



Application Note AN-EC-039

## 双光体同步射的光化学光研究

ECL monitoring of the resonance energy transfer (RET) system formed by luminol and fluorescein

Electrogenerated chemiluminescence, short electrochemiluminescence (ECL), is the emission of light arising from excited states generated by electron transfer reactions at the electrode surface.

This technique offers advantages such as high versatility, excellent sensitivity, a compact,

portable device. In addition, ECL allows precise temporal and spatial control of the reaction [1,2].

This Application Note describes the ECL response when more than one luminophore is present in a solution.

## INSTRUMENTATION AND SOFTWARE

ECL experiments are performed using the SpectroECL instrument equipped either with a microspectrometer cell (Figure 1) or with a photodiode cell (ECLPHOTODIODCELL) as detector.

Carbon screen-printed electrodes (SPEs, 110) are used for performing the ECL experiments.

The SpectroECL is controlled with the DropView SPELEC software, that allows the simultaneous collection of the electrochemical and the emitted light signal. Furthermore, the software includes tools for data treatment and analysis.

Table 1 lists all hardware and software used for this study.



**Figure 1.** The SpectroECL instrument and microspectrometer cell.

**Table 1.** Hardware and software equipment overview.

Equipment	Article number
Instrument	SPECTROECL
Cell	ECLPHOTODIODCELL
Gold SPE	110
Connection cable for SPEs	CAST
Software	DropView SPELEC

## ECL OF LUMINOL LUMINOPHORE

The electrochemical excitation of 0.002 mol/L luminol in the presence of 0.05 mol/L hydrogen peroxide in 0.1 mol/L PBS buffer (pH 8) is carried out using linear sweep voltammetry, scanning from 0.00 V to +1.00 V at 0.05 V/s.

The photodiode detector integrated in the

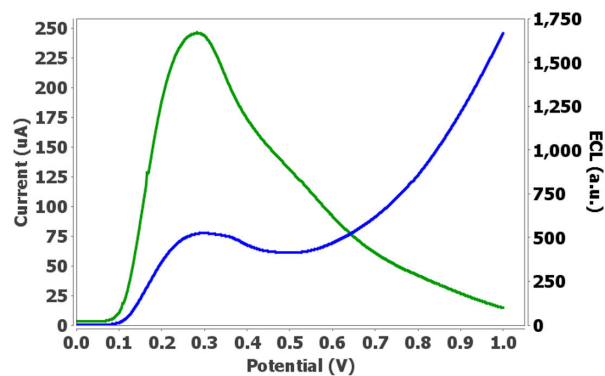
ECLPHOTODIODCELL records the total ECL signal, however it does not provide wavelength resolution.

This detector is highly sensitive, enabling the detection of very low concentrations of the lumiphore under study.

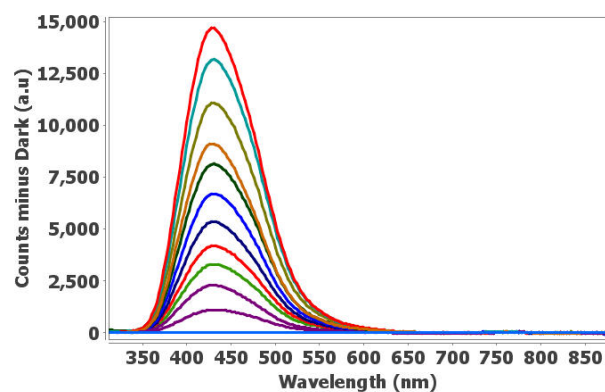
As can be observed in **Figure 2**, the linear sweep voltammogram (blue line) shows an oxidation peak at 0.30 V, corresponding to the electrochemical oxidation of luminol in the presence of hydrogen peroxide. It also generates the emission of light at 425 nm, which is recorded by the photodiode as total light emission (green line). The ECL and electrochemical peaks match exactly at 0.30 V. The ECL signal of luminol in the presence of hydrogen peroxide shows its characteristic behavior with the emission intensity increasing during the oxidation of luminol [3,4].

Repeating the experiment with the microspectrometer as detector shows the same electrochemical response (blue line **Figure 2**), but the optical response is different as the microspectrometer differentiates between wavelengths. The recorded spectrum displays a single emission band at 425 nm, as seen in **Figure 3a**.

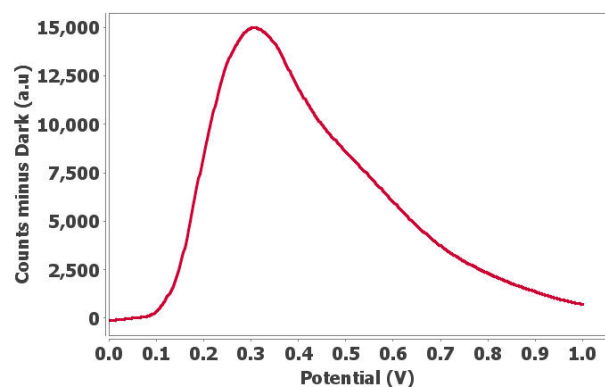
The evolution of the emission at 425 nm as a function of the potential can be obtained using the «Spectra vs EC» tool in DropView SPELEC. As can be seen in **Figure 3b**, the ECL behavior matches exactly with the ECL response obtained by the photodiode detector (green line **Figure 2**). The ECL emission reaches a maximum when the oxidation peak of luminol at 0.30 V is electrochemically observed.



**Figure 2.** Linear voltammogram (blue line) and ECL signal (green line) for 0.002 mol/L luminol and 0.05 mol/L hydrogen peroxide in PBS solution using the ECLPHOTODIODECELL.



**Figure 3a.** ECL signal for 0.002 mol/L luminol and 0.05 mol/L hydrogen peroxide in PBS solution using the microspectrometer cell.



**Figure 3b.** Evolution of luminol emission at 425 nm as a function of the potential.

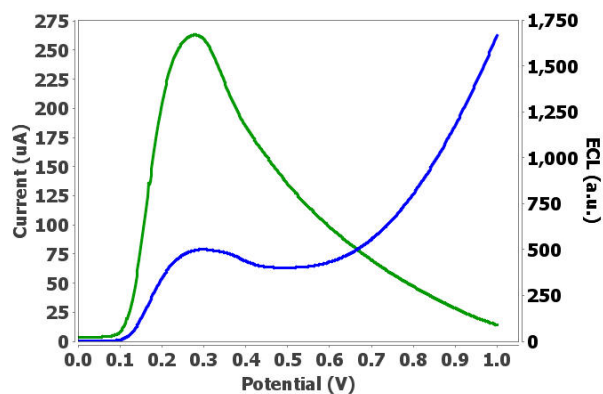
## ECL OF LUMINOL AND FLUORESC EIN LUMINOPHORES

The resonance energy transfer (RET) system formed by the luminophores luminol (donor) and fluorescein (acceptor) [5] is studied. According to RET mechanism, luminol generates luminescence upon its electrochemical oxidation

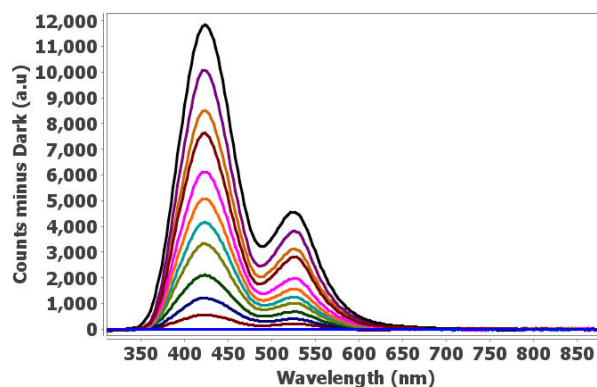
Analysis of this system using the ECLPHOTODIODE CELL provides the electrochemical (blue line) and ECL (green line) responses shown in **Figure 4**. As only luminol undergoes electrochemical oxidation, the voltammogram is identical to the one without fluorescein (**Figure 2**). The photodiode detector records an increase in total ECL intensity during the oxidation of luminol at 0.30 V. However, as photodiode does not record the emission spectra it cannot distinguish the luminol and fluorescein contributions.

When the experiment is repeated using the microspectrometer cell, the electrochemical behavior remains the same (blue line **Figure 4**), but the ECL displays two emission bands. **Figure 5a** shows the spectra recorded during the linear sweep voltammetry. The band at 425 nm corresponds to the luminol emission while the band at 530 nm is associated with the emission of fluorescein. This confirms RET from luminol to fluorescein and demonstrates the ability of the microspectrometer to differentiate between emissions from different luminophores.

in the presence of hydrogen peroxide. Part of this emitted light acts as excitation source for fluorescein, which re-emits light at a different wavelength.

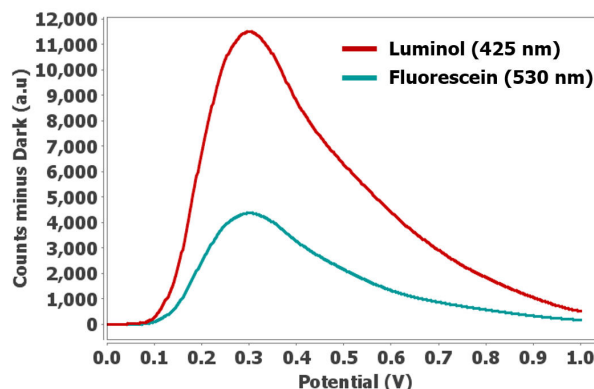


**Figure 4.** Linear voltammogram (blue line) and ECL signal (green line) for 0.002 mol/L luminol, 0.0001 mol/L fluorescein, and 0.05 mol/L hydrogen peroxide in PBS solution using the ECLPHOTODIODE CELL.



**Figure 5a.** ECL signal for 0.002 mol/L luminol, 0.0001 mol/L fluorescein and 0.05 mol/L hydrogen peroxide in PBS solution using the microspectrometer cell.

The potential-dependent evolution of the emissions is analyzed using the «Spectra vs EC» tool in DropView SPELEC. As can be observed in **Figure 5b**, the emissions of both luminol and fluorescein increase during the oxidation of luminol and reach their maxima at 0.30 V. The spectro-electrochemiluminescence response also allows the evaluation of each luminophore's contribution, showing that the luminol emission is higher than the fluorescein signal.



**Figure 5b.** Evolution of luminol emission at 425 nm as a function of the potential.

## CONCLUSION

The ECL system formed by luminol as luminophore and hydrogen peroxide as co-reactant as well as the RET ECL system based on two luminophores, luminol and fluorescein, and hydrogen peroxide as co-reactant have been studied using the SpectroECL with two different detectors.

The photodiode detector does not discriminate between wavelengths and records the total luminescence intensity for each electrochemical point. The photodiode cell is very useful for detection of very low concentrations of the

lumiphore under study and for research with only one marker species.

On the other hand, the microspectrometer detector provides wavelength resolution and allows the performance of spectro-electrochemiluminescence experiments since visible spectra are simultaneously recorded to the electrochemical signal. This cell is useful for multianalyte systems, development of new luminophores, and characterization of material properties.

## REFERENCES

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SpectroECL 将一个双恒位/恒流和一个微型光集成在一个用于网印刷的新池中。小型化的便携式器非常合行致化学光(ECL)量。



用于网印刷的池,可行化学光量。ABS 池包含一个前置放大器的硅光二管,其光范 340 - 1100 nm,峰敏度波 960 nm。

池与  $\mu$ Stat ECL 或  $\mu$ Stat SpectroECL 化学光器配合使用。



### i- $\mu$ Stat

接器将作我网印刷和瑞士万通 DropSens 恒位的接口

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### DropView SPELEC

DropView SPELEC 是一套光化学件及先的数据硬件件,可控制 SPELEC 光,能同步光学和化学量数据。