Chapter 6

Tetra Dansylated Calix[4]resorcinarene as Selective and Sensitive Turn Off Fluorescence Sensor for Fe(III), Co(II) and Cu(II) Ions
**Resume**

In this chapter a fluorescence receptor i.e. tetra dansylated calix[4]resorcinarene (TDCR) bearing dansyl group as fluorophore has been used to study its interaction behaviour with various metal ions e.g. Ag(I), Cd(II), Co(II), Fe(III), Hg(II), Cu(II), Pb(II), Zn(II), U(VI) (10$^{-4}$ M) by spectrophotometry and spectrofluorometry. Among various metal ions red shift in the absorption spectra was observed only in case of Fe(III), Co(II) and Cu(II) which led us to conclude that there is a selective complexation. These metal ions have also shown quenching with red shifts in the emission spectra. The maximum quenching in emission intensity was observed in the case of Fe(III) ions and its binding constant was also found to be significantly higher than that of Co(II) and Cu(II) ions. Quantum yield of metal complexes of Fe(III) ions was found to be lower in comparison with Co(II) and Cu(II) complexes.
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1. Introduction

The development of selective artificial fluorescence receptors for the detection of biologically and environmentally important metal ions has been an important goal in the field of supramolecular chemistry [1] and chemical sensors [2]. Fluorescent chemosensors typically consist of an ion recognition unit (ionophore) and a fluorogenic unit (fluorophore) linked through the proper spacer [3], are also called as fluoroionophore [4]. The ionophore is required for selective binding of the substrate, while the fluorophore provides the means of signaling this binding, either by fluorescence enhancement or quenching [5, 6]. The ideal fluorophore in chemosensors has high quantum yields of fluorescence, high extinction coefficients, long excitation and emission wavelengths, a long lifetime and photostability. Therefore, the choice of fluorophore is the most important factor in the design of new fluorescent chemosensors [7]. Different chemosensors have been used for investigation of their selective fluorescent responses upon complexation with various cations [8, 9]. Recently, there has been strong interest in dansyl derivatives as potential fluorophores [10-13]. Dansyl chloride is an attractive fluorophore due to the strong fluorescence, relatively long emission wavelength (Em = 400–600 nm), Stokes shift (Ex = 330–350 nm) and is also easy to introduce in a molecular skeleton to obtain fluorescent sensors [14]. Calixarenes [15, 16] which are cyclic oligomers of phenolic and methylene moieties, their preorganized binding sites, easy derivatization and flexible three dimensional structure make them perfect construction platforms for molecular design to generate fluorescent receptors [17, 18]. Calix[4]resorcinarenes which are analogous of calixarenes (resorcinol derived calixarenes) have a variable number of reactive positions for attaching
the ligating functions. Calix[4]resorcinarenes with appropriate appended groups have been found to be good candidates for recognition of cations and anions. Calix[4]resorcinarenes have received increased interest in synthesis of variety of chemosensors [19-21] bearing different fluorescent groups.

Among all metal ions, iron is very important for most organisms and play significant role in many biochemical processes. Iron is the most essential transition element responsible for carrying the oxygen in haem and acts as a cofactor in enzymatic reactions of mitochondrial respiratory chain. The deficiency of iron causes anaemia, hemochromatosis, liver damage, diabetes, Parkinson’s disease and cancer. As a consequence, intense research, efforts have been directed to develop sensitive and selective fluorescent sensors for such metal ions. Fluorescent sensor for Cu(II) is actively investigated, as it is a significant metal pollutant due to its widespread use, but it is also an essential trace element in biological systems [22]. Although Cu(II) toxicity for humans is rather low compared with other heavy-metal ions, certain microorganisms are affected by sub-micromolar concentrations of metallic materials.

With a view to develop selective calix[4]resorcinarene based fluoroionophore, the interaction behaviour of tetradansylated calix[4]resorcinarene with various cations Ag(I), Co(II), Fe(III), Zn(II), Cd(II), Hg(II), Pb(II), Cu(II) and U(VI) has been studied. Cation binding constants have also been determined from fluorescence titration data. Although there are multiple factors which affect the emission intensity and hence no single guideline can be used for their quantitative interpretation, an attempt has been made to explain the red shift in absorbance and emission spectra and quenching of
emission intensity of TDCR in the presence of Fe(III), Co(II) and Cu(II) metal ions.

2.0 Experimental section

2.1 Chemicals and reagents

Dansyl chloride, triethyl amine, metal nitrate salts and other chemicals were obtained from Sigma-Aldrich. Silica gel and fluorescence active TLC plates (F-2009) were purchased from the Merck. All aqueous solutions were prepared with quartz distilled deionized water, which was further purified by a Millipore Milli-Q water purification system. All the solvents employed for synthesis were commercially available and used as received without further purification.

2.2 Instruments

The melting points (uncorrected) were obtained from a VEEGO (Model; VMP-DS, Mumbai, India). Samples for infrared spectra were prepared as KBr pellets; spectra were recorded on Tensor Bruker 27 (Ettlingen, Germany) and expressed in cm⁻¹. Elemental analysis (C, H and N) was performed on varioMICRO-Variant elemental analyzer (Mt. Laurel, USA). Electrospray ionization (ESI) mass spectra (MS) were determined using MicromassQuarter2 (Utah, USA). NMR spectra were recorded on a model DPX 200 MHz and Avance II 500 MHz Bruker FT-NMR instruments (Ettlingen, Germany). UV-Vis spectra were recorded on a JASCO spectrophotometer (Easton, US). Fluorescence spectra were recorded on an EDINBURGH Uf-920H (Xenon lamp head Xe900) spectrometer (Livingston, US).
2.3. General procedure for the detection of cations by spectrophotometry and spectrofluorimetry measurements

The UV–Visible spectra of all the solutions containing metal ions (100 equivalents) were recorded and the same were compared to that of the original solutions to ascertain the chromogenic response of the ionophores in presence of various metal ions. A few ions exhibited significant changes in absorption spectra with detectable colour changes.

Stock solutions of the TDCR (2 x 10⁻⁶ M) and that of nitrate salts (2 x 10⁻⁴ M) of various metal ions Ag(I), Cd(II), Co(II), Fe(III), Hg(II), Cu(II), Mn(II), Pb(II), Zn(II), U(VI) were prepared in freshly purified methanol. Then 2.5 mL stock solution of the TDCR and 2.5 mL stock solution of each metal salts were taken in a 5 mL volumetric flask, so that the effective concentration of the TDCR is 1.0 x 10⁻⁶ M and that of the metal ions are 1.0 x 10⁻⁴ M (100 fold). The UV-Visible spectra of the resulting solutions and that of the original complex (1.0 x 10⁻⁶ M) were recorded with excitation at the absorption maxima (λ_max) of the TDCR, which is 332 nm. The spectra obtained upon addition of cations were compared with that of the original solution to ascertain the interactions of the metal ions with the fluoroionophore TDCR.

For emission titration study, the same stock solutions of the compounds were used and the metal nitrate solutions of desired concentration (1.0–100.0 equivalents) were prepared by proper dilution of the stock solution. Then 2.5 mL of each solution were mixed in a 5 mL volumetric flask to prepare reaction mixture with 1.0 to 100 fold molar equivalent of the concentration of metal ions with respect to the concentration of compound and the fluorescence spectra of
the resulting solutions were recorded. Binding constants of complex formation were calculated following the same literature procedure as described in the results and discussion section.

3. Results and Discussion

3.1. Absorption studies

**TDCR** shows a boat conformation with $C_{2v}$ symmetry. The four hydroxyl groups form hydrogen bonds to the sulfoxide oxygens of the dansyl moieties and stabilize the $C_{2v}$ conformation in solution [24], which is also indicated by four singlets of aromatic protons ($\delta$ 6.07-6.86) of resorcinarene in $^1$H-NMR. Usually, the dansyl group is attached through the S-atom to an amine, obtaining aminonaphthalensulfonate derivatives. The band is affected by the polarity of the solvent and shows a considerable charge-transfer character arising from the promotion of a lone-pair electron on the amino group into an antibonding orbital of the naphthalene ring [25].

The absorption spectra of **TDCR** ($10^{-6}$ M) upon addition of several metal ions (Ag(I), Co(II), Fe(III), Zn(II), Cd(II), Hg(II), Pb(II), Cu(II) and U(VI)) ($10^{-4}$ M), in excess (100 equivalent) were recorded in methanol. Only metal ions, Fe(III), Co(II) and Cu(II) shows exhibited significant changes in the spectra compared to other metal ions (Figure 1). The absorption band of **TDCR** was found at 332 nm. Changes in absorption spectra were noted in presence of metal ion like Fe(III) with a colour change from colourless to yellow and the band at 332 nm was shifted to 362 nm. In case of Co(II) it was seen that absorption bands shifted to 341 nm, while in case of Cu(II) it was shifted to 342 nm (Figure 2). This new band due to red-shift, indicates that these cations are interacting strongly with the **TDCR**, because when a cation is coordinated with the oxygen, the
excited state is more stabilized by the cation than the ground state, and this leads to a red shift of the absorption and emission spectra [23].

### 3.2. Fluorescence emission titrations

For emission titration studies, the same stock solutions of the TDCR and metal ions were used, which were used for absorption studies. The fluorescence spectra of the compounds (TDCR) were recorded in methanol in presence of 1-100-fold excess of various metal ions (Fe(III), Co(II) and Cu(II)) and the spectra thus obtained were compared with TDCR to ascertain the complexing ability of the fluoroionophore (TDCR). TDCR exhibits significant quenching in emission intensity in presence of Fe(III), Co(II) and Cu(II). When excited at 370 nm, TDCR gives emission bands with maxima at 472 nm. The effects of metal cations on the fluorescence spectra of TDCR shows in figure 3. Red shifts and strong quenching of the emission intensity were observed in the presence of Fe(III), Co(II) and Cu(II) because introduction of a harder O-dansyl electron donor instead of HN-dansyl fluoroionophores which shifts coordination preferences for these transition metal ions [26]. This results in strong complex formation with Fe(III), Co(II) and Cu(II), however other metal ions (Ag(I), Zn(II), Cd(II), Hg(II), Pb(II), and U(II)) did not induce any significant change in the emission spectra. Stronger complex formation will not only induce red shift in absorption and emission spectra rather it will also remove the hydrogen bonding of four hydroxyl groups which form hydrogen bonds to the sulfoxide oxygens of the dansyl moieties. This will facilitate the deprotonation of phenolic protons and simultaneous protonation of nitrogen of dimethylamino group which will reduce the available charge density on nitrogen and prevent charge transfer, thereby decreasing the emission intensity.
3.3. Binding constant and quantum yield

Cations, which exhibited substantial changes in emission intensity, were considered for emission titration to evaluate binding constant for TDCR following the literature procedure [27, 28].

\[
\frac{(F_0-F)}{(F-F_1)} = [M] \left(\frac{K_{\text{dis}}}{K_s}\right)^n
\]

A few representative spectra showing the changes observed in emission intensities upon addition of increasing concentration of ions are shown in (Figure 4-6). According to this procedure, the fluorescence intensity (F) scales with the metal ion concentration [M] through \((F_0-F)/(F - F_1) = ([M]/ K_{\text{dis}})^n\). The binding constant (Ks) is obtained by plotting \(\log\left(\frac{(F_0-F)}{(F-F_1)}\right)\) versus \(\log [M]\), where \(F_0\) and \(F_1\) are the relative fluorescence intensities of the complex without addition of guest metal ion and with maximum concentration of metal ion, respectively. The value of \(\log [M]\) at \(\log\left(\frac{(F_0-F)}{(F-F_1)}\right) = 0\) gives the value of \(\log (K_{\text{dis}})\), the reciprocal of which is the binding constant (Ks). The plots \(\log\left(\frac{(F_0-F)}{(F-F_1)}\right)\) versus \(\log [M]\) for selected metal ions are shown as insets in (Figure 4-6). The binding constants of all complexes are summarized in Table 1. Analysis of the data (Table 1) shows that, the binding constant of Fe(III) is significantly higher than that of Co(II) and Cu(II) with TDCR in methanol. These metal ions are hard acids, when compared to other metal ions used in the present study and will prefer to bind oxygen donor (hard base) atoms to form stronger complex.

Quantum yield is frequently used to characterize luminescent material. It corresponds to the ratio between the number of emitted photons at a given wavelength, usually in the UV-Visible region, and the number of absorbed photons.
Fluorescence quantum yields ($\phi_f$) were determined by the comparative method (Equation (2) [29, 30].

$$\phi = \phi_{std} \frac{F \cdot A_{std} \cdot \eta^2}{F_{std} \cdot A \cdot \eta^2_{std}}$$

Where $F$ and $F_{std}$ are the areas under the fluorescence emission curves of the metal (U(VI), Th(IV), and Fe(III)) complexes with TDCR and the standard TDCR, respectively. $A$ and $A_{std}$ are the relative absorbance of the sample and standard at the absorption wavelength respectively. $\eta$ and $\eta_{std}$ are the refractive indices of solvent (methanol) used for the sample and standard respectively. Both the sample and the standard were excited at the same relevant wavelength.

Quantum yield [31, 32] of fluoroionophores (TDCR) was obtained using emission spectra of Standard (dansyl chloride). Reported quantum yield of dansyl chloride is approximately 0.7 and that of fluoroionophore (TDCP) was found to be 0.78. It was observed that number of emitted photons decreases with the addition of increasing concentration of metal ions i.e. quenching takes place. Quantum yield of metal complexes of Fe(III) was found to be lower in comparison with Co(II) and Cu(II) complexes which are summarized in Table 1.

3.4. Stern-Volmer analysis

Stern-Volmer plots are useful for understanding the mechanism of emission quenching [33, 34] and hence were utilized to probe the nature of the quenching process in the complexation of Fe(III), Co(II) and Cu(II) with TDCR. From the data, dynamic or static quenching processes can be determined by plotting relative emission intensities ($I_0/I$) against quencher concentration [Q].
Expressed by the equation 3, the slope of the plotted line yields $K_{sv}$ (static quenching constant).

$$\frac{I_0}{I} = 1 + K_{sv}[Q] \quad 3$$

If the evolution of $I_0/I$ plots, according to the concentration of quencher, is linear for the whole range of quencher concentrations, fluorescence quenching can be attributed either to being purely dynamic or purely static, the latter mechanism being due to the formation of a ground-state non-fluorescent complex. In contrast, if the ratio $I_0/I$ is not linear and shows an upward curve at higher quencher concentrations, the fluorescence quenching mechanism can be attributed to the presence of simultaneous dynamic and static quenching [35]. In all our cases, typical linear plots for Fe(III), Co(II) and Cu(II) metal ions with TDCR were observed which indicate that fluorescence quenching is either purely dynamic, or purely static (Figure 7).

### 3.5. Stoichiometry of the metal ions complex

The stoichiometric ratio of the metal complex Fe(III), Co(II) and Cu(II) was determined by the modified Job’s method of continuous variation. The absorbance of complex $A_c$ of a series of solution having different concentration ratios of metal ions $(a)$ and TDCR $(b)$, keeping total the concentration $(a+b)$ constant, was measured at 332 nm. The absorbance of $b$ was subtracted from the observed absorbance $A_c$ to obtain the true absorbance $\Delta A$.

$$\Delta A = A_c - A_b$$

$\Delta A$ values were plotted against the molar ratio of metal ions Fe(III), Co(II) and Cu(II) $a/a+b$ (Figure 8). The maximum value for $\Delta A$ was clearly obtained for Fe(III) at $a/a+b = 0.58$, indicating 1:1, Co(II) at $a/a+b = 0.48$, indicating 1:1
and Cu(II) at a/a+b = 0.52, indicating 1:1 respectively stoichiometric ratio of metal ions and TDCR. The accuracy of this result is possible only if a single complex is formed. To verify this, measurement were taken at different selected wavelength, which gave the same value for a/a+b ratio.

Conclusion

Complexation behavior of TDCR with large number of metal ions was studied by spectrophotometry and spectrofluorimetry. Study reveals that TDCR has selectivity complexing ability with Fe(III), Co(II) and Cu(II) ions which leads to a red shift in the absorption and emission spectra. The maximum quenching in emission intensity was observed in the case of Fe(III) and its binding constant was also found to be significantly higher. Stern Volmer analysis indicates that the mechanism of fluorescence quenching is either purely dynamic, or purely static. Quenching is due to the formation of metal complex which helps removal of hydrogen bonding and thereby deprotonation of phenolic hydrogen and protonation of dimethylamino group which prevents charge transfer thereby decreasing the emission intensity.
**Figure 1:** Graph of TDCR (Methanol, $1.0 \times 10^{-6}$ mole L$^{-1}$) upon addition of various metal ions in methanol, $(1.0 \times 10^{-4}$ mole L$^{-1}$) 100eq.

**Figure 2:** UV-Visible spectra of TDCR ($1 \times 10^{-6}$ mole L$^{-1}$) upon addition of 100 equivalents of various metal ions in methanol, $(1 \times 10^{-4}$ mole L$^{-1}$)
Figure 3: Fluorescence spectra of TDCR (1 X 10⁻⁶ mole L⁻¹) upon addition of 100 equivalents of various metal ions in methanol.
**Figure 4:** The fluorescence spectral change of TDCR (1 X 10^-6 mole L^-1) upon addition of 0-100 equivalents of Fe(III) ions in methanol. Inset: linear regression fit (double-logarithmic plot) of the titration data as a function of concentration of metal ion.
Figure 5: The fluorescence spectral change of TDCP (1 X 10^{-6} mole L^{-1}) upon addition of 0-100 equivalents of Cu(II) ions in methanol, Inset: linear regression fit (double-logarithmic plot) of the titration data as a function of concentration of metal ion.
Figure 6: The fluorescence spectral change of TDCP (1 X 10^{-6} mole L^{-1}) upon addition of 0-100 equivalents of Cu(II) ions in methanol. Inset: linear regression fit (double-logarithmic plot) of the titration data as a function of concentration of metal ion.
Figure 7: Stern-Volmer plots of the TDCP in methanol by (A)Fe(III), (B)Co(III) and (C)Cu(II)
Figure 8: Job’s plot for the TDCR with metal ions (Fe(III), Co(II) and Cu(II))
**Graphical abstract**

Schematic representation showing fluorescence quenching of TDCR in presence of Fe(III), Co(II) and Cu(II) metal ions, resulting in a turn-off fluorescent assembly.
Table: 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\lambda_{\text{max}}$</th>
<th>$\lambda_{\text{em}}$</th>
<th>Binding constant ($K_s$) M$^{-1}$</th>
<th>Stokes shift (nm)</th>
<th>Quantum yield ($\phi$)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDCR</td>
<td>332</td>
<td>472</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TDCR + Fe(III)</td>
<td>362</td>
<td>535</td>
<td>$8.2 \times 10^4$</td>
<td>110</td>
<td>0.36</td>
<td>0.9928</td>
</tr>
<tr>
<td>TDCR + Co(II)</td>
<td>341</td>
<td>478</td>
<td>$5.6 \times 10^4$</td>
<td>131</td>
<td>0.58</td>
<td>0.9915</td>
</tr>
<tr>
<td>TDCR + Cu(II)</td>
<td>342</td>
<td>478</td>
<td>$5.2 \times 10^4$</td>
<td>130</td>
<td>0.62</td>
<td>0.9838</td>
</tr>
</tbody>
</table>

Emission maxima ($\lambda_{\text{em}}$) of the spectra recorded in presence of maximum concentration of guest ion
References

[34] H.M. Larsen RW, Everett WR, Jameson DM Ground- and excited-state characterization of an electrostatic complex between tetrakis(4-sulfanato-