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A new selective fluorogenic probe for trivalent cations†

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A new selective chromo-fluorogenic probe for Fe^{3+}, Cr^{3+} and Al^{3+} is reported. Detection limits are in the μM range and the fluorogenic sensing ability could be observed by the naked eye when illuminated with UV-light. No response is observed with divalent cations.

The design and synthesis of new chemosensors for transition and p-block metal cations remain an important subject in the field of supramolecular chemistry because of their impact on the environment and human health.[1–3] Even though a large number of chemosensors for divalent transition metal cations have been described, very few studies have been devoted to the development of organic probes that are sensitive to triple-charged metal cations.[4–6] However, trivalent cations have important biological properties and are directly involved in the cell function where there is a critical control of M^{3+} levels.[7] For instance, the trivalent form of chromium is an essential element in human nutrition and has a huge impact on the metabolism of carbohydrates, fats, proteins and nucleic acids as it can activate certain enzymes and stabilise proteins and nucleic acids. Cr^{3+} deficiency has been reported to disturb glucose levels and lipid metabolism.[8,9] At the same time, it is an environmental pollutant that causes concern in industry and agriculture.[9–11] Fe^{3+} is not only the most abundant transition metal in cellular systems, but is of outstanding biological importance given its presence in numerous enzymes and proteins. Iron is critically involved in both electron transfer reactions and oxygen transport due to its adequate redox potentials and its high affinity for oxygen. Finally, it is well-known that Al^{3+} is abundantly found in nature, causes drinking water contamination and can be toxic to humans in excessive amounts. Many symptoms of aluminium toxicity mimic those of Alzheimer's disease and osteoporosis. Moreover, gastrointestinal problems, interference with Ca^{2+} metabolism, decreased liver and kidney function can be caused by aluminium toxicity.[12–14] Thus, there is a pressing need to develop chemical sensors capable of detecting the presence of trivalent cation ions in environmental and biological samples.

Sensors based on ion-induced changes in fluorescence are especially suitable as they are easy to use and usually give an instantaneous response with high sensitivity.[15–17] Moreover in this field, the design of probes displaying changes in optical properties through a "turn-on" response is much preferred for designing efficient sensors than those showing a "turn-off" response. Some Fe^{3+} [18–20] and Al^{3+} [21–24] selective fluorogenic probes have been reported yet, surprisingly, very few sensors for Cr^{3+} have been described in the literature.[9,25] Consequently, the design of new probes bearing suitable multidentate chelating units, which can potentially sense these metal ions, is a timely area of research. Given our interest in developing new chemosensing systems,[26] we report herein a new probe based on a derivative of fluorescein for the detection of trivalent cations Fe^{3+}, Al^{3+} and Cr^{3+}. In all cases a remarkable enhancement of the fluorescence emission in acetonitrile was observed.

Fluorescent probe **1** (*vide infra*) was based on the well-known fluorophore fluorescein and has been used herein for the selective detection of trivalent cations. This choice was supported by our previous experience in using fluorescein as a signalling unit in different sensing systems.[27,28] Fluorescein is one of the most commonly used fluorophores due to its high molar absorptivity, large fluorescent quantum yields and high photostability. However, fluorescein's photophysical properties are strongly dependent on pH, which is why our preliminary studies were carried out in organic media (acetonitrile).

The synthesis of **1**, Fig. 1, began with a Fischer esterification of fluorescein to fix the open form of the lactone ring. The coordinating moiety was then built from the hydroxyl group by alkylation with bromoacetic acid *tert*-butyl ester and subsequent trifluoroacetic acid-mediated deprotection. Both steps proceeded easily, as previously described.[29] 2-Hydroxyethylamine was introduced by esterification of the BOC-protected derivative under optimised conditions.[30] BOC-deprotection was performed

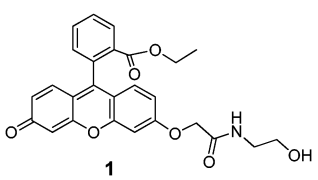

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Fig. 1 Ligand **1**.

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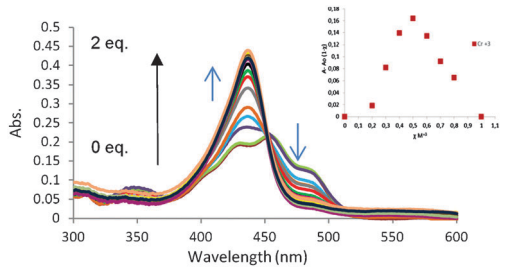

Fig. 2 UV-vis spectra of ligand **1** (10^{-5} M) upon titration of Cr^{3+} (0–2 equiv.) in CH_3CN. Inset: stoichiometry determination by the Job's plot from UV-vis data.

with trifluoroacetic acid to provide, through a rearrangement, the most thermodynamically stable compound **1** (44% overall yield) (see ESI†). The spectroscopic properties of **1** were evaluated in acetonitrile solution. As shown in Fig. 2, **1** exhibits maximal absorptions at 352 ($\varepsilon = 6.62 \times 10^3$ M^{-1} cm^{-1}), 430 ($\varepsilon = 1.80 \times 10^4$ M^{-1} cm^{-1}), 453 ($\varepsilon = 2.00 \times 10^4$ M^{-1} cm^{-1}) and 484 ($\varepsilon = 1.10 \times 10^4$ M^{-1} cm^{-1}) nm in accordance with the presence of the fluorescein moiety, which is responsible for probe **1**'s pale yellow colour. Upon addition of chromium (0–2 equiv.), the absorbance at 484 nm decreases gradually and simultaneously, and a new significant absorption band developed at 437 nm ($\varepsilon = 4.33 \times 10^4$ M^{-1} cm^{-1} for Cr^{3+} and $\varepsilon = 5.05 \times 10^4$ M^{-1} cm^{-1} for Fe^{3+}). A similar effect was observed in the presence of Fe^{3+} (see ESI†). Moreover, absorbance at 437 nm remained constant in the presence of more than 1 equiv. of Fe^{3+} or Cr^{3+}, strongly suggesting the formation of 1 : 1 ligand-to-metal complexes. This was also demonstrated *via* the corresponding Job's plots for both the **1**-Fe^{3+} and **1**-Cr^{3+} systems.

Ligand **1** (10^{-5} M) exhibited practically no fluorescence ($\lambda_{exc} = 453$ nm) in the 450–520 nm range, whereas a significant enhancement of emission at 475 nm emerged after addition of Fe^{3+} or Cr^{3+} (see ESI†). This band showed a linear enhancement with an increased cation concentration for ratios [cation]/[ligand] up to 1. A further increase of the cation concentration did not lead to any further emission enhancement. Probe **1** showed excellent selectivity for Al^{3+}, Fe^{3+} and Cr^{3+} ions over the relevant competing metal ions. The enhancement of fluorescence allowed detection limits to reach the micromolar range (*vide infra*). These fluorogenic results are in full agreement with the UV-vis data. When similar studies were carried out with Al^{3+}, similar changes in both UV-vis and fluorescence to those found in the presence of Fe^{3+} and Cr^{3+} were also observed (see ESI†). However for this cation, the formation of a 2 : 1 ligand-to-metal complex was determined. This 2 : 1 stoichiometry observed for **1** in the presence of Al^{3+}, in contrast to the 1 : 1 stoichiometry found for Fe^{3+} and Cr^{3+}, could be related to the smaller size of the former (ionic radius 0.50 Å for Al^{3+} *versus* 0.69 Å and 0.64 Å for Cr^{3+} and Fe^{3+}, respectively), which precluded an efficient coordination of Al^{3+} with only one ligand molecule. However, size could not be the only crucial factor as Liu and colleagues have developed selective fluorescent chemosensors, based on rhodamine, that show different stoichiometries for complexation of Fe^{3+} and Cr^{3+}.[31]

Moreover, complexation constants were determined from the corresponding UV-vis and fluorescence titration curves using the Spectfit program.[32] The log β values for the equilibria **1** + M^{3+} ⇄ $[M(\mathbf{1})]^{3+}$ for Cr^{3+} and Fe^{3+}, and for the equilibrium 2**1** + Al^{3+} ⇄ $[Al(\mathbf{1})_2]^{3+}$ are included in Table 1. Detection limits were evaluated and determined from the equation DL = $K \times Sb_1/S$, where $K = 3$, Sb_1 is the standard deviation of the blank solution and S is the slope of the calibration curve[33] (see ESI†). These values are also depicted in Table 1.

Table 1 Complexation constants and DL for **1** with trivalent cations

Cation	Log β UV	Log β fluorescence	Detection limit (UV)/μM	Detection limit (Fluor.)/μM
Cr^{3+}	3.5 ± 0.8	4.2 ± 0.2	2.5	0.5
Fe^{3+}	5.3 ± 0.3	5.1 ± 0.2	0.6	0.3
Al^{3+}	9.71 ± 0.07	9.81 ± 0.03	0.3	0.2

A detailed analysis of the UV-vis absorption spectrum of **1** in the presence of other metal cations was carried out. As shown in Fig. 3, the addition of 1 equiv. of Li^+, Cu^{2+}, Cd^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Fe^{2+}, and Hg^{2+} had no noticeable effect on the fluorescence emission at 475 nm. This is in contrast with the strong enhancement of the emission intensity upon addition of Fe^{3+}, Cr^{3+} and Al^{3+}. Competitive experiments in the presence of the above-mentioned metal cations also demonstrate the selective response of **1** to Fe^{3+} or Cr^{3+}or Al^{3+}. Probe **1**'s fluorogenic sensing ability could be observed by the naked eye when acetonitrile solutions of **1** in the presence of Fe^{3+} or Cr^{3+}or Al^{3+} were illuminated with 254 nm UV-light *via* the enhancement of a clearly observed pale blue emission (see Fig. 3, bottom). Quantum yields of 0.07, 0.10 and 0.16 were determined for the Cr^{3+}, Fe^{3+} and Al^{3+} complexes, respectively (using fluorescein, 0.1 M NaOH, $\Phi = 0.85$, as standard). The quantum yield difference is high enough to discriminate between these cations at concentrations higher than 5 μM. The fluorescence intensity decreases with an increase in the water content, however this could also be useful for the discrimination of the different cations. Thus, the Al^{3+} complex still remains fluorescent after addition of 4% water, whereas quenching of the fluorescence for Cr^{3+} and Fe^{3+} complexes was observed when more than 2% and 1% water, respectively, were added.

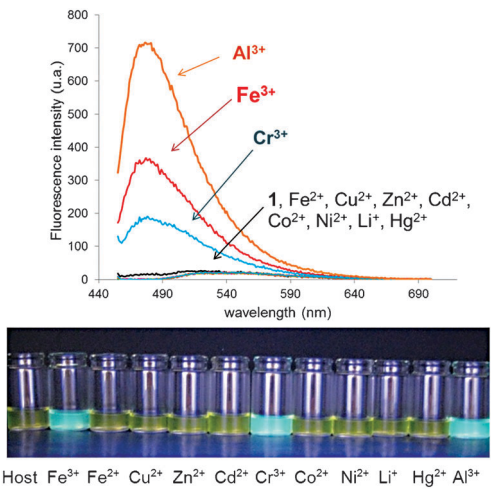

Fig. 3 (top) Fluorescence spectra of **1** (10^{-5} M) upon addition of 1 equiv. of Fe^{3+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Li^+, Hg^{2+}, Cr^{3+} and Al^{3+} in CH_3CN ($\lambda_{exc} = 437$ nm). (bottom) Visual changes ($\lambda_{exc} = 254$ nm) observed for **1** in the presence of different metal cations.

The ^{1}H NMR spectra of ligand **1** recorded in CD_3CN upon the addition of increasing concentrations of Al^{3+} showed significant spectral changes (see ESI†). The most important shifts of the signals of **1** were observed in the xanthene moiety, especially the α protons to the carbonyl group at 6.25 and 6.39 and the *ortho*-protons to the lateral chain at 7.19 and 6.95 ppm, which underwent a significant downfield shift upon the addition of Al^{3+}. On the other hand, the singlet signal at 4.67 ppm displayed a similar behaviour, with a downfield shift of 0.3 ppm. There were no appreciable changes in the peak positions of the ethylene chain next to the amide group. These data strongly suggest the direct involvement of the xanthene moiety and the lateral chain for Al^{3+} coordination. In order to suggest a complexation model for the 1 : 1 complexes, complementary NMR studies with Ru^{3+}, which showed to form 1 : 1 complexes with ligand **1**, were carried out (see ESI†). The similarity of the spectra modifications, with those observed with Al^{3+}, suggests that the same groups are involved in both kinds of complexes.

In summary, we report herein the synthesis and characterisation of a new probe for the fluorogenic detection of trivalent cations Fe^{3+}, Cr^{3+} and Al^{3+}. The choice of the fluorescein group allowed the optical detection of these ions, whereas the probe remained silent in the presence of monovalent and divalent cations such as Li^+, Cu^{2+}, Cd^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Fe^{2+} and Hg^{2+}. The acetonitrile solutions of **1** in the presence of Fe^{3+}, Cr^{3+} and Al^{3+} resulted in an associated "turn-on" response *via* the formation of the corresponding metal complexes. This chelation-enhanced fluorescence might be attributed to the change in polarity of the dye by increase of the donor–acceptor electronic delocalization after complexation. Fe^{3+} and Cr^{3+} were found to form 1 : 1 ligand-to-metal complexes with **1**, whereas the formation of 2 : 1 complexes was observed for Al^{3+}. A discrimination based on the fluorescence response according to the amount of water in solution can be envisaged.

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