doi:10.2533/chimia.2023.165 Chimia 77 (2023) 165–167

Chemical Education

A CHIMIA Column

Topics for Teaching: Chemical Analysis for High-school Students

Maturarbeit: Screening Strategy against an Inorganic Complex for the Rapid Investigation of Naked-eye Detection of Analytes

Kevin Gbessaya[a] and Thibaud Rossel*[ab]

*Correspondence: Dr. T. Rossel[ab], E-mail: thibaud.rossel@unine.ch
[a]Gymnase français de Bienne, rue du Débarcadère 8, 2503 CH-Bienne;
[b]University of Neuchâtel, Institute of chemistry, avenue de Bellevaux 51, 2000 CH-Neuchâtel

Abstract: We recently reported a copper-azophloxine complex for the naked-eye detection of pyrophosphate (E450) in water as solvent (Fig. 1). Here, both for a maturarbeit and the continuation of our study with a high school student, we investigate a new quick screening strategy of analytes as well as the discrimination abilities of our reported copper complex.

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

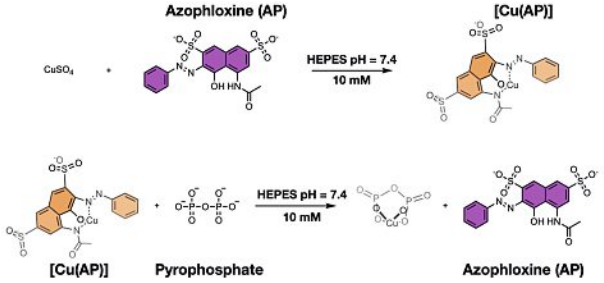

Fig. 1. Our reported multi-step reaction mechanism presenting the indicator displacement assay based on azophloxine (AP) added to copper sulfate forming a [Cu(AP)] complex at 250 µM in HEPES pH = 7.4 for the naked-eye detection of pyrophosphate.

Introduction

Indicator displacement assay is a seminal strategy for the naked-eye detection of analytes.[1] The system is often based on inorganic complexes, where the ligand is displaced by an analyte.[1] This triggers an optical signal change that can be detected visually. The developed sensors can be directed against different groups of analytes.[2] We can cite among others: sugars,[2–4] phosphorylated molecules,[2] amino acids,[5] *etc*. In this context, the reported selectivity of a sensor depends on the size of the matrix used to test its abilities. Often, the investigation of such matrices takes time and requires meticulous work for the strict respect of the precise concentrations of all the tested solutions. Chemists, therefore, restrict themselves to a limited investigation of the selectivity of their sensors, often based on the chemical proximity of the group of analytes,[6] even if high-throughput screening would be preferable.[7,8] Additionally, to get a clear output when screening, a small amount of analyte is often added in excess compared to the sensor (*e.g*. 5 equiv.).[9] However, only the best sensors can detect an analyte with a 1:1 ratio.[2] Sometimes efficient sensors can detect substances whose concentration is lower than the sensor.[2] Overall, the detection is governed principally by the affinity constant of the host (complex) for the guest (analyte). Good examples are extrusion systems where a host–guest partnership generates a coprecipitation.[9] Here for a maturarbeit (a high-school diploma project), we investigated a rapid screening strategy allowing us to explore the selectivity of a sensor. Our procedure is based on the preparation of a library by imprecise weighing. We also deliberately use a large excess of analyte versus sensor. The sensor is screened with this library. The hits are then identified, and the analyte stock is diluted and screened again (Fig. 2). The selection of hits and the incremental dilution make it possible to screen large libraries rapidly and ultimately investigate the selectivity of a sensor. Here, as a proof of concept, we show that our copper-based sensor can discriminate between amino acids versus sugars, but this concept can be easily extended to other groups of analytes.

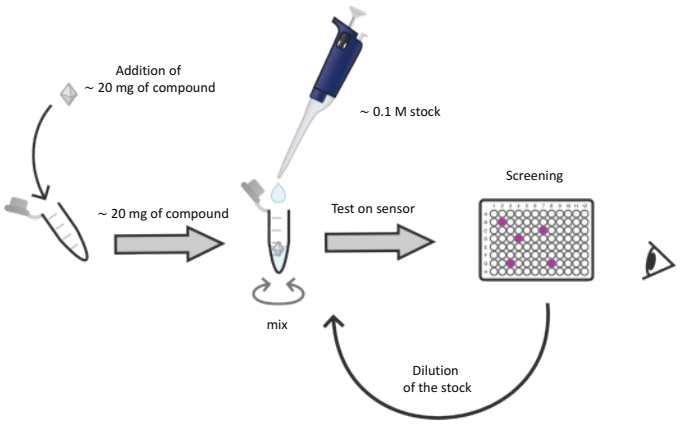

Fig. 2. Description of the screening and selectivity strategy procedure.

Materials and Methods

Screening Strategy

We recently reported on Cu-azophloxine complexes for the naked-eye detection of pyrophosphate (Fig. 1).[1] We dissolved and mixed azophloxine (AP, 250 µM, 100 µL) and copper sulfate ($CuSO_4$, 250 µM, 100 µL) in 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES, 10 mM, pH = 7.4) to afford [Cu(AP)] (250 µM, 200 µL), which is water-soluble and orange in colour at working micromolar concentrations (Fig. 3). The displacement of the receptor-bound AP by anions could be visually observed with a naked eye. The indicator displacement assay

(IDA) competition assay that we reported is illustrated schematically in Fig. 1. Of a collection of anions, only pyrophosphate was able to engage in an indicator displacement with [(Cu(AP)]. Here, in order to extend the formation of complexes using the ligand azophloxine, we screened a small collection of metals. Only the copper reacted, followed by a slight reaction with nickel. This suggests that azophloxine is a suitable ligand for copper only and confirmed our choice of [Cu(AP)] for the continuation of our study. Next, the screening of large libraries is time-consuming and requires rigour in the preparation of a solution with precise concentration. To address this problem, and to accelerate the preparation of potential tested solutions, we decided to apply a quick strategy (Fig. 2). We anticipated that most of our tested analytes have a molecular weight of about 100–1000 Da.

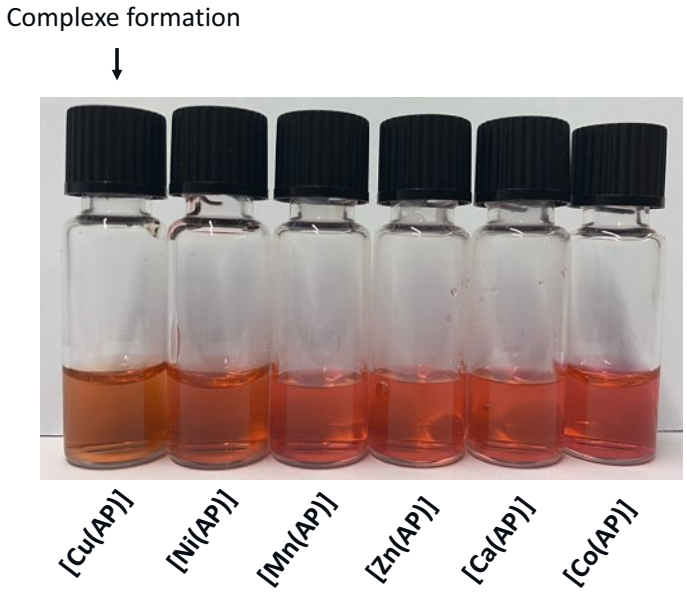

Fig. 3. Screening of various metals (Cu^{2+}, Ni^{2+}, Mn^{2+}, Zn^{2+}, Ca^{2+}, Co^{2+}) in the presence of azophloxine (AP) (total concentrations of complexes: 250 µM in 200 µL in 10 mM HEPES pH = 7.4)

Using that assumption, a 0.1 M solution of the compound is prepared by the addition of approximately 20 mg of analyte (with a molecular weight of about 200 Da) in 1 mL solvent. We, therefore, prepared a collection of potential analytes using this strategy in two groups 1) amino acids and 2) sugars. In the second instance, in order to minimise the effort in the screening process, we decided to add an excess of analyte to the chemosensor (respectively 300 equiv.). The occurrence of a colour change would suggest that the displacement assay took place: If no change in colour appears, the chemosensor is not further investigated for its detection abilities of the specific analyte. If detection occurs, the sensitivity of the sensor is further investigated in a second and third round of screening using 10 times, and 100 times dilution up to 3 equiv. of analyte versus sensor (see Figs 4, 5, 6). The solubility of each compound is scrutinised and referenced (Fig. 2). Finally, for the best combinations, a precise solution of analyte can be prepared and double-checked with the imprecise one to confirm the results. The validity of our strategy can be appreciated in Figs 4, 5 and 6.

Results and Discussion

Of all metals tested for the formation of inorganic complexes based on the ligand azophloxine, only two complex formation reactions occurred. Copper(II) has already been

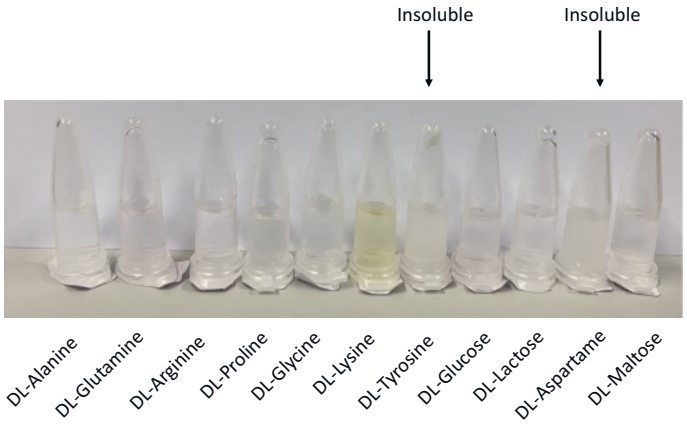

Fig. 4. Solubility test of various substances at ~0.1 M concentration.

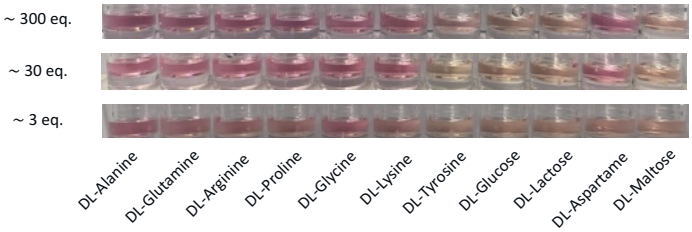

Fig. 5. Proof of principal screening of [Cu(AP)] in the presence of various analytes (~300 equiv. of analyte versus complex up to 3 equiv.).

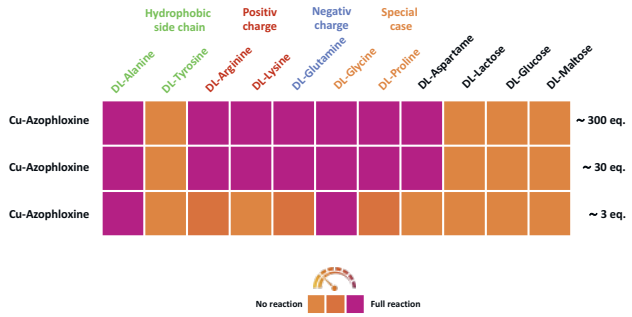

Fig. 6. Fingerprint of the screening of [Cu(AP)] in the presence of various analytes at various concentrations.

described by us and we found that nickel(II) reacted to only a small extent and was therefore discarded after the screening. All other metals did not react (Fig. 3). This confirms the solid selective constitution of the complex with only copper. The constitution of libraries with imprecise weighing is a time saver and allows for a quick construction of libraries. The dissolution of compounds forming stock solutions at 0.1 M gives rise in rare cases to precipitation. In this context, DMSO can be used as a replacement for water, for example. Ultimately, the analyte can be discarded in the screening process. Of our two groups of analytes tested, only the amino acids allow an indicator displacement while the sugars do not react with the complex. This makes it possible to show the discriminating power of the sensor and prove that the screening is quickly executed for a large matrix. The incremental dilution of aspartame shows, for example, that it does not react at all at 3 equiv. whereas it does at a higher concentration.

Conclusion

We have described a quick screening strategy for the investigation of [Cu(AP)] for the recognition of analytes. Our procedure allows us to explore extensively the potential selectivity of a chemosensor and ultimately discuss its potential for real-life applications. For students, it allows them to obtain results quickly without investing major time-consuming efforts in the constitutions of the analytes matrix. Finally, it allows to discuss the possible interactions between an analyte and a metal complex, therefore, encouraging critical thinking.

Acknowledgments and Contributions

The author would like to thank the Gymnase français de Bienne for supporting us through lab equipment as well as support in this project, the University of Neuchâtel and especially Prof. Dr. Bruno Therrien for discussions and support in the realisation of this project. Finally, thanks to all the students past and present who participated in this research program. Kevin Gbessaya performed the experiments. Thibaud Rossel conducted the research and wrote the manuscript.

Received: September 28, 2022

[1] A. Bieri, T. Rossel, *CHIMIA* **2022,** *76*, 368, https://doi.org/10.2533/chimia.2022.368.
[2] 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.
[3] R. Yadav, C. Kwamen, J. Niemeyer, *Isr. J. Chem.* **2021**, *61*, 231, https://doi.org/10.1002/ijch.202000104.
[4] P. Kumar, S. Pachisia, R. Gupta, *Inorg. Chem. Front.* **2021**, *8*, 3587, https://doi.org/10.1002/anie.202103545.
[5] A. Buryak, K. Severin, *J. Am. Chem. Soc.* **2005**, *127*, 3700, https://doi.org/10.1021/ja042363v.
[6] M. S. Han, D. H. Kim, *Angew. Chem. Int. Ed.* **2002**, *41*, 3809, https://doi.org/10.1002/1521-3773(20021018)41:20<3809::AID-ANIE3809>3.0.CO;2-N.
[7] S. A. Valenzuela, H. S. Crory, C. Y. Yao, J. R. Howard, G. Saucedo, A. P. de Silva, E. V. Anslyn, *Angew. Chem. Int. Ed.* **2021**, *60*, 1381913823, https://doi.org/10.1002/anie.202101004.
[8] T. Qin, X. Zhao, T. Lv, G. Yao, Z. Xu, L. Wang, X. Peng, *ACS sensors* **2022**, https://doi.org/10.1021/acssensors.2c00803.
[9] M. V. R. Raju, S. M. Harris, V. C. Pierre, *Chem. Soc. Rev.* **2020**, *49*, 1090, http://doi.org/10.1039/C9CS00543A.