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LETTER

Selective chromo-fluorogenic detection of trivalent cations in aqueous environments using a dehydration reaction†

Maria Lo Presti,[abc] Sameh El Sayed,[abc] Ramón Martínez-Máñez,*[abc]
Ana M. Costero,*[acd] Salvador Gil,[acd] Margarita Parra[acd] and Félix Sancenón[abc]

Trivalent cations (Al^{3+}, Fe^{3+}, Cr^{3+}, As^{3+}, In^{3+} and Ga^{3+}) induced a dehydration reaction of a chemodosimeter in water that is coupled with colour and emission changes.

The preparation of probes for the selective recognition of trivalent cations has received increased attention in the last few years due to the crucial roles played by these cations in many biological and environmental processes. For instance, Al^{3+} is the third most abundant element on earth and some aluminum derivatives are used in water purification, as food additives and in pharmaceutical formulations. Unfortunately, an overdose of Al^{3+} in human tissues and cells can induce diseases like Parkinson's, osteoporosis, headache, gastrointestinal problems and Alzheimer's.[1] Iron is the most abundant transition metal in cells. It is used as a cofactor in the electron transport system and in many biochemical processes at the cellular level.[2] Its deficiency can lead to anemia, hemochromatosis, diabetes, liver and kidney damage, Parkinson's and heart diseases.[3] Chromium is one of the essential elements in human nutrition and plays a fundamental role in the metabolism of carbohydrates, proteins, lipids and nucleic acids.[4] Gallium exists in nature only in its 3+ oxidation state and it has no known biological role. However, because gallium and iron salts behave in a similar way in biological systems, gallium ions often mimic iron in some medical applications; for instance, certain drugs and radiopharmaceuticals containing gallium have been used.[5]

All indium compounds should be regarded as highly toxic and are able to damage the heart, kidneys and the liver, and may be teratogenic.[6] Finally, arsenic is one of the most toxic elements known. Despite its toxic effect, inorganic arsenic can be found on earth naturally in small quantities. Humans can be exposed to arsenic through food, water and air. Exposure can also occur through skin contact with soil or water containing arsenic. High levels of arsenic can cause cardiovascular, neurological, dermatological and carcinogenic effects.[7]

For these reasons, the development of optical chemosensors able to discriminate trivalent from divalent and monovalent cations[8] is especially appealing. In particular, there are relatively few probes able to detect simultaneously several trivalent cations[9–19] and we have found in the literature only one probe that recognizes Al^{3+}, Fe^{3+}, Cr^{3+}, As^{3+}, In^{3+} and Ga^{3+} at the same time.[20] Moreover, most probes for trivalent cations display sensing features in organic solvents and it is rare to find probes for these cations in pure water.[21] Taking into account these facts, and our experience in the synthesis of molecular and material-based sensors for the optical recognition of chemical species,[22] we report herein the synthesis, characterisation and chromo-fluorogenic behavior toward cations of the chalcone-based probe **3**.

The synthesis of **3** is shown in Fig. 1. In the first step, 4-aminoacetophenone was reacted with ethylisothiocyanate, yielding derivative **1**. Then, 4-(*N,N*-dimethylamino)benzaldehyde was condensed with **1** to give the chalcone-based product **2**. Finally, reduction of the α,β-unsaturated carbonyl moiety in **2**, using sodium borohydride, yielded probe **3**. The most characteristic ^{1}H NMR signals (CDCl$_3$) of probe **3** were one doublet ($J = 4$ Hz) centered at 5.3 ppm, which was ascribed to the proton in the carbon atom functionalized with an hydroxyl moiety and attached directly to the *trans* double bond; one double doublet ($J = 14, 4$ Hz) at 6.1 ppm for the proton of the *trans* double bond linked directly to the carbon atom bearing the hydroxyl group; and another doublet ($J = 14$ Hz) at 6.5 ppm attributed to the other proton in the C=C bond. Moreover, the aromatic protons appeared,

^{a} *Instituto Interuniversitario de Reconocimiento Molecular y Desarrollo Tecnológico (IDM), Unidad Mixta Universidad Politécnica de Valencia-Universidad de Valencia, Spain*

^{b} *Departamento de Química, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022, Valencia, Spain. E-mail: rmaez@qim.upv.es*

^{c} *CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN),*

^{d} *Departamento de Química Orgánica, Facultad de Ciencias Químicas, Universidad de Valencia, Doctor Moliner 50, 46100 Burjassot (Valencia), Spain. E-mail: ana.costero@uv.es*

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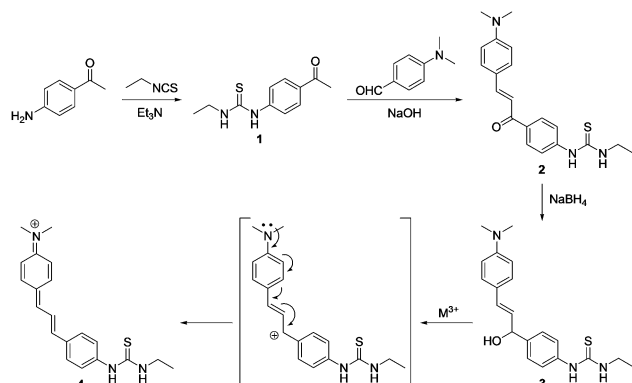
Fig. 1 Schematic representation of the synthetic route used to prepare probe **3** and structure of its dehydration product **4**.

Fig. 3 Color changes observed for water–acetonitrile 95:5 v/v solutions of probe **3** (1.0×10^{-4} mol L^{-1}) at pH 7.0 upon addition of 1 eq. of selected metal cations.

as doublets, at 6.6, 7.1, 7.3 and 7.4 ppm, whereas the singlet at 2.8 ppm was ascribed to the *N,N*-dimethyl moiety.

Water–acetonitrile 95:5 v/v (pH 7.0) solutions of probe **3** were colorless and only intense absorption bands in the UV region were observed. The optical response of probe **3** was tested upon addition of 2 eq. of selected anions (*i.e.* F$^-$, Cl$^-$, Br$^-$, I$^-$, AcO$^-$, HS$^-$, CN$^-$, NO$_3^-$) and cations (*i.e.* Li$^+$, Na$^+$, K$^+$, Cu$^+$, Ag$^+$, Mg^{2+}, Ca^{2+}, Al^{3+}, Fe^{3+}, Fe^{2+}, Ba^{2+}, In^{3+}, As^{3+}, Zn^{2+}, Ni^{2+}, Ga^{3+}, Cd^{2+}, Hg^{2+}, Cr^{3+}, Co^{2+}, Cu^{2+}, Pb^{2+}, Eu^{3+}, Gd^{3+}, Ce^{3+}, Nd^{3+}). Of all of them, only Al^{3+}, Fe^{3+}, Cr^{3+}, As^{3+}, In^{3+} and Ga^{3+} induced the appearance of a new absorption band after *ca.* 5 minutes in the visible region centered at 511 nm (see Fig. 2). The appearance of the new absorbance produced a marked color change from colorless to deep violet (see Fig. 3).

Having assessed the highly selective response of **3** toward trivalent cations, the sensitivity of the probe was studied by monitoring UV-visible changes in water–acetonitrile 95:5 v/v at pH 7.0 upon the addition of increasing quantities of Cr^{3+}, Fe^{3+}, Al^{3+}, Ga^{3+}, In^{3+} and As^{3+}. The UV-visible titration profiles obtained in the presence of the six trivalent cations were quite similar and, as an example, the complete set of spectra obtained for probe **3** and Cr^{3+} cations is shown in Fig. 4
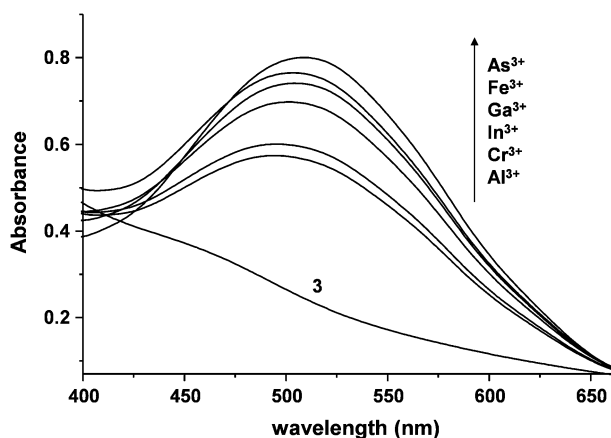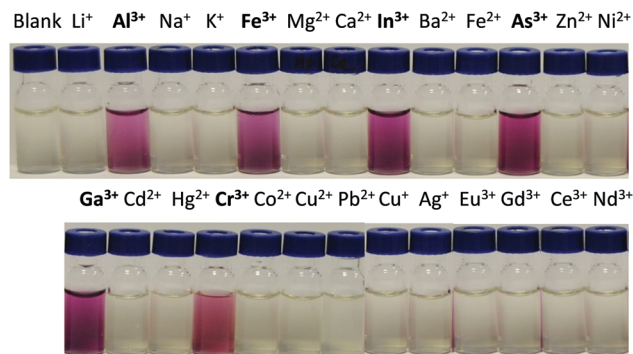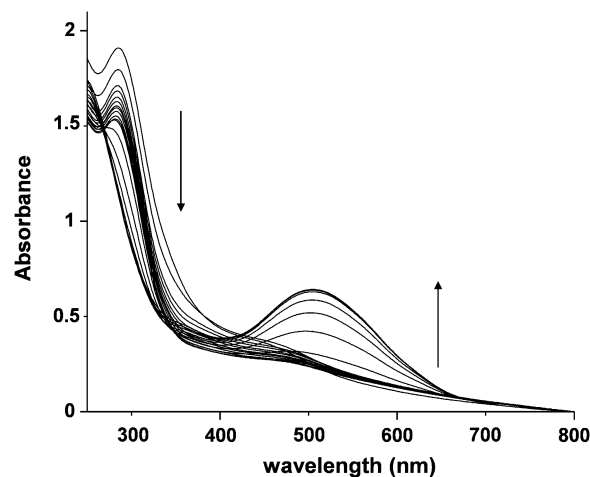

Fig. 4 UV-visible titration of probe **3** (1.0×10^{-4} mol L^{-1}) in water–acetonitrile 95:5 v/v at pH 7.0 upon addition of increasing quantities of Cr^{3+} cation.

(for titrations with the other trivalent cations see the ESI†). As seen in Fig. 4, upon addition of increasing quantities of Cr^{3+} to water–acetonitrile 95:5 v/v solutions of **3**, the absorbance at 516 nm progressively increased. Moreover, from these titration profiles with **3**, the limits of detection (LOD) were calculated (see Table 1). The LOD were in the μM range and were similar to those calculated for other recently published probes for trivalent cations.[9–21]

Moreover, we also found that water–acetonitrile 95:5 v/v solutions of **3** showed, when excited at 300 nm, an intense emission band at 397 nm. From all the cations tested, only the

Fig. 2 UV-visible spectra of probe **3** (1.0×10^{-4} mol L^{-1}) in water–acetonitrile 95:5 v/v (pH 7.0) alone and in the presence of trivalent cations (2 eq.).

Table 1 Detection limits for probe **3** with trivalent cations

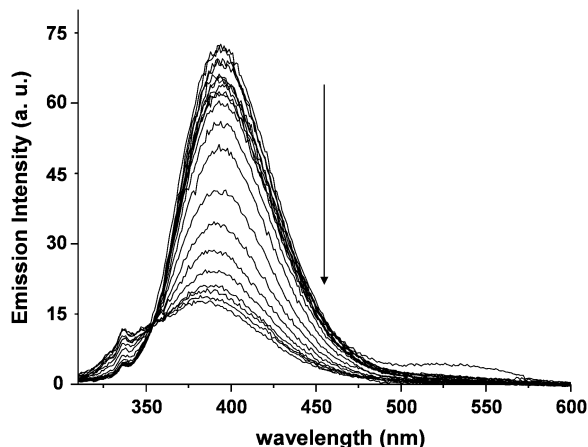| Cation	UV-visible (μM)	Fluorescence (μM)
Al^{3+}	9.7	9.9
In^{3+}	10.6	10.4
Ga^{3+}	10.9	10.0
Fe^{3+}	10.8	9.2
As^{3+}	10.4	10.7
Cr^{3+}	9.3	10.2

Fig. 5 Fluorescence titration of probe **3** (1.0×10^{-4} mol L^{-1}) in water–acetonitrile 95 : 5 v/v at pH 7.0 upon addition of increasing quantities of Cr^{3+} cation.

Fig. 6 ^1H NMR spectra of probe **3**, in CD_3CN, alone and in the presence of 20 eq. of Al^{3+} cations.

addition of Cr^{3+}, Fe^{3+}, Al^{3+}, Ga^{3+}, In^{3+} and As^{3+} induced a marked emission quenching (see Fig. 5 for the set of spectra obtained upon addition of increasing quantities of Cr^{3+}). The LOD obtained from fluorescence titration profiles are also shown in Table 1.

The fact that color and emission changes in the presence of Cr^{3+}, Fe^{3+}, Al^{3+}, Ga^{3+}, In^{3+} and As^{3+} were not instantaneous but time-dependent (*ca.* 5 min) pointed toward a chemical reaction as the mechanism for the chromo-fluorogenic response observed. Moreover, the appearance of the same visible band for the six trivalent cations seemed to indicate the occurrence of a similar chemical reaction in all cases. These trivalent cations are small, have a strong Lewis acid character and a high polarizing power. On the other hand, the appearance of a strong violet color upon reaction with Cr^{3+}, Fe^{3+}, Al^{3+}, Ga^{3+}, In^{3+} and As^{3+} indicated the formation of a final highly conjugated molecule. Taking into account both the facts, we proposed a cation-induced dehydration of probe **3** to yield the highly delocalized cationic molecule **4** (see Fig. 1) as the mechanism in control of the chromogenic modulations observed. This hypothesis was confirmed by the fact that addition of *p*-toluenesulfonic, hydrochloric or sulfuric acids (able to induce dehydration reactions) to water–acetonitrile solutions of probe **3** induced exactly the same chromo-fluorogenic changes (see the ESI†). Moreover, HRMS carried out for the compound obtained through *p*-toluenenesulfonic acid-induced dehydration of **3** showed a molecular fragment at *m*/*z* 338.1687 indicative of the formation of **4** (*m*/*z*: 338.1691 calculated for $C_{20}H_{24}N_3S^+$). Chemical reactions that induced an extension of conjugation were recently used as a mechanism for the preparation of fluorogenic probes for Hg^{2+} cations[23] and reactive oxygen species.[24]

Also, ^1H NMR measurements were carried out in order to assess the dehydration mechanism for the color changes observed in the presence of Cr^{3+}, Fe^{3+}, Al^{3+}, Ga^{3+}, In^{3+} and As^{3+}. In this respect, probe **3** was dissolved in CD_3CN and its ^1H NMR was registered before and after addition of 20 equivalents of Al^{3+} cations. The obtained results are shown in Fig. 6.
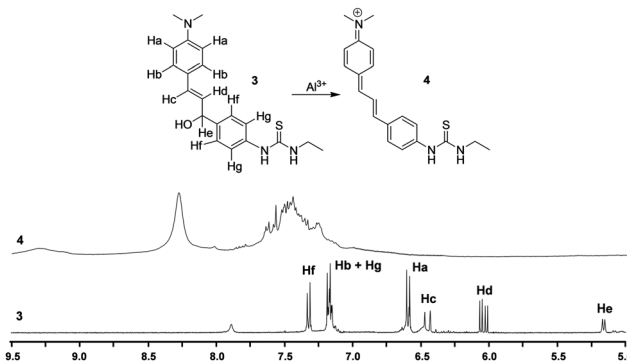
As a general trend, all the resonances of probe **3** were downfield shifted upon addition of Al^{3+} cations. The most remarkable shift was that of methyl groups that in probe 3 appeared as a singlet centered at 2.8 ppm, which was displaced to 3.3 ppm in the presence of Al^{3+} (data not shown in Fig. 6). This remarkable shift could be ascribed to the fact that the methyl groups in the reaction product were directly linked to a positively charged nitrogen atom. Dealing with the signals of the aromatic protons of probe **3**, all resonances showed marked downfield shifts upon addition of Al^{3+} cations. In fact, most of the signals of the final product appeared as a broad multiplet in the 7.0–7.7 ppm interval. However, the most remarkable shifts were suffered by protons He, Hd and Hc, located in the vicinity of the hydroxyl group of probe **3**. These shifts were ascribed to an Al^{3+}-induced dehydration reaction yielding compound **4**.

In summary, we reported herein the synthesis and characterization of a new simple probe able to selectively detect Al^{3+}, Fe^{3+}, Cr^{3+}, As^{3+}, In^{3+} and Ga^{3+} over monovalent, divalent and lanthanide trivalent cations and anions in nearly pure water. Trivalent cations were able to induce a dehydration reaction of **3** that resulted in colour changes from colorless to deep violet. Moreover, the addition of Cr^{3+}, Fe^{3+}, Al^{3+}, Ga^{3+}, In^{3+} and As^{3+} induced quenching of the emission of free **3**. The probe was also quite sensitive with LOD in the μM range. As far as we know, probe **3** displays a different sensing mechanism from those already reported for the detection of trivalent metal cations; moreover, this is also one of the few probes able to display sensing features for these cations in an aqueous environment.

Experimental section

Synthesis of 1

4-Aminoacetophenone (2.61 g, 19.31 mmol) was dissolved in THF (25 mL) and then ethyl isothiocyanate (1.75 mL, 19.38 mmol) and triethylamine (2.84 g, 19.31 mmol) were added to the initial solution. The resultant mixture was refluxed for 16 h. Later, the solvent was removed under reduced pressure and the residue was diluted with dichloromethane (30 mL), washed with HCl 10% (3 × 20 mL), dried with $MgSO_4$ and,

finally, the organic solvent was evaporated to yield **1** (3.43 g, 15.44 mmol, 80%) as a pale yellow solid. The ^{1}H and ^{13}C NMR data are coincident with those previously published.[20]

Synthesis of 2

4-(Dimethylamino)benzaldehyde (738.73 mg, 4.95 mmol) and compound **1** (1100 mg, 4.95 mmol) were dissolved in ethanol (16 mL). Then, NaOH 40% solution in H_2O–EtOH 4:1 v/v (3.24 mL) was added and the mixture was stirred at room temperature for 18 h. The crude was poured over 100 mL of water. The precipitate was filtered off and dried at 60 °C. Finally, the solid was recrystallized from MeOH to yield probe **2** (0.78 g, 2.22 mmol, 45%) as red crystals. The ^{1}H and ^{13}C NMR data are coincident with those previously published.[20]

Synthesis of 3

Compound **2** (356 mg, 1 mmol) and sodium borohydride (272.13 mg, 7.19 mmol) were dissolved in acetonitrile–MeOH 2.8:1 v/v (380 mL) and stirred for 21 hours. Later, the solvent was evaporated and the crude extracted with water and ethyl acetate (3 × 20 mL). The organic phases were collected, washed with saturated NaCl solution (3 × 20 mL), dried with $MgSO_4$ and, finally, the organic solvent was evaporated to yield **3** (213 mg, 0.6 mmol, 60%) as a yellow-orange solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.10 (t, J = 6–5 Hz, 3H), 2.80 (s, 6H), 4.0 (q, J = 6.5 Hz, 2H), 5.30 (d, J = 4 Hz, 1H), 5.85 (br s, 1H), 6.10 (dd, J = 14, 4 Hz, 1H), 6.55 (d, J = 14 Hz, 1H), 6.60 (d, J = 6.1 Hz, 2H), 7.10 (d, J = 6.3 Hz, 2H), 7.25 (d, J = 6.1 Hz, 2H), 7.40 (d, J = 6.3 Hz, 2H), 7.75 (br s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 14.0, 40.1, 74.5, 105.6, 125.8, 127.5, 128.0, 130.8, 142.5, 151.2, 181.4. Calcd. for $C_{20}H_{25}N_3OS$: 355.1718; found: 356.1787 ($M + H^+$).

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