

Synthesis High Sensivity ZnO Cholesterol Biosensor with One Dimensional Nanostructures

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Abstract. Cholesterol ZnO biosensors were synthesized by hydrothermal method with pre-deposited ALD-ZnO seed layers. The ZnO nanostructures were examined by SEM, XRD, AFM, respectively. The XRD is used to analyze the crystal structures of ZnO seed layers that were grown by ALD process, then using SEM and AFM to analysis the surface morphologies of them. Growing of one dimensional ZnO nanostructures on seed layer of 10nm thickness, reveals high sensitivity of $3.15 \text{ uA (mg/dl)}^{-1} \text{cm}^{-2}$ ($121.96 \text{ uAmM}^{-1} \text{cm}^{-2}$) and low K_m value of 0.29mM in 25-100mg/dl cholesterol concentration range. The response time could be as low as 10seconds. In the study, it is found that increasing the aspect ratio of ZnO one dimensional nanostructure also increases the sensitivity and expands the linear measureable range of detecting cholesterol in solution. The thickness of seed layer has a significant influence on sensitivity for one dimensional nano ZnO cholesterol biosensor.

1. Introduction

The biologic artifacts are chemoreceptors with good receptiveness to chemical substances. To detect external stimuli and signals, humans rely on not only taste and smell but also receptor proteins, cholesterol, enzymes, antibodies, antigens and many others. Neurohumours of the neurotransmitter systems are also involved in conveying tactile signals [1]. The development of biosensors started in 1962, and it was based on the enzyme electrode concept proposed by Clark and Lyons [2]. This enzyme electrode concept is about fixing glucose oxidase (GOD) on a dissolved oxygen electrode, once the GOD contacts with glucose, the glucose will be oxidized. Because oxygen in the solution is involved in the oxidation, its concentration will decrease, and this concentration change is detected by the dissolved oxygen electrode. In this case, the concentration of glucose in the solution can be estimated by measuring the amount of oxygen consumed. In recent years, nanostructures are often used as carriers; for example, the high surface area of zinc oxide (ZnO) nanostructures has been applied to achieve effective cholesterol oxidase absorption and to detect the concentration of cholesterol in order to enhance the efficiency of cholesterol biosensors and to prolong the lifespan of the sensors [3]. The characteristics of ZnO, such as good absorption and excellent biocatalytic efficiency, are suitable for biosensors. In addition, ZnO also possesses other excellent qualities, such as a higher isoelectric point (9.5), which gives ZnO a better bio-compatibility, better chemical



stability, excellent electronic transmission ability and no toxicity. Therefore, the application and development of ZnO materials as intermedium nanomaterials for sensing cholesterol are promising [4-7]. In this study, the atomic layer deposition (ALD) process and the hydrothermal method were used to synthesize the ZnO seed layers and ZnO nanostructures respectively. The ZnO nanostructures were then assembled as a cholesterol biosensor. It is suggested in the literature [8] that reducing the concentrations of zinc nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) and hexamethylenetetramine ($\text{C}_6\text{H}_{12}\text{N}_4$, HMTA) can increase the aspect ratio of ZnO nanorods during hydrothermal growth. Therefore, increasing the surface area of the nanostructure, would benefit for enhancing biosensor sensitivity.

2. Experimental Procedures

Firstly, the ITO glass was washed by acetone, isopropyl alcohol and deionized water sequentially. The precursor, $(\text{C}_2\text{H}_5)_2\text{Zn}$ (DEZn, Fullestly Corporation) and deionized water were set in a 1:1 pressure vapor ratio (40 mtorr:40 mtorr or 5.33Pa:5.33Pa) alternatively feeding into the vacuum reaction chamber for depositing 100nm, 30nm, and 10nm thickness of ZnO seed layers by atomic layer deposition (ALD). The process pressure was kept about 1 torr(133.32Pa). The process temperature was set at 150°C , and the feeding order of the precursor into the reaction chamber in one cycle was DEZn (1second) followed by Ar (purge, 5second) and then DI water (1second), and Ar (purge, 5second) and repeated again depending on how many cycles were set (see Figure 1).

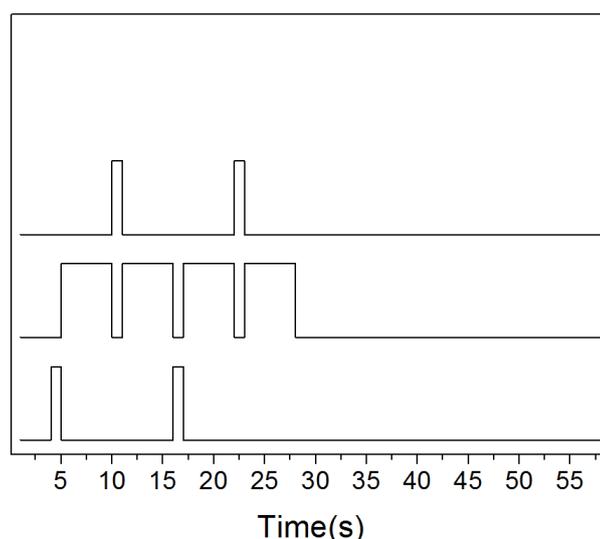


Fig.1 One cycle sequence of ALD-ZnO process.

The crystal structure of obtained ALD-ZnO seed layers had checked by XRD. Subsequently, hexamethylenetetramine ($\text{C}_6\text{H}_{12}\text{N}_4$, HMTA) and zinc nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) were used in a 1:1 molarity ratio (0.025M:0.025M) for growing ZnO nanostructures on the ALD-deposited seed layers hydrothermally. The growing temperature was maintained at 90°C for hydrothermal growth. For ZnO nanostructures, the SEM was used to observe the surface morphologies and the cross-section. As for cholesterol biosensors, the PBS solution with disodium hydrogen phosphate dehydrate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), sodium chloride (NaCl) and DI water was firstly prepared. Next, potassium hexacyanoferrate (III) ($\text{K}_3[\text{Fe}(\text{CN})_6]$) and potassium hexacyanoferrate (II) trihydrate ($\text{K}_4[\text{Fe}(\text{CN})_6]$) were added into PBS solution to be the electron transfer medium. The specimen of the cholesterol biosensor was prepared by pipetting 10uL of cholesterol oxidase (ChOx) onto the ZnO nanostructures for physical adsorption, and then it was placed in an environment of 25°C to dry naturally. The 15cc of well prepared PBS solution was taken, when

detecting the cholesterol in a solution. The tri-electrode electrochemical method was used for the cholesterol biosensor measurement. The schematically experimental process sequences for manufacturing ZnO cholesterol biosensor can be seen in Figure 2. Stage A: clean the substrates by acetone and de-ion water. Stage B: Using the ALD process to grow ZnO seed layers. Stage C: Hydrothermal grows ZnO seed layers structures from the mixture of HMT($C_6H_{12}N_4$) and $Zn(NO_3)_2$ solution at $90^\circ C$. Stage D: adsorption cholesterol molecules onto the surface of ZnO nano-structure s.

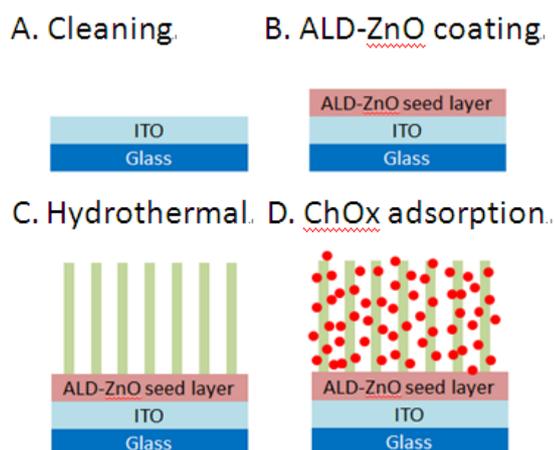
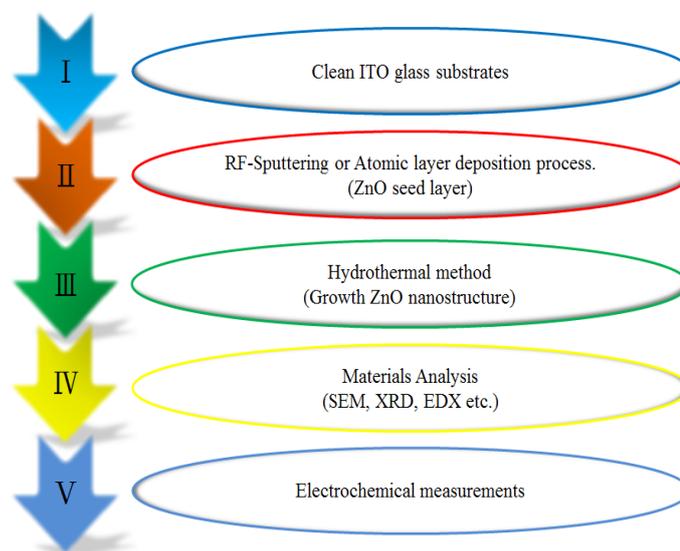


Figure 2 Experimental process sequences for manufacturing ZnO cholesterol biosensor

The overall experimental sequences can be shown by the flow chart shown below:



Flow chart

3. Results and Discussion

In the study, X-ray diffraction (XRD) was used to analyze the ZnO seed layers prepared by ALD and the preferred orientation of hydrothermally grown ZnO nanostructures. In figure 3, the diffraction peaks can be easily found by crystal planes having orientations of (100), (002), and (101) of ZnO seed layers, and the preferred orientation was not profound. After hydrothermally growing ZnO nanostructures, a strong diffraction peak can be found in the orientation of (002). A possible reason is

that when preparing the ZnO nanostructures from solution, the surface energy would reduce due to thermodynamic rearrangement. The thermodynamic rearrangement has three possible stabilization mechanisms: surface reconstruction, surface electron transferring, and surface nonstoichiometry. The surface reconstruction is the one that is most likely to happen for reducing the surface energy [9-14]. The surface reconstruction method may reduce the surface energy of crystals and thereby increase the uniaxial growth capacity by decreasing the surface area of the polar plane.

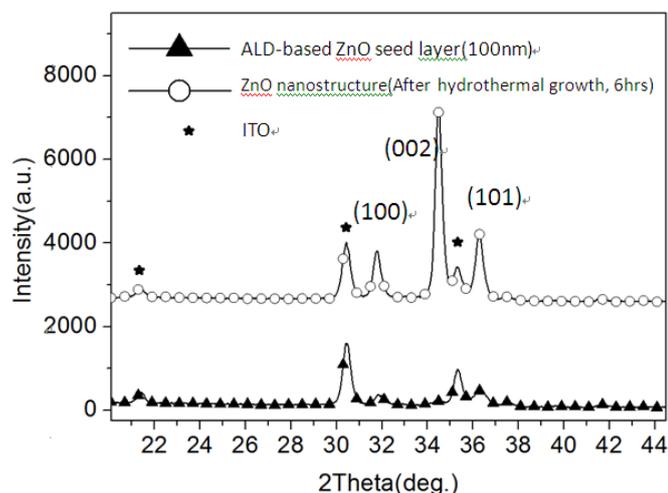


Figure 3 XRD patterns of ALD-ZnO seed layer and nanostructure after hydrothermal growth .

In this study, the SEM was used to observe the hydrothermally grown ZnO nanostructures on seed layers of 100nm, 30nm, and 10nm thickness respectively. In Figure 4, according to the literature[3], increasing the time of hydrothermal growth would increase the aspect ratio of ZnO nanostructures, which in turn would affect the amount of ChOx absorption on the surfaces. Therefore, hydrothermal growth periods of 3hr, 5hr, 9hr and 12hr were performed and the nanostructure formed at 12hr growth period which was chosen to be the best parameter of ChOx absorption. It can be found from the SEM diagram that as the time of hydrothermal growth increased, the length of nanostructures increased as well. Nonetheless, when the thickness of the seed layer was reduced (from 100nm to 30nm), nanostructures also became finer and denser, which might enhance the efficiency of the biosensor due to more surfaces creating. For the 10nm thickness seed layer, the nanostructure displayed a flower-like pattern and about 50 fold larger than previous microstructures. From the top view of SEM image, it can be found the microstructure distributed in a much more dispersed status, the flower-like crystals were about 5 μ m in diameter. Though it is dispersed, the flower-like crystals created more surfaces which may lead to a compromised contribution in the performance of ZnO biosensor.

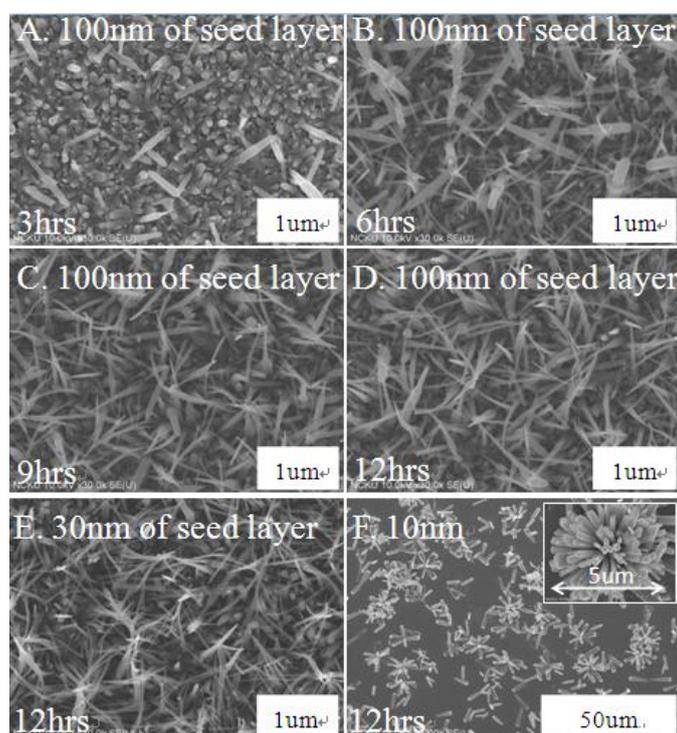
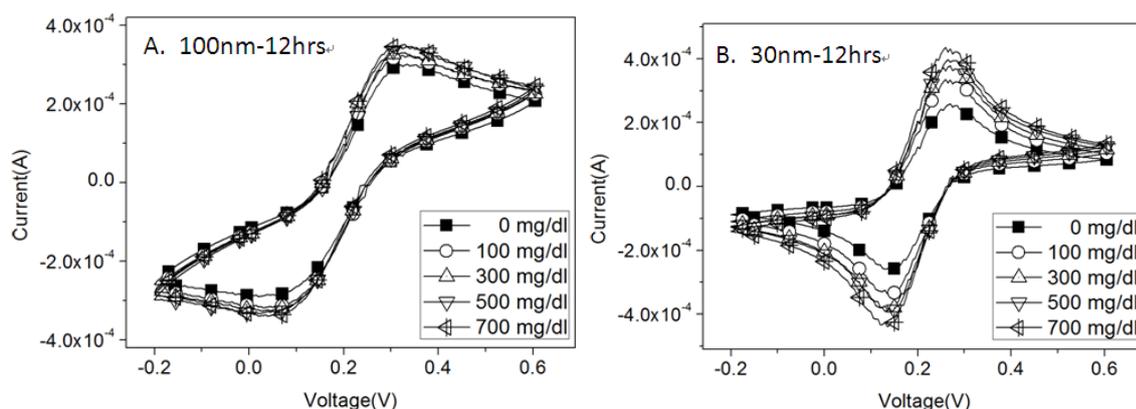


Figure4 HR-SEM images of ZnO nanostructures with different thickness and hydrothermal growth time (A)100nm ZnO seed layer and 3hrs growth time, (B)100nm ZnO seed layer and 6hrs growth time, (C) 100nm ZnO seed layer and 9hrs growth time,(D)100nm ZnOseed layer and 6hrs growth time, (E)30nm ZnO seed layer and 12hrs growth time, (F) 10nm ZnO seed layer and 12hrs growth time.,.

For cholesterol biosensor measurement (see Figure 5), we detected cholesterol concentrations ranged from 0mg/dl to 700mg/dl. Figure 5 shows the cyclic voltammetry measurement results of ZnO nanostructure grown on different thickness of ZnO seed layers.



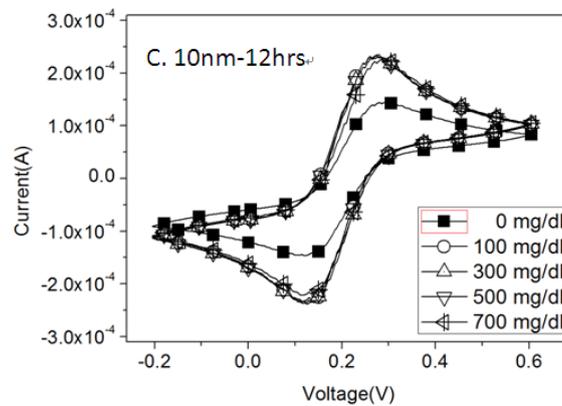


Figure5 Cyclic voltammograms of nano ZnO Cholesterol biosensors at various cholesterol concentrations with different thickness of ZnO seed layer hydrothermally growing ZnO nanostructures at 12hours (A)100nm (B)30nm (C) 10nm

Twelve hours hydrothermal growing time was chosen to grow ZnO nanostructures. According to figure 6 (100nm and 30nm seedyers), when the concentration of cholesterol increases, the anodic peak current will also increase. It shows that the sensor would be more reliable by using the linear interval detectable range due to the consistent responses of cholesterol concentration. As the cholesterol concentration increases to the point where the ZnO nanostructure surfaces are saturated by cholesterol molecules, the detectable range will not follow this linear response anymore. In other words, this interval detectable range data has its limits for the minimum and maximum cholesterol concentration that can be accurately detected.

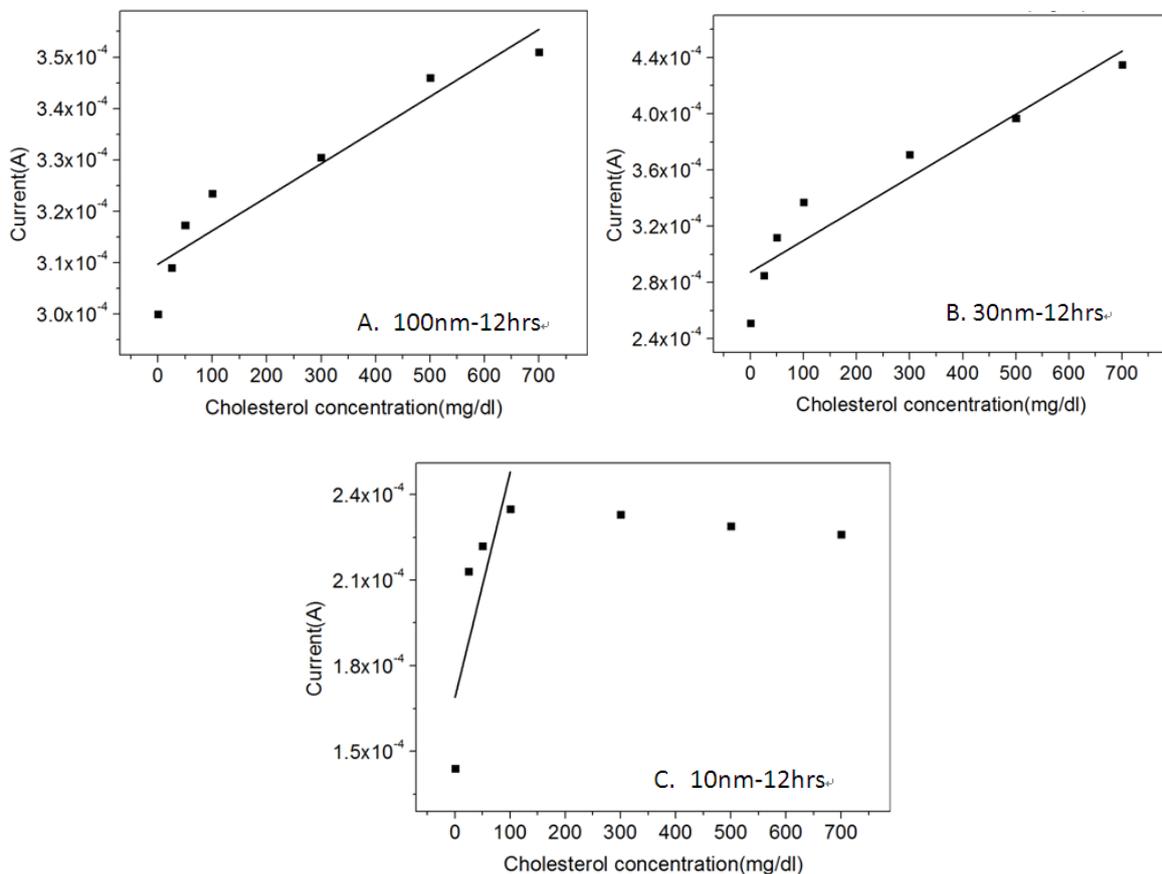


Figure 6 Current v.s Cholesterol concentration of nano ZnO Cholesterol biosensor with different thickness seed layer grown at 12hours

As for the 10nm seed layer, the anodic peak current began to drop when the cholesterol concentration reached 100mg/dl. It is possible that the absorption of ChOx was decreased because the flower-like nanostructure grown from 10nm seed layer distributed more loosely. Consequently, at higher cholesterol concentration condition, excess cholesterol molecules cannot react with ChOx and lead to generate extra electrical resistance, causing the anodic peak current to decrease. The sensitivity can be calculated in terms of the formula: sensitivity (S) = $[\Delta I_{pa}/\Delta C \cdot A]$, where ΔI_{pa} is the current peak variation of oxidization amount, ΔC is the amount of concentration variation, and A is the working area. The cholesterol biosensor using 100nm and 30nm as the base seed layer showed $0.26 \text{ uA}(\text{mg/dl})^{-1}\text{cm}^{-2}$ ($10.06 \text{ uAmM}^{-1}\text{cm}^{-2}$) and $0.90 \text{ uA}(\text{mg/dl})^{-1}\text{cm}^{-2}$ ($34.64 \text{ uAmM}^{-1}\text{cm}^{-2}$), respectively. It can be found that reducing the thickness of the seed layer was accompanied by increased sensitivity because with a thinner seed layer. The denser ZnO nanostructures formed which is beneficial for absorption ChOx and leads to high sensitivity. As growing ZnO nanostructure from 10nm thickness seed layer, the linear range was only between 0 mg/dl and 100mg/dl, hence this range was used to calculate the sensitivity and a value of $3.15 \text{ uA}(\text{mg/dl})^{-1}\text{cm}^{-2}$ ($121.96 \text{ uAmM}^{-1}\text{cm}^{-2}$) obtained. It can be found that the compactness and aspect ratio of ZnO nanostructure do affect the sensitivity of ZnO biosensors and the form of crystal is also another important factor.

The K_m^{app} value was determined by the analysis of the slope and intercept for the plot of the reciprocals of steady-state current vs. cholesterol concentrations, i.e., the Lineweaver–Burk plot of $1/\text{Current}$ vs. $1/\text{Concentration}$ [25]. According to Lineweaver–Burk, the K_m values of ZnO nanostructures grown on 100 nm, 30 nm, and 10nm thickness seed layers were 3.22mM, 2.85mM, and 0.29mM, respectively. The calculated results were demonstrated in Figure 7.

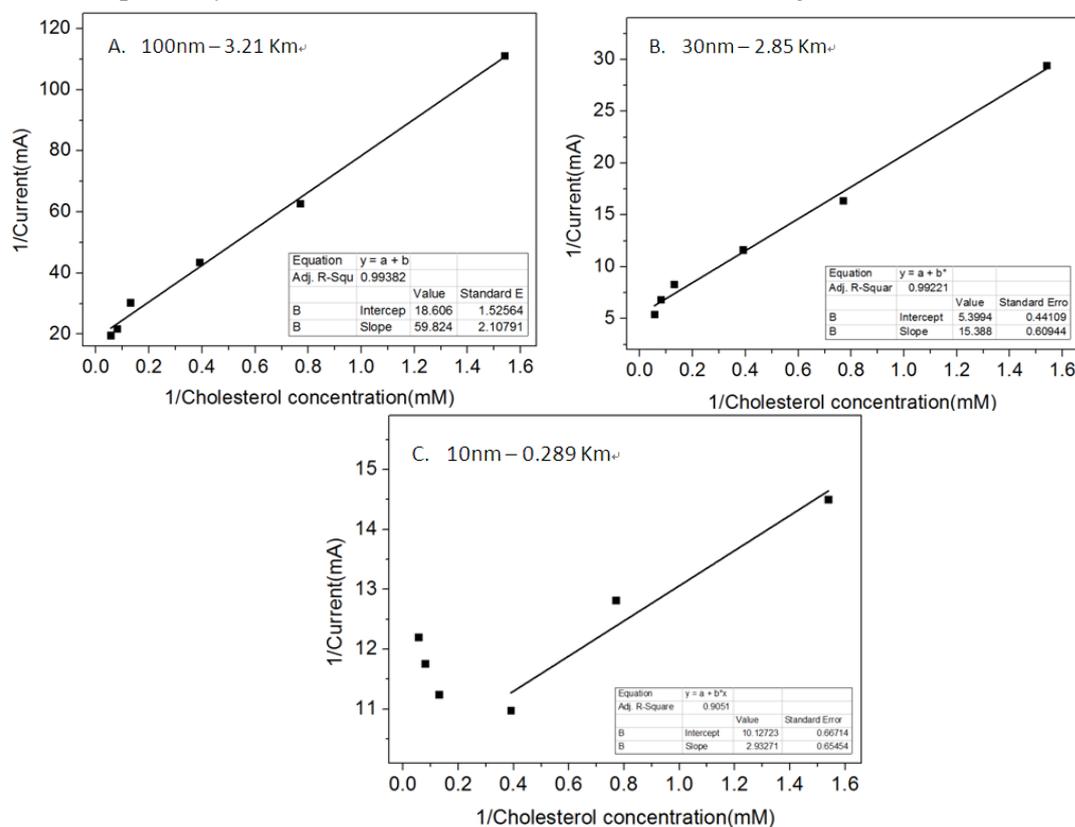


Figure7 Plot of $1/\text{Current}$ vs $1/\text{Cholesterol concentration}$ of nano ZnO Cholesterol biosensor hydrothermally growing for 12hours on different thicknesses of seed layers (A)100nm(B)30nm(C)10nm

The response time of ZnO cholesterol nano-biosensor grown on different seed layer thickness was measured by chronoamperometry. The response time is taken the initial flattening point from the curve of current vs. measuring time plot. As shown in figure 8, for seed layers of 100nm, 30nm and 10nm thickness, their response times were 25seconds, 10seconds, and 10seconds, respectively. It can be found that after reducing the thickness of the seed layer, the response speed increased. Nonetheless, for ZnO grown on 10nm thickness seed layer, no obvious improvement of response time was observed which may be due to looser crystal distribution. The response time of ZnO cholesterol biosensors were dependent on seed layer thickness. For thicker seed layers, electrons have to overcome larger resistance in the transportation according to ohm law. Therefore, the thickness of ZnO seed layers can significantly alter the nano ZnO cholesterol biosensor characteristics, not only the material properties but also the performances of the nano-biosensor, such as the sensitivity, response speed etc.

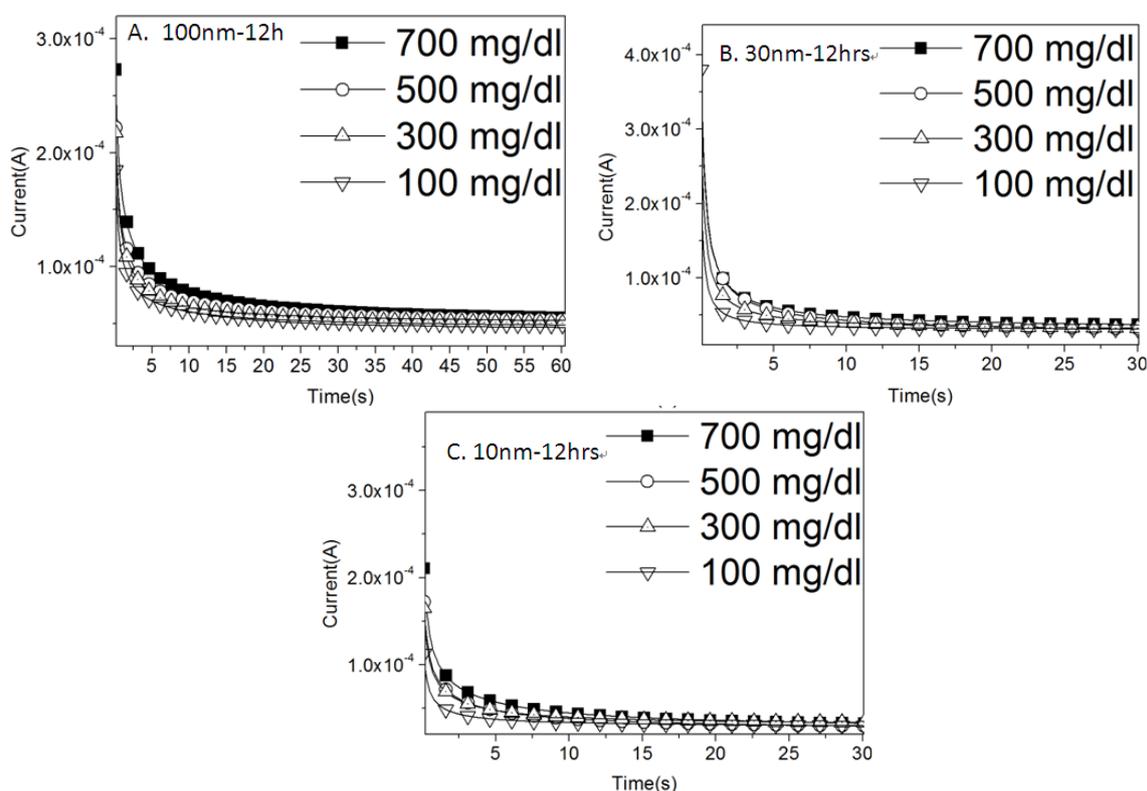


Figure8 Response times of nano ZnO cholesterol biosensor hydrothermally grown on different thicknesses of seed layers for 12 hours (A)25seconds(B)10seconds(C)10seconds

4. Conclusion

In this study, the atomic layer deposition (ADL) was applied to deposit ZnO seed layers of different thicknesses and they were subsequently used to grow ZnO nanostructures for manufacturing cholesterol biosensors with high sensitivity. It is found that using 100nm-thick ZnO seed layer to grow ZnO one dimensional nanostructures which had a sensitivity of $0.26 \text{ uA}(\text{mg/dl})^{-1}\text{cm}^{-2}$ ($10.06 \text{ uAmM}^{-1}\text{cm}^{-2}$) and a response time of 25seconds. While using 30nm and 10nm thickness ZnO seed layer, the sensitivity was $0.90 \text{ uA}(\text{mg/dl})^{-1}\text{cm}^{-2}$ ($34.64 \text{ uAmM}^{-1}\text{cm}^{-2}$) and the response time is 10seconds. The

thickness of seed layers affected not only on the form of crystal structures but also on the performance of cholesterol biosensors.

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