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Fabrication of ZnO Nanorods Based Biosensor via Hydrothermal Method

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***Abstract*—**Biosensors are playing vital role in industrial, clinical, and chemical analysis applications. Among other techniques, ZnO based biosensor is an easy approach due to its exceptional chemical and electrical properties. ZnO nanorods have positively charged isoelectric point which helps immobilize the negative charge glucose oxides (GOx). Here, we report ZnO nanorods based biosensors for the immobilization of GOx. The ZnO nanorods were grown by hydrothermal method on indium tin oxide substrate (ITO). The fabrication of biosensors was carried through batch processing using conventional photolithography. The buffer solutions of GOx were prepared in phosphate with a pH value of around 7.3. The biosensors effectively immobilized the GOx and result was analyzed by calculation of voltage and current on nanostructures.

***Keywords*—**Hydrothermal growth, zinc dioxide, biosensors.

I. Introduction

BIOSENSORS have a great research interest due to their wide range of applications in various fields. One of the important uses of biosensor is in medical [1]. Among available biosensors, glucose biosensors are becoming more important due to promising applications in a clinical lab, chemical analysis and environmental surface monitoring [2]. The enzymes are most suitable and actively responsive to any substrate [3], [4]. Thus the wide range of the glucose biosensors works on the bases of immobilization of enzymes on electrodes. The most used and realized analytical enzyme is the GOx [5]. The GOx is mainly analyzed by physical adsorption, sol-gels, cross-linkages, polymers and carbon paste [6].

Semiconductor nanomaterials have extra advantages on immobilization of enzymes due to its promising electrical and chemical properties. The immobilization of enzyme takes place on the nanomaterials by direct transfer of an electron from the surface to the surface [6], [7].

Biosensors are mostly based on semiconductor materials such as TiO_2, SnO_2 and ZnO [8]. The TiO_2 based biosensors have achieved excellent performance in terms of sensitivity and lower limit of GOx. As the charge mobility of TiO_2 is lower than that of SnO_2 and ZnO. A ZnO nanostructure has outstanding results in glucose biosensors due to its favourable isoelectric point (IEP). In various ZnO nanostructures, such as nanorods, nanowires, nanospheres, nanobelts, nanorings and nanoneedles, nanorods are of great research interest due to its promising electrical properties in biosensors [9]. The immobilization of analytical enzyme on the basis of the IEP of negatively charged GOx depends on the pH of GOx. Normally the IEP of GOx is 4.2 at a pH of 7.3 [10]. This difference in IEP of ZnO and GOx promotes the direct electron transfer from surface to surface and results in enzyme immobilization for the detection of glucose [11], [12]. Semiconductors and their composites have direct impact on the sensitivity and performance of biosensors. The morphology of semiconductor nanostructures has further enhanced the stability and sensitivity of biosensors [13]. A single crystal semiconductor nanocomb of ZnO grown by vapor-phase deposition method with mesoporous structures increases the sensitivity of biosensors [14]. This increase in the stability and sensitivity of nanostructures was due to large surface to volume ratio of mesoporous ZnO nanostructures, allowing deposition of more GOx for immobilization. The sensitivity of these mesoporous ZnO nanocombs is reported around 15.3 μA mM^{-1} cm^{-2} [14]-[16]. The growth of ZnO nanostructures on gold substrate also increases the performance of ZnO based biosensors. ZnO nanotubular based biosensors for the immobilization of GOx increase the sensitivity of device. This increase is due to larger aspect ratio and large surface to volume ratio for the loading of more enzymes or GOx [17].

The sensitivity of biosensors for the immobilization of GOx also depends on the 1D ZnO nanofibers of dimension 195-350 nm. The senility of the biosensors for immobilization of GOx increases to 21.7 μA mM^{-1} cm^{-2} [18].

In a composite semiconductor based biosensors, the efficiency of the device is further increased. Recently the carbon decorated ZnO (C-ZnO) nanostructures based biosensors have achieved higher efficiency with lower glucose detection limits. The C-ZnO nanowires have direct interaction of GOx for immobilization [19]. The enzymes or GOx interact with hexagonal face of ZnO nanowires which is more sensitive to GOx due to higher IEP of ZnO nanowires. Furthermore, highly sensitive GOx biosensors are based on the hybrid platinum-fullerene like ZnO nanospheres (50-200 nm) deposited on glassy carbon electrode for the immobilization of GOx. This biosensor exhibits higher stability and improved sensitivity of 43.5 μA mM^{-1} cm^{-2} [20]. However, composite of zinc oxide and carbon nanotube based sensors have been reported for the application of DNA immobilization. As the electrical conductivity of carbon nanotubes (CNTs) is much greater, its electrical response plays an important role in the sensitivity of the biosensors. This increase in the sensitivity of biosensors results in decreasing the lower limit of GOx and other enzymes for immobilization [17], [21]. Furthermore,

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ZnO nanorods based biosensors with large surface to volume ration and easy growth method have attained the researcher's interest [22]-[24]. The ZnO growth via hydrothermal method is the simplest and low cost fabrication method which in batch processing further decreases the cost of fabrication via conventional photolithography method [25], [26].

Here we report the ZnO nanorods based glucose biosensors. The growth of ZnO nanorods carried through a low-temperature hydrothermal method on ITO substrate [12]. The comb-like ZnO nanorods based biosensors were fabricated in a batch processing through conventional photolithography.

II. Experiment

Prior to a photolithography process, a thin layer of metal was deposited on a glass substrate for making the comb-like structure. A thin metal layer of gold was deposited by Plasma sputtering method.

In this method, the metal was deposited by the Ar plasma sputtering on glass slides substrate of dimension 3 x 3 cm. In organ plasma sputtering the glass substrates were placed in a chamber near the metal deposition plates and the chamber was evacuated at a pressure of 10^{-3} tor. The deposition of gold was deposited on a glass substrate for 1 min. The deposition of metal by plasma sputtering was smooth and plane.

A. Photomask Preparation

The photomask was used to transfer pattern on the metal deposited glass substrate. Initially, graphical images of various comb structures of desired shapes were designed in Adobe Photoshop and printed out on a transparent sheet. The pattern size of comb-like structures was about 50-60 mm in size as shown in Fig. 1.

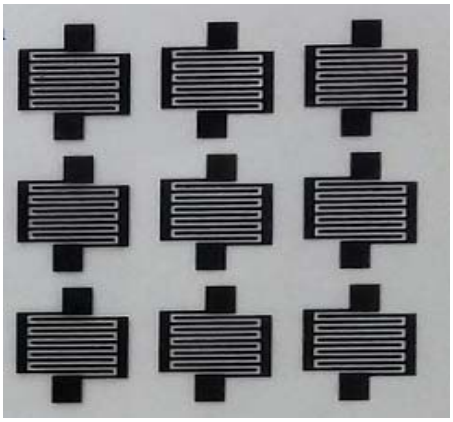


Fig. 1 Camera images of Photomasks of the comb-like structure

B. Photolithography

Photolithography process was used to obtain the required pattern on metal deposited glass substrates. Before the transferring the pattern from a mask to the substrates, a thin layer of photoresist was coated on the substrate spin coating method. The substrate was prebaked at 10 min at 90˚C followed by photomask alignment on a glass substrate. The Photomask was exposed to UV light for 1 min and then developed in 99% NaOH solution for 15 sec. The substrate was rinsed in Di water and post backed for 25min at 130 ˚C. The obtained pattern is shown in Fig. 2.

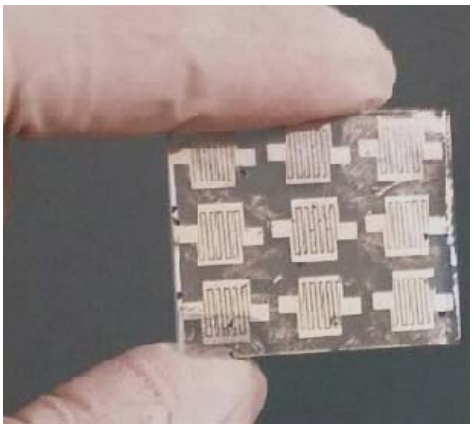


Fig. 2 Camera images of the batch process (3 x 3 arrays) of comb-like gold structure patterning on a glass substrate by a photolithography process

C. Hydrothermal Method

The patterned glass substrate was then prepared for the hydrothermal growth such that the nanorods by hydrothermal method were grown at the surface metallic comb-like structure. Before the hydrothermal growth of ZnO nanorods patterned glass substrate was cleaned in acetone ultrasonic bath.

The hydrothermal growth setup was designed locally for the growth of ZnO nanorods which maintained the required temperature during the experiment. The ZnO seeds layer was deposited through a seed solution. The seed solution was prepared using 0.15 M of zinc acetate dehydrate dissolved into ethanol solution. The ITO substrate was dipped into seeds solution for 5 min and annealed at 70^{o}C for 5 min. For a proper seed layer deposition, the process was repeated for 3 times.

The Precursor solution for the nanorods growth was prepared by mixing Zn $(NO_3)_2$ and HMT of 1:1in DI water. The solution was stirred for 30 mins at room temperature. The seeded ITO substrate was placed upside down in the precursor solution. The hydrothermal growth was carried at a constant temperature 85^{o}C. To maintain the solution concentration and temperature in a precursor container, a DC motor-based stirrer was used for stirring. The hydrothermal growth for ZnO nanorods was carried for 24h. After 24h of the growth process, ZnO/ITO substrates were cleaned in DI water and annealed at 130^{o}C for 15 min. The morphology of nanorods was examined by SEM analysis. The ZnO nanorods grown by hydrothermal methods are shown in Fig. 3. A dense growth of ZnO nanorods is shown in Fig. 3 (a). The diameters of nanorods are around 1µm and lengths of the nanorods are 12-15 µm as shown in Fig. 3 (b). The density of nanorods mainly depends upon the sol-gel concentration and deposition rate.

The aspect ratios of nanorods are directly proportional to its growth time, whereas the diameters of nanorods are controlled through the concentration of the precursor solution. It was also observed that the nanorods on the ITO substrate are mostly

normal to the substrate, which is promising for the ZnO nanorods based biosensor applications.

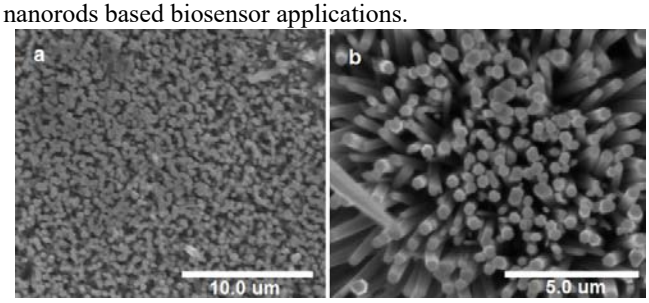


Fig. 3 SEM images of ZnO nanorods by hydrothermal method. (a) Dense growth of ZnO nanorods (b) Magnified image of ZnO nanorods

III. Result and Discussions

The grown ZnO nanorods were deposited with GOx solution for immobilization of glucose. A 5µL GOx solution prepared in a buffer solution of 0.01M phosphate with concentration 25mg/mL. The prepared buffer solution was doped on ZnO nanorods at 40°C for proper deposition [4]. The deposited sample with GOx solution was kept covered overnight for the smooth deposition of GOx thin film.

The ZnO nanorods have a high IEP of 9.5 as compared to that of GOx is about 4.1 at a pH 7.3. Thus, the surface of ZnO is positively charged and a negatively charged GOx on ZnO nanorods is suitable for immobilization of glucose [3]. The cyclic voltammograms of ZnO/GOx were obtained for biosensors as shown in Fig. 4.

It was also noted that the increase in pH value of buffer solution decreases the sensitivity of the biosensors. Furthermore, the increase in pH value also degrades the ZnO nanostructures which results into decrease in stability of the device.

On further analyses we observed that increase in the length of ZnO nanorods also increases the sensitivity and reduces the response time of biosensors. The morphology of ZnO nanostructures influences the sensitivity of biosensors, i.e. increasing the surface to volume ration increases the interaction of GOx molecules with ZnO nanostructures [4], [6], [10]. The reaction results in higher immobilization of GOx on the surface to ZnO nanostructures. Thick electroplating can be received by putting the electroplated material in a bath very precisely with the help of the clip patched at the edge. This patching also plays an important part in proper electroplating. The experimental results of the CV measurements can provide a quantitative result of the biosensor which effectively immobilized the GOx as shown in Fig. 4. The performance of biosensors analyzed are shown in graph with and without in the presence of glucose. Which shows that, the current increases in the presence of glucose exhibits the immobilization of GOx buffer solution. The pH value of the buffer solution were kept constant throughout the analyses of biosensors. It was further noted that the increase in the concentration of glucose in buffer solution decreases the stability of the biosensors, which needs to be addressed in future work. However, the stability issue could be easily resolved through composition of ZnO with other metals such as Al, Cu and Au [15], [22].

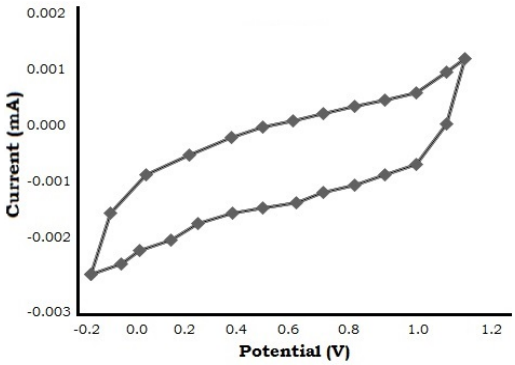


Fig. 4 Cyclic Voltammetry result showing the relationship between current and applied voltage

The performance of biosensors were also analyzed under varying temperature for optimal conditions. The temperature were set in the range of 15°C to 70 °C during performance analysis of biosensors as shown in Fig. 5. The optimal temperature for the saturation current is around 47 °C and then the current deceases and fluctuates up to 70 °C. This decrease in current is supposed to be due to degradation of ZnO nanostructures which reduces immobilization of GOx. It is also reported that decrease in temperature due to increasing temperature is degradation of GOx at a certain pH of buffer solution. The graph also represents the good performance of biosensors at room temperature.

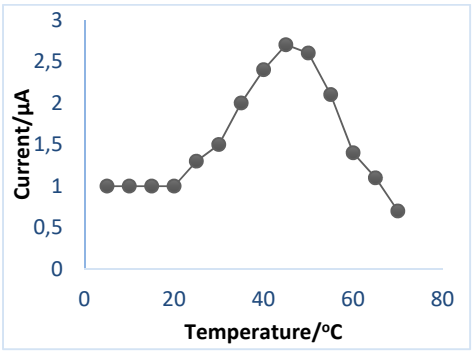


Fig. 5 The graph between Temperature and Amperometric current for the biosensors

IV. Conclusions

In conclusion, fabricated ZnO nanorods based biosensors result in a good sensitivity of 22.7 μAcm^{-1} mM^{-1}. The sensitivity of biosensors was directly proportional to the aspect ratio of nanorods. The response time recorded for the biosensors were less than 7s. The GOx immobilization further increases with increase in length nanorods and pH value of buffer solutions. The morphology of ZnO nanostructures would further optimize the performance of ZnO based biosensors. Due to higher electron mobility of ZnO nanorods and good electrical properties, ZnO nanorods based biosensors

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have a wide range of industrial applications. Fabrication of these type of biosensors is low cost and sensitive compared to fabrication of biosensors by magnetron sputtering or plasma sputtering.

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