Current decay might be an indicator to a fast oxidation reduction process that occurs between the two electrodes. In fact, this also could be because of the lower kinetic energy of the oxidant, which is the oxygen in the catalytic solution.

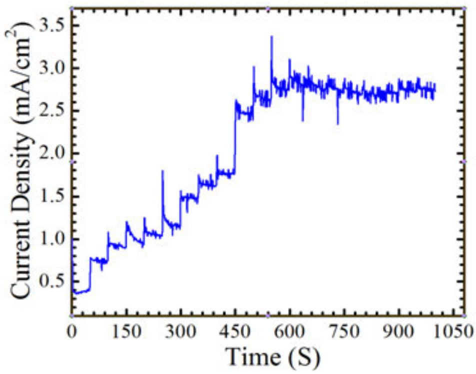

Figure. 4 shows the time response of the fabricated ZnO NRs electrochemical sensor.

The electric current was increasing linearly with changes in glucose concentrations. However, the current is saturated after 2.4 mM of glucose indicating that the current after 2.4 mM of glucose concentration does not depend on the amount of glucose but on the amount of the enzyme that the working electrode was immobilized with before running the experiment as it can be seen in figure 5. The fabricated ITO/ZnO/GO_x/Nafion sensor exhibited sensitivity around 1.06 mA/cm^2 mM and a lower detection limit ~ 291.6 µM which is the lowest concentration of glucose that can be detected by the sensor as in figure 6.

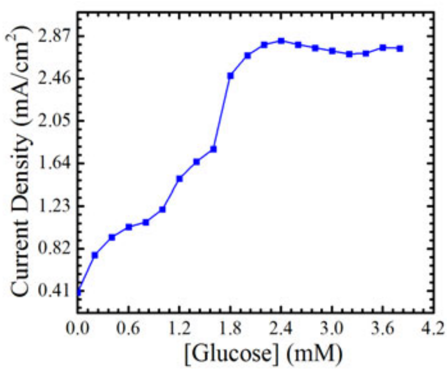

Figure. 5 illustrates the sensitivity of the fabricated sensor

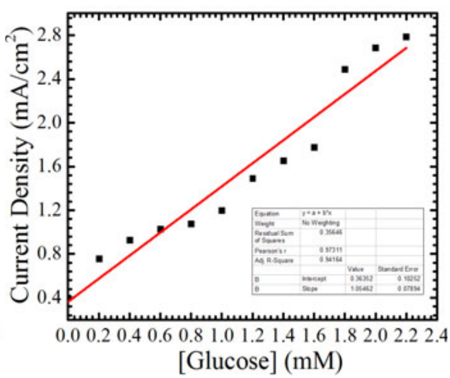

Figure 6 shows the linear relationship between the current density and the concentrations of glucose during the electrochemical reaction.

3. Conclusion:

Effects of annealing at 110 $^{\circ}$C on ZnO nanorods were investigated as it was shown in SEM images and spectrum of absorbance and Raman as well. Sol-gel and hydrothermal method was used to obtain high quality as-grown ZnO nanorods at low temperature. ITO substrate was used to grow the high quality of ZnO nanorods and to fabricate and immobilize the working electrode that was used for glucose detections. The fabricated device showed stability and selectivity toward glucose detection since the working electrode was immobilized with GO_x and nafion membrane. Nafion membrane helps in increasing the stability and fast ion exchange, while GO_x helps in increasing the specificity of the electrochemical sensor.

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