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The Analysis of Paracetamol – A Comparison between Electrochemistry, Electrochemiluminescence and GC-MSD

A. Habekost*

Department of Chemistry, Ludwigsburg University of Education, Reuteallee 46, D-71634 Ludwigsburg, Germany
*Corresponding author: A.Habekost@t-online.de

Abstract We present two electrochemical methods—cyclic voltammetry and electrogenerated chemiluminescence—to qualitatively and quantitatively detect paracetamol, a pharmaceutical preparation. The results are compared to those achieved with GC-MSD.

Keywords: *four-year undergraduate, beginner PhD student, analytical, electrochemistry, electrochemiluminescence, mass spectrometry, hands-on learning/manipulatives*

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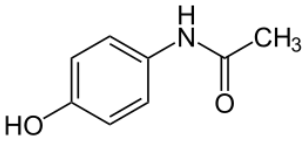
1. Introduction

Paracetamol (*N*-acetyl-*p*-aminophenol) (=acetaminophen) is an



analgesic that reduces fever and general pain. The half-life in plasma is 2–3 h. Paracetamol is metabolized in the human body via glucuronidation and sulphatation and partially via hydroxylation at the nitrogen atom. This leads to the toxic *N*-acetyl-*p*-benzochinonimine, which when in excess of 10 g may cause fatal liver necrosis [1]. The detailed mode of metabolic action of paracetamol is described by Wegner et al. [2].

In addition to acetylsalicylic acid and ibuprofen, paracetamol is one of the most common medicines. The total annual turnover of paracetamol in Germany alone reaches 80 million packs that have a commercial value of about 145 million euros [3].

It is not only the economic importance but also the simple synthesis and analytics that make paracetamol a very attractive compound to use in chemistry lessons [2,4,5,6].

In this paper we complement the routinely used analytical methods of chromatography and titration with the electrochemical methods of cyclic voltammetry (CV) and electrogenerated chemiluminescence (ECL). We also compare the analytical detection limits of the electrochemical methods used with those of Gas chromatography-mass spectrometry (GC-MSD).

2. Pedagogical Objectives

Electrochemistry plays an important role in curricula, textbooks, and everyday life, but several problems exist for learning electrochemistry, as already mentioned in this journal [7].

We think that the pedagogic benefit of the described experimental procedures is based on the combination of electrochemical and spectroscopic experiments. The electrode reactions are directly observable via ECL on the basis of $[Ru(bpy)_3]^{2+}$ and the emitted light can be detected as a function of the applied potential. This means that the ECL and electrical current can be measured synchronously. In addition, students can estimate the analytical strength of ECL and compare it with GC-MSD. The demonstrations of the ECL phenomenon are eye-catching. We would like to summarize the didactic reasons for presenting the ECL to an audience of chemistry students.

- Demonstrate ECL and correlate to CV predictions
- Investigate the electrochemical instruments (potentiostat, light detection)
- Use ECL in analytical techniques—in particular in investigating pharmaceutical substances such as paracetamol. We refer the reader to exhaustive reviews on this subject [8-11].

As mentioned in the keywords, the described experiments were conducted in a fourth-year undergraduate-level university course in analytical electrochemistry. Until now, thirty students (in three lab periods 2015/2017, in six courses) carried out the described experiments at the end of a practical electrochemistry course for advanced students.

We suggest that high-achieving students enjoyed the experiments, because the combination of three disparate

subjects—electrochemistry, light detection, and mass spectrometry—were unusual.

The aim of this article is to present a versatile experimental setup for measuring the (spectro) electrochemical characteristics of paracetamol and for testing the detection limits. The analytical validities of CV and ECL are compared to that of GC-MSD.

3. Experiments

Paracetamol can be analyzed with the Marquis reaction (i.e., the reaction with sulfuricacid and methanal (volume ratio 1:20)). For quantitative and qualitative detection, paracetamol is first hydrolyzed to *p*-aminophenol with sulfuric acid. Then *p*-aminophenol is titrated with $Ce^{4+}$to form *p*-quinonimine. At the equivalence point, the redoxindicator ferroin changes the color from blue to red.

Cyclic voltammetry (CV) and related method as differential pulse (DPV) and square wave voltammetry (SWV) are very meaningful and simple-to-use methods to qualitatively determine paracetamol and demonstrate the electrochemical behavior.

Several authors use different electrodes: Crispimet al used a Sonogel-Carbon electrode [12], Santos et al. [13] and Eisele [14] a cathodically pretreated boron-doped diamond electrode, Tyszczuk-Rotko [15] a boron-doped diamond electrode modified with Nafion and lead films, Fan [16] a TiO_2-graphene electrode, and Atta [17] a gold nanoparticles modified carbon paste electrode. These few recent examples will serve to illustrate the wide range of the activity to electrochemically detect paracetamol.

To the authors knowledge only Yuan et al [18] and Haslag et al [19] used electrochemiluminescence detection of paracetamol.

3.1. Cyclic Voltammetry of Paracetamol with Screen-printed Electrodes

Many texts about CV are available (see for example [20,21,22,23]). Here, we will present only the basic outline that is necessary for an understanding of the presented experiments.

CV is obtained by measuring the current between the working electrode and the counter electrode as a function of the potential (normalized to the potential of the reference electrode). To do this, a three-electron setup is used and the potential of an electrode (the "working" electrode), which is immersed in an unstirred solution, is varied. The resulting current is measured.

A triangular potential sweeps the potential of the working electrode between the starting potential and the switching potential and back again. The scan rate v (in V/s) is an important parameter: If the current peaks (anodic and cathodic peaks) appear to be sliding apart as a function of the scan rate, the process is quasi-reversible (or, in an extreme case, irreversible). If the difference between anodic and cathodic current peaks is about 59 mV, the (one) electron transfer process is reversible.

Reversible reaction: $59\ mV \leq \Delta E_{anodic\text{-}caathodic} \leq 62\ mV$

Quasi-reversible reaction: $62\ mV \leq \Delta E_{anodic\text{-}cathodic} \leq 200\ mV$

Irreversible reaction: $\Delta E_{anodic\text{-}cathodic} \geq 200\ mV$

The current flows in or out of the working electrode to or from a counter electrode. The potential of the working electrode is controlled versus a reference electrode (e.g., a saturated silver/silver chloride electrode). The reference electrode passes no current.

All these requirements can be fulfilled by a potentiostat.

In this study, a rapid, sensitive, and inexpensive detection method with different commercial screen-printed electrodes (SPEs)—based on graphite, platinum, carbon nanotubes, or single-walled carbon nanotubes SPEs—is used to quantitatively detect paracetamol.

A wide range of applications of SPEs exist for detecting pharmaceuticals [24]. An SPE is a sensor that is based on screen-printing technology and prints various types of inks on different substrates. Several devices are commercially available [25]. The main advantages of SPEs are their great utility, versatility, simplicity, and reliability, as well as the small instrumental setup and modest costs.

To check the quality of the SPE used, wetest the CV with the solvent to see whether any electrochemical activity occurs.

Chemicals and instruments:

The instruments include a potentiostat (DropSensµStat 400), SPE (DropSens): DS 100 (working electrode (WE): graphite), DS 550 (WE: platinum), DS SWCNT (WE: single walled carbon nanotubes), and DS CNT-GNP (WE: carbon nanotube decorated with Au nanoparticles). The chemicals included is tilled water, paracetamol (DropSens), and phosphate buffer (pH8).

Hazard

Paracetamol toxicity is caused by excessive use or overdose of the medication. Therefore, avoid uncontrolled swallowing; if swallowed, seek medical advice immediately.

Figure 1 shows the CV of paracetamol with different SPEs. The current and anodic and cathodic peak distances depend on the SPE used. This means that the electron transfer rate issignificantly dependent on the electrode material.

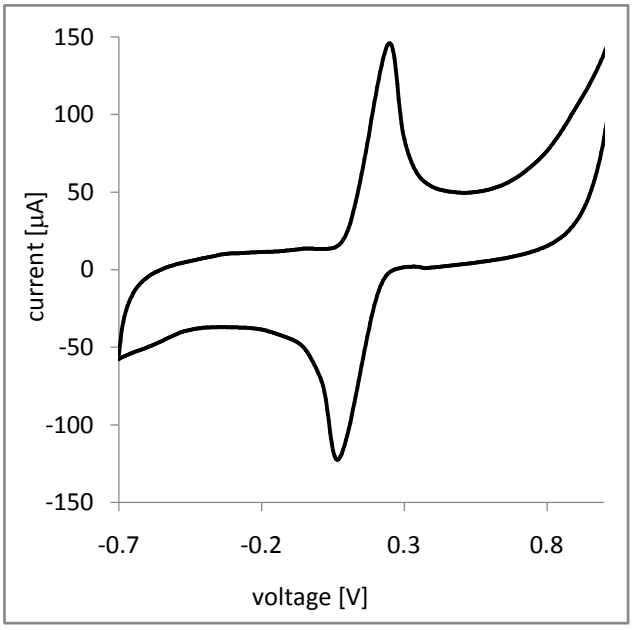


Figure 1a. CV of paracetamol (1 mmol, pH 8) with different SPEs (Figures with the same ordinate).SPE: CNT-GNP

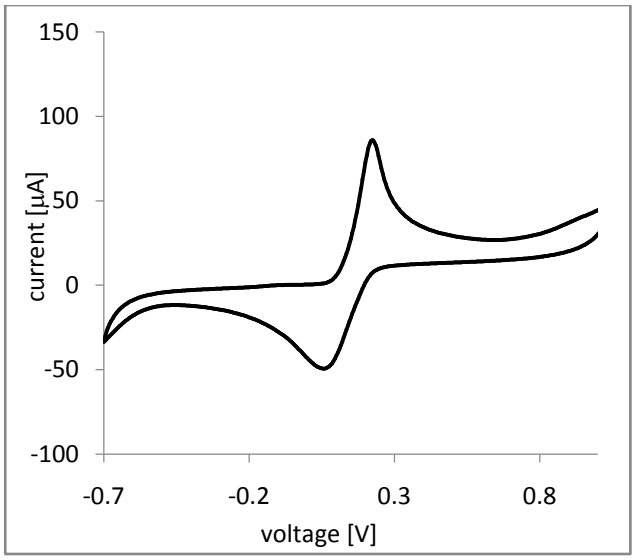


Figure 1b. SPE: SWCNT

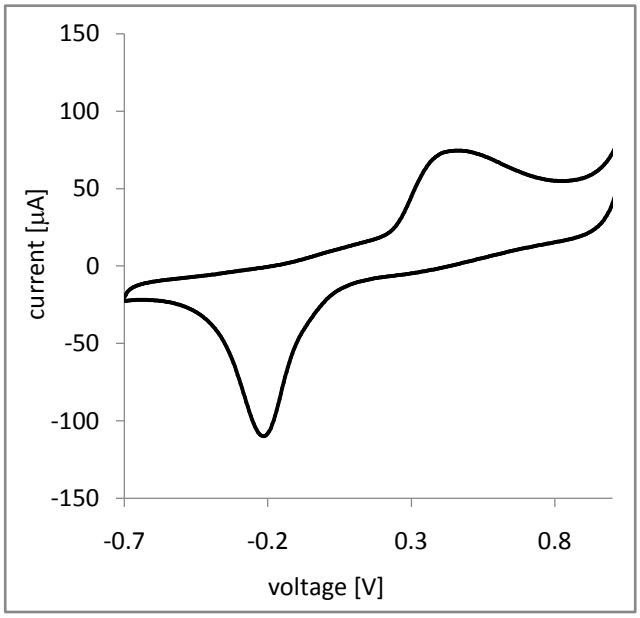


Figure 1c. SPE: platinum

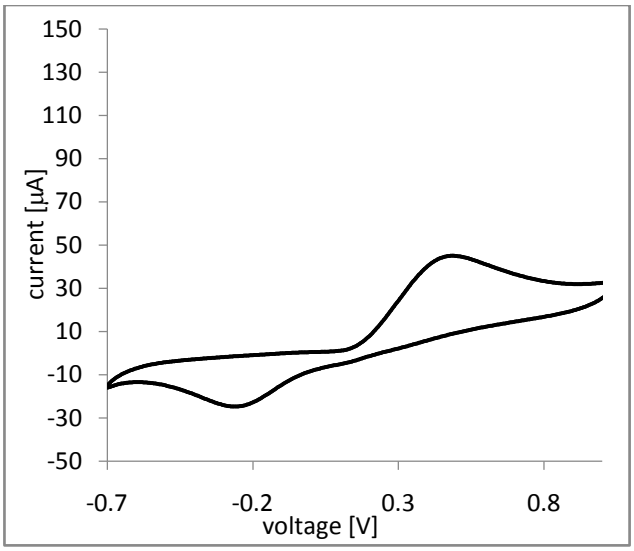


Figure 1d. SPE: graphite

In the following experiments we use the CNT-GNP SPE, because both the current and the electron transfer rate are maximal.

Figure 2 shows an electronmicroscope image of the electrode.

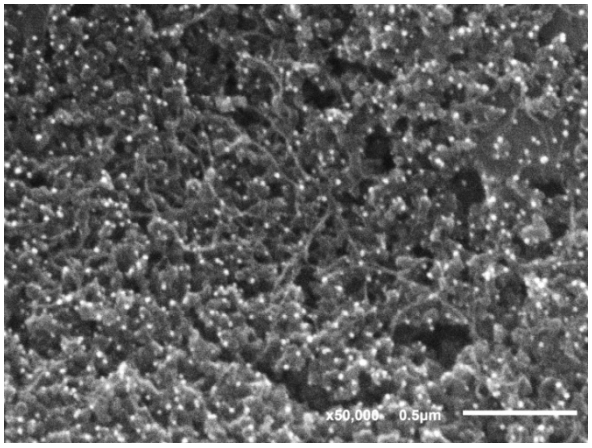


Figure 2. Electron microscope image of the CNT-GNP SPE. The white dots are the Aunanoparticles that coat the CNT surface

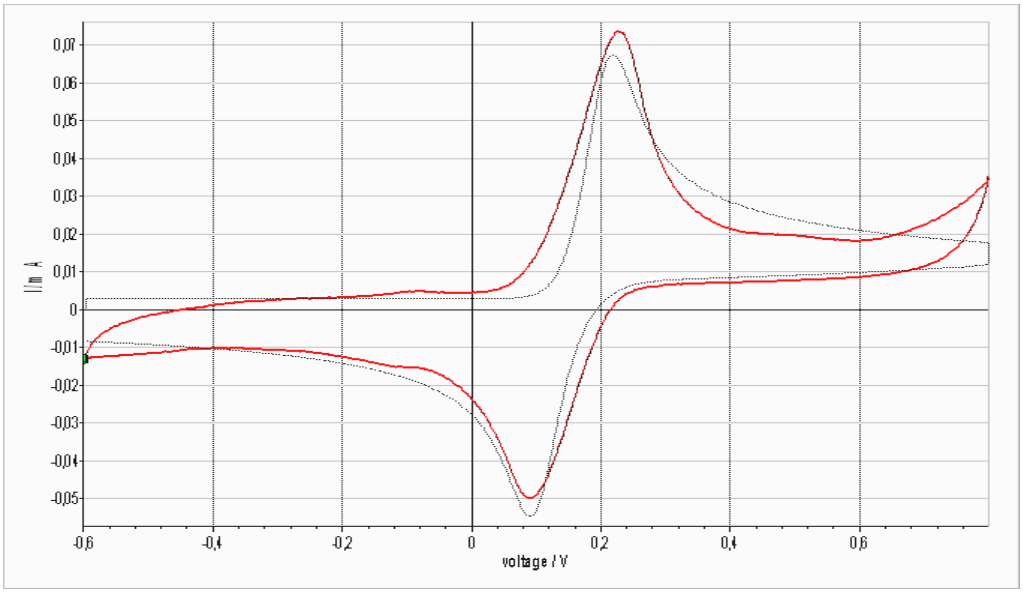


Figure 3. Experimental (red) and fitted (black) CV curves of paracetamol with CNT-GNP SPE

Fitting the experimental CV (program EC lab from BioLogic [26]) gives an electron transfer rate of k = $1.5 \cdot 10^{-4}$ s^{-1} and a standard potential of E_0 = 0.144V (vs Ag/AgCl). An analogfit of the CV with agraphite electrode gives a significantly lower transfer rate of k < $1 \cdot 10^{-6}$ s^{-1} (not shown).

Scan rate dependence of the CV

Figure 4 shows the scan rate dependence of the CV. For reversible reactions, $\Delta E_{anodic\text{-}cathodic}$ does not depend on the scan rate.

In the case of paracetamol, $\Delta E_{anodic\text{-}cathodic}$ increases with increasing scan rate. This is typical for quasi-reversible electron transfer reactions.

The Randles-Sevcikequation describes the current as a function of the scan rate v:

$$\mathbf{Ip} = 2,69 \cdot 10^5 \cdot \mathbf{n} \cdot \mathbf{A} \cdot \mathbf{c} \cdot \sqrt{\mathbf{n} \cdot \mathbf{D} \cdot \mathbf{v}}$$

Where I_p iscurrent peak in A, n is the number of transferred electrons (for paracetamol n=2), A is the electrode area(12.56 cm^2), c is the concentration in mol/cm^3, D is the diffusion coefficient in cm^2/s, and v is the scanrate in V/s.

Figure 5 shows the Randles-Sevcikplot forthe anodic current peaks.

The linear dependence indicates a diffusion-controlled process. This means that the electron transfer rate is faster than the diffusion.

The slope ofthe line gives the diffusion coefficient D = $5 \cdot 10^{-6} cm^2/s$.

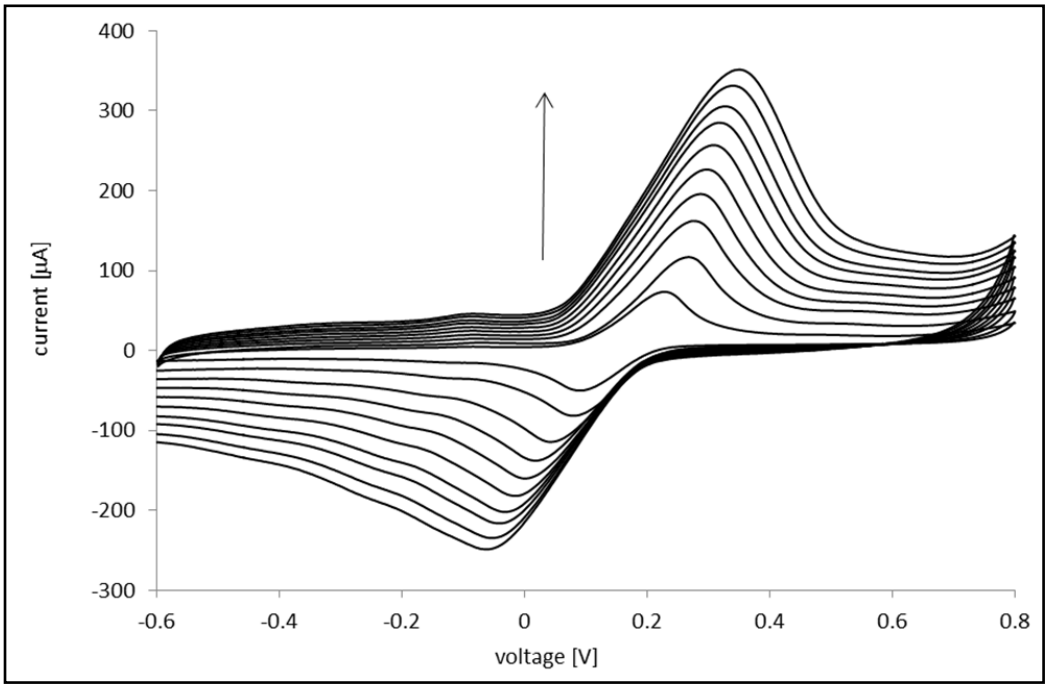


Figure 4. CV of paracetamol with a CNT-GNPSPE. Scan rate: 10–100 mV/s. Slower scan rates yield lower currents. The arrow indicates increasing scan rate

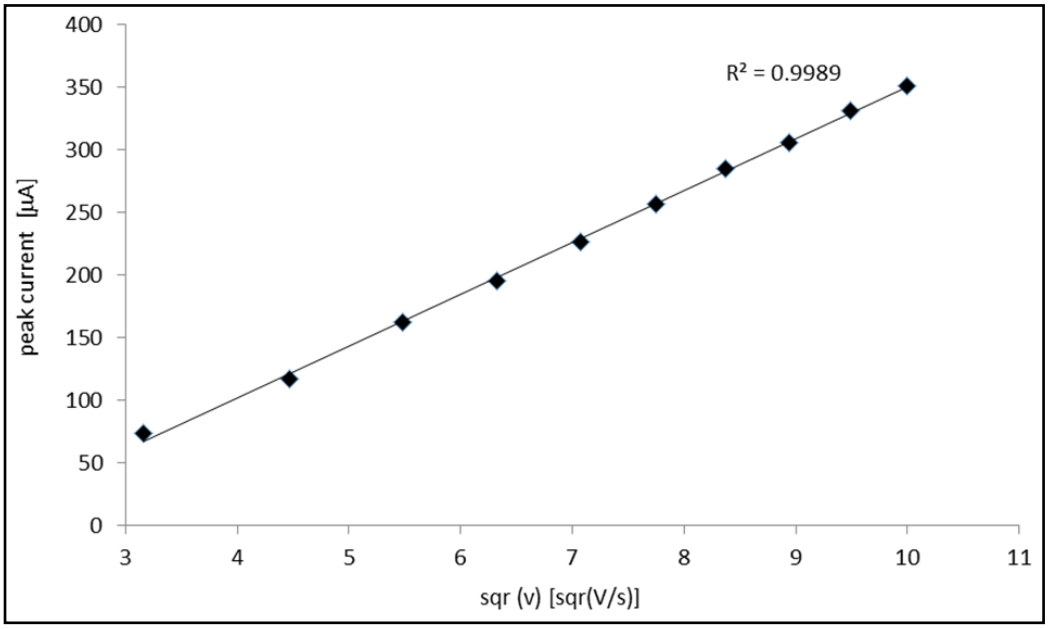


Figure 5. Randles-Sevcikplot

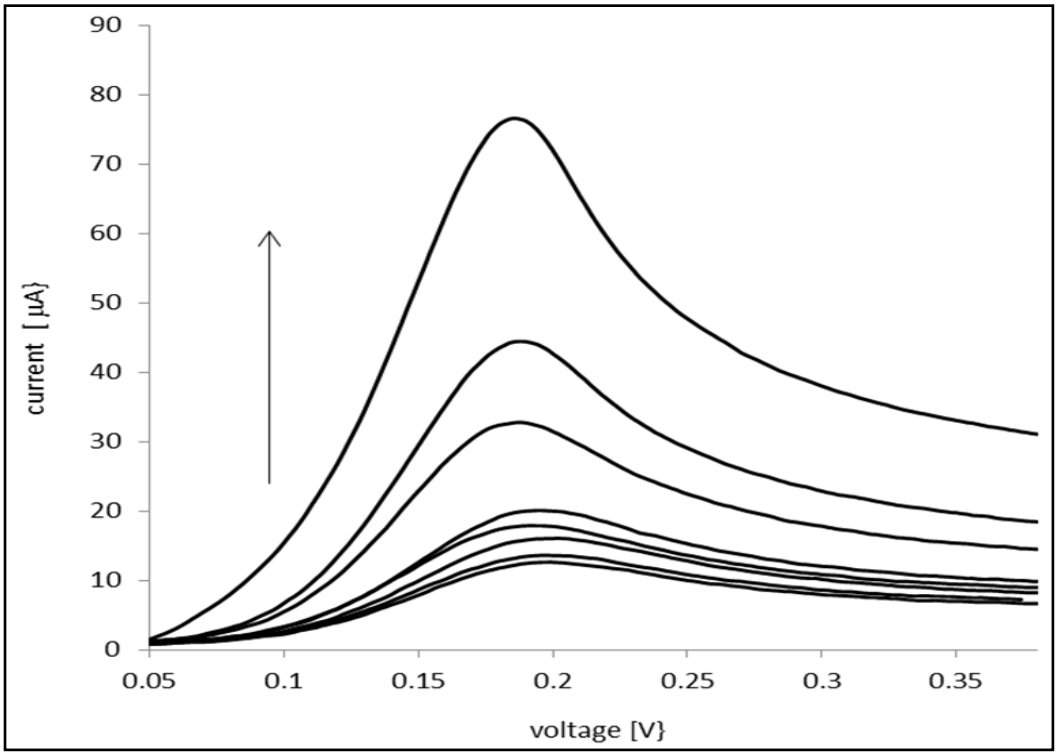


Figure 6a. Linear sweep voltammetry (LSV): Current as the function of different concentrations of paracetamol. The arrow indicates increasing concentration

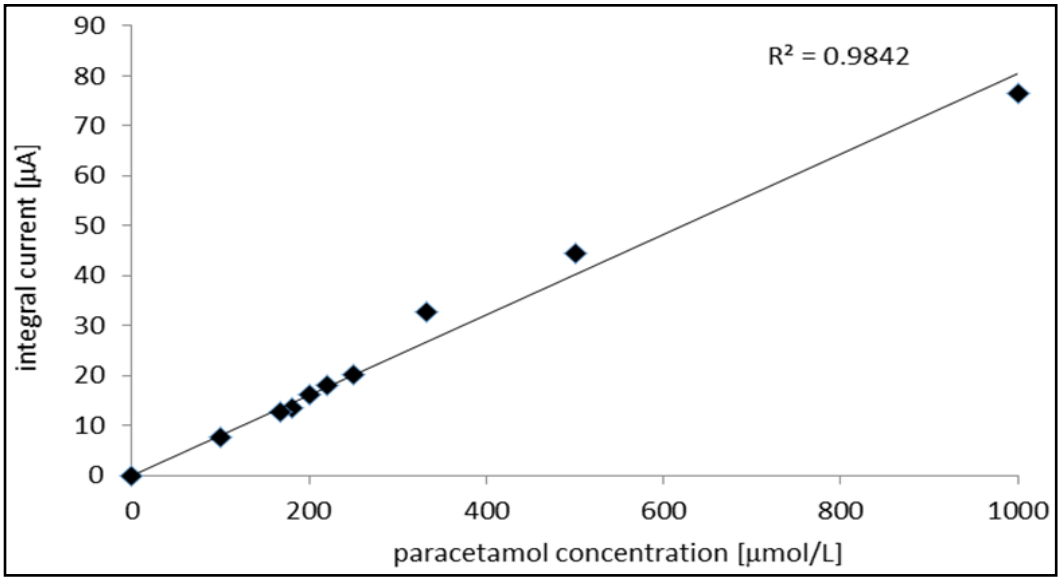


Figure 6b. Current peak as a function of the concentration of paracetamol (100–1000 µmol/L)

Concentration dependence of the anodic current peak

To quantitatively analyze paracetamol, the anodic current peak $I_p$is measured as a function of the paracetamol concentration c in the range of 100–1000 µmol/L. For simplicity, the linear sweep method is used instead of CV. In each measurement, 50 µL is dropped onto the SPE. According to the Randles-Sevcikequation, I_p depends linearly on c.

3.2. Cyclic Voltammetry and Electrogenerated Chemiluminescence

ECL has been previouslydescribed in detail [27,28,29]. Here we only summarize the fundamental aspects.

The most commonly used luminophore is tris(2, 2'-bipyridyl)ruthenium (II) $[Ru(bpy)_3]^{2+}$. $[Ru(bpy)_3]^{2+}$ is electrochemically oxidized to $[Ru(bpy)_3]^{3+}$ and reacts with a coreactant (here proline) forming an excited singlet state $[Ru(bpy)_3]^{2+*}$.

Under the emission of light, $[Ru(bpy)_3]^{2+*}$ thermalizes tothe ground state. The emission wavelength, which depends on pH, is around 600 nm.

The ECL system used is a combination of $[Ru(bpy)_3]^{2+}$ and proline (as coreactant), which produces one of the most intense ECL results [11]. In addition, this system has excellent water solubility and is less toxic than tripropylamine, one of the most common coreactants in ECL studies. Adding paracetamol, the ECL is quenched

[19]. Quenching can be used to quantitatively determine paracetamol. Figure 7 shows the used ECL cell with a fiber that guides the ECL directly into the photomultiplier, figure 8a shows the ECL quenching at two different paracetamol concentrations (5 µmol/L and 10 µmol/L).

For simplicity, the ECL curves are measured with a programmable frequency generator (not with a potentiostat), a two-electrode device, and a standard data acquisition system.

Chemicals and instruments:

SPEDropSens CNT-GNP, Power Cassy (LD didactic, Hürth, Germany)as frequency generator (triangle voltage 0-5 V in10 s), Sensor Cassy (LD didactic, Hürth, Germany) as data acquisition system, photomultiplier (R4220P, Hamamatsu), power supply forphotomultiplier (LKB, Bromma, USA), micropipette (Transferpette, Brand, Germany, 100 µL), fiber (diameter 4 mm), ECLcell (HPLC cell, Kontron).

The SPE is stuck below the measuring cell and the working and counter electrodes are connected with the frequency generator. The solution under investigation is pipetted into the cell (volume 80 µL) so that all electrodes are wetted.

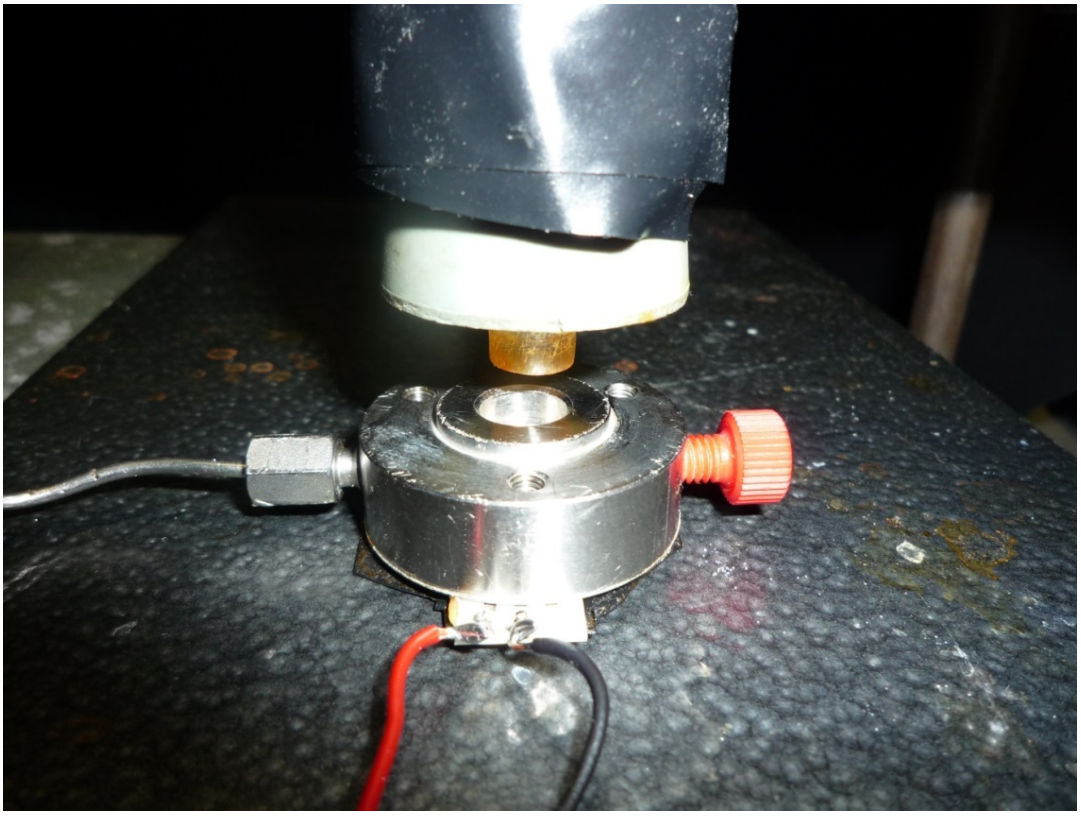


Figure 7. An ECL cell for ECL. The SPE is fixed below the cell. Volumeis about 80 µL. The fiber guides the ECL into the photomultiplier and is inserted into the hole of the cell

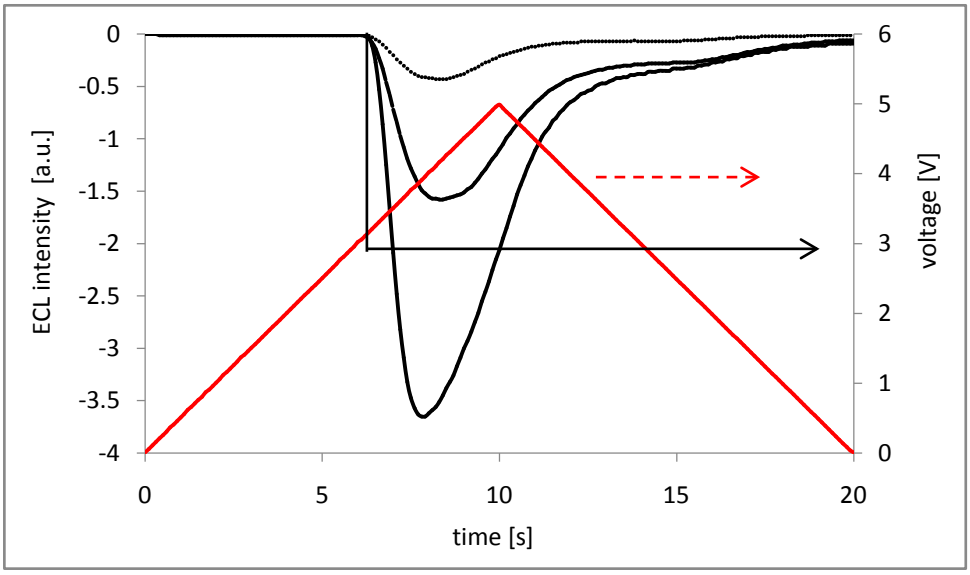


Figure 8a. ECL of $[Ru(bpy)_3]^{2+}$ - prolinealone (solid line) and with paracetamol (5µmol/L (dashed line) and 10 µmol/L (dotted line). The red curve represents the applied angular voltage (5V in 10 s)

Figure 8a shows the quenching effect for two different paracetamol concentrations (5 and 10 µmol/L dashed and dotted lines, respectively). The black curve shows the ECL of $[Ru(bpy)_3]^{2+}$ / proline alone (100 µmol/L) and the red curve shows the applied triangle voltage.

Figure 8b shows ECL quenching as a function of different paracetamol concentrations.

The lowest paracetamol concentration measured by ECL is about 5 µmol/L, which is less than the lowest concentration measured by CV (or LSV).

3.3. Gas Chromatography-mass Spectrometric Detection (GC-MSD)

GC-MD is one of the most established procedures for measuring substance concentrations.

Different concentrations of paracetamol in ethanol are injected and analyzed by GC-MSD. The temperature profile of the GC is 50°C in the first minute with 280°C as a final temperature. The temperature increase is at a rate of 15°C/minute. The MS detector starts after the solvent peak at 2.5 minutes.

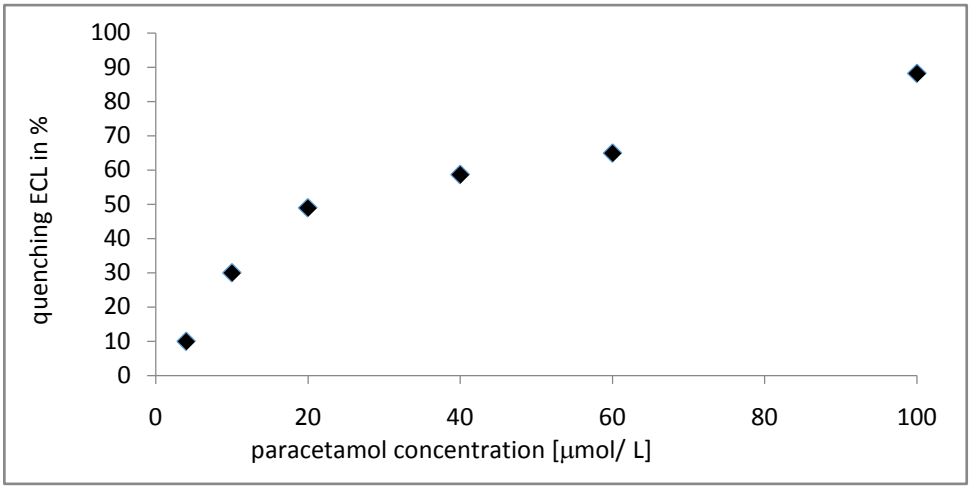


Figure 8 b. ECL quenching as a function of paracetamol concentration

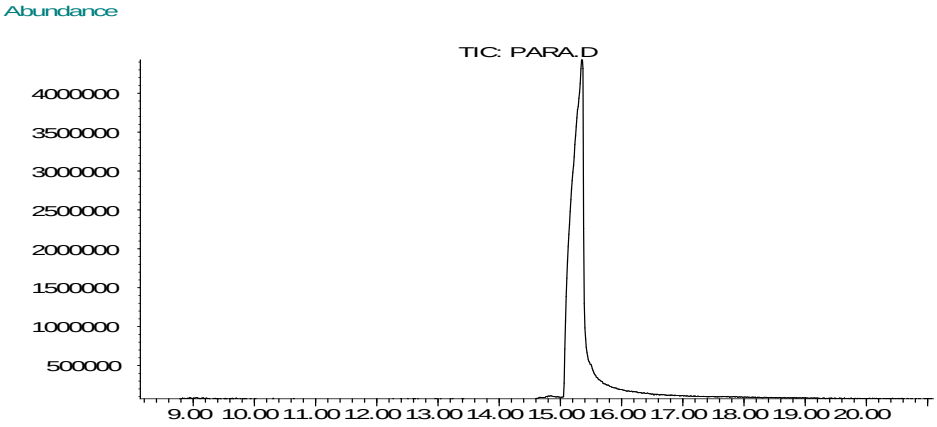


Figure 9a. Part of the GC-MSD of paracetamol (100 µmol/L, injection 1 µL).GC (Hewlett-Packard, 5890 SII), MS (Hewlett-Packard 5972) with capillary column (RTX-35 Restek);helium as carrier gas. Temperature profile: 50°C (1 min isotherm) to 280°C (temperature rate 15°C/min), 280°C (5 min isotherm); Autosampler (Hewlett-Packard, 7673)

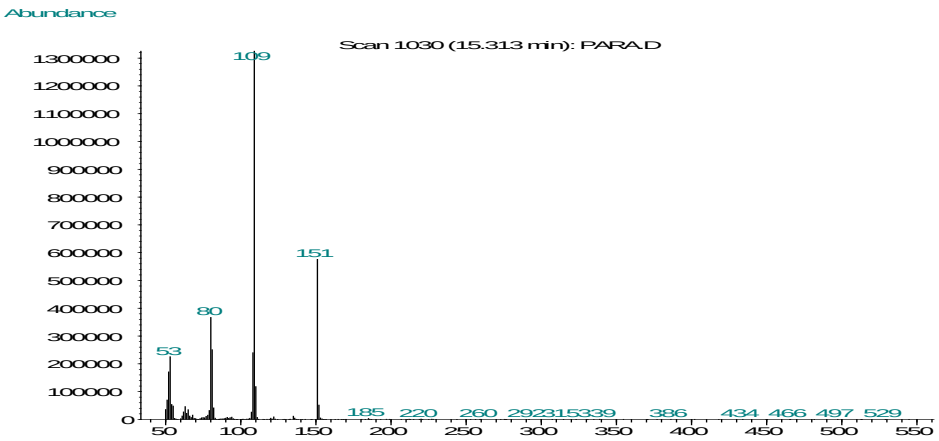
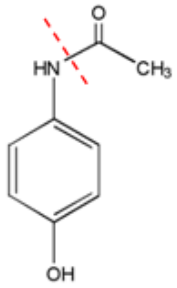


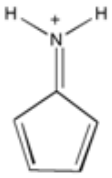
Figure 9b. EI-MS of paracetamol

m/e = 151 amu: paracetamol

109 amu:



80 amu: Cyclopentadienyliden ammonium cation



53 amu: unknown

Figure 9c. Assignment of fragments

The GC of paracetamol (Figure 9a) shows only one remarkable peak at 15.5 minutes (detection quality of the EI mass spectrum: 99% compared to the Wiley library), indicating that no degradation occurs on the column. Figure 9a shows a part of the GC and Figure 9b the fragmentation with the main fragments (151 amu, 109 amu, 80 amu, 53 amu, see Figure 9c).

Table 1 summarizes the detection limits ofthe methods used.

Table 1. Summary of the electrochemical and GC-MSD results

Method	Detection limit(mol)	Detection limit (g) ($M_{paracetamol}$= 151 g/mol)
CV (LV)	$5 \cdot 10^{-9}$	$7.6 \cdot 10^{-7}$
ECL	$3.5 \cdot 10^{-10}$	$5.3 \cdot 10^{-8}$
GC-MSD	$< 10^{-10}$	$< 1.5 \cdot 10^{-8}$

4. Conclusion

This paper has described (spectro) electrochemical methods (CV and ECL) for identifying paracetamol. The results were compared with the results obtained by GC-MSD. CV has a limit of detection (LOD) in the range of 5 nmol. Paracetamol has been shown to effectively quench the ECL of $[Ru(bpy)_3]^{2+}$-proline with the minimum quenching concentration of DCF being about 0.35 nmol. In contrast, the LOD of GC-MSD gives the value of lower than 0.1 nmol.

In summary, GC-MSD is still the best method for paracetamol analysis.

5. Further Work

In our further work, we will test whether the electrochemical and ECL-detection are satisfactory to analyze paracetamol in different solutions, too, e.g. in waste water or in urine: Are there any cross-sensitivities? In addition, we have to clarify the optimum pH for highest sensitivity.Amperometric detection (AD) is astandard electrochemical analysis technique in HPLC. We will test a new electrochemical HPLC cell (DropSens, Spain) to measure the limit of detection of paracetamol with the AD method.

Acknowledgements

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