



## Development of FET-Type Reference Electrodes for pH-ISFET Applications

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A reference field effect transistor (FET) fabrication method by using a perfluorosulfonated proton exchange membrane associated with ion-insensitive polymers is proposed. The single-layer film of the perfluorosulfonated polymer/photoresist composite among the eight films tested demonstrated the best performance of 5.8 mV/pH and 11.27 mV/pNa sensitivities. Meanwhile, the drift performances were 3.5 mV/h and less than 1 mV/h for the first and second 4 h tests. A high sensitivity of 52.1 mV/pH and a low interference of 4.61 mV/pNa were obtained in the range of pH 1–13 through the differential arrangement with ZrO<sub>2</sub> gate ion-sensitive field-effect transistors (ISFETs). Meanwhile, the transconductance match of the proposed reference FET/ISFET pair would simplify the differential readout circuits.

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Because the ion-sensitive field-effect transistors (ISFETs) were introduced by Bergveld<sup>1</sup> in 1970, they appear to be one of the potential sensors to be used for biological applications due to the advantages of fast response, high maturity, economical scale of semiconductor process, and feasibility of miniaturization, which is attractive for in situ measurements. However, a micro-ISFET requires a miniaturized on-chip reference system, which is challenging for fabrication processes. There are two categories of on-chip reference systems under development: The first type includes miniaturized Ag/AgCl reference electrodes with encapsulated liquid reference solutions;<sup>2–4</sup> however, the leakage of the reference solutions limits the device lifetime and affects the measurement accuracy. The other type is reference field effect transistor (REFET), which was first proposed by Matsuo and Esashi<sup>5</sup> and Janata and Huber.<sup>6</sup> Matsuo first introduced the concept of a REFET, which is an identical ISFET whose sensing layer is modified to be chemically insensitive. Janata incorporated the REFET into a system that consisted of an ISFET/REFET pair, a quasi-reference electrode (QRE), and differential measurement circuits. QRE is simply a noble metal that replaces a complicated reference electrode; however, the potential in the metal/liquid interface is unstable because the interface is polarized. Therefore, the interfacial potential is thermodynamically undefined, which becomes the common noise of the ISFET/REFET differential system. To eliminate this common noise and maintain measuring sensitivity, the development of REFETs should match with ISFETs to satisfy the requirements of differential measurements.

Several approaches to fabricate REFETs have been proposed. Matsuo and Nakajima<sup>7</sup> used an ion-blocking parylene as a chemically inert membrane. However, quasi-instability problems made parylene-gated REFET not practical in a differential ISFET/REFET measurement setup.<sup>8</sup> Another method to produce REFET was through a grafted membrane with a long alkyl chain silane;<sup>9</sup> nevertheless, the result shows that the gate surface sites cannot be captured sufficiently to obtain a low pH sensitivity. Van den Berg et al. reported that the total elimination of the pH-sensitive groups by chemical monolayer modification cannot be achieved.<sup>10</sup> A poly(vinyl chloride) membrane has also been used to form an ion-insensitive layer,<sup>11–17</sup> and normally their operating range is in between pH 2 and 9. Through kinds of modification, such as silylating pretreatment,<sup>13</sup> modifying a membrane by adding lipophilic cations,<sup>14</sup> and using a buffered poly(2-hydroxyethyl methacrylate)<sup>15</sup> or negative photoresist (PR)<sup>16</sup> layers, polymer-based low sensitivity ion-unblocking REFETs could be produced.

Nafion is a perfluorosulfonated material and is resistant to chemical attack. It has been widely used for ion exchange or immobiliza-

tion applications. Nafion was used as an outer layer to immobilize the chloride ion or nanoparticles while developing electrodes of pH sensors.<sup>18</sup> It was also used for pH measurement with a decrease in oxidation–reduction potential error.<sup>19</sup> Anh et al. utilized the Nafion membrane to reduce ion interferences from the electrolyte.<sup>20</sup> Nafion is also popular at biosensor fields,<sup>21,22</sup> where enzymes were immobilized with Nafion.

In this work, Nafion was introduced to combine with ion-insensitive polymers for REFET system fabrication. The sensitivity, electrical properties, and drift behavior were investigated.

### Experimental

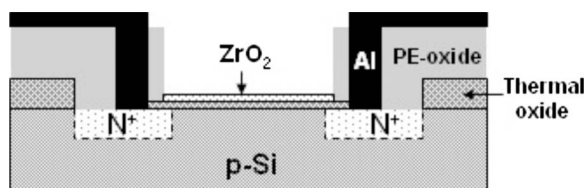
The Nafion solution (5 wt %) was obtained from DuPont, and pH buffer solutions were purchased from RDH (Frankfurt, Germany). Hexamethyldisilazane (HMDS) and PR (FH6400) were obtained from the Nano Facility Center, National Chiao Tung University. The preparation of the NaCl solution was for the Na<sup>+</sup> ion measurement. The NaCl salt was electronic grade, and solutions were prepared in deionized water with different mole concentrations, 10<sup>−3</sup>, 10<sup>−2</sup>, 10<sup>−1</sup>, and 1 M.

The fabrication of ZrO<sub>2</sub>-gated ISFETs was reported.<sup>23</sup> Figure 1 shows the schematic diagram of the ZrO<sub>2</sub>-gated ISFET fabricated by the metal-oxide-semiconductor field-effect transistor (MOSFET) technique. A 30 nm thick sensitive layer of the ZrO<sub>2</sub> membrane was deposited onto the SiO<sub>2</sub> gate ISFET by dc sputtering with 4 in. diameter and 99.99% purity of zirconium in oxygen atmosphere. The total sputtering pressure was 20 mTorr in the mixed gases Ar and O<sub>2</sub> for 200 min, while the base pressure was 3 × 10<sup>−6</sup> Torr, the radio-frequency power was 200 W, and the operating frequency was 13.56 MHz. To test the performance of REFETs, a QRE was also fabricated with Ti/Pt deposition by sputtering with a thickness of 150/350 Å.

Ion-insensitive polymer materials, PR (FH6400), and conductive polymer, poly(3-hexylthiophene) (P3HT), were incorporated to modify the ZrO<sub>2</sub> film. Two types of membranes were prepared and tested: One type was a double-layer structure, as shown in Fig. 2a; the Nafion and polymer-based materials, PR (FH6400) or P3HT, were deposited on the top of the original sensing layer (ZrO<sub>2</sub>) step by step. The other type was a single layer, as shown in Fig. 2b, with polymers entrapped in the Nafion solution and then casting on the top of the ZrO<sub>2</sub> film. To improve the adhesion of PR and P3HT with ZrO<sub>2</sub>, thin HMDS was pretreated on the ZrO<sub>2</sub> surface in advance. All of the above membranes were heated at 70°C for 1 min and then dried in air for over 12 h. The epoxy resin film, which is an excellent electrical insulator, was deposited by casting on the top of the ZrO<sub>2</sub> layer to form the ion-blocking membrane for the electrical property comparison test.

Figure 3 shows the measurement setup for the REFET system evaluation. A container was bonded to enclose the gate region of

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**Figure 1.** The schematic diagram of the  $\text{ZrO}_2$  gate ISFET fabricated by MOSFET technique.

ISFET by using epoxy resin. An HP4156A semiconductor parameter analyzer was used to investigate and collect the electrical characteristics of different types of polymer-based sensing layers. The  $I_{\text{ds}}-V_{\text{gs}}$  curves were obtained with constant drain-source voltage  $V_{\text{ds}} = 2 \text{ V}$ , and each condition was tested with three samples. For the drift performance test, the same device was soaked in the buffer solution for the first and second tests, which lasted for 4 h, respectively. The device was dried in air in between the two tests. To prevent from light influence, the measurements were entirely executed in a dark box.

### Results and Discussion

Figure 4a and b shows the pH and pNa sensitivities of the sole  $\text{ZrO}_2$  membrane and with Nafion coating, respectively. The pH and pNa sensitivities of the  $\text{ZrO}_2$  membrane with and without Nafion coating are the same. According to the study of Gorchkov,<sup>24</sup> the pH response of an ISFET would be influenced by the natures of the polymeric membranes, as the consequence of the “equivalent membrane charge.” Therefore, the slightly higher sensitivity of the  $\text{ZrO}_2$  membrane with the Nafion coating could be explained as extra cations stored in the Nafion membrane that leads to a surface potential increase. However, the effect is limited. Meanwhile, Nafion is a perfluorinated polymer that can be divided into three parts: a hydrophobic fluorocarbon backbone C-F, an interfacial region of relatively large fractional void volume, and clustered regions where the majority of the ionic exchange sites, counter ions, and absorbed water exist.<sup>25,26</sup> Accordingly, the Nafion membrane was a low im-

pedance film, which unblocks the hydrogen and sodium ions; those ions from the electrolyte can pass through the Nafion membrane with a low resist and an established potential on the  $\text{ZrO}_2$  surface. The pNa sensitivity was much lower than the pH sensitivity. The result reveals that it was mainly dominated by the selectivity property of the  $\text{ZrO}_2$  film, though the fact that the mobility of  $\text{H}^+$  was about 5 times that of  $\text{Na}^+$  in the Nafion membrane.<sup>27</sup> The selectivity coefficient can be determined by the methods based on the Nicolsky–Eisenman equation.<sup>28</sup> Meanwhile, the linearity of the pNa response was not as good as the pH response. The difference might be that the Nafion membrane has a slightly higher affinity for  $\text{Na}^+$  than for  $\text{H}^+$ ,<sup>27</sup> which affects the interface potential between the Nafion membrane and the  $\text{ZrO}_2$  film. As shown in Fig. 4c, the  $I_{\text{ds}}-V_{\text{gs}}$  curves were similar for both membranes except the threshold voltage shift; it revealed that  $\text{ZrO}_2$  films with or without Nafion coating have identical electrical characteristics. The REFETs produced by additional membrane coating on the top of the ISFETs should maintain the original electrical properties, such as transconductance, for differential measurements.

Table I and Fig. 5 show the measurement results of pH and pNa sensitivities for the tested conditions. Sensitivities to the hydrogen ions for all tested polymer-based REFETs were below 10 mV/pH except P3HT without HMDS pretreatment. However, pH sensitivities of all the REFETs were reduced. The reasons for the dramatic pH sensitivity decrease can be explained by employing a site-binding model.<sup>29,30</sup> The pH sensitivity of an ISFET can be expressed by Eq. 1 and 2

$$S = \frac{\partial \varphi_0}{\partial \text{pH}_B} = -2.3 \frac{kT}{q} \alpha \quad [1]$$

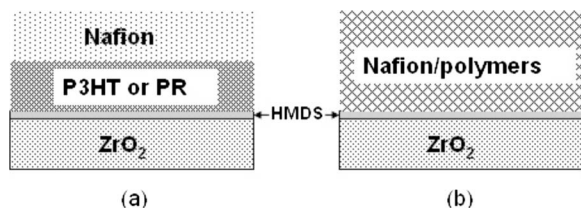
and

$$\alpha = \frac{1}{\frac{2.3kTC_{\text{dif}}}{q^2\beta_{\text{int}}} + 1} \quad [2]$$

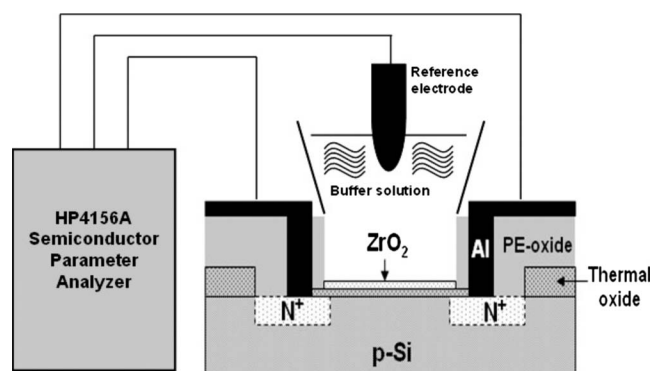
where  $\alpha$  is a dimensionless sensitivity parameter that varies between 0 and 1, which is determined by the intrinsic buffer capacity,  $\beta_{\text{int}}$ , of the sensing layer surface and the differential double-layer capacitance  $C_{\text{dif}}$ . According to Eq. 1 and 2, large values of  $C_{\text{dif}}/\beta_{\text{int}}$  would result in a lower pH sensitivity. This means that surfaces with small buffer capacity (small value of surface reactive sites and of surface charges) show low pH sensitivity. For the double-layer polymer/Nafion as shown in Fig. 2a, the sensing layers were PR and P3HT, whose surface site densities are smaller than  $\text{ZrO}_2$ ; therefore, the pH sensitivities were low. For the single-layer PR/Nafion composite, as shown in Fig. 2b, it was long chain hydrocarbon molecules which have the characteristic of a small buffer capacity and result in a dramatic decrease in the pH sensitivities. For the single-layer P3HT/Nafion composite, because P3HT is the conductive polymer that provides extra paths for electrons to pass through the membrane, the pH sensitivity was higher than that of the PR/Nafion composite.

HMDS is an adhesion promoter for hydrophobic polymers that attempt to attach on hydrophilic inorganic films. By comparing the pH sensitivity of the tested composite films 2 and 3, 4 and 5, and 6 and 7, all films with the HMDS layer have less sensitivity variation than those without the HMDS layer. It shows that the additional deposition of HMDS improved the stability of test films. Polymer-based HMDS also reduced the pH sensitivity. Figure 6 shows the  $I_{\text{ds}}-V_{\text{gs}}$  curves of the HMDS coated  $\text{ZrO}_2$  membrane. The sensitivity was decreased from 57.89 to 37.22 mV/pH, and the coating of HMDS caused more than a 35% reduction in sensitivity. Accordingly, the insensitivity character of REFET was not only dominated by the entrapped hydrophobic polymers but also contributed by HMDS. It was evident by comparing films 2 and 3, 4 and 5, and 6 and 7 that the films with the HMDS layer obtained a lower pH sensitivity than those without the HMDS layer.

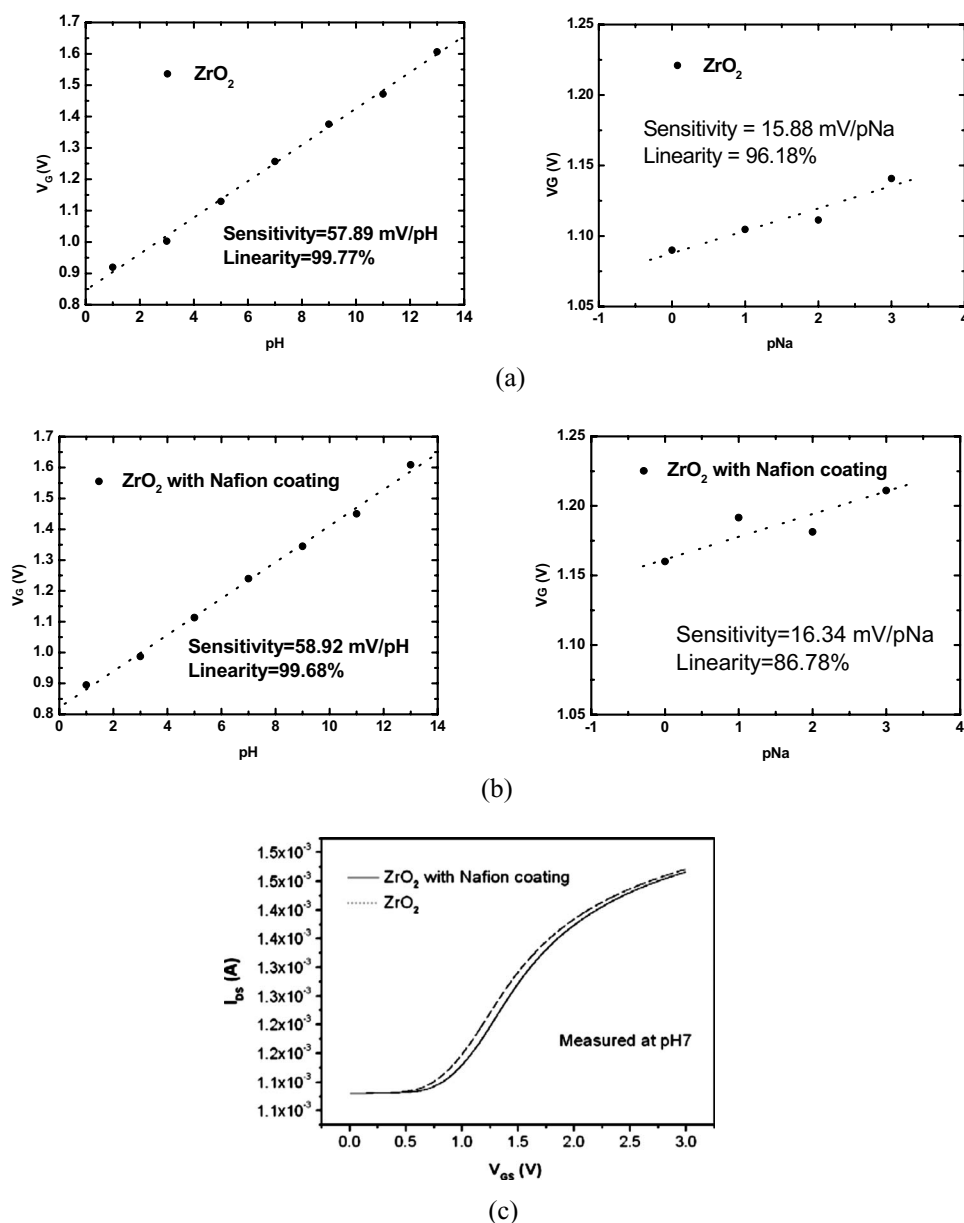
Among all the test films, the combination of Nafion-PR composite/HMDS has the lowest pH sensitivity, and the similar pNa



**Figure 2.** Tested membranes prepared with two methods: (a) Encapsulation and (b) entrapment.



**Figure 3.** Setup of measurement using HP4156A semiconductor parameter analyzer.



**Figure 4.** Hydrogen and sodium sensitivities of (a)  $\text{ZrO}_2$  and (b)  $\text{ZrO}_2$  with Nafion coating gated ISFETs. The pH and pNa sensitivities are (a) 57.89 and (b) 58.92 mV/pH and (a) 15.88 and (b) 16.34 mV/pNa, respectively. (c) The  $I_{\text{ds}}-V_{\text{gs}}$  curves of  $\text{ZrO}_2$  with and without Nafion coating.

response as the  $\text{ZrO}_2$  film, as shown in Fig. 7, demonstrated the capability to serve as a REFET. Comparing with Fig. 4b, the linearity became worse for the pH response but became better for pNa. The reasons for both cases are different. For pH, it is because the measured potential range was highly reduced that the measurement error becomes more significant. For pNa, a possible reason is that

the property of the Nafion film was altered by the mixed composition, which restricted the transport of  $\text{Na}^+$  and the potential variation and, therefore, became more stable.

Figure 8 shows the differential result of the pH and pNa mea-

**Table I.** Responses of hydrogen ions and sodium ions for tested structures.

Test structure	$\text{H}^+$ sensitivity (mV/pH)	$\text{H}^+$ linearity (%)	$\text{Na}^+$ sensitivity (mV/pH)	$\text{Na}^+$ linearity (%)
1 $\text{ZrO}_2$	57.89	99.8	15.88	96.2
2 NF/P3HT/ $\text{ZrO}_2$	36.11	99.6	20.17	81.8
3 NF/P3HT/HMDS/ $\text{ZrO}_2$	8.07	96.5	39.21	99.1
4 NF/PR/ $\text{ZrO}_2$	9.92	99.5	9.9	97.9
5 NF/PR/HMDS/ $\text{ZrO}_2$	7.43	94.3	28.27	96.9
6 NF-PR composite/ $\text{ZrO}_2$	8.47	98.5	19.89	97.9
7 NF-PR composite/HMDS/ $\text{ZrO}_2$	5.8	96.3	11.27	98.2
8 epoxy resin/ $\text{ZrO}_2$	4.7	93.9	—	—

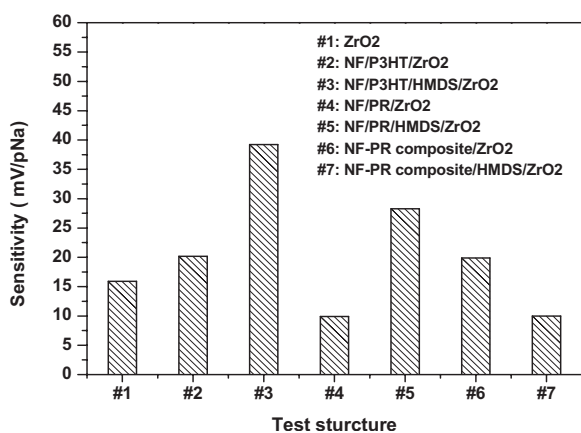
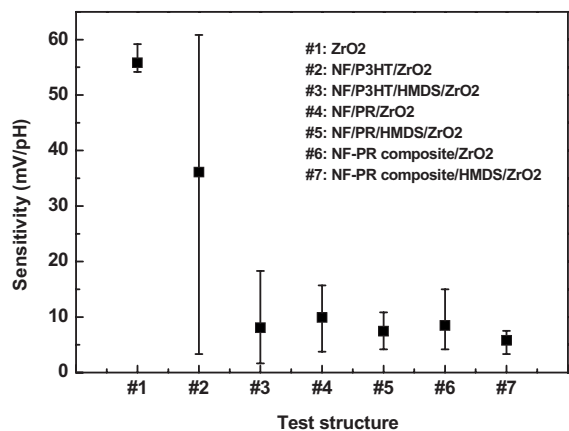


Figure 5. Measurement results of pH and pNa sensitivity for tested structures.

measurements for the ISFET/REFET arrangement; responses with more than 50 mV/pH sensitivity and less than 5 mV/pNa interference can be obtained in the range of pH 1–13.

Polymers entrapped in Nafion play the role of blocking the most paths of ion exchange to achieve a low ion sensitivity requirement for REFET. Nevertheless, the induced impedance change by the alteration of the overall membrane composition should be considered. Figure 9 describes the transconductance ( $g_m$ ) curves of the ISFET, the proposed REFET, and the REFET with epoxy coating membranes. The  $g_m$  curve with epoxy-REFET shows different behaviors from the other two. On the other hand, the curves of the

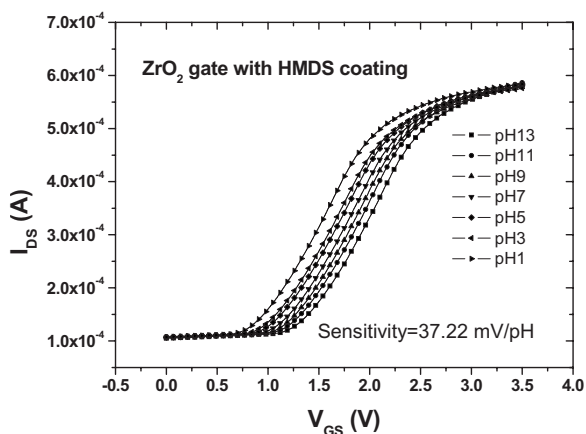


Figure 6.  $I_{ds}$ - $V_{gs}$  curves of HMDS coated  $ZrO_2$  membrane.

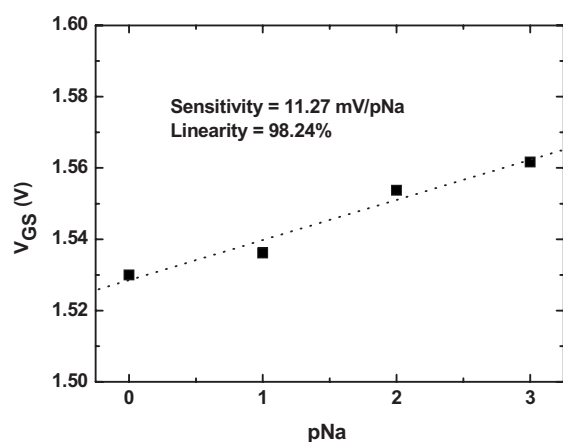
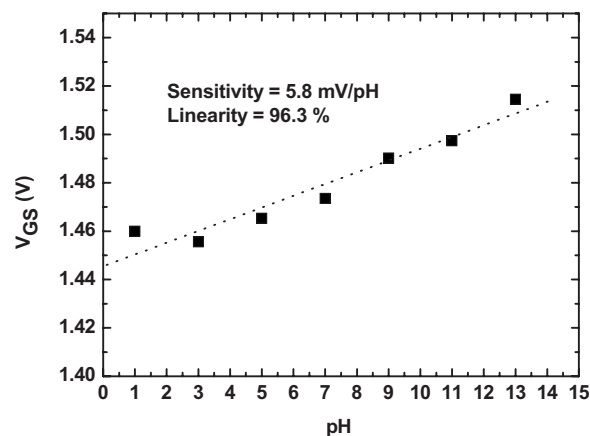
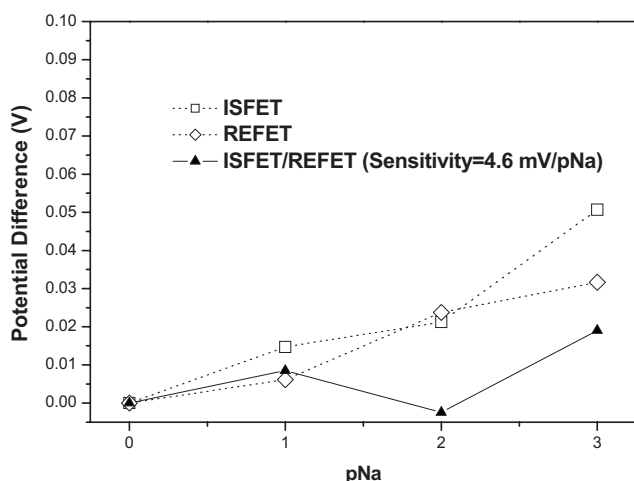
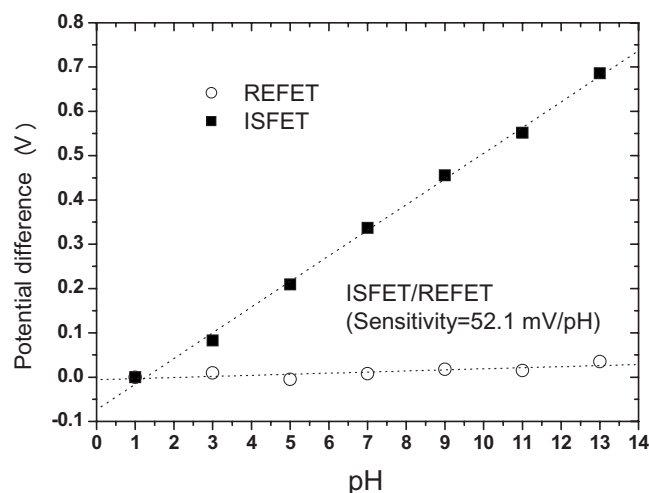


Figure 7. The pH and pNa responses of Nafion-PR composite/HMDS/ $ZrO_2$  ISFET.

proposed REFET and ISFET show similar curvatures in the linear region ( $V_{gs}-V_{th}:0-1.2$  V). The  $g_m$  curves represent the signal transformation characteristics of the device. An effective differential measurement requires similar  $g_m$  curvatures for ISFET and REFET in pair. Otherwise, mismatch issues would occur. Because the epoxy is an ion-blocking membrane that causes a polarizable interface whose potential difference changes as a consequence of the potential difference across the whole system, it would alter the original electrical properties of an ISFET. Consequently, the electrical mismatch of the ISFET/REFET differential pair would result in the common mode noises, which cannot be entirely eliminated. On the contrary, an ion-unblocking REFET as proposed causes a nonpolarizable interface, whose potential difference across it is virtually fixed. Meanwhile, it maintains the electrical properties of the original film and makes the ISFET/REFET differential pair match, which can easily eliminate the common mode noises with simple readout circuits. The  $g_m$  of the  $ZrO_2$  membrane was slightly altered due to the addition of PR; however, the electrical characteristic in most linear regions was not altered accordingly, which provided an operating region for the ISFET/REFET pair in wide dynamic pH measurements.

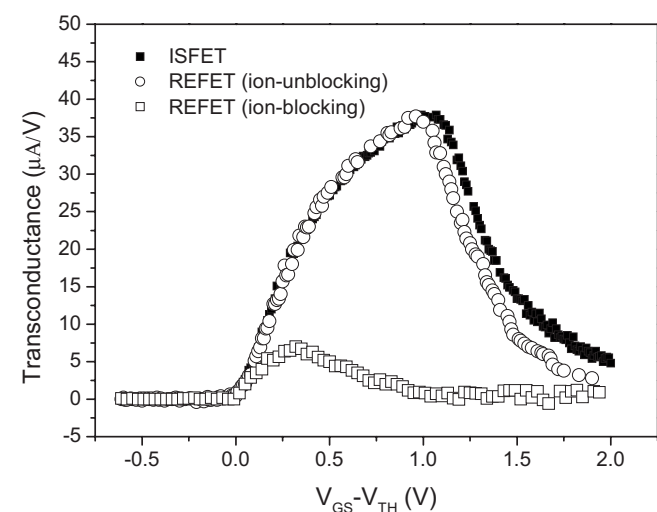
In this experiment, it was found that the measurement could not be conducted without the Nafion protection because P3HT and PR alone would dissolve in the buffer solution within a very short time period. The drift performances of the Nafion-PR composite/HMDS film were 3.5 mV/h and less than 1 mV/h for the first and second 4 h tests, as shown in Fig. 10. It demonstrated that the incorporation of the Nafion enhanced the stability of the proposed REFET.



**Figure 8.** Measurement results of pH and pNa response with ISFET/REFET differential arrangement.

### Conclusion

In this study, a method of fabricating reference ISFETs by combining Nafion and ion-insensitive polymers was proposed. With a



**Figure 10.** Drift performance of the proposed REFET.

differential arrangement, sensitivities of 52.1 mV/pH and 4.61 mV/pNa based on the  $ZrO_2$  sensing film were obtained in the range of pH 1–13; it demonstrated good performance for ISFET applications. Meanwhile, the drift performances were 3.5 mV/h and less than 1 mV/h for the first and second 4 h tests. With the high pH sensitivity and the low interference of pNa performed by the proposed functional polymer combination, a chemically protective ion-unblocking membrane associated with the ion insensitivity polymers provides a promising way for REFET fabrications. Meanwhile, the transconductance match of the proposed REFET/ISFET pair would simplify the differential readout circuits.

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**Figure 9.** Transconductance ( $g_m = dI_{ds}/dV_{gs}$ ) of the  $ZrO_2$ -gated ISFET and the proposed REFET (ion-unblocking) and REFET with epoxy coating on  $ZrO_2$  gate (ion-blocking).

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