

Review Article

Advances in bipolar electrochemiluminescence for the detection of biorelevant molecular targets

Laurent Bouffier^{1}, Dragan Manojlovic², Alexander Kuhn¹ and Neso Sojic¹*

Addresses

¹Univ. Bordeaux, CNRS, Bordeaux INP, Institut des Sciences Moléculaires, UMR 5255, F-33400 Talence, France

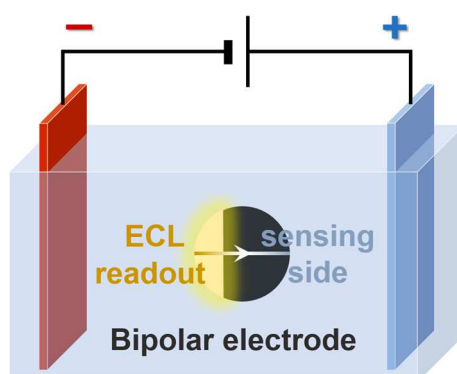
²University of Belgrade, Faculty of Chemistry, 11000 Belgrade, Serbia

Corresponding author: Laurent Bouffier (laurent.bouffier@enscbp.fr)

Abstract

Bipolar electrochemistry (BPE) contrasts very much with conventional electrochemistry because it is based on the control of the solution potential instead of the working electrode potential. In a typical setup, a piece of conducting materials is immersed inside an electrolyte and submitted to an electric field. Such conditions split the interfacial nature of the materials into cathodic and anodic domains where electrochemical reactions can readily take place. BPE has many potential applications and the present contribution aims to focus on recent analytical applications that involve electrogenerated chemiluminescence (ECL) detection. ECL is a special case of luminescence where the excited state of the luminophore is populated following a sequence of reactions that is triggered by an initial electron transfer step occurring at the electrode surface. The coupling between BPE and ECL is a powerful approach since it provides a unique opportunity to combine the intrinsic advantages of both techniques. BPE enables the spatial separation of sensing and reporting poles whereas ECL provides a simple and sensitive visual readout. This opinion paper will describe the experimental possibilities and the most recent applications of BPE/ECL coupling for the detection of biorelevant molecular targets.

Graphical abstract



Introduction

Context

In a conventional electrochemical cell, the role of the potentiostat is to bias the potential of the working electrode immersed in the electrolyte solution in order to promote a given electrochemical reaction. Bipolar electrochemistry (BPE) offers a rather different approach by controlling the solution potential with respect to a piece of conducting material that is positioned in the electrolyte (open-configuration case). Experimentally, a pair of feeder electrodes is connected to the external power supply to generate an electric field across the electrolyte solution. A linear evolution of the potential (denoted dE/dx in [Figure 1](#)) is established in solution whereas the conductor is equipotential. This leads to an interfacial polarization potential alongside the object versus the solution, thus establishing anodic and cathodic poles at both extremities of the object, justifying the denomination of bipolar electrode. Although being well known in specific domains such as corrosion or electrolysis, BPE remained relatively unexplored until the early 2000's when it has been intensively re-investigated by several groups. BPE has now found a whole range of new applications especially in the fields of materials science and electroanalytical chemistry [\[1-8\]](#).

BPE-based electroanalysis

BPE affords a unique way to induce a break of symmetry by an electrochemical mean. During the very last years, such a concept was used to enable the fabrication of organic/inorganic *Janus* particles [\[9,10\]](#), for the wireless formation of surface gradients [\[11-13\]](#), and also for the design and actuation of roving systems [\[14,15\]](#). However, the wider field of applications of BPE is dedicated to analytical chemistry. The obvious reason is that BPE offers a series of major advantages compared to conventional electrochemistry. Firstly, BPE is intrinsically wireless, meaning that no physical cable is required to connect the bipolar electrode to the external power supply. Consequently, either one or several bipolar electrodes can be indifferently addressed with a single pair of driving electrodes and a power supply avoiding the extra-cost of a multichannel potentiostat. Therefore, BPE was applied to power simultaneously a large number of bipolar electrodes for rapid screening and parallel sensing. Typically, metal spots patterned onto an insulating surface (i.e. electrode array) or ensembles of discrete conducting particles (i.e. dispersed electrodes) can be employed [\[1,16-18\]](#). Also, BPE is based on the coupling between the electrochemical processes that take place at both extremities of the bipolar electrode. This is another key-advantage because the exact same number of electrons afforded in the oxidation are necessarily involved in the reduction. This fundamental rule of charge neutrality enables a typical strategy where sensing and reporting can be effectively separated in space. This means that the electrochemical reaction of interest may occur at one pole of the bipolar electrode whereas a simple visual readout can be obtained at the opposite pole. Since the electrons involved in both Faradaic processes travel across the bipolar electrode, the amplitude of the reporting reaction reflects the amount of a given electroactive target. As a corollary, at a fixed driving force, the presence of an electroactive species is evidenced by an increased bipolar current due to an easier electrochemical coupling [\[19,20\]](#). If a threshold electric field is applied, the absence of the target can be discriminated because neither bipolar current nor reporting reaction are enabled in this condition.

Promotion of ECL by BPE

The most popular reporting strategy in analytical BPE is the emission of light by electrochemiluminescence (ECL). In brief, an electroactive luminophore is turned on by a sequence of reactions that is initiated by an electron transfer at one pole of the bipolar electrode. The principle was reported first in the early 2000's and has now been adapted to many bioassays [\[21,22\]](#). Generally, the bipolar platform serves to monitor a given reduction reaction by anodic ECL. The optical readout is the light emitted at a specific wavelength depending on the choice of the dye. The two most popular ECL systems used in that context are either tris(2,2'-bipyridine)ruthenium ($\text{Ru}(\text{bpy})_3^{2+}$) and tri-*n*-propylamine (TPA) or 3-aminophthalhydrazide (luminol) and hydrogen peroxide (H_2O_2) pairs, respectively.

Open-configuration/closed-configuration

From a chronologic point of view, the majority of the BPE experimental setups is based on the so-called “open-configuration”. This means that the bipolar electrode and both feeder electrodes are immersed together in the same electrolytic compartment (Figure 1). However, another experimental configuration is possible and referred to as “closed-configuration” [23,24]. In this rather different approach, the bipolar electrode bridges two separated vials that contain also one of the driving electrodes. It is like two half-cells that are solely connected by the bipolar electrode. Although being less widespread, the closed-configuration allows using chemicals that are not stable when mixed together, controlling independently the pH in both compartments or employing two immiscible solvents. Also, applying only a mild potential difference is sufficient in a closed-cell configuration (typically hundreds of millivolts or a few volts) compared to the open-configuration where the necessary applied voltage is much higher especially when dealing with microscale bipolar electrodes [25,26].

Sensing applications

Open-configuration ECL/BPE

The very first application of ECL reporting was proposed with a simple U-shaped Pt-based bipolar electrode positioned across a channel [21]. It was applied to the direct quantitation of $\text{Ru}(\text{bpy})_3^{2+}$ luminophore and the indirect detection of model amino acids dissolved in aqueous buffer solution. Here, the detection is referred to as indirect because the electrochemical sensing reaction does not participate to the ECL reporting although involving the same amount of electrons. This approach was then adapted for the detection of many biorelevant targets such as DNA, peptides, proteins receptors or enzymatic substrates. For DNA detection, a probe oligonucleotide is first immobilized at the extremity of a gold bipolar electrode. Hybridisation is performed with a complementary DNA target that is modified with a Pt nanoparticle. ECL detection is therefore coupled with catalytic oxygen reduction only in presence of the DNA target [1]. This principle was illustrated with simple metal band electrodes as well as integrated high-density bipolar microarray electrodes that can be addressed simultaneously. DNA detection was also proposed by using an antisense probe labelled with an ECL-active silica nanoparticle. The distance between the $\text{Ru}(\text{bpy})_3^{2+}$ ECL label and the metal surface depends on the hybridisation state, just as the resulting ECL signal [27]. Such a methodology was used to quantify mRNA or adapted to detect intracellular nucleic acid targets in tumor cells [28]. In the latter case, the presence of the target cancer cell displaces an ECL quencher away from the surface modulating thus the corresponding ECL intensity accordingly. BPE and ECL were also combined to provide a tool for recording an optical voltammogram where the information is contained in the spatial domain instead of the time domain [29]. Also, the localization of the ECL reaction can be controlled in a 2-dimensional space on the perimeter of a square bipolar electrode by adjusting the electric field direction [30]. More recently, in an effort to limit the fabrication cost of BPE analytical platforms that are very often based on microfabrication techniques and incorporate expensive metal electrodes, paper-based devices were proposed [31,32].

In the open-configuration, the driving force is the electric field applied across the electrolytic solution through both feeder electrodes. However, Xu's group proposed recently a very original twist by replacing the liquid electrolyte by a piece of conducting materials [33]. Conceptually, the electrical resistance of the conductor used in such an approach should not be too low and ITO appears to be a suitable candidate (Figure 2). The authors delimited on the ITO surface an array of 9 individual compartments acting as parallel bipolar cells. As a proof of principle, concentration-dependent detection of H_2O_2 was achieved with luminol-based ECL.

Even if the bipolar electrode is typically in a fixed position during BPE, the actuation of freely moving conducting pellets or beads is indeed possible. This was achieved in several capillary-based configurations but also in a fully open electrochemical cell [34,35]. The displacement of a suspension of bipolar electrodes could be controlled by a liquid flow and capillary forces or be directly electric field-driven. This latter locomotion mechanism involves the production of gas bubbles at the edge of the bipolar electrode that enable a directional thrust force. Typically,

H₂ gas formation was selected to take place at the cathode whereas either H₂O or a sacrificial species is oxidized at the anode. The generation of ECL by such propelled bipolar electrodes was reported for a purely academic purpose. The demonstration was done sequentially with Ru(bpy)₃²⁺ and luminol for red and blue ECL emissions, respectively [36,37]. Later, this approach was combined with an enzymatic system to quantify in time and space the presence of glucose [38]. The trick was to select a glucose dehydrogenase because the β-nicotinamide adenine dinucleotide (NADH) enzymatic co-product acts also as an ECL sacrificial co-reactant, enabling thus a substrate-dependent ECL signal. Apart from these systems, the influence of the orientation of a bipolar electrode mounted on a rotor was also investigated to demonstrate the feasibility of alternating ECL controlled by the rotation speed [39,40].

By essence, ECL is a surface-limited 2-dimensional phenomenon that is just produced at the immediate vicinity of the electrode. However, the coupling between ECL and BPE enables a true change of paradigm by addressing simultaneously a large ensemble of discrete conducting particles dispersed inside the electrolyte. In such a bulk experiment, intense light is emitted in the whole electrolytic volume allowing the first 3-dimensional ECL [17]. The proof-of-principle was achieved by using dispersible carbon microbeads or nanotubes. The quantity of single emitters should be optimized to avoid percolation issues and enable a collective ECL generation [41]. Again, the combination with enzymes was proposed for bioelectrochemical assays, illustrated with glucose and choline sensing, respectively. In the latter case, choline substrate is converted into betaine whereas H₂O₂ as an enzymatic co-product amplifies luminol ECL (Figure 3). Finally, the enzyme substrate was not homogeneously dissolved in solution but in a concentration gradient manner. In such a case, the chemical gradient can be instantaneously revealed by mapping the corresponding spatial distribution of ECL intensity.

Closed-configuration ECL/BPE

As mentioned above, the specificity of the closed-configuration is that the only current pathway bridging both electrolytic compartments is across the bipolar electrode. In fact, it was reported in the early 2010's that widespread carbon fiber microelectrodes backfilled with an electrolyte behave indeed as bipolar electrodes. The electrochemical behaviour of these microelectrodes was not yet investigated in the framework of BPE [24]. When an oxidation reaction does occur in the outer compartment, the extremity of the carbon fiber remaining in the inner compartment behaves as a bipolar cathode by reducing dissolved O₂. The understanding of this BPE-based mechanism has a major consequence on the electrochemical characteristics of these microelectrodes because the response depends on the limiting current occurring at each pole [42]. This is also true in the case of ECL reporting in the closed-configuration because the size and geometry (i.e. surface area) of both sensing and reporting poles may affect the overall ECL strength [43].

The closed-configuration was adapted by positioning one or several screen-printed microband electrodes across microfluidic channels [44]. The experimental advantage is the physical separation between the sensing and reporting channels, allowing the use of thermodynamically unstable mixtures of redox species that could not co-employ together in an open-cell configuration. With such a setup, the ECL intensity of Ru(bpy)₃²⁺/TPA in the reporting channel is modulated by the presence of Fe(CN)₆³⁻ target in the sensing counter-part. The strategy can then be implemented to detect a given bioanalyte. This was exemplify through the recognition of a glycated hemoglobin, which is an important glucose level marker. The electrochemical reactions promoted at the feeder electrodes that are in fact “counter-reactions” are as important to consider as the ones occurring at both anodic and cathodic poles of the bipolar electrode [45]. For this reason, the chemical composition of each compartment and/or channel as well as the size of the bipolar poles and feeder electrodes need to be taken into account. Finally, when dealing with BPE-based ECL, the photons also produced at the feeder anode can be detrimental to a sensitive measurement. In such a context, the closed-configuration offers an interesting alternative by avoiding to position the feeder and bipolar electrodes in close proximity [46]. The two-channel design was used to detect Ru(bpy)₃²⁺ luminophore and TPA co-reactant as well as additional redox-active species such as H₂O₂ which affects the bipolar coupling. The presence of dopamine was also quantified through ECL quenching caused by its oxidation product. An improved design exhibiting three independent channels cross-linked by two bipolar

electrodes was proposed for a sequential multi-analytes detection strategy [47]. Multiplex bioanalysis remains an essential challenge for point-of-care clinical diagnostic. In this context, a closed-configuration array of bipolar electrodes was designed for multiplexed cancer biomarker analysis [16]. Several molecular recognition strategies based on aptamers or antibodies were combined together to enable the detection of up to four different biomarkers such as adenosine triphosphate, prostate-specific antigen, α -fetoprotein and thrombin.

In a closed-cell configuration, the luminol/ H_2O_2 ECL system was also used in combination with the deposition of an electroactive inorganic layer [48]. In this report, the electrocatalytic conversion of glucose substrate is performed by an oxidase immobilized within a polymer matrix sitting on top of a Prussian blue layer. The ECL reporting (luminol/ H_2O_2 oxidation) is insured by the coupling with the reduction of enzymatically generated H_2O_2 . Here, the use of a closed-configuration is essential because the same reactant, namely H_2O_2 is involved on both sides of the bipolar electrode.

Even if only one single luminophore is involved in most analytical application, several dyes can also be simultaneously used. Such an approach was first demonstrated in an open-configuration by controlling the ECL generation alongside the anodic pole of a bipolar electrode from two different luminophores [49]. This spatially-resolved multicolour ECL was achieved by controlling the lateral position of the two corresponding ECL domains. Such a concept was recently adapted by Hogan's group to a closed-configuration, enabling heavy metal ion detection through a multi-colour encoding [50]. In this setup, the analytic pole of the bipolar electrode behaves as a cathode for Cu^{2+} or Cd^{2+} reduction whereas the ECL reporting occurs at the anodic pole. Mixing of $\text{Ir}(\text{ppy})_3$ and $\text{Ru}(\text{bpy})_3^{2+}$ ECL-active dyes allows controlling the light colour depending on the applied voltage (Figure 4) and to discriminate readily the chemical nature of the metal cations dissolved in the sensing compartment. Finally and just like in an open-configuration, it is also possible to adapt paper-based approaches to the closed-configuration [51]. Such a multicolour strategy with a voltage-dependant ECL emission wavelength was also proposed for detecting several biomarkers of prostate cancer [52].

Conclusions

BPE is currently a very active field of research. Among the possible applications, the case of analytical BPE is of major interest although it was only marginally exploited until the beginning of the 2010's. BPE offers indeed key-advantages compared to classic electrochemistry. The coupling with ECL reporting opens multiple analytical strategies depending on the nature of the (bio)chemical target. In the open-cell configuration, the number of bipolar electrodes can be tuned from a single one to up to thousands of sensing elements. Moreover, they are all simultaneously addressed with a single pair of feeder electrodes connected to a single external power supply. Collective ECL generation is therefore possible from large ensemble of particles. On the other hand, the closed-configuration offers the advantage of using two space-separated reservoirs that may contain incompatible chemicals and avoid potential interferences. The combination with microfabrication technology enables the incorporation of bipolar electrode arrays in microfluidic channels and to perform in-flow analysis. In fact, most of the analytical approaches reported in conventional electrochemistry can be potentially adapted to BPE. Also, new emerging concepts based on original ideas such as 3-dimensional ECL, self-propelled light-emitting objects, resistance-induced polarization potential or multicolour bipolar ECL analysis are still expanding the scope of this interesting BPE/ECL coupling.

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Recent papers of particular interest have been highlighted as:

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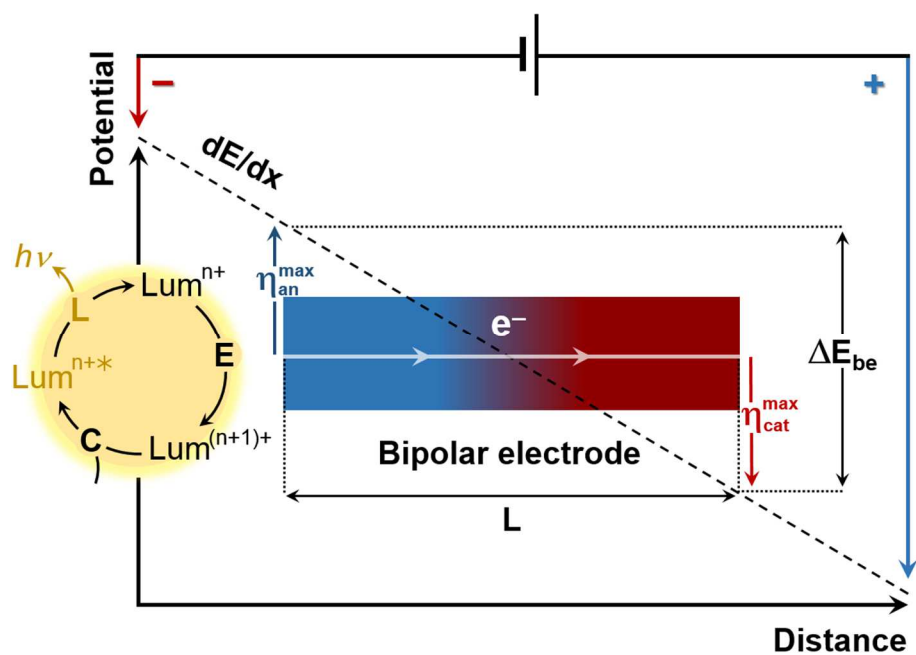


Figure 1. Principle BPE in an open-configuration. Feeder cathode and cathodic domain of the bipolar electrode are coloured in red whereas those of the anode are in blue. ECL does usually occur at the anodic side of the bipolar electrode.

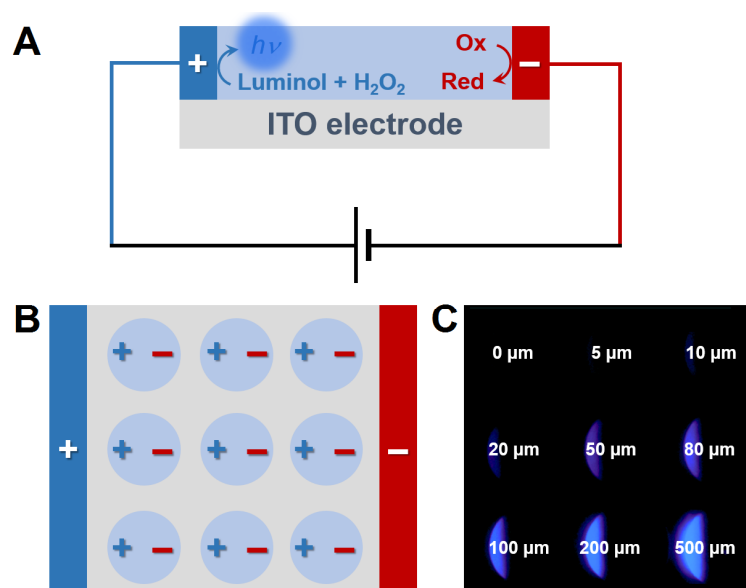


Figure 2. Principle of single electrode ECL detection based on the electrochemical oxidation of luminol (A). In such a setup, the polarization is solely due to the ohmic drop across the ITO. Scheme of the single ITO electrode with nine independent microcells (B). Corresponding ECL emission for increasing H₂O₂ concentrations (C). Adapted from ref. [33]. Copyright © (2018) Royal Society of Chemistry.

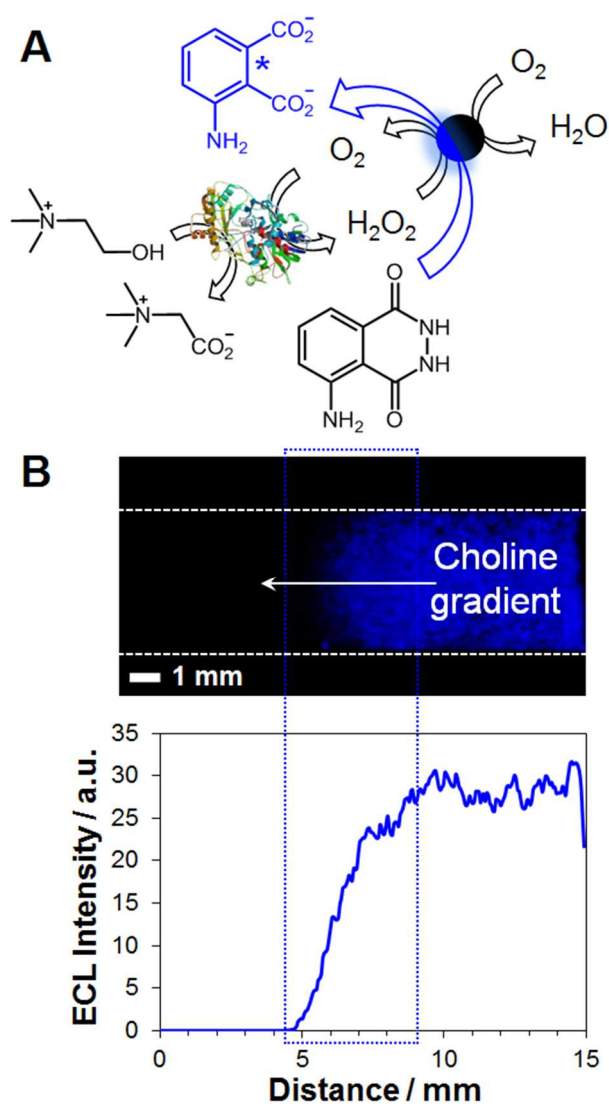


Figure 3. Enzymatic mechanism where choline is converted to betaine by choline oxidase. H_2O_2 promotes luminol-based blue light through the generation of an aminophthalate species (**A**). Snapshot ECL imaging and corresponding intensity profile of a concentration gradient of choline established inside a capillary filled with a suspension of carbon microbeads acting as a large ensemble of bipolar electrodes (**B**). Adapted from ref. [41]. Copyright © (2016) American Chemical Society.

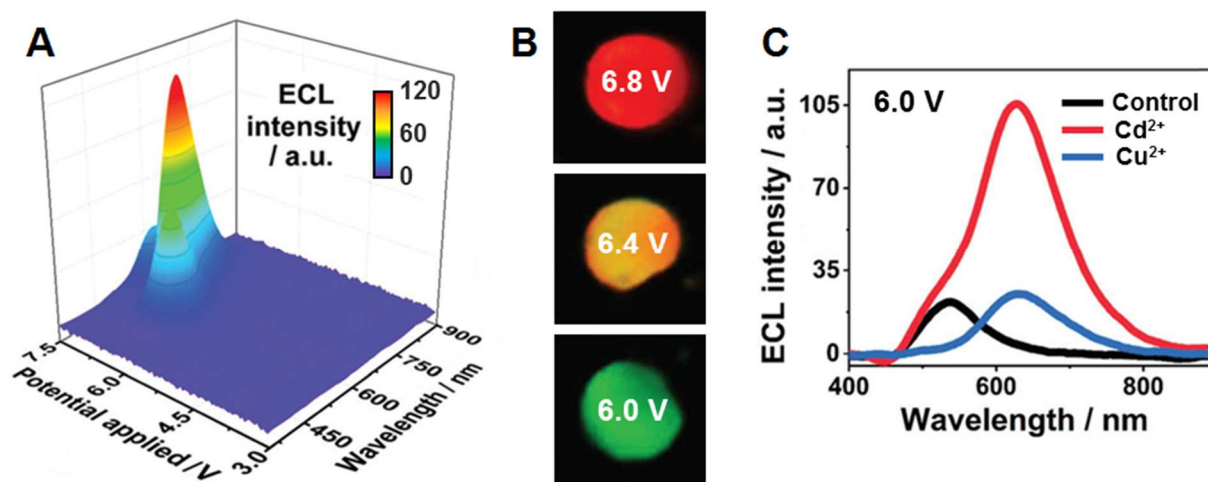


Figure 4. Potential-resolved BPE-induced ECL spectrum recorded with a mixture of Ir(ppy)_3 and Ru(bpy)_3^{2+} dyes (A). Series of potential-dependent images of the anodic pole of the closed bipolar electrode (B). Characteristic ECL spectra in the absence or presence of metal cations (C). Adapted from ref. [50]. Copyright © (2019) Royal Society of Chemistry