

**Self-quenching in electrochemiluminescence of  $\text{Ru}(\text{bpy})_3^{2+}$  using  
ascorbic acid as co-reactant**

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**ABSTRACT:**

In this study, electrochemiluminescence (ECL) of  $\text{Ru}(\text{bpy})_3^{2+}$  (bpy = 2,2'-bipyridyl) using ascorbic acid ( $\text{H}_2\text{A}$ ) as co-reactant was investigated in an aqueous solution. When  $\text{H}_2\text{A}$  was co-existed in a  $\text{Ru}(\text{bpy})_3^{2+}$  containing buffer solution, ECL peaks were observed at potential corresponding to oxidation of  $\text{Ru}(\text{bpy})_3^{2+}$ , and the intensity was proportional to  $\text{H}_2\text{A}$  concentration at lower concentration levels. The formation of the excited state  $^*\text{Ru}(\text{bpy})_3^{2+}$  was confirmed to be resulted from the co-reaction between  $\text{Ru}(\text{bpy})_3^{3+}$  and the intermediate of ascorbate anion radical ( $\text{A}^{\cdot-}$ ), which showed the maximum ECL at  $\text{pH} = 8.8$ . It is our first finding that the ECL intensity would be quenched significantly when the concentration of  $\text{H}_2\text{A}$  was relatively higher, or upon the ultrasonic irradiation. In most instances, quenching is observed with 4-fold excess of  $\text{H}_2\text{A}$  over  $\text{Ru}(\text{bpy})_3^{2+}$ . The diffusional self-quenching scheme as well as the possible reaction pathways involving in  $\text{Ru}(\text{bpy})_3^{2+}/\text{H}_2\text{A}$  ECL system, are discussed in this study.

**KEYWORDS:** Tris(2,2'-bipyridine)ruthenium(II); electrochemiluminescence; self-quenching; ascorbic acid; ultrasonic vibration.

## INTRODUCTION

Electrochemiluminescence (ECL) can be defined as the generation of light by means of homogenous electron transfer reactions in electrochemical processes (1). ECL from tris(2,2'-bipyridyl)ruthenium (II),  $\text{Ru}(\text{bpy})_3^{2+}$ , is generally generated by the co-reaction between the oxidized  $\text{Ru}(\text{bpy})_3^{3+}$  and some co-reactants in the vicinity of electrode, and produced the excited molecule  $\text{Ru}(\text{bpy})_3^{2+*}$  for light emitting. Because ECL of  $\text{Ru}(\text{bpy})_3^{2+}$  can emit light under room temperature in both aqueous and non-aqueous with relatively higher ECL efficiency, it is now being used widely as an analytical tool for many bioanalytical and environmental applications, including immunoassay or DNA-probe assay (2,3), and analysis of a variety of trace organic substances, like amines and so on (4,5,6).

The determination of ascorbic acid ( $\text{H}_2\text{A}$ ) has been studied extensively because of the significance of this compound in bioelectrochemistry and clinical diagnostics applications. Electrochemical methods with various chemically modified electrodes have been well demonstrated in this field (7). Application of  $\text{Ru}(\text{bpy})_3^{2+}$  based ECL in the detection of  $\text{H}_2\text{A}$  in liquid chromatography was first described by Chen and Sato (8). Although the ECL intensity in this system was found to be proportional to  $\text{H}_2\text{A}$  concentration, the detailed mechanism, however, is still not clear. Our subsequent studies showed that ECL intensity could be strongly quenched when the co-reactant of  $\text{H}_2\text{A}$  was existed in relatively higher concentration levels. The ECL scheme is thus necessary to be clarified because it can lead to the development of a validity ECL based analytical method in the determination of  $\text{H}_2\text{A}$ . Here we report the evidence of ECL quenching behavior in the presence of  $\text{H}_2\text{A}$ . The effect of the mass transport rate and the possible pathways involved in the ECL reaction are discussed in this paper for the first time.

## MATERIALS AND METHODS

### Chemicals

Tris(2,2'-bipyridyl)ruthenium(II) chloride [ $\text{Ru}(\text{bpy})_3\text{Cl}_2 \cdot 6\text{H}_2\text{O}$ ] was purchased from Aldrich and was used as received. The other reagents were of analytical grade purchased from Nacalai Tesque (Kyoto, Japan). Working standard solutions were prepared by precise dilution of stock solutions with water. Phosphate buffer solution (PBS) was prepared by equimolar amount of disodium hydrogenphosphate ( $\text{Na}_2\text{HPO}_4$ ) and potassium dihydrogenphosphate ( $\text{KH}_2\text{PO}_4$ ). The appropriate pH of the buffer was adjusted with orthophosphoric acid or sodium hydroxide. All solutions were with

distilled water purified by a WS200 distillation system (Yamato Scientific Co., Tokyo, Japan).

### **ECL and photoluminescence measurements**

The ECL measurements were conducted using a micro-ECL cell as was previously reported (9). A glassy carbon (GC) disk electrode ( $\phi 1.0$  mm) was used as working electrode, while a platinum wire and a silver (Ag) wire were employed as auxiliary electrode and quasi-reference electrode (QRE), respectively. The light emitting from the electrode surface was measured with a H7468-01 photomultiplier tube (PMT) module (Hamamatsu Photonics, Shizuoka, Japan) via an optical fiber which was positioned opposite to the working electrode surface. PMT was controlled by a notebook computer and a laboratory-written software package via RS-232C interface. The program was developed by Microsoft Visual Basic 6.0. Cyclic voltammetry and the potential control in ECL measurements were carried out with a model 660 CHI electrochemical analyzer (CH Instruments, Austin, TX, USA). In this study, a model MA-1000 ultrasonic tooth brush (1.2 MHz, Mrinu Co., Takamatsu, Japan) was used as an ultrasound source to vibrate the electrode. The electrode unit was mounted at the tip of the ultrasonic transducer (9). All ECL measurements were conducted in a light proof box. Photoluminescence spectra were obtained with a RF-5300PC fluorescence spectrophotometer (Shimadzu Co., Kyoto, Japan). A quartz cuvette with 1 cm path length was used.

## **RESULTS AND DISCUSSION**

### **Electrochemiluminescence in Ru(bpy)<sub>3</sub><sup>2+</sup>/H<sub>2</sub>A system**

Figure 1 shows the simultaneous cyclic voltammograms and the corresponding ECL signals of 0.50 mM Ru(bpy)<sub>3</sub><sup>2+</sup> in 0.1 M PBS (pH=8.8). A pair of redox waves of Ru(bpy)<sub>3</sub><sup>2+</sup> were observed around +1.15 V (vs. Ag QRE). When H<sub>2</sub>A was added in the solution, its oxidation peak ( $E_{\text{pox}}$ ) was appeared at +0.4 V, and in meanwhile, the oxidation peak current of Ru(bpy)<sub>3</sub><sup>2+</sup> was dramatically increased (Fig. 1a), accompanied with the light emitting from the electrode (Fig. 1b). As can be seen in Fig. 2, it gave only very weak ECL signal in acidic solutions, indicating the protonated form of H<sub>2</sub>A could not achieve an efficient light production. When the pH value was higher than 9.5, a distinguished background signal was observed, owing to the formation of the excite state upon the co-reaction between Ru(bpy)<sub>3</sub><sup>3+</sup> and OH<sup>-</sup> (10). As the normalized ECL intensity (intensity ratio of signal to background) reached maximum at pH 8.8, this pH value was used in subsequent experiments.

### ECL mechanism of Ru(bpy)<sub>3</sub><sup>2+</sup> using H<sub>2</sub>A as co-reactant

The electrochemical oxidation of H<sub>2</sub>A has been well investigated and is known to undergo two-electron transfer, followed by a chemical reaction to produce the electroinactive species, dehydroascorbic acid (DHA). However, the kinetic of electron transfer steps in the overall reaction is quite complicated, depending on pH of the electrolyte (11). In the pH region from neutral to weak basic, irreversible voltammograms of H<sub>2</sub>A were observed. The irreversibility of the reaction was indicated by an anodic shift of the oxidation peak potential ( $E_{pa}$ ) as function of the scan rate ( $v$ ), as is shown in Fig.3. The number of electrons transferred in the rate-limiting step for an irreversible wave can be determined from the following equation (Ref. 1; page 236).

$$E_{pa} = E^{0'} - \frac{RT}{\alpha n_a F} \left[ 0.78 + \frac{1}{2} \ln D_R - \ln k^0 + \frac{1}{2} \ln \left( \frac{\alpha n_a F}{RT} v \right) \right] \quad (1)$$

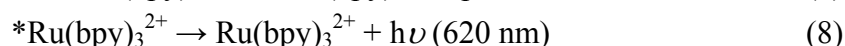
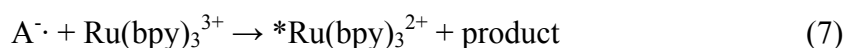
where  $E^{0'}$  is formal potential of H<sub>2</sub>A, T is temperature,  $\alpha$  is the transfer coefficient  $n_a$  is the number of electron transferred in the rate-determining step (rds),  $k^0$  is the electrochemical rate constant and  $F$  is the Faraday constant. A plot of  $E$  vs.  $\ln v$  yields a slope ( $dE_{pa}/d \ln v = RT/2\alpha n_a F$ ) of 0.0188. On the assumption of  $\alpha$  was 0.5 and at the temperature of 298 K, the value of  $n_a$  was calculated to be 1, suggesting that one electron transfer was occurred at the rate determining step as was shown in below.



At pH 8.8, H<sub>2</sub>A was dissociated to form ascorbate monoanion (HA<sup>-</sup>) according to the reported  $pK_a$  values ( $pK_{a1} = 4.17$ ,  $pK_{a2} = 11.57$ ) (12). In first electron transfer step, HA<sup>-</sup> was oxidized to the ascorbate radical (HA<sup>·</sup>). Since HA<sup>·</sup> is a very strong acid ( $pK_a = -0.45$ ) (12), it subsequently underwent a rapid decomposition, which led to the formation of a highly reducing species of ascorbate anion radical (A<sup>·-</sup>). The second electron transfer step resulted in the production of DHA, as was shown in equation (5).

Ru(bpy)<sub>3</sub><sup>2+</sup> was oxidized at +1.15 V. The excited state \*Ru(bpy)<sub>3</sub><sup>2+</sup> is thought to be produced by the energetic electron transfer between electrochemically generated Ru(bpy)<sub>3</sub><sup>3+</sup> and A<sup>·-</sup>.





$Ru(bpy)_3^{2+}$  that produced in the equation (8) was re-oxidized at the electrode, resulting in enhanced oxidation current as was shown Fig.1a. The ECL light emission spectrum exhibited a maximum band at 620 nm, which was identical to the photoluminescence spectrum of  $Ru(bpy)_3^{2+}$ .

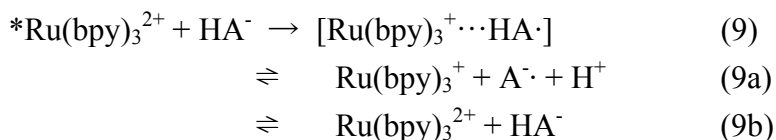
### Self-quenching of ECL intensity by H<sub>2</sub>A

Figure 4 shows the ECL intensity of 0.5 mM  $Ru(bpy)_3^{2+}$  as a function of the H<sub>2</sub>A concentration. The ECL intensity was found to be linearly proportional to the concentration of H<sub>2</sub>A from 0 to 1 mM, and then it decreased as the H<sub>2</sub>A concentration increased from 1 mM. The result implies that the ECL of  $Ru(bpy)_3^{2+}/H_2A$  system would be self-quenched by H<sub>2</sub>A in higher concentration region. In most instances, quenching is observed with 4-fold excess of H<sub>2</sub>A over  $Ru(bpy)_3^{2+}$ , and the ECL signal was completely quenched with 60-fold excess of H<sub>2</sub>A over  $Ru(bpy)_3^{2+}$  in the system. The generated  $*Ru(bpy)_3^{2+}$  species at the electrode surface might be scavenged by large portion of HA<sup>-</sup> diffused from the bulk solution, and resulted in the drop of ECL intensity.

The effect of mass transport on the ECL behavior in  $Ru(bpy)_3^{2+}/H_2A$  system was examined by using an ultrasonic vibrating electrode. Figure 5 depicts the ECL intensity-potential profiles obtained with and without ultrasonic vibration. For comparison, the ECL responses of  $Ru(bpy)_3^{2+}$  using tripropylamine (TPA) as co-reactant were also measured (Fig.5b). When the electrode was vibrated, substantially larger anodic currents for the oxidation of  $Ru(bpy)_3^{2+}$  could be observed in both of system, owing to enhances mass transfer rate in electrochemical systems (13). The resulted ECL profile was also enhanced in  $Ru(bpy)_3^{2+}/TPA$  system. In contrary, however, ECL signal in  $Ru(bpy)_3^{2+}/H_2A$  system was found to be suppressed greatly under the ultrasonic vibration. Because the ultrasound was used at very lower power output (ca. 1 W) with higher frequency (1.2 MHz), the effects associated with ultrasonic cavitation would not be evidenced here. The accelerated mass transfer rate was considered from the effect of acoustic streaming, which increased an effective collision frequency between  $*Ru(bpy)_3^{2+}$  and HA<sup>-</sup>. The result strongly supported that the ECL self-quenching behavior at the electrode surface is arisen from the diffusional HA<sup>-</sup> from the bulk solution.

### Photoluminescence measurements

It has been reported that photoluminescence of  $\text{Ru}(\text{bpy})_3^{2+}$  could be quenched by some co-existing organic reducing reagents (14). Similarly, the ECL quenching is interpreted in terms of electron transfer (ET) route (9), where the diffusional  $\text{HA}^-$  species served as electron donor.



The intermediate state,  $[\text{Ru}(\text{bpy})_3^+ \cdots \text{HA}\cdot]$  was assumed to be formed in diffusional control process. Once the intermediate state was formed, both the decomposition reaction (9a) and the back electron transfer reaction (9b) could occur.

The ET quenching route (9) the presence of  $\text{H}_2\text{A}$  was quantitatively examined by photoluminescence of  $\text{Ru}(\text{bpy})_3^{2+}$  in 0.1 M PBS (pH 8.8). As shown in Fig. 6, the emission intensity of 10  $\mu\text{M}$   $\text{Ru}(\text{bpy})_3^{2+}$  at 620 nm was quenched by the addition of  $\text{H}_2\text{A}$  in the solution, and the dependence of  $\text{H}_2\text{A}$  concentration on emission intensity was described by the Stern-Volmer equation:

$$\frac{I_0}{I} = 1 + K_{sv} [\text{H}_2\text{A}] \quad (10)$$

where  $I_0$  and  $I$  represent the emission intensity in the absence and the presence of  $\text{H}_2\text{A}$ , respectively. The Stern-Volmer constant,  $K_{sv}$  represents the quenching efficiency in ET route, which was calculated to be 11  $\text{M}^{-1}$  from the Stern-Volmer plot shown in the inset to Fig. 6. The Stern-Volmer relationship could also be used to estimate the quenching efficiency in ECL. Using the data presented in Fig.4,  $K_{sv}$  for ECL was calculated to be 884  $\text{M}^{-1}$ , which was 80 times larger than that of the photoluminescence quenching. Apparently, beside the ET route, the electrochemical intermediating reactions can contribute to decreasing the ECL intensity. For example, the electrochemically generated species like  $\text{Ru}(\text{bpy})_3^{3+}$  might be intercepted by  $\text{HA}^-$  before participating in the ECL reaction as shown in equation (6). These effects are now being examined in our laboratory.

## CONCLUSIONS

In conclusion, the ECL of  $\text{Ru}(\text{bpy})_3^{2+}/\text{H}_2\text{A}$  system is complicated which was elucidated to be resulted from the competition reaction between the chemiluminescence reaction (7) and the self-quenching processes. When the concentration of  $\text{H}_2\text{A}$  was low

(below 1 mM co-existed with 0.5 mM Ru(bpy)<sub>3</sub><sup>2+</sup>), equation (7) played a predominant role in ECL reaction. However, when the concentration, or the mass transfer rate increased, the excited state \*Ru(bpy)<sub>3</sub><sup>2+</sup> might be scavenged by large portion of HA<sup>-</sup> diffused from the bulk solution, and resulted in the drop of ECL intensity. These findings would help the development of a validity ECL based detection method for H<sub>2</sub>A determination.

### Acknowledgements

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## Figure Captions

**Figure 1.** Cyclic voltammograms (a) and the corresponding ECL responses (b) of 0.50 mM  $\text{Ru}(\text{bpy})_3^{2+}$  in PBS (pH 8.8) observed in the absence (dotted lines) and in the presence of 0.50 mM  $\text{H}_2\text{A}$  (solid lines).  $\nu = 100$  mV/s.

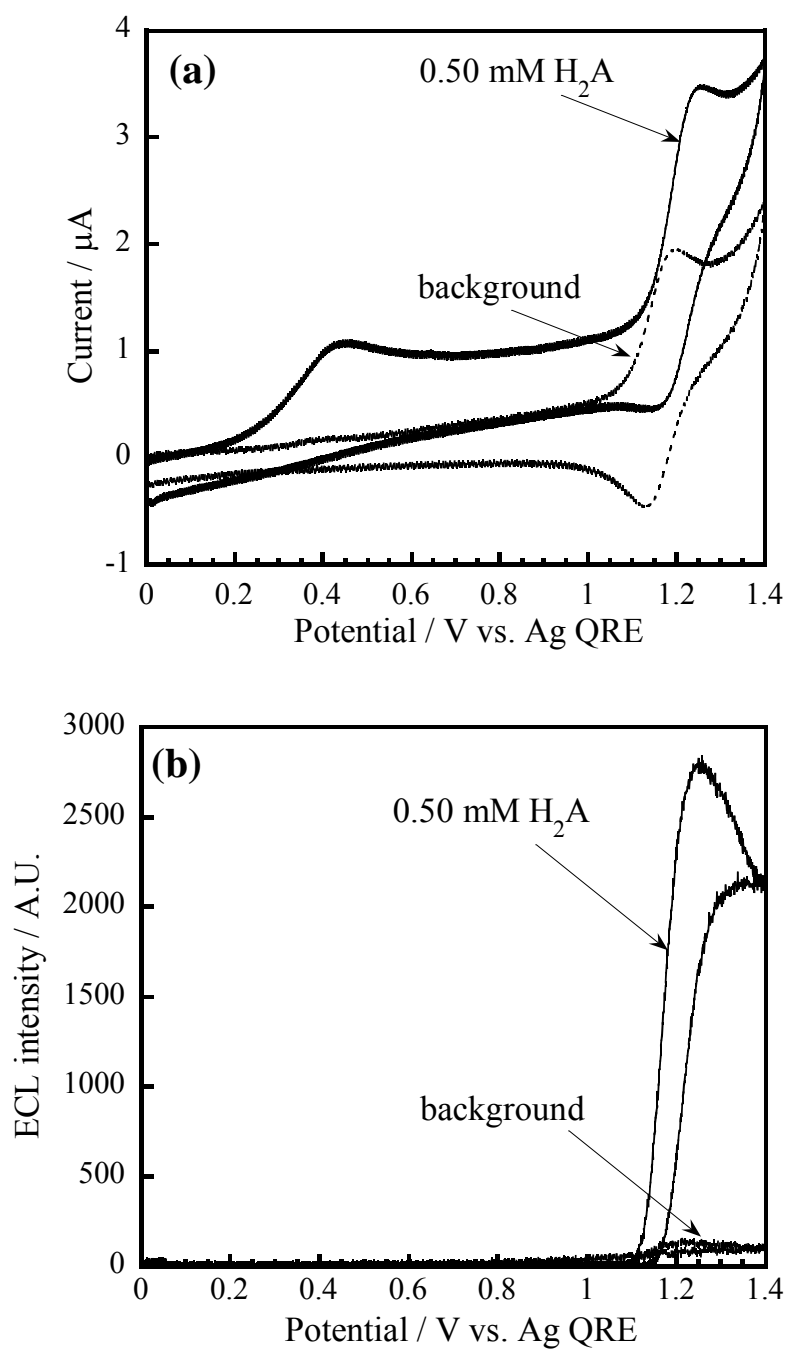
**Figure 2.** Effect of pH on the ECL intensities in  $\text{Ru}(\text{bpy})_3^{2+}/\text{H}_2\text{A}$  system. ( $\blacktriangle$ ) Background; ( $\bullet$ ) in presence of 0.5 mM  $\text{H}_2\text{A}$ . The other conditions were the same as in Fig.1.

**Figure 3.** Dependence of the peak potential ( $E_{\text{pa}}$ ) on the scan rate in the electrochemical oxidation process of  $\text{H}_2\text{A}$  at GC electrode in PBS (pH 8.8).

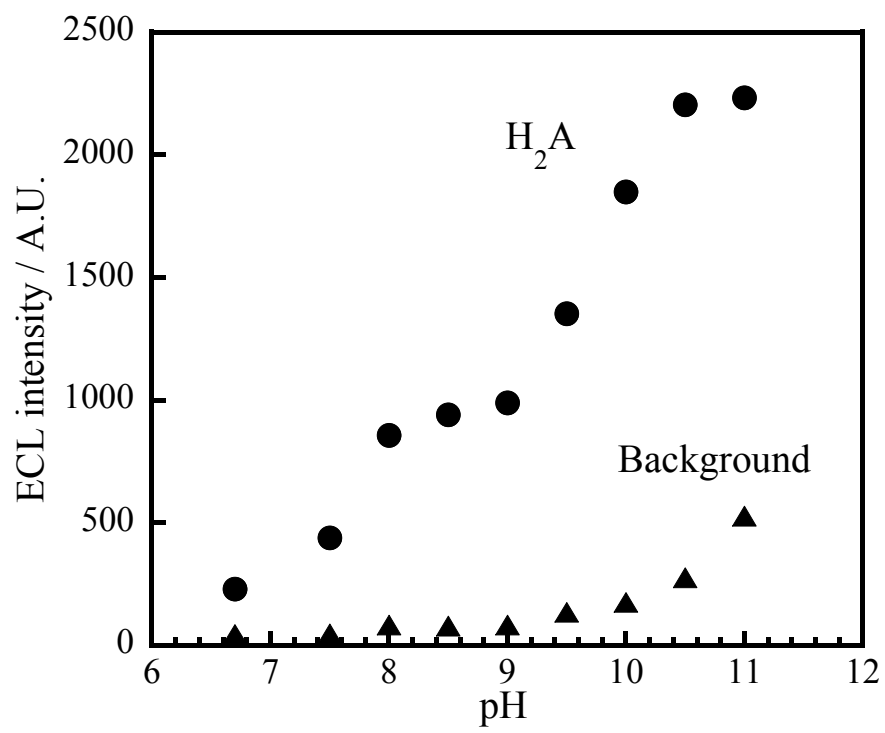
**Figure 4.** Effect of  $\text{H}_2\text{A}$  concentration on ECL intensity in PBS (pH 8.8) containing 0.5 mM  $\text{Ru}(\text{bpy})_3^{2+}$ . Inset is the plot in low concentration region (0 to 1 mM  $\text{H}_2\text{A}$ ).

**Figure 5.** Effect of ultrasonic vibration on ECL intensities of 0.5 mM  $\text{Ru}(\text{bpy})_3^{2+}$  in 0.1 M PBS (pH 8.8) containing 0.5 mM  $\text{H}_2\text{A}$  (a) and 0.5 mM TPA (b) as co-reactants, respectively. Potential scan rate: 100 mV/s.

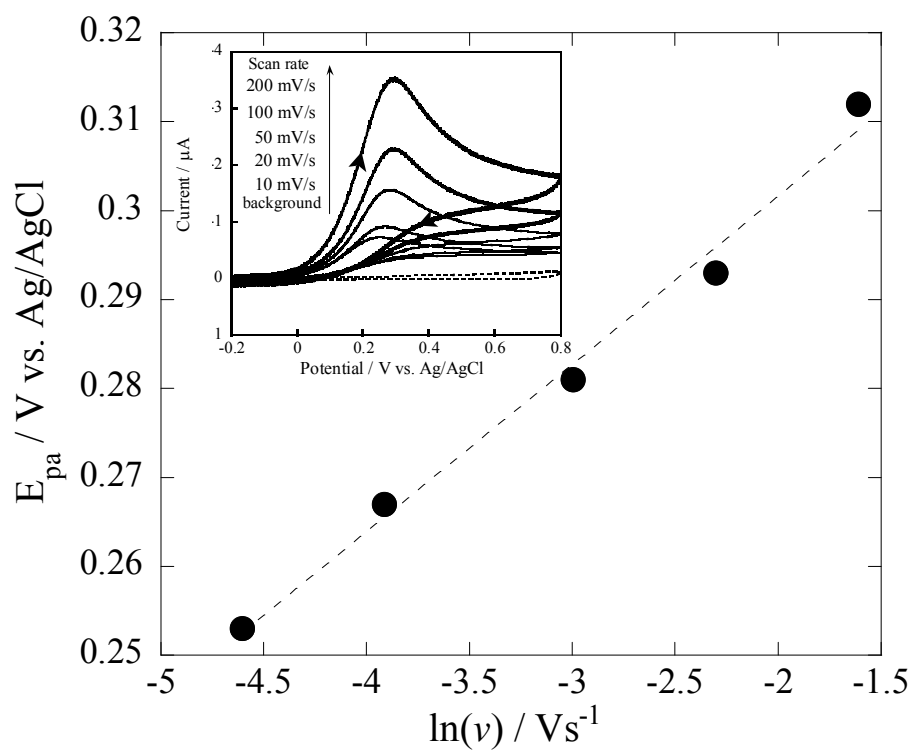
**Figure 6.** Photoluminescence spectra of 10  $\mu\text{M}$   $\text{Ru}(\text{bpy})_3^{2+}$  measured in presence of  $\text{H}_2\text{A}$  with various concentrations. Inset is the Stern-Volmer plot of photoluminescence quenching.



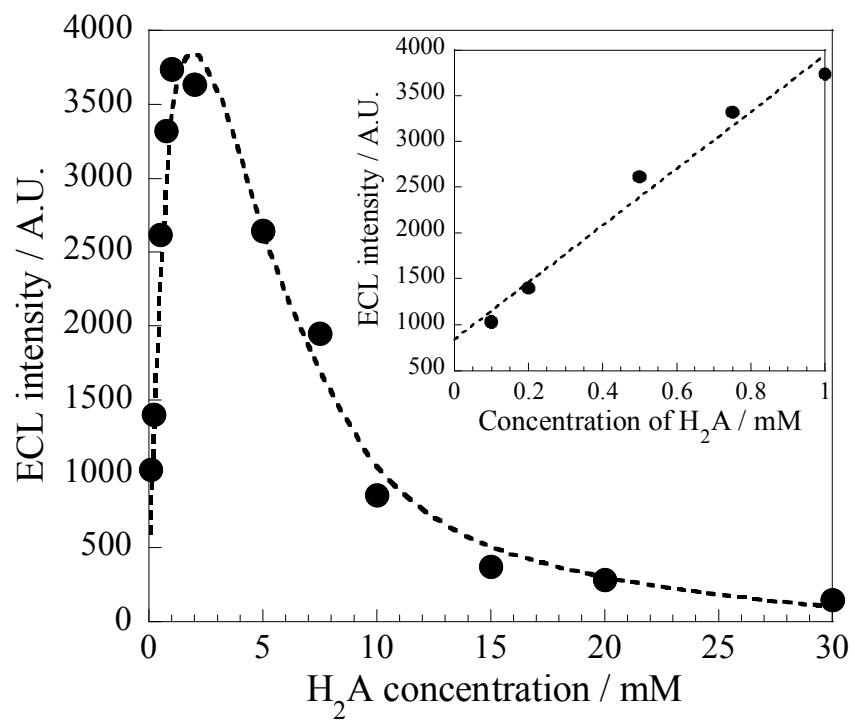
**Figure 1.** F. Takahashi



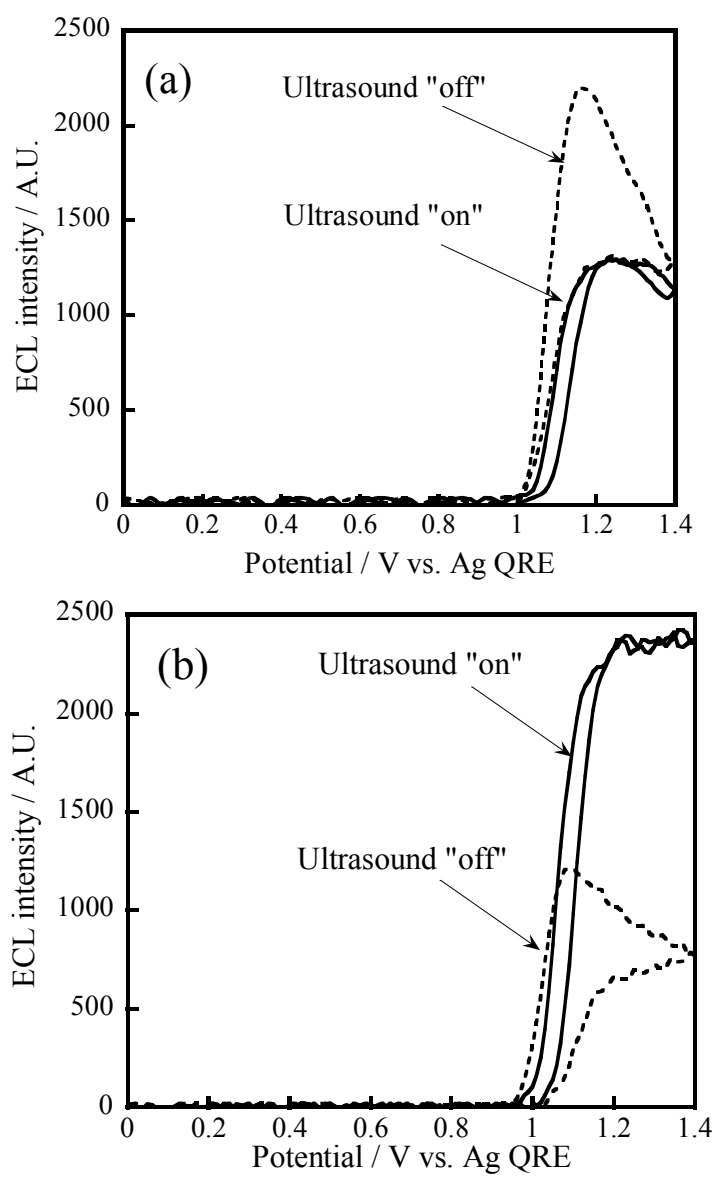
**Figure 2.** F. Takahashi



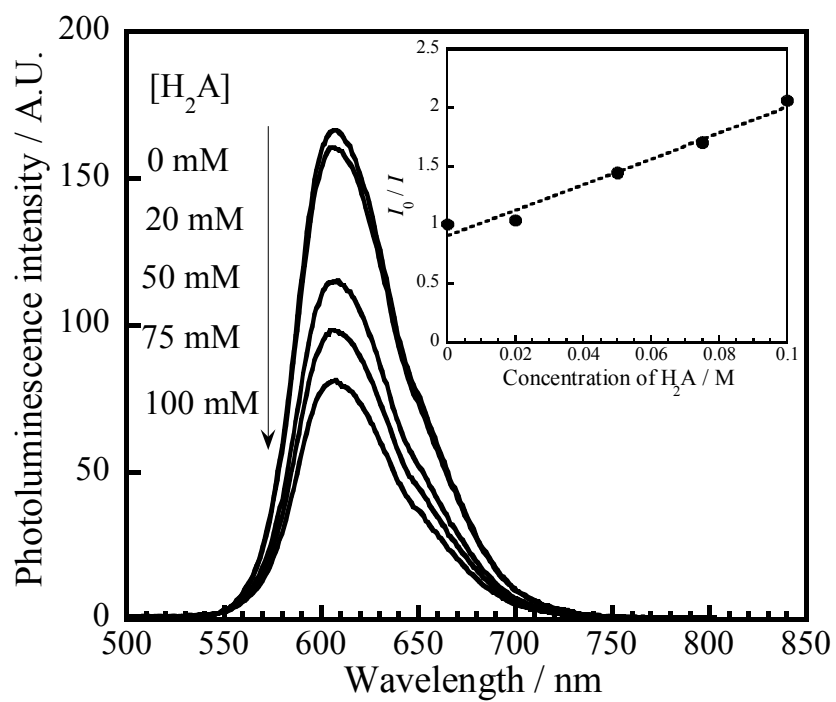
**Figure 3.** F. Takahashi



**Figure 4.** F. Takahashi



**Figure 5.** F. Takahashi



**Figure 6.** F. Takahashi