# A Conductometric Glucose Sensor Based on a Twin-Microband Electrode Coated with a Polyaniline Thin Film

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**Synopsis.** A conductometric glucose sensor was fabricated by coating a twin-microband electrode with a bilayer of a membrane composed of polyaniline and glucose oxidase/gluconolactonase films. The enzyme-catalyzed hydrolysis of glucose induces a change in the conductivity of the pH-sensitive polyaniline film. The conductivity change was detected by the current flowing between the two-band electrode. The sensor demonstrates clear responses to glucose up to 1 mM.

Enzyme sensors have been recognized as useful analytical tools for biologically important compounds. Most of them were based on amperometric or potentiometric responses induced by enzyme-catalyzed reactions.[1,2] We recently reported newly categorized enzyme sensors, i.e., NADH and penicillin sensors based on detection of conductivity changes of polypyrrole induced by the enzyme reactions.[3—5] Bartlett et al.[6] and Hoa et al.[7] followed this idea and independently fabricated conductometric glucose sensors using polyaniline. However, the response mechanisms of their devices were not specified in detail, because the conductivity of polyaniline is affected by pH as well as its oxidation state (or electrode potential).[8—11]

In this paper, we report a conductometric glucose sensor using a twin-microband electrode coated with polyaniline and enzyme films at fixed electrode potentials to obtain the responses induced by enzyme-catalyzed pH changes. We used the twin-microband electrode to prepare a thin, uniform polyaniline film,[12] which was essential to give reproducible results. Polyaniline is suitable for fabricating conductmetric sensors, since the conductivity is sensitive to pH.

## Experimental

Glucose oxidase (101 U $mg^{-1}$) (Toyobo), gluconolactonase (2720 U $mg^{-1}$) (Oriental Yeast), bovine serum albumin, glutaraldehyde (Wako Chemical Co.) and (+)-$\beta$-D-glucose (Sigma) were used as received. Aniline (Kanto Chemical Co.) was used immediately after being purified by distillation under a reduced pressure. We used a twin-microband electrode which has two Pt microbands on a glass substrate. Each band is 0.1 μm thick, 10 μm wide, and 5 mm long, and is separated from the adjacent band by 10 μm. The total electrode area exposed to the solution was ca. $1\times10^{-3}$ $cm^2$. The polyaniline-coated twin-microband electrodes were prepared by the procedure described in the previous report.[12]

In order to prepare a thin polyaniline film (<0.1 μm thick) at the microband electrode, the glass part of the electrode was hydrophobically pretreated by immersing into a ca. 20 mM octadecyltrichlorosilane/hexadecane solution for an hour.[12] Polyaniline was potentiostatically polymerized from a 0.1 M aniline/1 M $H_2SO_4$ aqueous solution (M=mol $dm^{-3}$) using a bipotentiostat. The polymerization potential for one of the two bands was set at 0.8 V and the other at 0.6 V vs. SCE. Polyaniline grows at only the band set at 0.8 V vs. SCE and interconnects with the other band. The polymerization was terminated immediately after the interconnection with polyaniline was observed[12] (total polymerization charge, ca. 90 μC).

A glucose sensor was fabricated by coating the polyaniline-deposited device with an enzyme membrane. The enzyme membrane was prepared from an aqueous mixture composed of equal volumes of 5 wt% glucose oxidase ($G_{OX}$), 1 wt% gluconolactonase ($G_{LA}$), 5 wt% bovine serum albumin and 10 wt% glutaraldehyde. After evaporation of the water, a thin layer of immobilized enzyme membrane was formed. The thickness of the enzyme membrane was measured by Surface Texture Analyzer (Dektak 3030) in a dry state.

The sensing characteristics of the device were investigated using a flow injection apparatus. The array device was placed in a flow-through cell; the total volume of the flow channel was ca. 20 $mm^3$. The mobile phase was a 1 M $Na_2SO_4$/5 mM Britton–Robinson buffer solution or a 1 M $Na_2SO_4$/5 mM acetate buffer solution. The solution was pumped with a JASCO Twincle at 2.0 mL $min^{-1}$. The potential of the one of the two bands ($E_1$) was set at 0.25 V vs. SCE and the other ($E_2$) was set at 0.23 V vs. SCE, using a bipotentiostat. A normalized conductivity or a change in conductivity can be evaluated from the ohmic current ($I_{ohm}$) driven by the potential difference between the two bands (20 mV).

## Results and Discussion

The electrical conductivity of polyaniline depends on the pH of the solution as well as on the potential. We have focused on the pH-dependence of the conductivity at a constant potential ($E_1$=0.25 V vs. SCE). Figure 1 shows pH-conductivity profiles of the polyaniline obtained from the steady $I_{ohm}$ values. The profile is a sigmoidal with an apparent inflection point at pH 4.5. The conductivity of polyaniline changes by 3 orders of magnitude as the pH changes from 4 to 5.4 (or 5.4 to 4). It is reported that polyaniline forms a conductive amine–imine structure at pH<4, and an insulating quinone–diimine structure at pH>6,[13] and the pH profile observed here evidently corresponds to this structure transition.

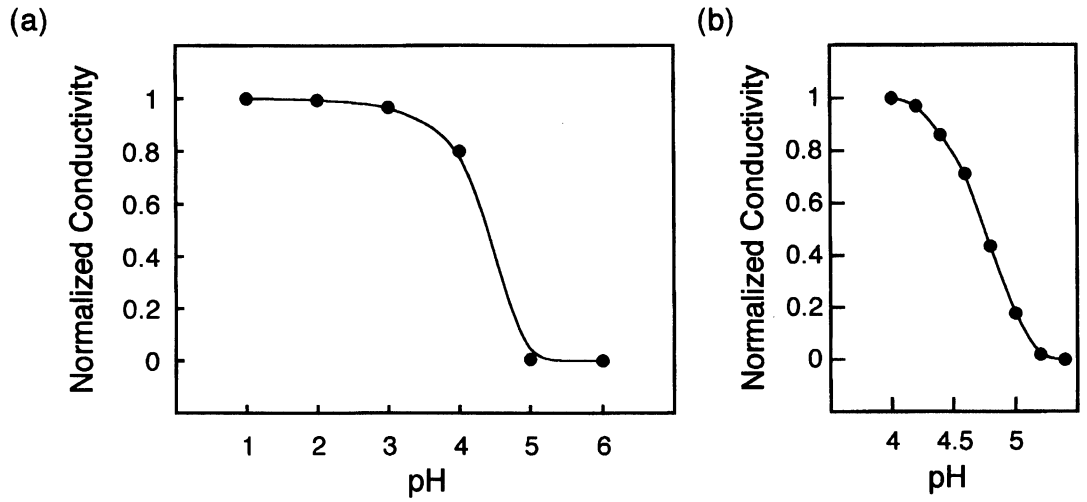


Fig. 1. Normalized conductivity vs. pH plot in the regions, (a) pH 1—6 and (b) pH 4.0—5.4. $E_1$=250 mV vs. SCE. $V_d$=20 mV.

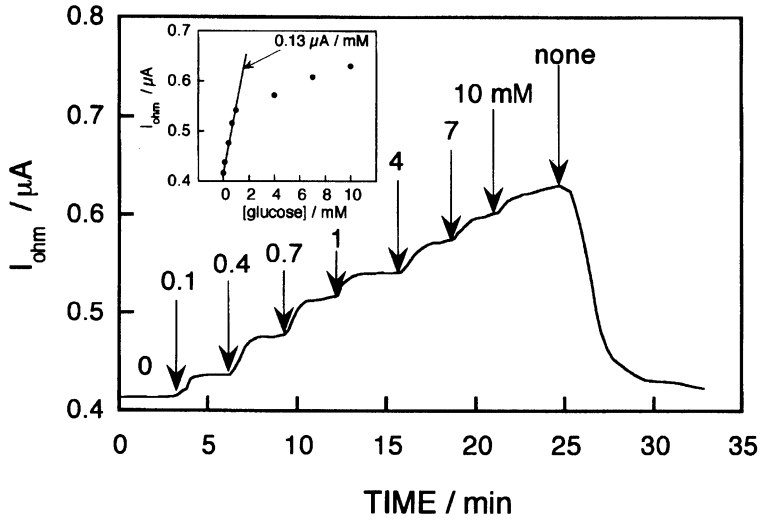


Fig. 2. Responses of the glucose sensor upon injection of various concentrations of glucose solutions. The thickness of enzyme membrane was ca. 0.8 μm. The pH of the flowing solution was fixed at 5.2.

We have applied the above-mentioned pH-sensitive device to a glucose sensor by coating the device with an enzyme membrane composed of $G_{OX}$ and $G_{LA}$. $G_{OX}$ catalyzes the oxidation of glucose to $\delta$-gluconolactone, followed by the $G_{LA}$-catalyzed hydrolysis to gluconic acid ($pK_a$; 3.7). These enzyme-catalyzed reactions acidify the polyaniline film at the twin-microband electrode. Figure 2 shows the $I_{ohm}$ responses to flowing of various concentrations of glucose solutions. The $I_{ohm}$ value reached a steady value within a minute. The change in $I_{ohm}$ increased linearly with concentration of glucose up to 1 mM (slope, 1.3 μA $mM^{-1}$), while $I_{ohm}$ responses were vague above concentration of 4 mM, probably due to the saturation of binding sites of $G_{OX}$ because the activity of $G_{OX}$ is 27 times lower than that of $G_{LA}$. No response was observed when the enzyme membrane contained only $G_{OX}$, indicating that the $G_{LA}$-catalyzed hydrolysis of $\delta$-gluconolactone is essential.[14]

The thickness of the enzyme membrane affected the

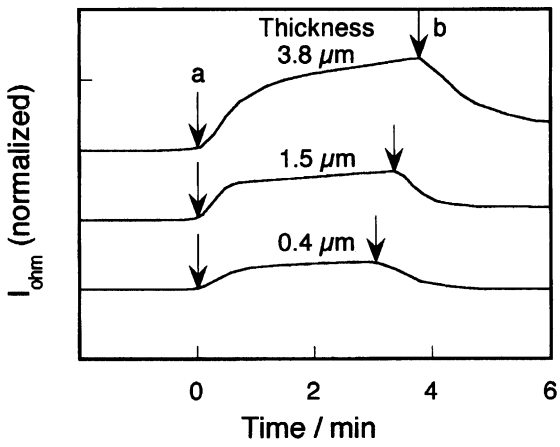


Fig. 3. Responses of the glucose sensors with upon injection of 1 mM glucose solution at a various thickness of enzyme membrane. Arrow a; injection of the solution with glucose. Arrow b; injection of the solution without glucose.

response of the device (Fig. 3). The device with a thin enzyme membrane showed a fast and reversible response but only a small change in the $I_{ohm}$ value. The response at the device with a thick membrane were found to be slow. Generally, this type of conductometric enzyme sensor is fabricable based on the same principle but using other hydrolases such as urease or phosphatase.

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