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4-*tert*-butylcalix[4]arene having nitrile pendant groups as Hg^{2+} selective receptors

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We synthesized a series of 4-*tert*-butylcalix[4]arene nitriles (**5–7**) by the reaction of 4-*tert*-butylcalix[4]arene (**1**) with 4′-(6-bromohexyloxy)biphenyl-4-carbonitrile (**2**), 4′-(10-bromodecyloxy)biphenyl-4-carbonitrile (**3**), and 4′-(12-bromododecyloxy)biphenyl-4-carbonitrile (**4**) and characterized their structures, respectively. The extraction abilities of newly synthesized **5–7** toward some selected heavy metal cations, such as Co^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, and Hg^{2+}, were first evaluated and compared by the solvent extraction method. The extraction results revealed that **5–7** were efficient and selective cation receptors for Hg^{2+} over-selected cations. On the other hand, the complexation behavior of Hg^{2+} with **5–7** was also investigated by using NMR, UV–vis, and IR spectroscopic methods.

1. Introduction

The presence of heavy metals in the environment has been of great concern because of their growing discharge, toxicity, and other adverse effects on receiving waters. Mercury, a commonly occurring environmental contaminant, is a major pollutant in land, water, living bodies, and so on. This metal is so volatile that it can easily be exposed to the human environment. Industrial wastes and mineral deposits are the major sources of mercury contaminations. Mercury hazards to living organisms such as marine samples are due to the accumulation and biomagnification characteristic of this toxic element that can influence the entire food chain and the humans who consume marine food [1–3].

From an environmental and economic viewpoint, there remains a need to discover more selective and effective extractants for such toxic heavy metals, especially Hg^{2+}. Calixarenes [4–9] are used as one kind of very efficient extractants for Hg^{2+} via coordination in general [10–15]. Calixarenes are metacyclophanes that consist of phenolic and methylene units and versatile ionophores, which provide a platform for the attachment of convergent binding groups that create host molecules primarily for the attraction of simple cations, anions, and small molecules [16–23]. Since their description by Gutsche in the 1980s, many calixarene derivatives have been described due to the ease of modification of the so-called upper and lower rims of the calixarene's central annulus. The most noteworthy achievement of these structural modifications has been the advent of many cation selective host compounds [24–28].

It is known that flexible calix[4]arenes exist in equilibrium of different conformations during complexation with metal cations in solutions; they tend to adopt the shapes which are the most appropriate for accommodation of the guests. In particular, the *cone* was found [29] to be one of the most preferred conformations of calix[4]arene derivatives in their complex with Hg^{2+}. The 4-*tert*-butylcalix[4]arene family that is substituted at the lower rim has proven especially popular for this purpose, yielding highly preorganized and symmetrical supramolecular structures that are often restricted to a *cone* conformation due to upper-rim bulky *tert*-butyl groups [4–9]. In previous cases, where nitrile groups were incorporated into a molecular backbone such as a calixarene or a bis-calixarene [11], these compounds were used (in monomeric form or attached to a polymer backbone) to perform multiphase extraction experiments of metals. Hg^{2+}, in particular, has been found to be extracted well in most cases. The best Hg^{2+} selectivity has been observed for nitrile substituents [30]. Moreover, due to their excellent binding characteristics (selectivity, reversibility, and rapid kinetics), their potential as active agents in chemical sensors has been recognized at an early stage and, in particular, several derivatives have provided the basis for successful ion-selective electrodes [31–36].

In general, the oxygen ligands are effective extractants for alkali and alkaline earth metals. By contrast, the nitrogen or sulfur ligands have shown a potential ability of forming stable

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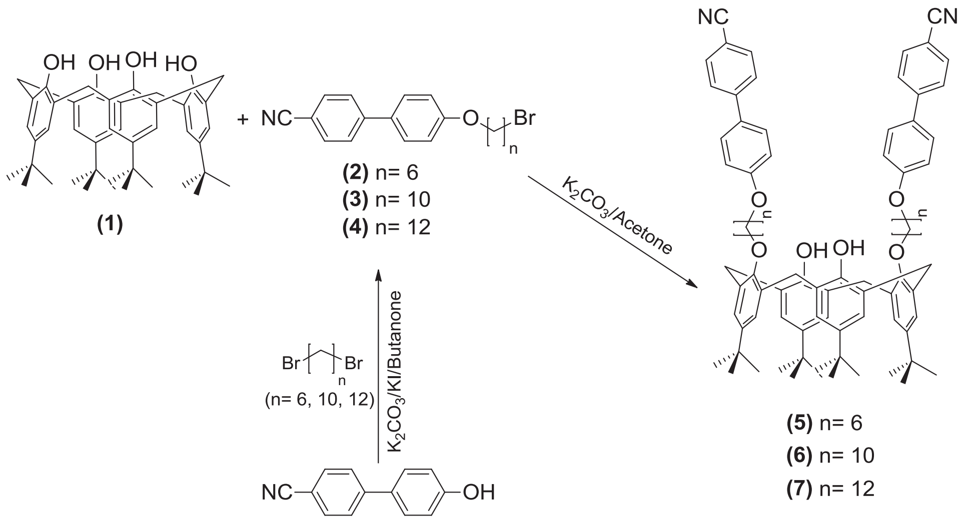

Scheme 1. Overall synthesis procedure .

complexes with soft metal cations [16–23], such as Hg^{2+} and Pb^{2+}. In view of the studies just referred to and our previous experiences [10–23], we report an investigation in this study regarding the synthesis and complexation of 4-*tert*-butylcalix[4]arenes bearing nitrile moieties (Scheme 1) for some selected heavy metal cations, such as Co^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, and Hg^{2+}.

2. Experimental section

2.1. Equipments and instruments

Melting points were determined using an Electrothermal 9100 apparatus in a sealed capillary and were uncorrected. NMR spectra were recorded on a Bruker 400 MHz spectrometer in $CDCl_3$, with TMS as the internal standard. IR spectra were obtained on a Perkin–Elmer Spectrum 100 IR spectrometer. Metal contents of samples in extraction studies were determined by Perkin–Elmer ICP-MS, Elan DRC-e. UV–visible spectra were obtained on PG Instruments T80+ UV–visible recording spectrophotometer. Elemental analyses were performed using a Leco CHNS-932 analyzer. FAB-MS spectra were taken on a Varian MAT 312 spectrometer. Analytical TLC was performed using Merck prepared plates (silica gel 60 F254 on aluminum). The chromatographic separations were performed on a Merck Silica Gel 60 (230–400 mesh). All reactions were conducted under a nitrogen atmosphere. All starting materials and reagents used were of a standard analytical grade from Fluka, Merck, and Sigma-Aldrich, and they were used without further purification. The commercial grade solvents were distilled and stored over molecular sieves. The drying agent employed was anhydrous sodium sulfate. All aqueous solutions were prepared with deionized water that had been passed through a Millipore milli-Q Plus water purification system.

2.2. Synthesis

2.2.1. General procedure

The preparation of **5–7** was carried out using the following general procedure. A solution of 4-*tert*-butylcalix[4]arene (1.0 mmol), 4′-(*n*-bromoalkyloxy)biphenyl-4-carbonitrile (3.0 mmol), K_2CO_3 (3.0 mmol), and dry acetone (75 mL) was refluxed for four days. The mixture was cooled and filtered off. The solvent was then evaporated under reduced pressure, and the residue was purified by column chromatography (CH_2Cl_2/MeOH, 10/0.5).

2.2.2. 5,11,17,23-Tetra-tert-butyl-25,27-di-[4′-hexyloxy-4-biphenylcarbonitrilo]-26,28-dihydroxycalix[4]arene (**5**)

Yield 47%; mp: 191 °C; IR: 2224 cm^{-1} (CN); ^1H NMR ($CDCl_3$, 400 MHz): δ 7.72 (s, 2H, ArOH), 7.67 (d, 4H, H1, J= 8.63 Hz), 7.60 (dd, 4H, H2, J= 8.65 Hz), 7.46 (d, 4H, H3, J=8.84 Hz), 7.06 (s, 4H, ArH), 6.95 (d, 4H, H4, J=8.85 Hz), 6.84 (s, 4H, ArH), 4.30 (d, 4H, $ArCH_2Ar$, J=12.85 Hz), 4.03 (t, 4H, H10, J=6.50 Hz), 3.99 (t, 4H, H5, J=6.44 Hz), 3.32 (d, 4H, $ArCH_2Ar$, J=12.93 Hz), 2.05 (p, 4H, H9, J=6.89 Hz), 1.90 (p, 4H, H6, J=6.99 Hz), 1.80 (p, 4H, H8, J=7.53 Hz), 1.65 (p, 4H, H7, J=7.41 Hz), 1.29 (s, 18H, Me_3C), 1.00 (s, 18H, Me_3C); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 159.74 (i), 150.75 (Ar), 149.88 (Ar), 146.80 (Ar), 145.20 (e), 141.39 (Ar), 132.71 (Ar), 132.54 (c), 131.13 (f), 128.27 (g), 127.79 (d), 126.99 (Ar), 125.49 (Ar), 119.10 (a), 115.05 (h), 110.0 (b), 76.14 (l), 68.00 (j), 33.98, 33.82 (Me_3C), 31.79, 31.72 (Me_3C), 31.07 ($ArCH_2Ar$), 29.97, 29.20, 25.85, 25.71 (k) (see supplementary material for letter assignments), FAB-MS m/z: 1226.44 $(M+Na)^+$; Anal. calcd for $C_{82}H_{94}N_2O_6$ (1203.63): C, 81.83; H, 7.87; N, 2.33. Found: C, 81.75; H, 7.80; N, 2.31.

2.2.3. 5,11,17,23-Tetra-tert-butyl-25,27-di-[4′-decyloxy-4-biphenylcarbonitrilo]-26,28-di-hydroxycalix[4]arene (**6**)

Yield 43%; mp: 144°C; IR: 2225 cm^{-1} (CN); ^1H NMR ($CDCl_3$, 400 MHz): δ 7.79 (s, 2H, ArOH), 7.68 (d, 4H, H1, J=8.46 Hz), 7.62 (d, 4H, H2, J=8.40 Hz), 7.52 (d, 4H, H3, J=8.72 Hz), 7.05 (s, 4H, ArH), 6.99 (d, 4H, H4, J= 8.74 Hz), 6.85 (s, 4H, ArH), 4.31 (d, 4H, $ArCH_2Ar$, J= 12.82 Hz), 4.04–3.92 (overlapped, 8H, H5, H14), 3.31 (d, 4H, $ArCH_2Ar$, J= 12.91 Hz), 2.03 (p, 4H, H13, J= 6.90 Hz), 1.81 (p, 4H, H6, J=6.94 Hz), 1.68 (p, 4H, H12, J=7.27 Hz), 1.56–1.33 (overlapped, 20H, H8, H9, H10, H11, H7, resp.), 1.29 (s, 18H, Me_3C), 1.01 (s, 18H, Me_3C); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 157.53 (i), 148.54 (Ar), 147.76 (Ar), 144.42 (Ar), 142.96 (Ar), 138.99 (e), 130.53 (Ar), 130.28 (Ar), 128.92 (c), 126.03 (f), 125.54 (g), 124.76 (d), 123.19 (Ar), 122.77 (Ar), 116.84 (a), 112.79 (h), 107.74 (b), 65.88 (j), 76.21 (l), 31.70, 31.54 (Me_3C), 29.58, 29.45 (Me_3C), 28.82 ($ArCH_2Ar$), 27.75, 27.37, 27.34, 27.25, 27.16, 26.97, 23.81, 23.74 (k) (see supplementary material for letter assignments); FAB-MS m/z: 1338.67 $(M+Na)^+$; Anal. calcd for $C_{90}H_{110}N_2O_6$ (1315.85): C, 82.15; H, 8.43; N, 2.13. Found: C, 82.07; H, 8.36; N, 2.10.

2.2.4. 5,11,17,23-Tetra-tert-butyl-25,27-di-[4′-dodecyloxy-4-biphenylcarbonitrilo]-26,28-di-hydroxycalix[4]arene (**7**)

Yield 41%; mp: 131 °C; IR: 2242 cm^{-1} (CN); ^1H NMR ($CDCl_3$, 400 MHz): δ 7.81 (s, 2H, ArOH), 7.69 (d, 4H, H1, J= 8.65 Hz), 7.63

(d, 4H, H2, $J=$ 8.56 Hz), 7.52 (d, 4H, H3, J=8.83 Hz), 7.05 (s, 4H, ArH), 6.98 (d, 4H, H4, J=8.81 Hz), 6.85 (s, 4H, ArH), 4.31 (d, 4H, $ArCH_2Ar$, J=12.85 Hz), 4.06–3.91 (overlapped, 8H, H5, H16), 3.31 (d, 4H, $ArCH_2Ar$, J=12.91 Hz), 2.03 (p, 4H, H13, $J=$ 6.86 Hz), 1.81 (p, 4H, H6, $J=$ 7.00 Hz), 1.66 (p, 4H, H12, J=7.39 Hz), 1.55–1.31 (overlapped, 28H, H7–H13), 1.29 (s, 18H, Me_3C), 1.01 (s, 18H, Me_3C); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 159.46 (i), 150.45 (Ar), 149.70 (Ar), 146.28 (Ar), 144.89 (Ar), 140.87 (e), 132.40 (Ar), 132.16 (Ar), 130.86 (c), 127.91 (f), 127.48 (g), 126.67 (d), 125.06 (Ar), 124.64 (Ar), 118.69 (a), 114.73 (h), 109.69 (b), 67.82 (j), 76.13 (l), 33.60, 33.57 (Me_3C), 31.47, 31.34 (Me_3C), 30.70 ($ArCH_2Ar$), 29.65, 29.37, 29.34, 29.32, 29.29, 29.26, 29.25, 29.18, 29.05, 28.88, 25.70, 25.66 (k) (see supplementary material for letter assignments); FAB-MS m/z: 1394.74 $(M+Na)^+$; Anal. calcd for $C_{94}H_{118}N_2O_6$ (1371.95): C, 82.29; H, 8.67; N, 2.04. Found: C, 82.19; H, 8.60; N, 2.02.

2.3. Solvent extraction studies

The extraction experiments of **2–7** were performed following Pedersen's procedure [37]. An aqueous solution of metal nitrate (10 mL of a 2.5×10^{-5} mol L^{-1}) and calixarene receptor (10 mL of 1.0×10^{-3} mol L^{-1}) in CH_2Cl_2 was shaken vigorously in a stoppered glass tube with a mechanical shaker for 2 min; it was then magnetically stirred in a thermostated water bath at 25 °C for 1 h; and, finally, left to stand for an additional 30 min. Blank experiments showed that no metal extraction occurred in the absence of 4-*tert*-butylcalix[4]arene nitrile derivatives. The concentration of the metal ion remaining in the aqueous phase after the extraction was then determined by ICP-AES [38].

3. Results and discussion

3.1. Synthesis

4-*tert*-butylcalix[4]arene **1** and nitrile derivatives **2–4** were prepared by the previously described methods [4–9,39], whereas the nitrile-substituted calix[4]arenes **5–7** were first synthesized in this work. The characterization of compounds for the confirmation of their structure and purity was made by various techniques, such as TLC, mp, IR, NMR, FAB-MS, and elemental analysis. The reaction of **1** with 4′-(*n*-bromoalkyloxy)biphenyl-4-carbonitrile derivatives (**2–4**) in the presence of K_2CO_3 in dry acetone gave **5–7** in 47, 43, and 41% yields, respectively. Following the "De Mendoza rule" [40], the compounds **5–7** were confirmed present in the *cone* conformation by a detailed study of their ^1H NMR spectra (doublets at 3.32 ppm and 4.30 ppm, at 3.33 ppm and 4.32 ppm, at 3.30 ppm and 4.30 ppm, J=12.8 Hz for $ArCH_2Ar$ protons, respectively). The other peaks in ^1H NMR and ^{13}C NMR spectra of **5–7** also confirmed the substitution of monomeric nitrile derivatives (**2–4**). Moreover, APT NMR spectra of **5–7** were recorded for the identification of the overlapped peaks (see Supplementary data).

3.2. Solvent extraction

A preliminary evaluation of the extraction efficiencies of **5–7** was carried out by the solvent-extraction method [37] from water into dichloromethane for transition metal cations, such as Co^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, and Hg^{2+}. The results (Fig. 1) revealed that all new calix[4]arene nitrile derivatives (**5–7**) showed selective affinity toward Hg^{2+} over selected metal cations; however, they had a small amount of affinity toward the other cations. To understand the chelating effect of both nitrile fragments in Hg^{2+} binding, non-cyclic monomeric analogs (**2–4**) were used. It was observed that Hg^{2+} cation was extracted only in a trace amount by **2–4**. Based on the results, we have concluded that the calix[4]arene unit plays a very important role in confirming the cooperative participation of both the peripheral nitrile groups.

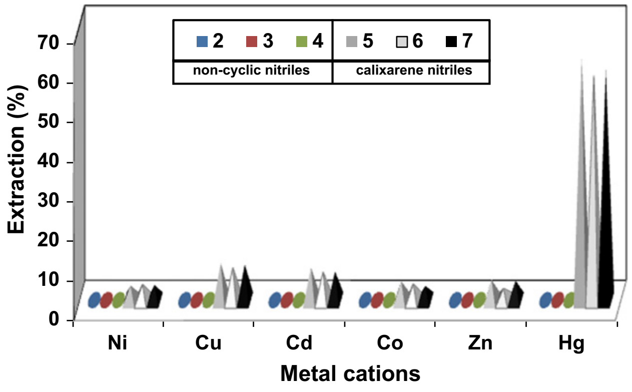

Fig. 1. Extraction percentages of selected metal cations by **2–7** at 25 °C.

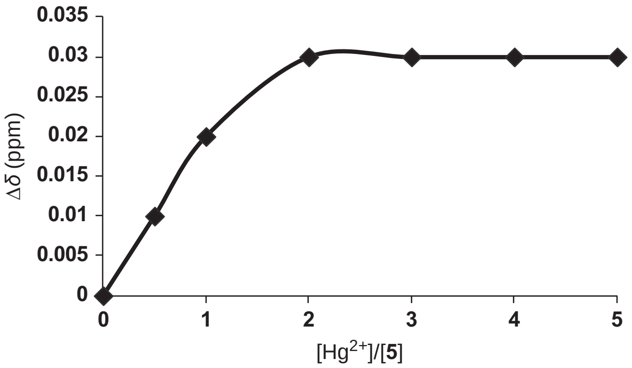

Fig. 2. ^1H NMR titration curve of **5** with Hg^{2+}.

From previous studies [10–15,30], it is apparent that nitrile functionalities containing calixarenes are selective extractants for Hg^{2+} due to the soft nature of Hg^{2+} as compared with competing cations. The effectiveness in transferring these cations by **5–7** indicates that the binding ability toward metal cations belongs to soft acids in Pearson's classification. This is consistent with the HSAB principle, because the nitrile group is more polarizable than are phenolic oxygens. Furthermore, the aromatic benzene rings may also reduce the selective nature of the nitrile functionalities. Consequently, this study reveals that new calix[4]arene nitrile derivatives present a high selective affinity toward Hg^{2+}, which supports its utility as an extractant or potential candidate material for fabricating membranes and sensors, which can separate or detect Hg^{2+} in a high selectivity.

To obtain further information on the Hg^{2+} binding behavior of new calix[4]arene nitriles, namely related to stoichiometry and/or binding mode of the complexation, ^1H NMR, UV–visible, and IR experiments were performed with **5** as a model compound. Variable amounts of Hg^{2+} were added to a solution of **5** in d_6-DMSO/$CDCl_3$, and ^1H NMR spectra were recorded after each addition. When Hg^{2+} was increased to the ligand in a molar ratio of 2:1, the biggest shifts of all the protons on the calix skeleton were observed. When the amount of Hg^{2+} was increased to 3:1 (metal:ligand), chemical shifts of all the protons were not further changed compared with those of ratio 2:1, suggesting that a 2:1 complex was formed (Fig. 2) (see Supplementary data).

UV–visible experiments were initially made to check out the complexation behavior of **5** in CH_3CN (1.0×10^{-5} mol L^{-1}) for Hg^{2+} as $Hg(ClO_4)_2$. The UV–visible spectra of **5** (free) show a strong band at 293 nm, which is attributed to $\pi\rightarrow\pi^*$ transition (Fig. 3). On the other hand, $Hg(ClO_4)_2$ does not exhibit a remarkable increase of absorbance under experimental conditions. Generally, an enhancement in the intensity of the band or the appearance of new bands after complexation is an informative sign of the complex formation. However, in this case, the addition of Hg^{2+} ion (10 equiv) to the solution of **5** causes a dramatic increase of absorbance at 293 nm. This increase could be assigned to metal–ligand charge transfer (MLCT) absorptions between Hg^{2+} and the nitrogen atoms of nitrile functions on a macrocycle.

We attempted to investigate the quantitative analytical behavior of **5** (1.0×10^{-5} mol L^{-1}) for Hg^{2+} complexation. The absorption profile as a function of metal ion concentration was obtained; this was followed by an increase in the intensity of absorbance with regard to increased Hg^{2+} concentration (Fig. 4). After 10 equiv, the intensity did not significantly change, which implies that **5** could quantitatively intimate the concentration of Hg^{2+} up to 1.0×10^{-4} mol L^{-1}. The continuous variation method was applied by varying the concentration of both ligand **5** and Hg^{2+} to determine the stoichiometry of **5**–Hg^{2+} complex. Fig. 4 shows typical Job's plots of **5**–Hg^{2+} complexation at 293 nm. The maximum point of the mole fractions was found to be about 0.66, which refers to a ligand–metal ratio of 1:2 in the complex.

Nonetheless, all the NMR and UV–visible studies related to the complexation behavior of Hg^{2+} by **5** suggest that new calix[4]arene nitriles may be used as potential Hg^{2+} selective ionophores. Thus, according to Job's plot analysis as discussed earlier, Hg^{2+} forms 1:2 (ligand:metal) complex with **5**. This is the first study when the stochiometries of previous calix[4]arene–Hg^{2+} complexes are considered, according to the best of our knowledge. Therefore, the proposed mechanism for metal–ligand interaction is shown in Fig. 5. In addition, the association constants of Ni^{2+}, Cu^{2+}, Cd^{2+}, Co^{2+}, Zn^{2+}, and Hg^{2+} with **5** were calculated from UV titration experiments by Benesi–Hildebrand plots [41], and presented in Table 1 (see Supplementary data).

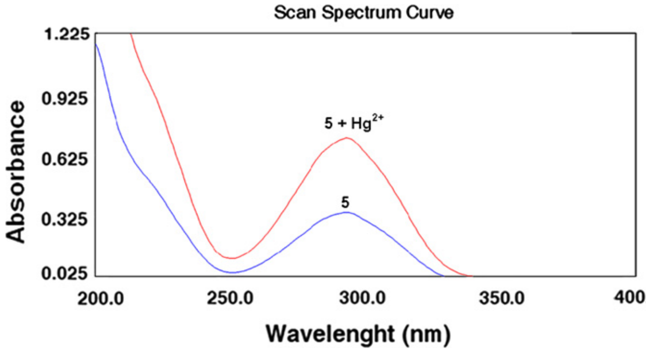

Fig. 3. Comparative UV–visible spectra of **5** (1.0×10^{-5} mol L^{-1}) before and after interacting with Hg^{2+} (10 eq).

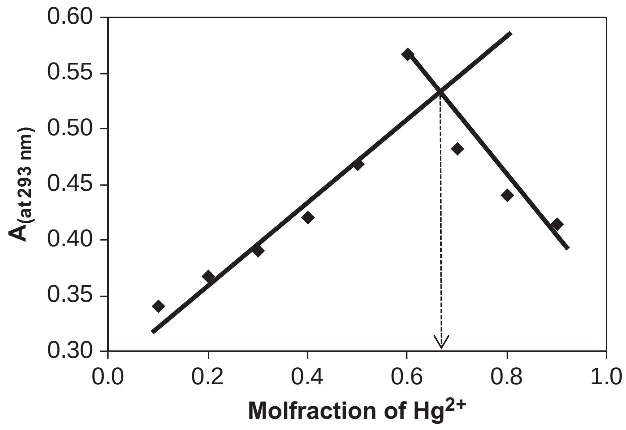

Fig. 4. Job's plot of **5** and Hg^{2+} evaluated from UV–visible titration experiment of **5** (1.0×10^{-5} mol L^{-1}) upon addition of various equivalents of $Hg(ClO_4)$.

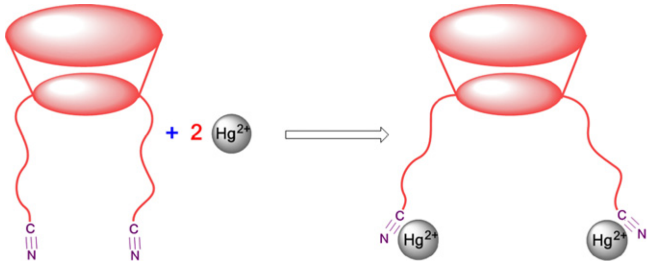

Fig. 5. Proposed structure of the complexes of Hg^{2+} formed with the calix[4]arene nitrile derivatives (**5–7**).

Table 1
Association constants (K_a) of **5** with Ni^{2+}, Cu^{2+}, Cd^{2+}, Co^{2+}, Zn^{2+} and Hg^{2+}.

Cations	Ni^{2+}	Cu^{2+}	Cd^{2+}	Co^{2+}	Zn^{2+}	Hg^{2+}
K_a	1100	2604	2420	1040	1196	4180

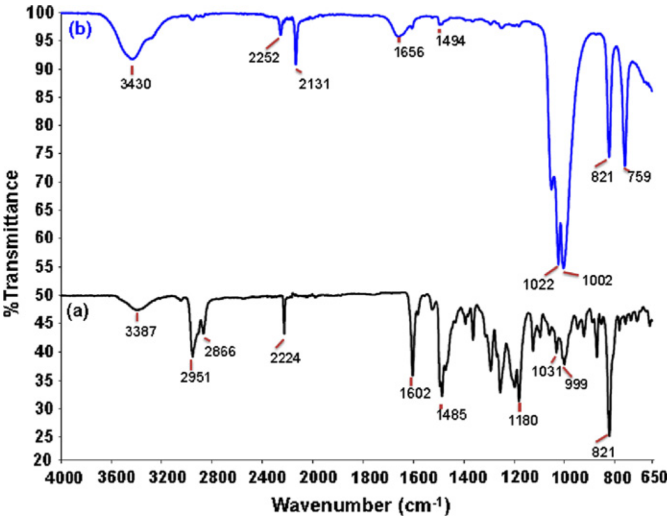

Fig. 6. Comparative IR spectral analysis of (a) compound **5** (——) and (b) **5**–Hg^{2+} complex (——).

The binding mode of **5** with Hg^{2+} was further confirmed through IR spectroscopic analysis, as it provides strong evidence of complexation between ligand and metal ion. The IR spectrum showed characteristic bands of this ligand (cm^{-1}) at 2951 $\upsilon(CH_2)$, 2866 $\upsilon(CH_2)$, and 2224 $\upsilon(CN)$. The coordination of the Hg^{2+} ion can be readily verified in the IR spectrum of the complexes. Fig. 6 clearly gives the stronger indication for complexation, because the shifting in various frequencies of specific functional groups occurred as a result of the introduction of Hg^{2+} into ionophoric cavity. For example, a prominent band at 2224 cm^{-1} was shifted to 2252 cm^{-1} for nitrile moieties, and the marked changes appeared in the region of 900 cm^{-1}, where a considerable decrease in the intensity of the band at 1485 cm^{-1} and the appearance of a new broad band at 1656 cm^{-1} complexation indicates the involvement of N donor atoms of nitrile groups in the complexation process with Hg^{2+}.

4. Conclusions

In conclusion, the chemosensing potential of new calix[4]arene nitrile derivatives (**5–7**) was found with high selectivity toward Hg^{2+} among a series of selected metal ions that attributed to the changes in the intensities of spectral lines observed in NMR, UV–visible, and IR spectra of **5**–Hg^{2+} complex. Moreover, the results revealed that the complexation stochiometry between calix[4]arene nitrile derivatives and Hg^{2+} was novel with 1:2 (ligand:metal) when it was compared with previous calix[4]arene–Hg^{2+} complexes. Consequently, the current study may be treated as a test for the detection of Hg^{2+}. It will find its applicability in various fields of analytical and environmental chemistry.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.12.021.

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