

Fundamental Studies of Synthesizing Novel Calix[4]arene Derivative as Host for Treatment of Toxic Metal Cations (Hg^{2+} , Cd^{2+} , Pb^{2+} and Ag^+)

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Abstract

Novel calix[4]arene derivative containing mixed pendant arms in its lower rim, 25, 27-bis (diethylamino) ethoxy- 26, 28-bis (bis-methoxyethoxy) calix[4]arene, L3, has been synthesized to target the heavy metal cations. ^1H NMR investigations seem to indicate that the receptor L3 interacts preferentially with Hg^{2+} , Cd^{2+} , Pb^{2+} and Ag^+ in acetonitrile. Complexation studies in acetonitrile show that the lower rim groups of the receptor L3 are the active sites of its interaction with metal ions. These findings are corroborated by conductance measurements in acetonitrile, plots of molar conductance against the ligand/metal cation ratio reveal the formation of 1:1 complexes between this ligand with these cations. Standard thermodynamics parameters of complexation ($\log K_s$, ΔH°_c , ΔS°_c , ΔG°_c) of L3 with Hg^{2+} , Cd^{2+} , Pb^{2+} and Ag^+ in acetonitrile were determined using the Nano ITC (isothermal titration calorimetry). For all the systems investigated, the complexation process between these metal cations and the receptor L3 was enthalpically controlled. The enthalpic and entropic contributions to the Gibbs energy associated with these processes are analysed.

Keywords: Calixarene receptor; Heavy metal cations; ^1H NMR investigations; Conductance measurements; Thermodynamics parameters of complexation

Introduction

The environmental and biological concern caused by the presence of the heavy metal cations encouraged the researchers to discover the possibilities of the calixarene derivative in dealing with these cations [1-10]. The heavy metals which are released continuously from industrial, agricultural to the environment will cause great problems to the biological life [1-3]. These elements cause significant effects on humans in all over the world [11-13]. These highly toxic metals are known to cause health problems such as brain damage [14-16], nephropathy [17-19], sluggishness [20,21], hyperirritability [22], restlessness [23], infertility [24] and a number of diseases in early aged children [19,25]. The removal of these trace heavy metal ions are tested by number of methods i.e., membrane filtration, osmosis, precipitation, oxidation/reduction, microbiological activity, ion exchange and extraction etc. [26-28].

The supramolecular chemistry has provided a much better solution to the search for molecular structures that can serve as building blocks for the production of sophisticated molecules by anchoring functional groups oriented in such a way that they delineate a suitable binding site. This was achieved with the development of macrocyclic molecules such as synthetic crown ethers, cryptands, spherands [29], natural cyclodextrins [30,31] and calixarenes [32,33].

The use of the calixarene compounds which has a high selectivity is one of the proposed methods. The design of calixarene-based receptors able to interact selectively with a given guest is a challenging area of research. The structure of the 'cone' *p-tert*-butylcalix[4]arene is shown in Figure 1.

The most common forms of calixarenes are insoluble in water, due to their aromatic components. They also possess limited solubility in organic solvents, thereby making their purification and characterization a formidable task. Most forms are sufficiently soluble to allow spectral analyses in a limited number of common organic solvents however [34].

Calix[4]arene-based macrocycles have attracted much attention

because of their unique molecular structure and simple single-pot synthesis in supramolecular chemistry [29,32,35,36]. These molecules are generally *ortho*-substituted calix[n]arenes capable of alkali, alkaline earth and heavy metal ion recognition [37-39].

It has been reported that ester function derived from calixarenes possess remarkable tendency to bind IA group cations with unique size selectivity [2,3,40] as well as with ions and molecules of biological and environmental relevance [26]. Recently, a number of studies have been explored on the subject of the liquid-liquid, extraction or complexation of targeted ions [41-45]. It has been explored earlier that a calixarene with diester and/or dinitrile functionalities at its lower rim (narrow rim) can selectively extract lead/mercury from an aqueous to the organic phase in liquid-liquid extraction processes [46]. Solangi et al. have explored Pb^{2+} selective extraction behavior of ester derivatives of calix[n]arenes [47]. They observed that the hexaester derivative of calix[6]arene is more selective and efficient ionophore as compared to tetraester derivative of calix[4]arene for the extraction of Pb^{2+} from aqueous to the organic phase. However, an early attempt was made by Roundhill et al. [48] to extract the toxic metals with calix[4]arenes modified with sulfur containing functionalities. Compounds were found to be effective extractants for Hg^{2+} . Arnaud-Neu et al. [49] used ionophores for complexation of Pb(II) , Hg(II) and Cd(II) cations by replacing hard oxygen-based binding group with softer sulfur based binders. Calixarenes with thioamide functions in lower rim have demonstrated good efficiency in the selective extraction of Cd(II) and Pb(II) ions. Solvent extraction of heavy metals with macrocyclic ligands based on calix[4]arenes was also studied by Dung and Ludwig [50].

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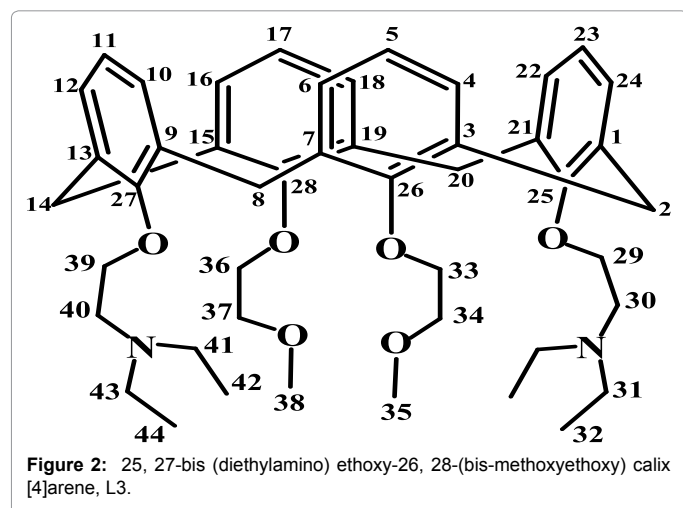
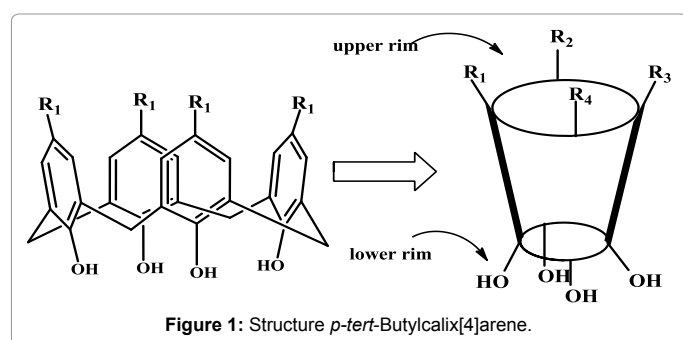
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In this investigation, fully substituted, a novel calix[4]arene derivative containing mixed pendant arms in its lower rim, 25, 27-bis (diethylamino) ethoxy-26, 28-(bis-methoxyethoxy) calix[4]arene, L3, has been synthesized for the detection of heavy metal ions (Figure 2). This receptor was characterized by elemental analysis and ¹H NMR and ¹³C NMR and ¹³C NMR-DEPT which confirmed that this receptor was successfully synthesized.

The capacity of a calixarene ligand to complex a specific guest species is determined by various structural factors, in particular, the type of donor groups and the shape of the cavity. A good response to cations was obtained by full functionalization of the lower rim with ether-amine groups. Calix(4)arene containing ether and amine functional groups at the lower rim have shown pronounced selectivity for metal cations in acetonitrile. It can be observed that the presence of the amino groups (basic) in the lower rim for this receptor makes this ligand attractive to explore their complexation with these metal cations.

Calixarenes are typical allosteric systems where changes on the one side lead to changes on the other side of macrocycle. The conformational changes that the calix[4]arene ligand undergoes upon complexation with ionic or neutral species can be assessed from ¹H NMR investigations [51]. Gutsche et al. [52] established that the conformations of calixarenes and their derivatives can be established by observing the resonance of the methylene protons (axial and equatorial) in the ¹H NMR spectrum. In the "cone" conformation $\Delta\delta_{ax-eq}$ is about 0.90 ppm while values of 0.50 ppm and higher than 0.90 ppm are found for calixarenes in their flattened and distorted conformation respectively.



Investigation on the complexation of the L3 with metal cations in acetonitrile using a variety of techniques such as ¹H NMR (to establish the binding sites and the conformational changes that these ligands undergo upon complexation with metal cations), conductance measurements (to establish the composition and the type and strength of the host-guest complexes). Nano ITC to derive the thermodynamics of complexation (stability constant, log *K*s, hence standard Gibbs energy, $\Delta_c G^\circ$; enthalpy, $\Delta_c H^\circ$; and entropy, $\Delta_c S^\circ$ of complexation).

Experimental Section

Chemicals

All chemicals were obtained from Sigma-Aldrich, Fluka and Fisher UK Scientific and were either analytical or reagent grade. Solid chemicals were used as received without further purification. All solvents were dried and purified as described in the literature [53]. Lead (II), cadmium (II), mercury (II), and silver tetrabutylammonium perchlorate 99%, were dried over P₄O₁₀ under vacuum for several days before use.

¹H NMR measurements

¹H NMR measurements were used to characterize the calix[4]arene derivatives and to provide information about its interaction with heavy metals and whenever possible to establish the site of interaction of the ligand. ¹H NMR measurements were recorded at 298 K on a Bruker DRX-500 pulse Fourier Transform NMR Spectrometer. The operating conditions involved pulse or flip angle of 30°, spectra width (SW) of 15 ppm, spectral frequency (SF) of 500.150 MHz, delay time of 0.3 s, acquisition time (AQ) of 3.17 s, and line broadening of 0.3 Hz. Solutions of the samples of interest (1 × 10⁻³ mol dm⁻³) were prepared in the appropriate deuterated solvent. These were placed in 5 mm NMR tubes using TMS (tetramethylsilane) as the internal reference.

Complexation studies: The complexation behavior of L3 toward metal cations at 298 K was studied using the ¹H NMR technique, by adding the metal-ion salt (2.5 × 10⁻³ to 5.0 × 10⁻³ mol dm⁻³) into the NMR tube containing the ligand dissolved in the appropriate solvent (1.0 × 10⁻³ to 2.0 × 10⁻³ mol dm⁻³). Stepwise additions of the metal-ion salt were made and chemical shifts were recorded. Changes in chemical shifts upon addition of the metal-ion salt relative to the free ligand were calculated.

Conductance measurements

A Wayne-Kerr Autobalance Universal Bridge type B642 was used for conductometric measurements. The Wayne-Kerr is connected to a platinum glass bodied electrode housed in a cylindrical glass vessel where the reaction takes place. A thermostated bath circulating water in the vessel jacket was used to maintain the temperature of the vessel at 298.15 K. A magnetic stirrer was used to keep homogeneous the solutions throughout the time of the experiment. The conductance cell was a Russell type glass bodied electrode with a cell constant (determined using 0.10 mol dm⁻³ aqueous KCl solution). For these experiments, the vessel was filled with metal cations in the appropriate solvent (25 cm³) and the conductance of the solution was measured. Then, a known volume of solution of calix[4]arene derivative in the same solvent was added stepwise into the vessel and the conductance measured after each addition.

Determination of the constant of the conductivity cell: The cell constant was determined by titrating a solution of KCl (0.1 mol dm⁻³) in deionised water (25 cm³) [54]. The cell was immersed in a thermostated bath at 298.15 K. The conductance of the solution was recorded after addition of KCl once the stability of the system was ensured.

Nano ITC (Isothermal Titration Calorimetry)

ITC measurements were performed in a Nano Isothermal Titration Calorimeter, models 5300 (TA Instruments). All measurements were carried out in acetonitrile solvent at a fixed temperature of 298.15 K. The basic principle of ITC is simply to measure the heat released or absorbed in a liquid sample after the addition of another liquid sample. This heat is proportional to the total amount of binding that occurs within the calorimeter cell. The instrument has a pair of identical cells (1.4 ml), denoted as the reference and sample cells. These cells, along with access stems, are enclosed in a temperature-controlled thermal jacket. The reference cell was filled with acetonitrile. The ligand solution (1 m M) placed in the sample or reaction cell. The metal cations (20 m M) loaded in the syringe. The interval time between two readings was set at 240 s. The experiments were designed for a total of 25 consecutive injections. The power (or heat) difference between the sample and reference cells is used to determine reaction stoichiometry or number of binding sites (n), stability constant (K_a), and the enthalpy (ΔH°) [55,56]. The first data point was removed from the data set prior to curve fitting. The data was analyzed to determine the heat of interaction by using Origin 7.0 and Nano Analyzer data analysis software's supplied by Microcal and TA Instruments, respectively with the 'independent sites' model.

Calibration of the equipment: To determine the accuracy of measurements carried out in the Nano ITC, a chemical calibration should be performed. The following equilibrium is established upon the addition of barium chlorideto 18-crown-6 ether in water at 298.15 K [57]. The sample cell was filled with an aqueous solution of 18-Crown-6 (1 × 10⁻³ mol dm⁻³) and titrated incrementally from the burette stirring system with BaCl₂ (0.015 mol dm⁻³). The thermodynamic parameters for the complexation of Ba²⁺ with 18-C-6 in aqueous medium show a good agreement with the values reported in the literature by Briggner and Wadso (Appendix B).

Synthesis

Synthesis of 25, 26, 27, 28-tetrahydroxycalix[4]arene via de-tert-butylation of p-tert-butylcalix[4]arene(L1): The removal of p-tert-butyl groups from p-tert-butylcalix[4]arene was achieved by a catalyzed retro friedel-Crafts reaction as described by Gutsche et al. [58].

Synthesis of 25, 27 dihydroxy 26, 28- bis(diethylamine) ethoxycalix(4)arene, L2: The preparation of this derivative was achieved by procedure reported in the literature [59].

Synthesis of 25, 27-bis (diethylamino)ethoxy-26,28-(bis-methoxyethoxy)calix[4]arene, L3: The 25, 27 dihydroxy 26, 28-bis(diethylamine) ethoxycalix(4)arene, L2 (1 g, 1.60 mmol), sodium hydride (1 g, 41.6 mmol) were suspended in 150 ml mixture of freshly refluxed THF and 30 ml of DMF dried on molecular sieves. Then 2-Bromoethylmethylether (0.64 g, 4.6 mmol) in DMF (10 ml) was syringed to the reaction mixture. Then the reaction was stirred and refluxed for 24 hr at 90°C. The reaction was monitored by TLC using DCM/Methanol (9:1) as developing solvent. After cooling down the reaction, the solvent was filtered through filter paper and removed under vacuum, to give an oily product which was dissolved by small amount acetonitrile followed by precipitation with drops of water afforded the pure compound as a white powder. Yield: 0.8 g, 1.08 mmol (67.80%). The compound was characterized by ¹H-NMR in CD₃CN at 298 K and microanalysis. ¹H-NMR (CD₃CN, 500 MHz); δ(ppm)=6.81 (d, H-1); 6.67 (t, H-2); 6.60(d, H-3); 6.52(t, H-4); 4.51(d, H-6(ax)); 3.19(d, H-5(eq)); 4.17 (t, H-7); 3.98(t, H-8); 3.84(t, H-9); 2.95(t, H-10);

3.35(s, H-11); 2.60(q, H-12), 1.02(t, H-13). ¹³C NMR(CDCl₃, 500 MHz); δ(ppm)=122.15 (s, Ar, 5, 12, 19, 26), 127.80(s, Ar, 11, 13, 25, 27), 128.55(s, Ar, 4, 6, 18, 20), 134.01(s, Ar, 1, 10, 14, 24), 135.90 (s, Ar, 3, 7, 17, 21), 155.48 (s, Ar, 15, 28), 157.17 (s, Ar, 8, 9), 72.88(C38), 72.62(C35), 58.72(C29), 52.56(C30), 47.61(C31), 30.95(C37), 30.87(C2), 11.96(C32). ¹³C NMR-DEPT: δ (ppm): 11.97 (C30), 30.97 (C31), 58.74 (C2), 122.17 (C29), 127.81 (C35), 128.56 (C36). Elemental analysis carried out, % found for C, 74.81, N; 3.82 and H; 8.45, % calculated, C; 74.79, N; 3.79 and H, 8.40.

Results and Discussion

¹H NMR characterization of the calix[4]arenes derivatives

The NMR spectra of these receptors (L1, L2 and L3) in CDCl₃ at 298 K were recorded (Appendix A). The ¹H NMR spectrum of L3 in CDCl₃ shows that this ligand has thirteen different sets of protons. Five triplets at 4.16, 3.98, 3.84 and 1.01 ppm corresponding to proton 7, 8, 9, 10 and 13 respectively. The signal observed at 3.35 ppm is those corresponding to proton 11 that is found on the terminal ethyl group respectively. The pair of doublets at 4.51 and 3.19 ppm corresponds to the axial and equatorial protons respectively. The difference between these chemical shifts (Δδ_{ax-eq}) was found to be 1.31 ppm. These results indicate that this macrocycle adopts a distorted 'cone' conformation. This might be due to the steric and electrostatic effects between the pendent arms at the lower rim. Thus, these groups try to move away as possible from each other to reduce the steric and electrostatic effects. Two pairs of doublets were found at 6.80 and 6.60 ppm corresponding to the aromatic protons in meta position and a multiple peak (6.67 and 6.52 ppm) corresponding to the protons in para position. One quartet found at 2.59 ppm is assigned to the protons in the terminal ethyl chain.

¹³C NMR of L3 in CDCl₃ at 298.15 K: ¹³C NMR spectrum of L3 in CDCl₃ at 298.15 K is shown in (Appendix A).

¹H NMR studies of L3 with tetrabutylammoniumperchlorate as counter ion in CD₃CN at 298 K: It is very important at the start, and to be very exact, to examine the interaction between the counter ion (tetrabutylammoniumperchlorate) to these toxic metal cations (the receptor L3). It must be found out whether a receptor - counter complexes will be formed. ¹H NMR spectrum of the receptor L3, with the counter ion (tetrabutylammoniumperchlorate) in acetonitrile at 298 K is shown in (Appendix A). It can be seen that no chemical shift changes are observed for L3 upon addition of the counter ion. It is indicate that the L3 receptor rejects the counter ion.

¹H NMR studies on the interaction of L3 with metal cations in CD₃CN at 298 K: ¹H NMR spectra of the metal- receptor complexes at 298 K are shown in (Appendix A). The relevant ¹H NMR chemical shift changes of the protons observed by the addition of heavy metal cations (as tetrabutylammoniumperchlorate) to L3, in CD₃CN at 298 K are listed in Table 1.

Table 1 shows the chemical shift changes of L3 induced by addition of heavy metal cation salts (as tetrabutylammoniumperchlorate) in CD₃CN at 298 K. These changes suggest that interaction of L3 and these metal cations is taking place. It can be seen that Δδ_{ax-eq} decreased from the free to the complexes ligand from 1.32 to 1.18, 1.07, 1.08, 1.09, 1.08 ppm for Ag⁺, Cd²⁺, Hg²⁺ and Pb²⁺ respectively, suggesting that the ligand adopts a "cone" conformation approximately upon complexation with these metal cations in this solvent.

Approximately, all protons for ligand L3 have been affected upon the addition of Ag⁺, Cd²⁺, Hg²⁺ and Pb²⁺ in CD₃CN at

298 K. Some protons have shielding effects and others have deshielding effects. It can be noted that the protons closest to the nitrogen and the oxygen atoms such as H-7, H-8, H-9, H-10, H-11 H-12 and H-13 have considerable chemical shift changes relative to others. This is an indication that the lower rim groups of the receptors interact with these metals. Protons such as H-1, H-2, H-3, H-4, H-5 and H-6 have been also affected as a result of this interaction. The significant shift is observed for the methylene protons of the amine groups $\text{CH}_2\text{NCH}_2\text{CH}_3$ (H-10) which exhibit high upfield shifts of 0.37, 0.81, 0.79 ppm for Ag^+ , Cd^{2+} , Hg^{2+} and Pb^{2+} respectively. These may indicate that the amine and possibly the ethereal oxygens are strongly involved in the complexation process, although the nitrogen atoms seem to interact more strongly. This upfield shift can be explained by assuming that the amine groups move inward the hydrophilic cavity toward the metal cation upon complexation which brings the CH_2 protons toward the exterior of the hydrophilic cavity where they experience a shielding effect in the presence of the aromatic rings.

Conductometric measurements

With the aim of establishing the composition of the metal-ion complex in acetonitrile at 298 K. Conductometric curves for the titration of heavy metal cations with L3 in acetonitrile at 298 K are shown in Figure 3. These are plots of molar conductances (Λ_m , $\text{S cm}^2 \text{mol}^{-1}$) at each step of the titration against the $[\text{L3}]: [\text{M}^{n+}]$ molar ratio.

Among the cations investigated, strong complexes of 1:1 stoichiometry were found for Ag^+ , Cd^{2+} , Hg^{2+} and Pb^{2+} . It can be seen that the conductometric titration curves shows an decrease in molar conductance of the complexes throughout the titration, until the ligand/ M^{n+} concentration ratio reaches 1:1. Then the molar conductance remains almost constant until the end of the experiment. This is due to the larger size of the complex relative to that of the free cation. Therefore, the mobility of the cation decreases leading to a decrease in the molar conductivity. Sharp break points are observed for Cd^{2+} , Hg^{2+}

and Pb^{2+} and L3 in this solvent at 1: 1 (ligand:metal cation) molar ratio suggesting that relatively strong complexes are formed in acetonitrile between this ligand and these cations. These findings are in agreement with ^1H NMR measurements where significant chemical shift changes were observed by the addition of heavy cations (Cd^{2+} , Hg^{2+} and Pb^{2+}) to the ligand in CD_3CN .

Thermodynamics of complexation

Standard thermodynamics parameters of complexation ($\log K_s$, ΔH° , ΔS° , ΔG°) of L3 with heavy metal cations in acetonitrile were determined using the Nano ITC. For this purpose the instruments were electrically and chemically calibrated prior to measurements. ITC has been used to quantify the affinity of compounds for metal cations. Sessler et al. reported that crown-6 calix[4]arene-capped calix[4] pyrrole contained both cation and anion-recognizing sites and was an ion-pair receptor for CsF through ITC and ^1H NMR investigations [60]. Calorimetric titration curves for the titration of metal cations with the receptor L3 in acetonitrile at 298 K were recorded (Appendix B).

Thermodynamic parameters of complexation of L3 with heavy metal cations in acetonitrile at 298.15 K: Titration calorimetry was used to obtain the $\log K_s$ and the enthalpy of complexation of L3 with cations in acetonitrile. Combination of Gibbs energies and enthalpies led to the calculation of the entropies associated to the complexation process. Thermodynamic data for the complexation of L3 with the cations in acetonitrile are summarized in Table 2.

For this ligand and these metal cations complexation, the stoichiometry n value was equal to 1, an ideal 1:1 metal ligand complex was formed. These findings are corroborated by conductance measurements in acetonitrile, plots of molar conductance against the ligand/metal cation ratio reveal the formation of 1:1 complexes between this ligand with these cations.

A general analysis of the thermodynamic parameters shows that the complexation process is favored in terms of enthalpy ($\Delta H^\circ < 0$) but

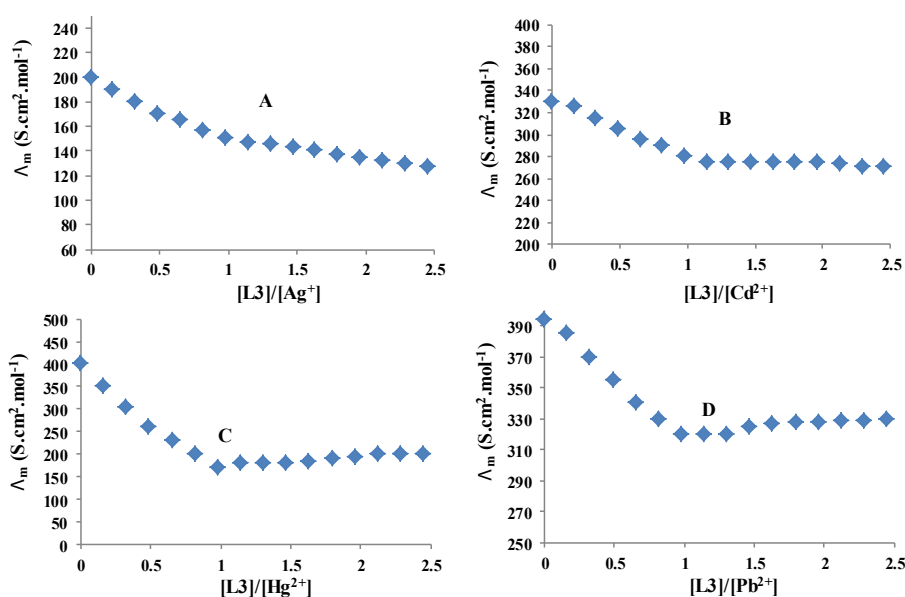


Figure 3: Conductometric curves for the titration of Ag^+ , Cd^{2+} , Hg^{2+} and Pb^{2+} (as tetrabutylammoniumperchlorate) with L3 in acetonitrile at 298.15 K, (A) Ag^+ +L3, (B) Cd^{2+} +L3, (C) Hg^{2+} +L3, (D) Pb^{2+} +L3.

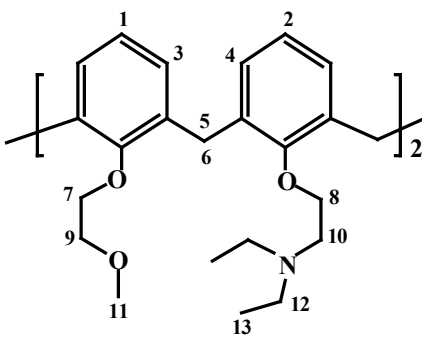
Receptor L3	δ in CD ₃ CN	L3	L3 + Ag ⁺	L3 + Cd ²⁺	L3 + Hg ²⁺	L3 + Pb ²⁺
	H-1	6.60	-0.39	-0.07	-0.12	-0.09
	H-2	6.52	-0.22	0.02	0.02	-0.01
	H-3	6.81	0.31	0.11	0.15	0.1
	H-4	6.67	0.26	0.1	0.15	0.09
	H-5	3.19	0.05	0.11	0.12	0.1
	H-6	4.51	-0.09	-0.13	-0.11	-0.14
	$\Delta\delta_{(ax-eq)}$	1.32	1.18	1.08	1.09	1.08
	H-7	4.16	0.16	0.02	0.04
	H-8	3.98	0.03	-0.23	0.19
	H-9	3.84	-0.23	-0.22
	H-10	2.95	0.37	0.81	0.79
	H-11	3.35	-0.01	-0.03	-0.03	-0.05
	H-12	2.59	0.24	1.02	1.03	1.03
H-13	1.01	0.20	0.30	0.32	0.3	

Table 1: ¹H NMR chemical shift changes observed via the addition of metal cation salts to L3 relative to the free ligand (L3) in CD₃CN at 298 K.

Complexes	Log K	$\Delta_c H^\circ$ (kJ. mol ⁻¹)	$\Delta_c G^\circ$ (kJ. mol ⁻¹)	$\Delta_c S^\circ$ (J. mol ⁻¹ .K ⁻¹)	n
L3 + Ag ⁺	4.80	-35.71	-27.40	-27.87	1.01
L3 + Cd ²⁺	5.73	-43.88	-32.71	-37.46	1.04
L3 + Hg ²⁺	6.50	-60.52	-37.10	-78.55	1.03
L3 + Pd ²⁺	6.01	-51.43	-34.30	-57.62	0.93

Table 2: Thermodynamics of complexation of L3 and metal cations in acetonitrile at 298.15 K.

not in terms of entropy ($\Delta S < 0$) in all the above systems. Therefore, the complexation process is enthalpically controlled.

Conclusions

From the above discussion on the calix[4]arene derivative, the following conclusion can be drawn. The ligand under investigation (L3) were successfully synthesised in good yields and characterized by ¹H NMR. From ¹H NMR studies, it is concluded that L3 interact with Ag⁺, Cd²⁺, Hg²⁺ and Pb²⁺. The presence of the amino groups (basic) in the lower rim for calix[4]arenes makes this ligand attractive for exploring their complexation with these cations. The ¹H NMR techniques was successfully used for establishing the binding sites and the conformational changes that this ligand undergoes upon complexation with the metal cations. The results obtained seem to indicate that the sites of interaction of this ligand with the heavy metal cations are amine group and ethoxy group. Indeed significant chemical shift changes in the proton close to the amine group and ethoxy group were observed. Conductometric measurements were carried out with the aim of determining the composition of the receptor - cation interaction and gaining information regarding the type and strength of interaction of this receptor with these cations in acetonitrile at 298 K. Nano isothermal titration calorimetry is the most powerful tool to determine the enthalpies of binding of various reactions, including cation - ligand binding. Isothermal titration calorimetry (ITC) provides the most accurate and direct measurement of the enthalpy of any reaction under isothermal and isobaric conditions. It is also the only method capable of determining the enthalpy, entropy, and the

Gibbs free energy of a reaction in a single titration experiment. Future work will involve the attachment of the receptor to a solid support to generate recyclable materials for cations removal.

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References

- Vicens J, Bohmer V (1991) Calixarenes: A Versatile Class of Macrocyclic Compounds. Kluwer Academic: Boston.
- Chang SK, Cho I (1984) New Metal Cation-Selective Ionophores Derived From Calixarenes. Chem Lett 13: 477-478.
- Chang SK, Cho I (1986) New metal cation-selective ionophores derived from calixarenes: their syntheses and ion-binding properties. J Chem Soc Perkin Trans 1: 211-214.
- Toumi N, Kajo F, Fournier D, Vocanson F, Lamartine R, et al. (2008) A useful approach towards solid-liquid extraction of metal cations with unsupported calixarenes. Mat Sci Eng C 28: 645-652.
- Benosmane N, Hamdi SM, Hamdi M, Boutemour B (2009) Selective transport of metal ions across polymer inclusion membranes (PIMs) containing calix[4]resorcinarenes. Separ Purif Technol 65: 211-219.
- Yilmaz A, Memon S, Yilmaz M (2002) Synthesis and study of allosteric effects on extraction behavior of novel calixarene-based dichromate anion receptors. Tetrahedron 58: 7735-7740.
- Benounis M, Jaffrezic-Renault N, Halouani H, Lamartine R, Dumazet-Bonnamour (2006) Detection of heavy metals by an optical fiber sensor with a sensitive cladding including a new chromogenic calix[4]arene molecule. Mater Sci Eng C 26: 364-368.

8. Khan AA (2006) Applications of Hg(II) sensitive polyaniline Sn(IV) phosphate composite cation-exchange material in determination of Hg²⁺ from aqueous solutions and in making ion-selective membrane electrode. *Sens Actuators B* 120: 10-18.
9. Marcos PM, Felix S, Ascenso JR, Santos MA, Segurado MAP, et al. (2002) Synthesis, conformational behaviour, alkali and alkaline-earth metal cation extraction and transport studies of p-tert-butylidihomooxalix[4]crowns. *Tetrahedron* 58: 9223-9230.
10. Yang WC, Yu XD, Yu AM, Chen HY (2001) Study of a novel cationic calix[4]arene used as a selectivity modifier in capillary electrophoresis with electrochemical detection. *J Chromatogr A* 910: 311-318.
11. Tong S, von Schirnding YE, Prapamontol T (2000) Environmental lead exposure: a public health problem of global dimensions. *Bull World Health Organ* 78: 1068-1077.
12. Baldwin DR, Marshall WJ (1999) Heavy metal poisoning and its laboratory investigation. *Ann Clin Biochem* 36: 267-300.
13. Horiguchi H, Teranishi H, Niiya K, Aoshima K, Katoh T, et al. (1994) Hypoproduction of erythropoietin contributes to anemia in chronic cadmium intoxication: clinical study on Itai-itai disease in Japan. *Arch Toxicol* 68: 632-636.
14. Antonio MT, Leret ML (2000) Study of the neurochemical alterations produced in discrete brain areas by perinatal low-level lead exposure. *Life Sci* 67: 635-642.
15. Antonio MT, Corredor L, Leret ML (2003) Study of the activity of several brain enzymes like markers of the neurotoxicity induced by perinatal exposure to lead and/or cadmium. *Toxicol Lett* 143: 331-340.
16. Lidsky TI, Schneider JS (2003) Lead neurotoxicity in children: basic mechanisms and clinical correlates. *Brain* 126: 5-19.
17. Fels LM (1999) Risk assessment of nephrotoxicity of cadmium. *Ren Fail* 21: 275-281.
18. Friberg L, Elinder CG, Kjellstrom T, Nordberg GF (1986) *Cadmium and Health: A Toxicological and Epidemiological Appraisal*. CRC Press, Boca Raton.
19. Satarug S, Nishijo M, Ujii P, Vanavanitkun Y, Moore MR (2005) Cadmium-induced nephropathy in the development of high blood pressure. *Toxicol Lett* 157: 57-68.
20. Klassen CD, Amdur MD, Dull J (1986) *Casarett and Doull's Toxicology*, 3rd edn, MacMillan, New York.
21. Vernberg FJ, Vernberg WB (1974) (Eds.), *Pollution and Physiology of Marine Organisms*, Academic Press, New York.
22. Fergusson JE (1990) *Heavy Elements: Chemistry Environmental Impact and Health Effects*, Pergamon Press, Oxford.
23. Kaim W, Schwederski B (1991) *Bioinorganic Chemistry: Inorganic Elements in the Chemistry of life, an Introduction and Guide*, Wiley-Interscience, New York.
24. Benoff S, Jacob A, Hurley IR (2000) Male infertility and environmental exposure to lead and cadmium. *Hum Reprod Update* 6: 107-121.
25. Grigg J (2004) Environmental toxins; their impact on children's health. *Arch Dis Child* 89: 244-250.
26. Deligoz H (2006) Azocalixarenes: Synthesis, Characterization, Complexation, Extraction, Absorption Properties and Thermal Behaviours. *J Incl Phenom Macrocycl Chem* 55: 197-218.
27. Gupta R, Ahuja P, Khan S, Saxena RK, Mohapatra H (2000) Microbial biosorbents: Meeting challenges of heavy metal pollution in aqueous solutions. *Curr Sci* 78: 967-973.
28. Feng D, Aldrich C, Tan H (2000) Treatment of acid mine water by use of heavy metal precipitation and ion exchange. *Miner Eng* 13: 623-642.
29. Asfari Z, Bohmer V, Harrowfield MMCB, Vicens MJ, Calixarenes (2001) Kluwer Academic Publishers, Dordrecht.
30. Crini G, Peindy HN (2006) Adsorption of C.I. Basic Blue 9 on cyclodextrin-based material containing carboxylic groups. *Dyes Pigments* 70: 204-211.
31. Del Valle EMM (2004) Cyclodextrins and their uses: a review. *Process Biochem* 39: 1033-1046.
32. Gutsche CD (1998) *Calixarenes Revisited*, The Royal Society of Chemistry, Cambridge.
33. Yilmaz M, Memon S, Tabakci M, Bartsch RA (2006) in: RK Bregg (Ed.), *New Frontiers in Polymer Research*, Nova Publishers Inc., New York.
34. Arduini A, Pochini A, Reverberi S, Ungaro R (1984) p-t-Butyl-calix[4]arene tetracarboxylic acid. A water soluble calixarene in a cone structure. *Journal of the Chemical Society, Chemical Communications* 15: 981-982.
35. Bohmer V (1995) Calixarenes, Macrocycles with (Almost) Unlimited Possibilities. *Angew Chem Int Ed Engl* 34: 713-745.
36. Vicens J, Harrowfield J (2007) (Eds.), *Calixarenes in the Nanoworld*, Springer, Dordrecht.
37. Vicens J, Bohmer V (1991) *Calixarenes: A Versatile Class of Macrocyclic Compounds*, Kluwer Academic: Boston.
38. Memon S, Yilmaz M, Roundhill DM (2004) Remediation and Liquid-Liquid Phase Transfer Extraction of Chromium(VI). *A Review Collect Czech Chem Commun* 69: 1231-1250.
39. Pathak DA, Street NC, Roundhill DM, Yilmaz M, Memon S, et al. (2006) Computations between Metalocalix[4]arene Host and a Series of Four Oil-Based Fuel Pollutant Guests. *Pak J Anal Environ Chem* 7: 48-55.
40. Chang SK, Kwon SK, Cho I, (1987) *Chem Lett Calixarene-Based Amide Ionophores for Group IIA Metal Cations*. 16: 947-948.
41. Sgarlata C, Zito V, Arena G, Consoli GML, Galante E, et al. (2008) A sinapic acid-calix[4]arene hybrid selectively binds Pb²⁺ over Hg²⁺ and Cd²⁺. *Polyhedron* 28: 343-348.
42. Toumi N, Kajo F, Fournier D, Vocanson F, Lamartine R, et al. (2008) A useful approach towards solid-liquid extraction of metal cations with unsupported calixarenes. *Mat Sci Eng C* 28: 645-652.
43. Pont N, Salvado V, Fontas C (2008) Selective transport and removal of Cd from chloride solutions by polymer inclusion membranes. *J Mem Sci* 318: 340-345.
44. Kadarkaraisamy M, Sykes AG (2007) Selective luminescence detection of cadmium(II) and mercury(II) utilizing sulfur-containing anthraquinone macrocycles (part 2) and formation of an unusual-crown ether dimer via reduction of Hg(II) by DMF. *Polyhedron* 26: 1323-1330.
45. Vigato PA, Tamburini S, Bertolo L (2007) The development of compartmental macrocyclic Schiff bases and related polyamine derivatives. *Coordination Chemistry Reviews* 251: 1311-1492.
46. Memon S, Yilmaz M (2000) Synthesis and complexation studies of ,3-dialkylated p-tert-butylcalix[4]arene telomers. *React And Funct Polym* 44: 227-233.
47. Solangi IB, Memon S, Memon N, I.Bhanger M (2008) Exploration of Pb²⁺-Selective Behavior of Calix[6]arene Ester Derivative. *J Macromol Sci Pure and Appl Chem* 45: 1003-1008.
48. Yordanov AT, Whittlesey BR, Roundhill DM (1998) Calixarenes Derivatized with Sulfur-Containing Functionalities as Selective Extractants for Heavy and Precious Metal Ions. *Inorg Chem* 37: 3526-3531.
49. Arnaud-Neu F, Barret G, Corry D, Cremin S, Ferguson G, et al. (1997) Cation complexation by chemically modified calixarenes. Part 10. Thioamide derivatives of p-tert-butylcalix[4]-, [5]- and [6]-arenes with selectivity for copper, silver, cadmium and lead. X-Ray molecular structures of calix[4]arenethioamide-lead(II) and calix[4]areneamide-copper(II) complexes. *J Chem Soc Perkin Trans 2*: 575-579.
50. Dung NTK, Ludwig R (1999) Solvent extraction of heavy metals with macrocyclic ligands based on calix[4]arenes. *New J Chem* 23: 603-607.
51. Ikeda A, Shinkai S (1997) Novel Cavity Design Using Calix[n]arene Skeletons: Toward Molecular Recognition and Metal Binding. *Chem Rev* 97: 1713-1734.
52. Gutsche CD, Dhawan B, Levine JA, No KH, Bauer LJ (1983) Calixarenes 9: Conformational isomers of the ethers and esters of calix[4]arenes. *Tetrahedron* 39: 409-426.
53. Furniss BS, Hannford AJ, Smith PWG, Tatchell AR (1989) *Vogels Textbook of Practical Organic Chemistry*, 5th Eds.; Logman Scientific and Technical, London.
54. Lind JE, Zwolenik JJ, Fuoss RM (1959) Calibration of Conductance Cells at 25° with Aqueous Solutions of Potassium Chloride. *J Am Chem Soc* 81: 1557-1559.
55. Wiseman T, Williston S, Brandts JF, Lin LN (1989) Rapid measurement of binding constants and heats of binding using a new titration calorimeter. *Anal Biochem* 179: 131-137.

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56. Gill DS, Roush DJ, Shick KA, Willson RC (1995) Microcalorimetric characterization of the anion-exchange adsorption of recombinant cytochrome b5 and its surface-charge mutants. *J Chromatogr A* 715: 81-93.
57. Briggner LE, Wadso I (1991) Test and calibration processes for microcalorimeters, with special reference to heat conduction instruments used with aqueous systems. *J Biochem Biophys Methods* 22: 101-118.
58. Gutsche CD, Lin LG (1986) Calixarenes 12: The synthesis of functionalized calixarenes. *Tetrahedron* 42: 1633-1640.
59. Alekseeva EA, Basok SS, Rakipov IM, Mazepa AV, Gren AI (2013) Specific features of the reduction of disubstituted amide derivatives of p-tert-butylcalix[4]arene. *Russian Journal of Organic Chemistry* 49: 1035-1041.
60. Sessler JL, Kim SK, Gross DE, Lee CH, Kim JS, et al. (2008) Crown-6-calix[4]arene-capped calix[4]pyrrole: an ion-pair receptor for solvent-separated CsF ions. *J Am Chem Soc* 130: 13162-13166.