DEVELOPMENT AND PHOTOPHYSICAL STUDY OF FLUORESCEIN BASED FLUORESCENT SENSORS AND THEIR DIFFERENT APPLICATIONS

A Thesis Submitted
In Partial Fulfillment of the Requirements
for the Degree of

Doctor of Philosophy

SWAPNADIP ROY

(Registration number: NITD/PhD/CH/2014/00523)

Under the Supervision of

DR. SUJIT SANKAR PANJA



DEPARTMENT OF CHEMISTRY
National Institute of Technology Durgapur
West Bengal 713209, India

August, 2022

ABSTRACT

Fluorescein and its related derivatives are extensively used as signalling handles for molecular imaging and chemosensing applications. As a fluorogenic moiety, fluorescein is an excellent chromophore with characteristics of large molar extinction coefficients, high fluorescence quantum yield values, easy of synthesis, high water solubility and excellent photostability.It has been given much less attention as compared to other fluorophores such as pyrene, rhodamine and coumarin derivatives. The unique photophysical feature of fluorescein derivatives lies in its two distinctive structural forms, closed spirocyclic and opened form with distinctly different spectroscopic properties. The interaction of colourless and non-fluorescent spirocyclic form of fluorescein derivatives with specific analyte triggers their conversion to highly conjugated open form which results in solution colour change and different fluorescence properties.

Chapter 4 describes the results of the investigation on a new thiophene-appended fluorescein-hydrazone-based chromo-fluorogenic sensor for the screening of Hg^{2+} ions in real water samples are reported and discussed thoroughly.Our research work sprouted from the observation that fluorescein derivatives play a vital role in the detection of various metal ions like Cu^{2+}, Fe^{3+}, Hg^{2+}, Al^{3+}, Pb^{2+}, Zn^{2+}, Co^{2+}, Cr^{3+} and Pd^{2+} by virtue of their unique photophysical properties with remarkable selectivity and sensitivity attributes. In this present study, we have endeavoured to design and synthesize thiophene appended fluorescein-hydrazone derivative and analyze its potential as real-time sensory system for Hg^{2+} ions in aqueous medium through different experimental and theoretical techniques. The present sensor **FT** was well characterized by single crystal X-ray structural analysis, FT-IR, HR-MS and elemental analysis. Initially, the UV-visible absorption spectrum of free probe **FT** was monitored which displays two strong absorption bands centered at 276 nm and 342 nm, which are the characteristics of thiophenyl and closed spirolactum form of fluorescein moieties respectively. The interaction of target analyte Hg^{2+} with **FT** was analyzed by performing UV-visible spectrophotometric titration. The initial characteristic of absorption bands of free probe gradually decreased in intensity, while a new broad absorption band at 456 nm was concomitantly appeared and its absorption intensity was increased with increasing the concentration of Hg^{2+} ions. The absorbance changes at 342 nm and 456 nm were plotted with the variation of Hg^{2+} concentrations which shows almost linear intensity changes in wide concentration range.Secondly,

fluorescence spectroscopy was employed to investigate fluorogenic sensing aptitude of **FT** towards Hg^{2+}. The weak fluorescence emission band of **FT** at 430 nm was vividly enhanced and shifted at 512 nm resulting in a 51 fold increase in emission intensity upon gradual 5 μM incremental addition of aliquots of Hg^{2+} ions in water (0–160 μM). The ratio of fluorescence intensities at 512 nm and 430 nm (I_{512}/I_{430}) plotted against varying concentration of Hg^{2+} ions also implies a linear trajectory of intensity change over a wide concentration range making the probe feasible enough to monitor wide concentrations of Hg^{2+} ions in practical samples. The mechanism of sensing Hg^{2+} ions by probe **FT** is explained in detail through a mechanistic scheme. The ratiometric fluorescence emission at two different emission wavelengths implies a change in emissive unit between free probe and complexed species. Complexation of Hg^{2+} ions with the chelating probe resulted in the formation of rigid host-guest molecular assembly which can effectively suppress non-radiative deactivation channels of the photoexcitedfluorophores through different quenching processes such as photo-induced electron transfer (PET), inhibition of C=N isomerization and rotation across single bonds, leading to efficient chelation enhanced fluorescence effect (CHEF).A slight spectral overlap between thiophenylemission with the absorption of fluorescein hydrazone - Hg^{2+} complex also encourages the two moieties to act as a donor-acceptor pair which results in energy transfer from thiophene donor to fluorophore acceptor resulting in opening of spirocyclic ring leading to FRET process.Thus, a synergistic effect of CHEF and FRET might be the predominant photophysical processes which direct the selective fluorogenic signaling mechanism of **FT** with Hg^{2+} ions.The limit of detection (LOD) value of the prepared sensor is found to be 137 nM. The association constant of FT with Hg^{2+} is estimated to be 5.076×10^{3} M^{-1} indicating a stable complexation between probe and the concerned analyte. The average fluorescence lifetime (τ_{avg}) increased from 1.53 ns to 3.95 ns upon addition of Hg^{2+} to **FT** which also corroborates with the 'turn-on' emission response of the sensor.

In **Chapter 5** we described the details of the investigation on the mechanism and working phenomenon of a judiciously designed and synthesized novel fluorescein-aminobenzaldehyde based fluorogenic probe for selective targeting of copper (II) ions in aqueous media. Here we have synthesized the probe **FAB** which could be facilely synthesized by one-step condensation reaction between equimolar amount of fluorescein hydrazone (FH) and 2-amino benzaldehyde. It was characterized in detail by FT-IR, HR-MS and elemental analysis.The probe did not exhibit any colorimetric response towards any investigated analytes. Next, the fluorescence sensing aptitude of the fluorophore **FAB** was ascertained by recording

fluorescence spectra of probe **FAB** (10 μM) in the presence of individual metal ions (20 μM each) to the probe solution. Contrary to all other metal ions where no specific response was recorded, addition of Cu^{2+} (1 equivalent) to probe solution (10 μM) significantly altered its fluorescence response with remarkable fluorescence emission enhancement at 535 nm (284 a.u., 4 fold) visually discernible with exclusive solution colour change from colourless to green monitored under UV light irradiation at 365 nm. Next, spectrofluorophotometric titration of **FAB** (10 μM) was performed by the incremental addition of Cu^{2+} (0-22 μM) upon excitation at 480 nm. The probe **FAB** displayed feeble emission band centered at 535 nm upon excitation at 480 nm. However, upon gradual addition of Cu^{2+} (0-22 μM) to the mixed ethanol/H_2O buffered medium of **FAB** (10 μM), the feeble emission band gradually raised in fluorescence intensity and became more sharper with maximum emission intensity at λ_{em}=535 nm resulting in 4.5 fold enhancement of fluorescence intensity at emission maxima.Limit of detection (LOD) was estimated by 3σ method and it was found to be 37 nM indicating remarkable sensitivity and potential of practical applicability of the probe in aqueous samples. The optimized ground state geometries for ligand and its complexation adduct with Cu^{2+} are depicted. The mechanism can be explained as follows. The dominating non-radiative processes like photo-induced electron transfer (PET) and active intra-molecular rotations (IR) quenches the light emission of the free fluorophore. However, complexation of probe with Cu^{2+} suppresses the non-radiative quenching processes like PET and intramolecular rotation due to formation of rigidified stable complex between **FAB** and Cu^{2+} and revive the fluorescence emission of the sensor through chelation enhanced fluorescence (CHEF) resulting in a 'turn-on' response.