— Technical Paper —

Electrochemiluminescence of Luminol Investigated by Electrochemical Impedance Spectroscopy

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The electrochemiluminescence (ECL) of luminol in a hydrogen peroxide solution was studied by an electrochemical impedance spectroscopy (EIS). Parallel Pt wire electrodes in a flow-type spiral cell were used for the measurements of ECL. A superoxide ion, O_2^- , was generated by electrooxidation of H_2O_2 on Pt, and a luminol was oxidized on Pt. The oxidized luminol fluoresces by a reaction with O_2^- . An ECL impedance L , which is the ratio of the luminescence amplitude to the current in the frequency domain, was measured. By comparing the theoretical equation with the experimental results of L , the rate constant for luminol luminescence was determined.

Key Words : Electrochemiluminescence, Electrochemical Impedance Spectroscopy (EIS), ECL Impedance, Luminol

1 Introduction

Fluorescence emission from molecules excited by a chemical reaction is called electrochemiluminescence (ECL).¹⁾ The ECL is expected as trace analyses of metallic ions and radical ions because the background signal in detection is quite small. Collinson *et al.*²⁾ performed ECL using a high-frequency potential pulse with a micro-electrode, and determined the ion-annihilation rate constant of 9,10-diphenylanthracene. Okajima *et al.*^{3, 4)} studied ECL by the reaction of O_2^- and 6-(4-Methoxyphenyl)-2-methylimidazo [1,2-a] pyrazin-9 (7H) -one (MCLA). They^{3, 4)} stated that the ECL of MCLA was controlled by a redox reaction of O_2/O_2^- . Oyama *et al.*⁵⁾ applied the stopped-flow method to the ECL by reactions of oxidized and reduced species generated on anodic and cathodic electrodes respectively. Arai *et al.*⁶⁾ developed the trace analysis of *N*-(4-aminobutyl)-*N*-ethylisoluminol (ABEI) by ECL with flow-injection analysis (FIA). They⁶⁾ determined ABEI of 6.0×10^{-15} mol/dm³ as the detection limit in a hydrogen peroxide solution.

The luminol is a highly sensitive reagent as CL analysis, because its luminescence quantum efficiency is extremely large. In the previous report,⁷⁾ a frequency-domain analysis was applied to the study of ECL of ABEI. The electrochemical impedance, which can characterize the ECL reaction mechanism in the frequency domain, was measured by imposing a sinusoidal potential perturbation. Moreover, an ECL impedance, which is a new transfer function corresponding to the ratio of luminescence amplitude to the current in the frequency domain, was determined. In the present study, the frequency-domain analysis is used for the detailed investigation of luminol luminescence, and the kinetics of ECL is discussed.

2 Experimental

The details of the electrochemical cell and the instru-

ments were described in the previous report.⁷⁾ Electrochemical measurements were performed by a two-electrode system. Both the working and counter electrodes were Pt wires whose diameter was 0.5 mm. The two parallel Pt wires were set in the spiral channel. The distance between two electrodes was 0.6 mm. The length of each electrode in contact with the electrolyte was 16 cm. The working electrode joined the solution inlet at the center of the spiral channel. The counter electrode was inserted into the channel from the solution outlet. This cell was located in front of a photomultiplier in the CL detector. The electrolyte was an aqueous solution containing H_2O_2 and luminol. The pH of the electrolyte was adjusted by 4×10^{-3} mol/dm³ Na_2HPO_4 and a 1.0 mol/dm³ NaOH solution. The electrolyte was prepared by doubly distilled water and analytical grade reagents. The electrolyte solution was flowed into the CL detector (Soma, S3400) by a microtube pump (Tokyo Rikakiki, MP3N). The CL detector was designed for high-frequency measurements. Since the time constant of the CL detection was 1 kHz, a reliable frequency response of ECL could be obtained below 100 Hz. The flow rate of the electrolyte solution was 2 cm³/min. The current and potential were controlled by a potentiostat-galvanostat (Hokuto, HA501G). In measuring the current-potential (*I-E*) curve, the current and the amplitude of the luminescence were recorded by two recorders (Nihon Denshikagaku, U228). In a frequency-domain measurement, the current, potential and luminescence amplitude were sent into a frequency-response analyzer (FRA) (NF, 5020).

In the measurement of *I-E* curve, the potential of the working electrode against the counter electrode was scanned at 0.1 V/min using a function generator (Hokuto, HB111). In a frequency-domain measurement, a 10 mV sinusoidal potential signal between 10 mHz and 10 kHz was superimposed on the working electrode potential. The potential and the current responses were sent into

FRA for an electrochemical impedance measurement. The current and the luminescence responses were sent into FRA for an ECL impedance measurement. The control of FRA and the data processing were performed by the desk-top computer (NEC, PC98).

3 Results and Discussion

3.1 *I-E* and *P-E* curves and luminescence reaction

Figure 1 shows *I-E* and *P-E* curves in a pH 12.5 solution containing 1×10^{-9} mol/dm³ luminol and various concentrations of H₂O₂. In the present paper, the dimension of the ECL amplitude is represented by V, which is the output signal of the CL detector. A oxidation current of H₂O₂ flowed from 0.0 V, and increased abruptly due to O₂ evolution by H₂O oxidation above 0.7 V. The luminescence was observed approximately above 0.2 V, and decreased at H₂O oxidation potential. Both the current and the luminescence amplitude increased with increasing H₂O₂ concentration. It was difficult to judge which luminescence originated from the working electrode or the counter electrode, because both the working and counter electrodes were located in front of the photomultiplier. Arai *et al.*⁶⁾ reported by a three-electrode system that the luminescence was caused by an anodic reaction in a solution containing ABEI and H₂O₂. Therefore, it is concluded that the luminol luminescence occurred by radical formation by H₂O₂ oxidation on the anodic working electrode at positive potentials. Comparing Figs. 1 (a) and (b), it was found that the oxidation potential (above 0.0 V) of H₂O₂ is different from the fluorescence potential (above 0.2 V). This discrepancy means that the fluores-

Fig. 1 *I-E* and *P-E* curves in a solution of pH 12.5 containing 1.0×10^{-9} mol/dm³ luminol. The concentrations of H₂O₂ are ① 1.0×10^{-5} mol/dm³, ② 1.0×10^{-4} mol/dm³ and ③ 1.0×10^{-3} mol/dm³. The scan rate of the potential was 0.1 V/min.

cence does not originate from only the reaction of luminol and oxidized H₂O₂. In order to investigate another reaction, *I-E* curves were measured in the solution containing no H₂O₂ and high concentration of luminol, and are shown in Fig. 2. Another oxidation wave is observed above 0.2 V, and this potential agrees with fluorescence potential in Fig. 1 (b). Moreover, the current between 0.2 V and 1.0 V increases with increasing the luminol concentration. These results indicate that the oxidation of luminol is necessary for the luminescence.

Figure 3 shows the effects of pH on *I* and *P* at 0.6 V in the solution containing 1.0×10^{-9} mol/dm³ luminol and 3.0×10^{-4} mol/dm³ H₂O₂. The peak of luminescence is around 0.6 V in the present condition. The current does not depend on the pH, and the maximum peak of luminescence is at pH 11. Therefore, the experiments described later are measured in the solution of pH 11.

The influences of the reactant concentrations on *I* and *P* were investigated. Figure 4 shows log-log plots of *I* and *P* against the H₂O₂ concentration at 0.60 V in a solution containing 1.0×10^{-9} mol/dm³ luminol. The values of *I* and *P* increase with the increase of H₂O₂ concentration, indicating that H₂O₂ was oxidized by an electrochemical reaction. Figure 5 shows plots of *P* and the luminol concentration at 0.60 V in a solution containing 3.0×10^{-4} mol/dm³ H₂O₂. The *P* increases with increasing luminol concentration. The current presented in Fig. 5 is not influenced by the luminol concentration because the con-

Fig. 2 *I-E* curves in a solution of pH 11 containing no H₂O₂. The concentrations of luminol are ① 0 mol/dm³, ② 1.0×10^{-6} mol/dm³ and ③ 1.0×10^{-5} mol/dm³, ④ 2.5×10^{-5} mol/dm³, ⑤ 5.0×10^{-5} mol/dm³ and ⑥ 1.0×10^{-4} mol/dm³. The scan rate of the potential was 0.1 V/min.

Fig. 3 Plots of current and ECL amplitude against pH at 0.6 V in a solution containing 1.0×10^{-9} mol/dm³ luminol and 3.0×10^{-4} mol/dm³ H₂O₂.

Fig. 4 Log-log plots of current and ECL amplitude against H_2O_2 concentration at 0.6 V in the solution of pH 11 containing $1.0 \times 10^{-9} \text{ mol/dm}^3$ luminol. The square and circle symbols denote the current and the ECL amplitude, respectively.

Fig. 5 Log-log plots of the current and ECL amplitude against the luminol concentration at 0.6 V in a solution of pH 11 containing $3.0 \times 10^{-4} \text{ mol/dm}^3 \text{ H}_2\text{O}_2$. The square and circle symbols denote the current and the ECL amplitude, respectively.

Fig. 6 Log-log plots of the current and the luminol concentration at 0.6 V in a solution of pH 11 containing no H_2O_2 .

centration of luminol is much smaller than that of H_2O_2 . Figure 6 shows the plot of I against the luminol concentration. The current increases with the luminol concentration in the case of the high concentration, indicating that the luminol was oxidized on Pt. From these results, it can be concluded that both H_2O_2 and luminol are oxidized on Pt, and that the luminescence was due to a re-

action of two products by electrochemical reactions. The ECL of luminol is divided into four processes as follows:⁸⁾

(a) Dissociation of luminol in alkaline solution.

The luminol dissociates by two steps in Eq. (1). Since $\text{p}K_{a1} = 6.2\text{--}6.7$ and $\text{p}K_{a2} = 15.1$, almost species are LH^- at pH 11.

(b) Generation of super-oxide radical $\text{O}_2^{\cdot -}$.

It was reported that the radicals $\text{O}_2^{\cdot -}$ and OOH were formed by the oxidation of H_2O_2 .⁹⁾ Watanabe *et al.*^{10,11)} stated in FIA study that the superoxide ion $\text{O}_2^{\cdot -}$ produced from H_2O_2 reacted with 1,10-phenanthroline and emitted luminescence.

(c) Oxidation of luminol:

(d) Reaction of the oxidized luminol and $\text{O}_2^{\cdot -}$.

The oxidized luminol reacts with $\text{O}_2^{\cdot -}$ and forms an excited aminophthalate ions $\text{AP}^{2- *}$. The $\text{AP}^{2- *}$ decays to base state emitting fluorescence. In the case of the present ECL of luminol, the step (b) and (c) are promoted by electrochemical reactions on Pt.

On the basis of the above-mentioned results, the following reactions are considered as the present ECL mechanism:

where $h\nu$ means a photon.

3. 2 Electrochemical and ECL impedance

When the small perturbation signal ΔE is imposed on the electrode reaction system in the frequency domain, the signal ΔI is responded. The ratio of ΔE to ΔI is the electrochemical impedance (Z) at each frequency, and electrochemical reaction can be analyzed by the Z ,

$$Z = \Delta E / \Delta I \quad (11)$$

In ECL, the amplitude of the luminescence (ΔP) is alternated with ΔI . An ECL impedance (L), which is the ratio

of ΔP to ΔI , was proposed in Ref. 7 as a new transfer function to investigate an electrode reaction. The ECL impedance corresponds to luminescence efficiency in the frequency domain,⁷⁾ and allows the kinetic analysis of the ECL.

$$L = \Delta P / \Delta I \quad (12)$$

Figure 7 shows Nyquist plots of the electrochemical impedance (Z) measured in a pH 11 solution containing 3×10^{-4} mol/dm³ H₂O₂ and 1.0×10^{-9} mol/dm³ luminol. The Z 's describe the loci in the forth quadrant whose real part is plus and imaginary part is minus. Since the amplitude of the sinusoidal potential signal was sufficiently small, the reaction can be regarded as being a linear system, and is interpreted by the equivalent circuit which has two time constants. The two time constants are due to the electrochemical reaction and double layer capacitance of the working and counter electrodes. However, it is impossible to determine which reaction resistance of the working electrode or the counter electrode

Fig. 7 Nyquist plots of electrochemical impedance Z in the solution of pH 11 containing 3.0×10^{-4} mol/dm³ H₂O₂ and 1.0×10^{-9} mol/dm³ luminol. The potentials of the experimental results are 0.0 V (○), 0.1 V (△) and 0.2 V (□) in (a), 0.3 V (○), 0.4 V (△) and 0.5 V (□) in (b), 0.6 V (○), 0.7 V (△) and 0.8 V (□) in (c), and 0.9 V (○), 1.0 V (△) and 1.1 V (□) in (d).

Fig. 8 Nyquist plots of ECL impedance L in the same solution as Fig. 9. The open and solid symbols denote experimental and simulated results, respectively. The potentials of the results are 0.0 V (○) in (a), 0.1 V (○) in (b), 0.2 V (○) in (c), 0.3 V (○) in (d), 0.4 V (○), 0.5 V (△) and 0.6 V (□) in (e), 0.7 V (○) and 0.8 V (△) in (f), and 0.9 V (○), 1.0 V (△) and 1.1 V (□) in (g). The parameters for the curve fitting are as follows: $a = 210 \Omega$, $k = 0.16$ dm³ mol⁻¹ s⁻¹ for 0.3 V, $a = 5000 \Omega$, $k = 0.10$ dm³ mol⁻¹ s⁻¹ for 0.4 V, $a = 31000 \Omega$, $k = 0.16$ dm³ mol⁻¹ s⁻¹ for 0.5 V, $a = 32000 \Omega$, $k = 0.10$ dm³ mol⁻¹ s⁻¹ for 0.6 V, $a = 8000 \Omega$, $k = 0.10$ dm³ mol⁻¹ s⁻¹ for 0.7 V.

is presented in Fig. 7 in this analysis.

Figure 8 shows Nyquist plots of the ECL impedance (L) measured in the same solution as shown in Fig. 7. The plots of L below 0.2 V gather the origin of the complex plane, indicating no fluorescence emission. The L describes the semicircle at the forth quadrant of the complex plane between 0.3 V and 0.7 V. Above 0.8 V, the L describes the spiral shape around the origin, and approaches the minus of the real axis. Assuming that the concentrations of O₂^{-*} is c in reactions (7) and (9), the mass balances of c is:

$$dc/dt + kc = \gamma \Delta I / F \quad (13)$$

In this analysis, the oxidation current of luminol was neglected because the concentration of luminol is much smaller than that of H₂O₂. According to the previous report,⁶⁾ the theoretical expression of L is derived as below,

$$L = a / (1 + j\omega/k) \quad (14)$$

$$a = K \gamma / F, \quad (15)$$

Fig. 9 Plots of reaction rate constant and potentials in the solutions of pH 11. The concentration of chemicals are as follows: ○: 6.0×10^{-4} mol/dm³ H₂O₂ and 1.0×10^{-9} mol/dm³ luminol, △: 3.0×10^{-4} mol/dm³ H₂O₂ and 1.0×10^{-9} mol/dm³ luminol, □: 6.0×10^{-4} mol/dm³ H₂O₂ and 1.0×10^{-9} mol/dm³ luminol.

where K is a constant concerning the sensitivity of the detector, j is an imaginary number, and ω is an angular frequency ($\omega = 2\pi f$, f is a frequency). The plot of L presented by equation (14) describes a semicircle on the complex plane. The experimental results of L were fitted by equation (14) at 0.3–0.7 V in Fig. 8. The parameter x for the curve fitting are written in the caption of Fig. 8. The obtained rate constants (k) are shown in Fig. 9. In various H₂O₂ concentrations, the k takes a value between 0.09 and 0.17 dm³ mol⁻¹ s⁻¹, and the k does not depend on the electrode potential since the reaction (9) is the chemical step. In Figs. 8 (f) and (g) the L shows spiral shape and approaches a negative value (negative real axis) in the low-frequency range above 0.8 V, indicating that the luminescence decreases with increasing the current. This result is in an agreement with the P - E curve in Fig. 1. The negative value of L can be explained by the efficiency decrease of reactions (6)–(9) to the total reaction. This luminescence requires the formation of O₂^{-*} in the reaction (7). However, the formation efficiency of O₂^{-*} may decrease above 0.8 V because O₂^{-*} is an in-

termediate of H₂O₂ oxidation and the final product is O₂.

4 Summary

The ECL of luminol was investigated by electrochemical impedance spectroscopy. By the comparison of ECL impedance with the theoretical expression, the rate constant of chemical step in ECL was obtained.

The CL analysis is a useful method to determine trace amount of chemicals because of the small background signal. Furthermore the ECL can be used in various analyses since the catalytic reaction can be precisely controlled. The frequency-domain analysis developed in the present paper allows information concerning not only the ECL mechanism, but also the kinetic parameters.

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