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Laboratories and Demonstrations

An Instrumental Analysis Laboratory Using Electrogenerated Chemiluminescence

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An undergraduate instrumental analysis laboratory exercise is presented for the characterization of light emission generated using electrochemiluminescence (ECL). ECL involves the electrochemical generation of excited states and as such is a sensitive probe of electrochemical, electron-transfer and energy-transfer processes at electrified interfaces. An objective of this experiment is to have students develop an understanding of the mechanisms and factors affecting ECL. Also, this exercise gives students experience in coupling two powerful analytical techniques: electrochemistry and spectroscopy. With the recent development of ECL technology for use in clinical diagnostics applications, this exercise also facilitates discussions on the importance of basic research and the practical aspects of taking a technology from the bench top to commercial reality.

One objective of this experiment is to develop an understanding of the mechanisms and factors affecting ECL.

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Introduction

While there are a large number of electrochemical and spectroscopic laboratory experiments to complement undergraduate instrumentation courses, there are few experiments utilizing a combination of these techniques; therefore, we have developed an experiment involving the electrochemical generation of excited states (electrogenerated chemiluminescence; electrochemiluminescence or ECL). Furthermore, the experiment revolves around the commercially important $Ru(bpy)_3^{2+}$/TPrA (bpy = 2,2′-bipyridine; TPrA = tri-*n*-propylamine) reaction sequence.

Traditionally, ECL was generated via annihilation, where the electron transfer reaction responsible for excited state formation is between an oxidized and a reduced species, both of which are generated at an electrode by alternate pulsing of the electrode potential [1, 2].

$$A + e^- \rightarrow A^- \qquad \text{(reduction at electrode)} \qquad (1)$$

$$D - e^- \rightarrow D^+ \qquad \text{(oxidation at electrode)} \qquad (2)$$

$$A^- + D^+ \rightarrow A^* + D \qquad \text{(excited state formation)} \qquad (3)$$

$$A^* \rightarrow A + h\nu \qquad \text{(light emission)} \qquad (4)$$

For example, the potential of the working electrode is quickly changed between two different values in order to generate the reduced, A^-, and oxidized, D^+, species (equations 1 and 2, respectively) that will react near the electrode surface to form the emissive state, A^* (equation 3). These types of reactions generally involve the use of rigorously purified and deoxygenated nonaqueous solvents (e.g., dimethylformamide and acetonitrile) because the available potential range in water is too narrow to generate the required energetic precursors. Many ECL reactions of this type have been investigated and their mechanisms are well understood [2, 3].

ECL can also be generated in a single potential step utilizing a coreactant (i.e., a species capable of forming energetic oxidants or reductants upon bond cleavage) [4]. For example, in the previously studied $Ru(bpy)_3^{2+}$/TPrA system, ECL is produced upon concomitant oxidation of the two reactants:

$$Ru(bpy)_3^{2+} - e^- \rightarrow Ru(bpy)_3^{3+} \qquad \text{(oxidation at electrode)} \qquad (5)$$

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$$\mathrm{TPrA} - e^- \rightarrow \left[\mathrm{TprA}^{\bullet +}\right] \rightarrow \mathrm{TPrA}^{\bullet} + \mathrm{H}^+ \qquad \text{(oxidation at electrode)} \quad (6)$$

$$\mathrm{Ru(bpy)_3}^{3+} + \mathrm{TprA}^{\bullet} \rightarrow^{*} \mathrm{Ru(bpy)_3}^{2+} + \text{products} \qquad \text{(excited state formation)} \quad (7)$$

$$\mathrm{Ru(bpy)_3}^{2+} \rightarrow^{*} \mathrm{Ru(bpy)_3}^{2+} + h\nu(\sim 2.0\,\mathrm{eV}, 610\,\mathrm{nm}) \qquad \text{(light emission)} \quad (8)$$

The ECL mechanism and subsequent signal generation in this system is believed to occur through an "oxidative–reductive" pathway. This involves the production of a strong reductant (presumably TPrA•) by an initial oxidation sequence [4]. Electrochemical studies of various aliphatic amines have indicated a possible reaction pathway for the oxidation of TprA [5]. Upon oxidation, the short lived TPrA radical cation (TPrA•$^+$) is believed to lose a proton from an α-carbon to form the strongly reducing intermediate TPrA• (equation 6). This radical can then reduce $Ru(bpy)_3^{3+}$ to $^*Ru(bpy)_3^{2+}$ (equation 7) [4, 6]. Other reaction mechanisms for the production of the excited state have also been proposed [4, 7]. For example, reduction of $Ru(bpy)_3^{2+}$ to $Ru(bpy)_3^{1+}$ by TPrA•, followed by annihilation:

$$\mathrm{Ru(bpy)_3}^{1+} + \mathrm{Ru(bpy)_3}^{3+} \rightarrow^{*} \mathrm{Ru(bpy)_3}^{2+} + \mathrm{Ru(bpy)_3}^{2+} \qquad \text{(excited state formation)} \quad (9)$$

This methodology and analogous pathways involving the generation of strongly oxidizing intermediates have allowed the generation of ECL in aqueous solution, a great advantage in terms of analytical applications and an essential component to this exercise.

ECL has found use in studying the properties of both organic and inorganic systems [8]. These include polyaromatic hydrocarbons [9], polymer assemblies [10], transition metal complexes incorporating such metals as Ru, Os and Pt [8, 11, 12], as well as rare earth systems [13]. The properties studied include characterizing the nature of the emitting states, discerning the mechanisms by which these states are formed, and determining the efficiency of excited state formation. There has also been recent interest in using ECL reactions as the basis for highly sensitive and selective analysis. In such schemes the ECL luminophore (e.g., $Ru(bpy)_3^{2+}$) is used as a label on the molecule of interest (e.g., DNA or an antibody) in the same way that photoluminescent (fluorescent) or radioactive labels are employed [14, 15]. ECL has the advantage over radioactive labels in not producing radioactive waste. When compared to fluorescent

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methods, those involving ECL do not require an excitation source and therefore are immune to interferences from luminescent impurities and scattered light making them more sensitive and selective. ECL appears very promising and is being commercially developed and marketed for use in the clinical analysis of biomolecules [14, 15].

The purposes of this laboratory exercise are to introduce students to ECL and to familiarize them with cyclic voltammetry and photon-counting detection. This experiment involves the characterization of light emission from the $Ru(bpy)_3^{2+}$/TPrA system as a function of potential and pH and is suitable for upper division instrumental and inorganic laboratory courses. Students generally work in groups of 2–3 and can successfully prepare solutions and perform ECL experiments in one three-hour laboratory session.

Experimental

Materials

Potassium phosphate monobasic (Fisher), $Ru(bpy)_3Cl_2$ (Strem Chemicals), and tri-*n*-propylamine (Avocado) are commercially available and were used as received. Triply distilled water was used throughout. An aqueous buffer stock solution of 0.2 F potassium phosphate monobasic (phosphate) and 0.05 F TPrA was prepared. It was necessary to stir the solution vigorously for several minutes to quantitatively dissolve the amine. This buffer solution was used in the preparation of all subsequent solutions. 0.5-mL of a 0.1 mM $Ru(bpy)_3Cl_2$ stock solution (prepared by dissolving 0.7 mg of $Ru(bpy)_3Cl_2$ in 10 mL of buffer) was pipetted into a 500-mL volumetric flask and diluted to the mark with the phosphate/TPrA buffer solution. The resulting concentrations of this working solution were 0.1 μM $Ru(bpy)_3^{2+}$, 0.2 F phosphate and 0.05 F TPrA. The pH of the solution was adjusted with 6 M NaOH or 6 M HCl. (CAUTION: $Ru(bpy)_3Cl_2$ is a heavy metal complex and Pr_3N is a highly flammable toxic irritant; therefore, proper care should be exercised in their use and Pr_3N should only be handled in a fume hood.)

Electrochemistry and ECL

ECL intensity versus potential profiles were monitored using a photomultiplier tube (Hamamatsu HC 135) in conjunction with a CH Instruments electrochemical analyzer. This arrangement resulted in both cyclic voltammograms (from the electrochemical analyzer) and intensity versus time data that were then combined using a spreadsheet

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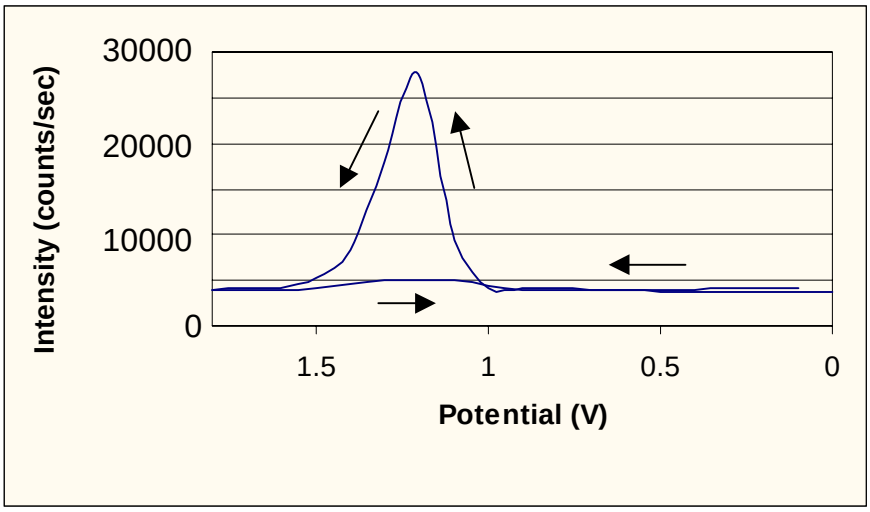

FIGURE 1. ECL INTENSITY VERSUS POTENTIAL PLOT FOR 0.1μM $Ru(bpy)_3^{2+}$ AND 0.05 M TPrA IN 0.2 M Kphos BUFFER AT pH = 7.5. SCAN RATE 100 mV s^{-1}. ARROWS INDICATE DIRECTION OF FORWARD AND REVERSE SCANS.

program to produce the intensity versus potential profiles (i.e., Figure 1). A more detailed schematic of the experimental setup, as well as recommendations for alternative setups, is provided in the supplementary material.

The experiments employed a conventional three-electrode configuration. An electrochemical cell was constructed of a 100-mL beaker and a machined Teflon cover (Bioanalytical Systems Inc.). The cover contained openings for three electrodes. Enough solution (~50 mL) was placed in the cell to immerse a platinum-mesh working electrode (5-mm diameter), a platinum wire counter electrode, and a saturated Ag/AgCl reference electrode (E^o = 0.199 V vs NHE). The working electrode was cleaned prior to and after each run by immersion in concentrated nitric acid, followed by rinsing with doubly deionized water. The cell was positioned so that the working electrode was facing the photomultiplier tube. The scan rate was 100 mV s^{-1} with a scan range from 0 to +1.8 V vs Ag/AgCl with an initial potential of 0 mV. Initial and final potentials were chosen to be at least 100 mV past the peak anodic and cathodic potentials.

Due to the catalytic nature of platinum for oxidizing water at potentials that obscure processes of interest (>+1.0 V) the cyclic voltammograms in Figure 2 were generated with a glassy carbon electrode. A Pt electrode was used in the ECL experiments

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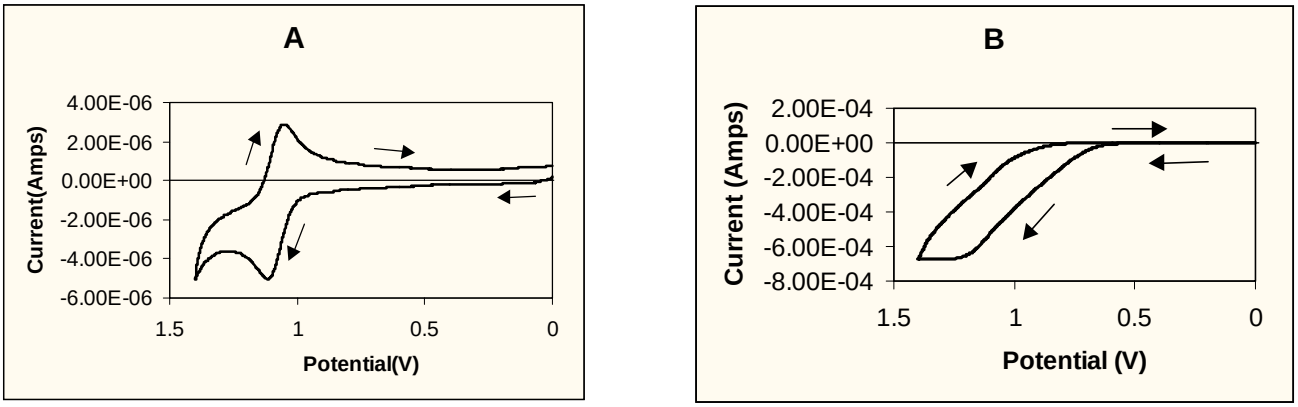

FIGURE 2. CYCLIC VOLTAMMOGRAMS OF (a) 1 mM $Ru(bpy)_3^{2+}$ IN 0.2 M Kphos AND (b) 0.1 mM $Ru(bpy)_3^{2+}$ AND 0.05M TPrA IN 0.2M Kphos BUFFER. SCAN RATE 100 mV s^{-1}. ARROWS INDICATE DIRECTION OF SCANS.

because it gives higher ECL intensities than either gold or carbon. The reasons for the increased ECL signal with Pt are still being investigated.

Results and Discussion

A useful experiment for understanding the basic chemistry of ECL is illustrated in Figure 1 where light intensity from the $Ru(bpy)_3^{2+}$/TprA system in aqueous buffered solution is plotted versus electrode potential. TPrA has an irreversible oxidation at ~+0.6 V vs Ag/AgCl while $Ru(bpy)_3^{2+}$ has a reversible couple at +1.4 V vs Ag/AgCl; therefore, light results at or near the electrode only when a sufficiently positive potential has been reached such that both $Ru(bpy)_3^{2+}$ and the coreactant TPrA are oxidized [4].

To understand the shape of the intensity versus potential plot it is helpful to look at the corresponding cyclic voltammograms (Figure 2). The voltammogram of $Ru(bpy)_3^{2+}$ in Figure 2a is an excellent example of a well-behaved, electrochemically reversible system. Cyclic voltammetry involves the linear application of a potential to a working electrode immersed in a still solution. Detailed discussions of the theory of cyclic voltammetry and the shape of resultant voltammograms can be found in the literature [16]. Briefly, the shape of the cyclic voltammogram results from the activity of the electroactive species at the surface of the electrode. For example, if the potential is scanned in the positive direction, anodic current flows when it becomes sufficiently positive to cause oxidation of the electroactive species. However, instead of leveling

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off at the maximum of the anodic wave, the current decreases as the potential is increased further. This is due to depletion of the electroactive species around the surface of the electrode. In other words, a diffusion layer develops, separating the electrode from the unoxidized species still in solution such that the current that is observed is due to the diffusion of the unoxidized species to the surface of the electrode. At a certain point the potential is switched to scan in the negative direction. When the potential becomes sufficiently negative to reduce the oxidized species at the surface of the electrode a cathodic current begins to flow. When the concentration of the electroactive species is once again depleted around the surface of the electrode, another maximum is reached at which point the cathodic current also decays as the last of the oxidant becomes reduced.

Addition of 0.05 M TPrA results in the voltammogram of Figure 2b and is defined by the irreversible nature of the coreactant (which is present in much higher concentrations than $Ru(bpy)_3^{2+}$); therefore, the shape of the intensity versus potential plot in Figure 1 can be understood as an extension of the above argument. Light emission is observed at potentials corresponding to oxidation of both the coreactant and the light-emitting molecule. A peak maximum is observed with the subsequent decrease being attributed to a depletion of electroactive (and ECL light generating) species near the electrode surface. Other factors may also contribute to loss of ECL at higher potentials, including passivation of the electrode by electrochemically generated products and the formation of dissolved oxygen in aqueous systems. The exact nature of these latter processes, however, is still an area of active investigation.

An ECL intensity versus solution pH profile is shown in Figure 3. Several factors appear to influence the sensitivity of ECL to solution pH. A crucial step in the proposed mechanism is the deprotonation of the $TPrA^{\bullet+}$ radical to form the strong reducing agent (TPrA•) [4]; therefore, the decrease in ECL at lower pHs can been attributed to protonation of the tertiary amine with a subsequent loss in TPrA activity.

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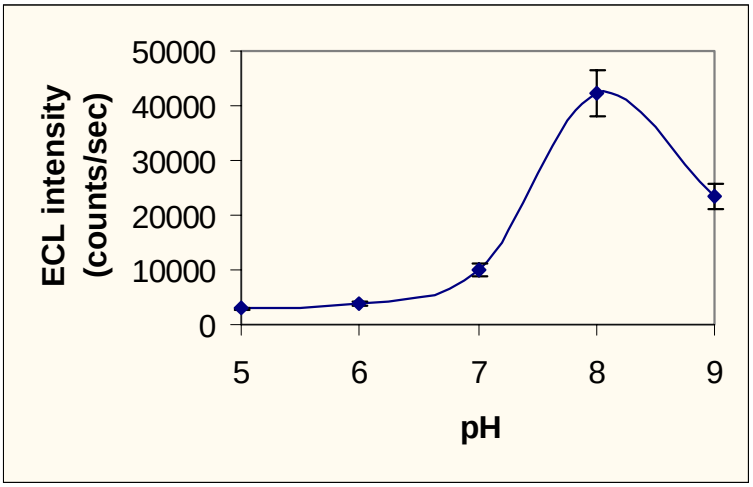

FIGURE 3. ECL INTENSITY VERSUS PH PLOT FOR 0.1 μM $Ru(bpy)_3^{2+}$ AND 0.05 M TPrA IN 0.2 M PHOSPHATE BUFFER. EACH POINT REPRESENTS THE AVERAGE OF AT LEAST THREE REPLICATE DETERMINATIONS.

Higher pHs (>9) result in the formation of an opaque solution (presumably due to interaction of the amine with NaOH). Despite these limitations, TprA is a unique coreactant in that it produces highly intense ECL at physiological pH (pH ~ 7–7.5), the pH range that is most useful for practical (e.g., clinical and environmental) analyses. Sub-picomolar detection limits have been reported for solution-phase ECL using Pr_3N as the coreactant [4]. This is one reason that ECL has been moved from the bench top to the commercial marketplace. When the ECL luminophore is bound to a magnetic particle (the particle is then captured on the surface of the electrode prior to electrochemical stimulation) detection limits as low as 10^{-18} M are attainable [14]. Therefore, the actual detection limits that one can obtain will depend on many factors, including instrumentation, solution conditions, and electrode materials. With the apparatus described in this paper and using Pt as the working electrode, we have been able to detect 10^{-11} M $Ru(bpy)_3^{2+}$ in aqueous media using Pr_3N.

The ECL emission with respect to [$Ru(bpy)_3^{2+}$] is linear over several orders of magnitude [4]. Figure 4 shows this trend in the sub- to mid-μM ranges. This can be used as the basis for a quantitative measurement of an unknown $Ru(bpy)_3^{2+}$ sample. We have yet to incorporate this into the laboratory, as it would require an additional 3-hour laboratory period.

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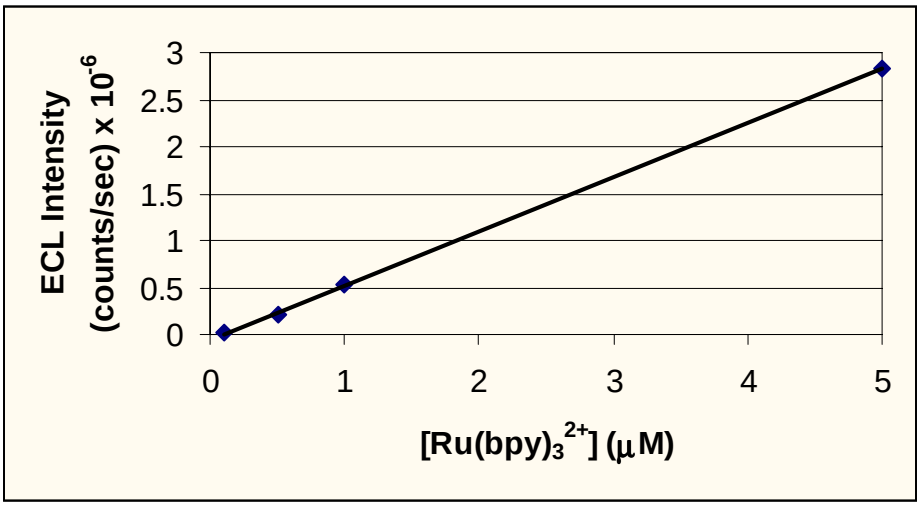

FIGURE 4. ECL INTENSITY AS A FUNCTION OF $[Ru(bpy)_3^{2+}]$ FROM 0.1–5 μM. 0.05 F Pr_3N IN 0.2 F POTASSIUM PHOSPHATE BUFFER. ERROR BARS HAVE BEEN OMITTED FOR CLARITY (ERROR OF EACH MEASUREMENT IS ~10%). CORRELATION COEFFICIENT (R^2) IS 0.9997.

Summary

The undergraduate laboratory exercise described above involves the characterization of electrogenerated chemiluminescence emission (ECL) as a function of potential and pH. One objective of this experiment is to develop an understanding of the mechanisms and factors affecting ECL. Another is to give students hands-on experience in coupling electrochemistry and spectroscopy.

Since the first detailed studies in the 1960s, over 500 papers, patents, and book chapters have appeared on ECL, ranging from the fundamental to the very applied. With the recent interest in using ECL reactions as the basis for highly sensitive and selective analysis, the prediction made by Faulkner and Glass in 1981 that “Continued research in this area will probably stress the development of ECL as a probe rather than as an end in itself” [3] appears to be coming to fruition. Unfortunately, such developments don’t happen overnight (this one took ~ 30 years) and without laying the solid foundations of the underlying science. We occasionally need to be reminded of this fact in a day and age when the push for ‘practical’ applications seems to take precedence over basic research.

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