Study of the Background Current through Polypyrrole / Glucose Oxidase Film for Glucose Sensing

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The electrodes modified with polypyrrole or polypyrrole / glucose oxidase films were fabricated by the electropolymerization method, and the background currents through the electrodes were investigated. The peak value of the background current through the electrode modified with the polypyrrole film depended on the amount of charges passed in the course of the electropolymerization, and it took longer time to attain a constant current when the amount of the passed charges increased. It was found that the model of the background current consisting of the capacitive current and the diffusion current agreed with the experimental results. On the basis of the proposed model, it was suggested that the glucose oxidase immobilized in the polypyrrole films caused an increase in the capacitive current and a decrease in the diffusion current.

Key words: glucose sensor / background current / polypyrrole / glucose oxidase

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1. Introduction

Analytical devices using biological systems have been developed for clinical analysis [1], environmental monitoring [2], and control of biochemical reactions [3]. These devices consist of a biomaterial and a transducer. For instance, the glucose sensor, which is composed of graphite electrode covalently attached with glucose oxidase (GOD), was prepared [4]. Recently, great interest has been taken in the researches for immobilization of enzymes in conducting polymers. The polypyrrole (PPy) film in which GOD was immobilized could be deposited on an electrode by the electropolymerization of pyrrole in aqueous solution containing GOD [5]. Such PPy film is prepared by one-step fabrication and therefore is very suitable for the development of the micro-enzyme electrode in in vivo measurements. It was also reported that the conducting polymers such as poly (N-methylpyrrole) [6], polyaniline [7] and polyindole [8] containing enzymes were electrochemically deposited on electrodes, and they had the ability of sensing substrates.

The immobilization of an oxidoreductase in the conducting polymer as a matrix will give rise to the direct electron transfer between the conducting polymer and the oxidoreductase. In the case that GOD is immobilized in PPy, electron transfer may occur between the GOD and the PPy, in addition to the well-known redox reaction of GOD described as follows:

\[ \text{GOD(FAD)} + \beta-D-glucose \rightarrow \text{GOD(FADH}_2) + \text{gluconolactone} \]

\[ \text{GOD(FAD)} + \text{electron acceptor(ox)} \rightarrow \text{GOD(FAD)} + \text{electron acceptor(red)} \]

where the electron acceptor is such as oxygen and \( \beta \)-benzoquinone which can be employed as a mediator [9]. Although a few papers reported that the current response based on enzyme reaction was observed without an electron acceptor [10], it is still uncertain whether the direct electron transfer occurs between enzymes and conducting polymers. Prior to the discussion on the direct electron transfer between GOD and PPy, it must be at least confirmed that the current response to the glucose arises from enzyme reaction in the measurement system.
However, a paper reported that the current detected in a solution with the PPy / GOD-modified electrode were not always attributed to the reaction between GOD and glucose [11]. Furthermore, a change in the background current \( I_b \) made the analysis of the current detected with the PPy / GOD-modified electrode so complex that the current measurement had to be carried out after the \( I_b \) was allowed to be constant. Umano suggested that \( I_b \) was related to the capacitance of polypyrrole film and faradaic current [12], but this idea was not dicussed in detail. In this paper, the true nature of \( I_b \) through the PPy / GOD-modified electrode at +0.8V \( \text{vs. S.C.E.} \) was discussed. The current is observed due to oxidation of hydrogen peroxide \( \text{(the reduced form oxygen, } i.e. \text{ the electron acceptor } \text{(red)}) \), was discussed.

2. Experimental

2.1 Reagents

Pyrrrole was purchased from Nacalai Tesque Co. and distilled under 42mmHg at 52°C.

Purified monomer was stored at -18°C in a glass tube filled with nitrogen gas.

Glucose oxidase (Grade II) was purchased from Toyobo Co. Ltd., KCl from Nacalai Tesque Co. was used as a supporting electrolyte.

2.2 Electrodes

An anode electrode was prepared by depositing gold in an area of 5mm \( \times \) 5mm on alumina plate \( (10\text{mm} \times 25\text{mm}) \) and annealed for 2hours at 500°C in air. Pyrrrole was electopolymerized [13] on the electrode in aqueous solution containing pyrrrole \( (0.2M) \) and KCl \( (0.1M) \) at the constant potential of +0.7V \( \text{vs. S.C.E.} \) supplied by a potentiostat / galvanostat (Hokuto Denko Model HA-301). The amount of passed charges were monitored by a coulomb / amperehour meter (Hokuto Denko Model HF-201). In the same manner, the electrode covered with PPy containing GOD was prepared by the electopolymerization of pyrrrole in the presence of GOD.

Fig. 1  Apparatus for measurement of the background current.

2.3 Measurements

Measurements of \( \text{Pb} \), between the working electrode and the counter electrode were carried out in 25ml of 0.1M phosphate buffer solution \( \text{(pH 7.6)} \) at the constant potential of +0.8V \( \text{vs. S.C.E.} \) with a potentiostat / galvanostat. The distance between the electrodes was 0.5cm. The \( \text{Ib} \) was recorded on a pen recorder (Chino works ltd. Model EB2P00). The apparatus used for the measurement is illustrated in Fig. 1.

3. Results and discussion

The time dependence of \( \text{Ib} \), through the electrode modified with PPy were measured in phosphate buffer solution at the constant potential of +0.8V \( \text{vs. S.C.E.} \). The result is shown in Fig. 2, compared with that of a gold anode electrode. It is seen from the result that the larger the amount of the charges passed in the course of the electropolimerization of pyrrrole was, the higher the peak value of the current through the modified electrode became. In addition, it took more than 2h to attain constant values of \( \text{Ib} \) through the PPy-modified electrodes, while \( \text{Ib} \) through the gold electrode became nearly equal to 0 at once. The time needed to attain the constant value of \( \text{Ib} \), through the PPy-modified electrode became longer with an increase in the amount of the passed charges.
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Fig. 2 Time dependence of the background current through the PPY-modified electrode prepared by the electropolymerization with the passed charges of 0.4C/cm² (a), 0.8C/cm² (b), and the gold electrode (c).

The measurement system is, generally, expressed as a serial equivalent circuit consisting of a capacitance and a resistance [14]. According to the simplified model, at first, a large capacitive current (I_c) flows and then rapidly becomes nearly equal to 0 or a small value. In the case of the gold electrode, the pattern of the current agreed with the above model. In the case of the PPY-modified electrode, however, a large I_c was observed followed by a marked increase in current after several ten minutes, and then the current was decreased rapidly via a maximum. It can be considered that the large I_c was due to an increase of the electrode surface area owing to the modification with porous PPY, and the succeeding current increase was due to the diffusion of ionic substances included in the PPy. Thus, the diffusion current (I_d) was added to I_c.

Eq. (1) can be set up by assuming that I_c consists of I_s and I_d.

\[ I_c = I_s + I_d \] (1)

The impedance of the measurement system is assumed to be composed of a resistance and a capacitance which are connected in series. In this equivalent circuit, I_c is given by Eq. (2).

\[ I_c = \frac{E}{R} \exp\left(-\frac{t}{RC}\right) \] (2)

where E, t, R and C are the applied potential, the time, the resistance and the capacitance of the measurement system, respectively. Eq. (2) means that I_c decreases gradually with the time.

I_c is defined as a current arising from ionic substances in the PPy, which diffuse into phosphate buffer solution.

According to Fick’s second law, the diffusion of chemical substances is given by

\[ \frac{\partial c(x,t)}{\partial t} = D \frac{\partial^2 c(x,t)}{\partial x^2} \] (3)

where c, x and D are the concentration, the distance and the diffusion coefficient in the buffer solution, respectively. In the present case, it is assumed that all the concerning ionic substances are on the working electrode at t=0, and the distribution of the concentration is expressed by use of the δ-function as

\[ c(x,0) = c_0 \delta(x) \] (4)

where c_0 is the initial concentration of the ionic substances on the working electrode. From Eqs. (3) and (4), Eq. (5) is obtained.

\[ c(x,t) = c_0 \frac{1}{\sqrt{4\pi D t}} \exp\left(-\frac{x^2}{4Dt}\right) \] (5)

According to Faraday’s law, the quantity of electricity Q is proportional to c, so Eq. (5) is converted into

\[ Q(x,t) = \frac{Q_0}{\sqrt{4\pi D t}} \exp\left(-\frac{x^2}{4Dt}\right) \] (6)

where Q_0 means the initial quantity of the electricity corresponding to c_0.

The differentiation of Eq. (6) gives I_d as expressed by Eq. (7).

\[ I_d = \frac{\partial Q(x,t)}{\partial t} = \frac{Q_0}{4\sqrt{\pi D}} \left[ \frac{x^2}{2D\sqrt{t}} - \frac{1}{\sqrt{t}} \right] \exp\left(-\frac{x^2}{4Dt}\right) \] (7)
Fig. 3 Calculated current: (1) the capacitive current $I_c$, $E/R=150\mu A$, $RC=1000s$; (2) the diffusion current $I_d$, $D=10^{-2}cm^2/s$, $x=0.5cm$, $Q_s=0.8C/cm^2$ (a) and 1.1C/cm$^2$ (b); (3) the total background current $I_b$ ($=I_c+I_d$), $Q_s=0.8C/cm^2$ (a) and 1.1C/cm$^2$ (b).

To compare the experimental result in Fig. 2 with the theoretical one calculated from Eqs. (1), (2), and (7), $E/R$, $RC$ and $c_s$ must be determined. Considering that the capacitive current is independent of the amount of charges passed in the course of the electropolymerization of pyrrole, $E/R$ and $RC$ are roughly estimated to be $100\mu A$ and 1000sec, respectively, on the basis of the time-current relationship in the initial stage shown in Fig. 2. The time dependence of capacitive current $I_c$ is calculated as shown in Fig. 3 (1). On the other hand, diffusion current $I_d$ is changed with the amount of the charges passed in the course of the electropolymerization. In order to calculate $I_d$, it must be considered that $D$ has a value of $10^{-2}cm^2/s$ [15] and $x$, the distance between the working and counter electrode, is 0.5cm. By fitting the maximum value of the current expressed by Eq. (7) to those represented by the curves (a) and (b) in Fig. 2, the corresponding values of $Q_s$ are estimated to be 0.8 and 1.1C/cm$^2$, and the time dependence of $I_d$ are calculated as shown in Fig. 3 (2). From the results of Figs. 3 (1) and 3 (2), the time-$I_c$ relationships are obtained as...
shown in Fig. 3. Comparing it with the experimental results shown in Fig. 2, the assumption that $I_1$ consists of $I_2$ and $I_3$ is thought to be quite reasonable.

Fig. 4 shows the time dependence of $I_3$ through the PPy / GOD-modified electrodes in phosphate buffer solution. Comparing the result with that of PPy-modified electrodes prepared by the electropolymerization in the absence of GOD with the same amount of passed charges (Fig. 2), the relaxation time $RC$ in Eq. (2) is found to have been increased by the GOD immobilized in the PPy film. It is considered, therefore, that the increase in $RC$ value was caused by an increase in the resistance of the film due to incorporation of GOD. On the other hand, $I_3$ was decreased by the GOD immobilized in the PPy film. The incorporation of GOD may prevent the migration of ionic substances in the film.

4. Conclusion

The current detected in glucose sensing with the PPy / GOD-modified electrode contains the background current $I_1$ independent on the reaction between GOD and glucose. In the present study, the PPy-modified and PPy / GOD-modified electrodes were fabricated by the electropolymerization method, and the reason for the generation of $I_3$ through the electrodes was investigated. It was demonstrated that the model of $I_3$ consisting of the capacitive current $I_2$ and the diffusion current $I_4$ agreed well with experimental results. The PPy-modified electrode gave both a large capacitive current and a large diffusion current. In the case of the PPy / GOD-modified electrode, the relaxation time of $I_3$ was increased by the GOD immobilized in the PPy, while $I_3$ was decreased. The incorporation of GOD in the PPy film is considered to result in increase in the resistance of the film, which increase the relaxation time, and to prevent the migration of ionic substances in the film.

5. References