Water depollution control : A gateway to reaction monitoring and analytical chemistry

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ABSTRACT

We describe here a 4-hr experimental session based on the reaction monitoring of the degradation of an organic pollutant *via* a Fenton-like reaction. An aqueous solution of *p*-nitrophenol is treated with hydrogen peroxide and magnetite, and the progress of the reaction is monitored by UV-Visible spectrophotometry and/or Gas Chromatography using an internal standard. During that session, undergraduate students discovered a new scope of heterogeneous catalysis, namely a green process for water depollution. Behind the simplicity of setting up the Fenton-like reaction, they were challenged by the exigence of replicability and precision of the quantitative tracking of the reaction. They appreciated being confronted with a realistic environmental problem, and seeing what role catalysis and modern chemistry could play to tackle the issue.

Since their formulation by P. Anastas and J. Warner in 1998[1], both research and teaching laboratory were infused with the 12 principles of green chemistry. Solvent-free reactions, catalysis, atom economy, renewable feedstocks,… all of them are henceforth fundamental notions in chemistry teaching from the first undergraduate years.[2] But we believe that teaching how chemistry can present only mild environmental impact thanks to these principles is not complete : it is necessary to demonstrate through experimental sessions that chemistry plays a key role in pollution removing processes.

As part of this global issue, organic pollution in fresh water is a major concern.[3] In France, as well as in many other developed countries, students show great concern through the news towards the presence of organic pollutants, such as pesticides, endocrine disruptors, antibiotics in the environment. Nonetheless, they are less aware of pollution control methods, which can be divided into two categories: physical adsorption, or chemical mineralization. Physical adsorption is mainly implemented by using activated carbons, zeolites or porous clays, depending on the pollutants and the environments.[4] Mineralizations are processes in which the organic pollutants are totally transformed into mineral chemicals, namely principally carbon dioxide and water. This can be achieved electrochemically, photocatalytically, or by oxidation.[5]

Fenton-like reactions correspond to the production of hydroxyl radicals $HO^{\bullet}$ and hydroperoxyl $HOO^{\bullet}$ radicals from hydrogen peroxide H_2O_2 and iron oxides which promotes the oxidation of them. In particular, the use of peroxide hydrogen with solid

magnetite Fe_3O_4 appears to efficiently remove phenol-derived and dye compounds in fresh water. R.K. Sharma *et al.* proposed an elegant laboratory experiment for two sessions of 3h each, during which students have to synthesize magnetite nanoparticules and to use them to degrade the Rhodamine B dye.[6] For the experiment session presented here, we chose to adapt the comprehensive study made by Sheng-Peng Sun and Ann T. Lemley, about *p*-nitrophenol (*p*-NP) degradation by an heterogeneous Fenton-like reaction on magnetite nanoparticles with an optimization of the reaction conditions to reach a 75% degradation within 2 hours.[7] We also adapted the reaction monitoring to the laboratory classroom: the students were asked to follow up the degradation both by UV-Visible spectrophotometry and Gas Chromatography (GC) with an internal standard method.[8] Both of these techniques required to use specific, and accurate equipment such as mechanical pipettes or syringes, whose use have to be mastered by upper-undergraduate students. Moreover, this session allows for the implementation of an internal standard, an essential technique in analytical chemistry.

EXPERIMENTAL OVERVIEW
Main protocol

The reaction procedure does not require any particular experimental skills. To 250 mL of a beforehand prepared solution (see Supp. Info.) of *p*-nitrophenol (0,20 $g.L^{-1}$) were added 2 g of Fe_3O_4 powder.

The solution was sonicated during 15 mn. Prior to any reaction with hydrogen peroxide, a "blank" solution study is carried out through UV-Visible and GC analyses (see below) with the freshly resulting solution (t=0). 10 mL of H_2O_2 (30% w/v) were then added under stirring, and the progress of the degradation was monitored by spectrophotometry and/or GC analysis over time every fifteen minutes from that moment.

It is noteworthy that S.-P. Sun *et al.* proposed to fix the initial pH value at 7 by adding small amounts of sodium hydroxide or hydrochloric acid to improve the reaction rate.[7] We found that it was not necessary to adjust it to reach the desired rates for the experimental session.

UV-Visible analysis

Para-nitrophenol can be considered as a colorimetric pH indicator, as it displays a maximum absorbance at 313 nm in acidic solution (pH<5.4), and at 405 nm in basic solutions (pH > 7.5).[9] Given that the pH of the media lies in the transition range, we decided to take advantage of the stronger absorbance of the basic p-nitrophenolate anion by adding sodium hydroxide solution to reach a pH>10 for spectrophotometric analysis. Thus, students have to withdraw a 1-mL portion of the reaction mixture with a syringe and release the content through a Millipore filter to remove any small particles that could hinder the analysis. With a micropipette, they precisely transfer 50 µL of the limpid sample it in a quartz cuvette, and they add 3 mL of NaOH 0.1 M solution. Then they proceed to a UV-visible measurement of the sample.

GC analysis

The kinetic study of the degradation process of *p*-NP was studied in comparison with a m-nitrophenol (m-NP) standard solution of same concentration. Contrary to the classical technique, in the case of this Fenton-type reaction, we cannot add the standard in the reaction mixture at the beginning of the experiment, simply because it would react as well as the p-NP. Therefore, elegantly, we proposed that the students add the standard just before the extraction of the aqueous reaction mixture with diethyl ether. We previously verified that this procedure is rigorously reliable, by performing a calibration line (see Supp. Info.)

Students have to prepare each sample for GC monitoring as follows: 5 mL of the beforehand prepared *m*-NP aqueous solution (0.2 g.L^{-1}) are precisely withdrawn and transferred in a small vial or test tube, and 5 mL of the reaction mixture are added. To decrease the solubility of *p*-NP and *m*-NP in the aqueous layer, a salting out is required : several spatulas of NaCl are added as well as 1 or 2 drops of 6M hydrochloric acid. (This additional precaution to reach an acidic pH enables to avoid the presence of *para* and *meta*-nitrophenolate anions which are more soluble in water than in organic solvents). Then 1 mL of diethyl ether is added. The container is stirred mechanically to foster the extraction, and 2 µL of the resulting organic layer of this mixture are taken with a microsyringe and injected in the GC apparatus.

The previously described *modus operandi* displays several upsides to contemplate an application during a lab session with students. Indeed, it offers an interesting use of precision instruments such as micropipettes to transfer and deliver accurate

volumes; as well as cutting-edge equipment namely Millipore filters one can find in most pharmaceutical and biotechnology industries and laboratories.

HAZARDS

All reactants, products, and solvents must be handled in a manner consistent with the information available on the material safety data sheets. Eye protection, laboratory coats, and gloves must be used throughout the course of the experiment. Students should perform the experiments in a well-ventilated fume hood. m-NP is harmful when in contact with skin and causes severe eye damage. p-NP is harmful when swallowed or in contact with skin, and it may cause damage to organs through repeated expositions. In our cases, the use of dilute aqueous solution of these products drastically reduces these hazards.

Diethyl ether is highly flammable and volatile, and harmful in contact with skin, eye, and if swallowed. Moreover needles and syringes must be handled very carefully to avoid any injury.

RESULTS AND DISCUSSION

The students achieved these experiments by pair and both were asked to implement the two monitoring techniques alternately during the session, to insure a good proficiency of all the experimental skills. The p-NP and m-NP solutions were prepared prior to the session, regarding both the handling of these harmful compounds and the time required to prepare the solutions (the solutions have to be heated or

sonicated to ensure a perfect dissolution, and we also verified that the solutions are stable during several weeks).

Furthermore, a calibration line was carried out to validate the GC internal standard method (see Supp. Info.)

UV-Visible monitoring

After a brief reminder of the proper use of millipore filters and micropipettes, each student pair achieved the UV-Visible spectrum of the initial solution before adding the hydrogen peroxide solution (blue curve). This spectrum confirmed that only the para-nitrophenolate is present in the basic solution, since the p-NP absorbance peak at 313 nm is not observed. After the addition of hydrogen peroxide, students observed a continuous decrease of the absorbance at ~400 nm, confirming the consumption of the p-NP (Figure 1).
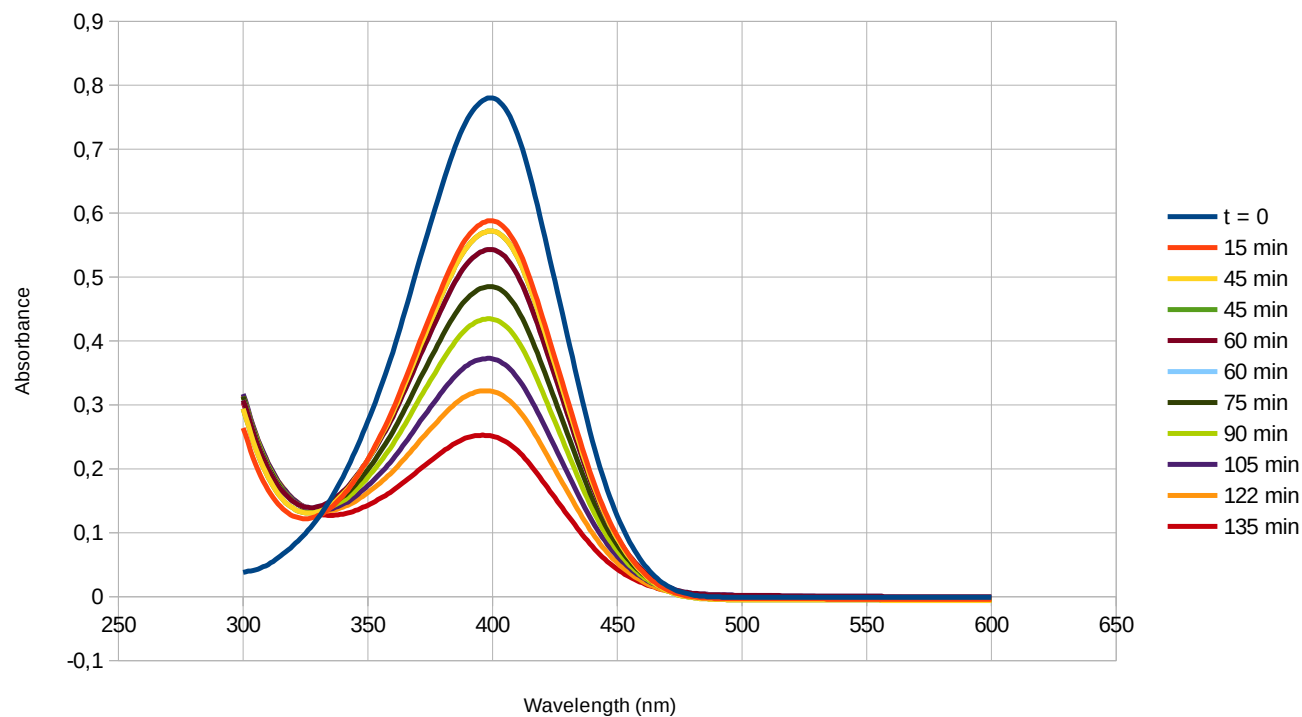
Figure 1. Superposition of the UV-visible spectra obtained from the reaction mixture during all the session, by a student pair.

Moreover, all the spectra showed an increase of the absorbance at 300 nm (it corresponds to the lower limit in our spectra). This was the starting point of very interesting and fruitful discussions with the students. That observation could be interpreted as the Rayleigh light scattering caused by very small particles of iron oxide (III), formed by oxidation of magnetite by hydrogen peroxide. Since the Rayleigh scattering intensity is proportional to λ^{-4}, this phenomenon only concerns the shorter wavelength, and does not perturbate our measurements.

Although student pairs rarely obtained the correct UV-Visible measurements for each of the samples (errors were mainly due to a misuse of the micropipette), all of them were able to draw the absorbance at 400 nm as a function of time. They noticed that

they obtained a straight line, indicating a zero order reaction in p-NP. This result is consistent with the previous study by Sun et $al.$[7]
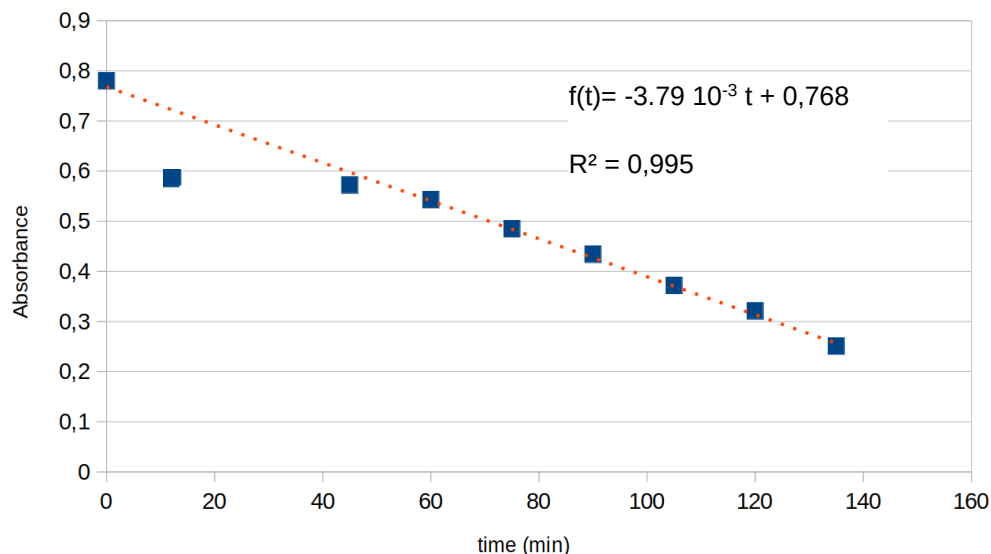

Figure 2. Absorbance at 400 nm of prepared samples in function of the reaction time (same student pair). The 15 min sample preparation has probably failed, and the 30 min sample is missing. The correlation line was drawn without the sample at 15 min.

Considering the first UV-Visible measurement, for the initial concentration at c = 0,2 g.L^{-1}, one can also easily access to a temporal evolution of concentration, by evaluating the mass attenuation coefficient.

GC monitoring

During their curriculum, students of our university had already used the gas chromatography for qualitative use only. This lab session was their first opportunity to use the GC with an internal standard. They were asked to get information about this technique prior to the session, and to understand the reasons why the implemented methodology differs a bit from the canonical one. They easily understood that the m-NP cannot be present in the reaction medium, since it would

be consumed as well as the *p*-NP. Students were also asked to clarify the taken precautions to reduce the solubility of the nitrophenol isomers (acidification of the aqueous layer and salt out) during the extraction with diethyl ether.

Otherwise, the GC method was optimized for short retention times (5 min max) and good separation of the two nitrophenol isomers (see supp. Inf. for details).

Three GC chromatograms (at t= 0 min, t = 45 min and t = 1h45 min) are shown in Figure 3. A GC chromatogram was realized 1 week after, showing the total mineralization of the *p*-NP.

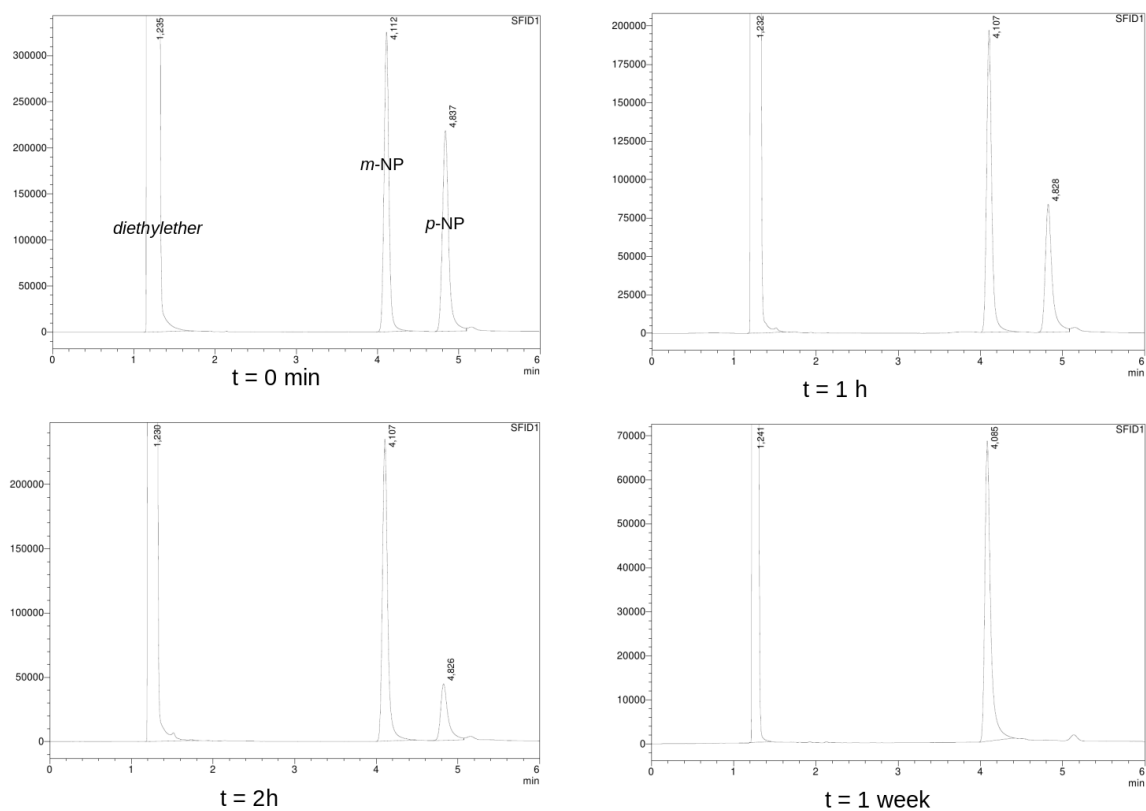Figure 3. Examples of chromatograms at t=0, t = 1h, t=2h, and after 1 week (same student pair).

For each sample, students measured the area and the height of the two peaks corresponding to the nitrophenols on the chromatogram (the first one corresponds to

the *m*-NP). The relevance of the comparison of the height of the peaks was thus discussed, especially with the first chromatogram at t=0 which showed similar areas but different heights.

Students were asked to draw the ratio Area(*p*-NP peak)/Area(*m*-NP peak) in function of time reaction (Figure 4), and they obtained again a zero-order towards *para*-nitrophenol.

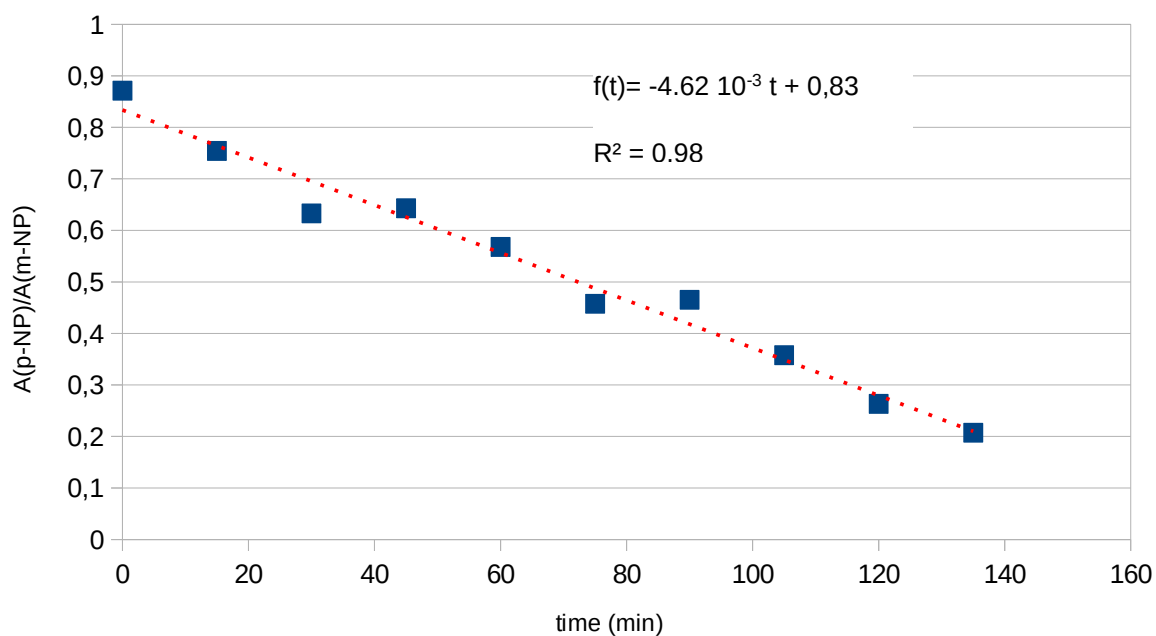Figure 4 : Ratio Area(*p*-NP peak)/Area(*m*-NP peak) in function of time reaction. (same student pair)

f(t)= -4.62 10^{-3} t + 0,83

R^2 = 0.98

CONCLUSION

This short experimental laboratory session (~ 4 hrs), that requires both UV-Visible and GC experimental skills for the mineralization of *para*-nitrophenol under Fenton-like conditions, presents several upsides. First, it was the opportunity for students to implement a realistic chemical depollution process, which appeared to be an efficient

driving force for their learning. Secondly, they were asked to work using specific materials (micropipette, millipore filter) and very precisely, since even slight imprecision resulted in inaccurate measurements. Thirdly, it was the first time they had implemented a internal standard technique At last, all the (classical) experimental precautions such as pH tuning or salt out could be perfectly rationalized, including the fact of knowing when and how to implement them. Furthermore, this experiment was a perfect opportunity for the students to train with motivation and to develop a know-how that successfully matches with the high standards of analytical chemistry and reaction monitoring.

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REFERENCES
1.   Anastas, P. T.; Warner, J. C. *Green Chemistry: Theory and Practice,* Oxford University Press: New York, **1998**.

2.   For recent examples or perspectives, see Zuin V. G., Eilks I., Elschami M., Kuemmerer K., *Green Chem.*, **2021**, *Accepted Manuscript;* O'Neil N.J., Scott S., Relph R., Ponnusamy E. *J. Chem. Educ.* **2021***, 98* (1)*,* 84–91.

3.   Sousa J. C., Ribeiro A. R., Barbosa M. O., Pereira M. F. R, Silva A. M. T. *J. Hazard. Mater.* **2018***, 344,* 146-162.

4.   Rashed M.N., *Adsorption Technique for the Removal of Organic Pollutants from Water and Wastewater, Organic Pollutants - Monitoring, Risk and Treatment,* IntechOpen, **2013**; 167-194.

5.   For example : Kusic H., Koprivanac N. Loncaric Bozic A. *Chem. Eng. J.* **2006**, *123*(3), 127-137; Wankhade Atul V., Gaikwad G. S., Dhonde M.G., Khaty N. T. Thakare S.R., *Res. J. Chem. Environ.* **2013**, *17*(1), 84-94.

6.   Sharma R. K., Yadav S., Gupta R., Arora G. *J. Chem. Educ.* **2019**, *96* (12), 3038–3044.

7.   Sun S.-P., Lemley A. T. *J. Mol. Catal. A: Chem.* **2011**, *349*, 71-79.

8.   For recent teaching examples of internal standard methodology implementation, see : Vuppala L.S., Vazquez T., Nguyen D., Knowlton M., Smith E., Evans-Nguyen T. *J. Chem. Educ.* 2020, 97, 8, 2260–2264; Parish-Virtue K., Johannisson-Wallman M., Pilkington L. I., Hsieh S., Barker D., and Fedrizzi B. *J. Chem. Educ.* **2020**, *97(*10), 3847–3851.

9.   Nitrophenol. Retrieved March 21, 2021, from Hazardous Substances Data Bank https://pubchem.ncbi.nlm.nih.gov/source/hsdb/1133