

Selective and Sensitive Chromogenic Detection of Trivalent Metal Cations in Water

Beatriz Lozano-Torres,^{1,2,3} Sameh El Sayed,^{1,2,3} Ana M. Costero,^{*1,3,4} Salvador Gil,^{1,3,4} Margarita Parra,^{1,3,4} Ramón Martínez-Máñez,^{*1,2,3} and Félix Sancenón^{1,2,3}



R. Martínez-Máñez

¹Centro de Reconocimiento Molecular y Desarrollo Tecnológico (IDM), Unidad Mixta Universidad Politécnica de Valencia-Universidad de Valencia, Spain

²Departamento de Química, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022, Valencia, Spain

³CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN)

⁴Departamento de Química Orgánica, Facultad de Ciencias Químicas, Universidad de Valencia, Doctor Moliner 50, 46100 Burjassot, Valencia, Spain

E-mail: rmaez@qim.upv.es, ana.costero@uv.es Received: December 3, 2015; Accepted: January 15, 2016; Web Released: January 22, 2016

Abstract

An azopyridine derivative for the selective and sensitive chromogenic detection of trivalent metal cations in water is described.

The application of supramolecular concepts for the synthesis and characterization of chromo-fluorogenic chemosensors for transition metal cations is a well stablished area in the field of supramolecular chemistry.¹ Most of the reported examples use the "binding site-signaling subunit" approach and have been developed for the colorimetric or fluorimetric detection of alkaline, alkaline-earth, or transition metal cations.² Inside this last type of cations, those containing three positive charges are especially appealing as they are involved in many biological and environmental processes.³ In this respect, Fe³⁺ is a cofactor of the electron transport system and it is present in a large number of enzymes and proteins.⁴ On the other hand, Al³⁺ is a common water pollutant and its accumulation could lead to a wide range of diseases such as Alzheimer, Parkinson, encephalopathy, and osteoporosis.⁵ Similar to Al³⁺, Ga³⁺ can be present in water, fruits and vegetables in small traces, and it could form derivatives that cause respiratory problems.⁶ Moreover, indium has been reported to damage heart, kidney, and liver. Additionally, the derivative indium pentetreotide has been used as a treatment for cancer that has spread to bones.⁷ Finally, As^{3+} is highly toxic due to its affinity for thiols



Figure 1. Schematic representation of the pathway followed to synthesize probe 6.

presented in several proteins, cofactors and enzyme active sites and its poisoning is linked with a variety of neurologic, dermatologic, cardiovascular, and carcinogenic effects.⁸

All the above-mentioned facts have boosted, in the last years, the synthesis of chromo-fluorogenic chemosensors able to selectively detect the presence of trivalent metal cations.^{9–13} In spite of this interest, the vast majority of the reported examples worked in organic solvents limiting applicability in real biological and environmental media. As far as we know, only two examples of probes for trivalent metal cations able to display sensing features in pure water have been reported to date.^{14,15}

Bearing in mind the above-mentioned facts and our interest in the design of molecular and material-based optical probes,¹⁶ we report herein the synthesis, characterization, and coordination behavior of an azopyridine chemosensor **6** which is able to chromogenically sense trivalent metal cations in pure water.

The synthesis of **6** is depicted in Figure 1. In the first step, 2-methoxyethanol (1) was mesylated with mesyl chloride yielding derivative **2**. Then, the Williamson ether synthesis was used to prepare the oligoethylene glycol derivative **4**. Finally, chemosensor **6** was obtained through a diazotization reaction between **4** and the diazonium salt of 4-aminopyridine (**5**). The overall yield of **6** was 21% (see Supporting Information for details). The synthesis of compound **6** was slightly modified from a previously described protocol.¹⁷ **6** was composed of an azopyridine scaffold (as signaling reporter) and two oligoethylene glycol chains which were included in the molecule to impart water solubility to the probe. Chemosensor **6** could potentially interact with metal cations due to the presence of four nitrogen atoms in its structure.

Water solutions of **6** at pH 7.0 showed an intense orange color due to the presence of a broad absorption band centered at 470 nm ($\varepsilon = 5.69 \times 10^3 \, \text{M}^{-1} \, \text{cm}^{-1}$) with a shoulder at 545 nm ($\varepsilon = 3.02 \times 10^3 \, \text{M}^{-1} \, \text{cm}^{-1}$). The UV–visible behavior of **6** upon addition of alkaline (Li⁺, Na⁺, and K⁺), alkaline-earth (Mg²⁺ and Ba²⁺), and transition metal (Co²⁺, Cu²⁺, Ni²⁺, Cd²⁺, Zn²⁺, Al³⁺, Ga³⁺, In³⁺, Fe³⁺, and As³⁺) cations was tested. The obtained results are showed in Figure 2. As seen, only addition of trivalent cations were able to induce a remarkable color change from orange to violet allowing the naked eye detection of these triple charged species. This selectivity is tentatively ascribed to the high polarizing power of these small and highly charged cations, when compared with other monovalent and divalent cations.



Figure 2. Color changes observed for water solutions of 6 $(1.0 \times 10^{-4} \text{ mol } \text{L}^{-1})$ at pH 7.0 upon addition of 1 equiv of selected metal cations.



Figure 3. UV-visible profile of the titrations of chemosensor 6 ($1.0 \times 10^{-4} \text{ mol } \text{L}^{-1}$) in water at pH 7.0 upon addition of increasing quantities of Al³⁺ cation (0–8 equiv). Inset: Job's plot for the complexation between 6 and Al³⁺ cation determined by UV-vis measurements in water at pH 7.0 with [6] + [Al³⁺] = $1.0 \times 10^{-4} \text{ mol } \text{L}^{-1}$.

Having assessed the highly selective response of **6** toward trivalent metal cations, the sensitivity of the probe was studied by monitoring UV–visible changes in water at pH 7.0 upon the addition of increasing quantities of Fe^{3+} , Al^{3+} , Ga^{3+} , In^{3+} , and As^{3+} . The UV–visible and emission profiles obtained in the presence of Fe^{3+} , Al^{3+} , Ga^{3+} , and In^{3+} cations were quite similar and, as an example, the complete set of spectra obtained for **6** and Al^{3+} is shown in Figure 3.

Upon addition of increasing amounts of Al^{3+} (0–8 equiv) to water solutions of **6** at pH 7.0 the absorbance at 470 nm decreased with the simultaneous growth of a new red-shifted absorption band centered at 545 nm with a clear isosbestic point at 458 nm. The appearance of a red-shifted absorption suggested that Al^{3+} cation interacted with the acceptor part of chemosensor **6**, namely the pyridine heterocycle or the diazo group.

However, the chromogenic response observed with As^{3+} cation is slightly different and no isosbestic points were observed in the set of spectra (Figure 4). Addition of increasing quantities of As^{3+} cation (0–0.5 equiv) to water solutions of **6** at pH 7.0 induced a progressive decrease of the absorbance of the band centered at 470 nm with the subsequent appearance of new red-shifted absorption (at 545 nm). However, addition of higher As^{3+} amounts induced the appearance of a new band



Figure 4. UV-visible profile of the titrations of chemosensor 6 $(1.0 \times 10^{-4} \text{ mol } \text{L}^{-1})$ in water at pH 7.0 upon addition of increasing quantities of As³⁺ cation (0–8 equiv).

Table 1. Complexation constants and limits of detection for6 with trivalent cations

Cation	$\log K$	Limit of detection/µM
Al ³⁺	6.21 ± 0.04	8.95
In ³⁺	5.04 ± 0.06	10.2
Ga ³⁺	4.99 ± 0.04	11.2
Fe ³⁺	2.42 ± 0.04	12.6
As ³⁺	5.16 ± 0.06	6.44

centered at 474 nm together with a marked decrease of the absorption at 545 nm). The appearance of the red-shifted absorption suggested also that As^{3+} cation interacted with the acceptor part of chemosensor **6**. The new band centered at 474 nm, which appeared at higher As^{3+} concentrations, was ascribed to a coordination of the cation with the 1:1 stoichiometry complex previously formed.

From UV–visible titrations the limits of detection (LOD) of **6** for Al³⁺, Fe³⁺, Ga³⁺, In³⁺, and As³⁺ cations were evaluated (Table 1). The LODs obtained are in the μ M range and are similar to those calculated for other recently published chromogenic probes for trivalent cations (Table SI-1 in Supporting Information).^{9–15}

Moreover, in order to determine the binding stoichiometry between **6** and the trivalent metal cations, Job's plots were performed. As an example the inset of Figure 3 shows the Job's plot determined for Al^{3+} that clearly shows the formation of 1:1 chemosensor-cation species. 1:1 stoichiometries were also determined for **6** with Fe³⁺, Ga³⁺, and In³⁺ (data not shown). In the case of As³⁺ only the set of spectra obtained at low metal cation concentrations (when only 1:1 stoichiometry complex was formed) was used for the estimation of the stability constant. Based in the Job's plots the corresponding stability constants for the formation of [M(**6**)]³⁺ complexes were determined using the HypSpec software (V 1.1.18) and adjusting the data to the equilibrium equation:

$$\mathbf{M}^{3+} + \mathbf{L} \rightleftharpoons [\mathbf{M}(\mathbf{L})]^{3+} \tag{1}$$

The estimated values of $\log K$ obtained using UV-visible titration profiles are shown in Table 1.

Probe 6 contains four nitrogen atoms (one in the aniline fragment, two in the azo moiety and one in the pyridine





heterocycle) that could potentially interact with trivalent metal cations. In order to establish the coordination mode ¹HNMR studies of solutions of 6 in D₂O upon addition of increasing quantities of Al³⁺ cation were carried out. The most remarkable changes were observed in the signals of the aromatic zone (Figure 5). Protons of the 1.4-disubstituted aniline ring in 6appeared as doublets centered at 7.02 (Ha) and 7.90 ppm (Hb) whereas the pyridine protons appeared, also as doublets, at 7.68 (Hc) and 8.68 ppm (Hd). As seen in the family of spectra shown in Figure 5, addition of increasing quantities of Al^{3+} cation induced moderate downfield shifts of Ha and Hd protons. However, the most remarkable changes were observed for Hb and Hc protons that suffered downfield shifts of ca. 0.1 and 0.4 ppm, respectively. We tentatively assigned these changes to the interaction of Al^{3+} cation with the azo group present in 6 (Figure SI-5 in Supporting Information for the ¹HNMR titration of **6** with As^{3+} cation).

In summary, we have reported the preparation of a simple chromogenic probe, based on a azopyridine derivative, for the colorimetric detection of Al^{3+} , Fe^{3+} , Ga^{+3} , In^{3+} , and As^{3+} over mono- and divalent cations in pure water with LOD in the μ M range. In the presence of trivalent metal cations the chemosensor was able to display a remarkable color change from orange to violet clearly visible to the naked eye. This is one of the few reported examples able to optically detect trivalent metal cations in pure water.

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Supporting Information

Full experimental details, compound characterization, and selected spectra for key compounds. This material is available on http://dx.doi.org/10.1246/bcsj.20150416.

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