Colorimetric and turn-on fluorescence detection of Ag$^+$ and Zn$^{2+}$ ions.
Colorimetric and turn-on fluorescence detection of Ag\(^+\) and Zn\(^{2+}\) ions.

Abstract

In the present work, we have synthesized the probes (1-(anthracen-9-yl)-N-(2-((2-((-anthracen-9-ylmethylene) amino)ethyl) disulfanyl) ethyl) methanimine (Cysan) and 2-((anthracen-9-ylmethylene)amino)ethyl)ethan-1-amine (Trian) based on anthracene platform. The probe was characterized using NMR spectroscopy, UV–visible and mass spectrometry. The probes were tested for its sensing behaviour towards metal ions like Na\(^+\), K\(^+\), Ba\(^{2+}\), Ca\(^{2+}\), Mn\(^{2+}\), Fe\(^{2+}\), Co\(^{2+}\), Ni\(^{2+}\), Al\(^{3+}\), Cu\(^{2+}\), Cu\(^+\), Cr\(^{3+}\), Zn\(^{2+}\), Pb\(^{2+}\), Cd\(^{2+}\), Au\(^+\), Hg\(^{2+}\) and Ag\(^+\) in the CH\(_3\)CH\(_2\)OH:H\(_2\)O mixture buffered with HEPES (pH 7.4) by UV–visible and fluorescent techniques. Cysan and Trian show respectively high selectivity towards sensing of Ag\(^+\) and Zn\(^{2+}\) ions. Importantly fluorescence enhancement at 440 nm was observed for probes upon complexation with Ag\(^+\)/Zn\(^{2+}\) ions over other metal ions. This fluorescence enhancement is attributable to the restriction of the C=N isomerization and the prevention of photoinduced electron transfer from nitrogen to anthracene fluorophore. These photonic studies indicate that the probes can be adopted as a selective, sensitive and reversible fluorescent chemosensor for Ag\(^+\)/Zn\(^{2+}\) ions.
5.1 Introduction

Ag$^+$ exhibits a rich biological chemistry by serving as an antibacterial agent, a transcriptional initiator in mammals, a specific target of plasmid-conferred resistance and a redox-inactive probe for Cu$^{2+}$/Cu$^+$ sites in metalloproteins\(^1\). Proposed roles of Ag$^+$ in biological system are (i) interaction and inactivation of vital enzymes, (ii) binding to DNA (iii) interaction with the cell membrane, and (iv) interference with electron transport\(^2\). Use of silver in photographic imaging, and electronics industries, and their nanoparticles in consumer products such as detergents and wound dressings inadvertently results in bioaccumulation in aquatic and terrestrial organisms since they are released into the sewage line and eventually reach the river stream\(^3\). Hence, the positive effects of the silver-related technologies may cause a potentially negative impact on the environment. Silver ion is not known to be a cumulative toxin, but it interacts and displaces essential metal ions like Ca$^{2+}$ and Zn$^{2+}$ in hydroxyapatite in bone. Blood silver (argyraemia) and urine silver excretions are useful indices of human silver exposure. Because of all these effects, Silver ions are assigned to the highest toxicity class of heavy metal pollution\(^4\).

Zinc is a second most essential trace element and involved in numerous biological processes in human body\(^5\). Though almost zinc is tightly associated with the active sites in enzymes and with DNA -binding proteins, pools of mobile zinc with a wide range of concentrations are present in tissues of pancreas, retina and brain\(^6\). In mammalian cells, the majority of zinc ions are stored in vesicles, and the zinc-ion concentration in the cytoplasm is approximately 1 nM\(^7\). Zinc plays a critical role in insulin biosynthesis, storage and secretion. Zinc pollution is suspected of causing cardiovascular, reproductive, immune, and respiratory problems\(^8\). Zn$^{2+}$ is involved in a variety of physiological and pathological diseases, such as Alzheimer’s disease, epilepsy, ischemic stroke, and infantile diarrhoea\(^9\). It is also reported that zinc
ion is a potent killer of neurons via oxidative stress. Therefore, it is essential but still a challenge to develop chemosensors that can discriminate $\text{Zn}^{2+}$ from $\text{Cd}^{2+}$ as zinc and cadmium are in the same group of the periodic table. They have similar properties, which usually cause similar spectral changes after interacting with chemosensor$^{10}$. Therefore, the design and development of a selective fluorescent chemosensor to zinc are of considerable interest.

$\text{Ag}^{+}$ and $\text{Zn}^{2+}$ belongs to the so-called “silent ions” since, unlike some biological metal ions (such as $\text{Cu}^{2+}$ or $\text{Fe}^{2+}$), they do not have an intrinsic spectroscopic or magnetic signal because of their d$^{10}$ electronic configuration. Therefore discrimination between $\text{Zn}^{2+}$/Ag$^{+}$ and chemically close ions presents a challenge. Traditional quantitative approaches that have been developed for the determination of Ag$^{+}$ to date, such as electro thermal atomic absorption spectrometry (ETAAS), voltammetry, inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma mass spectroscopy (ICP-MS) enabled the detection limit down to the ppb range$^{11}$. But, most of these methods are expensive and time-consuming in practice. Sensitive and selective optical sensors for Ag$^{+}$ with simple instrumental implementation and easy-operation have received much attention.

5.1.1 Some earlier reports of Silver and Zinc sensors

Zheng and Jang et al. reported, heptamethine cyanide motif containing adenine molecule 1 for Ag$^{+}$ detection$^{12}$. Compound 1 showed an emission around 546 and 731 nm in buffer solution. When 1 was titrated with Ag$^{+}$ a significant increase in the emission was observed. In the presence of Ag$^{+}$ enhancements of fluorescence are mainly attributable to cyanine aggregation in aqueous solution. ‘H’ aggregation leads to change in colour from blue to pink and no changes in the ratiometric behaviour.
Tseng et al. published C\textsubscript{20} nucleotides for highly selective and sensitive detection of silver and silver nanoparticles in 50 mM NaNO\textsubscript{3} at pH-7.0\textsuperscript{13}. The interaction of C\textsubscript{20} with Ag\textsuperscript{+} causes intramolecular change from a random coil to a folded structure leading to a fluorescence intensity of SG. It has a detection limit of 32 nM (Scheme 5. 1).

**Scheme 5. 1** Conformational change of C\textsubscript{20} nucleotide in the presence of Ag\textsuperscript{+}.

Duan et al. synthesised\textsuperscript{14} benzoimidazolium derivative 2 for the detection of Ag\textsuperscript{+}. Compound 2 exhibits a fluorescent chemodosimeter with high selectivity towards Ag\textsuperscript{+}. In the free receptor 2, the electrostatic interactions between the benzoimidazolium groups lead to a dipodal receptor stabilized in a trans-conformation, in which the two naphthyl lumophores are separated from each other and no excimer fluorescence is observed. In the presence of Ag\textsuperscript{+} coordination bonds between the metal ion and the carbine moieties (deprotonated benzoimidazolium) induce the dipodand receptor a cis-conformation, with both arms orientated in the same direction, bringing the
naphthalene lumophores into close proximity and leading to excimer fluorescence. Zeng et al. reported 3, a highly selective and sensitive fluorescent chemosensor for Ag\(^{+}\) based on a coumarin-Se\(_2\)N chelating conjugate. Due to the inhibition of the PET quenching pathway, fluorescence enhancement is observed in CH\(_3\)CH\(_2\)OH:H\(_2\)O solution\(^{15}\). The chemosensor 3 detects the Ag\(^{+}\) in 10\(^{-8}\) M range.

Ahn et al. developed a Rhodamine-based fluorogenic and chromogenic probe 4 for Ag\(^{+}\) ion in CH\(_3\)CH\(_2\)OH:H\(_2\)O system\(^{16}\). The sensing mechanism is based on irreversible tandem ring-opening and forming processes promoted by Ag\(^{+}\)-coordination to the iodide of the probe, which is accompanied by both color and turn-on type fluorescence changes. This probe 4 showed remarkable selectivity and could detect silver ion in 14 ppb range.

Akkaya’s group reported a ratiometric fluorescent Ag\(^{+}\) sensor 5 based on bodipy\(^{17}\). As a ratiometric chemosensor, the emission ratio intensities at 630/671 nm changes from 0.25 to 1.42 for the free and Ag\(^{+}\) based sensor.
Wang et al. designed a coumarin derivative\textsuperscript{18} 6 as a sensor for Zn\textsuperscript{2+}. The free probe 6 is almost non-fluorescent in CH\textsubscript{3}CN solvent because of the isomerisation of C=\textit{N} in the excited state. After addition of Zn\textsuperscript{2+} the fluorescence increased gradually due to the inhibition of C=\textit{N} isomerisation.

Quin et al. developed 8-carboxamidoquinoline derivative\textsuperscript{19} 7 as a water soluble ratiometric chemosensor for Zn\textsuperscript{2+}. Upon addition of Zn\textsuperscript{2+}, the carboxamido group is deprotonated, so that the intramolecular hydrogen bond of 8-aminoquinoxaline is broken and inhibit the intramolecular electron transfer process. Further addition of Zn\textsuperscript{2+} shows an 8-fold increase in the fluorescence and 75 nm red shift. It has been used as an imaging reagent for Zn\textsuperscript{2+} in living cells and in tissues.

Yang et al. designed bis-9-anthryl diamine ligands 8 for Zn\textsuperscript{2+} detection in buffer solution\textsuperscript{20}. In the presence of Zn\textsuperscript{2+}, 6.5 fold increase in fluorescence intensity was achieved due to blocking of PET. The enhancement of fluorescence is attributed to the releasing of PET. The probe 8 have remarkably higher selectivity towards Zn\textsuperscript{2+} over the other cations.
Arman et al. displayed anthracene-polyamine\textsuperscript{21} 9 as a sensor for Zn\textsuperscript{2+}, which is the first ratiometric fluorescence sensor for Zn\textsuperscript{2+} in aqueous solution. Addition of Zn\textsuperscript{2+} induces 4-fold fluorescence enhancement at 495 nm is mainly ascribed to the anthracene excimer formation.

Guo et al. reported a quinolone-hydrazide\textsuperscript{22} 10 as a sensor for Zn\textsuperscript{2+}. The probe exhibits an emission band at 405 nm. Binding of Zn\textsuperscript{2+} causes red shift to 100 nm as the binding induced deprotonation of the sensor causes electronic delocalisation with the more extensive π-system. The compound 10 shows higher selectivity towards Zn\textsuperscript{2+} based on CHEF or self-assembling fluorescence enhancement.

Qian group developed 4-amine-1, 8-naphthalimde\textsuperscript{23} 11 as a chemosensor for Zn\textsuperscript{2+}. The lone pair of electron from the aniline nitrogen quenches the fluorescence of the excited fluorophore by PET. Binding of Zn\textsuperscript{2+} to the electron donating aniline nitrogen blocked the PET process blocked to get a 6-fold fluorescence enhancement.
But, most of these methods are expensive and time-consuming in practice. Therefore the present work pertains to developing simple chemosensor for Ag$^{+}$/Zn$^{2+}$ ion.

**PART-A**

5. 2 Results and Discussions

We have designed and synthesized a simple bis-Schiff base derivative wherein anthracene acts as fluorophore and cystamine acts as a receptor (Scheme 5. 2). The probe was characterized by analytical and spectral techniques.

![Scheme 5. 2 Synthesis procedure of Cysan](image)

5. 2. 1 UV-Vis. response for selectivity of Ag$^+$ ion

The UV-Vis absorption spectrum of the receptor Cysan in CH$_3$CH$_2$OH/H$_2$O (1:9, v/v) buffered with HEPES (pH 7. 4) exhibited characteristic absorption bands at 351, 371 and 389 nm. Upon addition of aqueous solution of Ag$^+$ to the solution of Cysan the absorption band at 389 nm underwent a bathochromic shift to 415 nm. A well-defined isosbestic absorption point at 403 nm throughout the titration clearly indicates the formation of new species by the influence of Ag$^+$ ion with Cysan. This change
in the absorption spectrum is responsible for the naked eye perceptible colour change from yellow to colourless (Fig. 5.1). Selectivity of the receptor was checked in the presence of other metal ions. The addition of metal ions such as Na\(^+\), K\(^+\), Ba\(^{2+}\), Ca\(^{2+}\), Al\(^{3+}\), Cu\(^{2+}\), Co\(^{2+}\), Fe\(^{3+}\), Ni\(^{2+}\), Mn\(^{2+}\), Pb\(^{2+}\), Zn\(^{2+}\), Cd\(^{2+}\), Hg\(^{2+}\), and Cr\(^{3+}\) produces insignificant change even in the presence of excess amount (100 \(\mu\)M) (Fig. 5.2a). In particular, competitive metal ions such as Cu\(^+\) and Au\(^+\) neither induced any shift in the absorbance nor any colour change of the solution (Fig. 5.2b).

In order to gain insight into the signalling properties of the receptor towards Ag\(^+\), absorption titrations were carried out. During the aliquots addition of Ag\(^+\) [0-100 \(\mu\)l] the band at 389 nm shifted to 415 nm, whereas higher equivalent (>1eq) of Ag\(^+\) resulted in no significant change. All these results show that the probe Cysan has high sensitivity and selectivity towards Ag\(^+\) over the other competitive metal ions.

**Fig. 5.1** UV-Vis absorbance titration of receptor Cysan (10 \(\mu\)M) in CH\(_3\)CH\(_2\)OH/H\(_2\)O (1:9 v/v) buffered with HEPES (pH 7.4) solution upon the addition of Ag\(^+\) (0-10 \(\mu\)M).
Fig. 5.2 (a). UV-Vis absorption responses of Cysan (10 µM) to various metal ions including Ag⁺ in CH₃CH₂OH/H₂O (1:9, v/v) buffered with HEPES (pH 7.4). (b) UV-Vis absorption responses of Cysan (10 µM) to various metal ions Cu⁺ and Au⁺ including Ag⁺.

5.2.2 Fluorescence studies of Ag⁺ ion

Subsequently the metal-binding affinities and photonic responses of the probe Cysan in CH₃CH₂OH/H₂O (1:9, v/v) buffered with HEPES (pH 7.4) was studied by fluorescence spectroscopy. Initially the receptor Cysan showed very weak fluorescence (quantum yield = 0.012) because of the PET/C=N isomerisation mechanisms. More explicitly quenching of fluorescence was attributable to both the photo-induced electron transfer from nitrogen to anthracene (fluorophore) moiety and C=N isomerisation. When Ag⁺ was added to the solution of Cysan drastic fluorescence enhancement at 440 nm (quantum yield 0.24) was observed (Fig. 5.3). Addition of other metal ions caused no significant changes in the fluorescence. The selectivity of the probe Cysan towards Ag⁺ was checked in the presence of different metal ions such as Na⁺, K⁺, Ba²⁺, Ca²⁺, Al³⁺, Cu⁺, Au⁺, Cu²⁺, Co²⁺, Fe³⁺, Ni²⁺, Mn²⁺, Zn²⁺, Cd²⁺, Pb²⁺, Hg²⁺ and Cr³⁺ (Fig. 5.4a). It clearly indicates that the probe Cysan can selectively bind with the Ag⁺, compared to the other cations (Fig. 5.4b).
Fig. 5. 3 Fluorescence titration of probe Cysan (10 µM) upon addition of Ag⁺ in 
CH₃CH₂OH/H₂O (1:9, v/v) buffered with HEPES (pH 7.4). (λₑₓ = 350 nm, λₑᵐ = 440 
nm).

Fig. 5. 4a Fluorescence spectra of Cysan (10 µM) in CH₃CH₂OH/H₂O (1:9, v/v) 
buffered with HEPES (pH 7.4) in the presence of various species (50 µM) 
Fig. 5. 4b. Changes of fluorescence intensity of receptor TRIAN (10 µM) after adding 
(50 µM) of each of the other cations (λₑₓ = 350 nm, λₑᵐ = 440 nm).

Sensitivity is an important criterion in fluorescence sensor studies. To 
study the sensitivity with respect to Ag⁺, receptor Cysan was titrated with Ag⁺. 
Upon incremental addition of Ag⁺ to the receptor the fluorescence increased 
gradually. The binding of Ag⁺ with Cysan was confirmed as 1:1 stoichiometry 
by Job’s plot method (Fig. 5. 5). It was further supported by ESI-MS wherein 
the molecular ion peak at m/z = 637 corresponds to [Cysan+Ag⁺]+ (Fig. 5. 6).
From the fluorescence titration profile, the association constant\textsuperscript{24} and the detection limit\textsuperscript{25} were found to be $6.407 \times 10^2$ M\textsuperscript{-1} and $2.797 \times 10^{-7}$ M respectively (Fig. 5.7).

**Fig. 5.5** Job’s plot for Ag\textsuperscript{+} and Cysan in CH\textsubscript{3}CH\textsubscript{2}OH/H\textsubscript{2}O (1:9, v/v, 10 µM) buffered with HEPES (pH 7.4) ($\lambda_{\text{ex}} = 350$ nm, $\lambda_{\text{em}} = 440$ nm).

**Fig. 5.6** ESI-MS Spectrum of Cysan+Ag\textsuperscript{+}
Fig. 5.7 Fluorescence titration spectra of probe Cysan (100 µM) with Ag⁺ (0-100 µM) in CH₃CH₂OH/H₂O (1:9, v/v) buffered with HEPES (pH 7.4) (λₑₓ = 350 nm λₑₘ = 440 nm).

The reversibility of the chemosensor is an essential phenomenon in analytical methods. The interaction between Cysan and Ag⁺ was reversible, confirmed by the addition of Na₂S into the solution containing Cysan and Ag⁺. After adding Na₂S to Cysan.Ag⁺ fluorescence reverts to that of Cysan (Fig. 5.8). This observation indicates that the chemosensor is active to Ag⁺ and provides evidence for reversibility.

Fig. 5.8 Fluorescence spectra of Cysan.Ag⁺ mixture upon the addition of Na₂S in CH₃CH₂OH/H₂O (1:9, v/v) buffered with HEPES (pH 7.4) (λₑₚ = 350 nm λₑₘ = 440 nm).
The interference of various metal ions if any was monitored in the presence of Ag\(^+\) (10 µM) with the addition of other monovalent metal ions (K\(^+\), Na\(^+\), Cu\(^+\) and Au\(^+\)) divalent (Cu\(^{2+}\), Co\(^{2+}\), Ni\(^{2+}\), Mn\(^{2+}\), Zn\(^{2+}\), Pb\(^{2+}\), Cd\(^{2+}\), Hg\(^{2+}\), Ba\(^{2+}\) and Ca\(^{2+}\)) trivalent metal ions (Fe\(^{3+}\), Al\(^{3+}\) and Cr\(^{3+}\)) (100 µM). No significant variation of fluorescence was observed in the presence of other competing metal ions in comparison to probe solution containing only Ag\(^+\). This reveals that the probe has good selectivity towards Ag\(^+\) over other co-existing cations. In particular, Cu\(^+\) and Au\(^+\) do not interfere in the competing environment (Fig. 5.9).

**Fig. 5. 9** Fluorescence responses of Cysan (10 µM) to various metal ions in CH\(_3\)CH\(_2\)OH/H\(_2\)O (1:9 v/v) solution buffered with HEPES (pH 7.4). The cyan bars represent the emission intensities of Cysan in the presence of (100 µM) of monovalent metal ions (K\(^+\), Na\(^+\), Cu\(^+\) and Au\(^+\)) divalent (Cu\(^{2+}\), Co\(^{2+}\), Ni\(^{2+}\), Mn\(^{2+}\), Zn\(^{2+}\), Pb\(^{2+}\), Cd\(^{2+}\), Hg\(^{2+}\), Ba\(^{2+}\) and Ca\(^{2+}\)) trivalent metal ions (Fe\(^{3+}\), Al\(^{3+}\) and Cr\(^{3+}\)). The blue bars represent the change of the emission that occurs upon the subsequent addition of (10 µM) of Ag\(^+\) to the above solution. The intensities were recorded at 440 nm, excitation at 350 nm.
5. 2. 3 Mechanism and DFT calculation

Cysan receptor has no appreciable fluorescence emission in their free form and shows bright fluorescence on complexation with Ag\(^+\). The probe contains the heteroatom N in conjugation with anthracene moiety via imine linkage. It is envisaged that both photoinduced electron transfer (PET) from nitrogen to anthracene fluorophore and C=N isomerisation may be responsible for fluorescence quenching. When the metal Ag\(^+\) binds with probe through nitrogen atoms both PET and C=N isomerization as anticipated are prevented to restore fluorescence from anthracene moiety. To understand further the absorption and fluorescence behaviour of the probe and the complex, we carried out the DFT calculations with B3LYP and 6-31G basis set using Gaussian 03 program\(^26\). A similar calculation was performed for optimizing the Ag\(^+\)Cysan using B3LYP and LANL2DZ basis set. The TD-DFT calculations of probe Cysan shows a transition at 419, 415 and 410 nm. The dihedral angle 124.34° for C=N-C-C reveals the S-trans conformation of receptor whereas that for Cysan+Ag\(^+\) it is found to be 139.18° (Fig. 5. 10) Cysan readily forms chelate with Ag\(^+\) ion through the ligating atoms N and S. Hence both the photo induced electron transfer (PET) and C=N isomerisation are inhibited to enhance the fluorescence from the receptor Cysan.

![Fig. 5. 10 Optimized structure of Cysan and Cysan+Ag\(^+\)](image)

Fig. 5. 10 Optimized structure of Cysan and Cysan+Ag\(^+\)
5.2.4 Detection of Intracellular Ag$^+$ with Cysan

To further explore the potential biological application of this sensor, in vitro detection of Ag$^+$ ion in HeLa cells was evaluated. To the best of our knowledge, fluorescent probes reported for cellular Ag$^+$ are rare. In the present study, the HeLa cell was first treated with Cysan (10 µM) for 30 min at 37°C and then the HeLa cells were washed with 20 mM HEPES buffer solution (pH 7.4) containing NaNO$_3$ instead of NaCl to prevent intracellular precipitation of AgCl. The fluorescent changes of the cells were monitored by fluorescent microscopy. Cysan seems to have sufficient cell penetration ability possibly due to the hydrophobic nature of anthracene molecule. The cells were washed with buffer solution after adding AgClO$_4$ into the Cysan loaded cells, to wipe out the excess amount of metal solution. We observed a considerable enhancement of intracellular emission; a strong blue fluorescence was observed in the cells (Fig. 5.11). The results suggest that Cysan can penetrate the cell membrane and viable for imaging of Ag$^+$ ions in living cells.

![Figure 5.11](image)

**Fig. 5.11** The intracellular Ag$^+$ was imaged in HeLa cells at 37°C with use of confocal microscopy. (a) Fluorescence image of Cysan Loaded HeLa cells. (b) Bright field transmission image of HeLa cells incubated with probe Cysan (a) (c and d) Fluorescence and super imposed image of HeLa cells incubated with sensor Cysan and then treated with Ag$^+$. 
5.3 Summary

In summary we have synthesized and characterized a new anthracene-based chemosensor Cysan, which shows high sensitivity, reversibility and displays great selectivity towards Ag\(^+\) ion over other metal ions such as Na\(^+\), K\(^+\), Ba\(^{2+}\), Ca\(^{2+}\), Al\(^{3+}\), Cu\(^+\), Au\(^+\), Cu\(^{2+}\), Fe\(^{3+}\), Ni\(^{2+}\), Mn\(^{2+}\), Zn\(^{2+}\), Cd\(^{2+}\), Pb\(^{2+}\), Hg\(^{2+}\) and Cr\(^{3+}\). In addition to this the binding between Ag\(^+\) and Cysan has also been established by emission spectroscopy and ESI-MS analysis. Addition of Ag\(^+\) enhances the fluorescence intensity of Cysan through inhibition of PET/C=N isomerisation. The receptor forms a 1:1 complex with silver ions as evident from Job’s plot. The receptor has been shown to be feasible for selective determination of Silver ion.

Part-B

Based on chelate effect and HSAB principle\(^{27}\) it is inferred that the probe Cysan, by virtue of its sulphur ligating atoms, may preferentially interact with soft cations like Ag\(^+\). To further confirm the soft-soft and hard-hard interaction, it was intended to the sulphur group of Cysan by nitrogen atom. Hence Trian was synthesized by condensing anthracene aldehyde along with diethylenetriamine.

5.4. Results and discussions

We have synthesized the receptor by a one-step facile reaction between anthracene-9-carboxaldehyde and diethylenetriamine in ethanol (Scheme 5.3). For a better understanding of the chemosensor, different spectroscopic studies like \(^1\)H and \(^{13}\)C NMR and ESI-MS were performed.

Scheme 5.3 Synthetic procedure of Trian
The binding affinities of the receptor Trian towards various metal ions including Zn$^{2+}$ were examined by UV-visible absorption and fluorescence titrations in HEPES buffered aqueous [CH$_3$CH$_2$OH:H$_2$O (1:9, v/v)] solution. The absorption spectrum of free Trian exhibited an intense band positioned at 350, 372 and 399 nm due to the $\pi$-$\pi^*$ and n- $\pi^*$ transition of the anthracene unit (Fig. 5. 12). The addition of Zn$^{2+}$ ions leads to the appearance of a new band at 480 nm and at the same time, colour of the solution changes simultaneously from yellow to red, which could be detected by naked-eye (Fig. 5. 13). Addition of a series of biologically/environmentally relevant metal ions such as Na$^+$, K$^+$, Ca$^{2+}$, Ba$^{2+}$, Al$^{3+}$, Ag$^+$, Mg$^{2+}$, Cu$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Mn$^{2+}$, Cd$^{2+}$, Hg$^{2+}$, Pb$^{2+}$ and Fe$^{3+}$ to Trian resulted in insignificant changes in the absorption spectrum of Trian. These results summarize that the probe Trian can be successfully applied for the determination of Zn$^{2+}$ in the presence of other representative metal ions.

**Fig. 5. 12** UV-Visible absorbance spectra of Trian (1×10$^{-6}$ M) with (50 µM) various cations such as Na$^+$, K$^+$, Ca$^{2+}$, Ba$^{2+}$, Al$^{3+}$, Ag$^+$, Mg$^{2+}$, Cu$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Mn$^{2+}$, Cd$^{2+}$, Hg$^{2+}$, Pb$^{2+}$ and Fe$^{3+}$ in CH$_3$CH$_2$OH:H$_2$O (1:9, v/v) at pH 7.4 (HEPES buffer).

Upon successive addition of Zn$^{2+}$ to the Trian solution, absorption band at 297 nm increased while a new peak appeared at 480 nm. Simultaneously the peak at 399 nm decreased gradually along with increasing concentration of Zn$^{2+}$. Hence the receptor Trian is selective and sensitive sensor for Zn$^{2+}$ ion.
Fig. 5.13 UV-Visible spectra of Trian (1×10^{-6} M) in CH_3CH_2OH:H_2O (1:9, v/v) at pH 7.4 (HEPES buffer) in the presence of Zn^{2+} (0-1 equivalent) ion.

The emission spectrum of the free probe Trian shows a very weak emission with emission maximum positioned at 439 nm on excitation at 340 nm. The fluorescence behaviour of the probe was verified in the presence of various metal ions in HEPES buffer. Before the addition of Zn^{2+}, Trian showed very weak fluorescence because of the PET/C=N isomerization mechanisms. The quenching of fluorescence was due to both the photo-induced electron transfer from nitrogen to anthracene (fluorophore) moiety and C=N isomerization. Once the Zn^{2+} was added to the Trian fluorescence was enhanced. In the presence of other metal ions, probe did not show any change in the fluorescence spectra. The selectivity of Trian towards Zn^{2+} was examined by the fluorescence titration experiments of Trian with biologically relevant miscellaneous metal ions. The other metal ions such as Na^+, K^+, Ca^{2+}, Ba^{2+}, Al^{3+}, Ag^+, Mg^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+} and Fe^{3+} exhibited almost no fluorescence enhancement under identical condition (Fig. 5.14a and 5.14b). Hence it is obvious that the receptor Trian is highly selective fluorescent sensor for Zn^{2+} ions.
**Fig. 5. 14a** Fluorescence spectra of TRIAN (10 µM) with various cations and Zn$^{2+}$ (50 µM). **5. 14b** Changes of fluorescence intensity of receptor TRIAN (10 µM) after adding (50 µM) of each of the other cations in CH$_3$CH$_2$OH:H$_2$O (1:9, v/v) at pH 7.4 (HEPES buffer) $\lambda_{ex} = 340$ nm, $\lambda_{em} = 439$ nm.

The fluorescence titration experiments were performed by increasing the metal ion concentration with a receptor solution. Fluorescence intensity of the probe is increased with the increasing concentration of Zn$^{2+}$ (Fig. 5. 15a). Subsequently, the binding ratio between TRIAN and Zn$^{2+}$ was estimated at 1:1 via Job’s plot curve according to the fluorescent intensity change and stoichiometry for the TRIAN.Zn$^{2+}$ type complexation is further confirmed by the ESI-MS of the complex solutions, which correspond to the species [TRIAN. Zn$^{2+}$Cl$_2$+H$^+$]$^+$ (m/z = 614.44) (Fig. 5. 15b and 5. 16). Furthermore, the binding constant of TRIAN with Zn$^{2+}$ was determined from the emission intensity data following the steady-state fluorometric method (Fig. 5. 17). The association constant and detection limit was calculated to be $1.63 \times 10^2$ M and $3.19 \times 10^{-7}$ M$^{-1}$ respectively.
**Fig. 5.15a** Changes in the fluorescence spectra of Trian (10 µM) upon addition of Zn$^{2+}$ ion (0-1-equivalent) in CH$_3$CH$_2$OH:H$_2$O (1:9, v/v) at pH 7.4 (HEPES buffer) $\lambda_{ex}$ = 340 nm, $\lambda_{em}$ = 439 nm. **Fig. 5.15b** Job’s plot; fluorescence intensity of Trian at 439 nm versus the mole fraction of Zn$^{2+}$ ion.

**Fig. 5.16** ESI-MS Spectrum of Trian.Zn$^{2+}$
Moreover to ascertain the selective response of **Trian** towards Zn$^{2+}$, competitive experiments were performed. No significant change in the fluorescence intensity was found in the presence of other common interfering metal ions in comparison to the probe solution containing only Zn$^{2+}$ ion. It is concluded that the probe **Trian** has good selectivity and sensitivity for Zn$^{2+}$ when compared to the other competitive ions (Fig. 5. 18).
bars indicate the emission intensities after adding 10 µM of Zn$^{2+}$ ion to each of the above solutions.

The sensing process of a probe molecule must be reversible for real-time application. To analyse whether the sensing processes of Trian was reversible, two equivalents of disodium-ethylenediaminetetraacetate (Na$_2$EDTA) was added to probe+Zn$^{2+}$ solution reverted to that of pure Trian (Fig. 5. 19). These results suggest the high reversibility of Trian towards Zn$^{2+}$.

![Fig. 5. 19 Reversibility studies of Zn$^{2+}$ ion (5 µM) bound receptor Trian (1 µM) upon addition of EDTA (10 µM) in buffer solution.](image)

5. 4. 1 DFT calculations

The weak fluorescent intensity of Trian was ascribed to the quenching due to PET from the imine nitrogen to anthracene moieties. In order to understand the fluorescence enhancement process of Trian with Zn$^{2+}$, DFT/TD-DFT calculations were carried out with Gaussian 03 program. The geometries of Trian and Trian+Zn$^{2+}$ were optimized by B3LYP/6-31G and B3LYP/LANL2DZ methods respectively. The resulting optimized geometries of Trian and Trian.Zn$^{2+}$ are shown in Fig 5. 20. The dihedral angel of 117° between C=N-C-C is 117° discloses the S-trans conformation of receptor; whereas that for Trian.Zn$^{2+}$ is found to be 122°. Also obvious from the figure is that Trian readily forms chelate with Zn$^{2+}$ ion through the hard nitrogen
donors: this blocks both the photo induced electron transfer (PET) and C=N isomerisation thereby leading to fluorescence enhancement.

![Fig. 5.20 Optimised geometry of Trian and Trian+Zn²⁺](image)

**5.5 Summary**

In conclusion, we have developed an anthracene functionalized Schiff base ligand for selective detection of Zn²⁺ in aqueous medium at physiological pH. The orientation of the coordination of the three nitrogen atoms of the ligand and the chemical hardness of the ligating N atoms lead to the potential binding of Zn²⁺ over other metal ions. The receptor senses Zn²⁺ ions through arrest of PET/C=N process but without any interference from the other competitive metal ions in the experimental condition.

**5.6.1 Synthesis of Cysan**

Ethanolic solution containing anthracene-9-carboxaldehyde (0.154 g, 0.74 mmol), Cystamine dihydrochloride (0.08 g, 0.37 mmol) and two drops of triethylamine was refluxed for one hour. The excess solvent was distilled off under reduced pressure. The lime yellow residue was washed with diethylether. Recrystallized from chloroform to get dark yellow solid. Yield: 95%. Melting point: 250-251°C. \(^1\)H-NMR (300 MHz, CDCl₃): ppm δ 3.33 (t, 4H, J = 6.7Hz), 4.3 (t, 4H, J = 6.6Hz), 7.43-7.52 (m, 8H), 7.98 (d, 4H, J = 5.1Hz), 8.43-8.53 (m, 6H), 9.43 (s, 2H). \(^1^3\)C-NMR(75MHz, CDCl₃): 162.2, 131.2, 130.0, 129.4,
128.8, 127.9, 126.7, 125.2, 124.8, 61.2, 39.6. ESI-MS: calculated: 528. Found: 529 (Cysan +H)+ (Fig. 5. 21-23).

5. 6. 2 Synthesis of Trian

Anthracene-9-carboxaldehyde (0.11 g, 0.58 mmol), diethylenetriamine (0.03 g, 0.29 mmol) and two drops of glacial acetic acid were added into ethanol and stirred for 3h. After cooling to room temperature, the solvent was removed under reduced pressure. The formed orange solid was washed with diethylether to get a bright orange solid. Yield: 88%. 1H NMR (300 MHz, CDCl3) δ 9.41 (s, 2H), 8.47 – 8.42 (m, 4H), 8.38 (s, 2H), 7.92 (dd, J = 6.0, 3.6 Hz, 4H), 7.37 (dd, J = 6.7, 3.1 Hz, 8H), 4.12 (t, J = 5.6 Hz, 4H), 3.29 (t, J = 5.8 Hz, 4H), 1.94 (s, 1H). 13C NMR (75 MHz, CDCl3) δ 161.95, 131.49, 130.19, 129.63, 129.15, 128.37, 126.99, 125.51, 125.15, 63.35, 50.51. ESI-MS calculated: 479. Found: 480 (Trian+ H)+.

5. 7 In-vitro Cellular imaging procedure

Localization of Cysan and the ability of Cysan for Ag+ recognition were investigated in HeLa cell line. Briefly, HeLa (7×103 cells/well) was seeded on to 96 well Cell Carrier microplates (PerkinElmer, US). When the cells reached 80% confluence the media were changed. Cells were then treated with 10 µM of Cysan alone and supplemented with Ag+ (10 µM) and the plate incubated for 24 hrs in humidified incubator at 37°C with 5% CO2. The cells were washed twice with NaNO3 and the compound fluorescence localized in live cells using Operetta High Content Imaging System (PerkinElmer, US).

5. 8 References


Silver ion sensor

Chapter V


Fig. 5. 21 $^1$H NMR spectrum of Cysan in CDCl$_3$  

Fig. 5. 22 $^{13}$C NMR spectrum of Cysan in CDCl$_3$
b) Isotope stimulation with observed and Calculated:

Fig. 5. 23 (a) ESI-MS Spectra of Cysan. (b) Isotopic pattern of 529 (M+H)\(^+\) peak found and calculated.
**Fig. 5.24** $^1$H NMR spectrum of Trian in CDCl$_3$

**Fig. 5.25** $^1$H NMR spectrum of Trian in CDCl$_3$
Fig. 5.26 ESI-MS Spectrum of Trian