



# Chemical Education

## A CHIMIA Column

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.<sup>[1]</sup> The methodology is widely used for various applications such as, sensing,<sup>[2,3]</sup> determination of the enantiomeric excess<sup>[2]</sup> or monitoring the cellular metabolism,<sup>[4]</sup> 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.<sup>[5]</sup> 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 in chemistry to stimulate the next generations.<sup>[5]</sup> For example, the simple selective detection of phosphate<sup>[6]</sup> is still a major challenge given its chemical properties *e.g.* large size of the anion, hydrophilicity, multiple protonation states, *etc.*<sup>[7]</sup> Areas of science with fundamental problems can be solved with this approach. We, for example, reported chemosensors for the naked-eye detection of phosphate or glyphosate in the past.<sup>[5,7]</sup> Even if indicator displacement assay is more of a procedure practiced for research at universities, it proved to be useful for research at high school too by developing a subcategory in the screening matrix that can be explored. Here, for example, we report a copper-based inorganic complex for the selective naked-eye detection of pyrophosphate in water (Fig. 1).

The complex has not been reported in the literature so far for sensing this anion. This work has been carried out by a high school student for her maturarbeit demonstrating that relevant research contributing to the general knowledge of chemistry can be performed at high school using very modest and commercial resources.<sup>[8]</sup> This work can easily be explored and expanded in the future by the next generations of students thus providing an interesting piece of research using transgenerational collaborations driving curiosity.<sup>[9]</sup>

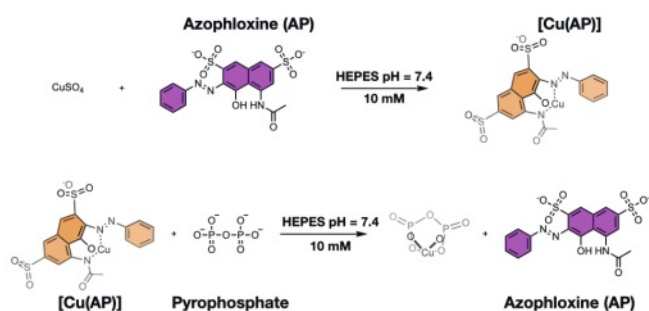


Fig. 1. The proposed multi-step reaction mechanism presenting the indicator displacement assay based on azophloxine (AP) added to copper sulfate forming [Cu(AP)] at 250  $\mu$ M in HEPES pH = 7.4 for the naked-eye detection of pyrophosphate.

#### Materials and Methods

Dissolving azophloxine (AP, 250  $\mu$ M) and copper sulfate ( $\text{CuSO}_4$ , 250  $\mu$ M) in 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (HEPES, 10 mM, pH = 7.4) affords [Cu(AP)] (250  $\mu$ M), which is water-soluble and orange at working micromolar concentrations (Fig. 1 and 2). Screening of various pHs from 5 to 8 allowed us to conclude that the best naked-eye discrimination between AP and [Cu(AP)] is obtained at pH = 7.4. We therefore selected pH = 7.4 for the continuation of our study. Next, we hypothesized that the [Cu(AP)] complex could be used to assemble a receptor for phosphate derivatives, exploiting metal-ligand interactions for selective target recognition. To the best of our knowledge, this mononuclear complex has never been described before in the literature as a selective chemosensor for anions, especially phosphate derivatives. In this case, the displacement of the 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 presence and in the absence of pyrophosphate (Fig. 3). 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).

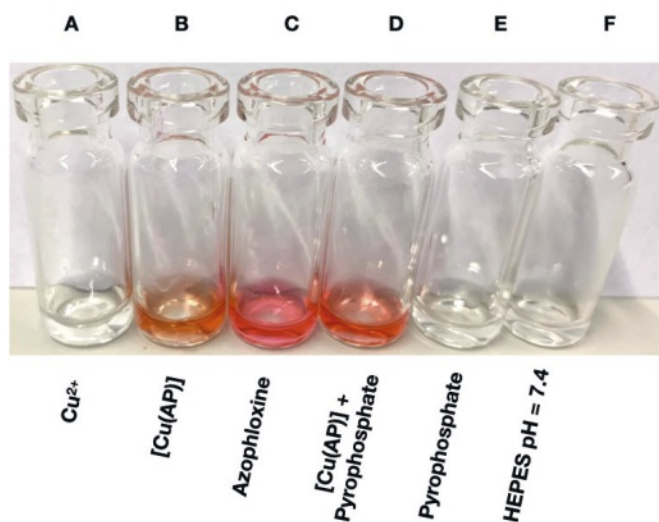


Fig. 2. Addition of copper sulfate (A, 250  $\mu$ M, HEPES pH = 7.4) to azophloxine (B, AP, 250  $\mu$ M, HEPES 10 mM pH = 7.4) affords an inorganic complex. The addition of pyrophosphate to B (5 equiv., HEPES 10 mM pH = 7.4) allows an indicator displacement assay (D, 250  $\mu$ M, HEPES 10 mM pH = 7.4). C, E and F are negative controls.

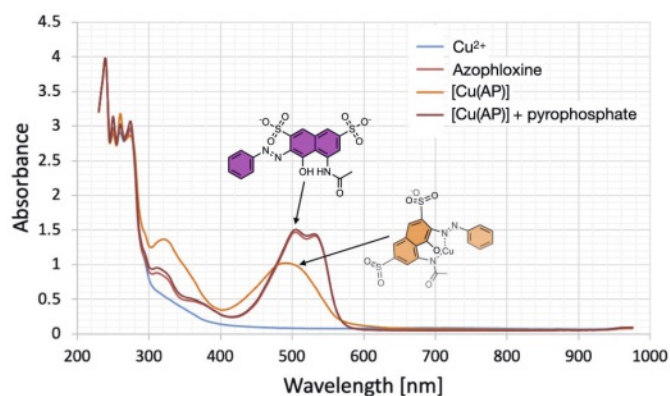


Fig. 3. Absorbance spectra of [Cu(AP)] at 250  $\mu$ M formed in HEPES pH = 7.4 and in the presence of pyrophosphate (5 equiv.).

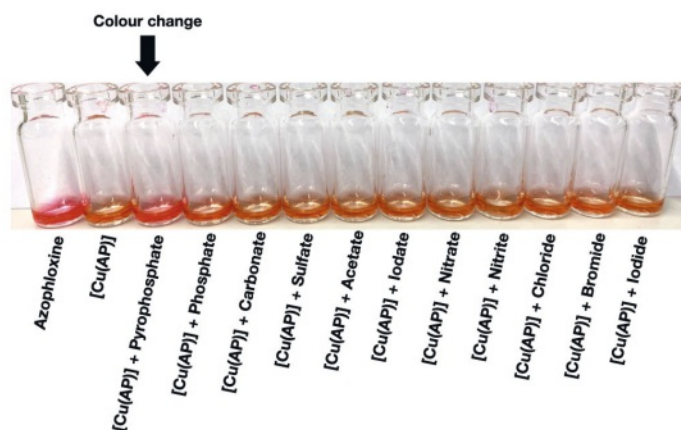


Fig. 4. Screening of [Cu(AP)] (250  $\mu$ M in HEPES 10 mM at pH = 7.4, aqueous solution) in the presence of various anions (1.25 mM, 5 equiv.).

## Results and Discussion

From a chemical point of view, the results obtained overall suggest that the complex [Cu(AP)] 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 [Cu(AP)] 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 maturation 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.<sup>[10]</sup> This is a key point that should be addressed nowadays due to the expansion of scientific literature and reported scientific misconduct.<sup>[11–13]</sup> 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.<sup>[14]</sup> 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.<sup>[15,16]</sup> 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.<sup>[17]</sup> This is a key point since women are still underrepresented in sciences in general.<sup>[18,19]</sup>

## 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 maturation 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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- [1] A. C. Sedgwick, J. T. Brewster, T. Wu, X. Feng, S. D. Bull, X. Qian, J. L. Sessler, T. D. James, E. V. Anslyn, X. Sun, *Chem. Soc. Rev.* **2021**, *50*, 9, <https://doi.org/10.1039/C9CS00538B>.
- [2] L. Zhu, Z. Zhong, E. V. Anslyn, *J. Am. Chem. Soc.* **2005**, *127*, 4260, <https://doi.org/10.1021/ja0435945>.
- [3] N.-N. Yang, L.-J. Zhou, P. Li, Q. Sui, E.-Q. Gao, *Chem. Sci.* **2019**, *10*, 3307, <https://doi.org/10.1039/C9SC00032A>.
- [4] A. Norouzy, Z. Azizi, W. M. Nau, *Angew. Chem. Int. Ed.* **2015**, *54*, 792, <https://doi.org/10.1002/anie.201407808>.
- [5] T. Rossel, M. Creus, *Chimia* **2019**, *73*, 599, <https://doi.org/10.2533/chimia.2019.599>.
- [6] P. Yadav, O. Blacque, A. Roodt, F. Zelder, *Inorg. Chem. Front.* **2021**, *8*, 4313, <https://doi.org/10.1039/D1QI00209K>.
- [7] T. Rossel, M. Creus, *Chem. Com.* **2019**, *55*, 14894, <https://doi.org/10.1039/C9CC04840E>.
- [8] G. M. Whitesides, 'Using Simplicity', **2014**, <https://theanalyticalscientist.com/techniques-tools/>
- [9] G. M. Whitesides, *Angew. Chem. Int. Ed.* **2018**, *57*, 4126, <https://doi.org/10.1002/anie.201800684>.
- [10] M. Baker, *Nature News* **2016**, *533*, 452, <https://doi.org/10.1038/533452a>.
- [11] D. Fanelli, *PNAS* **2018**, *115*, 2628, <https://doi.org/10.1073/pnas.1708272114>.
- [12] J. Berg, *Science* **2018**, *359*, 6371, <https://doi.org/10.1126/science.aar8654>.
- [13] J. J. V. Bavel, P. Mende-Siedlecki, W. J. Brady, D. A. Reinero, *PNAS*, **2016**, *113*, 6454, <https://doi.org/10.1073/pnas.1521897113>.
- [14] J. S. Torday, F. Baluška, *EMBO Rep.* **2019**, *20*, e49110, <https://doi.org/10.15252/embr.201949110>.
- [15] S. A. Seibert, *Teach. Learn. Med.* **2021**, *16*, 85, <https://doi.org/10.1016/j.teln.2020.09.002>.
- [16] L. Varenina, E. Vecherinina, E. Shchedrina, I. Valiev, A. Islamov, *Think. Skills. Creat.* **2021**, *41*, 100906, <https://doi.org/10.1016/j.tsc.2021.100906>.
- [17] T. A. Reese, T. A. Harris-Tryon, J. G. Gill, L. A. Banaszynski, *Sci. Adv.* **2021**, *7*, 9, <http://doi.org/10.1126/sciadv.abg9310>.
- [18] B. Avolio, J. Chávez, C. Vélchez-Román, *Soc. Psychol. Educ.* **2020**, *23*, 773, <https://doi.org/10.1007/s11218-020-09558-y>.
- [19] N. Ellemers, H. V. den Heuvel, D. D. Gilder, A. Maass, A. Bonvini, *Br. J. Soc. Psychol.* **2004**, *43*, 315, <https://doi.org/10.1348/0144666042037999>.