

## Developing a Solid-State pH Sensor for Wagyu-Rumen Monitoring

Lan Zhang,<sup>1\*</sup> Jian Lu,<sup>1</sup> Hirofumi Nogami,<sup>1,2</sup> Hironao Okada,<sup>1</sup> and Toshihiro Itoh<sup>1,3</sup>

<sup>1</sup>Research Center for Ubiquitous MEMS and Micro Engineering (UMEMSME),  
National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki 305-8568, Japan

<sup>2</sup>Department of Mechanical Engineering, Kyushu University, Fukuoka 819-0395, Japan

<sup>3</sup>Department of Human and Engineered Environmental Studies, The University of Tokyo,  
Kashiwa, Chiba 277-0882, Japan

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We have developed a solid-type pH sensor for dairy wagyu-rumen measurements. The pH sensor is composed of a sensing electrode of indium tin oxide (ITO) film and a metal–oxide–semiconductor field-effect transistor (MOSFET). In the solid-state sensor structure, the ITO electrode is a separated gate of the FET and the ITO electrode can be patterned with a suitable capture structure for the wagyu-rumen environment. A sensor system with a compact size can be fabricated by eliminating the reference solution. The pH sensors have a sensitivity of 10–15  $\mu\text{A}/\text{pH}$  under different sensing contact areas from 12 to 78  $\text{mm}^2$ . Long-term tests with interval experiments have been completed in 18 d, and the results demonstrate that the pH sensor has a stable performance for rumen pH measurements over a long time.

### 1. Introduction

The wagyu (Japanese cow) produces marbled meat with high levels of oleaginous unsaturated fat.<sup>(1)</sup> Wagyu beef is well known for its high quality, and it commands a high price. During the growth period of wagyu, their ruminal pH values are important physiological parameters because they affect the nutritional status of the cows. If a wagyu cow is fed a high level of rapidly digestible carbohydrates, its rumen pH value may decrease from the normal range of pH 7 (on average) to below pH 5.5. Low rumen pH (<5.5) causes the wagyu to experience rumen acidosis, which can develop into a life-threatening emergency if it is not discovered in time.<sup>(2)</sup> An urgent need exists to develop a novel method for sensing the daily range of the rumen pH to replace the traditional method of rumenocentesis.<sup>(3,4)</sup>

In the last decade, micro-electromechanical systems (MEMS) have been extensively developed. MEMS sensors have played very important roles and have been widely used in a variety of sensing applications in physics,<sup>(5)</sup> medicine,<sup>(6)</sup> biology, and chemistry.<sup>(7)</sup> Among the subjects investigated, a pH test has great significance, not only for field measurements but also in lab experiments, because a large number of chemical and biological processes depend on pH. There are many techniques that can be used to detect pH values, such as pH image sensors,<sup>(8)</sup> glass electrodes,<sup>(9)</sup> conductimetric pH sensors,<sup>(10)</sup> cantilevers,<sup>(11)</sup> optical fiber pH sensors,<sup>(12)</sup> and ion-sensitive field-effect transistor

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\*Corresponding author: e-mail: chou-ran@aist.go.jp

(ISFET)-based pH sensors.<sup>(13)</sup> Each experimental approach has its own merits and drawbacks.

Typical ISFET-based pH sensors may be solid state and small in size due to their fabrication and principle of glassless operation. However, because of the inherent structure of ISFETs, the components of drain, source, and gate are all integrated into one structure, which causes the ISFET-based pH to be measured over a relatively small sensing area (normally  $<1 \text{ mm}^2$ ). This feature restricts the application of ISFET-based sensors for measuring wagyu-rumen pH values. Because the wagyu rumen has a very complicated inner structure, an all-in-one structure sensor can easily malfunction during constant direct interaction with the gas and fluid in the rumen over a long time period. Moreover, the digested forage in the rumen normally consists of particles at the millimeter level in size, which is relatively large. Therefore, the digested forage can easily cover the small sensing area of a conventional ISFET sensor and causes the measurement accuracy to inevitably decrease.

Thus, to solve this problem and realize the long-term monitoring of pH in rumen, we developed a novel FET-based pH sensor of the solid type with a compact size. The pH sensor is composed of a sensing electrode of ITO film and a metal-oxide-semiconductor field-effect transistor (MOSFET). The ITO sensing film is a separate component and connects with the gate terminal of FET. In this way, the pH sensor can enjoy the advantages of both ITO and FET sensing techniques: the solid-state sensor structure is suitable for long-term pH measurement; the sensor system can be fabricated with a compact size by eliminating the reference solution, and the separate ITO sensing electrode can be patterned with a suitable capture structure for the environment in the rumen of wagyu. Figure 1(a) shows a schematic view of the sensing and reference electrodes. Figure 1(b) shows the assembled pH sensor bolus; in use, the measurement system is sealed. The cylindrical sensor bolus has a dimension of 78 mm length and 30 mm bottom diameter. Figure 1(c) shows the typical cross

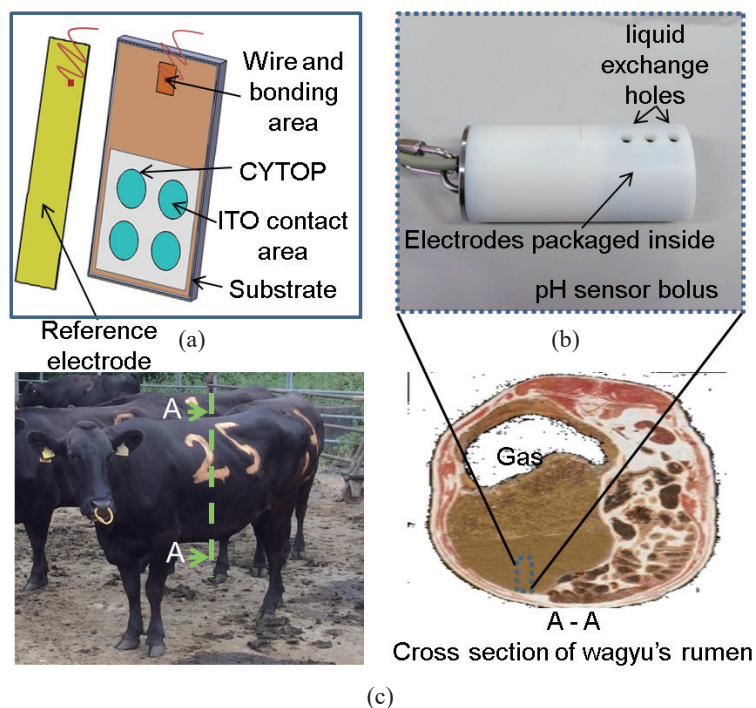


Fig. 1. (Color online) Rumen pH measurement system and sensor target testing position in a wagyu's rumen.

section of the wayu's rumen and the working position of the target sensor. In field measurements, the pH sensor should reside at the bottom of the rumen, which is a secure location with limited movement and a fluid-like content [see Fig. 1(c)]. The detection circuit and relative components are packaged into a polytetrafluoroethylene (Teflon) container. The filling material (Gel, Exseal Co., Ltd.) water-proofs the inner structure and also weighs down the sensor so it achieves the targeted position.

In this work, in the pursuit of high sensing sensitivity and high manufacturing yield, a comparison between the sensitivity and sensing area was made. To evaluate the working performance of the rumen pH sensor, the noise level was analyzed. Finally, a pH rumen sensor should work continuously for at least 2 weeks to provide the farmers with the information of acidosis (a rumen pH of less than 5.5) and help them to adjust their feeding strategy to avert the developing problem. Thus, to understand whether the developed sensor could maintain a stable baseline and remain stable over a long time, relatively long-term experiments with interval testing were carried out.

## 2. Experimental Methods

### 2.1 Fabrication of the pH sensor

Figure 2 shows the schematic view of the process to fabricate a typical pH sensing electrode. In this work, only three photomasks were used to complete the micromachining of the ITO sensing electrode. A 4-inch-diameter and 400- $\mu\text{m}$ -thick standard silicon wafer was used to make the sensor electrode substrate. As Fig. 2(a) shows, the silicon wafer substrate was immersed in a  $\text{H}_2\text{SO}_4 +$

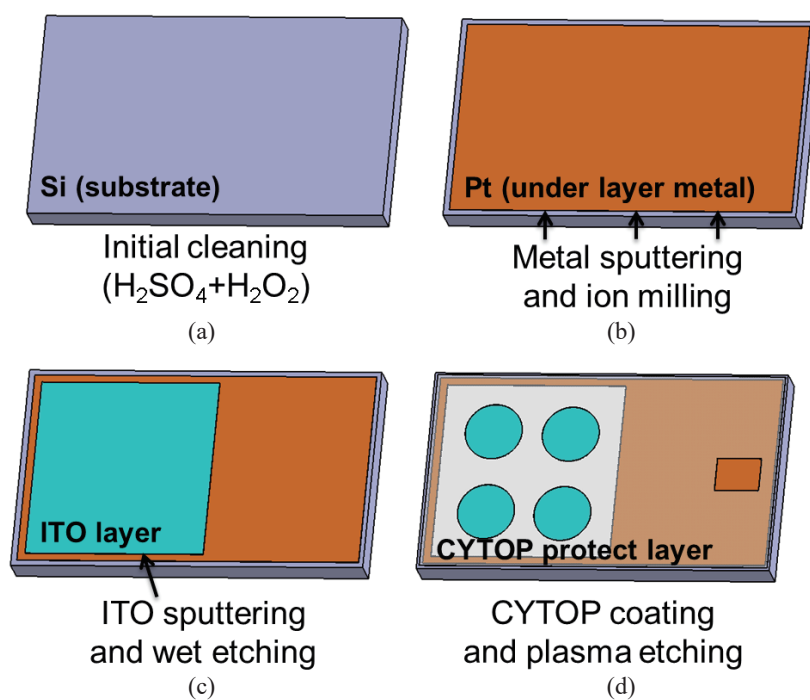


Fig. 2. (Color online) Schematic view of the fabrication sequence of the ITO electrode.

$\text{H}_2\text{O}_2$  solution at  $100\text{ }^\circ\text{C}$  for initial cleaning. To fabricate the underlayer metal electrode, as Fig. 2(b) shows, a 200-nm-thick Pt layer was generated and patterned by sputtering and ion milling processes, respectively. Then, an ITO (the proportion of  $\text{In}_2\text{O}_3$  to  $\text{SnO}_2$  was 9:1) film with a sheet resistance of  $10\ \Omega/\text{sq.}$  on average was coated on the target wafer by sputtering [Fig. 2(c)]. An HCl solution was used to wet-etch the ITO-sensing film with the desired pattern. Next, a 2- $\mu\text{m}$ -thick amorphous fluoropolymer (CYTOP®, Asahi Glass Co., Ltd.) layer was coated onto the ITO layer by spin coating and a post-bake treatment [Fig. 2(d)]. Finally, the CYTOP film was patterned by dry etching with an  $\text{O}_2$  plasma [Fig. 2(d)]. The patterned CYTOP layer in this MEMS device should protect the ITO sensing film in the complicated environment of the rumen and enable us to realize a sensing electrode with a desired contact area. As a result of that, the fabricated ITO sensing electrode has a dimension of 20 mm length and 15 mm width, and the contact areas are fabricated to be round with a total contact area of 12–78  $\text{mm}^2$ . The reference electrode is fabricated via a Ag film coated on a Si substrate; it has the same dimensions as the ITO sensing electrode.

## 2.2 Experimental setup

Figure 3 shows that the pH measurement system combines a MOSFET with a sensing electrode made of ITO film, an ITO electrode (which is a separate component) that connects the gate (G) terminal of the FET, and an n-type channel part (MOS transistor, Texas Instruments). A power supply provides the reference voltage ( $V_{\text{ref}}$ ) between the reference electrode and ground and working voltage  $V_{\text{dd}}$  for the FET. A source meter is used to provide the voltage ( $V_{\text{ds}}$ ) between the terminals of the drain (D) and source (S) and to simultaneously measure the current ( $I_{\text{ds}}$ ) between them. The sensor electrodes are immersed in three standard buffers with pH values of 4, 7, and 9, respectively. Phthalate, phosphate, and borate solutions with known pH values are used to calibrate the function of  $I_{\text{ds}}$  and the pH value.

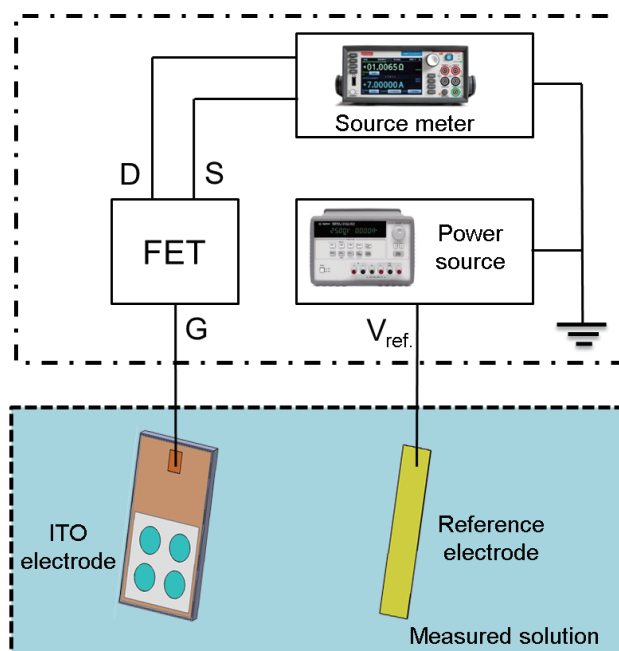


Fig. 3. (Color online) Experimental set up of pH-sensing system.

## 2.3 Experimental apparatus

A sputter (SME-200E, Ulvac Co., Ltd.) was used to generate the ITO and metal films on the Si wafer. Ion milling (M-8038, NS Co., Ltd.) equipment was used to etch the underlying metal film with a desired pattern, and a reactive ion etching device (RIE-10NRS, SAMCO Co., Ltd.) was used to etch the amorphous fluoropolymer of the CYTOP film. To investigate the effects of the wet-etching process on the ITO electrode, scanning electron microscopy (SEM S-3000H, Hitachi Co., Ltd.) and atomic force microscopy (AFM L-trace, SII Nanotechnology Co., Ltd.) were used to observe the pH sensor's surface. A touch-panel sourcemeter (2450, Keithley Co., Ltd.) was used to provide the voltage and measure the current between the drain and source of the FET. A dual-output DC power supply (E3620A, Agilent Co., Ltd.) was used to provide the reference and working voltages.

## 3. Results

### 3.1 SEM and AFM measurement of the pH sensing electrode

Figure 4 shows the SEM image of ITO film, and inserts are images of a pick-up sensing electrode and a local AFM scanning image. Using the image produced by AFM probing, the morphology of the ITO film after the etching process was investigated. Figure 4 (insert) shows that the surface roughness of the ITO coating films is in the range of several nanometers [the arithmetic average roughness ( $Ra$ ) and the square mean roughness ( $RMS$ ) are 1.237 and 1.635 nm, respectively]. As shown by the SEM and AFM images, the ITO film on the electrode has a smooth surface with few misalignment grains after the etching processes. The fabricated ITO-electrode surface remains fine and uniform; this phenomenon indicated that the microstructure of the thin film was well-protected during micromachining. This characteristic also represents an excellent feature because a smoothly coated film will have less effect on the succeeding procedural steps. In addition, the fabricated film with less morphological defects is considered to be very suitable for use in the

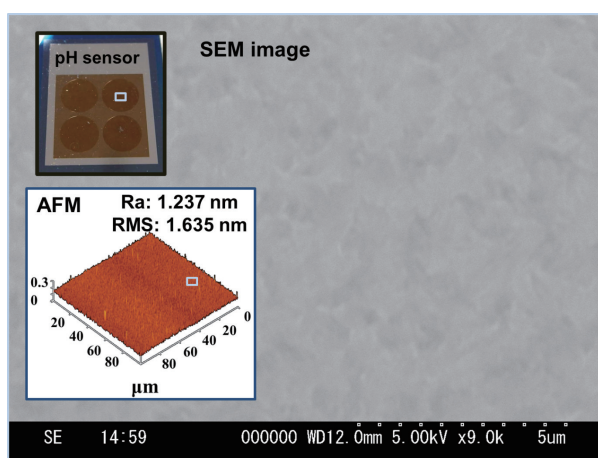


Fig. 4. (Color online) SEM image of the ITO film; inserts are images of a pick-up sensing electrode and a local AFM scanning image.

development of an electronic sensing device. Because in some cases, if the functional film has a rough grain surface, the intrinsic mechanical strength of the film itself will be decreased and the material intrinsic charge transport properties will also be obscured.

### 3.2 $V_{ds}$ - $I_{ds}$ characteristics

Figure 5 shows the  $V_{ds}$ - $I_{ds}$  characteristic of a separated ITO electrode connecting to the FET gate for a fixed reference voltage  $V_{ref}$  of 3.5 V and a  $V_{ds}$  value varied over a range from 0 to 0.6 V. The sensor electrodes are immersed in three standard pH buffered solutions with pH values of 4, 7, and 9. The output current was high in acid and low in alkali, obviously. The insert in Fig. 5 shows the  $I_{ds}$  output when  $V_{ds}$  is in the range from 0.2 to 0.4 V. The current curves had noticeable spaces among the different pH solutions with the different values of 33.75 and 22.5  $\mu$ A; they correspond to the differences between pHs 4 to 7 and pHs 7 to 9, respectively. Moreover, when  $V_{ds}$  was set in the range from 0 to 0.6 V, the output current of the FET operated in the ohmic region. If the FET operates in the ohmic region, it can provide the pH sensor with a linear output. A thorough analysis must consider both the desired high sensitivity and low power consumption; thus, the working voltage of  $V_{ds}$  can be maintained at 0.3 V.

### 3.3 Characteristics of sensitivity and contact area

Figure 6 shows a comparison of sensor sensitivity with the contact area of the ITO electrode. A change in the contact area of the ITO electrode in the range from 12 to 78  $\text{mm}^2$  for the sensor results in a sensitivity in the range from 10 to 15  $\mu\text{A}/\text{pH}$ . The sensitivity of the pH electrode increased as the contact area was increased, but the ratio of the change is not proportional to the change in ratio of the contact area. This phenomenon may be partially caused by the fact that the current sharing effect causes a decrease in the FET output signal. The pH sensor sensitivity of the small

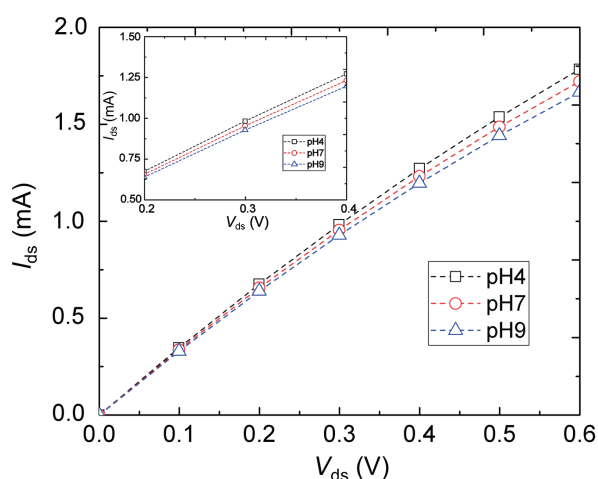


Fig. 5. (Color online)  $V_{ds}$ - $I_{ds}$  characteristics of the sensing electrode in solutions with different pH values.

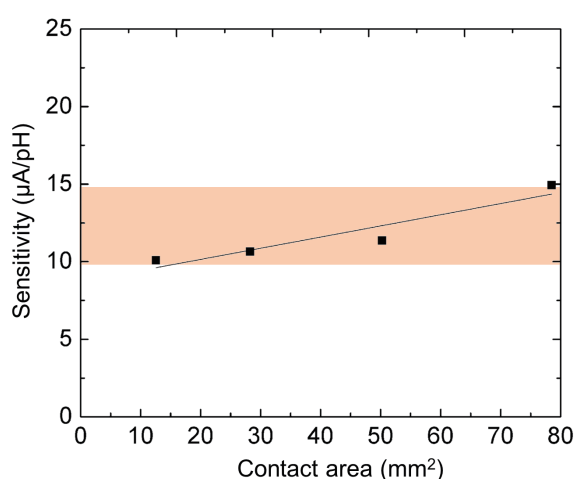


Fig. 6. (Color online) Changes in sensor sensitivity for different contact areas.

contact area on the FET-gate becomes lower. A balance between the desired high sensitivity and high manufacturing yield will have to be considered in the commercial fabrication environment. A contact area with an optimized value of 30 mm<sup>2</sup> provides a relatively large contact area with high sensitivity.

### 3.4 Characteristic noise level

Because the noise level affects the performance of a MEMS device directly, it is necessary to evaluate it. Figure 7 shows the  $I_{ds}$  characteristics of the separate ITO electrode connected to the FET gate in the time domain. The sensor electrodes were immersed in a standard solution at pH = 7, and the functional voltages of  $V_{ref}$  and  $V_{ds}$  were fixed at 3.5 and 0.3 V, respectively. After a stationary phase (normally 5 min), the output current of  $I_{ds}$  was recorded continuously by the sourcemeter. The output current was in the range from 862 to 863  $\mu$ A in the pH 7 standard solution. Corresponding to a given sensitivity of 10 to 15  $\mu$ A/pH, the pH sensor was confirmed to have an approximate noise level of  $\pm 0.04$  pH on average. Also, the baseline of 862.5  $\mu$ A at a pH value of 7 was obtained as a calibration value for the field measurement.

### 3.5 Long-term working function with interval measurement

Finally, we determined whether the sensor could maintain a stable baseline and output for pH interval testing. Figure 8 shows the  $I_{ds}$  output characteristics of the pH sensor compared with pH values for a long-term measurement. In the first phase of the test, the pH electrode was immersed in buffer solutions with varying pH values. The output current increased with decreasing pH values from 7.20 to 4.84, correspondingly. After the power supply was periodically turned off and on, the pH sensor could still measure a solution well at pH 4.58. Then, the sensor electrodes were continuously immersed in a solution with pH = 4.58 and the power supply was turned off for 18 d. As Fig. 8 shows, after 18 d, the pH sensor still maintained a constant output and measured different

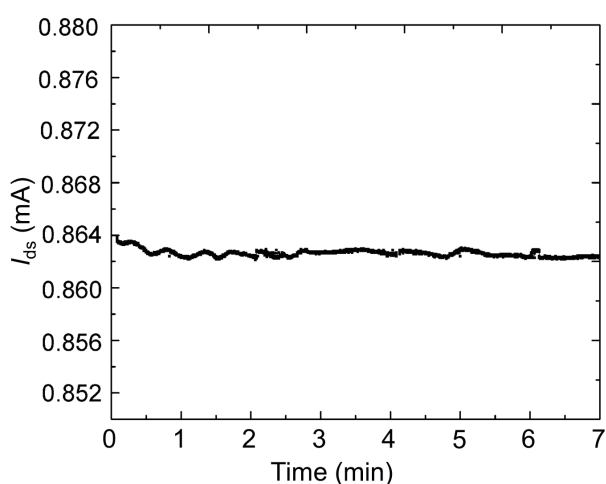


Fig. 7. Noise level of the solid-state pH sensor.

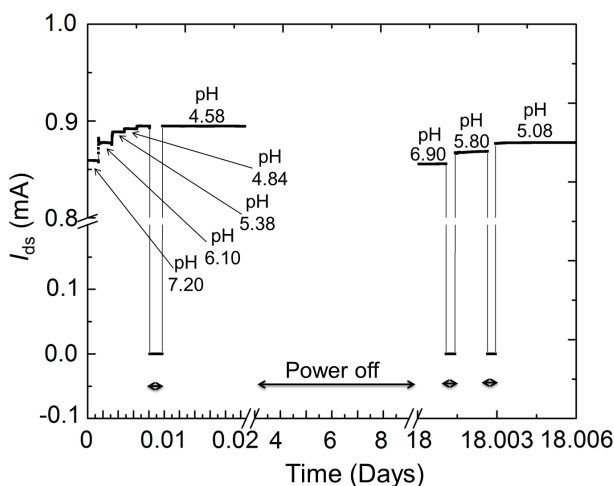


Fig. 8.  $I_{ds}$  output characteristics for measuring the pH of a solution when  $V_{ref}$  and  $V_{ds}$  have constant values of 3.5 and 0.3 V, respectively.

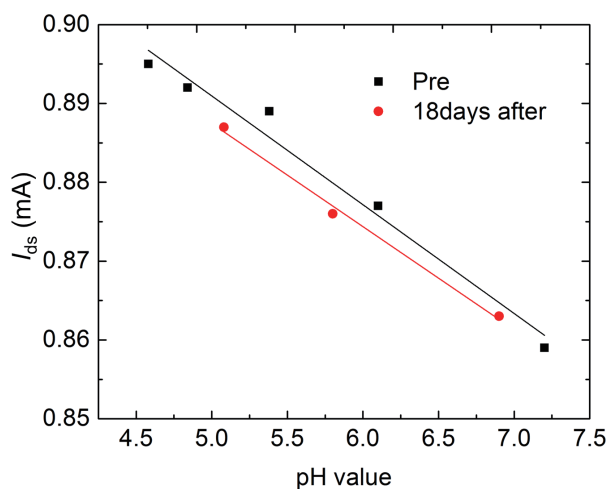


Fig. 9. (Color online) Linearity of the output current vs measured pH values.

pH values in the interval testing. Under field-test conditions, to realize a dynamic long-term sensing of rumen pH, the pH sensor system could be kept in sleep mode with minimum current flow into the LSI, and no useless power consumption would be incurred by the circuit and the electrodes.

Figure 9 shows the linearity of the output current versus known pH values. The pH sensor continuously worked well and had a very low drift after being held in a solution at low pH for a relatively long time (18 d in this study). Given a pH sensor with the smallest drift lower than 2  $\mu\text{A}$ , a minimum of drift error of  $<\pm 0.1$  pH could be achieved. In addition, we determined that the output current was linear with changing pH, i.e., the  $I_{ds}$  and pH values can be calculated using a first-degree polynomial equation. Thus, the pH sensor could be used to measure the pH of target solutions by fitting the output current to the pH value.

#### 4. Conclusions and Outlook

We developed a solid-type pH sensor for dairy measurement in the rumen of wagyu. The pH sensor was composed of a sensing electrode of ITO film and a MOSFET. In the solid-state structure, the ITO electrode was a separate gate of the MOSFET, and the ITO film could be patterned with a suitable capture structure for the complicated environment at the wagyu rumen. A sensor system was fabricated with a compact size by eliminating the reference solution, which is suitable for long-term pH measurement. The pH sensors had a measuring sensitivity of 10–15  $\mu\text{A}/\text{pH}$  for different contact areas over a range of 12–78  $\text{mm}^2$ . A long-term test with interval experiments was carried out, and the pH sensor demonstrated stable performance with a very small drift over a relatively long period of pH measurements.

In addition, we found that the latest conventional pH sensor of the Well Cow™ bolus (supported by Well Cow Ltd.) has a sensitivity of  $\pm 0.3$  pH. By comparing the response results, our pH sensor exhibited a desirable performance that was comparable to that of a high-quality commercial device. With appropriate packaging and adequate field experimental verification, the solid-state pH sensor may be adapted to sense the daily range pH value of rumen, and the sensor could help farmers efficiently monitor the health of wagyu cows.



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