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A simple fluorescent chemosensor for alkaline-earth metal ions

Luca Prodi [a,*], Roberto Ballardini [b], Maria Teresa Gandolfi [a], Roberta Roversi [a]

[a] *Dipartimento di Chimica 'G. Ciamician', Università di Bologna, Via Selmi 2, 40126 Bologna, Italy*
[b] *Istituto FRAE-CNR, Via Gobetti 101, 40129 Bologna, Italy*

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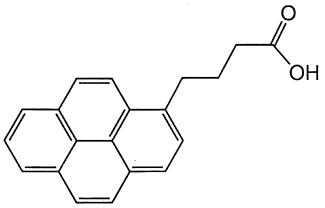
Abstract

In acetonitrile solutions, the deprotonated form of 1-pyrenebutyric acid (**1**) forms 1:2 (metal:ligand) complexes with Zn^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, and earth alkali metal ions. The complexation process leads two pyrene moieties in close contact, so that dimers are formed, causing strong changes in the absorption and fluorescence spectra. Taking advantage of these changes, very low concentrations of earth alkali metal ions can be detected, suggesting **1** as a good prototype of a new family of fluorescent sensors. © 2000 Elsevier Science S.A. All rights reserved.

Keywords: Fluorescent chemosensor; Alkaline-earth metal ions; Luminescence; Pyrene

1. Introduction

Typically, chemosensors are molecules that are able to bind selectively and reversibly an analyte with a concomitant change in some monitorable properties of the system, such as redox potentials, absorption or luminescence spectra [1]. The ability to monitor analyte concentrations in real-time and real-space using chemosensors finds application in many fields including medical diagnostics, environmental control, cell biology, and electronics [1,2]. The design, synthesis, characterisation, and application of many luminescent chemosensors have opened up many opportunities for solving complex analytical problems, thanks also to the great sensitivity typical of luminescence spectroscopy [1–7]. Among the different luminophores used for designing new chemosensors, pyrene provides many interesting features, such as a fluorescence emission with high quantum yield, a rather long excited state lifetime, and the possibility to form dimers and exciplexes with consequent remarkable changes in the luminescence spectrum [8–13]. Of particular interest has been the measurement of the concentration of alkaline-earth cations in biological samples. For example, Ca^{2+} and Mg^{2+} play important roles as intracellular messengers in the regulation of cell functions [14–16]. We report here the possibility to determine very low concentrations of earth alkali metal ions and Zn^{2+} using as fluorescent chemosensor 1-pyrenebutyric acid (**1**), that can take advantage of the above mentioned photophysical properties of the pyrene luminophore and of the coordinating abilities of the carboxylate group.



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2. Experimental

All the experiments were carried out in air equilibrated acetonitrile solutions at room temperature with equipment and procedures previously described [17]. The emission quantum yields of the fluorescence bands centered at 378 nm and of that one centered at 480 nm were obtained using as standards naphthalene in deaerated cycloexane (Φ=0.23 [18]) and quinine sulphate in 1N H_2SO_4 aqueous solution (Φ=0.546 [19]), respectively. The compounds used were commercially available products of analytical grade. All the metal ions were added as perchlorate or triflate salts.

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* Corresponding author. Tel.: +39-051-209-9541; fax: +39-051-209-9456.
E-mail address: lprodi@ciam.unibo.it (L. Prodi).

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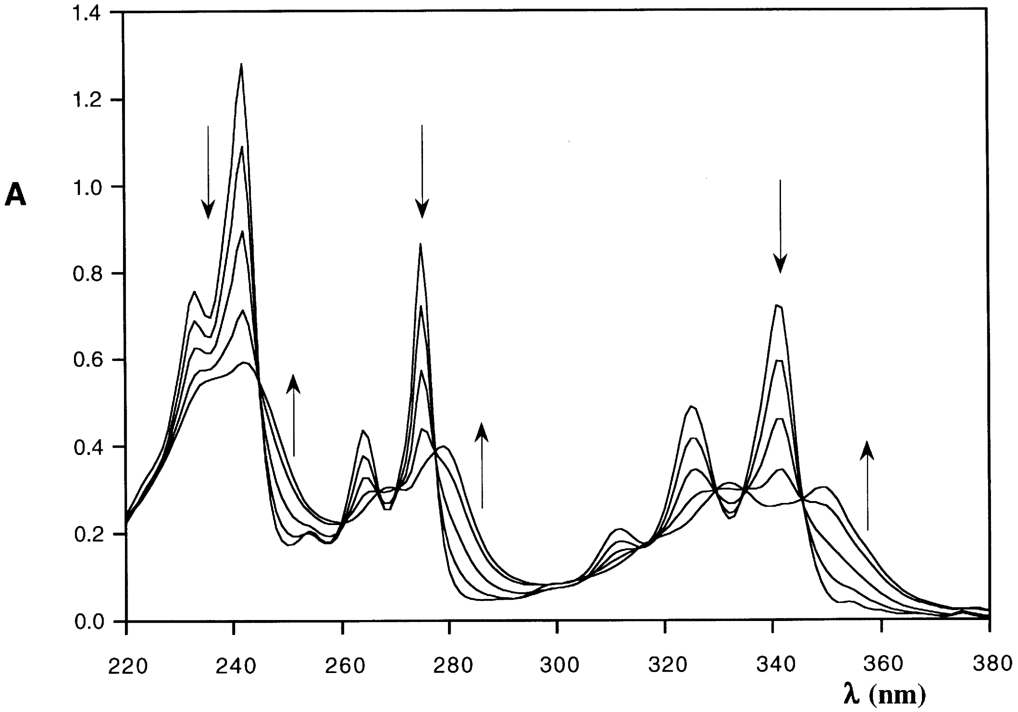

Fig. 1. Absorption spectrum of a 2.0×10^{-5} M acetonitrile solution of **1** and upon addition of 0.13, 0.26, 0.39, and 0.50 molar equivalents of $Ca(ClO_4)_2$.

3. Results and discussion

The absorption (ε_{340}=40,000 M^{-1} cm^{-1}, Fig. 1) and fluorescence (λ_{max}=378 nm, τ=18 ns, Φ=0.07, Fig. 2) spectra of an acetonitrile solution of **1** are very similar to those reported for the pyrene monomer, with only a small red-shift, due to the substitution in the 1 position with the alkyl chain. Addition of a stoichiometric amount of base able to deprotonate the carboxylic group, such as tributylamine or tetramethylammonium hydroxyde, does

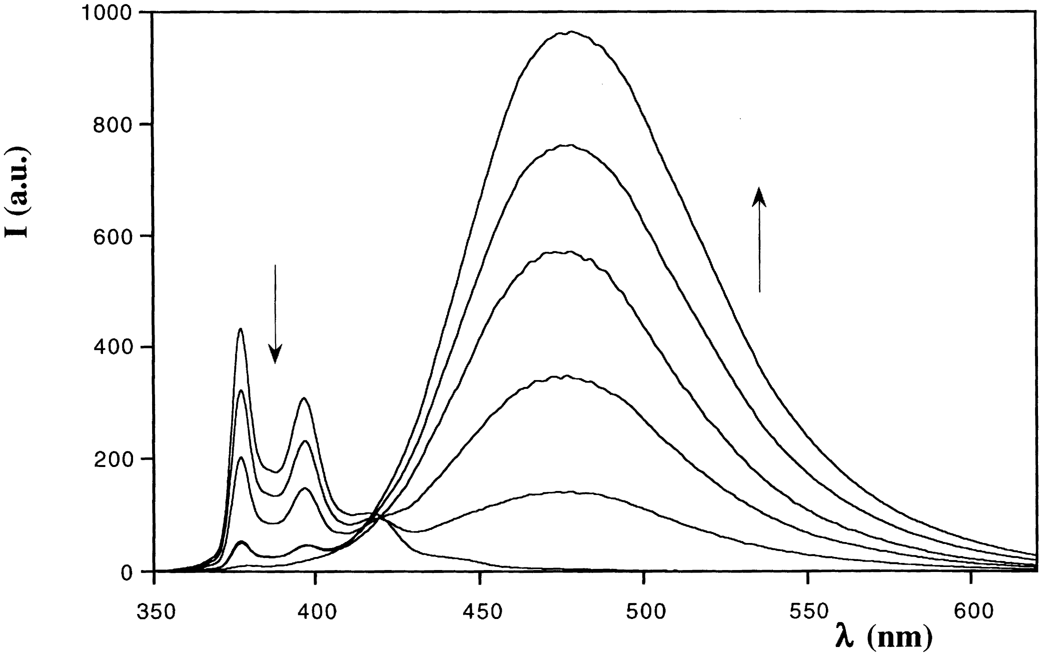

Fig. 2. Fluorescence spectrum of a 2.0×10^{-5} M acetonitrile solution of **1** and upon addition of 0.13, 0.26, 0.39, and 0.50 molar equivalents of $Ca(ClO_4)_2$ (λ_{exc}=330 nm, an isosbestic point).

Table 1
Photophysical properties[a] of **1** and its complexes with metal ions

Compound	Absorption[b]		Fluorescence	
	λ_{max} (nm)	ε ($M^{-1}\,cm^{-1}$)	λ_{max} (nm)	Φ
1	340	40000	378[c]	0.07
[Mg·**1**$_2$]	336	66000	479	0.17
[Ca·**1**$_2$]	343	28000	476	0.28
[Ba·**1**$_2$]	340	53200	479	0.22
[Zn·**1**$_2$]	342	50800	479	0.14
[Cu·**1**$_2$]	341	70000	–	–
[Fe·**1**$_2$]	340	57600	–	–
[Fe·**1**$_2$]$^+$	340	56600	–	–

[a] Acetonitrile solutions, room temperature.
[b] Structured band.
[c] Highest energy feature.

not cause any change in the absorption and fluorescence spectra. This is an expected result, because of the long distance between the pyrene moiety and the acidic function. However, when Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Zn^{2+}, Fe^{2+}, Fe^{3+}, Cu^{2+} are added to an acetonitrile solution containing equimolar amounts of **1** and tributylamine, strong changes are observed in the photophysical properties of the pyrene chromophore (Table 1). In particular, as can be observed in Fig. 1 upon addition of Ca^{2+}, the absorption spectrum undergoes a noticeable broadening of the structured band of the pyrene, up to a stoichiometric ratio of 1:2 (metal:ligand). Further addition of metal ions leads to a partial recovery of the initial absorption spectrum. The changes in the absorption spectrum are very similar to those observed after the formation of a ground state dimer of the pyrene chromophore, as observed, e.g. after inclusion of two molecules in a γ-cyclodextrin cavity [20,21]. Our findings can consequently be rationalised assuming that a complex with a 1:2 (metal:ligand) stoichiometry is formed (equilibria 1 and 2), in which the two pyrene units, lying very close to each other, can interact forming a dimer. It is important to stress that in all cases the global association constants (β_2) were very high, with a lower limiting value of $10^{15}\,M^{-2}$.

$$1^- + M^{2+} \rightleftarrows [M\cdot 1]^+ \qquad (1)$$

$$[M\cdot 1]^+ + 1^- \rightleftarrows [M\cdot 1_2] \qquad (2)$$

It is noteworthy that the entity of the changes observed in the absorption spectrum depends on the added metal ion (Table 1), since the geometry of the complex relies on the nature of the central ion. Addition of an excess of metal ion favours the formation of complexes with a 1:1 stoichiometry (Eq. (1)), and this can explain the partial recovery, observed in these conditions, of the intensity of the band typical of the monomeric form of pyrene. On the contrary, it is to note that addition of alkaline metal ions does not cause any spectral change, most probably because they are not able to form a 1:2 complex.

As far as the fluorescence spectra are concerned, very strong changes were observed upon complexation (Table 1 and Fig. 2 for Ca^{2+}). Addition of Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, and Zn^{2+} leads indeed to the progressive disappearance of the emission band typical of the monomeric form of the pyrene luminophore (λ_{max}=378 nm), and the appearance of

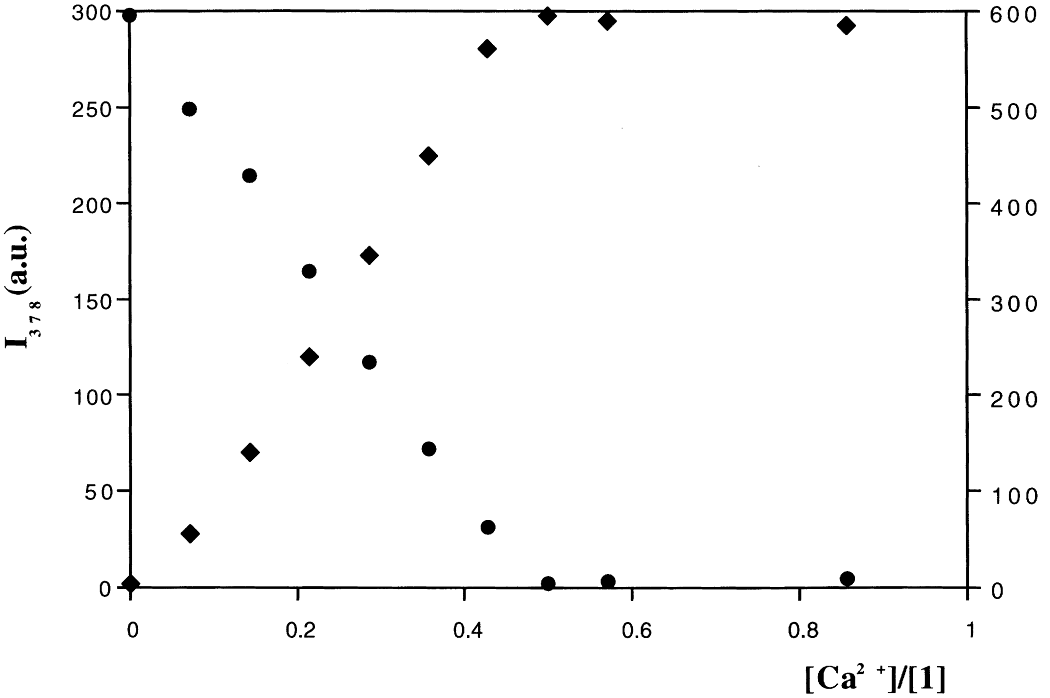

Fig. 3. Dependence of the fluorescence intensity (λ_{exc}=330 nm) at 378 nm (●) and at 476 nm (◆) of a 2.0×10^{-5} M acetonitrile solution of **1** upon addition of $Ca(ClO_4)_2$.

a new, large, and very intense band centred at 480 nm. The excitation spectra performed at $\lambda_{em}=480$ nm were almost identical to the absorption spectra of the complexes, confirming that, as discussed above, all the chromophores are already interacting in the ground state. Again, these findings are in agreement with the formation of pyrene dimers [2,8–13,22,23]. As already observed for the absorption, the fluorescence intensity and lifetime of the new band depend on the nature of the metal cation, reaching its maximum value for Ca^{2+} ($\Phi=0.28$, $\tau=62$ ns). The excited state lifetime for these complexes was not strictly monoexponential, indicating the presence of different conformers, as already observed in similar cases [9,10].

On the other hand, addition of Fe^{2+}, Fe^{3+}, and Cu^{2+} leads again to the disappearance of the fluorescence band with λ_{max} at 378 nm, without the concomitant appearance of the band typical of the dimeric form (λ_{max} at 480 nm). This result cannot be interpreted as a lack of formation of a complex, since the absorption spectra give clear evidence that 1:2 (metal:ligand) complexes do form; rather, it should be taken into account that these metal ions usually introduce easily accessible low energy levels that can give rise to energy- and electron-transfer processes [4,5], able to quench the fluorescent excited states of both the monomeric and dimeric form of pyrene. Again, no changes in the fluorescence spectra were observed upon addition of alkali metal ions.

Among all the different signals appearing after complexation of metal ions by the deprotonated form of **1**, the formation of the new fluorescence band with λ_{max} at 480 nm is the most relevant, since can allow a very sensitive detection of zinc and earth alkali metal ions without any interference from other species. In addition, the possibility to monitor at the same time the decrease of the fluorescence band typical of the monomeric form ($\lambda_{max}=378$ nm) and the intensity increase of that one typical of the dimeric form ($\lambda_{max}=480$ nm) can give additional advantages, since the ratio of the intensities at these two wavelengths can make the measure of analyte concentration independent on the absolute concentration of the chemosensor [1]. In Fig. 3 the fluorescence intensity changes observed at these two wavelengths upon addition of Ca^{2+} ions are reported. Using this methodology, a detection limit lower than 10^{-7} M (4 ppb) was obtained in acetonitrile for Ca^{2+} ions.

4. Conclusions

The deprotonated form of 1-pyrenebutyric acid (**1**), a very simple and commercially available species, has shown in acetonitrile good properties as fluorescent chemosensor. Indeed **1** forms complexes with Zn^{2+} and alkaline-earth metal ions having a 2:1 (ligand:metal) stoichiometry with very high association constants. The association process causes changes both in the absorption and, more interestingly, in the emission properties, i.e. the disappearance of the monomeric fluorescence emission of the pyrene chromophore, accompanied by the appearance of the intense fluorescence band typical of pyrene dimeric form. Notably, the ratio of the intensities of these two fluorescence bands can make the measure of analyte concentration independent on the absolute concentration of the chemosensor. This feature, together with the high affinity and sensitivity of **1** for Zn^{2+} and alkaline-earth metal ions, lead us to find an interestingly low detection limit ($<10^{-7}$ M). These findings show that **1**, a very simple species, can offer interesting opportunities and, at the same time, indicate that very efficient chemosensors can be developed using more appropriately designed receptor units, the aim of our future research.

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