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Electrogenerated chemiluminescence

Part 54. Electrogenerated chemiluminescence of ruthenium(II) 4,4′-diphenyl-2,2′-bipyridine and ruthenium(II) 4,7-diphenyl-1,10-phenanthroline systems in aqueous and acetonitrile solutions

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Abstract

The electrogenerated chemiluminescence (ECL) of $Ru(dp\text{-}bpy)_3^{2+}$ (dp-bpy = 4,4′-diphenyl-2,2′-bipyridine) and $Ru(dp\text{-}phen)_3^{2+}$ (dp-phen = 4,7-diphenyl-1,10-phenanthroline) in acetonitrile and aqueous solutions was studied. The diphenyl-substituted compounds produced a more intense emission than the nonsubstituted $Ru(bpy)_3^{2+}$ in acetonitrile. The ECL efficiencies, ϕ_{ECL}, of $Ru(dp\text{-}bpy)_3^{2+}$ and $Ru(dp\text{-}phen)_3^{2+}$ were estimated as 14% and 24% respectively. Similar results were obtained in aqueous solutions.

INTRODUCTION

The application of electrogenerated chemiluminescence (ECL) [1] to analysis or potential devices depends strongly on the magnitude of the light emission. For example, the sensitivity of analysis based on ECL-active labels [2] depends on the ECL efficiency of the label molecule. We discuss here increased ECL efficiencies in $Ru(bpy)_3^{2+}$-type (bpy = bipyridine) systems produced by suitable modification of the ligand.

Following the first report on metal chelate ECL in 1972 [3], there have been a number of studies of the ECL of $Ru(bpy)_3^{2+}$ in aprotic media [4–8]. For example, in acetonitrile (MeCN) solutions the excited $Ru(bpy)_3^{2+*}$ is produced by the annihilation reaction between $Ru(bpy)_3^{+}$ and $Ru(bpy)_3^{3+}$, as outlined in the following reaction scheme:

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

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

$$\mathrm{Ru(bpy)_3^{+} + Ru(bpy)_3^{3+} \rightarrow Ru(bpy)_3^{2+} + Ru(bpy)_3^{2+*}} \tag{3}$$

$$\mathrm{Ru(bpy)_3^{2+*} \rightarrow Ru(bpy)_3^{2+}} + h\nu \tag{4}$$

ECL can also be generated in aqueous solutions. Since aqueous solutions have a limited potential window compared with MeCN, the 3 + nor the 1 + forms of the ruthenium species cannot be generated simultaneously and alternative means must be used to produce suitable precursors to participate in the energetic electron transfer reaction that forms $\mathrm{Ru(bpy)_3^{2+*}}$. For example, oxidation of oxalate ion [2] or tripropylamine (TPrA) [9,10] can generate strong reductants that react with $\mathrm{Ru(bpy)_3^{3+}}$ to produced the excited state. We, and others, have also been interested in the determination of $\mathrm{Ru(bpy)_3^{2+}}$ in aqueous solutions, e.g., as a label in competitive binding assays [2]. For example, $\mathrm{Ru(bpy)_3^{2+}}$ in aqueous solutions containing TPrA(RH) can be detected at concentrations down to 10^{-11} M [9,10]. The ECL generation in this system occurs through an "oxidative–reduction" mechanism, in which a strong reductant (presumably $\mathrm{R^{\cdot}}$) is generated by an initial oxidation reaction. Electrochemical studies of various aliphatic amines suggest a possible reaction scheme for the oxidation of TPrA [11]. Upon oxidation, the short-lived TPrA radical cation $\mathrm{RH^{+}}$) is believed to deprotonate to form a highly reductive deprotonated TPrA radical $\mathrm{R^{\cdot}}$. This radical can then reduce the $\mathrm{Ru(bpy)_3^{2+}}$ to $\mathrm{Ru(bpy)_3^{+}}$, thus providing the 1 + species necessary for the annihilation reaction (3), or may reduce the $\mathrm{Ru(bpy)_3^{3+}}$ directly to the $\mathrm{Ru(bpy)_3^{2+*}}$:

$$\mathrm{Ru(bpy)_3^{3+} + R^{\cdot} \rightarrow Ru(bpy)_3^{2+*} + R^-} \tag{5}$$

This is analogous to the oxidation of oxalate, which in turn provides the $\mathrm{CO_2^{-\cdot}}$ anion radical after the decomposition of the $\mathrm{C_2O_4^{-\cdot}}$ species produced by oxalate oxidation [12,13].

The approach we have taken in increasing the ECL of the ruthenium labels is the use of ligand modification which has been shown to increase the photoluminescent yield in these compounds. For example, luminescence studies of many substituted derivatives of $\mathrm{Ru(bpy)_3^{2+}}$ and $\mathrm{Ru(phen)_3^{2+}}$ have been carried out by Cook and coworkers [14,15]. Of these derivatives, the diphenyl-substituted $\mathrm{Ru(dp\text{-}bpy)_3^{2+}}$ (dp-bpy = 4,4′-diphenyl-2,2′-bipyridine) and $\mathrm{Ru(dp\text{-}phen)_3^{2+}}$ (dp-phen = 4,7-diphenyl-1,10-phenanthroline) had the highest quantum efficiencies ϕ_m. In this paper we describe the ECL of $\mathrm{Ru(dp\text{-}bpy)_3^{2+}}$ and $\mathrm{Ru(dp\text{-}phen)_3^{2+}}$ in MeCN. We compare the results with those for the $\mathrm{Ru(bpy)_3^{2+}}$ system and show that enhanced ECL efficiencies are obtained for the diphenyl-substituted complexes. We also compare the ECL intensities of these complexes in an aqueous TPrA medium with that of $\mathrm{Ru(bpy)_3^{2+}}$ and discuss differences in behavior compared with $\mathrm{Ru(bpy)_3^{2+}}$ because of the hydrophobicity caused by the diphenyl substitution.

EXPERIMENTAL

All nonaqueous experiments were performed using tetra-*n*-butylammonium fluoroborate ($TBABF_4$) as the electrolyte and MeCN as the solvent. HPLC grade MeCN (Fisher Scientific Company, Fair Lawn, NJ) was purified and dried by continuous refluxing over P_2O_5 followed by distillation (all steps under nitrogen). The solvent was further dried and degassed by three vacuum distillations over P_2O_5 and stored over super I Woelm alumina N (ICN Biomedicals, Eschwege, Germany). Electrometric grade $TBABF_4$ (Southwestern Analytical Chemicals, Austin, TX) was recrystallized from ethyl acetate and then dried in a vacuum oven at 75–90 ° C overnight. The MeCN and $TBABF_4$ were then transferred to a glove-box (Vacuum Atmospheres, Hawthorne, CA), where they were stored under a dry oxygen-free, helium atmosphere.

$Ru(dp\text{-}bpy)_3Cl_2$ and $Ru(dp\text{-}phen)_3Cl_2$ were both prepared from potassium pentachlororuthenate(III) ($K_2(RuCl_5 \cdot H_2O)$, Alfa Chemical, Danvers, MA) according to published procedures [16]. These two complexes and $Ru(bpy)_3Cl_2$ (Sigma Chemical Co., St. Louis, MO) were converted from the chloride to the perchlorate salts by a metathesis reaction with an excess of sodium perchlorate (Aldrich Chemical Co. Inc., Milwaukee, WI). Each of the perchlorate salts was then recrystallized twice from an ethanol + acetone (5 : 1) solution. The resulting complexes were dried in a vacuum oven at 130 ° C and then transferred to the glove-box.

The aqueous experiments were performed using a 0.2 M phosphate buffer (pH 7.5). TPrA (Aldrich) was used as received at a concentration of 150 mM. Stock solutions of the $Ru(dp\text{-}bpy)_3^{2+}$ and $Ru(dp\text{-}phen)_3^{2+}$ were made by allowing the chloride salts of each to saturate a 100 ml sample of buffer + TPrA solution. A stock solution of $Ru(bpy)_3^{2+}$ was prepared with a concentration (10^{-5}–10^{-6} M) very near those of the other two complexes. The concentrations of the stock solutions were calculated from their absorbances (at about 450 nm). All subsequent solutions were made by diluting these stock solutions with buffer.

Apparatus and procedure

Cyclic voltammetric and ECL experiments were performed using a Princeton Applied Research (PAR, Princeton, NJ) model 175 universal programmer, model 173 potentiostat–galvanostat and model 179 digital coulometer. A charge-coupled-device (CCD) camera (Photometrics Ltd, Phoenix, AZ; model CH210) was used for all light measurements. Figure 1 shows the two arrangements used for light measurement. The total integrated light intensities were obtained on the CCD camera by focusing it on the electrode surface using a 100 mm Pentax macro lens. The integrated intensities were then obtained by calculating the average intensity of each pixel of the focused image (Fig. 1(a)). ECL spectra were recorded using a Chemspec 100S (American Holographic, Littleton, MA) spectrometer (focal length, 10 cm). The spectrometer was positioned such that the defracted

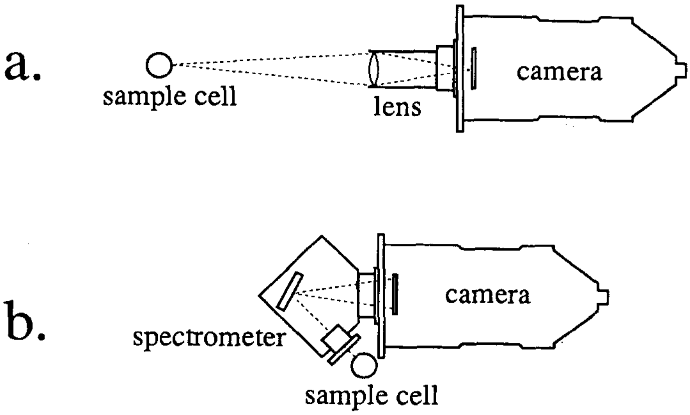

Fig. 1. (a) Schematic diagram of the experimental set-up for ECL imaging and light integration with the CCD camera; (b) set-up for ECL emission spectroscopy.

image was focused on the CCD detector (Fig. 1(b)). The camera was operated at −90 ° C and all exposures were corrected for any dark or background current.

The MeCN experiments employed a conventional three-electrode cell. The total cell volume was 1 ml and the cell was constructed to fit in front of the entrance slit of the spectrometer. A platinum disk electrode (0.03 cm^2) was used as the working electrode, and a platinum wire was used as the auxiliary electrode. A silver wire was used as a quasi-reference electrode (AgRE). The test solutions were then prepared by weighing suitable amounts of compound into the glass cell and then adding the appropriate amount of previously prepared electrolyte solution. All the solution preparations and cell preparations were conducted in the glove-box under a helium atmosphere. The ECL efficiencies were determined relative to Ru(bpy)$_3^{2+}$.

Cyclic voltammograms of Ru(dp-bpy)$_3^{2+}$ and Ru(dp-phen)$_3^{2+}$ are shown in Fig. 2, and are almost identical with that of Ru(bpy)$_3^{2+}$. The ECL was obtained by applying first a reducing and then an oxidizing potential. The reducing potential used for each compound was the potential E_{pc} of the first cathodic peak. Similarly, the oxidation potential used was the potential E_{pa} of the anodic peak. The positions of these potentials are shown in Fig. 2.

For the ECL efficiency measurements, the set-up shown in Fig. 1(a) was used and the potential was stepped to E_{pa} for 0.5 s and then to E_{pc} for 0.5 s. The camera recorded all light output from the electrode during this excitation, and the cathodic current was integrated for the double pulse.

For spectral measurements, the set-up shown in Fig. 1(b) was used with the potential continuously pulsed between E_{pa} and E_{pc} at a frequency of 10 Hz. A 2 s exposure was then made by the camera to give the resulting spectra. The spectrometer was calibrated using an Hg–Ar test lamp (Ultra-Violet Products, San Gabriel, CA). Experiments were performed in a darkroom and care was taken to eliminate stray light.

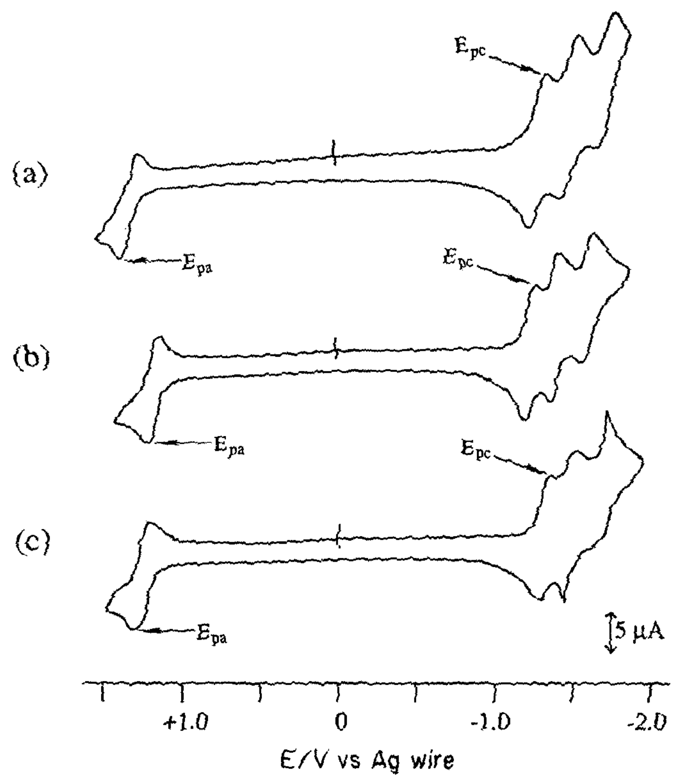

Fig. 2. Cyclic voltammograms of (a) $Ru(bpy)_3^{2+}$, (b) $Ru(dp\text{-}bpy)_3^{2+}$, (c) $Ru(dp\text{-}phen)_3^{2+}$, all approximately 1.0 mM in 0.1 M $TBABF_4$ in MeCN.

The aqueous experiments were performed using an ORIGEN I electrochemical analyzer (IGEN Inc., Rockville, MD) [10,17]. This analyzer employs a flow injection system which allows rapid and reproducible determinations of sequential samples. The detector was a photomultiplier tube positioned directly above the working electrode so that light from the electrode could be recorded and integrated during each measurement. Because of the low concentrations of the electroactive species used in these experiments, no cyclic voltammetry waves could be seen.

RESULTS AND DISCUSSION

ECL spectra and efficiencies in MeCN

The normalized emission spectra for the three compounds in MeCN produced by repetitive cycling are shown in Fig. 3. Clearly, the emission of the two diphenyl compounds is higher than that of the nonsubstituted $Ru(bpy)_3^{2+}$. The wavelengths for the emission peaks for all complexes, given in Table 1, were similar, with $Ru(dp\text{-}bpy)_3^{2+}$ shifted to the red compared with the other two. To obtain a more

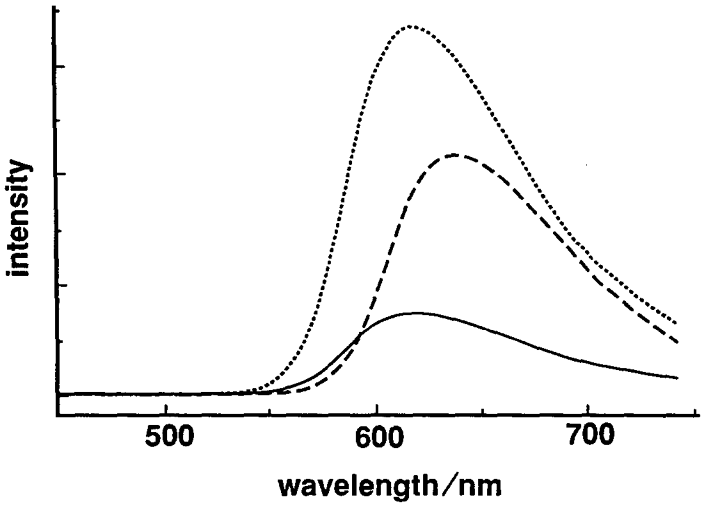

Fig. 3. ECL spectra of Ru(bpy)$_3^{2+}$ (———), Ru(dp-bpy)$_3^{2+}$ (----), and Ru(dp-phen)$_3^{2+}$ (·····). The solutions contained 0.1 M TBABF$_4$ in MeCN. Intensities shown have been normalized to the concentrations of the complexes.

quantitative measure of the relative ECL efficiencies, the integrated light intensity under the emission curve for a double-pulse experiment was determined; the results are shown in Table 1. In these experiments the ECL efficiencies were calculated relative to the ECL quantum efficiency of Ru(bpy)$_3^{2+}$, which was taken as 5% in MeCN [5,7]. The ECL quantum efficiency is defined as the ratio of the number of photons emitted to the number of annihilations between the 3 + and the 1 + forms of the ruthenium complex. The number of annihilations is somewhat smaller than the number of moles of reactant generated during a pulse, as

TABLE 1

Emission maximum, integrated intensity, integrated current, ECL and photoluminescence efficiencies in MeCN at 25 ° C.

Compound	λ_{max} /nm	Integrated intensity/ arbitrary units	Integrated current (one cycle) /μC	ECL efficiency ϕ_{ECL}	Photoluminescence efficiency ϕ_l
Ru(bpy)$_3^{2+}$	616	364(±33)	17.6	0.05 [a]	0.075 [a] 0.089 [b]
Ru(dp-bpy)$_3^{2+}$	635	761(±70)	14.1	0.14	0.31 [b]
Ru(dp-phen)$_3^{2+}$	615	1723(±153)	18.1	0.24	0.37 [c]

[a] From ref. 7.

[b] In ethanol + methanol (4:1 v/v) solution at 20 ° C [14].

[c] In ethanol + methanol (4:1 v/v) solution at 20 ° C [15].

calculated from the integrated current [18,19]. However, this difference is the same for all three complexes, so that, in estimating the relative efficiencies, the following equation can be employed:

$$\phi_{\mathrm{ECL}} = \frac{\int_0^t I \, \mathrm{d}t}{\int_0^t i \, \mathrm{d}t (N_{\mathrm{A}}/F)} \tag{6}$$

where I is the intensity in photons per second, i is the current in amperes (Coulombs per second), F is Faraday's constant and N_{A} is Avogadro's constant. Since the same electrode was used for each experiment and the concentrations of the test solutions were approximately the same, the charge passed for each compound was about the same, as shown in the integrated current values in Table 1. Each of the values shown in Table 1 is the average of at least five experimental trials.

ECL in an aqueous medium

The experiments performed in aqueous solutions of the ruthenium complexes and TPrA were complicated by several factors. The increased hydrophobicity of the diphenyl-substituted compounds decreased their solubility compared with $\mathrm{Ru(bpy)_3^{2+}}$ and their rate of dissolution. An excess of the chloride salt of each diphenyl compound was added to the buffer solution and allowed to equilibrate for several days. The concentrations were then estimated from absorbance measurements, using literature values of the extinction coefficients to calculate the concentrations. Measurements were made using TPrA, rather than oxalate, to generate the coreductant and ECL because the oxalate salts of the diphenyl compounds would slowly precipitate out of the solution. Moreover, the diphenyl-substituted compounds adsorbed strongly to the walls of the containers, both glass and polyethylene. This made it difficult to prepare a series of standard solutions at different concentrations. Reproducible results were only obtained when the experimental solutions were equilibrated with the walls of their polyethylene container before the absorbance measurements were made to determine their concentrations.

We also investigated the use of surfactants in solvating these hydrophobic species. The addition of surfactants (Triton X-100, Tween 20) did increase the solubility and decrease the adsorption to the walls of the container. However, the surfactants quenched the ECL of the diphenyl compounds, probably because they formed micelles which incorporated the complexes with the diphenyl ligands, encapsulating them and shielding them in the reaction producing ECL. The quenching of ECL of the diphenyl compounds by uncharged surfactant can be contrasted with the behavior of $\mathrm{Ru(bpy)_3^{2+}}$ in the TPrA system, where the addition of 0.5% Triton X-100 enhances the ECL emission [20]. Moreover, the addition of surfactant is useful in the flow injection system of the ORIGEN I analyzer, since

TABLE 2

Concentration, intensity, and relative ECL efficiency in 0.20 M phosphate buffer (pH 7.5) and 0.15 M TPrA.

Compound	Concentration μM	Intensity arbitrary units	No. of trials	Standard deviation	Relative ECL efficiency ϕ_{rel}
$Ru(bpy)_3^{2+}$	16	148	22	±14	1.0
$Ru(dp\text{-}bpy)_3^{2+}$	5.3	115	53	±40	2.4
$Ru(dp\text{-}phen)_3^{2+}$	4.0	30	24	±9.2	0.8

its use suppresses bubble formation and greatly improves the reproducibility of the measurements.

Since we could not use surfactants with the diphenyl compounds, the accuracy of the aqueous experiments was poorer than that of the $Ru(bpy)_3^{2+}$-based system. The relative intensities for the aqueous systems are given in Table 2. In making these measurements the same program was used for all three compounds, and the same potential, well beyond E_{pa}, was used for their oxidation. The relative efficiencies were obtained by normalizing the relative intensities by the concentration. It should be noted that, while the dp-bpy compound yields a relative efficiency compared with $Ru(bpy)_3^{2+}$ in the aqueous medium which was about the same as that in MeCN, that of the dp-phen compound is much lower. This may be caused by loss of the dp-phen compound by adsorption on the walls of the tubing and cell in the flow system.

CONCLUSIONS

The relative ECL efficiencies for the diphenyl-substituted compounds compared with $Ru(bpy)_3^{2+}$ in MeCN are quite close to those predicted from the photoluminescence efficiencies shown in Table 1. Earlier detailed studies of the $Ru(bpy)_3^{2+}$ system involving absolute emission measurements suggested that the yield of excited state on reaction between the 3+ and 1+ forms was close to unity. The results here imply a similarly high yield for the diphenyl-substituted compounds in MeCN. The significantly higher light emissions found in MeCN, and probably also in other nonaqueous solvents, suggest that they could be useful in ECL devices. However, it is doubtful whether these diphenyl species would offer any advantages as labels in aqueous ECL analysis systems. As discussed above, the reproducibility with these compounds was worse than that found with $Ru(bpy)_3^{2+}$, especially when the use of surfactants is precluded.

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