Chapter-2

An Ionic Receptor for Zn$^{2+}$ Metal Ion using Synthesized bis-formylpyrazole calix[4]arene and its Computational Study
Aim of the research

The aim of the research to synthesize a new calix[4]arene derivative receptor for metal ions. Calixarenes are the class of macromolecules and which have hydrophobic as well as hydrophilic part in their structures. The calixarenes are easily functionalized and their binding capacity and specificity with metal ions may be easily increased. This was aimed to functionalize at lower rim of the calix[4]arene with formylpyrazole group. That would give a solid support to the cavity of the calixarene with increased specificity for the metal ions. Transition metals are important part of chemistry and excess of any metal shows lethal effects to human and living species. Any receptor having good binding properties with these metals can be applicable in medicine chemistry as well as to remove the ionic toxicants. Taking this aim into account a receptor specific to Zn$^{2+}$ has been synthesized.

![Figure 2.1 Proposed Calix[4]arene with functional supporting groups for metal ions.](image)
Abstract

Receptor bis-(1,3-diphenyl-pyrazolyl methylene acetohydrazide)calix[4]arene (DPPMACA) has been synthesized by reacting calix[4]arene hydrazone with 4-formylpyrazole in presence of acid as a catalyst. The synthesized receptor DPPMACA shows strong recognition potential for Zn$^{2+}$ ions on evaluation with several metal sulfates such as Zn$^{2+}$, Y$^{3+}$, Eu$^{3+}$, Dy$^{3+}$, Na$^{+}$, Nd$^{3+}$, Fe$^{2+}$, Cd$^{2+}$, Al$^{3+}$, Be$^{2+}$, La$^{3+}$, Cu$^{2+}$ and K$^{+}$ using UV-visible spectroscopy. Receptor DPPMACA shows electronic absorption maxima at 230 nm and 280 nm. More interestingly, the receptor exhibited a bathochromic shift of 14 nm with Zn$^{2+}$ metal ion. The stoichiometry was found to be 1:1 with stability constant $6.438 \times 10^3$ M$^{-1}$. Additionally, computational visions were concentrated for studying the stability and spectroscopic analysis of the DPPMACA-Zn$^{2+}$ complex using docking, molecular dynamics simulations, and density functional theory (DFT) along with time-dependent density functional theory (TD-DFT). The calculations significantly supplement the findings and elucidate the structural geometry and mode of interactions in supramolecular complexation. Herein, it was obtained that DPPMACA was selectively stabilized by Metal-donor and Metal-acceptor contacts with Zn$^{2+}$ to generate a low-energy complex. These findings are of wide interest, especially because Zn$^{2+}$ is a well-known biologically important cation.

Keywords: Formylpyrazole, Calixarene, UV-visible, Zn$^{2+}$ sensor, DFT Calculation
2.1 Introduction

Calixarenes represent an important class of phenolic macrocyclic with an internal cavity which may be with hydrophobic and hydrophilic binding sites. Such sites are usually known as the upper and the lower rim of calixarenes\(^1\). The development of molecular receptors is very agile research area as it provides substantial binding sites for multiple ions\(^2,3\). Calixarenes provide framework as host molecules\(^4\) due to presence of cavity, functionalize sites and efforts are continuously made to design molecular receptors for recognition systems capable of sensing charged or neutral molecules\(^5\). These receptors reinforce theories on weak intermolecular interactions for sensing, separation, catalysis and for many other applications of supramolecular technology\(^6,7\). Mostly metals of d-block shows interactions with receptors and plays vital role in functioning of a vast number of widely differing bio-systems. When these metals are present in excess to biological processes they cause excessive damage\(^8\). Acute poisoning occurs due to ingestion of copper, cadmium and zinc salts. Further accumulation results in toxicity to liver, kidneys, brain, lungs, heart and central nervous system\(^9,10\).

Macrocyclic receptors are efficient for coordination with alkali and alkaline earth metals. But the derivatives of macrocyclic molecules (calix(aza-)crowns) are also able to interact with softer di- and trivalent cations. The Schiff-base of calix[4]arenes are also investigated to form complex with heavy transition metals and lanthanides. Calixarenes have also been investigated to act as a phosphodiesterase enzyme models\(^11,12,13\). Calixarenes with group containing nitrogen and sulfur atoms\(^14,15\) are also found as an ion-selective electrode sensitive\(^16,17\) to soft heavy-metal ions such as Ag\(^+\), Pb\(^{2+}\) and Hg\(^{2+}\).

Calixarene can be functionalized with phosphines, amides and imines. They come under attractive category that acts as host molecule for transition metal ions and for the formation of supramolecular building blocks\(^18,19,20\). The bis-(8-oxyquinoline)calix[4]arene, Di-aza-benzo crown ether derived from \(p\)-tert-butyl calix[4]arenes species is known to be effective receptor for Zn\(^{2+}\) ions\(^21\). Calix[6]arene-based receptor with hydrophobic imidazole cavity displays a remarkable set of biomimetic properties and binds with Zn\(^{2+}\) ion\(^22,23\).
Biological role of Zn$^{2+}$ metal ions

Zinc is a common element found in human and also a main component of natural environments. Zinc plays an important part in many biological processes and biochemical processes. Zinc in trace amount is essential for our body but in excess shows lethal effects. It is essential for the normal growth and the reproduction system in human, animals and plants. Zinc is responsible for growth and fulfils of immune function$^{24}$.

Zinc plays essential role in cellular metabolism$^{25}$. Zinc binds with proteins in major part; about 10% of human proteins potentially bind with zinc. In many enzymes catalytic activity zinc plays a very important role as they may not possible without zinc$^{26}$ and it also plays a vital role in immune function, wound healing, protein synthesis, DNA synthesis and cell division$^{27}$. In every growth stage of life zinc is required for proper sense of taste and smell and supports normal growth and development of human body$^{28}$.

Zinc is biological important element so proper it is not toxic in proper amount but excess may show adverse effects on human and living species; the excess may of overt toxicity symptoms (nausea, vomiting, epigastric pain, lethargy, and fatigue). The Recommended Dietary Allowance (RDA) (100-300 mg Zn/d vs an RDA of 15 mg Zn/d), is good and deficiency may result in many types of disorders in body also impaired immune function and adverse effects on the ratio of low-density-lipoprotein to high-density-lipoprotein (LDL/HDL) cholesterol$^{29}$. There are numerous techniques to study on the receptor metal cation binding phenomenon. However, spectroscopy techniques are found to be simple and fast and easy to handle$^{30}$.

Herein this chapter, synthesis and metal cation binding properties of bis-formylpyrazole calix[4]arene derivative have been revealed. The lower rim functionalization of calixarene with pyrazole has been chosen to design a moiety with the effective electron density. Oxygen and nitrogen atoms in calixarene are efficient in binding Zn$^{2+}$ ion. To know selective nature of receptor DPPMACA toward Zn$^{2+}$ ion UV-visible spectroscopy has been done and further binding of receptor DPPMACA with Zn$^{2+}$ was
studied by fluorescence spectroscopic, mass spectroscopic and cyclic voltammetric studies. In addition, computational visions were concentrated for studying the stability and spectroscopic analysis of the DPPMACA-Zn$^{2+}$ complex using docking, molecular dynamics simulations, and density functional theory (DFT) along with time-dependent density functional theory (TD-DFT).

2.2 Experimental

2.2.1 Chemicals and reagents

All reagents and solvents were used of HPLC or AR grade without further purification. Thin-layer chromatography was performed on silica gel 60 F254 silica-aluminium plates, and the plates were visualized using ultraviolet light.

2.2.2 Instruments

All glass wares were dried in an oven for 12 h before use. Open capillaries were used to determine melting point and are thus uncorrected. IR spectra were recorded on ABB MB 3000 IR Spectrophotometer. NMR data were recorded on a Bruker AV(III)-400 MHz. All samples were analyzed in CDCl$_3$. Mass spectra were recorded on a Xevo G2-S Q-TOF spectrometer (Waters, USA), capable of recording high-resolution mass spectrum (HRMS) in the electrospray ionization (ESI) modes. The abbreviations: s = singlet, d = doublet, m = multiplets have been used for multiplicities of coupled signals given in hertz. Electronic spectra of receptor and metal ions with receptor were recorded on T 90 (PG Instruments Ltd) UV/Vis spectrometer in the region 700–200 nm CH$_3$OH: H$_2$O in 1:1 as solvent system and emission spectra were recorded on Shimadzu-5301pc spectrofluorophotometer in THF:H$_2$O in 1:1 as solvent system. Cyclic voltammetric measurements were recorded on Ivium Stat Electrochemical Analyzer by using KCl as supporting electrolyte. Thermal Gravity Analysis was observed at Perkin Elmer TGA 4000 by heating at the rate of 5°C per minute.

2.2.3 Synthesis of receptor DPPMACA

4-formylpyrazole 4 was synthesized by the reported procedure$^{31}$. 

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The calix[4]arene hydrazone 5 was synthesized by reported procedure\textsuperscript{32,33}. The lower rim substituted bis-formylpyrazole calix[4]arene derivative DPPMACA was synthesized by refluxing 5 (0.8827 mmol) with formylpyrazole (1.7654 mmol) as in 1:2 molar ratio in ethanol with 2-3 drops of glacial acetic acid as catalyst for 30 h (yield = 70\%) scheme 2.2. After formation of product as observed by TLC in methanol-chloroform solvent system; the reaction mixture was cooled at room temperature and precipitated with yield 70\%. The solid obtained was recrystallized with methanol-chloroform. The precipitates were filtered with Whatman filter paper no.41 and dried in vacuum oven. The product DPPMACA formed was subjected to spectral analysis (IR, $^1$H NMR (Figure 2.2, Figure 2.3), $^{13}$C NMR (Figure 2.4) and mass spectroscopy (Figure 2.5).

Melting point 250-255\degree C; IR ($\nu_{\max}$, cm$^{-1}$): 3257, 2958, 1699, 1598, 1544, 1485, 1444, 1357, 1296, 1209, 1055, $^1$H NMR: $\delta$ (ppm) 11.37 (s, 2H, NH, D$_2$O exchangeable), 8.79 (s, 2H, pyrazole-\textbf{-H}), 8.66-8.62 (d, 4H, Ar-\textbf{H}), 8.43 (s, 2H, CH = N), 8.22 (s, 2H, OH, D$_2$O exchangeable), 7.84-7.73 (m, 4H, Ar\textbf{H}), 7.66-7.64 (d, 2H, Ar-\textbf{H}), 7.54-7.44 (m, 6H, Ar-\textbf{H}), 7.40-7.31 (m, 4H, Ar-\textbf{H}), 7.14, (s, 4H, Ar-\textbf{H}), 6.92(s, 4H, Ar-\textbf{H}), 4.19 (s, 4H, -OCH$_2$-), 3.86-3.82 (d, 4H, -Ar-CH$_2$-Ar-), 3.39-3.35 (d, 4H, -Ar-CH$_2$-Ar-), 1.33(s, 18H, -CH$_3$), 1.05(s, 18H, -CH$_3$). $^{13}$C NMR (75 MHz, CDCl3): $\delta$(ppm); 182.75, 132.00, 129.57, 128.95, 128.81, 128.75, 128.81, 128.75, 125.190, 119.42, 119.34, 117.20, 78.12, 77.34, 76.71, 34.18, 31.62, 30.94. MS-ESI-TOF (m/z) for $C_{80}H_{84}N_{8}O_{6}$ Calcd: 1252.6513 (M); Found: 1253.2372 (M+1)$^+$ (Figure 2.5).

Figure 2.2  $^1$H NMR spectrum of receptor DPPMACA in CDCl$_3$ at 25°C, recorded on a Bruker AV(III)-400 MHz.
Figure 2.3 $^1$H NMR spectrum of calixarene derivative receptor DPPMACA with protons position in $\delta$(ppm) recorded on a Bruker AV(III)-400 MHz.

Figure 2.4 $^{13}$C NMR spectrum of receptor DPPMACA in CDCl$_3$ at 25°C. NMR data recorded on a Bruker AV(III).
Mass spectroscopy studies are found to be in good agreement with the proposed structures of receptor **DPPMACA**.

### 2.2.4 Computational methodology

The Geometry were optimized using Gaussian at Density functional level (DFT) B3LYP/6-31G* of theory\(^\text{34}\). The Visualization of prior Geometry optimized structure and Geometry optimized structure were done using Schrodinger suite. The optimized structure was used as starting host and Zn\(^{2+}\) as a guest for docking in Hex 8.0 software\(^\text{35}\).

The Fast Fourier transformation (FFT) algorithm of rigid receptor approached with spherical polar Fourier (SPF) basis functions and grid sampling was accomplished to evolve the 1D, 3D and 5D orientations. The computational analysis was carried out to emphasize the best pair wise interactions between the host and guest molecules through...
rotational correlation over angular terms in a constrained search space approach. The inclusion complex of host and guest was utilized for the superimposition to decipher the 3D knowledge based shapes energy scores calculation. Thereafter, the best docked pose was retrieved from the top ten scored clusters. The lowest energy pose was taken as an input for Molecular dynamics stimulation using the Desmond Program version (academic version)\textsuperscript{36,37}.

To build the system, simple point charge (SPC) water was preferred as a solvent with optimization potential for liquid simulation (OPLS), all atoms force field\textsuperscript{38} 2005 in the cubic periodic box (10×10×10) size followed by neutralization process through Na\textsuperscript{+} and Cl\textsuperscript{-} counter ions. The docked host-guest complex was selected with 5036 atoms and 1619 water molecules proceeded by restraining steps through system minimization and pre-equilibrium to relax the system initially. 10 ns time interval was chosen for the molecular dynamic (MD) simulations with relaxation time of 2 ps at constant temperatures of 300 K, pressure 1.03215 bar with Nose-Hoover thermostat and Martyna-Tobias-Klein Barostat method having constant volume and shape ensemble (NVT). In the simulation process, Smooth Particle Mesh Ewald (PME) method\textsuperscript{39} (with a 10-9 tolerance limit) was employed to decipher the long range electrostatic interactions along with short-range cut-off distance of 9.0 Å, and long range electrostatic interactions. For detailed analysis, 1000 frames were generated during 10 ns in the trajectory and captured every 10 ps time step. Furthermore, the structural changes and dynamics behaviour of the complex were investigated by estimating the root mean square deviation (RMSD), total energy (E Total).

The Becke 3-parameter exchange functional together with the Lee-Yang-Parr correlation functional (B3LYP)\textsuperscript{40,41} was used for all the calculations in the density functional theory (DFT) method. In some previous reports, The B3LYP functional has been successfully utilized for geometry optimization of transition metal complexes\textsuperscript{42,43,44,45}. The general basis sets were used for the complex DFT calculation. The [B3LYP/6-31+G(d,p)/LANL2DZ] LANL2DZ relativistic pseudo potential was used for the zinc metal ion, while the C, H, N and O atoms were described by the 6-31+G(d,p)\textsuperscript{46,47}. The ligands and metal complexes were demonstrated using Avogadro
version 1.2.0 software\textsuperscript{48}. The gas phase geometry optimizations were carried out without symmetry constraint by using the Gaussian 09W software\textsuperscript{49}.

The electronic structure calculations, thermodynamic parameters were obtained from the geometry optimized structure. The frontier molecular orbital (FMO) energies, the energy of the lowest unoccupied molecular orbital ($E_{\text{LUMO}}$) and the energy of the highest occupied molecular orbital ($E_{\text{HOMO}}$), of the studied metal complexes are calculated. The absorption spectra calculated using TD-DFT level of theory. The effect of solvent (THF) was systematically monitored for all the steps via conductor-like polarizable continuum model (CPCM).

2.2.5 General procedure for the detection of cations by spectrophotometry

Stock solutions of DPPMACA (10 µM) and the sulfate salts (10 µM) of various metal ions [Zn$^{2+}$, Y$^{3+}$, Eu$^{3+}$, Dy$^{3+}$, Na$^+$, Nd$^{3+}$, Fe$^{2+}$, Cu$^{2+}$, Al$^{3+}$, Be$^{2+}$, La$^{3+}$, Cu$^{2+}$ and K$^+$ ions] were prepared in THF:H$_2$O (1:1 v/v) as the solvent system. In a 5-mL volumetric flask, 2.5 mL of the DPPMACA solution and 2.5 mL of metal salt stock solutions were combined so that the effective concentrations of both DPPMACA and metal ions were same. Absorption studies were performed using the as prepared solutions. A Benesi-Hildebrand plot (Figure 2.10) and Job’s plot (Figure 2.11) were also performed based on the absorption studies to establish the stoichiometry and association constant of receptor-metal. UV/VIS spectra (electronic spectra) of receptor and metal ions with receptor were recorded on T 90 (PG Instruments Ltd) UV/VIS spectrometer in the region 700–200 nm using THF:H$_2$O solvent system. Thermo Gravimetric Analysis was carried out on a Perkin Elmer TGA 4000 at heating rate of 5°C per minute.

2.3 Results and Discussion

The current section is organized as follows. Thermo gravimetical analysis TGA of DPPMACA was analyzed and then metal binding nature of DPPMACA was determined by evaluating different metal ions as their sulfates with the help of UV-visible spectroscopy. DPPMACA-Zn$^{2+}$ ion binding was further studied with the help of fluorescence spectroscopic, mass spectroscopic and cyclic voltammetric studies. The geometrical and energetic features associated with the binding of DPPMACA-Zn$^{2+}$
have been discussed on the basis of computational studies. Finally, it has been observed that the results of absorption spectra at TDDFT level of calculation and experimental UV-visible spectra change are almost similar for interaction between the DPPMACA and Zn$^{2+}$ ion.

2.3.1 Thermo Gravimetric Analysis (TGA)

Thermal study of DPPMACA was carried out in nitrogen environment (Figure 2.6). TGA data for all the samples were obtained in the standard form of weight% loss as function of temperature at heating rate 5°C min$^{-1}$ is shown in Figure 2.6. The thermal behaviour of DPPMACA was observed and discussed in stages as described, in first stage temperature 0-217°C there was no weight loss observed and from temperature 217-270°C, 2.5% of total weight was lost. The major weight loss was observed in the temperature range 270-527°C; in this temperature range total weight loss observed was 85.50% (calc. 81.79%) attributed to elimination of organic moiety of calixarene and pyrazole ring with mass loss. The remaining weight % loss i.e. 12% (calc. 18.20%) was lost in temperature range 527-800°C due to tert-butyl group of calixarene$^{50}$.

![Figure 2.6 TGA curves of DPPMACA at heating rates of 5°C min$^{-1}$.](image)

2.3.2 Evaluation of DPPMACA for ionic recognition

The synthesized molecular receptor DPPMACA was evaluated for cation binding ability by UV–visible spectroscopy using THF-water as solvent. The titration of
receptor DPPMACA were carried out in THF:H₂O (1:1 v/v) solvent system; by adding sulfate salts of Zn²⁺, Y³⁺, Eu³⁺, Dy³⁺, Na⁺, Nd³⁺, Fe²⁺, Cd²⁺, Al³⁺, Be²⁺, La³⁺, Cu²⁺ and K⁺ metal ions (Figure 2.7). The UV–visible absorption spectrum of the receptor DPPMACA (10 µM) exhibits good absorption bands λ_{max}, 230 nm and 280 nm (Figure 2.7). It was also observed that the receptor gave negligible response upon the addition of Y³⁺, Eu³⁺, Dy³⁺, Na⁺, Nd³⁺, Fe²⁺, Cd²⁺, Al³⁺, Be²⁺, La³⁺, Cu²⁺ and K⁺ metal ions as their sulfate salt. The change in spectra was observed only upon the addition of Zn²⁺ ions to receptor DPPMACA; it shows that the receptor is very selective for Zn²⁺ ions. In the UV-visible spectra a bathochromic shift was observed upon addition of Zn²⁺ ions to receptor DPPMACA from 280 nm to 294 nm and 230 nm to 244 nm with decrease in absorbance with isosbestic point 261 and 237 nm. The change in absorption spectra is an evidence for binding of receptor with Zn²⁺ ions to solution form, this decrease in absorbance and shifting in absorption maxima was noticed due to interaction of Zn---O and Zn---N of lower rim calixarene (Table 2.1).

![UV-visible spectra of receptor DPPMACA (10 µM) with Zn²⁺, Y³⁺, Eu³⁺, Dy³⁺, Na⁺, Nd³⁺, Fe²⁺, Cd²⁺, Al³⁺, Be²⁺, La³⁺, Cu²⁺ and K⁺ used their sulfates in THF:H₂O (1:1 v/v) as solvent system.](image)

Figure 2.7 UV-visible spectra of receptor DPPMACA (10 µM) with Zn²⁺, Y³⁺, Eu³⁺, Dy³⁺, Na⁺, Nd³⁺, Fe²⁺, Cd²⁺, Al³⁺, Be²⁺, La³⁺, Cu²⁺ and K⁺ used their sulfates in THF:H₂O (1:1 v/v) as solvent system.

2.3.3 Cations interference study

To know the interference of other metal cations; the effect of different metal ions with respect to zinc metal ion was also checked at the same concentration (Figure 2.8). The
results showed that the presence of other metal ions (Y$^{3+}$, Eu$^{3+}$, Dy$^{3+}$, Na$^+$, Nd$^{3+}$, Fe$^{2+}$, Cd$^{2+}$, Al$^{3+}$, Be$^{2+}$, La$^{3+}$, Cu$^{2+}$ and K$^+$) with Zn$^{2+}$ metal ions had a minimal effect on the absorption spectra of DPPMACA + Zn$^{2+}$ ions, with addition of these cations with respect to DPPMACA + Zn$^{2+}$, there was negligible change in absorption spectra was observed.

![Graph showing absorption spectra](image)

**Figure 2.8** Interference study of the cations Y$^{3+}$, Eu$^{3+}$, Dy$^{3+}$, Na$^+$, Nd$^{3+}$, Fe$^{2+}$, Cd$^{2+}$, Al$^{3+}$, Be$^{2+}$, La$^{3+}$, Cu$^{2+}$ and K$^+$ with DPPMACA + Zn$^{2+}$ ions (10 µM) in THF:H$_2$O (1:1 v/v) as solvent system at same concentration.

### 2.3.4 UV-visible titration

UV-visible titration gives valuable information of the complex formation. The slow change in absorption spectra with addition of guest ion and formation of inferred isosbestic point results to formation of new species in the solution$^{52}$. UV-visible titration was carried out by gradual addition of Zn$^{2+}$ ions (0–5 equivalent) to receptor DPPMACA, this led to a decrease in absorbance of band at 280 nm and 230 nm with appearance of a new band at 294 nm and 244 nm and isosbestic points at 261 and 237 nm (Figure 2.9). With decrease in absorbance there were formation of inferred isosbestic points indicating that a new species was formed and the transition between the free and the complexed species in the solution. It was envisioned that the synthesized cavity derived from diphenyl-pyrazolyl might be appropriate of 1:1 stoichiometry for DPPMACA-Zn$^{2+}$ ions, indicating an equilibrium between
DPPMACA and its Zn$^{2+}$ complex in THF:H$_2$O (1:1 v/v) as solvent system. The Benesi-Hildebrand (Figure 2.10) plot and Job’s plot (Figure 2.11) reveals the formation of a complex between DPPMACA and Zn$^{2+}$ ions with 1:1 stoichiometry. Also complexation of DPPMACA-Zn$^{2+}$ was determined by MS-ESI spectroscopy. DPPMACA (MS-ESI-TOF (m/z) for $C_{80}H_{84}N_8O_6$ Calcd: 1252.6513 (M); Found: 1253.2372 (M+1)$^+$ (Figure 2.5) and MS-ESI-TOF (m/z) for DPPMACA-Zn$^{2+}$ complex found 1314.1831 and m/z 1253.1522 for DPPMACA (Figure 2.12). Mass spectroscopy is found to be in good agreement with the proposed structures of receptor DPPMACA-Zn$^{2+}$ complex. The mass study of complex is evident for 1:1 complexation between DPPMACA-Zn$^{2+}$. The binding affinity, calculated by the Benesi-Hildebrand using the absorption spectra found to be $6.438 \times 10^3$ M$^{-1}$.

![Absorbance vs Wavelength graph](image)

**Figure 2.9** UV-visible spectra of receptor DPPMACA (10µM) with increasing concentration of Zn$^{2+}$ ions in THF:H$_2$O(1:1 v/v) solvent system.

2.3.5 Stoichiometry of complexation

2.3.5.1 Benesi–Hildebrand plot

To determine binding stoichiometry of complex and association affinity titration curve data obtained was utilized. The association constants have been calculated by the spectroscopic titration curve using Benesi-Hildebrand method employing following equations I for 1:1 stoichiometry.
\( I/(A - A_o) = I/(A - A_f) + I/K \ (A - A_f) \ [X] \) \( (I) \)

Where, \( K \) is the association constant, \( A \) is absorbance of free receptor, \( A_o \) is the observed absorbance of complex and \( A_f \) is the absorbance at saturation. \([X]\) is the concentration of ions. Receptor on titration by gradual addition of metal ion \([X]\), renders the spectrum that could be used to draw a plot between \( I/(A – A_o) \) vs. \( I/[Zn^{2+}] \) using above Benesi–Hildebrand equation. Linearity was observed in the Benesi–Hildebrand plot which indicated the formation of a 1:1 complex with association constant 6.438 \times 10^3 \text{M}^{-1}.

![Figure 2.10 Benesi-Hildebrand plot of receptor DPPMACA and Zn^{2+} ion at 280 nm, indicating the formation of a 1:1 complex with association constant 6.438 \times 10^3 \text{M}^{-1}.](image)

2.3.5.2 Job’s plot

To perform method of continuous variation (Job’s plot), stock solutions (100 ml) of receptor DPPMACA and Zn^{2+} metal salt of same concentrations were prepared. Then 10 solutions with altering mole fractions of each receptor and metal ions were prepared in THF:H_{2}O (1:1 v/v) as solvent system, keeping over all concentration constant (2\times 10^{-6} \text{ M}), UV-visible spectra were recorded to perform Job’s plot. The stoichiometry of DPPMACA-Zn^{2+} was determined by the method of continuous variation (Job’s plot). The total concentration of the receptor DPPMACA-Zn^{2+} was kept constant (20\times 10^{-6} \text{ M}), with a continuous variable molar fraction of guest Zn^{2+}. Figure 2.11 shows the Job’s plot of compound for DPPMACA-Zn^{2+} at 280 nm. The DPPMACA-Zn^{2+}
complex concentration approaches a maximum when the molar fraction of DPPMACA-Zn$^{2+}$ is 0.5. It means that DPPMACA-Zn$^{2+}$ complex formed a 1:1 (Host: Guest) complex with association affinity $K = 6.438 \times 10^3$ M$^{-1}$.

![Graph](image)

**Figure 2.11** Job’s plot for DPPMACA-Zn$^{2+}$ complex in THF-H$_2$O (v/v=1/1) solvent system. ([DPPMACA] + [Zn$^{2+}$]) = 20 $\times$ 10$^{-6}$ M at $\lambda_{\text{max}} = 280$ nm.

### 2.3.6 Mass spectroscopy

Mass spectroscopy is latest technique$^{53}$ for the study of host-guest complex formation and molecular recognition. Electrospray ionization mass spectrometry is found very efficient in the study of host-guest complexes and other non-covalent complexes formed in solution. With the development of electrospray ionization (ESI); it has covered the future of mass spectrometry in this important area of science$^{54}$. Mass spectra of DPPMACA and DPPMACA-Zn$^{2+}$ ion were recorded on Xevo G2-S Q-TOF spectrometer (Waters, USA), capable of recording high-resolution mass spectrum (HRMS) in the electrospray ionization (ESI) modes. DPPMACA (MS-ESI-TOF (m/z) for C$_{30}$H$_{54}$N$_8$O$_6$ Calcd: 1252.6513 (M); Found: 1253.2372 (M+1)$^+$ (Figure 2.5) and MS-ESI-TOF (m/z) for DPPMACA-Zn$^{2+}$ complex found 1314.1831 and m/z 1253.1522 for DPPMACA (Figure 2.12). The base peak for complex m/z 1314.183 and m/z 1253.1522 indicates the formation of 1:1 stoichiometry of complexation between DPPMACA-Zn$^{2+}$. Mass spectroscopy is found to be in good agreement with the proposed mass of receptor DPPMACA-Zn$^{2+}$ complex, it also suggest 1:1 stoichiometry of complexation between DPPMACA-Zn$^{2+}$. 42
2.3.7 Fluorescence spectra

The receptor DPPMACA acts as fluorophores and plays the central role in fluorescence spectroscopy\(^5\) and imaging on interactions with light. DPPMACA absorb energy of a specific wavelength and then re-emits energy with a different but equally specific wavelength. Upon addition of Zn\(^{2+}\) ion to DPPMACA; the intensity changes with hypsochromic shift due to change in both the fluorophore and the chemical environment of the fluorophore. In the fluorescence spectra, compounds DPPMACA (3×10\(^{-4}\) M) exhibited an emission from locally excited lowest state in THF:H\(_2\)O (1:1) solvent system. This emission from these compounds is due to photo induced electron transfer (PET) from the photo-excited part of receptor which leads to fluorescence quenching. The receptor DPPMACA shows maximum intensity near 402 nm at excited wavelength 285 nm. Upon addition of Zn\(^{2+}\) ions 1 eq. to receptor DPPMACA (3×10\(^{-4}\) M), fluorescence quenching was observed with new peak near 385 nm (Figure 2.13) due to mechanism of inhibition of electron transfer (eT)\(^5\). These observations indicate that the compound DPPMACA shows interaction with Zn\(^{2+}\) ions.
Scheme 2.3 Fluorescence mechanism of DPPMACA + Zn$^{2+}$ binding.

Figure 2.13 Fluorescence emission spectra of the DPPMACA and DPPMACA-Zn$^{2+}$, recorded on Shimadzu-5301pc spectrofluorophotometer in THF:H$_2$O (1:1) solvent system.

2.3.8 Cyclic voltammetric study

Electrochemical study$^{57}$ of DPPMACA (Figure 2.14) and DPPMACA-Zn$^{2+}$ ion (Figure 2.15) in solution, recorded in 3×10$^{-3}$ M in THF:H$_2$O (1:1 v/v) as solvent system using KCl as supporting electrolyte at room temperature with scan rate of 0.1 V/s in the
potential range -1.0 to +3.0 V. It was observed that oxidation and reduction potential are different for DPPMACA (Figure 2.14) and DPPMACA–Zn$^{2+}$ ion (Figure 2.15). The peaks observed for DPPMACA are $E_{pa} = -0.2450$ V and $E_{pc} = 0.0141$ V; and the peaks for DPPMACA–Zn$^{2+}$ ion are $E_{pa} = -0.4905$ V and $E_{pc} = -0.5314$ V. The huge change in the graphs observed and anodic cathodic peaks observed suggest reversible binding of DPPMACA with Zn$^{2+}$ metal ion (Figure 2.16).

**Figure 2.14** Cyclic voltammetric of DPPMACA ($3 \times 10^{-3}$ M) measurements recorded on a Ivium Stat Electrochemical Analyzer using KCl as supporting electrolyte.

**Figure 2.15** Cyclic voltammetric graph of DPPMACA–Zn$^{2+}$ ion ($3 \times 10^{-3}$ M) measurements were recorded on Ivium Stat Electrochemical Analyzer using KCl as supporting electrolyte.
2.3.9 Computational study

2.3.9.1 Docking

Geometry optimization (Figure 2.17) was carried out through the Gaussian 09 program package and Figure 2.18 confirms the optimization steps with energy fluctuation. Molecular docking was performed to evaluate the binding mechanism of DPPMACA complex with zinc metal ion to correlate the quenching mechanism. Figure 2.19 shows the top view as well as front view of docked complex. The \( \text{Zn}^{2+} \) molecules were reacted as a guest who bound with the host (DPPMACA). Five significant interactions (Table 2.1) were identified by docking analysis and four of them were metal acceptor while one was metal donor within the range of 1.65 to 2.41 Å (Table 2.1). The docked compound remained in the centre of the host compound which was the main aspect of the binding with metal acceptor groups. So, these results show the detection of metal ion with host compound. Figure 2.19 Different views allowed to decipher the binding affinity of \( \text{Zn}^{2+} \) compound with selected target very deeply.
Figure 2.17 Geometry optimized image of DPPMACA.

Figure 2.18 DPPMACA geometry optimization graph.
Figure 2.19 Molecular docking pose of DPPMACA-Zn$^{2+}$ complex.

Table 2.1 Docking result analysis.

<table>
<thead>
<tr>
<th>No</th>
<th>Interaction of molecule</th>
<th>Name of interaction</th>
<th>Distance (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Zn---O</td>
<td>Metal-Acceptor</td>
<td>2.41</td>
</tr>
<tr>
<td>2</td>
<td>Zn---O</td>
<td>Metal-Acceptor</td>
<td>2.15</td>
</tr>
<tr>
<td>3</td>
<td>Zn---O</td>
<td>Metal-Acceptor</td>
<td>1.87</td>
</tr>
<tr>
<td>4</td>
<td>Zn---O</td>
<td>Metal-Acceptor</td>
<td>1.85</td>
</tr>
<tr>
<td>5</td>
<td>Zn---N</td>
<td>Metal-Donor</td>
<td>1.65</td>
</tr>
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</table>
2.3.9.2 Molecular dynamics

Molecular dynamics (MD) simulations were implemented to gauge the structural stability and integrity of host-guest inclusion complex for 10 ns time trajectories. MD simulations were executed in water solvent with constant shape and volume at room temperature to understand the behaviour and interactions of host-guest inclusion complex during the whole event which reveals the total energy, potential energy, temperature, volume, pressure, root mean square deviation (RMSD), root mean square fluctuations (RMSF), intramolecular H-bond and radius of gyration (rGyr) at various time trajectories (Figure 2.20).

The average total energy value, potential energy value are given in Table 2.2; Figure-2.21 shows the event analysis of host-guest inclusion complex after the completion of MD simulation event which clearly depicts the major fluctuation in volume parameter within the range of 50,000 to 52,000 value while rest of all remained in between -204.861 to 500 value range. Different types of energies were obtained from event analysis, ranging from -200 kcal/mol to 500 kcal/mol which shows the changes occurred during the whole event were consolidated by the aforesaid range to make the complex stable. The most structural change was notified in RMSD values which had not affected the confirmation of host-guest inclusion complex and the average range was 0.75 Å to 4.8 Å. After the 9ns time interval the drastic change was observed in the confirmation of host-guest inclusion complex with the decreased numbers of RMSD values. Intra-hydrogen bonds were found during whole event that supports binding mechanism. Event analysis energy value during 0-10ns has been calculated. Furthermore, a steady RMSD trajectory value with a small change in the position in the Zn$^{2+}$ atoms clearly specified that the stability of the complex throughout the length of the simulation run. No major fluctuations in the rGyr, MolSA, SASA, and PSA plots were detected, which again established the compactness and steady exposure of Zn$^{2+}$ for a favourable conformational cavity of the DPPMACA (Figure 2.22).
Table 2.2 Simulation quality analysis result.

<table>
<thead>
<tr>
<th></th>
<th>Average</th>
<th>Std. Dev.</th>
<th>Slope (ps(^{-1}))</th>
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</thead>
<tbody>
<tr>
<td>Total Energy (kcal/mol)</td>
<td>-13014.535</td>
<td>47.058</td>
<td>-0.000</td>
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<tr>
<td>Potential Energy (kcal/mol)</td>
<td>-16032.925</td>
<td>36.867</td>
<td>-0.000</td>
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<td>Temp.(K)</td>
<td>298.793</td>
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<td>0.000</td>
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<tr>
<td>Pressure (bar)</td>
<td>-8.149</td>
<td>127.687</td>
<td>0.001</td>
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<tr>
<td>Volume (cm(^3))</td>
<td>51429.914</td>
<td>137.616</td>
<td>0.001</td>
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</table>

Figure 2.20 Simulation quality analysis graph.
Figure 2.21 Simulation event analysis graph.

Figure 2.22 Root mean square deviation (RMSD), radius of gyration (rGyr), molecular surface area (MolSA), solvent accessible surface area (SASA), and polar surface area (PSA) of the complex over a time period of 10 ns.
2.3.9.3 DFT calculations

Absorption spectra at the TD-DFT level of theory

The nature of the absorption spectra was highlighted via the quantum mechanical studies based on TD-DFT. Experimental and theoretical lowest energy transitions ($I_{max}$) were perceived by analyzing the nature of these transitions from the topologies of the Kohn–Sham orbitals. The frontier molecular orbitals (FMO’s) of DPPMACA can be found in Figure 2.23, and the maximum wavelength absorption ($\lambda_{max}$) is presented in Table 2.3.

![Molecular orbital representation of receptor DPPMACA obtained at the CPCM-TD-Cam-B3LYP/6-31+G(d) level of approximation.](image)

Figure 2.23 Molecular orbital representation of receptor DPPMACA obtained at the CPCM-TD-Cam-B3LYP/6-31+G(d) level of approximation.

It was clearly observed that the UV spectra emerged due to the electronic transitions from an amalgam of higher energy orbitals (HOMO-19, HOMO-10, HOMO-3, and HOMO) to lower energy orbitals (LUMO+1). Note that HOMO and LUMO was distributed on moiety substituents of DPPMACA. During the transitions, the distributions of LUMOs were not considerably affected even for other excited states.
The significant electronic conjugation between calixarene and moiety (DPPMACA-Zn$^{2+}$) is mainly due to the shifting of the electron density from HOMO-1 to LUMO+1 in the first excited state. However, other transitions were also possible that originated from HOMO and other lower energy orbitals. Also, the FMO of DPPMACA complex with Zn metal ion depicted in Figure 2.24, maximum wavelength absorption ($\lambda_{\text{max}}$) is presented in Table 2.3.

**Figure 2.24** Molecular orbital representation of receptor DPPMACA complexed with Zn$^{2+}$ obtained at the CPCM-TD-Cam-B3LYP/6-31+G(d) level of approx.
Table 2.3 Main Singlet Vertical Electron Transition Energies (ΔE), Wavelengths (λ), Oscillator Strengths (f), and Calculated at the TDDFT/CAMB3LYP Level

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name</th>
<th>Excitation (eV)</th>
<th>λ_exctation</th>
<th>Oscillator strength</th>
<th>Key transitions</th>
<th>% Contribution</th>
<th>λ_exp</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>DPPMACA</td>
<td>4.3858</td>
<td>282.7</td>
<td>0.9379</td>
<td>HOMO-19→HOMO HOMO-10→HOMO HOMO-3→HOMO HOMO-1→LUMO+1</td>
<td>2.03</td>
<td>280</td>
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<td>2</td>
<td>DPPMACA-Zn²⁺</td>
<td>4.1903</td>
<td>296.57</td>
<td>0.1033</td>
<td>HOMO-5→LUMO+6 HOMO-2→LUMO HOMO-2→LUMO+1 HOMO-2→LUMO+2 HOMO-2→LUMO+3 HOMO-2→LUMO+6 HOMO-2→LUMO+15 HOMO→LUMO+6</td>
<td>2.74</td>
<td>294</td>
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<td>2.02</td>
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</table>

It was perceived that the UV spectra emerged due to the electronic transitions from an amalgam of higher energy orbitals (HOMO-5, HOMO-2, and HOMO) to lower energy orbitals (LUMO, LUMO+1, LUMO+2, LUMO+3, LUMO+6, and LUMO+15). It was clearly identified that the UV-visible spectra of DPPMACA gives a bathochromic shift of 14 nm with Zn²⁺ metal ion due to complexation. The oscillator strength of these transitions was found to be feeble due to the non-bonded nature of the complex.

2.4 Conclusion

In this chapter an investigation has been carried out to determine the probability of DPPMACA as a receptor for Zn²⁺ ions. The synthesized Bis-(1,3-diphenyl-pyrazolyl methylene acetohydrazide)Calix[4]arene (DPPMACA) has been characterized by various spectral methods and it is found to show strong recognition potential for Zn²⁺ ions on evaluation with several metal sulfates. The stoichiometry was found to be 1:1 with stability constant $6.438 \times 10^3 \text{ M}^{-1}$. The host guest interactions were determined by computational methodology, molecular dynamics and DFT calculations. Although many techniques are available for the determination of Zn²⁺ but due to easiness in handling, low cost, good sensitivity, reliability and availability of instruments, the spectrophotometry has gained popularity over other techniques. Computational
discernments were provided to understand the interaction behaviour/mechanism of DPPMACA with Zn\textsuperscript{2+} ions via molecular docking followed by dynamics studies. Zn\textsuperscript{2+} was stabilized by the non-covalent interaction achieved by the cavity of DPPMACA. Importantly, the electronic transitions observed in the excitation spectrum were generated from the shifting of the electron density of HOMOs to LUMOs of Zn\textsuperscript{2+} to distribute at the arms of the DPPMACA. The proposed method using DPPMACA is highly qualified for determination of zinc. Further a method can be developed as a new, simple, sensitive and selective approach for the determination of Zn\textsuperscript{2+} ions in real samples. The choice of mix aqueous medium made the receptor more applicable.
References


49. Frisch, M. et al., Gaussian 09, rev. A. 02. Gaussian Inc., Wallingford, CT, **2009**.


