Electrochemical Light, From Laboratory Curiosity to Useful Analytical Technique

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Abstract: Electrochemiluminescence (ECL) is the process where species generated at electrodes undergo electron-transfer reactions to form excited states that emit light. Application of a voltage to an electrode in the presence of an ECL luminophore such as $Ru(bpy)_3^{2+}$ (where bpy = 2,2′-bipyridine) or diphenylanthracene results in light emission and allows detection of the emitter at very low concentrations ($\leq 10^{-12}$ mol dm^{-3}). By employing ECL-active species as labels on biological molecules, ECL has found commercial application for immunoassays and DNA analyses. The history of ECL is presented including the earliest, curiosity driven experiments and the development of ECL into an analytical technique for clinical diagnostic applications. The development and use of ECL sensors is an excellent example of how, over time, a laboratory curiosity can become a useful, powerful, and commercially viable technique.

Background

A wide variety of methods exist for the detection of chemical and biological analytes of interest. One of the most versatile, and one being commercially developed for the clinical diagnostic market [1] is electrogenerated chemiluminescence (also called electrochemiluminescence and abbreviated ECL). It is a means of converting electrical energy into radiative energy. ECL involves the production of reactive intermediates from stable precursors at the surface of an electrode. These intermediates then react under a variety of conditions to form excited states that emit light [2–5]. Furthermore, it is an excellent example of how a mere curiosity can, *over time*, become a useful, powerful and commercially viable technique.

It is important to distinguish ECL from chemiluminescence (CL). Both involve the production of light by species that can undergo highly energetic electron-transfer reactions; however, luminescence in CL is initiated by the mixing of reagents and controlled by the careful manipulation of fluid flow. In ECL, luminescence is initiated and controlled by switching an electrode voltage. Traditionally, ECL was generated via annihilation, where an electron-transfer reaction is between an oxidized and reduced species, both of which are generated at an electrode by alternate pulsing of the electrode potential (i.e., a double potential step) [4].

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

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

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

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

where $h\nu$ is a photon of light. For example, the potential of the working electrode is quickly changed between two different values in order to generate the reduced, $A^{\bullet -}$, and oxidized, $D^{\bullet +}$, species (eqs 1 and 2, respectively) that will react near the electrode surface to form the emissive state, A^* (eq 3). A typical reaction involves 9,10-diphenylanthracene (DPA, Figure 1). ECL is generated when a double potential step is applied to an electrode (typically platinum), producing the radical cation ($DPA^{\bullet +}$) upon anodic oxidation and the radical anion ($DPA^{\bullet -}$) upon cathodic reduction. The resulting electrogenerated products can then react and undergo annihilation (i.e., eq 3) to produce an excited state (DPA^*) that is then able to emit light.

$$DPA - e^- \rightarrow DPA^{\bullet +} \qquad (5)$$

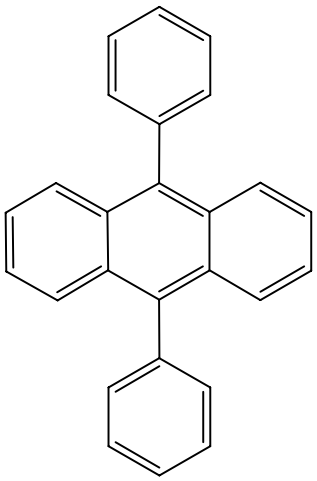
$$DPA + e^- \rightarrow DPA^{\bullet -} \qquad (6)$$

$$DPA^{\bullet +} + DPA^{\bullet -} \rightarrow DPA + DPA^* \qquad (7)$$

$$DPA^* \rightarrow DPA + h\nu \qquad (8)$$

For DPA the emission maximum (λ_{max}) occurs at about 512 nm, and the ECL spectrum (i.e., a plot of ECL emission versus wavelength) is identical to DPA photoluminescence. This indicates that the ultimate product of charge transfer, and hence luminescence, is the lowest singlet DPA species, $^1DPA^*$. There is considerable evidence that the reactions outlined in eqs 7 and 8 are oversimplifications, and that several mechanistic steps intervene between the electron transfer and photon emission steps (e.g., triplet–triplet annihilation). The identification of these mechanisms has been thoroughly reviewed elsewhere [3, 4].

The ECL system(s) described above use a single electrode to produce the electrogenerated species. It is also possible to use dual electrode systems to generate light emission. For example, a rotating-ring disk electrode (RRDE) can be employed where one reactant, such as $A^{\bullet -}$, is generated at the central disk and the other, such as $D^{\bullet +}$, is generated at the ring [6]. The reactants are then swept together by diffusion and convection.



9,10-diphenylanthracene

Figure 1. Diphenylanthracene.

Other dual electrode systems that use interdigitated electrodes have also been reported [7].

Although analytical uses for ECL are possible with annihilation systems (e.g., display devices), most require the use of rigorously purified and deoxygenated nonaqueous solvents because the available potential range in water is too narrow to generate the required energetic precursors. In essence, the stability range for the electrochemical oxidation and reduction of water is too small to conveniently generate both species (i.e., the radical anion and cation); therefore, and of more interest to practical applications, ECL can also be generated in a single potential step utilizing a coreactant [8–11]. A coreactant is a species that, upon oxidation or reduction, produces an intermediate that can react with an ECL luminophore to produce excited states. Usually, this occurs upon bond cleavage of the coreactant to form strong oxidants or reducants. For example, the oxalate ion ($C_2O_4^{2-}$) is believed to produce the strong reductant, $CO_2^{\bullet-}$, upon oxidation in aqueous solution [8]

$$C_2O_4^{2-} - e^- \rightarrow [C_2O_4^{\bullet-}] \rightarrow CO_2^{\bullet-} + CO_2 \qquad (9)$$

The oxidizing potential that leads to $CO_2^{\bullet-}$ may also oxidize an ECL luminophore (e.g., A).

$$A - e^- \rightarrow A^{\bullet+} \qquad (10)$$

$A^{\bullet+}$ and $CO_2^{\bullet-}$ may then react to produce an excited state capable of emitting light.

$$CO_2^{\bullet-} + A^{\bullet+} \rightarrow A^* + CO_2 \qquad (11)$$

$$A^* \rightarrow A + h\nu \qquad (12)$$

Oxalate is often referred to as an "oxidative–reductive" coreactant due to its ability to form a strong reducing agent upon electrochemical oxidation. Unlike annihilation schemes where a double potential step (e.g., oxidation followed by reduction) is required to generate the highly energetic precursors, in coreactant ECL the electrode typically only oxidizes *or* reduces the reagents in a *single* potential step. For example, in the oxalate system the electrode oxidizes both the oxalate and the ECL reactant A; the reducant, $CO_2^{\bullet-}$, is then generated upon bond cleavage of oxalate via eq 9. This strategy is used in most analytical and biotechnology applications, with the reactant A being $Ru(bpy)_3^{2+}$ (bpy = 2,2′-bipyridine). This methodology has allowed the generation of ECL in aqueous solution, a great advantage in terms of analytical applications. Without this ability, it is doubtful whether or not ECL would have moved beyond the laboratory phase.

Another example of an "oxidative–reductive" system is the commercially important $Ru(bpy)_3^{2+}$/TPrA system (TPrA = tri-*n*-propylamine). As with the oxalate system, this involves the production of a strong reductant (presumably $TPrA^{\bullet}$) by an initial oxidation sequence [10–12]

$$TPrA - e^- \rightarrow [TPrA^{\bullet}]^+ \rightarrow TPrA^{\bullet} + H^+ \qquad (13)$$

$$Ru(bpy)_3^{2+} - e^- \rightarrow Ru(bpy)_3^{3+} \qquad (14)$$

$$Ru(bpy)_3^{3+} + TPrA^{\bullet} \rightarrow Ru(bpy)_3^{2+*} + \text{products} \qquad (15)$$

$$Ru(bpy)_3^{2+*} \rightarrow Ru(bpy)_3^{2+} + h\nu \qquad (16)$$

ECL is produced upon concomitant oxidation of $Ru(bpy)_3^{2+}$ and TPrA (Figure 2). Electrochemical studies of various aliphatic amines have indicated a possible reaction pathway for the oxidation of TprA [8]. Upon oxidation, the short-lived TPrA radical cation ($TPrA^{\bullet+}$) is believed to lose a proton from an α-carbon to form the strongly reducing intermediate $TPrA^{\bullet}$. This radical can then reduce $Ru(bpy)_3^{3+}$ to $Ru(bpy)_3^{2+*}$.

Although the details of the coreactant ECL mechanism (eqs 13–16) to generate light emission are still under study [14], the origin of the light emission from $Ru(bpy)_3^{2+}$ has been well documented [3, 10, 12]. Because the photoluminescent and ECL spectra are nearly identical, the emission process in ECL involves the metal-to-ligand charge transfer (MLCT) state of $Ru(bpy)_3^{2+}$. This state may be formed if the reducing agent (i.e., $TPrA^{\bullet}$) transfers an electron to the π^* orbital of one of the bipyridine ligands. $Ru(bpy)_3^{2+*}$ can then decay to the ground state, producing the same luminescence as obtained from photoluminescence spectroscopy. Solution-phase coreactant ECL using TPrA and $Ru(bpy)_3^{2+}$ is quite sensitive with subpicomolar detection limits achieved [10, 15].

From Inauspicious Beginnings

The first detailed studies on ECL were begun in the mid-1960s but interest in light emitted during electrolysis was generated much earlier. In 1927 Dufford and co-workers [16] observed emission at an anode by applying between 500 and 1500 V to a cathode in a solution of Grignard compounds in anhydrous ether (The paper does not record how many explosions may have occurred!). The reaction conditions in these experiments were not very well defined and it is doubtful whether this process was actually ECL. At such high potentials, electroluminescence (the direct injection and removal of charge with the formation of an electron-hole pair), or electrode processes were probably responsible for the observed light emission; however, this initial report was followed two years later when Harvey published experiments on luminol (2,3-aminophthalhydrazide) in aqueous/alkaline

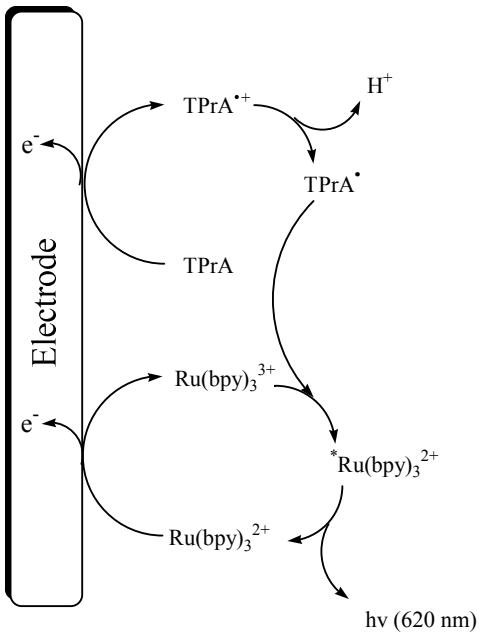


Figure 2. $Ru(bpy)_3^{2+}$–TPrA oxidative-reductive ECL sequence.

solution [17]. The potentials used to generate anodic light emission were much lower than those used by Dufford and coworkers [16] (2.8 V versus 500 to 1500 V, respectively). Several groups followed up on this [18], and in fact the luminol system continues to generate interest [19].

In the mid-1960s several research groups decided to study in detail the luminescence generated during electrolysis of polyaromatic hydrocarbons (e.g., anthracene, diphenylanthracene, rubrene (Figure 1)) in aprotic media (e.g., dimethylformamide) [20]. In essence, they wished to see if excited states could be generated electrochemically as well as photochemically. It was observed, both visually and spectroscopically, that the radiation emitted by sweeping to both negative and positive potentials (annihilation pathway, eqns 1–4) was identical to that generated during photoluminescence, indicating formation of excited states [21].

Throughout the sixties and early seventies, work continued on the polyaromatic hydrocarbons and was eventually extended to other systems, most notable among them the ruthenium chelates. Since the discovery by Paris and Brandt [22] that $Ru(bpy)_3^{2+}$ is photoluminescent, a large body of literature has appeared aimed at understanding both the ground and excited state properties of $Ru(bpy)_3^{2+}$, $Os(bpy)_3^{2+}$ and their polyazine derivatives [23, 24]; therefore, it is not surprising that these compounds have also played an important role in the development of ECL. The first report of ECL in a metal chelate was by Tokel and Bard [25] in 1972, in which the excited state of $Ru(bpy)_3^{2+}$ was generated in aprotic media by annihilation of the reduced, $Ru(bpy)_3^{1+}$, and oxidized, $Ru(bpy)_3^{3+}$, species (analogous to eqs 1–4, above).

The original coreactant, and thus the first report of ECL in aqueous solution, was oxalate ion ($C_2O_4^{2-}$; eqs 9–12) [8]. Subsequently, other species were shown to act as coreactants, among them peroxydisulfate ($S_2O_8^{2-}$) and tri-*n*-propylamine (TPrA; equations 13–16). The discovery of TprA [10] allowed efficient ECL not only in aqueous media, but also at physiological pH. Following the first report on TPrA, other species containing amine groups were proposed, among them many biologically important analytes (e.g., alkylamines, NADH, antibiotics, L,D-tryptophan, glucose, erthromycin, valine, HIV-gag gene). The list is quite extensive, and compilations up to 1998 have been published [11, 26].

To date, ECL has found use in studying the properties of both organic and inorganic systems [3, 11]. These include polyaromatic hydrocarbons [3, 4, 27], exciplexes [28], polymer assemblies [29], transition-metal complexes incorporating such metals as Ru, Os and Pt [2, 11, 25, 30, 31], as well as rare earth chelates [32] to name a few. $Ru(bpy)_3^{2+}$ is perhaps the most thoroughly studied ECL active molecule [33] and, as with other ECL systems, there was particular emphasis on characterizing the nature of the excited state, discerning the mechanisms by which these states were formed, and determining the efficiency of excited state formation. Various techniques were used and are still being used, including detailed electrochemical studies, spectroscopic and spin-resonance measurements as well as magnetic field effects [3–5, 11].

In the 1980s a method was developed [34] for the binding of $Ru(bpy)_3^{2+}$ to biological molecules of interest (e.g., antibodies, proteins, nucleic acids). The interest in using $Ru(bpy)_3^{2+}$ stems from its rather unique properties. Namely, it emits and is soluble at room temperature in aqueous solution and undergoes reversible one-electron transfer reactions at easily attainable potentials (≤ +1.5 V vs Ag/AgCl). Also, the ligands provide synthetic versatility. For example, by attaching *N*-hydroxysuccinimide (NHS) ester to one of the bipyridine ligands (Figure 3), the ECL label can bind to substances containing free amino groups [34]. The amino acid will attack the carboxylate ester, leading to displacement of *N*-hydroxysuccinimide. IGEN International, Inc. (Gaithersburg, Maryland, USA) began developing ECL for use in biosensor analyses in the early 1980s.

Applications of ECL

Coreactant ECL has been used in a wide range of analytical applications [11, 35]; however, recent comprehensive reviews of the ECL literature have been published so only a few representative examples will be discussed here [5, 36, 37]. Because ECL emission intensity is usually proportional to the concentration of the emitter [38] or coreactant [10], ECL can be used in the analysis of various species. For example, ECL from $Ru(bpy)_3^{2+}$ has been used to measure the concentrations of coreactants such as oxalate and peroxydisulfate to levels as low as 10^{-13} mol dm^{-3}) [39]. In fact, the ability of $Ru(bpy)_3^{2+}$ to undergo "oxidative-reductive" ECL in the presence of coreactants has led to the selective determination of oxalate in synthetic urine samples [40], and $Ru(bpz)_3^{2+}$ (where bpz = bipyrazine) has been used for the determination of peroxydisulfate with nanomolar (nM) detection limits [41]. Because the intensity of ECL is a function of both the coreactant and the emitter, ECL can be used to analyze for both. In these examples, ECL was measured in the presence of high, pre-determined concentrations of ECL emitters. These types of experiments can then be used as a means to assay for compounds that act as coreactants including a variety of amines [11, 35, 42]. ECL assays for amines find many applications because amine groups are prevalent in numerous biologically and pharmacologically important compounds including alkyl-amines, antibiotics, antihistamines, opiates, nicotinamide, and the reduced form of NADH (i.e., adenine dinucleotide) [5, 35]. In general, these compounds contain no

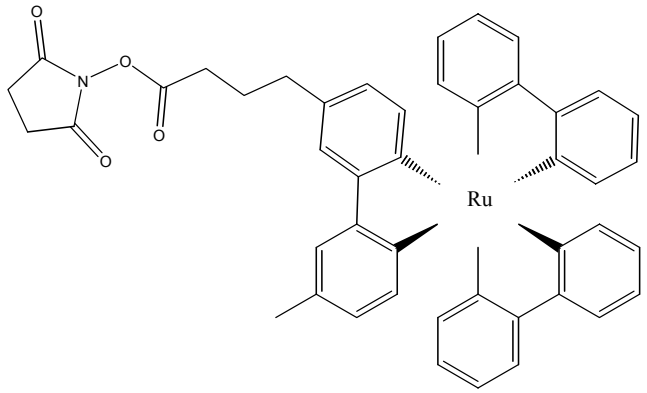


Figure 3. $Ru(bpy)_3^{2+}$–NHS ester for ECL labeling.

chromophore and therefore cannot undergo luminescence unless an ECL-active compound is present.

The most common and, arguably, the most important commercial application to date for ECL is its use in clinical diagnostic assays. In these applications, ECL emitters are typically used as labels in affinity binding assays that attach the ECL emitter to the analyte of interest [15]. The label is physically linked to one of the binding partners in the assay and provides the means for detecting the coupling of the binding partner to the analyte. Several classes of binding partners are used including antibody/antigen, enzyme/inhibitor, carbohydrate/lectin, and nucleic acid/complementary nucleic acid [43]. Commercial instruments are available for ECL assays of antibodies, antigens, and DNA [15, 44]. Assays that have been developed for these systems include alpha-fetoprotein, digoxin, thyrotopin, protein and steroidal hormones, cytokines, and various antibodies, to name a few.

Advantages and Limitations of ECL

As with other measurements based on the emission of light (e.g., photoluminescence, chemiluminescence) ECL labels have distinct advantages over detection methods such as radioactivity. For example, they are sensitive, nonhazardous, inexpensive, diagnostic of the presence of a particular label, linear over a wide range and incorporate simple and relatively inexpensive equipment. When compared to such light emission techniques as photoluminescence (PL) and chemiluminescence (CL), ECL also displays certain desirable qualities. In PL [45], excited state formation occurs upon absorption of electromagnetic radiation:

$$R + h\nu \rightarrow {}^{*}R \quad \text{(excited state formation)} \qquad (17)$$

The versatility of this technique lies in the number of species able to luminesce, the quantum efficiency of emission, and the ability to incorporate these molecules into a wide variety of formats. Unfortunately, this versatility also leads to limitations. For example, in clinical situations, typical biological fluids containing analyte may also contain a large number of potential luminophores. In ECL, for a complex to emit it must meet several stringent criteria, including stable redox chemistry and the ability to undergo energetic electron or energy transfer. Of course, this advantage of ECL is also a potential limitation, in that the number of efficient ECL labels is diminished. CL involves the generation of excited states due to an energetic chemical reaction. In a typical CL [46] reaction, reagents are pumped separately to the reaction site. In ECL, on the other hand, production of reagents occurs electrochemically in-situ from passive precursors, allowing localization of the emission near the electrode. This may result in enhanced sensitivity since the optics used for light detection can be focused on a relatively small area. Furthermore, amplification is possible in ECL due to the turnover of reactants at or near the electrode surface. However, as with any electrochemical process, stringent cleaning of the electrodes is required prior to and after each run to insure reproducibility.

Conclusions

This report has centered on the background and history of ECL, and its development into a biomedical research and clinical diagnostic tool; however, ECL is a versatile detection methodology and is being developed as a sensor and probe for other applications. For example, as a detector in normal and reverse-phase high-performance liquid chromatography [47], in food industry applications [48], in metal-ion coordination complex chemistry with nucleic acids [49], in environmental applications such as detecting aromatic hydrocarbon pollutants [50], and even in military applications [48, 51], to name a few.

With the interest in using ECL reactions as the basis for highly sensitive and selective analysis, the prediction made by Faulkner and Glass that "Continued research in this area will probably stress the development of ECL as a probe rather than as an end in itself" [52] appears to be coming to fruition. Unfortunately, such developments don't happen overnight (this one took 25 to 30 years from the first publication on ECL in 1964 to the introduction of a commercial instrument for biological assays) and without laying solid foundations of the underlying science, something that often appears to be forgotten in this day and age when the push for "practical" applications seems to be at the forefront. Since the first detailed studies hundreds of papers, patents, and book chapters have appeared on ECL, ranging from the very applied, to the curiosity driven, to the need to understand the underlying science. One wonders what the next 30 years hold for ECL, and science in general, and how many other laboratory *curiosities* will become useful analytical techniques! [53]

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