



The investigation of cooperative binding between *p*-sulfonatocalix[6]arene and fluorescein with transition metal ions by spectrometrically

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Abstract. The ternary complexes are formed by self-assembly through cooperative hydrogen bonding between *p*-SCX6-FL and M^{2+} through water molecule which is reinforced by columbic and electrostatic interactions. The binding efficiency of Cu^{2+} and Zn^{2+} is observed at a greater extent than Co^{2+} and Ni^{2+} . Furthermore, the kinetic study of *p*-SCX6-FL- Cu^{2+} and Zn^{2+} reveals that the process of complexation is slower than the binary system (FL+*p*-SCX6).

Keywords. *p*-sulfonatocalix[6]arene; ternary system; cooperative binding.

1. Introduction

The characterizing aspect of supramolecular chemistry is carefully designed synthetic structures (hosts) recognize target molecules (guests) and form a supramolecular complex through noncovalent interactions.¹ In the last few years, the inclusion complexation and molecular recognition are of huge interest in host-guest chemistry.² In this field, cyclodextrins (CDs),³ cucurbiturils (CBs)⁴ and calixarenes (CAs)⁵ as three most active synthetic receptors have been extensively studied. In conniving a suitable host, you have to consider parameters like host size, charge and character of the donor atom, according to the properties of target molecules. The calixarene chemistry is a well-established field within the supramolecular chemistry.^{6,7} Sulfonatocalix[n]arene (n = 4, 5, 6, 8), a well-known kind of water-soluble calixarene derivatives have been attracting increasing attention in supramolecular

chemistry and coordination chemistry. As compared to *p*-sulfonatocalix[4]arene, the study of *p*-sulfonatocalix[6]arene in the solid state and solution chemistry is less well developed.^{8,9} The cations for fluorescent sensors have constantly established their potential in a variety of fields, such as environmental sensors, biological probes, food safety, etc.^{10–12} Calixarenes are widely used in the field of ion-selective electrodes, sensors,¹³ optical sensors,¹⁴ self-assembly,¹⁵ catalysis,¹⁶ drug discovery⁶ and as molecular recognition devices for solid-phase, modifiers and as the stationary phase. The applications of these studies of metal complexes are required to design sensors for detection and to determine toxic metal ions, and their removal to protect our environment. The application of calixarene metal complexes in the elucidation of enzymatic processes is worth noting.¹⁷

Water-soluble calixarene-dye complexation study is well known because of its cavity size, hydrophobic

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cavity and hydrophilic ends which tend to increase the complexation ability towards the dye. Zhang and co-worker¹⁸ noted that, the restriction in rotation of Auramine O dye due to complexation with *p*-sulfonatocalix[6]arene which leads to 1:1 binding. As it has been discussed earlier the fluorescent molecular sensor for the detection of heavy metal ions were explored by many researchers, between them in 2009 Leray has been modified the calixarene crown moiety which offers a selective complexation unit for the detection of caesium ion.¹⁹ From the xanthene dyes, Fluorescein is rarely explored by the researcher. It is partially soluble in the mixture of water and alcohol. In the diagnosis of a number of eye problems, fluorescein is used. Ion recognition is a subject of considerable interest because of its implications in many fields: chemistry, biology, medicine, environmental, etc. In particular, selective detection of metal cations involved in biological processes (e.g., sodium, potassium, calcium, magnesium,²⁰ in clinical diagnosis (e.g., lithium, potassium, aluminium) or in environmental pollution (e.g., lead, mercury, cadmium) has received much attention.²¹ Among the various methods available for detection of ions and more generally organic and inorganic species, those based on fluorescence sensors offer distinct advantages in terms of sensitivity, selectivity, response time, etc.

p-sulfonatocalix[6]arene has been used by many researchers for investigating the study of supramolecular complex in solid-state. Cuiping Han²² and group synthesized-sulfonatocalix[6]arenes-modified gold nanoparticles in an aqueous media for detection of diaminobenzenes isomer^{23,24} for pollutant detection, silver nanoparticles are used for electrochemical sensor.²⁵ *p*-sulfonatocalix[6]arene-modified superparamagnetic behaviour of magnetite nanoparticles was investigated.²⁶ Both superparamagnetic and fluorescent properties are investigated for *p*-sulfonatocalix[6]arene coated superparamagnetic Fe₃O₄ nanoparticles.²⁷ Luis Garcia Rio²⁸ used *p*-sulfonatocalix[6]arene for studying cmc values of simple surfactants and micellization process. Single crystals of *p*-sulfonatocalix[6]arene/ytterbium(III) pyridine N-oxide was prepared and inclusion complex was studied by X-ray diffraction studies. Wuping Liao *et al.*,²⁹ synthesized copper/*p*-sulfonatocalix[6]arene/phenanthroline supramolecular compound and characterized by single-crystal X-ray diffraction. Werner Nau and co-workers explored the simple supramolecular approach to metalloenzyme models in aqueous solution, which is based on the dynamic self-assembly between macrocyclic host with cation receptor properties, organic guests and metal ions.³⁰ They proposed the

ternary complex where the guest is held in hydrophobic interactions with the host, while the metal ion experiences attractive coulombic interactions with negative charges positioned at the portal of the macrocycle (*p*-sulfonato groups at the upper rim). They also introduced the mechanism of bonding, if the guest functions as a weak ligand the host can assist the formation of metal-ligand bond with the guest which reinforces the ternary complex and result into the cooperatively.³¹

Thus, it is observed that binary inclusion complexation either with dye or metal ions with *p*-sulfonatocalixarenes is done by many researchers. However, to the best of our knowledge, less report has been available for the ternary system with few transition metal ions using *p*-sulfonatocalix[6]arene-fluorescein. To know more about complexation behaviour for the ternary system, an endeavour is made to carry out this study. The binding behaviour of these binary and ternary complexes is evaluated by spectrofluorometer, lifetime study, NMR spectroscopy and SEM.

2. Experimental

2.1 Reagents and materials

p-Sulfonatocalix[6]arene (*p*-SCX6) was purchased from TCI. Fluorescein (FL) and metal salts cobalt, nickel, copper and zinc were of A. R. grade procured from S.D. Fine chemicals. The stock solution of Fluorescein (dye) was made of 2×10^{-6} M and all-metal ions solutions were of 1×10^{-2} M and prepared by dissolving appropriate amount in Milli Q water. Double distilled water was purified further by Milli Q. All solvents used were of A. R. grade. For NMR studies D₂O and DMSO-d₆ were supplied by S. D. Fine which were 99.8% and used as it is.

2.2 Procedure

To 1.66×10^{-6} M fluorescein solution, which is taken in cuvette, 1×10^{-3} M solution of *p*-SCX6 was added in different volumes. The pH of the solution was maintained at 6.0. To the same solution, 5×10^{-3} M of M²⁺ (Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺) was added in different volumes till saturation is obtained. Absorbance and fluorescence intensity of the solution was measured by spectrophotometrically and fluorometrically. The concentrations were, FL (1.66×10^{-6} M), *p*-SCX6 (1×10^{-3} M) and M²⁺ (5×10^{-3} M). The parameters for all the fluorescence spectroscopy experiments were: bandwidth = 5 nm; sensitivity = low; scan speed = 200 nm/min.; data interval = 1 nm; response = 1 s; wavelength = 450 nm.

To explore the correct picture of complexation of binary and ternary system, NMR study is also carried out. The

mode of binding behaviour of *p*-SCX6 with fluorescein and metal ions is studied using ^1H NMR titration at 25 °C for binary and ternary systems. NMR data for binary and ternary systems were collected in deuterated solvents of an equal volume of D_2O and DMSO-d_6 . The lifetime of Cu^{2+} ternary system was also studied. The binding constants were determined for FL with *p*-SCX6 and M^{2+} with the aid of Valeur equation, (eq. 1).³²

The surface morphology of complexes is studied by evaporating the samples taken for complexation study for absorbance and fluorescence measurements. The changes in morphologies were studied by Scanning Electron Microscope (SEM). The samples are dropped onto a small silicon vapour and left at room temperature. An attempt was made to develop suitable crystals for X-ray diffraction by slow diffusion of 1:1 equivalent of (FL + *p*-SCX6), (*p*-SCX6 + FL + Cu^{2+}) and (*p*-SCX6 + FL + Zn^{2+}) in methanol. The crystallization experiments were carried out at room temperature in a sealed beaker.

3. Results and Discussion

3.1 Steady-state fluorescence studies

Steady-State fluorescence study of FL and *p*-SCX6 are carried out, which reveals the quenching in fluorescence of FL. In Figure 1a, it is clearly observed that bathochromic shift from 509 nm to 512 nm is due to hydrogen bonding between FL and *p*-SCX6 in polar protic solvent. This may be because the FL interacts with *p*-SCX6 through solvent molecule (Hydrogen bonding). The study of FL-*p*-SCX6 complex with some of the first transition series metal ions which exhibit heavy metal ion effects are discussed here.

3.2 Characterization of the M^{2+} complex

The study of complexes of few metal ions from the first transition series in aqueous medium are examined by various methods. Firstly, in steady-state fluorescence, the Cu (II), Zn (II), Co (II) and Ni (II) complexes were prepared by mixing 5 mM M^{2+} in FL-*p*-SCX6 complex at room temperature. The corresponding fluorescence spectra (Figures 2–5) of complexes show maximum emission at 512 nm with red shift, this may be due to a solvent molecule embedded into the cavity of *p*-SCX6. The cooperative binding between FL- *p*-SCX6 and Cu (II), Zn (II), Co (II) and Ni (II) is observed because there are no spectral changes seen upon addition of M^{2+} except that it shows heavy metal ion effect resulting in the quenching of fluorescence.

3.3 Determination of association constant

The association constant of FL- *p*-SCX6 and Cu (II), Zn (II), Co (II) and Ni (II) were measured from steady-state fluorescence by using the linear fitting method. The value of binding constant listed in Table 1.

The determination of binding constants by Valeur method is

$$\frac{I_0}{I_0 - I} = \frac{\varepsilon_L \phi_L}{\varepsilon_{ML} \phi_{ML}} \left(\frac{1}{K_s [M]} + 1 \right) \quad (1)$$

where, I_0 and I are initial and final fluorescence intensities.

In Table 1, the binding constant for (FL-*p*-SCX6- Cu^{2+}) and (FL-*p*-SCX6- Zn^{2+}) turn out to be 13.90 and

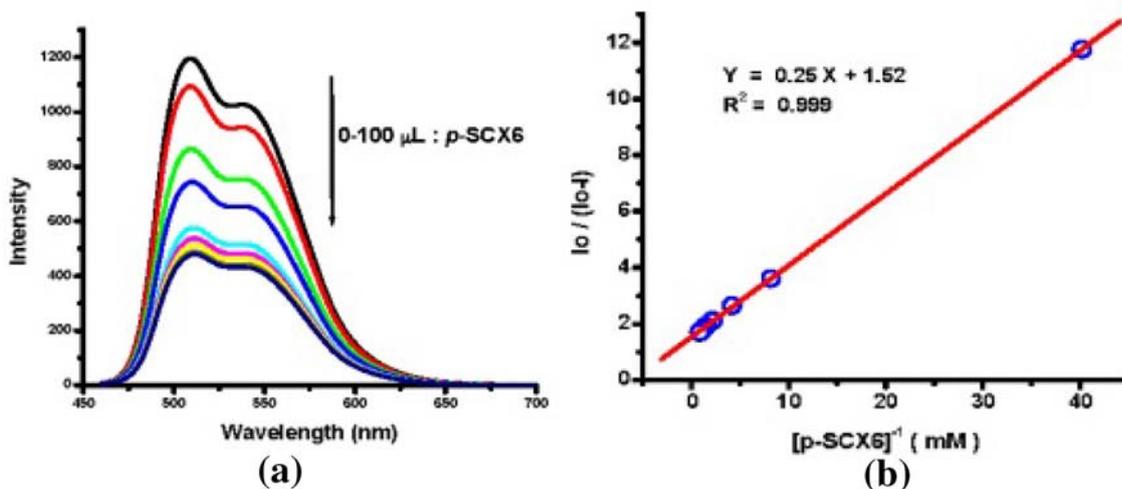


Figure 1. (a) Fluorescence spectra of FL (1.66×10^{-6} M) in the presence of (i) *p*-SCX6 (binary system) {*p*-SCX6/ (1×10^{-3} M)}: 1) 0 μL , 2) 1 μL , 3) 5 μL , 4) 10 μL , 5) 20 μL , 6) 40 μL , 7) 60 μL , 8) 80 μL , 9) 100 μL (b) Plot of $I_0 / (I_0 - I)$ vs $[p\text{-SCX6}]^{-1}$.

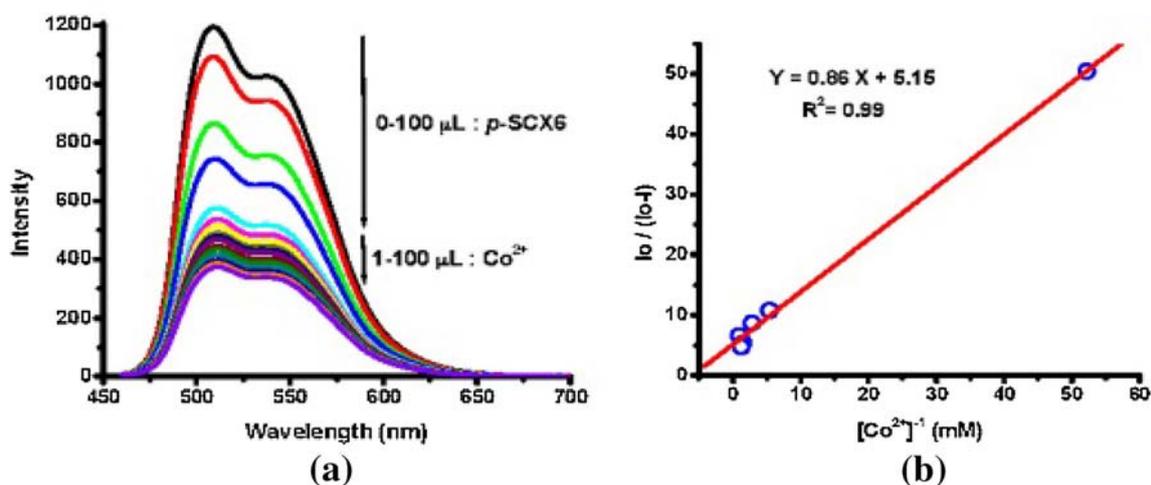


Figure 2. (a) Fluorescence spectra of FL (1.66×10^{-6} M) in the presence of (i) *p*-SCX6 (binary system) (*p*-SCX6/ (1×10^{-3} M): 1) 0 μ L, 2) 1 μ L, 3) 5 μ L, 4) 10 μ L, 5) 20 μ L, 6) 40 μ L, 7) 60 μ L, 8) 80 μ L, 9) 100 μ L) and (ii) FL-*p*-SCX6- Co^{2+} (ternary system), $\{\text{Co}^{2+}$ (5×10^{-3} M): 1) 1 μ L, 2) 5 μ L, 3) 10 μ L, 4) 20 μ L, 5) 40 μ L, 6) 60 μ L, 7) 80 μ L, 8) 100 μ L. (b) Plot of $I_0/(I_0-I)$ vs $[\text{Co}^{2+}]^{-1}$ of FL-*p*-SCX6- Co^{2+} system.

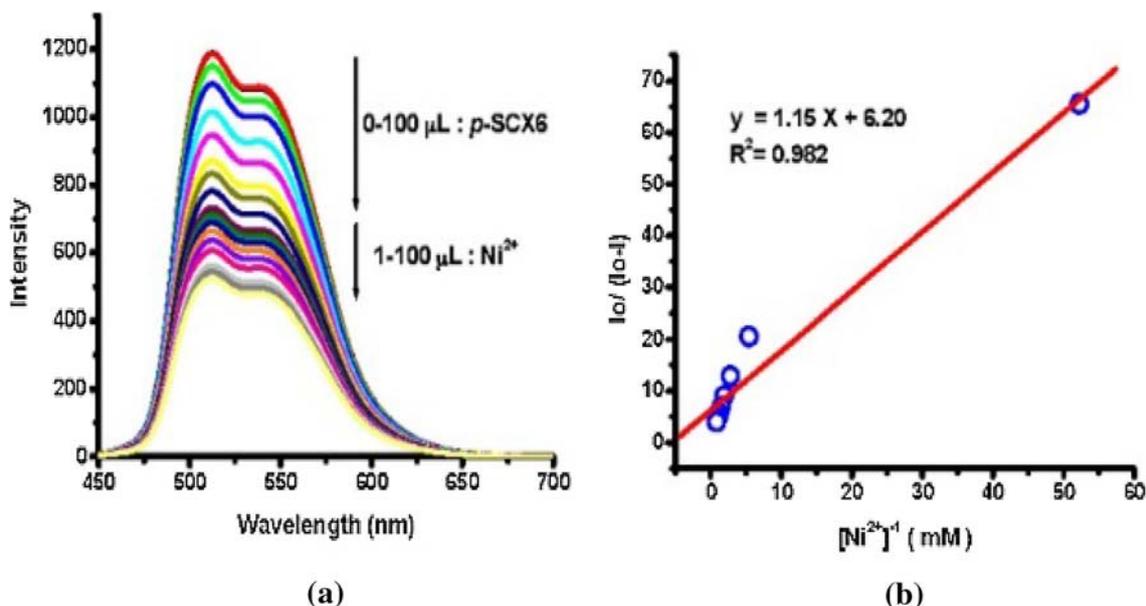


Figure 3. (a) Fluorescence spectra of FL (1.66×10^{-6} M) in the presence of (i) *p*-SCX6 (binary system) (*p*-SCX6/ (1×10^{-3} M): 1) 0 μ L, 2) 1 μ L, 3) 5 μ L, 4) 10 μ L, 5) 20 μ L, 6) 40 μ L, 7) 60 μ L, 8) 80 μ L, 9) 100 μ L) and (ii) FL-*p*-SCX6- Ni^{2+} (ternary system), $\{\text{Ni}^{2+}$ (5×10^{-3} M): 1) 1 μ L, 2) 5 μ L, 3) 10 μ L, 4) 20 μ L, 5) 40 μ L, 6) 60 μ L, 7) 80 μ L, 8) 100 μ L. (b) Plot of $I_0/(I_0-I)$ vs $[\text{Ni}^{2+}]^{-1}$ of (FL-*p*-SCX6- Ni^{2+}) system.

7.03×10^3 respectively. This may be due to the strong coulombic interaction of Cu^{2+} and Zn^{2+} with FL-*p*-SCX6.³³ The selectivity towards Cu^{2+} may be due to tetragonally-distorted geometry; copper is anomalously stable in the 3d-series and therefore it leads to stable complexation.³⁴ The driving forces responsible for this ternary binding are hydrophobic and CH- π interaction between FL and *p*-SCX6. In addition to this, the electrostatic interaction between $\text{M}^{2+} \cdots \text{SO}_3^-$ and hydrogen bonding between -OH group of *p*-SCX6

and coulombic interaction between the metal ions and binary system are also responsible for ternary complexation.³⁵

3.4 Determination of Stern-Volmer quenching constant

The change in fluorescence intensity has been portrayed as I_0/I vs $[Q]$ which gives a straight line

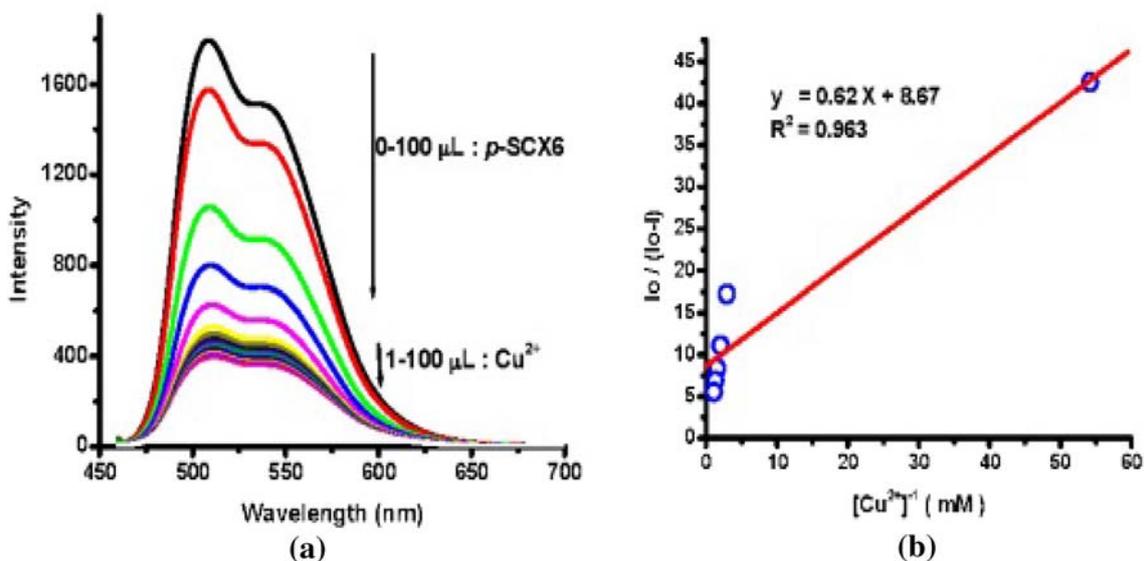


Figure 4. (a) Fluorescence spectra of FL (1.66×10^{-6} M) in the presence of (i) *p*-SCX6 (binary system) (*p*-SCX6/ (1×10^{-3} M): 1) 0 μ L, 2) 1 μ L, 3) 5 μ L, 4) 10 μ L, 5) 20 μ L, 6) 40 μ L, 7) 60 μ L, 8) 80 μ L, 9) 100 μ L) and (ii) FL-*p*-SCX6- Cu^{2+} (ternary system), $\{\text{Cu}^{2+}$ (5×10^{-3} M) $\}$: 1) 1 μ L, 2) 5 μ L, 3) 10 μ L, 4) 20 μ L, 5) 40 μ L, 6) 60 μ L, 7) 80 μ L, 8) 100 μ L (b) Plot of $I_0 / (I_0 - I)$ vs $[\text{Cu}^{2+}]^{-1}$ of (FL-*p*-SCX6- Cu^{2+}) system.

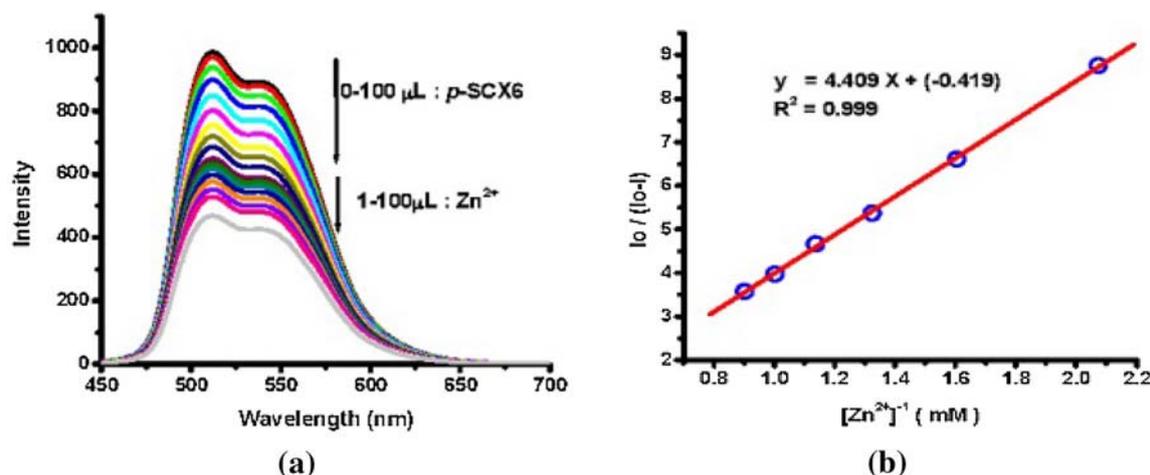


Figure 5. (a) Fluorescence spectra of FL (1.66×10^{-6} M) in the presence of (i) *p*-SCX6 (binary system) (*p*-SCX6/ (1×10^{-3} M): 1) 0 μ L, 2) 1 μ L, 3) 5 μ L, 4) 10 μ L, 5) 20 μ L, 6) 40 μ L, 7) 60 μ L, 8) 80 μ L, 9) 100 μ L) and (ii) FL-*p*-SCX6- Zn^{2+} (ternary system), $\{\text{Zn}^{2+}$ (5×10^{-3} M) $\}$: 1) 1 μ L, 2) 5 μ L, 3) 10 μ L, 4) 20 μ L, 5) 40 μ L, 6) 60 μ L, 7) 80 μ L, 8) 100 μ L (b) Plot of $I_0 / (I_0 - I)$ vs $[\text{Zn}^{2+}]^{-1}$ of FL-*p*-SCX6- Zn^{2+} system.

Table 1. Binding constants for the binary and ternary system by Spectrofluorometric.

Sl. No.	System	Binding constant (M^{-1})
1	FL- <i>p</i> -SCX6	6.08×10^3
2	FL- <i>p</i> -SCX6- Co^{2+}	5.68×10^3
3	FL- <i>p</i> -SCX6- Ni^{2+}	5.39×10^3
4	FL- <i>p</i> -SCX6- Cu^{2+}	13.90×10^3
5	FL- <i>p</i> -SCX6- Zn^{2+}	7.03×10^3

(Figure 6a–e). The Stern-Volmer constant is estimated from the slope listed in Table 2 which was calculated from eq 2.

$$I_0 / I = 1 + K_{sv}[Q] \tag{2}$$

3.5 Time-resolved fluorescence spectroscopy

The outcome from the emission spectral studies shows that the efficiency of binding is established in the ground state. To investigate the complexation, it is necessary to study the excited-state lifetime of the Fluorescein in presence of *p*-SCX6 on the addition of metal using time correlating single-photon counting technique. The excitation wavelength is fixed at

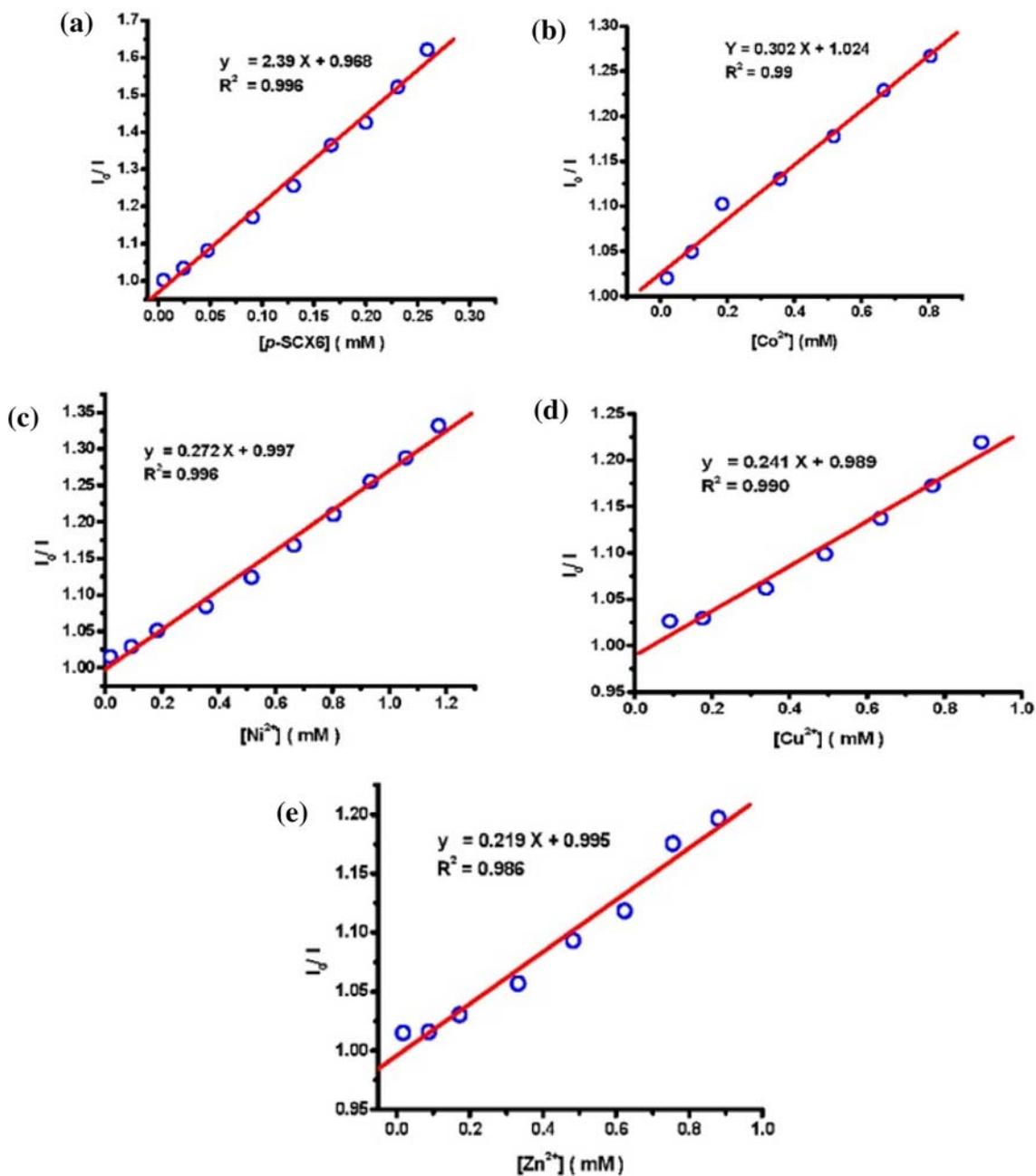


Figure 6. (a-e): Plot between I_0/I vs $[M^{2+}]$ for binary and ternary system. (a) FL + *p*-SCX6 (b) FL + *p*-SCX6 + Co^{2+} (c) FL + *p*-SCX6 + Ni^{2+} (d) FL + *p*-SCX6 + Cu^{2+} (e) FL + *p*-SCX6 + Zn^{2+} .

Table 2. Quenching constants of binary (FL-*p*-SCX6) and ternary (FL-*p*-SCX6- M^{2+}).

Sl. No.	System	Quenching constant (K_{sv}) M^{-1}
1	FL- <i>p</i> -SCX6	2.39
2	FL- <i>p</i> -SCX6 - Co^{2+}	0.30
3	FL- <i>p</i> -SCX6 - Ni^{2+}	0.27
4	FL- <i>p</i> -SCX6 - Cu^{2+}	0.24
5	FL- <i>p</i> -SCX6 - Zn^{2+}	0.21

Table 3. Lifetime measurement for (FL + *p*-SCX6 + Cu²⁺).

System	τ_0 (ns)	α_1	γ^2
FL	4.248	0.0475	1.179
FL + <i>p</i> -SCX6	3.181	0.0473	1.176
FL + SCX6 10 μ L Cu ²⁺	3.180	0.0469	1.051
FL + <i>p</i> -SCX6 + 100 μ L Cu ²⁺	3.177	0.0482	1.328
FL + <i>p</i> -SCX6 + 500 μ L Cu ²⁺	3.149	0.0487	1.280
FL + <i>p</i> -SCX6 + 1000 μ L Cu ²⁺	3.142	0.0488	1.436

479 nm. The decay of the excited state is shown in Figure S2 (Supplementary Information), and the lifetime data was collected and tabulated in Table 3 which shows, the decrease in lifetime from 4.248 ns to 3.142 ns due to addition of *p*-SCX6. This single exponential decay shows there is only one type of short-lived species, upon binding with metal ion. Moreover, we have also determined the bimolecular quenching constant for Cu²⁺.³⁶

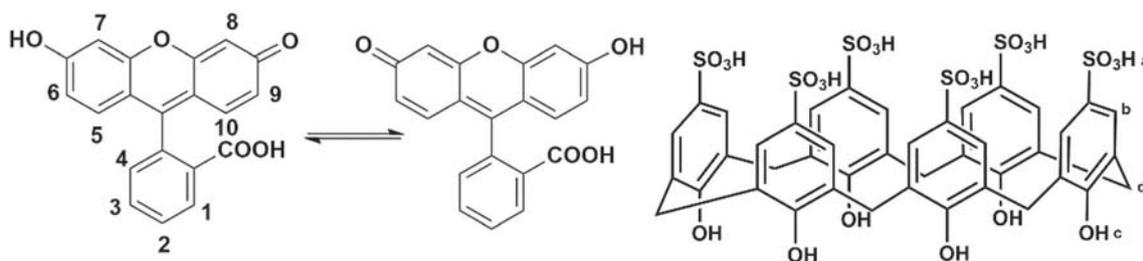
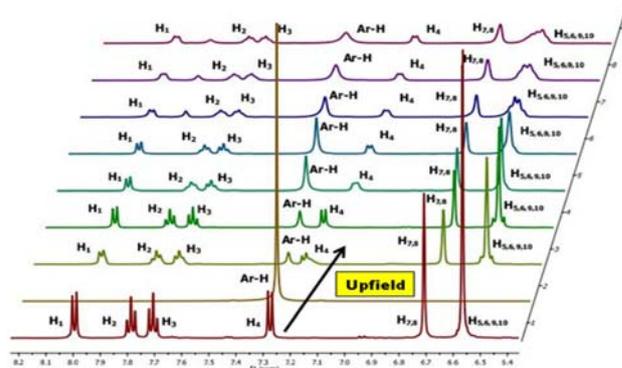
The lifetime of the binary complex (FL + *p*-SCX6) was estimated from equation 3 ($\tau_0 = 3.181$ ns). From the stability constant of Cu²⁺ complex the bimolecular quenching constant (k_q) for Cu²⁺ complex was calculated and it is found to be 0.075 M⁻¹s⁻¹.

$$K_{sv} = k_q \tau_0 \quad (3)$$

where k_q is the bimolecular quenching rate constant (proportional to the sum of the diffusion coefficients for fluorophore and quencher) and τ_0 is the excited state lifetime in the absence of quencher. From this data, it is inferred that the value of k_q is smaller than K_{sv} due to low quenching efficiency or steric shielding.³⁷

3.6 NMR analysis

¹H NMR spectra of FL+*p*-SCX6 along with complexes of Cu²⁺ and Zn²⁺ was acquired in the mixture of D₂O and DMSO-d₆. The protons are assigned to FL and *p*-SCX6 are shown in Scheme 1. In Figure 7 the

**Scheme 1.** (a) Equilibrium between lactone and carboxylate form of fluorescein (b) Structure of *p*-SCX6.**Figure 7.** The ¹H NMR spectral titration of: (1) Fluorescein (1×10^{-2} M), (2) *p*-SCX6 (1×10^{-2} M) (3) FL + 50 μ L *p*-SCX6, (4) FL + 90 μ L *p*-SCX6, (5) FL + 140 μ L *p*-SCX6, (6) FL + *p*-SCX6 + 20 μ L/0.01M Cu²⁺, (7) FL + *p*-SCX6 + 50 μ L Cu²⁺ and (8) FL + *p*-SCX6 + 90 μ L Cu²⁺ (9) FL + *p*-SCX6 + 140 μ L Cu²⁺ in D₂O-DMSO-d₆.

FL titrated against *p*-SCX6 which shows dramatic upfield shift in H₄ protons of FL it may be due to pendent aromatic ring of FL suspended inside the cavity of *p*-SCX6.³⁷ The aromatic protons of *p*-SCX6 interacting with H₄ protons through water molecule,³⁸ therefore after further addition of *p*-SCX6, the nature of doublet changed to singlet. In addition, the Cu²⁺ added to binary complex of FL+*p*-SCX6 which shows shift in ¹H NMR. This demonstrates the slow cation exchange has occurred in the case of Cu²⁺ and Zn²⁺ which is depicted in Figures 7 and 8.³⁹ These results indicate at a qualitative level that, the kinetics for the process of complexation is slower than the binary system (FL+*p*-SCX6) (Table 4).

3.7 Single crystal images

The observations of NMR spectral titrations indicate that FL interacted with *p*-SCX6 and M²⁺ but the characterization of the final product and exact mode of binding could not be completely ascertained as suitable crystals for single-crystal X-ray diffraction were not achieved. Very weak crystals were developed which could not

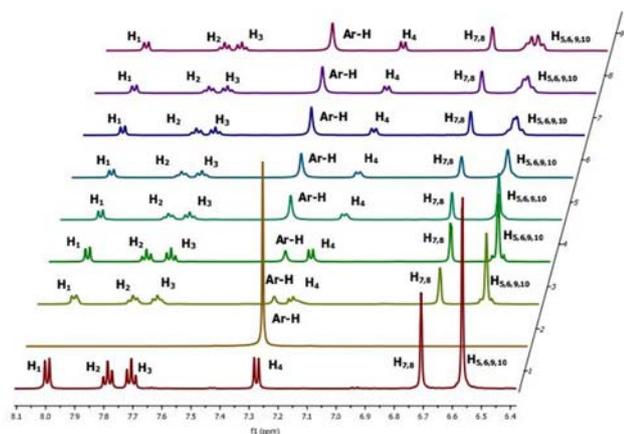


Figure 8. The ^1H NMR spectral titration of: (1) Fluorescein (0.01 M), (2) *p*-SCX6 (0.01 M) (3) FL + 50 μL *p*-SCX6, (4) FL + 90 μL *p*-SCX6, (5) FL + 140 μL *p*-SCX6, (6) FL + *p*-SCX6 + 20 $\mu\text{L}/0.01$ M Zn^{2+} , (7) FL + *p*-SCX6 + 50 μL Zn^{2+} and (8) FL + *p*-SCX6 + 90 μL Zn^{2+} (9) FL + *p*-SCX6 + 140 μL Zn^{2+} in D_2O -DMSO- d_6 .

diffract to collect sufficient data to publish. However, the images of crystals were obtained under Polarizing Microscope, Make-Leica 6 D, Magnification = $4\times$.

Distinguished images are obtained for (FL + *p*-SCX6), (*p*-SCX6 + FL + Cu^{2+}), (*p*-SCX6 + FL + Zn^{2+}) which may indicate different binding behaviour for each complex Figure 9. The cavity of *p*-SCX6 is flexible which also shows different conformational changes in room temperature. Therefore, for FL, it is hard to remain inside the cavity along with metal ion. As we discussed, the binding of FL and metal ion with *p*-SCX6 is through water molecule, consequently, the crystallization is not possible.

3.8 Scanning electron microscopy

The morphology of *p*-SCX6, (FL-*p*-SCX6) and (FL + *p*-SCX6 + Cu^{2+}) and (FL + *p*-SCX6 + Zn^{2+}) was studied. The particles observed in this study ranged from 2 μM to 5 μM . All the structures are distinct as compared to pure *p*-sulfonato-calix[6]arene. The change in morphology from FL to binary to ternary systems strongly indicates complexation. Also, the pattern for ternary systems: (FL + *p*-

Table 4. δ (ppm) of the ^1H NMR titration of Fluorescein, *p*-SCX6, (*p*-SCX6 + FL), (FL + *p*-SCX6 + Cu^{2+}) and (FL + *p*-SCX6 + Zn^{2+}) in a mixture of D_2O and DMSO- d_6 .

Sl. No.	Addition	H ₄	H ₃	H ₂	H ₁	H _{7,8}	H _{5,6,9,10}	Ar-H
1	Only FL	7.28	7.70	7.79	8.0	6.71	6.57	–
2	Only <i>p</i> -SCX6	–	–	–	–	–	–	7.29
3	50 μL <i>p</i> -SCX6 + FL	7.21	7.69	7.77	7.98	6.72	6.56	7.29
4	90 μL <i>p</i> -SCX6 + FL	7.16	7.67	7.75	7.97	6.74	6.56	7.30
5	140 μL <i>p</i> -SCX6 + FL	7.13	7.66	7.73	7.97	6.76	6.60	7.31
6	20 μL Cu^{2+} + <i>p</i> -SCX6 + FL	7.11	7.65	7.71	7.96	6.77	6.62	7.32
7	50 μL Cu^{2+} + <i>p</i> -SCX6 + FL	7.10	7.64	7.71	7.96	6.77	6.62	7.32
8	90 μL Cu^{2+} + <i>p</i> -SCX6 + FL	7.09	7.64	7.71	7.97	6.77	6.64	7.33
9	140 μL Cu^{2+} + <i>p</i> -SCX6 + FL	7.08	7.63	7.68	7.94	6.77	6.63	7.34
10	20 μL Zn^{2+} + <i>p</i> -SCX6 + FL	7.12	7.66	7.73	7.96	6.77	6.61	7.32
11	50 μL Zn^{2+} + <i>p</i> -SCX6 + FL	7.11	7.65	7.72	7.96	6.77	6.62	7.32
12	90 μL Zn^{2+} + <i>p</i> -SCX6 + FL	7.10	7.65	7.71	7.97	6.77	6.61	7.32
13	140 μL Zn^{2+} + <i>p</i> -SCX6 + FL	7.10	7.64	7.71	7.96	6.78	6.63	7.32

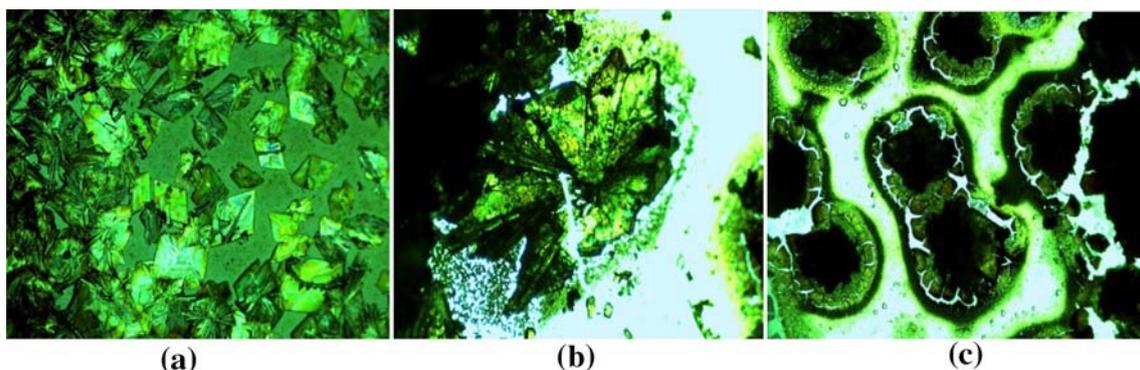


Figure 9. Single crystal images of (a) (FL + *p*-SCX6) (b) (*p*-SCX6 + FL + Cu^{2+}) (c) (*p*-SCX6 + FL + Zn^{2+}).

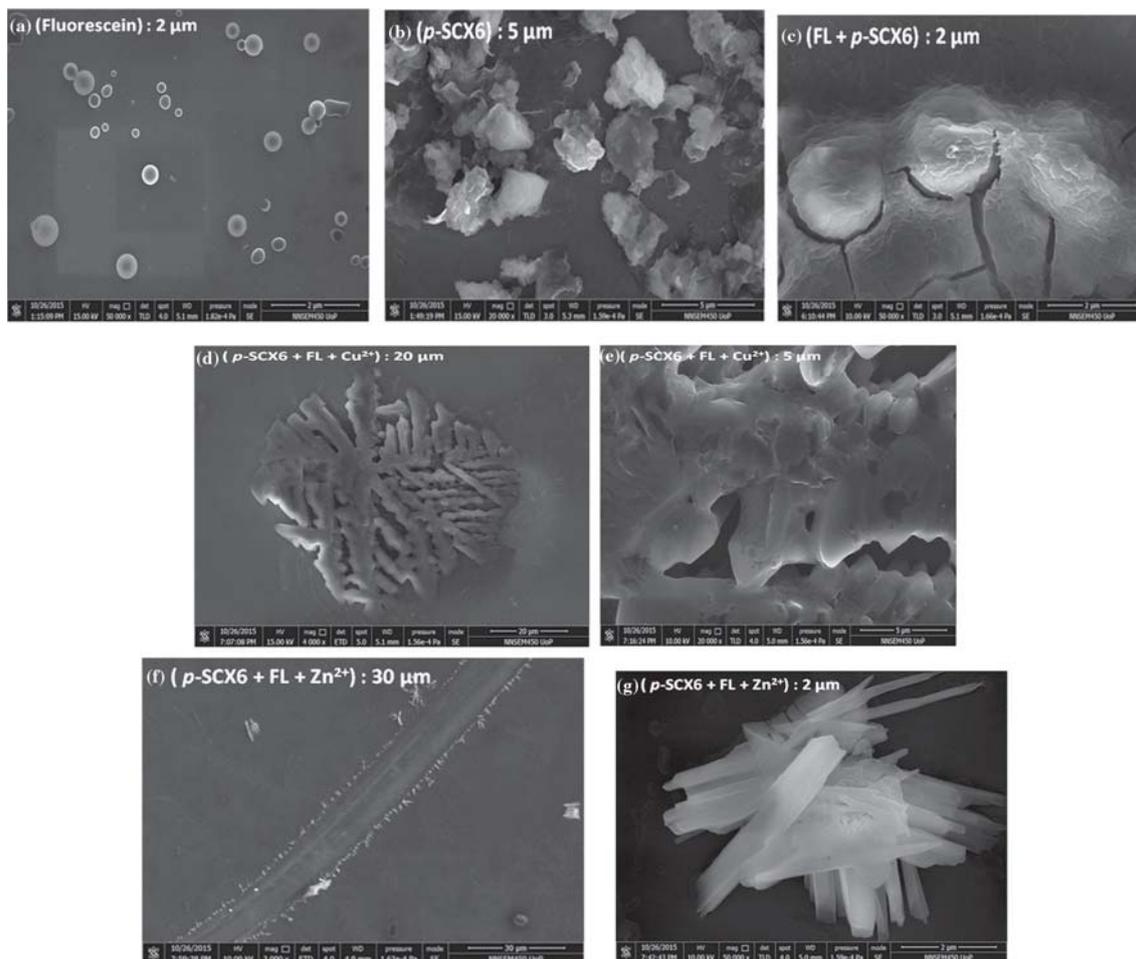


Figure 10. SEM images of (a) Fluorescein (b) *p*-SCX6 (c) (FL + *p*-SCX6) (d) (*p*-SCX6 + FL + Cu²⁺): 20 μm (e) (*p*-SCX6 + FL + Cu²⁺): 5 μm (f) (*p*-SCX6 + FL + Zn²⁺): 30 μm and (g) (*p*-SCX6 + FL + Zn²⁺): 2 μm.

SCX6 + Cu²⁺) and (FL + *p*-SCX6 + Zn²⁺) obtained at 20 μm is very different (Figure 10).

4. Conclusions

The study of inclusion complexation of bivalent metal ions into the binary complexes of FL-*p*-SCX6 has been carried out by steady-state fluorescence and ¹H NMR spectroscopy. The study reveals that the binding ability of Cu²⁺ and Zn²⁺ with FL-*p*-SCX6 is at a greater extent in comparison with Co²⁺ and Ni²⁺. From the lifetime of Cu²⁺ and Zn²⁺, the bimolecular quenching constant (k_q) were calculated which inferred that the k_q is smaller than the binding constant K_{sv} of respective metal ions owing to low quenching efficiency or steric shielding. Hitherto, the titration study by ¹H NMR demonstrations, the kinetics for the process of ternary complexation is slower than the binary system (FL+*p*-SCX6) in solution.

Supplementary Information (SI)

Effects of dilution are given in the Supplementary Information available at www.ias.ac.in/chemsci.

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