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Practising Chemical Research in High School

Maturarbeit: Towards a Copper-Azophloxine Inorganic Complex for the Selective Naked-eye Detection of Pyrophosphate in Water

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Abstract: For the purpose of a high school thesis, we investigate with a student the possibility of detecting pyrophosphate with an indicator displacement assay using a copper-based inorganic complex. Our results indicate that real research with socially relevant results can be conducted at high school using very modest resources.

Keywords: Azophloxine · Chemical education · Copper · High school · Indicator displacement assay

Introduction
Metal-based indicator displacement assays have proven their utility in chemistry.[1] The methodology is widely used for various applications such as, sensing,[2,3] determination of the enantiomeric excess[2] or monitoring the cellular metabolism,[4] for example. In this context, large combinatorial libraries with metal salts and indicators can be mixed and screened for a specific detection of analytes of interest.[5] Given the immense possibilities of the strategy, it is obvious that not all reactions have been reported in the literature so far. However, their discovery could be useful for various and specific applications and contribute to the general knowledge of chemistry. For example, reported chemosensors for the presence and in the absence of pyrophosphate (Fig. 1). The competition assay developed here receptor-bound AP by anions could be visually observed with the naked eye (Fig. 2). The competition assay developed here is illustrated schematically in Figs 1 and 2. Additionally, we measured absorbance spectra of the formed complex in the aqueous solution of this purple ensemble (Fig. 4). The proposed multi-step reaction mechanism presenting the indicator displacement assay based on azophloxine (AP) added to copper sulfate forming [Cu(AP)] at 250 μM in HEPES pH = 7.4 for the naked-eye detection of pyrophosphate.

Materials and Methods
Dissolving azophloxine (AP, 250 μM) and copper sulfate (CuSO4, 250 μM) in 2-[4-(2-hydroxyethyl)-1-piperazine]ethanesulfonic acid (HEPES, 10 mM, pH = 7.4) affords a copper-based inorganic complex [Cu(AP)]. Out of all the tested ions, only one was able to displace the AP ligand shows an absorbance peak at 505 nm [range of 400–1000 nm] whereas the formed complex displays an absorbance peak at 490 nm [range of 400–1000 nm]. The addition of 5 equiv. of pyrophosphate to [Cu(AP)] allows an indicator displacement assay thus recovering the initial absorbance spectra of AP. In the next step, we screened a collection of anions against the copper-based inorganic complex [Cu(AP)]. Out of all the tested ions, only one was able to displace the AP ligand: pyrophosphate. The addition of pyrophosphate anions to the aqueous solution of this purple ensemble (Fig. 4) resulted in the recovery of AP’s spectroscopic properties (Fig. 2 and 3). The present sensor exhibits excellent selectivity towards pyrophosphate ions over other anions, including phosphate and carbonate ions (Fig. 4).
Results and Discussion

From a chemical point of view, the results obtained overall suggest that the complex \([\text{Cu(A)}]\) can be used for the naked-eye detection of pyrophosphate. However, these preliminary data should be further investigated in many ways such as:

- Identifying the effects of the promiscuity of various groups of anions on the complex and extending the screening matrix with phosphorylated molecules to investigate the selectivity of the complex.
- Trying to determine the detection limit abilities of this system and the affinity constant of \([\text{Cu(A)}]\) for pyrophosphate, comparing it with chemosensors described in the literature.
- Trying to prove the mechanism of detection of this indicator displacement assay
- Analysing the effect of light and heat on this complex since the ligand is an azobenzene. Is an isomerisation possible?

These are some of the investigations that can be carried out by the next generations of students for their maturarbeit inspired by this research piece.

From a pedagogical point of view, this approach allowed the student to dive into chemistry research by exploring non-described compounds. She was able to use many fundamentals she learned during chemistry courses such as the basics of organic chemistry, inorganic chemistry, spectroscopy, stoichiometry, etc. She was also able to reproduce the results she obtained and therefore train and discuss the reproducibility of experiments.\[^{[10]}\] This is a key point that should be addressed nowadays due to the expansion of scientific literature and reported scientific misconduct.\[^{[11–13]}\] As an extension, she was also able to design and discuss positive and negative controls that are fundamental for reliable science, which may be a key point in the education of young scientists.\[^{[14]}\] She was also able to discuss the hypothesis of detection abilities of the complex against various anions and therefore train her mind to look the direction of inorganic chemistry concepts. This allows the development of scientific critical thinking.\[^{[15,16]}\] Finally, as a woman in science, she was able to acquire confidence in herself, and this project allowed her to acquire the motivation to solve interesting scientific problems with social interests.\[^{[17]}\] This is a key point since women are still underrepresented in sciences in general.\[^{[18,19]}\]

Conclusion

We have described a simple assay for the detection of pyrophosphate ions in an aqueous medium. The assay is based on commercially available chemicals (copper sulfate and a colorimetric indicator) and allows detection of pyrophosphate at low micromolar concentration. The interference of common anions is remarkably low. Moreover, the assay is very easy to perform and requires no prior synthesis. The research project was realized as part of a maturarbeit realized by a high school student proving its simplicity. The initial results suggest that interesting research with modest material can be performed with high school students. It also allows high school students to further the development of this project in various directions thus constructing interesting pieces of research that could be published ultimately. The project also allows the development of interesting and key pedagogical interests such as discussing reproducibility or positive and negative controls. Finally, it can also develop women’s interest in the sciences.

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Aria Bieri performed the experiments. Thibaud Rossel supervised the research and wrote the manuscript.

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