Title: Characterization of electrochemiluminescence of tris(2,2′-bipyridine)ruthenium(II) with glyphosate as co-reactant in aqueous solution

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Characterization of electrochemiluminescence of

tris(2,2′-bipyridine)ruthenium(II) with glyphosate as co-reactant in aqueous

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Abstract

Glyphosate, a phosphorus-containing amino acid type herbicide was used as a co-reactant for studying of electrochemiluminescence (ECL) reaction of tris(2,2'-bipyridyl)ruthenium(II) [$Ru(bpy)_3^{2+}$] in an aqueous solution. In a phosphate buffer solution of pH 8, glyphosate itself was known to be electrochemically inactive at glassy carbon electrode, however, it participated in a homogeneous chemical reaction with the electrogenerated $Ru(bpy)_3^{3+}$, and resulted in the producing the $*Ru(bpy)_3^{2+}$ species at the electrode surface. Kinetic and mechanistic information for the catalysis of glyphosate oxidation were evaluated by the steady-state voltammetric measurement with an ultramicroelectrode. The simulated cyclic voltammogram based on this mechanism was good agreement with that obtained experimentally. ECL reaction of $Ru(bpy)_3^{2+}$ / glyphosate system was found to be strongly dependent on the media pH. In a pH region of 5 ~ 9, an ECL wave appeared at ca. +1.1 V *vs*. Ag/AgCl, which was caused by the generation of $*Ru(bpy)_3^{2+}$ via a $Ru(bpy)_3^{3+}$-mediated oxidation of glyphosate. When pH >10, a second ECL wave was observed at ca. +1.35 V *vs*. Ag/AgCl, which was believed to be associated with a reaction between $Ru(bpy)_3^{3+}$ and the species from direct oxidation of GLYP at a GC electrode surface.

Keywords: Electrogenerated chemiluminescence of $Ru(bpy)_3^{2+}$; Glyphosate; EC' catalytic reaction; Ultramicroelectrode; Potential modulated ECL
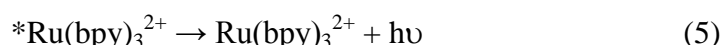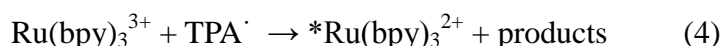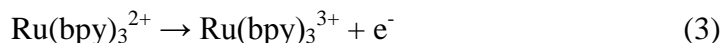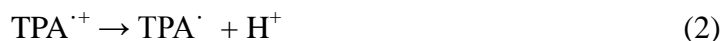
1. Introduction

Glyphosate [N-(phosphonomethyl)glycine] and glufosinate [DL-homoalanine-4-yl(methyl) and phosphinic acid] are phosphorus containing amino acid-type herbicides (see Fig. 1), which have been extensively used in the history of agriculture [1]. Although they are of comparatively low toxicity to humans and animals, their effects on non-target organisms and the environment have not been entirely investigated, questions regarding the environmental safety with their increasing use have to be addressed [2-4]. Glyphosate (GLYP) often represents an analytical challenge because of its relatively high solubility in water, insolubility in organic solvent, high polarity and low volatility. Most methods developed until now require pre- or post-column derivatization procedures to improve both the chromatographic behavior and the detection ability by gas chromatography (GC) or high-performance liquid chromatography (HPLC) [5-8]. There is an expanding need for analytical method able to provide rapid, sensitive, easy and reliable detection of GLYP at low concentrations and costs used.

Electrochemiluminescence (ECL), also known as electrogenerated chemiluminescence is a process where the species generated at electrodes undergo electron transfer reactions to form the excited state that emits light. Because it does not need an excitation light source like fluorometry, ECL provides the advantages of simple instrumentation and low background signal, and the technique has been received considerably attention in the field of analytical chemistry [9-11]. ECL reaction from Tris(2,2'-bipyridyl)ruthenium(II), $Ru(bpy)_3^{2+}$ can emit light at room temperature in both aqueous and non-aqueous solutions with relatively higher ECL efficiency. Tripropylamine (TPA) is often used as a coreactant in $Ru(bpy)_3^{2+}$ ECL system [9, 12],

and the reactions can be expressed by

Scheme 1

$$TPA \rightarrow TPA^{\cdot+} + e^- \tag{1}$$

$$TPA^{\cdot+} \rightarrow TPA^{\cdot} + H^+ \tag{2}$$

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

$$Ru(bpy)_3^{3+} + TPA^{\cdot} \rightarrow {}^*Ru(bpy)_3^{2+} + products \tag{4}$$

$${}^*Ru(bpy)_3^{2+} \rightarrow Ru(bpy)_3^{2+} + h\upsilon \tag{5}$$

The oxidation of TPA is believed to generate a strongly reducing specie of $TPA^{\cdot}$ in equation (2). This radical can then reduce the electrogenerated $Ru(bpy)_3^{3+}$ (in eq 3) to produce the excited state ${}^*Ru(bpy)_3^{2+}$ at the electrode surface[9]. In addition, ${}^*Ru(bpy)_3^{2+}$ can also been generated by the "catalytic routes" in which electrogenerated $Ru(bipy)_3^{3+}$ reacts with TPA to produce the additional $TPA^{\cdot+}$ intermediate. The details of the coreactant ECL mechanism have been recently documented by some researchers [11, 12].

In recent, Nieman et al reported the determination of GLYP with HPLC using a $Ru(bpy)_3^{2+}$-based ECL detecting system [13]. It was shown that GLYP could act as a coreactant capable of generation of ECL signals in alkaline aqueous solutions. Although the system has been of practical importance, the kinetics and the reaction routes involved have not been investigated in detail. Since GLYP is an electroinactive species, the coreactant ECL route should be different from Scheme 1, in which the different homogeneous electron-transfer steps should be taken account. In this work, comparative studies on the $Ru(bpy)_3^{2+}$ based - ECL system using TPrA, GLYP and glufosinate (GLUF) as coreactants were performed with respect to the kinetic and mechanistic information in the ECL processes.. The possible pathways of ECL reaction for

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Ru(bpy)$_3$$^{2+}$ / GLYP system are discussed in this paper for the first time.

2. Experimental

2.1. *Apparatus*

Voltammetry was performed with a model 660 electrochemical workstation (CH Instruments, Austin, TX, USA) or an EG&G/PAR 263A potentiostat / galvanostat. A conventional three-electrode cell was used, with a Pt wire as the counter electrode and an Ag/AgCl reference electrode (RE-1, BAS Japan). Glassy carbon disk (Tokai Carbon Co., Tokyo, Japan) working electrodes (geometric area, 0.20 cm^2) and a carbon fiber microdisk electrode with a diameter of 10 μm were used as working electrodes. The electrochemical measurements with microelectrode were conducted in a Faraday cage to minimize the interference from external electric noise.

In the ECL experiments, the electrode potential was controlled by an EG&G/PAR 263A potentiostat/ galvanostat. The electrochemical cell was placed in a thick iron dark box. The electrode surface of working electrode was 0.5 mm far from the optical window. A H7732-10 PMT photosensor module equipped with a C7319 signal preamplifier unit (Hamamatsu photonics, Shimokanzo, Japan) was placed in front of the optical window to detect the light emitting from the electrode. A C7169 power supply unit (Hamamatsu photonics, Shimokanzo, Japan) was used for driving the photosensor module.

In the potential modulated ECL (PMECL) experiments, a sinusoidal ac voltage with a frequency of 1 − 100 Hz and amplitude of 10 mV generated by a function generator, was superimposed upon a dc potential ramp during the potential scan. The

output ECL signal from the PMT photosensor module was amplified by a LI-574 lock-in amplifier (NF Circuit Block, Japan) as well as the current signal from the potentiostat were fed into a PowerLab data acquisition system (AD Instruments, NSW, Australia).

Digital simulations for electrochemical oxidation of $Ru(bpy)_3^{2+}$ with GLYP were run on a Windows PC with DigiSim software package (BAS, West Lafayette, IN, USA).

2.2. *Reagents and solutions*

Tris(2,2'-bipyridyl)ruthenium(II) chloride [$Ru(bpy)_3Cl_2 \cdot 6H_2O$] was purchased from Aldrich. Glyphosate (GLPY), glufosinate (GLUF) and tripropylamine (TPA) were of analytical grade purchased from Wako Pure Chemical Industries (Osaka, Japan). Phosphate buffer solution (PBS) was prepared by equimolar amount of disodium hydrogenphosphate (Na_2HPO_4) and potassium dihydrogenphosphate (KH_2PO_4). The appropriate pH of the buffer was adjusted with orthophosphoric acid or sodium hydroxide. All solutions were prepared with the distilled water purified by a WS200 distillation system (Yamato Scientific Co., Tokyo, Japan).

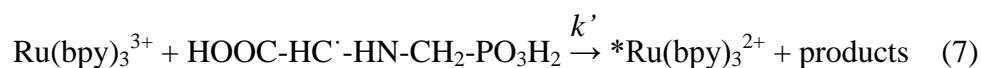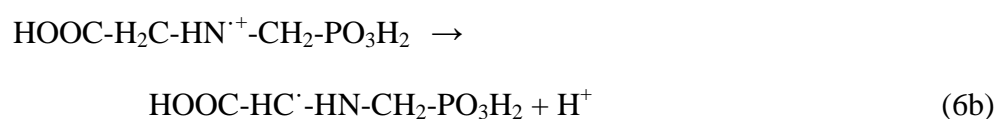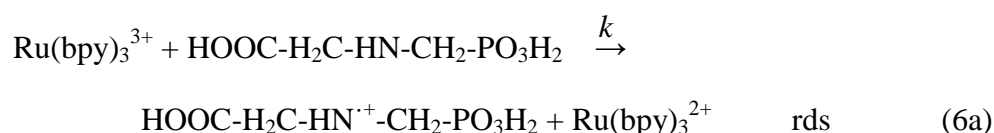## 3. Results and discussion

3.1. *ECL of $Ru(bpy)_3^{2+}$ using TPrA, GLYP and GLUF as coreactants*

Figure 2 shows the cyclic voltammograms (lower) and the corresponding ECL signals (upper) for 0.50 mM $Ru(bpy)_3^{2+}$ in 0.1 M PBS (pH 8.0) at GC electrode in the presence of (A) 0.5 mM GLYP, (B) 0.5 mM GLUF and (C) 0.5 mM TPA, respectively. The dashed lines are the responses of 0.5 mM $Ru(bpy)_3^{2+}$ in the absence of the

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coreactants. A pair of redox waves of Ru(bpy)$_3$$^{2+}$ were observed around +1.1 V *vs.* Ag/AgCl. When the coreactants were added into the solution, the anodic peak currents increased greatly, implying the catalytic mechanism in which the charge transfer reaction is followed by a homogeneous chemical reaction that regenerates the Ru(bpy)$_3$$^{2+}$ species at the electrode surface. The significant ECL signals were simultaneously observed from Ru(bpy)$_3$$^{3+}$/GLYP and Ru(bpy)$_3$$^{3+}$/TPA systems (Fig. 2A and Fig. 2C) around the oxidative potentials. But it gave only a very weak ECL signal for Ru(bpy)$_3$$^{3+}$/GLUF system (Fig. 2B). Since TPA is easier to oxidize than GLYP and GLUF, the direct electrochemical oxidation of TPA could generate the short-lived TPA radical species at GC electrode surface that initialized the ECL reaction according to Scheme 1. On the other hand, both GLYP and GLUF did not show the oxidative peak in the potential range studied. To explain the ECL generation for Ru(bpy)$_3$$^{2+}$/GLYP system, an EC' catalytic route which was mediated by Ru(bpy)$_3$$^{3+}$ was proposed [12].

Scheme 2

$$Ru(bpy)_3{}^{2+} \xrightarrow{k^o} Ru(bpy)_3{}^{3+} + e^- \tag{3}$$

$$Ru(bpy)_3{}^{3+} + HOOC\text{-}H_2C\text{-}HN\text{-}CH_2\text{-}PO_3H_2 \xrightarrow{k}$$

$$HOOC\text{-}H_2C\text{-}HN^{\cdot+}\text{-}CH_2\text{-}PO_3H_2 + Ru(bpy)_3{}^{2+} \qquad rds \tag{6a}$$

$$HOOC\text{-}H_2C\text{-}HN^{\cdot+}\text{-}CH_2\text{-}PO_3H_2 \rightarrow$$

$$HOOC\text{-}HC^{\cdot}\text{-}HN\text{-}CH_2\text{-}PO_3H_2 + H^+ \tag{6b}$$

$$Ru(bpy)_3{}^{3+} + HOOC\text{-}HC^{\cdot}\text{-}HN\text{-}CH_2\text{-}PO_3H_2 \xrightarrow{k'} {}^*Ru(bpy)_3{}^{2+} + products \tag{7}$$

$$^*Ru(bpy)_3{}^{2+} \rightarrow Ru(bpy)_3{}^{2+} + h\upsilon \tag{5}$$

In the EC' catalytic route, the amino group in GLYP was oxidized by the electrogenerated Ru(bpy)$_3$$^{3+}$ to produce the cationic radical GLYP$^{\cdot+}$ (= HOOC-H$_2$C-HN$^{\cdot}$$^+$-CH$_2$-PO$_3H_2$) in eq 6a. It was deprotonated immediately in water to form the strongly

reducing radical intermediate (GLYP$^{.}$ = HOOC-HC$^{.}$-HN-CH$_2$-PO$_3$H$_2$) in eq 6b. The radicals can reduce Ru(bpy)$_3^{3+}$ back to Ru(bpy)$_3^{2+}$ in an excited state *Ru(bpy)$_3^{2+}$ (eq 7). The stabilization of the radical formation would play an important role in the generation of Ru(bpy)$_3^{2+}$ ECL. In general, the ionization potential of the alkylamines can be ordered primary > secondary > tertiary [14, 15]. The secondary amine unit in GLYP was thought to contribute the stabilization of the intermediate radical, and consequently exhibited stronger ECL signal than GLUF in which only the primary amine unit is contained.

3.2. Voltammetric study of the Ru(bpy)$_3^{2+}$ / glyphosate EC' catalytic route

In the case of Scheme 2 (eqs 6a, 6b and 7), the electron transfer process is followed by a homogeneous chemical reaction regenerating the electroactive species. Microelectrodes exhibit a range of favorable characteristics making them ideally suited to the investigation of voltammetry under steady state conditions. These characteristics have led to considerable interest in the use of microelectrodes for studying electrochemical processes with coupled chemical reactions [16, 17]. A second-order (nonlinear) model was derived by G. Denuault and D. Pletcher for studying steady-state kinetics of an EC' (catalytic electrochemical) reaction at a microelectrode [18]. The method consists of comparing the variation of the steady-state limiting current (i_s) measured at a carbon ultramicrodisk electrode for the oxidation of Ru(bpy)$_3^{2+}$ in the absence of GLPY (i_s^0), and in the presence of GLPY (i_s^{cat}) as is shown in Fig. 3(A). The step for the generation of an excited state *Ru(bpy)$_3^{2+}$ in eq 7 is known to be relatively fast (a value of k' ≈ 10^{10} M^{-1}s^{-1} in Ru(bpy)$_3^{2+}$/TPA system) [19]. If the substrate of GLPY is electroinactive and eq 6a is supposed to be the rate determining step (rds), kinetic and mechanistic information can be derived from the variation of the

catalytic efficiency, defined as the ratio of the plateau currents i_s^{cat}/i_s^0, with the GLYP

and Ru(bpy)$_3^{2+}$ concentration [18].

$$\frac{i_s^{cat}}{i_s^0} = 1 - \frac{k\pi^2 a^2 D_{Ru^{2+}} C_{Ru^{2+}}^b}{32 D_{Ru^{3+}} D_{GLYP}} + \frac{1}{2}\left[\left(\frac{k\pi^2 a^2 D_{Ru^{2+}} C_{Ru^{2+}}^b}{16 D_{Ru^{3+}} D_{GLYP}}\right)^2 + \frac{k\pi^2 a^2 D_{Ru^{2+}} C_{GLPY}^b}{4 D_{Ru^{3+}}}\right]^{\frac{1}{2}} \quad (8)$$

where a is the radius of microdisk electrode, k is the homogeneous 2nd order rate

constant for equation 6a (rds); D_{Ru2+}, D_{Ru3+}, D_{GLPY} are the diffusion coefficients for

Ru(bpy)$_3^{2+}$, Ru(bpy)$_3^{3+}$ and GLPY; C_{Ru2+}^b and C_{GLPY}^b are the concentrations of

Ru(bpy)$_3^{2+}$ and GLYP in bulk.

As can be seen in Fig. 3(A), the catalytic efficiency depended on the GLYP

concentration (C_{GLPY}^b) in bulk if the Ru(bpy)$_3^{2+}$ concentration (C_{Ru2+}^b) was kept

constant. In this system, the diffusion coefficients $D_{Ru2+} = D_{Ru3+} = 2.6 \times 10^{-6}$ cm s^{-1} were

measured by chronoamperometry. If we assumed the value of D_{GLYP} was equal to $2.6 \times$

10^{-6} cm s^{-1}, the 2nd order rate constant k of eq 6a was calculated to be 621 M^{-1} s^{-1} at pH

= 8.0 by solving the nonlinear equation (8) with the Newton-Raphson's method [20].

Fig.3(B) shows a plot of i_s^{cat}/i_s^0 as a function of the second term in eq 8. ξ at

the horizontal axis refers to $\left[\left(\dfrac{k\pi^2 a^2 D_{Ru^{2+}} C_{Ru^{2+}}^b}{16 D_{Ru^{3+}} D_{GLYP}}\right)^2 + \dfrac{k\pi^2 a^2 D_{Ru^{2+}} C_{GLPY}^b}{4 D_{Ru^{3+}}}\right]^{\frac{1}{2}}$, which were

calculated with the variation of the GLYP concentration. The 2nd order rate constant k of

621 M^{-1} s^{-1} and the diffusion coefficients above were employed for the calculation. As

expected from eq 8, the value of catalytic efficiency (i_s^{cat}/i_s^0) increases linearly with

ξ, indicating that the ultramicroelectrode was preferred for the kinetic studies in this

system.

To verify the EC' mechanism in Scheme 2, the cyclic voltammograms were analyzed by a commercial digital simulation program DigiSim to find the best-fit between experimental and simulated cyclic voltammogram. Fig.4 shows the voltammograms of 0.5 mM $Ru(bpy)_3^{2+}$ and 0.5 mM GLYP in 0.1 M PBS (pH=8) at a GC electrode. Digital simulation of Scheme 2 using the rate constant above yields the open circle points in Fig. 4. The simulation was carried out assuming semi-infinite diffusion and planar electrode geometry. The standard electron transfer rate constant k^o, and transfer coefficient α in eq 1 were optimized to be 0.0012 cm s^{-1} and 0.47 through the fitting processes. The electrode area was of 0.00785 cm^2. The agreement is quite good over the whole part in the experimental results.

3.3. Influence of pH

Figure 5 illustrated the plots of the ECL intensity as a function of pH observed for $Ru(bpy)_3^{2+}$/TPrA, $Ru(bpy)_3^{2+}$/GLYP and $Ru(bpy)_3^{2+}$/GLUF systems. The ECL intensities were measured at +1.10 V and were subtracted form the background response. The pH dependence obtained for $Ru(bpy)_3^{2+}$/GLYP system is similar to that of $Ru(bpy)_3^{2+}$/TPA system which show maximum ECL intensity at pH 8.0. On the other hand, GLUF can not produce the pronounced ECL signal in the whole pH range probably because of the primary amine group in GLUF. It has been described in the previous studied that the tertiary amines like TPA would achieve higher ECL efficiency because they favor the formation of more stable aminoalkyl free radicals than the secondary amines [14]. Although GLPY has a secondary amine group, it achieved very high ECL efficiency comparable to TPA at pH 8. As was shown in Fig. 4, the ECL signals were very weak in acidic solutions because it was not basic enough to deprotonate the TPrA or the GLYP radicals according to eqs 2 and 6b. When the pH

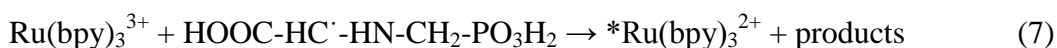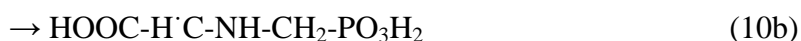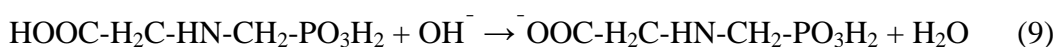10

value was higher than 10, a distinguished background signal was observed, owing to the competation co-reaction between $Ru(bpy)_3^{3+}$ and OH^- [21,22]. Meanwhile, enhanced ECL signals were observed for $Ru(bpy)_3^{2+}$/GLYP system at more positive potential range (> +1.3 V vs. Ag/AgCl). Jirka and Nieman reported modulated potential ECL (PMECL) with the capability of differentiating the analytical signal from background stray light [23]. To study the ECL – potential dependence with better potential resolution at higher potential range, a PMECL measurement technique was employed.

In the potential modulated ECL measurement, light emission intensity is modulated by modulating the potential of a working electrode which is used to generate a key species in the ECL reaction. Figure 6 shows the potential modulated ECL (PMECL) profiles of $Ru(bpy)_3^{2+}$/GLYP system obtained at the pH of 8 and 11, respectively. It was found that smaller potential modulation amplitude would provide better potential resolution by narrowing the potential range over which ECL emission. In this study, a sinusoidal ac voltage with a frequency of 10 Hz and amplitude of 10 mV was superimposed upon a dc potential ramp during the potential scan, and the light signal was detected synchronously with a lock-in amplifier. As expected, the analytical signal can be effectively differentiated from the background components by PMECL measurement, and thus resulted in a well resolved ECL – potential profile. In the neutral or weak alkaline media (pH 7 ~ 9), there was only one ECL peak observed at +1.1, while in alkaline media (pH > 11), a second ECL peak was appeared at +1.35 *vs.* Ag/AgCl with the relative intensity larger than the former. In an alkaline supporting electrolyte (pH =11), the voltammogram of 1mM GLYP exhibited a broad and relatively weak oxidation wave in a region between 1.3 and 1.5 V vs. Ag/AgCl. This can be due to the direct oxidation of GLYP governed by slow electron transfer process. The pK_a value

of amino group for GLPY was reported to be 10.3 [13]. After deprotonating in a basic

solution (at pH > pK_a), GLPY was supposed to be successively oxidized at the electrode

to produce GLYP radical, as were shown in eqs 10a and 10b.

In a potential range of 0.8–1.1 V *vs*. Ag/AgCl, the electrode potential was not

positive enough for direct oxidation of GLYP, but significant amount of $Ru(bpy)_3^{3+}$

could be formed. The ECL generation can be due to the $Ru(bpy)_3^{3+}$ mediated EC'

catalytic routed shown in Scheme 2. Beyond +1.1 V *vs*. Ag/AgCl, however, the direct

oxidation of GLYP at the GC electrode surface became important, and the second ECL

wave followed the mechanism as Scheme 3.

Scheme 3

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

$$HOOC\text{-}H_2C\text{-}HN\text{-}CH_2\text{-}PO_3H_2 + OH^- \rightarrow {}^-OOC\text{-}H_2C\text{-}HN\text{-}CH_2\text{-}PO_3H_2 + H_2O \tag{9}$$

$${}^-OOC\text{-}CH_2\text{-}NH\text{-}CH_2\text{-}PO_3H_2 \rightarrow {}^-OOC\text{-}H_2C\text{-}HN^{\cdot+}\text{-}CH_2\text{-}PO_3H_2 + e \tag{10a}$$

$$\rightarrow HOOC\text{-}H\dot{C}\text{-}NH\text{-}CH_2\text{-}PO_3H_2 \tag{10b}$$

$$Ru(bpy)_3^{3+} + HOOC\text{-}H\dot{C}\text{-}HN\text{-}CH_2\text{-}PO_3H_2 \rightarrow *Ru(bpy)_3^{2+} + products \tag{7}$$

$$*Ru(bpy)_3^{2+} \rightarrow Ru(bpy)_3^{2+} + h\upsilon \tag{5}$$

The dependence of MPECL intensities for the first and the second ECL waves on the

frequency of modulation are shown in Fig. 7. At lower modulation frequency range

(2~10 Hz), it were favorable to the generation of strong and stable PMECL signals.

However, the 2nd ECL wave decreased rapidly with increasing of the modulation

frequency. ECL intensity dependence in frequency domain can be associated with

factors such as the rate of the electrode reaction, the rate of the flowing chemical

reactions and mass transfer in the ECL processes [23, 24]. Although quantitative

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separation of these factors is difficult, the dependence of MPECL in frequency domain implies the slow electrochemical process for the generation the 2nd ECL wave. The quantitative characterizations are now in progress in our laboratory.

4. Conclusions

The electrochemical and ECL properties of GLPY, a phosphorus-containing amino acid type herbicide, were investigated in an aqueous solution in the presence of $Ru(bpy)_3^{2+}$. It was shown that the ECL intensity of $Ru(bpy)_3^{2+}$/ GLPY system strongly depended on the media pH. In a pH region of 5 ~ 9, GLPY itself did not give the oxidation wave at GC electrode but an ECL wave appeared at ca. +1.1 V *vs*. Ag/AgCl. The ECL reaction can be interpreted by a catalytic homogeneous electron transfer between $Ru(bpy)_3^{3+}$ and GLPY, and resulted in the producing the $*Ru(bpy)_3^{2+}$ species at the electrode surface. Kinetic and mechanistic information for the electrocatalytic oxidation process for glyphosate in the presence of $Ru(bpy)_3^{2+}$ were evaluated by the steady-state voltammetric measurement with an ultramicroelectrode. The results were in good agreement with the result by digital simulations. In an alkaline media (pH >10), a second ECL wave was observed at ca. +1.35 V *vs*. Ag/AgCl. The second ECL process can be attributed to the intervention of the direct oxidation of GLYP at the electrode.

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Figure Captions

Figure 1   Chemical structures of glyphosate and glufosinate.

Figure 2   Cyclic voltammograms (lower) and the corresponding ECL profiles (upper) for 0.5 mM Ru(bpy)$_3$$^{2+}$ in 0.1 M PBS (pH 8) in the presence of (A) 0.5 mM TPA, (B) 0.5 mM GLYP and (C) 0.5 mM GLUF, respectively. The dashed lines are the responses of 0.5 mM Ru(bpy)$_3$$^{2+}$ background. Potential scan rate: 50 mV s^{-1}.

Figure 3   (A) Linear sweep voltammograms of 2.0 mM Ru(bpy)$_3$$^{2+}$ at a carbon microdisk electrode (a = 5 μm) with a diameter of 10 μm in 0.1 M PBS (pH 8) in absence (dashed line) and in the presence of GLYP with the concentration variation from 0 to 2 mM. The potential scan rate was 10 mV/s. The dotted line is the blank of 0.1 M PBS (pH=8). (B) Plot of i_s^{cat}/i_s^0 as a function of ξ, which equals to

$$\left[\left(\frac{k\pi^2 a^2 D_{Ru^{2+}} C_{Ru^{2+}}^b}{16 D_{Ru^{3+}} D_{GLPY}}\right)^2 + \frac{k\pi^2 a^2 D_{Ru^{2+}} C_{GLPY}^b}{4 D_{Ru^{3+}}}\right]^{\frac{1}{2}}$$. k = 621 M s^{-1}; the all species were assumed to have a diffusion coefficient of 2.6×10^{-6} cm s^{-1}.

Figure 4   Cyclic voltammograms of 0.5 mM Ru(bpy)$_3$$^{2+}$ and 0.5 mM GLYP in 0.1 M PBS (pH=8). A 1-mm diameter GC electrode was used at a scan rate of 50 mV/s. The open circles show the regression data simulated on the basis of Scheme 2. Parameters used in the simulation were (see Scheme 2 for further information): electrode area, 0.00785 cm^2; $E^0_{Ru3+/Ru2+}$= +1.08 V, k^o = 0.01 (cm s^{-1}) and α = 0.47 in eq 1; k = 621 M s^{-1} in eq 6a; k' = 5.0×10^8 M s^{-1} in eq 7. The temperature was assumed to be 298 K.

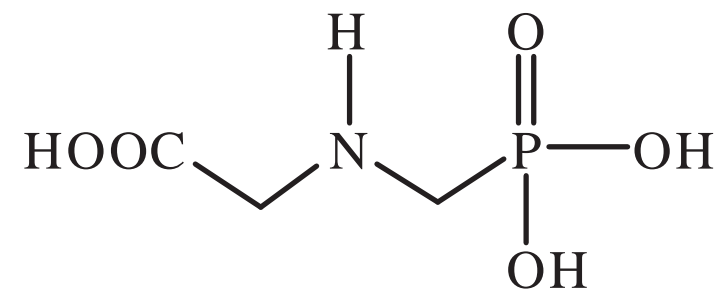**Figure 5**   Dependence of pH on ECL intensity for Ru(bpy)$_3$$^{2+}$/TPA (●), Ru(bpy)$_3$$^{2+}$

/GLYP (■) and Ru(bpy)$_3$$^{2+}$ /GLUF (♦) systems, respectively. The ECL intensities were subtracted from the background signal.
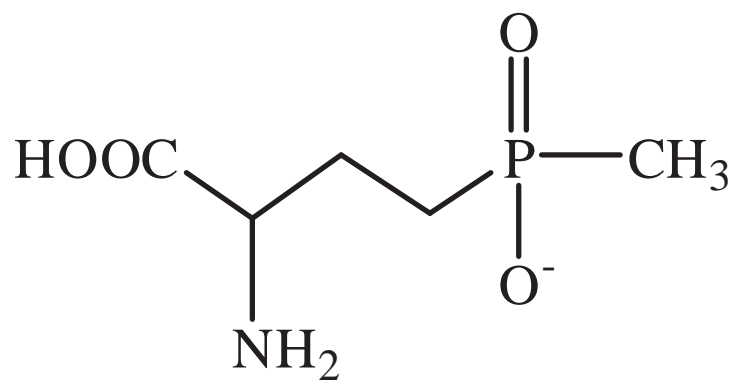
Figure 6 Potential modulated ECL profiles for Ru(bpy)$_3$$^{2+}$ /GLUF system at pH 8 (A) and pH 11 (B). C_{Ru2+}^{b} = C_{GLPY}^{b} =0.5 mM; scan rate: 20 mV/s; ac voltage frequency: 10 Hz; modulation amplitude: 10 mV.

Figure 7 MPECL intensity dependence on potential modulation frequency for (●) 1st ECL peak (at +1.1 V *vs*. Ag/AgCl) and (■)2nd ECL peak (at +1.35 V *vs*. Ag/AgCl), respectively. The other conditions were the same as in Fig. 6

Fig_1

Glyphosate : GLYP

Glufosinate : GLUF

Fig_2

(A) GLYP/Ru(bpy)$_3$$^{2+}$ system

(B) GLUF/Ru(bpy)$_3$$^{2+}$ system

(C) TPA/Ru(bpy)$_3$$^{2+}$ system

ECL 0.5 a.u.

0.5 a.u.

0.5 a.u.

CV 0.5 μA

0.5 μA

0.5 μA

0.6 0.7 0.8 0.9 1 1.1 1.2 1.3
Potential *vs.* (Ag/AgCl) / V

0.6 0.7 0.8 0.9 1 1.1 1.2 1.3
Potential *vs.* (Ag/AgCl) / V

0.6 0.7 0.8 0.9 1 1.1 1.2 1.3
Potential *vs.* (Ag/AgCl) / V
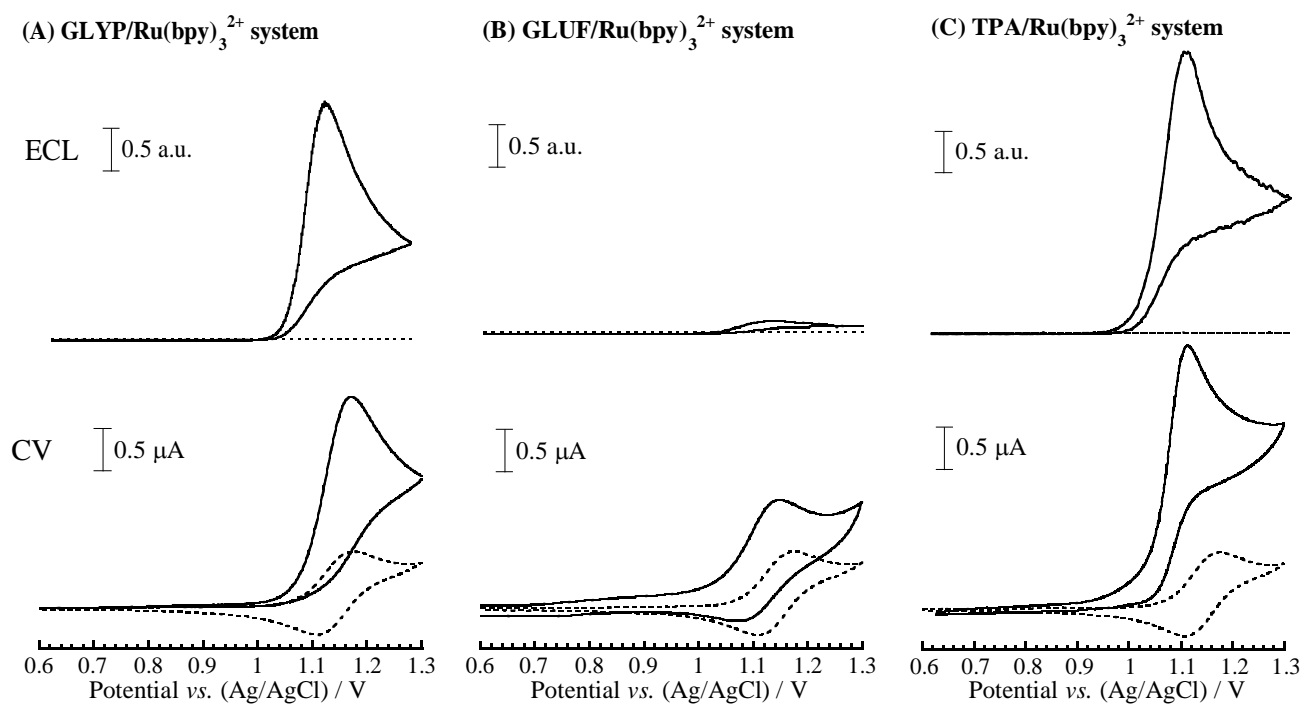
Fig. 2 J. Jin
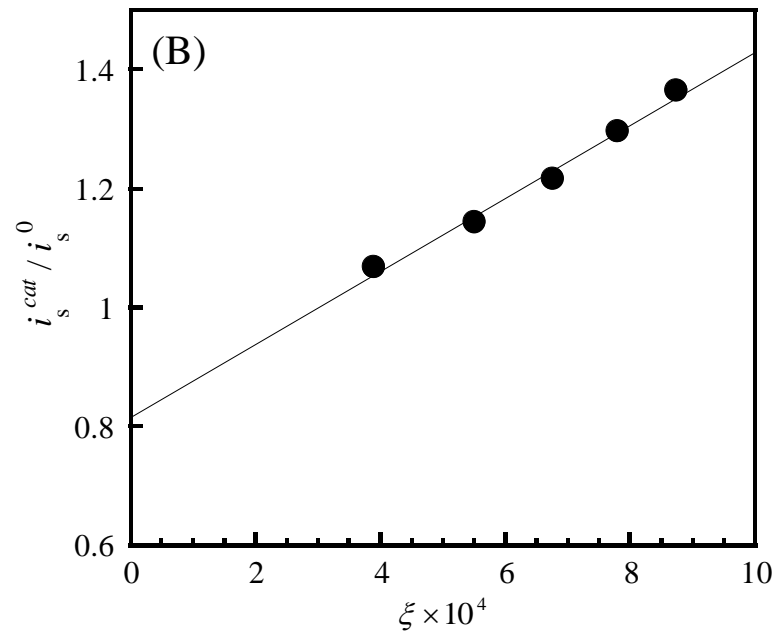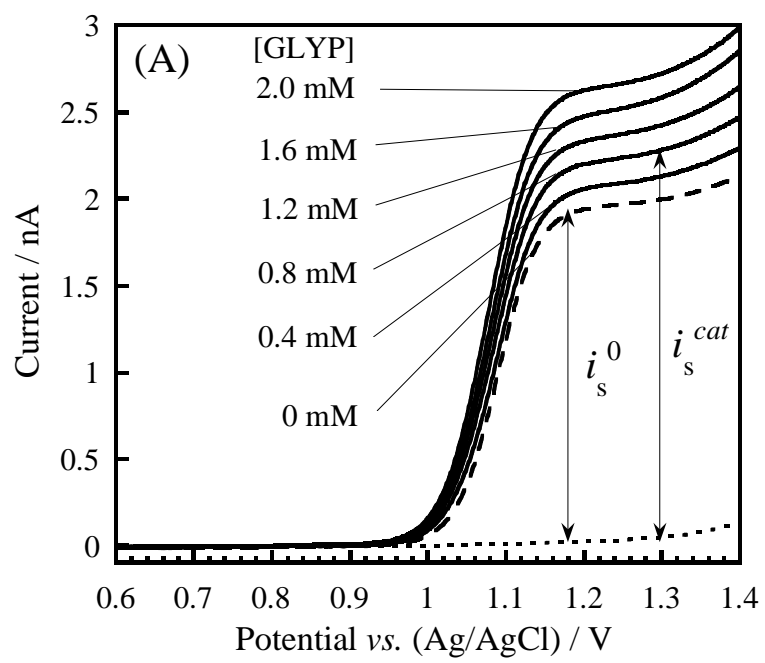
Fig_3

Fig. 3   J. Jin
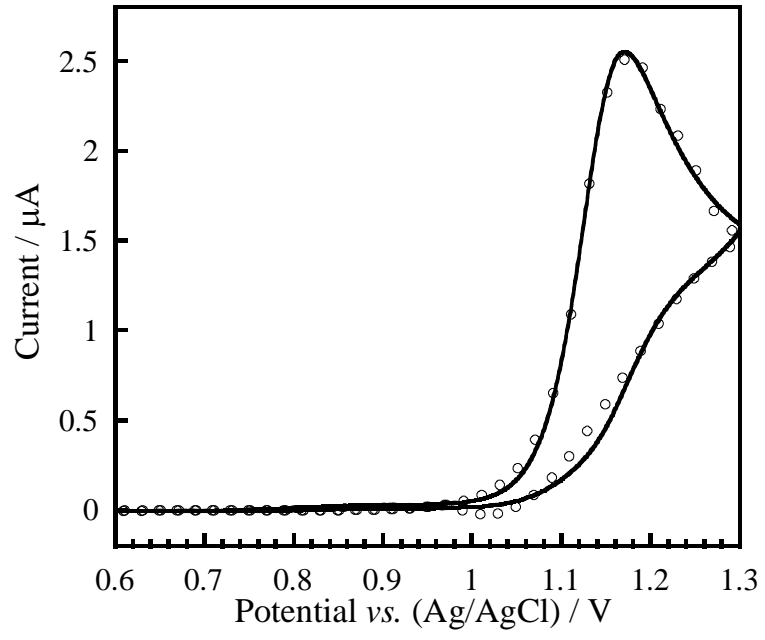
Fig_4

Fig. 4 J. Jin

Fig_5

Fig. 5 J. Jin

Fig_6

Fig. 6 J. Jin

Fig_7

Fig. 7   J. Jin