



A novel benzothiazole based azocalix[4]arene as a highly selective chromogenic chemosensor for Hg²⁺ ion: A rapid test application in aqueous environment

Haluk Bingol*, Erdal Kocabas, Erhan Zor, Ahmet Coskun

Selcuk University, Chemistry Department, 42099 Meram/Konya, Turkey

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ABSTRACT

A novel calix[4]arene derivative containing benzothiazole azo groups at the upper rim was synthesized as chromogenic chemosensor, and its binding and sensing properties with heavy metal ions (Pb²⁺, Hg²⁺, Ni²⁺, Cd²⁺, Cu²⁺, Zn²⁺, Co²⁺, Fe²⁺, Mn²⁺, Cr³⁺, Ag⁺) were investigated by UV–vis spectroscopy and voltammetric techniques. The results of spectroscopic and voltammetric experiments showed that the chromogenic chemosensor has high selectivity towards Hg²⁺ ion over the other heavy metal ions. Moreover, it was shown that the interaction between Hg²⁺ and the chromogenic chemosensor occurs by means of the benzothiazole azo groups at the upper rim by using differential pulse voltammetry. The stoichiometric ratio and the association constant were determined as 1:1 and $(6.1 \pm 0.3) \times 10^5 \text{ L mol}^{-1}$ for the complex between Hg²⁺ and the ionophore. Furthermore, we prepared a rapid test kit for early detection of Hg²⁺ in aqueous environment in the concentration range of 1×10^{-4} to 1×10^{-2} M.

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1. Introduction

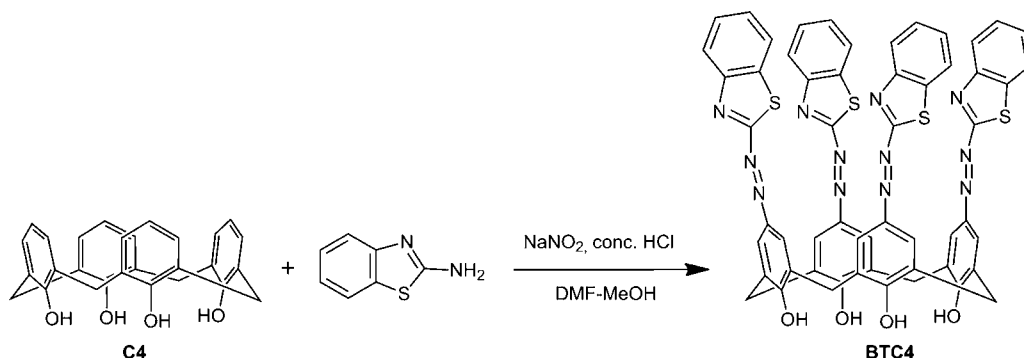
Since the pioneering work of Cram [1], “Host–guest chemistry” has been primarily correlated with the non-covalent interactions between host and guest. Hosts may be acyclic or macrocyclic compounds and may possess cavities into which the guest fits. There are various species of macrocyclic compounds, such as cyclodextrins [2], calixcrowns [3], crownethers [4,5] and calixarenes [6] in host–guest chemistry. The importance of the design and synthesis of new macrocyclic compounds to recognize metal ions has been a considerable subject due to their fundamental role in biological, environmental, and chemical processes. Especially, the using of them as chemosensors has been one of the most interesting recent studies [3,7,8]. One must not be forgotten that chemical sensors, which are defined as simple host molecules “sensors” by chemists, are supposed to be useful devices that have to perform a near-automated chemical analysis task in a relatively complex sample due to the concepts of their high selectivity, reversibility and reliability [9].

In the field of host–guest macrocyclic chemistry, calixarenes are shown one of the most useful molecular scaffolds for the develop-

ment of chromoionophores, especially for metal ion recognition [10]. The molecular recognition is an important subject defining relationships between host and guest compounds occurring in chemical reactions and physicochemical interactions [11]. In specific applications of chemical sensors, most of the calixarene derivatives are functionalized from the lower rim or upper rim [12–15] to control the conformations of the calixarenes, to increase their solubility and to enhance the selectivity and efficiency of their complexation behaviors. In addition to this, the derivatized chromogenic chemosensors show a color change upon specific metal ion complexation, so electrochemical transduction gains low operational costs and considerable reliability of the results due to the observing color changes which can be detected by “naked eye” [12,15].

While there are lots of studies about chromogenic chemosensors based on calix[4]arenes having selectivity upon alkali, alkaline earth and heavy metal ions [12,15–17], there are only a few reports on chromogenic sensing of soft heavy metal ion, such as Hg²⁺ ion [18,19]. We, herein, considered the information presented above, and synthesized a novel azocalix[4]arene derivative, benzothiazole azocalix[4]arene (BTC4), as chromogenic chemosensor having good selectivity towards Hg²⁺ ion. The optical and electrochemical behaviors of BTC4 in the presence of various heavy metal cations were investigated by UV–vis spectroscopy and voltammetric techniques which are cyclic voltammetry and differential pulse voltammetry.

* Corresponding author. Tel.: +90 332323 8220x5499; fax: +90 332323 8225.
E-mail addresses: halukbingol@gmail.com (H. Bingol), ekocabas50@gmail.com (E. Kocabas), zorerhan@gmail.com (E. Zor), acoskun42@gmail.com (A. Coskun).



Scheme 1. Synthesis of benzothiazole azocalix[4]arene (*BTC4*).

2. Experimental

2.1. Chemicals and apparatus

Unless otherwise noted, chemicals were obtained from global suppliers (Merck or Aldrich) and were used without further purification. Solvents were of HPLC or analytical grade and they were dried with molecular sieves (3 Å). All the heavy metal salts used as perchlorate compounds (MClO_4 , $\text{M} = \text{Pb}^{2+}$, Hg^{2+} , Ni^{2+} , Cd^{2+} , Cu^{2+} , Zn^{2+} , Co^{2+} , Fe^{2+} , Mn^{2+} , Cr^{3+} , Ag^+) were dried prior to use over P_2O_5 . Ultra-pure water was freshly obtained from Direct-Q3 UV water purification system (Millipore, USA).

Melting point was recorded on an EZ Melt-MPA120 capillary apparatus. Infrared measurements were performed on Perkin-Elmer 100. ^1H NMR spectra were recorded on a Varian 400. UV-vis absorption spectra were obtained on Shimadzu UV-1800 Double Beam Spectrophotometer. All of the electrochemical measurements were made with PAR 263/A2 (Princeton Applied Research, USA) potentiostat/galvanostat combined with BAS C3 electrochemical cell stand.

2.2. Procedure

2.2.1. Synthesis of benzothiazole azocalix[4]arene (*BTC4*)

Chromogenic calixarene, *BTC4*, was firstly obtained (Scheme 1) by the following procedure. A solution of benzothiazole diazonium chloride was prepared from 2-aminobenzothiazole (2.253 g, 15.0 mmol), sodium nitrite (1.035 g, 15.0 mmol) and conc. HCl (7 mL) in water (25 mL), and was added slowly into a cold (0–5 °C) solution of calix[4]arene (1.446 g, 3.41 mmol), coded as C4, and sodium acetate trihydrate (6.124 g, 45 mmol) in MeOH–DMF (15 mL, 5:8, v/v) to give an dark brown suspension. After allowing it to stand for 18 h at 0–5 °C, the suspension was acidified with aqueous HCl (50 mL, 2 M). The resulting dark brown precipitate was filtered and washed with water and methanol. The crude product was purified by chromatography column on silica gel ($\text{CHCl}_3/\text{MeOH}$: 10/1). Yield 1.494 g (41%), m.p. (decomp.) 298 °C. (Found: C, 61.76; H, 3.04; N, 14.87; S, 11.21%. $\text{C}_{56}\text{H}_{36}\text{N}_{12}\text{O}_4\text{S}_4$: requires C, 62.91; H, 3.39; N, 15.72; S, 12.00%; $\nu_{\text{max}}/\text{cm}^{-1}$: 3319, 3032, 2350, 1581. $\delta_{\text{H}}(400 \text{ MHz}; \text{DMSO-}d_6, \text{Me}_4\text{Si})$ 8.30 (4 H, s, Ar–OH), 7.74 (8 H, s), 7.09 (8 H, d, J 7.53, Ar–H), 6.62 (8 H, d, J 7.53, Ar–H), 3.89 (4 H, br s), 3.35 (4 H, br s).

2.2.2. UV-Vis spectroscopy

Stock solutions of *BTC4* and the heavy metal perchlorates were prepared 2×10^{-4} and 2×10^{-2} M in DMF, respectively. For the absorbance spectrum of *BTC4*, the concentration was diluted to 2×10^{-5} M with DMF. Then, we prepared a 3 mL volume of a solution containing the same final concentrations of *BTC4* and the desired amount of metal ions. The absorbance spectrum of the

mixed solution was recorded against DMF at the end of the completed reaction between metal ions and *BTC4*.

2.2.3. Electrochemical measurements

Cyclic and differential pulse voltammetry were performed in a glass cell with a conventional three-electrode configuration in DMF including 0.1 M tetrabutylammonium perchlorate (TBAP, Aldrich) as supporting electrolytes. As working electrode, a glassy carbon electrode (GCE) having surface diameter of 2 mm was used together with a platinum wire as counter electrode. In all cases, the reference electrode was Ag/Ag⁺ (0.1 M), separated from the solution by a vycor plug and was standardized for the redox couple, ferricinium/ferrocene, ($E_{1/2} = 0.480 \text{ V}$, $\Delta E_p = 61\text{--}67 \text{ mV}$ at 100 mV s^{-1}) under the experimental conditions [20]. The cyclic voltammogram (CV) was recorded at potential range of 1.70 to -2.00 V and at scan rates between 0.05 and 0.4 V s^{-1} , whereas the differential pulse voltammogram (DPV) was obtained at anodic range. Prior to each experiment, GCE was polished with 1.0, 0.3 and $0.05 \mu\text{m}$ alumina powder (PACE Technologies, USA) on a felt pad, and then rinsed with water. Residual alumina particles were removed by sonicating with water and methanol, respectively, in an ultrasonic cleaner (Sonorex Super RK 106, Germany) for 10 min. Then, the electrode was dried and washed with pure DMF before use.

Solutions of the heavy metal perchlorates and *BTC4* in DMF including 0.1 M TBAP were prepared. For electrochemical measurements, the desired amount of the metal solution was transferred into *BTC4* solution (1.0×10^{-3} M) each time. All solutions were purged with nitrogen steam for 30 min before measurement. The procedure was performed at room temperature and a nitrogen atmosphere was maintained over the solution during the measurements.

3. Result and discussion

3.1. Chromogenic properties

The colorimetric sensing ability of *BTC4* for heavy metal ions (Pb^{2+} , Hg^{2+} , Ni^{2+} , Cd^{2+} , Cu^{2+} , Zn^{2+} , Co^{2+} , Fe^{2+} , Mn^{2+} , Cr^{3+} , Ag^+) in DMF was monitored by naked eye (visual) and UV-vis absorption (optical) methods. The metal solutions were added into the solution of 2×10^{-5} M *BTC4* in DMF. The solution of *BTC4* showed dramatic changes in color from light orange to reddish only in the presence of Hg^{2+} . Addition of the solutions of the other metals did not result in appreciable change in color. Moreover, the color change in the presence of Hg^{2+} increased with the metal ion amount towards darker.

Fig. 1 shows the absorption behavior of *BTC4* and a series of UV-vis spectral experiments carried out to investigate the possible interactions between *BTC4* and each heavy metal ion. The

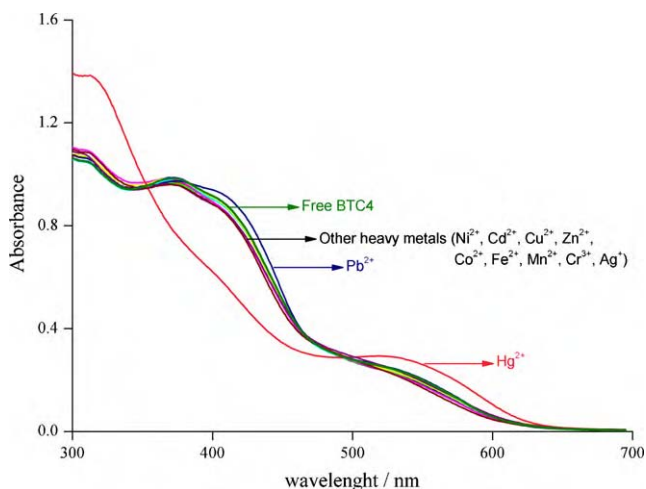


Fig. 1. Spectral changes of *BTC4* (4.0×10^{-5} M) with different heavy metal ions (5 eq.).

absorption spectrum of *BTC4* has a maximum absorption peak at 369 nm corresponding to π - π^* transitions of the $-N=N-$ bond of thiazole moieties in the absence of metal ions [19]. When *BTC4* (4×10^{-5} M) were treated with a series of heavy metal ions (2×10^{-4} M), bathochromic shifts were observed only for Hg^{2+} and Pb^{2+} ions. But, while the bathochromic shift in presence of Pb^{2+} has a negligible value (19 nm), the significant bathochromic shift (149 nm) and even a large absorbance quenching effect of the absorption spectrum of *BTC4* were observed for the case of Hg^{2+} ion. On the other hand, no significant change was observed upon the addition of the other heavy metal ions. The results can be attributed to the more suitable interaction between Hg^{2+} ion and negative charge center in *BTC4* [15,21,22].

In order to gain a further viewpoint to the binding properties of *BTC4* towards Hg^{2+} ions, a series of UV-vis titrations was carried out and the absorption profile as a function of Hg^{2+} concentration was obtained as shown in Fig. 2. The absorption peak at 369 nm gradually decreased upon addition of increasing amount of Hg^{2+} (0.0 – 2.0×10^{-3} M) into the solution of *BTC4*. Two well-defined isosbestic points, which were evident during the systematic titration with increasing concentration of Hg^{2+} , were observed at 348 and 499 nm. The isosbestic points indicate the presence of two species

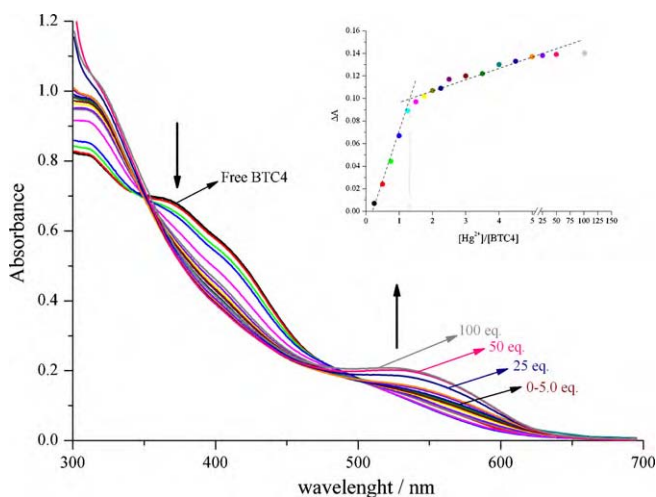


Fig. 2. UV-vis titrations of 2.0×10^{-5} M *BTC4* in DMF upon the increasing amount of Hg^{2+} : 0, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 25.0 and 100.0 equivalents Hg^{2+} . The inset shows the corresponding titration curve at 369 nm.

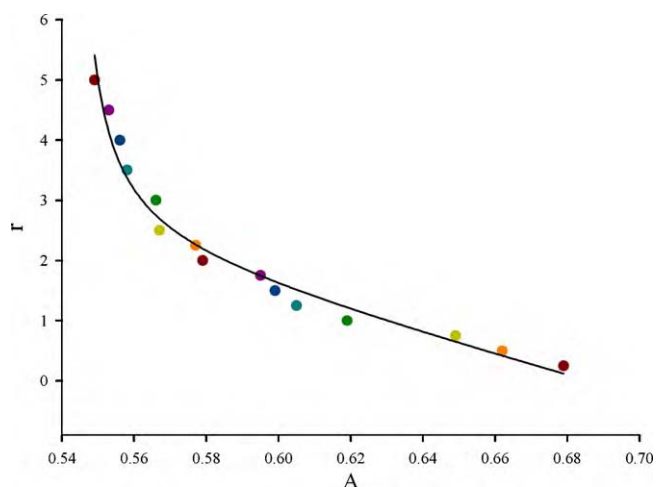


Fig. 3. The nonlinear curve fitting of Eq. (1) for the change in absorbance at 369 nm with respect to the added amount of Hg^{2+} ion in the range of 0.25–5.0 eq. The correlation coefficient (R) of the nonlinear curve fitting is 0.9892.

(*BTC4* and *BTC4*- Hg^{2+}) in equilibrium. On the other hand, both the decrease and increase of the peaks almost did not change after addition of 50 equivalents Hg^{2+} ion (1×10^{-3} M), and even they stayed stable. The stoichiometry between *BTC4* and Hg^{2+} ion was determined as 1:1 complex formation by mole fraction methods as shown in inset of Fig. 2. Also, the stoichiometry was confirmed by using Job's method (data not shown).

On the basis of UV-vis band changes upon the addition of metal ion, and complexation ratio between chromophore (*BTC4*) and metal ion (Hg^{2+}), the stability constant (K_a) can be determined by the nonlinear curve fitting procedure by using following equation [22]:

$$r = \frac{A - A_f^0}{A_c^0 - A_f^0} + \frac{A - A_f^0}{C_f^0 K_a (A_c^0 - A)} \quad (1)$$

where r is the added equivalents of cations, A_f^0 , A and A_c^0 are absorbance of the free chromophore, the chromophore plus r equivalents of cations and the chromophore plus large excess cation solutions, respectively and C_f^0 is the concentration of the free chromophore and K_a is the stability constant.

The obtained nonlinear fitting curve of Eq. (1) by using Sigma Plot 10 has been presented in Fig. 3. The stability constant (K_a) for 1:1 stoichiometry between Hg^{2+} and *BTC4* was determined as $(6.1 \pm 0.3) \times 10^5$ L mol $^{-1}$ from the curve fitting.

In addition, the stability constant value of the Hg^{2+} with *BTC4* demonstrates that *BTC4* exhibits good Hg^{2+} recognition probably due to having a more complementary structure compared to other heavy metal ions. Moreover, when it is compared to some chemosensors in the literature, *BTC4* has a higher association constant value. For example, *BTC4* has an approximately 20, 4 and 2 times higher association constant value for Hg^{2+} with respect to azo-8-hydroxyquinoline benzoate chemosensor [23], squaraine-based chemosensor [18] and benzoxadiazole-based chemosensor [24], respectively.

3.2. Voltammetric properties

The electrochemical behaviors of the parent calixarene (C4 in Scheme 1) and *BTC4* compounds were studied at room temperature by cyclic voltammetry and differential pulse voltammetry. The obtained cyclic voltammograms (CVs) for C4 and *BTC4* in DMF solution including 0.1 M TBAP have been shown in Fig. 4. As denoted in the previous literature [13,25] and can be seen from the inset

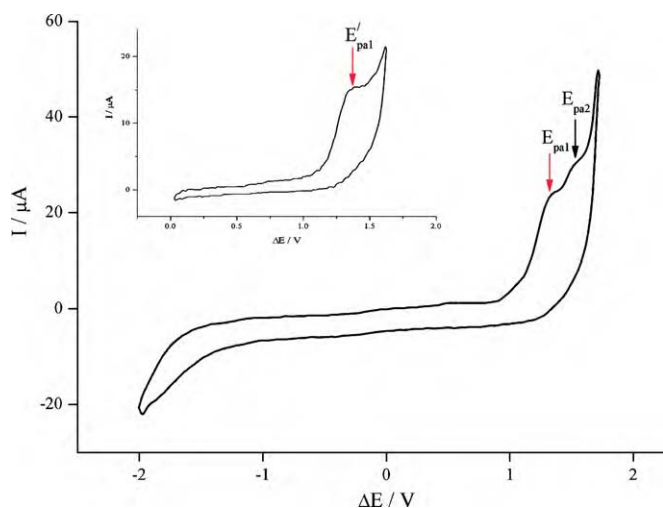


Fig. 4. The cyclic voltammogram (CV) of 1×10^{-3} M *BTC4*; inset shows the CV of 1×10^{-3} M *C4* (first scan), 0.1 M TBAP in DMF at GCE electrode and scan rate 0.1 V s^{-1} .

in Fig. 4, *C4* exhibits only one oxidation peak at 1.22 V (E_{pa1}) due to phenol groups. On the other hand, CV of *BTC4* has two anodic peaks having irreversible nature at 1.21 (E_{pa1}) and 1.42 V (E_{pa2}). The first peak has been expected to belong to the oxidation of the phenol parts in the *BTC4* moiety, as expected from the known electro activity of phenols as well as that (E_{pa1}) of *C4* in DMF solution. In addition, the latter one at 1.42 V can be attributed to benzothiazole azo groups at the upper rim of *BTC4*. For this reason, we decided to record differential pulse voltammograms (DPVs) of *BTC4* at anodic range (at 0–1.75 V) to obtain the interactions occurring between heavy metal ions and *BTC4*.

The DPV changes of *BTC4* in the presence of 3 equivalents of heavy metal ions in the solution have been shown in Fig. 5. As shown, when heavy metal ions except Hg^{2+} were added into *BTC4* solution, it caused no significant changes at the peaks as seen with the result of spectroscopic experiments. In the case of Hg^{2+} , while both the potential and current of the first peak changed only a few, the second peak current almost disappeared. The changes of the peaks in Fig. 5 show that Hg^{2+} ions exhibit an interaction compared to those of other heavy metal ions due to the cavity size of *BTC4*. In addition to this, the change of the second peak indicate that Hg^{2+} is accommodated within the region of the medium cavity of *BTC4* due to the interaction with benzothiazole azo groups at the upper rim of *BTC4* as shown in Scheme 2.

The further experiments were carried out to investigate the effect of amount of Hg^{2+} in detail. Fig. 6 shows DPVs of the addi-

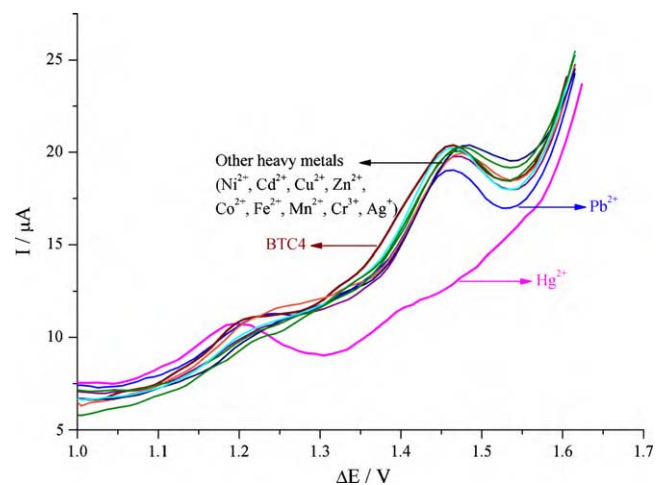


Fig. 5. The DPVs of *BTC4* (1×10^{-3} M) and *BTC4* with different heavy metal ions (3 equivalent) in DMF at GCE. Pulse amplitude 50 mV.

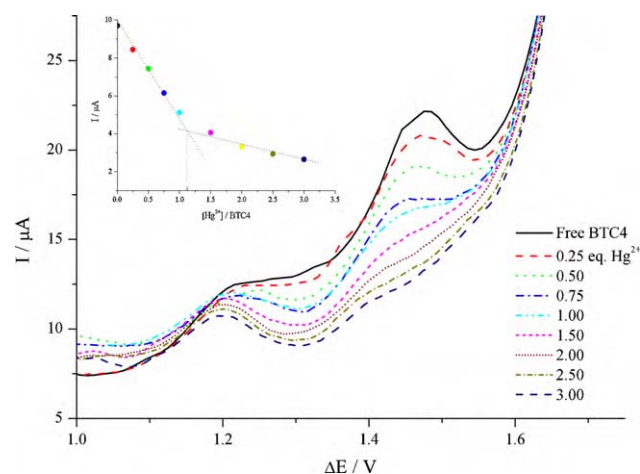
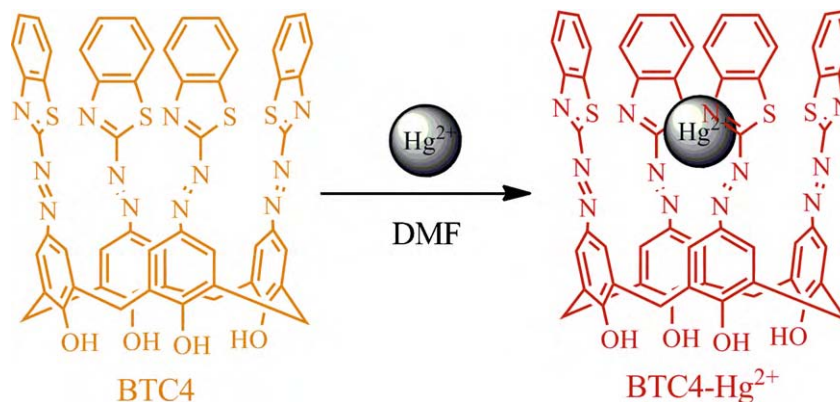


Fig. 6. The DPVs of *BTC4* upon increasing amount of Hg^{2+} ions in DMF including 0.1 M TBAP; pulse amplitude 50 mV. The inset shows the amperometric titration curve at 1.42 V.

tion of different equivalents of Hg^{2+} ion. As long as Hg^{2+} ion was added to the solution of *BTC4*, the changes at the first peak were a few as it might be negligible. On the other hand, the main change was obtained at the second peak, and its peak current (I_{pa2}) quantitatively decreased as long as the concentration of Hg^{2+} increased as shown in Fig. 6. But the greatest decrease value was monitored till around 1 equivalent. The obtained changes of I_{pa2} have



Scheme 2. The possible structure for the interaction between Hg^{2+} ion and *BTC4*.

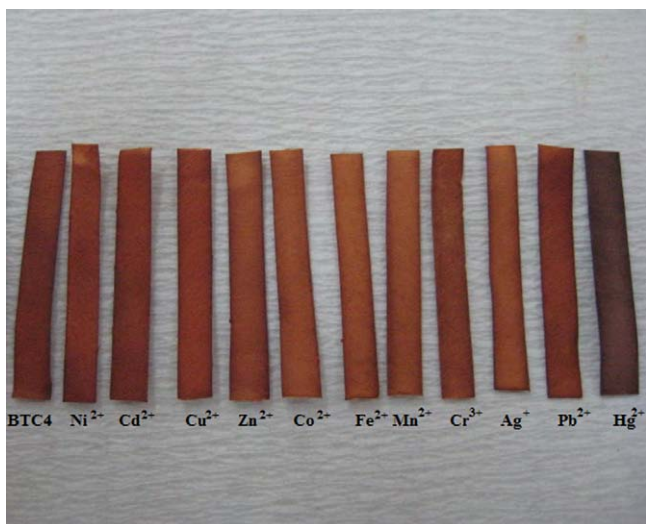


Fig. 7. The color changes observed after the test strips was dipped for 30 s in 1×10^{-3} aqueous solutions of the indicated metals.

been presented in inset of Fig. 6. The amperometric titration curve has confirmed that the interaction between Hg^{2+} and BTC4 occurs according to 1:1 stoichiometry as evaluated in the spectroscopic experiments.

3.3. Application of the rapid test kits for Hg^{2+} detection

In order for practical application of chemosensor, the test strips were prepared according to the methods in the literature [26,27] by immersing filter papers into a DMF solution of BTC4 (0.1 M) and then drying them for an hour in vacuum ($55\text{--}60^\circ\text{C}$, 60 mm Hg). After that, the different test strips were immersed into the aqueous solutions of different heavy metal ions (1×10^{-3} M) for 30 s. As can be seen in Fig. 7 obviously, immersion of these films in an aqueous solution of different metal ions results in a rapid colorimetric response only for Hg^{2+} ion. In order to gain a further insight to the sensing properties of BTC4 upon Hg^{2+} , the test strips were applied towards increasing amount of Hg^{2+} concentration (1×10^{-4} to 1×10^{-2} M). The color of the test strips changed from orange to dark brown shows that the rapidly recognizable concentration of Hg^{2+} may be as low 1×10^{-4} M.

4. Conclusion

In conclusion, we have synthesized a new calix[4]arene chemosensor including benzothiazole azo groups at the upper rim as the metal ion binding sites. The results of the experiments showed that it has high selectivity for Hg^{2+} in DMF. The Hg^{2+} ion detection gives rise to a large bathochromic shift in the absorption spectrum (from light orange to reddish), which is clearly visible to the naked eye. The stoichiometric ratio and the association constant

were determined spectrophotometrically. A series of experiments was carried out to obtain the binding properties by voltammetric techniques. Then, we applied the chromogenic chemosensor for Hg^{2+} detection by test kits. We believe that this application may be very useful for rapid detection of Hg^{2+} ion. Also, these studies may be helpful in the design of novel optical–electrochemical sensory devices for ionic and molecular recognition.

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