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Potential-modulated electrochemiluminescence of a tris(2,2′-bipyridine) ruthenium(II) / lidocaine system under 430 kHz ultrasound irradiation

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ABSTRACT

The electrochemiluminescence (ECL) of tris(2,2′-bipyridine)ruthenium(II) ($Ru(bpy)_3^{2+}$) in the presence of lidocaine was investigated under ultrasound (US) irradiation. The sonoelectrochemical experiments are conducted by indirect irradiation of ultrasound with a piezoelectric transducer operating at 430 kHz. In a supporting electrolyte at pH 11, the $Ru(bpy)_3^{2+}$/lidocaine system gave weak ECL peaks around +1.2 V and +1.45 V, respectively. The ECL signal at +1.2 V was attributed to redox reactions of the oxidative intermediates of $Ru(bpy)_3^{2+}$ and lidocaine, while the signal at +1.45 V was assumed to be caused by an advanced oxidation process due to the generation of hydroxyl radicals (·OH) at the electrode surface. In this study, the potential modulation approach is employed in the study of ECL process upon US irradiations because it can suppress the noise components from sonoluminescence effectly and improve the resolution of ECL-potential profiles. It is found ECL signals were greatly enhanced upon US irradiation at the output power of 30 W, however, the relative intensity of ECL signal at +1.2 V was larger than that obtained with a rotating disk electrode even though the mass transport effect is equilvalent. The experiment results suggest that the chemical effect (i.e., generation of ·OH) by 430 kHz US becomes remarkable in the electrochemical process. Detailed ECL reaction routes under US are proposed in this study.

1. Introduction

Ultrasound (US) is an acoustic wave that is above the range of human hearing. It shows potential for environmental, clinical, and industrial chemistry applications in diverse fields [1–3]. The combination of US to electrochemistry is currently attracting an amount of interest on account of a number of advantages, such as increasing of the mass transport, activation or *in situ* cleaning of the electrode surface, and the beneficial effects of US on electrochemical process have been reviewed by several researchers [4,5]. Some electrochemical sensing systems have been developed wit combination of US in order to improve the sensitivity [6–16].

Electrochemiluminescence (ECL) is light emitted from excited species in heterogeneous electrochemical reactions [17]. The ECL of $Ru(bpy)_3^{2+}$ (bpy = 2,2′-bipyridine) can be observed in aqueous solutions via an alternative means of utilizing coreactants, such as amines, polyphenols, and pesticides [18,19]. The coreactant systems have made it possible to adopt the ECL technique in analytical detections. In our previous study, it revealed that US could increase the $Ru(bpy)_3^{2+}$ ECL efficiency significantly when tripropylamine was used as coreactant. This has been attributed to the enhancement of mass transport effect by US which is advantageous to generate the $Ru(bpy)_3^{2+}$ excited state on electrode surface [20]. On the other hand, because ECL is the complex subsequent chemical reaction at the electrode surface, there has been little literature discussion regarding the chemical effect by US.

The chemical effects of US originate from acoustic cavitation in an aqueous solution. The generation of reactive chemical species following decomposition of H_2O is thought to occur as follows [21–23]:

$$H_2O \rightarrow H\cdot + \cdot OH \quad (1)$$

The ·OH has particularly high oxidative reactivity with high redox potentials, and can induce a subsequent decomposition reaction through intermediate radical generation [21,23–26]. So, it is possible to affect ECL reactions through the the formation highly reactive intermediate species. Up until now, however, few studies concerned with the chemical effect of US on ECL have been reported. To achieve a better understanding of the chemical effect by US, more detailed investigation is necessary.

In this study, we examined the ECL behavior of $Ru(bpy)_3^{2+}$ under 430 kHz US irradiation, because the cavitation efficiency (production rate of ·OH) is higher with this frequency. Lidocaine, a common local anesthetic, was was used as a coreactant in the present study. Lidocaine

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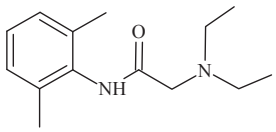

Fig. 1. Structure of lidocaine.

contains tertiary amine and amide groups (Fig. 1). Previous studies suggested that the excited state of $Ru(bpy)_3^{2+}$ could be produced primarily by lidocaine radical on the basis of lidocaine electrochemical oxidation [27,28], and the other radical intermediates induced by chemical decomposition with ·OH were also considered to contribute in the ECL reaction. To avoid background noise from sonoluminescence and improve the resolution, a potential-modulated (PM) technique was employed to examine the effect of US on the ECL. The evidence of chemical effect of US on ECL of $Ru(bpy)_3^{2+}$/lidocaine system is reported for the first time in this study.

2. Materials and methods

2.1. Chemicals

Tris(2,2′-bipyridine)ruthenium(II) chloride [$Ru(bpy)_3Cl_2$] hexahydrate was purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification. Lidocaine was purchased from FUJIFILM Wako Pure Chemical Corp. (Osaka, Japan). A standard stock solution (100 mM) was prepared in ultrapure water. Phosphate-buffered saline (PBS, 0.1 M) was selected as a buffered solution because the chemical compounds did not affect the ECL reactions. The PBS was prepared by mixing known volumes and concentrations of disodium hydrogen phosphate and potassium dihydrogen phosphate (Nacalai Tesque, Inc., Kyoto, Japan). The pH was adjusted with phosphoric acid or an aqueous solution of sodium hydroxide. Working standard solutions were prepared by precise dilution of the corresponding stock solution with PBS. All other reagents were of analytical grade and purchased from FUJIFILM Wako Pure Chemical Corp. Ultrapure water (resistivity > 18.2 MΩ) was obtained using a Milli-Q water purification system (Millipore, Billerica, MA, USA).

2.2. Apparatus and ECL measurement system with US irradiation

Fig. 2 shows a schematic illustration of the ECL measurement under US irradiation. A 430-kHz transducer was mounted on a stainless-steel plate at the bottom of the reactor (10 × 10 × 18 cm rectangular chamber) and driven by a QUAVA mini 30,110 generator (Kaijo Corp., Tokyo, Japan). Distilled water was added to a height of 15 cm from the bottom of the reactor. A cylindrical glass vessel of 3 cm diameter was used as a electrochemical cell and placed center at horizontal position in sonochemical reactor. In all experiments, the sample volume in the cell was 20 mL and the surface of the sample solution was adjusted so it was aligned with the water level in the sonochemical reactor. A 3 mm diameter glassy carbon electrode, an Ag/AgCl electrode (saturated with KCl), and a platinum wire were used as the working, reference, and counter electrodes, respectively. The working electrode in this study was a L-type glassy carbon disc electrode mounted in the Teflon holder. The distance between the electrode and the transducer was adjusted and measured manually with an accuracy of 0.5 mm. The light emission from the electrode was measured by a PMT module (H11901-01, Hamamatsu Photonics, Japan), which was set opposite to the electrode with a distance of 3 cm. Before the measurements, the working electrode was carefully polished using 0.30-μm aluminum powder, and finally sonicated in a Bransonic ultrasonic cleaner.

In the PM-ECL experiments, a sinusoidal AC potential (E_{ac}) was generated by a model WF1946A function generator (NF Co., Kanagawa, Japan). The AC potential was superimposed on a DC potential ramp generated by a model EG&G 263A potentiostat/galvanostat (Princeton Applied Research, Oak Ridge, Tennessee, USA), and the PM potential was applied to the working electrode. The ECL signals from the PMT module were amplified by an LI-574 lock-in amplifier (NF Circuit Block, Japan) with an AC reference input from the function generator. The output ECL signal from the lock-in-amplifier was recorded using a PowerLab data acquisition system (AD Instruments, Sydney, Australia).

2.3. Apparatus for GC–MS measurements and sample preparation

A Trace GC Ultra (Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with a Rxi-5Sil MS capillary column (30 m × 0.25 mm inner diameter, 0.25 μm film thickness, Shimadzu GLC Ltd., Kyoto, Japan), and Polaris Q mass spectrometer (Thermo Fisher Scientific) were used for GC–MS. Ultra-pure helium gas (> 99.99995%) was used as the carrier gas with a constant linear velocity of 40.0 cm/s. The oven temperature was initially held at 50 °C for 1.0 min, increased from 50 °C to 150 °C at 15 °C/min, then from 150 °C to 310 °C at 30 °C/min, and held at 310 °C for 4.0 min. The temperatures of the injector, transfer line, and ion source were 250 °C, 260 °C, and 200 °C, respectively. Mass spectra were obtained in electron ionization mode (positive) at 70 eV.

Lidocaine solution (20 mL, 500 μM) was submitted to ultrasound (US) irradiation at 430 kHz for 30 min. The solution was dispensed into two test tubes. In one test tube, 10 mL of the solution was basified with 1.0 mL of 2.5 M NaOH, and in the other, 10 mL of the solution was acidified with 1.0 mL of 1.0 M HCl. Ethyl acetate (1.0 mL) was added to each sample. The samples were shaken vigorously (300 rpm, 5 min) using a Mix-VR Invitro shaker (Taitec Co., Saitama, Japan), and then centrifuged at 2650×*g* for 5 min using a microcentrifuge (H-19α, Kokusan Co. Ltd., Tokyo, Japan). The ethyl acetate extracts from the acidified and basified samples were combined and 1.0 μL of the ethyl acetate mixture was injected into the GC–MS. The same procedure was carried out to prepare samples without US irradiation.

3. Results and discussion

3.1. Electrochemical and ECL behavior of the lidocaine/$Ru(bpy)_3^{2+}$ system

Fig. 3 shows ECL response (at a glassy carbon electrode in 0.1 M PBS (pH 11.0) containing 500 μM $Ru(bpy)_3^{2+}$ and 500 μM lidocaine obtained (A) at stationary condition and (B) under 430 kHz US irradiation. Under stationary condition (A), two ECL peaks were observed around +1.2 V and +1.45 V vs. Ag/AgCl, respectively. ECL at +1.2 V was corresponding with the potential for electrochemical oxidation of $Ru(bpy)_3^{2+}$. The ECL intensity for this system is proportional to the concentration of both $Ru(bpy)_3^{2+}$ and lidocaine and also depends on the solution pH. Lidocaine contains a tertiary amine group and is oxidized around +0.8 V in PBS (pH 11). The ECL reaction at +1.2 V should be similar to coreactant mechanism of $Ru(bpy)_3^{2+}$/tri-*n*-propylamine system [17], as shown below.

$$\text{Lid} \rightarrow \text{Lid}\cdot + \text{H}^+ + \text{e}^- \quad (2)$$

$$Ru(bpy)_3^{2+} \rightarrow Ru(bpy)_3^{3+} + \text{e}^- \quad (3)$$

$$Ru(bpy)_3^{3+} + \text{Lid}\cdot \rightarrow {}^*Ru(bpy)_3^{2+} + \text{products} \quad (4)$$

$${}^*Ru(bpy)_3^{2+} \rightarrow Ru(bpy)_3^{2+} + h\upsilon\ (610\ \text{nm}) \quad (5)$$

Lid stands for lidocaine in the equations. In pH 11 PBS solution, deprotonated lidocaine molecules first undergo one-electron oxidation to form lidocaine cation radicals ($\text{Lid}\cdot^+$), which rapidly deprotonate to form lidocaine free radicals (Lid·) (Eq. (2)) [29,30]. The excited state, $^*Ru(bpy)_3^{2+}$, was generated by the subsequent electron transfer between the electrochemically generated $Ru(bpy)_3^{3+}$ (Eq. (3)) and the reducing Lid· species, which emits a photon (Eqs. (4) and (5)). It was found that the second ECL peak around +1.5 V became more distinct, and the ECL peak potential shifted to the negative potential range with

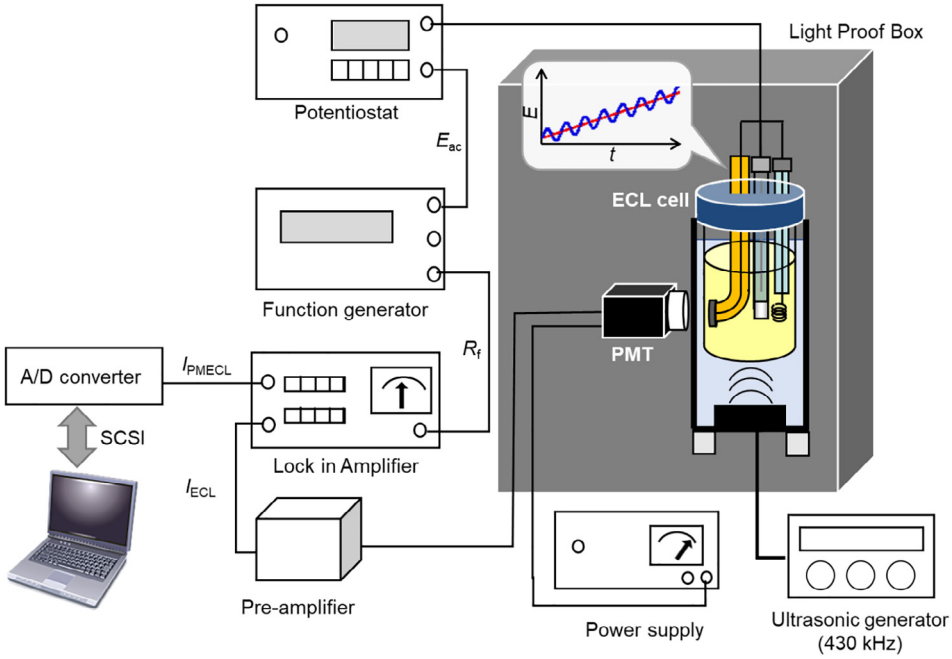

Fig. 2. Schematic illustration of the electrochemiluminescence (ECL) measurement system with ultrasound irradiation.

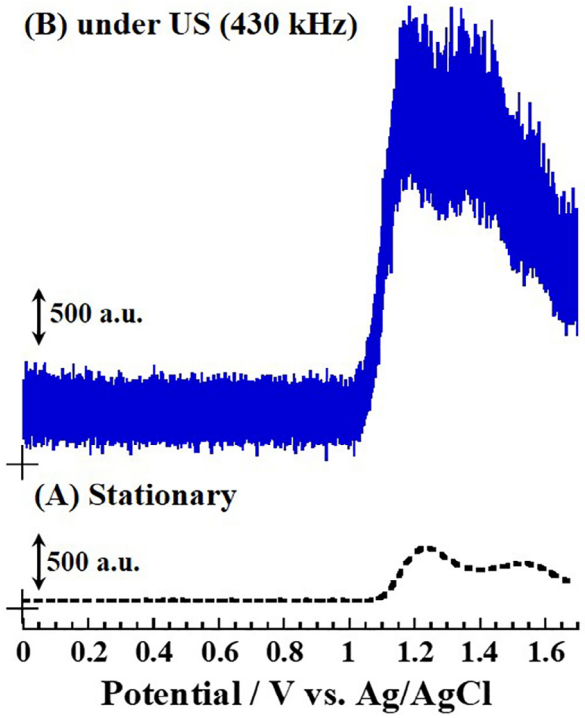

Fig. 3. (A) Electrochemiluminescence (ECL) responses for 500 μM $Ru(bpy)_3^{2+}$ in 0.1 M phosphate-buffered saline (PBS, pH 11.0) in the presence of 500 μM lidocaine, under (A) the stationary and (B) 430 kHz US condition. The scan rate was 50 mV/s and the photomultiplier tube (PMT) bias voltage was 650 V.

an increasing pH value of supporting electrolyte. The electrochemical oxidation of OH^- would lead to form reactive oxygen species such as ·OH or hydrogen peroxide (H_2O_2) at the electrode surface. We assumed that lidocaine was decomposed by ·OH to form some intermediate radicals, which contributed the second ECL reaction. The ECL intensity of $Ru(bpy)_3^{2+}$/lidocaine system was signaificantly enhaced upon 430 kHz US irradiation (Fig. 3(B)). On the other hand, larger background noises are recorded in ECL-potential profile. The noise component could be attributed to sonoluminescence from the acoustic cavitation effect [24,31–34], that make it difficult to examine the effect by US quantitatively.

3.2. Potential-modulated ECL measurements of the lidocaine/$Ru(bpy)_3^{2+}$ system under US

In order to suppress the effect from sonoluminescence and to improve the resolution of measurement, a potential modulation (PM) approach is employed in the study of ECL process upon US irradiations. In PM-ECL measurement, a sinusoidal ac voltage (E_{ac}) with a frequency of 10 Hz and amplitude of 100 mV was superimposed upon a dc potential ramp during the potential scan, and the light signal was detected synchronously with a lock-in amplifier. Fig. 4 shows PM-ECL profiles in PBS (pH11) containing 500 μM $Ru(bpy)_3^{2+}$ and 500 μM lidocaine, measured with the conditions of (A) stationary, (B) 430 kHz US irradiation (50 W) and (C) with a rotating disk electrode (RDE) with a rotation speed of 300 rpm, respectively. As expected, the analytical signal can be effectively differentiated from the background components by PM-ECL measurement, and thus resulted in a well resolved ECL – potential profiles. Under 430 kHz US irradiation, relative intensity of PM-ECL signal at +1.15 V exhibited higher on comparison with those obtained in stationary condition.

The enhancement of ECL intensity could also be caused by the mass transport effect by US. Fig. 5(A) shows cyclic voltammograms of 1.0 mM $Fe(CN)_6^{3-}$ in 0.1 M KCl for evaluation of the dependence on the US output power. A reversible redox pair of $Fe(CN)_6^{3-}$/$Fe(CN)_6^{4-}$ was observed without US irradiation (i.e., stationary condition, 0 W; (a)). Sigmoidal voltammograms were observed under US irradiation (Fig. 5(b) and (c)), and the steady-state current increased with increases the US output power. The steady-state current intensity was described as a limited-current density (i_L (μA/cm^2)) which could be used to evaluate the mass transport effect in hydrodynamic voltammograms [30]. It is known that ultrasound could increase the rate of mass transfer to the electrode raising the limiting current density and causing a reduction in the diffusion layer thickness. Cavitation at or near the electrode surface creates microstreaming, which is more effective than stirring in disrupting concentration gradients. The the limiting current increased with increasing of the output power. We found that the

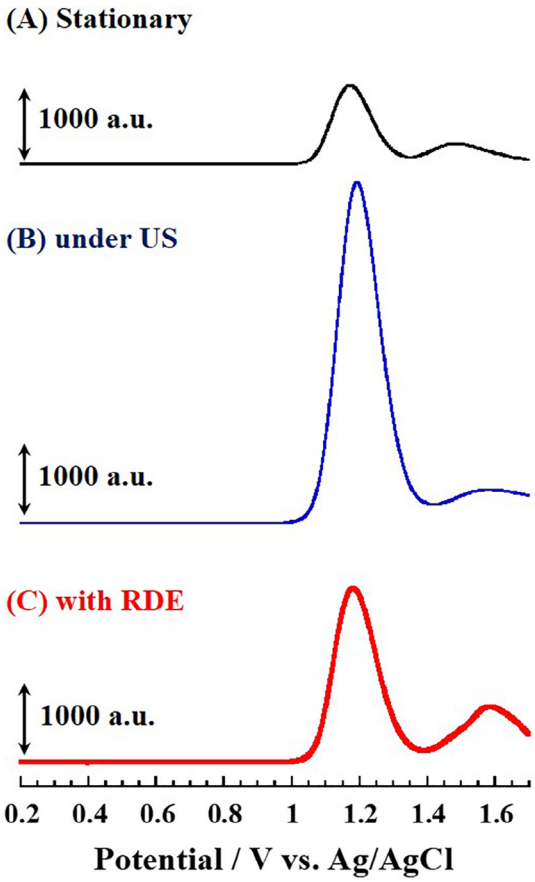

Fig. 4. Potential-modulated–electrochemiluminescence (PM-ECL) profiles of 500 μM $Ru(bpy)_3^{2+}$ and 500 μM lidocaine in phosphate-buffered saline (PBS) under (A) stationary, (B) ultrasound (US) irradiation (430 kHz, 50 W) and (C) with a rotating disk electrode (RDE, 300 rpm). The DC scan rate was 20 mV. The AC amplitude was 100 mV and the frequency was 10 Hz.

electrochemical responses under US irrdadiation were similar to those obtained by hydrodynamic voltammetry with RDE, and the mass transport effect by 430 kHz US at 50 W was equivalent to that obtained at the RDE with rotation rate of 300 rpm (Fig. 5(B)). On the other hand, the relative ECL intensity at +1.2 V measured under 430 kHz US was larger than that at RDE with the rotation rate of 300 rpm, as shown in Fig. 4. We suggest that the increasement under US irradiation is contributed from the the intermediate radicals which were formed by the reaction of lidocaine with ·OH.

3.3. ECL mechanism of the lidocaine/$Ru(bpy)_3^{2+}$ system under US irradiation

The content of the decomposition products of lidocaine by US were analyzed by gas chromatography (GC)–mass spectrometry (MS). Fig. 6(A) shows the selected ion monitoring (SIMs) chromatograms resuted from the decomposition products after US irradiation for 0 min, 10 min, 20 min, and 30 min. A peak at 9.6 min is ascribed to lidocaine (Fig. 6(B, b)), the molecular ion peak (*m/z* 234) was detected. The peak area decreased with increasing of US irradiation time, indicating that lidocaine was decomposed by US at 430 kHz. After US irradiation, a new peak appeared at 4.9 min, and the peak area increased with increasing of the irradiation time. Fig. 6(B, a) is the mass spectrum of this peak. The molecular ion peak, xylidine was comnfirmed as the final product in US decomposition. This provided an experimental evidence that the intermediate radical (R·) could be generated by the reaction of lidocaine with ·OH, as shown in Eqs. (6) and (7).

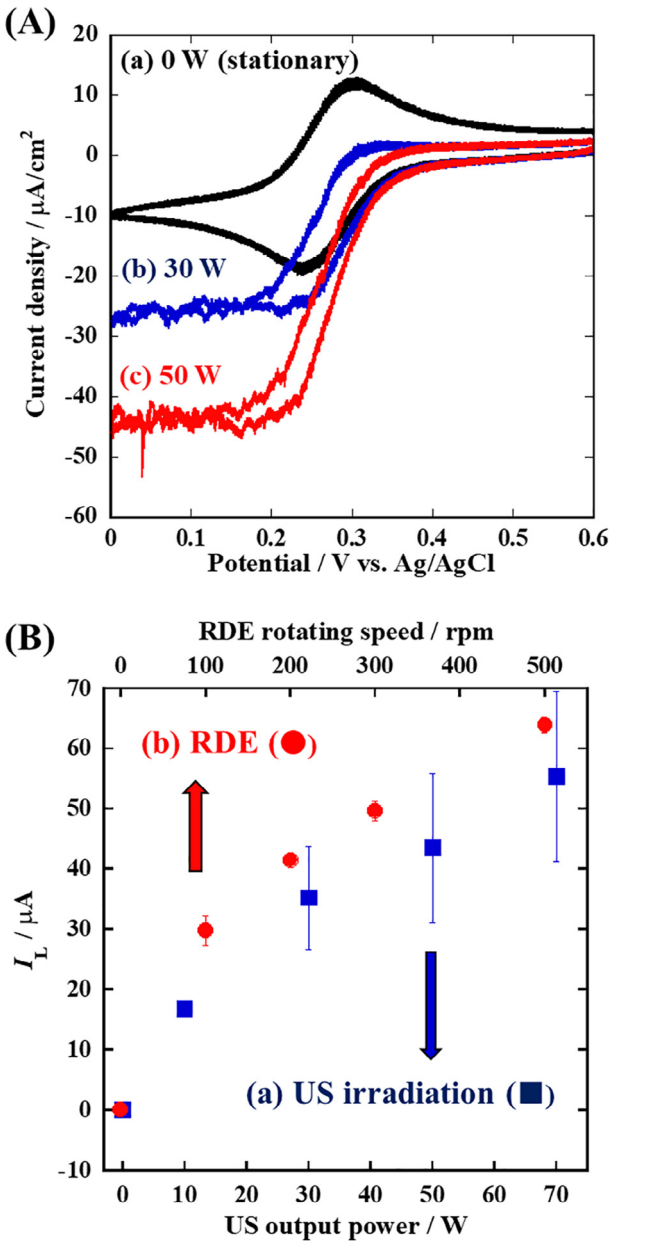

Fig. 5. (A) CVs of 1.0 mM $Fe(CN)_6^{3-}$ in 0.1 M KNO_3 under US irradiation at (a) 0 W (stationary condition), (b) 30 W and (c) 50 W. (B) The limiting-current density (I_L) versus the (a) US output power and (b) RDE rotation speed. Error bars represent the standard deviation (n = 5).

Lid + ·OH → R· (radical intermediate) (6)

→ → xylidine + products (7)

Fig. 7 compares the PM-ECL intensities at + 1.2 V measured under US irradiation and at RDE in a PBS solution containing 500 μM $Ru(bpy)_3^{2+}$ and 500 μM lidocaine, with different US powers and RDE rotation rates. The PM-ECL intensities increased with increasing of US output power or the rotation rate of RDE, but the intensities under US (Fig. 7(a)) were larger than those under the corresponding RDE conditions (Fig. 7(b)). The results suggests that the intermediate radical of lidocaine generated in Eq. (6) by US can contribute ECL generation at +1.2 V. Possible pathways for the ECL system under US irradiation are summarized in Fig. 8. In comparison to ECL at stationary condition, with Because an enhanced ECL signal could be observed in a lower potential region under US irradiation, a new strategy for analytical detection of lidocaine by ECL is expected to be developed in future

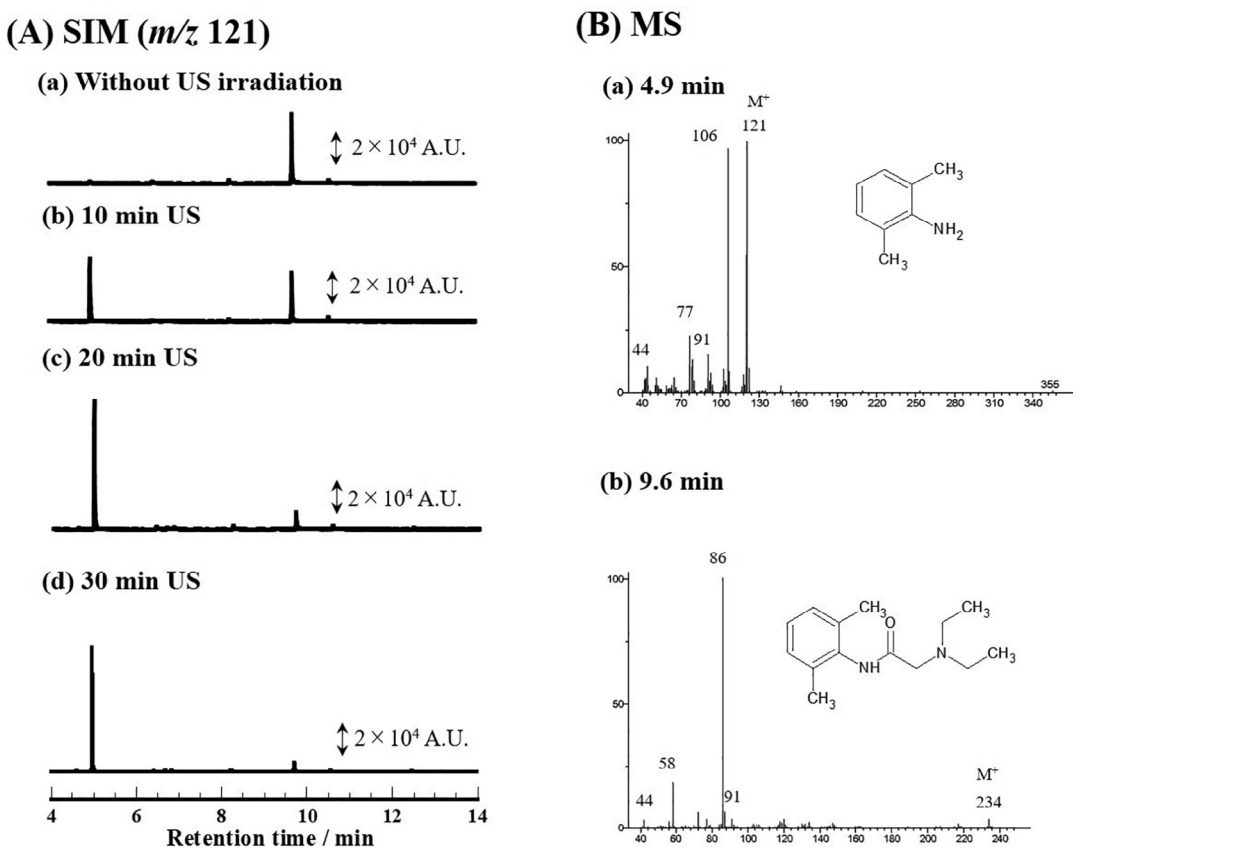

Fig. 6. (A) Selected ion monitoring (SIM) at *m/z* 121 of the extracted ethyl acetate phases obtained (a) without US and with US irradiation for (b) 10 min, (c) 20 min, and (d) 30 min. (B) Mass spectra (MS) for the peaks at (a) 4.9 min and (b) 9.6 min in the chromatograms.

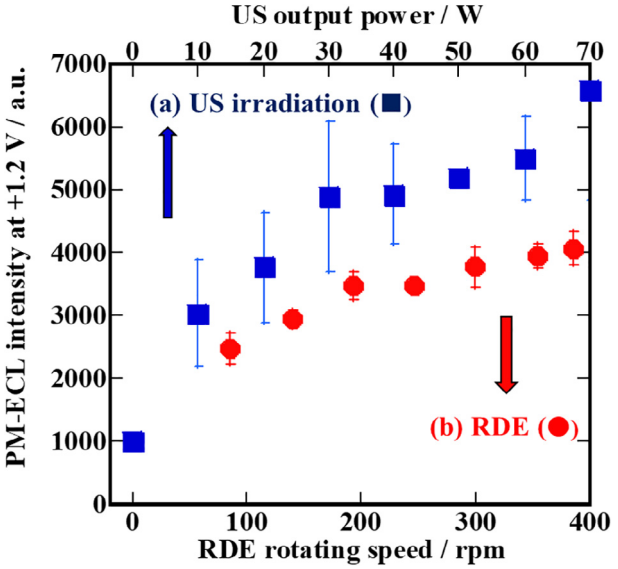

Fig. 7. Dependence of the potential-modulated–electrochemiluminescence (PM-ECL) intensity at +1.2 V on (a) US output power (squares) and (b) RDE (circles). The other conditions were the same as in Fig. 4. Error bars represent the standard deviation (n = 5).

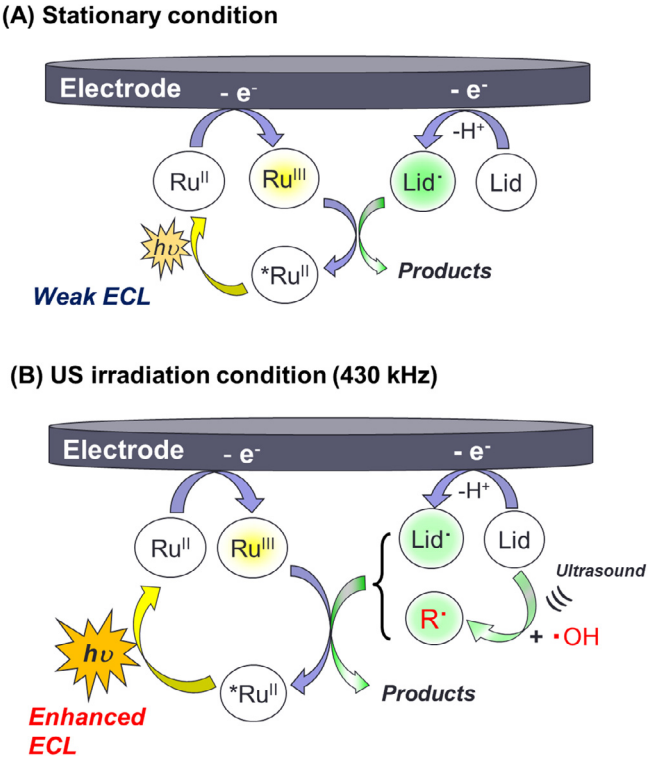

Fig. 8. Possible ECL reaction pathways of the $Ru(bpy)_3^{2+}$/lidocaine (Lid) system under (A) stationary condition and (B) 430 kHz US irradiation.

studies.

4. Conclusions

The evidence of chemical effect of US on ECL of $Ru(bpy)_3^{2+}$/lidocaine system was studied for the first time in this study. It was

demonstared that the ECL measurements using potential modulation approach could suppress the noise components from sonoluminescence effectly and improve the resolution of ECL-potential profiles. ECL signals of $Ru(bpy)_3^{2+}$/lidocaine system were greatly enhanced upon US irradiation. ECL reaction routes under US irradiations were characterized by both voltammetry and mass spectroscopy. In addition to the increasing of the rate of mass transport, the chemical effect by cavitational ·OH generation is suggested to contribute in the ECL reaction.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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