Spectral Interaction between ECL of Luminol and Different Luminophores - New Spectroelectrochemical Experiments for Students

A. Habekost[*]

University of Education Ludwigsburg, Ludwigsburg, Germany
*Corresponding author: Achim Habekost, A.Habekost@t-online.de

Abstract The resonant energy transfer from luminol / H_2O_2 to various luminophores is described in detail. The emission of light is triggered by the electrogenerated chemiluminescence of luminol. Luminophores can absorb the light and fluoresce. By using a mixture of different dyes, a wide range of emissions can be produced. The luminophore $[Ru(bpy)_3]^{2+}$ / tripropylamine can react in two ways: Either it can fluoresce by resonance energy transfer from the luminol, or it can emit by ECL itself.

Keywords: *Electrogenerated chemiluminescence, fluorovoltammetry, resonant energy transfer*

Cite This Article: A. Habekost, "Spectral Interaction between ECL of Luminol and Different Luminophores - New Spectroelectrochemical Experiments for Students." *World Journal of Chemical Education*, vol. 11, no. 3 (2023): 87-91. doi: 10.12691/wjce-11-3-11.

1. Introduction

Luminol is a chemical that emits light when it reacts with an oxidizing agent such as hydrogen peroxide. This reaction occurs at a certain electrochemical potential and creates an excited state of the oxidation product, 3-aminophthalate, which emits light on returning to its ground state: electrogenerated chemiluminescence (ECL).

There are different photophysical theories of energy transfer from donor to acceptor, called the Förster and Dexter theories. The energy transfer is assumed to be radiationless. In resonant energy transfer (RET), the excited luminol molecule transfers energy to a nearby dye molecule. The dye molecule becomes excited and emits light at longer wavelengths. The distance between the luminol and dye molecules r(donor-acceptor) and the spectral overlap between luminol emission and dye absorption determine the efficiency of the rate of the energy transfer $k_{donor->acceptor}$: The rate is proportional to the sixth-order Förster radius (the radius where 50% of the donor energy is transferred to the acceptor). At much smaller distances, even an electron can be transferred (Dexter mechanism). Our own measurements cannot resolve the nature of the energy transfer. Therefore, we use the non-specific term "resonant energy transfer" (RET). [1,2].

Several dyes can be used in RET experiments with luminol, including fluorescein, rhodamine and cyanine dyes. The desired spectral characteristics of the emitted light determine the choice of dye.

In this article, we distinguish between chemiluminescence to fluorescence energy transfer (CF-RET) and chemiluminescence to fluorescence energy-transfer following by chemiluminescence at a higher potential (CF-C-RET) [3].

Figure 2 shows the oxidation of luminol at the electrode and the subsequent reaction with H_2O_2, which produces an excited state of the product, 3-aminophthalate. This emits radiation.
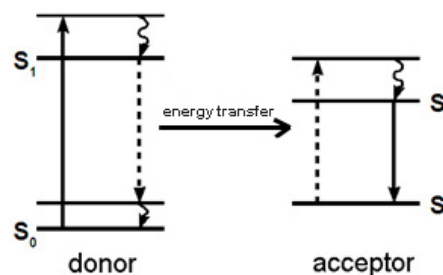

Figure 1. Scheme of energy transfer from donor to acceptor: After the electrochemical activation of the donor, it is oxidized and becomes chemiluminescent; the acceptor absorbs the emitted energy and, after vibrational relaxation, emits itself in the excited singlet state S1 (curved line).
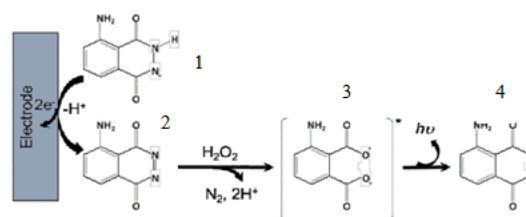

Figure 2. ECL of luminol (1): Electrochemical oxidation to diazoquinone (2), oxidation with H_2O_2 to 3-aminophthalate in an excited state (3) and after emission of light ground state of 3-aminophthalate (4).

The emission is broadband from 380 nm to about 600 nm [1].

The different types of energy transfer by different dyes are demonstrated here:

Transfer to fluorescein (CF-RET).

Transfer to a binary mixture of dyes ([Ru(bpy)$_3$]$^{2+}$ / fluorescein and riboflavin / rhodamine B) (CF-RET-double).

Transfer to another fluorescence and ECL system (CF-RET-C): [Ru(bpy)$_3$]$^{2+}$ / tripropylamine.

[Ru(bpy)$_3$]$^{2+}$, eosin, rhodamine B, rhodamine 6G, riboflavin and fluorescein have absorption bands around the luminol emission. So, it can be said that the external light source is the chemiluminescence of Luminol and the color of the chemical system can be changed by changing the dye.

In this thesis, ECL and fluorescence behaviour was investigated by two different experimental methods: Firstly, the emitted light is detected as a whole with a photodiode, and secondly, the emitted light is spectrally resolved using a spectrometer.

It is shown that spectral resolution is required to understand how energy is transferred.

For the measurement of the total emission we use a commercially available potentiostat-photodiode combination. However, we combine a potentiostat with a fibre spectrometer for spectral resolution measurements, rather than using a commercial spectroelectrochemical apparatus as used by Ibanez et al [4]. This is much less expensive, but has the disadvantage that two separate software packages have to be used for the measurements.

The separation of the potentiostat from the spectrometer has the didactic advantage of allowing the students to have a better understanding of the two different types of measurements: electrochemical (cyclic voltammetry) and spectroscopic (potential-dependent fluorescence and ECL).

2. Pedagogical objectives

We believe that the combination of electrochemical and spectroscopic experiments is the pedagogical value of the experimental procedures described. The reactions of the electrodes can be directly observed by means of ECL and the emitted light can be detected as a function of the applied potential. This means that ECL and cyclic voltammetry (CV) can be measured in a synchronized manner.

Energy transfer can only be observed by correlation of the cyclic voltammogram with spectrally resolved emission and not by detection of the emission as a whole. This is going to be discussed in this article.

3. Experiments

3.1. Instruments and Chemicals

Materials: Potentiostat (ECL-Stat from Metrohm / DropSens), ECL cell (Metrohm / DropSens), fiber spectrometer (Avaspec ULS 2048 from Avantes), Fiber (500 μm, Avantes), SPE: DRP 220 Au-Bt (Metrohm /

DropSens: working electrode: low-temperature gold ink, counter electrode: gold, pseudo reference electrode: silver), absorption and fluorescence spectrometer: SpectroVis, Vernier), absorption spectrometer: AvaLight DHC with cuvette holder, both from Avantes.

Software: DropView (Metrohm / DropSens), Avasoft (Avantes), Qtiplot 0.9.9, IONDEF.

Chemicals: Eosin (E6003, Merck), Rhodamin B (R6626, Merck), Rhodamin 6G (252441, Merck), Fluorescein (46955, Merck), Luminol (123072, Merck), Rutheniumtrisbipyridylchloride (Tris(2,2'-bipyridyl)ruthenium(II) chloride hexahydrate ([Ru(bpy)$_3$]$^{2+}$Cl$_2$*10H$_2$O) (T1655, TCI), H$_2$O$_2$ (35%, 108600, Merck), buffer pH 7,9.

Concentration of all dyes: 1 mmol in 100 mmol buffer, H$_2$O$_2$: 5 mmol in 100 mmol buffer.

A commercial potentiostat-photodiode (last in a light-blocking housing) is used to measure the total emission. For spectrally resolved emission, a potentiostat-fiber spectrometer combination is used. In this case, the emitted light is collimated by a lens into a fiber (500 μm) connected to the spectrometer. The volume of the solution is 60 μL, which is pipetted onto the SPE.

Figure 3 shows the experimental setup with the potentiostat-photodiode (top) and potentiostat-photometer combination (bottom).

Figure 3. Two used experimental setups. Top: Potentiostat and ECL cell with photodiode. Bottom: Potentiostat with spectrometer and homebuilt cell with lens, fiber and the adapter for SPE.

Figure 4 shows the software screenshot for the ECL measurement. The potentials of the cyclic voltammogram can be set on the left, and the amplification factor for the ECL intensity can be set on the right. Figure 4 shows the screenshot for combining spectral and CV measurements: On the left: the integration time and the measurement of the intensity or absorbance with a dark or reference spectrum for the baseline measurement.
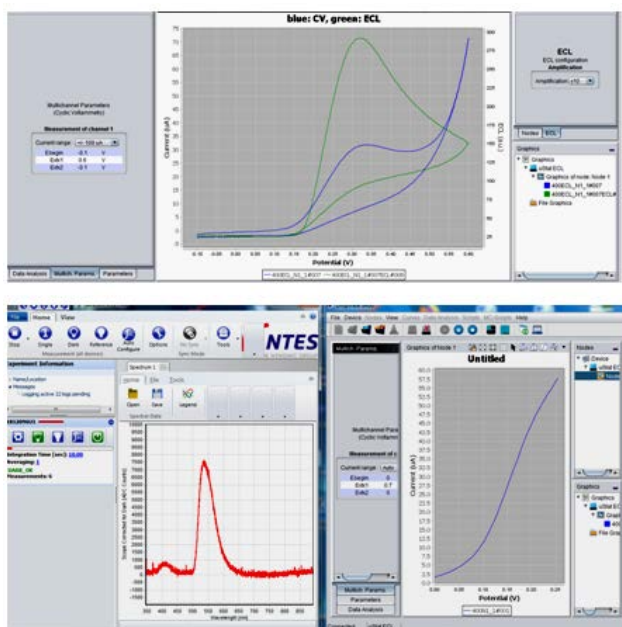

Figure 4. Top**:** Screenshot ECL-measurement: blue: CV, green: ECL. Bottom: Screenshot spectral (left) and the electrochemical curve (right).

3.2. Emission of luminol and absorbance of various dyes
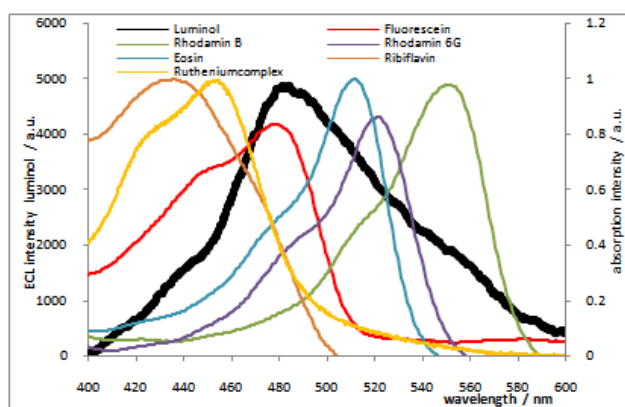
Figure 5. Spectral overlap between the emission of luminol (black line) and the absorption of fluorescence (colored lines).

Figure 5 shows the spectral emission of luminol (+ H_2O_2, black line) and the absorbance of several dyes such as rhodamine B, rhodamine 6G, eosin, fluorescein, riboflavin and $[Ru(bpy)_3]^{2+}$. Generally, the spectral overlap between the ECL of luminol and the absorbance is more or less marked. Figure 6 shows the CV (black line) and the potential dependent ECL (red line) of pure luminol / H_2O_2.
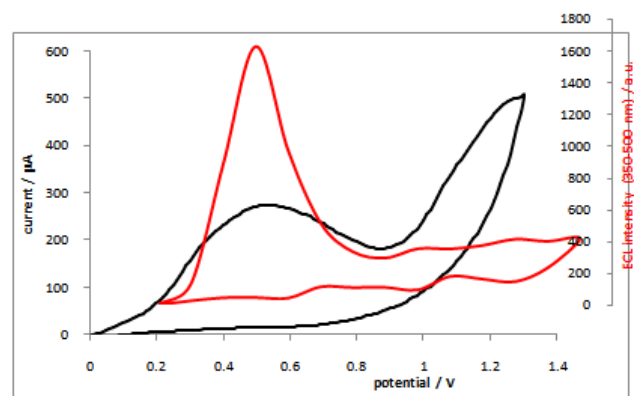

Figure 6. Luminol / H_2O_2: CV (black) and ECL intensity as a function of the potential (red).

It shows that the anodic peak begins at about 0.1V (black line), the onset of the luminol reaction in Figure 1. At the same potential, the intensity of the ECL (red line) increases. Figure 7 shows this potential dependent ECL spectrum in three dimensions.
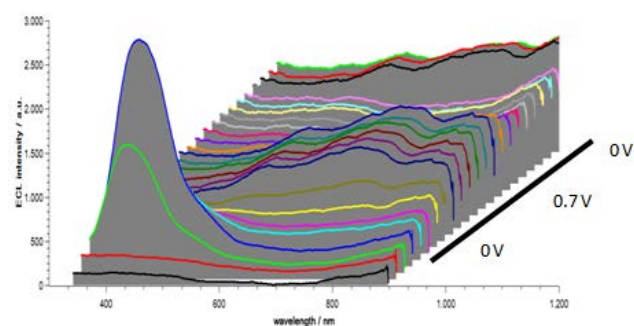

Figure 7. 3D image of the potential-dependent ECL spectrum of luminol from 0 V to 0.7 V and back.

3.3. Energytransfer to fluorescein (CF-RET).

Figure 8 shows the CV and the fluorovoltammogram (potential-dependent fluorescence) of fluorescein from 500 to 560 nm.
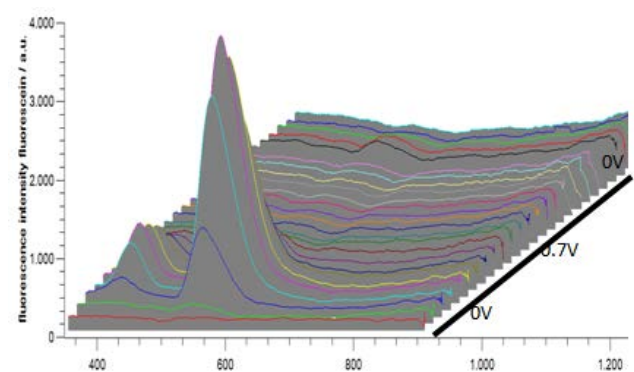

Figure 8. 3D image of the potential-dependent ECL (luminol) and fluorescence (fluorescein).

Fluorescein produces fluorescence (emitting around 530 nm) but not ECL. As can be seen in Figure 8, the fluorescence of fluorescein already starts at the potential of the luminol ECL (0.1 V). This means that there has to

be an energy transfer from the emission radiation of luminol to the absorption of fluorescein. Both ECL of luminol and fluorescence of fluorescein can be observed beginning at 0.1 V as shown in Figure 8. Higher potentials as 0.4 V cause both to decrease. The fluorovoltammogram (Figure 9) also shows this behaviour.
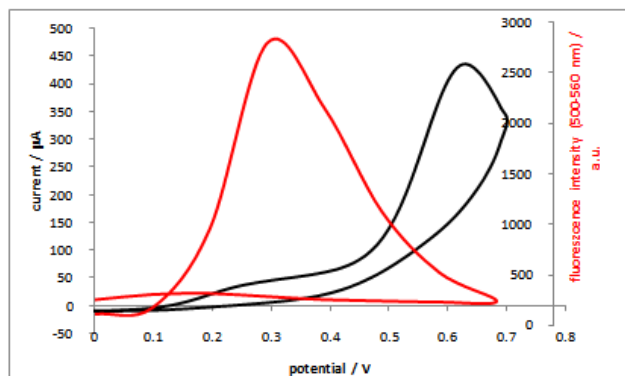

Figure 9. Fluorovoltammogram of luminol/fluorescein: CV (black line) and fluorescence of fluorescein (red line).

3.4. Energytransfer to a binary mixture of dyes (CF-RET-double)

The dyes studied have emission spectra that are very close together. Thus, in a mixture of luminol with two dyes $[Ru(bpy)_3]^{2+}$ / fluorescein or riboflavin / rhodamine B, the emission spectra cannot be fully resolved (Figures 10 and 11).
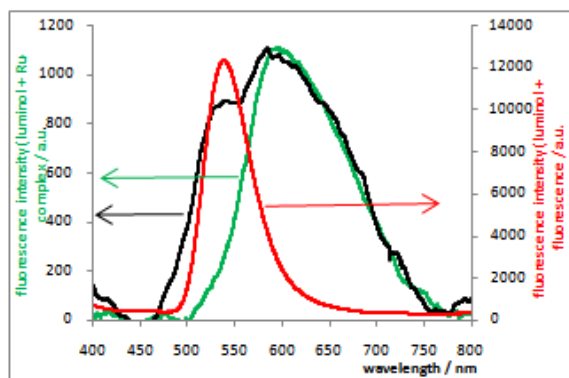

Figure 10. Emission spectra of luminol + fluorescein (red), luminol + [Ru(bpy)3]2+ (green) (all concentrations were 1 mmol/L) and luminol + fluorescein + [Ru(bpy)3]2+ (black). In the last mixture the concentration of fluorescein was 0.1 mmol/L. All spectra were recorded at 0.3 V for 20 s.
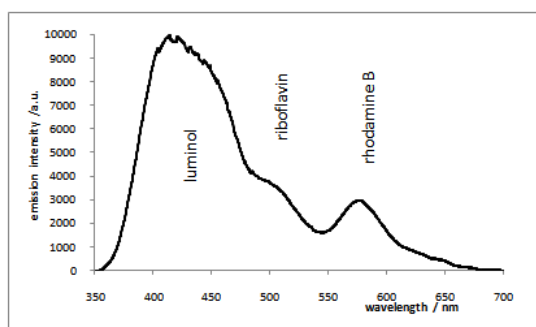

Figure 11. Emission spectrum of luminol + riboflavin + rhodamine B (all concentrations were 1 mmol/L). Spectrum was recorded at 0.3 V for 20 s.

A mixture of different dyes can be used to increase the bandwidth of the emission and thus the colour of the emission over a small range.

3.5. Energytransfer to another fluorescence and ECL system (CF-C-RET): $[Ru(bpy)_3]^{2+}$ / tripropylamine.

$[Ru(bpy)_3]^{2+}$ / tripropylamine is one of the best investigated ECL system [5,6,7,8].

Figure 12 shows the emission spectra of a mixture of Luminol / H_2O_2 and $[Ru(bpy)_3]^{2+}$ / tripropylamine. The total emission is not spectrally resolved. It is clear that the emission takes place at two different potentials: at the ECL of the luminol (starting at 0.15 V) and at the ECL of the $[Ru(bpy)_3]^{2+}$ / tripropylamine (starting at 0.7 V).

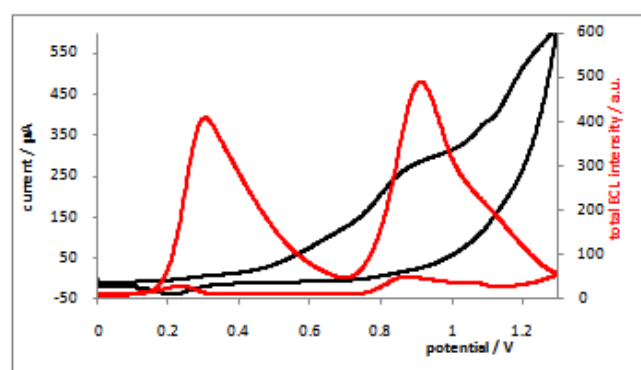**Figure 12.** CV (black) and total emission of luminol / H_2O_2 + $[Ru(bpy)_3]^{2+}$ / tripropylamine (red).

The spectrally resolved emission spectra as a function of potential are shown in the 3D image (Figure 12). From about 0.15 V, the energy of the luminol ECL emission is partially transferred to $[Ru(bpy)_3]^{2+}$ (emission around 600 nm). At about 0.7 V the $[Ru(bpy)_3]^{2+}$ - isopropylamine ECL occurs. Note that there are not three emission peaks between 0.1 V and 0.5 V. The dip around 450nm is due to $[Ru(bpy)_3]^{2+}$ absorption (see Figure 13).

In conclusion, spectro-electrochemiluminescence is an instructive method for the study of energy transfer from luminol to various dyes or dye mixtures. It has been shown that only a spectrally resolved emission can provide evidence of the transfer of energy from the luminol to the dyes.
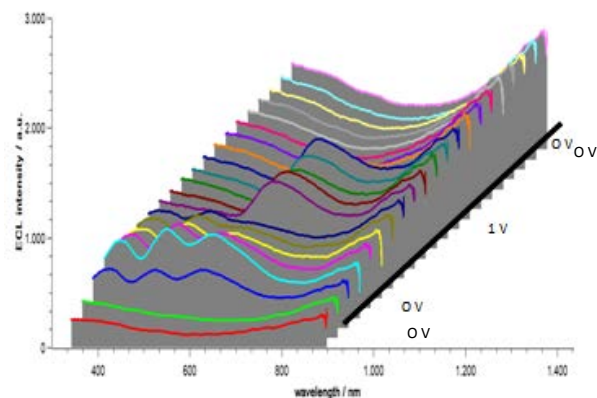

Figure 13. 3D image of the spectrally resolved emission of luminol / $[Ru(bpy)_3]^{2+}$ at potentials between 0 and1 V and back.

Acknowledgement

References

[1]   K. Hiramoto, E. Villani, E., T. Iwama, T., K. Komatsu, S. Inagi, K.Y. Inoue, Y. Nashimoto, K. Ino, H. Shiku, Recent Advances in Electrochemiluminescence-Based Systems for Mammalian Cell Analysis. Micromachines, 2020, 11, 530.

[2]   M. Tausch, Chemie mit Licht, Innovative Didaktik für Studium und Unterricht, Springer, Berlin, 2019, p121-122.

[3]   A. Roda, M. Guardigli, E. Michelini, Nanobioanalytical luminescence: Förster-type energy transfer methods, Anal. Bioanal. Chem. 2009, 393,109.

[4]   D. Ibanez, M.B. Gonzalez-Garcia, D. Hernandez-Santos, P. Fanjul-Bolado, Understanding the ECL interaction of luminol and $[Ru(bpy)_3]^{2+}$ luminophores by spectroelectrochemiluminescence, Phys. Chem. Chem. Phys. 2020, 22, 18261.

[5]   W. Miao, J.P. Choi, A.J. Bard, Electrogenerated Chemiluminescence 69: The Tris(2,2'-bipyridine)ruthenium(II), $(Ru(bpy)_3^{2+})$/Tri-n-propylamine (TPrA) System Revisited - A New Route Involving TPrA $\bullet$+ Cation Radicals, J. Am. Chem. Soc. 2002, 124, 14478.

[6]   A. Kapturkiewicz, Electrogenerated chemiluminescence from the tris(2,2'-bipyridine)ruthenium(II) complex, Chem. Phys. Lett. 1995, 236, 389.

[7]   D. M. Hercules, F. E. Lytle, Chemiluminescence from reduction reactions, J. Am. Chem. Soc. 1966, 88, 4795.

[8]   M. M. Richter, Electrochemiluminescence (ECL), Chem. Rev. 2004, 104, 3003.