

Electrochemically Generated Luminescence of Luminol and Luciferin in Ionic Liquids

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Electrochemiluminescence (ECL) is the generation of light triggered by an electrochemical reaction. ECL has been extensively studied in solvent-based electrolytes, but there is a lack of data on using electrode reactions to populate an excited-state light emitter in room temperature ionic liquids (RTILs). This work explores the current response, light intensity (photon counting), and spectral signatures of the cathodic ECL of luminol and firefly's luciferin in imidazolium-based RTILs. We have demonstrated that the cathodic (superoxide-triggered)

ECL of both luminol and adenylate-ester of firefly's luciferin is viable in RTILs, explored the effect of water contaminations, and importantly, shown that the ECL signal persists for up to about 700 s after the removal of the external cathodic pulse, which is probably due to the stabilization of superoxide by double-layer cation-rich structures. Long-lived RTIL double-layer structures and their endogenous fields are detected as stable and discrete open-circuit potential plateaus.

Introduction

The generation of light through electrochemical reactions has been actively researched since the 1920s, when it was first observed during the electrolysis of luminol in the presence of Grignard reagents.^[1] The systematic study of this branch of electrochemistry did however not start till the 1960s, with work done on radical ion annihilation electrochemiluminescence (ECL).^[2] ECL is currently exploited across science and technology, with applications ranging from food and water-quality testing, to immunoassays and biosensors for biological warfare.^[3] In comparison with chemiluminescence, ECL requires an electrical trigger, meaning that it has distinct advantages over its purely chemical counterpart, such as allowing for a fine control of the time and location of the reaction.^[3b,4] Furthermore, ECL can claim greater selectivity over chemiluminescence, as well as stability and simplicity.^[5]

Even though the number of ECL publications has been continuously rising since the 1990s,^[3b] the dominant reaction medium remains that of a molecular solvent added with an

electrolyte. ECL requires coupling electronic conduction in a solid electrode with ionic conduction in the electrolyte connecting at least two electrodes: the ECL emitter needs to be dissolved in an ionic conductor. ECL in a molecular, solvent-free ionic conductor, such as a room temperature ionic liquid (RTIL) is viable but under-explored, with only a limited number of studies having been published in the last two decades, along with their possible applications.^[6] The advantages of using an RTIL over a solvent-based electrolyte are several. Firstly, RTILs generally have very high boiling points, so that their use can mitigate undesirable solvent evaporation, hence solvent waste.^[7] Secondly, RTILs have a good electrochemical and thermal stability, large ionic conductivity, and are therefore an ideal medium for electrochemical applications.^[8] Thirdly, changes to the molecular nature of the solvent and electrolyte is a means of tuning the ECL emission wavelength,^[9] hence given the variety of RTILs now available (covering a broad range of viscosities, acidities, hydrophobicities, and polarizabilities^[8b]), it may be possible to harvest the large pool of RTILs to expand the spectral tuning of common ECL molecules.^[10]

Herein we explore the ECL of luminol and luciferin using widespread, commercially available and inexpensive imidazolium-based RTILs.^[11] Luminol is arguably one of the most popular ECL dyes. It has a wide number of applications ranging from immunoassays, biosensing, to non-clinical applications such as forensic detection of blood traces.^[3b,12] Luciferin is one of Nature's wonders, and is extremely fascinating due to its specie-specific breadth of colors, which cover almost all of the visible spectrum,^[13] its debated luminescence mechanism,^[14] and its several technological applications.^[15] Furthermore, luminol's anodic ECL in the presence of hydrogen peroxide has been used as proof-of-principle to demonstrate ECL spatiotemporal control,^[16] while fundamental studies on luciferin ECL, without its natural biocatalyst (luciferase-free luciferin ECL), have only recently begun.^[9]

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Results and Discussion

Luminol ECL is usually obtained under anodic biasing, and in the great majority of cases it relies on aqueous electrolytes and hydrogen peroxide as co-reactant.^[3b,17] However, luminol ECL may also be triggered under cathodic regimes,^[3b,18] where it relies on the formation of superoxide radical anion (superoxide hereafter) through the one-electron reduction of dissolved oxygen (Figure 1a).^[19] This reaction has been previously reported in RTILs,^[6a,b] but no spectral data are available. As shown in Figure 1b the application of a cathodic voltage to an oxygen-saturated solution of luminol in 1-ethyl-3-methylimidazolium ethyl sulfate ([EMIM][EtSO₄]) leads to a blue emission which is already detectable at -1.0 V (*vide infra* for a discussion on the ECL intensity-to-bias relationship). Similar results were obtained in other RTILs (Figures S1–S2). The emission of luminol in [EMIM][EtSO₄] peaks at 490 nm, which is analogous to the position observed in polar molecular solvents such as dimethyl sulfoxide (DMSO),^[18] and expected based on the polarity of [EMIM][EtSO₄].^[20] In [EMIM][EtSO₄] the luminol ECL persists well beyond the duration (30 s) of the cathodic -2.5 V pulse. As shown in Figure 1c, once the external potential is removed, light emission continues for over 700 s, while in a solvent-based electrolyte (Bu₄NClO₄/DMSO) it would fade off in ~ 45 s (Figure S3).

Figure 1d is an overlay plot of the simultaneous photon counting and current logging from the cyclic voltammetry experiment used to trigger the ECL. The plot shows that a small negative bias (-0.8 V) can trigger the ECL, but the photon

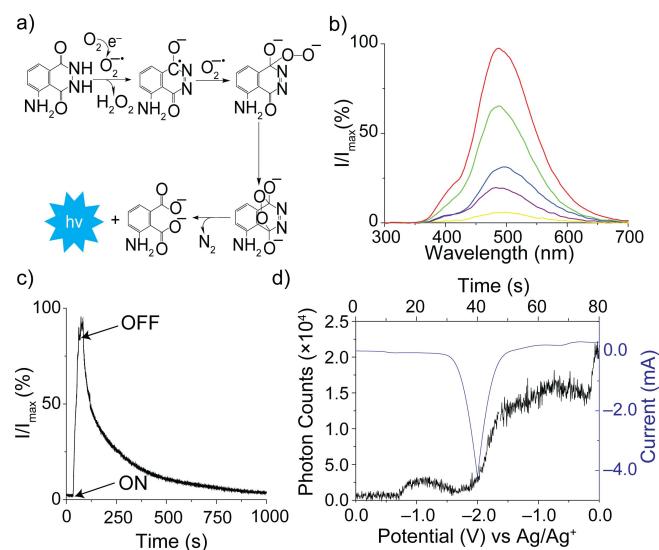


Figure 1. (a) Proposed mechanism for luminol cathodic ECL.^[4a,16] (b) ECL of luminol (1.0×10^{-3} M) in [EMIM][EtSO₄], recorded at a platinum mesh electrode biased at either -1.0 V (violet line), -1.5 V (yellow line), -2.0 V (blue line), -2.5 V (green line) and -3.0 V (red line). Spectra are normalized to the maximum intensity of the -3.0 V data set. (c) Time-resolved emission spectra (490 nm). The ON label indicates when the working electrode voltage bias is switched from open-circuit to -2.5 V. The OFF label indicates the end of the 30 s voltage pulse. (d) Photon counting experiments of luminol ECL during a cyclic voltammogram (the voltage was swept from 0.0 V towards -2.0 V, and back, at a scan rate of 0.05 V/s). Overlay plot of simultaneously acquired photon counts and current trace.

count begins to rise sharply only around -2.0 V. At the photon counts peak, the luminol system emits $\sim 2.0 \times 10^4$ photon/s, which is one order of magnitude less than what was found for the same system in Bu₄NClO₄/DMSO ($\sim 3.5 \times 10^5$ photon/s, Figure S4).

As shown in Figure 1a, the luminol cathodic ECL mechanism in a RTIL, similarly than in an aprotic molecular solvent such as DMSO,^[18] begins with the electrochemical formation of superoxide from oxygen, which in turn converts luminol to a diazasemiquinone radical. This radical intermediate then reacts in a cycloaddition reaction with a second superoxide, leading to the excited state of 3-aminophthalate*, which relaxes emitting light (Figure 1a).^[16,18] Superoxide formation is quenched by the presence of water, which leads to H₂O₂ via oxygen reduction. H₂O₂ at low concentrations causes an increase in ECL intensity,^[18] but as found experimentally, further addition of water to the RTIL causes a progressive drop in emission (Figure 2). The viability of generating electrochemically superoxide in RTILs has been already demonstrated,^[19,21] and despite the stability of superoxide in RTILs remaining unclear, it strongly depends on the nature of the cation.^[22] Ion-pairing of superoxide with the RTIL cations may enhance its stability without reducing its reactivity.^[23]

Notably, our data suggest more than one mechanism being involved, and luminol reacting with different species electro-generated at different potentials.

For example, the emission intensity at -1.0 V (Figure 1b) does not track the general trend in ECL rise with potential, probably because at this potential the emission is triggered by superoxide generated from oxygen, together with a contribution from a light path initiated by H₂O₂ and OH⁻. The latter requires water traces (see Karl-Fisher data in the experimental section). Consequently, the ECL intensity -1.0 V lies between those recorded at -1.5 V and at -2.0 V. A similar anomaly was also observed in other RTILs (Figures S1–S2). We believe therefore that an explanation of such outlier is the existence of multiple mechanisms for the cathodic ECL of luminol.^[18,24] It is possible that at -1.5 V (and at more negative biases), H₂O₂ is effectively reduced to OH⁻ instead of reacting directly with luminol, ($2 \text{ luminol} + \text{H}_2\text{O}_2 \rightarrow 2 \text{ luminol radical anion} + 2 \text{H}_2\text{O} + 2 \text{H}^+$) as it may happen at -1.0 V, and so the ECL intensity does track the increase in current. At -2.0 V (and beyond) the

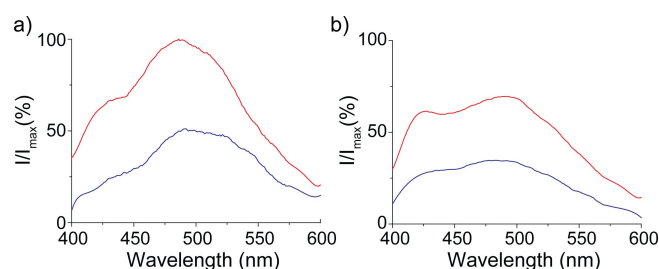


Figure 2. Electrogenerated chemiluminescence of luminol (1.0×10^{-3} M) in [EMIM][EtSO₄], obtained using a platinum mesh biased at either -2.5 V (blue line) or at -3.0 V (red line). The RTIL is deliberately spiked with water [5% v/v (a); 10% v/v (b)]. A further increase of water concentration to 15% v/v quenches completely the emission. Spectra are normalized to the maximum intensity of the -3.0 V, 5% v/v water data set.

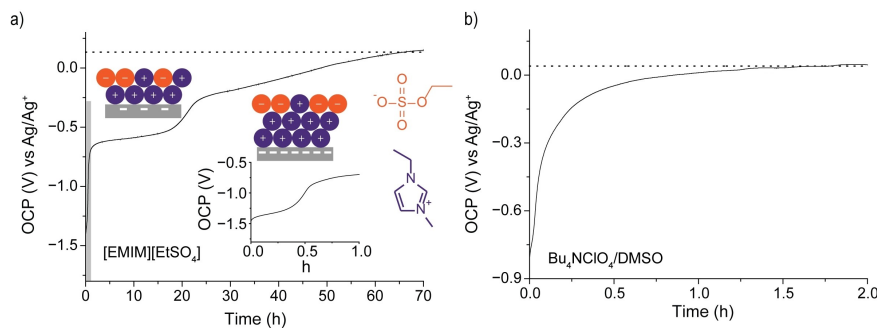


Figure 3. Open circuit potential (OCP)-time measurements recorded at a platinum mesh electrode immersed in neat [EMIM][EtSO₄] (a), and in 1.0×10^{-1} M Bu₄NClO₄ in DMSO (b). A negative potential step (60 s) was applied to the working electrode, away (-2.0 V) from the initial OCP rest value (dotted line) and the OCP relaxation monitored over time. In the molecular solvent the OCP relaxes rapidly and asymptotically back to the initial OCP (b). During the OCP relaxation in [EMIM][EtSO₄], discrete and long-lived OCP plateaus emerge between -0.65 V and -0.75 V. The inset in (a) highlights the shorter and more negative plateau located between -1.4 V and -1.5 V. This two plateaus have been ascribed to electrostatic signatures of crowding (first, more negative plateau) and overscreening (second, longer-lived plateau) double-layer structures.^[11]

current is so large that the ECL progressively increases despite the consumption of H₂O₂ (Figure 1b). Finally, in the return scan (towards anodic biases) there is sudden increase in ECL photon counting (Figure 1d). Here, at lower negative potential during the return scan, the superoxide radical anion is regenerated by H₂O₂ electro-oxidation to hydroperoxyl radical and then to superoxide radical anion,^[18,24,25] resulting in a rise the ECL emission. The delay between this peak and the current peak at -0.4 V (Figure 1d) may be attributed to a slower kinetic in RTILs, mainly due to their large viscosities.^[8a]

Since superoxide is complexed by large cations,^[26] we hypothesized that a cation-rich RTILs structure at the electrified interface may increase superoxide stability, causing luminol emission not to drop suddenly after the removal of the external voltage. Such ordered bilayer structure at the interface between RTILs and charged electrodes is well documented.^[11,27] This is particularly true at negative potentials, where stable, cations-rich, near-surface RTILs double-layer structures have been unambiguously demonstrated by atomic force microscopy (AFM).^[27e,f] However, AFM experiments with liquids are technically demanding and a simpler method to detect the presence of such cation-rich layer is by open circuit potentiometry (Figure 3a).^[11] Following a short (60 s) negative-potential pulse, the open-circuit potential (OCP) of a molecular solvent-based electrolyte/electrode system will rapidly and asymptotically return to its rest OCP value (Figure 3b). In sharp contrast to such rapid and asymptotic relaxation, in [EMIM][EtSO₄] we observed two stable OCP plateaus during the OCP relaxation measurements (Figure 3a). These two negative and consecutive OCP plateaus are interpreted as electrostatic signatures of the endogenous fields of overscreening and crowding structures (cation-rich near-surface structures formed after a negative pulse, see cartoons in Figure 3a).^[11,27a] The formation of a cation-rich crowding structure (the most negative OCP plateau) is proposed to be linked to the persistency of the ECL emission even after the removal of the external bias.

We then turned to a much less explored ECL luminophore: firefly's luciferin adenylate (AMP-luc hereafter).^[9] AMP-luc ECL is here performed without its natural biocatalyst, luciferase, in

contrast to most studies and applications of luciferin where luciferase is required to start the light path.^[28] The likely role of superoxide in the ECL reaction mechanism is shown in Figure 4a.^[9] Two superoxide molecules are needed for the AMP-luc light path: the first to remove the hydrogen atom on the carbon in alpha-position of the AMP ester, and a second to generate an endoperoxide which leads to the formation of the excited state (oxyluciferin, ox-luc) upon a decarboxylation.^[9] The ECL of AMP-luc in [EMIM][EtSO₄] peaks at an energy comparable to that observed in DMSO-based electrolytes (626 nm, Figure 4b).^[9] The

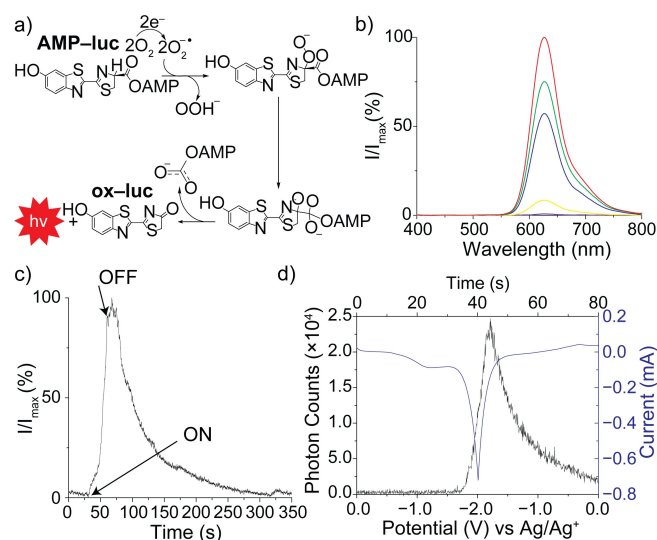


Figure 4. (a) Proposed mechanism for the firefly's luciferin ECL (extended mechanism in Figure S5).^[9] (b) ECL of AMP-luc (4.3×10^{-4} M) in [EMIM][EtSO₄] at a platinum mesh electrode biased at either -1.0 V (violet line), -1.5 V (yellow line), -2.0 V (blue line), -2.5 V (green line) and at -3.0 V (red line). Spectra are normalized to the maximum intensity of the -3.0 V data set. (c) Time-resolved emission spectra (626 nm). The ON label indicates the time which the working electrode voltage bias is switched from open-circuit to -2.5 V. The OFF label indicates the end of the 30 s (-2.5 V) voltage pulse. (d) Photon counting experiments of AMP-luc ECL during a cyclic voltammogram (the voltage was swept from 0.0 V to -2.0 V, and back, at a scan rate of 0.05 V/s). The simultaneously acquired photon counts and current trace plots are overlaid.

ECL of AMP-luc was also studied in other RTILs (Figures S6–S7). Interestingly a non-Gaussian shape is visible in the emission profile (Figure 4b), and it could be related to half-solvated molecules trapped at the interface.^[9] Data in Figure 4c show that the ECL of AMP-luc in [EMIM][EtSO₄] persists after the removal of the external bias, and as for luminol such persistence is possibly linked to the stability of the cation-rich crowding double-layer structure of [EMIM][EtSO₄] at platinum electrodes. As for luminol, the ECL of AMP-luc in DMSO drops fast once the external bias is removed (Figure S8). Figure 4d shows the simultaneous photon counting and voltametric current recording, with the emission beginning to rise at -1.8 V. At the photon counts peak, the AMP-luc solution emits 2.5×10^4 photon/s, which is roughly ~ 4.5 times less than in DMSO.^[9]

Conclusion

We have reported the ECL of luminol and luciferin (AMP-luc) in imidazolium-based RTILs. Luciferin ECL normally requires a biocatalyst, luciferase, but here we show that radical chemistry mediated by electrogenerated superoxide can trigger the light path in an enzyme-free environment. However, the emission intensity in RTILs is lower, probably due to the higher viscosity which limits mass transport. Demonstrating the feasibility of ECL in RTILs of a conventional dye (luminol) as well as of a relatively less explored luminophore (firefly's luciferin) we have shown how RTILs can be a good and a greener alternative to conventional molecular solvents. Moreover, photon counting experiments have shown that although ECL intensities are lower in RTILs than in molecular solvents, same photon count order of magnitudes are achieved.

Importantly, the ECL emission of both, luminol and luciferin, persists for several minutes after the removal of the cathodic pulse (~ 700 s for luminol, ~ 200 s for AMP-luc). We propose this long-lived emission to be linked to the stability of superoxide in the cation-rich (crowding) double-layer structure that persists for hours at the interface between platinum cathodes and several imidazolium-based RTILs.^[11] Such long-lived ECL emission is not observed in solvent-based electrolytes.

Experimental Section

Unless noted otherwise, all reagents were of analytical grade and utilized as received. 5-Amino-2,3-dihydrophthalazine-1,4-dione (luminol, $\geq 97\%$), 1-ethyl-3-methylimidazolium ethyl sulfate ([EMIM][EtSO₄], $\geq 95\%$), tetrabutylammonium perchlorate (Bu₄NClO₄, 98.0%), and anhydrous dimethyl sulfoxide (DMSO, $\geq 99.0\%$) were purchased from Sigma-Aldrich. 1-Butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([BMIM][NTf₂], 99.5%) and 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF₆], 99.5%) were purchased from Iolitec. Unless specified otherwise the water content, measured by a Karl-Fischer titrations, was ~ 600 ppm for [EMIM][EtSO₄], 300 ppm for [BMIM][NTf₂], and ~ 450 ppm for [BMIM][PF₆]. Firefly luciferin adenylate (AMP-luc) was synthesized and characterized according to procedures reported elsewhere.^[9] Electrolytic solutions used for the ECL experiments were 1.0×10^{-3} M luminol, and 4.3×10^{-4} M AMP-luc. Solutions for ECL were

oxygen-saturated by means of a 20 min oxygen-gas bubbling procedure (Coregas, $\geq 99.95\%$). Spectroelectrochemical experiments were carried out with a Cary Eclipse spectrophotometer (Varian, Palo Alto, California) operated in Bio/Chemi-luminescence mode at 25°C . The photomultiplier voltage was set to 800 V, and the emission slit to 20 nm. Each emission spectra was recorded in ~ 5 s. The spectroelectrochemical cell used for all ECL measurements was a quartz cuvette of 10 mm optical-path length (1/SX/10, Starna scientific, UK) fitted with a perforated PTFE cap/electrodes holder. A negative voltage bias was applied to a platinum mesh used as working electrode (011498 SEC-C Gauze, 80 mesh, 7×6 mm overall size, BASi), a platinum coil was used as counter electrode. A leakless Ag|AgCl electrode (ET072-1, eDAQ, with 3.4 M aqueous KCl as filling solution) was used as the reference electrode. All potentials are reported against the reference electrode. The spectroelectrochemical experiments were carried out under ambient air at room temperature by using a PalmSens4 (PalmSens BV, Houten, Netherlands) as potentiostat. Photon counting experiments were performed with a single-photon counting module (SPCM-AQR-14, Excelitas Technologies) interfaced with an avalanche photo-diode (APD) controller (Nanonics Imaging Ltd.). The APD controller time constant was set to 1.0 ms. The photon count output was recorded with a data logger (DrDAQ, Pico Technology, Figure S9). The photon count rate was corrected for the nominal wavelength-dependent efficiency of the APD module (Nanonics Imaging Ltd.), which is 70% for red light and 42% for blue light.

The water content was estimated by Karl-Fisher titration (Mettler-Toledo C20S compact coulometer, Honeywell HYDRANAL Coulomat AG reagent, Merck Water Standard 0.1%, USA), and with three samples run for each RTIL.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: electrochemically generated luminescence · luciferin · luminol · room temperature ionic liquids (RTILs) · spectroelectrochemistry

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