

## Publication II

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# Electrochemiluminescence and chemiluminescence of a carboxylic acid derivative of ruthenium(II) tris-(2,2'-bipyridine) chelate synthesized for labeling purposes

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## Abstract

Synthesis, purification and characterization of [4-ethoxycarbonyl-4'-carboxy-2,2'-bipyridine]bis(2,2'-bipyridine) ruthenium(II) hexafluorophosphate is described. This complex is shown to be electrochemiluminescent in aqueous solution during cathodic pulse polarization of thin insulating film-coated electrodes. Electrochemiluminescence (ECL) lifetime of the complex was observed to be ca. 40 μs at oxide-coated n-silicon electrodes; thus time-resolved detection is also possible. The ECL emission maximum of this carboxylate derivative is somewhat red-shifted when compared with an unmodified Ru(bpy)<sub>3</sub><sup>2+</sup>. Because the present complex can be easily covalently coupled with antibodies and oligonucleotides it is usable as an electrochemiluminescent label in various bioaffinity assays. The present chelates also produce strong chemiluminescence during dissolution of metallic magnesium in aqueous solution.

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**Keywords:** Electrochemiluminescence; Thin insulating film-coated electrodes; Chemiluminescence; Labels; Bioaffinity Assays

## 1. Introduction

We have previously shown that ruthenium(II) tris-(2,2'-bipyridine) chelate (Ru(bpy)<sub>3</sub><sup>2+</sup>) shows

cathodic electrochemiluminescence (ECL) at oxide-coated aluminum and silicon electrodes [1–3]. However, this chelate cannot be easily linked with biologically interesting compounds but must be modified to achieve easy coupling chemistry for practical applications. In the present case, a carboxylic acid derivative of Ru(bpy)<sub>3</sub><sup>2+</sup> is synthesized. This complex can be linked with primary amino groups of, e.g. antibodies and

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oligonucleotides through carboxyl functional group linked with the N-hydroxysuccinimide (NHS) ester as acylating agent, which enables the proteins, peptides and DNA to be labeled [4].

$\text{Ru}(\text{bpy})_3^{2+}$  can also be sensitively detected by other chemiluminescence methods [5–8]. Thus, the derivatives of  $\text{Ru}(\text{bpy})_3^{2+}$  usable as labels in bioaffinity assays are very useful and versatile and worth synthesizing by the researchers needing these labels. The commercially available variants are quite expensive and it has even occurred that these labels have not been sold to all of the researchers wishing to purchase the appropriate derivatives.

## 2. Experimental

### 2.1. Synthesis and characterization of complexes

General:  $^1\text{H}$  NMR spectrum was recorded on a Varian INOVA 400 MHz spectrometer, using TMS as internal standard. Electrospray ionization mass spectrometry (ESI-MS) was performed on a HP1100 LC/MS Detector with acetonitrile as solvent. 4,4'-bis(carboxyethyl)-2,2'-bipyridine [9] and *cis*- $\text{Ru}(\text{bpy})_2\text{Cl}_2 \cdot 2\text{H}_2\text{O}$  [10] were synthesized according to the literature, the corresponding halides were metathesized to the  $\text{PF}_6^-$  salts using  $\text{AgPF}_6$  dissolved in a minimal amount ( $< 10$  ml) of acetone. Silver hexafluorophosphate ( $\text{AgPF}_6$ ) and ammonium hexafluorophosphate ( $\text{NH}_4\text{PF}_6$ ) were purchased from Aldrich, silica gel (200–300 mesh) used for column chromatography was bought from Qingdao Ocean Chemical Factory. All solvents were reagent grade for synthesis or spectroscopic grade for optical measurements. They were used as received.

For synthesis of [4-ethoxycarbonyl-4'-carboxy-2,2'-bipyridine] bis(2,2'-bipyridine) ruthenium(II) hexafluorophosphate complex ( $\text{Ru}(\text{bpy})_2\text{L}_a(\text{PF}_6)_2$ , i.e.  $\text{L}_a = 4$ -ethoxycarbonyl-4'-carboxy-2,2'-bipyridine), a solution of 4,4'-bis(carboxyethyl)-2,2'-bipyridine (0.30 mmol, 90 mg in 40 ml of 50% ethanol/water (v/v)) was added into *cis*- $\text{Ru}(\text{bpy})_2(\text{PF}_6)_2$  solution (190 mg, 0.27 mmol) with magnetic stirring under Ar atmosphere. The resulting brown solution was heated and became clear orange after refluxing

for 2.5 h. The solvent was evaporated under a vacuum, and a red solid residue was obtained. The residue was redissolved in water (20 ml) and the unreacted bipyridyl ligand was removed by filtration. After the residue was washed with water (5 ml), the filtrates were combined and evaporated to dryness in vacuum at 70 °C.

Purification was conducted on column chromatography using  $\text{CH}_3\text{CN}:\text{H}_2\text{O}:\text{KNO}_3$  (saturated) 5:1:1 as eluent. The red fraction was collected and then the nitrate counter ion was exchanged to hexafluorophosphate by dissolving the fraction in minimum amount of water and adding a solution of saturated  $\text{NH}_4\text{PF}_6$ . Then the red precipitate was collected by filtration, washed with water and dried to give the desired [4-ethoxycarbonyl-4'-carboxy-2,2'-bipyridine] bis(2,2'-bipyridine) ruthenium(II) hexafluorophosphate complex.  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  1.41 (t, 3H,  $J = 7.1$  Hz,  $-\text{OCH}_2\text{CH}_3$ ), 4.45 (q, 2H,  $J = 7.1$  Hz,  $-\text{OCH}_2\text{CH}_3$ ), 7.47 (t, 2H,  $J = 6.0$  Hz, bpy-H), 7.53 (t, 2H,  $J = 6.4$  Hz, bpy'-H), 7.71 (t, 2H,  $J = 4.4$  Hz, bpy-H), 7.75 (t, 2H,  $J = 6.0$  Hz, bpy-H), 7.88 (dd, 2H,  $J = 5.4$ , 1.2 Hz, bpy'-H), 7.92–7.97 (d, 2H,  $J = 5.8$  Hz, bpy'-H), 8.13 (d, 2H,  $J = 7.2$  Hz, bpy-H), 8.16 (d, 2H,  $J = 7.2$  Hz, bpy'-H), 8.80 (d, 2H,  $J = 7.8$  Hz, bpy-H), 8.81 (d, 2H,  $J = 7.8$  Hz, bpy'-H), 9.17–9.18 (s, 2H, bpy'-H). ESI-MS positive  $m/z$  found mono-charged peak at 831.2 ( $[\text{M}-\text{PF}_6^-]^+$ ), double-charged peak at 343.0 ( $[\text{M}-(\text{PF}_6^-)_2]^{2+}$ ).

[4,4'-diethoxycarbonyl-2,2'-bipyridine] bis(2,2'-bipyridine) ruthenium(II) hexafluorophosphate complex ( $\text{Ru}(\text{bpy})_2\text{L}_b(\text{PF}_6)_2$ , i.e.  $\text{L}_b = 4,4'$ -ethoxycarbonyl-2,2'-bipyridine) was prepared according to literature [11].  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  1.41 (t, 6H,  $J = 7.2$  Hz,  $-\text{OCH}_2\text{CH}_3$ ), 4.46 (q, 4H,  $J = 7.2$  Hz,  $-\text{OCH}_2\text{CH}_3$ ), 7.47 (ddd, 2H,  $J = 7.6$ , 5.6, 1.2 Hz, bpy-H), 7.54 (ddd, 2H,  $J = 7.6$ , 5.6, 1.2 Hz, bpy'-H), 7.69 (dd, 2H,  $J = 5.6$ , 1.2 Hz, bpy-H), 7.74 (dd, 2H,  $J = 5.6$ , 1.2 Hz, bpy'-H), 7.88 (dd, 2H,  $J = 5.8$ , 1.6 Hz, bpy'-H), 7.96 (d, 2H,  $J = 5.8$  Hz, bpy'-H), 8.13 (dd, 2H,  $J = 7.8$ , 7.6 Hz, bpy-H), 8.17 (dd, 2H,  $J = 7.8$ , 7.6 Hz, bpy'-H), 8.81 (dd, 2H,  $J = 7.8$ , 1.2 Hz, bpy-H), 8.82 (dd, 2H,  $J = 7.8$ , 1.2 Hz, bpy'-H), 9.24 (d, 2H,  $J = 1.6$  Hz, bpy'-H). ESI-MS positive ( $m/z$ ) found mono-charged peak at 859.2 ( $[\text{M}-\text{PF}_6^-]^+$ ), double-charged peak at 357.0 ( $[\text{M}-(\text{PF}_6^-)_2]^{2+}$ ).

## 2.2. Measurements of photoluminescence, ECL and CL

Photoluminescence (PL) measurements were carried out using Perkin-Elmer LS 50B spectrometer. The apparatus for ECL experiments using a coulstatic pulse generator [12] as an excitation unit have been described elsewhere [13]. PL and ECL measurements were made in 0.05 M  $\text{Na}_2\text{B}_4\text{O}_7$  buffer at pH 9.2 and both complexes could be dissolved in this buffer with a stock solution concentration of  $1 \times 10^{-4}$  mol/l without the need of adding surfactants such as Tween 20, which can be used if higher concentrations are required.

Aluminum electrodes were made from a nominally 99.9% pure aluminum band (Merck Art. 1057, batch K18381957 539) and were covered with natural about 2–3 nm thick oxide film. Silicon electrodes were made from antimony-doped n-Si with an orientation (111) and a resistivity of 0.008–0.015  $\Omega\text{cm}$ . The silicon wafers were purchased from Okmetic Oy (Finland) and cut into  $14.0 \times 14.0$  mm pieces after thermal oxidation of 3.9 nm silicon dioxide film at 850  $^\circ\text{C}$  [3].  $\text{K}_2\text{S}_2\text{O}_8$  and  $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{-hydrate}$  were *pa.* products of Merck. Ruthenium(II) tris-(2,2'-bipyridine) chloride hexahydrate, and Tween 20 were purchased from Aldrich.

Chemiluminescence measurements under acidic conditions using magnesium metal as a strong reductant and potassium peroxodisulfate as a strong oxidant were carried out using Perkin-Elmer LS 50 B spectroluminometer in bioluminescence mode in a 1-cm quartz cuvette. Magnesium band (Merck Art. 5812) was used as a source of the reducing species.

## 3. Results and discussion

### 3.1. Cathodic electrochemiluminescence

Meyer and coworkers [11] reported that the synthesis of  $\text{Ru}(\text{bpy})_2\text{L}_\text{b}$  can be carried out in 15 h in darkness with the yield of 85%. When we repeated the reaction under ambient light, the  $\text{Ru}^{2+}(\text{bpy})_2\text{L}_\text{a}$  complex could be acquired in nearly 15% yield and the  $\text{Ru}^{2+}(\text{bpy})_2\text{L}_\text{b}$  complex

in 80% yield, but if the same reaction was run in darkness (wrapped the flask completely with aluminum foil), there was nearly no  $\text{Ru}^{2+}(\text{bpy})_2\text{L}_\text{a}$  produced. It seems that there is photocatalysis in the coordination process; the photocatalysis not only catalyzes the coordination, but also catalyzes the hydrolysis of one ester group on 4,4'-bis(carboxyethyl)-2,2'-bipyridine ring. The detailed mechanism is still under investigation in our laboratories.

$\text{Ru}^{2+}(\text{bpy})_2\text{L}_\text{a}$  displayed weaker PL than  $\text{Ru}(\text{bpy})_3^{2+}$  and its  $^3\text{MLCT}$  excitation maximum is somewhat red shifted as well as its emission maximum when compared with the PL spectra of unaltered  $\text{Ru}(\text{bpy})_3^{2+}$  (Fig. 1). These are consistent with the observations by other researchers [14]. In a corrected emission spectrum the emission maximum of  $\text{Ru}(\text{bpy})_3^{2+}$  would be about 610 nm [5]. However, the observed emission maxima wavelength of the other complexes are even smaller than the true emission maxima due to the blue sensitive PMT of LS 50B used, in which the sensitivity of the PMT decreased steeply as the wavelength increases.

[4,4'-diethoxycarbonyl-2,2'-bipyridine] bis(2,2'-bipyridine)ruthenium(II) ( $\text{Ru}(\text{bpy})_2\text{L}_\text{b}^{2+}$ ) has its emission and lowest excited state excitation

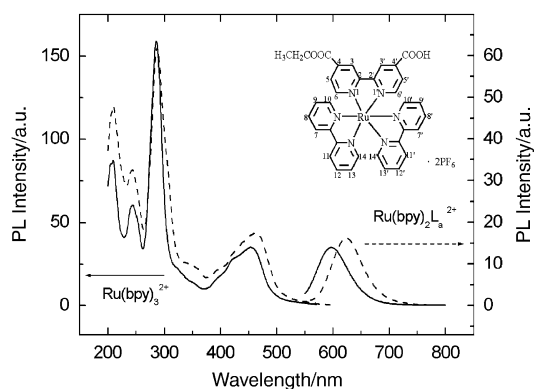


Fig. 1. Uncorrected PL excitation and emission spectra of (a)  $\text{Ru}(\text{bpy})_3^{2+}$  (solid line) and (b)  $\text{Ru}(\text{bpy})_2\text{L}_\text{a}^{2+}$  (dashed line) complexes. Conditions: 0.05 M  $\text{Na}_2\text{B}_4\text{O}_7$ , pH 9.2,  $1 \times 10^{-5}$  M complexes, both slits 10 nm, (a) Excitation wavelength 455 nm and emission wavelength 597 nm. (b) Excitation wavelength 465 nm and emission wavelength 626 nm. Inset: the structure of the complex  $\text{Ru}(\text{bpy})_2\text{L}_\text{a}^{2+}$ .

maxima at even longer wavelengths (Fig. 2). Thus, ethoxycarbonyl substituents have a stronger bathochromic effect in aqueous solution than carboxylate substituent.

$\text{RuL}_3^{2+}$  (denoted here as  $\text{RuL}_3^{2+}$  in which each L can be an unsubstituted or substituted 2,2'-bipyridine and all the ligands can either be identical or have different substituents) are in general characterized by a single, primarily metal-centered, oxidation occurring at rather positive potential in non-aqueous solutions, and a series of closely spaced, primarily ligand-centered reductions occurring at fairly negative potentials [14]. The existence of poly ethoxycarbonyl substituents in 4 and 4' positions were reported [14] to induce the oxidation potential to more positive direction and the first one-electron reduction potential to less negative direction in comparison with the unsubstituted  $\text{Ru}(\text{bpy})_3^{2+}$  [14].  $\text{Ru}(\text{bpy})_2\text{L}_b^{2+}$  is oxidized at 0.14 V more positive potential (i.e. 1.38 vs. SCE) than  $\text{Ru}(\text{bpy})_3^{2+}$  and the first one-electron reduction occurs at 0.34 V less-negative potential (i.e.  $-0.93$  V vs. SCE) in DMF [14].

Since ligand structures of  $\text{L}_a$  and  $\text{L}_b$  are similar, we assume that their redox potentials are close. Thus,  $\text{Ru}(\text{bpy})_2\text{L}_a^{2+}$  and  $\text{Ru}(\text{bpy})_2\text{L}_b^{2+}$  are a bit easier to one-electron reduce and only very slightly

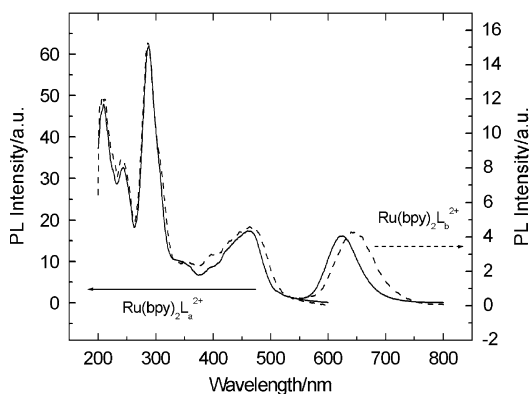
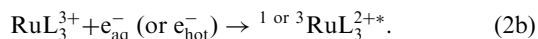
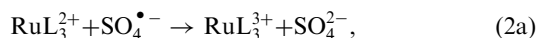


Fig. 2. Uncorrected PL excitation and emission spectra of (a)  $1 \times 10^{-5}$  M  $\text{Ru}(\text{bpy})_2\text{L}_a^{2+}$  (solid line) and (b)  $1 \times 10^{-5}$  M  $\text{Ru}(\text{bpy})_2\text{L}_b^{2+}$  (dashed line) complexes. Conditions: as in Fig. 1. (a) Excitation wavelength 465 nm and emission wavelength 626 nm. (b) Excitation wavelength 473 nm and emission wavelength 647 nm. Inset: the structure of the complex  $\text{Ru}(\text{bpy})_2\text{L}_2^{2+}$ .

more difficult to one-electron oxidize than the unsubstituted  $\text{Ru}(\text{bpy})_3^{2+}$ . Therefore, the traditional anodic ECL at noble metal electrodes [5,6] will also be a usable excitation method for the present complexes. Based on our previous study [1], hydrated electrons induced at insulating film-coated electrodes by pulse polarization are capable of reducing  $\text{Ru}(\text{bpy})_2\text{L}_a^{2+}$  or  $\text{Ru}(\text{bpy})_2\text{L}_b^{2+}$ . Thus, an obvious excitation pathway is a red-ox pathway: in the present case

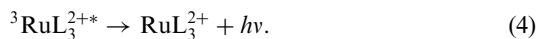


In addition, another obvious excitation pathway is ox-red excitation pathway when, again, peroxodisulfate ions are utilized as co-reactants. The reaction pathway is described in (2a) and (2b): sulfate radical is produced by one-electron reduction ( $k(e_{\text{aq}}^- + \text{S}_2\text{O}_8^{2-}) = 1.2 \times 10^{10} \text{ l mol}^{-1} \text{ s}^{-1}$  [15]), followed by one-electron reduction of  $\text{RuL}_3^{3+}$  species by an energetic electron [1]



As far as we know, the reaction rate constants of  $\text{Ru}(\text{bpy})_2\text{L}_a^{2+}$ ,  $\text{Ru}(\text{bpy})_2\text{L}_b^{2+}$ ,  $\text{Ru}(\text{bpy})_2\text{L}_a^{3+}$  and  $\text{Ru}(\text{bpy})_2\text{L}_b^{3+}$  in reactions with hydrated electron or sulfate radical have not been measured by pulse radiolysis or other methods. However, it is likely that the addition of the substituents to  $\text{Ru}(\text{bpy})_3^{2+}$  does not significantly alter the reactivity with hydrated or hot electrons and with sulfate radicals. Hence, the redox excitation of  $\text{Ru}(\text{bpy})_2\text{L}_a^{2+}$  or  $\text{Ru}(\text{bpy})_2\text{L}_b^{2+}$  should not be very different to that of  $\text{Ru}(\text{bpy})_3^{2+}$  studied previously [1].

Finally, either an intersystem crossing takes place (3) followed by a radiative transition (4) or, if the  ${}^3\text{RuL}_3^{2+*}$  is already earlier directly formed, emission from  ${}^3\text{MLCT}$  excited state occurs without the existence of excited singlet state intermediate (4):



The cathodic ECL spectra of  $\text{Ru}(\text{bpy})_3^{2+}$  and  $\text{Ru}(\text{bpy})_2\text{L}_a^{2+}$  are similar to the PL spectra of the

corresponding complexes (Figs. 1 and 3). This suggests that the same excited states are induced both by photo- and the present electrochemical excitation methods but, at the moment, it is not clear whether the red-ox or the ox-red excitation pathway is more favorable in the electrochemical excitation.

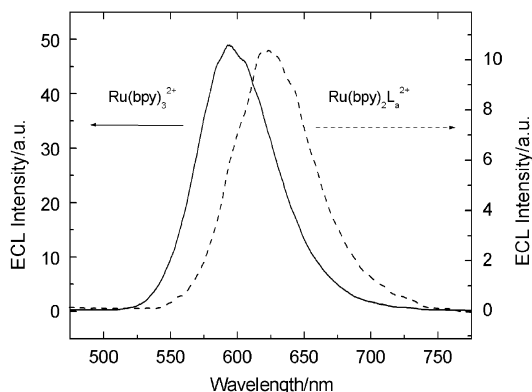


Fig. 3. Uncorrected ECL spectra of  $1 \times 10^{-5}$  M  $\text{Ru}(\text{bpy})_3^{2+}$  (solid line) and  $1 \times 10^{-5}$  M  $\text{Ru}(\text{bpy})_2\text{L}_a^{2+}$  (dashed line) complexes measured with the same instrument. Conditions: as in Fig. 1 except solution contained also 1.0 mmol/l  $\text{K}_2\text{S}_2\text{O}_8$  and emission slit 20 nm. Measured in bioluminescence mode, pulse charge  $120 \mu\text{C}$ , pulse frequency 100 Hz, pulse voltage 45 V.

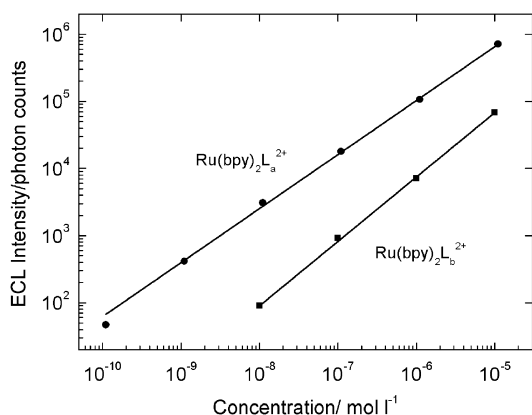


Fig. 4. Calibration curves of  $\text{Ru}(\text{bpy})_2\text{L}_a^{2+}$  (●) and  $\text{Ru}(\text{bpy})_2\text{L}_b^{2+}$  (■) complexes. Conditions: as in Fig. 3 except pulse frequency was 20 Hz and measurements were carried out with photon counting electrochemiluminometer equipped with 620 nm interference filter. ECL was integrated over 500 excitation cycles.

Fig. 4 shows that both  $\text{Ru}(\text{bpy})_2\text{L}_a^{2+}$  and  $\text{Ru}(\text{bpy})_2\text{L}_b^{2+}$  can be detected at oxide-covered aluminum electrodes, with linear calibration curves spanning over several orders of magnitude of concentration. Thus, at least  $\text{Ru}(\text{bpy})_2\text{L}_a^{2+}$  should be applicable as an electrochemiluminescent label. The results with  $\text{Ru}(\text{bpy})_2\text{L}_b^{2+}$  should be much better if a red sensitive detector and an appropriate optical filter would have been available during these studies because of the stronger red shift of its emission spectrum.

$\text{Ru}(\text{bpy})_2\text{L}_a^{2+}$  has a single exponential decay at oxide-coated n-silicon electrode with a luminescence lifetime of 40.0  $\mu\text{s}$  (Fig. 5). Therefore, this label can also be detected by time-resolved techniques when pulsed excitation is applied. The decay at oxide-covered aluminum electrode seems not to be a single exponential process (Fig. 5); it might be related to the features of electrode material itself [3]. However, time-resolved measurements are still also possible using oxide-covered aluminum electrodes.

### 3.2. Extrinsic lyoluminescence of magnesium under acidic conditions

We have previously studied chemiluminescence (CL) of luminol induced by dissolution of aluminum metal in alkaline aqueous solution in the presence

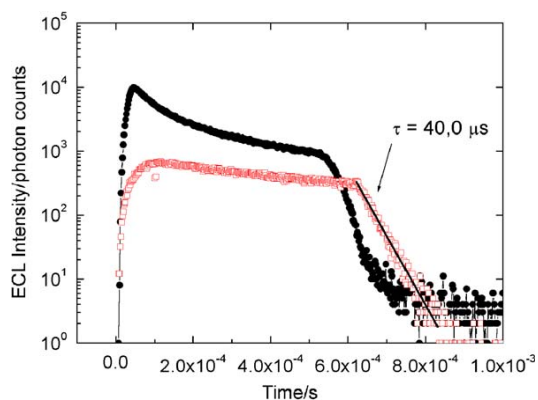


Fig. 5. Rise and decay of  $1 \times 10^{-5}$  M  $\text{Ru}(\text{bpy})_2\text{L}_a^{2+}$  ECL pulse at oxide-coated aluminum and n-Si electrodes. Conditions: as in Fig. 4. Silicon dioxide film thickness 3.9 nm (□) and aluminum oxide film thickness about 2 nm (●).

of peroxodisulfate and some other peroxides as coreactants [16]. This chemiluminescence can also be classified as extrinsic lyoluminescence (LL) of aluminum [17]. In this system, dissolution of aluminum can result in metallic aluminum acting as a reductant, but also short-lived Al(I) and Al(II) species, hydrogen atom and its conjugated base, hydrated electron can act as strong reductants [16]. Typical for most of these species is that they can behave as efficient one-electron reductants which naturally often results in generation of other radicals in the solution in addition to the primary radical species. In principle, dissolution of magnesium in aqueous solution could also be considered to form an analogous highly reactive Mg/aqueous solution interface in which at least Mg, Mg(I) and atomic hydrogen might act as very strong reductants.

Thus, some chemiluminescence generation experiments using the chelate  $\text{Ru}(\text{bpy})_2\text{L}_a^{2+}$  were carried out by dissolving metallic magnesium under acidic conditions in the presence of peroxodisulfate ions. Fig. 6 shows that the chelate exhibits strong CL also at dissolving Mg/aqueous solution interface in the presence of peroxodisulfate ions under strongly acidic condition regardless of the type of strong acid used. However, if the solution is

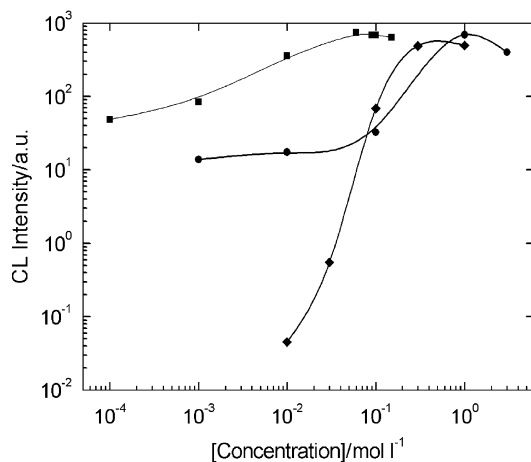


Fig. 6. Chemiluminescence intensity of  $\text{Ru}(\text{bpy})_2\text{L}_a^{2+}$  as a function of the concentration of sulfuric acid (◆), hydrochloric acid (●) and potassium peroxodisulfate (■). Conditions:  $1 \times 10^{-6}$  M  $\text{Ru}(\text{bpy})_2\text{L}_a^{2+}$ , slit width 20 nm, emission monochromator at 600 nm (blue sensitive PMT), Perkin-Elmer LS 50B, integration time 200 s.

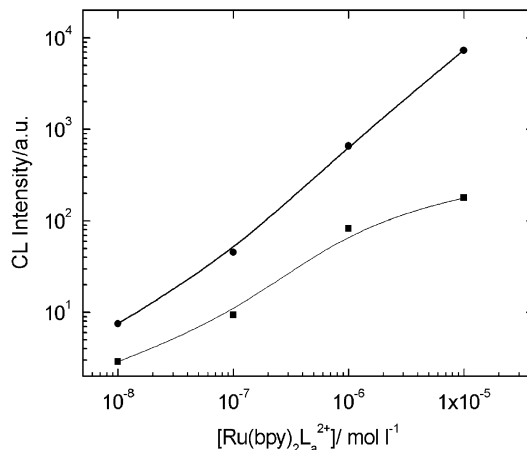


Fig. 7. Calibration curves of  $\text{Ru}(\text{bpy})_2\text{L}_a^{2+}$  in the presence of 0.06 M  $\text{K}_2\text{S}_2\text{O}_8$  (●) and 0.01 M  $\text{K}_4\text{P}_2\text{O}_8$  (■). Conditions: as in Fig. 6.

made acidic with sulfuric acid, much lower pH is needed to get electron transfer with magnesium and solution species started. The calibration curves of  $\text{Ru}(\text{bpy})_2\text{L}_a^{2+}$  (Fig. 7) look promising and, thus, a more detailed study of the system by using photon counting apparatus will probably result in the detection limit of about  $1 \times 10^{-10}$  M. Instead of peroxodisulfate ions, peroxodiphosphate ions were also tested; but sulfate radicals produced by reduction of peroxodisulfate ion seem to be better oxidants in the light-generating pathways than phosphate radicals under these conditions. This quite interesting and simple chemiluminescence system will be studied in a detailed way in our laboratory in the near future.

#### 4. Conclusions

Both  $\text{Ru}(\text{bpy})_2\text{L}_a^{2+}$  and  $\text{Ru}(\text{bpy})_2\text{L}_b^{2+}$  were observed to be cathodically electrochemiluminescent under the same experimental conditions as a simple  $\text{Ru}(\text{bpy})_3^{2+}$  chelate [1]. Their ECL spectra are similar to their PL emission spectra; hence, both emissions are induced by radiative transitions from the  $^3\text{MLCT}^*$  excited state of  $\text{Ru}(\text{bpy})_2\text{L}_a^{2+}$  or  $\text{L}_b^{2+}$ . Due to the carboxylic acid substituent of the present chelate,  $\text{Ru}(\text{bpy})_2\text{L}_a^{2+}$



can be covalently linked with biologically interesting molecules useful in various bioaffinity assays. It can also be detected with time-resolved ECL techniques, which might be usable in multiparametric assays in which, e.g. coumarine labels are used as another label having a very fast decay. Work is in progress in our laboratories to study the ECL of the present label in detail at different types of thin insulating film-coated electrodes to find optimal conditions for its ECL and to develop immunoassays in which  $\text{Ru}(\text{bpy})_2\text{L}_a^{2+}$  is used as an electrochemiluminescent label. Also, an immunoassay carried out on the surface of oxide-coated magnesium in basic conditions followed by the detection step under highly acidic peroxodisulfate solutions could be plausible. Coupling of  $\text{Ru}(\text{bpy})_2\text{L}_a^{2+}$  with bio-molecules probably induce an additional small red shift in the emission wavelength. Thus, it is better to use red sensitive PMTs, avalanche photodiodes or electron multiplying CCD detectors when this label is utilized.

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