

## Spectrophotometric and Electrochemical Study of Cu<sup>2+</sup>-Selective Azocalix[4]arene Bearing *p*-Carboxyl group

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The spectrophotometric and electrochemical properties of tetraazo(*p*-carboxy)phenylcalix[4]arene (*p*-CAC) in the presence of various metal ions were investigated by UV/Vis spectroscopy and voltammetry in CH<sub>3</sub>CN. *p*-CAC showed selective color changes to Cu<sup>2+</sup> over other metal ions from light orange to colorless, in accordance with hypsochromic shift in UV/Vis spectra. Voltammetric measurements also showed high selectivity for Cu<sup>2+</sup>.

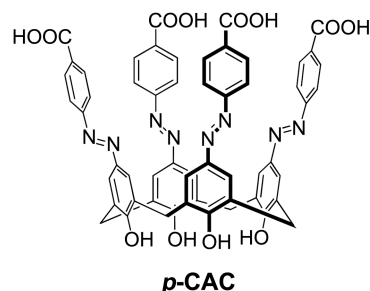
**Key Words :** Azocalixarene, Copper ion, Colorimetry, Voltammetry

### Introduction

Calix[4]arenes have been widely used for sensing an analyte through their conformational adaptability toward hosting guests along with easy derivatization of the lower rim (hydroxyl groups) and upper rim (*para* positions to the hydroxyl groups), and the possibility of locking a desired conformation (cone, partial cone, 1,2-alternate, 1,3-alternate) under specific reaction conditions.<sup>1-4</sup> Functionalization of the upper rim with azo groups confers chromogenicity, or fluorogenicity that can be employed for the development of molecular diagnostics and optical sensor materials for metal cations and other important targets.<sup>2</sup>

Many efforts over the past years have been made to synthesize and characterize the fully or partially substituted azocalixarenes with various functional groups for utilization as optical sensors.<sup>5-8</sup> Different studies on the synthesis of azocalixarenes for related applications have been reported in the literatures.<sup>9-11</sup> Our group has been also interested in the designing selective coloring sensors toward metal ions using azocalix[4]arene derivatives.<sup>12-16</sup> Among them, azocalix[4]arenes substituted in *ortho* position by ester and carboxyl groups, respectively, showed color changes each to only transition metal ions, and to alkaline earth and transition metal ions over various metal ions.<sup>12,14</sup> Consequently, with these two azocalix[4]arenes, one can establish a qualitative analytical protocol to screen alkali, alkaline earth and transition metal ions.<sup>14</sup> As one of our efforts to tune up the selectivity, we have also tried pH study of azocalix[4]arene with *ortho*-carboxyl groups to find the optimum conditions for the detection of specific metal ion, which leads to Pb<sup>2+</sup> selective azocalix[4]arene in basic media.<sup>13</sup>

In this context, our aim was to design the azocalix[4]arene possessing the ability to bind the specific metal ion by changing the position of carboxyl substituents. Resultantly, we prepared tetraazo(*p*-carboxy)phenylcalix[4]arene (*p*-CAC) with the *para*-carboxyl substituents (Scheme 1). To the best



**Scheme 1.** Structure of the tetraazo(*p*-carboxy)phenyl calix[4]arene (*p*-CAC).

of our knowledge, spectrophotometric and voltammetric study of *p*-CAC in the absence and the presence of alkali, alkaline earth and transition metal ions in CH<sub>3</sub>CN has not been reported. Present study reports *p*-CAC exhibiting a remarkably selective response to Cu<sup>2+</sup> ion over various metal ions in CH<sub>3</sub>CN by UV/Vis spectroscopy, colorimetry and electrochemical method. Cu<sup>2+</sup> as a third in abundance among the essential heavy metal ions in the human body can play a pivotal role, or be toxic to biological systems depending on the concentration.<sup>17</sup> Thus so, this work would be useful for development of Cu<sup>2+</sup> sensing system using simple spectrophotometric and electrochemical measurements.

### Experimental

**Chemicals.** All reagents were of analytical-reagent grade or the best grade, purchased from Aldrich and used without further purification. Synthesis and identification of *p*-CAC was described in the previous paper.<sup>18</sup>

**Spectrophotometric Measurements.** Absorption spectra were measured with Scinco-S3130 UV/Vis spectrophotometer (Korea). UV stock solutions of *p*-CAC ( $2 \times 10^{-4}$  M, in CH<sub>3</sub>CN) and metal perchlorate ( $2 \times 10^{-2}$  M, in CH<sub>3</sub>CN) were prepared. The metal solution was diluted 10 and 100

times to give  $2 \times 10^{-3}$  and  $2 \times 10^{-4}$  M solutions. Aliquots of metal solution were added to the *p*-CAC solution, and the final concentration and composition of the solution were adjusted to the desired value by adding extra CH<sub>3</sub>CN. The stability constant ( $K_a$ ) for complexation between *p*-CAC and Cu<sup>2+</sup> metal ion was determined on the basis of the nonlinear curve fitting procedure for a 1:1 complex:

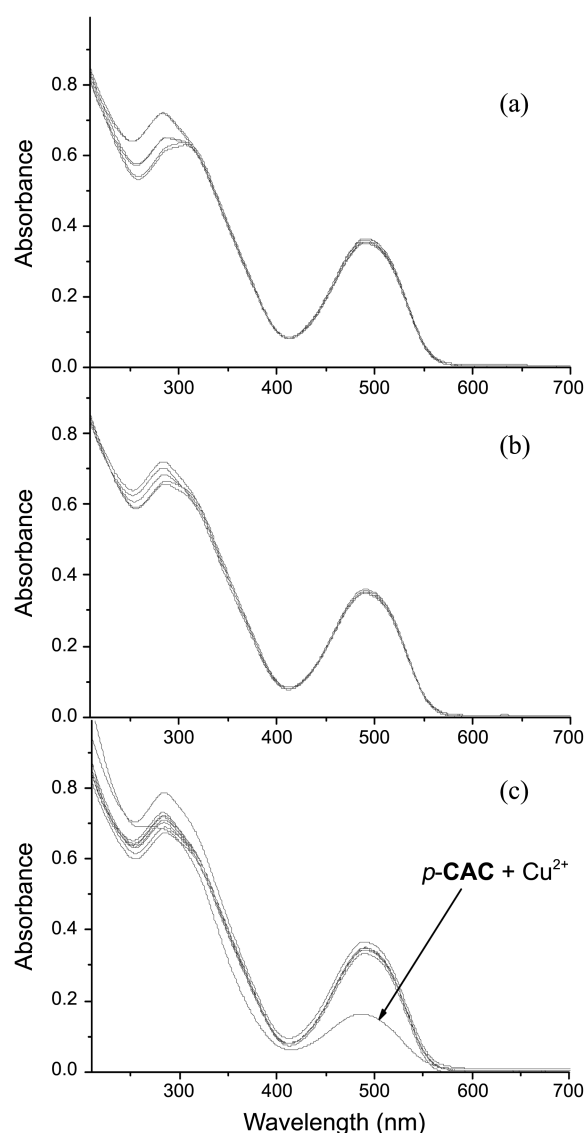
$$r = \frac{A - A_f^0}{A_c - A_f^0} + \frac{A - A_f^0}{C_f^0 \times K_a \times (A_c^0 - A)}$$

where  $r$  is the added equivalents of cations,  $A_f^0$ ,  $A$  and  $A_c^0$  are absorbance of the free ligand, the ligand plus  $r$  equivalents of cations and the ligand plus large excess cation solutions,  $C_f^0$  the concentration of the free ligand and  $K_a$  is the stability constant.<sup>19</sup>

**Electrochemical Measurements.** Voltammetry were carried out with a Model 660D electrochemical workstation (CH Instruments, Austin, TX, USA). The three-electrode system consists of a glassy carbon working electrode, an Ag/Ag<sup>+</sup> (in 0.1 M AgNO<sub>3</sub>) reference electrode and a Pt wire counter electrode. The surface of the working electrode was polished with 0.03 and 0.05  $\mu$ m alumina (CH Instruments, Austin, TX, USA) and rinsed with deionized water. Residual alumina particles were thoroughly removed by positioning the electrode in an ultrasonic bath for 15 min. Then, the electrode was dried and washed with pure acetonitrile before use. The supporting electrolyte was 0.1 M tetrabutylammonium hexafluorophosphate (TBAPF<sub>6</sub>) in acetonitrile solution (CH<sub>3</sub>CN). The concentration of *p*-CAC was 0.1 mM and stock solution of metal perchlorate salts with various concentrations were prepared using acetonitrile. Test solutions were prepared by placing 2 mL of the *p*-CAC solution into an electrochemical cell, adding appropriate aliquot of each metal stock solution with a microsyringe.

## Results and Discussion

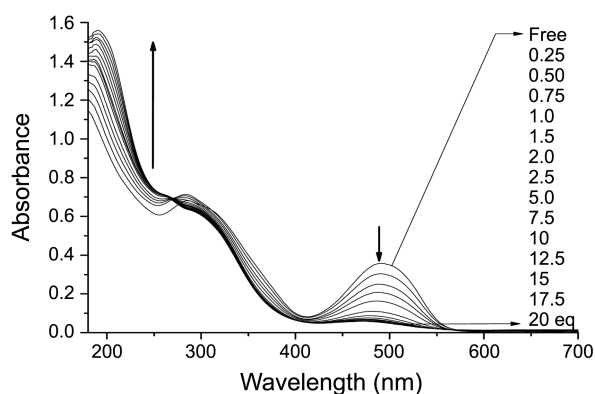
The chromogenic ionophore properties of *p*-CAC upon alkali, alkaline earth and transition metal ions were investigated by examining the UV/Vis absorption behaviors of the host in CH<sub>3</sub>CN solution (Figure 1). *p*-CAC showed two absorption bands and its absorption maxima ( $\lambda_{\max}$ ) located at 285 and 490 nm corresponding to  $\pi$ - $\pi^*$  and  $n$ - $\pi^*$  transitions, respectively, which are in accordance with typical azo spectra.<sup>12,14</sup> This indicates that *p*-CAC exists as a mixture of the two tautomeric forms, namely azophenol form and quinone-hydrazone form in CH<sub>3</sub>CN solution.<sup>14</sup> It is known that *para*-substituent in the phenylazo-1-naphthol reinforces a tautomerization between azophenol and quinone-hydrazone in polar solvent, and the quinone-hydrazone form is found to be highly favored in polar media. For example, the azo/hydrazone tautomerism is found to be more favored in *p*-nitrophenyl azophenol but is least favored in *p*-methoxyphenyl azophenol.<sup>19,20</sup> This is due to the ease in the resonance of *para*-nitro group which improves the stability of the quinone forms. In the case of *para*-carboxyl group



**Figure 1.** Absorption spectra of *p*-CAC with (a) alkali (b) alkaline earth and (c) other metal ions (Cr<sup>3+</sup>, Fe<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Pb<sup>2+</sup>) in CH<sub>3</sub>CN. [*p*-CAC] = [metal ion] =  $2 \times 10^{-5}$  M.

substituted azocalix[4]arene, *p*-CAC, *para*-carboxyl group is able to form various kinds of resonance structures like *para*-nitro group. Thus so, the ease in the resonance of *para*-carboxyl groups favors the quinone forms of azocalix[4]-arene.

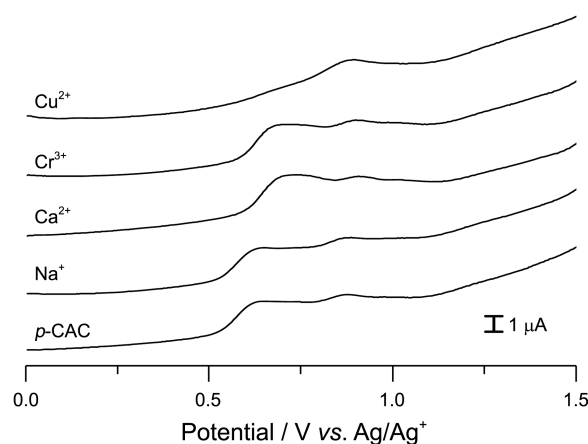
To obtain insight into the ability of *p*-CAC to selectively sense metal ions, we next investigated the affinity of *p*-CAC for a series of alkali, alkaline earth and transition metal ions. Absorption spectra of 0.02 mM *p*-CAC in the presence of one equivalent of metal ions in CH<sub>3</sub>CN are shown in Figure 1. The results show that Cu<sup>2+</sup> ion causes a significant change in absorption spectra of *p*-CAC, whereas upon the addition of alkali, alkaline earth and other transition metal ions, minor absorption changes are observed. In the absence of Cu<sup>2+</sup>, the absorption spectrum of *p*-CAC is characterized by the presence of absorption maximum peak at 490 nm. Upon the addition of Cu<sup>2+</sup> ion, the peak at 490 nm decreases while



**Figure 2.** Changes in absorption spectra as a function of [Cu<sup>2+</sup>]. [p-CAC] =  $2 \times 10^{-5}$  M in CH<sub>3</sub>CN.

a new peak appears around 200 nm. We here noticed that this hypsochromic shift of *p*-CAC–Cu<sup>2+</sup> complex is different from bathochromic shifts in the complexation of *ortho*-carboxyl-substituted azocalix[4]arene–Cu<sup>2+</sup> complex in CH<sub>3</sub>CN. Concerning complexation between azocalix[4]arene derivatives and metal ions, metal cation is able to interact with the phenol units of the lower rim and azo groups in the medium cavity. In the present case with *para*-carboxyl groups, the upper rim could also participate in the interaction with metal ions. In the case of azocalix[4]arene with *ortho*-carboxyl groups, due to the stabilization of the quinone-hydrazone form in *azo*[4]calixarene through six-membered coordination between metal ion, *ortho*-carboxyl group, and azo group, metal ions may be accommodated within the region of the medium cavity of the calixarene, thus leading to the bathochromic shifts.<sup>12,14</sup> However, the present case with hypsochromic shift indicates that the complexation of *p*-CAC–Cu<sup>2+</sup> causes the restriction of charge separation of the azo-benzene system, resulting from the binding to the four phenolic OHs at lower rim.

In order to have a further insight into the chromogenic behavior of *p*-CAC, the absorption profile as a function of metal ion concentration was obtained. Figure 2 shows characteristic absorption spectra in the spectrophotometric titration of *p*-CAC with Cu<sup>2+</sup> metal ion. A well-defined isosbestic point was observed. *p*-CAC exhibited a gradual increase in absorption intensity at the  $\lambda_{\text{max}}$  of 190 nm and a decrease at the  $\lambda_{\text{max}}$ s of 285 and 490 nm upon the addition of incremental copper ions. From the plot of the absorbance changes of *p*-CAC–Cu<sup>2+</sup> system, the stability constant was determined by nonlinear curve fitting procedure and found

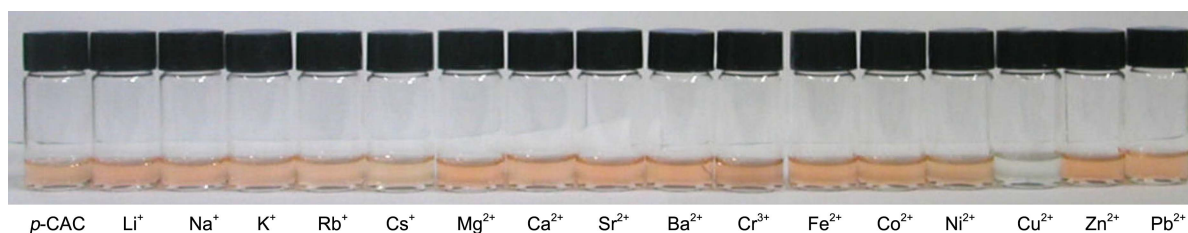


**Figure 4.** Differential Pulse Voltammograms (DPVs) of 0.1 mM *p*-CAC in the absence and presence of 0.1 mM Na<sup>+</sup>, Ca<sup>2+</sup>, Cr<sup>3+</sup> and Cu<sup>2+</sup> in 0.1 M TBAPF<sub>6</sub>/CH<sub>3</sub>CN. Pulse amplitude: 50 mV.

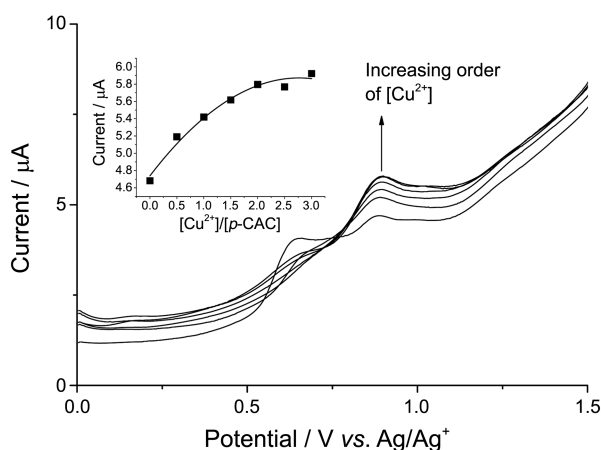
to be  $2.8 \times 10^5 \text{ M}^{-1}$  in CH<sub>3</sub>CN.

The visible color changes of *p*-CAC upon various metal cations was also observed and depicted in Figure 3 where only Cu<sup>2+</sup> metal ion clearly change the color of the *p*-CAC from light orange to colorless. When a complex forms between *p*-CAC and Cu<sup>2+</sup> ion, the excited state would be more stabilized by cation binding, resulting in a hypsochromic shift in the absorption maxima ( $\lambda_{\text{max}}$ ) as well as a color change.

Electrochemical properties of *p*-CAC (0.1 mM) in the presence of alkali, alkaline earth, and transition metal ions were also investigated by differential pulse voltammetry at glassy carbon electrode in 0.1 M TBAPF<sub>6</sub>/CH<sub>3</sub>CN, by taking advantage of the phenol moieties present at the lower rim. Differential pulse voltammogram (DPV) of *p*-CAC shows two anodic peaks at 0.64 and 0.89 V. This could be explained by redox behavior of phenols and intramolecular hydrogen bonding between phenol moieties in the lower rim of *p*-CAC.<sup>15,16</sup> In general, oxidation of phenol exhibits only one oxidation peak with two-electron and one-proton transfer in organic solution and phenols of *p*-CAC are essentially equivalent. However, the intramolecular hydrogen bonding between phenols of *p*-CAC results in one proton transfer more difficult and the other easier, leading to oxidation peaks at a less positive potential and a more positive potential, respectively. The complexation properties of *p*-CAC were also investigated by comparing the voltammetric behaviors of phenols in *p*-CAC with metal ions in DPV. In brief, a constant volume (10  $\mu\text{L}$  per injection) of the metal ion in 0.1



**Figure 3.** Color changes of *p*-CAC ( $2 \times 10^{-5}$  M) upon the addition of various metal ions ( $2 \times 10^{-3}$  M) in CH<sub>3</sub>CN.



**Figure 5.** DPVs of 0.1 mM *p*-CAC upon the addition of increasing amount of  $\text{Cu}^{2+}$  (0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 mM) in 0.1 M TBAPF<sub>6</sub>/CH<sub>3</sub>CN. Pulse amplitude: 50 mV. Inset is the amperometric titration curve at 0.9 V.

M TBAPF<sub>6</sub> supporting electrolyte solution was added into the cell to make 0.5 to 3.0 equivalent of metal ion in the solution. DPVs were recorded after adding stoichiometric equivalent of metal ions successively to the respective electrochemical solution. When one equivalent of alkali, alkaline earth and transition metal ions except  $\text{Cu}^{2+}$  was added to *p*-CAC, no significant change was observed in the peak current or potential (data not shown). However, *p*-CAC in the presence of  $\text{Cu}^{2+}$  shows significant voltammetric changes of DPV in both peak current and potential. Figure 4 shows typical DPVs of *p*-CAC in the absence and presence of representative examples of alkali, alkaline earth and transition metal ions,  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Cr}^{3+}$  and  $\text{Cu}^{2+}$ , respectively. The addition of one equivalent of  $\text{Cu}^{2+}$  to *p*-CAC caused first oxidation peak to disappear and second oxidation peak to increase. This result suggests that  $\text{Cu}^{2+}$  coordinates to *p*-CAC, and *p*-CAC is more stabilized in its complex with  $\text{Cu}^{2+}$ . Meanwhile, DPVs of *p*-CAC in the presence of other metal ions such as  $\text{Na}^+$ ,  $\text{Ca}^{2+}$  and  $\text{Cr}^{3+}$  show no significant changes. And this is in good accordance with spectroscopic data.

In order to confirm the electrochemical recognition phenomenon, the electroactivity of *p*-CAC was measured in the presence of increasing substoichiometric amount of  $\text{Cu}^{2+}$  cation. Figure 5 shows the effect of concentration of  $\text{Cu}^{2+}$ . With a function of  $[\text{Cu}^{2+}]$ , the oxidation peak of *p*-CAC at 0.64 V decreases but the oxidation peak at 0.89 V increases. The peak current ( $I_{p2}$ ) at 0.89 V decrease quantitatively by increasing  $\text{Cu}^{2+}$ . But it gradually reaches to the maximum value (Figure 5 inset).

### Conclusion

We have investigated the spectrophotometric and electro-

chemical properties of an azocalix[4]arene bearing *para*-carboxyl substituents in the absence and the presence of alkali, alkaline earth, and transition metal ions by UV/Vis spectroscopy, colorimetry and electrochemical measurements. The selectivity of the  $\text{Cu}^{2+}$  preference to other metal ions with *p*-CAC was observed by spectroscopic, colorimetric and voltammetric studies, which is presumably due to the preorganized conformation for  $\text{Cu}^{2+}$  ion binding in which carboxyl units of the upper rim, or hydroxyl units of the lower rim in *p*-CAC form a ligand proximity toward  $\text{Cu}^{2+}$  ion.

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