Application Note AN-EC-039

Spectro-electrochemiluminescence study of simultaneous emission from two luminophores

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].

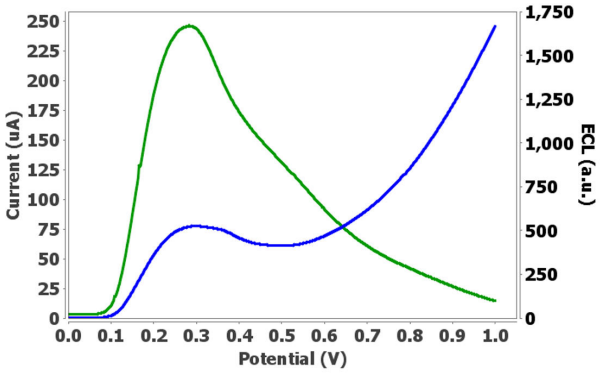

Figure 2. Linear voltammogram (blue line) and ECL signal (green line) for 0.002 mol/L luminol and 0.05 mol/L hydrogen peroxide in PBS solution using the ECLPHOTODIODCELL.

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.

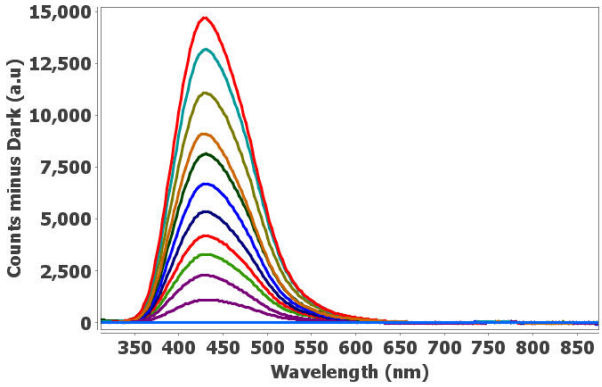

Figure 3a. ECL signal for 0.002 mol/L luminol and 0.05 mol/L hydrogen peroxide in PBS solution using the microspectrometer cell.

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.

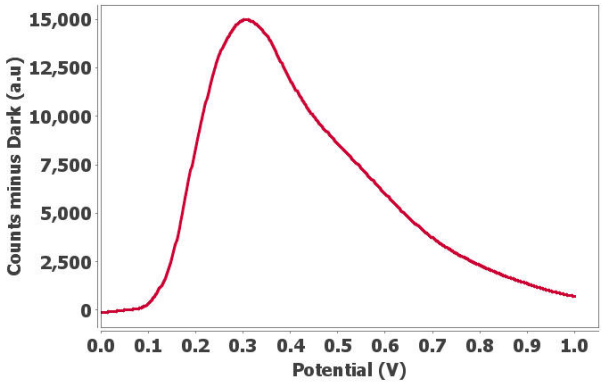

Figure 3b. Evolution of luminol emission at 425 nm as a function of the potential.

ECL OF LUMINOL AND FLUORESCEIN 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 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.

Analysis of this system using the ECLPHOTODIODCELL 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.

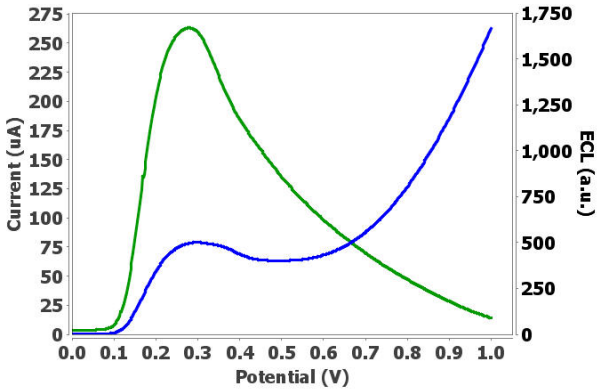

Figure 4. Linear voltammogram (blue line) and ECL signal (green line) for 0.002 mol/L luminol, 0.0001 mol/L fluorescein, and 0.05 mol/L hydrogen peroxide in PBS solution using the ECLPHOTODIODCELL.

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.

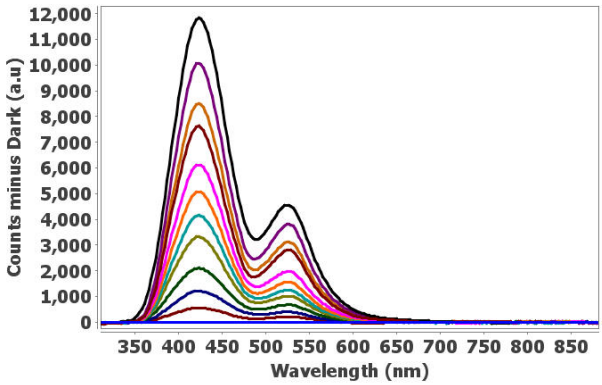

Figure 5a. ECL signal for 0.002 mol/L luminol, 0.0001 mol/L fluorescein and 0.05 mol/L hydrogen peroxide in PBS solution using the microspectrometer cell.

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.

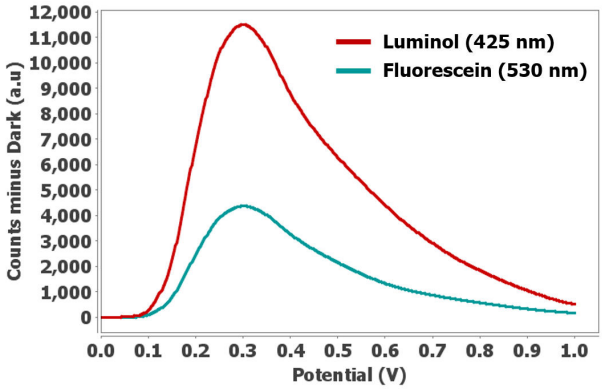

Figure 5b. Evolution of luminol emission at 425 nm as a function of the potential.

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

1. Richter, M. M. Electrochemiluminescence (ECL). *Chem. Rev.* 2004, *104*, 3003–3036. https://doi.org/10.1021/cr020373d.
2. Hu, L.; Xu, G. Applications and Trends in Electrochemiluminescence. *Chem. Soc. Rev.* 2010, *39*, 3275–3304. https://doi.org/10.1039/b923679c.
3. Ballesta-Claver, J.; Valencia-Mirón, M. C.; Capitán-Vallvey, L. F. Disposable Electrochemiluminescent Biosensor for Lactate Determination in Saliva. *Analyst* 2009, *134*, 1423–1432. https://doi.org/10.1039/b821922b.
4. Yildiz, G.; Tasdoven, U.; Menek, N. Electrochemical Characterization of Luminol and Its Determination in Real Samples. *Analytical Methods* 2014, *6*, 7809–7813. https://doi.org/10.1039/C4AY01281J.
5. Neves, M. M. P. S.; Bobes-Limenes, P.; Pérez-Junquera, A. et al. Miniaturized Analytical Instrumentation for Electrochemiluminescence Assays: A Spectrometer and a Photodiode-Based Device. *Analytical and Bioanalytical Chemistry* 2016, *408*, 7121–7127. https://doi.org/10.1007/s00216-016-9669-7.

CONTACT

Metrohm Romania
Str. Emil Racoviță nr. 25
041753 București
office@metrohm.ro

SpectroElectrochemiluminescence instrument for Screen-Printed Electrodes

SpectroECL combines in one equipment a biopotentiostat/galvanostat and a microspectrometer integrated in an innovative cell for Screen-Printed Electrodes. This miniaturized and portable alternative is perfect for performing Electrogenerated ChemiLuminescence (ECL) measurements

Photodiode cell for electrochemiluminescence measurements

Cell for screen-printed electrodes to perform electrochemiluminescence measurements. This ABS cell includes a Silicon photodiode with preamp with a spectral response range 340 - 1100 nm and peak sensitivity wavelength of 960nm.
This cell should be used in combination with µStat ECL or µStat SpectroECL electrochemiluminiscence instruments.

i-µStat Cable Connector for Screen-Printed Electrodes

Connector that act as an interface between our Screen-Printed Electrodes and Metrohm DropSens potentiostats.

DropView SPELEC Software

DropView SPELEC is a Spectroelectrochemical software that controls SPELEC instrument, offering a perfect synchronization of the optical and electrochemical measurements, as well as advanced tools for data treatment.