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# Urea Sensor Based on an Ion Sensitive Field Effect Transistor. II.[1,2] Effects of Buffer Concentration and pH on the Potentiometric Response

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The effects of pH and concentration of working buffer on the potentiometric response of a urea sensor based on an ion-sensitive field effect transistor (ISFET) were examined. The sensor showed a higher response in slightly acidic buffer solution (pH 6.0) than in buffer solutions of pH 7.1 and 8.0. The pH dependence of the response of the sensor can be explained in terms of the activity of urease immobilized on the ISFET. The buffer concentration also affected the response. Higher concentration buffers (20 and 50 mM) strongly disturbed the response due to the higher buffer capacity.

**Keywords**——urea sensor; enzyme sensor; ion-sensitive field effect transistor; urease; immobilized enzyme

The development of urea sensors has been investigated by many researchers using urease immobilized on gas- and ion-sensitive electrodes.[3-7] In this connection, we[1] and Miyahara *et al.*[8] have independently reported that an ion sensitive field effect transistor (ISFET) can function as a urea sensor if the gate surface is coated with a urease membrane. In the preceding paper[1] we described the effect of the membrane thickness on the performance characteristics of the sensor. The urea sensor is based on the urease-catalyzed reaction (1), in which urea is decomposed to ammonia and carbon dioxide with consumption of $H^+$. The amount of $H^+$ consumed through the reaction (1) can be detected as a pH change around the gate surface of the urease-bearing field effect transistor (FET). Accordingly, the pH and concentration of the working buffer are crucially important factors affecting the performance characteristics of the sensor. The purpose of this paper is to describe the effects of buffer concentration and pH on the potentiometric response of our sensor.

$$(NH_2)_2CO + 2H_2O + H^+ \xrightarrow{\text{urease}} 2NH_4^+ + HCO_3^- \quad (1)$$

## Experimental

**Fabrication of pH-Sensitive FET**——The pH-sensitive FET used in the present study was fabricated on a $p^-$-silicon wafer, the dimensions of which were 0.5 mm wide, 6.5 mm long and 0.2 mm thick. The silicon nitride gate (1000 Å) was grown on an $SiO_2$ layer by the chemical vapor deposition method using $SiH_4$ and $NH_3$. The procedure for fabricating the ISFET and its properties can be found elsewhere.[9]

**Reagents**——Urease (EC 3.5.1.5., Type III, 3.9 units/mg) and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. as lyophilized powders. Glutaraldehyde (GA) and urea were of reagent grade. Double-distilled water was used throughout.

**Preparation of a Urease Membrane on the ISFET**——Urease and BSA were dissolved in 1 mM phosphate buffer ($KH_2PO_4$–NaOH, pH 7.1) to prepare 10% solutions. *Ca.* 8% GA solution was also prepared. Equal volumes of these

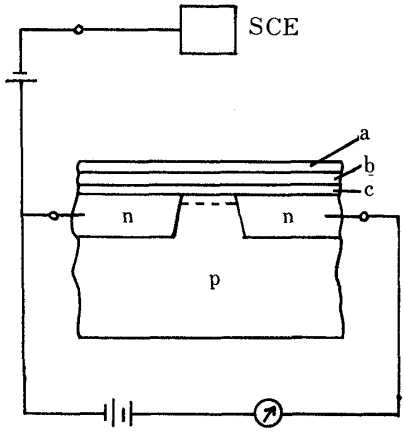


Fig. 1. Schematic Representation of Urease Membrane-Coated ISFET

a, urease membrane; b, $Si_3N_4$ layer; c, $SiO_2$ layer.

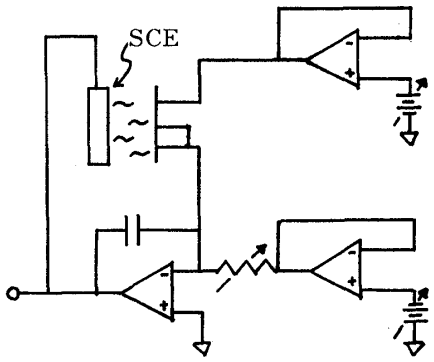


Fig. 2. Circuit for Operating the Urea Sensor

three solutions were mixed thoroughly and an appropriate amount of the mixture was applied to the ISFET gate before gelation began. The probe was air-dried for about 1 h, and was immersed in the working buffer for about 1 h before use. A schematic representation of the urea-sensitive FET is illustrated in Fig. 1. The thickness of the urease membrane was about 5 $\mu$m in the dry state.

**Measurements**——All measurements were conducted at 23 °C using a saturated calomel electrode as a reference electrode. Sample solutions were not stirred during the measurement, and the probe was rinsed with the buffer solution after each measurement. A circuit for operating the sensor in a constant drain current mode is shown in Fig. 2. As the drain current is held constant, the change in the gate voltage ($E_{gs}$) can be read out directly.

## Results and Discussion

Typical response curves of the sensor for 0.5—20 mM urea solutions of pH 6.0, 7.1 and 8.0 are depicted in Figs. 3 and 4. In all cases the gate voltage shifted markedly in the negative direction at the initial stage of measurements and declined to reach steady-state values after about 4—5 min.[10] In 1 mM phosphate buffer of pH 6.0, the sensor exhibited a linear response with the slope of *ca.* 50 mV/decade over the urea concentration range of 0.5—20 mM (Fig. 3A). The response, however, was disturbed appreciably in pH 7.1 and 8.0 buffer solutions (Figs. 3B and 3C). It should be noted that the potentiometric response for the samples of higher urea concentration was remarkably reduced in pH 7.1 and 8.0 media, but this was not the case for the lower concentration samples (0.5 and 1.0 mM). A similar effect of buffer pH was observed when the measurements were conducted in 5 mM phosphate buffers (Fig. 4). The pH-dependence of the response of the sensor may arise from the facts that the urease activity curve is bell-shaped with a maximum at pH 7.0—7.5, and that urease loses its catalytic ability precipitously around pH 9.0—9.5.[11] In the case of the urease-membrane coated ISFET, the pH value of the membrane interior shifts in the alkaline direction as a result of $H^+$ consumption through the enzyme reaction (1). For example, about −53 mV output voltage, obtained when the sensor was dipped into 20 mM urea in 5 mM phosphate buffer of pH 8.0 (Fig. 4C), corresponds to about one pH unit shift to the alkaline side from the buffer condition, *i.e.*, about pH 9.0 in the membrane. This is the reason why the potentiometric response was significantly reduced in pH 8.0 buffer. Thus, it is preferable to employ slightly acidic buffer conditions to obtain maximum performance of the sensor.

Figure 5 shows the relationship between the slope of the linear part of the calibration graph for urea solutions and the concentration of pH 6.8 phosphate buffers. A near-Nernstian response was obtained in the lower concentration buffers, but the response was severely reduced in the higher concentration buffers. This is due to the higher buffer capacity of the

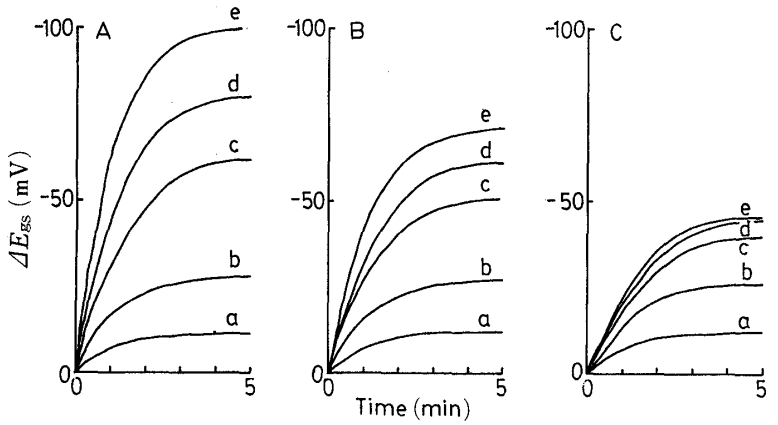


Fig. 3. Typical Response Curves of the Sensor for Urea Solutions in 1 mM Phosphate Buffers of pH 6.0 (A), 7.1 (B), and 8.0 (C)

Urea concentrations were: a, 0.5; b, 1.0; c, 5.0; d, 10; e, 20 mM.

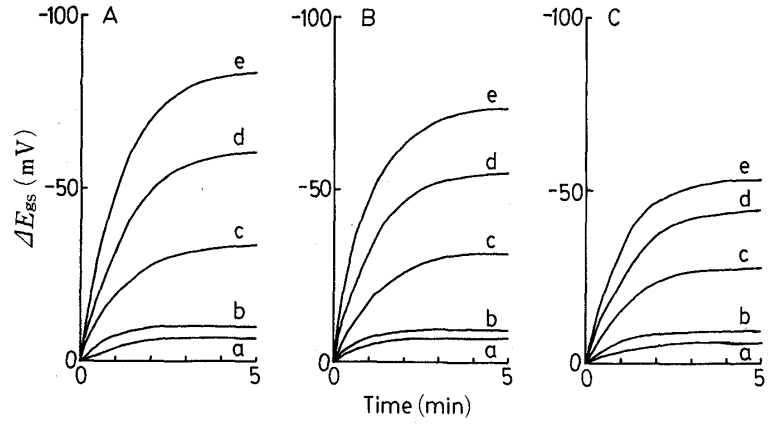


Fig. 4. Typical Response Curves of the Sensor for Urea Solutions in 5 mM Phosphate Buffers of pH 6.0 (A), 7.1 (B), and 8.0 (C)

Urea concentrations were: a, 0.5; b, 1.0; c, 5.0; d, 10; e, 20 mM.

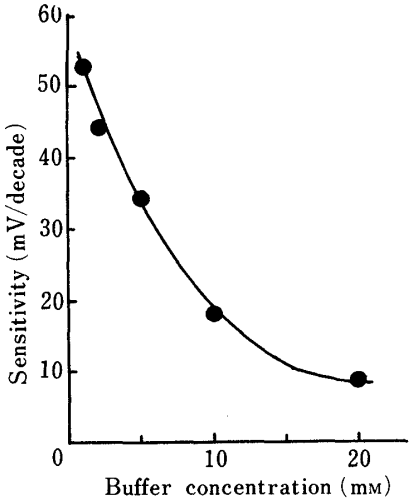


Fig. 5. Effect of the Buffer Concentration on the Response of the Sensor

Sensitivity (mV/decade) was estimated from the slope of the calibration graph.

media, which cancels the pH change originating from the enzyme reaction (1) at the gate surface.

The data in Figs. 3, 4, and 5 suggest that the ISFET-based urea sensor should be

operated in slightly acidic media with a rather weak buffer capacity. Based on these results, we are now trying to determine urea in human blood and urine.

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