APPLICATION NOTE ECL-001

Spectro-electrochemiluminescence monitoring of light emitted simultaneously by two luminophores

Electrogenerated chemiluminescence or electrochemiluminescence (ECL) consists of the emission of light due to the generation of excited states by electron transfer reactions at the electrode surface. This technique offers many advantages as its versatility, high sensitivity, simplified device, and good temporal and spatial control. This Application Note describes how the SpectroECL is used to study the ECL process when several luminophores are present in solution.

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INTRODUCTION

Electrochemiluminescence (ECL) or electrogenerated chemiluminescence is the combination of electrochemistry and chemiluminescence. It has been widely used for food and clinical analysis due to its versatility, simplified optical set-up, low background signal and high sensitivity [1,2]. Electrogenerated chemiluminescence signals are usually obtained from the excited states of a luminophore generated at the electrode surface due to the presence of a co-reactant during an electrochemical reaction.

Particularly interesting is the resonance energy transfer (RET) mechanism when at least two luminophores are present in solution, one luminophore acts as donor and the other one as acceptor [3]. In the absence of an external light source, donor luminophore emits light as consequence of its electrochemical excitation, part of this emission is absorbed by the acceptor to be excited and re-emits light.

In this Application Note, two ECL systems were studied using different detectors, the first one consists of luminol as luminophore and hydrogen peroxide as co-reactant, while the second one is a RET system based on luminol and fluorescein as luminophores and hydrogen peroxide as co-reactant.

INSTRUMENTATION AND SOFTWARE

ECL experiments are performed using SpectroECL instrument (Figure 1) with a microspectrometer cell as detector. Additionally, SpectroECL can be also used with DRP-ECLPHOTODIODCELL that includes a photodiode detector. SpectroECL is controlled by DropView SPELEC, a powerful software for data acquisition in real-time as well as for data treatment and analysis.

Figure 1. DRP-SPECTROECL.

RESULTS

The electrochemical excitation of 2mM luminol in presence of 50 mM hydrogen peroxide in 0.1 M phosphate buffer solution (pH 8) is carried out by linear sweep voltammetry, scanning the potential from 0.00 V to +1.00 V at 0.05 V/s.

Photodiode detector integrated in DRP-ECLPHOTODIODCELL is able to perform electrochemiluminescence experiments recording total ECL signal since it cannot discriminate different wavelengths. This detector is very sensitive and very low concentration can be detected. As can be observed in Figure 2a, the electrochemical response (blue line) shows an oxidation peak at +0.30 V associated with the luminol oxidation. This electrochemical process, in presence of hydrogen peroxide, produces the luminol emission of light at 425 nm. However, as the photodiode detector does not discriminate wavelengths, the evolution of the total light emitted is recorded (green line). ECL signal of luminol in presence of hydrogen peroxide shows its characteristic behavior and the emission of light increases during the oxidation of luminol [4,5]. ECL and electrochemical peaks match exactly at +0.30 V.

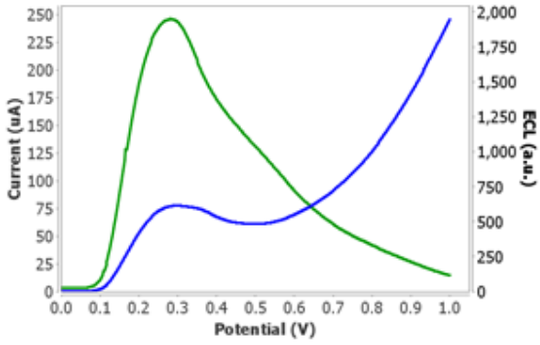
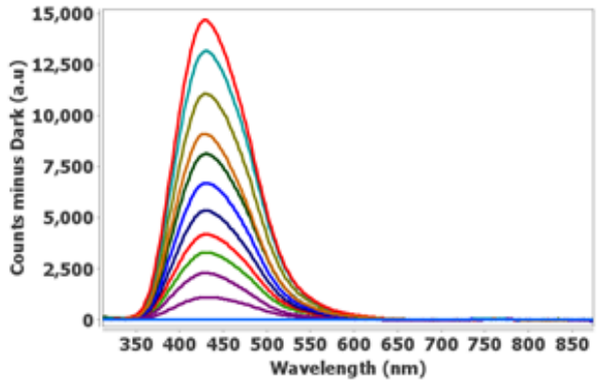


Figure 2. Linear voltammogram (blue line) and ECL signal (green line) obtained in luminol and hydrogen peroxide in PBS solution using DRP-ECLPHOTODIODCELL.

On the other hand, if the same experiment is performed using a microspectrometer as detector, the electrochemical response is exactly the same (blue line in Figure 2a) but the optical response is completely different. In this case, microspectrometer differentiates different wavelengths and as luminol oxidation produces its emission of light at 425 nm, one band located at this wavelength is observed in Figure 3a. Then, as only one luminophore is present in solution, only the emission at one wavelength is noticed.

Evolution of light emitted at 425 nm can be analyzed using "Spectra vs EC" tool of DropView SPELEC. As can be seen in Figure 3b, ECL behavior matches exactly with the ECL response obtained with photodiode detector (green line in Figure 2). ECL emission increases from the beginning of the experiment and it reaches a maximum when the oxidation peak of luminol at +0.30 V is electrochemically observed.

a)



b)

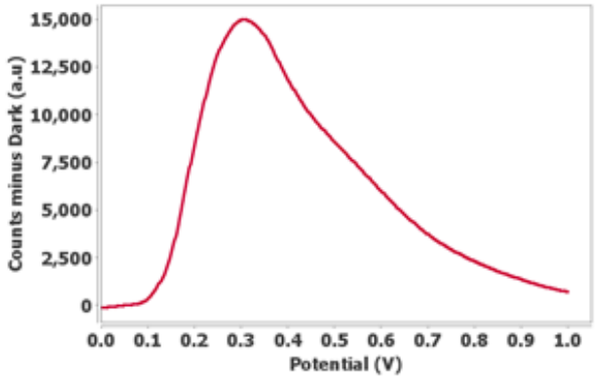


Figure 3. (a) ECL signal obtained in luminol and hydrogen peroxide in PBS solution using microspectrometer cell. (b) Evolution of luminol emission at 425 nm with potential obtained using "Spectra vs EC" tool.

In addition, RET system formed by two luminophores, luminol and fluorescein, and hydrogen peroxide as co-reactant was studied. According to RET mechanism, luminol generates luminescence due to its electrochemical oxidation in presence of hydrogen peroxide. Part of this light emitted is used as excitation source for fluorescein molecule and it re-emits light at a different wavelength than luminol.

Analysis of this system by DRP-ECLPHOTODIODCELL, provide the electrochemical (blue line) and ECL (green line) responses shown in Figure 4. As can be observed, linear voltammogram is the same than without fluorescein (blue line in Figure 2) because only the electrochemical oxidation of luminol takes place. Photodiode detector records ECL total intensity, it increases during the oxidation process of luminol at+0.30 V. However, contribution of luminol and fluorescein cannot be differentiated in the ECL signal due to photodiode does not record the emission spectra.

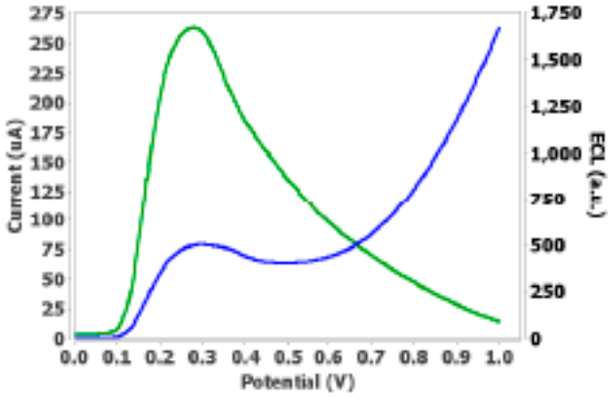
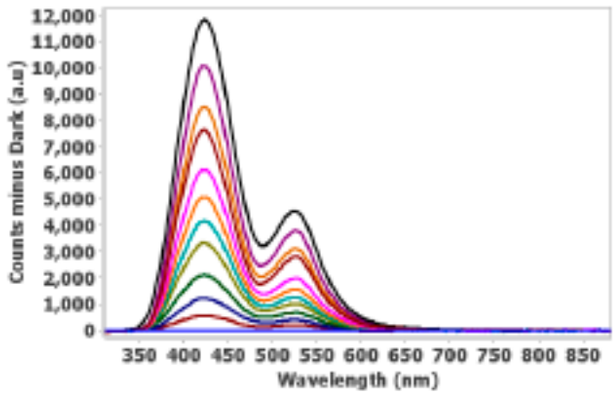


Figure 4. Linear voltammogram (blue line) and ECL signal (green line) obtained in luminol and hydrogen peroxide in PBS solution using DRP-ECLPHOTODIODCELL.

The same experiment carried out using microspectrometer cell shows the same electrochemical behavior (blue line in Figure 4), but the ECL is different than that obtained with DRP-ECLPHOTODIODCELL. Figure 5a shows the spectra simultaneously recorded to the linear sweep voltammetry. As can be observed, two bands are obtained, band at 425 nm corresponds to the luminol emission while band at 530 nm is associated with fluorescein light emitted. Microspectrometer allows us to differentiate luminescence emission coming from different luminophores.

Evolution of luminol and fluorescein emission with potential is analyzed by "Spectra vs EC" tool of DropView SPELEC. As can be noticed in Figure 5b, both luminol and fluorescein emissions increase during the electrochemical oxidation of luminol, reaching a maximum at +0.30 V. However, spectro-electrochemiluminescence response allows us to differentiate the contribution of each luminophore, showing than luminol emission is higher than fluorescein signal.

a)



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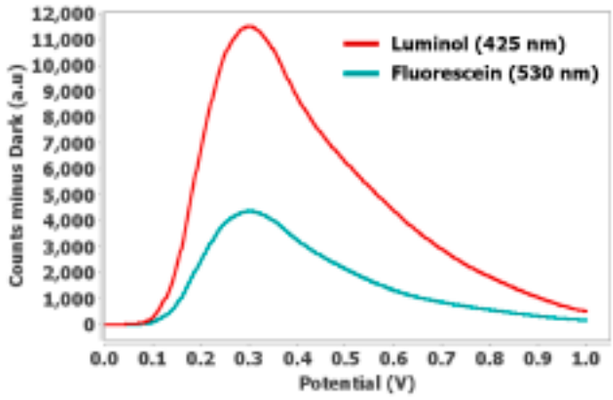

Figure 5. (a) ECL signal obtained in luminol, fluorescein and hydrogen peroxide in PBS solution using microspectrometer cell. (b) Evolution of luminol and fluorescein emission at 425 nm and 530 nm with potential, respectively, using the "Spectra vs EC" tool.

CONCLUSIONS

In this Application Note, ECL system formed by luminol as luminophore and hydrogen peroxide as co-reactant and RET-ECL system based on two luminophores, luminol and fluorescein, and hydrogen peroxide as co-reactant have been studied using SpectroECL with two different detectors. Photodiode detector does not discriminate different wavelengths and is able to perform electrochemiluminescence experiments. This cell records the luminescence total intensity for each electrochemical point. Photodiode cell is very useful for detection of very low concentrations and for research with only one marker species. On the other hand, microspectrometer detector differentiates all photons of each wavelength and allows us to perform spectro-electrochemiluminescence experiments since visible spectra are simultaneously recorded to the electrochemical signal. This cell is useful for multianalyte determination, development of new luminophores and characterization of material properties.

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Method:	**Electrochemistry, Spectroelectrochemistry**
Industry:	**R&D**