Hot Electron-Induced Electrogenerated Chemiluminescence of $Ru(bpy)_3^{2+}$ Chelate at a Pointed Active Metal Cathode in Fully Aqueous Solutions

Päivi Kuosmanen[a], Kalle Salminen[a*], Matti Pusa[a], Timo Ala-Kleme[b] and Sakari Kulmala[a]

[a]Laboratory of Analytical Chemistry, Department of Chemistry, Aalto University, P.O. Box 16100, FI-00076 Aalto, Finland

[b]Laboratory of Materials Chemistry and Chemical Analysis, Department of Chemistry, University of Turku, 20500 Turku, Finland

kalle.salminen@aalto.fi

Abstract

Tris(2,2'-bipyridine)ruthenium(II) chelate exhibits strong electrogenerated chemiluminescence during cathodic high-voltage pulse-polarization of pointed Pt electrode in aqueous solutions. The present method is based on a field emission or other type of tunnel emission of hot electrons into an aqueous electrolyte solution. The method allows the detection of tris(2,2'-bipyridine)ruthenium(II) and its derivatives below nanomolar concentration levels and yields linear log-log calibration plots spanning several orders of magnitude of concentration.

Keywords: Hot electron, Electrochemiluminescence, Bioaffinity assay, Immunoassay, DNA probe assay

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Introduction

It has been previously shown that tris(2,2'-bipyridine)ruthenium(II) chelate ($Ru(bpy)_3^{2+}$) can be sensitively detected on the basis of chemiluminescence in the presence of hydrated electrons and strongly oxidizing radicals such as sulphate radicals and chlorine atoms and dichlorineradical ions [1,2]. $Ru(bpy)_3^{2+}$ is a well-known label molecule in bioaffinity assays such as in immuno- and DNA-probing assays [3]. In general, salts of $Ru(bpy)_3^{2+}$ are very stable, water-soluble compounds that can be chemically modified with reactive groups on one of the bipyridyl ligands to form activated species with which for example proteins, haptens and nucleic acids are readily labelled [4]. Therefore, $Ru(bpy)_3^{2+}$ has been used as a label in different techniques but mainly in electrogenerated chemiluminescence (ECL) based analysis [5]. Traditionally, the anodic ECL of $Ru(bpy)_3^{2+}$ has been measured in the conventional three electrode cell using gold or platinum electrodes as both counter and working electrodes and Ag/AgCl as a reference electrode [6]. The ECL mechanism of $Ru(bpy)_3^{2+}$ normally utilized is based on the use of coreactants which produce reducing radicals upon one-electron oxidization. In commercial kits the coreactant is typically a tertiary amine, tripropylamine (TPA). Both $Ru(bpy)_3^{2+}$-label and TPA are oxidized at the surface of the working electrode (Au, Pt or carbon), forming $Ru(bpy)_3^{3+}$-derivative and $TPA^{+\cdot}$, respectively. In the appropriate pH range, the $TPA^{+\cdot}$ loses a proton forming strongly reducing $TPA^{\cdot}$, which successively reacts with strongly oxidizing $Ru(bpy)_3^{3+}$ resulting in the excited label molecule $Ru(bpy)_3^{2+*}$. The resulting ^{3}MLCT excited state decays to the ground state by emitting light at 620 nm. In principle, the excitation cycle may occur repeatedly several times for each label molecule [6].

An alternative system studied previously is based on the use of cathodically pulse-polarized disposable oxide-coated aluminum [7] or silicon [8] electrodes with a quite freely selectable counter electrode. According to previous studies, strongly oxidizing species such as sulphate and hydroxyl radicals can be cathodically generated at pulse polarized thin insulating film-coated metal electrodes in fully aqueous solutions [9,10,11,12]. We have recently shown that also hole injection is possible into aqueous electrolyte solutions by anodic voltage pulses in case of an oxide-coated metal electrode [13].

The cathodic pulse polarization of thin insulating film-coated metal or strongly doped semiconductor [14,15] electrodes induce as the primary step a tunnel emission (field-assisted direct tunnelling [16]) of hot electrons (e_{hot}^-) into aqueous electrolyte solutions. This probably results in a subsequent generation of hydrated electrons (e_{aq}^-) and oxidizing radicals such as sulphate radicals ($SO_4^{\cdot -}$) from added coreactants [17,18]. Hot or hydrated electrons can react with compounds that are difficult to reduce, and therefore cathodic reductions usually not possible to carry out in aqueous solutions can be accomplished when hydrated electrons act as mediators [19]. Analogous phenomena have also been observed at oxide-coated silicon electrodes [15,20]. It is unlikely that all of the emitted hot electrons are reacting at the aluminum oxide/solution interface with solute species during the high amplitude cathodic pulse-polarization. If tunnel-emitted electrons have enough energy, they can be injected into the conduction band of water and become hydrated electrons after thermalization and solvation processes [21,22]. This precludes that the concentration of the coreactant added to produce oxidizing radicals is not too high and electron species are not too efficiently scavenged by oxidizing radical precursors, such as, peroxodisulphate ion, hydrogen peroxide or molecular oxygen. Peroxodisulphate ions and hydrogen peroxide react near diffusion controlled rate with hydrated electrons and produce highly oxidizing sulphate or hydroxyl radicals upon one-electron reduction [23]. Hence, highly reducing and oxidizing conditions are simultaneously achieved in the vicinity of the electrode surface by appropriate selection of the concentration of the cathodic coreactant [17,18]. We have previously shown that $Ru(bpy)_3^{2+}$ can be very sensitively detected by hot electron-induced electrochemiluminescence (HECL) at oxide-coated aluminium or silicon electrodes in fully aqueous solutions [7,24].

$Ru(bpy)_3^{2+}$ is known to produce cathodic ECL in the presence of peroxodisulphate ions at active metal electrodes in acetonitrile and acetonitrile-water mixtures, but not in fully aqueous solutions [25]. Bard et al. have suggested that either $Ru(bpy)_3^+$ is not produced, or is a highly unstable species, or peroxodisulphate is always reduced with concerted two-electron transfer at active metal electrode in fully aqueous solutions [25]. We have recently demonstrated that hot electrons can be injected into aqueous electrolyte solutions from pointed metal cathodes resulting in HECL of an aromatic Tb(III) chelate not excitable on the basis of traditional electrochemistry at active

metal electrodes in aqueous solution [26]. The present work was carried out to study if analogous cathodic HECL of $Ru(bpy)_3^{2+}$ chelate in fully aqueous solution could be observed.

Experimental

Tris(2,2'-bipyridine)ruthenium(II) chloride hexahydrate, benzophenon-4-carboxylate and Tween 20 were purchased from Aldrich. $K_2S_2O_8$, $Na_2B_4O_7 \cdot 10H_2O$, H_2O_2, $NaNO_3$, $NaNO_2$, NaBr, NaI, NaN_3, NaSCN and HCOONa were pro analysi or suprapur products of Merck. Ethanol was supplied by Oy Alko Ab and suprapur Na_2SO_4 by Merck. Hexamminecobalt(III) chloride was a product of Ventron. Quartz-distilled water was used in all solutions. The safety precautions required in handling of the reagents have been presented previously [27,28].

The ECL measurements were made in 0.2 M boric acid buffer at pH 9.2. Boric acid buffer was used due to its unreactivity toward sulphate radicals and hydrated electrons. The methods of ECL excitation are described elsewhere [9,10,11,12,17,18]. Measurements were made either through 580-nm long pass filter or an interference filter having a transmission maximum of 620 nm and bandwidth of about 10 nm. The apparatus has been described earlier elsewhere [11,29]

Working electrode was a sharpened platinum wire electrode (0.5-mm diameter) and the counter electrode was a platinum cup electrode fabricated similarly as the aluminium cup electrodes earlier [11,29]. Effective Pt cup solution surface area was about 2.1 cm^2 and that of Pt wire electrode fixed to ca. 8 mm^2.

Results and Discussion

Presently used pointed Pt wire electrode against the same large area Pt cup counter electrode was used earlier in case of HECL of an aromatic Tb(III) chelate. In that study hot electrons were injected also into a peroxydisulphate solution, and the optimal peroxydisulphate concentration was observed to be 0.030 M [26]. When calibration plot of $Ru(bpy)_3^{2+}$ was measured in 0.03 M $K_2S_2O_8$ solution, a linear plot spanning several orders of magnitude of concentration was readily observed in borate buffer at pH 9.2 (Fig. 1). Obviously, the present system could be used also for

determination of peroxydisulphate as demonstrated previously [7], even if determination of peroxydisulphate is not often analytically important.

The present measuring system allows photomultiplier tube to detect luminescence regardless it is formed at the cathode or at the anode. I.e. the sum of possible anodic or cathodic ECL is always measured. Earlier, when the polarity of the Pt wire electrode and Pt cup electrode were reversed no HECL of the aromatic Tb(III) chelate could be obtained [26], but in the present case some very weak ECL could be observed (Fig.1). However, at concentration level of 1.0 x10^{-5} M $Ru(bpy)_3^{2+}$ the ECL intensity with a pointed cathode was more than 1000-fold stronger than with the large area Pt cup cathode. We believe that at a large area platinum cathode peroxydisulphate is practically exclusively reduced by concerted two-electron reduction and therefore no sulphate radicals are generated. However, a small portion of one-electron reduced $Ru(bpy)_3^{2+}$ chelate (the ruthenium oxidation state is not changed but the extra electron is added to the ligand-centered orbital resulting in an unstable species in aqueous solution) [4] at the cathode may have sufficient lifetime before decomposition in aqueous solution [4] to one-electron reduce peroxydisulphate generating a minute amount of peroxydisulphate in the system which can finally oxidize another one-electron reduced $Ru(bpy)_3^{2+}$chelate to its excited state.

Fig. 1

Next, it was confirmed that borate buffer (which is exceptional buffer since it is only mildly reactive with hydrated electrons and oxidizing radicals) [11] is also usable in the present case as shown in the (Fig. 2). On this basis, also the rest of the measurements were decided to be carried out in the borate buffer.

Fig. 2.

In anodic ECL of $Ru(bpy)_3^{2+}$ at noble metal electrodes surfactants such as Tween 20 are normally used mainly due to the need to increase the solubility of tripropyl amine [6]. When the effect of Tween was tested the performance of large area cathode became somewhat better as shown in Fig. 3. We assume that the presence of Tween could now increase the lifetime of one-electron

reduced $Ru(bpy)_3^{2+}$ chelate. Thus, the ECL intensity was somewhat higher and better response could be obtained for the high concentration of $Ru(bpy)_3^{2+}$.

Fig. 3.

When the hydrated electron scavengers were added in the presently studied system, they prevented HECL generation in the order of their second order reaction rate constants as observed earlier in the case of Tb(III) chelate in the similar cell, as well as, in the case of $Ru(bpy)_3^{2+}$ at oxide-coated aluminium electrode during cathodic pulse-polarization [30]. When the balance between oxidizing and reducing equivalents of the system were disturbed by addition of hydrogen peroxide no clear beneficial effect of hydroxyl radicals formed upon one-electron reduction of hydrogen peroxide could be observed. All the different types of fast hydrated electron scavengers (an aromatic compound, an inorganic anion, an inorganic oxidant, an inorganic metal chelate) prevented the HECL generation very efficiently at their high concentrations (Fig. 4).

Fig. 4.

When studying the effects of oxidizing radical scavengers (hole scavengers) it was observed that a small concentration of hole scavenger producing a reducing secondary radical (formate and ethanol) had a mild enhancing effect on HECL intensity (Fig. 5). This is assumed to mean that a small increase on the reducing equivalents of the system promotes more efficient reduction of $Ru(bpy)_3^{3+}$ in its excited MLCT singlet or triplet state which is its emitting state both in its photoluminescence and in the different types electrochemiluminescence [4]. Bromine atom produced by one-electron oxidation of bromide ion was almost equally good oxidant for the system as sulphate radical, but dibromine radical ions and molecular bromine prevailing at higher concentrations of bromide ion were not sufficiently efficient oxidants in HECL generation pathways. Azide and thiocyanate radical were behaving quite similarly to dibromine radical ions, but consumption of sulphate radicals to produce iodine radical ions had a very clear adverse effect on the HECL generation (Fig. 5). The production of weaker oxidants from the halide and pseudohalide series like iodine ions had a clear adverse effect on the HECL. As a conclusion of these measurements the sulphate radical is probably the best available oxidant for the present system.

Fig. 5.

The energy diagram of the present system is presented in Fig 6. The diagram has been drawn on the following basis. Cathodic pulse-polarization of pointed Pt electrode induces tunnel emission of hot electrons into aqueous electrolyte solution as the primary step. These hot high energy electrons can become hydrated electrons after thermalization and solvation processes as has been discussed in detail previously [11,17,18,19]. The energy diagram also contains the energetics of some of the secondary radicals produced by the free radical scavengers used in the system and presented as at a flat band potential. The redox potentials of inorganic couples and $Ru(bpy)_3^{2+}$ presented in the energy diagram are taken from references [4] and [23].

Fig. 6.

The mechanism of HECL is discussed below on the basis of the energy diagram (Fig. 6) and results presented above. When e_{aq}^- or other suitable one-electron reductans are available, sulphate radicals can be generated from peroxodisulphate ions according to reaction (1)

$$e_{aq}^- + S_2O_8^{2-} \rightarrow SO_4^{\cdot -} + SO_4^{2-} \qquad k_1 = 2.3\times10^{10}\ M^{-1}\ s^{-1}\ [23] \qquad (1)$$

After this, there are two obvious pathways to excite $Ru(bpy)_3^{2+}$ to its excited ^{1}MLCT or ^{3}MLCT states at energies of 2.7 and 2.1 eV, respectively [4]. The ECL excitation route of $Ru(bpy)_3^{2+}$ can occur by the reduction-initiated oxidative excitation (red-ox) pathway (reactions 2a and 2b), or the oxidation-initiated reductive excitation (ox-red) pathway (reactions 3a and 3b) and is analogous to that observed previously in case of ECL of $Ru(bpy)_3^{2+}$ at oxide-covered aluminum electrodes [7].

$$Ru(bpy)_3^{2+} + e_{aq}^- \rightarrow Ru(bpy)_3^{+} \qquad (2a)$$

$$Ru(bpy)_3^{+} + SO_4^{\cdot -} \rightarrow {}^1Ru(bpy)_3^{2+*} + SO_4^{2-} \qquad (2b)$$

$$Ru(bpy)_3^{2+} + SO_4^{\cdot -} \rightarrow Ru(bpy)_3^{3+} + SO_4^{2-} \qquad (3a)$$

$$Ru(bpy)_3^{3+} + e_{aq}^- \rightarrow {}^{1or3}Ru(bpy)_3^{2+*} \quad (3b)$$

$Ru(bpy)_3^{2+}$ is rapidly reduced by hydrated electron [$k(e_{aq}^- + Ru(bpy)_3^{2+}) = 8.2x10^{10}$ l mol^{-1} s^{-1}] [23], or alternatively oxidised by a sulphate radical. The next step is the formation of ${}^1Ru(bpy)_3^{2+*}$ or ${}^3Ru(bpy)_3^{2+*}$, i.e. the $Ru(bpy)_3^{2+}$ in its excited ^{1}MLCT or ^{3}MLCT state, by the oxidation of $Ru(bpy)_3^+$ (reaction 2b), or the reduction of $Ru(bpy)_3^{3+}$ (reaction 3b) with the second-order rate constants of $k_{3b} = 5.2x10^{10}$ l mol^{-1} s^{-1} but the reaction rate constant k_{2b} is unknown [23]. As discussed previously [RuAC), the calculated enthalpies for reactions 2b and 3b are ca. 4.5 eV and 4.0 eV, respectively, when the standard reduction potentials of $SO_4^{\cdot -}/SO_4^{2-}$, $Ru(bpy)_3^{2+}/Ru(bpy)_3^+$, $Ru(bpy)_3^{3+}/Ru(bpy)_3^{2+}$ and hydrated electron are 3.4 V, -1.28 V, 1.26 V, and -2.9 V vs. SHE [4,23]. Thus the excitation steps (2b and 3b) are sufficiently energetic to result in $Ru(bpy)_3^{2+}$ even in its ^{1}MLCT state at 2.7 eV above the ground state, but because of a fast intersystem crossing ($k_{ISC} > 10^{10}$ s^{-1}) [4], the ^{1}MLCT state is quite probably mainly relaxed (reaction 4) to the ^{3}MLCT state at 2.1 eV, which is further relaxed to the ground state by a radiative transition (reaction 5) displaying the 620 nm peak emission.

$${}^1Ru(bpy)_3^{2+*} \rightarrow {}^3Ru(bpy)_3^{2+*} \quad (4)$$

$${}^3Ru(bpy)_3^{2+*} \rightarrow Ru(bpy)_3^{2+} + h\nu \text{ (620 nm)} \quad (5)$$

As discussed earlier, in the simultaneous presence of hydrated electrons and sulphate radicals [7,24], we suggest again in the present that ox-red excitation route is the dominant one, although Mulazzani et al. [31,32] have proposed red-ox excitation pathway to be also an important excitation mechanism.

For the analytical applicability there seems to be three alternatives. First, bioaffinity assays could be carried out in small wells made in plastic at the surface of pointed cathode network. Secondly, bioaffinity assays could be carried out on the surface of latex particles and finally measured e.g. in holes trilled in stainless steel which are acting as anodes. Third obvious choice would be carry out the assay on the surface of plastic wells that are coated with a conductive polymer or carbon paste which act as anodes during detection step of the bioaffinity assay such as an immunoassay or a DNA probe assay.

Conclusions

It is suggested that hot electrons are first injected into the conduction band of water by field emission or by other high-field tunnelling mechanism and the electrons are thermalized and solvated in water producing hydrated electrons as highly reducing mediators. These mediators are able to produce sulphate radicals by one electron reduction of peroxydisulphate ion at diffusion controlled rate. Tris(2,2'-bipyridine)ruthenium(II) chelate is proposed to be excited mainly by oxidation initiated route where it is first one-electron oxidized by sulphate radical and then reduced by hydrated electron into its excited state. This method allows simultaneous excitation of derivatives of tris(2,2'-bipyridine)ruthenium(II) and Tb(III)-chelates. The former label compounds have a luminescence lifetime of the order of microseconds, while the latter compounds generally have a luminescence lifetime of around 2 milliseconds. Thus, the combined use of these labels easily provides the basis for two-parameter bioassays by either wavelength or time discrimination or their combination, such as in immunoassays or DNA-probe assays utilizing latex particles as reaction sites and carriers.

References

[1] S. Kulmala, A. Hakanen, P. Raerinne, A. Kulmala, K. Haapakka, Ruthenium(II) tris- (2,2´-bipyridine) -specific Extrinsic Lyoluminescences of X-ray Irradiation Colored and Electrolytically Colored Alkali Halides, Anal. Chim. Acta 309 (1995) 197-210.

[2] Q. Jiang, M. Kotiranta, K. Langel, J. Suomi, M. Håkansson, A.-M. Spehar, T. Ala-Kleme, J. Eskola, S. Kulmala, Ruthenium(II) tris(2,2'-bipyridine) chelate as a chemiluminophore in extrinsic lyoluminescences of aluminium and magnesium in aqueous solution, Anal. Chim. Acta 541 (2005) 179-186.

[3] P.G. Sammes, G. Yahioglu, Modern bioassays using metal chelates as luminescent probes, Natural Product Reports 13 (1996) 1-28.

[4] A. Juris, V. Balzani, F. Barigelletti, S. Campagna, P. Belser, A. Zelewsky, Ruthenium(II) polypyridine complexes: photophysics, photochemistry, electrochemistry, and chemiluminescence, Coord. Chem. Rev. 84 (1988) 85-277.

[5] M. M. Collinson, P. Pastore, K.M. Maness, R.M. Wightman, Disproportionation During Electrooxidation of Catecholamines at Carbon-Fiber Microelectrodes, J. Am. Chem. Soc. 116 (1994) 4095-4096.

[6] G. Blackburn, H. Shah, J. Kenten, J. Leland, R. Kamin, J. Link, J. Peterman, M. Powell, A. Shah, D. Talley, S. Tyagi, E. Wilkins, T.-G. Wu, J. Massey, Electrochemiluminescence detection for development of immunoassays and DNA probe assays for clinical diagnostics, Clin. Chem. 37 (1991) 1534-1539.

[7] T. Ala-Kleme, S. Kulmala, L. Väre, P. Juhala, M. Helin, Hot electron-induced electrogenerated chemiluminescence of $Ru(bpy)_3^{2+}$ chelate at oxide-covered aluminum electrodes, Anal. Chem. 71 (1999) 5538-5543.

[8] Q. Jiang, J. Suomi, M. Håkansson, A. J. Niskanen, M. Kotiranta, S. Kulmala, Cathodic electrogeneratedchemiluminescence of Ru(bpy)32+ chelate at oxide-coated heavily doped silicon electrodes, Anal. Chim. Acta 541 (2005) 159-165.

[9] S. Kulmala, J. Kankare, K. Haapakka, Electrogenerated luminescence of terbium(III) in aqueous solutions, Anal. Chim. Acta 252 (1991) 65-76.

[10] S.Kulmala, K. Haapakka, Mechanism of electrogenerated luminescence of terbium(III)-{2,6-bis[N,N-bis(carboxymethyl)aminomethyl]-4-benzoylphenol} chelate at an oxide-covered aluminum electrode, J. Alloy. Compd. 225 (1995) 502-506.

[11] S. Kulmala, A. Kulmala, T. Ala-Kleme, J. Pihlaja, Primary Cathodic Steps of Electrogenerated Chemiluminescence of Lanthanide(III) Chelates at Oxide-covered Aluminum Electrodes in Aqueous Solution, Anal. Chim. Acta 367 (1998) 17-31.

[12] S. Kulmala, T. Ala-Kleme, M. Latva, K. Loikas, H. Takalo, Hot Electron-Induced Electrogenerated Chemiluminescence of Rare Earth(III) Chelates at Oxide-Covered Aluminium Electrodes, J. Fluor. 8 (1998) 59-65.

[13] K. Salminen, P. Kuosmanen, M. Pusa, O. Kulmala, M. Håkansson, S. Kulmala, Electrogenerated chemiluminescence induced by sequential hot electron and hole injection into aqueous electrolyte solution, Anal. Chim. Acta 912 (2016) 24-31.

[14] M. Håkansson, Q. Jiang, M. Helin, M. Putkonen, A. J. Niskanen, S. Pahlberg, T. Ala-Kleme, L. Heikkilä, J. Suomi, S. Kulmala, Cathodic Tb(III) Chelate Electrochemiluminescence at Oxide-covered Magnesium and n-ZnO:Al/MgO Composite Electrodes, Electrochim. Acta 51 (2005) 289-296.

[15] Q. Jiang, J. Suomi, M. Håkansson, A. J. Niskanen, M. Kotiranta, S. Kulmala, Cathodic electrogenerated chemiluminescence of Ru(bpy)32+ chelate at oxide-coated heavily doped silicon electrodes, Anal. Chim. Acta 541 (2005) 159-165.

[16] D. DiMaria, E. Cartier, Mechanism for stress-induced leakage currents in thin silicon dioxide films, J. Appl. Phys. 78 (1995) 3883-3894.

[17] S. Kulmala, T. Ala-Kleme, A. Hakanen, K. Haapakka, F-centre luminescence from oxide-covered aluminium cathode induced by two-step reduction of peroxydisulfate anions, J. Chem. Soc., Faraday Trans. 93 (1997) 165-168.

[18] S. Kulmala, T. Ala-Kleme, L. Heikkilä, L. Väre, Energetic electrochemiluminescence of (9-fluorenyl)methanol induced by injection of hot electrons into aqueous electrolyte solution, J. Chem. Soc., Faraday Trans. 93 (1997) 3107-3113.

[19] S. Kulmala, T. Ala-Kleme, H. Joela, A. Kulmala, Hot electron injection into aqueous electrolyte solution from thin insulating film-coated electrodes, J. Radioanal. Nucl. Chem. 232 (1998) 91-95.

[20] T. Ala-Kleme, S. Kulmala, M. Latva, Generation of Free Radicals and Electrochemiluminescence at Pulse-polarized Oxide-covered Silicon Electrodes in Aqueous Solutions, Acta Chem. Scand. 51 (1997) 541-546.

[21] Y. Pleskov, Z. Rotenberg, Photoemission of Electrons from Metals into Electrolyte Solutions as a Method for Investigation of Double-Layer and Electrode Kinetics, in: H. Gerischer, C. Tobias (Eds.), Advances in Electrochemistry and Electrochemical Engineering, Wiley, New York, 1978, pp. 3-115.

[22] E. Hart, M. Anbar, The Hydrated Electron, Wiley, New York, 1970.

[23] G. Buxton, C. Greenstock, W. Helman, A. Ross, Critical review of rate constants for reactions of hydrated electrons, hydrogen atom and hydroxyl radicals ($^{\cdot}OH/^{\cdot}O^{-}$) in aqueous solution, J. Phys. Chem. Ref. Data 17 (1988) 513-886.

[24] Q. Jiang, H. Ketamo, A.J. Niskanen, J. Suomi, M. Håkansson, S. Kulmala, Effects of thermal oxidation conditions of silicon electrodes on cathodic electrochemiluminescence of $Ru(bpy)_3^{2+}$ chelate, Electrochim. Acta 51 (2006) 3332-3337.

[25] D. Ege, W. Becker, A. Bard, Electrogenerated chemiluminescent determination of tris(2,2'-bipyridine)ruthenium ion ($Ru(bpy)_3^{2+}$) at low levels, Anal. Chem. 56 (1984) 2413-2417.

[26] P. Kuosmanen, K. Salminen, S. Kulmala, Hot electron-induced Electrochemiluminescence at pointed active metal cathodes, J. Luminesc., submitted.

[27] S. Kulmala, T. Ala-Kleme, A. Kulmala, D. Papkovsky, K. Loikas, Cathodic Electrogenerated Chemiluminescence of Luminol at Disposable Oxide-Covered Aluminum Electrodes, Anal. Chem. 70 (1998) 1112-1118.

[28] C. Matachescu, S. Kulmala, T. Ala-Kleme, H. Joela, Mechanism and Analytical Applicability of Luminol-Specific Extrinsic Lyoluminescence of UV-Irradiated Potassium Peroxodisulfate, Anal. Chem. 69 (1997) 3385-3390.

[29] J. Kankare, K. Fäldén, S. Kulmala, K. Haapakka, Cathodically induced time-resolved lanthanide(III) electroluminescence at stationary aluminum disc electrodes, Anal. Chim. Acta 256 (1992) 17-28.

[30] P. Neta, R. Huie, A. Ross, Rate constants for reactions of inorganic radicals in aqueous solution, J. Phys. Chem. Ref. Data 17 (1988) 1027-1284.

[31] Q. Mulazzani, S. Emmi, P. Fuochi, M. Venturi, M. Hoffman, M. Simic, One-electron reductionof aromatic nitrogen heterocycles in aqueous solution. 2,2'-Bipyridine and 1,10-phenantroline, J. Phys. Chem. 83 (1979) 1582-1590.

[32] Q. Mulazzani, S. Emmi, P. Fuochi, M. Hoffman, M. Venturi, On the nature of tris(2,2'-bipyridine)ruthenium(1+) ion in aqueous solution, J. Am. Chem. Soc. 100 (1978) 981-983.

Figures and figure captions

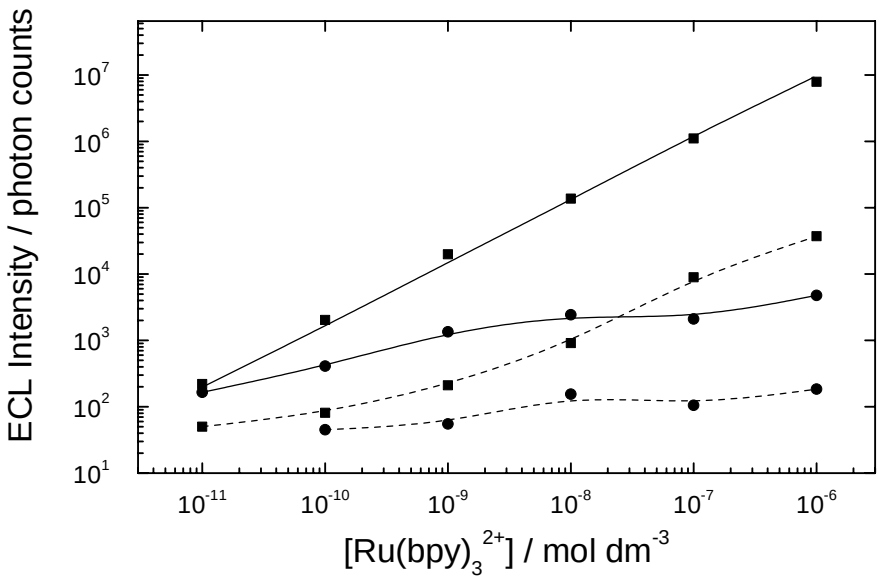

Fig. 1. Calibration plots of $Ru(bpy)_3^{2+}$ at a pointed and large-area Pt electrodes in a peroxydisulphate solution. Pointed area Pt electrode as a cathode and large-area Pt as an anode (■); pointed area Pt electrode as a anode and large-area Pt as a cathode (●), solid lines total signals (i.e. the sum of the delayed signal and the signal during the excitation pulse) and dashed lines time-resolved signal with a delay time of 1 µs and gate width of 200 µs. Conditions: measured through a 580-nm long pass filter, measuring buffer 0.05 M pH 9.2 $Na_2B_4O_7$ containing 3×10^{-2} M $K_2S_2O_8$, coulostatic pulse generator excitation with pulse voltage -70 V, frequency 40 Hz and pulse charge 200 µC, 1000 excitation cycles.

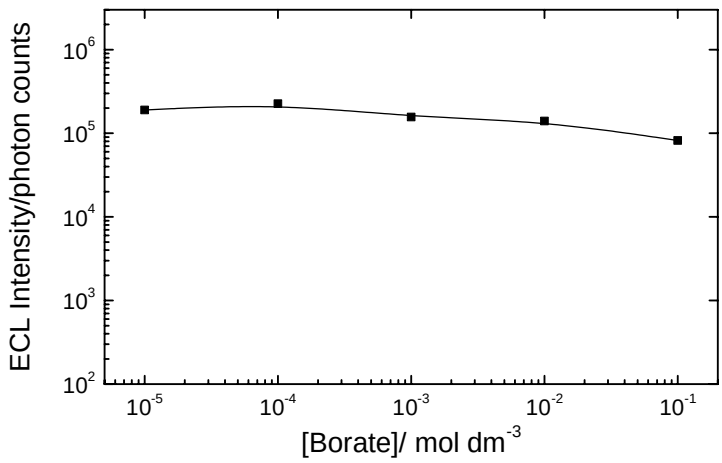

Fig. 2. The effect of borate concentration on the HECL of $Ru(bpy)_3^{2+}$ at a pointed Pt electrode. Pointed area Pt electrode as a cathode and large-area Pt as an anode. Conditions: 1.0×10^{-6} M $Ru(bpy)_3^{2+}$ measured in the presence of 1.0×10^{-3} M $K_2S_2O_8$ and 0.1 M Na_2SO_4, pH 7.1 adjusted with 0.1 M H_2SO_4, 100 excitation cycles other conditions as in the Fig. 1.

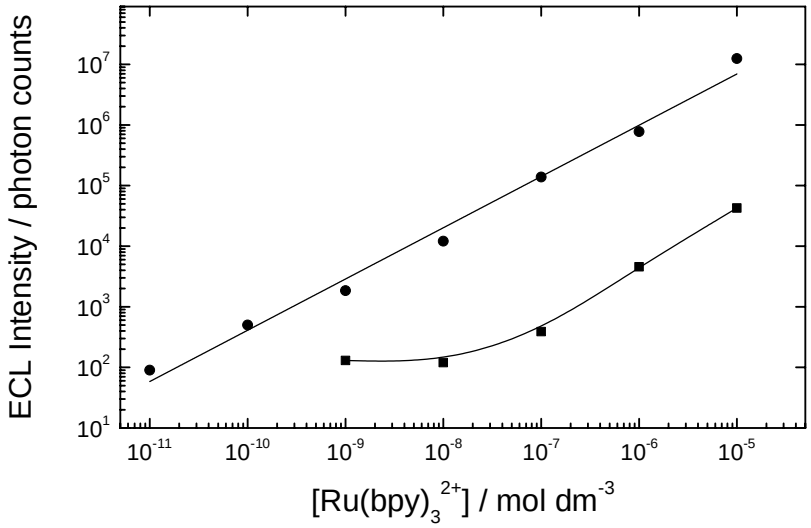

Fig. 3. Calibration plots of $Ru(bpy)_3^{2+}$ at a pointed (●) and large-area Pt electrodes (■) in a peroxydisulphate solution in the presence of Tween 20. Conditions: 0.20 M borate buffer at pH 9.2, 3×10^{-3} M $K_2S_2O_8$, pulse voltage -40 V, pulse charge 200 µC, pulse frequency 20 Hz, 620-nm Interference filter, 1000 excitation cycles.

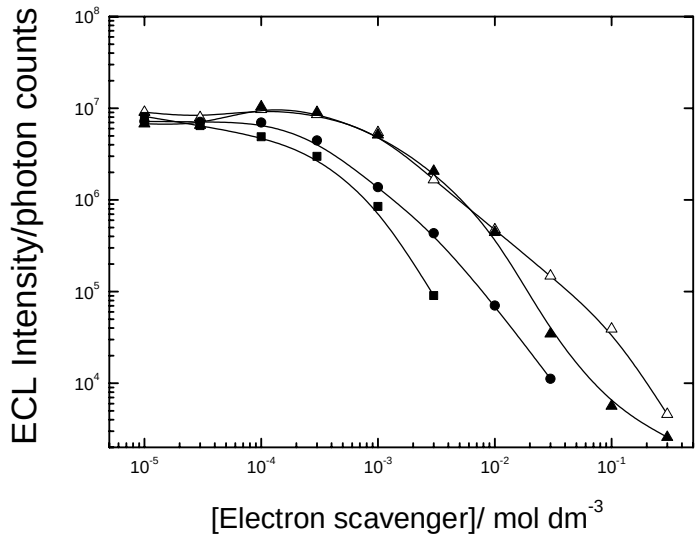

Fig. 4. The effect of various types of hydrated electron scavengers on the HECL of the present system. The ECL signal in the presence of electron scavengers during the pulse polarization of 1000 excitation cycles, $Co(NH_3)_6$ (squares), benzophenone-4-carboxylate (circles), H_2O_2 (solid trangles), $NaNO_3$ (open triangles). Conditions : 1.0×10^{-6} M $Ru(bpy)_3^{2+}$, 0.05 M $Na_2B_4O_7$, pH 9.2, 1×10^{-3} M $K_2S_2O_8$, coulostatic pulse generator excitation with pulse voltage -40 V, frequency 40 Hz and pulse charge 120 µC.

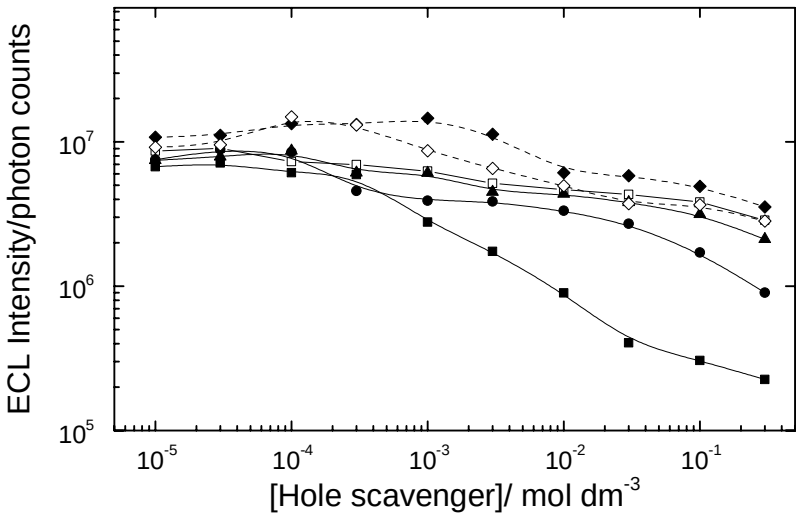

Fig. 5. The effect of various types of hole scavengers on the HECL of the present system. The ECL signals of the hole scavengers during the pulse polarization of 1000 excitation pulses NaBr (hollow squares), NaSCN (circles), NaN_3 (triangles), NaI (squares), HCOONa (hollow diamonds), EtOH (solid diamonds). Conditions: as in Fig 4.

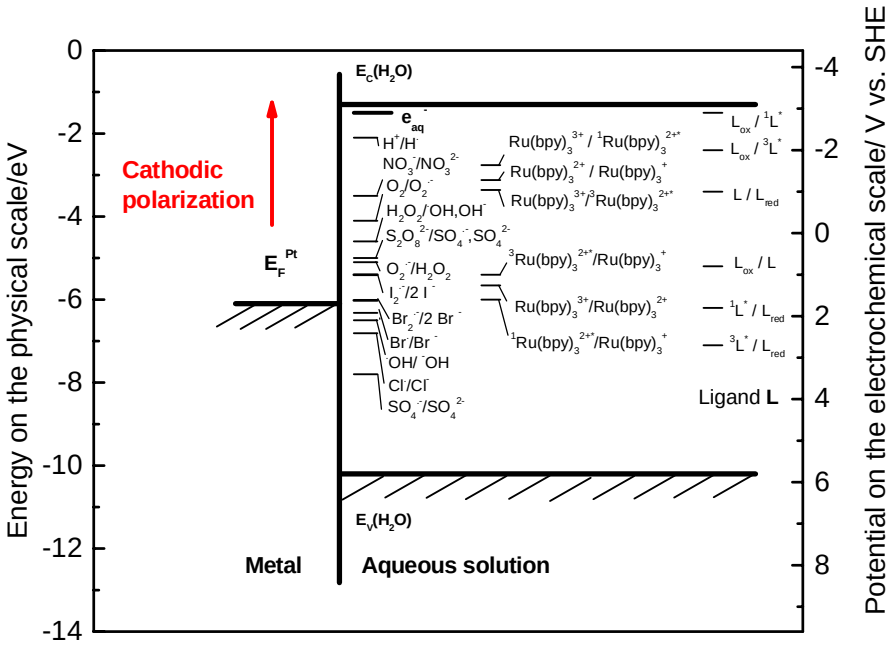

Fig. 6. Schematic energy diagram of the Pt/Electrolyte interface. The principles of the diagram are explained in the text.