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ORIGINAL ARTICLE

Study by fluorescence of calix[4]arenes bearing heterocycles with divalent metals: highly selective detection of Pb^{2+}

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Abstract The present work describes a study of complexation efficiency and selectivity of calix[4]arenes bearing benzoimidazolyl, benzothiazolyl, and benzoxazolyl heterocycles (**5–7**) towards several selected metal ions using fluorescence and UV–Vis spectroscopy. The binding ability of calixarene derivatives **5–7** toward selected divalent metal ions such as Ca, Cd, Pb, Ni, Mg, Mn, Co, Fe and Zn has been investigated by fluorescence spectroscopic techniques. Fluorescent chemosensor ability of three calixarene derivatives was highly selective for Pb (II) in contrast with other divalent metal studied. The highest association constant corresponds to benzoxazolyl calixarene derivative **7,** with a K_a of $(1.37 \pm 0.06) \times 10^4$ mol^{-1} L and detection limit for lead of 1.14 ± 0.05 mg L^{-1} in methanol being acceptable for the recognition of this metal at micromolar concentrations.

Keywords Fluorescence · Calix[4]arene · Metal ions · Complexation · Heterocycles · Pb(II)

Introduction

Noteworthy attention has been put on the design of supramolecules which have the ability of selectively recognizing cations through the naked eye or electrochemical, and optical responses. Optical fluorescence-based sensors seem especially attractive because they provide a more efficient and less expensive alternative to standard practices. A typical optical chemosensor for recognition of metal ions consists of a selective ligand (ionophore) with a covalently attached fluorophore which responds to the complexation-induced changes in the electron environment by spectral variation. These molecules are called fluoroionophores.

Fluoroionophores built on the calixarene scaffolds are a promising and quickly developed group [1–3]. Calixarenes are macrocyclic compounds composed of phenolic units, connected by methylene bridges, to form a hydrophobic cavity that is capable of generating inclusion complexes with a variety of molecules [4]. An important reason for the current widespread interest in calixarenes is the remarkably simple route used for the synthesis of the parent compound, *p-tert* Butyl calix[4]arene, made up by base-induced condensation of *p*-substituted phenols and formaldehyde [5]. Calix[4]arenes are greatly used as molecular scaffold in the design of artificial receptors because of its tunable and unique three- dimensional structure together with readily functionalization. In addition, they are readily accessible for chemical modification on both narrower and wider rims by attachment of a wide range of potential ligating groups and they are connected to multiple systems (crown ethers, cyclodextrines, porphyrines, amino acids, etc.). Since their discovery, the calixarenes have gained much attention and they have been widely used as building blocks focused to the synthesis of cation receptors and neutral molecules in the last two decades [6–9]. The expanded or extended cavity could be of benefice to the encapsulation and recognition properties. Furthermore, the extended calixarene skeleton with π-conjugated units could act as a chromophore or fluorophore. Upon binding with a guest, the change of the spectral properties would give rise to a sensing mechanism.

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The recognition and sensing of the environmentally metal ions have been growing in considerable ways in recent years. Some heavy metals such as mercury, cadmium, and lead are very toxic, and consequently, the detection and removal of these venomous constitute an important goal in the field of chemical sensors [10, 11]. Lead is the most abundant of the three, it is a well-known heavy metal that plays a critical role in environmental politics due to its high toxicity, and its long-term harmful effects are significant on humans and the environmental system, even upon exposure to a trace amount [12, 13]. Lead is a neurotoxin which accumulates both in soft tissues and bones. Lead pollution is a persisting problem, often encountered in the environment due to its use in batteries, gasoline, and pigments, among others. Even very low levels of lead exposure can cause neurological, reproductive, cardiovascular, and development disorders.

In the literature, quite a few examples of Pb^{2+} selective fluorescent chemosensors using a unique calixarene template [10, 14–16] and even less examples of calix[4]arenes bearing heterocycles are found [17–19]. Therefore selective detection and determination of lead and other metal ion, is an important task, although important achievements, there is still increasing pressure to develop promising novel fluoroionophores.

It has been reported that the introduction of soft donor atoms (S and N) in a calixarene framework promotes their complexation with heavy metal ions [7]. For this purpose, a novel series of calix[4]arenes bearing benzothiazole, benzoxazole, and benzoimidazole groups were obtained recently by our group [20] by reaction of the corresponding 2-mercaptoheterocycle with 5,11,17,23-tetra-*tert*-butyl-25,27-bis[2-(chloroacetamido)ethoxy]-26,28-di-hydroxycalix[4]arene. There, we introduced two benzo-azolyl units into a calix[4]arene through an amide bridge (an infrequent –S–CH_2–(C=O)–NH- feature) between the calixarene and the heterocycle. Herein, we report a study of complexation efficiency and selectivity of this calixarenes towards the selected metal ions using fluorescence and UV–Vis spectroscopy.

Synthesis of calixarene derivatives

For the synthesis of fluorescent chemosensor, 25,26-bis(2-aminoethoxy]-26,28-di-hydroxycalix[4]arene (**3**), was chosen as starting material, Scheme 1. Compound **3** was readily obtained in two steps from *p*-*tert*-butylcalix[4]arene (**1**), by reaction with bromoacetonitrile (an important reaction for the functionalization: O-alkylation) and reduction of the cyano function with $LiAlH_4$ [21]. We have described before, the effective transformation of the amino functions of compound **3** into the corresponding chloroacetamido groups as a potential gateway to the preparation of different calix[4]arenes derivatives [22]. Treatment of calixarene **3** derivative with chloroacetic anhydride, using ethyl acetate as solvent, led to dichloroacetamide derivative **4** in high yield (84 %). The two arms of compound **4** were efficiently linked by treatment with the corresponding 2-mercaptoheterocycle and sodium bicarbonate in refluxing acetonitrile, yielding the calixarene derivatives **5**–**7** in good yields. Spectroscopic data (^{1}H and ^{13}C NMR) indicates that these compounds are consistent with the *cone* conformation.

Experimental

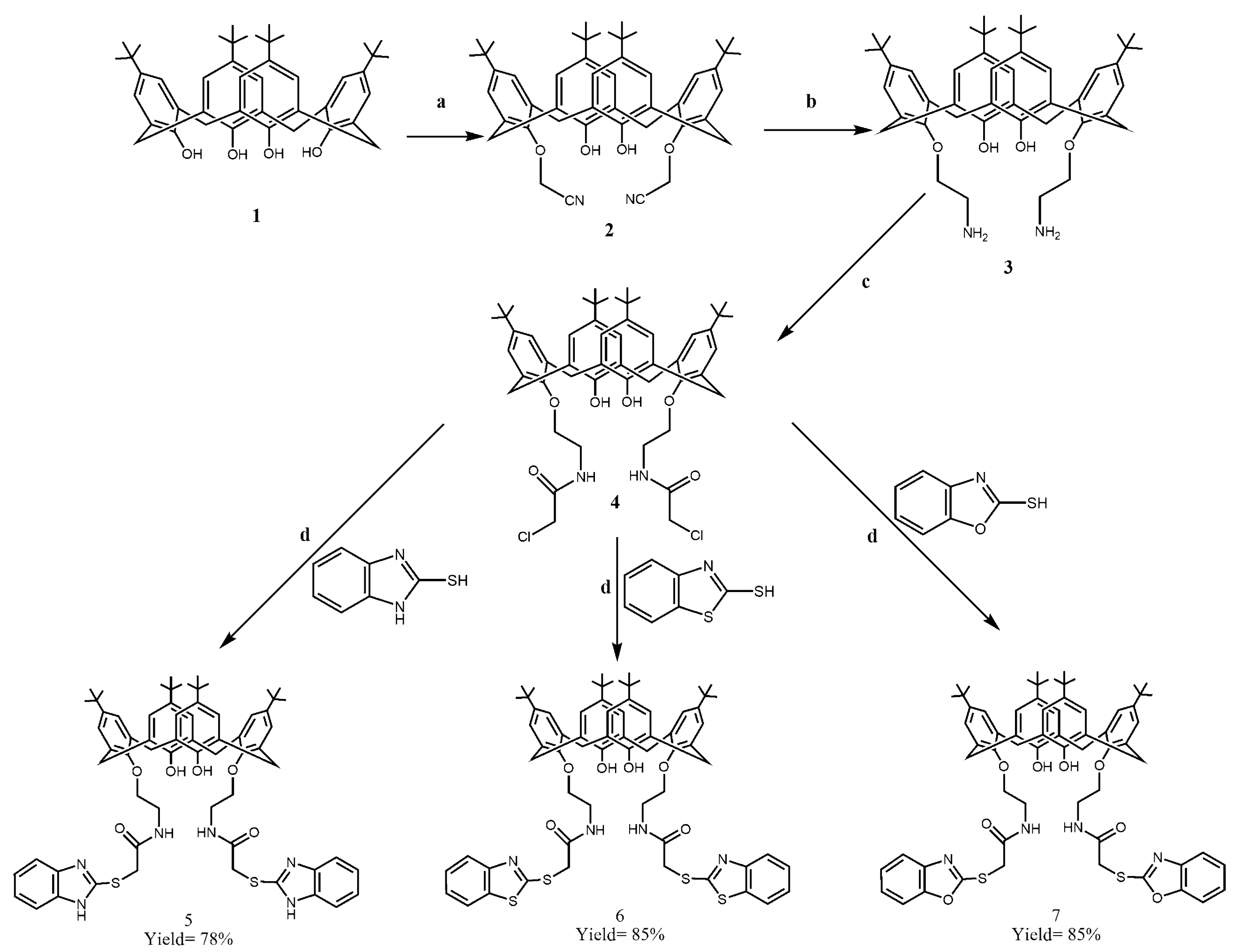
General experimental information

The UV absorption measurements were carried out on an Agilent 8453 spectrophotometer and Fluorescence studies of complexes (Calixarene–Metal) were performed on a LS 55 Perkin-Elmer spectrofluorometer equipped with a xenon lamp source using standard 1.00 cm quartz cells. All the reagents and solvents were of analytical grade and used without further purification. As described before [20] melting points were determined on a hot-stage apparatus and are uncorrected. Spectra were recorded using the following instruments: IR, FT-IR Bruker IFS55; ^{1}H and ^{13}C NMR, Bruker DRX-400 (400 and 100 MHz), using tetramethylsilane as internal reference. Microanalysis were determinated using Fisons EA 1108 analyser and were performed in CEPEDEQ (Centro Para el Desarrollo de la Química), Facultad de Ciencias Químicas y Farmaceuticas, Universidad de Chile. Mass spectra (MALDI-TOF) were obtained on a Bruker mod. AUTOFLEX spectrometer, using HCCA/DSO as matrix in Centro de Instrumentación Científica de La Universidad de Granada, España. Column chromatography was performed on Merck silica gel 60, 230–400 mesh, and TLC- on Merck silica gel G.

General procedure for the synthesis of calix[4]arenes **5**–**7**

To a solution of **4** (0.10 g, 0.122 mmol) in acetonitrile (5 mL) 2-mercaptobenzothiazole (0.268 mmol) and $NaHCO_3$ (50 mg) were added. The reaction mixture was refluxed by 6 h. Filtration and evaporation under reduced pressure gave a crude product that was triturated with ethyl acetate/hexane (1:1) [20].

*5,11,17,23-Tetra-tert-butyl-25,27-bis-{2-[2-benzothiazol-2-ylsulfanyl)acetamido]ethoxy}-26,28-dihydroxy calix[4]-arene (***5***)* Yield: 85 %; m.p. 227–229 °C. IR (KBr): $\nu = 3480, 1657, 1561, 1484, 1361, 1207\ cm^{-1}$. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.60$ (t, 2H:2NH), 8.15 (s, 2H,



(a) BrCH$_2$CN, K$_2$CO$_3$, reflux, 8h; (b) LiAlH$_4$, THF anh, 0ºC.
(c) (ClAc)$_2$O, AcOEt, 25ºC, 1h; (d) NaHCO$_3$, reflux, 8h.

Scheme 1 Synthesis of calixarene derivatives **5**–**7**

2OH), 7.78, 7.66 (2 d, 2H, J = 7.80 Hz), 7.37, 7.27(2 t, 2H, J = 7.80 Hz), 6.91, 6.43 (2 s, 8H:Ar), 4. 21 (s, CH$_2$S, 4H), 4.05 (d, J = 13.0 Hz, 4H:ArCH$_2$Ar), 4.01–3.98 (m, 4H:2OCH$_2$), 3.93–3.89 (m, 4H:2CH$_2$NH), 3.17 (d, J = 13.0 Hz, 4H:ArCH$_2$Ar), 1.23, 1.05 (s, 36H:4Me$_3$C). ^{13}C NMR (100 MHz, CDCl$_3$): δ = 167.9(CONH), 152.8, 149.6, 148.6, 147.9, 142.5, 135.7, 133.0, 127.7,126.0, 125.9, 125.4, 121.6, 121.1 (Ar), 75.3 (OCH$_2$), 40.0 (NHCH$_2$), 34.1, 33.9 (Me$_3$C), 32.0 (ArCH$_2$Ar), 31.6, 31.1(Me$_3$C); MS (MALDI-TOF) C$_{66}$H$_{76}$N$_4$O$_6$S$_4$: calcd for $[M+Na]^+$ 1171.453; found 1171.430; calcd for $[M+K]^+$ 1187.423; found 1187.414; C$_{66}$H$_{76}$N$_4$O$_6$S$_4$(1149,7): calcd. C 68.9, H 6.7, N 4.9, S 11.2: found C 69.1, H 7.0, N 4.9, S 10.8.

*5,11,17,23-Tetra-tert-butyl-25,27-bis-{2-[2-benzoimidazol-2-ylsulfanyl)acetamido]ethoxy}-26,28-dihydroxy calix[4]-arene (**6**)* Yield: 78 %; m.p.216–218 °C. IR (KBr) ν = 3460, 1654, 1512, 1485, 1361, 1206 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ = 12.54 (s, 2H, NH), 8.67 (t, 2H:2NH), 8.48 (s, 2H, 2OH), 7.40 (2 d, 4H), 7.37, 7.27 (2 t, 4H) 7.12 (s, 8H:Ar), 4. 17 (s, CH$_2$S, 4H), 4.0 (d, J = 13.0 Hz, 4H:ArCH$_2$Ar), 3.98–3.88 (m, 8H:2OCH$_2$ CH$_2$NH), 3.10 (d,J = 13.0 Hz, 4H:ArCH$_2$Ar), 1.16, 1.11 (s, 36H:4Me$_3$C). ^{13}C NMR(100 MHz, CDCl$_3$): δ = 167.9 (CONH), 152.8, 149.6, 148.6, 147.9, 142.5, 135.7, 133.0, 127.7, 126.0, 125.9, 125.4, 121.6, 121.1 (Ar),75.3 (OCH$_2$), 40.0 (NHCH$_2$), 34.1, 33.9 (Me$_3$C), 32.0 (ArCH$_2$Ar), 31.6, 31.1(Me$_3$C); MS (MALDI-TOF) C$_{66}$H$_{78}$N$_6$O$_6$S$_2$: calcd for

$[M+Na]^+$ 1137.606; found 1137.609; calcd for $[M+K]^+$ 1153.579; found 1153.572; $C_{66}H_{78}N_6O_6S_2$ (1115.6): calcd. C 71.05, H 7.06, N 7.53, S 5.75: found C 71.25, H 7.15, N 7.8, S 5.8.

5,11,17,23-Tetra-tert-butyl-25,27-bis-{2-[2-benzoxazol-2-ylsulfanyl)acetamido]ethoxy}-26,28-dihydroxy calix[4]arene (7) Yield: 80 %;m.p. 220–222 °C. IR (KBr) (yield: 85 %): ν = 3480, 1655, 1522, 1480, 1364, 1200 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.86 (t, 2H:2NH), 8.45 (s, 2H, 2OH),7.64, 7.44 (2 d, 2H, J = 7.80 Hz), 7.37–7.9 (2 t, 2H, J = 7.80 Hz) 7.09 (s, 8H:Ar), 4. 31 (s, CH_2S, 4H), 4.25 (d, J = 13.0 Hz, 4H:$ArCH_2Ar$), 4.16 (m, 4H:2OCH_2), 4.08 (m, 4H:2CH_2NH), 3.40 (d, J = 13.0 Hz, 4H:$ArCH_2Ar$), 1.35, 1.22 (s, 36H:4Me_3C). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.6 (CONH), 164.0, 152.1, 149.1, 148.5, 148.4, 143.0, 141.6, 138.1, 133.3, 127.8, 126.0, 125.5, 125.4, 124.1, 118.5, 116.4, 110.11(Ar), 75.5 (OCH_2), 40.1($NHCH_2$), 35.8 (CH_2), 32.0 ($ArCH_2Ar$), 31.6 (Me_3C), 31.5, 31.1(Me_3C). MS (MALDI-TOF) $C_{66}H_{76}N_4O_8S_2$ (1117.6): calcd. C 70.93, H 6.87, N 5.01, S 5.74: found C 71.1, H 7.0, N 4.8, S 5.8.

Analytical procedure

General procedure of fluorescence and UV–Visible studies

Stock solutions of **5**–**7** concentration of 1.5 mM and metal perchlorate salts (Ca^{2+}, Cd^{2+}, Pb^{2+}, Ni^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+}, Fe^{2+}, Zn^{2+}) concentration 15 mM were prepared in chloroform and methanol, respectively. The solutions of **5**–**7** for UV–visible, emission and excitation spectra were prepared by placing 15 µL of **5**–**7** into a cuvette diluting to 2600 µL with methanol.

Test solution for screening and selectivity studies were prepared by placing 15 µL of **5**–**7** into a quartz cell, adding aliquot (30 eq) of each metal stocks, and diluting the solution up to 2.6 mL with methanol. Test solution for titration and sensibility studies were prepared by placing 15 µL of **5**–**7** into a cuvette, adding appropriate aliquot from 0 to 60 eq of metal, and diluting with methanol to final volume of 2.6 mL. Fluorescence spectra measurements were scanned exciting at 225 nm with bandwidths for excitation and emission of 9 and 20 nm, respectively.

The stoichiometry of inclusion complex

The stoichiometry of inclusion complexes was determined by continuous variation method, Job′s Plot [23]. Equimolar solutions of **5**–**7** and cation were mixed to a standard volume varying the molar ratio but keeping the total concentration of the species constant, always the final volume is 3 mL. This study was conducted by fluorescence with the same experimental conditions.

Results and discussion

The characteristics of the calixarene **5**–**7** in emission and excitation spectra are almost the same, with a strong band at 210 nm for absorbance which is attributed to π–π* transition and at 280 nm a band due to *n*–π* transition, Fig. 1. The addition of metal ions does not change significantly the absorption spectra. The emission for **5**, **6** and **7** calixarene exciting at 225 nm, showed a band centered at 313, 330 and 313 nm respectively. The fluorescence response of calixarenes towards different divalent metals was investigated by fluorescence spectroscopy. The emission of **5**, **6** and **7** were recorded in methanol, exciting at 225 nm and reading the intensity at its maximum. This exciting

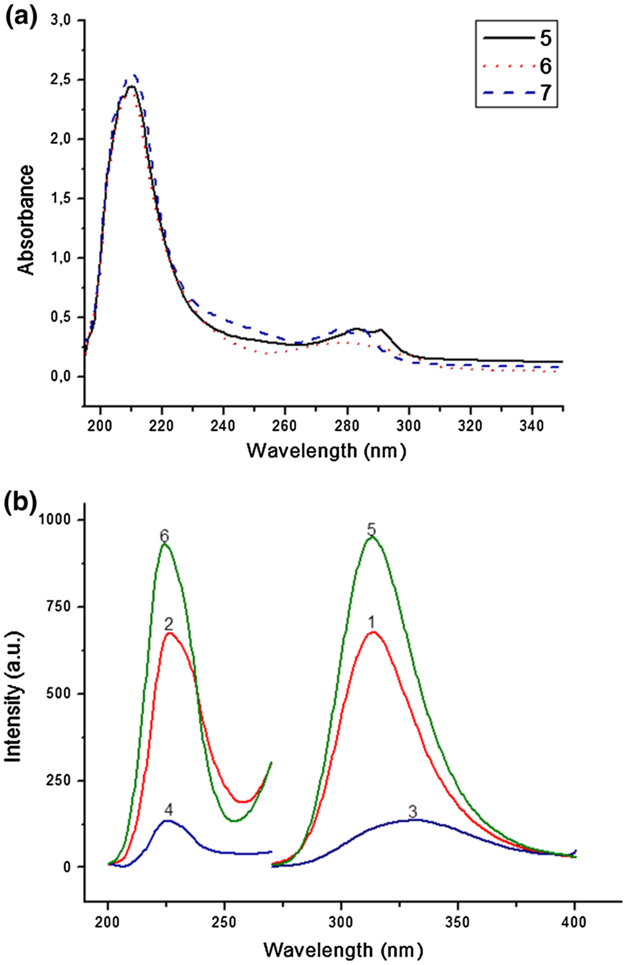
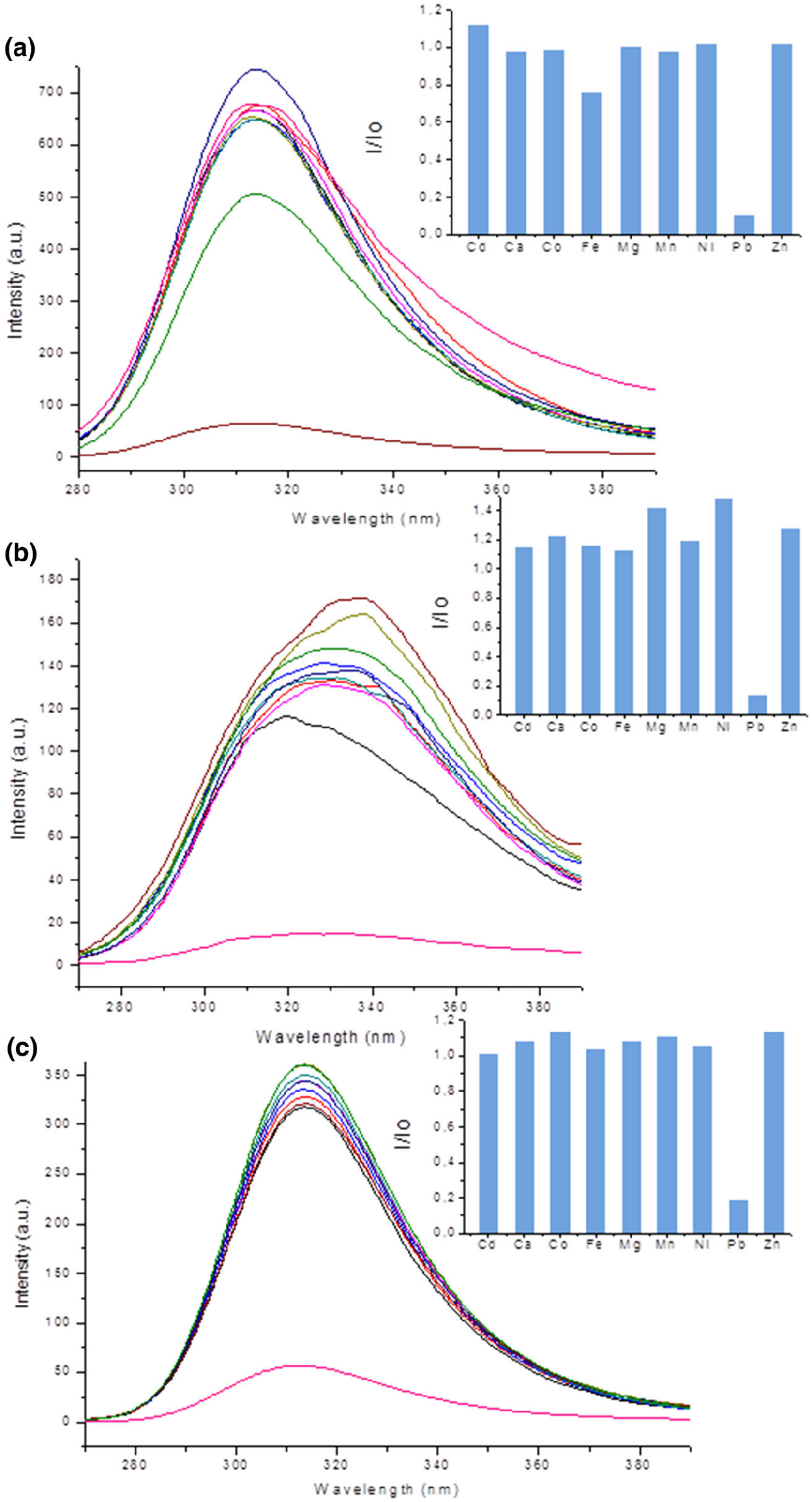


Fig. 1 **a** Absorption spectra of calixarene **5**–**7**, 8.6 µmol L^{-1} in methanol. **b** Excitation (*2*) and emission (*1*) spectra of **5**, excitation (*4*) and emission (*3*) spectra of **6**, and excitation (*6*) and emission (*5*) spectra of **7**

Fig. 2 **a**–**c** Change in fluorescence spectra of the host **5**–**7** (8.6 µmol L^{-1}) upon addition of 30 equivalents of different metals Fe^{+2}, Ni^{+2}, Mn^{+2}, Mg^{+2}, Zn^{+2}, Cd^{+2}, Cu^{+2}, Pb^{+2} and Co^{+2}



wavelength was selected because scanning excitation spectra show the maxima at 225 nm (Fig. 1b). Hence the band at 210 nm should be the superposition of two bands as reported by Rao and coworkers [24].

With the addition of the different cations Fe^{2+}, Ni^{2+}, Mn^{2+}, Pb^{+2}, Mg^{+2} Zn^{2+} Ca^{2+}, Cd^{2+} and Co^{2+}, to check its selective complexation, we can observe no displacement of the maximum for calixarene **5** and **7**, Fig. 2. However, the intensity of calixarene fluorescence is slightly influenced by the presence of each metallic cation, except for lead where a noticeable quenching is observed for the three of them. This suggests that Pb^{2+} is practically the only studied cation that binds strongly to calixarenes, causing significant fluorescence quenching permitting highly selective detection of Pb^{2+}. From the Stern–Volmer analysis for the complexation of Pb^{2+} by calixarene **5–7,** linear behavior was observed for Pb^{2+} ion concentration in the range from 0 to

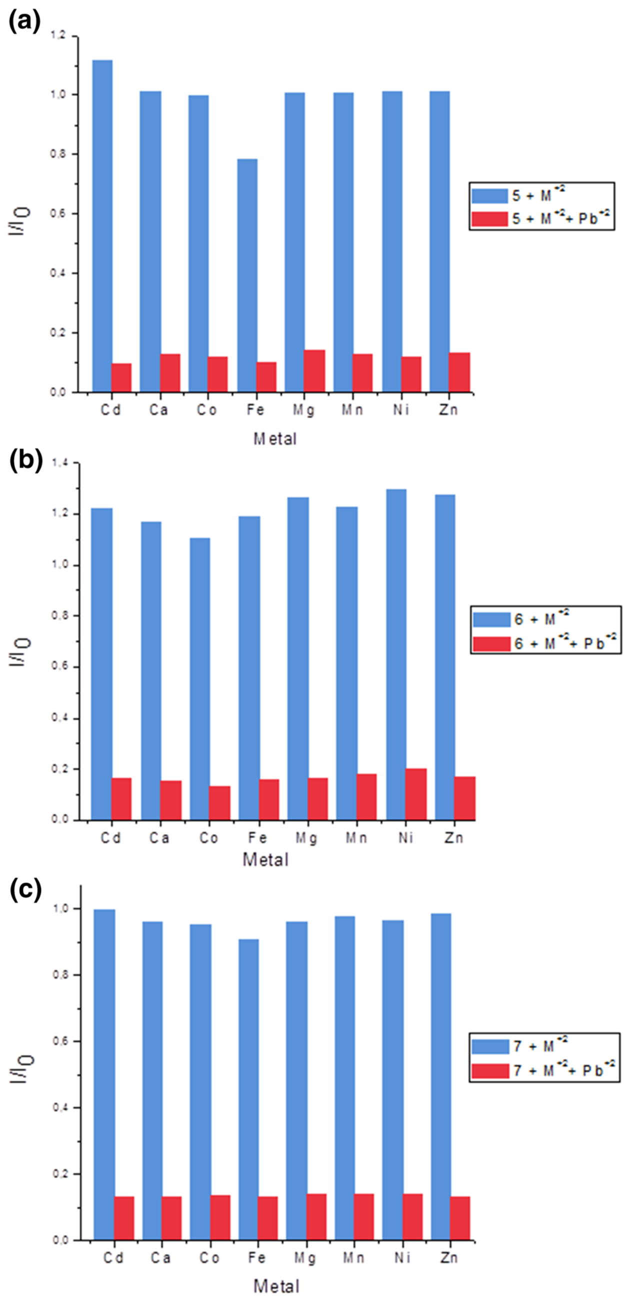


Fig. 3 a–c Fluorimetric ratiometric behavior (I/I_0) of **5–7** in the presence of different metal ions (30 equiv) upon addition of Pb^{2+} (30 eq) in methanol solution

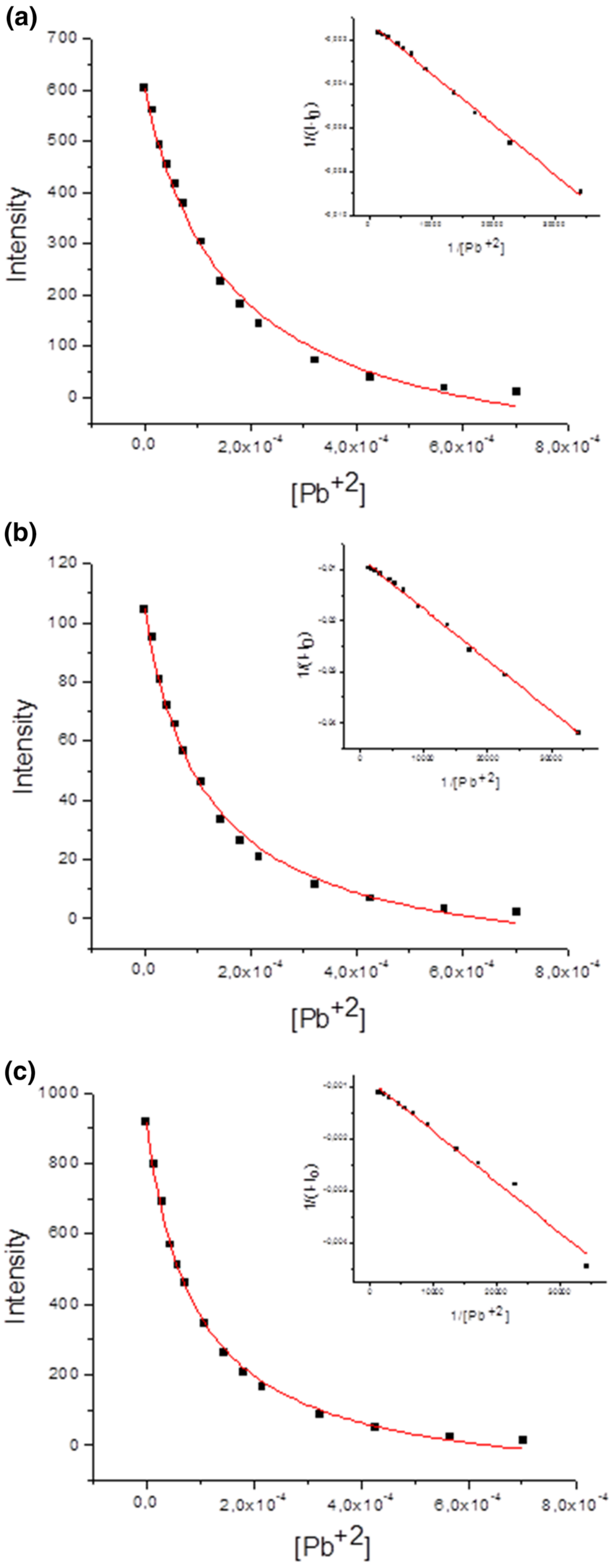


Fig. 4 a–c Nonlinear curve fitting of **5–7** (8.6 μmol L^{-1}) of the emission intensity upon addition of various equivalents of $Pb(ClO_4)_2$ in methanol ($\lambda_{ex} = 225$ nm). *Inset*: linear curve fitting according to Benesi–Hildebrand equation

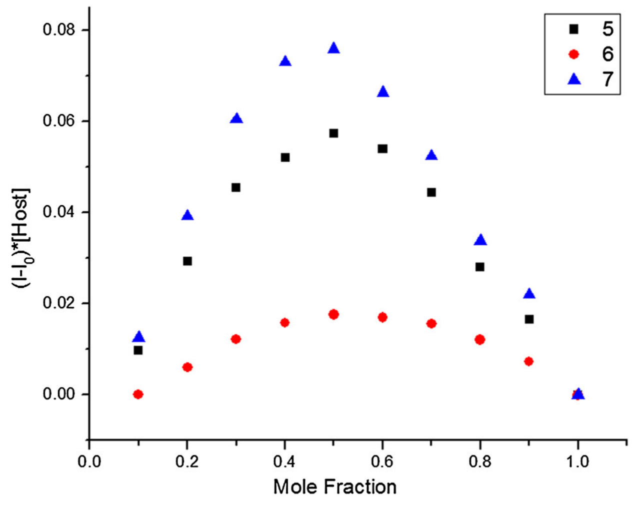


Fig. 5 Job plot of a 1:1 complex of calixarene **5**–**7** and Pb^{+2} ion. The total concentration of **5**–**7** and Pb^{+2} is 5×10^{-5} mol L^{-1}

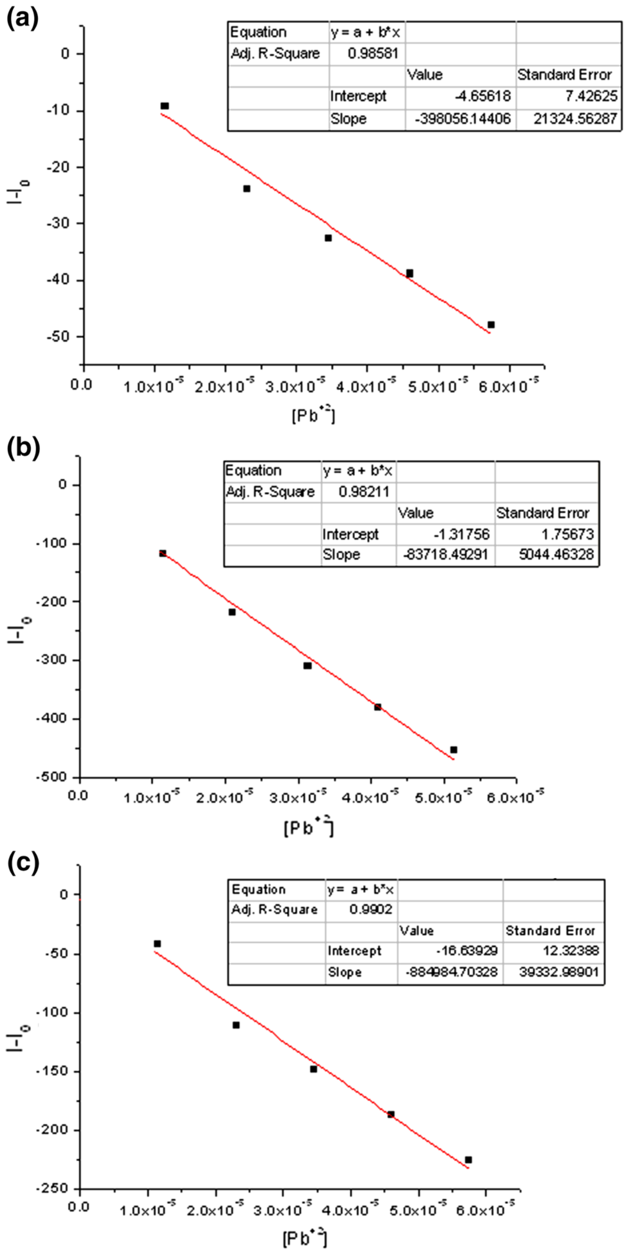
1.5×10^{-4} M obtaining a quenching constant K_{SV} of $(9.46 \pm 0.34) \times 10^{3}$ mol^{-1} L, $(1.48 \pm 0.10) \times 10^{4}$ mol^{-1} L and $(1.77 \pm 0.08) \times 10^{4}$ mol^{-1} L for calixarene **5**, **6** and **7**, respectively.

To study the influence of the other metal ions on Pb^{2+} binding with calixarene **5**–**7**, we performed competitive experiments. Fluorescence quenching caused by Pb^{2+} solution in the presence of the other cation, was similar to that caused by Pb^{2+} alone in the presence of calixarene **5**–**7**. As shown in Fig. 3, it can be seen that Fe^{2+}, Ni^{2+}, Mn^{2+}, Mg^{+2} Zn^{2+} Ca^{2+}, Cd^{2+} and Co^{2+} metal ions have small or no obvious interference with the addition of Pb^{2+} ion. These results suggest that calixarene **5**–**7** can be used as a potential selective fluorescent chemosensor for Pb^{2+}.

To choose the better calixarene to use as chemosensor, we determine the association constant, *K*a, for each calixarene. For this purpose, we used non-linear least-squares regression analysis [25] as an alternative and more appropriate approach than the graphical methods. The association constants, were calculated from the gradual changes in fluorescence intensity at any wavelength (F) of calixarene upon stepwise addition of lead to the solution by a non-linear regression described by the following equation:

$$F = F_o + \frac{(F_\infty - F_o)K_a\left[Pb^{+2}\right]_t}{1 + K_a\left[Pb^{+2}\right]_t} \qquad (1)$$

Experimental data of F as a function of $[Pb^{2+}]$ can be fitted to Eq. 1. As shown in Fig. 4 the intensity of the fluorescence emission of calixarene decreased as the concentration of Pb^{2+} ions increased. We can see that when 60 equivalents of lead were added, approximately 97 % quenching of the initial fluorescence of **5**, **6** and **7** was observed. For fitting the experimental data we have considered the presence of 1:1 calixarene–lead complex.



Equation	y = a + b*x		
Adj. R-Square	0.98581		
		Value	Standard Error
	Intercept	-4.65618	7.42625
	Slope	-398056.14406	21324.56287

Equation	y = a + b*x		
Adj. R-Square	0.98211		
		Value	Standard Error
	Intercept	-1.31756	1.75673
	Slope	-83718.49291	5044.46328

Equation	y = a + b*x		
Adj. R-Square	0.9902		
		Value	Standard Error
	Intercept	-16.63929	12.32388
	Slope	-884984.70328	39332.98901

Fig. 6 a-c Calibration curve of **5**–**7** with Pb^{+2} in methanol for determination limit of detection in the range lineal from 1×10^{-5} to 6×10^{-5} mol L^{-1}

The representation of the double reciprocal plot leads to a straight line indicating a 1:1 stoichiometry, inset Fig. 4. This was corroborated using the continuous variation method. The total concentration of calixarene and lead cation was kept constant while the molar fraction of lead was continuously varied. The result obtained indicates a maximum when the molar fraction is 0.5 for the three calixarenes, demonstrating that the formation of the lead complex has a stoichiometry of 1:1 for each one, Fig. 5.

The highest association constant obtained by fitting the experimental data to Eq. 1 correspond to calixarene **7** with

Table 1 Association constant, quantum yield (Φ) and limit of detection of the different calixarene with lead

Calixarene	Quantum yield[a]	Association constant		Limit of detection				
	Φ ($\times 10^3$)	K_a ($\times 10^{-4}$ mol^{-1} L)	R	LOD (mg L^{-1})	LOD (μM)	m ($\times 10^5$)	Sb	R
5	2.1 ± 0.3	0.79 ± 0.06	0.99	16.01 ± 0.86	77.25 ± 4.13	3.98 ± 0.21	10.25	0.982
6	4.3 ± 0.4	1.07 ± 0.07	0.99	7.61 ± 0.46	36.78 ± 2.12	0.84 ± 0.05	1.04	0.985
7	6.1 ± 0.3	1.37 ± 0.06	0.99	1.14 ± 0.05	5.48 ± 0.24	8.85 ± 0.39	1.61	0.990

K_a association constant, *LOD* limit of detection, *m* calibration sensitivity, *SD* standard deviation of the blank solution

[a] The fluorescence quantum yield of calixarene was determined exciting at 274 nm using tyrosine as standard (Φ=0.14)

a K_a of (1.37 ± 0.06) × 10^4 mol^{-1} L with a $R = 0.99$. In contrast, the other host–guest complexes **5** and **6** have an association constants of (0.79 ± 0.06) × 10^4 mol^{-1} L ($R = 0.99$) and (1.07 ± 0.07) × 10^4 mol^{-1} L ($R = 0.99$), respectively. This indicates that calixarene **7** is the selected one to use as chemosensor to detect lead, therefore determining the sensitivity is important. The limit of detection (LOD) for the analysis for lead is determined from the plot of fluorescence intensity as a function of the concentration of Pb^{+2} in the range lineal from 1×10^{-5} to 6×10^{-5} mol L^{-1} (Fig. 6a–c). The limit of detection is determined from the Eq. 2, where Sb corresponds to standard deviation of the blank measures, m corresponds to the calibration sensitivity and K corresponds to 3 for the method detection limit [26, 28].

$$\mathrm{LOD} = \frac{\mathrm{K} \cdot \mathrm{Sb}}{\mathrm{m}} \qquad (2)$$

The results are presented in Table 1, which indicates that host **7** has the best detection limit for lead (1.14 ± 0.05 mg L^{-1}) indicating that this results are acceptable for the recognition of lead in methanol at micromolar concentrations. This result is comparable to a PDA-based chemosensor system which has a detection limit of 0.8 mg L^{-1} [27].

Conclusion

The fluorescence response of calixarenes bearing heterocycles **5–7** towards different divalent metals was investigated by fluorescence spectroscopy. Fluorescent chemosensor ability of three calixarene derivatives was highly selective for Pb(II) in contrast with other divalent metal studied, including competition between ions. The highest association constant obtained corresponds to calixarene **7** with a K_a of (1.37 ± 0.06) × 10^4 mol^{-1} L, this demonstrates the effectiveness of the interaction between calixarene-lead compared to other molecules **5–6**. Moreover, the results indicate that host **7** has the best detection limit for lead (1.14 ± 0.05 mg/L), acceptable for the recognition of this metal in micromolar concentrations. Studies of selective recognition anion for these calixarenes derivatives are in progress. Finally, it can be concluded that the molecules studied allow the effective detection of Pb^{+2} ions.

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References

1. Asfari, Z., Böhmer, V., Harrowfield, M., Jack, McB, Vicens, J. (eds.): Calixarenes 2001. Kluwer Academic, Dordrecht (2001)
2. Kim, J.S., Quang, D.T.: Calixarene-derived fluorescent probes. Chem. Rev. **107**, 3780–3799 (2007)
3. Kim, J.S., Lee, S.Y., Yoon, J., Vicens, J.: Hyperbranched calixarenes: synthesis and applications as fluorescent probes. Chem. Commun. **32**, 4791–4802 (2009)
4. Stoddart, J.F. (ed.): Calixarenes Revisited. Royal Society of Chemistry, Cambridge (1998)
5. Gutsche, C.D., Iqbal, M.: p-*tert*-Butylcalix[4]arene. Org Synth **VIII**, 75–77 (1993)
6. Ludwig, R., Kim Dzung, N.T.: Calixarene-based molecules for cation recognition. Sensors **2**, 397–416 (2002)
7. Arora, V., Chawla, H.M., Singh, S.P.: Calixarene as sensor materials for recognition and separation of metal ions. Arkivoc **ii**, 172–200 (2007)
8. Sliwa, W., Girek, T.: Calixarene complexes with metal ions. J. Incl. Phenom. Macrocyclic. Chem. **66**, 15–41 (2010)
9. Joseph, R., Rao, C.P.: Ion and molecular recognition by lower rim 1,3-di-conjugates of calix[4]arene as receptors. Chem. Rev. **111**(8), 4658–4702 (2011)
10. Kim, H.N., Ren, W.X., Kim, J.S., Yoon, J.: Fluorescent and colorimetric sensors for detection of lead, cadmium, and mercury ions. Chem. Soc. Rev. **41**, 3210–3244 (2012)
11. Qazi, M.A., Qureshi, I., Memon, S.: Hg(II) selective complexation by chromoionophoric calix[4]arene derivative. J. Fluoresc. **21**, 1231–1238 (2011)
12. Toscano, C.D., Guilarte, T.R.: Lead neurotoxicity: from exposure to molecular effects. Brain Res. Rev. **49**, 529–554 (2005)
13. Papanikolaou, N.C., Hatzidaki, E.G., Belivanis, S., Tzanakakis, G.N., Tsatsakis, A.M.: Lead toxicity update. A brief review. Med. Sci. Monitor **11**, RA329–336 (2005)
14. Buie, N.M., Talanov, V.S., Butcher, R.J., Talanova, G.G.: New fluorogenic dansyl-containing calix[4]arene in the partial cone conformation for highly sensitive and selective recognition of lead (II). Inorg. Chem. **47**, 3549–3558 (2008)

15. Liu, J.-M., Bu, J.-H., Zheng, Q.-Y., Chen, C.-F., Huang, Z.-T.: Highly selective fluorescent sensing of Pb^{2+} by a new calix[4]-arene derivative. Tetrahedron Lett. **47**, 1905–1908 (2006)
16. Jeon, N.J., Ryu, B.J., Nam, K.C.: Pb^{2+} On-off switchable 1,3-alternate calix[4]arene chemosensor containing urea and pyrene moieties. Bull. Korean Chem. Soc. **33**(9), 3129–3132 (2012)
17. Jeong, Y., Yoon, J.: Recent progress on fluorescent chemosensors for metal ions. Inorg. Chim. Acta **381**, 2–14 (2012)
18. Joseph, R., Ramanujam, B., Acharya, A., Khutia, A., Rao, C.P.: Experimental and computational studies of selective recognition of Hg^{2+} by amide linked lower rim 1,3-dibenzimidazole derivative of calix[4]arene: species characterization in solution and that in the isolated complex, including the delineation of the nanostructures. J. Org. Chem. **73**, 5745–5758 (2008)
19. Bingol, H., Kocabas, E., Zor, E., Coskun, A.: A novel benzothiazole based azocalix[4]arene as a highly selective chromogenic chemosensor for Hg^{2+} ion: a rapid test application in aqueous environment. Talanta **82**, 1538–1542 (2010)
20. Saitz, C., De La Fuente, J., Jullian, C., Pessoa, H., Olea, C.: Synthesis of calix[4]arenes bearing benzothiazolyl, benzoxazolyl and benzoimidazolyl heterocycles. J Chem Res (S) **6**, 324–326 (2008)
21. Zhang W.C., Huang Z.T.: Synthesis of 4-*tert*-Butylcalix[4]arenes bearing two schiff-base units at lower rim. Synthesis, 1073–6 (1997)
22. Santoyo-Gonzalez F., Torres-Pinedo A., Saitz-Barría C.: An efficient synthesis of bis(calix[4]arenes), bis(crown-ether) substituted calix[4]arenes, aza-crown calix[4]arenes and thiaza-crown calix[4]arenes. Eur J Org Chem, 3587–93 (2000)
23. Job, P.: Formation and stability of inorganic complexes in solution. Ann. Chim. **9**, 113–203 (1928)
24. Joseph, R., Gupta, A., Ali, A., Rao, C.P.: Fluorescence and absorption studies on the selective recognition of iodide by lower rim 1,3-bis(aminoethoxy)-p-t-butyl-calix[4]arene derivate. Indian J. Chem **46A**, 1095–1100 (2007)
25. Connors, K.A.: Binding Constant: The Measurement of Molecular Complex Stability. Wiley, New York (1987)
26. Liu, S.-R., Wu, S.-P.: An NBD-based sensitive and selective fluorescent sensor for copper (II) ion. J. Fluoresc. **21**, 1599–1605 (2011)
27. Lee, K.M., Chen, X., Fang, W., Kim, J.-M., Yoon, J.: A dual colorimetric and fluorometric sensor for lead ion based on conjugated polydiacetylenes. Macromol. Rapid Commun. **32**, 497–500 (2011)
28. Huang, Y., Wang, J., Xue, S., Tao, Z., Zhu, Q., Tang, Q.: Determination of thiabendazole in aqueous solutions using a cucurbituril-enhanced fluorescence method. J. Incl. Phenom. Macrocyclic. Chem. **72**, 397–404 (2012)