CHAPTER V

Detection of Copper(II), Iron(III), Lead(II) and Silver(I) Ions Using Bis-Benzimidazolyl Diamide Ligands by Fluorescence Spectroscopy

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“Theory is general but the experiments are soldier....”

Leonardo da Vinchi
5.1 Introduction

Transition metal ions can be detected in low concentration by fluorescence spectroscopy and it is therefore of great importance in areas of human health and environment.\textsuperscript{1-8}

Copper and iron are essential transition metal ions for living systems and play an important role in a variety of major biological processes in living organisms.\textsuperscript{9} Their deficiency and excess is known to induce a number of diseases. A high level of Cu\textsuperscript{2+}, is found to be associated with severe neurodegenerative diseases, such as Wilson’s and Alzheimer’s diseases.\textsuperscript{10} Further, copper is usually found in the oxidized state and can be an environmental pollutant. Thus, the sensitive and selective detection of Cu\textsuperscript{2+} is crucial. Detection of copper(II) from various sources including those in waste water outlets, electroplating waste and other metal processing industries is therefore important. Fe\textsuperscript{3+} plays an important role in many biochemical processes at the cellular level.\textsuperscript{11} The change of the amount of Fe\textsuperscript{3+} is associated with many diseases, including certain cancers and dysfunction of organs.\textsuperscript{12} Detection of lead ions (Pb\textsuperscript{2+}) is important because they can have severe effects on human health and the environment.\textsuperscript{13} Even exposure to very low levels of Pb\textsuperscript{2+} ions (<100 \(\mu\)g/L in blood) can cause neurological, cardiovascular, and developmental disorders.\textsuperscript{14} Therefore the development of simple and inexpensive methods for the determination of concentrations of Pb\textsuperscript{2+} ions, in environmental, biological, and industrial samples is of utmost important.

Fluorescent sensors are powerful tools that are used to detect metal ions in environmental, biological, and chemical systems. A variety of designing strategies based on fluorescence techniques have been developed.\textsuperscript{15,16} Due to the paramagnetic nature of Cu\textsuperscript{2+} and Fe\textsuperscript{3+}, the fluorescent indication for Cu\textsuperscript{2+} and Fe\textsuperscript{3+} has limited applications. However in recent years, a number of sensors for detection of Cu\textsuperscript{2+} and Fe\textsuperscript{3+} have been reported.\textsuperscript{17,18,19,20}

A new area where organic molecules are utilized to act as sensors, switches, triggers and logic gates has also developed rapidly. Some of these systems utilize a fluorescent signal due to photoenduced electron transfer (PET) mechanism to understand a molecular recognition event.\textsuperscript{21} Further a number of fluorescent sensors exhibiting the turn “off-on-off” switch involving photo induced electron transfer (PET) phenomenon
for protons and metal ions, intramolecular charge transfer (ICT) process, Chelation Enhanced Fluorescence have also been reported.

In the present chapter, we describe the fluorescent properties of the bis-benzimidazolyl diamide ligands \( L_2, L_3 \) and \( L_4 \) with the biphenyl spacer. They show simultaneous fluorescence enhancement (turn on) and fluorescence quenching (turn off). Both benzimidazole and biphenyl are excellent fluorophores and their metal complexes show strong light emitting properties and long fluorescence lifetime. Further benzimidazole and biphenyl based compounds are useful as fluorescent sensors. The present series of ligands are capable of detecting \( \text{Fe}^{3+} \) and \( \text{Cu}^{2+} \) in presence of metal ions like \( \text{Ni}^{2+}, \text{Co}^{2+}, \text{Mn}^{2+}, \text{Mg}^{2+}, \text{Zn}^{2+}, \text{Pb}^{2+} \) and \( \text{Hg}^{2+} \). While with \( \text{Cu}^{2+} \) and \( \text{Na}_2\text{EDTA} \), a turn ‘off-on-off’ signaling behavior is observed for the fluorescent benzimidazolyl diamide ligands \( L_2 \) and \( L_3 \). To the best of our information, bis-benzimidazolyl diamide fluorescent sensors having a biphenyl spacer exhibiting the turn “off-on-off” behavior have not yet been reported.

5.2 Physical and spectral measurements

All the reagents and solvents were purchased from commercial sources. HPLC grade solvents and double distilled water was used for spectral work. Electronic spectra were obtained on a Shimadzu UV-Vis-1601 Spectrometer. \(^1\)H (400 MHz) NMR spectra were recorded on a JEOL ECX-400P NMR spectrometer using tetramethylsilane (TMS) as an internal reference. Fluorescence spectra were recorded in methanol as solvent on a Varian CARY Eclipse fluorescence spectrophotometer. The stock solutions of \( L_2, L_3 \) and \( L_4 \) (5000 \( \mu \)M) for electronic absorption and emission spectral studies was prepared using HPLC grade methanol. The solutions of all metal ions were prepared by dissolving nitrate salts in HPLC grade methanol and double distilled water (5000 \( \mu \)M). In the titration studies typically, 2.0 ml solution of \( L_2, L_3 \) and \( L_4 \) (100 \( \mu \)M) was taken in a quartz cell of 1 cm path length and 12.5 \( \mu \)M of metal ions were added gradually to the solution. The resulting solution was mixed thoroughly and immediately a fluorescence spectrum was recorded. For fluorescence intensity measurements, the excitation and emission wavelengths for all the three ligands were fixed at 277 nm and 285 nm, respectively. The slit widths were 5 nm/5 nm.
5.3 Results and discussions

5.3.1 Absorption spectral studies

The electronic absorption spectra of \( L_2 \) and \( L_3 \) show two sharp peaks in the UV region arising due to \( \pi-\pi^* \) transition in benzimidazolyl moiety.\(^{27}\) In diamides \( L_2 \) and \( L_3 \) these peaks are observed at 276 and 284 nm and are slightly shifted and appear at 271-280 nm upon addition of \( Cu^{2+} \), \( Fe^{3+} \), \( Ag^+ \) ions, while the shoulder around 249 nm disappears. The blue shift in the position of absorption bands in comparison to free ligands \( L_2 \) and \( L_3 \) may be attributed to the formation of a complex. A weak charge transfer band around 370 nm in case of copper and a strong charge transfer band around 350 nm in case of iron are also observed. With \( Pb^{2+} \), no significant changes are observed in the absorption spectrum (Figures 5.1a-5.1d). While other metal ions displayed insignificant changes. Thus absorption data shows that in case of metal ions like \( Cu^{2+} \), \( Fe^{3+} \) and \( Ag^+ \), binding is through benzimidazole imine nitrogen as it affects the \( \pi-\pi^* \) transition of the benzimidazole moiety.

5.3.2 \( ^1H \) NMR spectral studies

The change in \( ^1H \) NMR spectra of \( L_2 \) and \( L_3 \) upon addition of \( Cu^{2+} \), \( Fe^{3+} \), \( Pb^{2+} \) and \( Ag^+ \) was studied. \( ^1H \) NMR spectrum of \( L_2 \) was recorded upon addition of \( Ag^+ \), \( Cu^{2+} \) and \( Fe^{3+} \) ions and compared with the spectra of free \( L_2 \). In the free ligand \( L_2 \), the aromatic protons are bunched between 7.0-7.6 ppm, the linker –CH\(_2\) group adjacent to benzimidazole moiety are at 4.5-4.7 ppm and the \( N \)-ethyl chain protons are at 1.23 and 4.07 ppm. Addition of \( Ag^+ \) ions to the ligand \( L_2 \), causes a general broadening and a downfield shift of the protons in the aromatic region along with the methylene linker and \( N \)-ethyl chain protons (Figure 5.2a) with respect to the position of the protons in the free ligand \( L_2 \). While when paramagnetic ions like \( Cu^{2+} \) and \( Fe^{3+} \) are added, there are paramagnetic shifts and a general loss/broadening of the protons in aromatic region. The protons of the linker methylene group and amide (CONH) could also not be observed properly due to the effect of bound paramagnetic metal ion.\(^{28}\) As the most affected protons are the benzimidazole ring protons and the methylene linker protons adjacent to the amide (CONH) group. The above changes also indicate the preferred sites for the binding of \( Cu^{2+} \) and \( Fe^{3+} \) ions being the imine nitrogen of benzimidazole.
and oxygen of amide (CONH) group (Figure 5.2a). Similar changes were observed with L3 after adding Cu\(^{2+}\), Fe\(^{3+}\) and Ag\(^+\). Addition of Pb\(^{2+}\) did not result in any change in the position and shape of signals arising due to alkyl chain. However the signals due to aromatic protons were split and slightly shifted with respect to each other (Figure 5.2b).

Therefore from the absorption and NMR spectral studies, we can say that in case of metal ions like Cu\(^{2+}\) and Fe\(^{3+}\), the binding to metal ion is through both, imine nitrogen of benzimidazole and carbonyl oxygen of amide group. In case of Pb\(^{2+}\), binding may be only through carbonyl oxygen only and not through benzimidazole nitrogen as there are very weak changes are observed in the aromatic benzimidazolyl protons while with Ag\(^+\), binding may be through benzimidazole nitrogen and weakly through carbonyl oxygen.

### 5.3.3 Solvent dependence studies

It is well established that the energy difference between the 0–0 transitions depends on the different degrees of solvation in the two states. It should be greatest for those molecules showing large change in dipole moment on excitation to the first excitation state.\(^{29}\) For a given substance it should be greater in more polar solvents. By measuring the 0–0 band shift in a series of solvent, the dipole moment in the excited state can be evaluated.\(^{30,31}\) It has also been established earlier that as the electron in the excited state occupies a more extended orbital than in the ground state, the molecules in the excited state are more polarizable leading to small shift in the 0–0 band even in the absence of a change in dipole moment.

Figures 5.3a-5.3c gives the solvent dependence of ligand L2, L3 and L4 in solvents like MeOH, DMF, CHCl\(_3\) and mixed solvent systems MeCN/CHCl\(_3\) (9:1) and H\(_2\)O/MeOH (9:1). The intensity of the emission at 300 nm, can be attributed to arise from the Benzimidazolyl moiety.\(^{32}\) It is found that the \(\lambda_{\text{max}}\) of the emission band slightly shifts in various solvent systems, in the range of 296-300 nm for L2, 297-299 nm for L3 and 298-302 nm for L4 (Figures 5.3a-5.3c). These small shifts are indicative of the varying degree of solvation of the excited state and imply that there is no large change in dipole moment of the solute in the excited state, with varying
solvent system. The emission intensity for L2 is found to decrease in the order MeCN/CHCl3 > DMF > CHCl3 > MeOH > H2O/MeOH, showing a decrease of almost seven fold from MeCN/CHCl3 mixed solvent system to H2O/MeOH solvent system for L2 and almost a decrease of three and half fold from MeCN to MeOH for L3 and L4 (Figures 5.3a-5.3c).

In this connection it is worthwhile to consider the effect of photoinduced electron transfer, PET which is a well-known deactivation process involving an internal redox reaction between the excited state of the fluorophores and another species able to donate or accept an electron. PET strongly depends on the solvent polarity which affects the redox potentials. Higher solvent polarity causes an easier electron transfer as a result the PET-mediated quenching effect of fluorescence is larger in highly polar environment.21

For fluorophore L2, it is found that quenching is highest in MeOH and H2O/MeOH mixture (9:1) that are less polar than MeCN or DMF but more polar than CHCl3 while for L3 and L4 quenching is largest in MeOH and lowest in MeCN (Figures 5.3a-5.3c). Since PET mediated quenching is more rapid in polar solvents, the quenching effect in the present case cannot be attributed to a PET process.

Another factor that plays an important role in fluorescence quenching is H-bonding, for fluorophores containing groups such as -OH, -NH2 etc. H-bonded excited states can be produced by either route:

\[
A + S \xrightarrow{hv} AS \quad (5.1)
\]

\[
A^* + S \xrightarrow{} (AS)^* \quad (5.2)
\]

Oesterlin found that the separation of \(\pi^*-n\) and \(\pi^*-%\) singlet states determine the efficiency to fluoresce. Molecules having the \(\pi^*-n\) state as the lowest energy singlet state give little fluorescence, however if the energy difference between \(\pi^*-n\) and \(\pi^*-%\) states is small, weakly/moderately H-bonding solvents can act by lowering the \(\pi^*-%\) state by an increased interaction in the excited state (pathway 5.2). In the present case, a probable reason for quenching can be ascribed to the likely H-bonding of MeOH and H2O with the imine N-atom of benzimidazolyl moiety, a situation that does not
arise with solvents like CH$_3$CN, DMF and CHCl$_3$. Therefore we can conclude that the intensity quenching for fluorophores L$_2$, L$_3$ and L$_4$ is due to H-bonding in the excited state.$^{35}$

5.3.4 Wavelength excitation dependence studies

If molecules associate by processes not involving covalent bonds, they can give rise to dimeric or in general polymeric species in solution. Dimers can be generated by a reversible combination of two unexcited monomer molecules

\[
A + A \rightleftharpoons AA
\]

(5.3)

which are usually non fluorescent in the ground state. However excitation of such dimer may give rise to excited dimer (AA*), that may be long lived and would not dissociate rapidly. Such a stable dimer that could also be envisaged to form in the excited state as:

\[
^{1}A \xrightarrow{hv} ^{1}A^* \quad (5.4)
\]

\[
^{1}A^* + ^{1}A \rightarrow (A^*A) \quad (5.5)
\]

Such a dimer which is fluorescent in the excited state is referred to as an excimer.$^{36,37}$ Solution of compounds that give rise to both the monomer (A*) and dimer (A*A) in the excited state should give rise to two radiative transitions in its fluorescence spectrum characteristics of the excited species concerned. It has been shown that the energy of the quanta emitted by the monomer has higher energy in comparison to the dimer emission.$^{38}$

It has been established that wavelength excitation dependence study can give an insight to the monomer/excimer relationship and it is concluded that if the fluorescence is independent of wavelength of excitation, it is likely to be due to the monomer emission, while if the fluorescence dependent on the excitation wavelength, the band could originate from an ‘excimer’ or intermolecular ground state complex.$^{39}$
In the present case, we find that the emission band around 300 nm in all the ligands could be assigned to the ‘monomer’ emission as it does not show any wavelength dependence (Figure 5.4). However a small change is observed as we increase the N-ethyl chain to N-butyl (L2 to L3), wherein a new band starts to be observed for L3 around 375 nm. This band does show weak wavelength dependence, the intensity of the band increases as the excitation wavelength is changed from 260 to 280 nm (Figure 5.4).

![Figure 5.4 Emission spectra of (L2) (left) and (L3) (right) after excitation at different wavelengths in MeOH (c = 100 μM)](image)

### 5.3.5 Fluorescence quantum yield

The fluorescence spectra of the ligands L2, L3 and L4 are shown in Fig. 5.3a-5.3c. Quantum yields, ϕ (Table 5.1) were calculated by comparison of the spectra with that of anthracene (ϕ = 0.292) taking the area under the total emission. The fluorescence spectra of 100 μM solution in MeOH and H2O/MeOH mixture (9:1) for L2, L3 and L4 shows a band centred around 300 nm. The fluorescence quantum yield of the present ligands are similar to that reported for bis-benzimidazole type ligand40 but quite higher than for some of the nitrogen containing fluorophores41, implying that the lone pair of the benzimidazole nitrogen is only weakly involved in photo induced electron transfer (PET) to the aromatic fluorophores. The quantum yields have been calculated by utilizing the formula:

$$\Phi = \frac{\text{area}_{\text{ligand}}}{\text{area}_{\text{anth}}} \times \frac{\varepsilon_{\text{anth}}}{\varepsilon_{\text{ligand}}}$$  \hspace{1cm} (5.6)

where, $\varepsilon$ = extinction coefficient of the compound and anth = anthracene
Table 5.1 Quantum yield of ligands (c = 100 μM)

<table>
<thead>
<tr>
<th>Ligands</th>
<th>Solvent</th>
<th>Quantum yields (ϕ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>MeOH</td>
<td>0.060</td>
</tr>
<tr>
<td>L2</td>
<td>H₂O/MeOH (9:1)</td>
<td>0.024</td>
</tr>
<tr>
<td>L3</td>
<td>MeOH</td>
<td>0.073</td>
</tr>
<tr>
<td>L4</td>
<td>MeOH</td>
<td>0.062</td>
</tr>
</tbody>
</table>

5.3.6 Metal-binding experiments with ligands L2, L3 and L4

5.3.6.1 Copper(II)

Typically a large number of probes have been synthesized for divalent cooper sensing; however owing to its paramagnetic nature, most of the chemosensors reported generally undergo fluorescence quenching upon binding with Cu²⁺.¹²,¹³ It has been observed that fluorescence enhancement is more sensitive over fluorescence quenching.¹⁴ Thus more recently “turn-on” type chemosensors have been reported.¹⁵,¹⁶ It is also important to be able detect Cu²⁺ in aqueous media, rather than non-aqueous systems¹⁶ as most of Cu²⁺ which exists as a pollutant is found in aquatic system.

With the above background in mind, the fluorophores L₂, L₃ and L₄ have been employed for a selective detection of Cu²⁺ in aqueous and non-aqueous systems. Figure 5.5a depicts the effect of incremental addition of Cu²⁺ as nitrate salt (12.5 μM in each step) to a 100 μM solution of L₂ in MeOH. In the absence of added Cu²⁺, a strong monomer emission at about 300 nm is observed with intensity greater than 200 units (Figure 5.5a). Subsequent addition of copper(II) results in the gradual quenching of monomer emission at ~300 nm to ~50 units of intensity. This is attributed to the paramagnetic fluorescence quenching due to Cu²⁺ that allows the formally forbidden intersystem crossing (ISC) from an S₁ to a T₁ state, of the fluorophores. This state is deactivated by bimolecular non-radiative process,¹¹ which is also referred to as chelation enhanced quenching effect (CHEQ).

However, quite interestingly simultaneous to the monomer emission quenching, it is observed that a new band at about 375 nm starts to form and which gains in intensity with each incremental step of addition of Cu²⁺ from less than 50 unit intensity to
almost 150 unit in intensity. This ‘turn on’ of the fluorophore could be identified as chelation enhanced fluorescence effect (CHEF).

Quite significantly, when the same experiment is conducted in H$_2$O/MeOH (9:1) aqueous system, the initial monomer intensity is found to be relatively quenched with respect to that found in pure methanol. However, incremental addition of Cu$^{2+}$ causes a significantly larger CHEF and the new band at 375 nm increases from an intensity less than 40 units to greater than 180 units (Figure 5.5b). This shows that the present fluorophore L2, is not only capable of detecting Cu$^{2+}$ in non-aqueous and aqueous media but it shows two distinct mechanisms for detection, a ‘turn-off’ and a simultaneous ‘turn-on’.

Figure 5.5c shows the effect of incremental addition of Cu$^{2+}$ to fluorophore L3. Ligand L3 has an N-butyl chain in comparison to the N-ethyl chain for L2. It is observed that the phenomena of ‘turn off’ and ‘turn on’ is quite significant for this fluorophore also, the CHEQ as well as CHEF effects are relatively larger than L2. While with ligand L4, only quenching of 300 nm band was observed (Figure 5.5d) and no ‘turn-on’ of the new band could be significantly detected at 375 nm.

The presence of 375 nm band in both L2 and L3 has been attributed to an intraligand $\pi-\pi^*$ transition within the biphenyl spacer of the ligand.$^{47}$ This ‘enhancement’ of the 375 nm band could be ascribed to CHEF, that causes increased rigidity of the biphenyl ring, reducing the non radiative decay of the intraligand excited state.$^{48}$ The above fact is supported by a decrease in the dihedral angle between the plane of two phenyl rings, being 76.03(6)$^\circ$ in ligand L2 and dropping to 48.79(16)$^\circ$ upon complexation with Cu$^{2+}$ confirming greater rigidity in the copper bound ligand. The absence of a ‘turn-on’ type effect in the fluorophore L4, suggests that the presence of aromatic N-benzyl chain provide a pathway for the rapid decay of the intraligand excited state originating in the biphenyl system.

5.3.6.2 Iron(III)

Iron is one of the most important metals in the biological systems and plays a key role in many biochemical processes at the cellular level. Especially, ferric ion (Fe$^{3+}$) is widely retained in many proteins and enzymes either for structural purposes or as part
of a catalytic site. Recently, few examples of Fe\(^{3+}\) amplified fluorescent ‘turn-on’ systems in protic solvents have been reported, but most of the known Fe\(^{3+}\) sensors are based on the fluorescence quenching mechanisms due to the paramagnetic nature of ionic iron. It has been observed that fluorescence enhancement is more sensitive over fluorescence quenching. Thus more recently ‘turn-on’ type chemosensors have been reported.

With the above background in mind, the fluorophores L\(_2\), L\(_3\) and L\(_4\) have been employed for a selective detection of Fe\(^{3+}\) in aqueous and non-aqueous systems. Figure 5.6a depicts the effect of incremental addition of Fe\(^{3+}\) as nitrate salt (12.5 \(\mu\)M in each step) to a 100 \(\mu\)M solution of L\(_2\) in MeOH. In the absence of added Fe\(^{3+}\), a strong monomer emission at about 300 nm is observed with intensity greater than 200 units (Figure 5.6a). Subsequent addition of Fe\(^{3+}\) results in the gradual quenching of monomer emission at ~300 nm to ~100 unit of intensity. This is attributed to the paramagnetic fluorescence quenching due to Fe\(^{3+}\) that allows the formally forbidden intersystem crossing from an S\(_1\) to a T\(_1\) state of the fluorophore, causing subsequent deactivation through a bimolecular non-radiative process.

However, quite interestingly simultaneous to the monomer emission quenching, it is observed that a new band at ~375 nm starts to form and that gains in intensity with each incremental step of addition of Fe\(^{3+}\) from ~50 to 650 units. This spectacular 13 fold ‘turn on’ of the fluorophore could be attributed to chelation enhanced fluorescence effect (CHEF).

Quite significantly, when the same experiment is conducted in H\(_2\)O/MeOH (9:1) aqueous system, the initial monomer intensity is found to be relatively quenched with respect to that found in pure methanol. However, incremental addition of Fe\(^{2+}\) causes a significantly larger CHEF and the new band at 375 nm increases from an intensity ~40 to >1000 units, a remarkable enhancement of more than 20 fold (Figure 5.6b). This shows that the present fluorophore L\(_2\), is not only capable of detecting Fe\(^{3+}\) in non-aqueous and aqueous media but it shows two distinct mechanisms for detection, a ‘turn-off’ and a simultaneous ‘turn-on’. Further L\(_2\) is more sensitive to detect Fe\(^{3+}\) in comparison to Cu\(^{2+}\).
Figure 5.6c shows the effect of incremental addition of Fe$^{3+}$ to fluorophore L3. Ligand L3 has an N-butyl chain in comparison to the N-ethyl chain for L2. It is observed that the phenomena of ‘turn off’ and ‘turn on’ is quite significant for this fluorophore also, the CHEQ as well as CHEF effects are relatively similar to L2. While with ligand L4, the CHEF effect is lower than L2 and L3 (Figure 5.6d).

### 5.3.6.3 Lead(II)

Among the metal ions, Pb$^{2+}$ is one of the important targets because of the adverse health effects of lead exposure, particularly in children.\textsuperscript{52} Despite, efforts to reduce global emissions, lead poisoning remains the world’s most common environmentally caused disease.\textsuperscript{53} Thus more recently ‘turn-on’ type chemosensors have been reported that detect Pb$^{2+}$ in aqueous as well as in non-aqueous systems.\textsuperscript{54}

Figure 5.7a depicts the effect of incremental addition of Pb$^{2+}$, (12.5 μM in each step) to a 100 μM solution of L2 in MeOH. Upon adding increasing amounts of Pb$^{2+}$, (12.5-125 μM) as nitrate salt to solution of L2-L4, the emission at 300 nm was not quenched in all the three ligands (Figure 5.7a-5.7d). When L2 is taken in H$_2$O/MeOH (9:1) solution, there does arise the formation of a band at 375 nm, but the intensity increases by only two fold. However, the new band around 375 nm starts to arise significantly only in case of ligand L3 simultaneously upon the addition of Pb$^{2+}$ ions (Figure 5.7a-5.7d), which has been attributed to an intraligand π-π* transition within the biphenyl spacer of the ligand.\textsuperscript{47} It is quite interesting to observe that fluorophore L3 is more selective for Pb$^{2+}$ out of the three fluorophores L2, L3 and L4.

### 5.3.6.4 Silver(I)

In recent years, a large number of fluorescence probes have been synthesized for Ag$^+$ sensing involving the phenomena of fluorescence quenching as well as fluorescence enhancement in aqueous and non-aqueous media based on fluorescein derivatives\textsuperscript{55}, rosamine\textsuperscript{56}, boradiazaindacenes\textsuperscript{57}, 3,9-dithia-6-azaundecane\textsuperscript{58} and pyrene-functionalized heterocycle receptor.\textsuperscript{59}

Figure 5.8a depicts the effect of incremental addition of Ag$^+$, (12.5 μM in each step) to a 100 μM solution of L2 in MeOH. Upon adding increasing amounts of Ag$^+$, (12.5-
125 µM) as nitrate salt to solution of L2-L4, the emission at 300 nm was quenched in all the three ligands (Figure 5.8a-5.8c). Interestingly no new band around 375 nm starts to arise in all the ligands (Figure 5.8a-5.8c).

No such behavior was observed in case of other metal ions with ligands L2, L3 and L4 even when a large excess of metal ion (5 equiv.) were added, except for Zn^{2+} in methanol and Pb^{2+} in aqueous solution (90% H₂O) where a slight increase (10% and 25%, respectively) in the 375 nm band is observed with ligand L2 and except for Zn^{2+} and Mn^{2+} in methanol where a slight increase (15% and 20%, respectively) in the 375 nm band is observed with ligand L3 (Figures 5.9a-5.9g & 5.10a-5.10g, Figures 5.11a-5.11e and Figures 5.12a-5.12f).

Thus, we can say that the ligands behave quite differently towards different metal ions. The behavior of ligands L2, L3 and L4 towards Cu^{2+} and Fe^{3+} is similar but different from Pb^{2+} and Ag^{+}. The ligand L2 and L4 cannot detect Pb^{2+} at all but L3 can. With Ag^{+}, all the three ligands shows quenching of 300 nm band but non formation of 375 nm band.

5.3.7 Quenching and binding constants

The quenching and binding constants, log K (M⁻¹) of the ligands L2, L3 and L4 with Cu^{2+}, Fe^{3+}, Pb^{2+} and Ag^{+} were calculated by the Stern-Volmer and Benesi-Hildebrand plots, respectively.

The Stern-Volmer relationship, named after Otto Stern and Max Volmer⁶⁰ allows us to explore the kinetics of a photophysical intermolecular deactivation process. Processes such as fluorescence and phosphorescence are examples of intramolecular deactivation (quenching) processes. An intermolecular deactivation is where the presence of another chemical species can accelerate the decay rate of a chemical in its excited state. In general, this process can be represented by a simple equation:

\[
A^* + Q \rightarrow A + Q \quad (5.7)
\]

or

\[
A^* + Q \rightarrow A + Q^* \quad (5.8)
\]

where, A is one chemical species, Q is another (known as a quencher) and ‘*’ designates an excited state.
The kinetics of this process follows the Stern-Volmer relationship:

\[ \frac{F_0}{F} = 1 + K_q \tau_o [Q] = 1 + K_{SV}[Q] \]  

(5.9)

where, \( F_o \) and \( F \) are the fluorescence intensity in the absence and presence of quencher, respectively; \( K_q \) = bimolecular quenching rate constant; \( \tau_o \) = fluorescence lifetime of \( A \), without a quencher; \([Q]\) = concentration of the quencher and \( K_{SV} \) = Stern-Volmer quenching constant.

The ratio of \( F_o/F \) is directly proportional to the quencher concentration \([Q]\).

Evidently

\[ K_{SV} = K_q \tau_o \]  

(5.10)

\[ \frac{F_o}{F} = 1 + K_{SV}[Q] \]  

(5.11)

According to above equation a plot of \( F_o/F \) v/s \([Q]\) shows a linear graph with an intercept of 1 and slope of \( K_{SV} \).

The Benesi-Hildebrand (B-H) method was first developed by Benesi and Hildebrand in 1949 as a means to explain a phenomenon where iodine changes colour in various aromatic solvents. This was attributed to the formation of an iodine-solvent complex through acid-base interactions, leading to the observed shifts in the absorption spectrum. Following this development, the Benesi-Hildebrand method has become one of the most common strategies for determining association constants based on absorbance spectra. Although, initially used in conjunction with UV/Vis spectroscopy, many modifications have been made that allow the B-H method to be applied to other spectroscopic techniques involving fluorescence, infrared, and NMR. The binding constant \( K \) was calculated by the linear Benesi-Hildebrand equation as the following expression:

\[ \frac{F_0}{F} = F_0 K[L][M^{n+}] + F_0/[L] \]  

(5.12)

Where, \( F_0 \) and \( F \) are the fluorescence intensity of the solution of ligand (L) in the absence and presence of metal ions (M\(^{n+}\)), respectively

The quenching and binding constant, \( \log K \) (M\(^{-1}\)) of the ligands \( L_2, L_3 \), and \( L_4 \) with Cu\(^{2+}\), Fe\(^{3+}\), Pb\(^{2+}\), and Ag\(^{+}\) calculated by using Stern-Volmer and Benesi-Hildebrand plots (Figure 5.13-5.17) are listed in Table 5.2 and 5.3, respectively. The measured intensity \( F_o/F \) at 300 nm varies as a function of \([M^{n+}]\) (Figure 5.13-5.15) and \(1/(F – \)
\[ F_0 \] at 375 nm varied as a function of \( 1/[M^{\text{II+}}] \) in a linear relationship (Figures 5.16-5.17). The quenching and binding constants are found to be comparable with those reported with other ligating system.\(^{35,66}\)

**Figure 5.13** Stern-Volmer linear analysis plot of L2 (300 nm band), (a) at different Cu\(^{2+}\) concentration, (b) at different Fe\(^{3+}\) concentration and (c) at different Ag\(^{+}\) concentration in MeOH.

**Figure 5.14** Stern-Volmer linear analysis plot of L3 (300 nm band), (a) at different Cu\(^{2+}\) concentration, (b) at different Fe\(^{3+}\) concentration and (c) at different Ag\(^{+}\) concentration in MeOH.

**Figure 5.15** Stern-Volmer linear analysis plot of L4 (300 nm band), (a) at different Cu\(^{2+}\) concentration, (b) at different Fe\(^{3+}\) concentration and (c) at different Ag\(^{+}\) concentration in MeOH.
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Figure 5.16 Benesi-Hildebrand linear analysis plot of L2 (375 nm band), (a) at different Cu$^{2+}$ concentration in methanol, (b) at different Cu$^{2+}$ concentration in H$_2$O/MeOH (9:1) and (c) at different Fe$^{3+}$ concentration in MeOH

Figure 5.17 Benesi-Hildebrand linear analysis plot of L3 (375 nm band), (a) at different Cu$^{2+}$ concentration, (b) at different Fe$^{3+}$ concentration and (c) at different Pb$^{2+}$ concentration in MeOH

Table 5.2 Quenching constants of L2, L3 and L4 with various metal ions using stern-volmer relationship at 300 nm band

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Metal ions</th>
<th>Solvent</th>
<th>Log K (M$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>Cu$^{2+}$</td>
<td>MeOH</td>
<td>4.60</td>
</tr>
<tr>
<td></td>
<td>Fe$^{3+}$</td>
<td>MeOH</td>
<td>4.33</td>
</tr>
<tr>
<td></td>
<td>Ag$^+$</td>
<td>MeOH</td>
<td>4.19</td>
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<tr>
<td>L3</td>
<td>Cu$^{2+}$</td>
<td>MeOH</td>
<td>4.67</td>
</tr>
<tr>
<td></td>
<td>Fe$^{3+}$</td>
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<td>4.38</td>
</tr>
<tr>
<td></td>
<td>Ag$^+$</td>
<td>MeOH</td>
<td>4.70</td>
</tr>
<tr>
<td>L4</td>
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<td>MeOH</td>
<td>4.46</td>
</tr>
<tr>
<td></td>
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<td>4.74</td>
</tr>
<tr>
<td></td>
<td>Ag$^+$</td>
<td>MeOH</td>
<td>3.97</td>
</tr>
</tbody>
</table>

Table 5.2 Binding constants of L2, L3 and L4 with various metal ions using benesi-Hildebrand relationship at 375 nm band

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Metal ions</th>
<th>Solvent</th>
<th>Log K (M$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>Cu$^{2+}$</td>
<td>MeOH</td>
<td>4.69</td>
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<tr>
<td></td>
<td>Cu$^{2+}$</td>
<td>MeOH + Water (9:1)</td>
<td>4.72</td>
</tr>
<tr>
<td></td>
<td>Fe$^{3+}$</td>
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<td>L3</td>
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<td></td>
<td>Fe$^{3+}$</td>
<td>MeOH</td>
<td>4.87</td>
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<tr>
<td></td>
<td>Pb$^{2+}$</td>
<td>MeOH</td>
<td>5.02</td>
</tr>
</tbody>
</table>
5.3.8 Selectivity studies of ligands L2, L3 and L4

(a) To investigate the selective preference of L2, metal ions such as Cu^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Mg^{2+}, Zn^{2+}, Pb^{2+} and Hg^{2+} were added separately to the solution of L2 in MeOH and H2O/MeOH mixture (9:1). Upon addition of Cu^{2+} (108 µM) in methanol and water-methanol mixture (9:1) to L2 (100 µM), the fluorescence intensity at 375 nm is significantly enhanced (150 fold and 185 fold respectively) (Figure 5.18a & 5.18b). However, upon the addition of other metal ions (500 µM), the fluorescence enhancement is found to be lower than 50 fold (Figure 5.18a & 5.18b). Therefore L2 has preference only for Cu^{2+}.

(b) A competition experiment was also investigated by adding Cu^{2+} (100 µM) to the solution of L2 (100 µM) along with other metal ions (500 µM) (Figure 5.19a and 5.19b). It is found that other metal ions did not interfere in the detection of Cu^{2+}.

To investigate the selective preference of L3, metal ions such as Cu^{2+}, Fe^{3+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Mg^{2+}, Zn^{2+}, Pb^{2+} and Ag^{+} were added separately to the solution of L3 in MeOH. Upon addition of Fe^{3+} (108 µM) in methanol to L3 (100 µM), the fluorescence intensity at 375 nm is significantly enhanced (750 fold) (Figure 5.20). However, upon the addition of other metal ions (500 µM), the fluorescence enhancement was lower than 60 fold (Figure 5.20), except lead and copper which show some fluorescence enhancement (200 and 275 fold respectively). Therefore L3 has preference for Fe^{3+}.

To investigate the selective preference of L4, metal ions such as Cu^{2+}, Fe^{3+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Mg^{2+}, Zn^{2+}, Pb^{2+} and Ag^{+} were added separately to the solution of L4 in MeOH. Upon addition of Fe^{3+} (108 µM) in methanol to L4 (100 µM), the fluorescence intensity at 375 nm is enhanced (120 fold) (Figure 5.21a). However, upon the addition of other metal ions, the fluorescence enhancement was lower than 70 fold (Figure 19a). However if we consider fluorescence quenching rather than fluorescence enhancement, then Fe^{3+} shows maximum quenching followed by Cu^{2+} and then Ag^{+} (Figure 5.21b). Therefore L3 has preference for Fe^{3+}.

The Cu^{2+}, Fe^{3+}, and Ag^{+} complexes with ligand L3 were isolated and analyzed by ESI-MS. The [M+H]^+ peak for the Cu^{2+} complex is found at 676.6 which corresponds
to \([\text{L3} + \text{Cu}^{2+}]\), the \([\text{M} + \text{H}]^+\) peak for the \(\text{Fe}^{3+}\) complex is found at 669.5 which corresponds to \([\text{L3} + \text{Fe}^{3+}]\) and the \([\text{M} + 2\text{H}]^+\) peak for the \(\text{Ag}^+\) complex is found at 721.2 which corresponds to \([\text{L3} + \text{Ag}^+]\). The emission spectra of isolated complexes are shown in Figure 5.22a and 5.22b. The behavior of the spectra of isolated \(\text{Cu}^{2+}\) and \(\text{Fe}^{3+}\) complexes were also found to be similar (as shown in figures 5.5c, 5.6c and 5.8b) in the solution state where only 375 nm bands were observed while the 300 nm bands were found to be quenched. In case of \(\text{Ag}^+\), quenching of 300 nm bands was observed while the presence of 375 nm band was not observed (Figure 5.22a and 5.22b).

![Figure 5.22a and 5.22b Fluorescence spectra of isolated complexes of ligands L2 and L3 in MeOH (c = 100 μM). Excitation wavelength at 277 nm](image)

### 5.3.9 “Off-On-Off” switch

Both diamide ligands \(\text{L2}\) and \(\text{L3}\), under investigation exhibits reversible fluorescence enhancement (turn-on) in the presence of \(\text{Cu}^{2+}\) (375 nm band) and quenching (turn-off) in the presence of \(\text{Na}_2\text{-EDTA}\) as shown in Figure 5.23.

![Figure 5.23 Signaling ‘Off-On-Off’ behaviour of L2 (left) and L3 (right) towards Cu\(^{2+}\) and Na\(_2\)EDTA upon alternative addition to L2 and L3 (c = 100 μM) in MeOH](image)
References


Figure 5.1a Absorption spectra of 50μM solution of ligands L2 and L3 in methanol after adding Cu\(^{2+}\) ions

Figure 5.1b Absorption spectra of 50μM solution of ligands L2 and L3 in methanol after adding Fe\(^{3+}\) ions
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Figure 5.1c Absorption spectra of 50μM solution of ligands L2 and L3 in methanol after adding Ag⁺ ions

Figure 5.1d Absorption spectra of 50μM solution of ligand L3 in methanol after adding Pb²⁺ ions
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Figure 5.2a ¹H NMR spectra of L₂, L₂+Ag⁺, and L₂+Fe³⁺ in Methanol-d₄ and L₂+Cu²⁺ (DMSO-d₆+Methanol-d₄)

Figure 5.2b ¹H NMR spectra of L₃, L₃+Pb²⁺, L₃+Ag⁺ and L₃+Fe³⁺ in Methanol-d₄ and L₃+Cu²⁺ (DMSO-d₆+Methanol-d₄)
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**Figure 5.3a** Emission spectra of ligand L2 in different solvents ($c = 100 \, \text{μM}$)

**Figure 5.3b** Emission spectra of ligand L3 in different solvents ($c = 100 \, \text{μM}$)

**Figure 5.3c** Emission spectra of ligand L4 in different solvents ($c = 100 \, \text{μM}$)
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**Figure 5.5a-5.5d** Fluorescence titration of L2-L4 (c = 100 μM) with Cu$^{2+}$ with increasing Cu$^{2+}$ concentration (12.5-125 μM). (a) L2 in MeOH, (b) L2 in H$_2$O/MeOH (9:1), (c) L3 in MeOH and (d) L4 in MeOH

**Figure 5.6a-5.6d** Fluorescence titration of L2-L4 (c = 100 μM) with Fe$^{3+}$ with increasing Fe$^{3+}$ concentration (12.5-125 μM). (a) L2 in MeOH, (b) L2 in H$_2$O/MeOH (9:1), (c) L3 in MeOH and (d) L4 in MeOH
Figure 5.7a-5.7d Fluorescence titration of L2-L4 (c = 100 μM) with Pb²⁺ with increasing Pb²⁺ concentration (12.5-125 μM). (a) L2 in MeOH, (b) L2 in H₂O/MeOH (9:1), (c) L3 in MeOH and (d) L4 in MeOH

Figure 5.8a-5.8c Fluorescence titration of L2-L4 (c = 100 μM) with Ag⁺ with increasing Ag⁺ concentration in methanol (12.5-125 μM)
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Figure 5.9a-5.9g Fluorescence spectra of L2 (100 μM) after successive addition of various metal ions in methanol (500 μM), (a) Co²⁺ (b) Ni²⁺ (c) Pb²⁺ (d) Mn²⁺ (e) Mg²⁺ (f) Zn²⁺ (g) Hg²⁺

Figure 5.10a-5.10g Fluorescence spectra of L2 (100 μM) after successive addition of various metal ions in 9:1 H₂O/MeOH mixture (500 μM), (a) Co²⁺ (b) Ni²⁺ (c) Pb²⁺ (d) Mn²⁺ (e) Mg²⁺ (f) Zn²⁺ (g) Hg²⁺
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Figure 5.11a-5.11e Fluorescence spectra of L3 (100 μM) after successive addition of various metal ions in methanol (500 μM), (a) Co$^{2+}$ (b) Ni$^{2+}$ (c) Mn$^{2+}$ (d) Mg$^{2+}$ (e) Zn$^{2+}$

Figure 5.12a-5.12f Fluorescence spectra of L4 (100 μM) after successive addition of various metal ions in methanol (500 μM), (a) Zn$^{2+}$ (b) Ni$^{2+}$ (c) Mn$^{2+}$ (d) Mg$^{2+}$ (e) Co$^{2+}$ (f) Pb$^{2+}$
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Figure 5.18a Emission response of L2 (c = 100 μM) upon addition of Cu^{2+} (1 equivalent) and other metal ions (5 equivalents) in MeOH (left). Fluorescence enhancement factor (FEF) upon addition of Cu^{2+} (1 equivalent) and other metal ions (5 equivalents) (right) for excitation and emission wavelength at 277 nm and 375 nm respectively.

Figure 5.18b Emission response of L2 (c = 100 μM) upon addition of Cu^{2+} (1 equivalent) and other metal ions (5 equivalents) in H_{2}O/MeOH mixture (9:1) (left). Fluorescence enhancement factor (FEF) upon addition of Cu^{2+} (1 equivalent) and other metal ions (5 equivalents) (right) for excitation and emission wavelength at 277 nm and 375 nm respectively.
Detection of Copper(II), Iron(III), Lead(II) and Silver(I) Ions Using Bis-Benzimidazolyl Diimide Ligands by Fluorescence Spectroscopy

**Figure 5.19a** Fluorescence enhancement factor for the 375 nm emission band in MeOH, column 1, for L2 (c = 100 μM); column 2, for L2 (c = 100 μM in presence of other metal ions, (5 equivalents) except Cu²⁺ & Zn²⁺); column 3, for L2 (c = 100 μM, and Cu²⁺ = 100 μM) and column 4, for L2 (c = 100 μM & Cu²⁺ = 100 μM) in presence of other metal ions (5 equivalent) except Fe³⁺, Zn²⁺) and **Figure 5.19b** Fluorescence enhancement factor for the 375 nm emission band in Water-MeOH (9:1), column 1, for L2 (c = 100 μM); column 2, for L2 (c = 100 μM in presence of other metal ions, (5 equivalents) except Cu²⁺ & Pb²⁺); column 3, for L2 (c = 100 μM, and Cu²⁺ = 100 μM) and column 4, for L2 (c = 100 μM & Cu²⁺ = 100 μM) in presence of other metal ions (5 equivalent) except Fe³⁺, Pb²⁺). Excitation and emission wavelengths at 277 nm and 375 nm, respectively

**Figure 5.20** Emission response of L3, (c = 100 μM) upon addition of Cu²⁺, Pb²⁺ and Fe³⁺ (1 equivalent) and other metal ions (5 equivalents) in MeOH (left). Fluorescence enhancement factor (FEF) upon addition of Cu²⁺, Pb²⁺ and Fe³⁺ (1 equivalent) and other metal ions (5 equivalents) (right) for excitation and emission wavelength at 277 nm and 375 nm, respectively
Figure 5.21a Emission response of L4 ($c = 100 \, \mu M$) upon addition of Fe$^{3+}$, Ag$^+$ and Cu$^{2+}$ (1 equivalent) and other metal ions (5 equivalents) in MeOH (left). Fluorescence enhancement factor (FEF) upon addition of Fe$^{3+}$, Ag$^+$ and Cu$^{2+}$ (1 equivalent) and other metal ions (5 equivalents) (right) for excitation and emission wavelength at 277 nm and 300 nm respectively.

Figure 5.21b Emission response of L4 ($c = 100 \, \mu M$) upon addition of Fe$^{3+}$, Ag$^+$ and Cu$^{2+}$ (1 equivalent) and other metal ions (5 equivalents) in MeOH (left). Fluorescence quenching upon addition of Fe$^{3+}$, Ag$^+$ and Cu$^{2+}$ (1 equivalent) and other metal ions (5 equivalents) (right) for excitation and emission wavelength at 277 nm and 300 nm respectively.