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PREPARATION OF COATED-WIRE POTENTIOMETRIC ENZYME SENSORS

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A silver/silver chloride (Ag/AgCl) electrode coated with a tri-n-dodecylamine/poly(vinyl chloride) composite membrane was used to make potentiometric enzyme sensors. When the pH-sensitive membrane-coated Ag/AgCl electrode was further coated with a urease or penicillinase membrane, it responded potentiometrically to urea and penicillin. The performance of the enzyme sensors was comparable to conventional enzyme sensors.

KEYWORDS——enzyme sensor; coated wire electrode; pH-sensitive electrode; urea sensor; penicillin sensor

The development of biosensors is currently one of the most attractive fields in analytical science and technology.[1-3] Recently, attention is increasingly devoted to miniaturizing the sensors.[4-10] In this regard, we have been developing micro enzyme sensors using a semiconductor device, e.g., an ion sensitive field effect transistor (ISFET).[11-15] Another possible candidate for micro biosensors is the coated wire electrode. The advantage of the coated-wire electrode in miniaturizing the sensor body lies in the absence of internal reference solutions. Its ease of fabrication at a low cost is also advantageous. The present paper describes some preliminary data concerning the preparation of urea and penicillin sensors based on a polymer-coated Ag/AgCl electrode.

A schematic diagram of the sensor is shown in Fig. 1. The top of Ag wire (0.5 mm diameter) mounted in a Teflon rod was anodized in 0.1 M NaCl to deposit a AgCl layer on the surface. Then, the end of the resulting Ag/AgCl electrode was coated with a pH-sensitive polymer membrane composed of poly(vinyl chloride) (PVC) (32%), dibutyl phthalate (DBP) (64%), tri-n-dodecylamine (TDA) (3.5%), and sodium tetraphenylborate (0.5%). The polymer membrane was prepared from a tetrahydrofuran solution of the materials by dip-coating. The thickness of the polymer membrane was typically 0.2 mm. This polymer-coated electrode functions as a pH sensor, the potentiometric response of which will be described below.

The enzyme membrane was prepared on the surface of the polymer layer by pouring a mixture of equal amounts of 10% enzyme solution, 10% bovine serum albumin solution, and 8% glutaraldehyde. When the water was evaporated, a thin layer of the immobilized-enzyme membrane formed on the polymer layer. The sensitive layer was wrapped with nylon mesh. All potentiometric measurements were carried out at 23°C, using a reference saturated calomel electrode (SCE).

The pH-sensitive neutral-carrier electrode based on TDA was first reported by

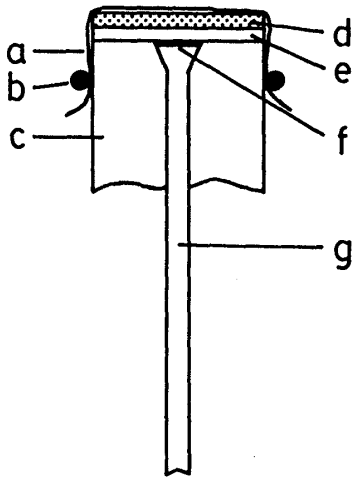

Fig. 1. Schematic Representation of the Enzyme Sensor

a)Nylon mesh, b)O-ring, c)Teflon rod, d)enzyme membrane, e)PVC/TDA membrane, f)AgCl, and g)Ag wire.

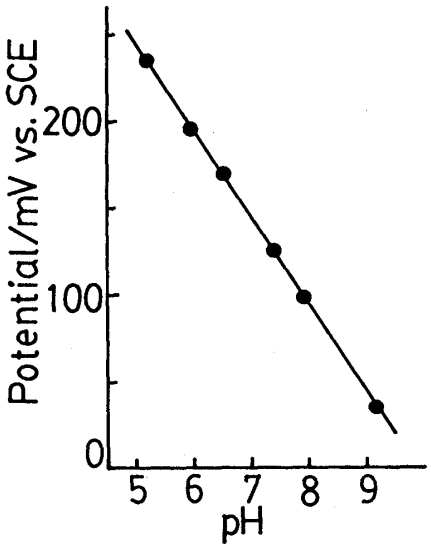

Fig. 2. pH Response of the PVC/TDA-Coated Electrode

The pH value of the solution was adjusted by adding varing amounts of NaOH to a 10 mM KH_2PO_4 solution.

Schulthess et al.[16,17] The coated wire type of electrode using the PVC/TDA membrane has been studied recently by Abe and Takezawa.[18] A typical pH response of the PVC/TDA-coated Ag/AgCl electrode used in the present study is shown in Fig. 2. Over the pH range 5.20-9.15, a linear calibration graph was obtained. The response time was 1 min or less. These characteristics of the pH electrode are suitable for constructing enzyme sensors.

A urea sensor was fabricated by covering the sensitive layer of the PVC/TDA-modified electrode with a urease membrane. Typical response curves of the urea sensor for 0.05-50 mM urea solutions are depicted in Fig. 3. When the probe was dipped into the sample solution, the electrode potential shifted in the negative direction and reached a steady-state value after ca. 15 min. The negative shift of the potential means that the pH value around the pH-sensitive layer of the electrode shifted from the buffer condition to alkaline side. This is due to the enzymatic reaction in the urease membrane (1) where urea is decomposed to ammonia and carbon dioxide and H^+ is consumed.

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

Figure 4 shows the calibration graph. The sensor has a linear response in the urea concentration range 0.5-10 mM, the slope being 43 mV/decade. The response characteristics and stability of the sensor are comparable to the conventional urea-sensitive electrodes fabricated with pH glass electrodes[19] and ammonia electrodes.[20,21]

Another example of an enzyme sensor with a PVC/TDA-coated electrode is the penicillin sensor. It was prepared by covering the pH-sensitive layer of the electrode with a penicillinase membrane. Figure 5 shows the potentiometric response

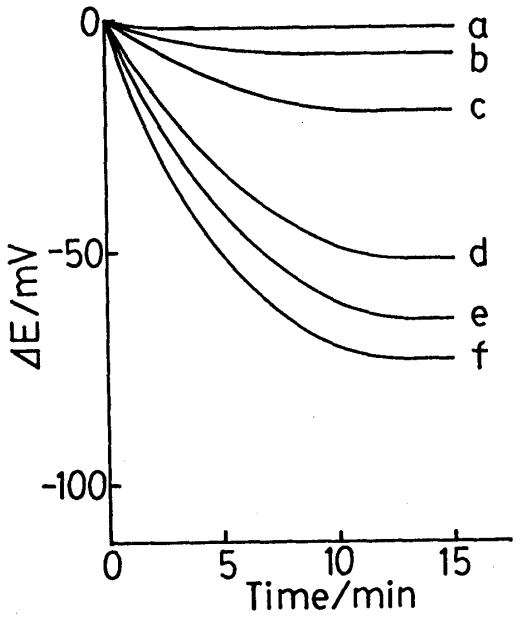

Fig. 3. Typical Response Curves of the Urea Sensor for Urea Solutions in 5 mM Phosphate Buffer (pH 7.20)

Urea concentration; a)0.05, b)0.5, c)1.0, d)5.0, e)10, and f)50 mM.

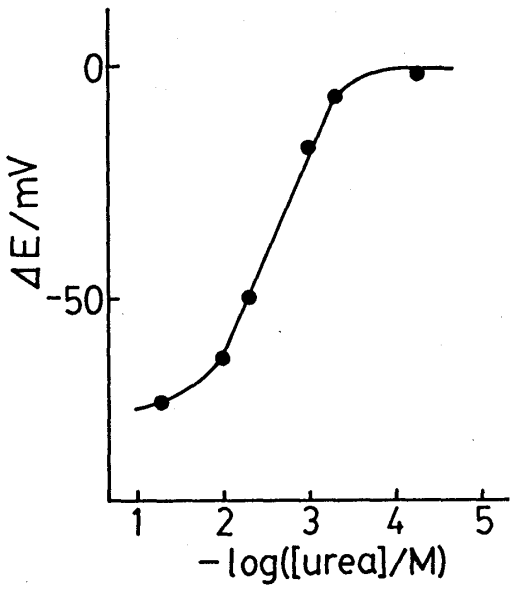

Fig. 4. Calibration Graph of the Urea Sensor in 5 mM Phosphate Buffer (pH 7.20)

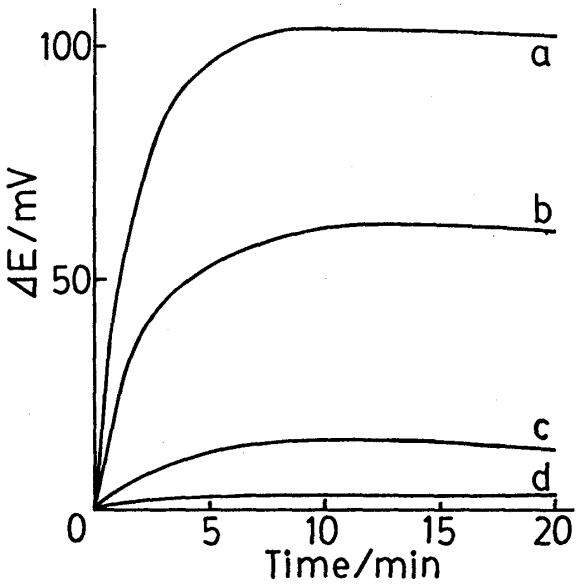

Fig. 5. Potentiometric Response of the Penicillin Sensor in 5 mM Phosphate Buffer (pH 7.20)

Penicillin concentration; a)10, b)5.0, c)1.0, and d)0.1 mM.

of the sensor to 0.1-10 mM penicillin. The electrode potential shifted in the positive direction with the increase of penicillin concentration. This is attributable to the increased acidity of the media around the pH-sensitive layer of the electrode, which, in turn, originates from the enzymatic reaction (2) in the penicillinase membrane.[22]

$$\text{penicillin} + H_2O \xrightarrow{\text{penicillinase}} \text{penicilloate} + H^+ \quad \text{——— (2)}$$

These preliminary results demonstrate that the coated wire electrode can be used successfully to make potentiometric enzyme sensors. The coated wire electrode may be especially suitable for making miniature sensors, since the effective surface area of the Ag/AgCl electrode used in the present study is only ca. 0.003 cm^2. Further development is now in progress in this laboratory to improve the response characteristics of the sensors.

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