Effects of N₂ and O₂ annealing on the multianalyte biosensing characteristics of CeO₂-based electrolyte–insulator–semiconductor structures

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A CeO₂–based multi-analyte electrolyte–insulator–semiconductor (EIS) biosensor has been fabricated on silicon substrate. To enhance the material quality and sensing performance, annealing treatment in N₂ and O₂ ambient has been incorporated. To examine the annealing effects, material analyses were conducted using X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM) to identify optimal treatment conditions. Furthermore, sensing performance for various ions of Na⁺, K⁺, urea, and glucose has also been tested. Results indicate that the membrane annealed at 800 °C in O₂ ambience exhibited a better performance with higher multianalyte sensitivity and a lower drift rate compared with the membrane annealed in N₂ ambience. Oxygen in O₂ ambience may cause stronger reflow and fill in the oxygen vacancy so that the material properties and sensing capability can be improved in O₂ annealing ambience.

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1. Introduction

Ion sensitive field effect transistors (ISFETs) used for pH detection were first demonstrated by Bergveld in the 1970s [1]. The device substituted the fragile glass electrode with metal oxides when measuring pH values for various concentrations [2]. Modern ISFETs offer specific advantages in terms of small size, low impedance, solid-state structure and multiple ion sensing capability, making them popular devices for ion activity vivo surveys in biomedical processes. Developed from ISFETs, electrolyte insulator semiconductor (EIS) sensors have neither source nor drain. Because of their simple structure and manufacturing ease, they are considered a fundamental device for silicon-based field-effect chemical and biological sensors [3]. To fabricate an EIS device, various kinds of high-k metal oxide materials including Al₂O₃, Ta₂O₅, and WO₃ have been used [4–7]. Unfortunately, their structures are not generally stable enough for long-term, high-temperature applications because of the interfacial layer between the high-k metal oxide and the silicon used in their manufacture. The interface layer is not only the most critical part of an EIS sensor that can affect performance, it is also vital to overall sensor properties, as a flaw in the interface layer can often render the sensor inoperable. It is therefore necessary to explore new materials and alternative processes to mitigate interfacial defects. Rare-earth (RE) metals provide an alternative to traditional metal oxides providing a thinner interfacial layer, higher capacitance value and lower leakage current [8,9]. Among the rare-earth oxides, CeO₂ allows a wide band gap of 3.19 eV, has great mechanical strength, good redox properties [10], and has been used for gas and humidity sensors [11,12].

Fabrication of CeO₂–based biosensors has been proposed by Ansari et al., who prepared glucose sensors using sol–gel methods [13], and Faisal et al., who proposed ethanol sensors consisting of CeO₂ [14]. However, a CeO₂ EIS sensing membrane has not yet been reported. In 2002, Nishikawa et al. demonstrated the high performance CeO₂ dielectrics, pointing to their good interfacial properties on Si substrate [15]. In this study, CeO₂ has been sputtered on Si substrate as the sensing membrane for EIS sensing applications. In addition to traditional pH sensing capability, the sensing performance of the CeO₂ EIS structure in various solutions containing Na⁺, K⁺, urea, and glucose [16–18] were examined for future industrial multi-analyte biosensor applications. Moreover, the effects of post rapid thermal annealing (RTA) treatment in
distinct ambience of N$_2$ and O$_2$ [19,20] on the material properties and the sensing performance were examined because appropriate annealing could improve the material quality and optimize the sensing performance [21]. According to previous reports, O$_2$ annealing could cause stronger atoms reflow during annealing [22]. Furthermore, oxygen vacaices could be filled-in by the oxygen in O$_2$ ambient [23]. Therefore, multianalyte sensing capability of the membrane with O$_2$ annealing performed better than the membrane with N$_2$ annealing treatment.

2. Experiment

To incorporate CeO$_2$ membranes on EIS structures, fabrication was performed on a 4-inch n-type (100) Si wafer with a resistivity of 5–10 Ω cm. To remove the native oxide, the wafers were cleaned using HF-dip (HF:H$_2$O = 1:100) before deposition on the CeO$_2$ film. Then, 50 nm CeO$_2$ was deposited on the silicon wafer by radio frequency (RF) reactive sputtering with a mixture of Ar and O$_2$ (Ar:O$_2$ = 25:0) ambient during sputtering. The RF power was 150 W and the ambient pressure was 20 mTorr. RTA was used to anneal the samples at different temperatures of 600, 700, 800 and 900 °C in O$_2$ ambient for 30 s. Other samples were annealed at the same RTA temperatures and conditions in N$_2$ ambient. An Al film of 300 nm in thickness was then deposited on the backside of the Si wafer. Next, a photosensitive epoxy (SU8-2005 of MicroChem Inc) was used to define the sensing area through a standard photolithography process. Finally, the samples were fabricated on the copper lines of printed circuit board (PCB) in silver gel. A detailed EIS structure is illustrated in Fig. 1.

3. Result and discussion

3.1. Material analysis

Fig. 2(a) and (b) show the XRD patterns of the cerium oxide layer treated with RTA in N$_2$ and O$_2$ ambient, respectively. The crystalline phase of CeO$_2$ of the as-deposited sample can clearly be observed to have the characteristic peaks of XRD and three diffraction peaks of (2 0 0), (2 2 0) and (3 1 1), which can be observed at 33.07°, 47.83° and 56.78° for both treatments. In Fig. 2(a) of the samples treated in N$_2$ ambient, when the temperature rose to 800 °C, the peaks at (2 0 0) and (2 2 0) increased in intensity. This phenomenon might be caused by the enhancement of lattice structures in different temperatures forming higher peak intensities. Moreover, the peak intensity increased as the temperature rose, becoming strongest when reaching an RTA temperature of 800 °C, clearly exhibiting a stronger peak at (2 0 0), and then suddenly decreasing at 900 °C. This could be attributed to likelihood of the bonds of cerium and oxygen being destroyed when the CeO$_2$ film annealed at 900 °C. To

Fig. 1. CeO$_2$—based EIS structure.

![Fig. 1](image)

Fig. 2. XRD of the CeO$_2$ film after annealing at various temperatures on single crystalline silicon (a) in N$_2$ ambient (b) in O$_2$ ambient.

![Fig. 2](image)

Fig. 3. The O 1s XPS results of CeO$_2$ film annealed (a) in N$_2$ ambient and (b) in O$_2$ ambient.

![Fig. 3](image)
Fig. 4. AFM images of (a) the as-deposited film, (b) the film annealed at 600 °C in N₂ ambient (c) the film annealed at 800 °C in N₂ ambient (d) the film annealed at 600 °C in O₂ ambient and (e) the film annealed at 800 °C in O₂ ambient. (f) Comparison of AFM analysis for CeO₂ annealed at various temperatures in different gas (N₂ or O₂) ambient on single crystalline silicon.

compare RTA treatments in N₂ and O₂ ambient. In Fig. 2(b) of the samples treated in O₂ ambient, it can be observed that during peaks (2 0 0) and (2 2 0) intensities increased more in the O₂ ambient, while peak (3 1 1) intensity increased much more than the samples treated in N₂ ambient. The drastic increase of peak (3 1 1) might be due to the fact that O₂ annealing caused oxygen atoms filled in more oxygen vacancies than N₂ annealing, leading to higher peak intensity [23]. By comparing Fig. 2(a) and (b), it can be confirmed

Fig. 5. (a) C–V curves for pH detection of the as-deposited CeO₂ sensing membrane and (b) the extracted sensing performance (c) C–V curves for pH detection of the CeO₂ sensing membrane annealed at 800 °C in N₂ ambient and (d) the extracted sensing performance (e) C–V curves for pH detection of the CeO₂ sensing membrane annealed at 800 °C in O₂ ambient and (f) the extracted sensing performance (g) sensitivity and linearity CeO₂ sensing membrane with various RTA temperatures in N₂ ambient (h) sensitivity and linearity CeO₂ sensing membrane with various RTA temperatures in O₂ ambient.
that the CeO$_2$ sensing membrane after post-RTA treatment in O$_2$ not only formed a stronger lattice structure, enhancing the peak intensity, but also better stabilized the crystalline structure than the post-RTA treatment in N$_2$ sample.

X-ray photoelectron spectroscopy (XPS) was employed to investigate the chemical-binding states in the CeO$_2$ sensing membrane on the Si substrate. Fig. 3(a) and (b) shows the O 1s spectra of the as-deposited samples and those formed by RTA annealing in N$_2$ and O$_2$ ambient, respectively. The O 1s spectra for the as-deposited and annealed films include the appropriate three-peak curve-fitting lines. In the spectra, the O 1s peak at 529.2 eV represents the CeO$_2$ bond, and the intermediate binding energy (531.9 eV) is attributed to Ce silicate represented by the Si–O–Ce bond. The highest binding energy (533 eV) is assigned to SiO$_2$ representing the Si–O bond. As the as-deposited samples show a strong Ce–silicate and low SiO$_2$ peak, and the samples which underwent post-RTA treatment in N$_2$ and O$_2$ at 800 °C show stronger bonding intensity for CeO$_2$ and a weaker O 1s peak for Ce–silicate than other samples. The results indicate that appropriate annealing at 800 °C both in N$_2$ and O$_2$ ambient could effectively suppress the formation of Ce silicate and SiO$_2$. However, as the annealing temperature increased to 900 °C, the concentration of Ce silicate and SiO$_2$ increased again both in N$_2$ and O$_2$ annealing because Si atoms might react with CeO$_2$ at a high annealing temperature of 900 °C [21].

To examine the surface morphologies for the CeO$_2$ films, atomic force microscopy (AFM) was used to monitor the surface. The surface morphologies of the sensing membranes were measured by Veeco D5000 AFM in tapping mode using an applied Nano silicon tip with a 50 N/m spring constant. The scan rate was 1 Hz, the scan area was 3 × 3 μm, and the set engagement ratio was 80%. To reveal the surface roughness, Fig. 4(a) shows the surface for the as-deposited film, Fig. 4(b) and (c) show the surface for the film annealed in N$_2$ ambient at 600 and 800 °C, and Fig. 4(d) and (e) show the surface for the film annealed in O$_2$ ambient at 600 and 800 °C. To illustrate the trend of the annealing conditions, Fig. 4(f) show the roughness of the CeO$_2$ films for the as-deposited and post-RTA films treated in N$_2$ and O$_2$ ambient, respectively. In both ambient, the CeO$_2$ film annealed at 800 °C had the highest surface roughness indicating, crystallization-induced large grains might be formed with annealing. Furthermore, the roughness of the CeO$_2$ film annealed in O$_2$ ambient was larger than the film annealed in N$_2$ ambient. The results imply that fewer dangling bonds and traps might exist in the film annealed in O$_2$ ambient than the film annealed in N$_2$ ambient because stronger reflow in O$_2$ ambient and repairing the oxygen vacancies by O$_2$ might improve material quality the CeO$_2$ film [22,23].

3.2. pH sensing capability

The flat band voltage shift of the C–V curve due to the change in the concentration of hydrogen ions was applied to detect the pH value of solutions. To evaluating the sensing performance, pH sensitivity of the EIS structures with CeO$_2$ sensing membranes without annealing, with annealing in N$_2$ ambient and O$_2$ ambient were measured, respectively. The C–V curves of the EIS structures with the as-deposited membrane, the membrane annealed at 800 °C in N$_2$ ambient and O$_2$ ambient are shown in Fig. 5(a), (c) and (e). Extracted from the CV, the reference capacitance shift of the CeO$_2$ membrane reveals that the sensitivity and linearity of the as-deposited membrane, the membrane annealed in N$_2$ ambient and O$_2$ ambient were 37.36 mV/pH and 97.56%, 55.22 mV/pH and 99.68%, and 58.76 mV/pH and 99.78%, as shown in Fig. 5(b), (d) and (f), respectively. In addition, the linearity and sensitivity of the EIS structure treated at various temperatures in N$_2$ ambient and O$_2$ ambient are shown in Fig. 5(g) and (h). The results indicate that annealing at 800 °C could enhance the pH sensing capability.

Furthermore, O$_2$ annealing had the best sensing performance, which was consistent with all the material analyses. O$_2$ annealing at 800 °C could effectively reduce the vacancies and eliminate the dangling bonds. Therefore, the pH sensing capability could be optimized.

To examine the hysteresis effects of the membrane in various annealing conditions, the samples were immersed in buffer solutions of different pH values in an alternating cycle (pH7, pH4, pH7, and pH10) for 5 min for each solution. The above samples were subjected to a pH loop of 7 → 4 → 7 → 10 → 7 over a period of 25 min. Hysteresis voltage is defined as the gate voltage difference between the initial and the terminal voltages measured in the above pH loop. Fig. 6(a) and (b) shows the hysteresis effects of the samples treated at various annealing temperatures in N$_2$ ambient and O$_2$ ambient. In N$_2$ ambient and O$_2$ ambient, the sample annealed at 800 °C had the lowest hysteresis deviation. Furthermore, the sample annealed at 800 °C in O$_2$ ambient had the smallest hysteresis voltage of 5.97 mV. Since the defects and vacancies of the membrane might cause ions to attach on the surface, which might delay the reference voltage response [24]. An appropriate annealing at 800 °C with oxygen treatment might fill in the oxygen vacancies and dangling bonds to reduce attached ions on the surface during the test.

To investigate the drift rate of the membrane for long-time operations, the samples were immersed in pH7 buffer solutions for 12 h. The drift rate of the CeO$_2$-based EIS structure with membrane
annealed at various temperatures in N₂ ambient and O₂ ambient are shown in Fig. 7(a) and (b). The drift rate of the as-deposited sample and the sample annealed at 800 °C in N₂ ambient and O₂ ambient were 6.32, 1.56, and 0.96 mV/h, respectively. Among all the annealing conditions, the CeO₂ membrane annealed at 800 °C had the smallest drift rate. Since the defects such as vacancies and dangling bonds might capture clusters of ions and cause the reference voltage drift based on a model of gate voltage drift of pH-ISFET [25,26], O₂ annealing could effectively remove those defects, which might worsen the sensing performance.

3.3. Multianalyte sensing performance

To investigate the properties of sensing film with different conditions in the potassium and sodium solution, 1 M NaCl/Tris–HCl and 1 M KCl/Tris–HCl were injected into buffer electrolyte by a micropipette. The concentrations of sodium and potassium ions were controlled in a range between 10⁻⁵ and 10⁻¹ M. The pNa of CeO₂ samples with as-deposited and annealed samples at 800 °C in O₂ and N₂ ambient were 11.62, 14.72, and 13.81 mV/pNa, and the pK of CeO₂ samples with as-deposited and annealed samples at 800 °C in O₂ and N₂ ambient were 11.46, 14.51, and 12.63 mV/pK. Consistent with pH sensing results, O₂ annealed samples had higher sensitivity and linearity than N₂ annealed samples. In addition, the CeO₂ sensing films annealed at 800 °C in O₂ and N₂ ambient were more responsive to H⁺ than to Na⁺ and K⁺, as shown in Fig. 8(a) and (b).

By incorporating suitable enzymes into the pH membranes, urea detection and glucose sensing biosensors can also be created [27–31]. Since enzymatic reactions usually produce ion species, ion-selective membranes with appropriate enzymes can measure variations in electrical signals caused by enzyme-catalyzed reactions, which can change ion concentration. After setting the process of enzyme immobilization by a covalent binding method, changes in pH hydrolysis of urea [32] or glucose [33] can be detected. The urea and glucose hydrolysis equations are written as Eqs. (1)–(3).

\[
\text{NH}_2\text{CONH}_2 + 3\text{H}_2\text{O} \xrightarrow{\text{urease}} 2\text{NH}_4^+ + \text{OH}^- + \text{HCO}_3^- \quad (1)
\]

\[
\beta-\text{D-glucose} + \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{glucose oxidase}} \text{D-glucose-δ-lactone} + \text{H}_2\text{O}_2 \quad (2)
\]

\[
\text{D-glucose-δ-lactone} \rightarrow \text{D-gluconoate} + \text{H}^+ \quad (3)
\]

To analyze the urea sensing properties of the CeO₂ sensing membrane on the EIS structure, the urea solution with a concentration in a range between 5 and 40 mM was prepared. The urea sensing properties are shown in Fig. 9(a) and (b). The sensibility values of the as-deposited sample and annealed samples with RTA at 800 °C in O₂ and N₂ ambient were 2.35, 6.43, and 3.76 mV/mM, respectively. The linearity values of the as-deposited sample and annealed samples with RTA at 800 °C in O₂ and N₂ ambient were 84.38%, 93.83%, and 93.96%, respectively. It can be seen that the CeO₂ sensing membrane after RTA treatment at 800 °C in O₂ ambient had higher urea

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**Fig. 7.** Drift voltages for pH detection of CeO₂ sensing membrane annealed with various RTA temperatures (a) in N₂ ambient (b) in O₂ ambient.

**Fig. 8.** H⁺, Na⁺, and K⁺ ion sensitivity of (a) the membrane annealed at 800 °C in N₂ ambient (b) the membrane annealed at 800 °C in O₂ ambient.
sensing properties than all the other samples. In addition, to investigate the glucose sensing properties of the CeO$_2$ sensing membrane on EIS structure, a glucose solution with concentration in a range between 2 and 7 mM was prepared. In Fig. 10(a) and (b), it can be seen that the sensibility values of the as-deposited sample and annealed samples with RTA at 800 °C in O$_2$ and N$_2$ ambient were 1.49, 3.73, and 3.55 mV/mM, respectively. The linearity values of the as-deposited sample and annealed samples with RTA at 800 °C in O$_2$ and N$_2$ ambient were 63.19%, 97.26%, and 89.18%, respectively. In line with H$^+$, K$^+$, and Na$^+$ sensing results, the sample annealed at 800 °C in O$_2$ ambient had the highest sensitivity and linearity for urea and glucose sensing because of the improvements of the material property.

4. Conclusion

In this study, we fabricated an EIS sensor with a CeO$_2$ sensing membrane treated with annealing in O$_2$ and N$_2$ ambient on a
References


Biographies

Chuyuan Haur Kao was born in Taipei, Taiwan, 1966. He received the M.S. degree from the Department of Electric Engineering, National Cheng Kung University, Taiwan in 1990 and the Ph.D. degree from the Institute of Electronics, National Chiao Tung University, Taiwan, in 1997. In 2005, he joined the faculty at Chang Gung University as an assistant professor in the Department of Electronics Engineering. He is a professor in that department and his current research areas focus on the high-k gate dielectrics, flash memories, and extended-gate field-effect transistors.

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