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Investigations of Some Reliable Electrochemiluminescence Systems on the Basis of tris(bipyridyl)Ruthenium(II) for HPLC Analysis

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Abstract Electrogenerated chemiluminescence (ECL) is not only an aesthetic phenomenon, but also of fundamental interest in analytical chemistry Several experiments are presented to introduce students to the capacity of ECL on the basis of tris(2, 2'-bipyridyl)ruthenium (II) ($Ru(bpy)_3^{2+}$) with different coreactants.. First, we summarize some spectroscopic and electrochemical features of $Ru(bpy)_3^{2+}$ that are important concerning ECL. Easily implemented experimental set-ups are presented, which show the correlation between ECL-intensity and cyclic voltammetry (CV) for different $Ru(bpy)_3^{2+}$ / coreactant systems. The time-dependence of the ECL-decay is measured on a millisecond time-scale with a conventional data acquisition system. Finally, we present an ECL detector that can be used in HPLC as an alternative to adsorption and fluorescence detection.

Keywords: *four-year undergraduate, beginner PhD student, analytical, UVVIS and fluorescence spectroscopy, mass spectrometry, electrochemistry, chemiluminescence, HPLC, hands-on learning/manipulatives*

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1. Introduction

Since the 1960s, the electrochemiluminescence (ECL) technique has become more and more attractive in analytical chemistry [1]. ECL involves the generation of an excited state of the commonly used and intensively investigated tris(2, 2'-bipyridyl)ruthenium (II) ($Ru(bpy)_3^{2+}$) on an electrode surface. First of all, the $(Ru(bpy)_3^{2+}$ is oxidized and then undergoes an electron-transfer reaction with a coreactant. During the latter process, an excited state is created that subsequently decays, and light is emitted. ECL represents a "marriage of electrochemical and spectroscopic methods" [2]. An excellent review of ECL is given by Miao [2], Kapturwicz [3], Hercules [4], Richter [5], Parveen [6] and Bard [7].

ECL can be used as a supplement to conventional HPLC detection methods (absorption and fluorescence) to quantitatively identify substances. ECL offers several advantages [4]: the experimental set-up is rather simple, the detection limit is low, and ECL needs no derivatization to incorporate a chromophore into the molecule under investigation..

$Ru(bpy)_3^{2+}$ is used for the identification of coreactants such as aliphatic amines [6], amino acids [8,9,10], derivatized amino acids [11,12] and glyphosate [13,14]. $Ru(bpy)_3^{2+}$ has a strong luminescence; it is readily soluble in water and in non-aqueous media at room temperature; it undergoes a one-electron transfer reaction at moderate potentials, thereby leading to stable reduced and oxidized species; and $Ru(bpy)_3^{2+}$) can be regenerated after the emission.

In the $Ru(bpy)_3^{2+}$ system the ECL-reaction can be expressed in the following way, here with tripropyleamine (Pr_3N) as a coreactant [15]:

Scheme: Reaction mechanism ECL

$$Ru(bpy)_3^{2+} \rightarrow Ru(bpy)_3^{3+} + e^- \quad (1)$$

$$Ru(bpy)_3^{3+} + Pr_3N \rightarrow Ru(bpy)_3^{2+} + Pr_3N^{\bullet +} \quad (2)$$

$$Pr_3N^{\bullet +} \rightarrow Pr_2NC^{\bullet}HEt + H^+ \quad (3)$$

$$Ru(bpy)_3^{3+} + Pr_2NC^{\bullet}HEt \rightarrow Ru(bpy)_3^{2+*} + Pr_2N^+ = CHEt \quad (4)$$

$$Ru(bpy)_3^{2+*} \rightarrow Ru(bpy)_3^{2+} + h\nu \quad (5)$$

(Pr: propyl, Et: ethyl, hν: energy of the emitted light).

Reaction (1) may be an electrode process or a direct oxidation with an oxidizing agent.

In contrast to the above scheme, there may be a direct oxidation of Pr_3N (sometimes as a competition process).

$Ru(bpy)_3^{2+}$ reacts with the $Pr_2NC{\cdot}HEt$ radical to form $Ru(bpy)_3^{2+*}$, a species in an excited state that undergoes radiative decay. In addition, Eqs. (3) shows that the forming of $Ru(bpy)_3^{2+*}$ with the subsequent emission of light is pH-dependent [13].

Table 1. Some striking photophysical, photochemical and electrochemical features of $Ru(bpy)_3^{2+}$

Feature	$Ru(bpy)_3^{2+}$
Absorption	In H_2O: 185 nm, 208 nm, 285 nm (all ligand π–π^* transitions) 323 nm, 345 nm, 238 nm, 250 nm (all metal d-d transitions) 452 nm (metal-ligand transition: charge transfer) Absorption tail above 500 nm (spin-forbidden metal-ligand transition: charge transfer), 0-0 band at 575 nm (2.12 eV)
Emission (at 298 K)	Around 607 nm (charge transfer, spin-forbidden emission)
Lifetime of the emission (in H_2O)	0.62 µs
Redox potentials (in H_2O)	E_0 (Ru(II/III) = 1.26 V (NHE) E_0 ($Ru(II^*/III)$ = -0.86 V (NHE)
Quenching (= deactivating by bimolecular reaction) oxidative quenching: $Ru(bpy)_3^{2+} \rightarrow$ $Ru(bpy)_3^{3+} + e^-$	Selected quenchers: 2.2'methylviologen (MV^{2+}) in CH_eCN: $k_{quenching}=1.6\cdot10^9\ mol^{-1}s^{-1}$ TMPD in CH_eCN: $k_{quenching}=1.0\cdot10^{10} mol^{-1}s^{-1}$ Triphenylamine in CH_3CN: $k_{quenching}=9.5\cdot10^5\ mol^{-1}s^{-1}$ Fe^{3+}-quencher: $Ru(bpy)_3^{2+*} + Fe^{3+} \rightleftharpoons Ru(bpy)_3^{3+} + Fe^{2+}$ $k_{forward} = 2.7\cdot10^9\ mol^{-1}s^{-1}$ $k_{back} = 5.2\cdot10^6\ mol^{-1}s^{-1}$ equals to a conversion rate of 25% of Ru(II) into Ru(III), depending on the solvent. Ferrocene in EtOH: $5.9\cdot10^9\ mol^{-1}s^{-1}$ Quencher $S_2O_8^{2-}$: $5.6\cdot10^8\ mol^{-1}s^{-1}$: (irreversible in 0.5 M H_2SO_4)
ECL, first observed	$Ru(bpy)_3^{3+} + OH^- \rightarrow Ru(bpy)_3^{2+*} + OH^-$ [4]
Photoredox system	$Ru(bpy)_3^{2+} + MV^{2+} \overset{h\nu}{\rightleftharpoons} Ru(bpy)_3^{3+} + MV^{+\cdot}$ (back-reaction can be prevented by TEOA, which is capable of reducing $Ru(bpy)_3^{3+}$ to the 2+ state.
Photoelectro-chemical cells	$Ru(bpy)_3^{2+} + Fe^{3+} \rightleftharpoons Ru(bpy)_3^{3+} + Fe^{2+}$ Potential change upon illumination because the composition changes by irradiation of the solution: Lin and Sutin [17] observed a photopotential of 160 mV upon visible light photolysis of $Ru(bpy)_3^{2+}$.
Luminescence of $Ru(bpy)_3^{2+}$ in micellar media	Biphasic decay of the $Ru(bpy)_3^{2+}$ luminescence in sodium lauryl sulphate (combination of an association of $Ru(bpy)_3^{2+}$ to the negatively charged micelles, and self-quenching, due to high local concentration of $Ru(bpy)_3^{2+}$)

In a review article [16], Kalyanasundaram refers to the salient features of the photochemical, photophysical, and electrochemical properties of $Ru(bpy)_3^{2+}$. Some of them are summarized in Table 1, in so far as they are relevant to our context.

A plethora of publications exist concerning ECL. Literature research in the database SciFinder has resulted in 8,171 references containing the concept of ECL. Most of these publications are concerned with the mechanism of the formation of electrochemiluminescence, and the application as a detection method in HPLC and capillary electrophoresis.

In this introduction, we will only refer to those publications that are associated with our own experiments.

Alternative to the above-mentioned electrochemical methods, Gerardi et al. [18] generated $Ru(bpy)_3^{3+}$ by chemical oxidation of $Ru(bpy)_3^{2+}$ with PbO_2 as an alternative to the electrochemical formation of Ru(III). They spectroscopically investigated the temporal stability of Ru(III) in water at different pH values.

Wightman et al [8] investigated the emission intensity, the rate constants for the above-mentioned processes (1) - (5), and the overall decay time of the ECL of $Ru(bpy)_3^{2+}$ with TPrA (commonly, Pr_3N is abbreviated as TPrA) and trimethylamine on a carbon-fiber electrode. They found that the deprotonation of the amine radical cation (see [3,7]) is remarkably slow. Therefore, deprotonation is the rate- determining step. The authors simulated this process and found a rate constant of 540 1/s (≈ 2 ms). This value is comparable with those measured by Bard et al [15]. With the assumption that the deprotonation is a first-order process, the authors estimated the rate constant of the deprotonation to be about 3,500 s^{-1}, which gives a half-life of about 0.2 ms.

Bard et al. [15] described the ECL-process of $Ru(bpy)_3^{2+}$/TPrA on different electrode surfaces, too. They noticed that the direct oxidation of TPrA takes place on a glassy carbon electrode surface at about +0.6 V, followed by the Ru-TPrA reaction (Scheme). On a platinum electrode, however, the situation is quite different: Beginning at about 0.2 V, the platinum electrode forms adsorbed OH and O layers, and these OH / O films are able to catalyze the oxidation of TPrA, but the catalytic rate is quite low. Therefore, Bard measured a low current-flow of up to about 0.6 V.

After studying the $Ru(bpy)_3^{2+}$ ECL-processes with TPrA in detail, the research focuses on systems of more practical interest. One example is glyphosate, a broad-spectrum systemic herbicide used to kill several types of grassy weeds. Ridlen et al. [13] and Adcock [14] investigated glyphosate and some other structurally related substances and measured the pH-dependence of the ECL signal. They found an optimum of the ECL intensity in the alkaline region. In their HPLC-studies, they described a detection limit for glyphosate of about 0.01 µM, with a linear working range of more than five orders of magnitude. Experiments were done twice: $Ru(bpy)_3^{2+}$ (as the coreactant of glyphosate) was incorporated directly into the mobile phase, and post-column. The authors concluded that the on-column injection of $Ru(bpy)_3^{2+}$ simplifies the experimental set-up and leads to better results if the method runs isocratically (better resolution and decrease of the retention time; therefore, the analytical throughput can be enhanced).

Jin et al. [19] investigated the same system and measured the pH-dependence as well. Gyphosate is not electroactive up to 1.1 V. Nevertheless, glyphosate causes the excitation of $Ru(bpy)_3^{2+}$ at low potentials. Jin showed

that the maximum ECL-intensity is at pH 8. Above pH 10 and at higher potentials, however, another ECL is observed. Jin interpreted this additional ECL signal by the reaction of $Ru(bpy)_3^{3+}$ with directly oxidized glyphosate on the glassy carbon microelectrode used. The authors assumed an EC-mechanism (electron transfer with subsequent chemical reaction) and calculated the electron transfer rate k_0 (0.01 cm/s) and a fast chemical reaction rate k (621 mol/s), based on the commercial cyclic voltammetry simulation program (DigiSim).

Chuang et al [20] developed a rapid screening method for glyphosate. They used a flow-injection system coupled with ECL on the basis of $Ru(bpy)_3^{2+}$. The electrode was a thin-layer cell with an ITO working electrode. The sample throughput was 100 injections/h with a detection limit of about 0.03 mg/L. Chiu et al [21] coupled capillary electrophoresis with ECL, and detected glyphosate and aminomethylphosphonic acid (AMPA), the degradation product of glyphosate when applied to weeding. The detection limit in water is 0.06 µg/mL, and 4.04 µg/mL for glyphosate and AMPA, respectively. With this method, the authors were able to detect glyphosate as an impurity in soybeans.

Derivatization of amino acids with 1-dimethylaminonaphthalene-5-sulphonyl chloride (dansyl chloride) is a common method to add a chromophore moiety to amino acids. In general, dansyl derivated amino acids can be detected either by absorption around 254 nm or by fluorescence between 460 –495 nm (after excitation at 385 nm) [11,12]. After derivatization, the dansyl amino acids can be mixed post-column with $Ru(bpy)_3^{2+}$, and the resulting ECL is detected. A direct injection of $Ru(bpy)_3^{2+}$ into the mobile phase gives slightly different retention times of the dansyl amino acids. A comparison between pre- and postcolumn injection of $Ru(bpy)_3^{2+}$, however, gives no significant differences among the detection limits [11,12].

2. Pedagogical Objectives

The phenomenon of light-emitting chemical systems is well known from (organic) light-emitting diodes (OLEDs) [22,23]. In a didactical sense, this topic is very motivating for students. However, a voltage-induced redox reaction that forms a species in a radiative excited state is an extension and needs as much knowledge about electrochemistry as about photochemistry.

The aim of this article is to present a versatile experimental set-up to measure the electrochemical and spectroscopic characteristics of the most used system in ECL, $Ru(bpy)_3^{2+}$, in combination with different coreactants. We think that the pedagogical benefit of the described experimental procedures is a combination of the electrochemical and spectroscopic experiments — i.e., the use of cyclic voltammetry together with the detection of the emitted light as a function of the applied potential. As an application, ECL can be used as a detector in HPLC. The comparison between the ECL method and, e.g., absorption and fluorescence detection can provide information about different experimental set-ups. Therefore, students can estimate the sensitivities of the different HPLC-detection methods.

3. Experiments

3.1. Absorption and Fluorescence of $Ru(bpy)_3^{2+}$

Chemicals and instruments:

Tris(2, 2'-bipyridyl)ruthenium (II) solution in a phosphate buffer (prepared by equimolar amounts of disodium hydrogenphosphate Na_2HPO_4 and potassium dihydrogenphosphate KH_2PO_4, acetonitrile (Sigma Aldrich, 34967).

Spectrovis (Vernier), GCMSD with autosampler (GC: Hewlett Packard 5890, MSD: Hewlett Packard 5972), HPLC (Kontron system 400 with UV-detector 430, column: Kieselgel 60, Merck).

Figure 1 shows the absorption and emission of a $Ru(bpy)_3^{2+}$ solution. The absorption and emission maximums are about 450 and 622 nm, respectively, so the Stokes shift is quite pronounced (about 170 nm).

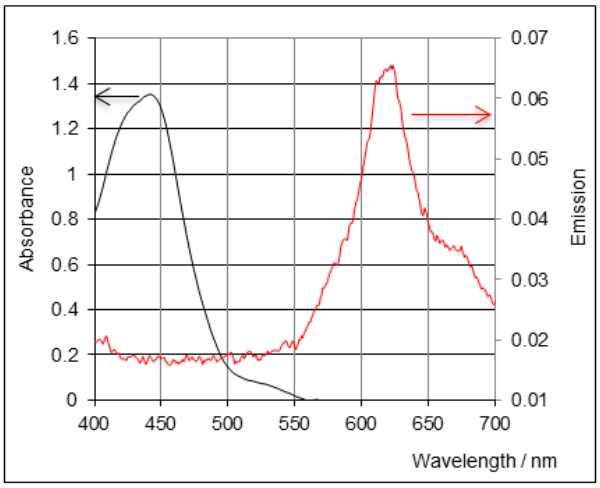

Figure 1. Absorption and emission of $Ru(bpy)_3^{2+}$ solution in a phosphate buffer (pH = 8)

3.2. Cyclovoltammogram and ECL-spectrum of $Ru(bpy)_3^{2+}$ with Different Coreactants

A convenient method in measuring the potential dependence of ECL is cyclic voltammetry. This method is described in this journal in [23].

Chemicals and instruments:

TPrA (Sigma Aldrich, 143979) 2-(Dibutyamino)ethanol (DBAE) (Sigma Aldrich, 550035), L-arginine (Sigma Aldrich, A 5006), tris(2, 2'-bipyridyl)ruthenium (II) solution in a phosphate buffer, double-distilled water.

Potentiostat (µStat 400 from DropSens) with screen-printed electrodes (DS 550: working electrode: Pt, counter electrode: Pt, reference electrode: Ag), photomultiplier (1P28A), power supply for the multiplier, alternatively: lux sensor (Leybold didactic, 666243), Sensor Cassy (data acquisition system).

Procedure: Set up the experiment shown in Figure 2. In detail: Connect the screen-printed electrodes with the potentiostat. The cyclic voltammogram (CV) parameters are: Start potential: 0.7 V, reverse potential: 1.3 V, final potential: 0.7 V, scan rate: 10 mV/s. The fiber glass rod is placed directly above the WE (1 mm above the solution); the ECL signal is collected with the fiber and fed into the photomultiplier (operating voltage between about -1,000

V). The output of the photomultiplier is digitized (Sensor Cassy) and the diagrams "intensity vs voltage" and "current vs potential" are recorded.

Figure 2 shows the experimental set-up to simultaneously measure the ECL-spectrum and the CV. The probe is dropped onto the screen-printed electrodes, and a fiber rod in a tube leads the light directly to the photomultiplier tube (see also Figure 3)

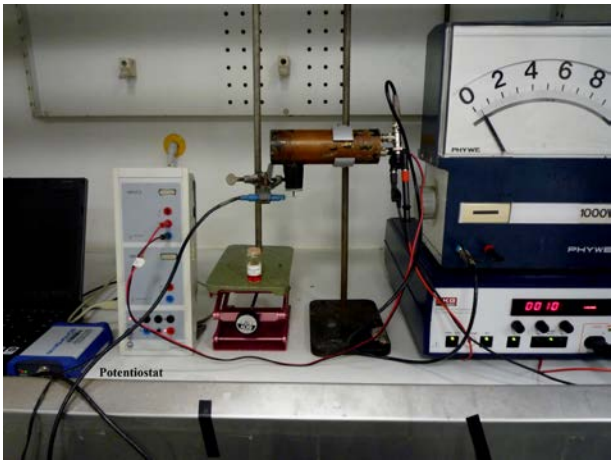

Figure 2. Experimental set-up for simultaneously determining cyclic voltammetry and ECL. From left to right: Potentiostat, CASSY, photomultiplier, power supply

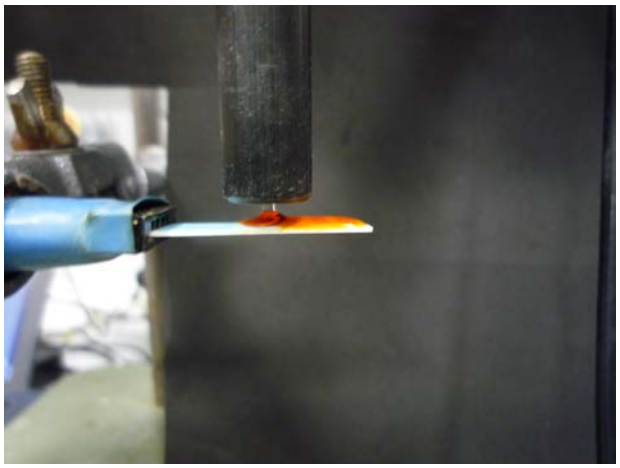

Figure 3. Detail of Figure 2: Electrode, glass fiber, housing for the fiber

By varying the potential of the WE, one can see a bright glow between about 1 V and 1.3 V (Figure 4). This glow disappears in the reversed scan.

Figure 4. Reddish glow above the WE at a voltage of 1.1 V

In the following, we will describe several ECL-systems in detail. The measuring procedure and the experimental equipment are the same as described.

a) $Ru(bpy)_3^{2+}$ / TPrA

First, the CV of $Ru(bpy)_3^{2+}$ is recorded. Afterward, a droplet of the solution of the coreactant (e.g., TPrA, 1 mmol in the buffer solution) is put into the $Ru(bpy)_3^{2+}$ solution, which is stirred carefully. The excess of the solution is pipetted so that only a thin layer of about 1 mm of the solution remains. Then, the electrodes are placed 1 mm below the glass fiber. We also use the fiber to avoid a possible damage of the potentiostat due to the electrostatic voltage of the photomultiplier.

Figure 5, top, shows a comparison between the ECL signal of $Ru(bpy)_3^{2+}$ with (black) and without TPrA (red). Figure 5, bottom, shows the corresponding CVs.

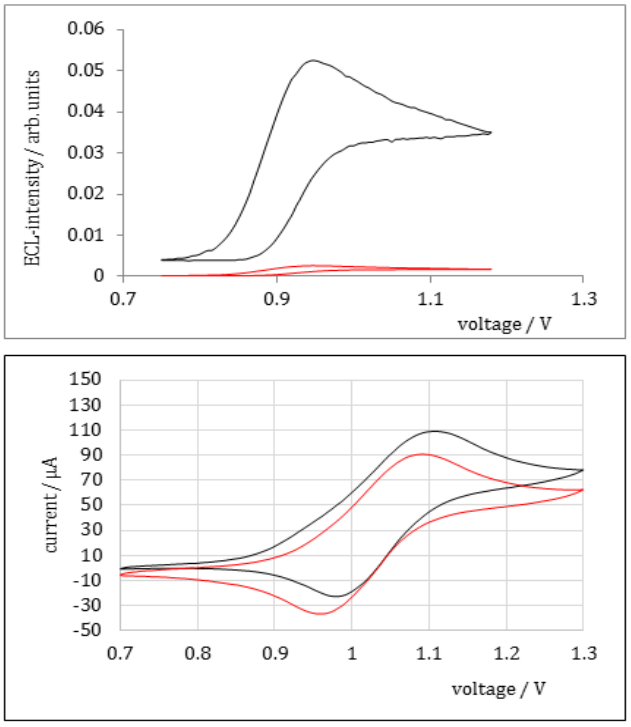

Figure 5. Top: ECL-intensity of $Ru(bpy)_3^{2+}$ (100 mmol) with TPrA (black, 1 mmol) and without TPrA (red) vs. potential. Bottom: CV of the same system. Scan rate: 10 mV/s

The redox waves in the CV ($Ru(bpy)_3^{2+}$ / $Ru(bpy)_3^{3+}$) are clearly observed (Figure 5, bottom, red curve). However, in the presence of TPrA, the anodic peak increases (Figure 5, bottom, black curve), implying that the electron transfer is followed by a chemical reaction of TPrA with $Ru(bpy)_3^{3+}$ to regenerate (more) $Ru(bpy)_3^{2+}$ on the electrode surface. In contrast, the reduction peak decreases because the concentration of $Ru(bpy)_3^{3+}$ is lowered (see Eq. 2) and therefore only a low quantity of $Ru(bpy)_3^{3+}$ can be reduced to $Ru(bpy)_3^{2+}$. The ECL-signal results significant only in the presence of TPrA (see the red curve in Figure 5, top).

With the theory of Nicholson and Shain [see, e.g., [24]], one can estimate the standard electron rate constant for the electron transfer of $Ru(bpy)_3^{2+}$ / $Ru(bpy)_3^{3+}$ from the difference of the cathodic and anodic current peaks (100mV). This results in $k_0 = 2 \cdot 10^{-3}$ cm/s (with an assumed diffusion coefficient of 10^{-5} cm^2/s and a scan rate of 10 mV/s). This means that the electron transfer must be quasi-reversible.

For testing the temporal stability of the ECL signal, we apply a rectangular voltages (0 to 1.3 V) to the $Ru(bpy)_3^{2+}$ / TPrA-system, and measure the ECL and the CV:

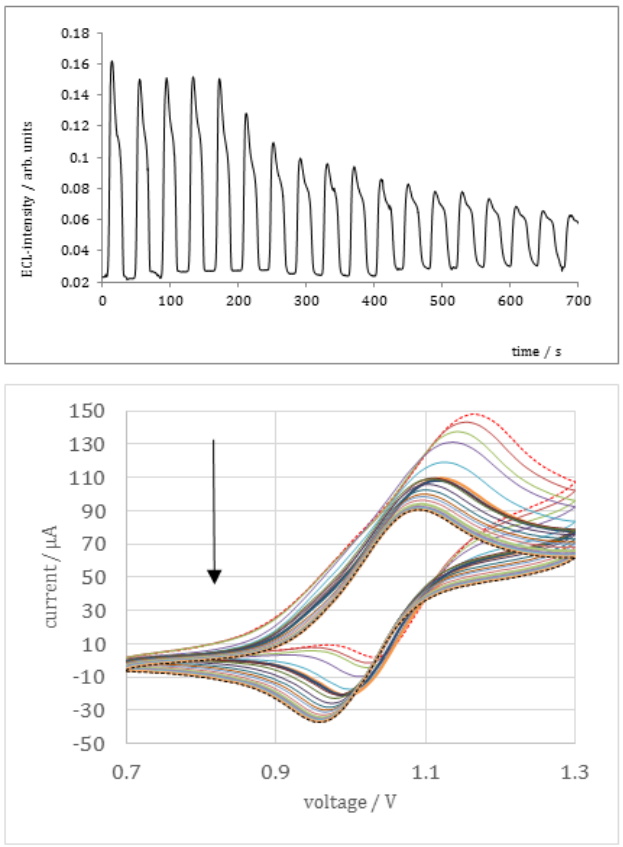

Figure 6. top: Time dependence of the periodical Ru-TPrA-ECL- signal. Bottom: CV several times in a row. The arrow indicates the increase of the CV cycle number

It is obvious that the CV signals decrease due to the vaporization of the TPrA and also due to the reaction of TPrA during the scans. In the CV, one can see that the cathodic peak increases simultaneously because at least all TPrA evaporate and / or react. After several cycles, the cyclic voltammogram is reduced to that of the redox couple $Ru(bpy)_3^{2+}$ $Ru(bpy)_3^{3+}$.

b) $Ru(bpy)_3^{2+}$ / DBAE

In contrast to TPrA, DBAE is less toxic and therefore more suitable to demonstrate ECL. In Figure 7, the voltage cycles are recorded seven times. It is obvious, that the curve shapes are similar to those of $Ru(bpy)_3^{2+}$ / TPrA (Figure 7).

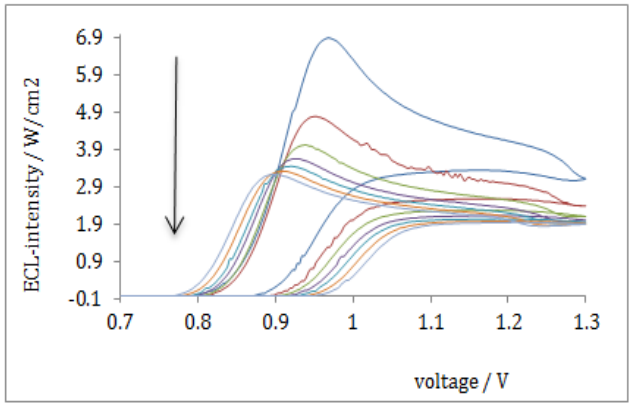

Figure 7. DBAE as coreactant, scans between 0.7 V and 1.3 V

If one applies rectangular pulses of 1.2 V once a second over a period of 6,000 s, one can observe a slow decay in the ECL intensity due to the surface reaction of DBAE (Figure 8).

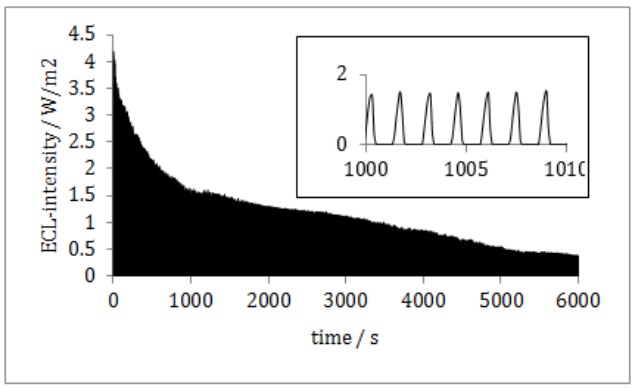

Figure 8. Cycles of the ECL-intensity of $Ru(bpy)_3^{2+}$ / DBAE after voltage-pulses between 0 V and 1.3 V (frequency: 8 Hz). Insert: The ECL-signal between 1,000 and 1,010 s in detail

c) $Ru(bpy)_3^{2+}$ / Amino acids

If a nonvolatile substance is used—e.g., arginine—the ECL-signal decreases, too. Now, the vaporization plays a minor role, and the chemical reaction dominates the temporal reduction of arginine.

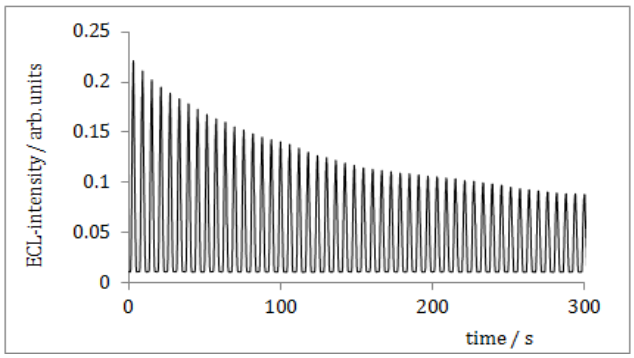

Figure 9. Time dependence of the periodical ECL-signal of Ru-Arginine. The rectangular voltage is applied with a frequency of about 0.2 Hz

d) ECL-time decay

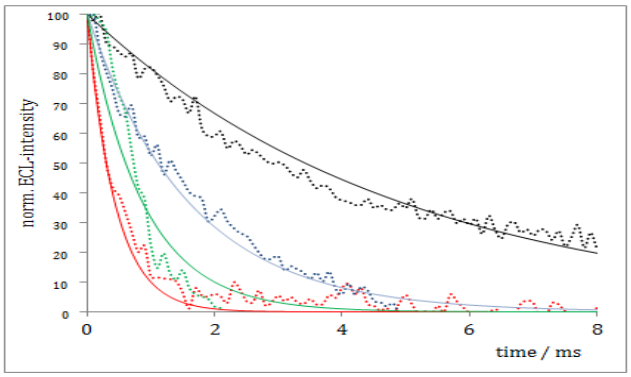

Figure 10. Time dependence of the ECL-decay of $Ru(bpy)_3^{2+}$ / TPrA (black dotted curve), $Ru(bpy)_3^{2+}$ / Arginine (red dotted curve), $Ru(bpy)_3^{2+}$ / DBAE (blue dotted curve), and $Ru(bpy)_3^{2+}$ / glyphosate (green dotted curve). Curve fitting (solid lines): τ (TPrA) = 4.5 ms, τ (arginine) = 0.3 ms, τ (DBAE) = 1.5 ms, τ (glyphosate) = 0.6 ms. The curve fitting was done with the Excel solver program

Procedure: Experimental procedure as above. Time base of Sensor Cassy: 10 ms, resolution: 10µs. Apply a

1.1 V pulse of the potentiostat to the WE, and measure the ECL-decay with the photomultiplier as a function of time.

After the voltage is switched off, the ECL of the different coreactants decays on different time scales: TPrA: 3.2 ms, Arginine: 0.3 ms, DBAE: 1.1 ms and glyphosate: 0.6 ms (black, red, blue and green curves in Figure 10). The absolute intensities differ: the ECL of e. g. TPrA is three times higher than the ECL of Arginine.

e) ECL as a detection method in HPLC: DBAE and TprA

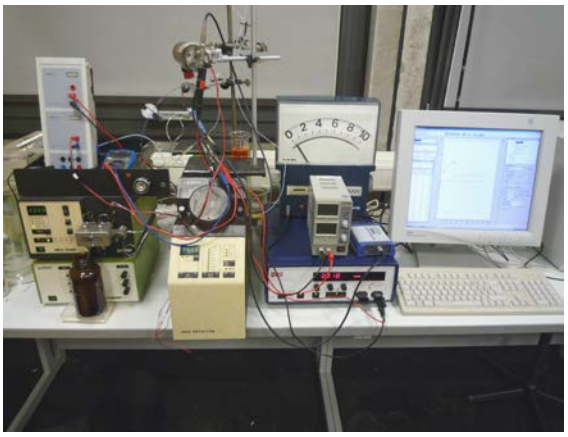

Figure 11. Complete set-up, HPLC with ECL-detection

Chemicals and instruments:

TPrA (Sigma Aldrich, 143979) 2-(Dibutyamino)ethanol (DBAE) (Sigma Aldrich, 550035), 2'-bipyridyl)ruthenium (II) solution in a phosphate buffer, double-distilled water,

Potentiostat or simple power supply, HPLC (Kontron), photomultiplier (1P28A), power supply for the multiplier, Sensor Cassy (data acquisition system), absorption through cuvette (Kontron, 8 µL volume), homebuilt ECL-cuvette (8 µL volume).

Figure 11 shows the complete set-up of the HPLC with an adsorption- (Kontron) und an ECL-detector (homebuilt).

Figure 12 shows the two used ECL cuvettes. We use the first one for didactical purposes to demonstrate the chemiluminescence, which can be directly observed. The screen-printed electrodes are bonded onto a glass wafer. A rubber o-ring seals the electrodes at the opposite side to a second glass wafer. All of this is held together by four screws. Two needles ensure the flow of the solution in and out of the cuvette.

The smaller cuvette was originally built as an absorption cell in HPLC. We modify the cell by removing the glass bottom and insert the screen-printed electrodes, which are bonded on the bottom of the stainless steel cell. Onto the other side, we mount the photomultiplier tube and protect it from stray light with a black tape.

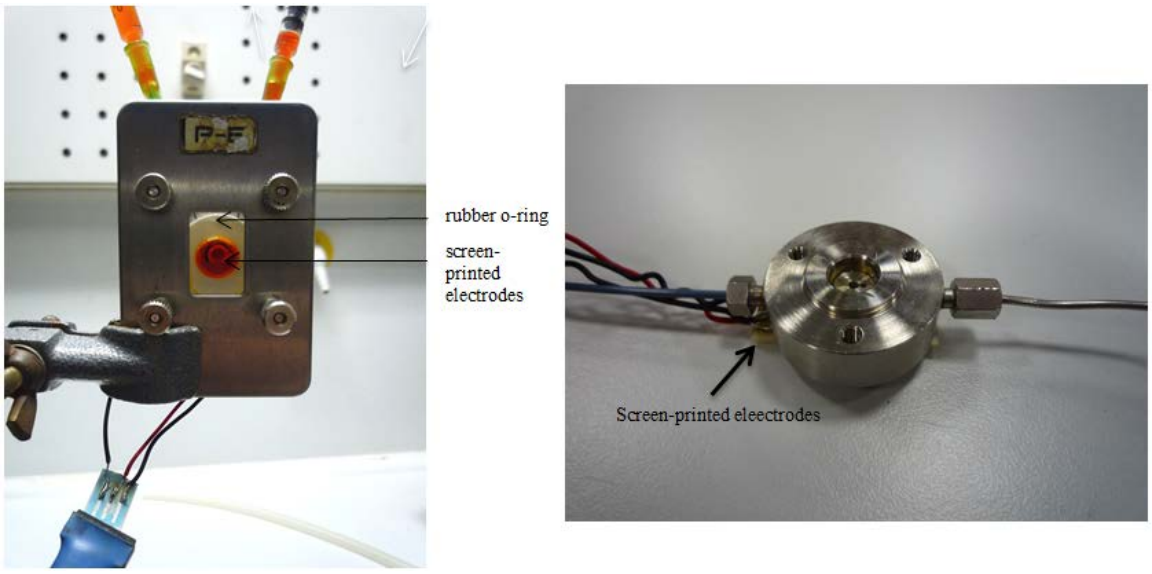

Figure 12. top: (Didactical) flow-through cuvette (Volume: 3 mL); bottom: Modified HPLC flow-through cuvette (Volume: 8 µL)

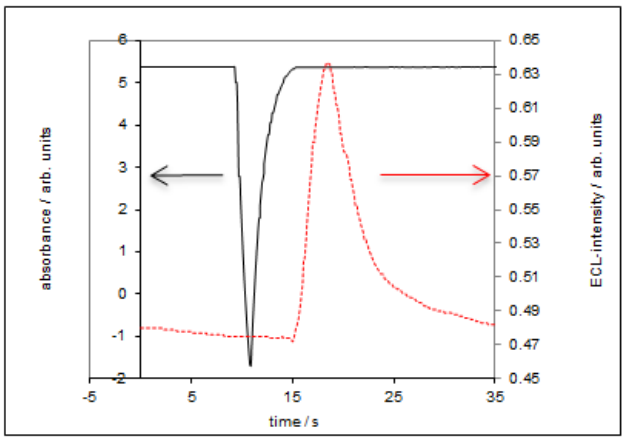

Figure 13. TPrA as coreactant.

Procedure: First, we purify the HPLC with double-distilled water. Afterwards, we fill the HPLC with 10 mmol of the aqueous $Ru(bpy)_3^{2+}$ solution by purging with the HPLC-pump (flow 1 mL/min). The ECL-detection cell is mounted directly after the conventional HPLC-absorption cell. TPrA as the coreactant is injected with a rheodyne valve into a 20 µL loop and then loaded into the HPLC column. The decay of the $Ru(bpy)_3^{2+}$ absorption signal (at λ_{max} = 450 nm) can de measured when the coreactant fills the absorption cell (black curve in Fig. 13). After a time delay of about 20 s, the ECL-signal rises (red, dashed curve).

f) HPLC-detection of alanine and derivatized alanine

Chemicals and materials:

Trifluoroacetic anhydride (SigmaAldrich, No. 106232), trifluoroethanol (SigmaAldrich, No. T 63002)

GC-MSD with autosampler (GC: Hewlett Packard 5890, MSD: Hewlett Packard 5972, autosampler: Hewlett Packard 6890), Column: RTX-35, Carrier gas: He 5.0, 50-mL round-bottom flask, Reflux condenser, Capillary air bleed for solvent evaporation, UVVIS-spectrometer (Lambda XLS+: Perkin-Elmer).

Procedure for derivatization of Arginine with trifluoroethanol / trifluoroacetic anhydride: The derivatization procedure with dansyl chloride can be followed in accordance with Lee et al. [12]. Alternatively, a much simpler derivatization procedure of alanine can be carried out following Börjesson et al [25]:

10 µg of the amino acid is mixed with 200 µg TFAA (trifluoroacetic anhydride) and with 100 µg TFE (trifluoroethanole) in a 50-mL round-bottom flask. The mixture is heated to 90 C with a controlled heater and refluxed for about one hour.

Afterwards, the mixture is flushed with clean air for two minutes to evaporate the solvents. The residue is then mixed with 10 mL ethyl acetate and directly analyzed with GC-MSD and UVVIS (Figure 14).

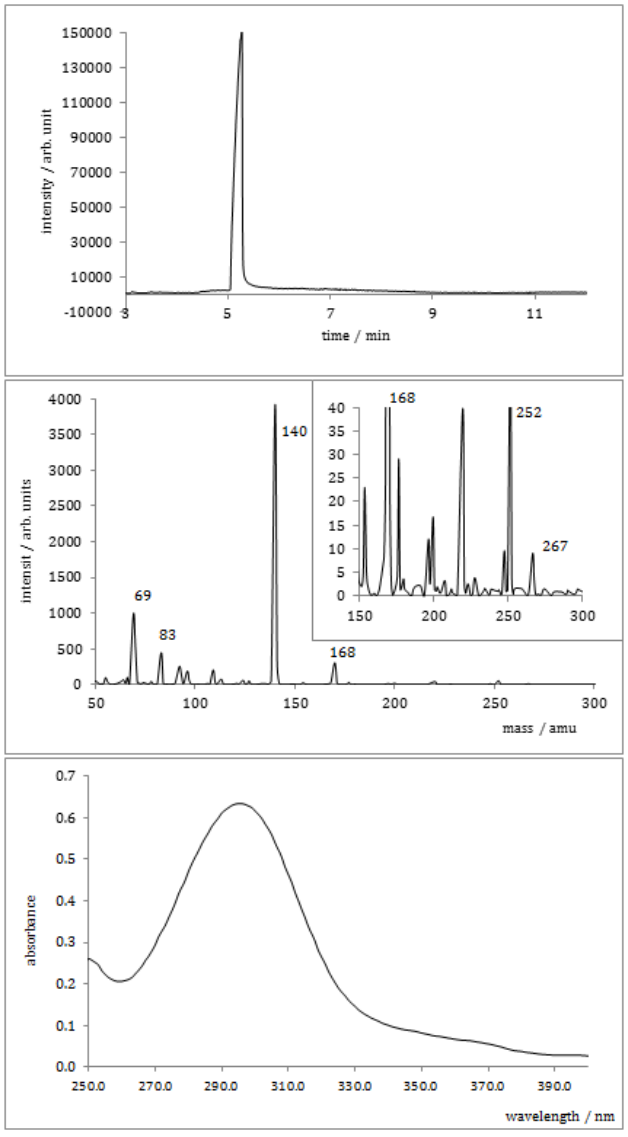

Figure 14. GCMSD of TFE-TFAA-alanine, top: GC, middle: EI-MS of the product (insert *100), bottom: UVVIS-spectrum, λ_{max} 295 nm

In the EI-MS, one can identify the (very small) parent-ion (mass 267 amu) and the main fragments (252 amu: loss of CH_3, 168: loss of TFE, 83 amu: CF_3CH_2, and 69 amu: CF_3). Fragment 140 amu is unknown.

Figure 15 shows the HPLC-absorption spectrum of TFE /TFAA-derivatized alanine (10 mmol in ethyl acetate) and the HPLC-ECL spectrum of alanine (underivatized, 10 mmol in water). In both cases, the injection volume is 50 µL and the Ru-concentration 20 mmol. In the first case, the detector is set to the absorption wavelength of TFE /TFAA-alanine (295 nm).

We think that the direct detection of alanine with ECL (potential 1.1 V) is much easier than the derivatization followed by the absorption detection of the derivatized alanine. As Fig. 15 shows, the signal-to-noise ratio in the first case is significantly better.

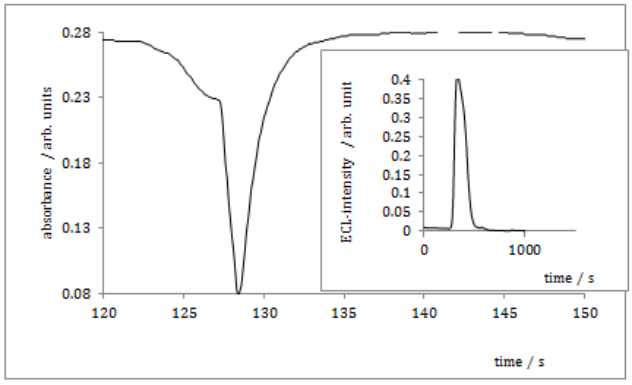

Figure 15. HPLC of TFE/TFAA-derivatized alanine (absorption wavelength: 295 nm, eluent: ethylacetate). Insert: ECL-intensity of alanine (same concentration as TFE/TFAA-derivatized alanine)

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