Enzymatic Glucose Biosensor Based on TbYxOy Electrolyte-Insulator-Semiconductor

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We report herein a glucose biosensor based on glucose oxidase (GOD) immobilized on TbYxOy sensing film grown by reactive cosputtering. The structural properties of TbYxOy sensing films annealed at different temperatures (600, 700, 800, and 900 °C) were performed using X-ray photoelectron spectroscopy and atomic force microscopy to identify the optimal annealing condition. The TbYxOy electrolyte-insulator-semiconductor (EIS) sensor annealed at 900 °C exhibited a higher sensitivity of 59.79 mV/pM, a lower hysteresis voltage of 1 mV, and a smaller drift rate of 0.26 mV/h than those prepared at the other annealing temperatures. We attribute this behavior to the formation of a honeycomb-like structure and a decrease in the amount of lattice defects improving the stoichiometry of TbYxOy film. Furthermore, we compared two surface modification techniques with 3-aminopropyltetraethoxysilane (APTES) and APTES+glutaraldehyde (GA) to functionalize the TbYxOy film surface and thus the enzymatic GOD was covalently immobilized on the modified surface of a TbYxOy EIS sensor. The TbYxOy glucose biosensor treated with APTES+GA has a high sensitivity (19.85 mV/M) in solutions containing glucose at concentrations in the range 2–8 mM. This TbYxOy EIS biosensor is adequate for general clinical examination of blood glucose.

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Diabetes mellitus (DM) has reached epidemic proportions, and it is estimated that over 350 million people worldwide are afflicted by this disease.1 DM is a leading cause of death (about 3.5 million people every year) and disability, the monitoring of blood glucose to keep it in the normal range of 80–120 mg/dl (4.4–6.7 mM) is critical. The diagnosis and management of DM need a tight monitoring of blood glucose levels. Electrochemical biosensors play an important role for glucose detection. For several decades, much attention has been focused on the silicon-based biosensors in the field of biological or biomedical applications due to their favorable characteristics, including sensitivity, speed, miniaturization, and low cost.2 An ion-sensitive field-effect transistor (ISFET) is one of the most popular electrical biosensors and has been introduced as the first miniaturized silicon-based chemical sensor.3 The ISFET device is essentially similar to the metal-oxide-semiconductor field-effect transistor (MOSFET) where the metal gate electrode has been replaced by an electrolyte solution and a grounded reference electrode. The response of this device to pH is explained by considering H+ specific binding sites at the surface of the gate insulator exposed to the electrolyte.4 The electrolyte-insulator-semiconductor (EIS) device has the advantages of simple structure and facile fabrication in comparison with an ISFET sensor. The gate insulator is one of the most important component parts in ISFET or EIS devices, because this insulator is placed directly in an aqueous electrolyte solution. There are many materials applied on the ion sensing membrane, such as SiO2,5 Si3N4,6 Al2O3,7 ZrO2.8 But SiO2, ZrO2 and Al2O3 are considered to be poor sensing materials because of their low sensitivity and narrow measurement pH range.9 In addition, they have very weak points at nonlinear phenomena such as the hysteresis effect and the drift phenomenon.6,9 Various high-dielectric constant (high-k) metal oxide materials, including Ta2O5,9 HfO2,10 TiO2,11 and Y2O3,12 have been recently investigated as pH-sensitive gate membranes in ISFET or EIS devices due to their high pH response.

Rare-earth (RE) oxide films are an interesting group of materials with high application potential. They are thermodynamically very stable refractory materials.13 RE oxides (e.g. Er2O3, Gd2O3) have also other favorable properties, such as high refractive index13 and small lattice mismatch.14 Consequently, RE oxide thin films are of great interest in developing a new material replacement for SiO2 gate dielectrics in advanced CMOS applications because of their high relative permittivity, large bandgap, and good thermal stability.16,17

Kitai18 demonstrated that terbium oxide (Tb2O3) film as a gate dielectric has a large capacitance value and a low leakage current due to its high dielectric constant and good thermodynamical stability in contact with silicon. Nevertheless, the biggest concerns when using RE oxide films as the sensing membrane are the moisture absorption and oxygen vacancies,19 which affect their sensing performance. To address these problems, the incorporation of other elements (Ti, TiO2, or Y) into the RE dielectric films can reduce the oxygen vacancies in RE oxide film and thus prevent the moisture absorption of RE oxide film.19–21

A full understanding of biological layers using immobilization techniques normally employed in the microelectronic device is of pivotal importance. When biological molecules are employed, the fabrication process must be optimized and the biological layers must be characterized using immobilization techniques that will maintain their chemical—physical properties, i.e., their native structure. In 1980, Caras and Janata22 developed the first pH-ISFET type sensor based on penicillinase for the detection of penicillin. A variety of approaches have been investigated in the immobilization of glucose enzyme onto the biosensors.23 Covalent immobilization of enzymes may promote very interesting stabilizing effects: (a) the immobilized enzyme is not able to undergo any intramolecular process, e.g. autolysis, proteolysis, aggregation; (b) the immobilized enzyme is not able to interact with large hydrophobic interfaces, e.g. air/oxygen bubbles, immiscible organic solvents.24 The enzyme immobilization on a semiconductor surface is mostly preceded by the silanization procedure.25 This procedure allows chemically reactive groups to be introduced.26 In the case of silanization performed with 3-aminopropyltriethoxysiloxane (APTES), the reactive groups are of the amine type (NH2). These groups are used to form covalent bonds between the amino groups of enzyme and glutaraldehyde (GA).

Up to date, the structural and sensing properties of TbYxOy films as a pH-sensitive gate membrane for EIS sensors are still unclear. In this study, we investigated the structural properties and sensing characteristics of TbYxOy thin films deposited on Si substrates through reactive co-sputtering. Moreover, X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM) were used to analyze the quality of the TbYxOy sensing films and provide information for optimal annealing condition. We determined the sensing characteristics (pH sensitivity, hysteresis, and drift) of the TbYxOy films. Moreover, based on well-established pH sensing performance, the glucose biosensor using a pH-sensitive TbYxOy EIS and the method of chemical immobilization of glucose oxidase (GOD) on the TbYxOy surface with the use of APTES and APTES+GA are described. The imine
linkages are formed in the reaction between the carboxyl groups of GOD and the amine groups on the modified surface of TbYxOy and the combination of the amine groups of GOD and the aldehyde groups on the functionalized surface of TbYxOy.

**Experimental**

The EIS sensor structure having a TbYxOy sensing film was fabricated on 4-in p-type (100) Si wafers. Before the deposition of TbYxOy film, the wafers were cleaned using a standard RCA process and then they were dipped in 1% HF to remove the native oxide. A ∼40 nm TbYxOy film was deposited on the Si substrate through co-sputtering from both terbium and yttrium targets in diluted O2. Next, the samples were subsequently treated with rapid thermal annealing (RTA) in O2 ambient for 30 sec at various temperatures (600, 700, 800, and 900 °C) to form TbYxOy. After that, the back side contact (a 400-nm-thick Al film) of the Si wafer was deposited using a thermal coater. The dimension of the deposited TbYxOy film was defined by using a robotic dispensing system, in which an adhesive silicone gel (S181) acted as an isolating layer. TbYxOy EIS devices were then fabricated on the copper lines of a printed circuit board by using a silver gel to form conductive lines. An epoxy package was used to encapsulate the EIS device and the Cu line. Fig. 1 illustrates the structure of the fabricated TbYxOy EIS device.

The surface morphologies of the TbYxOy sensing films after RTA at various temperatures were examined by AFM. The compositional features of the TbYxOy films were investigated using XPS. The pH sensitivity of the TbYxOy sensing films was determined by measuring the capacitance–voltage (C–V) curves of the EIS devices. The C–V curves for different pH buffer solutions (Merck) were measured through Ag/AgCl reference electrode (commercial liquid-junction electrode) using an Agilent 4284A precision LCR meter operated at an ac voltage with the amplitude of 20 mV and signal frequency of 500 Hz. One electrode of the EIS sensor is connected to the high potential (H$_{pot}$) and high current (H$_{cur}$) points in the 4284A LCR meter and the other Ag/AgCl reference electrode is connected to the low potential (L$_{pot}$) and low current (L$_{cur}$) points onto the 4284A LCR meter. The 4284A LCR meter was controlled by a computer via an IEEE-488 interface. The measurement scheme of an EIS device is shown in Fig. 1. To avoid light and noise interference, all of the experimental setups were kept in the dark and performed at room temperature.

The fabricated TbYxOy EIS sensors were employed to measure glucose concentration in solutions. Hydrolysis hydrogen ions may be used for glucose detection. Enzyme-catalyzed reaction can cause the variations of output electrical signals by the sensor. A glucose biosensor is fabricated with immobilization of GOD onto EIS sensors by covalent bonding methods. The enzyme immobilization procedure using APTES and GA consists of three steps: (1) silanization using 3-aminopropyltriethoxysilane (APTES), (2) linker molecule deposition and (3) enzyme coupling. These enzyme immobilization steps are shown in Fig. 2. In the first step the samples were immersed in a solution of APTES (2%) at 70 °C for 30 min. This immobilization step was modified to the surface of TbYxOy sensing membrane. The linker molecule deposition was carried out using glutaraldehyde (2.5%) at 70 °C for 10 min. Finally, samples were immersed in phosphate-buffered saline (PBS) solution containing GOD (2 mg/ml) overnight at room temperature. The samples were dried under a gentle nitrogen flow and stored at room temperature until measurement. Another GOD immobilization method using APTES comprises only (1) and (2) steps. To test the feasibility of the proposed sensor for glucose detection, the prepared EIS sensor was sequentially immersed in glucose solutions with different concentrations in the range from 2 to 8 mM. The glucose standard solutions with different concentrations were prepared using PBS to solve glucose powder and the resulting pH levels of each case were checked to be equal by a commercial pH meter. In the sensing experiments of glucose, an EIS sensor was incubated in the analyte for 5 min and then C–V curve was recorded. The device was thoroughly rinsed with R.O. water after each measurement.

**Results and Discussion**

**Structural properties.**—Fig. 3 shows the AFM surface images of the TbYxOy films annealed at various temperatures. The surface roughness of the TbYxOy films clearly increased upon increasing the RTA temperature, except 900 °C. The TbYxOy film annealed at 600 °C showed a smooth surface (0.83 nm), while the film annealed at 800 °C had a rough surface (1.63 nm). This behavior may be attributed to an increased self-diffusion of terbium, yttrium and oxygen during high-temperature annealing resulting in the enhancement of the clustering of grains, thus increasing the surface roughness of the TbYxOy film. Nevertheless, Fig. 3d displays the honeycomb-like surface of the TbYxOy film annealed at 900 °C. The honeycomb-like pattern may increase the formation of surface site density to improve pH sensitivity.

We used XPS to examine the structural and compositional changes of the TbYxOy films after RTA at different temperatures (600, 700, 800, and 900 °C). Fig. 4 displays the Tb 3d$_{5/2}$, Y 3d, and O 1s spectra for the TbYxOy films annealed at various temperatures. Each fitting peak followed the general shape of the Lorentzian-Gaussian functions. The binding energy of the Tb 3d$_{5/2}$ peak at 1241.5 eV is assigned to
the Tb$_2$O$_3$ structure.\textsuperscript{27} The Tb 3d spectra in Fig. 4a can be deconvoluted to two chemical states: the high binding energy state (at 1243.3 eV) can be related to O atoms in the Tb(OH)$_x$ and the low binding energy state (at 1240.6, 1240.5, 1240.4, and 1240.3 eV for 600, 700, 800 and 900°C, respectively) to O atoms in the TbY$_x$O$_y$. For the 600°C-annealed sample, the shift in the Tb 3d$_{5/2}$ peak position to lower binding energy (by 0.9 eV), relative to the Tb$_2$O$_3$ reference position suggests the presence of crystal defects in the film. Furthermore, the intensity of the O 1s peak corresponding to Tb(OH)$_x$ for the film annealed at 600°C exhibited larger than other temperatures. This result may be attributed a poor Tb$_2$O$_3$ film to the hydroxide formation after exposure to an air ambient. The moisture absorption of RE oxide film may be associated with the metal defects or oxygen vacancies in the films.\textsuperscript{19} The shift in the position of Tb 3d$_{5/2}$ peak to lower binding energy increased with increasing RTA temperature. We attribute this behavior to the reactions of oxygen with the Tb and Y atoms to form Tb–OH and Tb–O–Y bonds, respectively. The O 1s peak at 528.8 eV is assigned to the Tb$_2$O$_3$,\textsuperscript{27} whereas the high binding energy at 529.5 eV is the Y$_2$O$_3$.\textsuperscript{28} In the two sets of spectra under 600°C condition, the O 1s peaks at 531.8 and 528.7 eV (529.1, 529.1 or 529.3 eV for 700, 800 or 900°C, respectively) represent the Tb–OH and Tb–O–Y bonds, respectively. The O 1s peak intensity corresponding to Tb(OH)$_x$ for 600°C-annealed sample had larger compared to other temperatures. The shift in the position of O 1s peak corresponding to TbY$_x$O$_y$ to higher binding energy clearly increased with increasing RTA temperature. This behavior is consistent with the reaction of O and Tb with Y atoms, forming a well-crystallized TbY$_x$O$_y$ film.

Sensing characteristics.—In a pH ISFET or EIS sensor, the sensing element is the surface of the gate oxide that is put in direct contact with an aqueous solution. Under these conditions silanol sites (formulated as M–OH) develop at the oxide surface. The pH effect on such sites can be rationalized by the site-dissociation model,\textsuperscript{29} which assumes that the silanol site is able to go through both acid dissociation

Figure 2. Scheme of the two elementary activation reactions. (a) The TbY$_x$O$_y$ surface was modified using APTES solution, the amine group of APTES was then reacted with the carboxyl group of GOD to immobilize on the YbY$_x$O$_y$ surface. (b) The amine group of APTES modified on the YbY$_x$O$_y$ surface was combined with GA to produce aldehyde group. The GOD was immobilized on the aldehyde groups-modified YbY$_x$O$_y$ surface.
and protonation as follows:

\[
\text{MOH} \leftrightarrow \text{MO}^- + \text{H}^+, \quad k_a = \frac{[\text{M}--\text{O}^-][\text{H}^+]}{[\text{MOH}]} \tag{1}
\]

\[
\text{MOH} + \text{H}^+ \leftrightarrow \text{MOH}_2^+, \quad k_b = \frac{[\text{MOH}_2^+]}{[\text{MOH}][\text{H}^+]} \tag{2}
\]

where \([\text{H}^+]\) is the hydrogen ion concentration near the interface. Due to the reaction of M–OH sites with the hydrogen ions, a double electric layer forms at the insulator/solution interface.\(^30\) It comprises on the one side of the ionic sites at the oxide surface and, on the other side, of solution anions attracted by the positive charges at the surface. Therefore, a potential difference \(\Delta \psi_s\) builds up at the interface of the oxide and the solution. This determines the surface concentration of the hydrogen ion at the surface as a function of the bulk concentration \([\text{H}^+]_b\) according to the Boltzmann equation:

\[
[\text{H}^+]_h = [\text{H}^+]_b e^{-\frac{q \psi_s}{kT}} \tag{3}
\]

where \(q\) is the elementary charge, \(k_B\) is the Boltzmann constant, and \(T\) is the absolute temperature.

The M–OH site can act as either an acid (reaction 1) or base (reaction 2), which represents an amphoteric character. Hence, the assembly of ionogen sites forms a surface-confined pH buffer system. The buffer capacity of this system \((\beta_s)\) plays an important role in determining the pH response. The buffer capacity measures the ability of the buffer to neutralize a pH change when hydrogen ions are added or removed. This parameter relies on the above equilibrium constants and the total number of active sites per unit area \((N_i = [\text{M}--\text{O}^-] + [\text{M}--\text{OH}_2^+] + [\text{M}--\text{O}^-])\), as follows:

\[
\beta_s = \frac{N_i \sqrt{k_a k_b}}{\gamma} \tag{4}
\]

where \(\gamma\) is a specific parameter of the oxide. If \(\beta_s \gg \psi_s / RT\), where \(R\) is the universal gas constant, the following linear equation relates the surface potential to the solution pH:

\[
\Delta \psi_s = 2.303 \frac{RT}{F} \frac{\beta_s}{\beta_s + 1} (pH_0 - pH) \tag{5}
\]

where \(F\) is the Faraday constant and \(pH_0\) is the pH value at which \(\Delta \psi_s = 0\); it is determined by the equilibrium constants \([pH_0 = -\log(k_a/k_b)^{1/2}]\). The crucial issue here is that the response sensitivity is evaluated by \(\beta_s\), and a Nernstian response is determined only if \(\beta_s \gg 1\), that is, if there is a high surface buffer capacity. This condition is performed when the surface density of ionogen sites is very high. Moreover, the oxide layer has to be stable in contact with the solution so as to prevent solution reaching the semiconductor surface. Consequently, an oxide layer that develops a high ionogen site density by conditioning in water forms the hydrogen-responsive element of this pH sensor. By ion exchange with the solution it leads to an interface potential that is a linear function of the solution pH.

The flatband voltage \((V_{FB})\) contains terms that reflect the properties of interfaces between the liquid and the gate oxide on one side, and the liquid and the reference electrode on the other side. The work function of the gate metal in a conventional MOSFET is here replaced by the reference electrode potential relative to vacuum \((E_{re})\). The H\(^+-\) sensitive oxide layer is set in contact with the test solution and a gate voltage is applied via a reference electrode. The change in this oxide interface potential \((\Delta \psi_s)\) is reflected in the \(V_{FB}\), it can be expressed...
The pH ranging from pH 2 to pH 12. Figs. 5a–5d demonstrate the curves of the TbYxOy EIS devices annealed at different temperatures, after RTA at various temperatures, we measured a set of hysteresis voltage was defined as the difference in $V_{\text{REF}}$ between the initial (first pH 7) and terminal (last pH 7) voltages measured in the above pH loop. Fig. 6a shows the hysteresis voltage of the TbYxOy EIS devices annealed at different temperatures. The hysteresis voltages of TbYxOy EIS devices annealed at 600, 700, 800, and 900 °C were 25, 18, 5, and 1 mV, respectively. Results demonstrate that the hysteresis voltage decreased with increasing the RTA temperature. Previous studies reported that the clinging of ions caused by the dangling bonds might impede the diffusion of the reacting ions and thus delay the reference voltage response. Since the high annealing temperature might repair the dangling bonds and fill in the vacancies to reduce defects in an oxide film; therefore the TbYxOy sensing film annealed at 900 °C exhibited the lowest hysteresis voltage. Fig. 6b displays the drift characteristics of the TbYxOy EIS devices annealed at various RTA temperatures and measured in pH 7 buffer solutions for 12 h. The change in the reference voltage ($\Delta V_{\text{REF}}$) here defined as $\Delta V_{\text{REF}} = V_{\text{REF}}(t) - V_{\text{REF}}(0)$. The degradation slope of the reference voltage variation reflects the EIS stability. The drift rate of the TbYxOy EIS sensors annealed at 600, 700, 800, and 900 °C was 1.04, 0.92, 0.34, and 0.26 mV/h, respectively. The TbYxOy sensing film annealed at 900 °C had the best long-term stability among these annealing temperatures. This is because higher-annealing treatment may reduce the traps and the dangling bonds, which may gradually capture clusters of ions and result in the $V_{\text{REF}}$ drift of the device. The dangling bonds, which accumulate at the poles, attract the ions in the electrolyte so that extra amount of ions are needed to neutralize the dangling bonds and lattice defects. Consequently, dangling bonds affect the stability of the sensing performance between the membrane and electrolyte. The decrease of these dangling bonds could reduce the reaction of the ions with the sensing membrane in the electrolyte.

**Glucose biosensor.**—In order to achieve the best enzyme immobilization, we used a covalent binding technique to immobilize the GOD on the EIS surface, as briefly described in the experimental. The APTEs whose chemical formula is $(\text{NH}_2-\text{(CH}_2)_3-\text{Si(OCH}_3)_3)$ is used to functionalize the TbYxOy EIS devices annealed at various RTA temperatures and measured in pH 7 buffer solutions for 12 h. The change in the reference voltage ($\Delta V_{\text{REF}}$) here defined as $\Delta V_{\text{REF}} = V_{\text{REF}}(t) - V_{\text{REF}}(0)$. The degradation slope of the reference voltage variation reflects the EIS stability. The drift rate of the TbYxOy EIS sensors annealed at 600, 700, 800, and 900 °C was 1.04, 0.92, 0.34, and 0.26 mV/h, respectively. The TbYxOy sensing film annealed at 900 °C had the best long-term stability among these annealing temperatures. This is because higher-annealing treatment may reduce the traps and the dangling bonds, which may gradually capture clusters of ions and result in the $V_{\text{REF}}$ drift of the device. The dangling bonds, which accumulate at the poles, attract the ions in the electrolyte so that extra amount of ions are needed to neutralize the dangling bonds and lattice defects. Consequently, dangling bonds affect the stability of the sensing performance between the membrane and electrolyte. The decrease of these dangling bonds could reduce the reaction of the ions with the sensing membrane in the electrolyte.
Figure 5. Responses of the C–V curves for TbYxOy EIS sensors annealed at (a) 600 °C, (b) 700 °C, (c) 800 °C, and (d) 900 °C. Inset: Reference voltage as a function of pH for TbYxOy EIS sensors annealed at (a) 600 °C, (b) 700 °C, (c) 800 °C, and (d) 900 °C. 

\[
\text{β-D-glucose} + \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{glucose oxidase}} \text{D-glucono-δ-lactone} + \text{H}_2\text{O}_2
\]  

as follows:

\[
\text{D-glucono-δ-lactone} \rightarrow \text{D-gluconate} + \text{H}^+
\]

The GOD catalyzes the oxidation of β-D-glucose to D-glucono-δ-lactone by using molecular oxygen as an electron acceptor with simultaneous production of hydrogen peroxide (H₂O₂). D-glucono-δ-lactone will produce D-gluconate with an H⁺ ion. Therefore, one glucose molecule yields one H⁺ ion, and it changes the local hydrogen ion activity of the solution. Changes in the local hydrogen ion activity are reflected in the C–V curves. 

Figure 6. (a) Hysteresis voltages of TbYxOy EIS devices annealed at various temperatures during the pH loop of 7 → 4 → 7 → 10 → 7. (b) Drift rates of TbYxOy EIS sensors annealed at different temperatures and measured in a solution at pH 7.
EIS biosensors. In this study, we employed silanization by APTES and activated by APTES+GA to modify the surface of TbY<sub>x</sub>O<sub>y</sub> sensing film. The TbY<sub>x</sub>O<sub>y</sub> EIS biosensor treated with APTES+GA shows a higher sensitivity (19.85 mV/mM) in solutions containing glucose at concentrations in the range 2–8 mM, compared to that with APTES. The TbY<sub>x</sub>O<sub>y</sub>-based EIS sensor could pave the way for future medical biosensing applications.

Acknowledgments

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Table I. Comparison of immobilization method and sensing performance for enzymatic glucose EIS biosensors fabricated with a Dy<sub>2</sub>TiO<sub>5</sub>, Nb<sub>2</sub>O<sub>5</sub>, CeO<sub>2</sub>, and ZnO sensing film.

<table>
<thead>
<tr>
<th>Sensing film</th>
<th>Immobilization method</th>
<th>Sensitivity</th>
<th>Linearity</th>
<th>Dynamic range</th>
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<tbody>
<tr>
<td>Dy&lt;sub&gt;2&lt;/sub&gt;TiO&lt;sub&gt;5&lt;/sub&gt;</td>
<td>alginate microbead</td>
<td>12.19 mV/mM</td>
<td>99.44%</td>
<td>2–8 mM</td>
</tr>
<tr>
<td>Nb&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;5&lt;/sub&gt;</td>
<td>covalent binding</td>
<td>10.59 mV/mM</td>
<td>97.69%</td>
<td>2–7 mM</td>
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<tr>
<td>CeO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>covalent binding</td>
<td>6.43 mV/mM</td>
<td>97.26%</td>
<td>2–7 mM</td>
</tr>
<tr>
<td>ZnO</td>
<td>Nafion</td>
<td>0.395 mV/μM</td>
<td>96.70%</td>
<td>20–100 μM</td>
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<tr>
<td>TbY&lt;sub&gt;x&lt;/sub&gt;O&lt;sub&gt;y&lt;/sub&gt;</td>
<td>covalent binding</td>
<td>19.85 mV/mM</td>
<td>99.34%</td>
<td>2–8 mM</td>
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Conclusions

In this research, we have developed a glucose EIS biosensor featuring TbY<sub>x</sub>O<sub>y</sub> sensing membranes grown on Si substrates through reactive co-sputtering. We applied XPS and AFM to confirm the presence of the TbY<sub>x</sub>O<sub>y</sub> structures in these EIS devices. Consistent with structural analyses, the results indicate that the TbY<sub>x</sub>O<sub>y</sub> EIS device annealed at 900 °C exhibited a higher sensitivity of 59.79 mV/pH compared with other annealing temperatures. In addition, all other sensing devices, including the hysteresis voltage (1 mV) and drift rate (0.26 mV/h), could be achieved at an optimal temperature of 900 °C. Improvements caused by annealing might contribute to an increase in surface sites and elimination of crystal defects. Furthermore, the utilization of enzyme molecules as a biological recognition element in pH-EIS sensing systems can extend their application for glucose biosensing. In this study, we employed silanization by APTES and activated by APTES+GA to modify the surface of TbY<sub>x</sub>O<sub>y</sub> sensing film. The TbY<sub>x</sub>O<sub>y</sub> EIS biosensor treated with APTES+GA shows a higher sensitivity (19.85 mV/mM) in solutions containing glucose at concentrations in the range 2–8 mM, compared to that with APTES. The TbY<sub>x</sub>O<sub>y</sub>-based EIS sensor could pave the way for future medical biosensing applications.

References