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The Analysis of Nicotine by Electrogenerated Chemiluminescence (ECL) - Experiments for Undergraduates

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Abstract This study highlights the effectiveness of electrogenerated chemiluminescence (ECL) techniques for the analysis of nicotine, both in its pure form and within cigarettes and smoke. The measurements are simple to perform, and the required equipment is relatively inexpensive, making these experiments suitable for inclusion in undergraduate chemistry laboratory courses. Unlike absorbance and fluorescence methods, which can quantify nicotine in isolation but struggle within cigarettes due to interference from other compounds-particularly carotenoids, which fluoresce more strongly than nicotine-ECL selectively detects nicotine. Carotenoids, unlike nicotine, do not respond to ECL, making this technique well-suited for nicotine analysis in complex matrices like tobacco and smoke. This approach is further explored in the article.

Keywords: *Nicotine, electrogenerated chemiluminescence*

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1. Introduction

Nicotine is a harmful substance found in tobacco. Upon inhalation, nicotine rapidly reaches the brain, prompting the release of neurotransmitters such as dopamine, which reinforces the cycle of dependence [1]. Over time, nicotine addiction can result in the chronic use of tobacco products, which are associated with a range of serious health risks, including cardiovascular disease, stroke, and respiratory problems [2]. Furthermore, nicotine can have a detrimental impact on the developing brain, rendering it a particularly hazardous substance for adolescents and pregnant women [3]. In addition to its addictive properties, nicotine contributes to the overall toxicity of tobacco, thereby exacerbating the health risks associated with its use.

Detecting nicotine is crucial for several important reasons. In the field of public health, the accurate measurement of nicotine levels in products such as cigarettes, e-cigarettes, and nicotine replacement therapies (nicotine patches) are of great importance. This enables the regulation of their content and the protection of consumers from excessive exposure. Furthermore, it ensures compliance with safety standards and supports the enforcement of laws related to tobacco and nicotine-containing products. In research, the detection of nicotine is essential for studying its effects on health, understanding patterns of addiction, and developing effective cessation methods. Additionally, monitoring nicotine in various environments, such as workplaces or public spaces, is crucial for assessing secondhand exposure and its potential health risks.

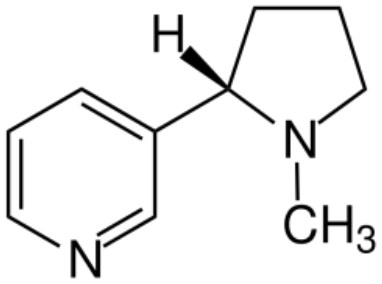


Figure 1. Structure of nicotine

There are various methods for the detection of nicotine, each with different degrees of sensitivity, accuracy and applicability, dependent on the context in question. The following methods are commonly employed: Gas chromatography (GC) (in many cases, gas chromatography (GC) is coupled with mass spectrometry (GC-MS), Liquid chromatography (LC), in particular high-performance liquid chromatography (HPLC), Enzyme-Linked Immunosorbent Assay (ELISA), Infrared (IR) spectroscopy.

All these methods vary in complexity, cost, and the type of sample they can analyse. The selection of a method is contingent upon the specific requirements of the detection task, such as the desired sensitivity, the matrix

of the sample, and the context in which the detection is performed.

It is our contention that both the equipment and the interpretation of the data are complex, and therefore these methods are not suited to undergraduates.

In contrast, we will present and analyse a straightforward technique: electrochemical chemiluminescence, based on $[Ru(bpy)_3^{2+}]$.

2. Experiments

2.1. Instruments and Chemicals

Materials: Photomultiplier R 4632 with the power supply socket C6270 from Hamamatsu, Mobile-CASSY 2 for data aquisition (ld-didactic, Germany), potentiostat (Metrohm/DropSens), ECL cell (Metrohm / DropSens), screen-printed electrode (SPE, DRP 550 Pt-Bt Metrohm / DropSens: working electrode: low-temperature platinum ink, counter electrode: platinum, pseudo reference electrode: silver), fiber (Thorlabs 200 µm), Hamilton 5 µL syringe (S.G.E. Australia, 0.1 µL precision)

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

Chemicals: (-)-nicotine, Roth, Germany (8746.1), West, red (American blend), $[Ru(bpy)_3^{2+}]$, TCI (T 1655, aqueous solution of 1 mmol/L)

Procedures and calculation of the different nicotine concentrations:

A) Nicotine alone: 20 mg nicotine (molecular weight 162 g/mol) = $1.23*10^{-4}$ mol. This is solved in 10 mL of distilled water ($1.23*10^{-2}$ mol/L). Therefore 1 µL of this solution contains 12.3 nmol nicotine. Various volumes are added to the $[Ru(bpy)_3^{2+}]$ solution and analysed.

B) West cigarette: One West cigarette contains 0.6 g tobacco. This is solved in 10 mL of distilled water. Stir for 10 minutes. 1 µL is added to 60 µL $[Ru(bpy)_3^{2+}]$ solution and is pipetted onto the SPE to wet all the electrodes and analysed.

C) Smoke of a West cigarette: The smoke was drawn into a bubbler containing 10 mL distilled water via a pump (Figure 2). 20 µL of the aqueous solution was added to 60 µL $[Ru(bpy)_3^{2+}]$ solution and analysed.

Figure 2. Apparatus for washing cigarette smoke. From left to right: cigarette, bubbler, pump

Figure 3 shows the ECL apparatus.

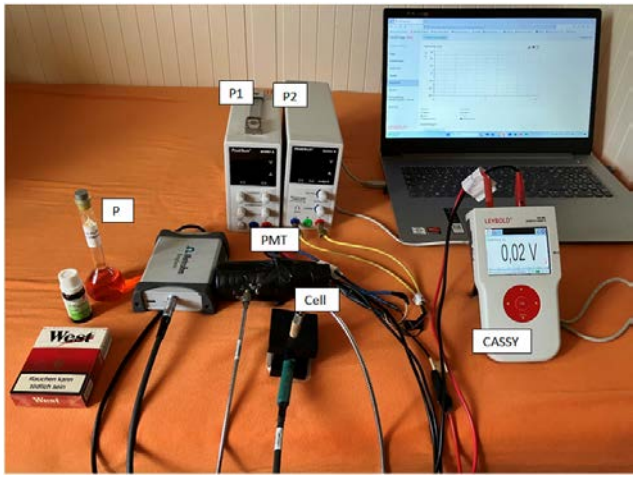
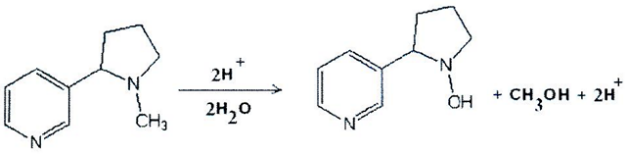


Figure 3. ECL apparatus: potentiostat (P), photomultiplier (in housing, PMT) with power supplies (P1 and P2), ECL cell with SPE holder and fibre (cell), SPE inside the cell, data acquisition system (CASSY)

2.2. CV and ECL Measurements

Cyclic voltammograms (CV) were used to assess the redox behaviour of nicotine. At approximately 0.75 V, nicotine underwent oxidation. No reduction process was observed in the back scan, indicating that the oxidation of nicotine is irreversible (electrochemically or chemically). The CVs are pH-dependent, with the oxidation peak shifting to higher potentials as the pH increases (not shown here).

In acidic solutions, Radhi et al. proposed that nicotine oxidizes to form the hydroxide nicotine (scheme 1) [5].



Scheme 1. Oxidation of nicotine in an acidic solution.

Figure 4 illustrates a diminishing oxidation signal with each scan, suggesting that the oxidation product of nicotine is adsorbed on the electrode surface (platinum).

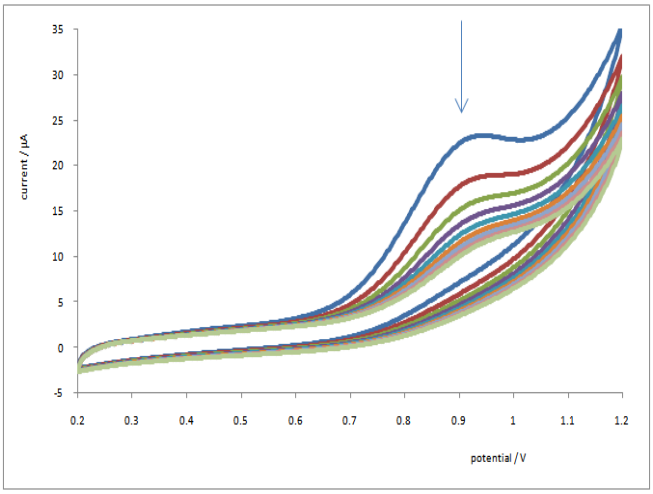


Figure 4. CV of nicotine (in aqueous sodium sulphate solution). Nine times in a row. The arrow indicates the decrease of the oxidation signal with each scan

Since the 1960s, electrochemiluminescence (ECL) has gained increasing popularity in analytical chemistry [5]. ECL involves generating an excited state of the widely used and extensively studied tris (2,2'-bipyridyl) ruthenium(II) complex, $[Ru(bpy)_3]^{2+}$, on an electrode surface. The process starts with the oxidation of the $[Ru(bpy)_3]^{2+}$ complex, which then engages in an electron transfer reaction with a coreactant. This reaction creates an excited state, which subsequently decays and emits light around 600 nm. ECL is often described as a "marriage of electrochemical and spectroscopic methods" [6]. For comprehensive reviews of ECL, see works by Miao [6], Kapturwicz [7], Hercules [8], Richter [9], Parveen [10], and Bard [11].

Jackson and Bobbit [12] have demonstrated that the ECL method is particularly well-suited for detecting tertiary amines. ECL offers several advantages, including a relatively simple experimental setup and a low detection limit.

$[Ru(bpy)_3]^{2+}$ itself exhibits moderate luminescence, is readily soluble in both water and non-aqueous media at room temperature, undergoes an one-electron transfer reaction at moderate potentials—resulting in stable reduced and oxidized species—and can be regenerated after emission.

Figure 5 shows the potential-dependent ECL of $[Ru(bpy)_3]^{2+}$ alone (black line) and in the presence of various concentrations of nicotine (colorful lines). The photomultiplier tube (PMT) measures the light-dependent voltage, which is transferred to the data acquisition system (CASSY®), displayed as a graph, and recorded in a table. This data can be easily analysed using Excel, and the integral can be calculated using the trapezoidal rule [13].

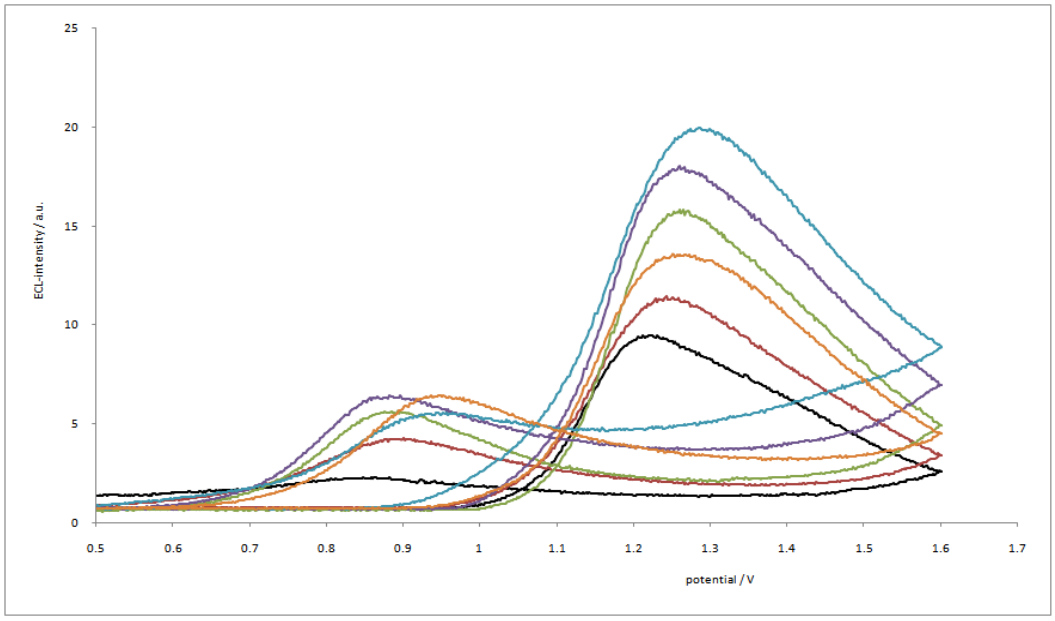


Figure 5. Potential-dependent ECL. The dotted line represents $[Ru(bpy)_3^{2+}]$, while the solid lines show $[Ru(bpy)_3^{2+}]$ with 5, 20, 30, 35, and 48 nmol nicotine

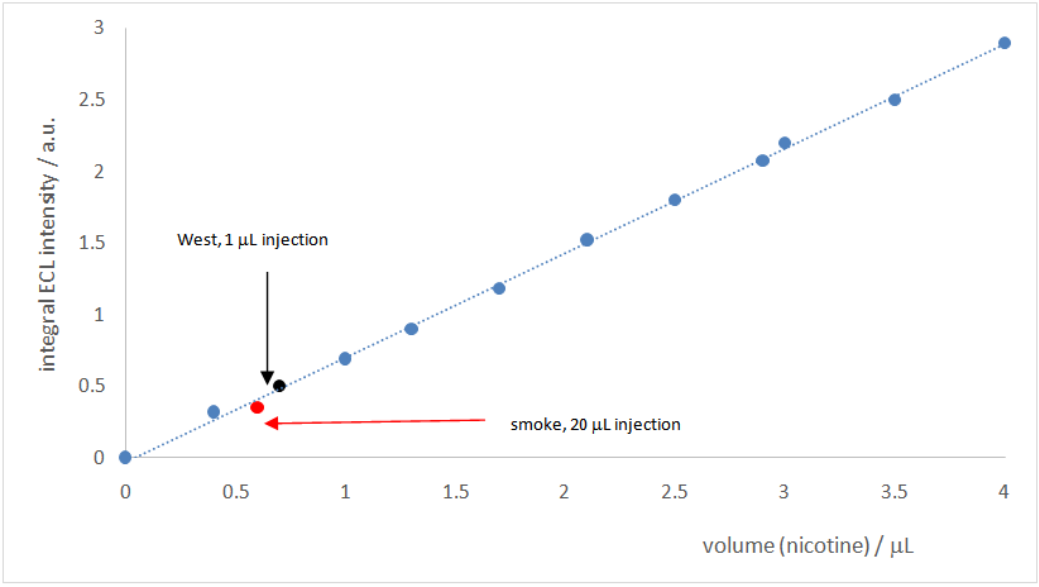


Figure 6. ECL signal as a function of the nicotine concentration. Red point: ECL intensity of smoke (20 µL injection), black point: ECL intensity of West cigarette (1 µL injection)

Figure 6 shows the linear dependence of the integral ECL intensity on nicotine mass up to 4 µL.

Analysis of West tobacco using the ECL method (after extraction with distilled water) yields a mass of approximately 13.6 mg. This is based on an ECL response of 0.5 a.u. for an injected 1 µL sample containing 8.4 nmol of nicotine, equivalent to 1.36 µg, with a molecular weight of 162 g/mol. Therefore, the total mass of nicotine in a whole cigarette dissolved in 10 mL of solution is calculated to be 13.6 mg. This result aligns with the literature [14].

Cigarette smoke, however, contains a lower concentration of nicotine compared to tobacco. The analysis shows a mass of approximately 0.6 mg, based on an ECL response of 0.35 a.u. for an injected 20 µL sample containing 7.2 nmol of nicotine, equivalent to 1.17 µg. Consequently, the total mass of nicotine in smoke dissolved in 10 mL of solution is 0.6 mg (calculated as 1.17 µg / 20 µL * 10 mL). This value is slightly lower than the 0.9 mg indicated on the West cigarette package. The discrepancy may be due to incomplete dissolution of nicotine in water during the extraction of smoke, as well as some nicotine being released into the air during smoking.

Table 1. Conversion injected volume – mole – mass for nicotine alone into 60 µL $[Ru(bpy)_3]^{2+}$ solution

Injection of nicotine alone µL	nmol	µg
0	0	0
0,4	4,8	0,7776
0,6	7,2	1,1664
0,7	8,4	1,3608
1	12	1,944
1,3	15,6	2,5272
1,7	20,4	3,3048
2,1	25,2	4,0824
2,5	30	4,86
2,9	34,8	5,6376
3	36	5,832
3,5	42	6,804
4	48	7,776

As shown in Figure 6, the ECL analysis of nicotine from a single West cigarette yields an estimated nicotine mass of approximately 1.34 µg. Considering the dilution factor (1 µL of a 10 mL solution is analyzed), the calculated total nicotine mass is around 13 mg, which is consistent with the data available online [14]. However, the ECL response for West cigarette smoke is slightly lower than the expected nicotine mass of 0.9 mg (based on the analysis of 20 µL from the 10 mL solution) [4]. This discrepancy is likely due to some of the smoke not being captured by the bubbler and instead dispersing into the air.

Conclusion

The Ludwigsburg University of Education has updated its curriculum to include new analytical methods. Students should learn about the ECL technique in chemistry lectures and gain hands-on experience through practical work. They can also engage in discussions about the advantages and limitations of these experiments, particularly the effectiveness of ECL measurements for analyzing nicotine.

The ECL method offers the advantages of being both inexpensive and easy to use. It has a detection limit suitable for analyzing cigarette smoke, with a sensitivity of 1 µg in a 60 µg solution, corresponding to a total detection limit of 6 nanomoles.

However, a key drawback is that the screen-printed electrode (SPE) must be carefully cleaned after each measurement by rinsing it with ethanol and water.

ACKNOWLEDGMENTS

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