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Multianalyte biosensor based on pH-sensitive ZnO electrolyte–insulator–semiconductor structures

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Multianalyte electrolyte–insulator–semiconductor (EIS) sensors with a ZnO sensing membrane annealed on silicon substrate for use in pH sensing were fabricated. Material analyses were conducted using X-ray diffraction and atomic force microscopy to identify optimal treatment conditions. Sensing performance for various ions of Na⁺, K⁺, urea, and glucose was also tested. Results indicate that an EIS sensor with a ZnO membrane annealed at 600 °C exhibited good performance with high sensitivity and a low drift rate compared with all other reported ZnO-based pH sensors. Furthermore, based on well-established pH sensing properties, pH-ion-sensitive field-effect transistor sensors have also been developed for use in detecting urea and glucose ions. ZnO-based EIS sensors show promise for future industrial biosensing applications. © 2014 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4874182>]

INTRODUCTION

After the first ion-sensitive field-effect transistor (ISFET) was invented by Bergveld in 1970, various kinds of chemical sensors have been designed to measure pH values.¹ Key benefits of ISFETs include small sizes, fast reactions, and reduced manufacturing prices. An ISFET comprised an electrolyte and a sensing dielectric, with the electrolyte in contact with the dielectric. A reference electrode is immersed in the electrolyte solution to replace the metal gate, creating an electrolyte-insulator-semiconductor (EIS) sensor. Variation in the threshold voltage provides information on ion changes. Recently, ISFET devices have been extensively investigated because of their high compatibility for integration with complementary metal-oxide-semiconductors (CMOSs).² Compounds, such as Al₂O₃, Ta₂O₅, and WO₃, have each been studied as sensing membrane materials.^{3–6} In addition to traditional dielectric materials, transparent conductive oxide (TCO), such as SnO₂ and ZnO, have been demonstrated as sensing membranes.^{7,8} Using TCO to fabricate EIS sensors provides possible applications, whereby sensors can be integrated with optoelectronic devices such as light emitting diodes,⁹ solar cells,¹⁰ and liquid crystal displays.¹¹ Notably, ZnO with a wurtzite structure, wide bandgap of 3.37 eV, and a large exciton bandgap of 60 meV, has also been used as a sensing material.^{12–15} Studies have also reported ZnO pH sensing membranes synthesized in liquid solutions¹³ and deposited by molecule beam epitaxy.¹⁴ Furthermore, sensors with nanowire or nanorods have been intensively studied recently.¹⁵ In contrast to the previous studies, ZnO sensing films sputtered on top of silicon with post RTA annealing as an alternative way to form sensing membranes for pH measurements were fabricated for this study.

Atomic force microscopy (AFM) and X-ray diffraction (XRD) were used to monitor the material quality of the sensing film and provide information for optimal annealing conditions. Based on these material analyses, a sputtered sensing membrane with post annealing at 600 °C demonstrated a comparatively high sensitivity of 42.45 mV/pH compared with other ZnO-based pH sensors from all previous studies. Furthermore, after measuring sensing capabilities including linearity, hysteresis, and drift rate, it can be confirmed that ZnO films treated with annealing at 600 °C in O₂ ambient can obtain high sensitivity, low hysteresis voltage, and low drift rate for pH detection. Based on well-established pH sensing properties, pH-ISFET sensors have also been developed for use in detecting urea and glucose ions.¹⁶ For potential industrial applications, multianalyte EIS-based biosensors can monitor blood glucose level for diabetes patients¹⁷ and blood/urine concentration for gout patients.¹⁸ Since the response time for glucose and urine concentration is much shorter, the EIS-based biosensor may replace the traditional medical urine or glucose test in the future. Therefore, sensing capability for different ions of Na⁺, K⁺, urea, and glucose was also evaluated for the ZnO-based EIS sensors in this study.

EXPERIMENTAL

The EIS structures consisting of ZnO membranes were deposited on a 4-in. n-type Si wafer with a resistivity of 5–10 Ω cm. To etch away the native oxide, the wafers were first cleaned with a HF-dip process (HF:H₂O = 1:100). Next, a 50-nm-thick ZnO layer was grown by radio frequency (RF) reactive sputtering on an n-type silicon wafer at 100 W and 20 mTorr of pressure in an ambient with an Ar:O₂ of 23:2. After sputtering, samples were annealed at temperatures of 600 °C, 700 °C, 800 °C, and 900 °C by rapid thermal annealing (RTA)

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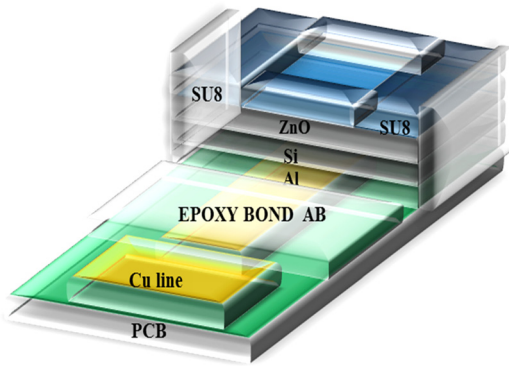
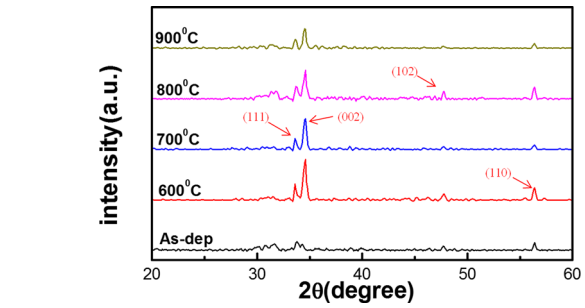
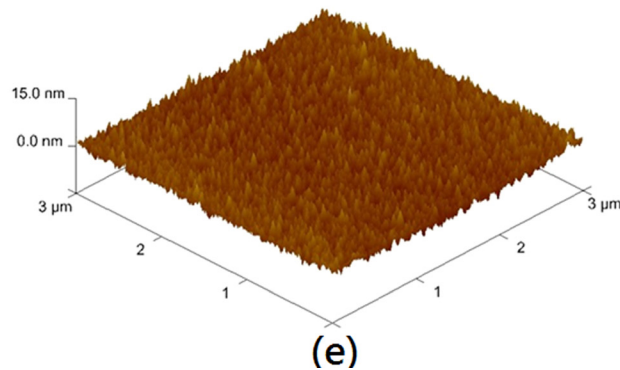
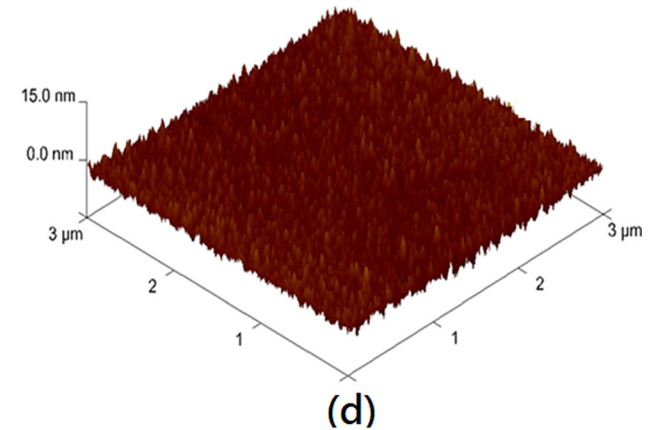
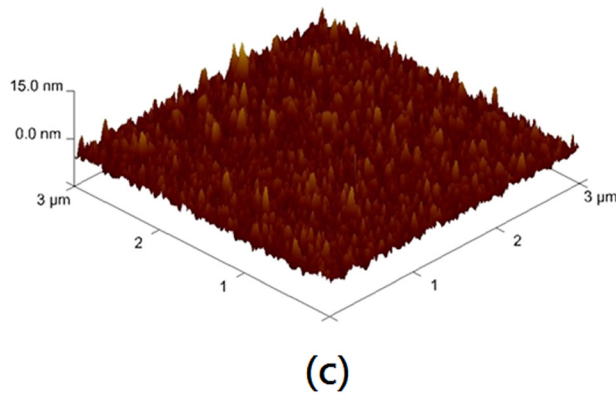
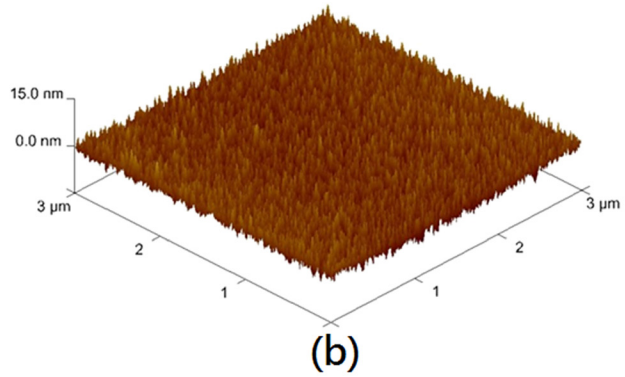
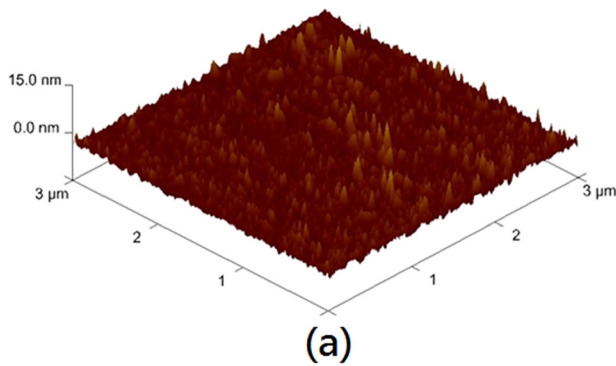


FIG. 1. EIS with ZnO membrane structure.

for 30 s in O_2 ambient. Then, a 300-nm-thick Al film was evaporated on the backside for use as the contact. The sensing area was then defined by a standard photolithography process

FIG. 2. XRD image of the ZnO film after annealing at various temperatures on single crystalline silicon in O_2 ambient for 30 s.

using a photosensitive epoxy (SU8-2005, Micro-Chem). Finally, the samples were fabricated on the copper lines of a printed circuit board (PCB) using silver gel. A detailed EIS structure is shown in Fig. 1.

FIG. 3. AFM image of high- k ZnO surface on single crystalline silicon after RTA at (a) As-deposited, (b) 600 °C, (c) 700 °C, (d) 800 °C, (e) 900 °C.

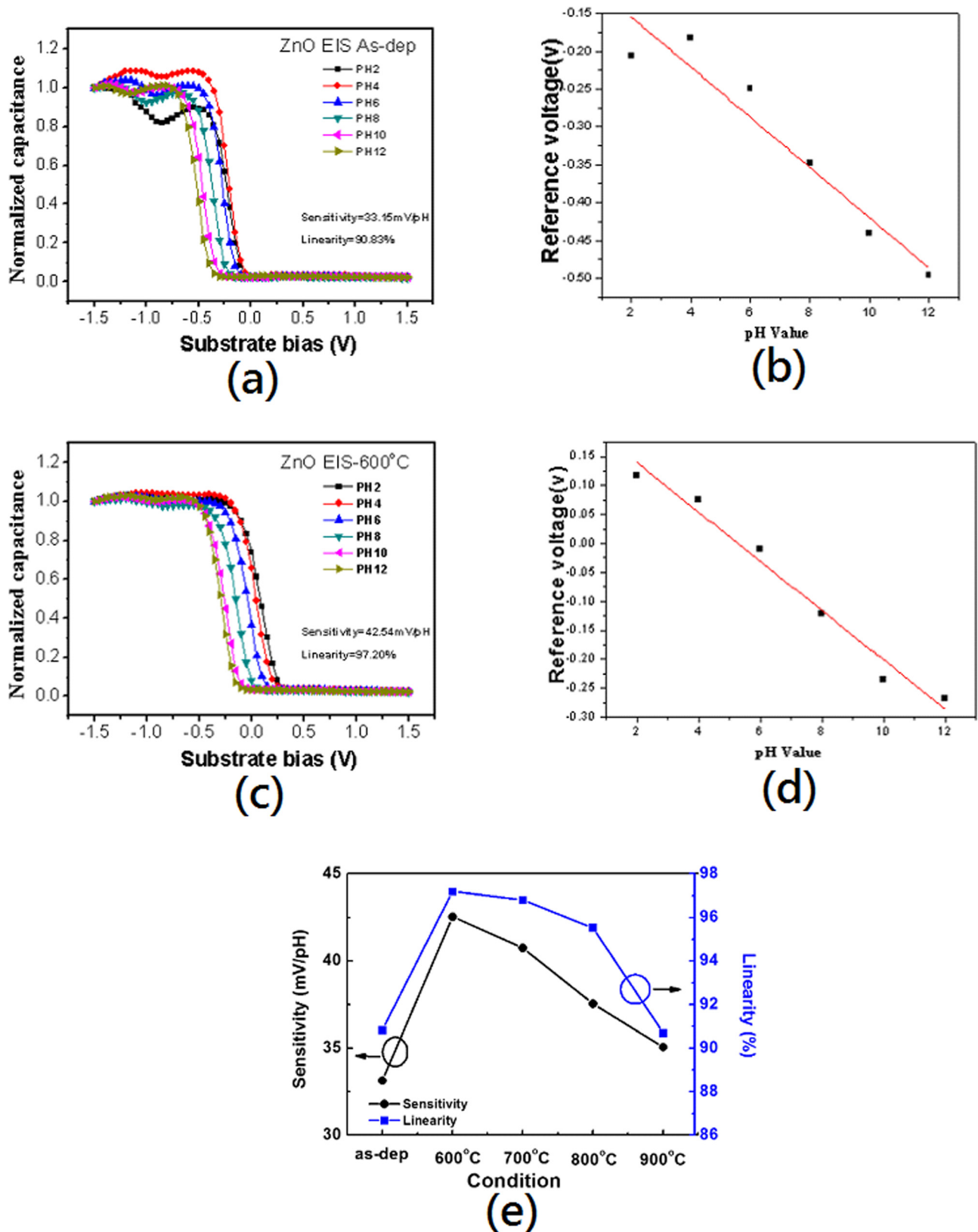


FIG. 4. (a) Normalized C-V curve of the ZnO as-deposited sensing membrane. (b) The pH sensing capability of the ZnO as-deposited sensing membrane. (c) Normalized C-V curve of the ZnO sensing membrane annealed at 600 °C. (d) The pH sensing capability of the ZnO sensing membrane annealed at 600 °C. (e) The sensitivity and linearity of the ZnO sensing membrane with different temperatures.

RESULTS AND DISCUSSION

To examine the crystalline structure and evaluate the optimal temperature for the sensing film, XRD was used to

study the annealing effects. Figure 2 shows XRD patterns after RTA treatment at different temperatures. Four diffraction peaks of ZnO (111), (002), (102), and (110) can be observed at 33.50°, 34.37°, 47.56°, and 56.78°, respectively. Compared

with the as-deposited sample, the intensity of peaks (111), (002), and (110) became higher after RTA treatment at 600 °C. However, the peak intensity suddenly decreased as the annealing temperature rose to 700 °C. It can therefore be confirmed that the ZnO sensing membrane after post-RTA treatment at 600 °C possessed a better crystalline structure with stronger peak intensity.

In order to study the effect of ZnO films after annealing at various temperatures, AFM was used to analyze the roughness of the films. Figures 3(a)–3(e) show AFM images of the ZnO samples. Analysis shows that the root mean square roughness (R_{rms}) values of the ZnO sensing membrane for as-deposited and samples annealed at 600, 700, 800, and 900 °C were 0.959 nm, 1.40 nm, 1.35 nm, 1.21 nm, and 1.11 nm, respectively. When the sample was treated at 600 °C, the film surface became rougher. Crystallization likely induces large grains and passivates defects and dangling bonds. Moreover, the rougher the surface is, the more surface sites exist. Based on the site binding theory,¹⁹ more surface sites result in higher degrees of sensor sensitivity and linearity.

To investigate the pH sensing performance of ZnO membranes, pH sensitivity, pH linearity, hysteresis voltage, and drift rate were measured using a HP4284A high precision LCR meter. Figure 4(a) shows the C-V curves of ZnO sensing membranes without annealing immersed in buffer solution of various pH values, and Fig. 4(b) shows the pH sensing capability extracted from the C-V curve. As for the annealed sample, Fig. 4(c) shows the C-V curves of ZnO sensing membranes annealed at 600 °C immersed in buffer solution with various pH values, and Fig. 4(d) shows the pH sensing capability extracted from the C-V curve. As shown in Fig. 4(e), the sensitivity of the as-deposited sample and the samples annealed at 600, 700, 800, and 900 °C were 33.15, 42.54, 40.75, 37.55, and 35.07 mV/pH, respectively. The sample annealed at 600 °C showed the highest sensitivity of all samples as well as the highest linearity. These results can be attributed to the increase in the number of surface sites during the 600 °C annealing process. Annealing at an optimal temperature improves ZnO sensing membrane

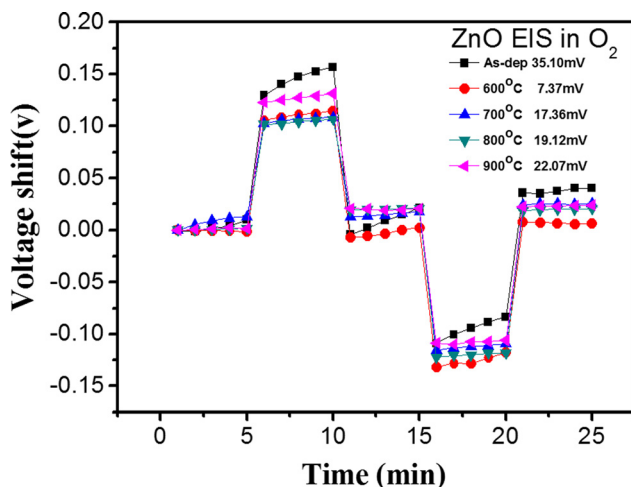


FIG. 5. Hysteresis measurements of the ZnO sensing membrane with various RTA temperatures during a pH loop of 7 → 4 → 7 → 10 → 7 over a period of 25 min.

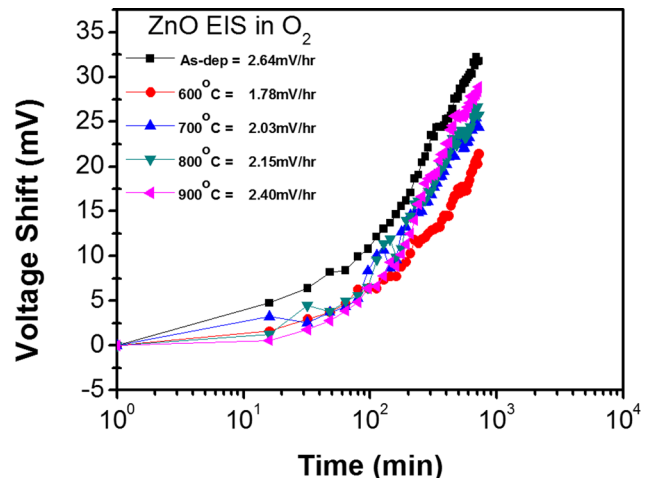


FIG. 6. Drift voltage of the ZnO sensing membrane annealed at various RTA temperatures in O_2 ambient and then dipped in a pH 7 buffer solution for 12 h.

surfaces and maintains good sensing stability in different buffer solutions.

To evaluate the hysteresis effects of the membrane, the samples were immersed in buffer solutions of different pH values in an alternating cycle. All the samples were subjected to a pH loop of 7 → 4 → 7 → 10 → 7 over a period of 25 min. The hysteresis voltage was defined as the gate voltage difference between the initial and terminal voltages measured in the above pH loop. Figure 5 shows the hysteresis voltage of the ZnO samples. The hysteresis voltage of the as-deposited sample and the samples annealed at 600, 700, 800, and 900 °C was 35.10, 7.37, 17.36, 19.12, and 22.07 mV, respectively. Previous studies indicated that the clinging of ions caused by the dangling bonds might hinder the diffusion of the reacting ions and delay the reference voltage response. Since proper annealing at 600 °C might fix the bond connections and fill in the vacancies to reduce defects in an insulator film, the ZnO sensing membrane annealed at 600 °C demonstrated the lowest hysteresis deviation.²⁰

To study the drift rate of the membrane for long-time reliability, the samples were immersed in pH 7 buffer

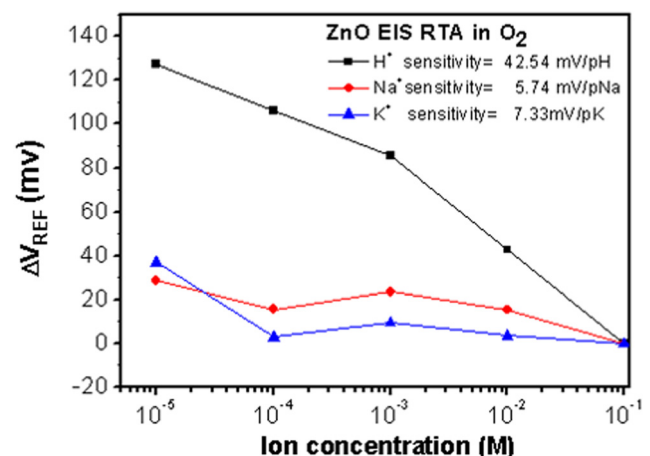


FIG. 7. The H^+ , Na^+ , and K^+ ion sensitivity of the ZnO sensing membrane annealed at 600 °C in O_2 ambient.

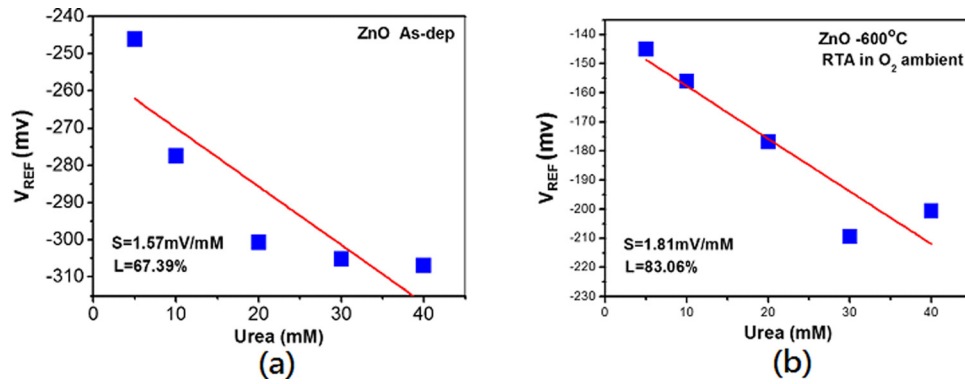


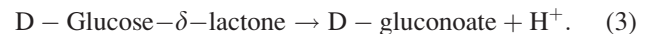
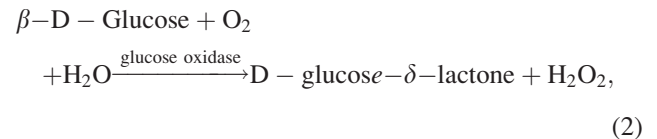
FIG. 8. Urea responses of enzyme-immobilized ZnO from (a) the as-deposited sample and (b) the sample annealed at 600 °C in O₂ ambient.

solutions for 12 h. The drift rates of the ZnO sensing membrane as measured by C-V curve are shown in Fig. 6. The drift rate of the as-deposited sample and the sample annealed at 600, 700, 800, and 900 °C were 2.64, 1.78, 2.03, 2.15, and 2.40 mV/h, respectively. Results show that the ZnO sensing membrane after post-RTA treatment at 600 °C had the lowest drift rate. This is because oxygen treatment may reduce the traps and the dangling bonds, which may gradually capture clusters of ions and cause the reference voltage drift of the device. The dangling bonds, which accumulate at the poles, attract the ions in the electrolyte so extra amount of ions are required to neutralize the dangling bonds and defects.¹⁸ Therefore, dangling bonds worsen the stability of the sensing performance between the electrolyte and the membrane. Eliminating these dangling bonds could cause the reaction between the ions in the electrolyte and the membrane to be more stable.

To analyze the properties of sensing film in the potassium and sodium solution, a micropipette was used to control the concentrations of sodium and potassium ions in a range between 10⁻⁵ and 10⁻¹M by injecting 1M NaCl/Tris-HCl and 1M KCl/Tris-HCl into buffer electrolyte. We also studied the sodium and potassium ion sensitivity of the ZnO sensing membrane under different annealing conditions. The pNa of ZnO samples with as-deposited and annealed samples at 600 °C were 3.41 and 5.74 mV/pNa, while the pK of ZnO samples with as-deposited and annealed samples at 600 °C were 4.97 and 7.33 mV/pK. The sensing capability for potassium and sodium ions could also be improved with proper

annealing. Based on the measurements, ZnO sensing film annealed at 600 °C was more responsive to H⁺ than to Na⁺ and K⁺, as shown in Fig. 7. Furthermore, the film annealed at 600 °C demonstrated higher H⁺, Na⁺, and K⁺ selectivity than films treated under other conditions.

By incorporating suitable enzymes into the pH membranes, urea detection and glucose sensing biosensors can also be created. 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 or glucose can be detected.¹⁷ The urea and glucose hydrolysis equations are written as follows:



To study the urea sensing properties of the ZnO 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 Figs. 8(a) and 8(b).

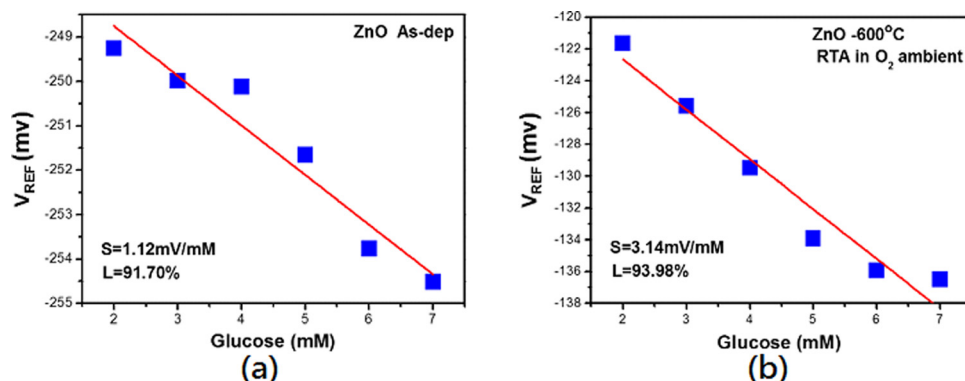


FIG. 9. Glucose responses of enzyme-immobilized from (a) the as-deposited sample and (b) the film annealed at 600 °C in O₂ ambient.

The sensibility and linearity values of the as-deposited sample were 1.57 mV/mM and 67.39%, while the sensibility and linearity values of the sample treated with RTA at 600 °C were 1.81 mV/mM and 83.06%, respectively. As a result, the ZnO sensing membrane after RTA treatment at 600 °C had higher urea sensing properties than the as-deposited sample.

In order to investigate the glucose sensing properties of the ZnO sensing membrane on EIS structure, a glucose solution concentrated in a range between 2 and 7 mM was prepared. The glucose sensing properties are shown in Figs. 9(a) and 9(b). The sensitivity and linearity values of the as-deposited sample were 1.12 mV/mM and 91.70%, while the sensibility and linearity values of the sample treated with RTA at 600 °C were 3.14 mV/mM and 93.98%. It can be seen that the ZnO sensing membrane for glucose detection annealed at 600 °C also had higher glucose sensing properties than the as-deposited sample.

CONCLUSION

In this research, we fabricated an EIS sensor with a ZnO sensing membrane treated with annealing on a silicon substrate for pH sensing. The material properties of ZnO films were examined by XRD and AFM analyses. Consistent with material analyses, the results indicate that the EIS sensor with a ZnO membrane annealed at 600 °C exhibited good sensing performance with a high sensitivity of 42.45 mV/pH compared with all other reported ZnO-based chemical sensors. In addition, all other sensing capability, including the hysteresis voltage and the drift rate, could be achieved at an optimal temperature of 600 °C. Improvements caused by annealing might contribute to an increase in surface sites and elimination of defects. Additionally, sensing performance of ZnO-based EIS sensors for various ions of Na⁺, K⁺, urea, and glucose was tested. ZnO-based EIS sensors show promise for future industrial biosensing applications.

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