<Title>

Potentiometric coulometry using a liquid-film-modified electrode as a reversible surface-confined system

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<Abstract>

A reversible surface-confined system for potentiometric coulometry studies was fabricated by the deposition of a liquid film on an electrode surface, and the system was used to detect charge accumulation due to the peroxidase (POD)-catalyzed reduction of H₂O₂. The components of the liquid film were 1-ethyl-3-methylimidazolium bis(nonafluorobutanesulfonyl)imide as the electrolyte, dibutyl phthalate as the medium, and ferrocene as the redox-active mediator. When the thickness of the liquid film was less than that of the diffusion layer in the minimum potential step of the apparatus, a symmetric voltammogram, according to the Nernst equation, was recorded. The reduction of H₂O₂ by POD was carried out on the liquid-film-modified electrode and studied by potentiometric coulometry. The addition of H₂O₂ shifts the electrode potential positively, due to the oxidation of ferrocene. The change in the potential is in agreement with the Nernst equation. Thus, potentiometric coulometry, carried out on the liquid-film-modified electrode, enables the determination of the substrate without the requirement of a calibration curve.

<Keywords>

Potentiometric coulometry

Liquid film

Reversible surface-confined system

Bioelectrocatalysis

1. Introduction

A biosensor is an electrochemical sensor combined with an enzymatic reaction, with a multitude of applications in various fields [1-3], due to its rapid detection ability, simplicity, and cost-effectiveness. In usual amperometric biosensing, the response of the sensor corresponds to the reaction rate of the rate determining step in the bioelectrocatalytic reaction. The calibration curve links the current with the concentration of the substrate [4-6]. If the rate determining step is the enzymatic reaction, the calibration curve also depends on the electrodes, and will change with repeated usage due to the instability of the enzyme. On the other hand, in coulometric biosensing, the substrate in the system is consumed by the enzymatic reaction and generates a charge corresponding to the amount of the substrate in the test solution [7-8]. Thus, the generated charge is not affected by the change in enzyme activity. However, low concentrations of substrate are difficult to evaluate since the generated current is very small compared to the charging current in the non-Faradaic process.

Mizutani et al. have proposed a coulometric method coupled with bioelectrocatalytic charge accumulation in a redox layer prepared at the electrode surface [9]. Bioelectrocatalytic charge accumulation enables the coulometric detection of dilute samples. However, the large non-Faradaic current involved in potential step chronoamperometry causes serious interference in the estimation of the total accumulated charge.

Recently, a technique coupling potentiometric coulometry with bioelectrocatalytic charge accumulation has been developed [10], in which the amount of charge accumulated on an immobilized redox polymer on the electrode is determined potentiometrically. The change in electrode potential corresponds to the change in the ratio of the reduced state to the oxidized state of the side chains in the polymer. The accumulated charge is evaluated directly from the total number of redox-active sites and the redox potential. In this method, the interference of the non-Faradaic processes in the coulometric method can be eliminated. However, the response of the redox polymer deviated from the Nernst equation because of the strong electrostatic interactions that exist between the redox sites in the polymer. Therefore, the determination of the amount of accumulated charge required a calibration curve based on the voltammogram [10]. Hence, the minimization of this electrostatic interaction is essential for potentiometric coulometry without the calibration curve.

In order to decrease the interaction between the redox species, we fabricated a liquid film from an electrolyte solution containing a redox-active species. It was

expected that higher ionic strength in the liquid film would decrease the electrostatic interaction between the redox-active species. Hydrophobic ionic liquids (ILs) are suitable for liquid films and retain high ionic strength. In this work, we fabricated a liquid film with 1-ethyl-3-methylimidazolium bis(nonafluorobutanesulfonyl)imide ([emim][Nf₂N]) as an electrolyte, dibutyl phthalate as a medium and ferrocene (Fc) by reference to the literature [11, 12]. The liquid-film-modified electrode provided cyclic voltammograms with the reversible surface-confined characteristics. Potentiometric coulometry was carried out using the liquid-film-modified electrode for the determination of H₂O₂.

2. Experimental

2.1. Chemicals

1-Ethyl-3-methylimidazoliumchloride ([emim]Cl) and Fc were obtained from Sigma-Aldrich Co. (USA), and a 30% solution of bis(nonafluorobutanesulfonyl)imide (H[Nf₂N]) from Mitsubishi Materials Electronic Chemicals Co. (Japan). Horseradish peroxidase was purchased from Oriental Yeast Co. (Japan). Other chemicals were sourced from Wako Pure Chemical Industries (Japan).

2.2. Preparation of [emim][Nf₂N]

The preparation of [emim][Nf₂N] was carried out by the reported ion exchange procedure [13]. In a round-bottomed flask, 46.48 g (24 mmol) of a 30% aqueous solution of H[Nf₂N], and 3.52 g (24 mmol) of [emim]Cl were dissolved in 20 mL of methanol. The solution was thoroughly mixed, following which the methanol and hydrogen chloride were removed under vacuum. Adequate water was added to the residue under constant agitation, and the water was decanted after a while. This sequence of addition/decantation of water was repeated 35 times. A small amount of water that remained in the resulting IL was removed under reduced pressure. The purified [emim][Nf₂N] was a clear and colorless liquid (11.73 g, 71% yield).

2.3. Construction of the liquid-film-modified electrode

A glassy carbon rod (3 mm diameter) was polished successively on three grades of alumina (5, 1, and 0.3 μm), and washed thoroughly with ultrapure water after each polishing step. As shown in Fig. 1A, the carbon rod was equipped with two plastic tubes to prevent reaction of the sides of the rod. Five μL of 50 μM Fc in acetone containing 0.5% v/v [emim][Nf₂N] and 0.5% v/v dibutyl phthalate, or 0.1% v/v

[emim][Nf₂N] and 0.1% v/v dibutyl phthalate, was cast onto the electrode surface as a drop. The acetone in the solution was dried in air at room temperature (~ 25 °C). Assuming that the liquid film uniformly covers the electrode surface, the thickness of the liquid films on the two electrodes was estimated to be 7 and 1.4 μm from the cast volume of the non-volatile liquid. The final concentrations of Fc in the liquid films with thicknesses of 7 and 1.4 μm are 2 and 10 mM, respectively.

2.4. Voltammetry experiments

Cyclic voltammetry of the liquid-film-modified electrode was carried out using an electrochemical analyzer (CHI701E, CH Instrument, USA) under three-electrode conditions at 25 °C. A 100 mM phosphate buffer solution (pH 7.0) saturated with [emim][Nf₂N] and containing 10 mM H[Nf₂N] as a common ion with the liquid film, was used to control the phase boundary potential (PBP) between the liquid film and the buffer solution [13]. An Ag|AgCl|sat. KCl and a platinum wire, were inserted into the buffer solution as the reference electrode and the counter electrodes, respectively. Cyclic voltammograms were recorded at a scan rate (ν) of 2 mVs⁻¹.

2.5. Coulometric potentiometry studies

A 4 μL drop of the solution containing a regulated amount of H₂O₂ and 10 mg mL⁻¹ POD was cast onto the electrode surface (Fig. 1A) under the open circuit conditions. After 10 min, the electrode was filled with 20 μL of the buffer solution. The electrode potential (E) was measured against the reference electrode (Fig. 1B). The measurements were individually carried out with each electrode. The statistical processing was applied to three data set recorded under various conditions.

3. Results and Discussion

The reversibility of the redox reaction of Fc in various liquid films were checked to select the film components. The mixture of [emim][Nf₂N] and dibutyl phthalate showed better performance of the Fe redox reaction at a liquid-film-modified electrode compared with the pure [emim][Nf₂N] or the mixture of [emim][Nf₂N] and 2-nitrophenyl octyl ether. In the following, the liquid film was prepared with the mixture of [emim][Nf₂N] and dibutyl phthalate.

Fig. 2A shows a cyclic voltammogram of the liquid-film-modified electrode with a thickness of 7 μm at $\nu = 2 \text{ mV s}^{-1}$. The positive and negative currents correspond to the oxidation of Fc to Fc⁺, and the reduction of Fc⁺ to Fc, respectively. The

voltammogram showed characteristics of the diffusion of Fc in the liquid film. One is a peak-separation of about 54 mV. The second is a residual current (diffusion tail) at 400 mV. These features are ascribed to the diffusion of Fc in the liquid film. Therefore, this liquid film is too thick to realize a reversible surface-confined system.

A cyclic voltammogram of the liquid-film-modified electrode with a thickness of 1.4 μm (Fig. 2B) at $\nu = 2 \text{ mV s}^{-1}$ showed cathodic and anodic waves that were symmetrical with each other. The full-width-at-half-maxima (FWHM) of the cathodic and anodic peaks were 100 and 97 mV, respectively, with a peak separation of 8 mV. Eq. (1) for the reversible surface-confined system [14] was fitted to the experimental data.

$$i = \pm \frac{n^2 F^2 A \nu \Gamma_t \exp\left\{\frac{nF}{RT}(E - E^{\circ'})\right\}}{RT \left[1 + \exp\left\{\frac{nF}{RT}(E - E^{\circ'})\right\}\right]^2}, \quad (1)$$

where n , F , A , Γ_t , R , T , and $E^{\circ'}$, are the number of electrons in the redox reaction at the surface, Faraday constant, electrode surface area, total surface concentration of the redox-active species, gas constant, temperature, and formal potential of the redox center, respectively. $E^{\circ'}$ and $A\Gamma_t$ were used as adjusting parameters. The refined curve (broken line in Fig. 2B) is very close to the experimental one. The values of $A\Gamma_t$ and $E^{\circ'}$ obtained by the fitting were $230 \pm 10 \text{ pmol}$ and $185 \pm 7 \text{ mV}$ (90% confidence interval, degree of freedom (DOF): 2). The estimated value of $A\Gamma_t$ is close to the amount of Fc embedded in the liquid film (250 pmol). The agreement of the characteristics of the experimental voltammogram with those of the theoretical voltammogram indicates that the Fc-containing thin liquid film displays characteristics of the reversible surface-confined system under the conditions investigated. The estimated charges for the oxidation and reduction in Fig. 2B are 23.1 and 22.9 μC , respectively. The recovery ratio in one single reached 99%; it indicates that the elution of Fc^+ from the liquid film is negligible if any.

A comparison of the two liquid-film-modified electrodes (with thicknesses of 7 μm (Fig. 2A) and 1.4 μm (Fig. 2B)) is relevant to understand the characteristics of the reversible surface-confined voltammogram. In a semi-infinite linear diffusion, the thickness of the diffusion layer (δ) is time-dependent and formulated as:

$$\delta = \sqrt{\pi D_{\text{Fc}} t}, \quad (2)$$

where D_{Fc} is the diffusion coefficient of Fc in the liquid film and t is time. From cyclic voltammograms of Fc in the mixture (data not shown), the value of D_{Fc} was estimated to be $4 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$ in the liquid film. The resolution of the potential control of the

electrochemical analyzer employed is 0.1 mV. Therefore, the time step for the potential control is 50 ms at 2 mV s⁻¹, and δ is estimated to be 2.4 μm at $t = 50$ ms. Hence, in the liquid film with a thickness of 1.4 μm , the entire composition of the liquid film will reach redox equilibrium at each step. On the other hand, in the liquid film with a thickness of 7 μm , the composition at the interface between the solution and the liquid film, is different from that at the interface between the liquid film and the electrode, and thus shows the diffusive characteristics. Thus, when the thickness of the liquid film is less than δ , the film is regarded as a reversible surface-confined system.

It has been reported earlier that the strong repulsive interaction of the oxidized mediator caused an asymmetric broadening of the voltammogram [10]. The FWHM in the voltammogram recorded at the liquid-film-modified electrode shows the existence of a rather weak interaction between the redox species [15], as the large amount of ionic liquid decreases the electrostatic interaction between the charged mediators. The elimination of this interaction is very important for the direct conversion of the change in potential to the amount of the charge, based on the Nernst equation.

When an electrode potential in the liquid film changes from E_i to E_f , the loaded charge for the oxidation of Fc (Q) is given by:

$$E_i = E^{\circ'} + \frac{RT}{nF} \ln \left(\frac{\Gamma_{\text{ox}}}{\Gamma_{\text{red}}} \right) \quad (3)$$

$$E_f = E^{\circ'} + \frac{RT}{nF} \ln \left(\frac{\Gamma_{\text{ox}} + Q/nF}{\Gamma_{\text{red}} - Q/nF} \right) \quad (4)$$

$$Q = \frac{-nFA\Gamma_i}{1 + \exp \left[\frac{nF}{RT} (E_f - E^{\circ'}) \right]} + \frac{nFA\Gamma_i}{1 + \exp \left[\frac{nF}{RT} (E_i - E^{\circ'}) \right]}, \quad (5)$$

where Γ_{ox} and Γ_{red} are the amounts of Fc⁺ and Fc in the liquid film, respectively.

This relationship makes it possible to conduct coulometry from the potential change, that is, potentiometric coulometry. In an enzymatic reaction, the total amount of the substrate (N) is given by:

$$N = \frac{Q}{n'F} \quad (6)$$

where n' is the number of electron for the redox reaction of the substrate. Hence, Eqs. (5) and (6) are reduced to:

$$E_f = E^{\circ'} + \frac{RT}{nF} \ln \left\{ \frac{nA\Gamma_t \exp\left[\frac{nF}{RT}(E_i - E^{\circ'})\right] + n'N \left\{1 + \exp\left[\frac{nF}{RT}(E_i - E^{\circ'})\right]\right\}}{nA\Gamma_t - n'N \left(1 + \exp\left[\frac{nF}{RT}(E_i - E^{\circ'})\right]\right)} \right\} \quad (7)$$

Eq. (7) represents the potential change of the liquid film, containing a mediator modified electrode, with the enzymatic reaction. The filled diamonds in Fig. 3A show the potential of the liquid-film-modified electrode (thickness 1.4 μm) after the reaction for different amounts of H_2O_2 ($N_{\text{H}_2\text{O}_2}$). The addition of H_2O_2 shifts the electrode potential positively, demonstrating that the bioelectrocatalytic charge accumulation in the liquid film occurs at the interface between the solution and the liquid film. Fig. 3B shows the calculated voltammogram from Eq. (1) at $A\Gamma_t = 250$ pmol, $E^{\circ'} = 185$ mV, and $\nu = 2$ mV s^{-1} . E_i and E_1 to E_5 correspond to the measured electrode potential at $N_{\text{H}_2\text{O}_2} = 0$ and 20 to 100 pmol, respectively. The amount of Fc^+ calculated from Eq. (5) is shown in Fig. 3C. The solid line in Fig. 3A demonstrates a theoretical curve given by Eq. (7) at $A\Gamma_t = 250$ pmol, $E^{\circ'} = 186$ mV, and $n' = 2$. At $N_{\text{H}_2\text{O}_2} = 20, 40$, and 60 pmol, the potentiometric responses are in very good agreement with the theoretical values. Fig. 3D shows the relation between the injected amount of $N_{\text{H}_2\text{O}_2}$ and the estimated amount of N . The dotted line in Fig. 3D corresponds to the relation for the conversion efficiency of 100%. At $N_{\text{H}_2\text{O}_2} = 20, 40$, and 60 pmol, the estimated values are almost identical with to the injected values, indicating that the analytical system is free from the influence of non-Faradaic currents and that the liquid film in the present form is able to detect up to 60 pmol of H_2O_2 by potentiometric coulometry without a calibration curve.

At $N_{\text{H}_2\text{O}_2} = 80$ and 100 pmol, the estimated final potentials are slightly more negative than the theoretical value, and the estimated values of N become lower than the injected values (Fig. 3D). This error is attributable to five possible causes: 1) the dissolution of Fc^+ from the liquid film into the buffer, 2) the change in PBP between the liquid film and the buffer solution due to an increase in the Fc^+ concentration, 3) the catalytic decomposition of H_2O_2 with Fc , 4) the decomposition of Fc^+ with oxygen, and 5) the mixed potential affected by some other redox contaminant.

The excess of $\text{H}[\text{Nf}_2\text{N}]$ in the buffer solution must minimize the dissolution of Fc^+ in the buffer phase, because $[\text{Nf}_2\text{N}]$ anion in the solution determines the PBP and promotes the distribution of Fc^+ into the liquid film. Judging from the molecular weight, Fc^+ seems to be more hydrophobic than $[\text{emim}]$. Therefore, effect of the generation of Fc^+ on the PBP would be quite small. The catalytic decomposition of H_2O_2 with Fc is

well known [16]. However, the reaction would be negligible in this system, because these species locate in the different phases. The half-life time of Fc^+ is reported in the range from 20 min [17] to 14 h [18]. The decomposition of Fc^+ if any will cause a negative shift in the electrode potential. From the viewpoint of the stability of the mediator, the further optimization will be possible.

As a conclusion, the most possible cause of the error is the mixed potential due to other redox contaminant, because most of the Fc molecules in the liquid film is oxidized and the redox capacity decreases drastically at elevated concentrations of H_2O_2 (Fig. 3B). Therefore, the electrode potential becomes unstable in the presence of the redox contaminant. When E°' of another redox contaminant is more positive than that for the Fc^+/Fc couple, the electrode potential negatively deviates from the Nernst equation for Fc to the positive potential side. The redox capacity of the modified electrode is a very important factor to govern the analytical dynamic-range, the sensitivity, and the stability of modified electrodes in potentiometric coulometry.

4. Conclusion

In order to make the fullest use of coulometric potentiometry, we fabricated a liquid film with a hydrophobic ionic liquid, organic medium, and a mediator. The liquid-film-modified electrode acts as a reversible surface-confined system without specific interaction. The dynamic range of the liquid-film-modified electrode could be regulated on the basis of the amount of the mediator. The total amount of the accumulated charge can be evaluated from the Nernst equation without a calibration curve. Thus potentiometric coulometry could be carried out free from the interference of non-Faradaic processes and the calibration curve.

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Figure captions

Fig. 1. A schematic illustration of (A) the accumulation process and (B) the

measurement of the electrode potential for a liquid-film-modified electrode.

Fig. 2. Cyclic voltammograms recorded at liquid-film-modified electrodes with a thickness of (A) 7 μm and (B) 1.4 μm , at $\nu = 2 \text{ mV s}^{-1}$. The broken line in panel B is the best fitted theoretical curve of the reversible surface-confined system.

Fig. 3. (A) Potentiometric responses of the liquid-film-modified electrode on the addition of H_2O_2 (diamonds) and the theoretical curve calculated from Eq. (7) (solid line). (B) The voltammogram calculated from Eq. (1) at $A\Gamma_t = 250 \text{ pmol}$, $E^{\circ'} = 185 \text{ mV}$, and $\nu = 2 \text{ mV s}^{-1}$. E_1 to E_5 and E_i are the measured potential from the result of (A). (C) The potential-dependence of the oxidized amount of Fc calculated from Eq. (5) at $A\Gamma_t = 250 \text{ pmol}$, $E^{\circ'} = 185 \text{ mV}$, and $E_i = 105 \text{ mV}$. (D) The plot of the injected values vs. the estimated values of H_2O_2 . The error bars show the 90% confidence level for the individually fabricated electrodes (DOF: 2). The dotted line in the panel D corresponds to the conversion efficiency of 100%.

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Fig. 2. Katsube et al.

Fig. 3. Katsube et al.

Graphical abstract

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