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Urea biosensor based on an extended-base bipolar junction transistor

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Abstract. In this study, a urea biosensor was prepared by the immobilization of urease onto the sensitive membrane of an extended-base bipolar junction transistor. The pH variation was used to detect the concentration of urea. The SnO2/ITO glass, fabricated by sputtering SnO2 on the conductive ITO glass, was used as a pH-sensitive membrane, which was connected with a commercial bipolar junction transistor device. The gels, fabricated by the poly vinyl alcohol with pendent styrylpyridinium groups, were used to immobilize the urease. This readout circuit, fabricated in a 0.35-um CMOS 2P4M process, operated at 3.3V supply voltage. This circuit occupied an area of 1.0 mm × 0.9 mm. The dynamic range of the urea biosensor was from 1.4 to 64 mg/dl at the 10 mM phosphate buffer solution and the sensitivity of this range was about 65.8 mV/pUrea. The effect of urea biosensors with different pH values was considered, and the characteristics of urea biosensors based on EBBJT were described.

Keywords: Extended-base bipolar junction transistor, urea, SnO_2, ITO, CMOS

1. Introduction

Urea is a general marker for evaluating uremia levels.. The normal condition of urea in serum is from 15 to 40 mg/dl. Patients suffer from the renal insufficiency, if urea concentration in serum is from 180 to 480 mg/dl. Moreover, the hemodialysis is required once the elevated level is above 180 mg/dl [1-2]. Reliable and simple approaches for detecting urea are required in any physician's office or clinical laboratory. The spectrophotometric methods are available for urea determination. However, this method can not be used for real-time control. This inconvenience of the spectrophotometric determination can be overcome by using electrochemical methods, in which biosensors are applied [3-5].

Several types of biosensors have been used for urea determination. The enzyme field effect transistor (ENFET) was first proposed by Caras and Janata [3]. The urea biosensors based on the ENFET have two kinds of the determination. One is a pH-based urea biosensor [1-5] and the other is a $NH4^+$-based urea biosensor [8]. The much drawback of the $NH4^+$-based urea biosensor is the interference caused by other cations in the serum. The pH-based urea biosensor detects pH changes by the enzymatic reaction. Therefore, the effect of urea biosensors with different pH values must be considered. Ion sensitive field-effect transistors (ISFETs) based on a metal oxide field effect transistor (MOSFET)

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have been developed to detect H^+-ion [9]. Extended gate field effect transistor (EGFET) is another structure to isolate MOSFET from chemical environment [10-14]. However, the source and body often can be at different voltages in real application. It is common to observe that the MOSFET with non-zero voltage between the source and body does not only affect linearity through bulk modulation, but also introduce undesirable temperature-dependent effect [15-17]. Therefore, the extended base bipolar junction transistor (EBBJT) was proposed to detect the pH variation.

The EBBJT was separated into two parts: one was sensing structure containing a sensitive membrane, and the other one was bipolar junction structure. The EBBJT configuration has several advantages: firstly, it has a lower cost than a traditional extended-gate H^+ ion sensitive field effect transistor; secondly, the transistor can be tested and characterized without the need for contacting with solutions; thirdly, the device can avoid the influences of bulk modulation; fourthly, the bipolar junction transistor only requires about 25% of the current of a MOSFET under the same transconductance. This is a considerable advantage for portable applications, where battery power is very important; finally, the output response is linear in dB owing to device characteristics. The conditions of the portable and disposable biosensor are low power and low cost. Therefore, the EBBJT configuration is useful to develop disposable and portable biosensors for clinical applications.

2. Experiment

2.1. Chemical and materials

The urease (EC 3.5.1.5) came from Jack beans, type, at activity 20,000-40,000 units, were obtained from Sigma. The photo-crosslinkable poly vinyl alcohols bearing styrylpyridinium groups (PVA-SbQ) was purchased from Toyo Gosei Kogyo Co. Ltd., Japan. Deionized (D.I.) water was used for all the electrolytes and the buffer solutions. Tin oxide (SnO_2) thin films were formed using the radio frequency sputtering system at a substrate temperature of 150℃. The ITO glasses (50-100Ω; ITO coating thickness, 23 nm) were purchased from the Wintek Corporation.

2.2. Sensor fabrication

The EBBJT was separated into two parts. One was a sensing membrane and the other one was a bipolar junction transistor (BJT). The SnO_2/ITO glass, fabricated by sputtering tin oxide on the conductive ITO glass, was used as the EBBJT sensing membrane. Before deposition, the ITO glass was washed in methyl alcohol and D.I. water for 20 and 10 min, respectively. Mixed sputtering gases included O_2 and Ar. The O_2 concentration was twenty percent of the total mass flow rate. Moreover, the thin films were formed by the radio frequency sputtering system at a substrate temperature of 150℃. The thickness of the SnO_2 thin film was about 2000 Å. Conducting line was bound from the ITO layer and packaged by epoxy after the thin film was deposited. Reference electrode used as input terminal of the EBBJT provided an electrical contact to the solution, from which the solution potential was defined. The complete encapsulation section diagram of SnO_2/ITO glass sensing structure is shown in Figure 1.

The operation principle of BJT has been studied in [18-19]. Like a general BJT, the transfer function for an EBBJT operating in an active region is given by

$$I_C = I_S^* \cdot \exp(\frac{qV_{BE}}{KT}) \tag{1}$$

Where, T is the absolute temperature; I_C is the collector current; V_{BE} is the base-emitter voltage; q is the electron charge; and K is the Boltzmann's constant.

The characteristic for I_S* is given by

$$I_S^* = \frac{qA_{EB}D_n n_{EBBJT}^2}{Q_S} \tag{2}$$

Where, Q_S is the integrated charge in the base; n_{EBBJT} is the effective carrier concentration; A_{EB} is the base-emitter junction area; and D_n is the diffusion constant for electrons. Regarding the EBBJT-based BJT saturation current I_S*, the terms in Eq. (2) are similar to that of the standard BJT, except the variation of the effective carrier concentration. Therefore, the operation current of the EBBJT is exponential to the H^+ ion concentration without complex function. The SnO_2/ITO glass EBBJT shows linear pH response about 58.3 mV/pH between pH 2.0 and 10.0.

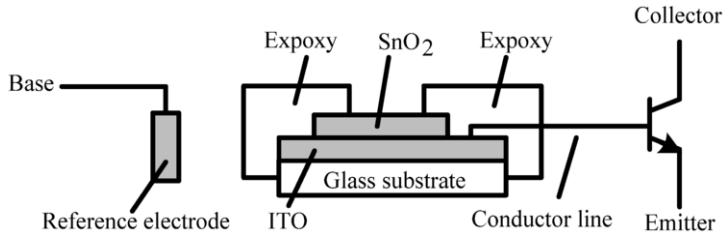

Fig. 1. Schematic diagram of the EBBJT.

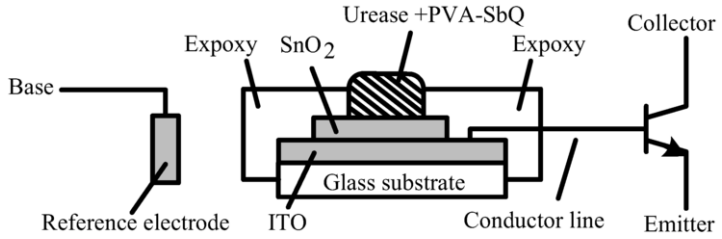

Fig. 2. Schematic of the urea biosensors based on the EBBJT.

2.3. Enzyme immobilization

The EBBJT with immobilized urease is proposed to detect the pH variation as a result of enzymatically catalyzed hydrolysis of the urea. The equation for the enzymatic reaction is as follows:

$$CO(NH_2)_2 + 3H_2O \xrightarrow{\text{urease}} 2NH_4^+ + HCO_3^- + OH^- \tag{3}$$

Before the immobilization, the EBBJT sensing membrane was washed in D.I. water for 15 min. A liquid pre-polymer solution was obtained by mixing the urease and PVA-SbQ in 5 mM phosphate buffer solution. The pH value of the phosphate buffer solution was 7.0. Then, the pre-polymer solution

was dropped at the sensitive membrane, which was exposed to UV light at 365 nm for 20 minutes. Therefore, the polymeric film entrapped the urease. After the immobilization, the samples were stored in the dark at 4 ℃ for four hours. Then, the non-polymeric PVA-SbQ was washed by immersing in the phosphate buffer solution for 30 minutes. The complete encapsulation section diagram of the sensing structure is shown in Figure 2.

3. Interface circuit

The sensor readout circuit based on pseudo EBBJT differential pair, which is shown in Figure 3, was used to compensate the common mode noise from chemical environment. The basic principle of the readout circuit is that the collector current is kept constant by applying compensating feedback voltage to the emitter of the EBBJT. Then, a differential amplifier following the pseudo EBBJT differential pair was used to reject the input common mode noise.

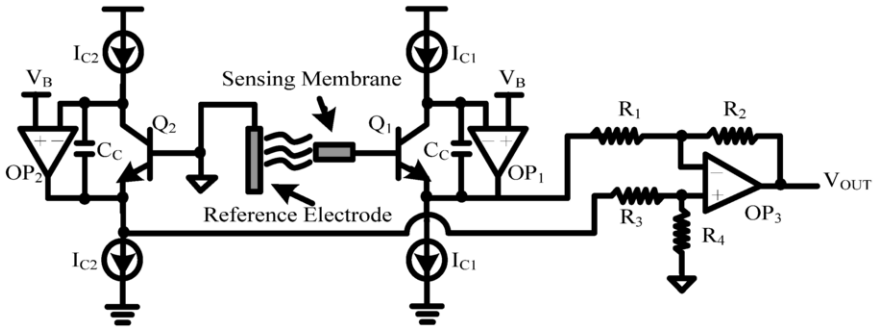

Fig. 3. Schematic of the urea biosensors based on the EBBJT.

Considering the circuit implementation, the EBBJT operated in an active region was biased by a constant collector current and claimed by a constant voltage V_{CE1} via the amplifier OP_1. The emitter voltage of the EBBJT was forced to settle to a steady value produced by the pH value of the solution where the EBBJT was dipped. Besides, the emitter follower structure also acted as a voltage buffer for driving the next stage electronics. C_C was designed to compensate the stability issue of the feedback loop. Reference electrode provided an electrical contact to the solution, from which the solution potential was defined. The action of the current sources ensured that the ratio of the collector currents of Q_1 and Q_2 remains constant. The resistors were designed so that $R_1 = R_2$ and $R_3 = R_4$. Neglecting the influence of base-width modulation, the output response of the EBBJT with interface circuit become

$$V_{OUT} = (V_{REF} - V_{BE2}) - (V_{REF} - V_{BE1}) = \frac{KT}{q} \ln\left(\frac{I_{C1}}{I_{C2}} \frac{I_S}{I_S^*}\right) \tag{4}$$

Where, V_{REF} is the potential of the reference electrode; V_{BE1} is the forward base-emitter bias voltage of the EBBJT; V_{BE2} is the forward base-emitter bias voltage of Q_2, I_{C1} is the collector current of the EBBJT; and I_{C2} is the collector current of Q_2. When choosing $I_{C1} = I_{C2}$, the expression of Eq. (4) can be simplified to

$$V_{OUT} = \frac{KT}{q} \ln\left(\frac{I_S}{I_S^*}\right) \tag{5}$$

Two-stage CMOS operational amplifier with rail-to-rail input and output ranges was adopted in this measurement system. The class-A input stage allowed to properly design bandwidth and noise. A large size input device was used to reduce flicker noise. Moreover, a class-AB output stage was used, since it increased the slew rate and reduces the slew-rate-induced distortion. The combined summing circuit and class-AB control was biased by a simple floating current source [20]. The complete CMOS operational amplifier circuit is shown in Figure 4. The DC gain of the class AB amplifier is about 80dB, and its phase margin is more than 85°. The unity gain bandwidth is designed by 10 MHz.

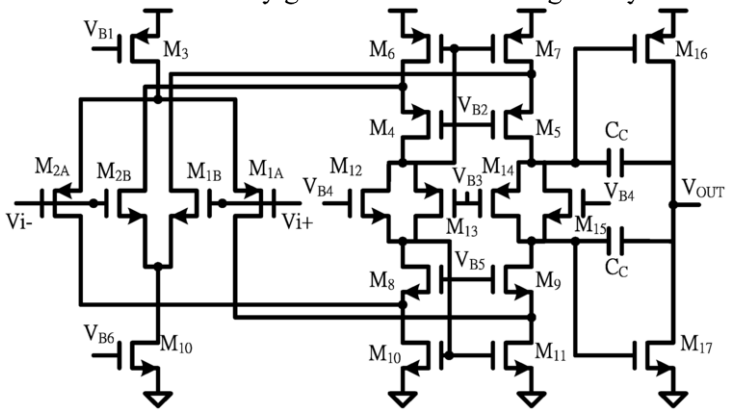

Fig. 4. CMOS operational amplifier with the class-AB output stage.

4. Results and discussion

All measurements were made at room temperature (around 25℃), and the readout circuit was as presented in Figure 3. This EBBJT interface circuit had been fabricated in TSMC CMOS 0.35 um process technology, with the micrograph shown in Figure 5. The measurement system worked with 3.3 V supply. The output response of the measurement circuit was monitored by Fluke 87 handheld multimeter. The commercial Ag-AgCl electrode S120C was employed as a reference electrode obtained from Sensorex Company. The commercial pH meter SP2200 measured the pH value of test solution, which was purchased from Suntex Company. The urea biosensor was first placed in the phosphate buffer solution until a stable potential was obtained before measurement. The desired urea concentrations at pH 6.0 were measured to determine the amount of urea from the calibration curve of the urea biosensor.

To prove the sensing characteristics of the EBBJT-based urea biosensor with interface circuit, the urea biosensor was immersed into 10 mM pH6.0 phosphate buffer solution to identify the output response of the EBBJT-based urea biosensor. This urea biosensor was dipped in the urea concentration solution, and then was taken back to the phosphate buffer solution after dipping the EBBJT-based urea biosensor in the phosphate buffer solution for 30 seconds. The output response is shown in Figure 6. The sensitivity of this configuration is about 65.8 mV/pUrea between 1.4 mg/dl and 64 mg/dl. Furthermore, the output response of the urea biosensor was decreased by increasing the capacity of the phosphate buffer solution. The influence of the phosphate buffer solution on the response of the urea biosensor is shown in Figure 7. The phosphate buffer solution was changed from 1 to 50 mM and the pH value of the phosphate buffer solution was kept in pH 6.0. Decrease of the buffer concentration results in shift of the linear range to the higher urea concentrations. According to the experiment re-

sults, 10 mM was chosen as the measurement environment, since the signal response was more monotonic and linear.

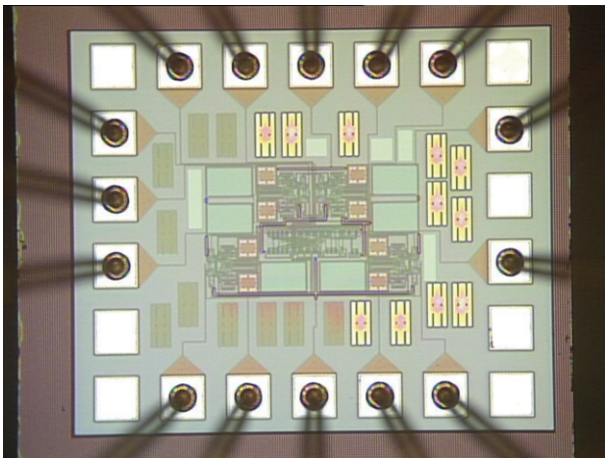

Fig. 5. Micrograph of the integrated EBBJT interface circuit.

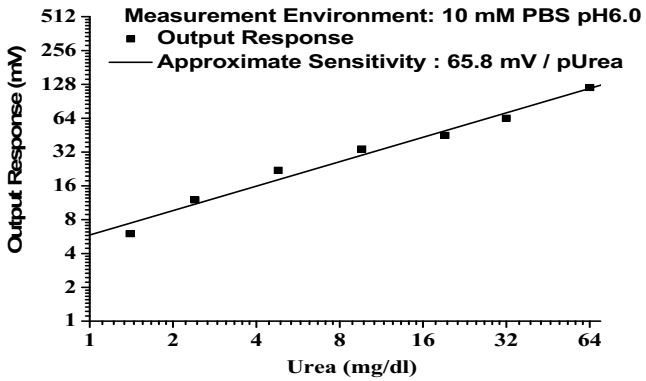

Fig. 6. Output response of the urea biosensor with the readout circuit.

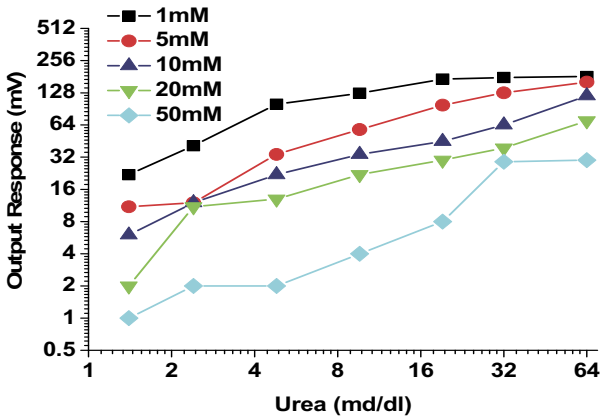

Fig. 7. Influence of the phosphate buffer concentration on the EBBJT.

The pH value of measurement environment is an important consideration, since the output response of the EBBJT depends on the value of pH. Additionally, the concentration of H^+-ion also influences the base current. The base current of the EBBJT consists of two major components. One is the recombination effect of holes and electronics in the base. This current type is proportional to the minority carrier charge in the base. The second major component of base current is due to injection of holes from the base into the emitter. This current component depends on the gradient of minority-carrier holes in the emitter. The calibration curves of the phosphate buffer solution at pH 6.0 to pH 8.0, plotted in Figure 8, reveal that the output response of the pH-sensitive EBBJT-based urea biosensor depends strongly on the pH of the solution. Figure 8 reveals that a higher pH of the phosphate buffer solution degrades the output response. Besides, the detection range is limited since the output signal has been saturated. According to the experiment results, pH 6.0 was chosen as the measurement environment, since the output response was monotonic without saturation.

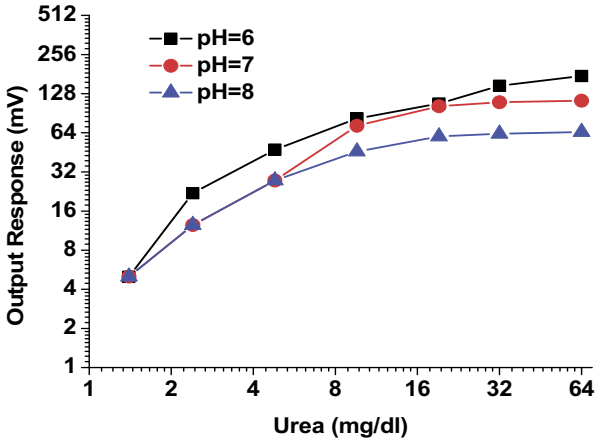

Fig. 8. Influence of the pH of the phosphate buffer on the EBBJT.

5. Conclusions

In this study, the EBBJT was successful to realize the urea biosensor. The EBBJT-based urea biosensor was separated into two parts: one was sensing structure containing a sensitive membrane, and the other one was bipolar junction transistor. The urea biosensor based on the EBBJT exhibits the advantages of low cost, simple packaging and wide range of applications. The detection range of the urea biosensor was from 1.4 to 64 mg/dl at the 10 mM phosphate buffer solution. The sensitivity of the urea biosensor based on the EBBJT was 65.8 mV/pUrea. The experiment results show decrease of the buffer concentration results in: (1) shift of the linear range of the EBBJT-based urea biosensor to the higher urea concentrations; and (2) reduction of the sensitivity. Furthermore, the output response of the urea biosensor was also decreased by increasing the pH value of the phosphate buffer solution. This measurement circuit, fabricated successfully in a 0.35-um CMOS 2P4M process, operated at 3.3 V supply voltage. The circuit occupied an area of 1.0 mm × 0.9 mm. The total power consumption was 1.2 mW.

6. Acknowledgment

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