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Quantification of Electrogenerated Chemiluminescence from Tris(bipyridine)ruthenium(II) and Hydroxyl Ions

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In this work, we quantify the electrogenerated chemiluminescence arising from the reaction of electrogenerated tris(bipyridine)ruthenium(III) with hydroxyl ions, in terms of emission intensity and reaction rate. Different electrode materials (glassy carbon and boron-doped diamond) and different supporting electrolytes (perchlorate, phosphate, and carbonate) were investigated under pH variation. Relative quantification of the electrogenerated chemiluminescence was proposed against the Ru(bpy)$_3^{2+}$/tri-n-propylamine system, taken as reference, with relative emission as low as 600 and 230 times at the same coreactant concentration and the same pH, respectively. The kinetic was investigated by foot of the wave analysis of cyclic voltammetry to measure the turnover frequency of the reaction.

Introduction

The luminescent reaction of tris(bipyridine)ruthenium(III), abbreviated here Ru(III), with hydroxyl ions was first reported by Hercules and Lytle in 1966.[1] The chemical reduction of Ru(III) leads to the formation of a metal-to-ligand charge transfer (^{3}MLCT) triplet excited state,[2] which emits at around 610 nm.[3,4] Not only hydroxyl ions, but many molecules react with Ru(III) to give luminescence, making this reaction a powerful tool for analytical applications,[5,6] and detect amines,[7] amino acids,[8] and heteroaromatic compounds.[9] If Ru(III) is generated electrochemically from Ru(bpy)$_3^{2+}$, abbreviated here Ru(II), this procedure as a whole is now an electrogenerated chemiluminescence (ECL) reaction, since it comprises an electrochemical step that trigger the light emission.[10-12] Examples of this application includes the detection of ascorbic and dehydroascorbic acids,[13] oxalate, ethanol and sulfite,[14] formaldehyde and formic acid,[15] thiocholine,[16] hydrazine,[17] methamphetamine,[18] and sarcosine,[19] a prostate cancer marker. In the mentioned examples, Ru(II) is free to diffuse in solution and the molecules detected by ECL can also act as coreactants, since they can be oxidized at electrode, resembling the usual ECL system Ru(II) and tri-n-propylamine (TPrA).[20] Since all these applications are conducted in water solution the luminescence reaction of Ru(III) with hydroxyl ions should be observed in some extent, and might generated a detectable background signal which cannot be neglected. Many ECL research papers[21] refer to this chemiluminescence reaction described by Hercules and Lytle to justify the background emission concurrent to the oxidation of Ru(II) in aqueous solutions. Instead, we present a measure of this light emission directly by ECL. Here, we investigated the reaction kinetic and the effect of pH on the reaction of Ru(III) with hydroxyl ions trough the ECL of Ru(II) in water solution, by comparing different electrolytes and electrode materials. A relative ECL emission was quantified against the well-known Ru(II)/TPrA system. We must recall that hydroxyl ions do not react at the electrode to give the ECL reaction, only Ru(II) is oxidized, following Eqs. 1 and 2, similar to the catalytic mechanism of Ru(II)/TPrA.[22]

$$Ru(bpy)_3^{2+} - e^- \rightarrow Ru(bpy)_3^{3+} \quad (1)$$

$$Ru(bpy)_3^{3+} + OH^- \rightarrow Ru(bpy)_3^{2+*} + products \quad (2)$$
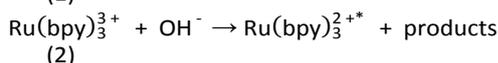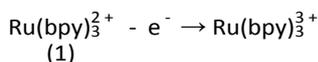
The reaction mechanism, described by eqs. 1 and 2, might be an oversimplification of the real mechanism leading to light emission, however justified in order to rationalise the kinetic of the bimolecular reaction between Ru(III) and OH$^-$ (see ESI, Part 1 and 2).

Experimental

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Ru(bpy)$_3$Cl$_2$•6H$_2$O, NaClO$_4$, Na$_3$PO$_4$, Na$_2$SO$_4$, Na$_2$CO$_3$, H$_3$PO$_4$, HClO$_4$ and NaOH were purchased from Wako Pure Chemical (JP), TPrA from Sigma Aldrich (USA), and used without further purification. All solution are prepared in pure double distilled water (ddw) with resistivity 18 MΩ cm, from a SimplyLab water system (DIRECT-Q3 UV, Millipore). Before each measurement, the GC (Tokai Carbon, JP) electrode was cleaned with 0.5 μm alumina powder suspension on cloth tape, then sonicated in ddw for 5 min, rinsed in ddw, and dried in a nitrogen stream. BDD fabrication and ECL instrumentation were explained in detail in ref. 23. The BDD electrode (1% B/C) was cleaned by sonication in isopropanol for 5 min, rinsed in ddw, and dried in a nitrogen stream. Prior to each measurement, the BDD surface was pretreated electrochemically, by performing 10 voltammetric cycles between -2.0 and 2.0 V followed by 10 cycles between 0 and -2.0 V in a 0.1 M NaClO$_4$ solution at a scan rate 0.3 V/s. All potentials throughout the text are referred to Ag/AgCl (saturated KCl) electrode. ECL spectra were collected by a SEC2000 Spectra system UV-visible spectrophotometer (ALS Co., JP). NaOH was used to adjust the pH of the NaClO$_4$ solution, HClO$_4$ for the pH of Na$_2$CO$_3$ solution and H$_3$PO$_4$ for the pH of Na$_3$PO$_4$ solution. All measurements were made in triplicate.

Results and discussion

The ECL of Ru(II) by cyclic voltammetry with glassy carbon (GC) electrode in perchlorate and phosphate electrolytes is shown in Figure 1.
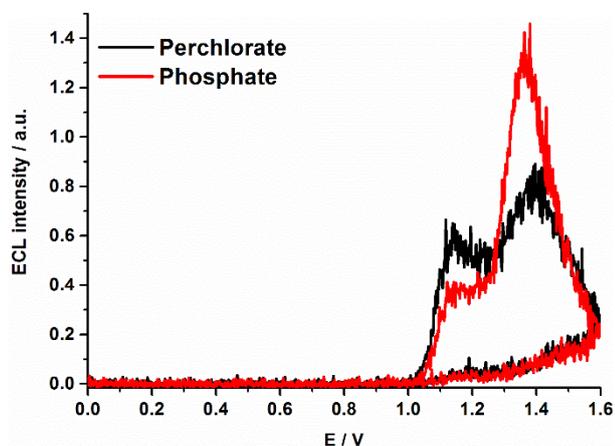
Fig. 1 ECL intensity of 100 μM Ru(II) in different electrolytes at GC electrode: perchlorate (black) pH 13 and phosphate (red) pH 12.5, solutions 100 mM each. Scan rate 100 mVs^{-1}.

In both electrolytes, ECL starts at the oxidation potential of Ru(II) (E$_{1/2}$ = 1.07 V, see SI), then confirming the participation of Ru(III) in the mechanism, with two peaks around 1.1 and 1.4 V.

The effect of pH was investigated from the pH of the pure phosphate solution to pH 5 by acid addition. Perchlorate solution was added with hydroxide, from pH 5 to 13.

The measure of ECL emission as a function of pH is reported in Fig. 2 and Fig. S1. Perchlorate and phosphate result in the

same trend of ECL for the first peak, while a clear effect of the buffer capacity of phosphate is evident for the second peak, with a shift of ECL emission to lower pH of 0.8 unit (Fig. 2 and Fig. S1). The double peak shape is very reproducible, furthermore seems to depend on the relative Ru(II)/OH$^-$ ratio (Fig. S3). For phosphate a detectable ECL emission (i.e., higher that the measurement noise) is clear from pH 7, a usual value in ECL measurements, which can contribute to ECL background.

Carbonate has also been used as electrolyte, however it can be oxidized in the same potential range of ECL emission, which impairs a correct measurement of the light intensity (Fig. S2).

Comparison of the ECL with the most used Ru(II)/TPrA system permits to give a relative quantification of the emission (Fig. S3).
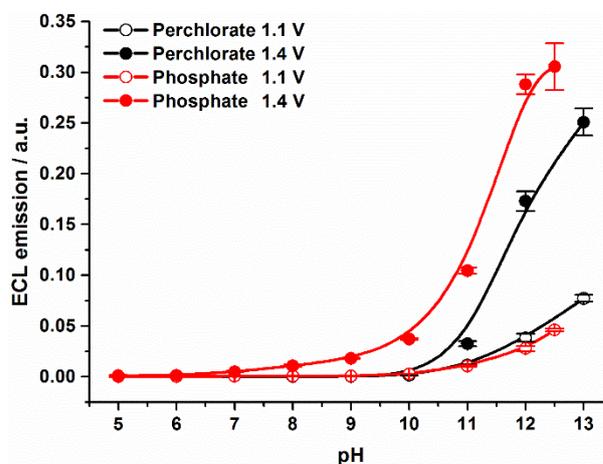
Fig. 2 ECL emission intensity at different pH, from cyclic voltammetry with scan rate 100 mVs^{-1}: perchlorate (black) and phosphate (red) 100 mM solutions; first peak at 1.1 V (empty symbols) and second peak at 1.4 V (filled symbols). Ru(II) is 100 μM. Lines are drawn only as guides for the eye.

Almost similar ECL signals are measured for large different concentrations of luminophore and coreactant, 1 mM Ru(II)/1 M OH$^-$ and 100 μM Ru(II)/100 μM TPrA (Fig. 3).

At the same pH and Ru(II) concentration the ECL from OH$^-$ in phosphate buffer is about 230 times lower compared to the Ru(II)/TPrA, which makes the Ru(II)/OH$^-$ system unlikely to give any relevant contribution to the background emission at neutral pH.[24]
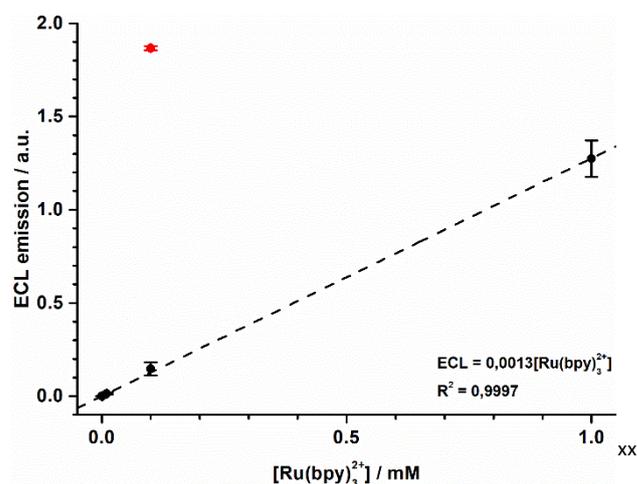
Fig. 3 Calibration of Ru(II) in 1 M NaOH, integrated ECL signal from 0 V to 1.6 V; the red point is from 100 μM Ru(II) and 100 μM TPrA in 0.2 M phosphate buffer at pH 7.5 (Fig. S3).

Finally, we resume the data from ECL quantification to highlight the finding of this investigation in Table 1.

Table 1. Comparison of Ru(II)/TPrA and Ru(II)/OH⁻ ECL emission. [a]Same [TPrA] and [OH⁻]. [b]Same pH and electrolyte (PO_4^{3-}).

	ECL	Ratio
[TPrA] / 100 μM	1.87 ± 0.01	
ClO_4^- / pH 10[a]	0.0031 ± 0.0004	600 ± 80
PO_4^{3-} / pH 7.4[b]	0.0080 ± 0.0010	230 ± 30

Furthermore, the TPrA concentration used in this case is lower than commonly used in ECL with Ru(II), hundreds of mM, which can make the contribution of Ru(II)/OH⁻ ECL even much less relevant.**Error! Bookmark not defined.**

An opposite result was observed when boron doped diamond (BDD) electrode was used. BDD is a very well-known electrode material able to oxidize OH⁻ to OH•.[25,26] The same experiments as of Figure 1 show no ECL emission in perchlorate and phosphate electrolytes (Fig. 4), while a detectable emission is measured for carbonate, although 5 times lower (Fig. S4). In this case, we speculate that the depletion of OH⁻ in the diffusion layer is enough to quench the ECL emission. This evidence is also an indication that hydroxyl radical (OH•) does not take part in the ECL reaction mechanism, since this is the main product of OH⁻ oxidation at the BDD electrode.
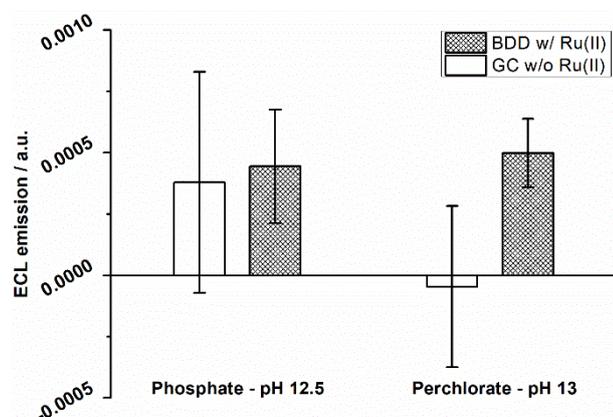
Fig. 4 Integrated ECL signal from CV-ECL, GC without and BDD with 100 μM Ru(II) in 100 mM supporting electrolyte at the specified pH.

We ruled out the interference of counter electrode and the effect of dissolved oxygen on ECL emission with dedicated experiments (Fig. S5).
Finally, ECL spectra comparison of NaOH and TPrA coreactants permitted to identify the emitting excited state (Fig. S6). ECL is emitted from the ³MLCT excited stated of Ru(II) with a peak centered around 610 nm, in line with previous experiments of Ru(II) ECL.[23]

This confirm that both peak lead to the generation of Ru(II)*, however the overall mechanism is still unclear (ESI Part 2). The fact we cannot identify exactly the real step involved in the generation of the excited state makes difficult any prediction on the energetic, and then the efficiency of this reaction.
In order to describe the reaction kinetic, we tentatively investigated the reactivity of Ru(III) toward hydroxide by foot-of-the-wave analysis of cyclic voltammetry. This approach, developed by Savéant and coworkers,[27-31] allows us to obtain the relationship between turnover frequency (TOF) and overpotential (η), as showed in the catalytic Tafel plot (Fig. 5, ESI Part 1). The reaction analyzed is depicted in Scheme 1, however if it may involve several steps, they are equivalent to an overall reaction with an apparent rate constant k_{OH}.[27] Moreover, this approach is applicable and reliable when side-phenomena are encountered, (e.g., consumption of the substrate, deactivation of the catalyst, inhibition by products), which permits to focus on the catalytic parameters, while discarding of side-effects.[27] This methodology was successfully tested on ruthenium catalysts for water oxidation[32] and proved to be in agreement with constants derived spectroscopically,[33] which further validates this analysis. The CVs and their elaboration to obtain the TOF-η plot is available in the Supporting Information (Fig. S7-S9).

Scheme 1. Model of the catalytic reaction based on one-electron/one-step process,[30]**Error! Bookmark not defined.** to be applied for foot-of-the-wave analysis. $E_{1/2}$ is the half-wave potential of the couple Ru³⁺/²⁺ = 1.07 V, and k^0 is the heterogeneous electron transfer constant (0.06 cm s⁻¹).[34] k_{OH} is the apparent catalytic rate constant. k_{ph} is the rate constant for
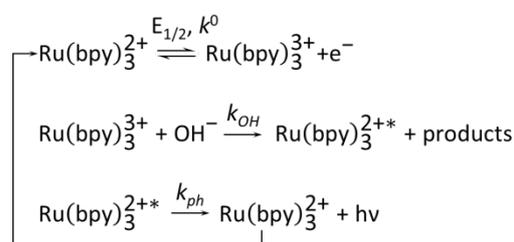
phosphorescence decay (6.5×10⁴ s⁻¹).[35]
The mechanism resemble the water nucleophilic attack (WNA), in analogy with Ruthenium-oxo complexes for water oxidation, where the primary pathway involves a nucleophilic attack of an uncoordinated water molecule.[36-39] The TOF for reaction given in Scheme 1 was measured for different concentration of NaOH, and the results are showed in Figure 5 and Table 2.
Ru(II) results in very low TOF values. For the sake of comparison, we report the TOF values of a ruthenium-oxo complex[32] for water oxidation. The intrinsic turnover frequency (TOF₀), which is the TOF at zero overpotential and describes the intrinsic performance of the electrocatalyst, for Ru(II) is five order of magnitude lower,[32,40] and reaching TOF_{MAX} requires higher overpotential.
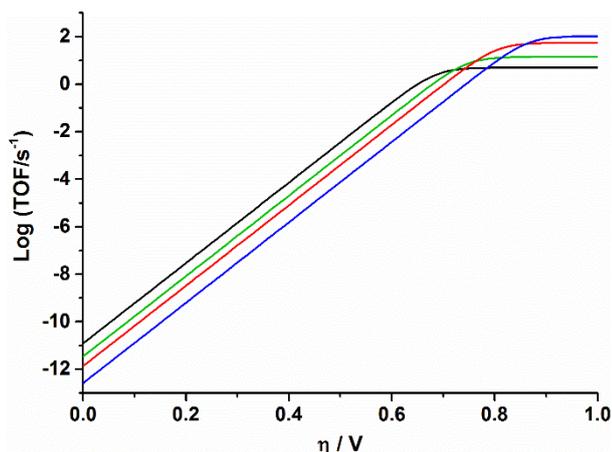
Fig. 5 Catalytic Tafel plot of Ru(II) in NaOH 1 (black), 10 (green), 100 (red), and 1000 (blue) mM with Na_2SO_4 100 mM supporting electrolyte.

Table 2. Values of TOF from Figure 5. a) Ruthenium-oxo complex at pH 7, from ref. 32.

OH^-/ M	1	0.1	0.01	0.001	$^aRu^{IV}$
TOF_{MAX} / s^{-1}	105	55	14	5	7700
Log (TOF_0 / s^{-1})	-12.5	-11.9	-11.5	-10.9	-6.5

However, in this case the TOF for Ru(II) does not represent a reaction kinetic of water oxidation, since O_2 formation is more the exception than the rule,[41] rather it is an information about the rate of the bimolecular reaction between Ru(III) and OH^-, which is similar to the WNA mechanism of Ruthenium-oxo catalysts.

The results are compared with the catalytic constant (k_{obs}) of production of Ru(II) from the reduction of Ru(III) in alkaline solution (Fig. 6), as obtained by stopped-flow spectrophotometry by Creutz and Sutin.[42]

Discrepancies may arises because the two techniques are different (spectroscopic or electrochemical),[33] moreover the largest difference is obtained at concentration out of the range of validity of the spectroscopic derived constant at 1 M of hydroxide.
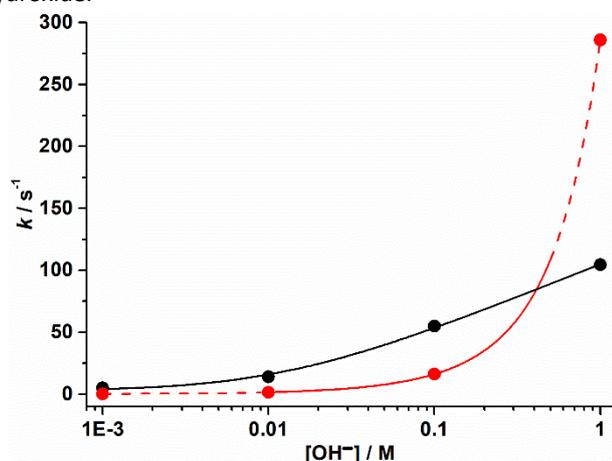
Fig. 6 Catalytic constants (k): Ru(II) production from the reduction of Ru(III) by hydroxyl ions within the range of validity

(red line, full), and beyond the range of validity (red line, dashed), adapted from ref. 42 (k_{obs}/s^{-1}); TOF_{MAX}/s^{-1} (black dots), line is only as guide for the eye.

Conclusions

We presented a quantification of the reaction of electrogenerated tris(bipyridine)ruthenium(III) in aqueous alkaline solutions, in terms of ECL emission and reaction rate, which could be useful in the interpretation of results obtained by ECL in aqueous solution involving freely diffusing Ru(II). ECL intensity is far below the standard Ru(II)/TPrA system, i.e., 600 and 230 times at the same coreactant concentration and the same pH, respectively. The comparison of different electrolytes and electrode materials show the effect of buffer capacity and hydroxyl ions on the ECL emission.

Description of the reaction rate by cyclic voltammetry analysis showed an extremely low intrinsic TOF_0 ($\approx 10^{-11}$-10^{-13} s^{-1}), while it reaches a maximum value at high overpotential, which is comparable with the kinetic constants derived from spectrophotometric measurements by Creutz and Sutin.

Conflicts of interest

There are no conflicts to declare.

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