

Electrochemiluminescence from $\text{Os}(\text{phen})_2(\text{dppene})^{2+}$ (phen = 1,10-phenanthroline and dppene = bis(diphenylphosphino)ethene)

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The electrochemiluminescence (ECL) of $\text{Os}(\text{phen})_2(\text{dppene})^{2+}$ (phen = 1,10-phenanthroline and dppene = bis(diphenylphosphino)ethene) is reported in mixed $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (50:50 v/v) and aqueous (0.1 M KH_2PO_4) solutions with tri-*n*-propylamine (TPRA) as an oxidative–reductive coreactant. ECL efficiencies (ϕ_{ecl} = photons emitted/redox event) of 2.0 in aqueous, and 0.95 in mixed for $\text{Os}(\text{phen})_2(\text{dppene})^{2+}$ were obtained using $\text{Ru}(\text{bpy})_3^{3+}$ (bpy = 2,2′-bipyridine) as a relative standard (ϕ_{ecl} = 1). Photoluminescence (PL) efficiencies of 0.094 and 0.053 were obtained in aqueous and mixed solutions, respectively, as compared to $\text{Ru}(\text{bpy})_3^{3+}$ (ϕ_{em} = 0.042). The ECL spectra were identical to photoluminescence spectra ($\lambda_{\text{max}} \cong 584$ nm), indicating formation of the same metal-to-ligand (MLCT) excited states in both ECL and PL. The ECL is linear over several orders of magnitude in aqueous and mixed solution, with theoretical detection limits (blank plus three times the standard deviation of the noise) of 16.9 nM in H_2O and 0.29 nM in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (50:50 v/v).

Electrochemiluminescence (ECL) involves the formation of excited states at or near electrode surfaces and, as such, is a sensitive probe of electron- and energy-transfer processes at electrified interfaces.^{1,2} ECL is also being commercially developed for use in clinical analyses (e.g., immunoassays, DNA probes) using $\text{Ru}(\text{bpy})_3^{3+}$ (bpy = 2,2′-bipyridine) and a coreactant to generate an ECL signal.³ ECL coreactants are species that, upon electrochemical oxidation or reduction, produce intermediates that react with other compounds to produce excited states capable of emitting light.^{4–6} For example, in the $\text{Ru}(\text{bpy})_3^{3+}/\text{TPRA}$ (TPRA = tri-*n*-propylamine) system,⁷ an anodic potential oxidizes $\text{Ru}(\text{bpy})_3^{3+}$

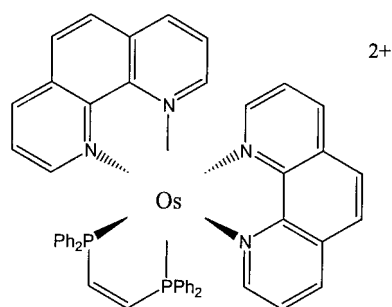


Figure 1. $\text{Os}(\text{phen})_2(\text{dppene})^{2+}$ (Ph = phenyl group).

to $\text{Ru}(\text{bpy})_3^{3+}$. The coreactant is also oxidized and decomposes to produce a reducing agent (presumably TPRA[•]) upon deprotonation of an α -carbon from one of the propyl groups.⁷ This strong reducing agent can then interact with $\text{Ru}(\text{bpy})_3^{3+}$ to form the excited state (i.e., $^*\text{Ru}(\text{bpy})_3^{2+}$).

Extension of $\text{Ru}(\text{bpy})_3^{3+}$ ECL to osmium systems has been somewhat limited due to the larger spin–orbit coupling in osmium systems that results in shorter excited-state lifetimes and weaker emission efficiencies.^{8–10} However, the incorporation of phosphine ligands into the framework of osmium polypyridyl systems (e.g., $\text{Os}(\text{phen})_2(\text{dppene})^{2+}$ where phen = 1,10-phenanthroline and dppene = bis(diphenylphosphino)ethene (Figure 1)) has resulted in excited-state photoluminescent lifetimes in excess of 200 ns.¹¹ The development of osmium-based sensors would be advantageous, since osmium systems are more photostable than their ruthenium analogues. In addition, polypyridyl osmium complexes usually oxidize at less anodic potentials than analogous ruthenium systems, and this could be important in designing DNA-labeling agents.¹²

In this work, the electrochemiluminescence of $\text{Os}(\text{phen})_2(\text{dppene})^{2+}$ is reported, opening up a new class of systems for fundamental and applied investigations. The use of $\text{Os}(\text{phen})_2(\text{dppene})^{2+}$

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(dppene)²⁺ and similar compounds as ECL labels in diagnostics is also proposed.

EXPERIMENTAL SECTION

Os(phen)₂(dppene)(PF₆)₂ was prepared and characterized by the literature method.¹³ Ru(bpy)₃Cl₂ (98%, Strem Chemical Inc, Newbury Port, MA), acetonitrile (Burdick and Jackson spectro-quality), potassium phosphate monobasic hydrate (99.0%, EM Science, Gibbstown, NJ), and tri-*n*-propylamine (98%, Avocado Research Chemicals, Ward Hill, MA) were used as received.

Potassium phosphate buffer solutions, 0.20 M KH₂PO₄·7H₂O, were prepared with deionized water that had been passed through a Barnstead/Thermolyne filtration system. Buffer solutions containing TPrA (0.05 M) were prepared in a similar manner, except that it was necessary to stir vigorously to completely dissolve the amine. The pH of these buffer solutions was adjusted to 8.0 ± 0.1 M (for electrochemical, spectroscopic, and ECL studies) and between 3 and 10 (for ECL vs pH studies) with either 6 M H₂SO₄ or 6 M NaOH.

Methods. Electrochemical and ECL instrumentation and experimental methods have been described elsewhere.¹⁴ All electrochemical and ECL experiments were referenced with respect to a Ag/AgCl gel electrode (0.20 V vs NHE).¹⁵ The platinum mesh (27 mm²) working electrode was cleaned prior to each experiment by repeated cycling (+2.0 to −2.0 V) in 6.0 M sulfuric acid, followed by sonication in 2 M nitric acid and rinsing in deionized water.

Solutions used to obtain the ECL were 1 nM–0.1 mM in metal complex and 0.05 M TPrA with 0.1 M potassium phosphate as the electrolyte. Photoluminescence and ECL spectra were obtained with a Shimadzu RF-5301 spectrofluorophotometer (slit widths, 5 nm). Excitation for photoluminescence studies was at 452 and 368 nm for Ru(bpy)₃²⁺ and Os(phen)₂(dppene)²⁺, respectively, with detection between 500 and 700 nm. ECL efficiencies (ϕ_{ECL} = photons generated/redox event) were obtained by the literature methods^{6,16} using Ru(bpy)₃²⁺ (ϕ_{ECL} = 1) as the standard.

RESULTS AND DISCUSSION

Electrochemistry. Cyclic voltammetric data for Os(phen)₂(dppene)²⁺ in all solvents is presented in Table 1. The oxidative wave is assigned to the Os^{II/III} couple.¹³ The redox chemistry shows an i_a/i_c of 1.13, indicating a reversible-to-quasireversible system and a peak-to-peak separation (ΔE_{pp}) of 80 mV. ΔE_{pp} is less than or equal to that observed for ferrocene^{+/0} under similar conditions, indicating a one-electron process.

Absorption and Photoluminescence. UV–vis spectra of Os(phen)₂(dppene)²⁺ are characterized by a series of ligand-based transitions in the UV with metal-to-ligand charge transfer (MLCT) bands in the visible region. Absorption bands centered at 370 nm have been assigned as MLCT transitions¹³ and are not solvent-dependent. Excitation into this broad visible absorption band produces room-temperature photoluminescence for Os(phen)₂(dppene)²⁺ in mixed and aqueous solution. An emission band is

Table 1. Spectroscopic and Electrochemical Data for Os(phen)₂(dppene)²⁺

solvent	$E_{1/2}(+1/0)$, V ^a	$\lambda_{\text{abs}}(\text{nm})$	$\lambda_{\text{em}}(\text{nm})$	ϕ_{em}^b	ϕ_{ECL}^c
CH ₃ CN/H ₂ O (50:50 v/v) ^d	1.02	368	585	0.053	0.95
H ₂ O ^d	1.16	368	589	0.094	2.0

^a All electrochemical and ECL experiments were referenced with respect to a Ag/AgCl gel electrode (0.20 V vs NHE).¹⁵ ^b Photoluminescence efficiency with respect to Ru(bpy)₃²⁺ (ϕ_{em} = 0.042).⁹ ^c Relative ECL efficiency with respect to Ru(bpy)₃²⁺ (ϕ_{ECL} = 1) at pH = 7.0 ± 0.1.^{6,16} Reported values are the average of at least three scans, with a relative standard deviation of ±5%. ^d 0.1 M KH₂PO₄ as electrolyte.

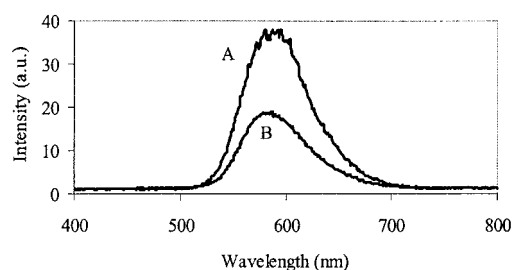


Figure 2. (A) Photoluminescence spectra of 0.01 mM Os(phen)₂(dppene)²⁺ in aqueous solution (0.1 M KH₂PO₄). Excitation wavelengths were at 368 nm with slit widths of 5 nm. (B) ECL spectra of 0.01 mM Os(phen)₂(dppene)²⁺ in aqueous solution (0.05 M TPrA, 0.1 M KH₂PO₄).

observed at 585 nm in 50:50 (v/v) CH₃CN/H₂O, and at 589 nm in aqueous solution (Figure 1). The photoluminescent emission spectra match well with those reported in the literature¹³ and have been assigned as MLCT transitions. Photoluminescence efficiencies (ϕ_{em} ; photons emitted/photons absorbed) for Os(phen)₂(dppene)²⁺ relative to Ru(bpy)₃²⁺ (ϕ_{em} (H₂O) = 0.042)¹⁷ are reported in Table 1 and are solvent-dependent, showing the sensitivity of these compounds to microenvironmental effects of the solvent media. The efficiencies of Os(phen)₂(dppene)²⁺ in mixed CH₃CN/H₂O and aqueous solutions are higher than Ru(bpy)₃²⁺ under identical conditions.

Electrochemiluminescence. Higher photoluminescence efficiencies for Os(phen)₂(dppene)²⁺, coupled with stable oxidative redox chemistry and solubility in both aqueous and nonaqueous solvents makes this system of interest in fundamental and applied ECL studies. TPrA was used as an “oxidative–reductive” coreactant^{5–7} to generate ECL because of the reversible-to-quasireversible nature of the Os^{II/III} anodic redox couple. ECL was observed for Os(phen)₂(dppene)²⁺ in aqueous (H₂O) and mixed solvent (i.e., 50:50 (v/v) CH₃CN/H₂O) solutions containing 0.05 M TPrA at a Pt interface (0.1 M KH₂PO₄ as the electrolyte). The ECL intensity peaks at potentials of ~+1.1 V, indicating oxidation of both TPrA (E_a ~+0.5 V vs Ag/AgCl) and Os(phen)₂(dppene)²⁺ (E° ~+1.0 V) has occurred. ECL emission spectra in each solvent (Figure 2) are identical to photoluminescence spectra, indicating the same MLCT excited state is formed in both experiments.

Both photoluminescence (ϕ_{em}) and ECL (ϕ_{ECL}) efficiencies for Os(phen)₂(dppene)²⁺ in mixed solvent and aqueous solutions are comparable to or higher than Ru(bpy)₃²⁺ (Table 1). Most striking

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are the results in aqueous solution, where a 2-fold increase in ϕ_{ECL} compared to $\text{Ru}(\text{bpy})_3^{2+}$ are observed. This is comparable to the ECL of the bimetallic ruthenium system $[(\text{bpy})_2\text{Ru}]_2(\text{bphb})^{4+}$ ($\text{bphb} = 1,4\text{-bis}(4'\text{-methyl-2,2'-bipyridin-4-yl})\text{benzene}$).¹⁶ The ligand bphb is capable of binding two independent metal centers through a "bridging ligand" framework. This bimetallic species produced more intense emission (2–3-fold) than $\text{Ru}(\text{bpy})_3^{2+}$ in aqueous and nonaqueous solution using annihilation and coreactant methods. With the goal of increasing the magnitude of ECL emission and, therefore, increasing ECL sensitivity and lowering detection limits, the bimetallic work was extended to dendrimeric systems containing eight $\text{Ru}(\text{bpy})_3^{2+}$ units at the periphery¹⁸ of a carbosilane dendrimer platform. The ECL of the $\text{Ru}(\text{bpy})_3^{2+}$ dendrimer was five times that of the reference monometallic species. Multimetallic compounds such as these show much promise for use in analytical applications. However, it has yet to be shown whether these types of labels will change nucleic acid hybridization or affinity binding of antigens and antibodies in diagnostic applications. ECL with $\text{Os}(\text{phen})_2(\text{dppene})^{2+}$ may present some advantages over multimetallic systems as a result of the similar reactivity of osmium and ruthenium compounds as well as charge and size similarities when comparing $\text{Os}(\text{phen})_2(\text{dppene})^{2+}$ with $\text{Ru}(\text{bpy})_3^{2+}$.

ECL is linear with respect to $[\text{Os}(\text{phen})_2(\text{dppene})^{2+}]$ in 50:50 (v/v) $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ and aqueous solution with the concentration of $\text{Os}(\text{phen})_2(\text{dppene})^{2+}$ being varied from 2 μM to 1 nM. Correlation coefficients (r^2) of 0.9986 (7 data points; standard deviation, $\pm 5\%$) and 0.9995 (8 data points; standard deviation, $\pm 5\%$) were obtained in aqueous and 50:50 (v/v) $\text{CH}_3\text{CN}/\text{H}_2\text{O}$, respectively, with theoretical detection limits (blank signal plus three times the standard deviation of the noise) of 16.9 nM and 0.29 nM.

The ECL emission is pH-dependent in both aqueous and 50:50 (v/v) $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ solutions (Figure 3), with maximum intensities observed around pHs 9 and 10, respectively. The increased intensities observed at pHs > 9 in mixed solvent solution, as compared to purely aqueous solution, are likely due to the increased solubility of TPrA in the mixed organic/aqueous solvent. In aqueous buffered solution at $[\text{TPrA}] > 0.50 \text{ mM}$ and pHs ≥ 9.4 , solubility becomes an issue.⁷ Similar trends are observed for $\text{Ru}(\text{bpy})_3^{2+}$ using TPrA as a coreactant and indicate that deprotonation of the TPrA radical cation (i.e., $\text{TPrA}^{+\cdot}$) is

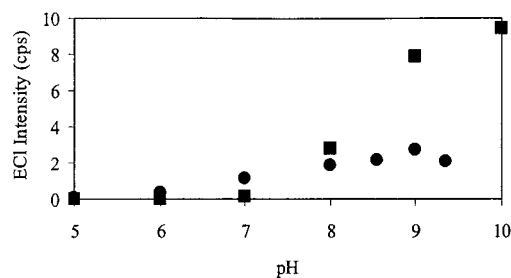


Figure 3. pH dependence of ECL using 0.05 M TPrA and 0.1 μM $\text{Os}(\text{phen})_2(\text{dppene})^{2+}$ in (■) mixed solvent (50:50 (v/v) $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (0.1 M KH_2PO_4) and (●) aqueous buffered (0.1 M KH_2PO_4) solution. Each point is the average of at least three scans, with a standard deviation of $\pm 7\%$ (error bars omitted for clarity).

critical to the generation of ECL in both osmium and ruthenium systems. This is important for potential applications, since the pH of environmental and biological systems is ~ 7.4 and would require less sample preparation prior to analysis.

CONCLUSIONS

This study illustrates that $\text{Os}(\text{phen})_2(\text{dppene})^{2+}$ (and, by analogy, other osmium(II) polypyridyl systems containing phosphine ligands) exhibits electrochemiluminescence in aqueous and mixed aqueous/nonaqueous solutions. In fact, the ECL emission quantum efficiency is 2-fold greater than $\text{Ru}(\text{bpy})_3^{2+}$ in aqueous solutions. This may prove useful in diagnostic or environmental applications in which greater sensitivity and detection limits than those that $\text{Ru}(\text{bpy})_3^{2+}$ can provide are required.

The lower potentials required to excite osmium systems, as compared to $\text{Ru}(\text{bpy})_3^{2+}$, may also prove useful in DNA diagnostic applications; however, it will be necessary to develop other osmium phosphine systems that can oxidize at even lower potentials, since it has been well-documented that oligonucleotide sequences undergo irreversible oxidative damage at potentials $\geq 1 \text{ V}$.¹²

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