Chemistry 681: Student Seminar Series

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Analytical Applications of Electrogenerated Chemiluminescence

I. Introduction

The study of analytical chemistry is largely based upon the necessity to transduce chemical reactions to measurable and quantifiable signals. The controlled emission of light as a result of a chemical reaction offers one such an analytical detection technique. Electrogenerated chemiluminescence, or electrochemiluminescence (ECL), is in effect a potential-controlled chemiluminescent (CL) reaction, which maintains all of the benefits of selectivity and sensitivity from traditional CL while incorporating the ability to control the location and generation of the signal. It should also be noted that while most CL reactions occur as an irreversible redox process in the bulk solution, in ECL the luminescent species is often regenerated at the electrode surface and recycled to produce additional photons in a process known as "amplification".

Chemiluminescence and ECL have been widely used for probing bulk solution-phase reactions however, several researchers have further utilized the spatial control of ECL to investigate reactions which occur at the liquid/liquid and liquid/solid interfacial junctions.^{1,2} In addition to functioning as a location-specific transducer, the ability to dynamically control the generation of the excited state species with ECL is an advantage in comparison to conventional CL for applications such as display devices and potential laser sources.^{3,4}

The relaxation from the excited state via either fluorescent or phosphorescent emission of a photon occurs as a spontaneous process in both ECL and CL. However, it is the actual excitation step which distinguish the two techniques and it is the control of this excitation which gives rise to the breadth of applications for ECL.

II. ECL mechanisms

There are two mechanisms for the electrogeneration of the excited state ECL products, organic ion-annihilation and inorganic coreactant-mediated. The annihilation process is based upon the alternating electrochemical oxidation *and* reduction of a compound at the electrode to

produce both the radical-cation and radical-anion species (Equations 1-2).⁵ In the coreactant mechanism, the electrode is poised to either oxidize *or* reduce the luminescent molecule (emitter) and its associated coreactant. In both cases this completes the electrochemical interaction and the products diffuse away from the electrode surface to react in solution yielding the excited state product and ultimately photon emission.

Annihilation ECL

The necessity of producing both the radical-cation and radical-anion at the electrode imposes the requirement that either an A.C. potential is applied or that multiple electrodes are utilized, this can also be accomplished with a rotating ring disk electrode.⁶ There are two routes available for the excitation step to achieve sufficient energy to result in photon emission. If the electron transfer reaction shown in equation 3 contains a redox potential which is sufficiently large to populate the singlet (S₁) state the process is termed "energy-sufficient ECL" if it is not sufficient yet an accessible triplet state exists then it is termed "energy-deficient ECL". The relaxation mechanism from the excited singlet state to the ground state via emission of a photon is known as the "S-route" (Equation 4).⁵ Although it is illustrated with only a single reactant, this process is also feasible with two different reactants (Equation 5-6).⁵ 9,10-Diphenyl anthracene is an example of a common annihilation ECL compound.

$$\mathbf{R} + \mathbf{e}^{-} \rightarrow \mathbf{R}^{-} \tag{1}$$

$$\mathbf{R} - \mathbf{e}^{-} \rightarrow \mathbf{R}^{+} \tag{2}$$

$$\mathbf{R}^{*+} + \mathbf{R}^{*-} \rightarrow {}^{1}\mathbf{R}^{*} + \mathbf{R}$$
(3)

$${}^{1}R^{*} \rightarrow R + h\nu \tag{4}$$

For the energy-deficient case where an accessible triplet state of either reactant is available (Equation 7)⁵, it is possible that a triplet-triplet annihilation will occur with the production of an excited state singlet (Equation 8).⁵ This exited singlet formation pathway is known as the "T-route" and it is from this singlet that photon emission is observed. Figure 1,⁷ illustrates the two routes.

$$A + e^{-} \rightarrow A^{-} \tag{5}$$

$$B - e^{-} \rightarrow B^{+} \tag{6}$$

$$B^{\bullet+} + A^{\bullet-} \xrightarrow{3} A^* + B \tag{7}$$

$${}^{3}A^{*} + {}^{3}A^{*} \rightarrow {}^{1}A^{*} + A \tag{8}$$



Figure 1. Energy level diagram for the DPA-TMPD ECL system.

The annihilation techniques of ECL were first investigated in the 1960's however, they have not been adopted into current analytical applications or commercial systems because of their reliance upon the use of aprotic solvents which must be stringently deaerated to reduce quenching of the CL reaction routes. However, the emergence of the field of

coreactant ECL in the early 1970's with the utilization of $\text{Ru}(\text{bpy})_3^{2+}$ salts in aqueous buffer solutions has allowed the application of ECL to enter such domains as biological and pharmacological assays.⁸

Coreactant ECL

Unlike the annihilation methods, coreactant ECL does not need to simultaneously produce the oxidation and reduction products of the luminescent species. A single working electrode can be poised at a potential adequate to oxidize both the emitter (Ru(bpy)_3^{2+}) and the coreactant (NADH) molecules as is shown in Figure2.⁹ The oxidized coreactant then functions as a reductant to the emitter producing the excited state product. One resulting benefit of this simplified approach is that coreactant ECL is capable of producing a continuous emission of photons since the electrode is not alternating between reduction and oxidation of the reactants.



Figure 2. Proposed ECL Reaction of NADH and $Ru(bpy)_3^{2+}$.

Figure 3. Schematic of a simple Electrochemical ECL flow cell.

This reaction has been developed into commercial bioassay instruments by IGEN International Inc.⁹⁻¹² Figure 2 also illustrates the previously mentioned regeneration of the luminescent species following the relaxation step. This allows the ruthenium complex to be reexcited to further generate additional photons in a process known as "amplification" which increases the sensitivity of the method and reduces the consumption of an expensive reagent. While figure 2 depicts the process occurring in quiescent solution, the use of a flow cell (Figure 3)⁵ is of greater analytical utility and is the heart of automated commercial assay instruments. The flow cell design employs a standard three-electrode electrochemical system with the incorporation of a photodetector (PMT, CCD or photodiode) to measure the resulting photon emission. The emitter and coreactant can be both present in the analyte stream entering the flow cell, resulting in photon emission as they are oxidized at the electrode, or the emitter may be immobilized on the surface of the electrode resulting in emission only when the coreactant is present in the stream.

The coreactant-mediated ECL emission can be utilized as a detector for the presence of the coreactant or as a detector for the emitter. A common approach is to covalently label a molecule of interest with an emitter in a flowing stream of excess coreactant. If this approach is employed in a separations-type experiment, the ECL emission will be observed only when the labeled analyte passes through the flow cell. Alternatively, if an upstream reaction product can be used as a coreactant, then the emitter can be immobilized on the electrode surface electrochemically poised to be excited as the coreactant passes through the flow cell.¹¹ These approaches offer very low background signal since no photons are generated unless both species are present and since the excitation step is a selective reaction.

III. Analytical ECL Applications

The uses of ECL in analytical applications can be broadly defined into two distinct categories, sensors and sources. Applications where the resulting photo-emission is primarily utilized as a transducer of chemical information shall be considered sensors, in the cases where the primary goal *is* the generated illumination the process will be termed a source.

Sensors

IGEN International Inc. was the first developer of commercial ECL assay instruments, intended to function as a direct replacement for labor-intensive ELISA techniques. Because of the on-going need for non-radioactive replacements for existing bio-pharmaceutical analysis

IGEN developed a commercial instrument to utilize coreactant ECL techniques suitable for flow injection analysis (FIA). Due to the primary interest in biologically relevant liquids and pH, annihilation ECL has not been adopted by commercial developers. IGEN has incorporated the use of magnetic beads into their design along with an electromagnet present behind their working electrode. Functioning as a rapid-screening technique the analyte of interest is first labeled with the $Ru(bpy)_3^{2+}$ species and is then captured onto a para-magnetic bead. The beads are carried into the flow cell where they are magnetically collected by the magnet positioned behind the electrode. The coreactant is present in large excess within the solution, when the oxidizing potential is applied a signal intensity results which is directly related to the quantity of analyte present in the aliquot. With the magnetic field removed the beads can be washed out of the cell preparing the cell for the next assay.^{5,13} Utilizing the same principles from the IGEN system Perkin-Elmer has developed an instrument (QPCR Sytem5000) for post-PCR (polymerase chain reaction) product quantitation (Figure 4).¹⁴ In one particular experiment, three PCR products (110bp, 441bp & 1829bp) amplified from a human ß-globin target gene were analyzed using ECL (Figure 5)¹⁴ with reported detection limits ranging from aprox. 10 amol to 200 amol. This variance in detection limit was attributed to a decreased bead-binding efficiency due to the longer DNA fragments.



ECL measurements of the gas-phase hydrazine, have been investigated by the NRL using $Ru(bpy)_3^{2+}$ excitation.¹⁵ A gold-coated cellulose membrane is adapted for use as a working electrode for the oxidation of the ruthenium complex. Using a sampling pump, analyte-containing air is passed across the surface of the cellulose membrane. As the gas-phase analytes reduce the $Ru(bpy)_3^{3+}$ species photon emission occurs which is then detected by the integrated

PMT. Detection of hydrazine via this ECL technique are shown in the 10ppb range with an estimated detection limit of less than 1ppb. This instrumental approach should prove generally applicable to gas-phase analytes which are sufficient reductants of the oxidized ruthenium.

While the majority of research utilizing ECL can be classified as a FIA technique, Preston and Nieman have developed a sensor similar in approach to the immersion of a pH electrode.¹⁶ Their sensor device incorporates a stirring mechanism to facilitate diffusion of the radical species and a fiber-optic bundle to channel the emitted photons out of the solution to the attached PMT detector. They investigated two separate coreactant mechanisms (luminol and $Ru(bpy)_3^{2+}$) using their immersion probe. The key benefit of this system is portability due to the omission of the flow system. They reported a detection limit of 1μ M for H₂O₂ using 100μ Mluminol emitter, which they found to compare favorably with traditional flow system equipped methods.

One of the earliest researchers in the field of ECL, Bard, A. J., has continued to find new applications for this analytical method. Bard and co-workers have combined scanning electrochemical microscopy (SECM) with ECL to demonstrate the possibility of using the ECL photon emissions as a source for optical imaging of surfaces.¹⁷ Figure 6¹⁷ is an illustration of the experimental design. An SECM ultramicroelectrode was rastered across a substrate consisting of alternating insulating and conducting materials. SECM-controlled ECL was shown to be generated by both annihilation and coreactant methods when the electrode was positioned over the conducting material. Figure 7, ¹⁷ is the raw data image collected from the PMT detector as the



Figure 6. Schematic of SECM/ECL System.

Figure 7. Raw SECM/ECL image of substrate.

SECM tip was rastered across the substrate containing an interdigitated array of conducting and insulating materials. The optical resolution achieved in this preliminary experiment was on the micrometer scale however, it is hoped that the combination of SECM/ECL will provide a method to investigate the kinetics of the coreactant ECL route which has not been possible by other electrochemical methods.

Sources

In addition to using the photon emission properties of ECL as a signal of reaction dynamics, there is an expanding interest in the materials science field for all forms of luminescent materials which can be implemented as possible display devices. Ideal materials will have the characteristics of being light-weight, thin films which consume minimal energy. One system which has been investigated by Rubner and co-workers by manufacturing multilayer films containing alternating layers of polymerized Ru(bpy)₃²⁺ and various polyanions.¹⁸ These multilayer films were formed upon a patterned ITO substrate and then an aluminum electrode was thermally evaporated on top of the thin film. Not surprisingly, it was discovered that the different polyanions, utilized as coreactants in this scheme, yielded varying efficiencies in terms of photon emission. Ultimately, the researchers were able to develop a device which was capable of yielding 3.2% (photons/electron) with a maximum output of 1350nW with lifetimes of approximately 50h. This is in contrast to values upto 24% (photons/electron) which have been previously reported for liquid ECL.¹⁹



Figure 8. Diagram of ECL Laser

An extreme example of ECL emission has been reported by Hatakenaka and co-workers. They observed actual lasing phenomenon from an ECL-derived 9,10diphenylantrhracene annihilation experiment.⁴ Figure 8⁴ is a diagram of the device they developed which utilizes mirrored and half-mirrored Pt-coated quartz substrates to establish the necessary standing wave in order to achieve lasing. It is interesting to note that due to the small distance between the anode and cathode the researchers were able to generate ECL without the use of a supporting electrolyte which otherwise may have excessively quenched the reaction. Despite the proximity of the two electrodes and the efforts of the researchers to continuously replace the solution content of the chamber, the signal intensity was observed to drop off as a function of time as the process became mass transport limited. Efforts to control this signal decay were investigated by tailoring the applied potential and the flow rate. Despite these limitations, the researchers were able to achieve lasing of the DPA molecules resulting in emission of coherent blue light with a wavelength of 420nm.

IV. Conclusion

The wide variety of applications for ECL which were presented here are indicative of a research field which is still growing and developing after almost 40 years from its inception. It has been the intention to illustrate a very diverse and unrelated series of recent applications developed which make use of the unique transducer characteristics of ECL. By altering the implementation of a relatively simple redox reaction, researchers have been able to probe analyte molecules present in gas, liquid and solid media. Possible applications as luminescent display devices using thin-films of emitter molecules are of great interest as well.^{3,18} As an analytical approach to investigating localized chemical species and for high-throughput assay analysis, ECL sensors will continue to be a powerful tool because of their spatial resolution and inherently low signal background.

V. References

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