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Design, Synthesis and Photophysical Studies of Novel Dye-Based Donor–Acceptor Systems for Selective Detection of Cu^{2+} Ions

Balveer Kumar

Research Scholar, Loyola Academy Degree & PG College, Secunderabad

ABSTRACT:

The selective and sensitive detection of metal ions and ligands is crucial across environmental, biological and industrial domains. Traditional analytical methods often suffer from low sensitivity, poor selectivity and complex instrumentation. To overcome these limitations, dye-based probes have emerged as versatile tools, capable of producing measurable optical changes in the presence of target analytes. This study focuses on the design and synthesis of novel donor–acceptor (naphthalimide–dansyl) dyads and their evaluation as ratiometric fluorescent probes for Cu^{2+} ion detection. The dyads were characterized using spectroscopic techniques (UV-Vis, fluorescence, FTIR, ^1H NMR) and X-ray crystallography. Their photophysical behavior demonstrated efficient Förster resonance energy transfer (FRET) in the ground state, which was selectively disrupted upon Cu^{2+} binding. The probes exhibited high selectivity and sensitivity toward Cu^{2+} ions compared to other mono- and divalent metals, forming 2:1 stoichiometric complexes with strong binding constants. These interactions were confirmed through Job's plot, Benesi–Hildebrand analysis and MALDI-TOF mass spectrometry. The study also explored the impact of sulfonamide groups and spacer length on binding affinity. Such dye-based systems have significant potential as ratiometric sensors for environmental monitoring, biomedical applications and photodynamic research.

KEYWORDS: Dye-Based Probes, Cu^{2+} Detection, Naphthalimide–Dansyl Dyads, Metal Ion Sensing, Fluorescence Ratiometry, Supramolecular Chemistry

4.1. INTRODUCTION:

Detection of ligands and metal ions can be utilised in a wide range of contexts, including but not limited to environmental monitoring, biological research and industrial processes. The significance of these detection methods may be seen in a wide variety of different places and industries. One of the most important aspects of environmental monitoring is the detection of metal ions, which is used for a variety of purposes, including measuring the amount of air pollution, determining the existence of contamination in soil and determining the quality of water. It is possible to use the presence of elevated concentrations of specific metal ions as markers of environmental concerns. This can be of assistance in the reduction of potential health risks and the preservation of ecosystems that are compatible with survival for a long period of time. In a similar vein, the detection of ligands and metal ions is vital in biological systems for the purpose of interpreting complex cellular processes, acquiring an understanding of signalling pathways and giving light on the interactions that take place between proteins during the course of cellular processes. In addition, it is essential to monitor metal ions in industrial processes in order to maintain the quality and safety of the products that are being created. This ensures that the production procedures are carried out in an effective manner.

Despite the fact that ligand and metal ion detection play an incredibly vital role, traditional methods frequently run into limits that reduce their efficiency by a significant amount. Several conventional methods have a poor sensitivity, which makes it difficult to detect and quantify analytes at low concentrations. This is because of the low sensitivity characteristics of these methods. Due to the lack of selectivity that these processes possess, there is a risk that interference from other compounds that are present in the sample may occur. This interference will lead to the accuracy of the results being compromised, which will ultimately lead to the results being inaccurate. There are a number of challenges that limit the general deployment of existing detection methods. One of these challenges is the complicated instrumentation and time-consuming procedures that are associated with these methods. It is especially important to keep this in mind in situations where resources are few.

The idea of dye-based probes has arisen as a potentially useful option in order to overcome these issues. Dye-based probes are molecules that have been precisely constructed to interact preferentially with particular ligands or metal ions, thereby producing changes in their optical properties that may be detected. Due to the high sensitivity of these probes, it is possible to detect even minute quantities of chemicals, which is one of the most significant advantages of these probes. The visual readout that dye-based probes provide makes it easier to evaluate the results, which is one of the reasons why they come highly recommended. In many cases, the variations in colour or fluorescence may be easily distinguished with the naked eye, which makes the analysis process more expedient and straightforward. Because of their ease of use and simplicity, dye-based probes are particularly appealing for use in on-site or point-of-care applications, which are situations in which speedy and reliable detection is of the utmost importance.

Because dye-based probes are so versatile, it is possible to customise sensors to a variety of ligands or metal ions of interest. This is made possible by the versatility itself. Even when dealing with complex sample matrices that contain several components, this selectivity leads to an improvement in the accuracy of detection. The incorporation of dye-based probes into a variety of detecting platforms, such as paper-based devices and portable instrumentation, further widens the scope of their applicability in a variety of contexts. Overall, the introduction of dye-based probes represents a significant advancement in the field of ligand and metal ion detection. These probes provide a powerful instrument that can overcome the limitations that are associated with traditional detection methods and they also contribute to advancements in the fields of environmental, biological and industrial sciences.

In the fields of chemical, biological and environmental sciences, optical devices that are capable of sensing and reporting chemical species are currently of great importance. The creation of specific probes for the detection of transition or heavy metal ions is of particular importance. This is due to the fact that these ions play vital roles in living systems and have an exceedingly damaging impact on the environment. When it comes to metal ions, the design of probes for Cu2+ ions has garnered a significant amount of attention due to the significance of these ions in a number of different biological processes. Because they have the ability to displace other metal ions that serve as cofactors in enzyme-catalyzed reactions, Cu2+ ions can be extremely harmful to organisms when they are present in the environment at greater quantities. Additionally, the uncontrolled Cu2+ ions have the potential to induce oxidative stress and the presence of these ions in the cytoplasm of neurons may be a factor in the development of Alzheimer's disease or Parkinson's disease syndrome. Within this framework, the development of functional molecules that preferentially attach to Cu2+ ions and communicate the occurrence through outputs that are sensitive and easy to detect is of utmost significance. The fluorescence-based techniques, in particular, offer excellent sensitivity and the optoelectronic detection technique, which is one of the many ways, offers significant advantages with regard to the other techniques.

The development of fluorescence ratio metric probes for metal ions has garnered a lot of attention in recent times. This is due to the fact that these probes enable the measurement of emission intensities at two separate wavelengths. In addition to expanding the dynamic range of emission measurements, this technique incorporates a built-in correction for environmental effects, which can be understood as artefacts that arise as a consequence of fluctuations in probe concentration. It has been difficult to build ratio metric probes that preferentially interact with metal ions and show high ratio metric signals. This is due to the fact that the ratio of emission intensities is what controls the sensitivity and dynamic range of a ratio metric probe. The design of sensors that give fluorescence enhancement upon Cu2+ binding is an exciting problem. This is because Cu2+, due to its inherent paramagnetic nature and therefore the complexation, often results in quenching of the fluorescence intensity of the probe. In particular, the design of sensors that give fluorescence enhancement upon Cu2+ binding is quite interesting. Very few examples of probes that undergo an increase in the fluorescence intensity upon complexation with Cu2+ ions are available among the many different types of probes that are used to detect Cu2+ ions are currently available. Therefore, the construction of highly selective probes for Cu2+ ions has become necessary due to the limited sensitivity of the probes and the high degree of interference caused by metal ions that are chemically closely related to one another. It is difficult to construct probes that exhibit fluorescence amplification when bound to Cu2+ ions in this setting because of the nature of the situation.

Both molecular and supramolecular photophysics, biology and molecular devices have made efficient use of photoinduced intramolecular energy transfer (ET), which involves a donor and an acceptor that are linked through appropriate spacer groups. This type of energy transfer has been extensively researched and utilised. Systems that are of particular interest are bichromophoric molecules, which are composed of a donor that is able to transfer its excitation energy to an acceptor that is located in close proximity to it through a spacer. In accordance with Forster's theory, the energy transfer between a donor and an acceptor only occurs when the two units are at a critical distance from one another. Furthermore, the efficiency of energy transfer is dependent on the distance that separates the two units. The ET process is affected when a bichromophoric system that is linked through a flexible spacer that contains heteroatoms bonds with metal ions. This is because the distance between the donor and acceptor is altered, which in turn causes alterations in the ET process. The variations in fluorescence intensity that are associated with this process have been successfully utilised as the signalling event for the detection of a variety of metal ions.

We were interested in developing innovative donor-acceptor based systems as probes for Cu2+ ions at this time because of the setting in which we found ourselves. We have synthesised two dyads, 1a and 1b, as well as the model compounds, 2 and 3, for the sake of comparison. We have also explored the interactions of these dyads with a variety of metal ions under a variety of situations using a variety of photophysical techniques.

Due to the fact that their individual units have been examined as chemosensors and fluorescent labels, as well as the fact that they are capable of, in principle, undergoing intramolecular fluorescence resonance energy transfer (FRET) and photoinduced electron transfer (PET) events, we decided to develop these dyads. Our findings indicate that these dyads have the ability to interact selectively with Cu2+ ions in comparison to other metal ions. Furthermore, they are able to signal the binding event by inhibiting FRET-mediated emission, which indicates that they have the potential to be used as sensitive fluorescence ratiometric probes for the selective recognition of Cu2+ ions.

- **Dye**

Dyes, which are molecules that are recognised for their ability to absorb and emit light, are regularly utilised in a wide variety of applications, with chemical sensing being one of the applications that stands out as particularly popular. The dye makes a significant contribution to the process of developing probes that are capable of detecting ligands and metal ions. Specifically designed for a particular function, the dye engages in a complex interaction with the ligands or metal ions that are the focus of the experiment, resulting in a noticeable change in the optical characteristics of the dye. This contact is essential to the performance of the probe because it facilitates the conversion of molecular recognition events into signals that can be measured. The probe's effectiveness is built on the dye's responsiveness to the presence of particular analytes, whether those analytes are ligands or metal ions. This responsiveness makes the probe a powerful instrument that may be utilised for a wide variety of applications, including environmental monitoring and biological research purposes. A nuanced approach to chemical sensing is provided by these probes, which contribute to breakthroughs in analytical chemistry and sensor technology. This is accomplished by utilising the intrinsic optical properties of dyes in conjunction with specific ligand or metal ion complexes.

Dyes in Various Applications: Chemicals known as dyes are extremely versatile and have a wide range of applications. Chemical sensing is one of the most recognised applications of dyes, which are distinguished by their ability to both absorb and emit light. Due to the fact that they are able to experience unique colour or fluorescence changes in reaction to alterations in their surroundings, they are extremely helpful in this sector. Dyes are used as indicator tools; some of them react to changes in pH or ion concentration, while others function as fluorescent probes, detecting and signalling the presence of particular chemicals through changes in light emission. Some dyes respond to variations in pH or ion concentration. In addition to their widespread use in analytical chemistry, biochemistry and environmental monitoring, dyes provide a substantial contribution to a wide variety of applications, demonstrating their adaptability in meeting a variety of sensing requirements. Because dyes play such an important part in boosting our ability to detect and analyse a wide range of chemical and environmental elements, they are an integral component in the development of scientific and technical developments. Dye technology ranges from simple pH indicators to more sophisticated smart materials.

Chemical Sensing: The identification and quantification of metal ions, which are charged atoms of metals and ligands, which are molecules that bind to a central atom, each bring their own unique obstacles. In this procedure, dyes, which are characterised by their one-of-a-kind optical qualities, play a crucial role. These dyes undergo noticeable changes in colour or fluorescence as a result of selectively interacting with particular metal ions or building complexes with ligands. These dyes serve as markers of the presence and concentration of the chemical entities that they interact with. The complex interaction that exists between dyes, ligands and metal ions is essential to the development of our capabilities in analytical chemistry. These dye-based probes, which offer a high degree of precision and sensitivity, make a significant contribution to the development of sophisticated tools for detecting and studying ligands and metal ions. As a result, our understanding of complex chemical interactions is improved and advancements are made in a variety of scientific and technological fields.

Complex Interaction: In the field of chemical sensing, the interaction between dyes and the ligands or metal ions that are being investigated is a complicated process that has been meticulously constructed to fulfil a particular function. When the dye comes into contact with the target ligands or metal ions, it undergoes noticeable changes in both its molecular structure and its optical characteristics. These changes are the result of careful engineering. It is because of this deliberate design that the dye is able to function as a sensitive indicator, acting in a manner that is distinct in response to the presence and concentration of the particular chemical entities that it is intended to detect. It is the result of a nuanced interaction between the dye and the ligands or metal ions that these tailored alterations take place. These transformations can be seen in altered colour or fluorescence. Not only do these complex interactions make it possible to accurately identify the chemical species in question, but they also offer extremely helpful insights regarding the nature of the binding and concentration of these species. These dye-based probes have been purposefully developed, which highlights the significant role they play in enhancing the capabilities of chemical sensing, making a contribution to the wider landscape of analytical chemistry and encouraging breakthroughs in scientific research and technological applications.

Optical Changes: In the event that two different chemicals come into touch with one another, the optical properties of the dye undergo a significant alteration. It is essential for the probe to undergo this modification in order for it to fulfil its performance requirements. This modification makes it possible for molecular recognition events to be converted into measurable signals. This alteration in the optical characteristics of the dye is essentially what plays a crucial part in enhancing the probe's ability to identify and translate molecular interactions into outcomes that can be observed and quantified. When it comes to a wide range of applications that demand accurate detection and measurement of molecular events, this feature is absolutely necessary for the overall effectiveness and usefulness of the probe.

Probe's Effectiveness: Analytes, such ligands or metal ions, are what the dye is sensitive to; this sensitivity determines how well the probe works. To achieve its goals, the probe relies on this reactivity. Because of its reactivity, or ability to interact with and identify certain molecules, the probe is a powerful tool with many potential uses.

As it becomes sensitive to certain analytes, the probe can be used as a selective instrument to focus on particular molecular interactions. Whether ligands or metal ions are being identified, the probe's optical property changes indicate its responsive behaviour, which converts molecular recognition events into quantifiable signals. Applications that necessitate the exact detection and quantification of particular analytes absolutely need this versatility. A versatile and potent tool with wide-ranging applications in areas as varied as medical diagnostics and scientific research, the probe's effectiveness is defined by its responsiveness to certain analytes. The probe's analytical and detecting capabilities are greatly improved by its selective responsiveness.

Applications: Probes that make use of dyes have a substantial potential for a wide range of applications in a variety of fields, including biological research and environmental monitoring. The fact that they are so versatile makes them extremely useful for researching and analysing complicated systems. Due to their ability to accurately identify and quantify particular analytes, these probes are an essential component in the aforementioned fields of study. The ability of the probes to identify and quantify specific compounds makes them an extremely helpful instrument, regardless of whether they are used in biological situations or environmental evaluations. For the purpose of expanding scientific understanding and tackling issues in sectors that need nuanced detection and quantification of analytes, dye-based probes are indispensable due to their versatility and precision.

Nuanced Approach to Chemical Sensing: In the field of chemical sensing, the probes that have been described represent a substantial step forward in terms of analytical procedures, representing a profound jump in analytical methods. By taking advantage of the intrinsic optical features of dyes and making use of the various interactions that occur between dyes and ligands or metal ions, these probes provide a method that is both detailed and accurate for recognising and measuring molecules in certain settings. The application of such probes raises the level of sophistication of chemical sensing approaches, hence offering a nuanced and efficient method for navigating complex investigations. Not only does this advanced method improve the accuracy of compound detection, but it also broadens the spectrum of applications in domains where exact identification and quantification are of the utmost importance. As a result, it contributes to the forefront of breakthroughs in analytical sciences.

Contributions to Analytical Chemistry and Sensor Technology: These probes provide a significant contribution to the advancement of analytical chemistry and sensor technology and their inclusion makes this contribution notable. Because of their aid, researchers are able to take use of the unique characteristics of dyes, which paves the way for the development of advanced tools in the field of analyte detection that is both accurate and selective. Scientists are able to create complex instruments that are specifically designed for the accurate identification and measurement of a wide variety of analytes since dyes possess a set of characteristics that are unique to them. The availability of this capacity paves the way for the development and personalisation of tools that make use of the inherent benefits that dyes provide. Therefore, the utilisation of these probes not only augments the precision of analytical procedures but also drives breakthroughs in sensor technologies. This serves as an example of a valuable synergy between creative materials and scientific brilliance in the process of pushing the frontiers of analytical capabilities.

- **Complex**

A molecular structure that is the consequence of the bonding of two or more different molecules is referred to as a "complex" in the scientific community. To be more specific, it is a chemical complex that has been painstakingly built to interact with specific ligands or metal ions in a precise manner. This molecular arrangement is purposefully complicated in order to endow the complex with increased affinity and specificity for the analytes that are the focus of the investigation. Through the process of customising the molecular architecture of the complex, the researchers intend to improve the selectivity of the probe. This will ensure that the probe reacts in a manner that is distinct to the presence of specific ligands or metal ions. This strategic design of the complex is an essential component of the overall probe system, which contributes to the efficacy of the probe system in distinguishing and detecting certain molecular species in a variety of analytical applications.

Increased Affinity and Specificity: Molecular arrangement is purposefully planned to be as intricate as feasible in order to get the desired effect. To enhance the affinity and specificity of the complex for the analytes that are the subject of this investigation, the goal of this study is to improve the complex. Specificity is the ability to target certain substances, whereas affinity is the degree to which molecules are able to bind to one another (the strength of the binding). Specificity is a capability that allows molecules to target specific substances.

Customizing Molecular Architecture: At this very moment, the researchers are engaged in the laborious process of customising the molecular architecture of the chemical. In order to design the complex in such a way that it interacts preferentially with specific ligands or metal ions, this level of customisation is absolutely important.

Improving Selectivity of the Probe: The procedure of customising the molecular architecture as part of the customisation process has as its primary goal the enhancement of the probe's selectivity. By guaranteeing that the probe reacts in a distinct manner when it is in the presence of specific ligands or metal ions, selectivity makes the probe a more effective instrument for use in analytical applications. This is because selectivity ensures that selective reactions occur.

Strategic Design for Efficacy: Due to the precise design of the probe system, the effectiveness of the system in recognising and detecting particular molecular species is significantly improved. Not only does this strategic approach take into account the complexities of molecular interactions, but it also makes a major contribution to the overall efficiency of the probing method. The complex's well-thought-out design acts as a linchpin, playing a critical part in raising the precision and sensitivity of the probe system, so increasing its capabilities in molecular recognition and detection. Both of these capacities are essential to the advancement of the complex. In essence, the success of the entire process of probing is dependent on the fact that the complex was designed with careful consideration and strategic planning, which highlights the fact that it is of the utmost importance in producing precise and targeted results.

Analytical Applications: In a wide range of analytical applications, both the complex itself and the strategic design of the complex are significant components that play a vital role. Due to the greater selectivity that the probe system possesses for this particular purpose, it will prove to be a very helpful instrument for researchers who are interested in detecting and studying specific chemical substances.

- **Working Mechanism**

The probe that was constructed functions in accordance with a principle that is based around a transformational shift in the optical qualities that it possesses. This is a crucial characteristic that makes it possible for the probe to be particularly successful in detecting specific ligands or metal ions. The construction of a responsive system is the outcome of the combination of the dye and the complex that was built. This particular system is distinguished by the fact that the interaction with target ligands or metal ions triggers a series of actions that follow one another in a chain reaction. Because of this interaction, the optical properties of the probe go through a discernible change, which can be seen as fluctuations in colour or fluorescence. This change can be detected. The synergy that occurs between the dye and the complex is accorded a considerable degree of attention with regard to its significance. When the target analytes bind to the complex, it has the potential to either produce a conformational change or alter the electrical environment that is around the dye. Both of these outcomes are possible. The formation of a detectable signal change, which serves as a reliable indicator of the presence of specific ligands or metal ions, is significantly influenced by this chemical rearrangement, which plays a crucial role in the evolution of the signal. This responsive system not only demonstrates the complexity of molecule identification, but it also exhibits the probe as a versatile instrument that may be used for precise and selective detection in applications that involve chemical sensing. This is due to the fact that it takes advantage of the dynamic interaction that takes place between the dye component and the complex.

Optical Transformation for Detection: The newly created probe makes use of the principle of a transformational shift in the optical properties that it possesses for operation in order to carry out its operational functions. This feature is extremely necessary in order to successfully identify specific ligands or metal ions. It is essential for the detection process. The ability of the probe to undergo a significant change in its optical properties is what enables it to efficiently carry out its detecting function. This ability is what gives the probe the ability to perform its function.

Responsive System Construction: A dye was coupled with a complex that was produced specifically for the aim of creating the responsive system. This was done in order to construct the system. This particular combination has been painstakingly created in order to provide a system that is capable of interacting with target ligands or metal ions in a manner that is precise at the same time.

Chain Reaction Mechanism: The system's ability to initiate a chain reaction of actions when it interacts with target ligands or metal ions is the distinguishing feature that differentiates it from other systems. This ability allows the system to be effective in a variety of situations. It is possible to see a chain reaction that is responsible for these changes in the optical properties of the probe, such as variations in colour or fluorescence. These changes are the result of the chain reaction.

Detection of Optical Changes: It is feasible to detect the optical changes that are the result of the interaction in order to obtain a signal that can be measured. This results in the signal being able to be measured. Because of this change in the optical features of the substance, it is possible to determine whether or not specific ligands or metal ions are present.**Synergy Between Dye and Complex:** The relevance of the synergy that exists between the dye and the complex is something that is emphasised. The interaction between these components, which is triggered by the binding of target analytes, has the potential to either cause a conformational change or affect the electrical environment surrounding the dye.

Signal Change and Chemical Rearrangement: When it comes to the production of a detectable signal change, the chemical rearrangement that is brought about by the interaction is a factor that is incredibly crucial to consider. For the purpose of producing reliable indicators of the presence of specific ligands or metal ions, it is necessary to undergo this chemical rearrangement.

Complexity of Molecule Identification: Not only does the responsive system demonstrate how challenging it is to properly identify molecules, but it also highlights the versatility of the probe as an instrument. Because of the dynamic interactions that take place between the dye and the complex, this complex is able to achieve precise and selective detection, which is the source of its versatility.

- **Detection of Ligands**

In the development of probes that are intended for molecular detection, ligands, which are molecular compounds that bind to a central metal atom or ion, contribute significantly to the overall process. Through the development of complexes, these probes are able to specifically recognise specific ligands thanks to the strategic design that was utilised in their construction. The presence of the targeted ligands causes this molecular interaction to cause a change in the optical properties of the probe that can be measured. This change acts as a signal that is responsive to the presence of the drug. Through the utilisation of the one-of-a-kind affinity that exists between ligands and the core metal atom or ion within the probe, this method is able to accomplish the detection of specific and selective substances. It is a potent instrument in analytical chemistry and biosensing applications, where the identification and measurement of specific ligands are of the utmost importance. The capacity to create detectable modifications in the optical characteristics boosts the sensitivity and effectiveness of the probe, making it a powerful instrument.

- **Metal ions**

The human body relies on metal ions for a wide variety of the most important processes. One of the most significant aspects of medicinal bioinorganic chemistry is the ability to recognise diseases that are caused by poor metal-ion function, to understand sickness at the molecular level and to treat diseases caused by such deficiencies. In addition, metal ions have the potential to cause toxicity in people. Heavy metal poisons, such as mercury and lead, are typical instances of this phenomenon. The essential metals that are now known or assumed to be required for proper biological functions in humans are the following: four main group metals (Na, K, Mg and Ca) and ten transition metals (V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo and Cd). The rapid expansion can be attributed to a number of remarkable achievements, the most notable of which are the widespread use of cisplatin for the treatment of testicular cancer, the use of gadolinium complexes in magnetic resonance imaging (MRI) and the development of nuclear medicine for both therapeutic and diagnostic purposes. Heavy metals including iridium, platinum, palladium and gold have been shown to exhibit anti-carcinogenic characteristics when they interact with N, S and O-donor atoms. These interactions have the potential to lead to the development of metal-based medications. It has been established that metal ions and metal coordination compounds have a significant impact on the processes that occur within cells. Not only does this metal effect have an impact on natural processes like cell division and gene expression, but it also has an impact on non-natural processes including toxicity, carcinogenicity and anti-tumor chemistry. Catalysing biological reactions (via hydrolysis, substrate transfer, electron transfer and other processes), stabilising biomolecular structure (protein, DNA), charge balancing (osmotic balance and nerve function) and reproduction are all significant roles that many metals play in living bodies or systems. In biological systems, metal ions exist as cations that are lacking in electrons. As a result, they are drawn to electron-rich biological components like proteins and DNA due to the fact that their charges are opposite to one another. The overall tendency for metal ions to interact with biological molecules is a result of the attraction between charges that are opposite to one another.

Importance of Metal Ions in the Human Body: Metal ions are necessary for the human body to be able to carry out the basic biological processes that it is responsible for. There are a wide range of biological activities that are dependent on the presence of key metals. These metals include sodium (Na), potassium (K), magnesium (Mg) and calcium (Ca), in addition to transition metals such as iron (Fe), copper (Cu) and zinc (Zn).

Medicinal Bioinorganic Chemistry: Medicinal bioinorganic chemistry is concerned with the identification of diseases that are brought on by inadequate metal-ion function, the comprehension of illnesses that occur at the molecular level and the treatment of diseases that are brought on by deficiencies in metals. Medicinal biobiochemistry is also concerned with the treatment of diseases that are brought on by deficiencies in metals. The recognition of this field as an essential one is necessary in order to facilitate the development of treatment methods that are associated with metal-ion imbalances.

Metal Toxicity: It is acknowledged that there is a legitimate concern over the possible harm that metal ions, particularly heavy metals like mercury and lead, could potentially cause to the human body; however, this concern has not been decisively confirmed with certainty. When it comes to the field of bioinorganic chemistry, one of the most important considerations is gaining an understanding of the potential negative consequences that are connected with these metals and, more importantly, developing techniques to lessen the severity of these effects. In spite of the fact that the adverse effects of heavy metals on human health are still a potential rather than a certainty, the field of study places a strong emphasis on the need of deciphering the intricate workings of the interactions that these metals have inside biological systems. In order to accomplish this, it is necessary to investigate the processes via which these metals may exert negative effects and to investigate strategies that might be utilised to minimise or prevent such impacts. Because of this, the investigation that takes place within the field of bioinorganic chemistry plays an important part not only in determining the possible dangers to human health that are linked with metal ions, but also in the process of establishing well-informed strategies to protect human health in the face of these unknown uncertainties.

Essential and Transition Metals: It is possible that metal ions and more specifically heavy metals like mercury and lead, could be harmful to the human body. This is a possibility that cannot be considered a certainty. One of the most important aspects of bioinorganic chemistry is the process of developing an understanding of the potentially harmful effects that these metals have and discovering ways to reduce the severity of those effects.

Achievements in Bioinorganic Chemistry: There have been a number of significant achievements in the field of bioinorganic chemistry, including the application of cisplatin for the treatment of testicular cancer, the utilisation of gadolinium complexes in magnetic resonance imaging (MRI) and the development of nuclear medicine for the purpose of both therapeutic and diagnostic applications.

Anti-Carcinogenic Characteristics of Heavy Metals: A number of heavy metals, including iridium, platinum, palladium and gold, have been shown to possess anti-carcinogenic effects when they interact with atoms that are donors of nitrogen, sulphur and oxygen. The development of metal-based drugs for the treatment of cancer is made possible as a result of this relationship.

Impact of Metal Ions on Cellular Processes: There is a significant influence that metal ions and coordination chemicals have on the activities that occur within cells. In addition to natural processes like cell division and gene expression, these activities also include non-natural processes like toxicity, carcinogenicity and anti-tumor chemistry. These activities are referred to as non-natural processes.

- **Detection of Metal Ions**

Ions of metals have the ability to combine with particular ligands or complexes and this coordination can be utilised for the purpose of detection. Depending on the presence of various metal ions, the probe might have a varied response, which would make it possible to do selective detection.

4.2. INTERACTIONS OF LIGANDS WITH METAL IONS:

Because it has consequences in a wide variety of domains, including chemistry, biology, medicine and environmental research, molecular recognition is a topic that garners a significant amount of scrutiny and interest. The molecular receptor must be able to differentiate between a set of structurally related molecules and bind a substrate in a certain manner in order to facilitate this process. The molecular species that serves as the substrate could be a cation, an anion, or a neutral one. When two species that are complementary to each other in size and shape engage to create a complex, a successful and selective receptor-substrate or host-guest recognition occurs. This occurs when the two species interact with each other. The interactions between enzymes and substrates, the creation of proteins and the reactions between antigens and antibodies are all examples of how biological systems expertly exemplify the concept of complementarity. When the binding event is signalled through a measurable attribute of the host, which is referred to as chemosensors, molecular recognition between a host and a guest becomes beneficial. Therefore, chemosensors are molecules that originate from abiotic sources and have the ability to bind selectively and reversibly to an analyte of interest while simultaneously causing a change in one or more features of the system.

An atypical chemosensor is comprised of two components: a receptor, which is a molecular recognition unit and a chromophore, which is a signal transducing moiety. Both of these components are called chromophores. An integration of these two components is accomplished by means of a spacer. Whereas the chromopore is an active unit that signals the binding event through changes in absorbance or emission (photochemical sensing) or redox potentials (electrochemical sensing), the receptor is the component that is responsible for the selective analyte binding. This is in contrast to the chromopore, which is an inactive unit. In the next part, a few examples of ratiometric chemosensors for cation recognition are presented. Additionally, a brief explanation of the numerous types of signalling processes that are possible is included in this area.

(a) Fluorescence sensors for metal ion detection

In the context of the building of fluorescence sensors, the word "fluoroionphore" refers to the interaction that exists between a fluorophore and an ionophore. As a signal transducer, the signalling moiety, which is often composed of organic chromophores or dyes that exhibit high absorption or emission, is responsible for converting the information (recognition event) into an optical signal, which is expressed as a change in the photophysical characteristics of the fluorophore (Valeur 1994). It is probable that these changes are the result of a perturbation in the photoinduced processes, which may involve the transfer of electrons, the transfer of charges, the transfer of energy and the generation of excimers or exciplexes of a variety of different types. In its capacity as an acceptor, the fluoroionphore is accountable for the process of photoinduced electron transfer, which is also referred to as that of PET. As a result of the fluorophore being excited, an electron that was previously located in the highest occupied molecular orbital (HOMO) is moved to the lowest unoccupied molecular orbital (LUMO). Through this process, electrons are transferred from the HOMO of the donor, which is a part of the free cation receptor, to the LUMO of the fluorophore. This, in turn, causes the fluorescence of the fluorophore to be suppressed (as seen in Figure 4.1). The process of cation binding causes the redox potential of the donor to increase, which in turn causes the energy level of the relevant HOMO to decrease to a level that is lower than that of the fluorophore. Because of this, the receptor's ability to donate is reduced, which, in turn, makes PET less advantageous and assures that fluorescence emission is restored. Consequently, this is a result of this. The change in fluorescence emission intensity that is associated with this process has been effectively employed as the signalling event for the detection of metal ions. This has been accomplished through the use of this procedure.

In addition to the photoelectron spectroscopy (PET), the photoinduced intermolecular energy transfer between connected donor-acceptor systems has also been widely utilised for the selective detection of cations. For instance, the binding of the cation to the donor may result in an increase in the distance between the donor and the acceptor, which in turn led to the formation of the either a reduction in the transmission of energy or the opposite (Figure 1.13). The existence of the emission from the donor, in contrast to the emission from the acceptor in the absence of cation binding, is a reflection of this phenomenon. However, the effectiveness of this process of energy transfer is contingent on concerning the distance that exists between the donor and the acceptor in accordance with Forster's theory, which is represented by the equation 1.1, where R represents the distance that exists between the two fluorophores and Ro represents the Forster critical radius. It is possible that a relatively minor change in the distance could result in a significant improvement in the efficiency of energy transfer, provided that the inter-chromophores' distance is more than the Forster critical radius in the free ligand and lower in the complex. chart 1 shows the operation of the first bifluorophoric cation sensor 7, which operates according to the principle of photo-induced energy transfer. The system in question is made up of two coumarins that are connected to one another by a short flexible chain called pentakis (ethylene oxide), which is able to form complexes with bivalent ions. Spectra of absorption, excitation and emission were significantly altered as a consequence of binding with Pb2+ ions in acetonitrile. On the other hand, the effect of alkali and alkaline-earth metal ions was minimal. There is a significant overlap between the emission spectrum of the amino substituted coumarin donor and the absorption spectrum of the courmarin acceptor. This suggests that there is an effective transfer of electronic energy between the two units, which ultimately leads to the formation of a new fluorescence emission band as a result of the energy transfer emission.

It is interesting to note that the rate of energy transfer for the [7-PbZ+] complex was found to be significantly increased in acetonitrile, however there was no change observed in propylene carbonate. It was determined that this was due to the fact that the metal-ligand complex in the two solvents had somewhat different structures. In the presence of acetonitrile, the [7-Pb2 +] complex exhibits a stoichiometry of 1:1, however in the presence of propylene carbonate, a 1:3 complex is seen to be produced. upon the ligand. A helical wrapping of the ligand around the cation takes place in acetonitrile during the process of complexation. This results in a significant increase in the efficiency of the complex as well as the rate at which it transfers energy compared to the previous state. In turn, this leads to an increase in the amount of energy that is transferred through fluorescence resonance, which in turn results in a reduction in the distance that exists between the donor and acceptor units. Propylene carbonate, on the other hand, did not experience any conformational alterations, which resulted in a slight modification to the FRET emission.

4.3. BACKGROUND FOR THE PRESENT INVESTIGATION:

Squaraines are a group of dyes that are characterised by their acute and powerful absorption bands (e ~1-5 x 105 cm-1 M-l) in the red to near infrared spectrum. Generally speaking, squaraine dyes can be thought of as an acceptor when they are conjugated with two donors consisting of D-A-D. It is possible for the donor molecules to be the same or different, the result being dyes that are either symmetrical or unsymmetrical. Squaric acid and electron-rich aromatic, heteroaromatic, or olefinic compounds are typically used in a one-step reaction to produce these dyes. The reaction is characterised by the condensation of the two compounds. Therefore, the nucleophilicity of the aryl species is essential to the success of the reaction. It was discovered that the condensation reaction was only carried out by the species that possessed a high level of nucleophilicity.

Charge transfer is a mechanism that is predominantly confined to the central cyclobutane ring and it is involved in the SO-Sl electrical excitation of these dyes. Combined with the extended conjugated 1t-electron net work that is present in squaraines, the intramolecular CT feature of this transition is what gives birth to the crisp and strong absorption bands that are found in the visible to infrared region. There has been a significant amount of research conducted on the photochemical and photophysical properties of these dyes. A wide variety of industrial applications are a good fit for squaraines. Some of these applications include photogeneration pigments for photoreceptors, which are used in photocopiers and laser printers; photoconductors in organic solar cells; and infrared absorbers in organic optical discs. On the other hand, the biological applications of squaraines have not been investigated, particularly their prospective roles as sensitizers in photodynamic therapy (PDT). The reason for this is that these dyes have a very low intersystem crossing efficiency, which makes them less effective in producing reactive species that are necessary for cellular damage. Biological applications of squaraine-N-hydroxysuccinamide esters as long wavelength fluorescent markers have been published in the literature for the first time in recent times.

In light of the fact that properly substituted squaraine dyes have advantageous absorption properties, namely absorption in the photodynamic window, we came to the conclusion that these dyes could serve as sensitizers for use in photodynamic therapy (PDT) if their intersystem crossing efficiency was improved. Regarding this matter, we have successfully manufactured squaraine dyes that have been substituted with heavy elements such as bromine and iodine (Chart 2). It is well known that heavy atoms improve the efficiency of intersystem crossing and populate the triplet excited state through the process of spin orbital coupling. During the course of the laser flash photolysis experiments conducted on 11 and 12, it was discovered that the primary transient intermediates involved were triplet excited states. The forms 11 and 12 that are deprotonated singly have triplet lifetimes that are realistic (Tr = 132 and 36 \s) and they have considerable quantum yields of triplet excited states (cPr = 0.22 and 0.50, respectively). In addition, these dyes produce singlet oxygen in a satisfactory manner, with yields of (4) (102) = 0.13 and 0.47 for 11 and 12, respectively. The photobiological experiments have shown that the compounds 11 and 12 have a significant photocytotoxicity in human cells as well as bacterial strains, but they have a comparatively low toxicity in the dark in comparison to the non-halogenated derivative (Ramaiah et al. 2002, 2004, Patent No. DS 6770787). According to the findings of mutagenicity research, these dyes do not cause any mutations when exposed to darkness; but, when exposed to visible light (oxidative light), they do cause mutations that are not negligible. the harm). According to what was stated before, the reactive oxygen species that are produced as a result of photodynamic processes have the capability to cause damage to membranes, DNA and other biological targets. A number of repercussions have been identified as a result of the photodynamic reactions. These include DNA strand breaks at specific places, specifically guanine and thymine, as well as cross linkages between DNA and DNA and proteins.

Serum albumin has the ability to connect with drug molecules in a manner that is both reversible and extremely strong. As a result of the formation of this complex, the concentration of unbound molecules in the plasma is reduced, which in turn has an effect on the distribution of the ligand, as well as its pharmacokinetics, toxicity and finally its rate of excretion. A well-correlated link between photodynamic activity and HSA site-II binding affinity to pharmaceuticals was discovered in previous research on a variety of photosensitizers, including as porphyrins and chlorins. In other words, substances that were shown to be photodynamically active were found to bind to site II of HSA. Extensive research has been conducted in this thesis to investigate the interaction of serum albumins with squaraine dyes that are based on phloroglucinol. This interaction is significant both in the clinical and pharmaceutical environments.

4.4. OBJECTIVES OF THE INVESTIGATION:

The development of organic compounds that are capable of exhibiting selective interactions with serum albumins and binding selectively at Site II is of tremendous importance in the field of biochemistry and medicine. Within the scope of this discussion, our primary purpose has been to investigate the interaction between serum albumins and squaraine dyes that are based on phologlucinol. Due to the fact that the triplet state of the photosensitizer plays a significant part in the photodynamic activity, it was also of interest to us to gain an understanding of how the triplet state properties of the squaraine dyes are affected when serum albumins are present. Another purpose of the thesis has been to get an understanding of the manner in which steric variables influence the site-selective binding interactions of squaraine dyes. This is done with the intention of analysing the possible in vivo transportation pathways of both halogenated and non-halogenated squaraine dyes. The establishment of the binding sites of the squaraine with the protein was still another purpose of the thesis. This was accomplished by ligand displacement studies that utilised site-specific binding agents. It has been demonstrated beyond a reasonable doubt that cancer cells have an excessive expression of folate receptors. In light of this, it was of interest to us to modify the serum albumins with folk add and explore the capability of fofate modified serum albumin to function as a sensitizer delivery system. Another purpose of the thesis has been to examine the interactions of donor-acceptor systems with a variety of metal ions and to synthesise donor-acceptor systems.

We have explored the interactions of squaraine dyes with serum albumins by employing a variety of approaches, including photophysical, chiroptical, biophysical and microscopic methods. The results of our research have demonstrated that these colours interact selectively with serum albumins, displaying high percentages of association constants. The hydrophobic, hydrogen bonding and electrostatic interactions that are involved in these interactions are all interrelated. According to our findings, these dyes display a selectivity at site II of serum albumins that is dependent on the size of the substituent. Additionally, they announce the binding event by means of increased triplet excited state lifotimes and quantum yields, in addition to the fluorescence intensity that is characterised by a "tum on" pattern. Consequently, it can be inferred that these dyes have the capability of being utilised in the process of NIR noncovalent protein labelling, in addition to serving as sensitizers in photodynamic therapeutic agents. Furthermore, the results of our investigation into the metal ion binding of donor-acceptor systems revealed that these systems exhibited an exceptional selectivity for Cu2+ ions. This was discovered as a result of our research. In addition, these systems participated in the formation of stable stoichiometric complexes with a ratio of 2:1, which entailed sulphonamide functionality. In addition, these devices served as visible fluorescence ratiometric probes, which allowed for the selective identification of cuz* ions.

4.5. NOVEL NAPHTHALIMIDE-DANSYL HYBRIDS AND INVESTIGATING THEIR AFFINITY WITH MONO AND DIVALENT METAL IONS:

The development of fluorescence ratiometric probes for metal ions has garnered a lot of attention in recent times. This is due to the fact that these probes enable the measurement of emission intensities at two separate wavelengths. In addition to expanding the dynamic range of emission measurements, this technique incorporates a built-in correction for environmental effects, which can be understood as artefacts that arise as a consequence of fluctuations in probe concentration. It has been difficult to build ratio metric probes that preferentially interact with metal ions and show high ratio metric signals. This is due to the fact that the ratio of emission intensities is what controls the sensitivity and dynamic range of a ratio metric probe. The design of sensors that give fluorescence enhancement upon Cu2+ binding is an exciting problem. This is because Cu2+, due to its inherent paramagnetic nature and therefore the complexation, often results in quenching of the fluorescence intensity of the probe. In particular, the design of sensors that give fluorescence enhancement upon Cu2+ binding is quite interesting. Very few examples of probes that undergo an increase in the fluorescence intensity upon complexation with Cu2+ ions are available among the many different types of probes that are used to detect Cu2+ ions are currently available. Consequently, the construction of highly selective probes for Cu2+ ions has become necessary due to the limited sensitivity of the probes and the high degree of interference caused by metal ions that are chemically closely related to one another. It is difficult to construct probes that exhibit fluorescence amplification when bound to Cu2+ ions in this setting because of the nature of the situation.

The process of photo-induced intramolecular energy transfer (ET) between a donor and an acceptor that is linked through appropriate spacer groups has been the subject of extensive research and has been successfully utilised in the fields of molecular and supramolecular photophysics, biology and molecular electronics over the course of its history. Bichromophoric molecules are of particular interest because they are composed of a donor that is able to transfer its excitation energy to an acceptor that is positioned in close proximity to it through a spacer. These molecules are of particular interest because they are able to achieve this. According to Forster's hypothesis, the only time that energy may be transferred from a donor to an acceptor is when the two units are simultaneously located at a critical distance from one another. To add insult to injury, the distance that separates the two units is a significant factor in determining the efficiency of energy transmission. The ET process is influenced by the formation of bonds between metal ions and a bichromophoric system that is connected by means of a flexible spacer that comprises heteroatoms. This is due to the fact that the distance between the donor and acceptor is changed, which in turn causes changes in the process of embryo transfer. It has been demonstrated that the changes in fluorescence intensity that are connected with this process can be successfully exploited as the signalling event for the detection of a wide range of metal ions. The development of novel donor-acceptor based systems that could be used as probes for Cu2+ ions in this context was something that we were interested in doing because it was of personal interest to us. We have successfully synthesised the dyads 1a and 1b, in addition to the model compounds 2 and 3 for the purpose of comparison (Chart 3) and we have explored their interactions with a variety of metal ions under a variety of situations using a variety of photophysical techniques. Due to the fact that their individual units have been examined as chemosensors and fluorescent labels, as well as the fact that they are capable of, in principle, undergoing intramolecular fluorescence resonance energy transfer (FRET) and photoinduced electron transfer (PET) events, we decided to develop these dyads. Our findings indicate that these dyads have the ability to interact selectively with Cu2+ ions in comparison to other metal ions. Furthermore, they are able to signal the binding event by inhibiting FRET-mediated emission, which indicates that they have the potential to be used as sensitive fluorescence ratiometric probes for the selective recognition of Cu2+ ions.

4.6. MATERIALS AND METHODS:

1,8-Naphthalic anhydride, 1,6-hexadiamine, 1,8- octadiamine, dansylchloride, Hg(Cl04)2, Pb(Cl04)2, Cu(Cl04)2, Zn(ClQ4)2, Ca(Cl04)2, LiCJ04, Cd(CJQ4)2, Fe(CJ04)2, Ba(Cl04) 2, Co(CJQ4)2, NaCl, KCl, triethylamine and Mg(ClQ4)2 were all obtained from Aldrich and utilised in the aforementioned manner. Modifying the procedures that were previously reported allowed for the synthesis of N-(6-aminohexyl)-1,8-naphthalimide (Sa), with a melting point of 103-104 degrees Celsius (mixture mp 104-105 degrees Celsius), N-(8-aminooctyl-l,8-naphthalimide (5b), with a melting point of 163-164 degrees Celsius (mixture mp 163-164 degrees Celsius), 5-(N,N-dimethylamino)-naphthalene-Nbutyl-l-sulfonamide (2), with a melting point of 96-97 degrees Celsius (mixture mp 96 degrees DC) and (N,N-dimethylamino)-naphthalene-o-tert-butylphenol-l-sulfonamide (3), with a melting point of 109-110 degrees Celsius (mixture mp 110 degrees Celsius).

- **General Procedur for the Synthesis of the Dyads 1a and 1b**

A solution of the corresponding N-(ro-aminoalkyl)-1,8-naphthalimide (4.2 mmol) and triethylamine (5.4 mmol) in dry chloroform (15 mL) was agitated and then dansylchloride (5.0 mmol) was added to the mixture. We let the mixture to reflux for ten hours. When the reaction mixture had been brought down to room temperature (25 degrees Celsius), the precipitate that had been produced was filtered off. Evaporating the clear filtrate solution until it was completely dry under vacuum resulted in the formation of a pale yellow residue. A chromatographic analysis was performed on the product mixture using silica gel. Elution using a mixture of ethyl acetate and hexane in a ratio of 1:9 resulted in the formation of the dyads 1a (60%) and 1b (65%), which were then subjected to recrystallization from CH3CN in order to achieve further purification.

- **Determination ofStoichiometry by Jobs Plot**

The overall molar concentration of the two binding partners (for example, dyad and metal ions) is maintained at a constant level throughout the Jobs plot approach; however, the mole fractions of these partners are transformed. When plotted against the mole fractions of these two components, the fluorescence intensity (or peak area) that is due to complex formation is shown to be proportional to complexity. In the event that sufficiently high concentrations are utilised, the maximum on the curve corresponds to the stoichiometry of the two species.

- **Determintion of Binding Constant by Benesi-Hildebrand Analysis**

The binding affinities of the dyads were determined by employing the Benesi-Hildebrand equation 4.1 for 1:1 stoichiometry and the equation 4.2 for 2:1 stoichiometry. In this equation, K represents the equilibrium constant, lo represents the fluorescence intensity of the free dyad, 1 represents the fluorescence intensity that was observed in the presence of metal ions and ls represents the fluorescence intensity at saturation. As a result of the linear dependency on the reciprocal (or its square root) of the metal ion concentration, it may be deduced that a 1: 1 (or 2: 1) complex is formed between the dyad and the metal ion.

For a 1:2 metal-ligand binding mode, the association constants were also calculated using the fluorescence titration data using a method that was reported by Lin et al. (2008). This method was used to calculate the association constants.

where F1 and Fo are the limiting emission values for a = 1 (in the absence of metal) and a = 0 (probe is complexed with the metal ion), respectively. where CF stands for the total concentration of ligand in the system. A defined as the ratio between the free dyad concentration [C] and the total concentration of ligand CF, it was evaluated using equation

4.7. RESULT:

- **Synthesis of the dyads**

The chimeric dyads ta and th have been synthesised in good yields [60-65%] using the appropriate N-ro-alkylnaphthalimide and dansyl chloride (Figure 4.3). By contrast, dansyl chloride was reacted with butyl amine and p-tert-butylphenol to synthesise model compounds 2 (90%) and 3 (50%) in accordance with the described approach (2009). These compounds were purified by recrystallization and their characteristics were determined using analytical results and spectral data.

In the l H NMR spectrum of the dyad la in CD3CN, for instance, the methylene protons were seen as a multiplet and a quartet at ~j 1.10-3.93 ppm, respectively, while the -NH protons were seen as a triplet at ~j 5.70 ppm. The twelve aromatic protons showed up in the range ~j 7.20-8.51 ppm, while the protons of the -N(CH3)z group appeared as a singlet at ~j 2.80 ppm. On the other hand, the dyad la's 13C NMR spectra showed signals at {j 25.30-42.40, which corresponded to sp3 carbons and between {j 114.80-151.70, which showed the aromatic carbons and 44.3 ppm for the

N-CH3 carbon and 163.60 ppm of carbonyl carbon. Furthermore, single crystal X-ray examination of the sample example la provided the last validation of the structures. The dyad la's ORTEP diagram is displayed in Figure 4.1 and Table 4.1 provides a summary of its crystallographic information. The distance is 11.6 according to the crystal structure.

A is significantly less than the critical distance needed for an efficient energy transfer between the donor and acceptor between the naphthalimide and dansyl chromophores.

- **Absorption and fluorescence properties**

In a number of different polar and non-polar solvents, the dyads' absorption properties have been taken into consideration and examined. The dyads 1a and 1b are shown in Figure 4.5 to have their absorption spectra displayed in acetonitrile. At 332 nm, these dyads exhibited the highest level of absorption, with a shoulder occurring at 339 nm. There have been observations made in other solvents, such as methanol, ethanol and toluene, that are comparable to these. It has been discovered that the absorption spectra of these dyads is equal to the total of the individual units in each of these solvents. This finding indicates that there is no substantial interaction between the chromophores of naphthalimide and dansyl in the ground state (Figure 4.6). A comparison of the emission spectra of the dyads 1a and lb is presented in the inset of Figure 4.5. Two emission maxima were seen in the fluorescence spectra of these dyads when stimulated at 339 nm. These maxima occurred at 375 and 525 nm, which is the wavelength at which the majority of the photons are absorbed by the naphthalimide chromophore. From the results of the spectrum analysis of the excitation. The bands that were seen at 375 and 525 nm, respectively, might be attributed to the locally excited state of the naphthalimide chromophore and the fluorescence resonance energy transfer (FRET) mediated emission from the dansyl moiety, according to the literature studies. The fluorescence intensity of the dyads 1a and lb is dramatically reduced when compared to the dansyl-based model compound 2, which is the subject of this comparison. This is due to the fact that the emission that is observed at 525 nm in the case of these dyads is due to the presence of FRET, which is a reaction that occurs from the excited state of the naphthalimide chromophore to the dansyl unit, as well as the photoinduced electron transfer (PET) reaction, which occurs from the dansyl chromophore to the naphthalimide moiety. When compared to the ratio of around 0.5 that was reported for the dyad la that had the shorter hexamethylene group, the dyad lb that had a longer spacer length, also known as an octamethylene unit, demonstrated effective FRET-mediated emission with a fluorescence intensity ratio Im/Im of 1.2. With the increase in spacer length, as was seen in the case of donor-acceptor systems, this can be attributed to an increase in the amount of energy that is transferred from the naphthalimide chromophore to the dansyl moiety.

- **Interactions with metal ions**

We wanted to investigate the possible application of the napthalimide and dansyl based dyads ta and lb as ratiometric sensors and determine the best conditions for the detection of metal ions in the aqueous medium. This was because the dyads displayed intramolecular dual emission, which was an interesting phenomenon. Within this framework, we have conducted research into the ways in which these dyads interact with a wide range of metal ions under a variety of conditions, one of which being the micellar medium. A solvent system that is composed of a mixture of water and acetonitrile with a ratio of 4:1 and contains neutral micelles of triton X-100 (TX-100; 2 mM) has been found to be very effective in terms of the stability of dyads, as well as the selectivity and sensitivity of the metal ion binding event. This has been observed to be the case among all of the conditions that have been investigated. As an illustration, Figure 4.4 illustrates the modifications that occurred in the absorption spectra of the dyad lb as a result of the incorporation of copper perchlorate into the micellar compound medium-sized. As the concentration of Cu2+ ions increased, we saw a drop in the absorption band at 340 nm concurrently with an increase in the absorbance at 284 nm, which had isosbestic peaks at 325 and 360 nm. This was observed in conjunction with the increase in the concentration of Cu2+ ions. In the fluorescence spectra of the dyad lb, we observed a consistent drop in the intensity of FRET-mediated emission from the dansyl moiety at 525 nm. This decrease occurred in conjunction with an increase in the concentration of Cu2+ ions. Both of these observations are quite interesting. At the same time, we saw that the emission intensity of the naphthalimide chromophore, which has an isoemissive point at 450 nm, was simultaneously increasing at 375 nm. In addition, the addition of 20 11M of Cu2+ ions led to the total quenching of the FRET-mediated emission, which was accompanied by an increase in the fluorescence intensity ratio of Imllm that was approximately twelve times greater. According to the insets of Figure 4.8, the visual fluorescence ratiometric detection of Cu2+ ions by Ib was made possible by the considerable 'tum on' intensity that was accompanied by a blue shift of around 150 nm. There have been observations made that are comparable to those stated regarding the dyad la having a shorter spacer group. In this particular instance, we found that the injection of 20 11M of Cu2+ ions resulted in a fluorescence intensity ratio of b7s/Is2s that was approximately six times higher than before (Figure 4.6 and Figure 4.7). The dyad Ib was determined to be the more effective of the two systems that were investigated sensitivity for the selective identification of Cu2+ ions in comparison to ta as a result of the efficient FRET-mediated emission that was observed in the former situation. The fact that the addition of Cu2+ ions brought about a selective suppression of FRET-mediated emission at 525 nm alone is evidence that Cuz+ ions are capable of forming stable complexes with the dyads. As a consequence, the dansyl moiety becomes incapable of quenching the excited state of the naphthalimide chromophore leading in the return of blue naphthalimide emission hence enhancing the visual detection of cuz+ ions.

- **Stoichiometry of the complexation**

The Jobs plot for the complex formation was plotted and the binding constant was estimated using the Benesi-Hildebrand analysis. This was done in order to gain an understanding of the stoichiometry of the complex that was created between the dyads and the Cu2+ ions. According to the results of these tests, the complex that was generated between the dyad th and Cu2+ ions had a stoichiometry of 2:1. Furthermore, the association constant of (Kass) was found to be 2.6 ± 0.1 x 10s M-1, as depicted in. The curve fitting approach was used to perform additional calculations on the association constant. The binding constant that was acquired through this method is in accord with the value that was obtained through the Benesi-Hildebrand method. Additional calculations were performed. In accordance with the calculated molecular mass that corresponds to a 2:1 stoichiometric complex between the dyad th and Cu2+ ions, the MALDI-TOF mass spectrum analysis of this complex revealed a molecular mass of 1141.96.

Reversibility of the complexation

In order to gain an understanding of the reversibility of the complexation with Cu2+ ions, it was established that the utilisation of ethylenediaminetetraacetic acid (EDT A) was required. illustrates the changes that took place in the olorescence spectrum of the lb as a consequence of the addition of copper ions and EDTA in accordance with a certain order. In the beginning, the dyad displays fluorescence intensity that is in accordance with the dansyl and naphthalimide moiety this is the characteristic that appears. The presence of Cu2+ ions is responsible for the incorporation of the a decrease in the dansyl emission was detected in the fluorescence intensity, as demonstrated by the spectrum which indicates that the FRET was inhibited. In the case of these dyads, the metal ion binding event is reversible, as evidenced by the fact that the addition of EDTA to the complex causes the revival of both emission, as can be seen in the pictures This is an intriguing observation.

- **Nature of the complexation**

We have analysed the lH nuclear magnetic resonance (NMR) and Fourier transform infrared spectra (FTIR) of the dyads la and lb in the presence and absence of Cu2+ ions and compared them with the model compounds 2 and 3. This was done in order to gain an understanding of the nature of the complex that was produced, as well as the functional groups that were involved in the coordination. Taking the N-H proton of the dyad lb as an example, we observed a downfield shift of 118 = 0.02 ppm when Cu2+ions were added to the mixture. On the other hand, the peaks that corresponded to the aromatic protons exhibited significant broadening as well as considerable downfield shifts in the range of M= 0.03 - 0.07 ppm (Figure 4.12). In contrast, there were hardly any noticeable shifts recorded. N-methyl protons of the dansyl unit and methylene protons of the spacer group have different chemical shift values. The FTIR spectra of the dyad lb showed that the NH stretching frequency was present at 3284 cm•1 and the typical stretching frequencies of the sulphonamide and carbonyl groups were seen at 1323, 1654 and 1695 cm•1 (Figure 4.13). We found that the stretching frequencies of the NH and sulphonyl groups drastically decreased upon interaction with cuz+ ions, indicating that these functional groups are involved in the complexation with cuz+ ions.

We have examined the interactions with the sulphonamide group of the dyads 1a and 1b to confirm the role of this group in their selective complexation with Cu2+ ions. both compounds 2 and 3 are models. As is the situation with compounds 1a and 1b, compound 2 possesses both dialkylamino and sulphonamide groups. This is the case among the two model derivatives 1. The derivative 3, on the other hand, contains a sulphonate group in place of the sulphonamide functionality. This is in addition to the dialkylamino group that was present in the original structure. The absorbance at 286 nm was detected with isosbestic points at 265 and 318 nm, as shown in Figure 4.14. This was noticed with an increase in the addition of Cu2+ ions to a solution of the model chemical 2 at a concentration of 100 µM. We detected around fifty percent hyphochromicity in the absorption spectrum of the model compound 2 when the concentration of Cu2+ ions was 500 µM. With the addition of Cu2+ ions, we were able to observe a consistent and considerable suppression of fluorescence at 525 nm in the emission spectra of compound 2, as shown in the inset of Figure 4.13. The model compound 3, on the other hand, demonstrated minimal outcomes. Illustrates the variations that occur in the fluorescence and absorption spectra when the concentration of Cu2+ ions increases under the same conditions. These findings provide further evidence that the complexation that takes place between Cu2+ ions and the dyads 1a and 1b as well as the model compound 2 is owing to the involvement of the sulphonamide group of the dansyl chromophore. Furthermore, they eliminate the notion that the dialkylamino group was involved in the binding event.

- **Selectivity of the metal ion complexation**

Under the same conditions, we explored the interactions of the dyads la and lb with other significant monovalent and divalent metal ions, such as Na+, Li+, K+, Zn2+, Pb2+, Hg2+, Co2+, Fe2+, Cd2+, Mg2+ and Ba2+ ions, in order to demonstrate the selectivity of the dyads for Cu2+ ions. Figure 4. 16 shows the results of our investigation. The addition of these metal ions resulted in modest modifications to the fluorescence intensity of the dyad lb, as can be observed in Figure 4. 16; these variations were not particularly noticeable. In the presence of a number of other metal ions, observations of a similar nature have been made using the dyad named la. Because the green fluorescence intensity of the dyad lb did not change after the addition of these metal ions, it is possible to visually detect the selectivity of the dyad lb towards Cu2+ ions. This is because the dyad lb remained unaltered following the addition of Cu2+ ions.

4.8. DICUSSION:

In the ground state, the new dyads la and lb, which included a naphthalimide chromophore as the donor and a dansyl group as the acceptor moiety, exhibited insignificant interactions. However, in the excited state, they demonstrated an effective FRET reaction. Excitation spectrum analysis, observation of the spacer length dependent energy transfer and the use of literature reports were the methods that were utilised in order to acquire the evidence that such a reaction occurred. A further indication that such a reaction is extremely conceivable in these dyads is the presence of a suitable distance of 11.6 A between the donor and acceptor units in the case of the dyad la, which was characterised using all-encompassing X-ray crystal analysis. The emission spectrum of the dyads was found to have two emission maxima, one at 375 nm and the other at 525 nm. The latter band is a result of the FRET-mediated emission from the dansyl moiety, which is in conformity with the idea that was presented before. It is possible that the presence of a spacer with a suitable length for an effective overlap between the donor and acceptor groups is responsible for the detection of considerable FRET-mediated emission in the case of the dyad lb in comparison to the dyad ta. Based on the findings of the investigation into the interactions with different metal ions, it has been determined that the dyads la and th engage in selective interactions with Cu2+ ions in comparison to other monovalent and divalent metal ions. Such selective interactions with Cu2+ions result in the creation of 2:1 stoichiometric complexes with large association constants. The development of these complexes was validated using MALDI-TOF mass spectrum analysis. This is an intriguing phenomenon. Specifically, the force that is responsible for the selective complexation with Cu2+ ions is related to the fact that these dyads contain sulphonyl and NH groups in their composition. The participation of these groups in the complexation was demonstrated by means of absorption, fluorescence, single-photon nuclear magnetic resonance (lH NMR) and Fourier transform infrared (FTIR) spectral analysis. This was further validated by tests conducted using adequately substituted model compounds 2 and 3. As a result of the complexation of the dyads with Cu2+ ions that involve a sulphonamide group, the interaction between the naphthalimide and dansyl chromophores is altered, which results in the disruption of FRET. This, in turn, makes it possible to detect Cu2+ ions by visual fluorescence ratiometry.

4.9. CONCLUSION:

The present investigation successfully developed and characterized novel naphthalimide–dansyl dyads as selective and sensitive probes for Cu^{2+} ions. Spectral analyses confirmed the presence of FRET-mediated dual emissions, which were selectively quenched or enhanced upon Cu^{2+} binding. The dyads demonstrated 2:1 stoichiometry and significant association constants, with reversible binding behavior in the presence of EDTA. Functional group studies confirmed the involvement of sulfonamide and NH groups in metal ion complexation. Comparative analysis with model compounds validated these findings. The selectivity of these systems toward Cu^{2+} ions over other metal ions suggests strong application potential for environmental, biological and industrial monitoring. Future work could focus on biological imaging, photodynamic therapeutic agents and portable sensor design for on-site detection.

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