PROJECT REPORT

On

MICROWAVE ASSISTED SYNTHESIS AND PHOTOPHYSICAL STUDIES OF 1,8-NAPHTHALIMIDE AND ITS N-ETHYL DERIVATIVE

Submitted by

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In partial fulfillment for the award of the

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DEPARTMENT OF CHEMISTRY AND CENTRE FOR RESEARCH

ST. TERESA'S COLLEGE (AUTONOMOUS)
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B.Sc. CHEMISTRY PROJECT REPORT

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This is to certify that the project " **MICROWAVE ASSISTED SYNTHESIS AND PHOTOPHYSICAL STUDIES OF 1,8-NAPHTHALIMIDE AND ITS N-ETHYL DERIVATIVE**" is the work done by **MALAVIKA C S**, **NAMRATHA SANTHAKUMAR, UNNIMAYA UNNIKRISHNAN, VARSHA K S.**

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Submitted to the Examination of Bachelor's Degree in Chemistry

Date:…….....

Examiners:……..

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DEPARTMENT OF CHEMISTRY AND CENTRE FOR RESEARCH

ST. TERESA'S COLLEGE (AUTONOMOUS)

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CERTIFICATE

This is to certify that the project work entitled " **MICROWAVE ASSISTED SYNTHESIS AND PHOTOPHYSICAL STUDIES OF 1,8-NAPHTHALIMIDE AND ITS N-ETHYL DERIVATIVE**" is the work done by **MALAVIKA C S**, **NAMRATHA SANTHAKUMAR, UNNIMAYA UNNIKRISHNAN, VARSHA K S** under my guidance in the partial fulfilment of the award of the Degree of Bachelor of Science in Chemistry at St. Teresa's College (Autonomous), Ernakulam affiliated to Mahatma Gandhi University, Kottayam.

Dr. ELIZABETH KURUVILLA
Project Guide

DECLARATION

We hereby declare that the project work entitled " **MICROWAVE ASSISTED SYNTHESIS AND PHOTOPHYSICAL STUDIES OF 1,8-NAPHTHALIMIDE AND ITS N-ETHYL DERIVATIVE**"submitted to Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous) affiliated to Mahatma Gandhi University, Kottayam, 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 and this project work is submitted in the partial fulfilment of the requirements for the award of the Degree of Bachelor of Science in Chemistry.

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Chapter 1

Introduction

ABSTRACT

In an era where 'seeing is believing', it is essential that non-invasive methods like fluorescence imaging be adopted to look into the body/cells in order to diagnose or treat diseases. Today, fluorescent probes based on small organic molecules have become indispensable tools in modern biology because they can provide information on the localization and quantity of the molecules of interest without the aid of genetic engineering. Fluorescence has been used in the research for the last hundred years. Developments and discoveries in fluorescence chemistry has fuelled the synthesis of different kinds of fluorophores – molecules that exhibit fluorescence. Fluorescence can be defined simply as the emission of light by a substance that has absorbed light or other electromagnetic radiation. The emitted light has a longer wavelength and lower photon energy than the absorbed radiation. Fluorescence has become an important method in biotechnology, medicinal chemistry and clinical diagnostics. It is often relied upon as it offers high sensitivity and selectivity, non-invasive, real - time monitoring, rapid response and low detection limits. Thus, fluorescence and fluorophores are considered to be important tools in a researcher's toolbox.

Most Cellular components are colourless and cannot be distinguished under a microscope. Some proteins or small molecules in cells like tryptophan**,** phycoerythrin**,** etc. are naturally fluorescent. Hence they are called intrinsic fluorophores. Alternatively, specific proteins, nucleic acids, lipids, etc. cannot be distinguished easily and are to be labelled with other fluorescent molecules called extrinsic fluorophores. Several other techniques also exist to explain the additional properties of fluorophores such as 'Fluorescence-Resonance Energy Transfer' in which energy is passed on non-radiatively to a particularly neighbouring dye/protein allowing proximity and thereby detecting protein activation. Thus fluorophores are of utmost significance. Among fluorophores, 1,8-naphthalimide is an old fluorophore useful in a wide range of applications. Presently, this class of fluorophores have proved themselves to be useful in fluorescent sensing of ions, cellular imaging, DNA binding and associated anticancer activities. Some of these are currently being investigated for their potential as anticancer and antibacterial agents.

This project explores the synthesis, characterisation, photophysical studies and pH dependence of 1,8-naphthalimide and its ethyl derivative.

1. PHOTOLUMINISCENCE

'Lumen', which means 'light' in Latin, is the root of the phrase luminescence. Luminescence was originally used, to refer to any light

phenomena that are not exclusively conditioned by temperature rise in 1888 by the German scientist and science historian Eilhard Wiedemann. The current definition of luminescence is as follows: When a species is electrically or vibrationally excited and out of thermal equilibrium with its surroundings, it can spontaneously emit radiation, a phenomenon known as luminescence. The different forms of luminescence are categorized based on the way in which they are excited. Specifically, photoluminescence is the light emission that results from the emitting specie's direct photoexcitation. Phosphorescence, delayed fluorescence, and fluorescence are all properly recognized types of luminosity in light. Chemiluminescence, bioluminescence, electroluminescence, cathodoluminescence, radioluminescence, sonoluminescence, thermoluminescence, and triboluminescence are only a few of the several additional forms of luminescence that vary depending on the method of excitation.[1], [2] The difference between fluorescence and phosphorescence was determined for a considerable amount of time following the term's introduction by G. G. Stokes in the middle of the nineteenth century. Fluorescence was thought to as a light emission that ends along with the excitation, while with phosphorescence, the light emission lasts longer than the excitation. However, this criterion is insufficient because there exist phosphorescences with short durations (like the violet luminescence of zinc sulfide) and fluorescences with lengthy durations (like divalent europium salts) that have comparable durations (many hundreds of nanoseconds). As said for the excited species, phosphorescence typically requires that it pass through an intermediate state prior to emission.

More specifically, within the context of molecular photochemistry, we now state that, in the case of fluorescence, spin multiplicity is preserved, but phosphorescence necessitates a change in spin multiplicity usually from triplet to singlet or the other way around.[3]

1.1 FLUORESCENCE AND PHOSPHORESCENCE

The two forms of photoluminescence are fluorescence and phosphorescence. When a photon absorbs and is followed by the emission of a lower energy photon, this phenomenon is known as photoluminescence. The temporal interval between photon emission and absorbance is the primary empirical distinction between phosphorescence and fluorescence. When a material absorbs a photon and nearly instantly produces a lower energy photon, this phenomenon is known as fluorescence. Because phosphorescence necessitates an illegal transition, it takes place over a longer time.Fluorescence is caused by the excitation of atoms in a material. The initial excitation is usually caused by absorption of energy from incident radiations or particles, such as X-rays or electrons. The atoms then re-emit the light almost immediately within a span of 10^{-9} seconds. Fluorescence takes place when excited energy is released and the molecule comes back to the ground state from the singlet-excited state. It stops as soon as we take away the light source. The absorbed amount of energy is completely released back during the process.

The difference between fluorescence and phosphorescence at the atomic level is determined by the electron spin state at energy level

transitions. This electron spin state is sustained throughout fluorescence transitions. On the other hand, phosphorescence necessitates a change in the spin state upon photon absorption. When the subsequent emission takes place, this is reversed. One significant distinction is that phosphorescent materials can glow for some time after the light source has been withdrawn, whereas fluorescence only happens when light is incident on the material. Phosphorescence is the light that a material subjected to radiation emits and that continues to exist as an afterglow once the stimulating radiation has been eliminated. In contrast to fluorescence, which releases light spontaneously approximately 10^{-8} seconds after stimulation, phosphorescence needs more excitation to generate radiation and, depending on the situation, might continue anywhere from 10^{-3} seconds to days or even years.[4]

When fluorescence occurs, a light photon or other form of radiation raises an electron's energy from a baseline known as the ground level to an excited level. With radiation of the same energy as that which was absorbed, the electron can spontaneously transition back to its ground state. The return, within around 10^{-8} seconds, is almost coincident, according to electromagnetic theory. Phosphorescence presents a different situation. Because a transition between the metastable level and other levels is prohibited (very unlikely), a level of intermediate energy known as the electron trap, or metastable level, exists between the excited level and the ground level in phosphorescence. After an electron has transitioned from its excited state into its metastable state. States that are electronically stimulated are unstable. Returning to their ground state are electrons. The excitation energy is released once more at the same moment. One

makes a distinction between degradation processes that radiate and those that don't. Most of the time, internal conversion (IC), quenching with nearby molecules, or vibrational relaxation are examples of non-radiative decay. Radiative decay can occasionally manifest as fluorescence and phosphorescence. Photons or electromagnetic radiation are released as the energy. Because some of the energy has already been released by non-radiative decay, the emitted light has a longer wavelength and less energy than the absorbed light. This explains why stimulation with non-visible UV light can result in an emission in the visible range. This change in direction towards a longer wavelength is known as Stokes shift.[5], [6]

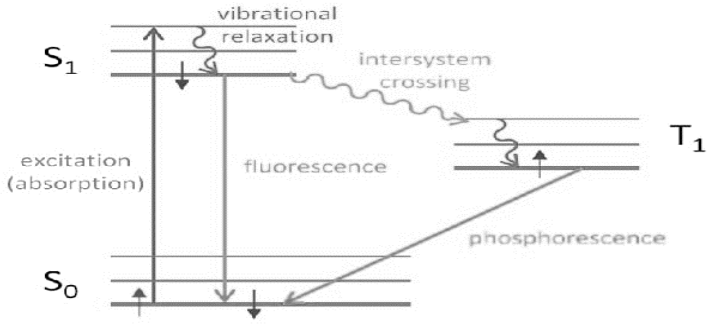


Fig: 1.1 Jablonski Diagram

1.2 FLUOROPHORES

Fluorophores are molecules capable of exhibiting fluorescent properties. Such molecules absorb photons and briefly enter an excited state before either dispersing the energy non-radiatively or emitting as a photon. Fluorophores can be classified into 3 categories: organic dyes, biological fluorophores and quantum dots. Organic dyes include fluorescein**,** rhodamine**,** AMCA (7-amino-4-

ethyl-3-coumarinylacetic acid), acridine orange, etc…. One of the most prominent and naturally occuring fluorophore is GFP (Green Fluorescent Protein). It is a biological fluorophore present in the jelly fish 'Acquoreavictoria' that exhibits green fluorescence when exposed to light (Blue-UV). GFP is composed of 238 amino acids. Biologists make use of GFP to study cells in embryos and fetuses during developmental processes. It is used as a marker protein that can attach and mark another protein with fluorescence, enabling scientists to detect the presence of a particular protein in an organic structure. Quantum dots also fall under the category of fluorophores.[7] They are fluorescent semiconductor nanoparticles which are more photostable than other types of fluorophores. When quantum dots get excited, they emit fluorescence at a particular wavelength based on the size of the particle. Fluorescent molecules can be used as dyes, probes, bio-sensors, etc. Fluorescent dyes can absorb wavelengths in the non-visible part of the spectrum but reflect back light in the visible spectrum. Probes detect protein location and activation, identify protein-complex formation and monitor biological processes in vivo. [8]

1.2.1 SOME COMMONLY USED FLUOROPHORES

Commonly used fluorophores include :

1. Fluorescein isothiocyanate (FITC)
2. Rhodamine
3. Acridine Orange
4. mCherry

Fluorescein isothiocyanate (FITC)

Fluorescein isothiocyanate (FITC) is a fluorescence dye that's used to label biomolecules like proteins, peptides, and nucleic acids. The isothiocyanate group interacts with amino terminal and primary amines in proteins. It has been employed for labeling proteins, including antibodies and lectins. It's also used in flow cytometry experiments to stain proteins, nuclei, and subnuclear particles.

FITC is a fluorescent probe that can be conjugated to proteins and tissue. It's used as a label in fluorescent antibody staining procedures and protein - amino acid binding techniques.

FITC is also used in non-microscopic immunoassays and physiological tracing experiments.[9]

Rhodamine

Rhodamine fluorophores and their derivatives are used in a variety of fields, including essence ion discovery. Rhodamine fluorophores are ideal for essence ion examinations due to their low cost, easy revision, and rich spectral parcels. Biomedical imaging Rhodamine fluorophores are used as pulpits for bioconjugates and reactive markers that are used in luminescence- grounded assays and biomedical imaging. Super-resolution imaging Rhodamine fluorophores and their derivations are of particular interest forsuper-resolution imaging grounded on stimulated emigration reduction(STED) microscopy. This is due to their high brilliance, excellent print- stability, and easy chemical derivatization. Water tracing Rhodamine B is a water-answerable, largely fluorescent color that's frequently used as a dick color to determine the rate and direction of water inflow and transport. It's also used to study aeration

and dissipation, measure time of drip, and understand the movement of water.[10]

Acridine Orange

Acridine orange (AO) is a fluorescent dye used in flow cytometry and epifluorescence microscopy. It can differentiate between different types of cells, such as white blood cells and bacterial cells.[11] AO emits green light when bound to DNA and red light when bound to RNA and lysosomes.[12] It's used in a variety of staining applications, including:

- Flow cytometry
- Epifluorescence microscopy
- Sperm quality analysis
- Microorganism detection
- Cell cycle analysis

mCherry

mCherry is a bright red fluorescent protein that is used as a fluorescent reporter. It is often used as a fluorescent tracer in transfection and transgenic experiments.

It is derived from proteins found in Cnidarians, such as corals, sea anemones, and jellyfish. It is a member of the fluorescent protein chromophores group, which are used to visualize and analyze genes in experiments.

It’s fluorescent and emissive properties allow it to function as an intracellular probe. It is commonly used as a labeled tag for genes, cells, or organelles of interest. It is also used to view constitutive gene expression.

It matures quickly, allowing results to be seen soon after transfection or transcription activation. It is also highly resistant to photobleaching and photostable.

To detect the mCherry signal, excite with a yellow-green laser at 561 nm and detect in the PE-TexasRed channel with a 610/20 bandpass filter.

Results can be observed shortly after transfection or transcription activation since mCherry develops quickly. It is also very photostable and resistant to photobleaching.[13]

1.2.2 APPLICATIONS OF FLUOROPHORES

Fluorophores are used in a variety of analytical methods, including fluorescent imaging and spectroscopy. They are used to stain tissues, cells, or materials. Fluorophores have several applications based on their property of being strongly colored. This is due to their ability to absorb certain wavelengths of light better than others.[14]

Fluorophores are used in: dyes, paints, and optical brighteners, non-destructive testing, water tracing, leak detection, antifreeze, adhesives, car wash soaps, detergents.

Fluorophores are also used in biology, for:

- ❖ Bioimaging
- ❖ Biosensing
- ❖ Phototherapy

It is also used in clinical practice to detect early stages of precancer and cancer.

- ➢ Fluorescence imaging has made significant advances in comprehending the complexity of biological systems, yet in vivo deep-tissue imaging remains difficult due to the optical opacity of biological tissue.[15] Recent advances in laser and detector manufacturing have

enabled the development of nonlinear and linear fluorescence imaging to the hitherto unexplored "tissue-transparent" second near-infrared (NIR-II; 1000-1700 nm) window, creating new prospects for optical access deep within opaque tissue. Molecular fluorophores have long played a significant role in fluorescence bioimaging. Designing novel molecular fluorophores is becoming increasingly necessary in order to fully realize the potential of NIR-II imaging.[16]

- In many cases, fluorescence diagnostics offers a quick and non-invasive way to image precancerous and cancerous tissues. The broad disciplines of cancer, dermatology, laryngology, pulmonology, gynaecology, and gastroenterology have all discussed numerous applications of this technique. This approach has been characterized as highly sensitive and specific in numerous investigations, particularly when it comes to the diagnosis of minute alterations that are invisible by white light endoscopic techniques.[17]

- A growing amount of interest has been drawn to molecularly fluorophores for nanotheranostics, which bridge in vivo imaging and therapeutic capacity inside a unimolecular system. Near-infrared (NIR) fluorescence imaging has several advantages over visible fluorescence imaging, such as reduced background, deep penetration, and less harm to biological tissues. There is progress on molecularly NIR fluorescent probes for in vivo chemotherapy and phototherapy based on the standard high-performance fluorophores, with special attention to their functionalization and design approach.[18]

1.2.3 1,8-NAPHTHALIMIDE AND ITS DERIVATIVES

We are already aware that fluorescent compounds have a broad range of applications such as multipurpose dyes, photoelectric materials, etc. It was found that organic fluorophores that are based on a naphthalene nucleus are specifically used as fluorescent sensors and probes.[19] Normally, to tune the photophysical properties of organic molecules, an electron rich donor and an electron deficient acceptor are introduced to a π linker. This π linkage between the donor and acceptor results in the formation of a D-π-A system. And the properties of such a system can be varied by changing the strength of the donor or the acceptor unit or by changing the π linker.1, 8- naphthalimide and its derivatives form one such class of compounds which exhibit excellent fluorescent properties.[20] Today, this class of fluorophores have been found to be useful in fluorescent sensing of ions**,** cellular imaging and DNA binding and associated anti-cancer activities. Besides these, they are also used in fluorescent solar energy collectors**,** as liquid crystal additives and as fluorescent markers.[21]

The major reason behind 1,8-naphthalimide exhibiting strong fluorescence could be explained from its structure. 1,8-naphthalimide has a unique structure with an extended conjugated aromatic system. It comprises of of two subsystems; a naphthalene nucleus and a dicarboximide (OC-NR-CO) linkage in a six membered cycle. The polarisation of the system arises as a result of the donor-acceptor interaction between the electron-acceptor groups of the imide structure and the substituent at the 4^{th} position of the naphthalene nucleus. This polarisation leads to fluorescent

emissions of different shades of blue**,** yellow-green**,** and orange-red.[22] Keeping this idea in mind**,** a number of such compounds can be synthesised with different fluorescent emission intensities.

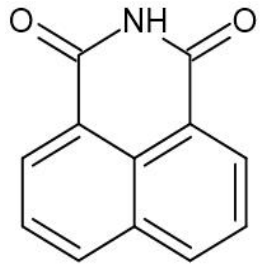


Fig : 1. 2 1.8- naphthalimide

1.2.4 APPLICATIONS

1. As DNA binders - 1, 8-naphthalimides bind through DNA via intercalation , and exert their antitumor activities through Topoisomerase inhibition , photoinduced DNA damage or related mechanism. Fluorescent proteins that attach to DNA are helpful probes for a variety of biological uses. DNA binding proteins expressed within a living cell can be directly and real-time visualized to examine DNA binding dynamics and patterns thanks to fluorescent protein (FP)-tagging. Furthermore, using a fluorescent microscope to image individual proteins bound to enormous, elongated DNA molecules is now possible thanks to FP-tagged DNA binding proteins (FP-DBP). While there are many DNA binding proteins, only a fraction of them have been used to create FP-DBPs for in vitro single-molecule visualization or to examine molecular motion.[23]

2. As Anticancer drugs –1, 8-naphthalimides have been found to be effective against cancer cells and many of them have also been approved for clinical trials. The effectiveness of anticancer medications used in clinical practice is compromised by poor pharmacokinetics, adverse effects, and, in particular, the quick evolution of drug resistance. Consequently, finding new and useful medications continues to be a top priority. One of the most potent anticancer drugs is the phthalimide family, which is based on an efficient DNA intercalator.

There have been reviews illustrating how a profile of anticancer activity can be obtained by modulating the moiety impacting the naphthalimide molecule for DNA binding. By using the intercalation mode of molecular recognition, imide and ring substitution at naphthalimide, bisnaphthalimide, and naphthalimide-metal complexes are bound to DNA. Thus, this synthetic/natural small molecule can operate as a medicine when activation or inhibition of DNA function, is necessary to treat or control the cancer disease. The current work reviews the developments in the field of 1,8-naphthalimide research, concentrating on the way in which these derivatives intercalate into DNA to exert their anticancer effects.[24]

3. As OLEDs – Naphthalimide derivatives have been utilized in laser active media, fluorescence switchers, etc. Their rich photophysical properties enable them to be used as prime molecular entities for use in optoelectronic devices. Large conjugate structures and the energy gap rule limit the efficiency of red organic light-emitting diodes (OLEDs), which have long been a source of criticism. Two important goals for producing efficient OLEDs are high photoluminescent

quantum yields (PLQYs) and high exciton utilization efficiency (EUE), which can be achieved through the aggregation-induced emission (AIE) phenomenon and the reverse intersystem crossing mechanism. Patient's access to precise diagnosis is hampered by the high cost and weight of conventional fluorescence sensing equipment. Point-of-care (POC) testing-compatible small fluorescence sensing could be made possible by organic light-emitting diodes (OLEDs). However, attaining adequate sensitivity may be difficult due to OLEDs' broad emission spectrum and restricted brightness. Here, high conductivity, high brightness, and restricted spectrum co-host microcavity OLEDs are produced for fluorescence sensing. Pulsed mode driving is used to achieve a more consistent and brighter light output from the OLEDs.[25], [26]

5. **For cell imaging** - Naphthalimide based fluorescence probes are used to monitor biological processes in vivo, identify protein complex formation and conformational changes, and detect the location and activation of proteins. Using immunofluorescence techniques, this representative image was produced.

Live-cell imaging has shown to be a valuable analytical instrument in the fields of pharmacology, neurology, and cell biology. One effective method for studying the biological functions of biomolecules and the dynamic cellular processes of cells is fluorescence microscopy, which may be used to see the structure of cells.

Fluorescent probes are an effective means of observing cellular activities in a spatiotemporal manner within crowded and complicated systems. They also allow us to visualize cellular and subcellular structures, their dynamics, and cellular chemicals in living cells.

Apart from fluorescent proteins that are widely used, a vast array of small-molecule dyes have been created by close collaboration with the interdisciplinary fields of chemistry and biology. These dyes can be used to label cellular components like organelles or to identify intracellular biochemical, biophysical, and signaling processes. Self-labeling technologies, like as the SNAP-tag system, have made it possible to attach dyes to certain proteins or cellular domains, and as a result, they are now being used in plant studies.[27]

6. **For drug delivery-** The creation of fluorescent probes has drawn a lot of attention in recent years. Fluorescence signaling makes it possible to image living things in real time with high spectrum resolution without causing injury, which is very helpful for contemporary medicinal applications. The fundamental photophysical concepts and design techniques for fluorescent probes as visualization aids in medication delivery and medical diagnosis systems have gained wide acceptance nowadays. Several photophysical phenomena are referred to as platforms for fluorescence sensing and imaging in vivo and in vitro. These phenomena include Fluorescent Resonance Energy Transfer (FRET), Aggregation-Induced Emission (AIE), Photoinduced Electron Transfer (PET), Twisted Intramolecular Charge Transfer (TICT), and Intramolecular Charge Transfer (ICT).[28]

1.3 OBJECTIVES

- To synthesise 1,8-naphthalimide and its N-ethyl derivative (N-ethyl-1,8-naphthalimide) using microwave assisted organic synthesis.
- Characterization of the products thus synthesized by means of spectroscopic methods.
- To study the pH dependence of the derivatives.

Chapter 2

Materials and Methods

This chapter gives a brief description of the materials and the experimental procedures adopted for the synthesis of 1,8-naphthalimide and its ethyl derivative.

2.1 MATERIALS

2.1.1 1,8- naphthalic anhydride manufactured by Sigma Aldrich Co. It was used for the synthesis of both 1,8- naphthalimide and its ethyl derivative.

2.1.2 Ammonia, Chloroform manufactured by Nice Chemicals (P) Ltd.

2.1.3 Ethyl Amine manufactured by manufactured by Sisco Research Laboratories Pvt. Ltd.

2.2 EXPERIMENTAL METHODS

2.2.1 Synthesis of 1,8-naphthalimide

10 g of 1,8-naphthalic anhydride was mixed with excess of ammonia to form a yellow slurry. The yellow slurry was then heated to 70°C with stirring for 2 hours. After heating, the mixture was washed with distilled water until the pH of the wash water was neutral. A yield of about 95% was recorded.[29]

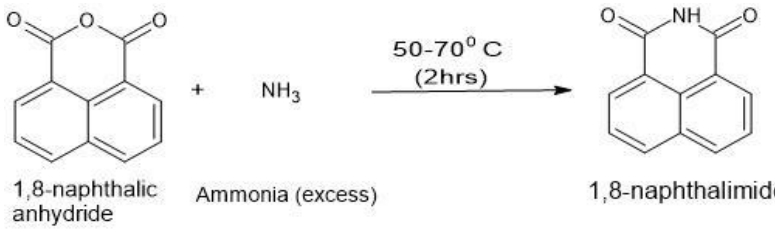
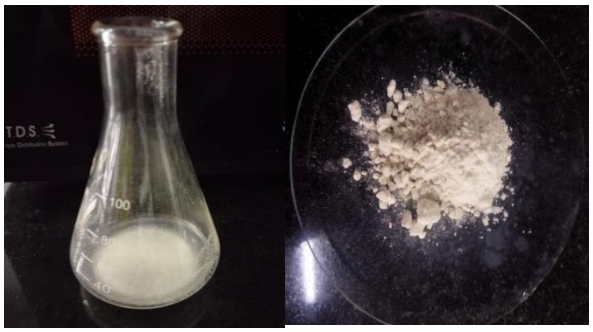


Fig 2.1 : Scheme for the preparation of 1,8-Napthalimide



2.2.2 Synthesis of N-ethyl-1,8-naphthalimide

0.2 g of 1,8-naphthalic anhydride and 2 g of ethyl amine was reacted in a microwave oven for 3 minutes 10 seconds. The reaction mixture was then subjected to TLC to ensure the complete consumption of 1,8-naphthalic anhydride.

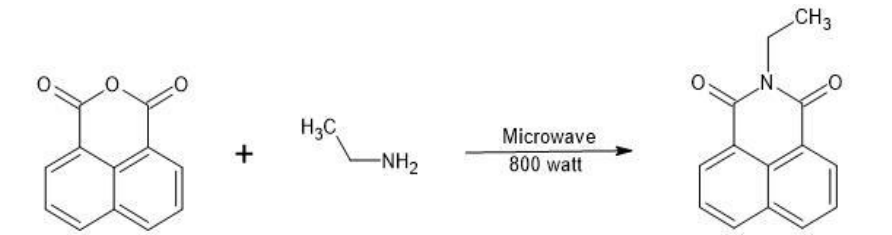


Fig 2.2: Scheme for the preparation of N-Ethyl-1,8-Napthalimide

The product was then filtered and 5 ml of ethanol was added followed by 10 ml of chloroform and 5 ml of water. The organic layer thus formed was seperated using a separating funnel. A little of anhydrous sodium sulphite was added to remove the water left

in the organic layer. The product was then subjected to TLC using 7:3 chloroform hexane solvent system to ensure the purity of the product. A yield of about 75% was recorded.[30]

2.2.3 Characterization Techniques Employed

2.2.3.1 FTIR (Fourier - transform infrared spectroscopy)

FTIR was recorded in a Thermo Nicolet iS50 4000 cm^{-1} to 100 cm^{-1} at STIC CUSAT.

2.2.3.2 $_1H^1$NMR (Proton NMR spectrocopy)

JEOL JNM-ECZR 500 MHz NMR spectrometer at NIT Calicut instrumentation facility was used for recording the proton NMR spectrum.

2.2.3.3 UV Visible spectroscopy

Thermoscientific Evolution 201 UV-Visible spectrophotometer was used to record the absorption spectra.

Chapter 3

Results and discussion

3.1 CHARACTERISATION OF 1,8 - NAPHTHALIMIDE

3.1.1 Infrared spectroscopy

IR spectrum of 1,8 - naphthalimide

The characteristic peaks corresponding to N-H stretching found at 3447 cm^{-1}, C=O stretching at 1670 cm^{-1}, and C-H stretching at 2843 cm^{-1}, in the FTIR spectra confirms the formation of 1,8 – naphthalimide .

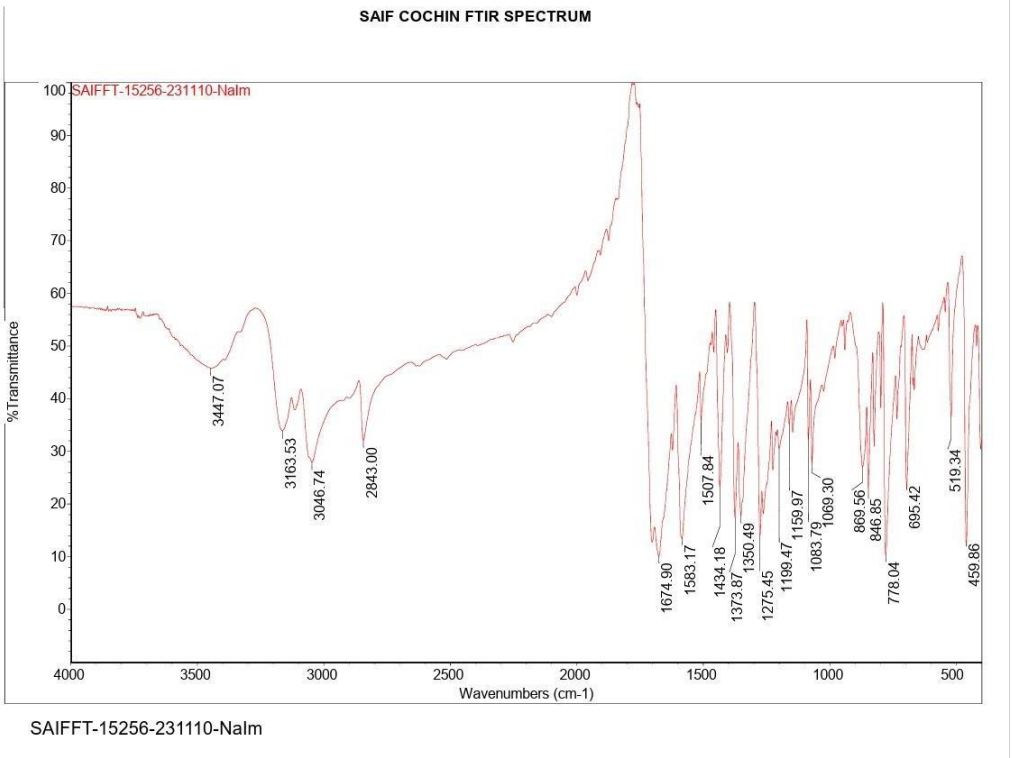


Fig 3.1 IR Spectrum of 1,8-naphthalimide

3.1.2 DETERMINATION OF MELTING POINT

The melting point of 1,8-naphthalimide was determined using the Digital Melting Point Apparatus and the melting point was found to be in between 298-301℃. The melting point of the product synthesised was found to be in conformation with that of the known value.

Fig 3.2 Melting Point Apparatus used.

3.2 CHARACTERISATION OF N-ETHYL 1,8 - NAPHTHALIMIDE

3.2.1 Infrared spectroscopy

Infrared spectrum of N-ethyl 1,8-naphthalimide

The characteristic peaks of C-H stretching corresponding to 2964.77 cm^{-1} and C=O corresponding to 1696.57 cm^{-1}in the FT-IR confirm the formation of N-Ethyl-1,8-naphthalimide.

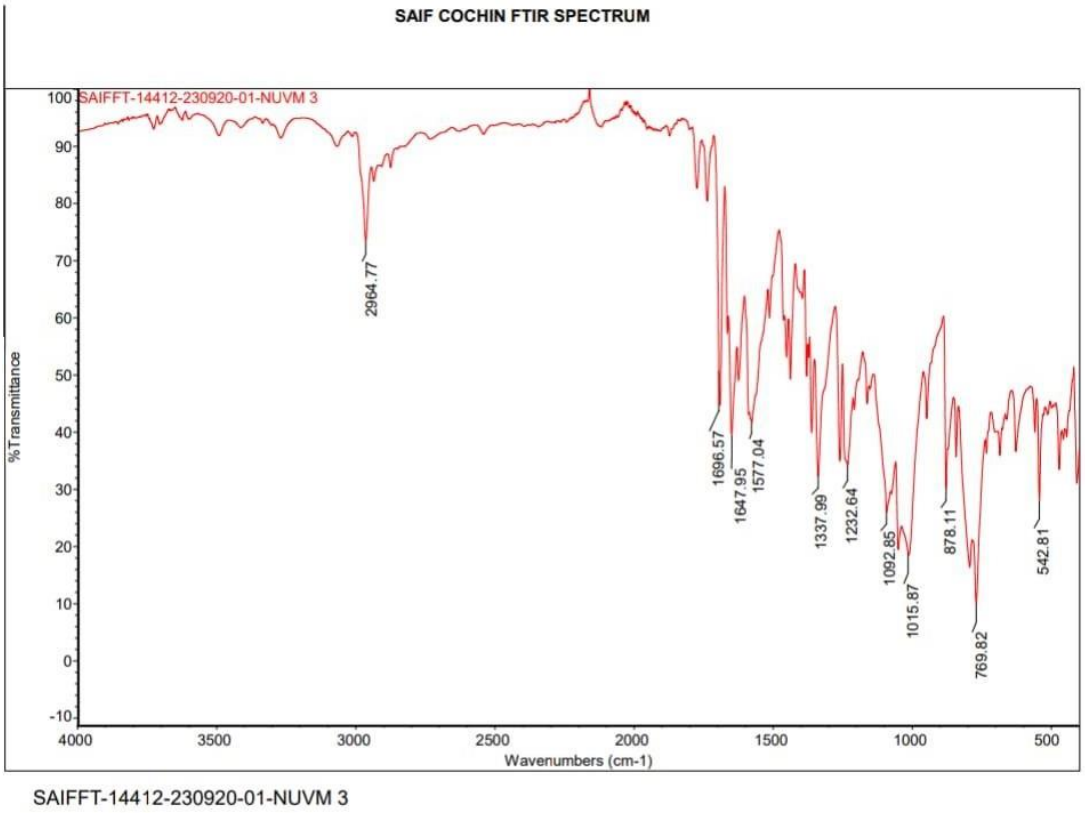


Fig : 3.3 IR Spectrum of N-Ethyl 1,8-Naphthalimide

3.2.2 NMR SPECTROSCOPY

NMR of N-ethyl 1,8 – naphthalimide

A triplet at δ 1.2 ppm and a quartet at δ 4.2 ppm confirms the presence of N-ethyl derivative in the proton NMR spectrum of N- ethyl 1,8-naphthalimide. The two sets of doublets and a set of triplet in the region δ 7.5 ppm to δ 8.5 ppm correspond to the aromatic protons in the naphthalimide.

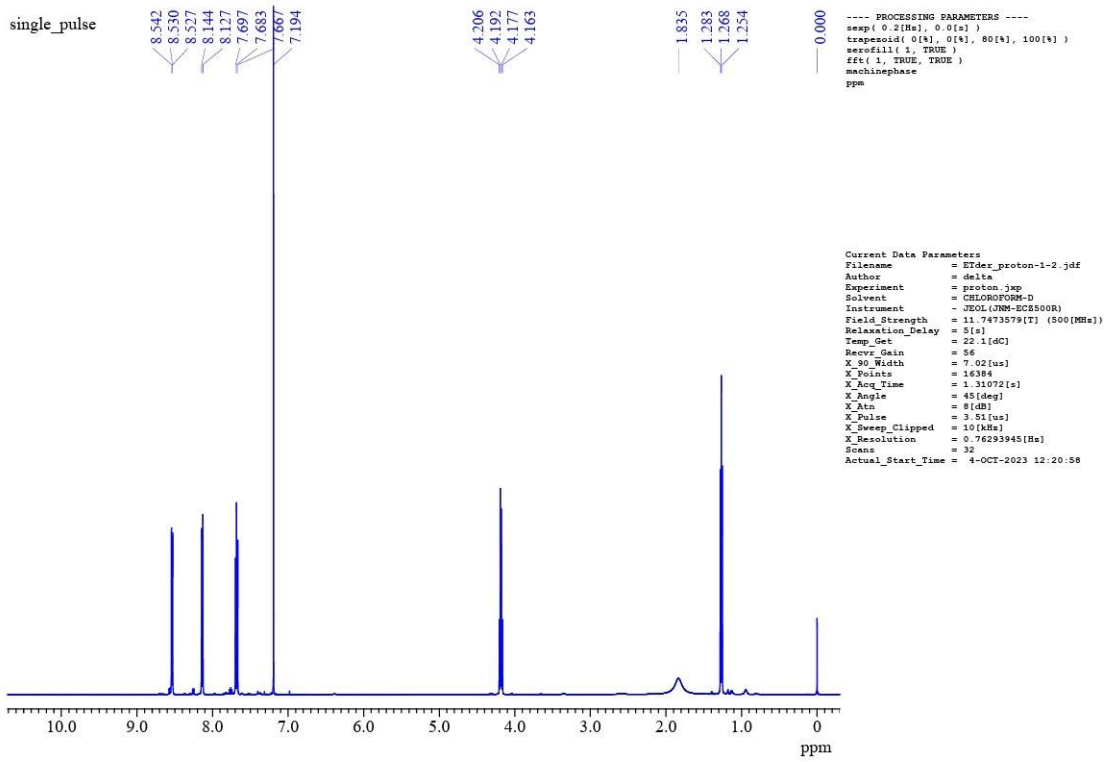


Fig 3.4 NMR Spectrum of N – ethyl-1,8-naphthalimide

3.2.3 MELTING POINT

The melting point of N -ethyl 1,8 – naphthalimide was determined using the Digital Melting Point Apparatus and the melting point was found to be in between 168-172℃. The melting point of the product synthesised was found to be in conformation with that of the known value.

3.2.4 ABSORPTION SPECTRA

3.2.4.1 Absorbance of 1,8-naphthalimide

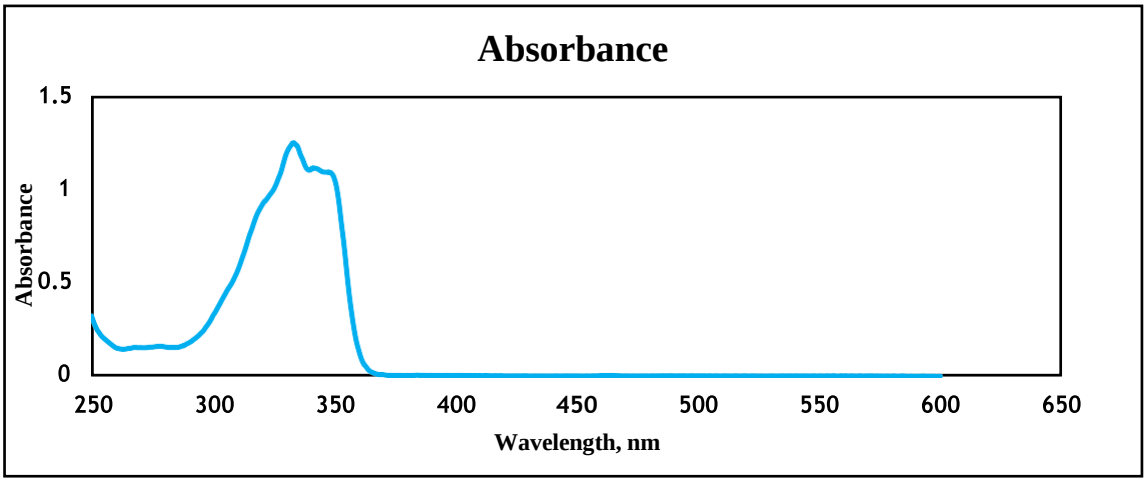


Fig 3.6- Absorption spectrum of 1,8 – naphthalimide in $CHCl_3$

The above figure shows the absorption spectrum of 1,8- naphthalimide in $CHCl_3$. It has an absorbance from around 250 – 370 nm with an absorption maximum at 333 nm and a shoulder at 349 nm.

3.2.4.2 Absorbance of N- ethyl 1,8-naphthalimide

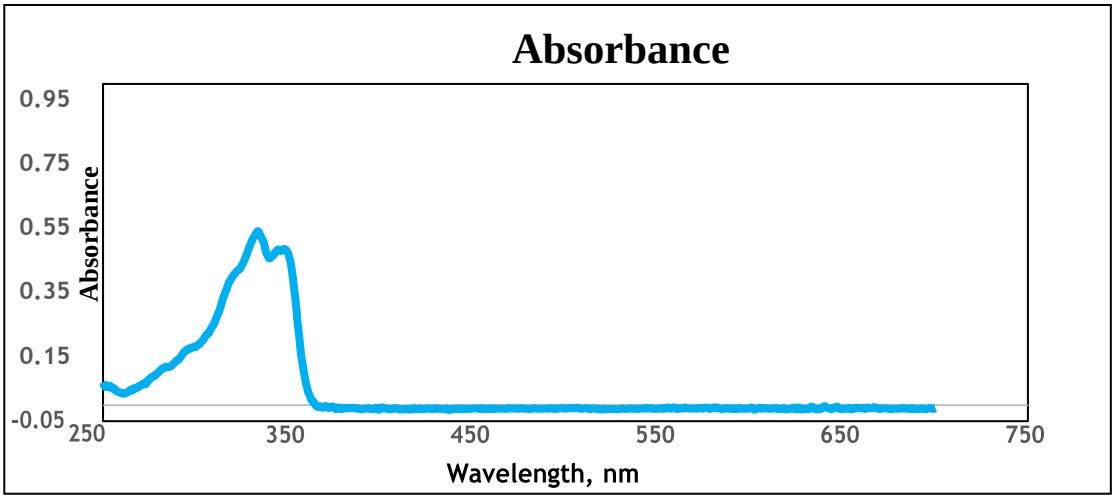


Fig 3.6- Absorption spectrum of N- Ethyl-1,8-Naphthalimide in $CHCl_3$

The above figure shows the absorption spectrum of N-ethyl-1,8-naphthalimide. It shows an absorption from around 250-360 nm, exhibiting vibrational structures with absorption maximum at 335 nm and a shoulder at 348 nm.
The N-ethyl derivative appears to have a blue shift of around 1 nm compared to the absorption maximum of 1,8- napthalimide.

3.2.5 FLUORESCENCE SPECTRA

3.2.5.1 Fluorescence of 1,8-naphthalimide

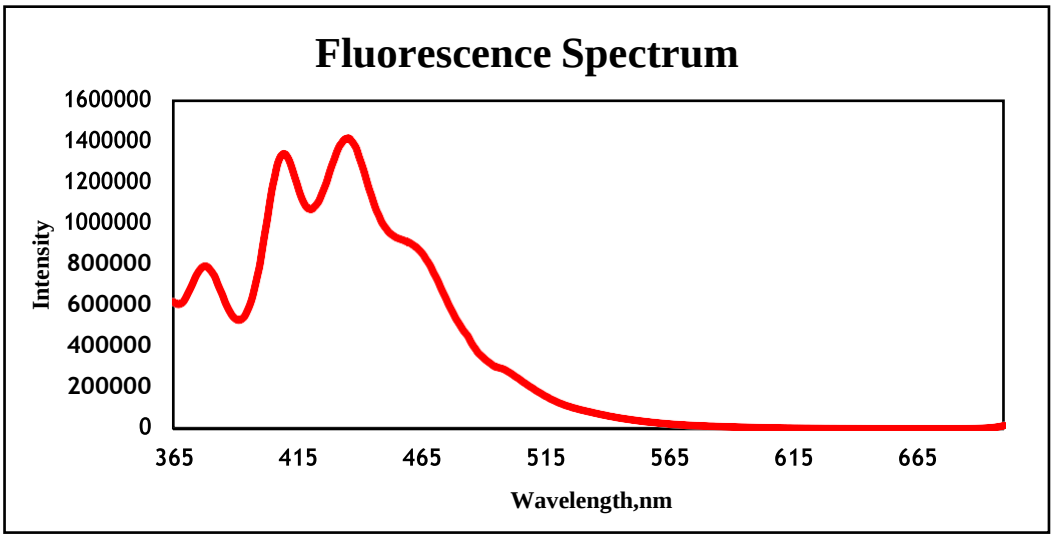


Fig 3.7 - Fluorescence spectrum of 1,8 – naphthalimide in $CHCl_3$

The above figure shows the fluorescence emission spectrum of 1,8-naphthalimide in chloroform. It was excited at a wavelength of 355 nm and the emission was recorded from 365 to 700 nm with bands at 378 nm, 410 nm and 436 nm and a shoulder at 460 nm. A weak emission band at around 500 nm may be due to formation of excimer, which is a complex formed between two fluorophore molecules of the same type-one in the excited state and the other in the ground state, typically by the π–orbital interaction.

3.2.5.2 Fluorescence of N-ethyl 1,8-naphthalimide

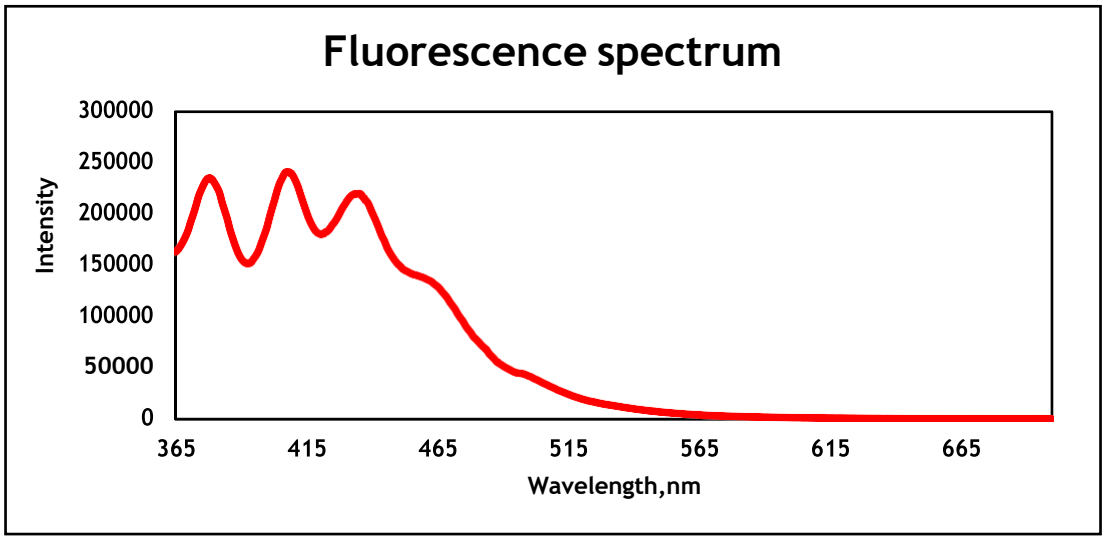


Fig 3.8 - Fluorescence spectrum of N- ethyl 1,8 – naphthalimide in $CHCl_3$

The above figure shows the fluorescence emission spectrum of N-ethyl 1,8-naphthalimide in chloroform. It was excited at 355 nm and bands at 378 nm, 408 nm, 434 nm were recorded.

3.3 Study of pH Dependance

pH dependence of the products synthesised were studied at different pH conditions.

3.3.1 Absorption Spectra

3.3.1.1Absorbance of 1,8-naphthalimide at pH 3

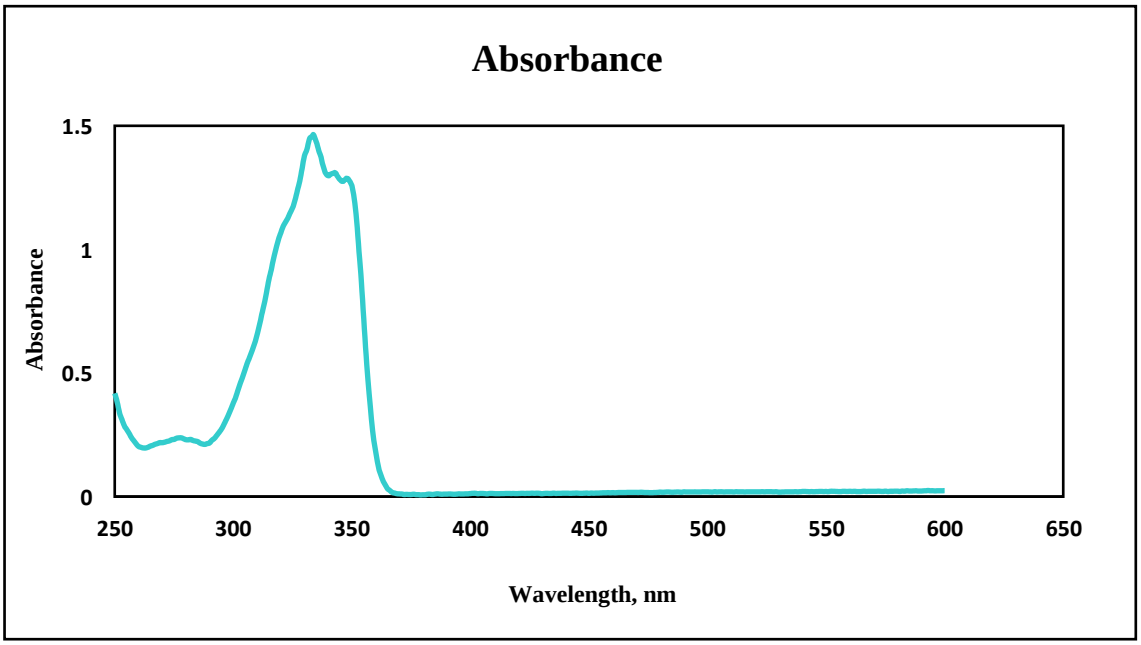


Fig 3.7 – Absorption spectrum of 1,8-naphthalimide+Acid at pH 3

The above figure shows the absorption spectrum of 1,8-naphthalimide at an acidic pH 3. It has absorption maxima at 334 nm and a shoulder at 348 nm.
The absorption spectrum of 1,8-naphthalimide at basic pH was not recorded since salt formation was evident.

3.3.1.2Absorbance of N- Ethyl 1,8-Naphthalimide at pH 3

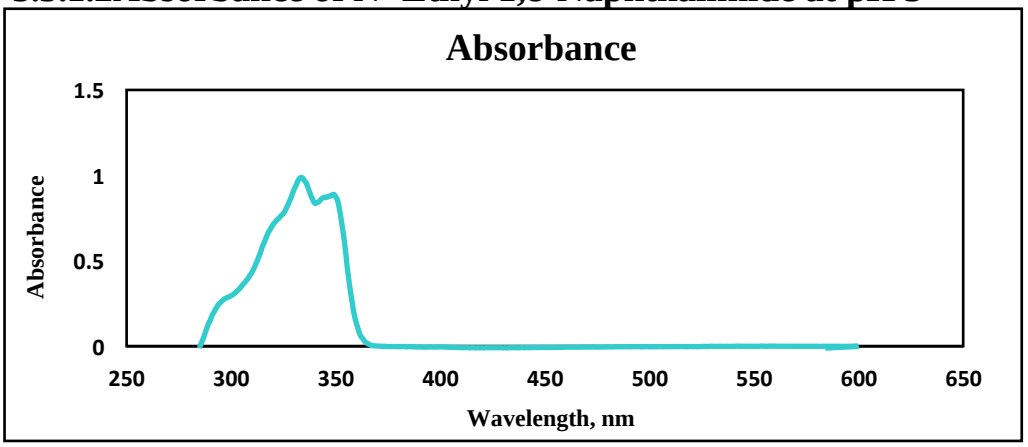


Fig 3.8 - Absorption spectrum of N- ethyl 1,8-naphthalimide+Acid at a pH 3.

The above figure shows the absorption spectrum of N –ethyl-1,8-naphthalimide at an acidic pH value of 3 . It shows an absorbance from 250 nm to 370 nm with an absorption maximum at 334 nm and a shoulder at 349 nm. There a slight blue shift of 1 nm in the absorption maximum at acidic pH when compared to the neutral one.

3.3.1.3 Absorbance of N- ethyl 1, 8-naphthalimide at pH 12

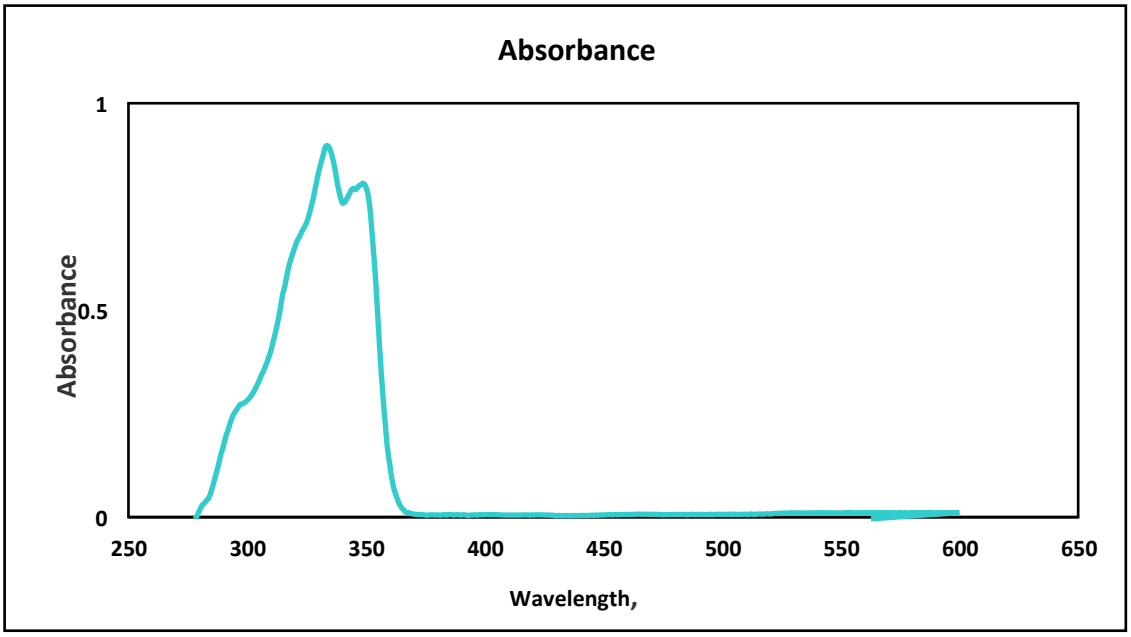


Fig 3.9 - Absorption spectrum of N- ethyl- 1,8-naphthalimide+Base at pH12.

The above figure shows the absorption spectrum of N-ethyl-1,8-naphthalimide at a basic pH of 12. It shows absorbance from 250 nm to 370 nm with absorption maxima at 334 nm and a shoulder at 349 nm. This too shows a blue shift from the neutral one.

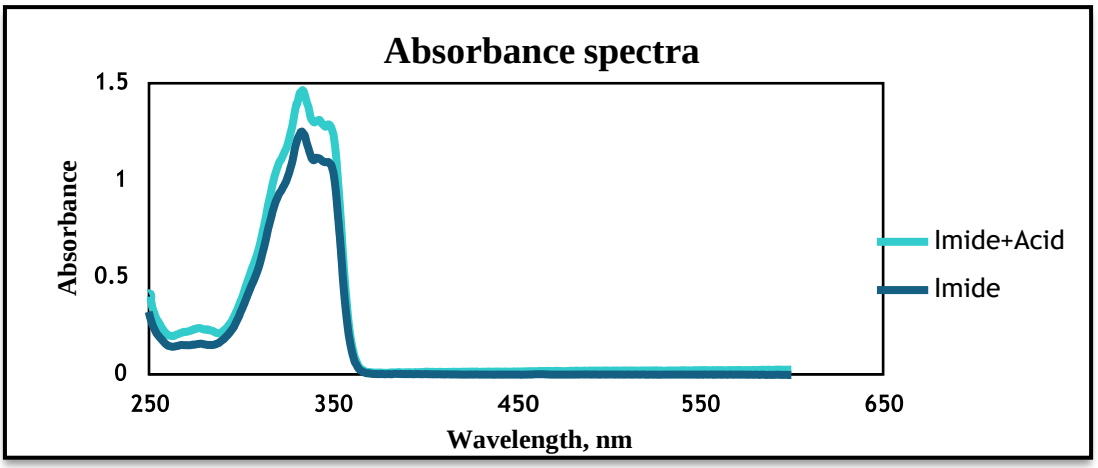


Fig 3.10 Absorbance Spectra of Imide at different pH.

The above figure shows the change in absorbance of 1,8-naphthalimide and 1,8-naphthalimide at an acidic pH value of 3. It is seen that at an acidic pH we get an absorption maximum at 334 nm i.e. a red shift of 1 nm to the absorption maximum of 1,8-naphthalimide is observed. There is also a slight increase in the absorbance at 334 nm at pH 12.

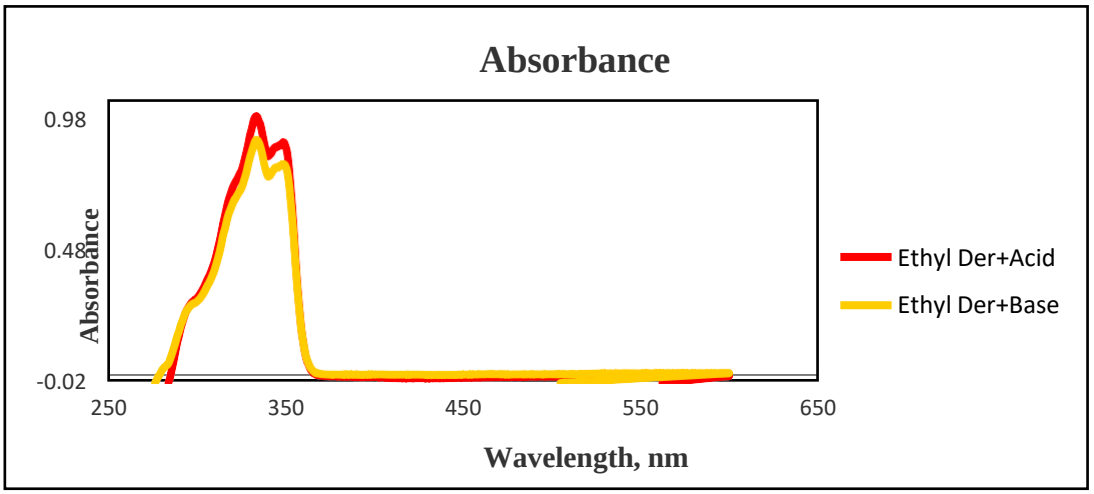


Fig 3.11 Absorbance Spectra of the ethyl derivative

The above figure shows the change in absorbance of N-ethyl- 1,8-naphthalimide and N-Ethyl- 1,8-naphthalimide at acidic and basic conditions. It is seen that at the acidic pH we get an absorption maximum at 334 nm which is the same at the basic pH as well. The absorption intensity doesn't show significant changes.

3.3.2 Fluorescence Spectra

3.3.2.1Fluorescence of 1,8-Naphthalimide + Acid

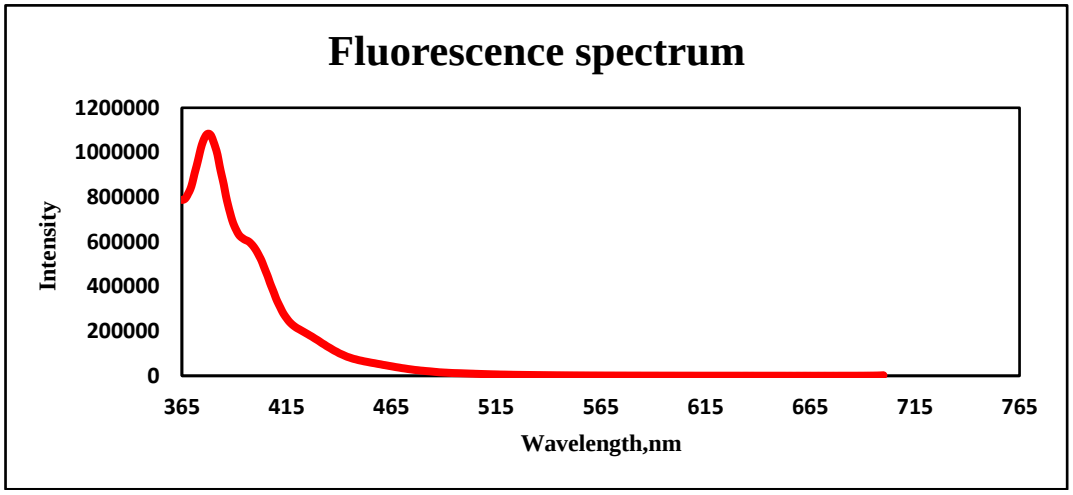


Fig 3.12- Fluorescence spectrum of 1,8 – naphthalimide + Acid at pH 3.

The above figure shows the fluorescence emission spectrum of 1,8-naphthalimide with an acidic pH value of 3 in chloroform. It was excited at 355 nm and a band at 378 nm was obtained.

3.3.2.2Fluorescence of N-ethyl 1,8-naphthalimide+Acid

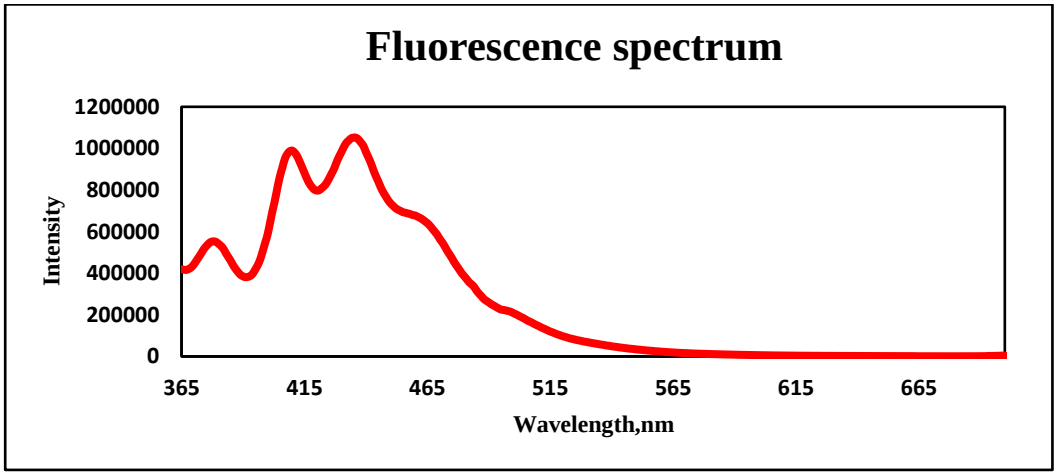


Fig 3.13- Fluorescence spectrum of 1,8 – naphthalimide + Acid at pH 3

The above figure shows the fluorescence emission spectrum of N-ethyl 1,8-naphthalimide with an acid of pH 3 in chloroform. It was excited at 355nm and bands at 378 nm, 410 nm, 435 nm were obtained.

3.3.2.3Fluorescence of N-ethyl 1,8-naphthalimide+Base

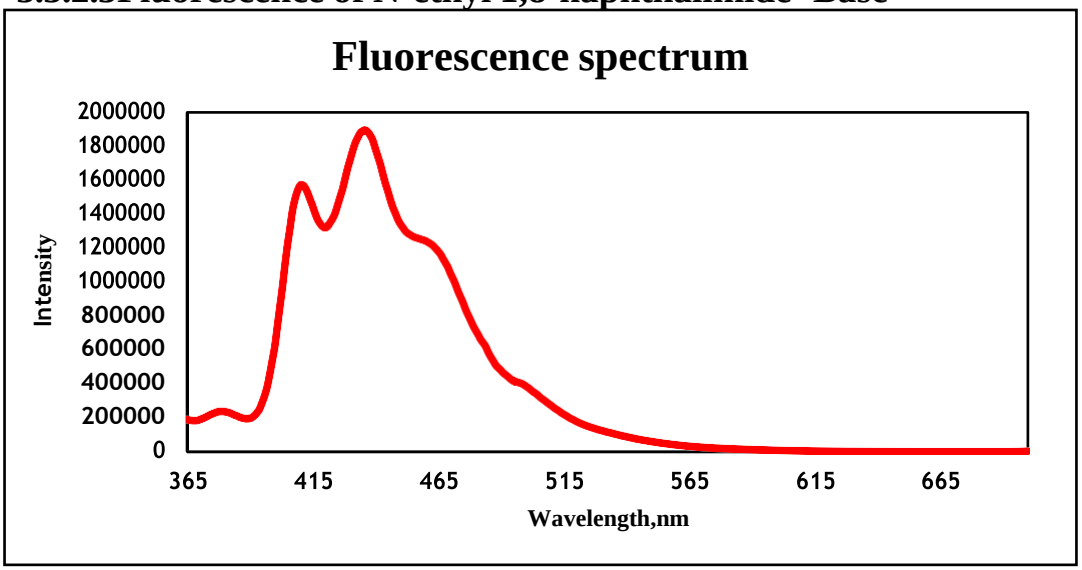


Fig 3.14– Fluorescence spectrum of N Ethyl 1,8 – naphthalimide + base at pH

The above figure shows the fluorescence emission spectrum of N-ethyl 1,8-naphthalimide with a base of pH12 in chloroform. It was excited at 355 nm and bands at 379 nm, 411 nm,436 nm were obtained.

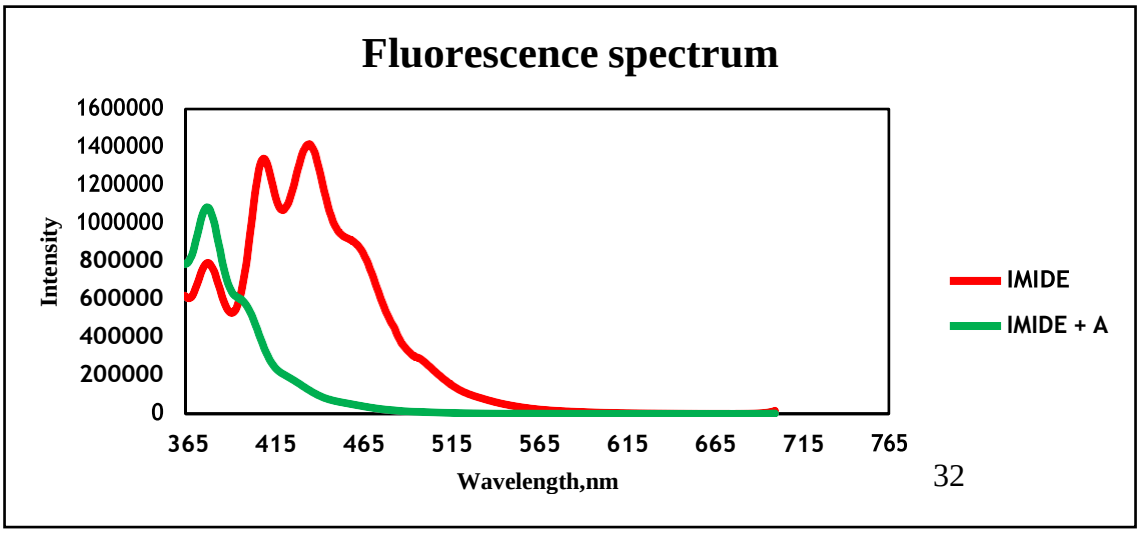


Fig 3.15: Fluorescence Spectra of imide

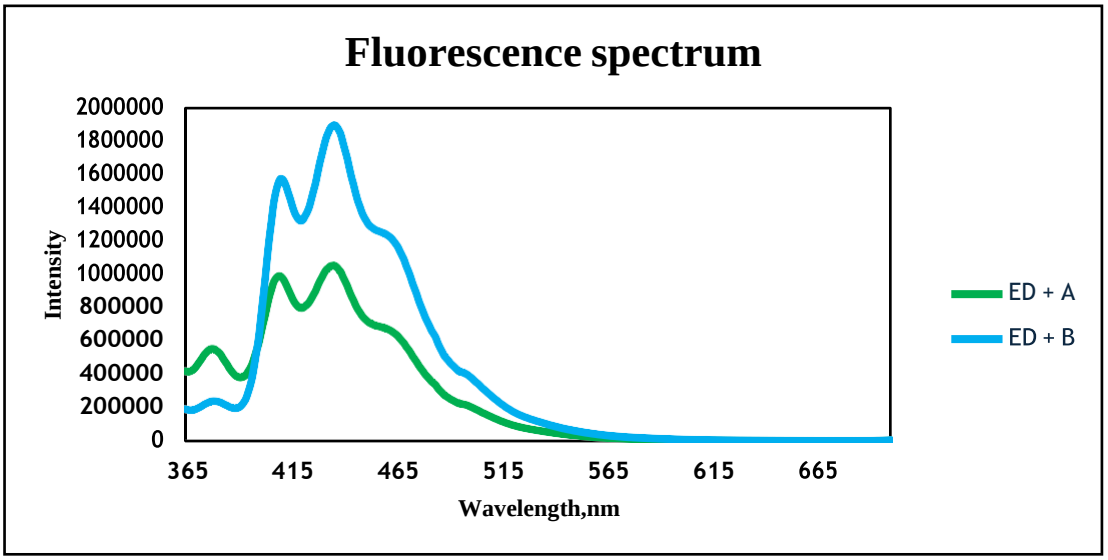


Fig 3.16: Fluorescence spectra of the N-ethyl derivative

The above figure reveals that the fluorescence intensity of N-ethyl-1,8-naphthalimide increases almost double fold at a wavelength of about 439 nm at the basic pH compared to the intensity at the acidic pH.

Chapter 4

CONCLUSION

1,8-Naphthalimide was synthesised with an yield of 90%. The N-ethyl derivative of 1,8-naphthalimide was synthesised by means of microwave assisted solvent free reaction. We obtained the n-ethyl derivative in 75% of yield and the product was recrystallised and purified with chloroform-hexane mixture in the ratio 7:3. The compounds were characterised using NMR and IR spectroscopy. Absorbance and fluorescence spectrum of the compounds were obtained The change in absorbance and fluorescence at different pH was studied. N-ethyl derivative is observed to show a 2 fold increase in intensity as pH increases from 3 to13. There were no significant changes in the absorption spectrum. These studies shows that the naphthalimide derivatives can be developed as fluorescent pH sensors.

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