

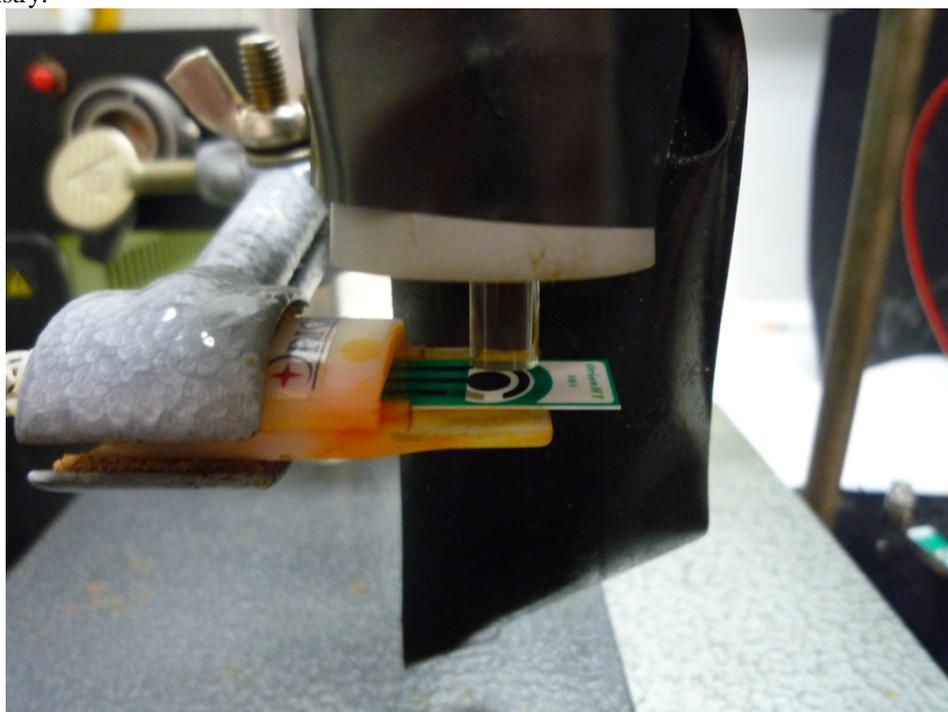
# Rapid and Sensitive Spectroelectrochemical Detection of Lidocainehydrochloride and Caffeine with Screen-Printed Electrodes

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**Abstract** In analytical and environmental chemistry there is a persistent need for rapid, inexpensive and sensitive detection of harmful organic compounds such as medicinal products, e.g. lidocaine, that can cause low blood pressure and an irregular heart rate and that often contaminate waste water, or caffeine, a screening-parameter for determining the quality of drinking water because it enters drinking water reservoirs only via the contamination of waste water. This article presents some reliable and easily performed spectroelectrochemical measurements, such as electrogenerated chemiluminescence (ECL), to identify lidocaine and caffeine. The main features of the spectroelectrochemical method are screen-printed electrodes (SPE) that use gold as the working electrode. In addition, this article compares the results of a low-cost experimental set-up ideal for classroom experiments with professional ECL-equipment. The experiments were conducted in an undergraduate-level university course in electrochemistry.



**Keywords:** upper-division undergraduate, hands-on learning/manipulatives, electrochemistry, mass-spectrometry

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## 1. Introduction

Since 1960s, electrogenerated chemiluminescence (ECL) techniques have become increasingly attractive for analytical chemistry because the method is cheap, fast and

sensitive [1,2,3]. ECL involves generating an excited state in the commonly used and extensively investigated tris(2,2'-bipyridyl)ruthenium(II)  $[\text{Ru}(\text{bpy})_3]^{2+}$  on an electrode surface.  $[\text{Ru}(\text{bpy})_3]^{2+}$  is first oxidized before undergoing an electron transfer reaction with a coreactant. During this latter process, an excited state is reached that subsequently decays and emits light, and finally,

$[\text{Ru}(\text{bpy})_3]^{2+}$  can be regenerated after the emission. The main features of  $[\text{Ru}(\text{bpy})_3]^{2+}$  were already reported in [4]. In addition, Gerardi [5] and Fährlich [6] provide a comprehensive review of the analytical application of ECL of  $[\text{Ru}(\text{bpy})_3]^{2+}$ .

This paper will show that spectroelectrochemical methods constitute a promising method of quickly identifying lidocaine and caffeine in batch experiments. Caffeine was extracted from commercial coffee powder before the ECL analysis. ECL can be done using screen-printed electrodes with working electrodes made from gold or other materials such as copper, silver, bismuth, nickel, carbon nanotubes or graphene. The detection limit (LOD) of the ECL method is compared with gas chromatography - mass-spectrometry (GC-MS) measurements.

## 2. Pedagogical Objectives

Electrochemistry plays an important role in curricula, textbooks, and everyday life, but there exist several problems in learning electrochemistry [7]. In 1990s, Garnett et al. [8] and Ogude et al. [9] determined some of the common misconceptions held by students studying electrochemistry. It was found that students based their reasoning on five wrong concepts:

- During electrolysis, the electric current produces ions;
- electrons migrate through the solution from one electrode to the other;
- the cathode is always the minus pole, the anode the plus pole;
- the plus and minus poles carry charges;
- the electrode reactions cannot be identified.

There have been numerous efforts to reduce these misconceptions. Sanger and Greenbowe [10] asserted that only conceptual change strategies have a positive effect on dispelling students' misconceptions. Huddle et al. [11] showed concrete models that represent the electrode reactions. Acar und Tarhan [12] showed 24 different misconceptions concerning electrochemistry, and proposed a cooperative learning model.

We think that the pedagogic benefit of the described experimental procedures lies in the combination of electrochemical and spectroscopic experiments (more information on the important aspects of the primary method used in electrochemistry, cyclic voltammetry, can be found in [13]). The electrode reactions are directly observable, and the emitted light can be detected as a function of the applied potential, meaning ECL and electrical current can be measured synchronously. In addition, students can estimate the analytical strength of ECL and compare it with GC-MS.

The demonstrations of the ECL-phenomenon are eye-catching. We will not pursue a discourse on the importance of stimulating experiences as a motivational aid – that has been discussed at length in other venues. Rather we would like to summarize the didactical reasons for presenting the ECL to an audience of chemistry students.

- Demonstration of ECL and correlation to electrochemical and spectroscopic theory predictions.
- Correlation of an easily observable phenomenon – a reddish colored chemiluminescence – with an

emission spectrum in the red region of the spectrum.

- Interpretation of the observed cyclic voltammetry curve and correlation with the observed color change.
- Investigating of the electrochemical and spectroscopic instruments.
- Use of the chemiluminescence in analytical techniques – in particular in investigating chemical (and biochemical) substances such as lidocaine and caffeine. We refer the reader to exhaustive reviews of this subject in [1,2,3,4].

As mentioned above, the described experiments were conducted in an undergraduate-level university course in electrochemistry. Until now twenty students (in two lab periods 2015/2016, in four courses) carried out the described experiments at the end of a practical electrochemistry course for advanced students. The theoretical information was taught one semester before in a lecture about physical chemistry. Before conducting the experiments students had to predict what would happen and to provide reasons for their prediction. The experiments were done, and the students wrote down their observations.

Finally, the students had to remember their predictions and explain their observations: What was the purpose of the experiment? What kind of reaction occurs at the electrodes? Do you see any advantage in the combination of electrochemical processes and spectroscopic observation? Compare the limit of detection of ECL and GCMS ...

Without empirical background we can resume that high-achieving students enjoyed the experiments, because the combination of two disparate subjects (electrochemistry and spectroscopy) were unusual.

## 3. Experimental Materials and Procedures

### Chemicals and Instruments

Lidocainehydrochloride (Caelo, Hilden, Germany No. 4376, CAS No. 137-58-6), Caffeine (Caelo, Hilden, Germany, 2186, CAS No. 58-08-2), double-distilled water, Methylenchloride (Carl Roth, Karlsruhe, Germany, CAS No. 75-09-2), phosphate buffer (pH 6.9, 0.1 mol/L).

Screen-printed electrodes (DropSens: DRP 250AT (Au as working electrode, Pt as counter electrode, Ag as reference electrode)), electrochemical reflection flow cell TLFCL 510-CIR (DropSens), luminescence spectrometer (Perkin Elmer LS 50B).

**Low-cost equipment (“Low-cost”):** photomultiplier (R 4220P, Hamamatsu), programmable pulse generator (Power Cassy, Leybold, Germany), data acquisition system (Sensor Cassy, Leybold, Germany).

**Expensive equipment (“STAT-ECL”):** ECL-potentiostat (STAT-ECL, DropSens). This device can measure different electrochemical procedures such as linear-sweep voltammetry, cyclic voltammetry, and square wave voltammetry, and can simultaneously detect the resulting chemiluminescence.

### Hazard

Lidocainehydrochloride is toxic if swallowed; if swallowed seek medical advice immediately.

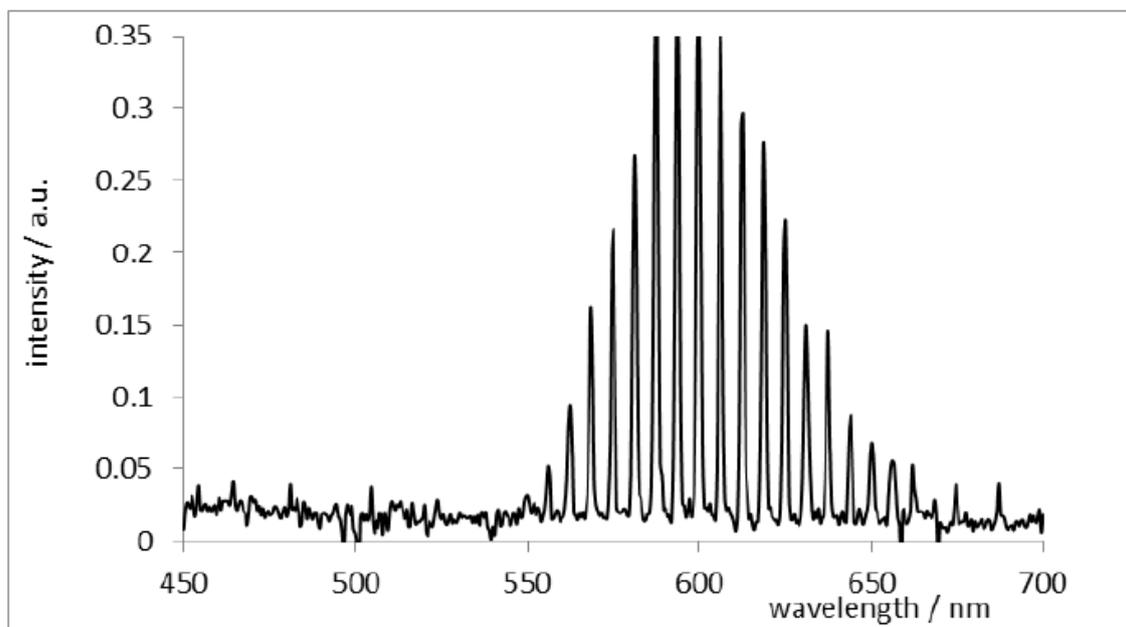
Caffeine is harmful if swallowed, and may cause indisposition.

### 3.1. Chemiluminescence Spectrum of $[\text{Ru}(\text{bpy})_3]^{2+}$ /Lidocainehydrochloride

*Procedure:* The electrochemical thin layer reflection cell is filled with a mixture of 200  $\mu\text{L}$  aqueous

$[\text{Ru}(\text{bpy})_3]^{2+}$  solution (100  $\mu\text{mol/L}$ ) and 200  $\mu\text{L}$  aqueous lidocaine solution (10  $\mu\text{mol/L}$ ). The cell is installed in front of the sample holder of the luminescence spectrometer.

Figure 1 shows the spectrally resolved ECL signal of  $[\text{Ru}(\text{bpy})_3]^{2+}$  / lidocainehydrochloride. The wavelength at maximum intensity was about 600 nm and resulted from the emission of  $[\text{Ru}(\text{bpy})_3]^{2+}$ .



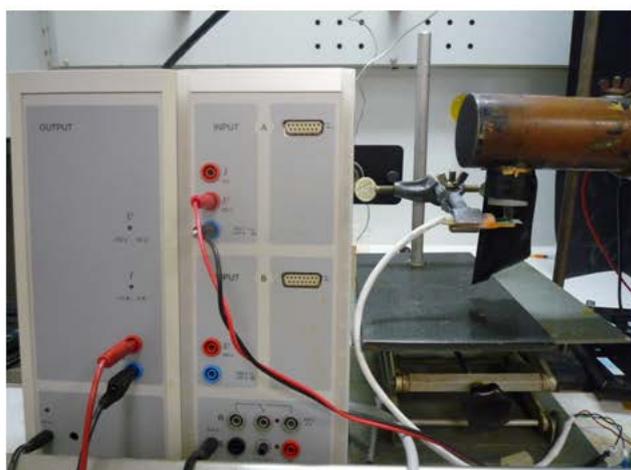
**Figure 1.** Chemiluminescence spectrum of  $[\text{Ru}(\text{bpy})_3]^{2+}$  (100  $\mu\text{mol/L}$ ) with lidocainehydrochloride (10  $\mu\text{mol/L}$ ) as coreactant. The chemiluminescence spectrum was recorded as follows: Wavelength scan 450-700 nm; scan speed 300 nm / min; electrical excitation pulse from a pulse generator (width 0.1 s; frequency 0.5  $\text{s}^{-1}$ ; pulse height 1-3.54 V)

### 3.2. ECL of $[\text{Ru}(\text{bpy})_3]^{2+}$ /Lidocainehydrochloride and Caffeine (Low-Cost)

Figure 2 shows the experimental setup for measuring ECL with the low-cost equipment:

A triangular voltage from a pulse generator is applied to the SPE (it is important to emphasize that the pulse

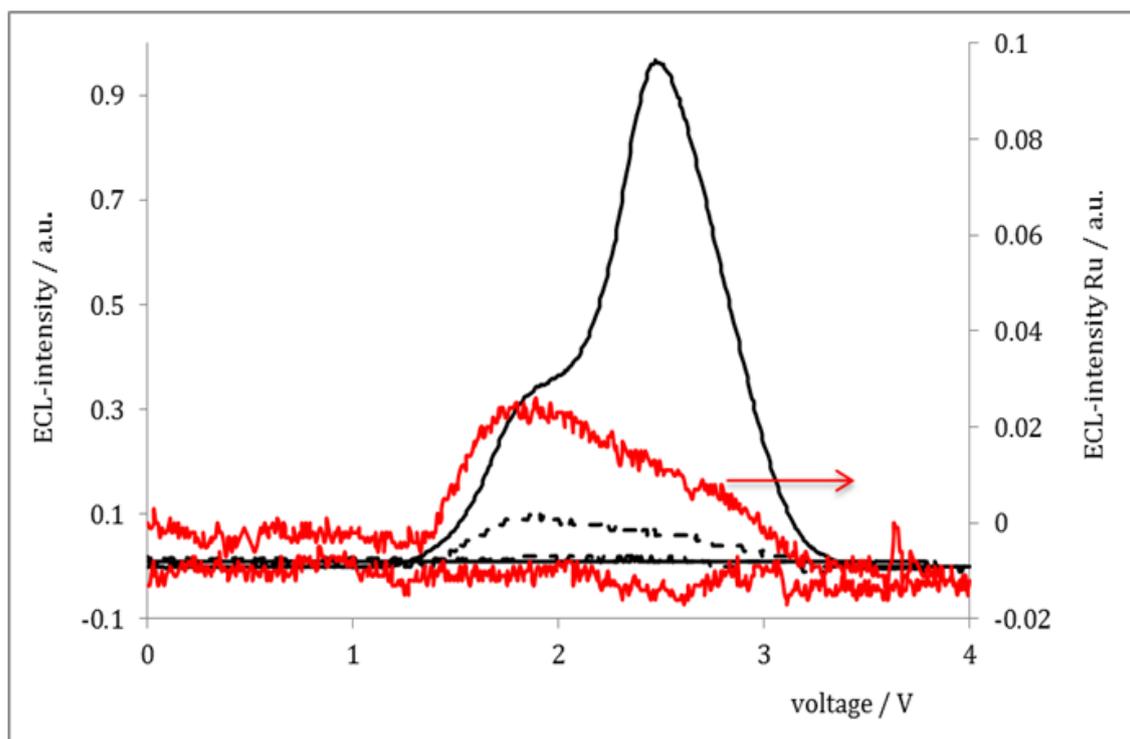
generator is not a potentiostat. Therefore, no CV can be recorded!). Only slightly above the working electrode (4 mm in diameter), a glass fibre guides the ECL into a photomultiplier (applied voltage: -1000 V). The photomultiplier converts the ECL intensity into a current that is digitized by a data acquisition system.



**Figure 2.** Experimental setup. Top: From left to right: programmable pulse-generator (Power Cassy), data acquisition system (Sensor Cassy), photomultiplier tube with glass fiber above the SPE. Bottom: Part of photo above with clearly seen SPE and glass fiber

Figure 3 shows the ECL as a function of the applied voltage of  $[\text{Ru}(\text{bpy})_3]^{2+}$  alone (70  $\mu\text{L}$ , 1 mmol, red curve),  $[\text{Ru}(\text{bpy})_3]^{2+}$  with caffeine (1 mmol/L, black dashed

curve), and  $[\text{Ru}(\text{bpy})_3]^{2+}$  with lidocainehydrochloride (black curve). The curves reflect the higher ECL-sensitivity of lidocaine.



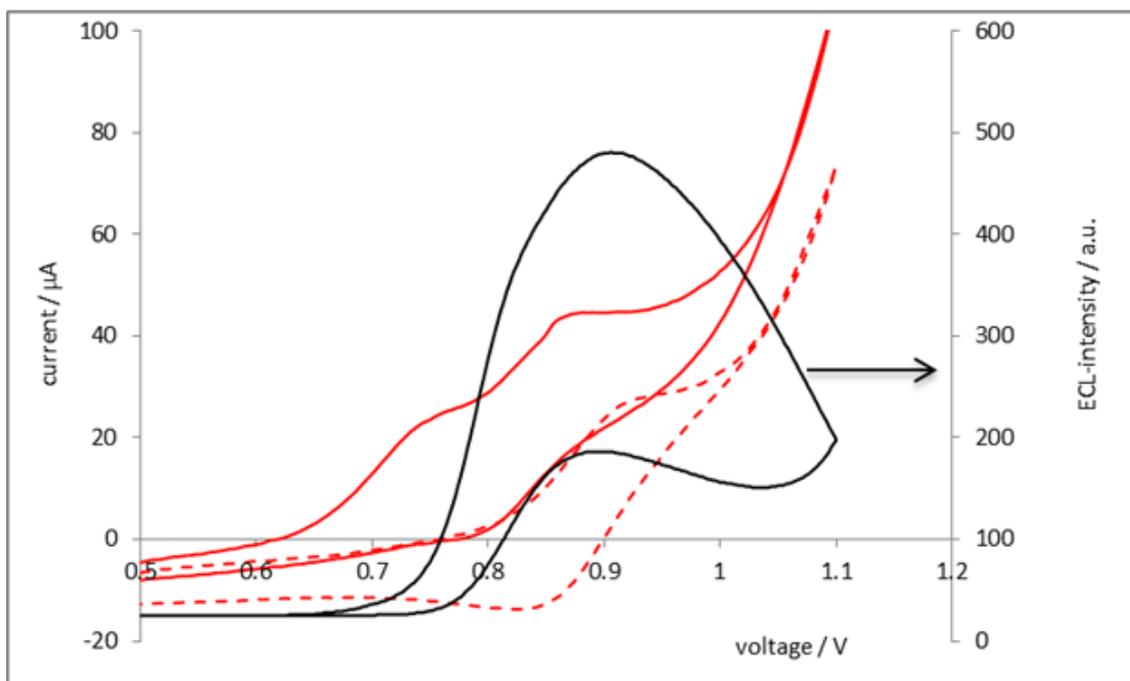
**Figure 3.** ECL-intensity as a function of the applied voltage. Red line: only  $[\text{Ru}(\text{bpy})_3]^{2+}$ , black dashed line:  $[\text{Ru}(\text{bpy})_3]^{2+}$  + caffeine (1 mmol), black line:  $[\text{Ru}(\text{bpy})_3]^{2+}$  with lidocaine (1 mmol)

Brune [7] suggested that due to the stability of the intermediate radical cation tertiary amines show strong ECL intensities while primary or secondary amines show weak ECL intensities. Although both substances are tertiary amines the difference in the ECL-intensity is enormous: As one can see, the ECL intensity of caffeine is much lower than that of lidocaine at the same concentration.

### 3.3. CV and ECL with the Expensive Equipment (“STAT-ECL”)

#### 3.3.1. $[\text{Ru}(\text{bpy})_3]^{2+}$ / Lidocainehydrochloride

Figure 4 shows the cyclic voltammogram and the ECL intensity curve of the expensive equipment with the STAT-ECL equipment (here the ECL curve and the CV can be recorded simultaneously):



**Figure 4.** Cyclic voltammogram of  $[\text{Ru}(\text{bpy})_3]^{2+}$  (1 mmol) with lidocaine (1 mmol, red line) and without lidocaine (red, dashed line). ECL-intensity (black line). Scan rate: 50 mV/s

In the presence of a co-reactant, the anodic peak increases, implying that the electron transfer is followed by a chemical reaction of lidocaine hydrochloride with  $[\text{Ru}(\text{bpy})_3]^{3+}$  to regenerate (more)  $[\text{Ru}(\text{bpy})_3]^{2+}$  on the electrode surface. In contrast, the reduction peak decreases because the concentration of  $[\text{Ru}(\text{bpy})_3]^{3+}$  is lowered, and therefore only a limited quantity of  $\text{Ru}(\text{bpy})_3^{3+}$  can be reduced to  $[\text{Ru}(\text{bpy})_3]^{2+}$ . The ECL signal results were

significant in the presence of lidocaine hydrochloride (see Figure 4).

Figure 5 shows an estimation of the LOD using the ECL method with "STAT-ECL". Lidocaine solutions were prepared with different concentrations and mixed with  $[\text{Ru}(\text{bpy})_3]^{2+}$  (100  $\mu\text{mol/L}$ ), respectively.

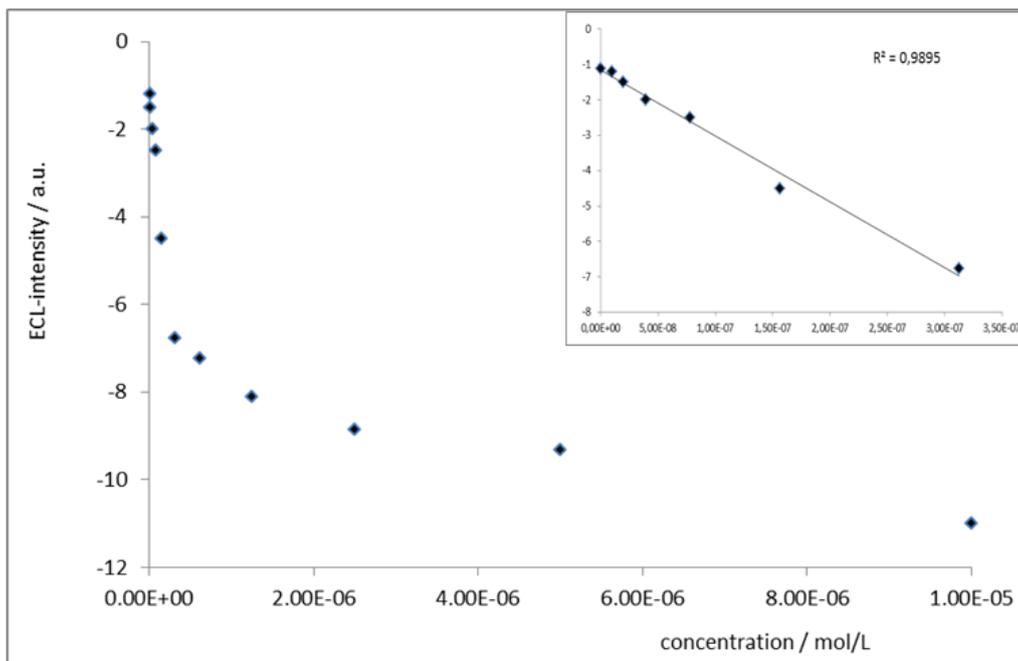


Figure 5. ECL-intensity as a function of the lidocaine concentration. Inset: expansion for low concentrations

Lidocaine concentrations lower than 0.1  $\mu\text{mol/L}$  can easily be detected. Between 0 and 0.3  $\mu\text{mol/L}$  the concentration dependence of the ECL signal is linear.

Unfortunately, other contaminants of drinking water, e.g. antibiotics and hormones, can affect the analytical results. Before lidocaine could be determined in drinking water the substance must be isolated e.g. with an appropriated method (HPLC or capillary electrophoresis).

### 3.3.2. Cyclic Voltammograms and ECL of $[\text{Ru}(\text{bpy})_3]^{2+}$ / Caffeine and Caffeine-Extract

*Procedure:* Mix an aqueous solution of caffeine (100  $\mu\text{L}$ , 1 mmol) with an aqueous solution of  $[\text{Ru}(\text{bpy})_3]^{2+}$  (100  $\mu\text{L}$ , 1 mmol) and drop it onto the SPE.

Figure 6 shows the cyclic voltammogram and the ECL curve of  $[\text{Ru}(\text{bpy})_3]^{2+}$  / caffeine.  $[\text{Ru}(\text{bpy})_3]^{2+}$  alone shows two current peaks, an anodic at about 0.91 V and a cathodic at about 0.91 V and 0.89 V, respectively.

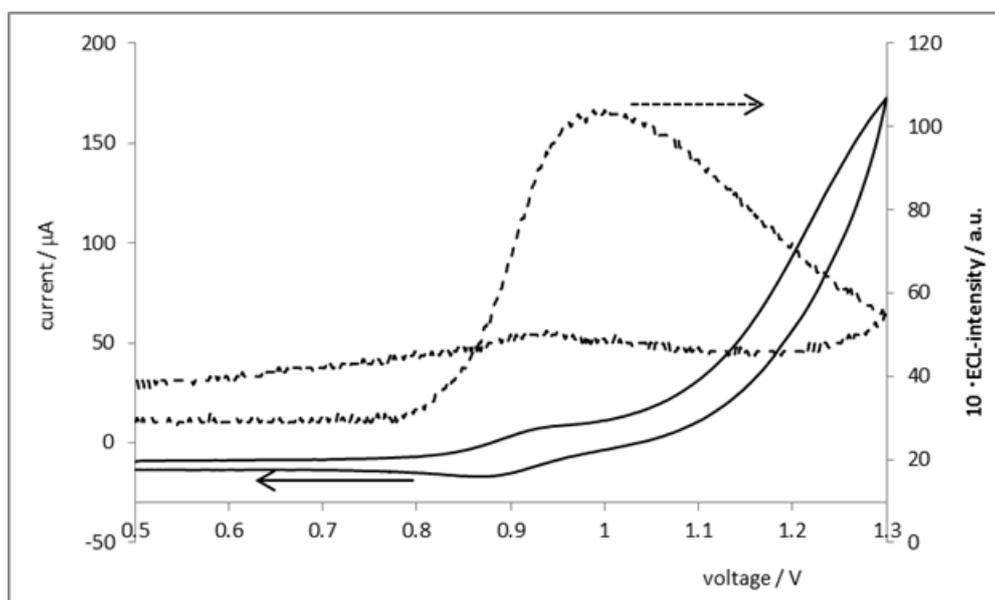
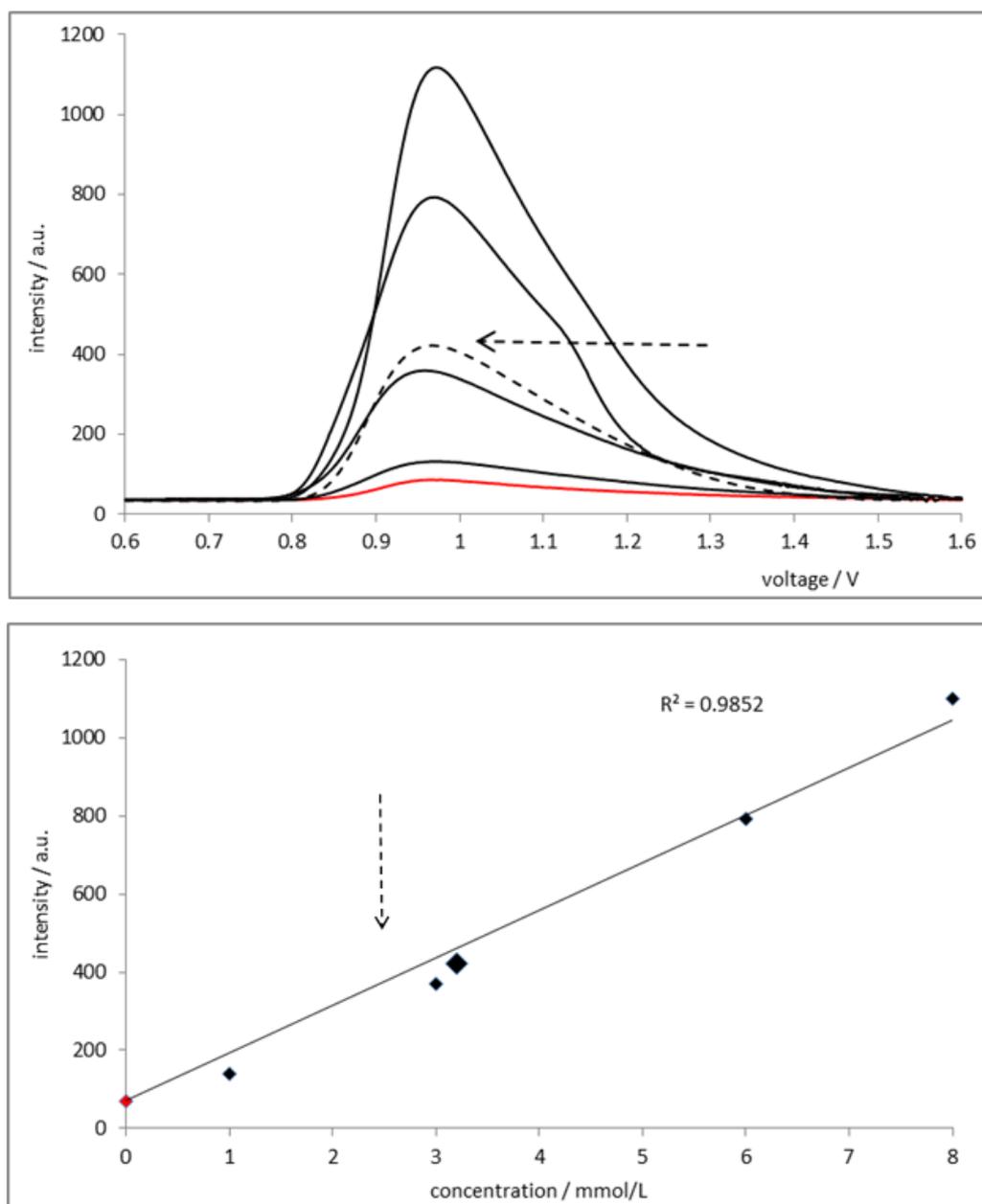


Figure 6. Top: Cyclic voltammograms and ECL of  $[\text{Ru}(\text{bpy})_3]^{2+}$  (1 mmol/L) with caffeine (1 mmol/L). Scanrate: 50 mV/s. ECL-intensity: 10

To determine the concentration of caffeine in coffee powder, one first has to take the calibration curve. For this purpose one mixes 100  $\mu\text{L}$  of different concentrations of the caffeine-buffer solution ( $\text{pH} = 6.9$ , 1 mmol/L – 8 mmol/L) with 300  $\mu\text{L}$  of 1 mmol/L  $[\text{Ru}(\text{bpy})_3]^{2+}$  solution and place 70  $\mu\text{L}$  of it onto the SPE.

Then 2 g of the coffee powder in 150 mL methylenchloride is extracted in a Soxhlet extractor for one hour. The extract was next concentrated with a rotary

evaporator, and dried in a drying oven at  $40^\circ\text{C}$  for two hours. Then 5 mL methylenchloride is added, and analyzed with GC-MS. After further evaporation 10 mL buffer solution ( $\text{pH} = 6.9$ ) is added and 100  $\mu\text{L}$  of the solution is analyzed by linear sweep voltammetry - ECL measurements. Figure 7 shows the ECL results of the different caffeine concentrations. The dashed line illustrates the ECL of the caffeine-extract.



**Figure 7.** Top: ECL-curves from different concentrated caffeine concentrations. Dashed line: ECL-curve from extracted caffeine. Red curve: without caffeine. Bottom: Calibration curve; the arrow shows the ECL-intensity of the coffee extract

One can estimate the significance of the result in the following manner: According to the manufacturer, 100 g of conventional coffee powder contains about 40 mg caffeine. Therefore, the extracted 2 g coffee must contain 0.8 mg caffeine or 41  $\mu\text{mol}$  (molecular weight of caffeine: 194 g/mol). 41  $\mu\text{mol}$  was then added to 10 mL buffer solution and analyzed: Concentration of 4.1 mmol/L. Therefore, the measured value of about 3 mmol / L caffeine is quite satisfactory.

Comparing the low-cost with the STAT-ECL equipment one can conclude

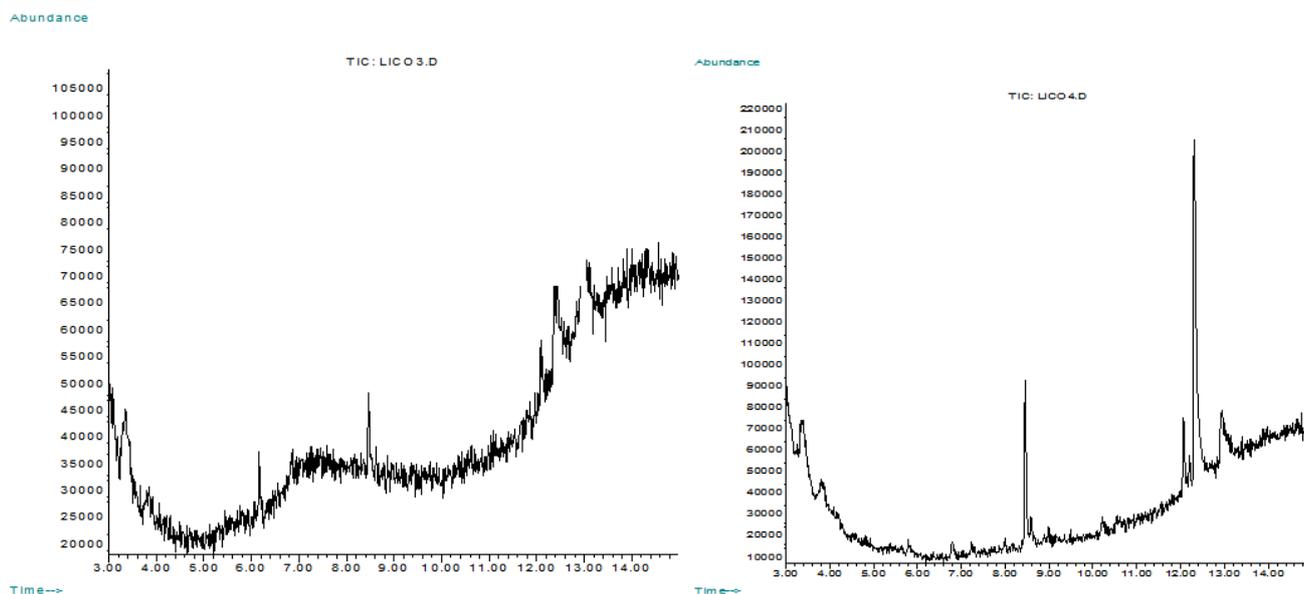
- STAT-ECL can record CV and ECL simultaneously,
- the S:N ratio for both methods are quite similar: for 1 mmol caffeine: S:N (“STAT-ECL”)  $\approx$  S:N (“low-cost”)  $\approx$  2 (see Figure 3 and Figure 7),
- the ECL method has the advantage of being sensitive, and the SPE used are cheap (€1-3 each), but the

cost of  $[\text{Ru}(\text{bpy})_3]^{2+}$  is a disadvantage. With a commercial STAT-ECL system the measurements are very fast, the sensitivity is high, but the high price of the device can be off-putting (€10,000),

- in contrast, the low-cost equipment gives more than satisfactory results, keeping in mind that the experimental effort is higher than with the “STAT-ECL” equipment.

### 3.4. Detection Limit of GC-MS

Figure 8 shows the GC-MS of lidocaine and caffeine, 10  $\mu\text{mol}$ , respectively. The S:N ratio is about 5:1 for lidocaine and 3:1 for caffeine. Therefore, the LOD for caffeine is comparable with that of the ECL method. For lidocaine, however, the ECL method is clearly more sensitive.



**Figure 8.** GC-MS for 10  $\mu\text{mol/L}$  of lidocaine (12.5 min: “L”) and caffeine (13 min: “C”). Experimental conditions: Column: RTX-35 (Restek), carrier gas: Helium, temperature-profile: 100°C (1 min isotherm), temperature-rate: 15°C/min, final temperature: 280°C, 5 min isotherm. Left: Injection volume: 2  $\mu\text{L}$ , right: Injection volume: 10  $\mu\text{L}$

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