World Journal of Chemical Education, 2016, Vol. 4, No. 3, 64-66
Available online at http://pubs.sciepub.com/wjce/4/3/3

DOI:10.12691/wjce-4-3-3

Short Communication: Electrochemiluminescence on the Basis of tris(bipyridyl)ruthenium(II) – Further Analytical Experiments

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Abstract Electrogenerated chemiluminescence (ECL) is an analytical process that is of fundamental interest for determining low concentrations of amino acids and related compounds (e.g., the broadband herbicide glyphosate). Nano zinc oxide or titanium (IV) oxide can enhance the analytical sensitivity by a factor of about 10.

Keywords: *four-year undergraduate, beginner PhD student, analytical, electrochemistry, chemiluminescence, HPLC, hands-on learning/manipulatives*

Cite This Article: A. Habekost, "Short Communication: Electrochemiluminescence on the Basis of tris(bipyridyl)ruthenium(II) – Further Analytical Experiments." *World Journal of Chemical Education*, vol. 4, no. 3 (2016): 64-66. doi: 10.12691/wjce-4-3-3.

1. Introduction

In [1] I presented illustrative experiments with electrogenerated chemiluminescence (ECL) for introducing students to the capability of ECL on the basis of tris(2,2'-bipyridyl)ruthenium (II) ($Ru(bpy)_3^{2+}$) with different co-reactants. Easily implemented experimental setups were presented that show the correlation between ECL intensity and cyclic voltammetry (CV) for different $Ru(bpy)_3^{2+}$ / coreactant systems. Furthermore, ECL can be an interesting means to quantitatively identify amino acids and related compounds such as glyphosate. In this paper we describe experiments that can detect the abovementioned substances at extremely low concentrations.

2. Pedagogical Objectives

A voltage-induced redox reaction that forms a species in a radiative excited state is not only an aesthetic phenomenon, but of fundamental interest in analytical chemistry. Understanding ECL needs as much knowledge about electrochemistry as about photochemistry.

In this paper I present some significant ECL results for quantitatively determining selected amino acids and glyphosate with the most commonly used reagent in ECL, tris(bipyridyl)ruthenium^{2+} ($Ru(bpy)_3^{2+}$). The experimental equipment is easy to set up, and the experiments are suitable for introducing students to modern analytical approaches that investigate organic substances with an amino group.

3. Experiments

As the experimental setup has already been characterized in [1], in this paper I will only describe the extending aspects such as the application to quantitatively identify, for example, amino acids in a very low concentration range.

3.1. ECL Detection Limit for Different Amino Acids [2,3]

Chemicals and instruments:

Tris(2,2'-bipyridyl)ruthenium (II) (TCI, T 1655) solution in a phosphate buffer (prepared by equimolar amounts of disodium hydrogen phosphate Na_2HPO_4 and potassium dihydrogen phosphate KH_2PO_4), zinc oxide nanopowder (544906, Sigma Aldrich, 34967), glyphosate (Sigma Aldrich, 54521), titanium (IV) oxide (Sigma Aldrich, 718467), and double-distilled water.

Potentiostat µ-Stat 400 (DropSens), three electrode device (screen-printed electrodes DRP-250AT: Au as working, Pt as counter, Ag as reference electrode), data acquisition system (Sensor Cassy, Leybold didactic, Germany), power supply (Power Cassy, Leybold didactic, Germany), photomultiplier (R4220P, Hamamatsu), power supply for photomultiplier (LKB, Bromma, USA), micropipette (Transferpette, Brand, 100 mL), fiberglass rod (diameter: 4 mm), modified HPLC cell, HPLC flow cell (80 µL), HPLC detector (Kontron, 432), Rheodyne valve 7497, HPLC pump (Kontron, 422).

Procedure: In detail: The screen-printed electrodes are connected to the potentiostat for cyclic voltammograms (CV) or the Power Cassy pulse generator. Typical CV parameters are: start potential 0.7 V; reverse potential 1.3 V; final potential 0.7 V; and scan rate 10 mV/s to 0.5 V/s.

Power Cassy is a programmable interface that can generate pulses with different forms (rectangular, sinusoidal, triangular) or DC, different potentials, and different pulse widths. Typical pulse widths are 200 to 300 ms for rectangular pulses. The voltage ranges between 1 V (low

signal) and 3.3 V (high signal). The electrochemical oxidation of $Ru(bpy)_3^{2+}$ occurs at about 3.2 V vs mass.

A fiberglass rod is placed directly above the working electrode (WE) (1 mm above the solution, realized with a stepping-motor driven laboratory jack). The ECL signal is collected with the fiberglas and fed into the photomultiplier (operating voltage between -800 V and about -1,200 V). The output of the photomultiplier is digitized via Sensor Cassy, and the "ECL intensity vs time" diagrams are recorded. The volume of the solution is about 50 µL.

Alternatively, I use a modified HPLC absorption cell (volume: 80 µL). We mill the bottom in such a way that a step of about 0.1 mm arises. The screen-printed electrodes are bonded and the working electrode (4 mm diameter) is directly below the fiberglas that is mechanically fixed in a 4 mm hole. The inlet and outlet of the cell, rectangular for the detection, is closed with appropriate screws (see Figure 1).

To support the reaction scheme, Brune [2] made stoichiometry studies to measure the optimum mixing ratio between $Ru(bpy)_3^{2+}$ and co-reagents. Within experimental error, their measurements suggest a 2:1 stoichiometry. We took this into account in our own experimental approaches. In all test series $Ru(bpy)_3^{2+}$ is significantly in excess.

Before each measurement, the reagents are freshly prepared: 200 µL of the aqueous 40 mmol Ru-solution is mixed with 100 µL of an aqueous solution of amino acid (resp. glyphosate, see 3.2) in different concentrations. For some measurements, zinc oxide or titanium (IV) oxide nanopowder (0.1 mg, respectively) is added and the suspension stirred thoroughly.

Figure 1 shows the screen-printed electrodes with the photomultiplier tube and the modified ECL cell.

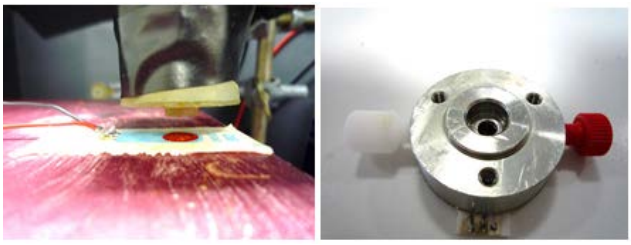

Figure 1. Left: Screen-printed electrodes with photomultiplier tube. Right: Modified ECL cell; at the bottom of the cell are the screen-printed electrodes

Figure 2 top shows the results of the ECL signal of alanine, proline, and serine as a function of concentration. Figure 2 bottom shows only proline at low concentrations.

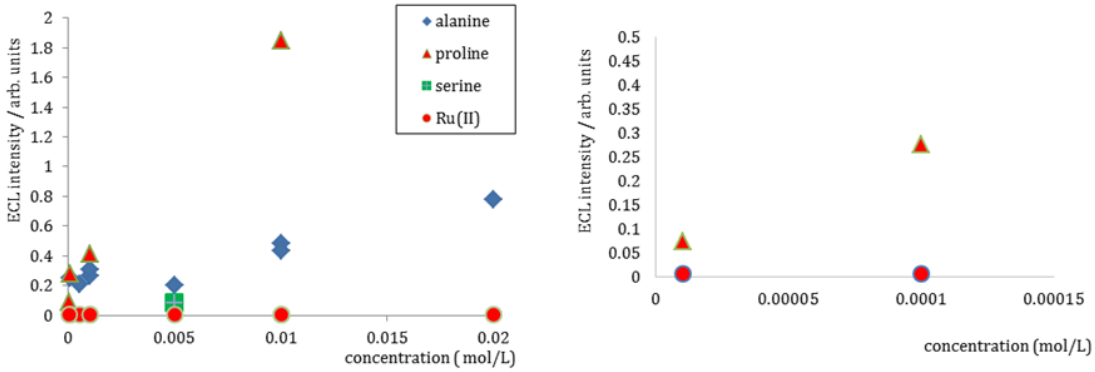

Figure 2. Top: Concentration-dependence of the ECL signal of three amino acids. The background (only Ru(II)) is quite low. Bottom: ECL intensity of proline (and $Ru(bpy)_3^{2+}$) at low concentrations.

The results are in accordance with Brune's experiments [2]: Proline, a secondary amine, shows the highest sensitivity and serine the lowest sensitivity. Brune *et al.* interpret this behavior with the electron donating and withdrawing effect of the different chains of amino acids: While the OH-group in serine (withdrawing character) leads to a lowering of the ECL, proline shows the opposite effect. In our measurements the lowest detectable concentration of proline is about $3*10^{-6}$ mol/L. This means that the volume of 100 µL contains $3*10^{-10}$ mol or about 33 ng proline (molecular weight: 115 g/mol). The detectable limiting concentration of serine is 10^{-4} mol/L.

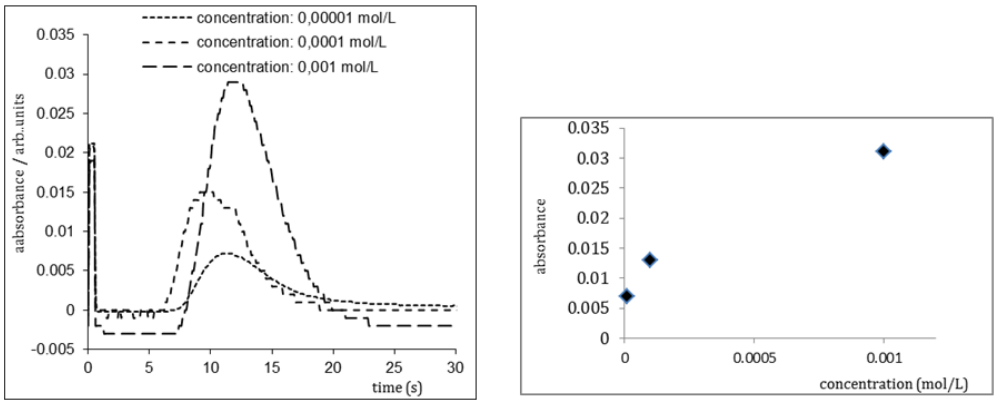

Figure 3. Top: Detection of the absorption of the proline-ninhydrine complex (different proline concentrations) at 420 nm. Bottom: Absorption vs. concentration

To compare the ECL with the absorption sensitivity, proline is mixed with ninhydrin (tenfold excess) heated to about 60°C for 15 minutes and then analyzed in an absorption flow cell of an HPLC absorption detector. The solution is inserted with a readyne valve into a hose system and pumped through the cell with an HPLC pump (pump speed: 1 mL/min, distilled water as eluent).

A comparison between Figure 2 and Figure 3 suggests that the sensitivities of ECL and absorption are comparable.

3.2. ECL Detection Limit for Glyphosate [4-7]

At present, glyphosate is of exceptional interest to the general public. The German Environmental Institute in Munich has shown that some species of bears are affected by glyphosate (http://www.umweltinstitut.org/aktuelle-meldungen/meldungen/umweltinstitut-findet-glyphosat-in-deutschem-bier.html). The testing method was an enzyme-linked immunosorbent assay (ELISA) with a sensitivity of about 1 µg/L.

Chiu *et al*. [4] coupled capillary electrophoresis with ECL and detected glyphosate and aminomethylphosphonic acid (AMPA); the latter is the degradation product of glyphosate when applied to weeding. The detection limit for glyphosate and AMPA in water is 0.06 mg/L and 4.04 mg/L, respectively.

Therefore, we tested the ECL detection limit of glyphosate. Fig. 4 shows the ECL intensity of a 10^{-5} mol/L glyphosate solution after several excitation voltage pulses in a row (half width 200 ms). The red dashed line is the detection limit (only Ru(II)) of glyphosate, which is lower than 1.7 mg/L (molecular weight: 169 g/mol). This result is quite sufficient, but demonstrates that this method is more insensitive than ELISA.

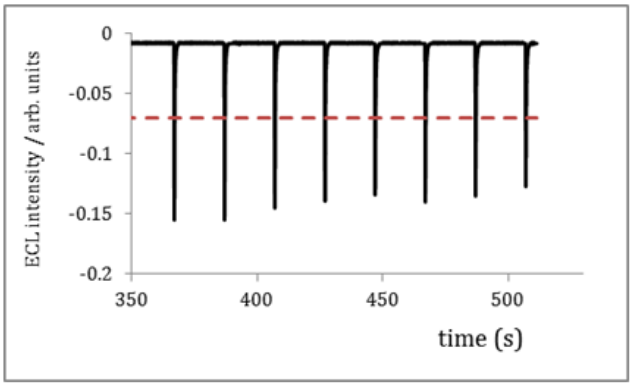

Figure 4. ECL signal of glyphosate solution (10^{-5} mol/L). Dashed red line: ECL of $Ru(bpy)_3^{2+}$ (background).

3.3. Enhancement of the Detection Limit with ZnO Nanopowder and with TiO_2

The detection limit can be enhanced by adding zinc oxide nanopowder to the solution under investigation. We mixed the amino acid (or glyphosate)–ruthenium system (overall 300 µL) with different quantities of ZnO and put the suspension either onto the screen-printed electrodes or into the cell. We do not observe markable differences between the methods. Depending on the quantity of ZnO used, the enhancement of the ECL signal is up to a factor of 10 for glyphosate (see Figure 5) and 3 for TiO_2. Quantities higher than 1 mg produce light scattering effects that reduce the ECL intensity toward the detector.

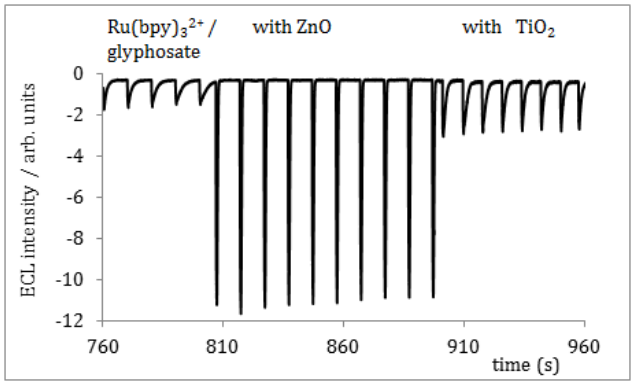

Figure 5. ECL intensity of 0.05 mol/L glyphosate solution without (760–800 s) and with (800–900 s) ZnO (1 mg) and with (900–960 s) TiO_2 (1 mg). Photomultiplier voltage: -1,200 V

It is obvious that the ECL peak width becomes lower with additional ZnO.

A possible reason of this phenomenon may be enhancement of the active electrode surface with the semiconductor ZnO, together with the formation of an additional redox system: ZnO has a band gap of about 3.2–3.4 eV. Therefore, at a voltage of 3.3 V an electron-hole pair separation occurs that can affect the $Ru(bpy)_3^{2+}$ co-reactant system. The effect of TiO_2 is less pronounced. We think that this can result from the lower surface area compared to ZnO, because we did not use TiO_2 nanoparticles.

As a resume the “supported ECL method” with ZnO is about a hundred times less sensitive for glyphosate than the ELISA test but much cheaper, uncomplicated, and fast.

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