Development of ZNR-calcium biosensor application

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Abstract

For sensitive Ca²⁺ biosensor fabrication, zinc oxide nanorods (ZNR) were coated with calcium ionophore membranes to detect Ca²⁺ ions concentration. For preparing ZNR samples two different layers were deposited by consecutively using two different methods: the former method was the sol-gel method and the later one was the aqueous chemical growth (ACG) method. Biosensors with four different substrate kinds, conducting plastic substrate (P), silicon substrate (S), silver wire substrate (W) and borosilicate glass capillary tube tip substrate (T) were used as working electrodes for calcium concentration determination Ca²⁺ biosensor were initially checked potentiometrically in two solutions: the CaCl₂ solution ranging between 100 mM and 100 nM and cells serum in human blood. Four prepared working electrodes morphologies and structures were characterized by field emission high resolution scanning electron microscopy.

Keywords: zinc oxide, nanorods, sol-gel, calcium biosensors

Classification numbers: 2.04, 4.06, 5.08, 6.09

1. Introduction

In medical physics, metal ions play main roles, then it is very interesting to detect their concentration for cell biology. The most promising ion is the Ca²⁺ ion, which is used in the present work. Each ion has its fundamental biology and its own chemistry with different chemical and physical characteristics [1, 2]. Each of them has its specific role in living systems by acting as cofactors in enzymes, current carriers, lipids and proteins stabilizers and integrator, and consequently as osmotic regulators [3]. In addition, nanostructured zinc oxide nanorods (ZNR)-ion selective biosensors were obtained by effective and simple techniques for measuring the ion concentrations in extra/intracellular media.

Calcium ions play main roles in muscle contraction, neuronal activity, vesicle exocytosis and regulating enzyme activity, cell development and death [4, 5]. Using these applications the Ca²⁺ ions is the one of the most important elements for sensing. Moreover, Ca²⁺ ion can be used industrially for measurements in fertilizers and soils boiler water.

Intra/extra-cellular Ca²⁺ determination was large interest and ZnO nanorods technology has potential for such measurements [6].

For Ca²⁺ biosensor fabrication, ZNR coated with calcium ionophore polymeric membranes were highly selective and sensitive to detect the concentration of Ca²⁺ ions [7]. The selective intra-extra-cellular calcium measurement methods used two electrodes: (i) a ZNR working electrode is coated with calcium ionophore polymeric membrane and (ii) reference electrode (Ag/AgCl electrode). For measuring the electrochemical surface potential difference generated near the electrodes, its recorded response should be measured. The ZNR are grown on the same conducting surface having the same potential [8–10].

The aim of the current study is focusing on the ZnO nanorods-based biosensor demonstration suitable for extra and intracellular selective Ca²⁺ detection in CaCl₂ solution...
and in human blood serum. Our main effort has been directed towards the construction of biosensors with conducting plastic substrate (ZNRP), silicon substrate (ZNRS), silver wire (ZNRW) and borosilicate glass capillary tube tip (ZNRT) for calcium ions concentrations measurement in test solutions.

2. Materials and methods

2.1. Preparation of ZnO nanorods thin film

Semiconductor ZNR were obtained by depositing two different layers by means of two methods: the sol-gel and the aqueous chemical growth (ACG) methods.

2.1.1. Pretreatment of the substrate. Substrates in plastic (P), silicon (S), silver wire (W) and borosilicate glass capillary tube tip (T) forms were used for the mentioned prepared ZNR thin film. Cleaning of the substrate (conducting plastic, silicon and silver wire) prior to synthesis of ZnO nanostructure was performed to reach the needed morphology. The substrates were ultrasonically cleaned in ethanol solution and de-ionized water in sequence from 10 up to 15 min. The cleaning substrate step removed impurities such as unwanted chemicals from its surface to be ready for thin film deposition.

2.1.2. Seed solution prepared by sol-gel method. To modify the surface of the substrate, thin film from the seed solution was deposited on it. It provides nucleation sights for the nanorods growth and enhance the density as well as particles homogeneity. For preparation the seed solution, different precursors and solvents were used. It was prepared by using zinc acetate dehydrate $\text{Zn(CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ (Oxford) dissolving in mono-ethanolamin (MEA) $\text{NH}_2\text{CH}_2\text{CH}_2\text{OH}$ (Oxford) and 2-methoxyxyethanol (2ME) (Merch) at temperature (about 25 °C), The molar ratio of zinc acetate to MEA was 1:1 and the concentration of zinc acetate was 0.5 M. The prepared seed solution was stirred at 50°C for 2 h until yielding a homogeneous and clear solution. The mixed solution was aged at room temperature for 24 h. Then, spin coater using sol-gel method on different kinds of substrate such as conducting plastic and silicon with a speed of 3000 rpm for 30 s coated the solution. While the deposition on the surface of a silver wire with 0.25 mm in diameter and borosilicate glass capillary glass tube tip with 0.5 and 0.7 μm as inner and outer diameters has carried out by simply dipping it into the same solution to obtain nanocrystalline ZnO (seed layers) on the mentioned surfaces. Before that, borosilicate glass capillary tube was placed on a flat support inside a vacuum chamber of evaporation system. We had evaporated 30 nm thickness of titanium followed by 120 nm of silver. The coating process in seed solution was repeated three time and dried at room temperature (about 25°C) and finally placed in pre-heated laboratory oven at 150°C for conducting plastic substrate and at 250°C for silicon substrate, silver wire and borosilicate glass capillary tube tip for annealing to decompose the zinc acetate dehydrate into ZnO nanoparticles. Moreover, the seed layers provide a good control on the density and alignment of the nucleation points that affect the synthesized nanorods diameter [11, 12].

2.1.3. Growth of ZNR by aqueous chemical growth method. After coating zinc oxide seed layers on the substrates, the nanorods of zinc oxides were grown at low temperature (90°C–95°C) using aqueous chemical growth (ACG) method. The ZnO nanorods growth was achieved by immersing the substrates with ZnO seed-layer in 150 ml of aqueous solution composed of zinc nitrate $\text{Zn(NO}_3)_2$ (Sigma-Aldrich) and hexamethylenetetramine HMT, $\text{C}_6\text{H}_{12}\text{N}_4$ (Sigma-Aldrich) with concentration 0.025 M. The reaction temperature was kept at 90 °C–95°C for 4–6 h, 4 h in silver wire and borosilicate glass capillary tube tip and 6 h in flat substrates. The substrates position inside the solution does affect on the growth process, generally substrates are being placed in the solution with face toward the bottom of the beaker. Then, the substrates were removed from the solution, immediately rinsed with deionized water to remove any residual salt from the surface, and dried at room temperature (about 25 °C) in air. The ZNR structure and surface morphology were characterized by x-ray diffraction (XRD) and field emission scanning electron microscope (FESEM) respectively [11].

2.2. ZNR-based calcium (Ca²⁺) biosensors

Zinc oxide nanorods on all substrates ZNRP, ZNR S, ZNR W and ZNR T were coated with calcium ionophore polymeric membrane by procedure as follow, 120 mg of powdered polyvinyl chloride (PVC) was dissolved in 5 ml tetrahydrofuran together with 10 mg of dibutylphtalate (DBP) plasticizer and 10 mg of Ca²⁺ specific ionophore (DB18C6). All chemicals were from Sigma-Aldrich-Fluka. After preparing the solution, the all mentioned ZnO-coated substrate were dipped twice into the solution until membrane thin film were attached to their surfaces then they will dried at room temperature.

All calcium biosensors working electrodes (ZNRP, ZNR S, ZNR W and ZNR T) were stored at 4 °C in dry condition when not in use.

After finishing these steps, the biosensors were initially checked potentiometrically in two solutions, the former solution is the $\text{CaCl}_2$ with different concentration of calcium ranging from 100 mM to 100 nM and the latter is human blood serum.

2.3. Electrochemical measurements with calcium biosensors-based on ZNR

The electrochemical potential cell (electromotive force) changed when the test solution composition was altered. These changes related to the calcium concentration ions in the test solution via a calibration procedure. The actual electrochemical potential cell can be described by the diagrams for each kind of substrates as follows

- For conducting plastic substrate: $\text{ZNRP} \parallel \text{CaCl}_2 \parallel \text{Cl}^- \parallel \text{AgCl} \parallel \text{Ag}$,
- For silicon substrate: $\text{ZNRS} \parallel \text{CaCl}_2 \parallel \text{Cl}^- \parallel \text{AgCl} \parallel \text{Ag}$,
- For silver wire: $\text{ZNRW} \parallel \text{CaCl}_2 \parallel \text{Cl}^- \parallel \text{AgCl} \parallel \text{Ag}$,
- For tube tip: $\text{ZNRT} \parallel \text{CaCl}_2 \parallel \text{Cl}^- \parallel \text{AgCl} \parallel \text{Ag}$.
3. Results and discussion

3.1. FESEM images for ZNR

The ZNR-P, ZNR-S, ZNR-W and ZNR-T surface morphologies were investigated by using field emission scanning electron microscopy (FESEM) as shown in figures 1–4, respectively. The obtained ZNR-P FESEM photos were employed to explore the nanorods size and alignment at 12 000 × and 24 000 × as showing in figures 1(a) and (b), respectively, while figure 2 shows the ZNR-S FESEM image at constant magnification equal to 60 000 ×. It is clearly seen from the mentioned figures that the nanorods were distributed uniformly in diameters range between 26 and 38 nm.

Figures 3 and 4 show the ZNR-W and ZNR-T FESEM images of panoramic view for ZnO nanorods and by focusing in small area for both of them. It is displaying from the figures that the nanorods were found to be perpendicular to the substrate and well alignment, however its diameter is in the range between 22 and 24.2 nm for ZNR-T.

We can conclude that the figures of the ZNR-P, ZNR-S, ZNR-W and ZNR-T are in rodshapes, hexagonal cross section and have homogeneous distribution perpendicular to the substrate with small diameter nano-rods [1, 11–13].

3.2. Extracellular Ca^{2+} selective biosensor based on ZNR

The four fabricated calcium biosensor kinds ZNR-P, ZNR-S, ZNR-W and ZNR-T were used for Ca^{2+} concentrations measurement in extracellular solutions. They were used to study calcium ion selectivity by using biosensors based on ZNR coating with ionophore membrane. The working electrode (Ca^{2+}) potentiometric response was evaluated by immersing it in CaCl₂ aqueous solutions having concentration range between 100 nM and 0.1 M.

Figures 5 shows the calibration curves for the potential difference between ZNR-P, ZNR-S, ZNR-W and ZNR-T as calcium biosensor working electrodes with reference electrode (Ag/AgCl) versus log Ca^{2+} concentrations in CaCl₂ solution with concentration ranging from 100 nM to 0.1 M [14]. From the curves we detected the calcium concentration in human blood serum (dashed lines). All these curves show the linear dependence. Moreover, the electrodes are found to be very sensitive to calcium ions giving a slope calculated from linear equations curve and biosensors efficiency $R^2$ (regression coefficient), as depicted in table 1.

After successfully using the ZNR-P, ZNR-S, ZNR-W and ZNR-T as Ca^{2+} biosensors for calcium concentration measurements in CaCl₂ solution, we can use them for the same measurement in the human blood serum.

For ZNR-P, ZNR-S, ZNR-W and ZNR-T calcium biosensors, the measured potential responses were found to be equal to 63.9, 95.7, 121 and 116.8 mV in y axis corresponding to −2.63 in x axis as depicted in table 2, which all are equivalent to 2.3 mmol × l⁻¹ calcium concentration as detected from figures 5(a)–(d), respectively.

From the obtained results we noted that the experimental calcium concentration in human blood serum value
Figure 3. ZNRW FESEM images (a) panoramic view and (b) highly magnified by focusing at small area.

Figure 4. ZNR{T} FESEM images: (a) panoramic view, and (b) highly magnified by focusing on small area at 60 000x.

Figure 5. The electrochemical potential difference calibration curves of (a) ZNR{P}, (b) ZNR{S}, (c) ZNR{W}, (d) ZNR{T} as calcium biosensor versus logarithm concentration of calcium (logC_Ca) range in CaCl_2 solution. Ca^{2+} concentration in human blood serum was marked at the x and y axis dashed line.
measured by the mentioned four fabricated Ca$^{2+}$ biosensors are located at a normal range of calcium human concentration, where the normal value is ranging from 2.2 to 2.6 mmol $\times l^{-1}$.

### 4. Conclusion

The work demonstrated a ZnO nanorods study aselectrochemical biosensor for Ca$^{2+}$ in tested solutions. We have achieved good performance in selectivity and stability by coating the working electrodes surface (ZN$_R$P, ZN$_R$S, ZN$_R$W and ZN$_R$T) by ionophore polymeric membrane. The potentials difference gives linear in the following logarithmic concentration range (100 nmol to 0.1 mol). The obtained results confirmed the accuracy of the biological capability relevant measurements in CaCl$_2$ solution and in human blood serum using functionalized ZnO nanorods.

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