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Enzyme Sensors Based on a Coated-Wire Electrode. Effects of Buffer Concentration and pH on the Potentiometric Response of Penicillin Sensor

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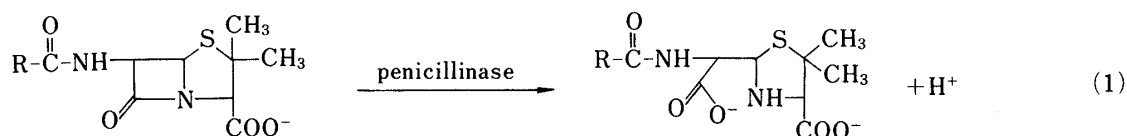
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The effects of pH and concentration of working buffer on the potentiometric response of a penicillin sensor based on a carboxyl-substituted poly(vinyl chloride)-coated Ag electrode were examined. The sensor showed a good response in buffer solutions of pH 6.5—8.5. The buffer concentration affected the response of the sensor. Higher concentration buffer (30 mM) strongly disturbed the response due to the higher buffer capacity.

Keywords—enzyme sensor; penicillin sensor; penicillinase; coated-wire electrode; carboxyl-substituted poly(vinyl chloride)

We have reported that urea and penicillin sensors could be prepared by the use of an Ag electrode coated with a poly(vinyl chloride) (PVC)/tri-*n*-dodecylamine (TDA) composite membrane which is coupled with an enzyme/albumin cross-linked membrane.^{1,2)} Though the sensors showed satisfactory response, the durability of the enzyme/albumin membrane was not adequate. A poor surface adhesion between the polymer membrane and the enzyme membrane was also a drawback. In the preceding paper³⁾ we showed that the poly(vinyl chloride) derivative (PVC-COOH) which contains 1.8 wt% carboxyl residue could be used as a polymer matrix for a pH-sensitive coated-wire electrode and that, using the electrode, a penicillin sensor could be prepared by immobilizing penicillinase on the polymer surface mainly through electrostatic attraction. The carboxyl residue in PVC-COOH is essentially required to bind penicillinase on the membrane surface without inactivation. The principle of operation of the sensor was that the change of H⁺ concentration arising from the enzymatic reaction (Eq. 1) at the surface of the polymer membrane was detected as a local pH change by the pH-sensitive coated-wire electrode. Accordingly the pH and concentration of the working buffer should be 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 the penicillin sensor based on the PVC-COOH-/TDA-coated Ag electrode.



Experimental

Materials—The carboxyl-substituted poly(vinyl chloride) (PVC-COOH), nominally 1.8 wt% carboxyl residue, was used as received from Aldrich Chemical Co. Sodium tetraphenylborate (NaTPB), tri-*n*-dodecylamine

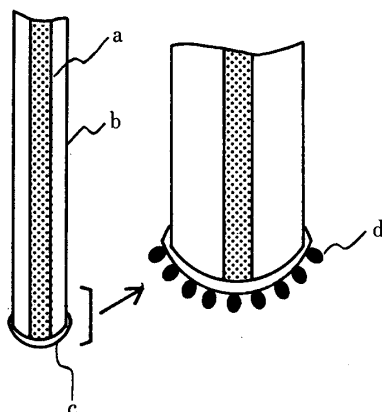


Fig. 1. Schematic Representation of Penicillin Sensor Based on Coated-Wire Electrode

a, Ag wire; b, Teflon rod; c, PVC-COOH/TDA layer; and d, penicillinase.

(TDA), and *o*-nitrophenyl octyl ether (NPOE) were of extra pure reagent grade. Penicillinase was purchased from Miles Chemical Co. Penicillin G potassium salt was obtained from Wako Chemical Co.

Electrode Preparation—Figure 1 shows a schematic representation of the electrode. The top of the Ag wire (0.5 mm diameter) mounted in a Teflon rod was coated with a pH-sensitive solvent polymeric membrane composed of PVC-COOH (31%), NPOE (63%), TDA (5.5%), and NaTPB (0.5%). The polymer membrane was prepared by dropping an appropriate amount of tetrahydrofuran solution of the materials onto the top of the electrode. The thickness of the polymer membrane thus prepared was about 0.2 mm. Prior to enzyme immobilization of the membrane surface, the pH response of the electrode was checked. Then, the polymer layer of the electrode was further modified with penicillinase by immersing the top of the electrode in 0.5% penicillinase solution (1 mM phosphate buffer, pH 7.0⁴). After treatment for about 15 h at 5–10 °C, the probe was rinsed adequately with the working buffer.

Measurements—All potentiometric measurements were carried out at 23–25 °C, using a digital multimeter (TR 6843, Takeda Riken Co.) and a saturated calomel electrode. Solutions were not stirred during the measurements.

Results and Discussion

It has been shown that a plasticized PVC/TDA membrane can serve as a pH-sensitive layer for membrane electrodes.^{5–7} The use of PVC-COOH for the preparation of a coated-wire electrode was first reported by Satchwill and Harrison.⁸ They demonstrated that the potentiometric response of the K⁺-sensitive electrode coated with the PVC-COOH/valinomycin membrane is comparable to that of the parent PVC-based device.

As reported previously,³ the PVC-COOH/TDA-coated Ag electrode changed its electrode potential depending on the solution pH, with a sensitivity of about 50 mV/pH, and this property was scarcely influenced by the ionic strength (*i.e.*, buffer concentration) of sample solutions.

Typical response curves of the penicillin sensor for 1–10 mM penicillin G solutions (5 mM phosphate buffer, pH 7.5) are shown in Fig. 2. In all cases the electrode potential shifted rapidly in the positive direction at the initial stage of measurements and then declined to reach steady-state values after 4–6 min. The difference between the electrode potential in the buffer and that at the steady state in the sample solution was defined as ΔE .

Calibration graphs of the sensor in pH 6.5, 7.5, and 8.5 solutions (5 mM buffer) are depicted in Fig. 3. The effect of buffer pH, within the pH range tested, on the response was small, although the ΔE value for 10 mM penicillin G in the pH 6.5 medium seems somewhat low. This might arise from the shift of pH value around the electrode surface, as a result of progress of the enzymatic reaction, to a more acidic region where penicillinase partly loses its catalytic activity. For the range of 1–10 mM substrate, a super-Nernstian response was observed in pH 7.5 and 8.5 media, the slope of the graphs being *ca.* 80 mV/decade. This is presumably because the catalytic activity of the enzyme on the electrode surface is enhanced by the local pH change arising from the progress of the enzymatic reaction on the membrane surface (*i.e.*, a positive feedback effect).

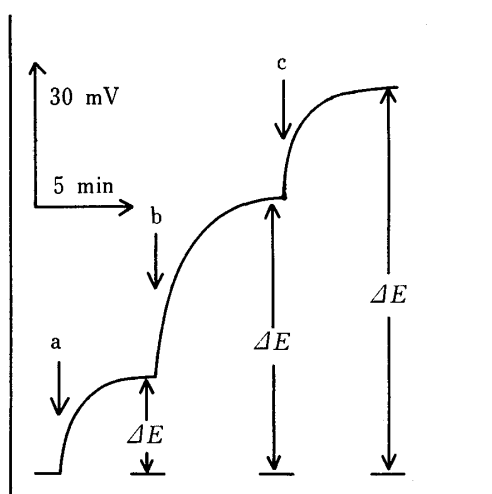


Fig. 2. Time Response of the Penicillin Sensor
a, 1 mM; b, 3 mM; and c, 10 mM penicillin G.

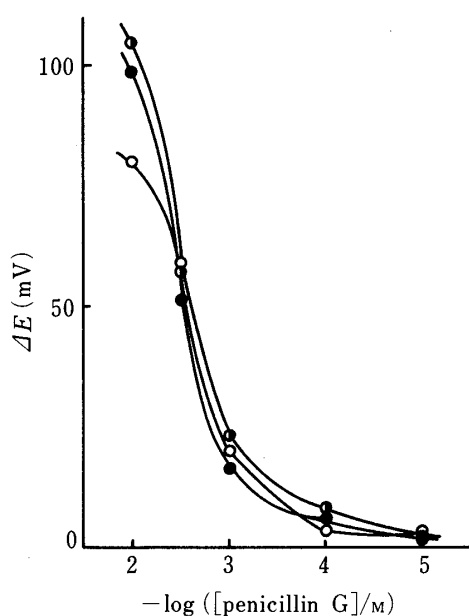


Fig. 3. Effect of Buffer pH on the Response of the Penicillin Sensor
pH 6.5, —○—; pH 7.5, —●—; pH 8.5, —○—.

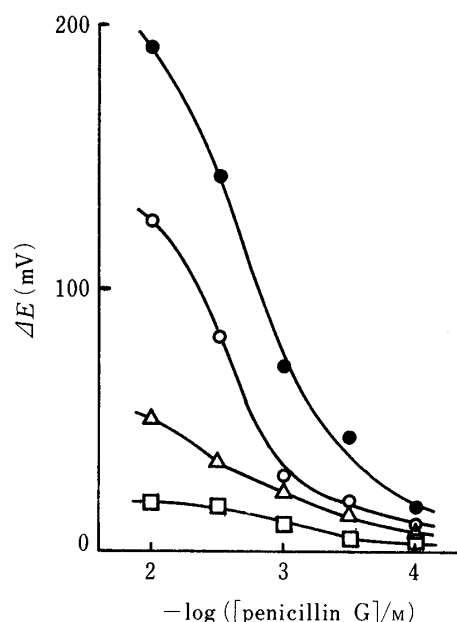


Fig. 4. Effect of Buffer Concentration on the Response of the Sensor
Buffer concentration was 2 mM, —●—; 5 mM, —○—; 10 mM, —△—; or 30 mM, —□—.

Figure 4 shows the effect of buffer concentration on the response of the sensor in pH 8.5 solutions. The response depended drastically on the concentration of working buffer. A super-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 strong buffer capacity of the media, which cancels the pH change originating from the enzyme reaction (Eq. 1) at the electrode surface. It is recommended to use buffer solutions with a rather weak buffer capacity.

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