ARTICLE TYPE

Ferrocene-pyrylium dyad as a selective colorimetric chemodosimeter for the toxic cyanide and hydrogen sulfide anions in water††

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The ferrocene-pyrylium dyad 1 behaves as a selective redox and colorimetric chemodosimeter for CN⁻ and HS⁻ anions, exibiting a clear perturbation in the redox potential of the ferrocene unit and in the absorption band, in the presence of
10 **these anions.**

The development of simple and sensitive anion sensors continues to be a research area of considerable interest because of the important roles that these species play in biological, pathological or environmental processes. Increasing attention within the
15 chemical sensor community is turning to the development of multi-ion responsive unimolecular systems, which has also become a challenging task in the field of supramolecular chemistry. In this context, molecular logic gates[1]molecular keypad lock devices,[2] lab-on-molecule type devices[3] and
20 investigation of approaches that utilize known successful receptors for detection of and discrimination among a range of high priority toxic industrial chemicals[4] represent exciting new prospects in this field.

Cyanide is known as one of the most rapidly acting and
25 powerful poisons, and the toxicity results from its propensity to bind the iron in cytochrome *c* oxidase, inhibiting the mitochondrial electron-transport chain and resulting in hypoxia.[5] Also, even very small amounts of the cyanide can affect many functions in the human body, including vascular, visual, central
30 nervous, cardiac, endocrine and metabolic systems.[6] Indeed, the concentration of cyanide in drinking water cannot be greater than 1.9 μM according to the World Health Organization.[7] The widespread use of cyanide in industrial settings (1.5 million tons per year)[5a] and potential threats for terrorism continue to
35 engender significant research efforts directed toward its detection under biologically relevant conditions. The detection of cyanide through various supramolecular approaches has been extensively reported.[5b] However, most cyanide sensing molecules are insoluble in water because of their organic nature and,
40 consequently, only few examples of sensing this anion in aqueous solutions have been described.[8]

On the other hand, hydrogen sulfide (H_2S) has long been known as a toxic gas. It can cause blood poisoning, and at high enough concentrations may lead to death, typically levels as high
45 as 600 μg dm^{-3} in blood serum can cause fatalities.[9] Hydrogen sulfide which is a weak acid in aqueous solutions (pK_{a1} = 7.04, pK_{a2} = 11.96),[10]equilibrating mainly with HS⁻ at physiological pH, is also known as a mitrochondrial toxin and inhibits

cytochrome oxidase, leading to histotoxic hypoxia.[11] However,
50 recent studies have regarded H_2S as the third gasotransmitter, together with nitric oxide (NO) and carbon monoxide (CO),[12] for regulating cardiovascular, neuronal, immune, endocrine and gastrointestinal systems.[12b, 13] Nevertheless, abnormal levels of H_2S are associated with many types of diseases including
55 Alzheimer's disease,[14] liver cirrhosis,[15] Downs's syndrome,[16] and diabetes.[17]

Therefore, for detailed studies of the physiological/toxicological functions of H_2S, as well as of the toxicological effects of the cyanide anion, selective and
60 convenient methods for the detection of these analytes are required.

From the general approaches to design optical sensors for anions,[5b] the "chemodosimeter" approach is based on the use of a molecular system which selectively undergoes guest-induced
65 chemical reactions coupled to suitable optical events.[18] Most of the reaction-based anion sensors often employ sophisticated structures, requiring complicated synthetic steps. However, despite their inherent interest, the use of pyrylium ions for the anion detection has been little developed. The only salts used for
70 this purpose are those substituted at the 2-, 4- and 6-positions by alkyl or aryl groups.[19]

We report in this paper the use of the already reported (4-ferrocenyl-2,6-dimethylpyrylium) hexafluorophosphate[20] as a new chemodosimeter for the selective detection and
75 discrimination of toxic cyanide and hydrogen sulfide anions in water solutions, which constitutes a particularly important and difficult challenge. Although the ferrocene unit has largely proved to be a simple and remarkable redox-signaling unit, used in the preparation of a wide variety of chemosensors,[21] reports
80 about pyrylium cations incorporating the ferrocene moiety are relatively few.[22] Thus, the integration of this motif into the pyrylium rings is an attractive proposition in the field of anion sensing.

The synthesis **1** was by sequential treatment of ferrocenyl
85 lithium with 2,6-dimethyl-γ-pyrone and subsequent reaction of the resulting intermediate with HPF₆.[20]
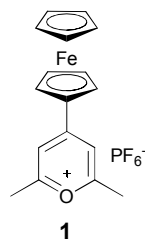
Scheme 1 Structure of receptor **1**

Then, the anion sensing properties of **1** were analysed by electrochemical experiments, and absorption and ^{1}H NMR spectroscopies. At first, the electrochemical properties of **1** as its own as well as in the presence of variable concentrations of cyanide anion were examined under cyclic (CV) and Osteryoung square wave (OSWV) voltammetries. This chemodosimeter exhibited a reversible one-electron redox wave,[23] typical of a ferrocene derivative, at a $E_{1/2}$ = 850 mV, calculated *versus* the decamethyl ferrocene (DCMF) redox couple, and which is identical as that obtained from the corresponding OSWV peak. The stepwise addition of CN^{-}, as its tetrabutylammonium (TBA) salt, to an electrochemical solution of **1** (c = 5×10^{-4} M in CH$_3$CN), containing 0.1 M [n-Bu$_4$N]PF$_6$ as supporting electrolyte, revealed a clear evolution of the wave from $E_{1/2}$ = 850 mV to $E_{1/2}$ = 565 mV ($\Delta E_{1/2}$ = -285 mV), the maximum perturbation being obtained when 1 equiv of cyanide anion was added (Figure 1).
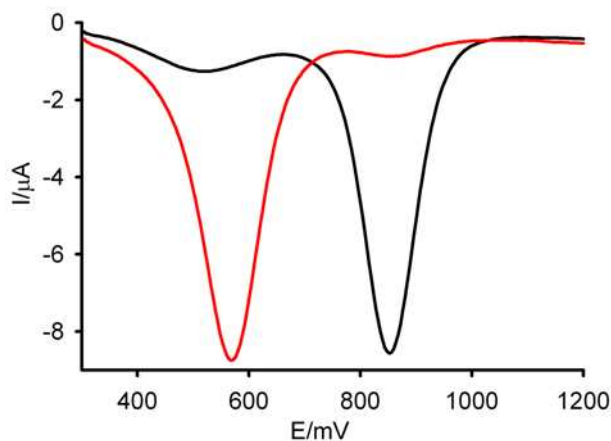

Fig. 1 Evolution of the OSWV of **1** (c = 5×10^{-4} M) in CH$_3$CN (black) upon addition of increasing amounts of CN^{-} (in H$_2$O), until 1 equiv (red).

Titration experiments followed by absorption spectroscopy were initially performed in acetonitrile solution (c = 1×10^{-4} M). The Uv-vis spectrum of **1** shows four absorption bands at λ = 342 nm (ε = 14630 M^{-1} cm^{-1}), λ = 386 nm (ε = 2650 M^{-1} cm^{-1}), λ = 405 nm (ε = 2430 M^{-1} cm^{-1}), and λ = 600 nm (ε = 3230 M^{-1} cm^{-1}). The addition of cyanide aliquots (c = 2.5×10^{-2} M in H$_2$O) caused, as the most prevalent changes, the progressive disappearance of the band at λ = 600 nm while a new weaker band at λ = 500 nm (ε = 1250 M^{-1} cm^{-1}) grew up simultaneously, until 1 equivalent of anion was added (see ESI). These preliminary experiments showed that, in a poorly competitive medium such as acetonitrile, receptor **1** can effectively sense cyanide anions. Taking into account that only few cyanide detection systems operate in

genuine aqueous environment[24] and although the water could decrease the anion's nucleophilicity by hydrogen bonding to the cyanide, we also studied the chemodosimeter behaviour of **1** in a pure aqueous medium. Interestingly, no significant changes were observed in its UV-vis spectrum when compared to that obtained in acetonitrile. Moreover, the addition of increasing amounts of cyanide anion (c = 2.5×10^{-2} M in H$_2$O) to a solution of **1** in H$_2$O (c = 1×10^{-4} M) induced the same changes observed when the titration was carried out in the organic solvent (Figure 2). The detection limit[25] of cyanide in a solution of **1** in pure H$_2$O was found to be 3.75 ppm. As a result of the above mentioned spectral changes, the solution colour of **1** changed from blue to pink, indicating that **1** can serve as a "naked-eye" sensor for cyanide anion in pure water solutions
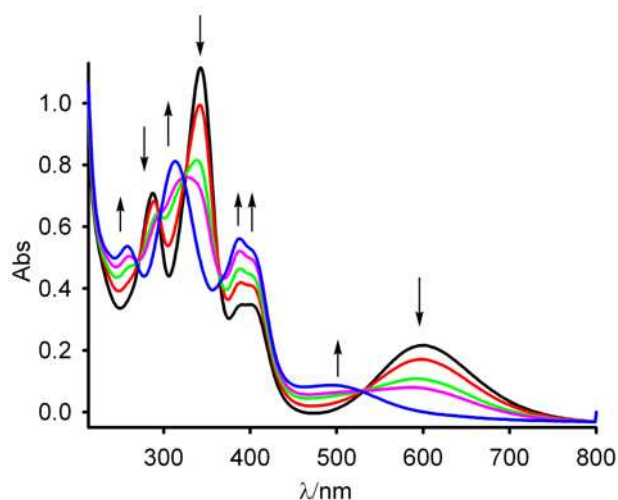

Fig. 2 Evolution of the absorption spectrum of **1** (c = $1. \times 10^{-4}$ M) in H$_2$O (black) upon addition of increasing amounts of CN^{-} (in H$_2$O), until 1 equiv. Arrows indicate the absorptions that increase and decrease during the reaction.

The anion recognizing ability of **1** for HS[-] anions was also determined by the changes in its absorption spectra measured upon addition of aliquots of NaHS (c = 2.5×10^{-2} M in H$_2$O) to a solution of **1** in both acetonitrile (c = 1×10^{-4} M) or H$_2$O (c = 2.5 $\times 10^{-2}$ M). However, the most significant changes observed upon addition of this anion to the aqueous solution of **1** involve the disappearance of the band at λ = 600 while a new band at λ = 500 nm (ε = 1900 M^{-1} cm^{-1}) appeared, with a concomitant colour change from blue to pink. By contrast, the same titration experiments carried out in CH$_3$CN solutions gave rise to a UV-vis spectra in which the highest energy band appeared at λ = 403 nm, promoting a change in the color of the solution from blue to yellow (see ESI). The detection limit was found to be 3.95 ppm in the aqueous solution.

It is very well known that OH^{-} is a common interfering anion in most samples with high cyanide concentrations because it is used to avoid formation of toxic HCN vapors. Similarly, S^{2-} is also described as a very relevant interfering ion with cayanide.[26] Thus, we have also carried out competition experiments among these anions, to further elucidate the selectivity of **1** toward these anionic species.

Interestingly, in preliminary experiments we observed that the spectral and color changes obtained on addition of TBAOH (c =

2.5 x 10^{-2} M) to **1** (c = 1x 10^{-4} M), both in H_2O and CH_3N, were the same as those resulting upon addition of HS^- anion to **1** under the same conditions (see ESI).
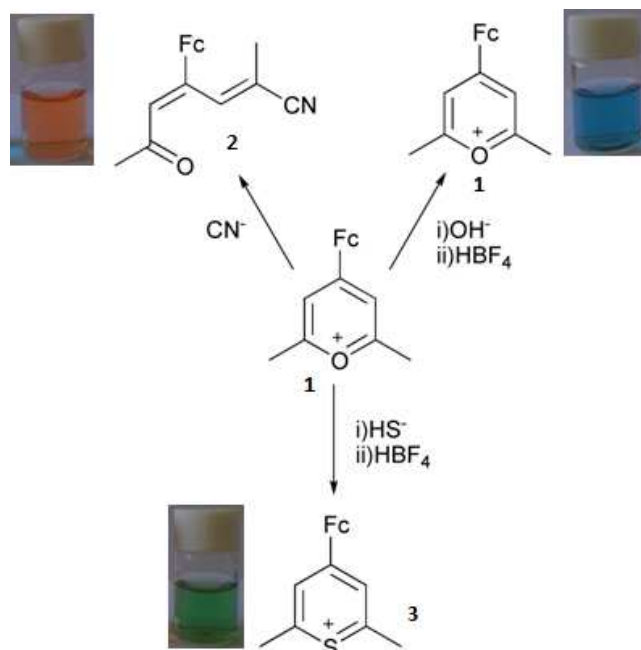
5   **Scheme 2** Evolution of **1** upon treatment with anionic species

    In all the cases, the reaction between **1** and the nucleophiles takes place according to the usual addition of nucleophilic agents to the α-position of the pyrylium cation, giving rise to the corresponding acyclic intermediates. However, such open-chain
10 derivatives undergo significant and different behaviour when they were treated with HBF_4. When the nucleophile is an hydroxide anion, the ring opening reaction of the pyrylium is fully reversible and upon addition of HBF_4, the pent-2-en-1,5-dione intermediate formed, undergoes ring closure to generates again
15 the pyrylium ring **1** with a concomitant color change of the solution from pale yellow to blue. The open chain derivative obtained upon addition of HS^- (pale yellow λ = 405 nm), upon addition of HBF_4 undergoes ring closure to give the corresponding thiopyrylium salt **3** (deep green, λ = 640 nm)
20 which was isolated and characterized by standard analytical and spectroscopic techniques (see ESI). By contrast, the intermediate **2**, obtained upon addition of CN^- anion (red, λ = 640 nm), does not react with HBF_4 and, consequently, no visual or spectroscopic changes were observed (Scheme 2).
25   The formation of the open chain derivative **2** was further confirmed by mass spectrometry. Thus, the ESI mass spectrum of chemodosimeter **1** in the presence of KCN shows a peak at m/z 320.07 corresponding to the $[M^++1]$ of the neutral species **2** formed (see ESI) during the recognition process.
30   It should be also mentioned that the chemosensor behaviour of **1** towards amines was also examined. Unfortunately, the experiments only work in pure acetonitrile medium and do not discriminate between the set of amines tested because, in all cases, the same changes in both the electrochemical responses
35 and UV-vis spectrum of **1** were detected (see ESI).
    The responses of receptor **1** upon addition of CN^-, OH^-, and HS^- anions have also been investigated by ^1H-NMR experiments

in CD_3CN, providing further evidence for the ring-opening of the pyrylium ring by these anions. In the case of the CN^- anion
40 (Figure 3), the progress of the reaction can be easily observed following the chemical shift change of the singlets corresponding to the pyrylium protons H3 and H5 (red signal at δ = 7.87 ppm) and that of the CH_3 substituent at 2 and 4 position (green signal at δ = 2.70 ppm) which in both cases are split into two different
45 signals (δ = 7.22 and δ = 6.67 ppm for the H3 and H5 protons and at δ = 2.18 and δ = 2.11 for the CH_3). Moreover, the signals associated to the ferrocene moiety were also upfield shifted when the corresponding open structure **2** was formed.
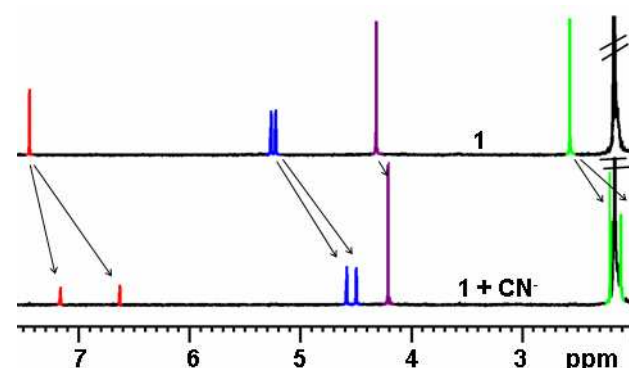

50   **Fig. 3** Evolution of ^1H NMR in CD_3CN of **1** (top) upon addition of CN^- anion, in H_2O (bottom). The black signal corresponds to the H_2O present in the medium.

Conclusions

In conclusion, the ferrocene-pyrylium dyad behaves as a selective
55 chemodosimeter for the toxic cyanide and hydrogensulfide anions. A salient feature of this simple receptor, is the presence of a reactive pyrylium ring able to react with these anionic species in water to give acyclic products, exhibiting an easily detectable signal change in the redox potential of the ferrocene/ferrocinium
60 redox couple and a remarkable perturbation in the absorption spectrum, which can be used for "naked eye" detection of these anions.

Supporting Information Available General comments. Electrochemical, UV-vis, and ^1H NMR data

Notes and references
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†† In memoriam of Prof. A. R: Katritzky, Professor at the Universities of East Anglia (Norwich, UK) and Florida (Gainesville, USA).

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