

Chapter 2

Generation Pathways of Electrogenerated Chemiluminescence

Abstract ECL continues to be an area of active research. This chapter provides a brief way for understanding fundamentals of ECL. An overview of selected key ECL mechanisms for the production of ECL is given. Studies on finding new ECL co-reactants, disclosing the relationship between ECL efficiencies and structure of co-reactants, and improving ECL efficiencies are also discussed.

Keywords Annihilation • Pathway • Electron-transfer reaction • Gibbs free energy • Energy sufficient reaction • Reductive–oxidation co-reactant • Oxidative–reduction co-reactant • Hot-electron ECL

Electrogenerated chemiluminescence (ECL) can be generated by two principal routes, namely the annihilation and co-reactant pathways. In each case, two species are generated electrochemically, and those two species undergo an electron-transfer reaction to produce an emissive species [1].

2.1 Annihilation ECL Pathway

The early ECL studies originated with ion annihilation ECL [2]. Proceeding by annihilation pathway, ECL can be generated on reaction between oxidized and reduced species produced either at a single electrode by using an alternating potential or at two separate electrodes. In later case, one electrode is set at a reductive potential and the other at an oxidative potential and they are placed in close proximity to each other [3]. Most of the annihilation ECL processes have been investigated in organic solvents or partially organic solutions because annihilation reactions are very energetic and aqueous solutions usually have too narrow potential range to allow convenient electrolytic generation of both the oxidized and reduced ECL precursors [4]. Such reactions performed in organic solutions require a supporting electrolyte such as tetrabutylammonium salt and the absence of dissolved oxygen as this oxygen may sometimes react with radical intermediates and quenches the reaction.

A system having radical cations and anions from different molecules may also exhibit annihilation processes. Gibbs free energy for annihilation process can be calculated from the redox potentials using Eq. (2.1).

$$\Delta G = -nF(E_{\text{reduction}}^{\circ} - E_{\text{oxidation}}^{\circ}) \quad (2.1)$$

where ΔG is Gibbs free energy, F is Faraday's constant, while $E_{\text{reduction}}^{\circ} - E_{\text{oxidation}}^{\circ}$ are the reduction and oxidation potentials, respectively. As enthalpy is directly related to Gibbs free energy, enthalpy can be calculated based on Eq. (2.2).

$$\Delta G = \Delta H - T\Delta S \quad (2.2)$$

Enthalpy plays a significant role in describing which path to be followed, i.e.,

1. The enthalpy exceeds the energy required to produce the lowest excited states from the ground state. In this case, the reaction is to follow the singlet route "S-route" and $1R^*$ will be directly generated. The reaction is recognized as "energy sufficient," and DPA system is the notorious example of this case.
2. The enthalpy is lower than the energy required to produce the lowest excited state, still exceeding the triplet state energy, $3R^*$, which in turn produces $1R^*$ by subsequent annihilation of $3R^*$ (triplet-triplet annihilation, TTA). ECL of ruthenium tris-bipyridyl-type derivatives falls in this category. Additionally, ion annihilation can also follow "E-route," ensuing the formation of excimers (excited dimers) and exciplexes (excited complexes).

The key advantage of the annihilation process is its simplistic approach as it requires only the ECL species, solvent, and supporting electrolyte in order to generate light [5]. The mechanism of annihilation method is generalized as shown in Scheme 2.1, where A is a polyaromatic hydrocarbon (PAH), B is either the same or another PAH or an aromatic derivative, $1A^*$ is the singlet excited state, and $3A^*$ is the triplet excited state [6].

Let us have a common example of the above annihilation method. First of all, Bard's group [7] in 1972 published an article related to the ECL of $\text{Ru}(\text{bpy})_3^{2+}$

Scheme 2.1 Mechanism of annihilation method [6]

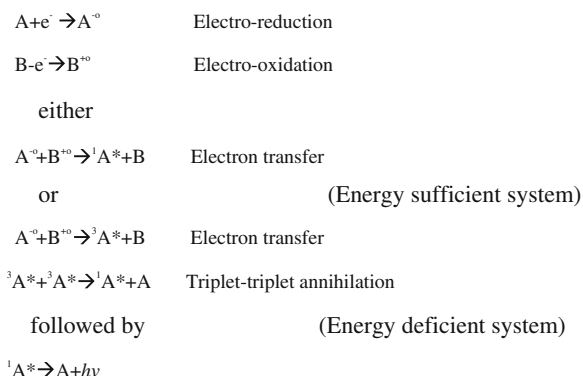
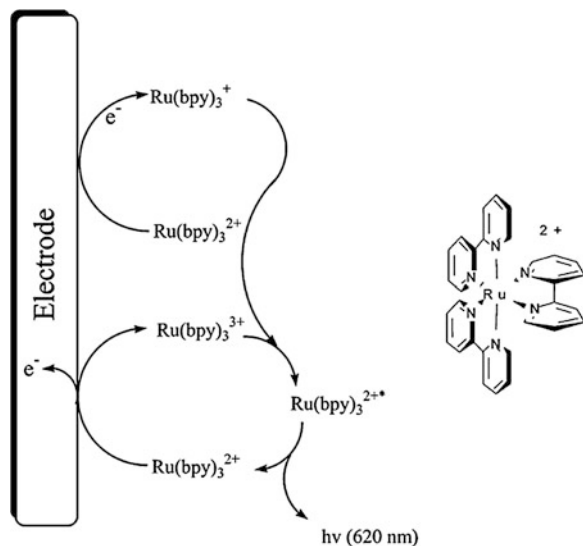
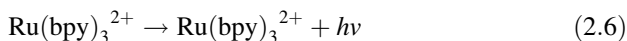
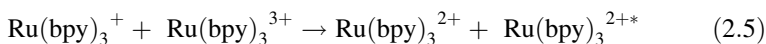
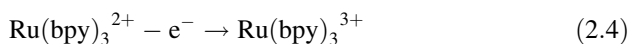
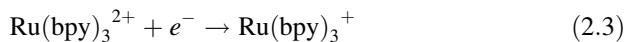


Fig. 2.1 Structure of $\text{Ru}(\text{bpy})_3^{2+}$ and proposed mechanism for $\text{Ru}(\text{bpy})_3^{3+}/\text{Ru}(\text{bpy})_3^+$ ECL system (Reprinted with permission from Ref. [34]. Copyright 2004 American Chemical Society)



(Fig. 2.1). They generated ECL by alternatively pulsing the electrode potential to form oxidative $\text{Ru}(\text{bpy})_3^{3+}$ and reductive $\text{Ru}(\text{bpy})_3^+$ in acetonitrile (Eqs. 2.3–2.6) [8].



As $\text{Ru}(\text{bpy})_3^+$ is exceptionally unstable in aqueous solutions, so it was found that annihilation ECL cannot be observed in aqueous solution by changing the potential successively to generate $\text{Ru}(\text{bpy})_3^{3+}$ and $\text{Ru}(\text{bpy})_3^+$. However, annihilation ECL of $\text{Ru}(\text{bpy})_3^{2+}$ can more likely be attained in aqueous solution employing a carbon-interdigitated microelectrode array of 2 μm width and spacing [9]. These electrodes are kept so close that ECL can be obviously seen with the unaided eye in normal room lighting. The experimental concentration is kept greater than 1 mM in a generation/collection biasing mode. Later, this system was applied by Michel and group for analysis of protein [10].

2.2 Co-reactant ECL Pathway

Currently, overwhelming majority of commercially available ECL analytical instruments is based on co-reactant ECL technology [2]. The reason for its becoming popular and easy availability lies in the key advantage of the co-reactant approach which includes its assistance in ECL generation in aqueous solution, opening a gateway to a wide range of assays for molecules of diagnostic or biological significance. Moreover, co-reactant ECL requires one-directional potential scanning at an electrode in a solution having luminophore species and a reagent (co-reactant), whereas electrolytic generation of both the oxidized and reduced ECL precursors is required in ion annihilation ECL [4, 11]. In ion annihilation ECL, all starting species can be regenerated after light emission, whereas in a co-reactant ECL system, only luminophore species can be regenerated, whereas the co-reactant is consumed via electrochemical–chemical reactions. In situ-generated co-reactant intermediates, as indicated by their standard redox potentials, are either strong reducing agents (in oxidative–reduction ECL) or strong oxidizing agents (in reductive–oxidation ECL) [2]. Noteworthy, co-reactant is consumed during the chemical (or electrochemical) reactions, while only the luminophore species can be regenerated at the electrode. Suitable co-reactants can be easily oxidized or reduced, which undergo a rapid chemical reaction to form an intermediate species with sufficient oxidizing or reducing power to create the excited state of the luminophore [5]. Therefore, to select a good ECL co-reactant, many factors should be kept in mind [12], including solubility, stability, electrochemical properties, kinetics, quenching effect, ECL background. Of these, electrochemical properties of the co-reactant are foremost important factor. The co-reactant should be available for easy oxidation or reduction with the luminophore species at or near the electrode and undergo a rapid chemical reaction to form an intermediate possessing sufficient reducing or oxidizing energy to react with the oxidized or reduced luminophore to form the excited state.

Four processes are usually involved in co-reactant ECL systems (as presented in Table 2.1) such as (a) redox reactions at electrode, (b) homogeneous chemical reactions, (c) excited-state species formation, and (d) light emission. Two types of redox reactions, namely heterogeneous and homogeneous redox reactions of co-reactants, are possible, which depend on the redox potential of the co-reactant and nature of the working electrode [2].

Both, the luminophore and the co-reactant, in co-reactant pathway, can be first oxidized or reduced at the electrode to form radicals by considering the polarity of the applied potential. The intermediates thus formed from the co-reactant then decompose to produce a strong reducing or oxidizing species that reacts with the oxidized or reduced luminophore to produce the excited states that emit light. The term “oxidative–reduction” ECL is usually used when highly reducing intermediate species are generated after an electrochemical oxidation of a co-reactant, and “reductive–oxidation” ECL is the phenomenon where highly oxidizing intermediates are produced after an electrochemical reduction [5].

Table 2.1 General mechanisms of co-reactant ECL systems^a

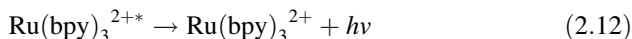
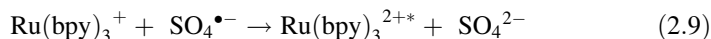
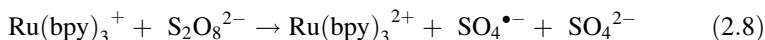
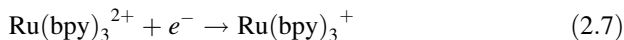
| Reaction process | Oxidative-reduction ECL | Reductive-oxidation ECL |
|---------------------------------|---|---|
| Redox reactions at electrode | $R - e \rightarrow R^{\bullet+}$ $C - e \rightarrow C^{\bullet+}$ | $R + e \rightarrow R^{\bullet-}$ $C + e \rightarrow C^{\bullet-}$ |
| Homogeneous chemical reactions | $R^{\bullet+} + C \rightarrow R + C^{\bullet+}$ $C^{\bullet+} \rightarrow C^{\neq}_{Red}$ $C^{\neq}_{Red} + R \rightarrow R^{\bullet-} + P$ | $R^{\bullet-} + C \rightarrow R + C^{\bullet-}$ $C^{\bullet-} \rightarrow C^{\neq}_{Ox}$ $C^{\neq}_{Ox} + R \rightarrow R^{\bullet+} + P$ |
| Excited-state species formation | $R^{\bullet+} + R^{\bullet-} \rightarrow R + R^* \text{ or } R^{\bullet+} + C^{\neq}_{Red} \rightarrow R^* + P$ | $R^{\bullet+} + R^{\bullet-} \rightarrow R + R^* \text{ or } R^{\bullet-} + C^{\neq}_{Ox} \rightarrow R^* + P$ |
| Light emission | $R^* \rightarrow R + h\nu$ | $R^* \rightarrow R + h\nu$ |

^a R luminophore, C co-reactant, C^{\neq} co-reactant intermediate with subscript "Red" for reducing agent and "Ox" for oxidizing agent, P product associated with C^{\neq} reactions

2.2.1 “Reductive–Oxidation” Co-reactants

2.2.1.1 Peroxydisulfate (Persulfate, $\text{S}_2\text{O}_8^{2-}$) System

ECL in the “reductive–oxidation” systems is usually produced by applying a very negative potential. Consequently, it seems quite difficult to study stable ECL in these systems in aqueous solutions because of serious hydrogen evolution upon applying very negative potentials [13]. Persulfate produces ECL on reaction with $\text{Ru}(\text{bpy})_3^+$ in DMF or acetonitrile– H_2O mixed solution (Eqs. 2.7–2.12) [10, 14]. It inhibits ECL emission in both solutions, if present in high concentrations. In former solution, inhibition resulted due to side reactions rather from quenching reaction in DMF, while quenching reaction occurred in the acetonitrile– H_2O mixed solution. The ECL emission was not observed in purely aqueous solutions due to instability of $\text{Ru}(\text{bpy})_3^+$ or due to the rapid quenching by persulfate in aqueous solution which may be the result of serious hydrogen evolution upon applying very negative potentials.



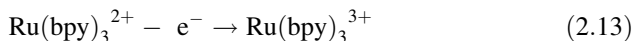
In 1984, Bard first of all reported $\text{Ru}(\text{bpy})_3^{2+}$ -labeled ECL [14] with a linear range of 10^{-13} to 10^{-7} M in acetonitrile– H_2O mixed solution containing persulfate. Composition of electrode has profound effect on ECL intensity, as reaction occurs in the proximity of electrode. Quenching by persulfate can be slow down by using carbon paste electrodes as they are made of carbon materials and organic solvents and can improve the stability of electrogenerated $\text{Ru}(\text{bpy})_3^+$ by supplying organic phase at the electrode surface. To overcome the problem of serious hydrogen evolution upon applying very negative potentials, enhanced ECL emission in purely aqueous solution has been observed by using carbon paste electrode. Storage time of carbon paste electrode sometimes takes part in enhancement of ECL intensity. Amazingly, the ECL emission from $\text{Ru}(\text{bpy})_3^{2+}$ and persulfate is greater at freshly prepared carbon paste electrode, whereas the ECL emission intensity from $\text{Ru}(\text{phen})_3^{2+}$ and persulfate was not affected by the storage time [15]. Xu’s group while studying ECL emission in aqueous solution by using bismuth electrode realized that the quenching effect of high concentration persulfate was weak at bismuth electrode. Owing to their high hydrogen

overpotential, bismuth electrodes are expected to be powerful electrodes for cathodic ECL studies in aqueous solutions [16]. Moreover, the ECL emission of persulfate can also be observed in aqueous solutions by using easily reducible metal complexes $\text{Ru}(\text{bpy})_3^{2+}$ or $\text{Cr}(\text{bpy})_3^{3+}$ [17].

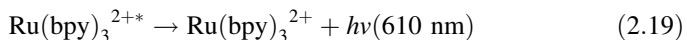
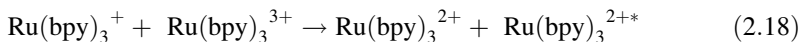
2.2.2 “Oxidative–Reduction” Co-reactants

2.2.2.1 Oxalate System

Bard and co-workers in 1977 observed ECL emission from oxalate in acetonitrile [8]. Later, they observed it in aqueous solutions following mechanism as shown in Eqs. (2.13)–(2.19) for the detection of oxalate and $\text{Ru}(\text{bpy})_3^{2+}$ [10, 14]. In the following years, a fiber-optic-based ECL sensor for oxalate detection was constructed [18], and the reaction mechanism of the oxalate system was further investigated and applied to study the electron transfer at liquid/liquid interface [19]. Simultaneous electrochemical and ECL detection of oxalate was reported in 2000 by Forster et al. [20].



or



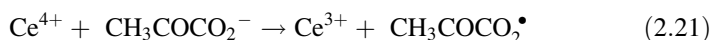
A clear ECL response was reported from the $[\text{Ru}(\text{bpy})_2\text{dcb}]^{2+}$ SAMs deposited on optically transparent fluorine-doped tin oxide (FTO) electrodes in the presence of oxalate and amino acids as co-reactants (dcb = 4,4-dicarboxy-2,2-bipyridine) [21]. Few years back, Forster and co-workers studied the use of metallopolymers as a novel ECL platform using oxalate, TPA, and other small molecules as co-reactants [22]. $\text{Ru}(\text{II})$ diimine complexes having phosphonic acid substituents adsorbed to TiO_2 -modified ITO electrodes can undergo co-reactant ECL with $\text{C}_2\text{O}_4^{2-}$ [23]. In order to improve the performance of existing systems and designing new ECL systems, a series of ruthenium bipyridine complexes and oxalate as a co-reactant were used to study the structure–activity relationships.

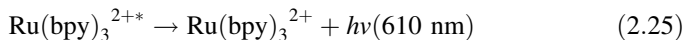
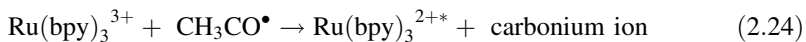
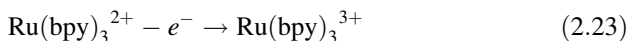
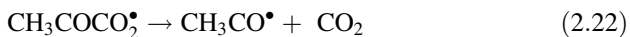
ECL generated by oxidation of $\text{Ru}(\text{bpy})_3^{2+}$, $\text{Ru}(\text{phen})_3^{2+}$, $\text{Ru}(\text{bpy})_2(\text{dmbp})^{2+}$, $\text{Ru}(\text{dmphen})_3^{2+}$, or $\text{Ru}(\text{dmbp})_3^{2+}$ (dmbp) 4,4'-dimethyl-2,2'-bipyridine (dmphen), 4,7-dimethyl-1,10-phenanthroline, and oxalate in aqueous solution was attributed to the driving force for the electron-transfer reactions and the different pathways for co-reactant (i.e., $\text{CO}_2^{\bullet-}$) reaction [19].

Some other papers reported microenvironmental effects of micelles on the electrochemical and ECL behavior of $\text{Os}(\text{bpy})_3^{2+}$ [24]. For the one electron oxidation of $\text{Os}(\text{bpy})_3^{2+/3+}$ near +0.6 V, aqueous solution using oxalate was used to generate $\text{Os}(\text{bpy})_3^{2+*}$. A study involving electron/hole annihilation through electron transfer between sterically stabilized silicon nanocrystals (NCs) and redox-active co-reactants was reported. Higher ECL intensity light emission was observed from the NC solutions in the presence of oxalate or persulfate as co-reactant [25]. Oxalate and peroxydisulfate in aqueous solution were determined at subpicomolar levels [14] based on $\text{Ru}(\text{bpy})_3^{2+}$ to generate ECL and are applied for the selective determination of oxalate synthetic urine samples [26]. The role of oxalate direct oxidation to the overall ECL behavior is dependent on many factors, e.g., surface property of the electrode, the concentration of oxalate, and the electrode potential applied [19, 27, 28]. ECL generation is also very sensitive to the solution pH. A maximum of pH ~ 6 in unbuffered (aqueous) solutions at Pt [4], constant from pH 4 to 8 in phosphate buffer solutions at GC [14, 29], and from pH 5 to 8 in phosphate buffer solutions at ultramicroelectrodes [19] has been described for $\text{Ru}(\text{bpy})_3^{2+}$ /oxalate system.

2.2.2.2 Pyruvate System

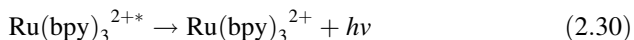
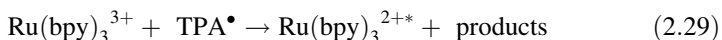
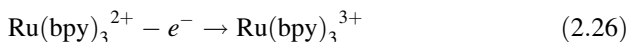
Another example of the “oxidative–reduction” system is the determination of pyruvate by $\text{Ru}(\text{bpy})_3^{2+}$, which was reported by Knight and Greenway. ECL from pyruvate either at the electrode or by electrogenerated $\text{Ru}(\text{bpy})_3^{3+}$ cannot be observed, as the oxidation of pyruvate is suppressed by the presence of an oxide layer on the Pt electrode surface. At a reduced Pt electrode, the difference in pyruvate and $\text{Ru}(\text{bpy})_3^{2+}$ oxidation potentials is negligibly small. As a result, the electrochemically produced $\text{Ru}(\text{bpy})_3^{3+}$ is not strong enough to oxidize the pyruvate in solution, so no ECL is generated. Addition of Ce(III) to an acidic solution containing $\text{Ru}(\text{bpy})_3^{2+}$ and pyruvate facilitates the ECL reaction upon anodic oxidation. Ce(IV) produced as a result of the oxidation of Ce(III) at the Pt electrode at higher potentials than $\text{Ru}(\text{bpy})_3^{2+}$ oxidation is a strong oxidant and can effectively oxidize pyruvate as shown in Eqs. (2.20)–(2.25), ensuing in the formation of the strongly reducing intermediate $\text{CH}_3\text{CO}^{\bullet}$. This species behaves like $\text{CO}_2^{\bullet-}$ and participates in electron-transfer reactions with $\text{Ru}(\text{bpy})_3^{3+}$ and $\text{Ru}(\text{bpy})_3^{2+}$, resulting in the emission of ECL [30].





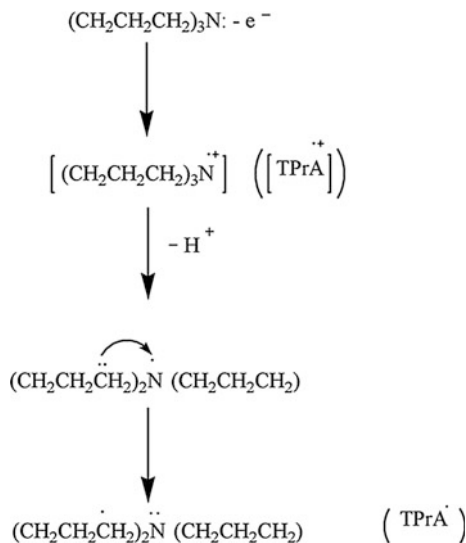
2.2.2.3 Amine System

$\text{Ru}(\text{bpy})_3^{2+}$ /tripropylamine (TPA) system, the most common ECL system, is currently the basis for the commercial ECL immunoassays and DNA analyses. Its mechanism has been explained in detail in recent years. There are four distinct possible reaction mechanisms [31, 32] of this system as shown in Fig. 2.2. During a detailed study on reaction mechanisms, it was revealed that there are some difficulties with these mechanisms, such as the lifetime of the excited species (R_3N^+ cation) generated by oxidation seemed to be either too long or too short on the basis of electron paramagnetic resonance measurements and on the basis of voltammetric measurements [1]. Possibilities related to pH values were also discussed by Pastore [15]. As for pH values less than 5, the deprotonation of the R_3NH^+ was the rate-limiting step, whereas above pH 5, the formation of an amine neutral radical was the rate-limiting step [33]. In Scheme 1 of Fig. 2.2, the ECL reactions occur as follows (Eqs. 2.26–2.30). Oxidation of both $\text{Ru}(\text{bpy})_3^{2+}$ and TPA takes place at the electrode surface, and $\text{Ru}(\text{bpy})_3^{3+}$ is reduced by TPA to produce the excited state.



In Scheme 2 of Fig. 2.2, $\text{Ru}(\text{bpy})_3^{2+}$ is reduced by TPA^\bullet , and $\text{Ru}(\text{bpy})_3^+$ reacts directly with $\text{Ru}(\text{bpy})_3^{3+}$ to form the excited state, ensuing the emission of light. In Scheme 3 of Fig. 2.2, only $\text{Ru}(\text{bpy})_3^{3+}$ is generated at the electrode surface, and TPA is oxidized by the generated $\text{Ru}(\text{bpy})_3^{3+}$. $\text{Ru}(\text{bpy})_3^{2+}$ concentration is a factor on which the overall ECL intensity depends; hence, this process is not favoured when $\text{Ru}(\text{bpy})_3^{2+}$ concentrations are low. The process of Scheme 4 of Fig. 2.2 shows the direct oxidation of TPA at the electrode to generate $\text{TPA}^{\bullet+}$ and TPA^\bullet . The following reaction between TPA^\bullet and $\text{Ru}(\text{bpy})_3^{2+}$ generates $\text{Ru}(\text{bpy})_3^+$, which in turn reacts with $\text{TPA}^{\bullet+}$ to form the excited-state $\text{Ru}(\text{bpy})_3^{2+*}$. The ECL route in

Fig. 2.3 Proposed tri-*n*-propylamine oxidation/reaction sequence with abbreviations in parentheses (Reprinted with permission from Ref. [34]. Copyright 2004 American Chemical Society)



the ECL intensity occurs at around pH 7.5, which is exceptionally suitable for applications in biological systems. Sensitivity of the system may also be significantly increased by the addition of some surface-active agents [35–38]. These agents are in fact adsorbed on the surface of electrodes by which electrode surface becomes more hydrophobic, thus facilitating the direct oxidation of TPA. These results are supported by the effects of non-ionic chain length on $\text{Ru}(\text{bpy})_3^{2+}/\text{TPA}$ ECL [35] and the dependence of the surfactant effect on the electrode materials [36].

Non-ionic surfactants, fluorosurfactants [37], and the ionic surfactants [38] can also enhance ECL intensity up to 8-fold, 50-fold, and 30-fold, respectively. Hydroxylic solvents including fluorinated alcohols also play important role to enhance $\text{Ru}(\text{bpy})_3^{2+}/\text{TPA}$ ECL intensity remarkably [39]. Due to increase in polarity, hydrogen bonding and dipole forces dramatically change the ground- and excited-state properties in $\text{Ru}(\text{bpy})_3^{2+}$, thus increasing the energy of maximum PL and ECL emission. Moreover, dramatic increases in ECL efficiencies using mixed alcohol/water solutions were observed ranging from 6- to 270-folds. Solution pH also affects the ECL intensity of $\text{Ru}(\text{bpy})_3^{2+}/\text{TPA}$ system [40, 41]. It shows dramatic increase in pH till pH > 5.5 with maximum pH value at 7.5. The exact reason of this phenomena might be indistinct; however, causes related to the deprotonation reactions of TPAH^+ and $\text{TPA}^{\bullet+}$, the stability of the intermediates formed, and the solubility decrease in TPA at high pH are also reported. Moreover, pH values higher than 9 should be avoided, as at this pH a significant ECL background signal is produced by $\text{Ru}(\text{bpy})_3^{3+}$ reaction with hydroxide ions at electrode. ECL behavior of $\text{Ru}(\text{bpy})_3^{2+}/\text{TPA}$ in MeCN shows that the ECL generation in this case is predominately associated with the direct oxidation of $\text{Ru}(\text{bpy})_3^{2+}$ at the electrode [42–45]. ECL with TPA in fluorinated and non-fluorinated alcohols and alcohol/water mixtures is also found in literature [39].

A number of articles [17, 34, 41, 46–49] have been published for the last few years for describing the correlation of ECL efficiency with the amine structure. Generally, ECL intensity enhancement occurs in the order primary < secondary < tertiary amines. For the amines to be ECL efficient, these should have α -hydrogen, so that upon oxidation, newly formed radical cation species can deprotonate easily to form a strongly reducing free radical species [41]. The nature of substituents and other functional groups on amine molecule also plays role in determining the ECL intensity. Usually, electron-withdrawing substituents tend to cause a reduction in ECL activity, and electron-donating groups have the opposite effect. Aromatic amines, aromatic substituted amines, and amines with a carbon–carbon double bond that can conjugate the radical intermediates consistently give a very low ECL response [50]. The ECL reaction mechanism of $\text{Ru}(\text{bpy})_3^{2+}$ with six tertiary aliphatic amines, namely tri-*n*-butylamine (TBuA), tri-isobutylamine (TisoBuA), TPA, methyl-di-*n*-propylamine (MeDPrA), triethylamine (TetA), and trimethylamine (TMeA), was studied in aqueous solution, and their ECL was examined using fast potential pulses at carbon-fiber microelectrodes and with simulation techniques to obtain information on the E° value of the amine redox couples [33].

Although having marvelous features and attractiveness, TPA has several shortcomings such as toxicity and volatility, and high concentrations (usually up to 100 mM) are needed to be used to obtain good sensitivity. 2-(dibutylamino)ethanol (DBAE), a more environmentally-friendly co-reactant was introduced with approximately 10 and 100 times greater ECL enhancement at Au and Pt electrodes, respectively [51] (Fig. 2.4). This improved ECL intensity was attributed to the catalytic effect of hydroxyl group toward the direct oxidation of DBAE at the electrode. The sensitivity of the $\text{Ru}(\text{bpy})_3^{2+}$ /DBAE system is about an order of magnitude better than that of the $\text{Ru}(\text{bpy})_3^{2+}$ /TPA system. DBAE is much less toxic and less volatile than TPA and more soluble in aqueous solutions, and 20 mM DBAE is more effective than 100 mM TPA. These features make DBAE a very promising co-reactant for $\text{Ru}(\text{bpy})_3^{2+}$ ECL immunoassay and DNA probe assays. *N*-butyldiethanolamine containing two hydroxyl groups was found even more effective than DBAE containing one hydroxyl at Au and Pt electrodes and is the most effective co-reactant reported so far. While in search of more efficient co-reactants, it was revealed that monoamines exhibited much higher ECL than the diamines, which may result from considerable side reactions of diamines [52]. These results may be helpful for new chemists in investigating new highly efficient co-reactants.

2.3 Hot-Electron ECL or Cathodic Luminescence

ECL can also be generated via hot electrons emitted into electrolyte solution from an oxide-covered electrode surface, sometimes called a conductor/insulator/electrolyte surface [53]. This special light emission pathway in ECL is commonly known as hot-electron-induced ECL [54–56], where hot electrons are defined as

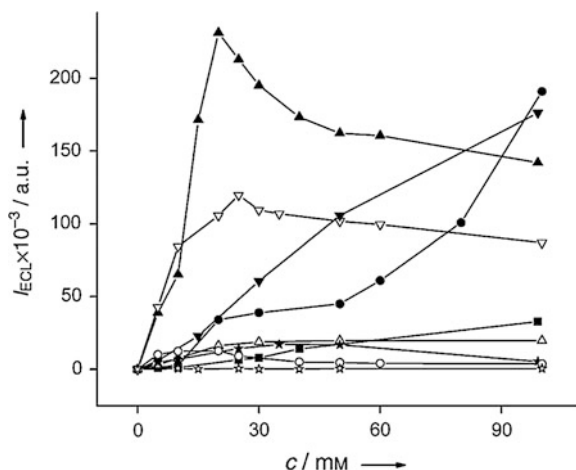


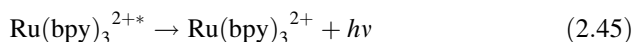
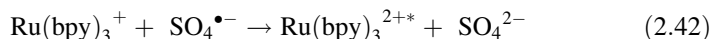
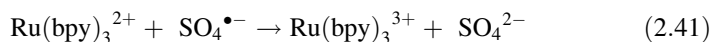
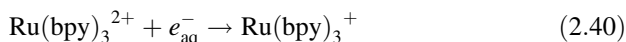
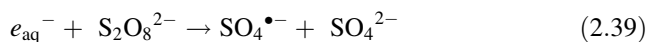
Fig. 2.4 Dependence of the ECL peak intensity on the concentrations of 2-(dibutylamino)ethanol (DBAE, *filled triangle*), triethanolamine (*empty square*), N,N-diethylethanolamine (*filled inverted triangle*), TPA (*filled circle*), triethylamine (*filled square*), N,N-diethyl-N'-methylethylenediamine (*empty triangle*), 3-diethylamino-1-propanol (*filled stars*), nitritotriacetic acid (*empty circle*), and ethylenediaminetetraacetic acid (*empty stars*), measured at the Au electrode in 0.1 m phosphate buffer solution (pH 7.5) containing 1 mM $[\text{Ru}(\text{bpy})_3]^{2+}$. The potential was stepped from 0 to 1.35 V (Reprinted with permission from Ref. [51]. Copyright 2007 Wiley-VCH)

electrons possessing thermal energies greater than the thermal energy of a phase or as electrons at energy far above the Fermi energy of a phase [57, 58].

The insulating layer of metal oxide used for generation of hot electrons prevents electron transferring from the metal to a solution species, until a large electric field is present across the oxide and the Fermi level in the metal is above that of the conduction band of the metal oxide. Current flow across the oxide film can then produce a hot solution-phase electron [56]. Strong reductants are generated due to the strong reductive ability of the hot electrons which then react with the emitter to generate an excited state and then emit light. For example, both $\text{Ru}(\text{bpy})_3^{2+}$ and luminol exhibit strong hot-electron-induced ECL on an oxide-covered aluminum electrode based on a tunnel emission of hot electrons into an aqueous solution [56, 59]. By using this technique, subnanomolar detection of $\text{Ru}(\text{bpy})_3^{2+}$ and luminol labels with a dynamic range of several orders of magnitude is realized. ECL emission of $\text{Ru}(\text{bpy})_3^{2+}$ or the mixed solution of $\text{Ru}(\text{bpy})_3^{2+}$ and persulfate on oxide-covered aluminum electrode was described by Kulmala et al. [52].

Soon afterward, a detection method for $\text{Ru}(\text{bpy})_3^{2+}$ based on the ECL of $\text{Ru}(\text{bpy})_3^{2+}$ and persulfate on oxide-covered aluminum electrode [60] and $\text{Ru}(\text{bpy})_3^{3+}$ in acetonitrile and aqueous solution on oxide-covered tantalum electrode was developed [11]. The ECL intensity showed linearity in the concentration range of 10^{-10} M and 10^{-5} M $\text{Ru}(\text{bpy})_3^{2+}$ in the presence of 1 mM persulfate. The sensitivity was improved by an order of magnitude in the presence of Tween 20, and this technique was applied to immunoassays. The ECL emission on oxide-covered

aluminum or tantalum electrode may be generated by the reaction between $\text{SO}_4^{\bullet-}$ and $\text{Ru}(\text{bpy})_3^+$, the reaction between hot electron and $\text{Ru}(\text{bpy})_3^{3+}$, and the reaction between $\text{Ru}(\text{bpy})_3^+$ and $\text{Ru}(\text{bpy})_3^{3+}$ (Eqs. 2.39–2.45) [60].



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Electrogenerated Chemiluminescence

Protocols and Applications

Parveen, S.; Aslam, M.S.; Hu, L.; Xu, G.

2013, XIII, 152 p. 54 illus., 22 illus. in color., Softcover

ISBN: 978-3-642-39554-3