

Analysis of p-Nitrophenol Reduction

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Abstract p-Nitrophenol (p-Np) is a chemical compound that causes various problems in the environment. The main aspects of the environmental problem are [1]: Toxicity to humans, animals and aquatic organisms and persistence in the environment. p-Np is a relatively stable compound and therefore degrades slowly in the environment (up to years in anaerobic environments). Through industrial effluents and pesticide applications (e.g. degradation products of parathion) it can accumulate in ecosystems and become a long-term problem. Due to its solubility in water, p-Np can easily penetrate groundwater and endanger drinking water supplies. Modern waste water treatment with appropriate measures is therefore necessary. Purification methods such as adsorption, photocatalysis and electrochemical oxidation are used to eliminate p-Np in wastewater [1]. Their monitoring helps to identify the sources of pollution, to safeguard water quality and thus to protect the health of ecosystems and people in the long term. In particular, electrochemical and spectroscopic methods have been developed for the detection and quantification of p-Np [1]. This article presents two analytical methods for the identification of p-Np: UV-VIS and Raman spectroscopy. The combination with electrochemical methods such as linear sweep voltammetry (LSV) and differential pulse voltammetry (DPV) allows the spectroscopic monitoring of the redox reactions of p-Np.

Keywords: Reduction of p-Nitrophenol, UV-VIS spectroscopy, SERS-Raman spectroscopy

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1. Introduction

The electrochemical reduction of p-Np under acidic conditions proceeds in several steps to p-aminophenol (p-AP). The reaction scheme is shown in Figure 1.

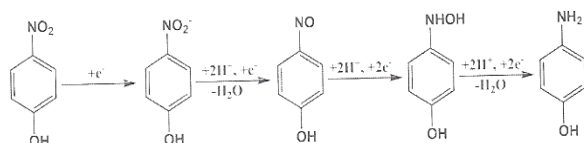


Figure 1. Reduction of p-Np (left) to p-AP (right) [1,2]

The stepwise reduction of p-Np (4-Np) to p-AP (4-AP) involves the sequential reduction of the nitro group (-NO₂) to an amino group (-NH₂) through the formation of intermediates such as nitroso (-NO) and hydroxylamine (-NHOH). The process typically takes place in the presence of a reducing agent or electrochemically at the cathode. The reducing agent donates electrons and protons from water as the solvent to the nitro group, breaking one of the N=O bonds to form a nitroso group. The nitroso group (-NO) is then further reduced to form a hydroxylamine group (-NHOH). The hydroxylamine group is finally reduced to an amino group (-NH₂), completing the conversion. The hydroxyl group (-OH) attached to the nitrogen is removed as water, and the remaining nitrogen atom accepts hydrogen atoms to form the amino group (-

NH₂).

Attempts to reduce p-Np to p-AP using sodium borohydride (NaBH₄) are only successful when different nanoparticles are added, as shown by Liu et al [3].

The oxidation of p-Np in the presence of the TiO₂ photocatalyst has been extensively studied by Mendez et al [4]. Under the influence of UV radiation, an electron-hole separation takes place in TiO₂. The electron is lifted into the conduction band and the hole remains in the valence band. The absorbed p-Np reacts with the hole to form free organic radicals and, in the presence of water, hydroxide radicals. The electrons from the conduction band also react with the oxygen physically dissolved in water to form hydroxide radicals. Both radicals eventually react with p-Np to form CO₂ [5].

2. Pedagogical Objectives

The experiments described are not suitable for school chemistry courses, but for university courses, because the price (about 40,000 USD) is very high.

With low-cost equipment, experiments are limited to fluorovoltammetry:

All you need is an LED and a cheap photomultiplier. If available, low-cost USB potentiostats or the open hardware Rodeostat (<https://iorodeo.com/collections/cheapstat-open-source-potentiostat/cheapstat>) are a cheap alternative to the potentiostat used in our experiments.

The author is not aware of a cheap alternative to the

commercial Raman spectrometer, but there may be some attempts to use simple laser pointers with an appropriate notch filter and a spectrometer. The disadvantage of this proposal is the time-consuming construction of the apparatus [6].

The University of Education Ludwigsburg has updated its curriculum to include new analytical methods. Students should learn about Raman and electrogenerated chemiluminescence (ECL) techniques in chemistry lectures and gain hands-on experience through practical work. They can also discuss the advantages and limitations of these experiments and the experimental problems.

This paper is aimed at university lecturers who wish to introduce students to the principles of spectroelectrochemistry, in this case fluorovoltammetry, ramanvoltammetry and ECL, in order to improve their knowledge of electrochemical methods.

Fluorovoltammetry can be extended to absorption voltammetry by using non-transparent electrodes with bifurcated light guides with separate excitation and emission fibers. This requires a continuous light source such as a laser (pointer) or LED light source.

We believe that the pedagogical value of the described experimental procedures lies in the combination of electrochemical and spectroscopic experiments (linear sweep or cyclic voltammetry and fluorescence and Raman spectroscopy). The electrode reactions, i.e. anodic and cathodic current peaks, are directly correlated with the emission of light and with a dramatic increase in Raman intensities.

The phenomenon of both ECL and surface-enhanced Raman spectroscopy (SERS) plays an important role in analytical chemistry: The oxidation of the precious metal gold will surprise students, as will the increase in Raman signals after the simple electrochemical formation of a finely dispersed surface.

The experiments described have been carried out in a university course in electrochemistry. So far, more than twenty students have carried out these experiments at the end of an advanced electrochemistry course. The students received the theoretical information in a parallel lecture on physical chemistry, and the students' misconceptions in electrochemistry were addressed in different didactic lectures. The students were not freshmen. The experiments were carried out and the students wrote down their observations. The teacher gave instructions through lecture and discussion, while the students took notes and asked questions. The teacher also solved problems related to spectroscopy and electrochemical concepts. At the end, the students had to remember their predictions and explain their observations: What was the purpose of the experiment? What is the role of the substances used? Consider the electrode reactions. Do you see any advantage in combining electrochemical processes with spectroscopic observation? Without any empirical background, we can say that high achieving students enjoyed the experiments because the combination of two different subjects (electrochemistry and spectroscopy) was unusual.

The advantage of studying p-Np can be summarised as follows:

Spectroscopic studies of p-Np are useful for didactic purposes in university teaching, as they clearly illustrate

basic chemical concepts and analytical methods:

- p-Np shows characteristic absorption bands in the UV-visible region due to its electronic structure, $\pi \rightarrow \pi^*$ transitions: An Introduction to the Interaction of Light and Matter.

- The nitro and hydroxyl groups in p-Np provide an opportunity to discuss the role of functional groups in electronic properties and interaction with light.

- The change in molecular structure in redox reactions is easily achieved by changing the potency. Raman spectroscopy is a suitable tool to follow this change.

- The pH dependence combined with a colour change shows that p-Np has an acid and a base dependent structure, which have different absorption properties and can be visualised by a distinct colour change. This makes p-Np suitable for explaining pH values, protolysis equilibria and the importance of the pKa value.

The disadvantage of using p-Np is that all experiments must be carried out under a fume hood as p-Np is harmful.

3. Experiments

3.1. Spectroelectrochemical Monitoring of the Reduction

The DPV of p-Np on a silver screen-printed electrode (SPE) (Figure 2) shows four reduction current peaks between 0 V and -1.2 V at -0.1 V, -0.4 V, -0.6 V and -0.85 V. Four reduction peaks are also found on an gold electrode, but shifted to higher potentials compared to Ag. This indicates that the reductions on silver are thermodynamically favoured [7].

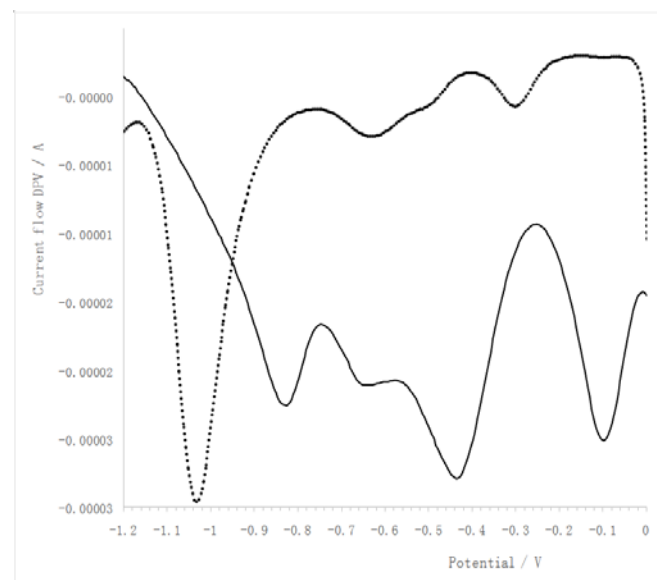


Figure 2. DPV of p-Np on an Ag-SPE (solid line) and on an Au-SPE (dotted line) at pH=5. DPV parameters: Modulation amplitude: 0.025 V, modulation time: 0.05 s, interval time: 0.5 s

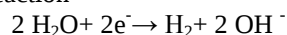
The absorption spectrum of p-Np is pH dependent, as shown in Figure 3 for two different pH values.

The p-Np anion is formed in the alkaline medium. The interaction between the aromatic ring and the non-bonding electrons leads to a red shift in the spectrum. The anion is much more yellow than the neutral p-Np. In addition,

another peak appears at around 260 nm. The Raman spectrum of p-Np shows no difference in the symmetric NO_2 stretching oscillation at different pH values (Figure 4). Therefore, Liu et al. suggest phenolate rather than NO_2^- as the anion [3].

During electrolysis of the aqueous p-Np solution at -2 V, the peak at 320 nm gradually disappears and the peak at

400 nm appears simultaneously (Figure 5). Due to the reaction



in the increasingly alkaline cathode chamber. Even with a long electrolysis time, the spectrum remains practically unchanged (see inset, Figure 5).

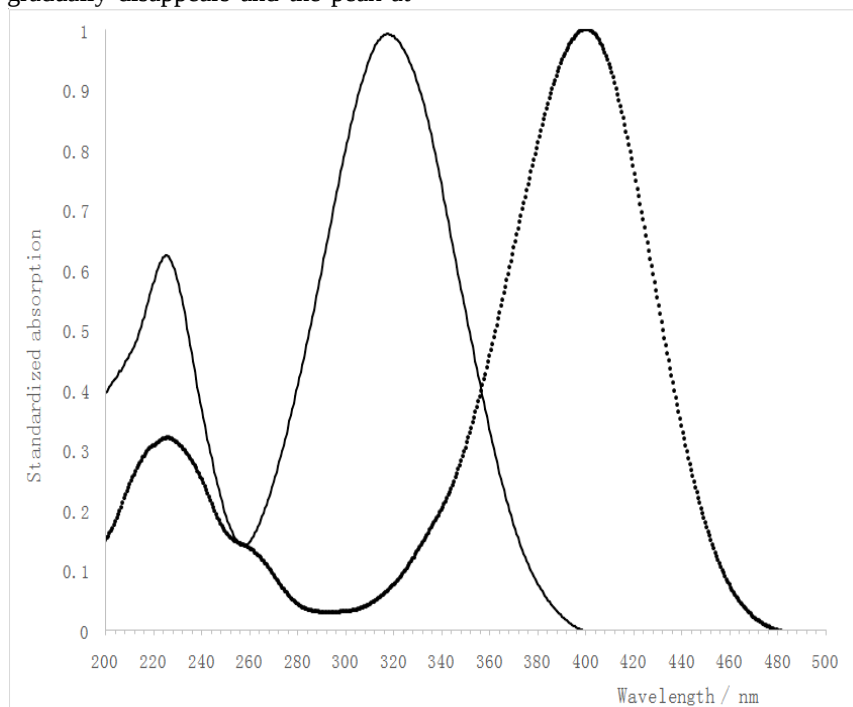


Figure 3. Absorption spectra of p-Np at pH=1 (solid line) and at pH=9 (dotted line)

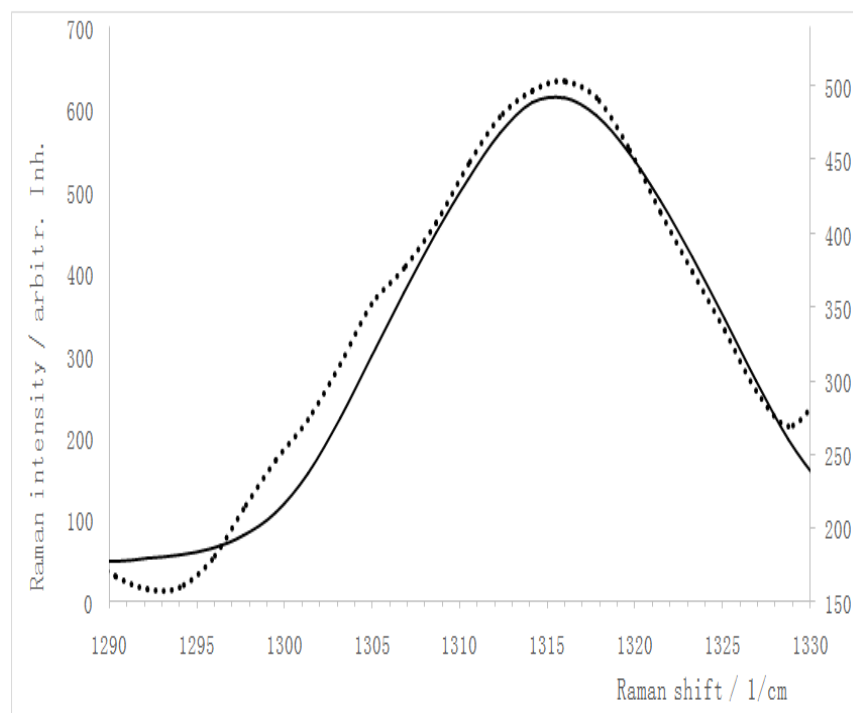


Figure 4. Raman transition of the symmetric NO_2 stretching vibration at 1315 cm^{-1} of p-Np in acid (solid line) and alkaline (dotted line)

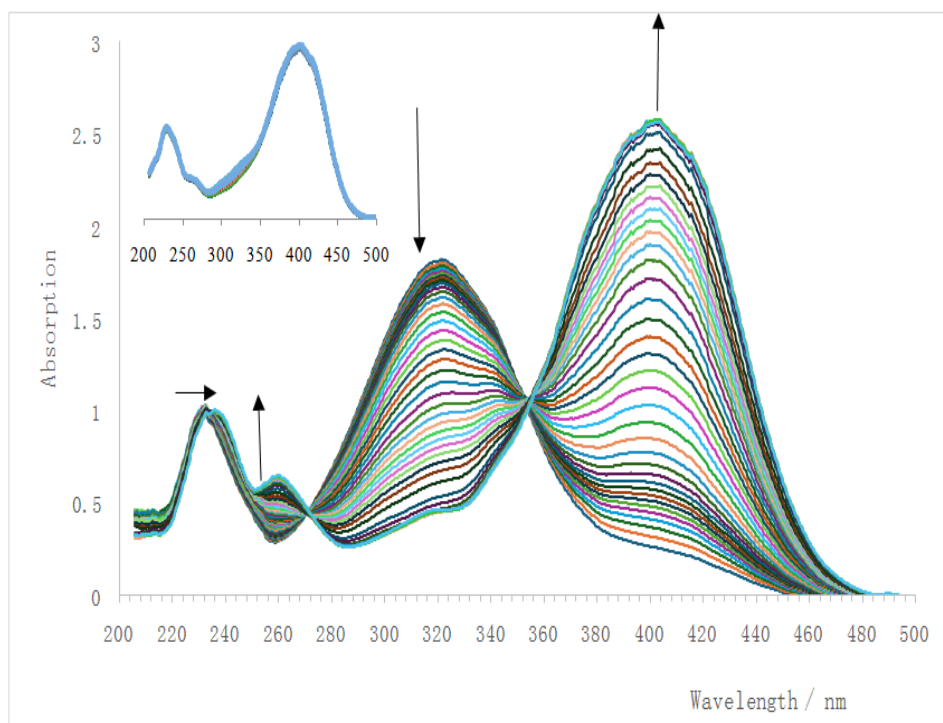


Figure 5. Absorptovoltammogram of p-Np from 0 V to -2 V on Pt-WE. Increase in alkalinity. Inset: Twenty spectra, each recorded after 10 minutes

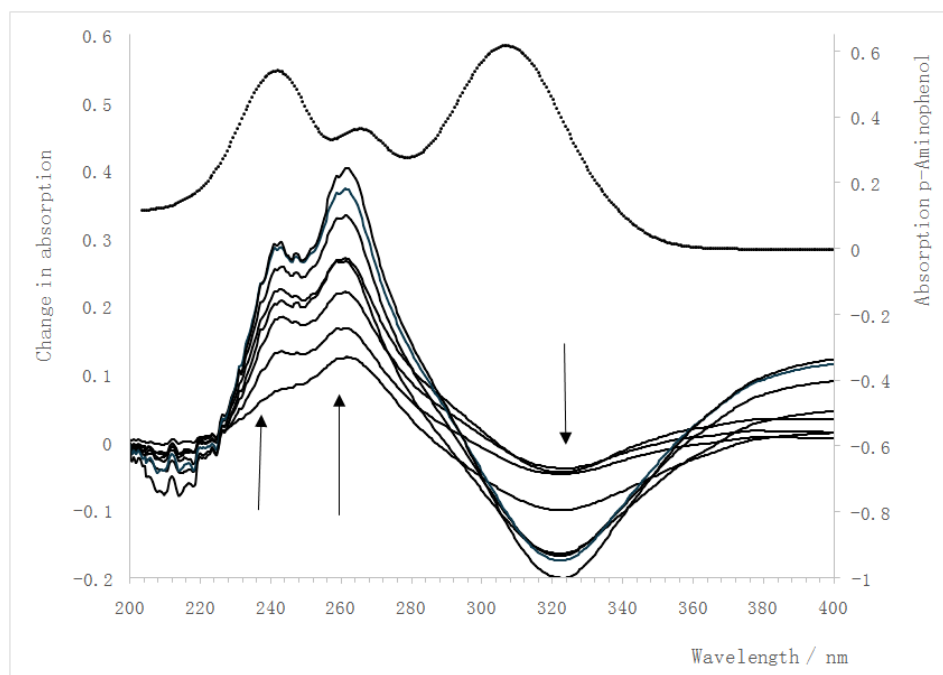


Figure 6. Absorbance change of p-Np at -3 V on a carbon electrode with microholes covered with an Ag-Np. Spectra were recorded every five minutes

When aqueous NaBH_4 solution is added, the same shift in the spectrum is observed because the cathode space also becomes alkaline ($\text{BH}_4^- + 2 \text{H}_2\text{O} \rightarrow \text{BO}_2^- + 4 \text{H}_2 + \text{OH}^-$). However, the reduction potential of NaBH_4 (about -1.2 V in the alkaline solution) is not sufficient to reduce the p-Np.

For the reduction of p-Np to p-Ap, Liu et al [3] additionally used gold nanoparticles on Fe_3O_4 nanoclusters, Ashrat et al [8] gold and silver nanoparticles on cellulose, Zhang et al [9] fly ash with palladium nanoparticles, Muniz-Miranda [10] silver-doped TiO_2 nanoparticles, Krishna et al [11] silver-cobalt on reduced graphene oxide. In all cases, the change in UV-VIS spectrum was used as an indicator of p-Ap formation.

We used Ag nanoparticles (Ag-Np, prepared according to Lee and Meisel [12]). The aqueous solution of Ag particles together with p-Np was placed on a carbon SPE with microholes and the change in absorbance was measured approximately every minute for ten minutes. The result is shown in Figure 6, which shows a decrease in absorbance at 320 nm, a slight increase in absorbance at 240 nm and a more marked increase in absorbance at 260 nm. Figure 6 also shows the absorption spectrum of p-Ap for comparison.

The different changes in absorbance at 240, 260 and 320 nm are striking against the background of the absorption spectrum of p-Ap also shown. The lower

absorbance at 230 nm is due to the fact that p-Np also absorbs in this wavelength range, so the change in absorbance due to the formation of p-Ap is less than at 260 nm. The same applies to the region around 320 nm. Here p-Ap absorbs, so the change in absorbance due to the reduction of p-Np is less.

3.2. Electrochemical Oxidation

Figure 7 shows the change in absorbance at 320 nm due to oxidation of p-Np in the presence of TiO_2 during irradiation at 255 nm. For comparison, the electrochemical reduction of p-Np at +2V is shown. It can be seen that in both cases the absorbance is significantly reduced over a period of 30 minutes. The result is in good agreement with that obtained by Mendez et al. [4]. These authors also formulate the mechanism for the oxidation of p-Np, which should proceed via the intermediates of p-benzoquinone and maleic acid to CO_2 . Both intermediates have been identified by GC-MSD.

3.3. SERS Raman Spectroscopy

To follow the reduction of p-Np over time, SERS-Raman spectra [13] were recorded on an electrochemically prepared nanostructured silver electrode. The fabrication of such an electrode has been described in detail by Martin-Yerga [14]. Figure 8 shows microscopic images of the silver electrode before (left) and after electrochemical activation (right). The increase in surface homogeneity is clearly visible. Martin-Yerga used electron microscopy to show that the surface consists of Ag clusters of 80 to 240 nm in size. This nanostructuring leads to a significant enhancement of the Raman effect. The change in the SERS Raman spectrum of p-Np between -0.3 V and -1.5 V is shown in Figure 9.

Table 1 shows selected Raman transitions of p-Np.

The potential dependence of two selected transitions (740 and 1315 cm^{-1}), the so-called Raman voltammogram, is shown in Figure 10. It can be seen that the Raman intensities of both transitions increase up to about -0.7 V due to the nanostructuring of the Ag surface; the intensity

of the transition at 1315 cm^{-1} (symmetric NO_2 stretching oscillation) then decreases. The LSV (at Pt) shows that the decrease in Raman intensity of the transition at 1315 cm^{-1} directly correlates with the reduction of p-Np. The transition at 740 cm^{-1} cannot be attributed to either p-Np or p-Ap. We therefore assume that this transition can be assigned to one of the intermediates.

When the p-Np solution is electrolysed together with Ag nanoparticles at -2.5 V for ten minutes, the SERS Raman spectrum in Figure 11 (solid line) is obtained. The SERS Raman spectra of p-Np and p-Ap are shown for comparison.

It can be seen that some Raman transitions of the reduction product coincide with those of p-Ap (640 , 840 , 955 , 1165 , 1249 , 1305 and 1321 cm^{-1}), but some still coincide with p-Np (1040 , 1165 , 1320 cm^{-1}). Obviously, the reduction from p-Np to p-Ap has partially taken place after ten minutes.

Table 1. Raman transitions of p-Np, solid and p-Np, SERS and name of transitions (ip: in plane)

Raman (solid) ($1/\text{cm}^{-1}$)	EC SERS-Raman ($1/\text{cm}^{-1}$)	Vibration
1614	1610	Ring stretch
1585	1565	Ring stretch
1520		NO_2 antisymm. stretch
1500	1500	C-H ip bending
1450	1425	C-H ip bending
1326	131	NO_2 symm. stretch
1284	1275	C-H ip bending
1217		O-C stretch
1172	1165	C-H ip bending
1112	1105	C-H ip bending
1070	1060	C-C-C bending
1008	1045	C-H ip bending
964	960	C-H op bending
870	870	NO_2 bending
756	740	NO_2 op bending
694		C=O bending
636 628	620-650 broad	C-C-C bending, CO bending
534	535	NO_2 ip bending

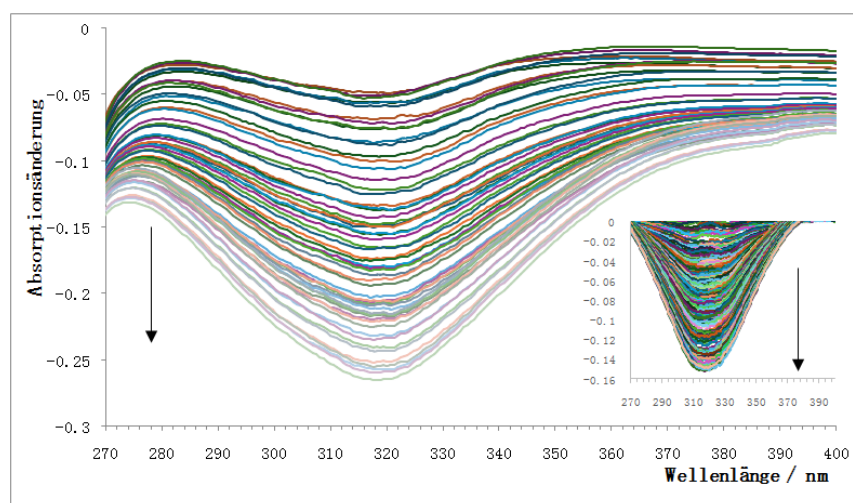


Figure 7. Change in absorption of p-Np during oxidation with $\text{TiO}_2/255\text{ nm}$. Insert: Electrochemical oxidation at +2 V

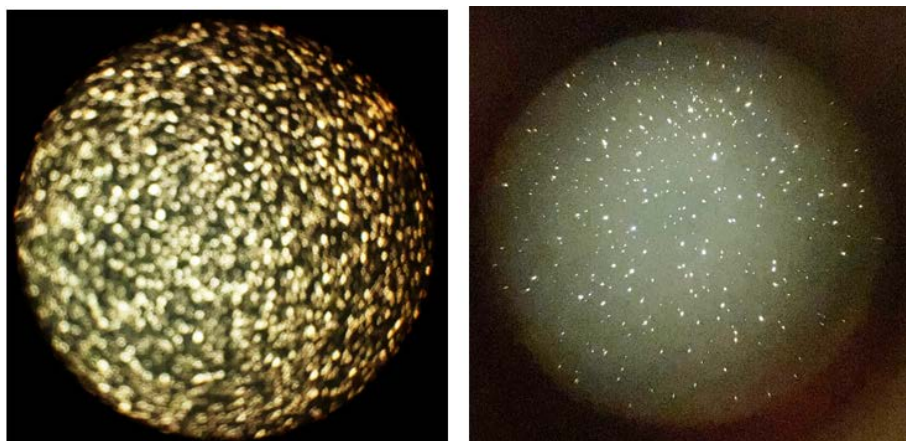


Figure 8. Ag-SPE used. Left before, right after electrochemical activation

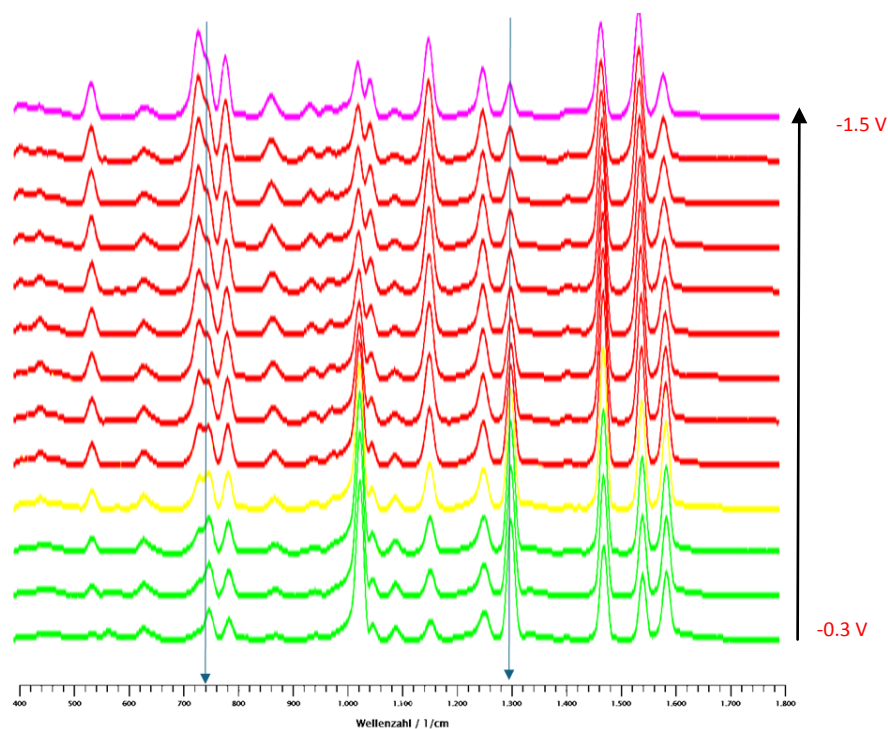


Figure 9. Raman transitions of p-nitrophenol from -0.3 V to -1.5 V (Small arrows: 740 cm^{-1} and 1315 cm^{-1})

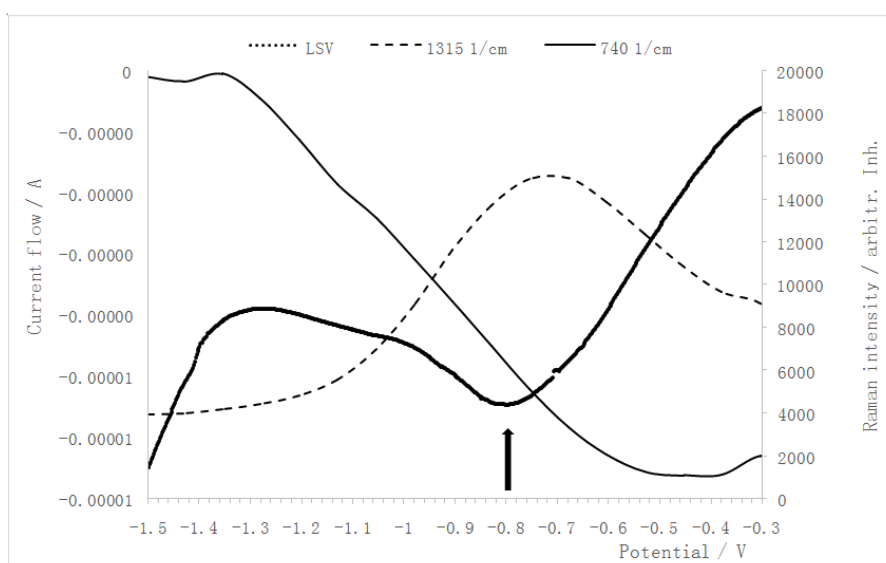


Figure 10. Raman voltammetry of selected Raman transitions. Working electrode: Pt network. Arrow: reduction of p-Np

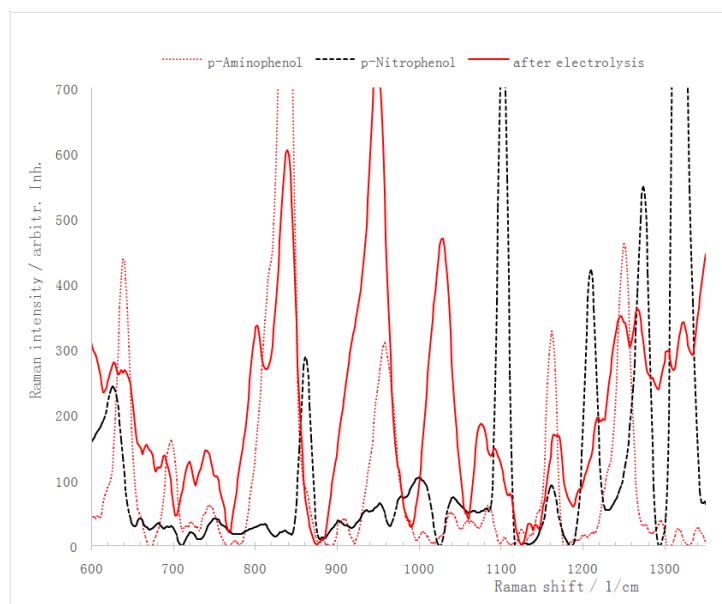


Figure 11. Raman spectrum of the reduction product after electrolysis of p-Np at -2.5 V for ten minutes (solid line). For comparison, the Raman spectra of p-Np (dashed line) and p-Ap (dotted line) are shown

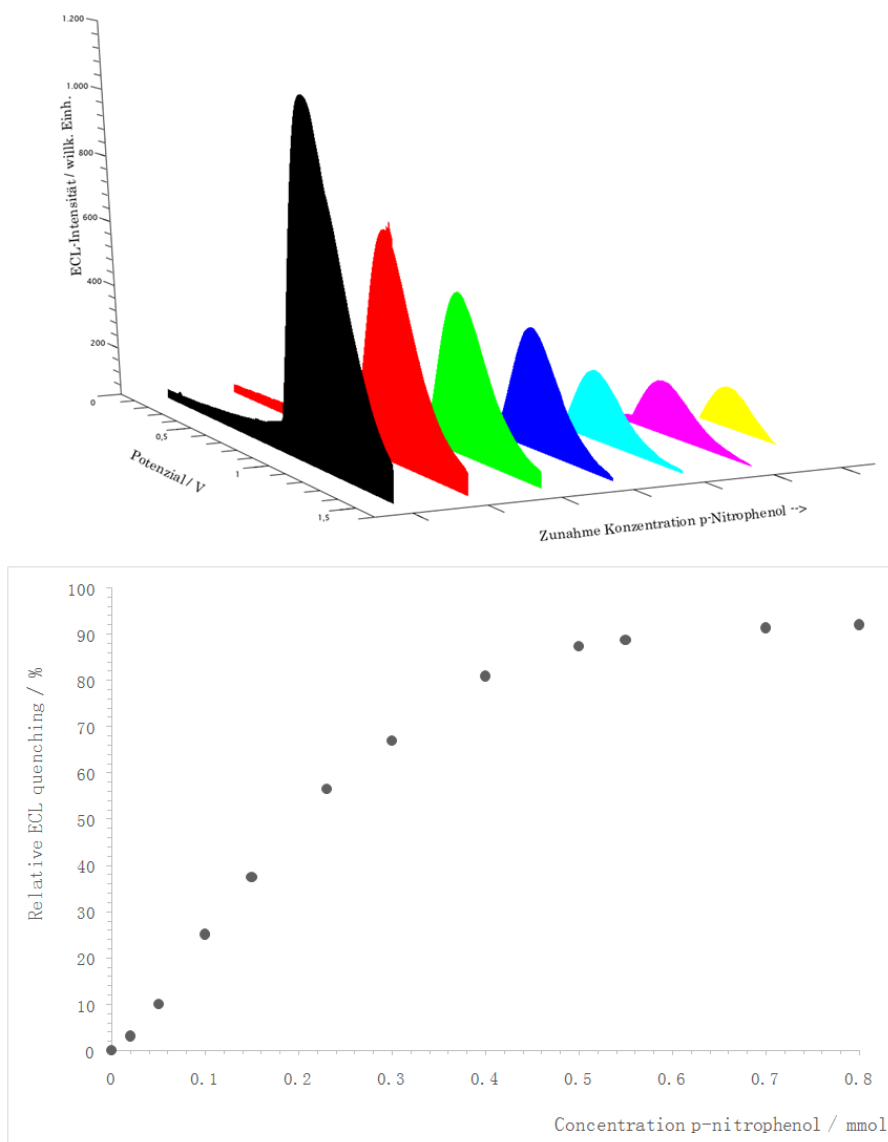


Figure 12. Top: ECL of $[\text{Ru}(\text{bpy})_3]^{2+}$ / proline for different concentrations of p-Np. Bottom: Percentage quenching of the ECL of $[\text{Ru}(\text{bpy})_3]^{2+}$ / proline by p-Np

3.4. Electrochemically Generated Chemiluminescence (ECL)

McCall and Richter [15] investigated various substituted phenols with regard to their quenching behaviour of the ECL of a $[\text{Ru}(\text{bpy})_3]^{2+}$ solution. This prompted us to investigate the quenching of the ECL of $[\text{Ru}(\text{bpy})_3]^{2+}$ / proline by p-Np [16,17,18]. This was done using the commercial STAT-ECL system, which consists of a potentiostat and a photosensitive photodiode. It was found that p-Np also effectively quenches the ECL of $[\text{Ru}(\text{bpy})_3]^{2+}$ / proline, as shown in Figure 12 for different p-Np concentrations. The limiting concentration (of a 1 mmol $[\text{Ru}(\text{bpy})_3]^{2+}$ / proline solution) of still measurable ECL is approximately 0.02 mmol p-Np (Figure 11).

Discussion and Conclusion

p-Np is a harmful substance that causes various diseases when dispersed in the environment. It is therefore necessary to detect p-Np even at low concentrations using user-friendly equipment. This can be done by various spectroelectrochemical methods such as absorbance voltammetry and Raman voltammetry.

Reduction or oxidation processes are the "gold standard" for detoxification of p-Np. Spectroelectrochemical methods provide a good tool for observing these processes as a function of applied potential over time.

For the detection of p-Np at low concentrations, ECL may be a good choice.

Due to the toxic nature of p-Np, it is necessary for students to carry out experiments under a fume hood and to follow all necessary safety conditions.

Further research is needed to detect p-Np in the natural environment of water and soil.

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Supporting Information

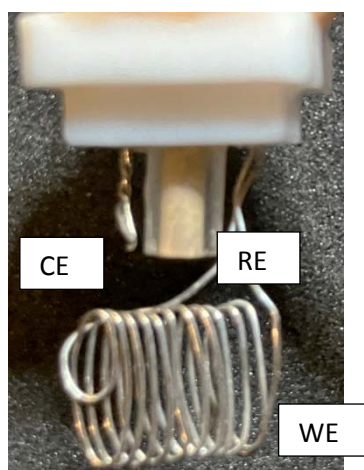
Materials

- Potentiostat: PGSTAT204 Autolab, Metrohm with Nova 2.1.17 software
- ECL potentiostat: STAT-ECL, Metrohm/DropSens with DropView software

- SPE for DPV and Raman measurements: DRP-010 (Ag as working electrode, C as counterelectrode, Ag as reference electrode), Metrohm/DropSens, DRP-220AT (Au as working and counterelectrode, Ag as reference electrode), Metrohm/DropSens
- SPE for redox measurements: C-SPE with 19 microholes DRP-MH-110 (Metrohm/DropSens)



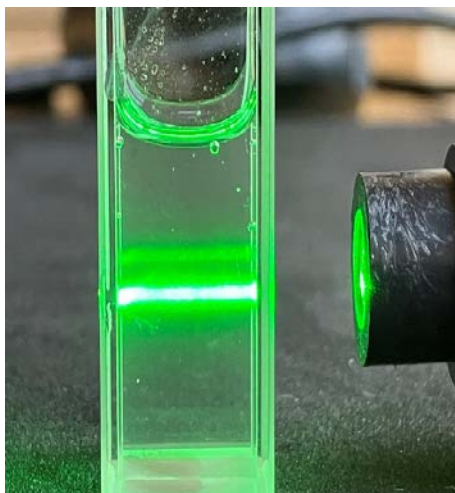
- SPE for ECL measurements: DRP-220Bt (Au as working and counterelectrode, Ag as reference electrode), Metrohm/DropSens
- Quartz cell with Pt working electrode (WE), Ag/AgCl reference electrode (RE) and Pt counter electrode (CE): PTGRID TRANSCELL (Metrohm/DropSens):



- Raman spectrometer: AvaRaman (785 nm laser, 500 mW with Raman probe RPB 785 from Inphotonics), Avantes with software Avasoft 8.16 and Spectragryph 1.2 from Dr. Menges.
- Raman cell for SPE: Metrohm/DropSens
- Eppendorf pipette: 100 μ L
- Analysis software: QtiPlot data analysis and scientific visualisation (<https://www.qtiplot.com/>)
- Fibre optics: double 400 μ m, Thorlabs
- 255 nm LED: Laser components

Chemicals:

- p-Nitrophenol: SigmaAldrich (No. 241326): H302 + H312 + H332, H373, H373, P260, P280, P301 + P312, P302 + P352 + P312, P304 + P340 + P312, P314
- p-Aminophenol: SigmaAldrich (No 800421): H302, H317, H341, H373, H400, H410
- Tris(2,2'-bipyridyl)ruthenium(II) chloride hexahydrate: TCI T 1655, 10 mM solution in 0.1 M KCl aqueous solution: No hazard pictogram, no signal word, no risk phrase, no precautionary statement required.
- Titanium dioxide: SigmaAldrich (No. 14021): Not a dangerous substance or mixture according to Regulation (EC) No 1272/2008.
- Sodium borohydride: SigmaAldrich (No 452882): H301, H314, P260, P280, P303 + P361 + P353, P304 + P340 + P310, P305 + P351 + P338, EUH014
- Ag nanoparticles: Preparation according to Lee and Meisel 11) from citric acid (SigmaAldrich, No. 251275) and silver nitrate (SigmaAldrich, No. 209139)



Tyndall effect of the prepared Ag nanoparticles