Functionalised zinc oxide nanotube arrays as electrochemical sensors for the selective determination of glucose

S.M.U. Ali[1,2], *M. Kashif*[3], *Z.H. Ibupoto*[1], *M. Fakhar-e-Alam*[1], *U. Hashim*[3], *M. Willander*[1]

[1]*Physical Electronics and Nanotechnology Division, Department of Science and Technology, Campus Norrköping, Linköping University, SE-60174 Norrköping, Sweden*
[2]*Department of Electronic Engineering, NED University of Engineering and Technology, Karachi-75270, Pakistan*
[3]*Nano Biochip Research Group, Institute of Nano Electronic Engineering (INEE), University Malaysia Perlis (UniMAP), 01000 Kangar, Perlis, Malaysia*
E-mail: syeal@itn.liu.se

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In the present study, highly oriented single-crystal zinc oxide nanotube (ZnO-NT) arrays were prepared by a trimming of ZnO nanorods along the *c*-axis on the gold-coated glass substrate having a diameter of 100–200 nm and a length of ~1 μm using a low-temperature aqueous chemical growth process. The prepared (ZnO-NT) arrays were further used as electrochemical enzyme-based glucose sensors through immobilisation of glucose oxidase by the physical adsorption method in conjunction with a Nafion coating. The electrochemical response of the sensor was found to be linear over a relatively wide logarithmic concentration range from 0.5×10^{-6} to 12×10^{-3} M. The proposed sensor showed a high sensitivity of 69.12 mV/decade with $R = 0.9934$ for sensing of glucose. A fast-response time less than 4 s with good selectivity, reproducibility and negligible response to common interferents such as ascorbic acid and uric acid prevailed.

1. Introduction: Glucose biosensors are by far the most widely studied type of biosensors and numerous designs have been proposed. However, converting the biological signal to an easily processed electronic signal is challenging owing to the complexity of connecting an electronic device directly to a biological environment. Electrochemical biosensors provide an attractive means to analyse the content of a biological sample owing to the direct conversion of a biological event to an electric signal. Over the past decades, several sensing concepts and related devices have been developed. The inherent advantages of electrochemical biosensors are their robustness, easy miniaturisation, excellent detection limits, also with small analyte volumes, and the ability to be used in turbid biofluids with optically absorbing and fluorescing compounds [1, 2]. Among these biosensors, most glucose biosensors follow amperometric principles because of good sensitivity and a low detection limit. However, on applying a high polarising voltage (Vapp. = 0.6–0.8 V), interfering substances such as ascorbic acid and uric acid, which are commonly present in biological fluids, are also oxidised, leading to non-specific signals [3]. Several artificial redox mediators have been investigated as electron acceptors to solve these problems [4–13]. Additionally, the sensor electrodes have been modified to enhance the performance of amperometric glucose biosensors [14–18]. The interferences mentioned above are avoided in thermometric biosensors such as enzyme thermistor [19]. In addition, their excellent stability makes them particularly suitable for long-term monitoring. Compared to amperometric biosensors, since no extra potential is required, potentiometric biosensors have an advantage in selectivity and simplicity. However, a limitation of ion-sensitive electrodes is that only charged molecules can be directly detected. This obstacle can be overcome by letting the analyte undergo a reaction, such as an enzyme reaction, which produces a detectable ion in an amount proportional to the concentration of the analyte in the sample. In the enzyme field effect transistor, this is taken a step further by combining the enzyme reaction with an ion-sensitive field effect transistor and was first introduced by Caras and Janata [20]. Many potentiometric devices are based on various forms of field effect transistor (FET) devices to measure pH changes, selective ion concentrations and the kinetics of biocatalytic reactions involving enzymes [21]. The conversion of an FET into a sensing device normally involves the replacement of the metal gate electrode by a biochemically sensitive surface like an analyte-selective membrane, an enzyme layer or an ion-conductive solution etc., which is brought into contact with the analyte solution [22]. Also present in the analyte solution is a reference electrode that completes the circuit via the gate voltage bias [23, 24].

The use of nanomaterials has allowed the introduction of many new signal transduction technologies in biosensors resulting in improved sensitivity and performance. Owing to their submicrometre dimensions, nanosensors, nanoprobes and other nanosystems have allowed simple and rapid analyses *in vivo*. Their implementation as highly sensitive electrodes is one obvious example, such as the platinum electrode network proposed by Wang *et al.* for glucose detection [25]. Among the nanostructures, zinc oxide (ZnO) is of special interest to biological sensing owing to many favourable properties. ZnO is a distinguished material with some special properties owing to the wide direct bandgap (3.37 eV) and large exciton binding energy (60 meV). Recently, extensive research efforts have been focused on the synthesis, characterisation and device application of ZnO nano- and micromaterials. 1D ZnO nanostructures can have significant applications in optics, optoelectronics, sensors and actuators because of their remarkable semiconducting and piezoelectric properties [26–30]. The ZnO nanomaterials can be used in a variety of electrochemical bio-sensing schemes due to their unique advantages in combination with immobilised enzymes. Owing to such unique properties, these ZnO nanosensors offer some significant advantages owing to their small size and high surface area to volume ratios allowing larger signals, better catalysis and the more rapid movement of analyte through sensors show higher sensitivity and a lower limit of detection (LOD) as compared to those prepared from bulk ZnO devices. As compared to ZnO nanorods and nanowires, ZnO nanotube (ZnO-NT) structures possess lots of interesting unique properties such as porous structures and large surface areas and there have been reports on the use of ZnO tubular structures as sensors with improved performance and higher sensitivity compared to ZnO nanorods and nanowires [31–34]. In general, nanostructures such as ZnO nanowires, nanotubes and non-porous are attractive for their versatile roles in bioelectronics and nanoelectronics applications. They are increasingly being used as building blocks for biosensing purposes owing to the remarkable properties like non-toxicity, bio-safety, excellent biological compatibility, high-electron

transfer rates, enhanced analytical performance, increased sensitivity, easy fabrication and low cost. Moreover, ZnO has a high isoelectric point (IEP) of about 9.5, which should provide a positively charged substrate for immobilisation of low IEP proteins or enzyme such as glucose oxidase (GOD) (IEP $\simeq$ 4.5) as described in our earlier investigations [35–38]. In addition, ZnO has high ionic bonding (60%), and it dissolves very slowly at biological pH values.

In this study, we have successfully demonstrated the determination of glucose with improved electrochemical response by using the ZnO-NT array-based biosensor fabricated by a two-step aqueous chemical growth (ACG) method. This method is advantageous by being a low-cost, simple with high yield, low-temperature deposition process and also proves to be less hazardous compared to other methods. The enhanced electrochemical response of the ZnO-NT array-based sensor as compared to our earlier investigation with ZnO nanowires [28] can be attributed to the unique properties of our sensor electrode like the vast surface-to-volume ratio due to the porous structure of ZnO-NT arrays which can provide a favourable microenvironment for the immobilisation of enzyme GOD, the enzyme catalysis of the glucose oxidation on electrode and excellent electrical contact between the gold electrode and the ZnO-NTs. In addition, owing to the large surface-to-volume ratio of the porous structures of the ZnO-NTs, the sensor electrode enhances the sensitivity for analytes as demonstrated by the detection of glucose without the presence of a mediator.

2. Experimental details

2.1. Materials: GOD (E.C. 1.1.3.4) from *Aspergillus niger* 360 U/mg (BBI Enzymes (UK) Ltd.). Bovine serum albumin ($\geq$98%), glutaraldehyde (50% solution), Nafion (5 wt.%), *d*-(+)-glucose (99.5%), potassium chloride, zinc nitrate hexahydrate and hexamethylenetetramine were purchased from Sigma–Aldrich. Phosphate-buffered, 10 mM solution (PBS) was prepared from Na_2HPO_4 and KH_2PO_4 (Sigma–Aldrich) with sodium chloride in 0.135 mM, the pH was adjusted to 7.4. Glucose stock solution was kept at least 24 h after preparation for mutarotation. All chemicals used (Sigma–Aldrich) were of analytical reagent grade.

2.2. Fabrication of (ZnO-NT) arrays on gold-coated glass: To prepare the ZnO-NT array-based sensor electrodes, the glass was used as a substrate and cleaned with acetone, isopropanol and deionised water. After cleaning, a titanium (Ti) thin film with 20 nm thicknesses was evaporated as an adhesive layer, then a gold (Au) thin film with 100 nm thickness was evaporated on a glass substrate as a gold electrode. To obtain the well-aligned hexagonal ZnO-NT arrays on the prepared electrode surface, we followed the low-temperature ACG method described in [39–41]. In the ACG method, prepared substrates were spin coated with seed solution and annealed at 200°C for 20 min and zinc nitride hexahydrate [(Zn (NO^3)$26H_2O$)] was mixed with hexamethylenetetramine [C6H12N4] using the same molar concentration of 0.05 M for both solutions. Then, the substrates were placed in the prepared solutions using Teflon sample holders and kept in an oven preheated up to at 90°C for 4–6 h. During the growth process of ZnO nanorods, a small part of the gold-coated glass substrates was covered and used as a contact area. After the growth was completed, the grown nanorod arrays were cleaned in deionised water and dried at room temperature. In the second step, the fabrication of ZnO-NT arrays was carried out as described in [42] by suspending the prepared sample with the ZnO nanorods upside down in 100 ml aqueous solution of potassium chloride (KCl). The experiment was repeated several times while varying growth parameters like temperature (the temperature of the solution was kept at 85°C), concentration of precursor (KCl) from 0.2 to 4.5 M, as well as etching time from 3 to 15 h. For obtaining the desired etching and fine structure control of the ZnO-NT arrays, all these variables must be optimised. After carefully performing the chemical etching of ZnO nanorods along the *c*-axis, we finally obtained the ZnO-NT arrays 100–200 nm in diameter and ~1.3 μm in length. The scanning electron microscopy (SEM) image and the schematic diagram of the obtained ZnO-NT arrays are shown in Figs. 1*a* and *b*.

2.3. Enzyme (GOD) immobilisation on ZnO-NT arrays: To investigate the electrochemical response of the ZnO-NT arrays, we prepared six ($n = 6$) sensor electrodes for the experiments. GOD solution was prepared by using GOD (E.C. 1.1.3.4) from *Aspergillus niger* by dissolving 10 mg/ml GOD in PBS at pH 7.4. Before the immobilisation of enzymes (GOD) on the surface of the sensor electrode, the sensor electrode was rinsed with PBS to generate a hydrophilic surface. To immobilise the enzymes (GOD) on the tailored ZnO-NT arrays on gold-coated electrode, 5 μl of prepared GOD solution was deposited and left in air for 2 h to dry. The cross-linking procedure was carried out by adding 2 μl aqueous solution containing 2.5% glutaraldehyde and 0.5% Nafion onto the electrode surface. After drying at room temperature, 2 μl of 0.5% Nafion solution was further applied onto the electrode surface to prevent possible enzyme leakage and eliminate foreign interferences. The immobilised ZnO-NT arrays are shown in Fig. 1*c*. All enzyme electrodes were stored in dry condition at 4°C when not in use. After completing these steps, the sensors were initially checked potentiometrically in 100 μl of 0.5 μM glucose solutions with an Ag/AgCl reference electrode purchased from Metrohm (3MKCl). A pH meter (Model 215, Denver Instrument) was used to measure the potentiometric output voltage of the sensors presented here. For the time response measurements, model 363A potentiostat/galvanostat (EG & G, USA) was used.

3. Results and discussion

3.1. Electrochemical measurements with ZnO-NT sensors: The electrochemical cell voltage (electromotive force) changed when the composition of the test electrolyte was altered. These changes can be related to the concentration of ions in the test electrolyte

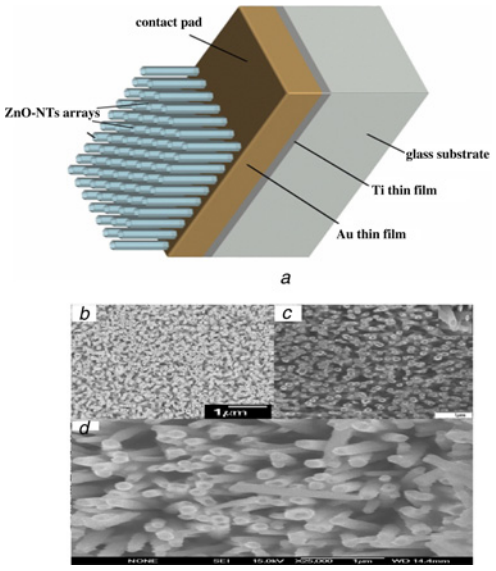

Figure 1 *SEM images and the schematic diagram of the obtained ZnO-NT arrays*
a Schematic diagram of ZnO-NT array sensors
b Typical SEM image of ZnO-NT arrays grown on gold-coated glass using low-temperature chemical growth. The Figure shows that the diameter of the ZnO-NT arrays is in the range of 100–200 nm
c Immobilised ZnO-NT arrays with inset SEM image showing the magnifying image of ZnO-NT arrays
d SEM image of ZnO-NTs after measurement

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via a calibration procedure. The electrochemical potential cell can be described by following representation:

$$ZnOs|Zn^{+2}\ PBS_{sol}||Ag/AgCl\ PBS_{sol}|Ag$$

The electrochemical response of the ZnO-NT arrays sensor against an Ag/AgCl reference electrode was measured at room temperature (23 ± 2)°C. The sensor as fabricated is sensitive to the concentration changes of glucose in PBS. The measurements started after conditioning the sensor electrode in PBS buffer at pH 7.4 for 30 min and when the electrodes were inside the PBS, a constant potential was observed. When freshly prepared 0.5 μM glucose solution was tested, a change in the signal was observed. The response of the electrochemical potential difference of the ZnO-NT-based sensor to the changes in buffer electrolyte glucose was measured for the range of 0.5 μM to 12 mM. This showed that this glucose dependence is linear and has a sensitivity equal to 69.12 mV/decade at around 23°C (Fig. 2*a*). This linear dependence implies that such sensor configuration can provide a large dynamic range. A very fast response time was noted over the whole concentration range with 95% of the steady-state voltage achieved within 4 s as shown in Fig. 2*b*. The tested sensor configuration showed large dynamic ranges with an output response (EMF) that was linear against the logarithmic concentration of glucose ranging from 15 mV for 0.5 μM to 315 mV for 12 mM. This corresponds to slopes of around 69.12 mV/decade with a regression coefficient $R = 99.34$ as shown in Fig. 2*a*. An electrochemical response from ZnO-NT array-based sensor in the 100 μM glucose solution was observed around 160 mV. The response stayed around 160 mV, regardless of the analyte solution volume. During all experiments the ZnO-NT array-based sensor followed Nernst's expression:

$$E = E_0 - 0.05916\,V/n \log [\text{Reduced}]/[\text{Oxidised}]$$

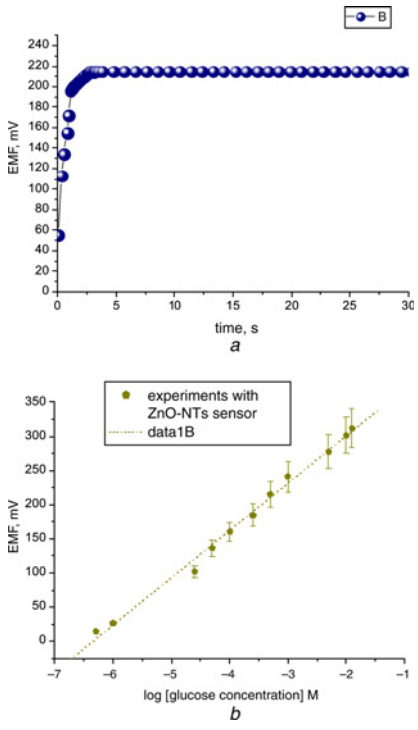

Figure 2 *Response of the electrochemical potential difference of the ZnO-NT-based sensor*
a Time response of the sensor electrodes in 500 μM glucose solution
b Calibration curve of the ZnO-NT arrays sensor electrode reveals the linear relationship between the of output response (EMF) and glucose concentrations with a Ag/AgCl reference electrode

It is very important to note that ZnO-NT arrays are relatively stable around a neutral pH 7.4 and this gives these sensors much more biocompatibility in biological fluids and species since most of the biological fluids is around pH 7.4. The sensing mechanism of most electrochemical glucose sensors is based on an enzymatic reaction catalysed by GOD as schematically illustrated in the experimental setup shown in Fig. 3. Fig. 3 describes the sensing mechanism of the glucose using immobilised GOD enzymes on ZnO-NT array sensor electrodes. As a result of this reaction, δ-gluconolactone and hydrogen peroxide are produced. These two products and the oxygen consumption can be used for the glucose determination. With the availability of H_2O in the reaction, gluconolactone is spontaneously converted to gluconic acid, which, at neutral pH, form the charged products of gluconate$^-$ and proton (H^+), according to the (1) and (2) given below

$$H_2O + O_2 + \beta - D - \text{glucose} \xrightarrow{GOD} \delta - \text{gluconolactone} + H_2O_2 \quad (1)$$

$$\delta - \text{gluconolactone} \xrightarrow{\text{spontaneous}} \text{gluconate}^- + H^+ \quad (2)$$

3.2. Reproducibility and influence of temperature: The reproducibility and long-term stability were evaluated by using six different ZnO-NT array sensor electrodes constructed independently; the sensor-to-sensor reproducibility in 1 mM glucose solution was tested during periodic measurements after being kept in a refrigerator at 4°C for three weeks. The sensors retained around 90% of its original response with good reproducibility and repeatability in pH 7.4 PBS solution as shown in Fig. 4*a*. The influence of the varying temperature on the ZnO-NT array sensor response was also examined between 20 and 75°C. As shown in Fig. 4*b*, the EMF response gradually increases with the increase in temperature and reaches its maximum value at around 50°C. This is because each enzyme has maximum activity at optimum temperature condition. After 50°C, the response decreases which is caused by the natural thermal degradation of the enzymes. Although the ZnO-NT array sensor shows a maximum response at 50°C, room temperature (23 ± 2)°C is still chosen for this work to prevent possible solution evaporation at higher temperature and to ensure ease of operation.

3.3. Study of interferences and stability: The selectivity of a glucose sensor depends on two major factors that are the enzyme–analyte reaction and selective measurements. The enzyme–analyte reaction is very specific because of the nature of the enzyme (GOD) functionality. The GOD reaction with β-D-glucose is highly specific without any major interfering reaction with other types of sugars. It could, however, be useful to check possible interferences from reducing agents such as ascorbic acid and uric acid, which are

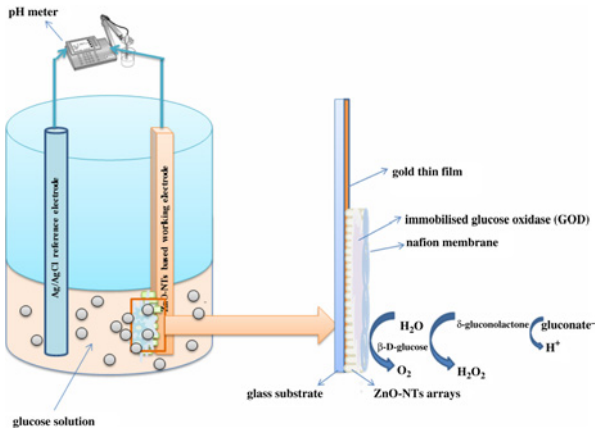

Figure 3 *Schematic diagram showing the measuring setup and sensing mechanism of the glucose*

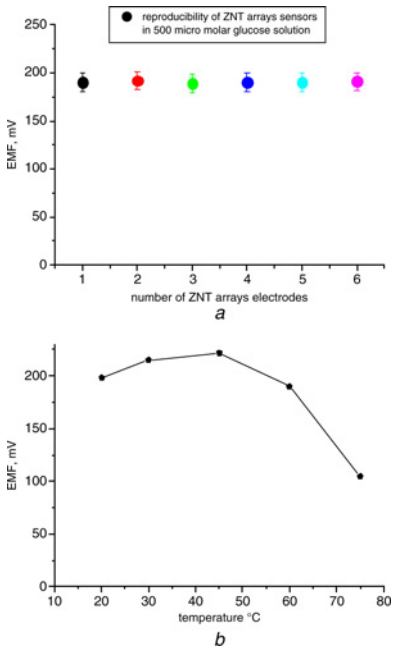

Figure 4 *Reproducibility and long-term stability evaluated using six different ZnO-NT arrays*
a Sensor-to-sensor reproducibility of six ($n = 6$) ZnO-NT array sensor electrodes in 500 μM glucose solution
b EMF response with the influence of varying temperature

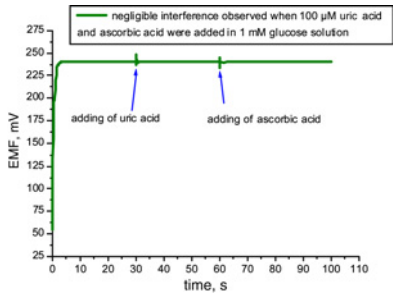

Figure 5 *Calibration curve showing the study of interferences with time trace line of output response (EMF) change with time after adding 100 μM ascorbic acid (AA) and uric acid (UA) in 1 mM glucose solution*

well-known interferents with amperometric glucose measurement methods. As clearly seen from the output response of the sensor, the addition of these potential interferents does not substantially change the signal. Addition of 100 μM of ascorbic acid or uric acid to 1 mM glucose only generated some extra noise as shown in Fig. 5. We suggest that the good selectivity of the present biosensor can be attributed to the permselective (charge-exclusion) property [43, 44] of Nafion films coated on the electrode. The proposed ZnO-NT-based sensor demonstrated an excellent response to the glucose. Therefore based on our obtained results during the experiments, we proposed that instead of fabricating the ZnO nanorods/nanowires/nanotubes on the gate area inside the transistor (e.g. on the MOSFET/AlGaN/GaN HEMT devices), ZnO nanorods/nanowires/nanotubes can be interfaced/integrated as an extended gate [36, 45]. In this way, the chemically sensitive gate is then separated from the rest of the transistor construction, and the sensing area increases significantly as compared to the gate areas of some published sensors based on transistors, for example, HEMT [46]. Thereby, the biosensor construction is much facilitated as the enzyme can be readily immobilised on the nanomaterials, and applied in a variety of different sensors or flow system designs without problems arising from, for example, encapsulation of the electronics etc.

4. Conclusion: In conclusion, we have successfully demonstrated a glucose biosensor using immobilised ZnO-NT arrays. Our experimental results showed that the proposed sensor electrode has sensitivity around twice as high as that of determined by zinc oxide nanowires reported elsewhere in the literature. This can be ascribed to the fact that small-dimensional ZnO-NT arrays have a higher surface area, subsurface oxygen vacancies and provide a larger effective surface area with higher surface-to-volume ratio as compared to zinc oxide nanowire arrays and thus enables the sensor with a higher sensitivity. The good performance in terms of improved sensitivity, stability, selectivity, reproducibility, negligible interference and rapid response (EMF) by our proposed sensor also makes it suitable for externally integrating/interfacing a nanosensing element to commercial (low-threshold) FET devices, adding to the advantages of simplicity and low cost for the enzymatic detection of biochemically important substances. All these advantageous features can make the proposed biosensor applicable in wireless monitoring of physiological parameters, environmental, food or other areas.

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