

Lead and Mercury Sensing by Calixarene-Based Fluoroionophores Bearing Two or Four Dansyl Fluorophores

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Abstract: A detailed study on the photophysical and complexing properties of calixarenes bearing two and four dansyl derivatives (Calix-DANS2 and Calix-DANS4) in a CH₃CN/H₂O mixture (60:40 v/v) is reported. Calix-DANS2 shows a high selectivity towards Hg²⁺ over interfering cations (Na⁺, K⁺, Ca²⁺, Cu²⁺, Zn²⁺, Cd²⁺ and Pb²⁺) and a sensitivity in the 10⁻⁷ molL⁻¹ concentration range. The

complexation of mercury ion induces a strong fluorescence quenching due to a well-defined electron transfer process from the fluorophore to the metal center. Calix-DANS4 exhibits an extremely high affinity for Pb²⁺ with a

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high selectivity over various competing ions. The unprecedented detection limit (4 µg L⁻¹) is fully compatible with the level defined by the World Health Organisation. The affinity of Calix-DANS4 for Pb²⁺ can be rationalized by the activation of the inert pair of electrons on Pb²⁺. The number of fluorophores involved in the complexation can be determined from a careful time-resolved fluorescence characterization.

Introduction

Hg²⁺ and Pb²⁺ are the most toxic heavy metal ions causing adverse environmental and health problems. A wide variety of symptoms, including digestive, cardiac, kidney and especially neurological diseases, suggests that they affect multiple targets in vivo. The release of these detrimental ions into the environment originates from numerous natural and man-made sources, such as fossil fuel combustion and the electronic industry. Lead is also present in tap water as a result of dissolution from household plumbing systems. For these reasons, the level of heavy metal ions in drinking water is the object of strict health official norms and must not exceed 1 and 10 µg L⁻¹ for Hg²⁺ and Pb²⁺, respectively.^[1] While sophisticated analytical techniques (atomic absorption or atomic emission spectroscopies for instance) are currently used in applications relevant to environmental contamination,^[2] there is still a significant need to provide

inexpensive and real-time monitoring methods for the detection of trace amounts of mercury and lead in drinking water, in polluted areas and in industrial waste streams. With this aim, emphasis was placed in recent years on the development of optically responsive sensor materials for the detection of heavy metal ions which offer a robust and high-performance sensor platform with remote detection capability.^[3] One major challenge involves creating Hg²⁺ and Pb²⁺ sensors that function in water and are highly sensitive as well as selective against a background of possible competing cations.

In optical sensors, the use of fluorescence as detecting method offers distinct advantages in terms of sensitivity, selectivity and response time. Fluorescent molecular sensors have attracted considerable interest because of their intrinsic sensitivity and selectivity.^[4,5] They are also called fluoroionophores because they consist in a recognition moiety (ionophore) linked to a transducing moiety (fluorophore). The choice of the fluorophore is of major importance because it governs the sensitivity of the system. The fluorophore converts the recognition event into an optical signal owing to the change of its photophysical characteristics due to the perturbation by the bound cation of various photoinduced processes (electron transfer, energy transfer, charge transfer). The recognition moiety is responsible for the efficiency and the selectivity of binding. Among ionophores for metal ions, calixarene-based ligands offer numerous advantages because of the rigidity of the complexing unit and tuneable strategies to obtain molecules with appropriate substituents.^[6]

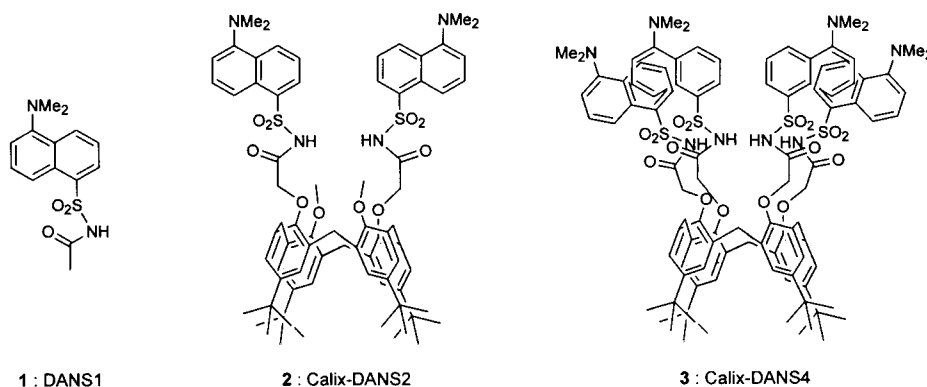
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In contrast to the large number of fluoroionophores for alkali and alkaline-earth cations that have been developed for many years,^[4,7] only few recent examples concern the detection of heavy metal ions. In particular, some fluorescent molecular sensors have been designed for Hg²⁺ sensing, but most of them display strong drawbacks in terms of sensitivity, selectivity or lack of water solubility for potential applications in environment monitoring.^[8] Nevertheless, a fluoroionophore recently described, composed of a quinoxalione^[9] linked to a crown ether, permits selective detection of Hg²⁺ in the 10⁻⁷ mol L⁻¹ concentration range. Another system consisting of a fluorescein bearing a sulfur-based chelating agent shows also a good affinity and selectivity for Hg²⁺.^[10] In contrast, to the best of our knowledge, no highly selective and sensitive system is available for the detection of Pb²⁺. A tetrapeptide bearing a dansyl fluorophore offers the possibility of ratiometric measurements upon lead complexation but with insufficient selectivity and sensitivity.^[11] Some fluoroionophores, including a crown ether as a complexing unit, were shown to respond to Pb²⁺ binding but a lack of sensitivity was observed in a partially aqueous solution or water.^[12] In this context, the use of a calixarene frame with pending fluorophores should offer a number of advantages in terms of sensitivity and selectivity. Bartsch and co-workers showed that a calix[4]arene bearing N(X)sulfonylcarboxamide exhibits a good extraction selectivity for Hg²⁺ and Pb²⁺ over alkali and transition-metal ions.^[13] A calix[4]arene (**2**) with two dansyl groups as part of the

N(X)sulfonylcarboxamide (Calix-DANS2) was used for selective Hg²⁺ extraction in acidic solutions in the millimolar concentration range,^[14] but no attention was paid to the possible detection of Hg²⁺ at low concentration.

In a preceding paper, we reported preliminary results on the synthesis and the photophysical properties of a new calixarene Calix-DANS4 (**3**) bearing four dansyl groups which has been designed for the sensitive and selective detection of Pb²⁺.^[15] The photophysical properties of Calix-DANS2 (**2**), Calix-DANS4 and the model compound DANS1 (**1**) were previously investigated in order to characterize the different photoinduced processes (charge transfer, energy transfer, proton transfer) that occur in these ligands.^[16]

In the present work, we report a thorough investigation of cation-induced photophysical changes and complexing properties of Calix-DANS2 and Calix-DANS4 in a partially aqueous solvent (CH₃CN/H₂O mixture) with the aim of mercury and lead sensing at very low level. Special attention will be paid to the selectivity against possible interfering cations (Scheme 1).



Scheme 1. Structural formulae of DANS1, Calix-DANS2 and Calix-DANS4.

Abstract in French: Cet article présente l'étude des propriétés photophysiques et complexantes de calixarènes substitués par deux ou quatre fluorophores dansylamides (Calix-DANS2 et Calix-DANS4) dans un solvant partiellement aqueux CH₃CN/H₂O (60:40 v/v). Le fluoroionophore Calix-DANS2 se montre très sélectif du mercure vis-à-vis de cations tels que Na⁺, K⁺, Ca²⁺, Cu²⁺, Zn²⁺, Cd²⁺ et Pb²⁺, avec une limite de détection de l'ordre de 10⁻⁷ mol L⁻¹. La complexation avec le mercure, accompagnée d'une complète extinction de fluorescence, a révélé un mécanisme de transfert d'électron très rapide des fluorophores vers le centre métallique. La sélectivité et la sensibilité du composé Calix-DANS4 pour le plomb sont exceptionnelles: ses propriétés ont permis d'atteindre une limite de détection très intéressante de 4 µg L⁻¹, compatible avec la norme définie par l'Organisation Mondiale de la Santé pour les eaux de boisson, et sans interférence avec les cations habituellement compétitifs. L'affinité particulière de Calix-DANS4 pour le plomb est expliquée par l'activation de la paire inerte du plomb. Le nombre de fluorophores impliqués dans la complexation est déterminé par l'étude précise des déclins de fluorescence.

Results and Discussion

Before reporting the complexation effect of cations on the photophysical properties of Calix-DANS2 and Calix-DANS4, it is worth recalling the photophysical properties of the reference compound DANS1 and the two calixarenes Calix-DANS2 and Calix-DANS4 without cation.

The absorption and fluorescence spectra of DANS1 in a mixture CH₃CN/H₂O (60:40 v/v) are pH dependent. Two acidity constants of DANS1 were determined by pH titration (pK_A 2.4 and 6.3) and three acido-basic forms of DANS1 were characterized: the acid form LH₂⁺ corresponds to the protonation of the amino group whereas the basic form L⁻ is due to the deprotonation of the sulfocarboxamide function.^[16] The absorption spectra of the LH₂⁺, LH and L⁻ forms and the emission spectra of LH and L⁻ resulting from the pH titration by excitation at 350 nm are displayed in Figure 1. As previously reported for dansyl derivatives, the absorption and emission spectra of the neutral form are the most red-shifted.^[17] The emitting state of dansylamide is a mixing of the ¹L_a and ¹L_b states of naphthalene with a charge-transfer state arising from the promotion of a

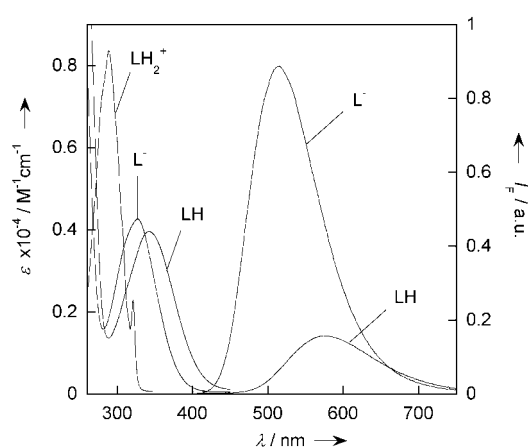


Figure 1. Absorption and fluorescence spectra ($\lambda_{\text{exc}}=350$ nm) of the different acido-basic forms of DANS1 in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (60:40 v/v). LH_2^+ is not excited at 350 nm.

lone-pair electron of the amino group into a π -antibonding orbital of the naphthalene ring. This provides considerable charge-transfer character of the absorption and fluorescence bands of dansylamide.^[18] The basic form exhibits blue-shifted absorption and emission spectra, as compared to the neutral form, which is explained by a reduction of the acceptor character of the sulfonyl group. A significant enhancement of the fluorescence quantum yield is also observed through deprotonation of the sulfocarboxamide group. The protonation of the dimethylamino group causes a strong blue shift of the absorption spectrum which is similar to naphthalene derivative because of the loss of the charge-transfer character of the transition. Steady-state and time resolved fluorescence experiments showed that upon excitation at 286 nm, LH_2^+ undergoes photoinduced deprotonation.^[16]

Moreover, we previously showed that in both Calix-DANS2 and Calix-DANS4, non-radiative energy transfer occurs from a dansyl fluorophore in the basic form to another one in the neutral form.^[16] The transfer efficiencies are 0.1 and 0.5 for Calix-DANS2 and Calix-DANS4, respectively. The fractions of dansyl fluorophores in the basic and neutral forms were determined by both steady-state and time-resolved fluorescence data analysis. At pH 4.0 in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ mixture (60:40 v/v), a 93% fraction of the Calix-DANS2 contains two neutral dansyl groups and a 7% fraction is composed of molecules with one neutral form and one basic form. In the case of Calix-DANS4 at pH 5.2, there are one basic and three neutral dansyl fluorophores.

Cation-induced photophysical changes in Calix-DANS2 and properties of its complexes

Complexation with mercury ion: With the aim of solvent extraction, Calix-DANS2 was already used in chloroform to remove mercury(II) from highly acidic solutions.^[14] This compound showed excellent selectivity for Hg^{2+} over a wide range of cations, and extraction of Hg^{2+} was accompanied by a strong quenching of the fluorescence. Unfortunately, the detection limit of this technique, determined in the millimolar concentration range, was too high for a usable appli-

cation of mercury detection under environmental conditions where the concentrations of mercury are usually in the nanomolar to micromolar range. Thus, the present paper investigates the ability of Calix-DANS2 to bind Hg^{2+} directly in a partially aqueous solvent ($\text{CH}_3\text{CN}/\text{H}_2\text{O}$ 60:40 v/v) which permits solvation of both organic and inorganic species.

Complexation study of mercury(II) with Calix-DANS2 was performed in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (60:40 v/v) at pH 4.0 in order to observe the strongest photophysical effects. Figure 2 displays the evolution of the emission spectra of Calix-DANS2 (1.6×10^{-5} M) upon complexation with Hg^{2+} . The inset in Figure 2 shows the decrease of the fluorescence intensity at 575 nm on progressive addition of Hg^{2+} . Analysis of the whole emission spectra upon mercury binding reveals the formation of a complex with a 1:1 stoichiometry and an apparent stability constant of 1.5×10^7 . A drastic quenching of the fluorescence intensity and a slight blue-shift of the emission band of 20 nm are observed upon complexation with Hg^{2+} . The quantum yield of the 1:1 complex is estimated to be only 2.5% of the fluorescence quantum yield of Calix-DANS2. The strong quenching upon mercury binding can be tentatively explained in terms of electron transfer from the excited dansyl fluorophore to the complexed mercury cation, which is an additional two-step way of non-radiative deactivation of the fluorophore.^[19] Actually, the electrochemical potentials of dansylamide were previously measured in acetonitrile: $E(\text{DANS}^{\bullet+}/\text{DANS})=0.9$ V/SCE and $E(\text{DANS}/\text{DANS}^-)=2.0$ V/SCE.^[20] The energy of the DANS1 excited state, as estimated from the average of the first absorption-band and emission-band maxima, is about 2.9 eV. It follows that the excited state of the dansyl unit is a weak oxidant $E(\text{DANS}^{\bullet+}/\text{DANS}^-)=0.9$ V/SCE and a strong reductant $E(\text{DANS}^{\bullet+}/\text{DANS}^{\bullet})=-2$ V/SCE. Thus the excited dansyl fluorophores are able to reduce Hg^{2+} ($E=0.68$ V/SCE). This reaction is therefore thermodynamically allowed and the free energy can be estimated around $\Delta G_{\text{ET}} \sim 2.7$ eV. A following back electron transfer can then occur between the two separated ions of such a generated intermediate species in order to retrieve the ground state mercury(II) complex. The free energy of the back electron transfer is also positive ($\Delta G_{\text{BET}} \geq 0.2$ eV).

The nature of the quenching mechanism was confirmed through fluorometric investigations carried out on a frozen solution at liquid nitrogen temperature (100 K). The emission spectrum of Calix-DANS2 was first measured in a EtOH/MeOH mixture (9:1 v/v). At room temperature, as observed in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ mixture, the complexation of Hg^{2+} induces a large quenching of Calix-DANS2 emission ($I_{\text{F}}/I_0=23\%$). In contrast, in a glass at 100 K, the fluorescence emission of $\text{Hg}^{2+} \subset \text{Calix-DANS2}$ complex is partially restored: the ratio I_{F}/I_0 is equal to 82% (where I_0 corresponds to Calix-DANS2 fluorescence under the same conditions). Such a temperature effect is characteristic of quenching mechanism through an electron-transfer process.^[21] In a frozen matrix, immobilization of the solvent molecules prevents solvent reorganization surrounding the generated charge separated molecules and raises the energy of the ion-pair species. This process explains the revival of fluores-

cence intensity of the complex due to the absence of electron transfer in rigid matrix.

Time-resolved fluorescence measurements were performed upon addition of various quantities of mercury(II) ions in order to obtain further information on the quenching

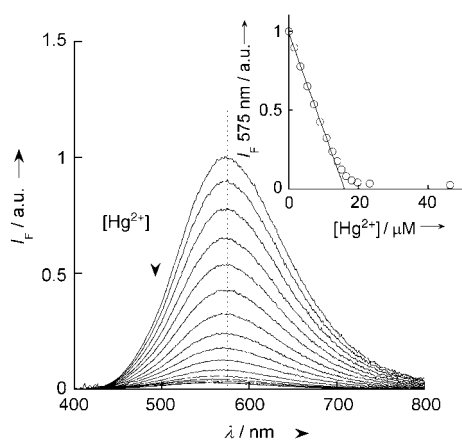


Figure 2. Corrected emission spectra of Calix-DANS2 (1.6×10^{-5} M) in the presence of increasing concentration of Hg^{2+} in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (60:40 v/v) at pH 4.0. $\lambda_{\text{exc}} = 350$ nm. Inset: Calibration curve as a function of mercury concentration.

process upon mercury complexation. The fluorescence decay of the free ligand Calix-DANS2 at pH 4.0 is biexponential. The shortest lifetime (3.7 ns) is attributed to the neutral form LH while the longest decay time (14.4 ns) corresponds to the basic form L^- of the fluorophore.^[16] Upon complexation with Hg^{2+} , the preexponential factors of the decay time of 3.7 and 14.4 ns gradually decrease to attain a value close to zero when full complexation is achieved (Figure 3a). In parallel, a very fast decay time appears and its preexponential factor reaches approximately one after addition of two equivalents of Hg^{2+} . As shown by the fit displayed in Figure 3a, these evolutions are perfectly compatible with the value of the apparent stability constant previously determined by absorption and steady-state fluorescence. The complete disappearance of the lifetimes corresponding to the basic and the neutral forms of the dansyl units implies that the two fluorophores of Calix-DANS2 undergo non-radiative deactivation by electron transfer. Consequently, the two fluorophores are close enough to the mercury cation to allow this process and then, in all probability, they are involved in the coordination of the central mercury cation (Scheme 2).

In order to improve the accuracy of the fast time-constant, the fluorescence decay of the complex ($[\text{Hg}^{2+}]/[\text{Calix-DANS2}] = 2$) was recorded in a short timescale (Figure 3b). The average short decay time was estimated to be 50 ± 15 ps. Such a value allows the estimation of the quenching rate k_Q by using Equation (1):

$$k_Q = \frac{\tau_0/\tau - 1}{\tau_0} \quad (1)$$

$$k_Q = \frac{\Phi_0/\Phi - 1}{\tau_0} \quad (2)$$

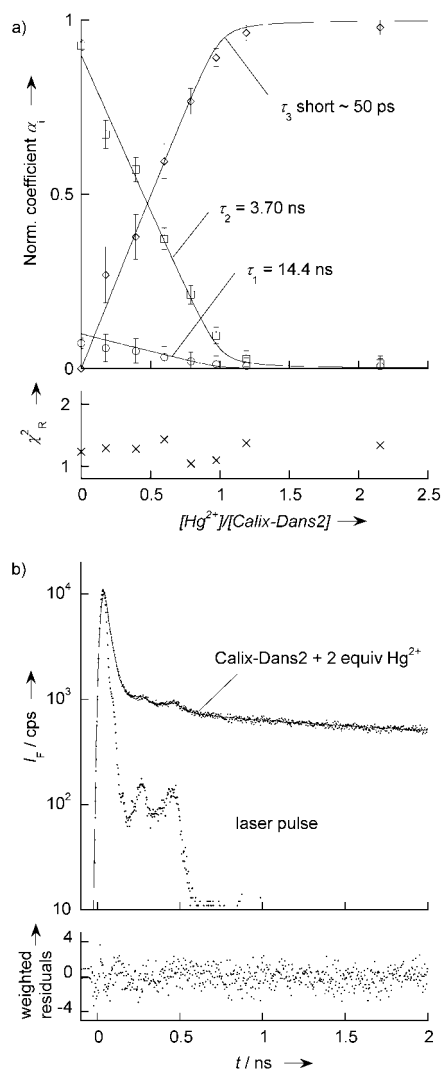
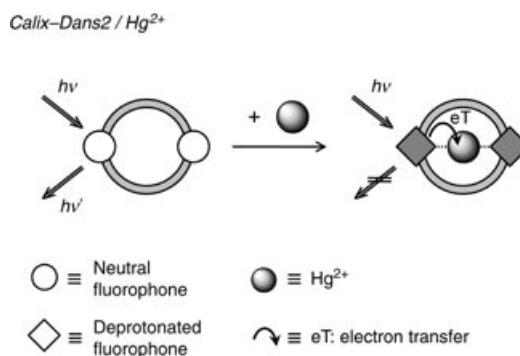


Figure 3. a) Global analysis of fluorescence decays of Calix-DANS2 upon increasing concentration of Hg^{2+} in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (60:40 v/v) at pH 4.0. $\lambda_{\text{exc}} = 330$ nm, $\lambda_{\text{em}} > 375$ nm, channel width = 44 ps. The analysis was performed with a sum of three exponentials. The solid lines represent the fits from the apparent stability constant; b) fluorescence decay of Calix-DANS2 with two equivalents of Hg^{2+} . Channel width = 3.2 ps.



Scheme 2. Schematic representation of the $\text{Hg}^{2+} \subset \text{Calix-DANS2}$ complex and involved photoinduced electron transfer.

k_Q was found to be equal to $2.2 \pm 0.7 \times 10^{10} \text{ s}^{-1}$. This result is compared to the one calculated from the steady-state fluorescence measurements [Eq. (2): $k_Q = 1.4 \pm 0.3 \times 10^{10} \text{ s}^{-1}$].

The agreement between these two values corroborates the static fluorescence quenching by fast electron transfer process of the Hg^{2+} –Calix-DANS2 complex. It should be emphasized that fluorescence quenching by heavy atom effect cannot be completely excluded. But the quenching of dansyl-amide fluorophores, observed in the case of the mercury complex, was lacking for other heavy cations such as cadmium and even lead (see below). This observation leads us to believe that quenching by heavy atom effect is negligible in the present case.

The conformational structure of the Hg^{2+} –Calix-DANS2 complex was investigated through ^1H NMR spectroscopy in a $\text{CD}_3\text{CN}/\text{D}_2\text{O}$ (60:40 *v/v*) solvent at pD 4.0. The typical spectra in the 5–3 ppm region of the ligand and the complex, obtained with a large excess of mercury, are shown in Figure 4. All the signals corresponding to the Ar-CH₂-Ar,

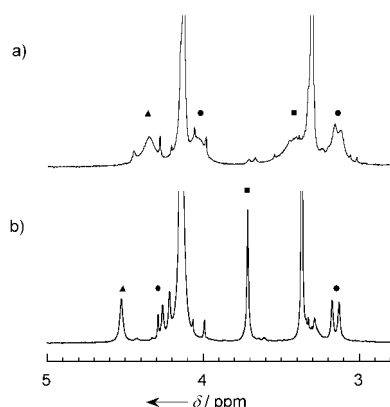


Figure 4. Partial ^1H NMR spectra of a) the free ligand Calix-DANS2 (5×10^{-3} M) and b) the 1:1 Hg^{2+} –Calix-DANS2 complex, in a $\text{CD}_3\text{CN}/\text{D}_2\text{O}$ (60:40 *v/v*) solvent at pD 4.0. Circles, squares and triangles denote the signals of Ar-CH₂-Ar, OCH₃ and OCH₂ functions, respectively. The two peaks at $\delta = 4.15$ and 3.35 ppm are characteristic of the protons from the solvent mixture.

OCH₃ and OCH₂ functions are strongly broadened for the ligand (Figure 4a) and indicate that there is a fast interconversion between the cone, partial cone and 1,3-alternate conformations of the calixarene.^[22] These signals are conversely thin and well-defined for the 1:1 complex and shifted to lower magnetic field (Figure 4b). Especially, the doublet/doublet signal splitting attributed to the methylene protons of the calixarene clearly confirms the presence of a well-defined cone conformation. This demonstrates that the Hg^{2+} cation fixes the calixarene into the cone conformation via coordination with the oxygen atoms of the methoxy functions, and also excludes any possibility of π -interactions between the metal centre and the calixarene subunit.

Practical determination of mercury ion with Calix-DANS2:

The calibration curve shown in the inset of Figure 2 represents the fluorescence intensity at 575 nm as a function of Hg^{2+} concentration. From the calculation of three times the standard deviation of the background noise, the detection limit was found to be $0.30 \mu\text{M}$ ($60 \mu\text{g L}^{-1}$). Under experimental conditions, the response is linear from 0 to $12 \mu\text{M}$. Thus,

accurate measurements of Hg^{2+} concentration are possible under almost two orders of magnitude. Such a sensitivity of Calix-DANS2 is greatly improved by a factor 10^4 with respect to the previous Bartsch's study on solvent extraction of mercury(II) in highly acidic solutions with the same molecule.^[14]

The binding ability of Calix-DANS2 over a range of other cations including Na^+ , K^+ , Ca^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} and Pb^{2+} was evaluated. Calix-DANS2 exhibited noticeable binding properties with only two of them, Na^+ and Pb^{2+} . Figure 5 displays the absorption and fluorescence spectra of Calix-DANS2 and its complexes, determined by titration experiments and interpolation of the whole spectrum. While a significant increase of the molar absorption coefficient was only noted for the mercury(II) complex, a blue-shift of 12 nm was observed for the absorption spectra of both sodium and lead(II) complexes (Figure 5a). Concerning the fluorescence spectra, blue-shifts of 20, 55 and 50 nm were observed for Hg^{2+} , Na^+ and Pb^{2+} , respectively. The spectra of the deprotonated dansyl fluorophore are precisely shifted to shorter wavelengths due to destabilization of the charge-transfer excited state as shown in Figure 1. By comparison, these blue-shifts indicate that metal binding causes deprotonation of the sulfocarboxamide functions of the fluorophores. On the other hand, complexations with sodium and lead result in a 2.1- and 2.9-fold enhancement of the fluorescence quantum yield, respectively. This observation confirms the concomitant deprotonation of the fluorophores upon complexation. Nevertheless, the quantum yield of the sodium and lead(II) complexes are lower than that of the basic form of the dansyl fluorophores. Since a photoinduced electron transfer process is highly improbable, especially in the case of the Na^+ –Calix-DANS2 complex, this can be tentatively explained by a reduction of the charge density of the anionic nitrogen atoms due to the interaction with the metal cations. Concerning the Hg^{2+} complex, the lack of absorption shift and the weak fluorescence blue-shift reveal a much more covalent Hg–N bond, which is in agreement with the common behaviour of mercury cations.^[23,24]

The apparent stability constants of the 1:1 complexes are reported in Table 1. The selectivity towards Hg^{2+} , expressed as the ratio of the apparent stability constants, was found to be satisfactorily higher than ~ 500 with respect to sodium and lead. This behaviour is coherent to the fact that a linear coordination with two nitrogen donor atoms is generally favoured in the case of mercury complexes. The effective selectivity of the ligand Calix-DANS2 for Hg^{2+} over Na^+ and Pb^{2+} is in fact a combination of both the photophysical properties (Figure 5) and the thermodynamic data (Table 1) of the complexes. Such a selectivity was evaluated experimentally via the competition-based fluorescence effect profiles for all the tested cations at concentration level of the detection limit. The resulting diagram is shown in Figure 6. These cations induced no significant effect on the fluorescence of Calix-DANS2 and did not inhibit the fluorescence response of Calix-DANS2 to mercury(II). The Hg^{2+} response of Calix-DANS2 was then unaffected by a background of environmentally relevant metals. Pb^{2+} was the only cation which slightly interfered with mercury by reducing its ability

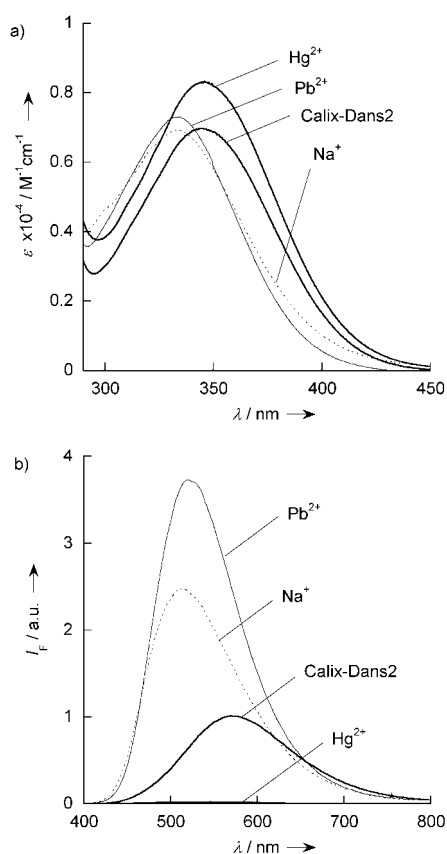


Figure 5. a) Absorption and b) emission spectra ($\lambda_{\text{exc}}=350$ nm) of Calix-DANS2 and its complexes in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (60:40 v/v) at pH 4.0.

to quench the fluorescence of Calix-DANS2. Finally, we highlighted the performance of Calix-DANS2 which can be used as a sensitive and selective fluoroionophore for mercury sensing.

Table 1. Stability constants of Calix-DANS2 complexes in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (60:40 v/v) at pH 4.0, determined by both absorption and fluorescence spectroscopy.

	$\log K_{\text{app}}$ (ML)	Selectivity $K(\text{Hg}^{2+})/K(\text{M}^{n+})$
Hg^{2+}	7.18 ± 0.28	–
Na^+	4.49 ± 0.05	490
Pb^{2+}	4.02 ± 0.10	1450

Cation-induced photophysical changes in Calix-DANS4 and properties of its complexes

Complexation with various cations: With the aim of generating the largest photophysical changes upon cation binding, the complexation study of Calix-DANS4 was performed at pH 5.2 using a non-interfering lutidine buffer. In contrast to Calix-DANS2, the ligand Calix-DANS4 exhibited complexing properties over a wide range of metal cations. Table 2 reports the apparent stability constants of the complexes formed with Na^+ , K^+ , Ca^{2+} , Cu^{2+} , Cd^{2+} , Hg^{2+} and Pb^{2+} , calculated from titration experiments. No effect was noticed upon addition of a very large excess of zinc(II). Except for

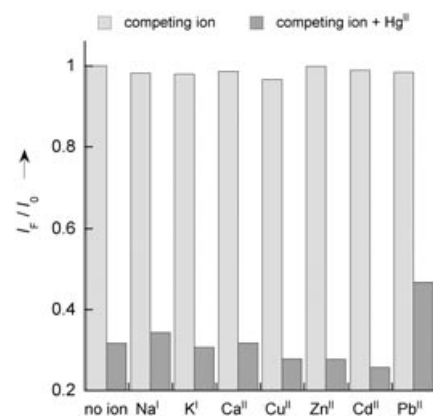


Figure 6. Fluorescence response I_f/I_0 of Calix-DANS2 (0.3 μM) in the presence of various metal ions (0.3 μM), in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (60:40 v/v) at pH 4.0. The light bars represent the response of Calix-DANS2 in the presence of the cation of interest. The dark bars represent the response upon an addition of 0.3 μM of Hg^{2+} to a solution of Calix-DANS2 and the cation of interest. I_0 corresponds to the emission of Calix-DANS2 without cation. $\lambda_{\text{exc}}=350$ nm and $\lambda_{\text{em}}=575$ nm.

Pb^{2+} , all the studied cations formed 1:1 complexes with the ligand. The behaviour of Pb^{2+} is interestingly specific, as mentioned in our previous paper.^[15] In this particular case, the evolution of the fluorescence titration revealed that a 2:3 complex and a 1:1 complex are successively formed upon Pb^{2+} addition.

Table 2. Stability constants of Calix-DANS4 complexes in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (60:40 v/v) in lutidine buffer, pH 5.2.

	$\log K_{\text{app}}$ (ML)	Selectivity ^[a] $K(\text{Pb}^{2+})/K(\text{M}^{n+})$
Pb^{2+}	$33.5 \pm 1.5/10.0 \pm 0.5^{\text{[b,c]}}$	–
Hg^{2+}	$5.89 \pm 0.07^{\text{[d]}}$	1.3×10^4
Cd^{2+}	$7.30 \pm 0.08^{\text{[e]}}$	500
Cu^{2+}	$4.80 \pm 0.09^{\text{[b]}}$	1.6×10^5
Zn^{2+}	–	–
Ca^{2+}	$7.23 \pm 0.28^{\text{[b]}}$	590
K^+	$3.69 \pm 0.05^{\text{[e]}}$	2.1×10^6
Na^+	$4.86 \pm 0.04^{\text{[e]}}$	1.4×10^5

[a] Selectivity calculated with $\log K_{\text{app}}(\text{Pb}^{2+})=10.0 \pm 0.5$. [b] Determined by both absorption and fluorescence spectroscopies. [c] $\log K_{\text{app}}$ values for M_2L_3 and ML complexes. [d] Determined by fluorescence spectroscopy. [e] Determined by absorption spectroscopy.

Figure 7 displays the absorption and emission spectra of the 1:1 complexes formed with Calix-DANS4. On one hand, Hg^{2+} and Cu^{2+} complexes show low fluorescence quantum yields. According to the oxidative character of mercury(II) and copper(II), both of these complexes might be prone to photoinduced electron transfer process. In the case of copper(II) only, energy transfer from the dansyl fluorophores to the bound Cu^{2+} may also be invoked to explain this fluorescence quenching.^[25] In other respects, the fluorescence quenching in the Hg^{2+} -Calix-DANS4 complex is not complete ($I_f/I_0=13\%$) and reveals a less efficient non-radiative deactivation by electron transfer than in the case of the Hg^{2+} -Calix-DANS2 complex. Moreover, only slight blue-shifts of the emission spectra were noticed for Hg^{2+} and

Cu^{2+} complexes, which can be explained by the covalent character of the $\text{M}^{n+}-\text{N}$ bond. On the other hand, a noticeable blue-shift extent of the spectra and an increase of the fluorescence quantum yield can be observed for Na^+ , K^+ , Ca^{2+} , Cd^{2+} and Pb^{2+} complexes. As explained in the case of Calix-DANS2 complexes (see above), this effect is rationalized by the deprotonation of the sulfoxycarboxamide functions upon cation binding. As a crucial parameter, the number of fluorophores involved in the complexation is actually unknown for these various cations. However, it is worth noting that the complexes with the largest photophysical effects (Ca^{2+} , Cd^{2+} and Pb^{2+}) correspond to the highest stability constants. This correlation led us to conclude that the strength of the $\text{M}^{n+}-\text{N}$ bond is also of major importance in this system. Indeed, the strongest the ionic bond between the cation and the deprotonated fluorophore, the highest the stability constant and the most pronounced the blue-shift of the absorption and the fluorescence spectra.

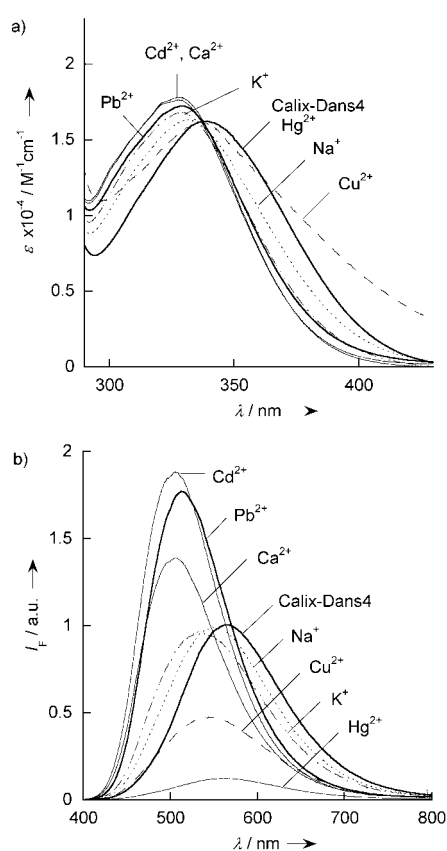


Figure 7. a) Absorption and b) emission spectra ($\lambda_{\text{exc}} = 350$ nm) of Calix-DANS4 and its complexes in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (60:40 v/v) in lutidine buffer, pH 5.2.

The stability constants of the lead(II) complexes reported in Table 2 are very high compared with the other cations. Of particular interest, the value of the 2:3 complex is exceptional ($\log K_{2:3} = 33.5 \pm 1.5$). Finally, Calix-DANS4 is perfectly well-suited for Pb^{2+} sensing.^[15] Since the selectivity towards Pb^{2+} , defined as the ratio of the stability constants $K(\text{Pb}^{2+})/K(\text{M}^{n+})$, is impossible to evaluate rigorously for

complexes of different stoichiometry, we only calculated the selectivity for 1:1 complexes, while bearing in mind that such a selectivity is largely under-estimated due to the highly stable 2:3 lead(II) complex. But whatever the cation, the selectivity is anyway higher than 500, even in the case of Ca^{2+} and Cd^{2+} which possess large stability constants. Such an outstanding selectivity of Calix-DANS4 towards Pb^{2+} over all the cations tested justifies further detailed investigations in order to clarify the properties of its lead(II) complexes.

Properties of lead complexes: In contrast to Calix-DANS2, the extremely high stability of the Pb^{2+} complexes with Calix-DANS4 can be interpreted in terms of the higher affinity of lead(II) for nitrogen donor groups of the sulfocarboxamide functions. In fact, it is well-known in coordination chemistry that ligands with a large number of nitrogen donors induce a dramatic change in complexing properties of Pb^{2+} : the inert pair of electrons on Pb^{2+} may become stereochemically active, leading to a decrease in the Pb^{2+} ionic radius, a shortening of the $\text{Pb}-\text{N}$ bond length and a much more covalent bonding.^[26] Consequently, if the valence shell of Pb^{2+} contains at least three nitrogen donor atoms in the Calix-DANS4 complexes, a lone pair stereochemical activity would explain such high stability constants.

In order to obtain further information on the coordinative binding of Pb^{2+} in the 1:1 and 2:3 complexes, fluorescence decays of Calix-DANS4 with increasing Pb^{2+} concentration were recorded. As reported in Table 3, the fluorescence

Table 3. Fluorescent decay components α_i and τ_i of Calix-DANS4 and its 1:1 and 2:3 complexes with Pb^{2+} .

	α_1	τ_1/ns	α_2	τ_2/ns
free ligand (L) ^[a]	0.45 ± 0.03	8.09 ± 0.18	0.55 ± 0.03	4.60 ± 0.14
2:3 complex (M_2L_3) ^[b]	0.65 ± 0.01	8.90 ± 0.12	0.35 ± 0.01	4.60 ± 0.11
1:1 complex (ML) ^[b]	0.72 ± 0.02	9.20 ± 0.15	0.28 ± 0.02	4.60 ± 0.12

[a] Measured ($\chi^2_{\text{R}} = 1.11$); [b] obtained by fitting the evolutions displayed in Figure 8 using the Equations (3), (4) and (5).

decay of the free ligand at pH 5.2 was found to be biexponential. The shortest lifetime (4.60 ns) is attributed to the neutral form of the fluorophore and corresponds precisely to the lifetime of the neutral form of the model compound DANS1. The longest decay time (8.09 ns) is due to a significant proportion of fluorophores in the basic form in Calix-DANS4. Taking into account the energy-transfer process between basic and neutral fluorophores, this proportion of deprotonated fluorophores was previously estimated from the preexponential coefficients and found to be ~25%, the transfer efficiency being 0.51.^[16] This result means that Calix-DANS4 at pH 5.2 bears three neutral dansyl groups and one deprotonated fluorophore. When Pb^{2+} was progressively added to the solution of Calix-DANS4, the fluorescence decays remained biexponential. Figure 8 shows that, while the shortest lifetime was unaffected ($\tau_2^{\#} = 4.6$ ns), a lengthening of the longest decay time ($\tau_1^{\#}$) was observed, with a simultaneous increase of its preexponential coefficient. This long time constant, between 8.09 and 9.20 ns, is

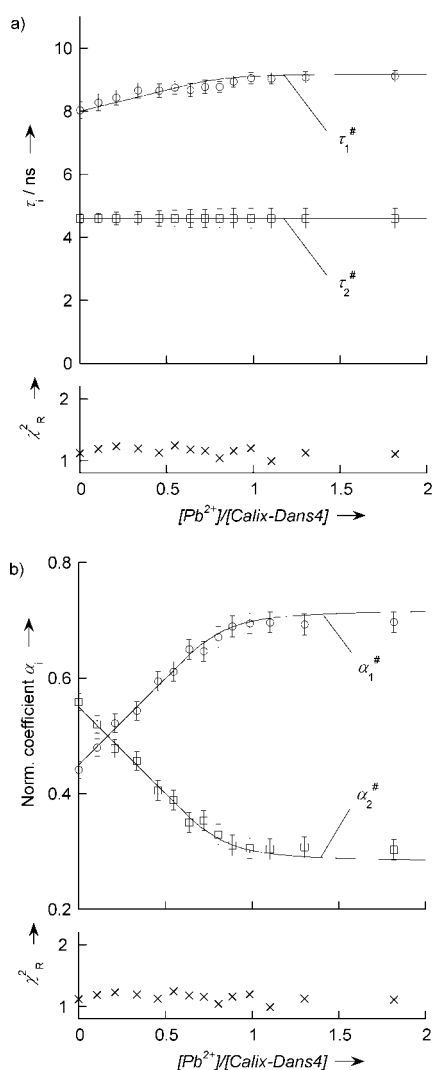


Figure 8. Global analysis of fluorescence decays of Calix-DANS4 upon increasing concentration of Pb^{2+} in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (60:40 v/v) in lutidine buffer, pH 5.2. $\lambda_{\text{exc}} = 330$ nm, $\lambda_{\text{em}} > 375$ nm, channel width = 44 ps. The analysis was performed with a sum of two discrete exponentials: a) evolution of the time constants and b) of the preexponential coefficients as a function of Pb^{2+} concentration. The solid lines represent the evolution fitting with the parameters showed in Table 3.

attributed to that of deprotonated dansyl groups. In fact, it is an average time constant corresponding to both deprotonated/uncomplexed fluorophores of the free ligands and deprotonated fluorophores bound to Pb^{2+} cation of the lead(II) complexes. Thanks to the apparent stability constant determined by spectrophotometry and steady-state fluorometry, the evolutions of the decay time $\tau_1^{\#}$ and preexponential coefficients $\alpha_1^{\#}$ and $\alpha_2^{\#}$ were fitted using the following relations:

$$\tau_1^{\#} = x_L \tau_1(\text{L}) + x_{\text{ML}} \tau_1(\text{ML}) + x_{\text{M}_2\text{L}_3} \tau_1(\text{M}_2\text{L}_3) \quad (3)$$

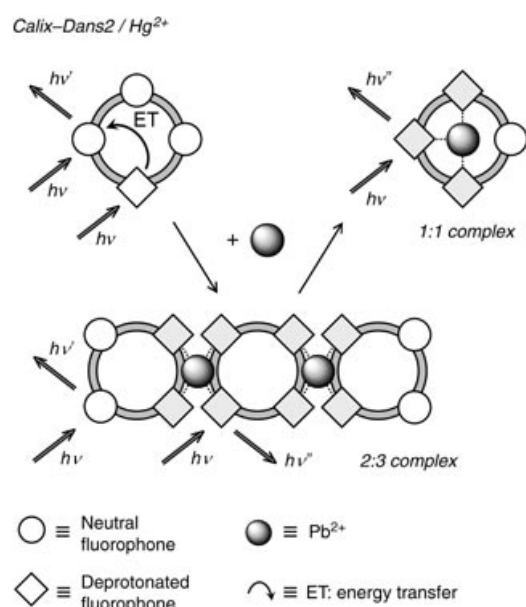
$$\alpha_1^{\#} = x_L \alpha_1(\text{L}) + x_{\text{ML}} \alpha_1(\text{ML}) + x_{\text{M}_2\text{L}_3} \alpha_1(\text{M}_2\text{L}_3) \quad (4)$$

$$\alpha_2^{\#} = x_L \alpha_2(\text{L}) + x_{\text{ML}} \alpha_2(\text{ML}) + x_{\text{M}_2\text{L}_3} \alpha_2(\text{M}_2\text{L}_3) \quad (5)$$

where $\tau_1^{\#}$ is the longest measured time constant, and $\alpha_1^{\#}$ and $\alpha_2^{\#}$ are the preexponential coefficients for a given $[\text{Pb}^{2+}]/[\text{Calix-DANS4}]$ ratio. $\tau_1(\text{L})$, $\alpha_1(\text{L})$, $\tau_1(\text{ML})$, $\alpha_1(\text{ML})$, $\tau_1(\text{M}_2\text{L}_3)$, and $\alpha_1(\text{M}_2\text{L}_3)$ are the time constants and preexponential coefficients of each species L, ML and M_2L_3 . x_L , x_{ML} and $x_{\text{M}_2\text{L}_3}$ are the proportions of the different species related to the given concentration of Pb^{2+} and the stability constants K_{app} showed in Table 2.

Satisfactory fits were obtained and permitted the extraction from this decay-curves network of the fluorescent decay components of the two lead(II) complexes reported in Table 3. By neglecting the energy-transfer process from the complexed basic form to the neutral form (see below), the fraction of neutral fluorophores within the complexes is directly given by the preexponential coefficient of the short decay time (4.60 ns). This proportion is $\sim 1/3$ (35%) for the 2:3 complex and $\sim 1/4$ (28%) for the 1:1 complex. As the 2:3 complex, composed of two Pb^{2+} cations complexed by three ligands, bears a total fluorophore number of twelve, this preexponential coefficient implies that eight dansyl groups are deprotonated and involved in the complexation, while four other neutral dansyl fluorophores are not bound to the Pb^{2+} cation. In the most probable case, each Pb^{2+} cation is thus complexed by four dansyl groups from different calixarene units, forming a large supramolecular entity where the three calixarene moieties are strongly linked together by two Pb^{2+} cations. Likewise, the 1:1 complex contains one unbound dansyl groups and Pb^{2+} is complexed by only three deprotonated fluorophores. Such structures of lead(II) Calix-DANS4 complexes are illustrated in Scheme 3. As reported in Table 3, the lifetime of the complexed fluorophores is otherwise shorter when the coordination number of Pb^{2+} is higher. This lifetime goes from 8.90 ns in the 2:3 complex (each cation is bound to four dansyl groups) to 9.20 ns in the 1:1 complex (each cation is complexed by three fluorophores). This trend is confirmed by the lifetime of the $\text{Pb}^{2+} \subset \text{Calix-DANS2}$ complex (10.7 ns), where the two fluorophores are involved in the complexation. As the lifetime of the basic form of the model compound DANS1 was measured (16.5 ns),^[16] we can easily conclude that the higher the coordination number of Pb^{2+} , the more covalent the Pb–N bond character due to lone-pair stereochemical activity of lead(II), and the larger the difference between the lifetime of bound fluorophores and uncomplexed deprotonated ones.

Since the fluorescence of the complexed dansyl groups is blue-shifted with respect to the fluorescence of the neutral ones, the question of non-radiative energy transfer between the former and the latter within lead(II) complexes must now be discussed. This possibility is of current interest, but no definitive answer can be provided. Actually, the impact of such an energy transfer is usually evaluated through the estimation of the Förster radius using a model fluorophore. In our case, DANS1 showed no affinity to Pb^{2+} and then a model compound is missing. Only a rough estimate of this value was obtained owing to the photophysical properties of the $\text{Pb}^{2+} \subset \text{Calix-DANS2}$ complex, in which the two fluorophores are involved in the complexation. This method showed us that the transfer efficiency should not exceed



Scheme 3. Schematic representation of the structures of Pb²⁺⊂Calix-DANS4 complexes and the involved photophysical processes.

~12%, and certainly even much lower. Finally, although this process cannot be completely excluded, its impact is probably negligible.

Practical determination of lead ion with Calix-DANS4:

The observed blue-shift of the fluorescence spectra of Calix-DANS4 upon Pb²⁺ addition (Figure 9) allows a ratiometric measurement. The choice of the emission ratio I_{515}/I_{565} as the response of the sensing system has considerable interest (Figure 9, inset). Indeed, the ratio of these fluorescence intensities at two different wavelengths is independent of the total concentration of Calix-DANS4, the fluctuations of the source intensity and the sensitivity of the instrument.^[4] A very low detection limit is in this manner reachable. The detection limit, calculated as three times the standard deviation of the background noise from the calibration curve, was found to be 0.02 μM (4 μg L⁻¹), and a linear measurement is possible until 440 μM with high accuracy. Thus, the range of accessible lead(II) concentration is very large, the detection limit is unprecedented and fully compatible with the level defined by the World Health Organisation.^[1]

Moreover, such a ratiometric measurement permits a minimization of the cation-induced response upon Na⁺, K⁺, Cu²⁺ and Hg²⁺ complexation. The slight blue-shifts and the absence of increase of the fluorescence quantum yield associated to the complexes lead actually to weak modifications of the ratio I_{515}/I_{565} (Figure 7b). It should be also recalled that the thermodynamic selectivity of Calix-DANS4 towards Pb²⁺, reported in Table 2, is higher than ~500 against all the other possible interfering cations. Nevertheless, with the aim of evaluating the effective selectivity of Calix-DANS4 combining the thermodynamic and the photophysical properties of the complexes, the experimental diagram of fluorescence responses of Calix-DANS4 to various competing cations at concentration level of the detection limit is showed in Figure 10. Through a first observation, the effects induced

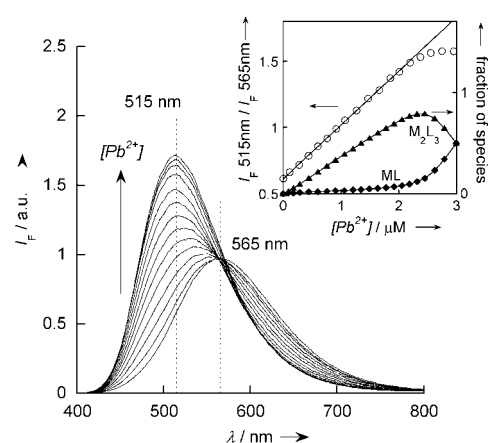


Figure 9. Corrected emission spectra of Calix-DANS4 (3.6×10^{-6} M) in the presence of increasing concentration of Pb²⁺ in CH₃CN/H₂O (60:40 v/v) in lutidine buffer, pH 5.2. $\lambda_{\text{exc}} = 350$ nm. Inset: Ratiometric calibration curve (○), and fraction of the species M₂L₃ (▲) and ML (◆) as a function of lead concentration.

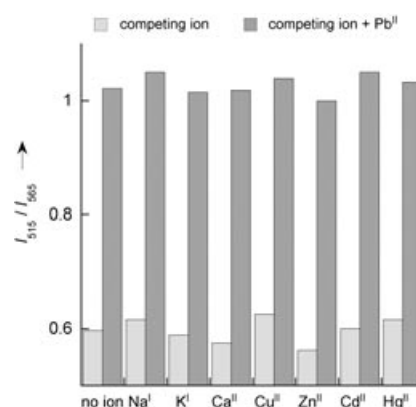


Figure 10. Ratiometric response I_{515}/I_{565} of Calix-DANS4 (0.02 μM) in the presence of selected metal ions (0.02 μM), in CH₃CN/H₂O (60:40 v/v) at pH 4.0. The light bars represent the response of Calix-DANS4 in the presence of the cation of interest. The dark bars represent the response upon an addition of 0.02 μM of Pb²⁺ to a solution of Calix-DANS4 and the cation of interest. $\lambda_{\text{exc}} = 350$ nm.

by an addition of one equivalent of competing metal ions were negligible. Secondly, the full response was obtained with an addition of one more equivalent of Pb²⁺, which indicates that no significant interference results from the presence of these cations. Calix-DANS4 finally showed, in addition to a high-performance sensitivity in the nanomolar concentration range, an efficient selectivity towards Pb²⁺ against a wide background of environmentally relevant metal ions. Quantitative Pb²⁺ sensing is indeed possible with Calix-DANS4, at a very low level and in a partially aqueous solvent.

Conclusion

The fluoroionophores Calix-DANS2 and Calix-DANS4, described in the present paper, showed remarkable sensitivity and selectivity to mercury(II) and lead(II) metal ions, respec-

tively, in acetonitrile/water. The fluorescence of Calix-DANS2 is completely quenched upon Hg^{2+} addition through an ultra-fast photoinduced electron transfer (50 ± 15 ps) from the excited state of the dansyl units to the mercury(II) cation with a detection limit of $0.3 \mu\text{M}$. The selectivity of Calix-DANS2 towards Hg^{2+} was confirmed in a partially aqueous solvent, which allows quantitative detection of Hg^{2+} in polluted areas where its concentration is within $1 \mu\text{M}$. Concerning Calix-DANS4, two distinct 2:3 and 1:1 complexes with Pb^{2+} are successively formed with very high stability constants. Careful examination of the decay times and the preexponential coefficients obtained by time-resolved fluorescence experiments provided the coordinative properties of Pb^{2+} in these complexes. We demonstrated that only three fluorophores in the 1:1 complex are involved in the complexation. The supramolecular 2:3 complex is composed of three Calix-DANS4 ligands which complex two Pb^{2+} cations, where eight fluorophores bind the metal ions. The blue-shift of the fluorescence spectrum induced by Pb^{2+} binding permits a ratiometric measurement to reach an outstandingly low detection limit in the nanomolar range (20 nm), which is, to the best of our knowledge unprecedented and entirely compatible with the level defined by the World Health Organisation in drinking water.^[1]

Experimental Section

Solvents and salts: Acetonitrile from Aldrich (spectrometric grade), absolute ethanol from SDS (spectrometric grade) and millipore filtered water (conductivity $< 6 \times 10^{-8} \Omega^{-1} \text{cm}^{-1}$ at 20°C) were employed as solvent for absorption and fluorescence measurements. Sodium thiocyanate, potassium thiocyanate, calcium perchlorate, copper(II) perchlorate, zinc perchlorate, cadmium perchlorate, mercury(II) perchlorate, mercury(II) nitrate and lead(II) thiocyanate, from Aldrich or Alfa Aesar, were of the highest quality available and vacuum dried over P_2O_5 prior to use. The solution at pH 4.0 was prepared with perchloric acid (99.99%) in water. 2,6-Lutidine (99%) was distilled prior to use as a pH 5.2 buffer.

NMR experiment: ^1H NMR spectra were recorded at room temperature on a Bruker AC300 spectrometer using tetramethylsilane as reference. Deuterated acetonitrile and water, from Aldrich, were used as solvent. Deuterated perchloric acid was employed to set the acidity of the solution.

Spectroscopic measurements: UV/Vis absorption spectra were recorded on a Varian Cary5E spectrophotometer. Corrected emission spectra were obtained on a Jobin-Yvon Spex Fluorolog 1681 spectrofluorometer. The fluorescence quantum yields were determined by using quinine sulfate dihydrate in sulphuric acid (0.5 n) as a standard ($\Phi_f = 0.546$).^[27] The complexation constants were determined by global analysis of the evolution of all absorption and/or emission spectra by using the Specfit Global Analysis System V3.0 for 32-bit Windows system. This software uses singular value decomposition and non-linear regression modelling by the Levenberg–Marquardt method.^[28]

Fluorescence intensity decays were obtained by the single-photon timing method with picosecond laser excitation using a Spectra-Physics set-up composed of a Titanium Sapphire Tsunami laser pumped by an argon ion laser, a pulse detector, and doubling (LBO) and tripling (BBO) crystals. Light pulses were selected by optoacoustic crystals at a repetition rate of 4 MHz. Fluorescence photons were detected through a long-pass filter (375 nm) by means of a Hamamatsu MCP R3809U photomultiplier, connected to a constant-fraction discriminator. The time-to-amplitude converter was purchased from Tennelec. Data were analysed by a non-linear least-squares method using Globals software (Globals Unlimited, University of Illinois at Urbana-Champaign, Laboratory of Fluorescence Dynamics).

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