Spectrophotometric and voltammetric characterization of a novel selective electroactive chemosensor for Mg$^{2+}$

Erhan Zor$^{1,2}$, Ahmet Ozgur Saf$^2$, Haluk Bingol$^{2*}$

$^1$Selcuk University, Science Institute, 42003 Selcuklu/Konya, Turkey
$^2$Chemistry Department, Ahmet Kelesoglu Education Faculty, Necmettin Erbakan University, 42099 Meram/Konya, Turkey

Received 9 September 2012; Accepted 27 November 2012

Abstract: A novel azocalix[4]arene derivative, 5,11,17,23-tetrakis[(acetophenone)azo]-25,26,27,28-tetrahydroxycalix[4]arene (APC4), containing acetophenone azo groups at the upper rim was synthesized as a chemosensor. Its binding and sensing properties with alkali and alkaline earth metal ions (Li$^+$, Na$^+$, K$^+$, Rb$^+$, Cs$^+$, Mg$^{2+}$, Ca$^{2+}$, Sr$^{2+}$, Ba$^{2+}$) were investigated by UV-vis spectrophotometric and voltammetric techniques. The stoichiometric ratio and the association constant were determined spectrophotometrically as 1:1 and (1.94±0.31)×10$^5$ L mol$^{-1}$ for the complex between Mg$^{2+}$ and the chemosensor, respectively. Moreover, it was shown that the interaction between Mg$^{2+}$ and the APC4 occurred by means of the phenol groups at the lower rim by voltammetric methods. The results of spectrophotometric and voltammetric experiments showed that the chromogenic chemosensor has high selectivity towards Mg$^{2+}$ among the other used metal ions, especially the interfering Ca$^{2+}$ ion.

Keywords: Chemosensor • Azocalix[4]arene • Magnesium ion • Spectrophotometric method • Voltammetric method

1. Introduction

The investigation of highly sensitive and selective molecular sensory materials for ions has become a growing field of research with the advent of supramolecular chemistry [1,2]. Specific ion detection performed using optically and electrochemically active supramolecules has received considerable attention in the area of chemical sensors (chemosensors) in recent years. Chemosensors which are composed of a receptor unit and a signaling unit have become useful molecules that bind selectively and reversibly with an analyte with a concomitant change in one or more properties of the system, such as color, enhancement or inhibition of absorbance or fluorescence, or redox potentials [1,3,4]. Among different types of chemosensors, chromogenic chemosensors are especially interesting for ion determination. When a selective interaction occurs between a chromogenic chemosensor and an ion, a distinctive color change arises which provides simple naked-eye readout without use of expensive equipment [5,6].

Many macrocyclic systems such as cyclodextrin [7], calixarene [8], calixcrown [9], and crown ether [10] have been developed for the improvement of effective chemosensors to detect metal ions. Calixarenes are one of the highly selective macrocyclic scaffolds used for detection of metal ions. The structural properties of calixarenes, such as their macrocyclic cores and easily organizability into a number of topographies, give them the capacity for selective detection of metal ions [11]. Additionally, they can be selectively functionalized at their upper and lower rims for different analytes. In this view, calixarenes functionalized by chromophoric azo groups (–N=N–), known as azocalixarenes, and have been extensively studied in selective colorimetric detection of metal ions [11-14].

Magnesium is one of the most needed abundant elements in the human body. It plays important roles...
in processes such as neuronal activity, neuromuscular transmission, cardiac excitability, and blood pressure [15,16]. In industrial processes, magnesium is used for the cathodic protection of other metals and for making machine parts moving at high speed – in aerospace applications, for example – due to its light weight and high strength [15]. All of these properties provide sufficient impetus to fabricate chemical sensors for the selective and efficient detection of magnesium in biological, clinical, industrial, environmental and food management applications. However, selective detection of Mg$^{2+}$ is usually difficult due to the interference of other alkaline earth metal ions, especially Ca$^{2+}$. Velu et al. have reported the synthesis of an aza-crown ether acridinedione-functionalized gold nanoparticles (ACEADD-GNPs) chemosensor, and have found that the chemosensor interacts with Mg$^{2+}$ and Ca$^{2+}$ among the used metal ions in the spectrophotometric, fluorometric and colorimetric experiments [17]. Suresh et al. have synthesized a new coumarin-based sensor molecule and have observed that it binds Ca$^{2+}$ and Mg$^{2+}$ more effectively as compared to other used metal ions [18]. Therefore, the choice of sensing molecule is crucial for the development of an effective sensor for selective detection of Mg$^{2+}$.

In this paper, the synthesis of a novel azocalix[4]arene derivative, 5,11,17,23-tetrakis[(acetophenone)azo]-25,26,27,28-tetrahydroxycalix[4]arene (APC4), as a chemosensor is reported. The optical and electrochemical behavior of APC4 in the presence of various metal ions (Li$^+$, Na$^+$, K$^+$, Rb$^+$, Cs$^+$, Mg$^{2+}$, Ca$^{2+}$, Sr$^{2+}$, Ba$^{2+}$) was investigated by spectrophotometric and voltammetric methods, respectively. The stoichiometry and association constant of the occurring complex were determined spectrophotometrically. APC4 exhibited highly selective binding for Mg$^{2+}$ among other metal ions. To the best of our knowledge, several kinds of chromogenic chemosensors having selectivity towards Mg$^{2+}$ have been reported [19-21], however, there have been no highly Mg$^{2+}$-selective azocalix[4]arene derivatives in the literature.

2. Experimental procedure

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). The metal salts were used as perchlorate compounds (MClO$_4$, M= Li$^+$, Na$^+$, K$^+$, Rb$^+$, Cs$^+$, Mg$^{2+}$, Ca$^{2+}$, Sr$^{2+}$, Ba$^{2+}$) and dried prior to use over P$_2$O$_5$. $^1$H NMR spectra were recorded on a Varian 400 NMR spectrometer. Chemical shifts were measured in relation to the internal reference, tetramethylsilane (TMS).

The melting point was recorded on an EZ MeltMPA120 capillary apparatus. Infrared measurements were performed on a Perkin Elmer 100 FT-IR spectrometer. UV-vis absorbance spectra were obtained on a Shimadzu UV-1800 double beam spectrophotometer. Electrochemical measurements were performed with a PAR 263/A2 (Princeton Applied Research, USA) potentiostat/galvanostat combined with a BAS C3 electrochemical cell stand. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) data were recorded in a glass cell including a conventional three-electrode configuration in CH$_3$CN (acetonitrile) with 0.1 M tetrabutylammonium perchlorate (TBAP, Aldrich) as the supporting electrolyte. A glassy carbon electrode (GCE) with a surface diameter of 2 mm was used as the working electrode with a platinum wire as the counter electrode. In all cases, Ag/Ag$^+$ (0.1 M) in CH$_3$CN was used as the reference electrode and standardized by the redox couple ferrocenium/ferrocene. Prior to each experiment, GCE was polished with 1.0, 0.3 and 0.05 μm alumina powder (PACE Technologies, USA). Residual alumina particles were removed by an ultrasonic cleaner (Sonorex Super RK 106, Germany) in ethanol.

The electrode was dried and washed with pure CH$_3$CN before use. The other electrodes were cleaned by well-established methods. To avoid undesired effects, the electrochemical cell setup was freshly prepared before each experiment. The electrochemical measurements (CV and DPV) were recorded anodically at a potential range of 0.0 to 2.0 V. All experiments were carried out at room temperature.

2.2. Synthesis of Chemosensor (APC4)

Tetrakis[(acetophenone)azo]-25,26,27,28-tetrahydroxycalix[4]arene (APC4) was synthesized according to the following procedure, as given in Scheme 1. A solution of diazonium chloride prepared from 4-aminoacetophenone (0.324 g, 2.4 mmol), sodium nitrite (0.165 g, 2.4 mmol) and concentrated HCl (1.68 mL) in water (6 mL) was added slowly into a cold (5°C) solution of calix[4]arene (0.1 g, 0.24 mmol) and sodium hydroxide (0.192 g, 4.8 mmol) in MeOH-DMF (13 mL, 5:8, v/v) to give a yellow suspension. After allowing it to stand for 3 h at room temperature, the suspension was acidified with aqueous HCl (40 mL, 2 M). The resulting yellow precipitate was filtered and washed with water and MeOH to yield a crude product, which was purified by column chromatography on silica gel using chloroform/MeOH (10:1) to give the pure product as a yellow solid. m.p. (decomp.) 261°C.
Spectrophotometric and voltammetric characterization of a novel selective electroactive chemosensor for Mg$^{2+}$

C, 71.04; H, 4.68; N, 10.94%. C$_6$H$_{48}$N$_8$O$_8$: requires C, 71.42; H, 4.79; N, 11.10%. ν$_{max}$/cm$^{-1}$: 3324, 3028, 2341, 1734. $^1$H NMR(CDCl$_3$) δ 2.64 (s, 12H, CH$_3$), 3.52 (broad s, 4H, ArCH$_2$Ar), 4.20 (broad s, 4H, ArCH$_2$Ar), 6.70-6.75 (m, 16H, ArH), 7.04-7.12 (m, 8H, calixaren ArH), 10.19 (s, 4H, ArOH).

2.3. Procedure

2.3.1. UV–vis measurements

Stock solutions of APC4 and metal perchlorates were prepared as 5.0×10$^{-4}$ M and 2.0×10$^{-2}$ M, respectively, in CH$_3$CN. In order to obtain the absorbance spectrum of APC4, the stock solution was diluted to 1.0×10$^{-4}$ M with CH$_3$CN. Then, a 3-mL volume of a solution containing the desired amount of APC4 and metal ions was prepared. The absorbance spectra were recorded against CH$_3$CN as the blank solution at the end of the completed reaction between metal ions and APC4.

2.3.2. Electrochemical measurements

Stock solutions of the metal perchlorates and APC4 were prepared at 2.0×10$^{-2}$ M and 5.0×10$^{-3}$ M, respectively, in CH$_3$CN including 0.1 M TBAP. The electrochemical measurements were performed with an APC4 solution (1.0×10$^{-3}$ M) that included the desired amount of the metal solution. All solutions were purged with argon for 30 min before measurements, and the experiments were performed at room temperature with an argon atmosphere maintained over the solution.

3. Results and Discussion

3.1. UV-vis experiments

The binding behavior of the synthesized azocalix[4]arene derivative (APC4) towards the used metal ions was investigated by spectrophotometric experiments in a CH$_3$CN solution. Fig. 1 shows the absorbance spectra of APC4 in the presence and absence of metal ions. In the absence of metal ions, the absorbance spectrum of APC4 has absorbance maxima located at 361 nm and 451 nm corresponding to π-π$^*$ and n-π$^*$ transitions, respectively, which are in accord with typical diazo spectra [22]. In order to obtain the affinity of APC4 for the used metal ions, the absorbance spectra of APC4 (1.0×10$^{-4}$ M) were recorded in the presence of the used...
metal ions (1.0×10^{-3} M). No obvious change was observed upon addition of Li^+, Na^+, K^+, Rb^+, Cs^+, Ca^{2+}, Sr^{2+}, Ba^{2+}, whereas a decrease was observed for the absorbance spectrum of APC4 at 451 nm in the presence of Mg^{2+}. In addition, the peak at 361 nm showed an absorbance increase (hyperchromic effect) in the presence of Mg^{2+}. The Mg^{2+} sensors reported in the literature demonstrate poor selectivity for Mg^{2+} over Ca^{2+} and are useful only where the concentrations of Mg^{2+} are much higher than those of Ca^{2+} [4]. Interestingly, addition of Ca^{2+} ions did not alter the absorbance properties of APC4. Moreover, the spectrophotometric measurements showed that the APC4 chemosensor exhibits excellent selectivity towards Mg^{2+} even in the presence of Ca^{2+}.

In order to gain further insight on the binding properties of APC4 towards Mg^{2+}, a series of UV-vis titration experiments was carried out. The absorbance profile as a function of Mg^{2+} concentration was obtained, as shown in Fig. 2. The absorbance peak at 451 nm gradually decreased with increasing amounts of Mg^{2+}. In addition, a clear isosbestic point was observed at 392 nm, confirming that the stable species is present at equilibrium and that a stable complex forms between APC4 and Mg^{2+}. On the other hand, both the decrease and increase of the peaks plateau after addition of 50.0 equivalents Mg^{2+} (purple line), after which they remained stable. The inset in Fig. 2 shows the complex stoichiometry between APC4 and Mg^{2+} determined with a value of 0.99 ([Mg^{2+}]/[APC4]) at 451 nm. On the basis of this mole-ratio value, the occurring complex mechanism was proposed as 1:1 stoichiometry.

Taking into consideration UV-vis spectra changes and the complexation ratio (1:1 stoichiometry) between APC4 and Mg^{2+} ion, the nonlinear curve fitting procedure was used to determine the association constant (K_a) by applying Eq. 1 [6,22].

\[
r = \frac{A - A_f}{A_c - A_f} + \frac{A - A_f}{C_f K_a (A_c - A)}
\]

where r indicates the added equivalents of metal ion. A_f, A and A_c are absorbencies of the free chromophore, the chromophore plus r equivalents of metal ion and the chromophore plus large excess metal ion solution, respectively. C_f is the concentration of the free chromophore and K_a is the association constant.

Fig. 3 presents the nonlinear fitting curve of Eq. 1 obtained using Sigma Plot 10. The association constant (K_a) for 1:1 stoichiometry of the occurring complex between Mg^{2+} and APC4 was calculated as (1.94±0.31)×10^{5} L mol^{-1} from the curve fitting. This association constant value is higher than that of some reported papers. For example, Singh et al. calculated an association constant value of (1.4±0.1)×10^{4} L mol^{-2} for 2:1 complexation occurring between a Schiff base sensor molecule and Mg^{2+} [19]. Song et al. have synthesized a calix[4]arene diamide derivative and have investigated its selectivity behavior for Mg^{2+} with an association constant value of 2.97×10^{4} L mol^{-1} [21]. When comparing these values, the association constant value of the complex occurring between Mg^{2+} and APC4 demonstrates that APC4 exhibits good Mg^{2+} recognition, probably due to having a more complementary structure.
**3.2. Voltammetric experiments**

Chemists increasingly apply electrochemical methods to the investigation of their systems, in particular towards a better understanding of molecular properties such as molecular interaction in supramolecular chemistry [23]. In order to investigate the electrochemical behavior of the parent calixarene (C4 in Scheme 1) and APC4, cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques were carried out in the anodic range (0.0-2.0 V) at room temperature. The cyclic voltammograms recorded for C4 and APC4 (1.0×10⁻³ M) in CH₃CN solution including 0.1 M TBAP have been shown in Fig. 4. As previously reported [6,11,24], and as can be seen from the inset in Fig. 4, C4 exhibits only one peak (Eₚₐ₁, approximately at 1.22 V) in the anodic range in organic media due to the oxidation of phenol groups at the lower rim. The voltammogram of APC4 has two irreversible anodic peaks at 1.23 V (Eₚₐ₁) and 1.52 V (Eₚₐ₂), as shown in Fig. 4 (solid line). While the first peak was attributed to the oxidation of phenol in the APC4 moiety, as expected from the known electro-activity of phenols in organic media, the second peak was attributed to acetophenone groups at the upper rim of APC4. The cyclic voltammogram of APC4 was recorded in the presence of Mg²⁺ (the dotted line in Fig. 4) to determine complexation behavior of APC4 with Mg²⁺. As can be seen from the voltammogram, no significant change was observed at the second peak while the first peak disappeared dramatically. In order to verify the results obtained with CV, DPV was also...
carried out. Fig. 5 shows the pulse voltammograms of APC4 in the absence and presence of Mg$^{2+}$. In accord with CV measurements, APC4 exhibited two pulse peaks in the anodic range in the absence of Mg$^{2+}$. When a differential pulse voltammogram was recorded in the presence of Mg$^{2+}$, no significant change was observed at the second peak while the first peak disappeared, as observed with CV measurements. The change at the first peak indicates that Mg$^{2+}$ is accommodated within the region of the medium cavity of APC4 due to the interaction with phenolic groups at the lower rim of APC4 as shown in Scheme 2. This result stands in contrast to previously reported papers which indicate that the metal ions interact with the upper rim of azocalix[4]arene derivatives [6,11]. The data obtained from the electrochemical studies corroborate the results of the spectrophotometric experiments. As demonstrated, the CV technique can be used as a cheap alternative to some methods, such as NMR, in order to carry out a structural analysis for interaction between an analyte and sensor molecule.

4. Conclusion

In summary, the results of the spectrophotometric and voltammetric experiments have indicated that the novel synthesized azocalix[4]arene derivative (APC4) shows excellent selectivity towards Mg$^{2+}$ among the used alkali and alkaline earth metal ions in CH$_3$CN. The stoichiometric ratio and the association constant have been determined by spectrophotometric methods. A series of experiments have been carried out to obtain the structural binding properties by voltammetric techniques. Due to the simplicity, low cost and efficiency of CV and DPV techniques, and the opportunity they

Figure 5. The differential pulse voltammograms of APC4 (1.0×10$^{-3}$ M) in the absence (solid line) and presence (dotted line) of Mg$^{2+}$ (5 eq.) in CH$_3$CN at GCE (first scan). Pulse amplitude 50 mV.

Scheme 2. The possible interaction structure for the complexation between Mg$^{2+}$ and APC4 according to the voltammetric results.
provide to perform structure analysis for complexation between ions and sensor molecules, these techniques can be used as powerful alternative methods. The voltammetric results show that Mg²⁺ interacts with phenolic groups at the lower rim of azocaltalic[4]arene derivative. The electrochemical results show important structural implications for the development of new selective amperometric sensors for metal ions.

Acknowledgements

The authors are grateful for the grant sponsored by the Scientific Research Projects (BAP-10401048) of Selcuk University.

References