

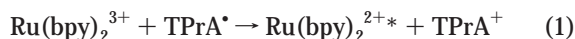
Electrogenerated Chemiluminescence. 68. Detection of Sodium Ion with a Ruthenium(II) Complex with Crown Ether Moiety at the 3,3'-Positions on the 2,2'-Bipyridine Ligand

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We show here how a Ru(bpy)₂(CE-bpy) complex can be used for the ECL detection of sodium ion in both aqueous and nonaqueous media. Recognition of Na⁺ by the crown ether moiety in CE-bpy results in a significant increase in the ECL emission intensity of the complex.

We report here an approach using electrogenerated chemiluminescence (ECL) for the determination of species that do not show redox activity, for example, alkali metals and ligands, within the potential limits of most solvents. In general, ECL has been used as an analytical tool for either species that themselves produce emission in an energetic redox reaction or for coreactant molecules (e.g., oxalate, aliphatic amines) that produce a redox-active molecule.^{1,2} For example, Ru(bpy)₃²⁺ (bpy = 2,2'-bipyridine) has been extensively used as a label for biological molecules (antibodies, DNA) in aqueous media in which the excited state of Ru(bpy)₃²⁺ is generated via a coreactant scheme, as shown in eq 1, where TPrA^{*} represents the radical formed upon deprotonation of the cation radical of tri-*n*-propylamine. In nonaqueous media with a wider potential window, radical ion annihilation of the electrogenerated 3+ and 1+ forms produces the excited state, as shown in eq 2.



Currently, there are no methods describing the ECL detection of alkali metal ions or other species that do not participate in the redox reaction generating the excited state. Systems capable of sensing such ions would be useful in clinical assays that involve a panel of tests, for example, immunoassays, in which determi-

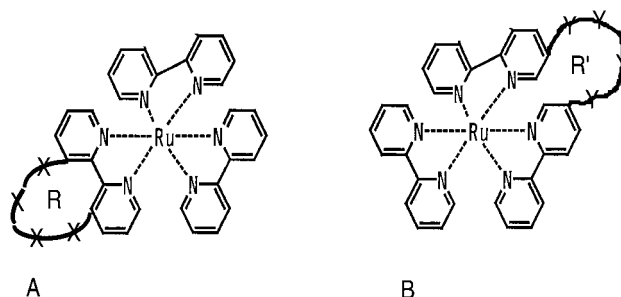


Figure 1. Structures of modified Ru(bpy)₃²⁺ species: A, substituent on one bpy ring at 3,3' positions; B, substituent across two bpy rings.

nation of electrolytes or metal ions might also be of interest. The strategy we propose here for the determination of such ions involves modification of the basic Ru(bpy)₃²⁺ structure (or the structure of another ECL active species) so that the emission characteristics change after interactions with a metal ion (or ligand) of interest. Consider the molecules shown schematically in Figure 1. This represents a Ru(bpy)₃²⁺ molecule in which a chain with recognition groups extends either within a single bipyridine (bpy) (A) or between two bpy's (B). Such modifications can cause steric or electronic effects that can decrease the luminescence efficiency of Ru(bpy)₃²⁺.^{3,4} For example, substitution of methyl groups on one of the bpy's in a position where steric interactions are important could cause a significant decrease in photoluminescence quantum efficiency.^{5,6}

We illustrate this approach with the ECL detection of an alkali metal ion via Ru(bpy)₂(CE-bpy)²⁺ (Figure 2) in both aqueous and nonaqueous environment; the structural changes and relevant ECL responses provide alkali metal ion recognition. Similar approaches for photoluminescence and electrochemical studies of alkali metal binding to CE-modified Ru(bpy)₃²⁺ have been reported previously.⁷ The reaction of interest is illustrated in Figure 2, in which it is

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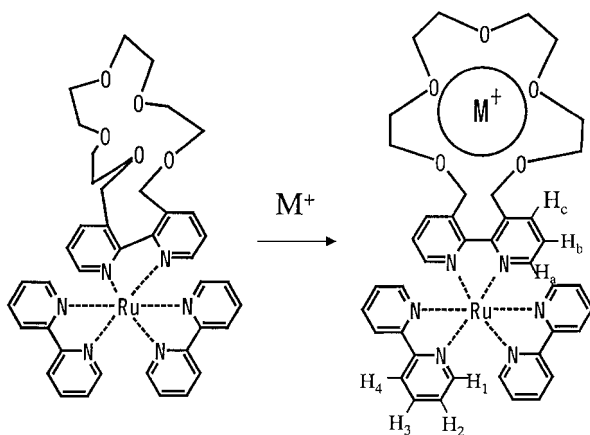


Figure 2. Scheme showing complexation of $\text{Ru}(\text{bpy})_2(\text{CE-bpy})^{2+}$ by metal ion, suggesting structural changes.

implied that the reaction of the crown ether (CE) moiety on one bpy with Na^+ causes a structural change that affects the luminescence efficiency of the $\text{Ru}(\text{bpy})_3^{2+}$. This general phenomenon demonstrates a new class of ECL sensors based on similar Ru(II) complexes in which the steric or electronic effects of bpy substituents can be changed by an interaction, such as the incorporation of guest ions, which is then accompanied by observable changes in the photophysical, spectroscopic, and ECL properties of the label. Muegge and Richter⁸ have recently reported an analogous strategy with a $\text{Ru}(\text{bpy})$ molecule modified with a macrocycle for ECL determination of Pb^{2+} , Cu^{2+} , or Hg^{2+} .

EXPERIMENTAL SECTION

$\text{Ru}(\text{bpy})_2(\text{CE-bpy})^{2+}$ was synthesized by procedures given previously.⁹ Tetra-*n*-butylammonium perchlorate (TBAP), tri-*n*-propylamine (TPrA), sodium perchlorate, tris(hydroxymethyl)aminomethane (Tris) and acetonitrile (MeCN) (anhydrous, spectrophotometric grade) were obtained from Aldrich (St. Louis, MO) and used as received. All nonaqueous experiments were performed using TBAP as the electrolyte and acetonitrile as the solvent. Aqueous experiments were performed in 0.1 M Tris buffer (pH 7.00).

Cyclic voltammograms were recorded on a CH Instruments (Austin, TX) Electrochemical Work Station. A platinum disk electrode (0.03 cm^2) was used as the working electrode, and a platinum wire was the auxiliary electrode. A saturated silver/silver chloride electrode was utilized as a reference electrode for all aqueous experiments. A silver wire was used as a quasireference electrode for the nonaqueous experiments. All potentials were calibrated versus an aqueous SCE by the addition of ferrocene as an internal standard taking $E^\circ(\text{Fc}/\text{Fc}^+) = 0.424 \text{ V vs SCE}$.¹⁰

All ECL measurements were performed as previously reported.¹¹ Measurements were obtained with solutions containing 0.3 mM $\text{Ru}(\text{bpy})_2(\text{CE-bpy})^{2+}$ and 30 mM TPrA in 0.1 M TBAP in MeCN for the nonaqueous system and 0.1 M Tris buffer (pH 7.0) for the aqueous system. To generate the reaction, the working electrode was pulsed between 0 V and the oxidation peak potential

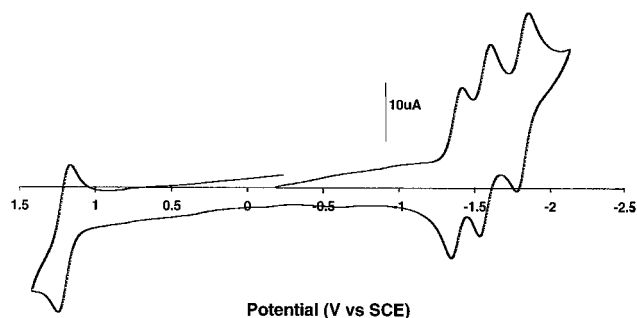


Figure 3. Cyclic voltammogram of a 1 mM solution of $\text{Ru}(\text{bpy})_2(\text{CE-bpy})^{2+}$ in MeCN at a platinum electrode (0.1 M TBAP; scan rate, 100 mV/s).

of $\text{Ru}(\text{bpy})_2(\text{CE-bpy})^{2+}$ with a pulse width of 0.1 s. The resulting emission spectra were obtained with a charge-coupled-device (CCD) camera (Photometrics CH260, Photometrics—Roper Scientific, Tucson, AZ) that was cooled to -100°C . Integration times were 2 min. The CCD camera and grating system were calibrated with a mercury lamp.

RESULTS AND DISCUSSION

Electrochemistry of $\text{Ru}(\text{bpy})_2(\text{CE-bpy})^{2+}$. Similarly to other ruthenium (II) tris(2,2'-bipyridine) compounds, $\text{Ru}(\text{bpy})_2(\text{CE-bpy})^{2+}$ fulfills the same electrochemical and stability criteria to show ECL. A typical cyclic voltammogram of $\text{Ru}(\text{bpy})_2(\text{CE-bpy})^{2+}$ in MeCN, which is characterized by a one-electron oxidation peak at 1.28 V (vs SCE) and three consecutive one-electron reduction peaks at -1.44 V , -1.63 V , and -1.87 V (vs SCE), is shown in Figure 3. The close correspondence of these potentials to those of $\text{Ru}(\text{bpy})_3^{2+}$ which is characterized by a one-electron oxidation at peak potential of 1.25 V (vs SCE) and three consecutive one-electron reductions at peak potentials of -1.47 V , -1.62 V , and -1.89 V (vs SCE), suggests that the energy levels of the Ru(II) center and the bpy's are not greatly perturbed by the substitution. Analysis of the voltammograms also indicates that the 3+ and 1+ forms are stable. Scan rate studies showed that the peak current ratio ($i_{\text{pa}}/i_{\text{pc}}$) of the oxidation peak and the first reduction peak were approximately unity down to a scan rate of 10 mV/s, indicating that the oxidation and the first reduction are nernstian. However, due to ohmic (iR) drop, the peak separation is slightly larger than the nernstian value. Additionally, the anodic and cathodic peak currents (i_{pa} , i_{pc1}) of the oxidation wave and the first reduction wave were proportional to the square root of scan rate ($v^{1/2}$), but the corresponding peak potentials (E_{pa} , E_{pc1}) did not vary with scan rate (v).

ECL of $\text{Ru}(\text{bpy})_2(\text{CE-bpy})^{2+}$. ECL could be generated via annihilation, which involves a cyclic double-step potential alternating between 1.28 V and -1.44 V (vs SCE), the potentials for the oxidation and first reduction of the complex. Alternatively, ECL could be generated by the use of a coreactant, such as TPrA. Oxidation of this species is known to generate a strong reducing agent that reacts with the $\text{Ru}(\text{bpy})_2(\text{CE-bpy})^{3+}$ to generate an excited state.² This is the only approach that can be used for aqueous solutions in which radical ion annihilation is not possible because of the limited potential window of aqueous media. In the results reported here, TPrA was utilized as the coreactant in all ECL measurements for better comparison between the aqueous and nonaqueous systems. Results obtained in experiments per-

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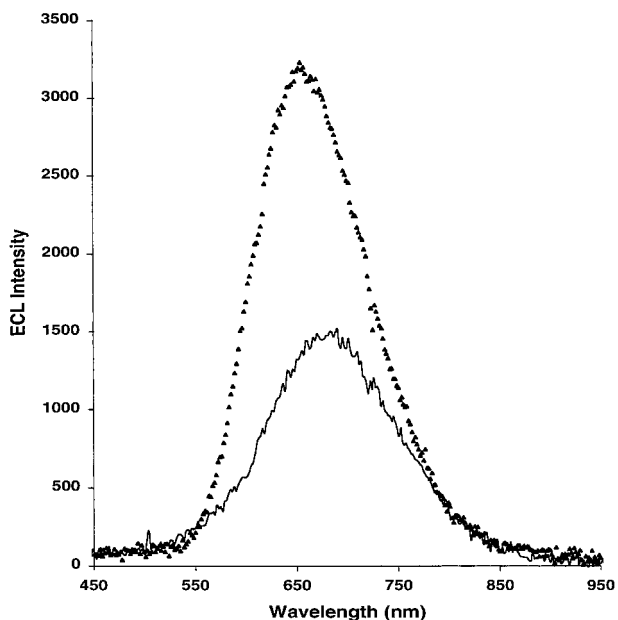


Figure 4. ECL spectra of 0.3 mM of $\text{Ru}(\text{bpy})_2(\text{CE-bpy})^{2+}$ (30 mM TPRA) in 0.1 M, pH 7.0 Tris buffer (\blacktriangle) and 0.1 M TBAP in MeCN ($-$), both in the absence of sodium and with pulsing (0.1 s) between 0 and 1.25 V (vs SCE).

formed in MeCN via radical ion annihilation showed that the ECL signal intensities obtained were comparable to those using TPRA as the coreactant.

Figure 4 shows the ECL spectra of $\text{Ru}(\text{bpy})_2(\text{CE-bpy})^{2+}$ in aqueous and nonaqueous media, both using TPRA as the co-reactant. Both spectra shown here were collected under the same conditions and in the absence of sodium ion. Previous work has shown that in the absence of a guest ion, the coordination structure would be distorted from an octahedral symmetry as a result of the steric hindrance between the disubstituted bipyridine; thus, very weak photoluminescence is observed at room temperature.^{14,15} As shown in Figure 4, the ECL signal intensity was much smaller in the MeCN solution than it was in the aqueous medium. This can be attributed to the solvation effects of water, which can hydrogen bond to the crown ether oxygens in the complex and change the structure. Recent research has shown that crown ethers and their derivatives are capable of intra- and intermolecular hydrogen bonding in the presence of guest ions in aqueous media.^{12,13} Thus, we speculate that the larger ECL signal intensity in the aqueous system is due to the presence of hydrogen bonding between crown ether and water. Hydrogen bonding could distort the structural conformation of $\text{Ru}(\text{bpy})_2(\text{CE-bpy})^{2+}$ from a highly sterically hindered structure to a less sterically hindered conformation, even in the absence of a guest ion. This structural change would alter the compound's emission properties and lead to higher ECL emission, as observed in the aqueous system.

Effect of Na^+ on the ECL of $\text{Ru}(\text{bpy})_2(\text{CE-bpy})^{2+}$. Structural changes in the crown ether upon ion recognition should decrease the steric repulsion within the disubstituted bipyridine and alter the compound's emission properties significantly.^{14,15} As a result,

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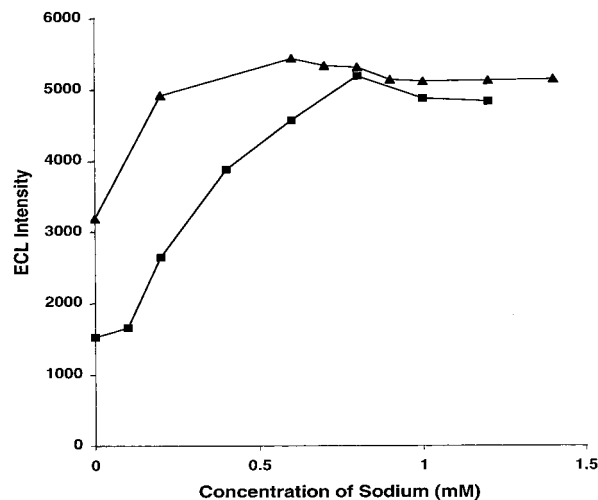


Figure 5. ECL signal intensity as a function of sodium concentration for solutions containing 0.3 mM $\text{Ru}(\text{bpy})_2(\text{CE-bpy})^{2+}$ and 30 mM TPRA in 0.1 M TBAP, MeCN (\blacksquare); and 0.1 M, pH 7.0 Tris buffer (\blacktriangle).

higher photoemission is observed in the presence of the guest ion, Na^+ , which makes this compound an interesting photochemical ion sensor.^{7c} Similar behavior is observed in ECL. As shown in Figure 5, the ECL signal intensity increases in both MeCN and aqueous media upon the addition of Na^+ , but control experiments showed that Na^+ does not affect the ECL efficiency of $\text{Ru}(\text{bpy})_3^{2+}$ without a crown ether moiety. As determined from the graphs, the point of saturation for either system was higher than 0.3 mM, which was the concentration of the ruthenium complex in the solution. This does not support a one-to-one ratio between the host and the Na^+ , which is often seen in systems involving crown ethers and alkali metals. However, it is difficult to determine the saturation point from the graph for either of the systems, because the signal intensity is dependent on the time the measurement is made after addition of the Na^+ (as opposed to the time-independence of the ECL in the absence of Na^+). ECL measurements in MeCN taken as a function of time showed a gradual increase in signal intensity from the time sodium was added to at least 90 min after the addition. As a result of this time-dependency, the graphs can only be taken to show a concentration dependence; they cannot be used to establish stoichiometry or an equilibrium constant for either system. The sluggish kinetics of this reaction are surprising and perhaps suggest that structural changes in the crown ether upon ion recognition are very slow. Although this specific compound seems to have kinetic problems that hinder it from being a practical ion sensor, this study demonstrates the basic principle that a similar or modified $\text{Ru}(\text{bpy})_2(\text{CE-bpy})$ complex could provide a new approach to an ECL-based ion detector. Selectivity of ions must also be investigated. More detailed investigations of these reactions and modifications of this type of compound are contemplated.

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