PROJECT REPORT

On

SYNTHESIS, CHARACTERISATION AND PHOTOPHYSICAL STUDIES OF 1,8-NAPHTHALIMIDE DERIVATIVES

Submitted by

ASWATHY ASHOK
(AM23CHE001)

In partial fulfillment for the award of the Post graduate Degree in Chemistry

DEPARTMENT OF CHEMISTRY
AND
CENTRE FOR RESEARCH

ST. TERESA'S COLLEGE (AUTONOMOUS)
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DECLARATION

I hereby declare that the project work entitled **"SYNTHESIS, CHARACTERISATION AND PHOTOPHYSICAL STUDIES OF 1,8-NAPHTHALIMIDE DERIVATIVES"** submitted to Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous) affiliated to Mahatma Gandhi University, Kottayam, Kerala is a record of an original work done by me under the guidance of **Dr. Elizabeth Kuruvilla, Assistant Professor** Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous), Ernakulam (Internal Guide). This project work is submitted in the partial fulfillment of the requirements for the award of the Degree of Master of Science in Chemistry.

ASWATHY ASHOK

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Chapter 1

Introduction

1.1 Fluorescence

For more than a century, physicists have studied about fluorescence, however the findings of these investigations have only slightly extended through biology and medicine, in part because of communication barriers and because of the compounds, temperatures, and solvents employed were not particularly interesting from a biological perspective(1).

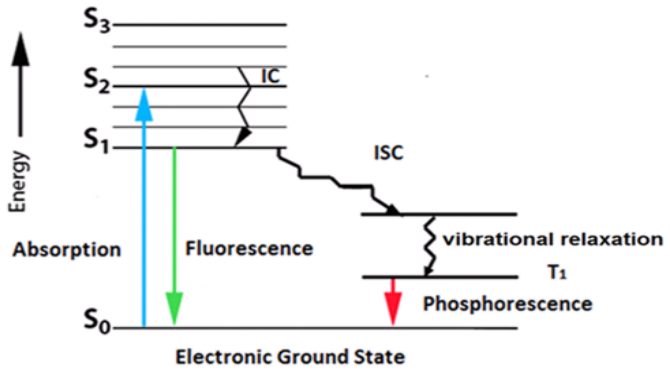


Fig 1.1 Jablonski diagram

The processes that underlie the absorption and emission of photons by a molecule involve transitions between its different electronic levels, where each level has various vibrational energy states and each vibrational state involves a collection of rotational levels(2).

Jablonski diagrams provide a clear representation of the electronic states and transitions of a molecule. It can be used to identify the key photophysical processes that occur in a molecule such as fluorescence and phosphorescence. It is also used to design new materials and devices with specific photophysical properties.

A Jablonski diagram typically consists of a series of horizontal lines each representing a different electronic state of the molecule. The lines are labeled with the energy of the state and the transitions between states are indicated by arrows. The lowest energy state of the molecule represented by the bottom line (S_0), higher energy states of the molecule represented by lines above the ground state (S_1, S_2, S_3 etc). States with a different spin multiplicity than the singlet states are triplet states presented as lines between the S_0 and S_1 states. Transitions from the ground state to higher energy states by the absorption of radiation is indicated by upward arrows. Non-radiative transitions between states of the same spin multiplicity is known as internal conversion (IC) and is indicated by wavy lines. Non-radiative transitions between states of different spin

multiplicity known as inter system crossing (ISC) is indicated by single waved line.

Transitions from higher energy states to lower energy states due to emission is indicated by downward arrows. A transition from the S_1 state to the S_0 state results in the emission of a photon known as fluorescence and a transition from the T_1 state to the S_0 state results in the emission of a photon is phosphorescence.

When a beam of light is incident on a certain substances they emit visible light or radiations known as fluorescence. Fluorescence starts immediately after the absorption of light and stops as soon as the incident light is cut off. The substances showing this phenomenon are known as fluorescent substances.

Fluorescent compounds have two characteristic spectra, an excitation spectrum (wavelength and amount of light absorbed) and an emission spectrum (wavelength and amount of light emitted). These spectra are often referred as a compound's fluorescence signature or fingerprint. No two compounds have same fluorescence signature. This makes fluorometry a highly specific analytical technique.

All molecules that absorb radiations will not show fluorescence rather organic molecules that have rigid framework and not many loosely coupled substituents through which vibronic energy can flow will show fluorescence.

1.2 Fluorescent Compounds

Substances exhibiting fluorescence are typically aromatic or have conjugated double bonds. A chemical is likely to exhibit fluorescence if it includes π-electrons. If a substituent is added to the compound which increases the freedom of these electrons, the substituted product will probably be more fluorescent than the unsubstituted parent compound. However, fluorescence will be reduced or eliminated if the substituent has a tendency to localize the π –electrons(1).

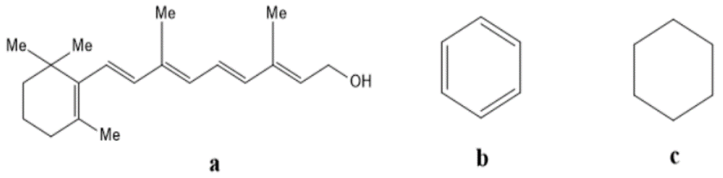


Fig 1.2 a. Vitamin A(non-fluorescent) b. Benzene(mild) c. Cyclohexane(fluorescent)

Let's use the straight forward examples of vitamin A, benzene and cyclohexane. Cyclohexane is non-fluorescent and free of conjugated double bonds. Benzene is a mildly luminous aromatic chemical. Despite not being aromatic, vitamin A is luminous due to its five conjugated double bonds.

Benzene, aniline, and nitrobenzene are examples of how substituents affect fluorescence. Benzene is a mildly luminous while nitro-benzene is not luminous, aniline is 40–50 times more fluorescent than benzene, in diluted solutions.

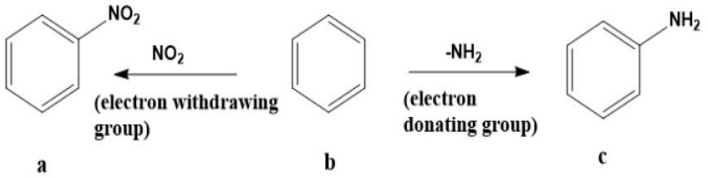


Fig 1.3 a. Nitro benzene(non-fluorescent) b. Benzene(mild) c. Aniline(fluorescent)

The NH_2 group in aniline tends to activate the benzene ring and thus increases the freedom of the π-electrons. The NO_2 group in nitro-benzene on the other hand deactivates the ring by tending to withdraw the π-electrons from the ring thus reducing their freedom by increasing their localization. Now the NH_2 group is one of the classical ortho-para directing groups and the NO_2 group is one of the classical meta-directing groups, so that one might conclude that mono-substituted benzene containing ortho-para directing groups are fluorescent whereas those containing meta-directing groups are non-fluorescent.

The impact of a single substituent on benzene fluorescence has been studied however, the influence of many substituents must now be taken into account, as some meta-directing groups typically eliminate benzene fluorescence. In contrast to ortho-para directing groups alone, which typically enhance benzene fluorescence, this combination may create more or less fluorescence. When the highly meta-directing nitro group is joined with an amino group as in p-nitro aniline, the nitro group's action is enough to render p-nitro aniline non-fluorescent. Nitro-benzene is also non-fluorescent. Therefore, the mobility of the pi-electrons is affected when many substituent's are present on a compound. When combined with strong ortho-para directing groups (NH_2, OH), weak meta-directing groups like SO_3H and SO_2NH_2 can frequently boost fluorescence by increasing the mobility of the π -electrons.

The nature of heteroatoms and the substituents will affect the fluorescence of heterocyclic systems. Examples include -N=, -NH, -O-, and -S-. By attracting the π-electrons to the ring, doubly bonded nitrogen tends to deactivate the ring in a heterocyclic system and are referred to as pi-deficient rings. Heterocyclic systems with nitrogen that is doubly bonded typically don't glow unless they have substituents that nullify the effect.

The electron-donating OH group in 3-hydroxypyridine causes it to be luminous, while pyridine is not.

a **b**

Fig 1.4 a. Pyridine(non-fluorescent)
b. 3-Hydroxy pyridine(fluoresent)

1.3 Factors affecting fluorescence

There are several factors that influence a compound's fluorescence intensity, but the most crucial ones are concentration, solvent, pH, temperature, and light stability.

1.3.1 Concentration

In quantitative work, the concentration of the substance to be assayed is crucial because the intensity of fluorescence is only proportional to the concentration of the fluorescent compound in extremely diluted solutions. A higher concentration of the molecule results in a larger percentage of the fluorescence that is reabsorbed. At high dilutions, where the number of molecules present is small enough to show the extent of re-absorption irrelevant in comparison to the amount of fluorescence emitted, linearity between fluorescence intensity and concentration may be predicted.

1.3.2 Effect of solvent

Fluorescence intensity and wavelength can be influenced by solvent changes however the results are frequently unanticipated. Three factors will be useful to take into account when talking about the solvent effect. Solvent purity, non-aqueous solvents, and aqueous solvents. Since fluorescence is a very sensitive method it is crucial that the solvents used are free of fluorescent contaminants and non-fluorescent themselves. Examples of solvents used in include ether, benzene, ethylene dichloride, hexane, heptane, and simple alcohols ranging from methanol to butanol.

1.3.3 The effect of pH

The impact of pH on a compound's fluorescence is significant and variations in fluorescence caused by medium pH changes can be advantageous in a number of ways.

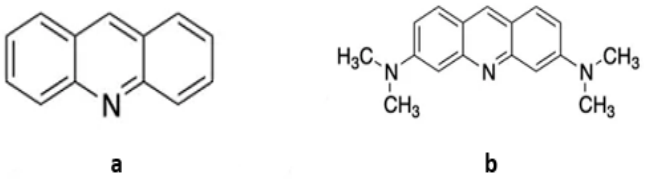
1.3.4 Temperature

When the temperature drops fluorescence intensity tends to peak and then drop to zero at high temperatures. Although it is unclear exactly how temperature affects fluorescence, it is likely related to how molecules flow through the medium. A higher temperature causes molecules to move more and increases the likelihood of collisions. Some of the energy that may have been emitted as fluorescence would be lost as a result. Temperature variations of a few degrees are almost insignificant, but one should be mindful of them since

certain chemicals exhibit extremely sensitive fluorescence intensity at different temperatures.

1.4 Fluorophore

Fluorophores are structures with chromophores (-N=N-, -C=O, -C=C-…) and they are the part of molecules with conjugated systems of double bonds. Fluorochromes are substituents that enhances fluorescence. They are electron donors such as –OH, NH_2 etc which enhances the transition probability or intensity of colour.



a b

Fig 1.5 a. Acridine orange (non-fluorescent)
b. Acridine orange (fluorescent)

The fluorescein isothiocyanate (FITC) is a widely used fluorophores. Additional fluorophores include coumarin, cyanine, 1,8-naphthalimide, and rhodamine derivatives.

1.4.1 Fluorescein isothiocyanate (FITC)

The most used fluorescent probe for creating conjugates with biological molecules is FITC. It's water solubility makes conjugates easy to prepare and reasonably large extinction coefficients, high quantum yields after conjugation make it brightly fluorescent, and its low nonspecific binding with the majority of biological tissues make this xanthene dye especially helpful(3).

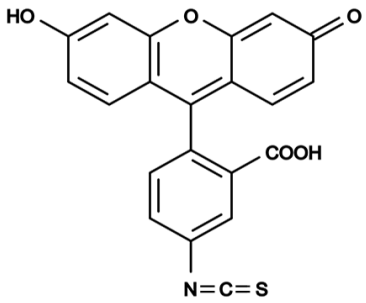


Fig 1.6 Fluorescein isothiocyanate (FITC)

1.4.2 Rhodamine

Rhodamine is a yellow anthracene tricyclic fluorescent dye with red to brown colour. It has a large conjugated structure with non-toxicity, good biocompatibility, high fluorescence quantum yield, good light stability, and long excitation and emission wavelength. Therefore, it is often used as fluorescent probes(5).

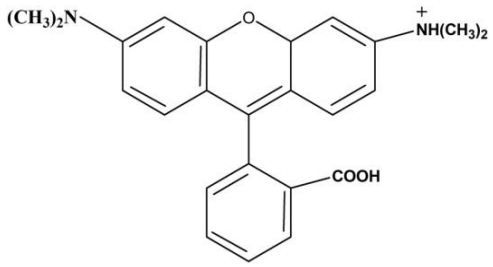


Fig 1.7 Rhodamine B

1.4.3 Coumarin

Benzopyran-2-one, another name for coumarin, is a common natural compound. It has a range of biological actions and is often linked to low toxicity(6). It is a member of the flavonoid class of secondary metabolites found in plants(7). Although coumarin is found in many plant parts, it is most concentrated in fruits, seeds, roots, and leaves. Because of their chelation, hydrophobicity, and extreme size variability, coumarin derivatives can be employed as fluorescent probes for metal ions. Numerous industries, including medicine and environmental protection, have made extensive use of coumarin fluorescence probes(6).

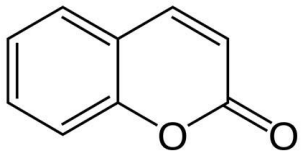


Fig 1.8 coumarin

1.4.4 Cyanine

Cyanines containing reactive functional groups can be made with absorption maxima ranging from < 500 nm to > 750 nm. This property opens additional regions of the spectrum for experiments involving the simultaneous multi-color analysis of different fluorescent probes(8). They are mainly cationic and tend to localize in the mitochondria of cancer cells.

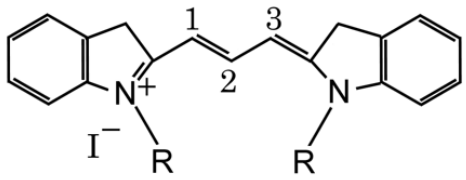


Fig 1.9 Cyanine-3

1.5 Fluorometer

Fluorescence is measurable by fluorometers. A fluorometer is an instrument designed to measure the different limits of fluorescence including its strength and wavelength distribution of the emission after excitation. Chemists use this to identify properties and the amount of particular molecules in a sample. Fluorometry is normally more sensitive than absorbance measurements(9). It is a widely accepted and powerful way of doing things that is used for a variety of environmental, industrial, medical, DNA quantitative and qualitative analysis, and biological uses.

There are two main types of fluorometers, filter fluorometers and spectro fluorometers.

1.5.1 Filter fluorometers

In filter fluorometers it uses filters to select wavelengths. They are often cheaper to build or buy, but they are less sensitive and have less ability to measure very small things than spectro fluorometers. Filter fluorometers can only operate at the wavelengths of the available filters(10).

1.5.2 Spectrofluorometers

A spectrophotometer is an instrument containing a monochromator, a device which produces a light beam containing wavelengths in a narrow band around a selected wavelength and a means of measuring the ratio of that beam's intensity(11).

1.6 Applications of Fluorescence

1.6.1 Lighting

The term mercury vapor discharge lamp is another name for a fluorescent lamp. As the name suggests a tiny amount of gas (mercury) is contained within the tube. It electrifies the gas as an electric current flows through it. Consequently, the tube’s atoms lose electrons, releasing photons that are primarily in the ultraviolet spectrum. The tube’s interior contains phosphor, which glows in the visible spectrum of light while absorbing ultraviolet light. Energy-efficient fluorescent lamps outperform filament lamps. The color

produced by the lamp, however, might differ significantly from that produced by daylight or incandescent light bulbs, according to a number of complications. The mercury vapor light spectrum does, however, have a distinctive line that is always centered at 254 nm. Other lines are centered at 436 nm, green at 546 nm, and yellow-orange at 579 nm. When looking through a hand spectroscope, these three lines appear in the white background color when white fluorescent lamps are being used. Together with trivalent europium and trivalent terbium emission lines, as well as the emission continua of divalent europium in the blue region, the same visible lines comprise the less continuous light emission in the contemporary trichromatic phosphor systems used in a variety of compact fluorescent spectrometers(12).

1.6.2 Spectroscopy

Typically, the setup of a fluorescence assay involves a light source that can emit many different wavelengths of light. Generally, a single wavelength is required for proper analysis. To selectively filter the light, it is passed through an excitation monochromator and then the selected wavelength is passed through the sample cell. After the energy is absorbed and re-emitted, many wavelengths can be created due to the Stokes shift and various electron transitions. To separate and analyze them the fluorescence radiation is passed through an emission monochromator and selectively observed by a detector(13).

1.6.3 Lasers

Lasers most often use the fluorescence of certain materials as an active medium, such as the red glow of a ruby (chrome sapphire), the infrared light of titanium sapphire, or the unlimited color range of organic dyes. These materials typically fluoresce through a process called spontaneous emission, in which the light is emitted in all directions and often on many discrete spectral lines at the same time. In many lasers, the fluorescent medium is "pumped" by exposing it to an intense light source, creating a population inversion, meaning more of its atoms go to an excited state (high energy) rather than the ground state (low energy). When this happens, the spontaneous fluorescence can cause the other atoms to emit their photons in the same direction and at the same wavelength creating stimulated emission. If some of the spontaneous fluorescence is trapped between two mirrors, almost all of the medium's fluorescence can be excited to emit along the same line creating a laser beam(14).

1.6.4 Biochemistry and medicine

Fluorescence is generally used in life sciences as a non-destructive method for tracking or analyzing biological molecules using fluorescence emission at a specific frequency where there is no background from the excitation light because relatively few cell components naturally fluoresce (so-called intrinsic or autofluorescence)(15). In fact, a protein or other component can be "tagged" with an

extrinsic fluorophore, a fluorescent dye that can be a small molecule, a protein, or a quantum dot, which has wide use in many biological applications(13).

1.6.5 Signage

Fluorescent colors are often used in signage, particularly traffic signs. Fluorescent colors are generally detectable from greater distances than their non-fluorescent counterparts with fluorescent orange being particularly noticeable. This property has led to its frequent use in safety signs and labels(16).

1.6.6 Optical brighteners

Chemical substances known as optical brighteners making up the lack of blue and purple light reflected by the material with the fluorophore's blue and purple optical emission, are frequently used to improve the color of fabric and paper, creating a "whitening" effect that makes naturally yellow or orange materials appear less. Cosmetics, high-visibility clothing, laundry detergent and glossy paper are just a few products that use optical brighteners(17).

1.7 1, 8 - Naphthalimide

In recent years, considerable efforts have been paid to develop 1,8-naphthalimide derivatives as fluorescent probes, fluorescent dyes, gene vectors, and anticancer agents(18). 1,8-Naphthalimide-based fluorescence probes have been widely used for sensing cations (Cu^{2+}, Zn^{2+}, Hg^{2+}, Ag^{+}, and

Pb^{2+})(19) (20), anions (F^-, CN^-, AcO^-, and PO_3^{-4}) (21)(22), and biomolecules (ATP, ADP, amino acid, and protein)(23). Many excellent examples of 1,8-naphthalimide-based probes have been reported, and some of them have been successfully applied in live-cell imaging research(24). Furthermore, it is well-known that development of safe and efficient gene vectors is important to gene therapy(25). Organic functional molecule as a new type of non-viral vector has received more and more attention because of its easy preparation, low immunogenicity, and good biodegradability(26). 1,8-naphthalimide-based functional molecules not only exhibit high transfection efficiency but also can be applied in real-time fluorescence tracking, which makes it possible to study the mechanism of gene delivery(27). It is thus no surprise that the 1,8-naphthalimide structure has made rapid development in applications for non-viral vectors, fluorescence probes, and anticancer agents in recent years(18).

The optical and photophysical properties of 1,8-naphthalimides are very sensitive to any changes in the chemical structure of the aromatic ring via the addition of substituents(28). In particular, the introduction of amines to the 1,8-naphthalic-anhydride core has been extensively explored because the proton input results in(29) alteration of fluorescence properties. In addition to chemical modifications, the emission spectra of many fluorophores are sensitive to the polarity of the surrounding environment (solvent effects) (30). This phenomenon also affects their

ability to switch fluorescence and the overall magnitude of fluorescence enhancement or quenching that can be observed (31).

The compound 3-Amino-1, 8-naphthalimide has many uses, including as a fluorescent probe in biological research, protein labeling, DNA detection, and cell imaging. It also serves as a precursor for the production of pigments and dyes, such as photographic, laser, and fluorescent dyes(32). Derivatives of 3-amino-1, 8-naphthalimide have also been studied for possible medicinal uses, such as anti-inflammatory, antiviral, and anticancer properties. Additionally, the compound finds application in the development of fluorescent materials, polymer chemistry, optoelectronic devices, and sensors and detection systems for metal ions, anions, and small molecules(33).

1.8 Applications

1.8.1 Fluorescent probes

1,8-Naphthalimide derivatives are used as fluorescent probes in biological research such as cell imaging, protein labeling, DNA detection. selective and sensitive detection of peroxynitrite in living cell imaging (34) fluorescent sensors for enzymes (35) two proton–receptor fluorescent probes (36).

1.8.2 Dyes and pigments

1.8 – Naphthalimide is used as a precursor for the synthesis of dyes and pigments including fluorescent dyes, laser dyes, photodyes. Fluorescent Dispersed Dyes for Cotton Fibers(37) potential for light-harvesting (38) azo dyestuffs for the dyeing of polyester fibres(39).

1.8.3 Pharmaceuticals

1,8-Naphthalimide has been studied for its potential therapeutic applications including anticancer agents, antivirals, and anti-inflammatory agents. Antibacterial Activity of Several New 3,4-Dimethyl Maleimides (40) DNA targeting binders, anticancer and fluorescent cellular imaging agents (41) as potential anticancer agents(42).

1.8.4 Materials Science

1,8-Naphthalimide is used in the development of fluorescent materials, optoelectronic devices, polymers chemistry, metal ions, anions, small molecules bioimaging containing different chromophores(43). Organic dyes based optical and electrical devices (44), highly emissive luminophors with various mechanofluorochromism and aggregation-induced Characteristics(45).

1.8.5 Biotechnology

1,8-Naphthalimide is used in biotechnological applications such as gene expression analysis, protein purification, and cell sorting. Antitumor Activity (46) modified

Polyamidoamine Dendrimer as Fluorescent Disperse Dye (47).

1.8.6 Biosensing

Molecular entities or devices that enable biosensing are generally referred to as biosensors. The primary challenge of creating biosensors is transducing the nanometer-scale event of a bio-recognition process into an observable change in a macroscopic property such as color or fluorescence hue(48). The first biosensor was reported by Clark in 1956. (49). IUPAC defined a biosensor as a specific type of chemical sensor comprising a biological or biologically derived recognition element either integrated within or intimately associated with a physicochemical transducer (48).

Biosensors based on fluorescence have many advantages such as simple operation, fast response, simple instrument, multiple analysis, high sensitivity, and good selectivity. In most fluorescent biosensors, the fluorescence intensity is recorded as the readout signal for the analytes. In order to obtain better performance of the fluorescent biosensors, several parameters, including the analyte recognition units, signal transducers, and fluorescent tags should be considered carefully. To improve the fluorescence signals of biosensors, a brighter fluorescent tag and signal amplification technology are usually applied during the construction(50).

Chapter 2

Literature Survey

1. Synthesis, characterization, Photophysical and biological properties Of novel antimicrobial fluorescent naphthalimide derivatives was investigated by H. Shaki1, A.Khosravi1, K.Gharanjig and A.Mahboubi. This paper presents a series of novel naphthalimide dyes incorporating sulfonamide heterocycles. The dyes and their intermediates were characterized using various methods, including DSC, FTIR, elemental analysis, TLC, 'H-NMR, 1C-NMR, LC-mass spectroscopy, UV-Vis, and fluorimetry. The dyes were tested for their antibacterial and antifungal properties against E. coli, M. luteus, B. subtilis, B. cereus, and C. albicans. The compounds showed higher antimicrobial properties against gram-positive bacteria than gram-negative bacteria. Dye 10 had the most significant antimicrobial activity against bacteria. A series of 4-amino-N-substituted-1,8-naphthalimide and 4-allylamino-N-substituted-1,8-naphthalimide derivatives were synthesized from intermediate 4-nitro-1,8-naphthalimide. The dyes exhibited fluorescent emission and biological properties due to the presence of sulfonamide groups in their structure. The photophysical characteristics of the dyes were also assessed,

with Stokes shift values ranging between 3860 and 4469 cm−1(51).

2. Synthesis and spectroscopic studies of some naphthalimide based disperse azo dyestuffs for the dyeing of polyester fibres investigated by A. Khosravi a, S. Moradian a, K. Gharanjig b, F. Afshar Taromi. A series of naphthalimide derivatives were prepared, including 4-nitro-1,8-naphthalic anhydride, which can be converted into various derivatives through nucleophilic substitution with different amines. The solvent plays a crucial role in these substitution reactions. Naphthalimides with different substituents in the 9-position have also been prepared. Naphthalimides with different substituents in the 9-position have been prepared. The use of N-substituted naphthalimide as an intermediate for monoazo disperse dyestuffs was found to be suitable for dyeing polyester. The dyestuffs were identified using various techniques, including elemental analysis, DSC, FTIR, H NMR, and UV-visible spectroscopic techniques. The spectrophotometric properties of the dyestuffs were also examined. The dyestuffs were tested on polyester fabrics, revealing good optical and dyeing properties, light fastness, wash fastness, and sublimation fastness(39).

3. Synthesis and characterization of naphthalimide-based dyes for dye Sensitized solarcells studied by Ankita Saini, K. R. Justin Thomas, Yi-June Huang, Kuo-Chuan Ho. In this

study a set of D–A–π–A dyes with a carbazole donor, naphthalimide auxiliary acceptor, and spacers has been synthesized and characterized. The spacers affect the optical and photovoltaic properties, with naphthalimide and cyanoacrylic acid having a significant effect on absorption. The dyes have high-lying LUMO and low-lying HOMO, facilitating electron injection and regeneration(52).

4. Synthesis and Application of Some Alkali-clearable Azo Disperse Dyes Based on Naphthalimide Derivatives investigated by Parvizi, A. Khosravi, S. Moradiana and K. Gharanjig. In this paper a series of naphthalimide-based alkali-clearing azo disperse dyes with a fluorosulfonyl group has been prepared using diazo components and coupling components. The dyes were synthesized from N-acetylsulfanilyl chloride and characterized using various techniques. The dyes were then dyed on polyester fabrics to investigate their dyeing properties and color gamut. The aim was to convert the dyes to water-soluble sulfonate dyes without breaking the azo bond. The fluorosulfonyl group in these dyes offers desirable build-up, excellent wash fastness, and the selection of alkali clearance for high wash fastness(53).

5. Synthesis and Properties of Some Fluorescent 1,8-Naphthalimide Derivatives and Their Copolymers with Methyl Methacrylate investigated by T.N. Konstantinova, P. M. Miladinova. This study identifies six compounds,

including three dyes and three FWAs with a TMP stabilizer fragment, which can co-polymerize with MMA to create self-colored or whiten polymers with intense fluorescence. These compounds can dye or whiten textiles with good color characteristics and fluorescence. They have a good stabilizing effect on photodegradation of PMMA, especially those with a TMP fragment and are suitable for one-stemer whitening and stabilization of PMMA (54).

6. Synthesis and Investigation of Derivatives of 1,8-Naphthalimide With a Red Emission via an Aromatic Nucleophilic Substitution

   Reaction studied by Chul-Hyun Jeong, Aatiya Ahmad , Hannah C. Schmitz, Haishi Cao. Four novel Nis derivatives (1-4) were synthesized and studied for their photophysical properties in six solvents. These derivatives showed a long absorption and emission wavelength, potentially addressing autofluorescence issues in biological applications. They also showed high solubility in water, enabling direct use in bio-samples without cosolvent interference. This research offers a new approach to developing Nis derivatives for biological applications (55).

7. Blue organic light emitting materials: Synthesis and characterization Of novel 1,8-naphthalimide derivatives synthesized by Hidayath Ulla, B.Garudachari, M.N. Satyanarayan, G. Umesh, A.M. Isloor. A series of 1,8-naphthalimides were synthesized with high electron affinity for blue light emission. Optical, thermal, and electrochemical studies were conducted on the naphthalimides. Results showed broad-bands with red shifts in UV-Vis absorption spectra, excellent PL properties, high melting points, and good thermal stabilities. The naphthalimides also exhibited low-lying energy levels, making them promising candidates for non-doping blue emitters with electron-transport/hole-blocking properties for OLED applications(56).

8. Naphthalimide-Dyes Bearing Phosphine and Phosphorylamide Moieties: Synthesis and Optical Properties studied by Massimo Tosolini, Chiara Alberoni, Mani Outis, António Jorge Parola, Barbara Milani, Paolo Tecilla, and João Avó. The study focuses on the preparation of mono- and di-phosphinated 1,8-naphthalimide dyes, phosphino/amino mixed derivatives, and their corresponding Pd(II) complexes. The optical properties of these compounds were assessed using absorption and emission spectroscopy. The electron-donating character of phosphorus-based groups was found to be lower than nitrogen-bearing counterparts, and the presence of a phosphine moiety led to unusually low PLQY values. The

Pd(II) complexes did not show luminescence in solution, but a novel 1,8-naphthalimide dye with a cyclic phosphoryl amide moiety was formed due to the deprotonation of the highly acidic secondary amine moiety in complex 6. This dye showed a long emission lifetime, showing potential for fluorescence imaging(57).

9. Synthesis, Characterization, and Photo-Physical Properties of Dendrimers Modified With 1,8-Naphthalimide Derivatives as Novel Fluorescent pH Sensors investigated by Mohammad Dodangeh, Kamaladin Gharanjig, and Mokhtar Aram. In this paper two new 4-(amino and acetylamino)-N-PAMAM-1,8-naphthalimide were synthesized and studied for their photo-physical properties in organic solvents. The fluorescence and absorbance spectrophotometric properties showed that the solvent and substituents in the 4-position were crucial. The fluorescence quantum yields were low in 1,4-dioxan but enhanced in DMF. The solvent effect on fluorescence intensity is complex and depends on the substituent's nature. The compounds A2 and A3 have potential as fluorescence(58).

10. Synthesis and spectral properties of fluorescent dyes Based on 4-styryl-1,8-naphthalimide studied by Pavel A. Panchenko, A. N. Arkhipova, Marina A. Zakharko, Gediminas Jonusauskas, Yuri V. Fedorov, Olga A. Fedorova. The paper presents a study on the synthesis and spectroscopic analysis of novel N-butyl-4-styryl-1,8-

naphthalimide dyes. The compounds exhibit positive solvatochromism, high Stokes shift values in polar solvents, and fluorescence in the visible range. These findings suggest potential applications as fluorescent dyes in biochemistry. The Lippert-Mataga equation was used to estimate the changes in dipole moments caused by excitation, with compound 3 suggesting twisted states with charge transfer(59).

11. Synthesis and photochemical properties of novel 4-diarylamine-1,8-naphthalimid derivative studied by Wei Jiang, Yueming Sun, Xiaoliang Wang, Qi Wang, Wenlian Xu. This study involving diarylamine-substituted 1,8-naphthalimide derivatives was conducted, revealing high thermal stability and asymmetry in the compounds. The compounds were characterized using FT-IR, 1H, 13C NMR, mass spectra, and elemental analyses. UV-visible absorption and photoluminescent spectra were investigated in n-hexane, tetrahydrofuran (THF), and CH2Cl2. The lowest absorption band, centered at z450 nm, was assigned to the charge-transfer transition in nonpolar solvents and 578-624 nm in polar solvents(60).

Chapter 3

Materials and Methods

3.1 Materials

1,8-naphthalic anhydride was obtained from Sigma-Aldrich. Sodium nitrate, concentrated sulphuric acid, aniline, 2-hydroxy aniline, stannous chloride, aqueous sodium bicarbonate, sodium chloride, charcoal, sodium sulphate, salicylaldehyde, sodium hydroxide, ethyl acetate, are provided from nice chemicals(p) Ltd, in Kochi. Ethanol was provided from Changshu Hongsheng Fine Chemicals Co.Ltd.

3.2 Methods

3.2.1 3-Nitro-1, 8-naphthalic anhydride

Nitration of 1,8-naphthalic anhydride was carried out by adding sodium nitrate (2.60 g, 30 mmole) portion wise to a solution of 1, 8-naphthalic anhydride (6.00 g, 30 mmole) in 60 ml of the concentrated sulfuric acid in a 500 ml beaker. The reaction mixture was heated for 2 hr on a water bath, then poured onto ice. The precipitate formed was filtered, washed with water and dried at 110°C. The crude

product was purified by crystallization from acetic acid to obtain **1** as a yellow solid (61) . M.P, – 242-244°C.

Fig 3.1 3-Nitro-1,8-naphthalic anhydride

3.2.2 N-phenyl-3-nitro-1, 8-naphthalimide

3-nitro-1, 8-naphthalic anhydride (1.944 g, 8 mmole) and 50 ml of ethanol were introduced into a round bottomed flask. The resulting suspension was heated to reflux, and aniline (3 ml, 16 mmol) was added. After 2 hr the mixture was cooled, filtered, washed with ethanol and air-dried to obtain **2** as light peach colored solid. M.P – 132 °C.

Fig 3.2 N-phenyl-3-nitro-1,8-naphthalimide

3.2.3 N-phenyl-3-amino-1,8-naphthalimide

N-Phenyl-3-nitro-1,8-naphthalimide, **2** was reduced by refluxing 3.34g, of **2** with 22.55g, 0.1 mmole of $SnCl_2.2H_2O$ in 40 ml of absolute ethanol in a round bottomed flask for 30 minutes. After 30 min the solution is allowed to cool down and then poured into ice. The pH is made slightly basic (pH 7-8) by the addition of 5% aqueous sodium bicarbonate before being extracted with ethyl acetate. The organic phase is thoroughly washed with brine, treated with charcoal and dried over sodium sulphate, Evaporation of the solvent leaves **3** as orange crystals(62).

Fig 3.3 N-phenyl-3-amino-1,8-naphthalimide

Fig 3.4 N-phenyl-3-amino-1,8-naphthalimide (Fluorescence)

IR: 3448.55, 3356.75, 3061.33, 2919.49, 2849.51, 1652.99, 1625.98, 1625.59, 1595.16, 1577, 1315.40, 1306.12, 1196.62, 1140.92, 872.68, 817.12, 778.98, 646.84, 509.40 cm^{-1}.

^{1}H NMR :δ8.051, δ8.046, δ8.028, δ7.947, δ7.942, δ7.601, δ7.584, δ7.499, δ 7.481, δ7.432, δ7.330, δ 7.312, δ 7.293, δ 7.287, δ 5.990, δ2.461.

^{13}C NMR :δ164.698, δ164.247, δ 148.570, δ 136.520, δ 134.062, δ 132.164, δ 129.687, δ129.376, δ 128.609, δ 127.493, δ 125.964, δ 122.292, δ 112.33.

3.3 Characterization Techniques

3.3.1 FT-IR Spectroscopy

The IR spectra were recorded on a Thermo Scientific Nicolet 12A0712 iS5 FT-IR spectrometer, within the extend 4000-500cm^{-1} utilizing KBr pellets at the Centralized instrumentation facility Bharat-Mata College, Thrikkakara, Ernakulam.

3.3.2 ^{1}H and ^{13}C NMR Spectroscopy

NMR spectra were recorded utilizing DMSO as solvent on JEOL (JEM-ECZ400S) NMR Spectrometer at Centralized Common Instrumentation Facility (CCIF) NMR Lab, Thycaud, Thiruvananthapuram.

3.3.3 UV- Visible Spectroscopy

UV-Vis spectroscopy measures the absorption of ultraviolet and visible light by samples. It is based on the absorption of the electromagnetic radiation in UV/Vis region, with the wavelength ranges of 200–400 nm, called 'ultraviolet spectroscopy,' and 400–800 nm, called 'visible spectroscopy. When used qualitatively the absorption spectrum reveals information about the molecular structures. When used quantitatively it determines the analyte concentrations. We have recorded the absorbtion spectrum from TICC, at St.Teresas College, Ernakulam.

3.3.4 Fluorescence Spectroscopy

Fluorescence spectroscopy is a technique that measures the fluorescence of a substance when exposed to light. It is used to analyze the chemical composition of a sample and its measurements are categorized as either emission or excitation. Fluorescence measurements were taken in TICC, at St.Teresas College, Ernakulam.

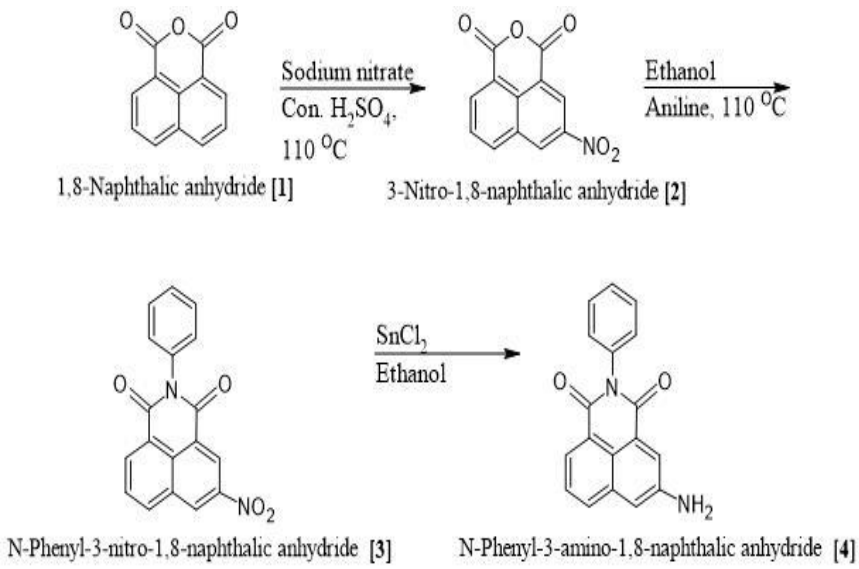
Chapter 4

Results and discussion

4.1 Characterization N-phenyl-3-amino-1,8-naphthalimide

N-Phenyl-3-amino-1,8-naphthalimide **4** was synthesized starting from 1,8-naphthalic anhydride **1** which was first nitrated to give the 3-nitro-1,8-naphthalic anhydride **2** in 90% yield. Compound **2** was treated with aniline to give the N-phenyl-3-nitro-1,8-naphthalimide **3** in 60% yield which was subsequently reduced with Sn/HCl to yield, N-phenyl-3- amino-1,8-naphthalimide **4** in 40 % yield.

Compound **4** was purified by recrystallisation from ethanol. The purified compound was characterized using IR and NMR spectroscopy.



1,8-Naphthalic anhydride **[1]**

3-Nitro-1,8-naphthalic anhydride **[2]**

N-Phenyl-3-nitro-1,8-naphthalic anhydride **[3]**

N-Phenyl-3-amino-1,8-naphthalic anhydride **[4]**

Scheme 1. Schematic representation of the synthesis of compound 4

4.1.1 FT-IR

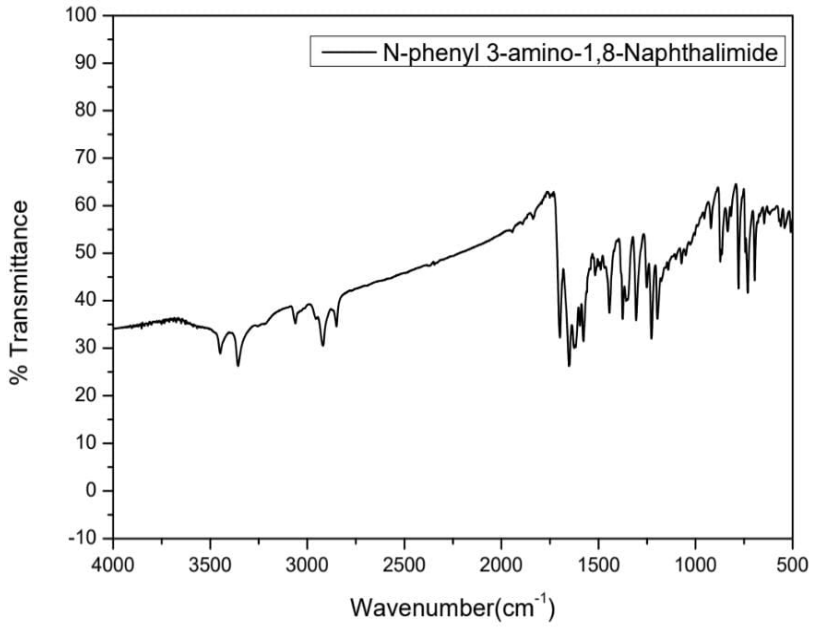


Fig 4.1 IR-spectrum of compound 4

The FT-IR spectrum shows two peaks of medium intensity at 3356 cm^{-1} and 3449 cm^{-1}, which corresponds to the symmetric and asymmetric N-H stretch. The peaks at 2850, 2919 and 3060 cm^{-1} is due to the aromatic C-H stretch. The peaks in the 1625 -1577 cm^{-1} is due to the C-C stretch of the aromatic ring. The peaks from 650-900 cm^{-1} can be attributed to the out of plane bending of the aromatic C-H. The carbonyl peak of the naphthalimide comes at 1653 cm^{-1} which is shifted to lower wave number due to the conjugation possible with the nitrogen and aromatic ring. The peak at 1190 cm^{-1} is attributed to the C-N stretch.

4.1.2 ^{1}H-NMR

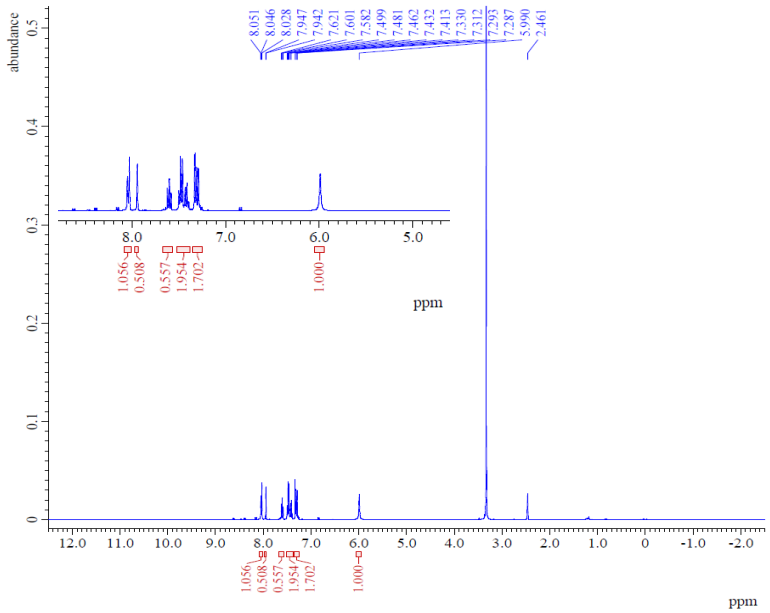


Fig 4.2 ^{1}H NMR of compound 4

The ^{1}H NMR of compound 4 in DMSO - d^{6}, is shown in Figure 4.2. The -NH_2 protons appeared as a slightly broad peak at δ 5.99 ppm. The phenyl and napthyl protons come in the region from δ 7.287 to δ 8.051 ppm as multiplets.

4.1.3 ^{13}C NMR

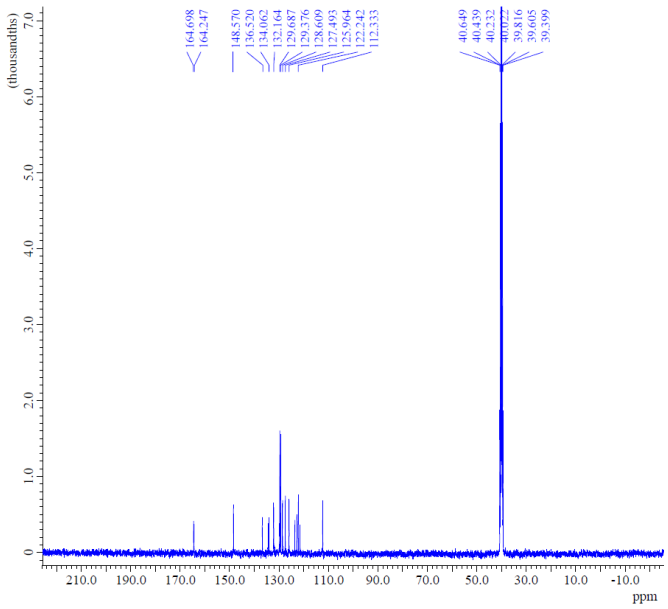


Fig 4.3 ^{13}C NMR of compound 4

The ^{13}C NMR (Fig 4.3) gives peaks corresponding to the two carbonyl at δ 164.69 and δ 164.24 ppm. The carbon attached to the imide-N appears at around δ 148.57 ppm. The carbon attached to the -NH_2 comes at around 136.52 due to deshielding. The phenyl and the naphthalene ring protons comes in the range 112.3 – 134 ppm.

4.1.4 Absorption and fluorescence properties

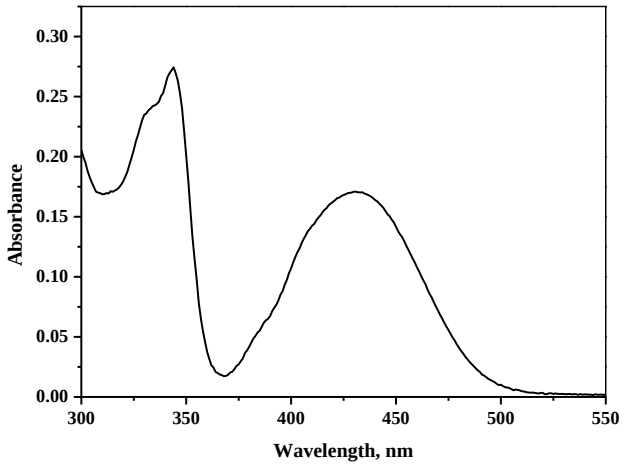


Fig 4.4 UV-visible spectrum of compound 4

Figure 4.4 shows the absorption spectrum of compound **4** in ethanol. The absorption extends upto 500 nm with two bands with λ_{max} at around 345 nm and 430 nm.

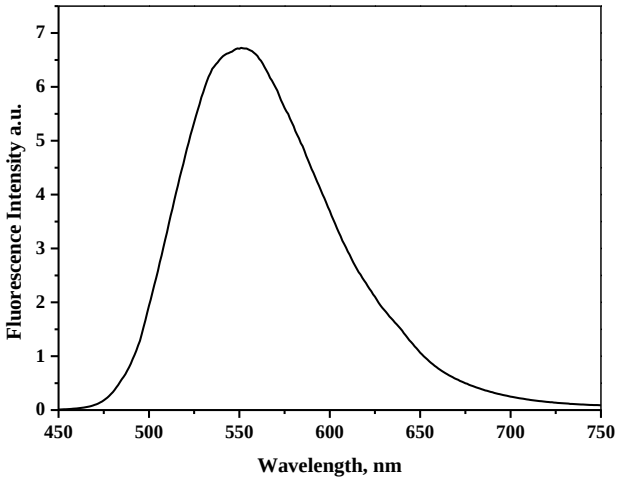


Fig 4.5 Emission spectrum of compound 4

The fluorescence emission spectrum of **4** in ethanol is shown in Fig 4.5. The compound **4** was excited at 430 nm. The fluorescence emission spectrum was observed from 450 – 740 nm with maximum emission intensity at 550 nm.

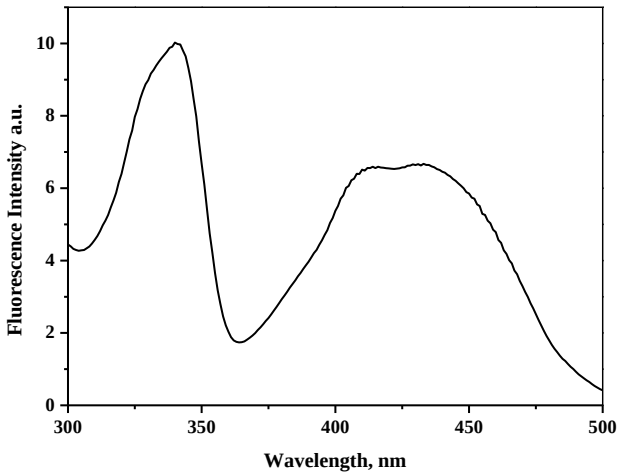


Fig 4.6 Excitation spectrum of compound 4

Figure 4.6 shows the excitation spectrum of **4** recorded by monitoring the emission at 550 nm. The excitation spectrum matches the absorption spectrum indicating that the molecule follows Kasha's rule which states that the emission wavelength is independent of excitation wavelength.

4.2 Solvent dependent absorption and fluorescence properties

The solvent dependent absorption and fluorescence changes are shown in figure 4.7 and 4.8 respectively. The spectrum were recorded in ethanol,acetone and chloroform. Ethanol is more polar than acetone followed by chloroform.

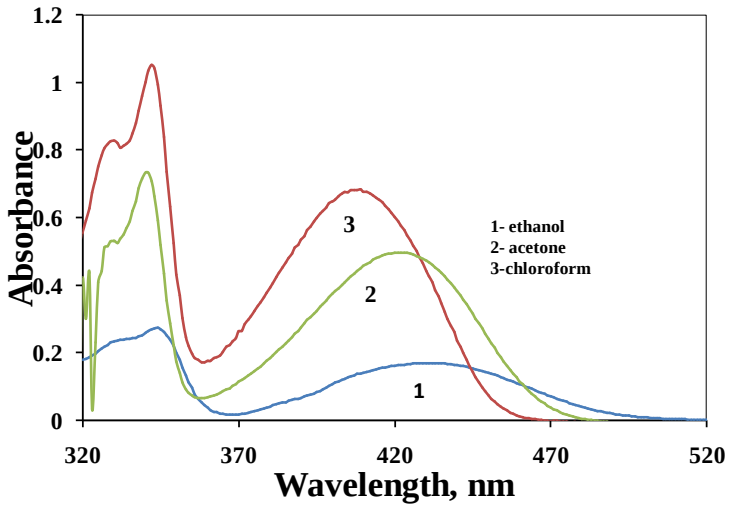


Fig 4.7 Absorbance of compound 4 in ethanol, acetone, and chloroform

The compound showed two absorption maxima, one in the range 330-350 nm and comparitively lower intensity band in the range 400-450 nm. The higher energy band in 330 nm is attributed to the transition π-π* while the longer wavelength absorption is due to the intramolecular charge transfer (ICT) as the presence of electron donation amino group and electron deficient imide unit makes the naphthalimide derivative a push-pull type of fluorophore. On

excitation the ICT process results in a large excited state dipole which is affected by the solvent polarity. From figure 4.6, it can be seen that with increasing solvent polarity the long wavelength absorption maximum showed a red shift. The π-π* band doesn't show any significant change in increasing solvent polarity. Also a broadening of the ICT band is observed.

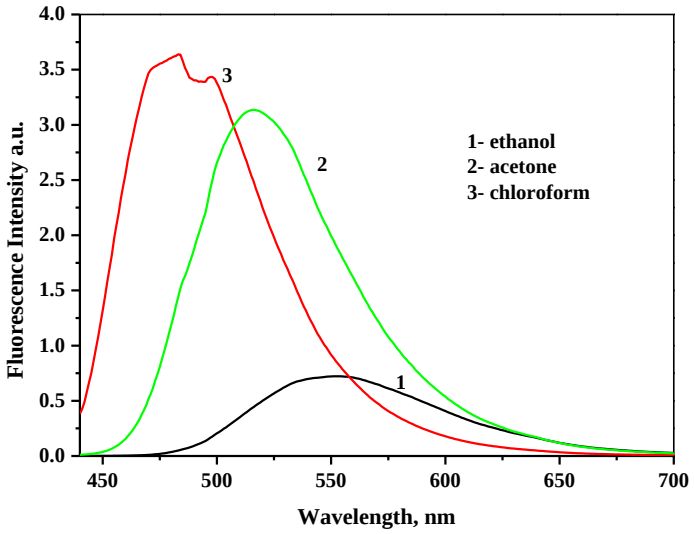


Fig 4.8 Emission of compound 4 in ethanol, acetone and chloroform

Figure 4.8 shows the changes in the emission spectrum of 4 with increasing solvent polarity. The emission maximum shifts to longer wavelength with spectral broadening. An increased stock shift is also observed with increasing solvent polarity. A stocks shift of 70 nm, 98 nm and 116 nm was observed in chloroform, acetone and ethanol

respectively. This shows that the excited state is stabilized in a polar medium.

4.3 Calculation of Molar extinction coefficient

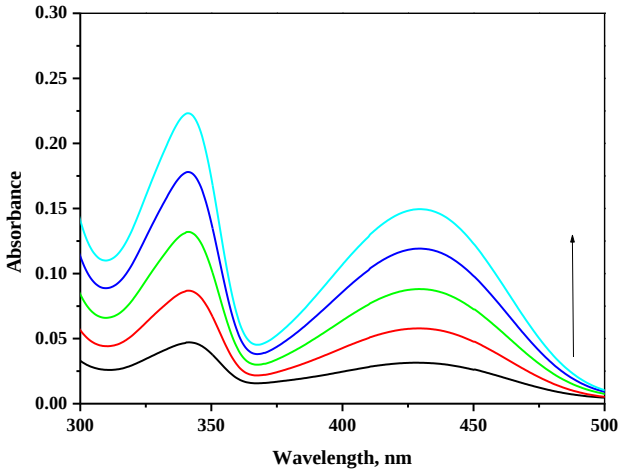


Fig 4.9 Molar Extinction Coefficient plot

In order to find out the molar extinction coefficient of 4, a series dilution of the compound was done in ethanol. Absorption spectrum at each dilution was measured and the absorbance at 341 nm and 429 nm was used to calculate the Molar extinction coefficient (λ max) using the Beer-Lambert equation.

$$A = \text{Ɛ}Cl$$

where,

A – Absorbance; ε - Molar Extinction Coefficient;

C – Concentration; l - Path length [1 cm cuvette]

The calculated values are given in the table 4.1 and 4.2. The molar extinction coefficient were found to be 6177 $M^{-1}cm^{-1}$ and 4325 $M^{-1}cm^{-1}$ at 341 nm and 429 nm respectively in ethanol.

Sl. no	Absorbance	Concentration	Molar extinction coefficient
1	0.04711	0.72646×10^{-5}	6480
2	0.08682	0.14505×10^{-4}	5986
3	0.13199	0.21721×10^{-4}	6076
4	0.17804	0.28913×10^{-4}	6157
5	0.22319	0.36082×10^{-4}	6185
Mean - 6177 $M^{-1}cm^{-1}$			

Table 4.1 Molar Extinction Coefficient At 341 nm

Sl. no	Absorbance	Concentration	Molar extinction coefficient
1	0.03146	0.72646×10^{-5}	4330
2	0.05771	0.14505×10^{-4}	3971
3	0.08806	0.21721×10^{-4}	4054
4	0.11915	0.28913×10^{-4}	4121
5	0.14948	0.36082×10^{-4}	4142
Mean = $4325 M^{-1}cm^{-1}$			

Table 4.2 Molar Extinction Coefficient at 429 nm

4.4 pH dependance of absorption and emission spectra

The changes in the absorption and fluorescence of 4 was studied in phosphate buffer solution with pH 4,5,6,7,8 and 11 which is shown in figure 4.10 and 4.11 respectively. While no significant changes observed in the absorption spectrum.

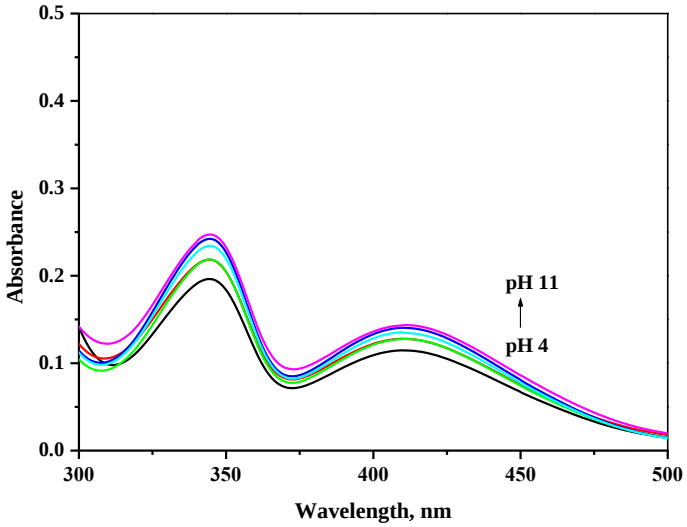


Fig 4.10 Change in absorbance of 4 with pH

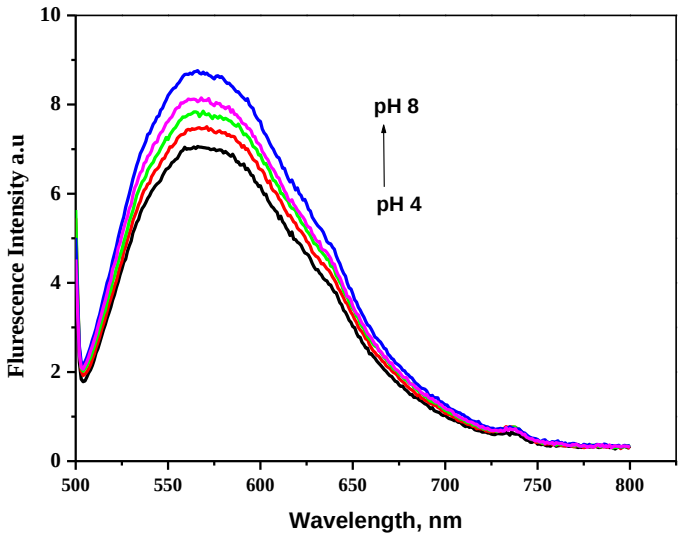


Fig 4.11 Change in fluorescence of 4 with pH

The fluorescence intensity increased 1.25 times as the pH was increased from 4 to 8. At pH 11, the emission was observed to be blue shifted with a slightly reduced intensity. It is possible that at lower pH the amino group get protonated there by reducing the charge transfer efficiency which leads to the observed reduction in the fluorescence intensity at lower pH.

4.5 Absorption and fluorescene properties with metal ions

Figures 4.12 - 4.16 shows the changes in fluorescence of 4 in the presence of increasing concentration of biologically important metal ions such as Fe^{3+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, and Zn^{2+}. The changes in absorption is given as insets in the respective graphs. The absorption spectra did not show significant changes.

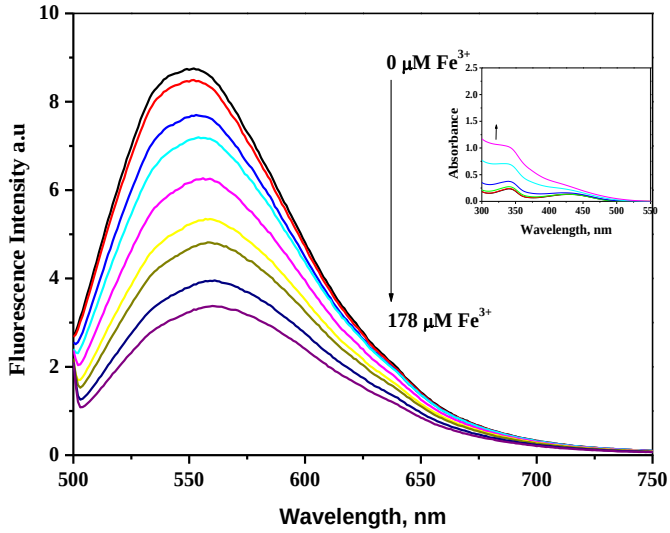


Fig 4.12 Changes in fluorescence and absorbance (inset) of compound 4 (35.5 µM) with increasing concentrations of Fe^{3+}

Fe^{3+} had absorption in the 300-400 nm region, hence it showed hyperchromicity in that absorption range with increasing concentration of iron.

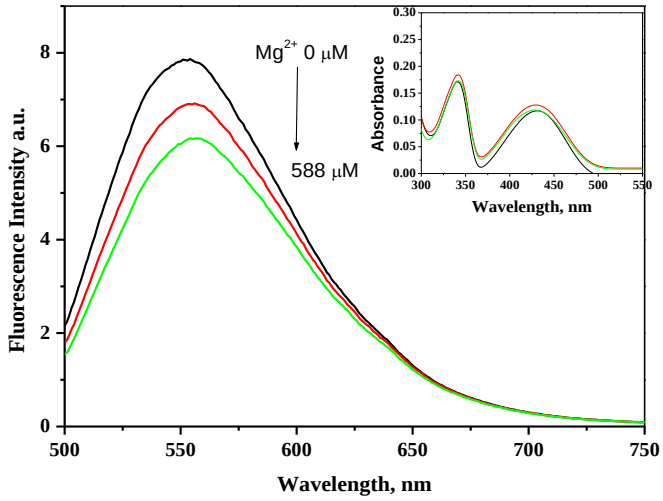


Fig 4.13 Changes in fluorescence and absorbance (inset) of compound 4 (35.5 μM) with increasing concentrations of Mg^{2+}

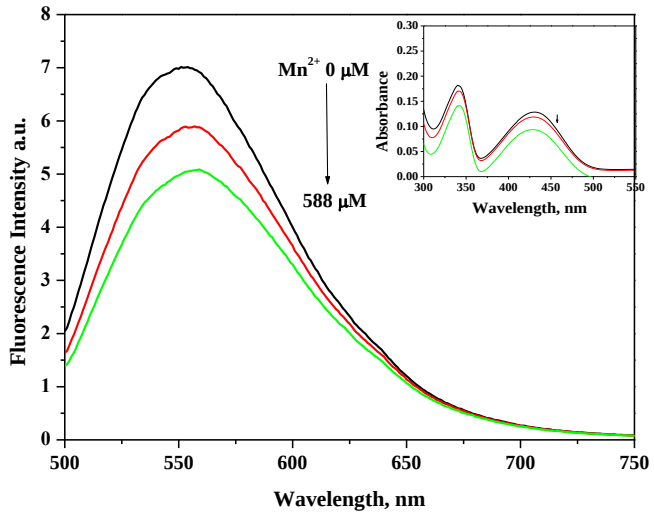


Fig 4.14 Changes in fluorescence and absorbance (inset) of compound 4 (35.5 μM) with increasing concentrations of Mn^{2+}

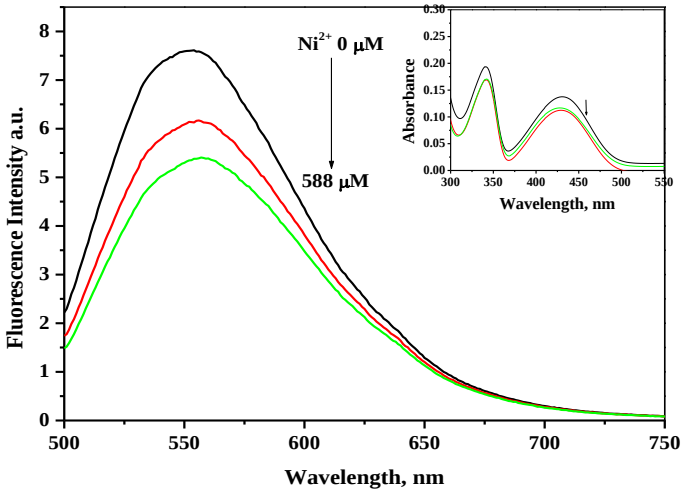


Fig 4.15 Changes in fluorescence and absorbance (inset) of compound 4 (35.5 µM) with increasing concentrations of Ni^{2+}.

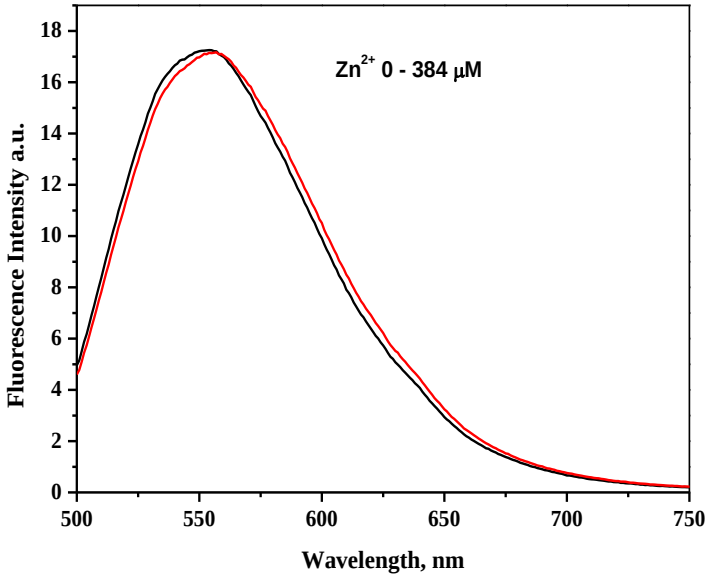


Fig 4.16 Changes in fluorescence of compound 4 (35.5 µM) with increasing concentrations of Zn^{2+}.

However significant changes was observed in the fluorescence spectrum with increasing concentration of the metal ions. The fluorescence intensity of 4 showed quenching in the presence of 148 μm Fe^{3+} solution. Mg^{2+}, Mn^{2+}, and Ni^{2+} respectively. Zn^{2+} showed no changes in fluorescence emission at similar concentration. The selectivity for Fe^{3+} can be due to its stronger complexation with the compound **4.**

Chapter 5

Conclusions

In this work we have synthesised fluorescent N-phenyl derivative of 3-amino-1,8-naphthalimide from 1,8-naphthalic anhydride. Nitration of 1,8-naphthalic anhydride using 1:1 mixture of concentrated nitric acid and acetic acid gave the 3-nitro derivative which on refluxing with aniline gave N-phenyl substituted 3-nitro-1,8-naphthalimide. This was then reduced with stannous chloride to give N-phenyl substituted 3-amino derivative. The IR values obtained at 3448.55, 3356.75 indicated the presence of NH Stretching of amines, peak at 3061.33, 2919.49, 2849.51, showed the presence of aromatic =C-H stretching , peaks between 1652.99-1625.59 indicated C=O stretching of ketone, peaks at 1315.40, 1306.12 indicated C-N stretching. Bands at 1196.62, 1140.92 indicated the presence of primary amine and peaks between 872.68- 646.84 indicated meta disubstituted ring. The chemical shift values in $_1H^1$ NMR values found at δ8.051, δ8.046, δ8.028, δ7.947, δ7.942, δ7.601, δ7.584, δ7.499, δ 7.481, δ7.432, δ7.330, δ 7.312, δ 7.293 and δ 7.287 indicated the presence of aromatic protons. Also the peak at δ 5.990 shows the amine protons. ^{13}C NMR values at at δ164.698 and δ164.247 ppm is due to the carbon of two carbonyl groups and all the peaks at δ 148.570, δ 136.520, δ 134.062, δ

132.164, δ 129.687, δ129.376, δ 128.609, δ 127.493, δ 125.964, δ 122.292, δ 112.33 ppm indicates the presence of carbon present in the aromatic ring. Hence the spectroscopic data confirmed the structure of the derivative.

The solvent dependent absorbtion and fluorescence studies showed significant red shift in absorption spectra. Similarly the fluorescence emission spectra also showed significant shift to longer wavelength with increasing solvent polarity.

The pH dependent studies showed 1.25% enhancement in fluorescence intensity on increasing the pH from 4 – 8.

Moreover, the N-phenyl 3-amino-1,8-naphthalimide derivative **4** showed significant quenching in its fluorescence in the presence of Fe^{3+} ion when compared with metal ions such as Mg^{2+}, Mn^{2+}, Ni^{2+}, and Zn^{2+}. This selective interaction with Fe^{3+} indicates its potential use as a fluorescence based sensor for Fe^{3+} ion.

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