Vertically aligned TiO$_2$ nanotube arrays decorated with CuO mesoclusters for the nonenzymatic sensing of glucose
3. Vertically aligned TiO$_2$ nanotube arrays decorated with CuO mesoclusters for the non-enzymatic sensing of glucose

In the realm of nanomaterials based direct electro-oxidation of glucose, Ni and Cu based materials have shown better catalytic effect due to the redox couples mediated by Ni(OH)$_2$/NiOOH [258] and Cu(OH)$_2$/CuOOH [259]. CuO, being a p-type semiconductor, shows excellent electrochemical activity which led to its immense use in the development of gas [260] and glucose sensors [68,71,74,79,81,82]. The mechanism of oxidation of glucose on Cu and CuO modified electrodes does not simply produce gluconic acid instead it involves the complete cleavage of C-C bonds. This results in greater sensitivity of the non-enzymatic sensors based on CuO [86]. Sensitivity of an electrochemical sensor is directly related to the surface area and hence a great amount of effort has been directed to increase the surface area of the sensor electrode. Metal nanoparticles electrodeposited TiO$_2$ nanotube arrays have been widely used for sensing of biomolecules. Considering the excellent electrocatalytic activity of CuO towards glucose oxidation and the large surface area of the vertically aligned TiO$_2$ nanotube arrays, this study aims at integrating these two materials to obtain a glucose sensor exhibiting superior characteristics. TiO$_2$ nanotube arrays were prepared by electrochemical anodization in a fluoride containing electrolyte. Cu mesoclusters were electrodeposited on the TiO$_2$ nanotube arrays and oxidized to CuO mesoclusters. The sensor electrode was characterized morphologically and electrochemically and tested with glucose and other commonly interfering molecules. Results obtained with the sensor electrode for blood samples are comparable with that of the commercially available sensor strips.

3.1 Experimental

3.1.1 Synthesis of TiO$_2$ nanotube arrays

Prior to anodization, the titanium foil was cut into small strips and was washed sequentially with Millipore water, ethyl alcohol, and acetone and then dried in nitrogen atmosphere. Teflon tape was used to mask Ti strip selectively to expose an area of 0.09 cm$^2$. Titanium was potentiostatically anodized in an electrolyte of glycerol and water (90:10 v/v) containing 0.15 mol L$^{-1}$ ammonium fluoride at 20 V for 5 h [126]. The anodized specimen was washed in Millipore water, dried and annealed at 500 °C for 3 h.
3.1.2 Fabrication of sensor electrodes

The electrodeposition of Cu on TiO$_2$ electrode was carried out at a constant potential of - 0.8 V from a solution containing 50 mmol L$^{-1}$ copper sulphate and 0.1 mol L$^{-1}$ lithium perchlorate. This experiment was carried out for different time durations (10, 20, 30, 40, 50 and 60 s) to optimize the amount of copper on the electrode for best response. The deposited Cu was electrochemically oxidized to CuO by applying a constant potential of 0.8 V in a stirred solution of 0.1 mol L$^{-1}$ NaOH for 60 s. It is denoted as CuO/TiO$_2$. In the similar fashion, CuO was obtained on bare titanium electrode (CuO/Ti) and glassy carbon electrode (CuO/GC) and used for comparision.

3.1.3 Electrochemical experiments

All electrochemical reactions were performed in 0.1 mol L$^{-1}$ NaOH. Electro-oxidation of glucose was studied using linear sweep voltammetry (LSV) at a potential window of 0 to 0.9 V with a scan rate of 100 mV s$^{-1}$. Amperometric detection of glucose was carried out at a constant potential by injecting different concentrations of glucose into a constantly stirred solution of 0.1 mol L$^{-1}$ NaOH. The effect of interfering species was studied by injecting ascorbic acid (AA), uric acid (UA), dopamine (DA) and sugars (galactose and lactose) for their possible interference under similar experimental conditions.

3.1.4 Real sample analysis

Blood sample was collected from three volunteers and centrifuged with a force of 15294 g for 30 minutes at room temperature. The serum obtained was collected and used for further analysis. Amperometry was carried out by adding 40 μL of 5 mmol L$^{-1}$ glucose and 40 μL of the serum into a constantly stirred solution of 4 mL 0.1 mol L$^{-1}$ NaOH. The additions were carried out at a time interval of 50 seconds. Each volunteer sample was tested for four times on the CuO/TiO$_2$ electrode.

3.2 Result and Discussion

3.2.1 Surface characterization

The SEM image of the anodized titanium foil (Figure 3.1A) shows the presence of highly ordered, vertically aligned TiO$_2$ nanotube arrays. The diameter of the tube was found to be about 60 nm. A cross sectional view of the TiO$_2$ nanotube modified electrode (Figure 3.1B) revealed the thickness of the anodized layer to be 1.58 μM. Figure 3.1C
depicts FESEM image of TiO$_2$ nanotube arrays with copper deposited for 50 seconds followed by electrochemical oxidation. From this image, it is clear that the copper nanoparticles are uniformly deposited on the TiO$_2$ nanotube. From Figure 3.1D, the size of the mesoclusters was estimated to be about 500±3 nm. The quantity of copper deposited on TiO$_2$ of 0.09 cm$^2$ at -0.8 V was calculated to be 210.9 μg cm$^{-2}$.

![Figure 3.1: SEM images of (A) annealed TiO$_2$, (B) cross sectional view of the annealed TiO$_2$, (C) CuO/TiO$_2$ obtained after 50 seconds of copper deposition and (D) magnified image of CuO/TiO$_2$ obtained after 50 seconds of copper deposition.](image)

The mechanism of formation of TiO$_2$ nanotubes in a fluoride medium is well established by many groups [261]. Briefly, the formation is due to (i) field assisted oxidation of Ti metal to form titanium dioxide, (ii) field assisted dissolution of Ti metal ions in the electrolyte, and (iii) chemical dissolution of Ti and TiO$_2$ due to etching by fluoride ions, all of which occur simultaneously. The formation of titanium nanotube arrays can be represented by the following equations.

\[
\begin{align*}
2\text{H}_2\text{O} & \rightarrow \text{O}_2 + 4\text{e}^- + 4\text{H}^+ \quad \text{.........(1)} \\
\text{Ti} + \text{O}_2 & \rightarrow \text{TiO}_2 \quad \text{..........................(2)} \\
\text{TiO}_2 + 6\text{F}^- + 4\text{H}^+ & \rightarrow \text{TiF}_6^{2-} + 2\text{H}_2\text{O} \quad \text{...(3)}
\end{align*}
\]

Here equations 1 and 2 lead to the formation of oxide layer and 3 leads to the dissolution of the oxide layer.
Elementary analysis (Figure 3.2) using energy dispersive X-ray spectroscopy (EDS) revealed the presence of oxygen, titanium and copper at atomic weight percentage of 48.1%, 16.4% and 35.3% respectively (Table 3.1).

### Table 3.1: Elementary composition of CuO/TiO$_2$ electrode surface

<table>
<thead>
<tr>
<th>Elements</th>
<th>Weight %</th>
<th>Atomic %</th>
</tr>
</thead>
<tbody>
<tr>
<td>O K</td>
<td>20.2</td>
<td>48.1</td>
</tr>
<tr>
<td>Ti K</td>
<td>20.7</td>
<td>16.4</td>
</tr>
<tr>
<td>Cu K</td>
<td>59.0</td>
<td>35.3</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

#### 3.2.2 Electrocatalytic oxidation of glucose on CuO/TiO$_2$

The electrocatalytic oxidation of glucose was carried out on CuO/GC, CuO/Ti and CuO/TiO$_2$ electrodes and results were compared. From Figure 3.3 A, it is evident that the CuO/TiO$_2$ exhibited maximum current for 6.25 mmol L$^{-1}$ of glucose. The increased current response can only be attributed to the presence of TiO$_2$ nanotube arrays. The effect of the amount of copper on the current response was studied by varying the time of deposition as 10, 20, 30, 40, 50 and 60 s resulting in 173.6, 181.2, 194.7, 202.1, 210.9 and 217.7 μg cm$^{-2}$ of copper respectively. The electrocatalytic activity of all the modified electrodes was studied in 0.1 mol L$^{-1}$ NaOH containing 6.25 mmol L$^{-1}$ of glucose and
found that the electrode obtained after 50 s of copper deposition is superior (Figure 3.3 B). This may be attributed to the size of CuO mesoclusters being optimal for the electro-oxidation of glucose. Hence all further experiments were carried out with the CuO/TiO₂ electrode obtained after 50 s of copper deposition.

Figure 3.3: (A) Comparison of LSVs obtained in 0.1 mol L⁻¹ NaOH towards 6.25 mmol L⁻¹ of glucose on CuO/GC (a), CuO/Ti (b), CuO/TiO₂ (c) and (B) effect of varying amount of copper deposited on the response current in 0.1 mol L⁻¹ NaOH containing 6.25 mmol L⁻¹ of glucose.

Figure 3.4: LSV on CuO/TiO₂ electrode in 0.1 mol L⁻¹ NaOH with increasing concentrations of glucose from 1.25 mmol L⁻¹ to 12.5 mmol L⁻¹ at a scan rate of 100 mV/s (inset: plot of peak current vs concentration with equation \( I_p(mA) = 0.15328C(mmol L^{-1}) + 0.11404 \), \( \sigma = 0.03814 \), \( n = 10 \) and \( r = 0.9980 \)).
Figure 3.4 represents LSVs recorded on the modified electrode in 0.1 mol L\(^{-1}\) NaOH with increasing glucose concentration. In the absence of glucose (curve a) no characteristic peak was obtained, but a small shoulder can be seen around +0.45 V which may be due to the redox wave of Cu(II)/Cu(III) as reported [86]. But, in the presence of glucose, a distinct peak at +0.50 V was obtained (curve b) and this can be attributed to the oxidation of glucose. During each addition of 1.25 mmol L\(^{-1}\) of glucose (b-m), the peak current increases with a regression equation of \(I_p\) (mA) = 0.15328 C + 0.11404, where C is in mmol L\(^{-1}\), \(\sigma = 0.03814\), n=10 and \(r = 0.9980\). The standard error between each addition was found to be less than 3%.

The mechanism of glucose oxidation on copper electrode in an alkaline medium has been studied by different groups. According to Kano et al.,[262] CuO is responsible for the direct electro-oxidation of glucose through six catalytic cycles each involving two-electron transfers leading to the formation of formic acid. The formation of Cu(II)/Cu(III) surface states and the oxidation peak of glucose occur at around +0.40 to +0.80 V. Hence it is believed that the Cu(III) species is acting as a mediator for the electrocatalytic oxidation of glucose. This is entirely different from that of chemical and biochemical glucose oxidation that involves gluconic acid intermediate formation.

The oxidation of glucose on the CuO modified electrode is a diffusion controlled process that is sensitive to the glucose concentration. A peak current is obtained at a potential when the concentration of glucose molecules is equal to the quantity of Cu(III) sites. But as the concentration of glucose increases, more number of Cu(III) sites are required for oxidation which are made available at a higher potential. Hence a small positive shift in anodic peak current is observed with increase in glucose concentration[125].

LSV obtained on the modified electrode in 0.1 mol L\(^{-1}\) NaOH containing 5 mmol L\(^{-1}\) glucose with different scan rates (a to o) revealed that the faradaic current increased linearly with square root of scan rate (Figure 3.5) with a regression equation \(I_p\) (mA) = 0.1025\(v^{1/2}\)(mV/s) + (-0.02836), \(\sigma = 0.01003\), n = 16 and \(r = 0.9937\). This confirms that the mechanism of glucose oxidation on the CuO/TiO\(_2\) electrode is a diffusion controlled mechanism as reported in earlier studies [85,263].
Figure 3.5: LSV on CuO/TiO$_2$ electrode in 0.1 mol L$^{-1}$ NaOH containing 5 mmol L$^{-1}$ glucose at different scan rate from 10 mV/s to 150 mV/s (a to o) (inset: peak current vs. scan rate with $I_p$(mA) = $0.1025\sqrt{\nu}$(-0.02836), $\sigma = 0.01003, n = 16$ and $r = 0.9937$).

3.2.3 Amperometric detection of glucose

The steady state response current on the modified electrode at various potentials was studied (results not shown). It was found that the response current for unit concentration of glucose is maximum at +0.75 V compared to the other applied potentials. The electrode showed a rapid response to glucose with a detection time of less than 2 s. Hence all amperometric studies were carried out at +0.75 V. Figure 3.6 shows two linear ranges of current response with concentration. For glucose concentration up to 6.25 mmol L$^{-1}$, the regression equation is, $I_p$(mA) = 0.16538C(mmol L$^{-1}$) + 0.01253 with $r = 0.9984$ and $\sigma = 0.01834$ for $n = 10$. The sensitivity was calculated from the slope of the calibration curve as 1836 µA mmol$^{-1}$ L cm$^{-2}$. The other linear range is from 6.87 mmol L$^{-1}$ to 12.5 mmol L$^{-1}$ with a regression equation, $I_p$(mA) = 0.12755C(mmol L$^{-1}$) + 0.29517, $r = 0.9980$ and $\sigma = 0.1616$ for $n = 10$, and the sensitivity was calculated to be 1416 µA mmol$^{-1}$ L cm$^{-2}$. The lower limit of detection was found to be 3.4 µmol L$^{-1}$ (S/N=3).
Figure 3.6: Amperometric response obtained on CuO/TiO$_2$ electrode in 0.1 mol L$^{-1}$ NaOH with additions of 0.625 mmol L$^{-1}$ of glucose at +0.75 V (Inset: plot of current vs glucose concentration for 0.625 mmol L$^{-1}$ to 6.25 mmol L$^{-1}$ (a) and 6.87 mmol L$^{-1}$ to 12.5 mmol L$^{-1}$).

Figure 3.7: Amperometric response at +0.75 V on the CuO/TiO$_2$ with glucose concentrations from 6.25 µmol L$^{-1}$ to 6.25 mmol L$^{-1}$ in 0.1 mol L$^{-1}$ NaOH. (Inset: Magnified image of glucose response for 6.25 µmol L$^{-1}$ and 0.0625 mmol L$^{-1}$ additions (a), plot of current vs concentration (b)).
Figure 3.7 represents the amperometric response towards 6.25 µmol L\(^{-1}\), 0.0625 mmol L\(^{-1}\), 0.625 mmol L\(^{-1}\) and 6.25 mmol L\(^{-1}\) of glucose. Over this wide range (6.25 µmol L\(^{-1}\) to 9.71 mmol L\(^{-1}\)) of concentrations, the sensor electrode showed a linear response with a regression equation \(I_p(\text{mA}) = 0.1618C(\text{mmol L}^{-1}) + 0.00127\), \(\sigma = 0.08871\), \(n = 16\) and \(r = 0.9979\). The sensor is able to exhibit a linear response from a very low concentration to very high concentration with a regression value close to 1 which is an important characteristic.

**Table 3.2:** Comparison of the CuO/TiO\(_2\) sensor electrode with other recently reported CuO based non-enzymatic glucose sensors.

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Potential (V)</th>
<th>NaOH (Con. mol L(^{-1}))</th>
<th>Sensitivity (µA mmol L(^{-1}) cm(^{-2}))</th>
<th>Linear range of detection (mmol L(^{-1}))</th>
<th>Lower limit of detection (µmol L(^{-1}))</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>TiO(_2)/CuO</td>
<td>0.5</td>
<td>0.1</td>
<td>79.79</td>
<td>upto 2</td>
<td>0.1</td>
<td>[264]</td>
</tr>
<tr>
<td>Graphene-CuO nanocomposite</td>
<td>0.4</td>
<td>0.02</td>
<td>1480</td>
<td>0.002 to 0.6</td>
<td>0.029</td>
<td>[265]</td>
</tr>
<tr>
<td>Cu-CuO composite</td>
<td>0.75</td>
<td>0.025</td>
<td>598</td>
<td>Upto 3</td>
<td>3</td>
<td>[266]</td>
</tr>
<tr>
<td>CuO/MWC NT</td>
<td>0.4</td>
<td>0.1</td>
<td>2596</td>
<td>upto 1.2</td>
<td>0.2</td>
<td>[123]</td>
</tr>
<tr>
<td>CuO nanorod/Graphite</td>
<td>0.6</td>
<td>0.1</td>
<td>371.43</td>
<td>0.004 to 8</td>
<td>0.4</td>
<td>[74]</td>
</tr>
<tr>
<td>CuO nanoflower/Graphite</td>
<td>0.6</td>
<td>0.1</td>
<td>709.52</td>
<td>non linear</td>
<td>0.4</td>
<td>[74]</td>
</tr>
<tr>
<td>CuO mesocluster/TiO(_2)</td>
<td>0.75</td>
<td>0.1</td>
<td>1836</td>
<td>0.625 to 6.25</td>
<td>3.4</td>
<td>This Work</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1416</td>
<td>6.87 to 12.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The Table 3.2 represents the comparison between CuO/TiO₂ sensor developed in this study and other CuO based glucose sensors reported recently [74, 123, 264-266]. As evident from Table, the combination of CuO mesoclusters and TiO₂ nanotube is superior in exhibiting a good balance between sensitivity and linear range. For example, the sensitivity of the CuO nanorod/MWCNT is 2596 µA mmol⁻¹ cm⁻² with linearity only up to 1.2 mmol L⁻¹. Similarly the CuO nanorod/graphene exhibited linearity in the range of 0.004 to 8 mmol L⁻¹ but its sensitivity was low (371.43 µA mmol L⁻¹ cm⁻²). The CuO/TiO₂ electrode also exhibits superiority in many aspects such as sensitivity and linearity in the physiological range of glucose in comparison to many sensor electrodes based on copper and other metal-metal heterogeneous systems which were compared by Guangfeng Wang et al.[267].

3.2.4 Reproducibility and storage stability

The CuO/TiO₂ electrode was tested for its repeatability and reproducibility by making 10 CuO/TiO₂ electrodes under similar conditions and testing them amperometrically at +0.75 V with 5 mmol L⁻¹ glucose in 0.1 mol L⁻¹ NaOH. A variation of less than ±3% was observed. This indicates that the electrode modification is highly reliable. The sensor electrodes were stored in distilled water at room temperature (25 ± 2°C) when not in use. The amperometric response of the sensor to 5 mmol L⁻¹ glucose was tested once in every three days for a period of one month. The decrease in current response was found to be less than 4%. These studies clearly indicate that the sensor has very good reproducibility and storage stability.

3.2.5 Effect of interfering species

Biomolecules like ascorbic acid, uric acid and dopamine are present in blood at very low concentrations and are found to interfere with the detection of glucose as these are oxidized at a relatively lower potential than glucose. Sugars such as galactose and lactose, though present in low concentrations, are known to interfere with detection of glucose. Hence, to ascertain the selectivity of the developed sensor, it is important to test the interference of these biomolecules along with glucose at physiological concentrations. For this purpose response current was measured at +0.75 V with successive additions of 0.1 mmol L⁻¹ dopamine, 0.1 mmol L⁻¹ uric acid, 0.1 mmol L⁻¹ ascorbic acid, 0.2 mmol L⁻¹
galactose and 0.029 mmol L\(^{-1}\) lactose along with 5.0 mmol L\(^{-1}\) of glucose. The results obtained are represented in Figure 3.8. For ease of comparison, the response of CuO/TiO\(_2\) sensor towards 5 mmol L\(^{-1}\) glucose was considered as 100% and the response of interfering biomolecules was calculated accordingly. Interference from uric acid and lactose was found to be very negligible whereas dopamine and ascorbic acid exhibited interference of 2.06% and 2.13% while galactose, showed an interference of 3.55%. From this it is clear that the CuO modified TiO\(_2\) nanotube array electrode is highly selective and sensitive towards glucose under physiological concentrations of interfering molecules.

![Figure 3.8](image)

**Figure 3.8:** Sensitivity profile of the CuO/TiO\(_2\) electrode to glucose and interfering species.

### 3.2.6 Real sample analysis

**Table 3.3** Determination of glucose concentrations in human serum samples

<table>
<thead>
<tr>
<th>Samples*</th>
<th>OneTouch SelectSimple Glucometer (mmol L(^{-1}))</th>
<th>CuO/TiO(_2) (mmol L(^{-1}))</th>
<th>RSD (%)</th>
<th>Added glucose (mmol L(^{-1}))</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.74</td>
<td>4.87</td>
<td>4.2</td>
<td>0.5</td>
<td>99</td>
</tr>
<tr>
<td>2</td>
<td>5.51</td>
<td>5.39</td>
<td>6.8</td>
<td>0.5</td>
<td>101</td>
</tr>
<tr>
<td>3</td>
<td>5.03</td>
<td>5.21</td>
<td>5.7</td>
<td>0.5</td>
<td>97</td>
</tr>
</tbody>
</table>

*Each sample was tested four times
To validate the reliability of the developed CuO/TiO$_2$ sensor toward physiological samples, amperometric measurements were carried out at +0.75V for three serum samples (each sample was tested four times). The results obtained were compared with those obtained from commercially available glucose strips (OneTouch SelectSimple) and was found to be highly comparable (Table 3.3). Moreover from Table 2 it is observed that the RSD values fall well within the 3 to 10% exhibited by commercially available disposable glucose sensors with a high recovery rate [268].

3.3 Conclusion

The development of a highly sensitive and selective non-enzymatic glucose sensor based on CuO mesocluster modified TiO$_2$ nanotube array was successfully carried out. The electrode showed an excellent sensitivity of 1836 and 1416 µA mmol$^{-1}$ L cm$^{-2}$ for glucose concentrations ranging from 6.25 µmol L$^{-1}$ to 6.25 mmol L$^{-1}$ and 6.87 to 12.5 mmol L$^{-1}$ respectively. The sensor exhibited good storage stability and reproducibility. Physiological concentrations of commonly interfering molecules had negligible effect on glucose detection. Testing of glucose concentration in blood serum was successfully carried out and was found to be in good agreement with the commercially available glucose sensor strips.