# PROJECT REPORT

**On**

**"STUDY OF THE EFFECT OF SOLVENTS ON ELECTRONIC SPECTRA OF AROMATIC AZO COMPOUNDS BY EXPERIMENTAL AND DFT CALCULATIONS AND THEIR ANTIBACTERIAL PROPERTY BY DOCKING"**


Submitted by

**JENIFER JOHN**

**(AM22CHE005)**


***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)**
**ERNAKULAM**


**2023-2024**

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This is to certify that the project "**STUDY OF THE EFFECT OF SOLVENTS ON ELECTRONIC SPECTRA OF AROMATIC AZO COMPOUNDS BY EXPERIMENTAL AND DFT CALCULATIONS AND THEIR ANTIBACTERIAL PROPERTY BY DOCKING**" is the work done by **JENIFER JOHN.**

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**Submitted to the Examination of Master's degree in Chemistry**

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### CERTIFICATE

This is to certify that the project work titled "**STUDY OF THE EFFECT OF SOLVENTS ON ELECTRONIC SPECTRA OF AROMATIC AZO COMPOUNDS BY EXPERIMENTAL AND DFT CALCULATIONS AND THEIR ANTIBACTERIAL PROPERTY BY DOCKING**" is the work done by **Jenifer John** under the guidance of **Dr.Maria Linsha P.L, Assistant professor,** Department of Chemistry and Centre for Research, St. Teresa's College, Ernakulam in partial fulfilment of the award of the Degree of Master of Science in Chemistry at St. Teresa's College, Ernakulam affiliated to Mahatma Gandhi University, Kottayam.

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## CERTIFICATE

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Dr.Maria Linsha P.L

Project Guide

# DECLARATION

I hereby declare that the project work entitled "**STUDY OF THE EFFECT OF SOLVENTS ON ELECTRONIC SPECTRA OF AROMATIC AZO COMPOUNDS BY EXPERIMENTAL AND DFT CALCULATIONS AND THEIR ANTIBACTERIAL PROPERTY BY DOCKING**" 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.Maria Linsha P.L, 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.

JENIFER JOHN

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| | |
|---|---|
| NAA | 1-(4-nitrophenylazo)-2-naphthol |
| NAR | p-nitro benzene azo resorcinol |
| NAY | 5-[(p-Nitrophenyl)azo]salicylic acid |
| E-coli | Escherichia coli |
| S.aureus | Staphylococcus aureus |

# Chapter 1

## Introduction

When an electron moves from the HOMO orbital, which covers the donor portion of the molecule, to the LUMO orbital, which covers the acceptor region of the molecule, this is known as a charge transfer transition. The mode of coupling between the two regions determines the excitation energy of the charge transfer bands. The excitation energy will be smaller if there is weak coupling between the donor and acceptor regions. Intensely coloured compounds will display this charge transfer transition event. This can be clarified using the ultraviolet-visible spectroscopy principle.

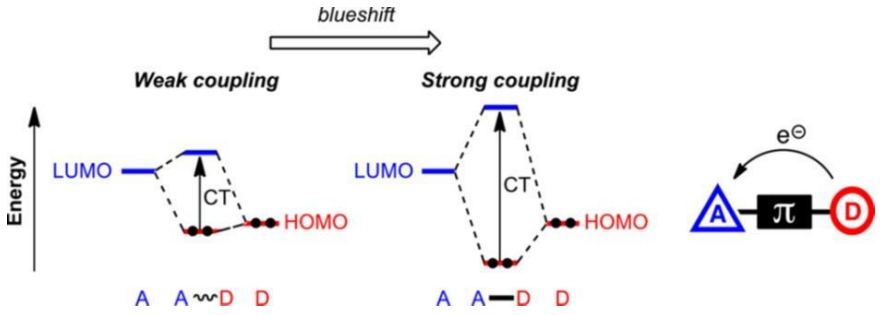



**Fig1. HOMO -LUMO transition**

In order to excite electrons to higher anti-bonding molecular orbitals , molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb energy in the form of ultraviolet or visible light. The longer the wavelength of light it can absorb, the more easily excited the electrons are (i.e., lower energy gap between the HOMO and the LUMO) [1].

## 1.1 Solvatochromism

A chemical substance's capacity to change colour as a result of a shift in solvent polarity is known as solvatochromism [2]. The term "solvatochromic effect" or "solvatochromic shift" describes how strongly the polarity of the solvent affects the absorption and emission spectra. The molecule's absorption wavelength and excitation energy are more significantly impacted by the influence of a solvent. Since the solvent molecules' interactions with the substrate molecules alter the HOMO and LUMO orbitals' energy gaps, the excitation energy is dependent on the solvent. The solute and solvent molecule interact more strongly when a solvent with a far higher polarity is used. In such a case, the two orbitals have a smaller energy gap and are strongly coupled. . Hence the wavelength of the light absorbed ($\lambda_{max}$) will be maximum[3].

Positive and negative solvatochromism are the two main forms that are seen. A hypsochromic shift with increasing solvent polarity is correlated with negative solvatochromism.Positive solvatochromism is the term used to describe the corresponding bathochromic shift. The dipole moment difference between the chromophore's excited and ground states determines the solvatochromism's sign.

Mohanad Shakoor .and co-workers have been studied the solvatochromic behaviors of two newly synthesized pyrido coumarins of different substituents. A new band appeared in the visible range when the nitro-substituted pyrido coumarin was compared to the other H-pyrido coumarin. A new band appeared in the visible range when the nitro-substituted pyrido coumarin was compared to the other H-pyrido coumarin. The new band displayed negative solvatochromic behaviour, meaning that as solvent polarity increases, λmax, decreases [3].

Azo compounds, known for their high photo-induced anisotropy, are excellent substrates for liquid crystals and efficient photorefractive media. Their photosensitivity and superior structuring properties are attributed to the lability of substituents binding to the N=N groups. Azo dyes typically show positive solvatochromism, but negative solvatochromism has been reported in neutral azo dyes with both electron-donating and electron withdrawing moieties.

.

### 1.2 Azo Dyes

Any member of a broad class of artificial dyes whose molecules have two nitrogen atoms next to each other instead of carbon atoms. More than 60% of all dyes are made up of azo dyes, the most significant synthetic colourants that are used extensively in the production of textiles, paper, and printing [4]. Azole dyes make up about 70% of all dyes used in industry. The functional group (-N¼N-) that unites two symmetrical, asymmetrical, identical, or non-azo alkyl or aryl radicals is what distinguishes these compounds. The detrimental effects of azo dyes on aquatic life and humans have prompted urgent calls for the treatment of effluents containing azo dyes in order to remove them or transform them

into safe and useful product.[5]. The azo group could potentially bound to aromatic heterocycles, naphthalene, benzene rings, or enolizable aliphatic groups. These, in their various shades of varying intensities, are what give the dye its colour. An azo dye's backbone, auxochrome groups, chromophoric groups, and solubilizing groups generally represent its chemical structure. The azo bonds and the chromophores and auxochromes they are connected to determine the colour of the azo dyes.

## 1.3 Aromatic azo compounds

Diazine derivatives known as azo compounds have hydrocarbyl groups in place of both hydrogen atoms. Aromatic azo dyes are any organic molecules that have a -N=N- linkage and nitrogen atom ends connected by one or more aromatic groups. They are a common class of colourants used in consumer goods, foods, cosmetics, and tattoos. aromatic in addition to this commercial use. When the R groups in aromatic azo compounds are amine rings, their structures are more stable than when the R groups are alkyl groups. This is due to the fact that the –N=N– group joins an expanded delocalized system that includes the arene groups. Because of their vibrant colours, aromatic azo groups are frequently utilised as dyes a form aromatic azo compounds.

## 1.4 Diazonium salt

The only somewhat stable diazonium salts are the aromatic ones, and even they are not very stable [6][7]. The diazonium salts themselves are very unstable. This is because the ±N≡N group is stabilised by the presence of

the benzene ring, which has a high electron density. One type of diazonium salt is benzene diazonium chloride:

A solution of arylamine in concentrated acid (below 5°C) is mixed with a cold solution of sodium nitrate in a diazotization reaction. First, the acid interacts with the Nitric acid, an unstable nitrous acid, is created by adding sodium nitrate:

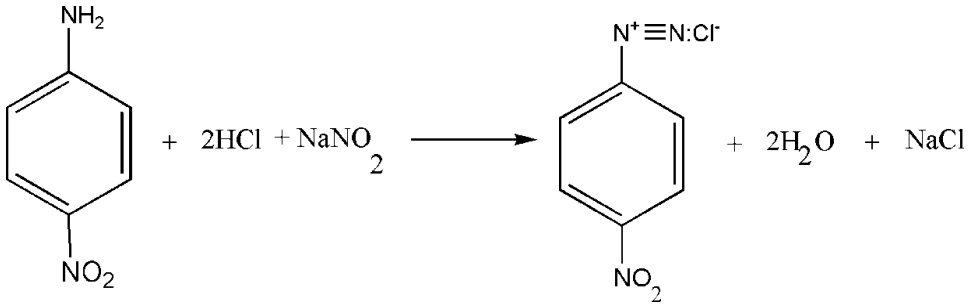



**Fig2. diazotization reaction mechanism**

The arylamine and nitrous acid then reacts.

### 1.5 Diazo coupling reactions

Diazo coupling is a facile electrophilic substitution reaction that is limited to substrates that have been activated. The diazonium salt combines with another arene (the coupling agent) in a diazo coupling reaction. As an electrophile, the diazonium salt reacts with the coupling agent's benzene ring. An azo compound, many of which are dyes, precipitates in a coloured form when the ice-cold solution of the diazonium salt is added to

a solution containing the coupling agent. The benzene ring's two or four positions of which one is occupied by the functional group are where the coupling agent always reacts. Depending on which coupling agent is interacting with the diazonium salt, a particular compound will have a different colour.

A wide variety of azo compounds can be created by reacting amines with distinct diazonium salts. When phenols and diazonium salts are coupled, azo compounds with the azo group -N=N-are produced.

## 1.6 Pharmacological properties

Recent years have seen a great deal of research on aromatic azo phenol derivatives because of their wide range of pharmaceutical applications, primarily because of their cost, a simple, reproducible synthetic approach that works well. Combining aromatic substituted amines with coupling agents like this could result in a variety of azo compounds with adaptable biological characteristics.

The biological properties of phenolic compounds, which are simple, naturally occurring compounds with an aromatic ring and one or more OH groups, have drawn a lot of attention.These properties include anticarcinogen, antimicrobial, and anti-diabetic properties. A class of substances known as hydroxytriazenes, which have an alpha hydroxyl group in comparison to a diazo group, have a variety of pharmacological effects, such as lowering cholesterol and acting as antidiabetic, antioxidant, anti-inflammatory, and antimicrobial agents[8]. Pharmacological chemistry has paid much attention to phenolic

compounds fused with azo moiety due to their diverse therapeutic properties and the feasibility of synthesizing azo derivatives.

## 1.7 Applications of solvatochromism

In theory, solvatochromism can be utilised to create molecular switches and sensors in molecular electronics. Solvatochromic dyes are utilised in the measurement of solvent parameters, which can be utilised to forecast the appropriate solvents for specific applications and explain solubility phenomena of solvatochromism.

## 1.8 Antibacterial Activity

A molecule's ability to inhibit the growth of bacteria and viruses or kill them locally without causing significant harm to surrounding tissues is known as its antibacterial and antiviral activity. Antibacterial substances are the most important ones in the fight against infectious diseases. However, because antibacterial medications are so widely used and abused, the emergence of bacterial resistance to these medications has become a serious problem for the pharmaceutical industry. Developmental events, like taking antibiotics, are the most common cause of resistance because they produce inheritable resistance. The bacteria's increasing resistance to antibiotics has resulted in serious health issues in recent years. Most harmful bacteria are resistant to at least one common antibiotic used to treat the infection. This problem motivates the search for new materials that can effectively inhibit the growth of microorganisms. We studied anti-bacterial activity of our three azo derivatives against

E.coli ,gram negative and streptococcus aureus, gram positive using well diffusion method.

## 1.9 UV spectroscopy

Electronic transitions between energy levels are related to the UV and visible spectra of substances. The transitions typically occur between bonding or lone pair orbitals and non-bonding or antibonding orbitals. The relationship between the absorption intensity and the light's absorbed UV/VIS spectrum.

### 1.9.1 UV-visible spectrophotometer

The instrument used in ultraviolet-visible spectroscopy is a UV/Vis

Spectrophotometer. It calculates the intensity of one light beam that passes through a Sample and compares it to the intensity of another beam of the same wavelength that does not pass through the sample (Io). The transmittance is defined as the ratio I/Io.

## 1.10 IR spectroscopy

The infrared section of the electromagnetic spectrum, or light with a longer wavelength and a lower frequency than visible light, is an aspect of infrared spectroscopy. Infrared spectroscopy is the study of a molecule's interaction with infrared light.

Three methods can typically be used to analyse the idea of IR spectroscopy: reflection, emission, and absorption measurements.The primary application of infrared spectroscopy is to identify the functional

groups of molecules, which are important for both organic and inorganic chemistry[9].

### 1.11 NMR ;Nuclear magnetic resonance spectroscopy

According to the NMR principle, many nuclei have spin and all nuclei are electrically charged. It is possible to move energy from the base energy to a higher energy level by applying an external magnetic field. Many nuclei are electrically charged and have spin. Energy can move more easily from base energy to higher energy levels when there is an external magnetic field present. A wavelength that corresponds to the radio frequency is used to transfer energy. At the same frequency, energy is also released when the spin returns to its base level. Measuring the signal corresponding to this transfer yields the NMR spectrum processing for the relevant nucleus.

### 1.12 computational chemistry

A subfield of chemistry called computational chemistry makes use of computer simulation to solve chemical puzzles. It computes the structures and characteristics of molecules and solids by using theoretical chemistry techniques integrated into effective computer programs. The primary premise is that molecules properties can be computed by using the Schrodinger equation to solve the molecule. Although the data from chemical experiments is typically supplemented by computational results, in certain situations, unobserved chemical phenomena can be predicted. It is frequently utilised when creating novel compounds.

The techniques used address both static and dynamic scenarios. The size of the system under study is always accompanied by a sharp increase in

computer time and other resources (like memory and disc space). A single molecule, a collection of molecules, or a solid can make up that system. The foundation of ab initio (from first principle) methods is quantum mechanics and fundamental physical constants. Because they use extra empirical parameters, other approaches are referred to as empirical or semi-empirical. The Born-Oppenheimer approximation is based on both ab initio and semi-empirical methods. It assumes that nuclei remain in place during the calculation, unlike electrons, which greatly simplifies the underlying Schrödinger equation. As the number of approximations is decreased, ab initio methods should, in theory, eventually converge to the exact solution of the underlying equations. The specifics of the electronic structure may not always matter.

### 1.12.1 Gaussian software

Gaussian is a computational chemistry computer programme that was first made available as Gaussian 70 by Carnegie-Mellon University researcher John Pople and his team. It has been regularly updated ever since. The term refers to Pople's decision to employ Gaussian orbitals instead of Slater-type orbitals in order to accelerate Hartree-Fock calculations on the then-available computer hardware's constrained processing power. It was created and licensed by Gaussian, Inc. and was initially accessible through the Quantum Chemistry Programme Exchange. Gaussian gained popularity and widespread use as an electronic structure programme very quickly[10]. The package's development was pushed by Prof. Pople, his students, and post-docs, among others, so that they could conduct cutting-edge research in quantum chemistry and other areas.

### 1.12.2 Density functional theory (DFT)

In physics and chemistry, DFT is a quantum mechanical modelling technique used to study the electronic structure of molecules. According to this theory, functionals, or functions of another function—in this case, the spatially dependent electron density can be used to determine the properties of a many-electron system. Because of this, the theory known as density functional theory derives its name from the functionals of electron density. The two theorems put forth by Hohenberg and Kohn form the basis of this theory: (1) any molecule's ground state property is functionally related to its electron density; and (2) any trial electron density function will yield an energy greater than the true ground state energy. In the fields of computational physics, computational chemistry, and condensed-matter physics, DFT is one of the most widely used and adaptable techniques. While requiring less computer time, DFT methods with hybrid functionals are as accurate as high-level ab intio methods. The most well-known hybrid functional was created in 1993 by Becke, Lee, Yang, and Parr and is called B3LYP[11].

### 1.12.3 Basis set

A basis set is a collection of mathematical operations that, when combined linearly, produce molecular orbitals. Atomic nuclei are typically the focal point of the functions. The orbitals in standard basis sets for electronic structure computations are formed by linear combinations of Gaussian functions. Numerous pre-defined basis sets are available in Gaussian, and they can be categorized based on the variety of basis functions they include. To approximate an atom's orbitals, basis sets assign a set of basis functions to each atom in a molecule. The Gaussian functions that make

up these basis functions are called primitives. These Gaussian functions are combined in a linear fashion[10]. Uncontracted is the term used to describe a basis function that consists of a single Gaussian function.

**1.12.3.1 STO-3 G:** This is called a minimal basis set. It has just enough contacted Gaussian functions for each atom. 3 Gaussian primitives per function. A single Gaussian gives a poor representation of a slater function, but this approximation can be improved by using a linear combination of Gaussians.

H;1s : C;1s,2s,2px,2py,2pz

**Double zeta basis set**

Two sizes of contacted functions are used for each atomic orbital.

**Split valence basis set**

Multiple contracted Gaussians are used for valence atomic orbitals.

E.g.: 3-21 G

The basis set split each valence orbital into two parts, an inner shell and an outer shell. The basis function of the inner shell is represented by two Gaussians, and that of the outer shell by one Gaussian; the core orbitals are each represented by one basis function, each composed of three Gaussians. Thus H and He have a 1s orbital split into ( 1s inner) and ( 1s outer), for a total of two basis functions.

6-31G

6 primitive Gaussians in one contracted core function. 2-contracted functions in valence region; one consisting of 3 gaussians and the other consisting of 1 primitive Gaussian.

### 1.12.3.2 Polarized basis functions

Molecules can be more accurately described by supplementing the split valence basis set with d functions, called polarization function. The term arises from the fact that d functions permit the electron distribution to be polarized. The SCF process can create a more anisotropic electron distribution thanks to polarisation functions.

The 3-21G basis set augmented where appropriate with d functions is called the 3-21G* or 3-21G(d) basis; the asterisk indicates polarization functions (d in this case), and the parentheses mean that the extra polarization functions are present only beyond the first row**.**

### 1.12.4 B3LYP

Density functional theory (DFT) uses a class of approximations known as hybrid functionals to approximate the exchange correlation energy functional. These approximations combine a portion of exact exchange from Hatreefock theory with exchange and correlation from other sources (either empirical or ab initio, like LDA). Known as an implicit density functional, the exact exchange energy functional is expressed not in terms of the density but rather in terms of the Kohn Sham orbitals. The 3-parameter Lee-Yang-Parr, or B3LYP, version of Becke is one of the most widely used ones.

## 1.13 Molecular docking

Putting molecules in the right arrangements so they can interact with a receptor is a process known as molecular docking. A cell's natural process of molecular docking takes place in a matter of seconds. The study of how two or more molecular structures fit together is known as "molecular docking" in molecular modelling.

For the creation of new drugs and the research of protein-ligand interactions, computational docking is frequently utilised.[12] Usually, the procedure begins with a target whose structure is known, like the crystal structure of an enzyme of potential medical value.The bonded conformation is then predicted using docking. tiny molecule targets are bound by their binding free energy.

### 1.13.1 Auto Dock Vina Software

Auto Dock is a collection of open-source, free tools for virtual receptor screening and computational docking of small compounds. There are now a number of supplementary tools in the suite[13].The molecular docking software Auto Dock Vina is open-source. In the Molecular Graphics Lab at The Scripps Research Institute, it was initially created and put into use by Dr. Oleg Trott.

## 1.14 Scope and Possibilities

Due of the recognised consequences on a variety of common and general diseases like cancer, allergic reactions, cardiovascular disease, infectious

diseases, parasitic diseases, type 2 diabetes mellitus, and others, interest in and attraction to natural substances are steadily growing. The most significant in vitro and in vivo biological actions, such as antibacterial, antimalarial, antidiabetic, anti-cancer, and antifungal, were highlighted in this study. Derivatives of chalcones have demonstrated antibacterial action against both Gram-negative and Gram-positive germs, as well as anticancer activity against a number of cancer cell lines. Clinical trials with chalcones have demonstrated good plasma concentrations, a lack of side effects in individuals with chronic venous insufficiency, and a reduction in clinical signs and symptoms although conducted in small number. However, further clinical research is required to completely comprehend the cellular mechanisms of action and establish links between their structure and pharmacological actions, particularly anticancer activity.

Although they demonstrated a variety of intriguing biological effects and allowed for a wide range of preclinical testing, their exact mode of action is unknown. Future research must focus on creating new synthesis techniques that enable the investigation of novel biological properties, a deeper understanding of molecular mechanisms of action, and in particular the identification of the action's target. In order to discover new drugs and pharmaceutical forms using modern methods, particularly new nano-formulations, in order to increase their bioavailability, prolonged effect, or transport to the target of action, this successful story of the promising therapeutic effects of chalcones is applicable.

EFFECT OF SOLVENT POLARITY ON THE ELECTRONICABSORPTION SPECTRA OF SOME PHENYL AZO MOIETIES OF2-NAPHTHOL, RESORCINOL AND 1,2DIHYDROXYANTHRAQUINONE By HAMILTON-AMACHREE A. AND MENEGBO LEDESI ISRAEL is one among the work which was an inspiration for our project.[11].1-(4-carboxy phenylazo)-2-naphthol is an example of a compound which shows solvatochromic effect. It is an azo compound. They have done the effect of solvents on these compounds by dissolving in ethanol and acetonitrile. It shows bathochromic shift: λmax increases with increase in solvent polarity.

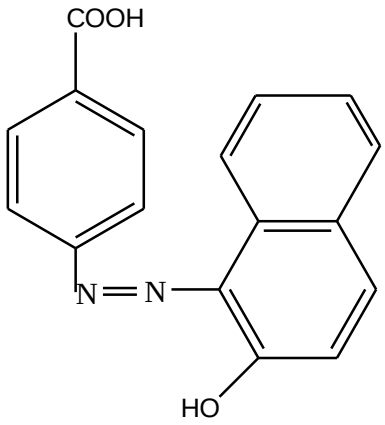



**Fig3. 1-(4-carboxy phenylazo)-2-naphthol**

## 1.15 Objectives of current work

- To study the effects of solvent on the electronic spectra of p-nitroaniline derivatives of azo compounds by recording the UV-VIS spectra of these compounds in various solvents.
- To optimize the ground state structures by DFT calculations to compare the HOMO-LUMO gaps and Absorption maxima in the solvents.
- To study the antibacterial activity of azo compounds by experimental method.
- To Compare the Antibacterial activity of azo compounds by experimental and Molecular Docking method.

# Chapter 2

## Literature Survey

MAMDOUH S.MASOUD and his coworkers have studied solvatochromic behavior of the electronic spectra of azo derivative[14]. The intramolecular charge transfer between the compounds and their tautomerism is also explained. The combination of electron-donating and -withdrawing moieties in the molecules determines the UV-Vis absorption bands of azo dyes because they contain intramolecular charge-transfer chromophores. Strong electron-donating and withdrawing moieties are present in the azo dyes under investigation.

ISA SIDIR and colleagues conducted a study using electronic absorption spectra to examine the solvatochromic behaviour of certain mono azo derivatives. UV -vis spectral shifts which are solvent dependent and other parameters like refractive index and dielectric constant were analysed.Using linear regression analysis, the electronic transitions are assigned, and the solvent-induced spectral shifts are examined in connection to various solute-solvent interaction mechanisms. They were able to approximate the contribution of each kind of interaction to the spectral shift in the molecule under study with the aid of fitting coefficient results that came from the analysis[15]. The electronic nature of the solvent's chemical makeup and the electronic nature of its substituents are determined to be the primary factors contributing to the observed solvatochromism.

RUTH SAHILU and her coworkers done a study on anti-bacterial activities on azo dye derivatives combined with in-silico molecular docking and DFT analysis. Eight azo dye derivatives were created by condensing 1-naphthol, resorcinol, and phenol with p-nitroaniline, o-toluidine, and p-chloroaniline. Four of these derivatives are novel, and UV-Vis, 1H, 13C, and DEPT-135 NMR were used to characterize their structures. Using penicillin as the reference medication, the dyes in vitro antibacterial activities against Gram-positive and Gram-negative bacterial species were evaluated at concentrations of 50, 75, and 100 mg/ml. When tested against these tested bacteria, the majority of the synthesised dyes demonstrated encouraging activity. Molecular docking on these compounds done by Auto dock vina software[16] . These ligand dye compounds show good docking efficiency with DNA gyrase with binding affinity -7.4kcal/mol.

Another detailed investigation on the solvatochromism and potentiometric studies on active nitroso and nitroso-azo- compounds.Studies on the stability of biologically active N,N~-bis-[4,4}-(1,3-diphenyltriazine)], 2,4-dinitrosoresorcinol, and o-carboxy phenylazo-dinitrosoresorcinol-diacetamide, 2-amino-3-hydroxy-pyridin-6-ylazo, 2-amino-6-phenylazo-pyridin-3-ol, 4-(2-amino-3-hydroxy-pyridin-6-ylazo)-benzoic acid .Both N-[4-(2-amino-3-hydroxy-pyridin-6-ylazo)-phenyl] and the ethyl ester of benzoic acid-acetamide combinations were investigated by HESHAM MOSTAFA and coworkers. Potentiometric analysis was used to determine the dissociation constants. The dissociation's thermodynamic parameters were assessed. The various parameters are correlated using

regression analysis. The findings aid in determining the solvatochromic potential and solute-solvent interactions of the compounds under investigation[17]. The main causes of the observed solvatochromism are the chemical makeup of the solvent and the electronic nature of the substituent.

DRAGOS LUCIAN ISAC and his coworkers have done a theoretical study on charge transfer excitations in azobenzene compounds. When the maleimide (MI) functional group was substituted, CT transitions in azobenzene (AB) were seen. In this work, the excited states of the AB–MI structures of eight azo derivatives are systematically studied theoretically. Our calculations reveal a CT between the azo moiety as a donor and the MI group as an acceptor, in addition to the two main azo transitions ($\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$). The number and location of the MI fragments can be used to describe the CT mechanism. Low-energy transitions caused by the MI groups in the azo structure introduce a CT character and alter the main transitions' order[18]. The CT character of these derivatives is confirmed by calculations using both advanced molecular orbital theories and density functional theory (DFT).

A detailed study on intramolecular charge transfer of azo dyes were done by BAILI CHEN and coworkers. Azo dye molecules were synthesised and their various intramolecular charge transfer (ICT) properties were examined. Thermogravimetric analysis (TGA) was used to investigate their thermal stabilities. UV-Vis and fluorescence spectroscopy were used to examine their linear optical properties[19]. Using the optical method and △-Self-consistent-field density functional theoretical (SCF-DFT, or

simply △SCF) calculation, the ICT strength of the azo Dyes was examined. As ICT strength increases, the fundamental gap, energy gaps (E.g.) between HOMO and LUMO, and optical gap decreases.

UV spectroscopic studies and charge transfer properties of azo benzene compounds along with experimental and density functional theoretical study were done. This study investigates the fluorescence emission and UV-Vis absorption of newly synthesised iron hexafluorophosphate (Fc-azo), η6-4-(4-nitrophenylazo) phenoxy benzene, and η5-cyclopentadienyl. Density functional (DFT/B3LYP and TD-DFT) calculations were used to determine the orbital energy, geometrical structure, absorption spectra, and first hyperpolarizability (β) values of the The hydroxyl azobenzene compound and Fc-azo were contrasted.The calculated values and the observed spectra were found to be in good agreement and this work was done by GUANG LEI LI and coworkers[20]. Charge transfer within the molecule was demonstrated by the positive solvatochromism of the UV–Vis absorption of Fc–azo upon the increase in solvent polarity from the experiment and the computed HOMO and LUMO energies.

An extensive study on the computational and solvatochromic UV -Vis absorption and antibacterial studies on azo compounds were done by DARA MUHAMMED.A .A standard azo coupling procedure was used to create the novel azo-azomethine derivatives (3a-j) at a temperature of 0–5 °C. The structure elucidation was carried out precisely using a number of spectroscopic techniques, such as FT-IR, NMR, and HRMS. Computational analysis of global reactive descriptors, Mulliken atomic charges, and molecular geometry was done to gain a better understanding

of the molecular properties. Additionally, reduced density gradient analysis (RDG) and molecular electrostatic potential (MEP) were investigated. A solvatochromic UV-Vis absorption study was carried out using different solvents. The type and energy of synthetic molecule binding to bacterial proteins were examined using a molecular docking study[21]. The compounds were found to have excellent antibacterial activity upon antibacterial activity testing.

A study on the electronic spectra, solvatochromic behaviour and acid-base properties of some azo compounds were done by NASR M RAGEH. Three azo cinnoline derivatives electronic spectra have been examined in both pure and mixed organic solvents with various properties, along with the impact of the compounds' concentration in each solvent. The appropriate electronic transition has been assigned to the various bands that have been observed. An intermolecular CT transition is responsible for the longer wavelength band that the para nitro cinnoline derivative in dimethylformamide (DMF) solution displayed. We looked into the solvated H-bonding complexes that developed between the para nitro derivative and DMF. The values of Kf and $\Delta G$ for these complexes have been established. The spectra in an aqueous-methanolic solution with different pH values were used to calculate the acidity constants of the para nitro compound[22]. Temperature's impact on p-NO2's longer wavelength visible band has been investigated.

ANIMESH KARMAKUMAR and associates synthesised, characterised spectroscopically, and theoretically investigated the charge-transfer complex of 4-(2-thiazolylazo) resorcinol (TAR) in conjunction with 3, 5-

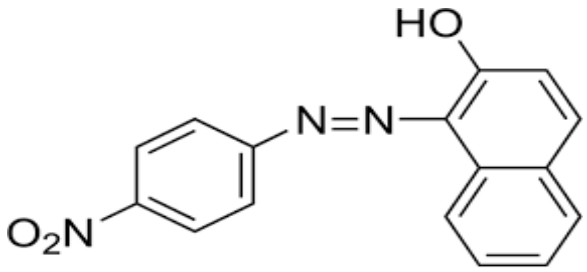
dinitrosalicylic acid (3,5-DNSA), picric acid (PA), and chloranilic acid (CLA) [20]. Using the mole ratio method, the prepared complexes molecular composition was ascertained. In methanol solvent, it was discovered that the stoichiometry of all prepared complexes is 1:2 (donor: acceptor). High complex formation constant values indicated a high degree of complex stability. Using UV-Vis spectroscopy in the same solvent, various spectroscopic parameters were determined, such as the donor's ionisation potential (ID), oscillator strength (f), transition dipole moment (μEN), etc[23]. These compounds show good antibacterial and antifungal properties.HOMO and LUMO interactions were performed by optimized structures of complexes.

# Chapter 3

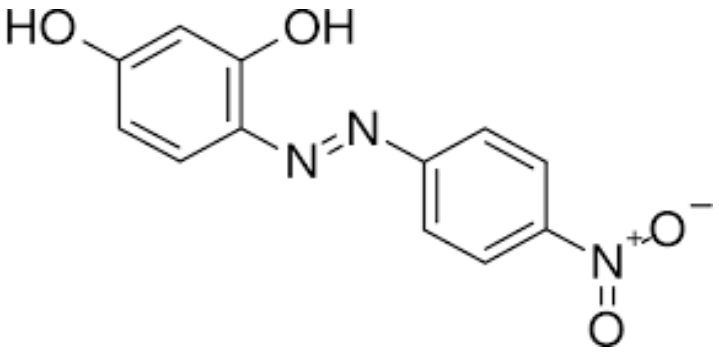
## Materials and Methods

### 3.1 The azo compounds we chosen are:

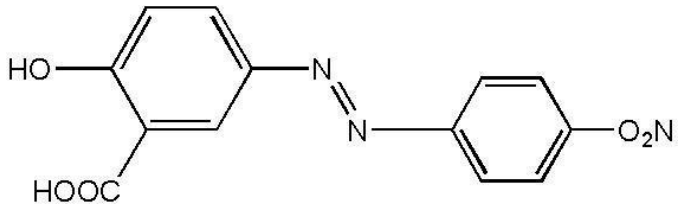
1. NAA : 1-(4-nitrophenylazo)-2-naphthol




2. NAR : p-nitrobenzeneazoresorcinol



3. NAY : 5-[(p-Nitrophenyl)azo]salicylic acid




### 3.1.1 Chemicals required

- Conc.HCl
- P-Nitroaniline
- NaNO2
- Salicylic Acid
- 1-Naphthol
- Resorcinol
- NaOH
- NaCl

## 3.2 Procedure

### 3.2.1 Basic step in the preparation of our samples; diazotization reaction

- In a test tube, add 1.5 mL of water and 1.5 mL of conc. HCl and place the test tube in an ice water bath.
- In a 25 mL RBF, add 0.7 g of nitroaniline (5 mmol), 0.38 g (5.5 mmol) of sodium nitrite (NaNO2), 1.5 mL of water and a magnetic stir bar. Stir the contents rapidly using a stirrer/hotplate.
- Remove the test tube from the ice water bath and place the RBF in the bath. Add the contents of the test tube to the RBF and stir gently for 10 minutes.
- Filter the solid into a test tube using a glass funnel and a small cotton plug [24].

### 3.2.2 synthesis of NAA

- In another RBF add 0.74 g of 1-napthol and 2.5 M NaOH and mix them by keeping in a magnetic stirrer.
- Add contents from test tube slowly with stirring and continue stirring for 10 minutes by keeping them in an ice bath.
- Slowly add 1.5 ml conc. HCl.
- Then add 1g of NaCl and heat the RBF in a thermowell until dissolved.
- Cool it in room temperature and keep in ice bath for 15 minutes.
- Filter, it using vacuum filtration with a Buchner funnel and wash the solid with 5 ml of water.

- Dry the solid we extracted[24].

**Fig4. NAA**

### 3.2.3 synthesis of NAR

- In another 25 mL RBF with a magnetic stir bar, dissolved 0.56g of resorcinol (5.1 mmol) in 10 mL of 2.5 M aq. NaOH and placed in an ice- water bath.
- Added the contents of the test tube slowly while stirring and continued stirring for 10 minutes while in the ice-water bath.
- Slowly added 1.5 mL of conc. HCl. Added 1 g of NaCl and heated the RBF until dissolved.
- Cooled the reaction to room temperature then placed in an ice-water bath for 15 minutes.
- Finally filtered the solid using vacuum filtration with a Buchner funnel and washed the solid with approximately 5mL of water[24].

**Fig5. NAR**

### 3.2.4 synthesis of NAY

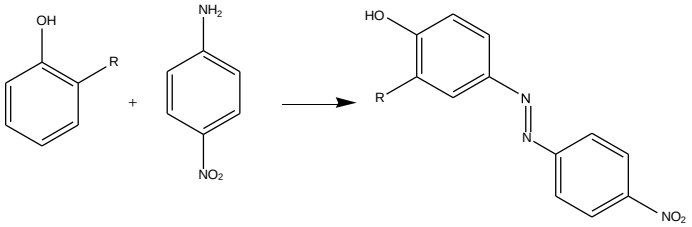
- In another 25 mL RBF with a magnetic stir bar, dissolve 0.74 g of salicylic acid (5.4 mmol) in 10 mL of 2.5 M aq. NaOH and place in an ice-water bath.
- Add the contents of the test tube slowly while stirring and continue stirring for 10 minutes while in the ice-water bath.
- Slowly add 1.5 mL of conc. HCl.
- Add 1 g of NaCl and heat the RBF using a thermowell until dissolved (check with the instructor to ensure the set-up is correct).
- Cool the reaction to room temperature then place in an ice-water bath for 15 minutes.
- Filter the solid using vacuum filtration with a Buchner funnel and wash the solid with approximately 5 mL of water.

- . If no solid is precipitated, only keep the filtrate. If solid is precipitated, let the solid air dry and keep both the filtrate and the solid[24].

**Fig6. NAY**

## 3.3 General Reaction

### 3.3.1 NAR & NAY




R = OH, COOH

**Fig7. NAR & NAY synthesis reaction**

### *3.3.2 NAA*

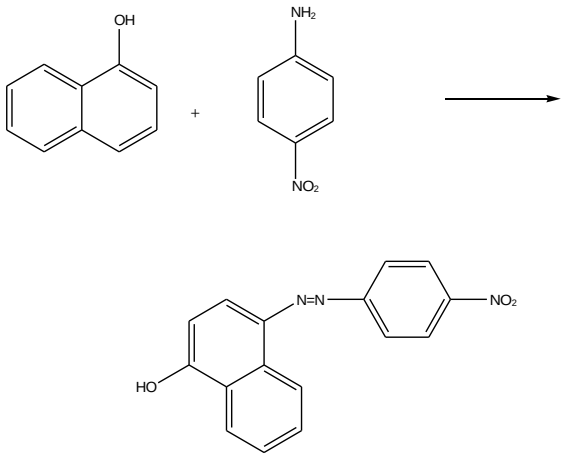



**Fig8. NAA synthesis reaction**

## 3.4 Method

### 3.4.1 UV Visible Spectroscopy

The UV and visible spectra of compounds indicate electronic transitions between energy levels, typically between bonding or lone pair orbitals and non-bonding or antibonding orbitals, with the absorption intensity influencing the spectral wavelength.

**Beer's law**: It states that the faction of the incident light absorbed is proportional to the quantity of absorbing molecules in the light-path, which will rise as concentration or sample thickness.

**Lambert's law**: It states that the fraction of the monochromatic light absorbed by a homogenous medium is independent of the intensity of the incident light and each successive unit layer absorbs an equal fraction of light incident on it.

From these two laws, the following empirical expression, known as Beer-Lambert law, may be formulated.

Log ($I_0$ /I) = Ɛcl= A

Where,

$I_0$= intensity of incident light

I = intensity of emergent light

Ɛ =molar absorptivity

C = concentration of solute in moles/liter

L =path length

A =absorbance

### 3.4.1.1 UV Visible Instrumentation

In ultraviolet-visible spectroscopy, a UV/Vis spectrophotometer is a tool used to measure the intensity of light passing through a sample and compare it to another light beam with the same wavelength and intensity. By comparing the intensity of the light beam with the light that did not pass through the sample, the transmittance ratio is computed. A light source, sample holder, diffraction grating, prism, and detector make up a spectrophotometer. LEDs, Deuterium arc lamps, Xenon arc lamps, and tungsten filaments are examples of radiation sources. A detector is a tool used to gather light from different sources, including arrays, charge coupled devices (CCDs), photomultiplier tubes, and photodiodes. CCDs and photodiode arrays, which can simultaneously gather light at various wavelengths on individual pixels or groups of pixels, are utilised in conjunction with fixed monochromators.

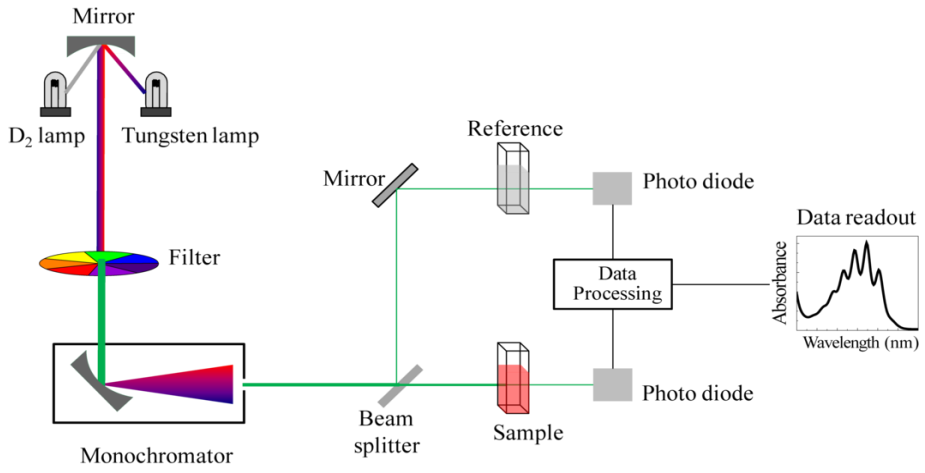



**Fig9. UV Instrumentation**[25]**.**

### 3.4.2 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) is a widely used technique to identify functional groups in materials like gas, liquid, and solid by measuring the absorption of infrared radiation by each bond in the molecule, resulting in a spectrum. Materials with covalent bonds absorb electromagnetic radiation in the IR region, which is lower energy and higher wavelength than UV-visible light and shorter than microwave radiation. To determine functional groups in a molecule, it must be IR active, with a dipole moment. When IR radiation interacts with the covalent bond of materials with electric dipoles, the bond starts back and forth oscillation, causing the change in the net dipole moment of the molecule. Atoms and symmetrical molecules cannot absorb IR radiation due to their lack of chemical bonds and zero dipole moment.

Infrared radiation absorbs light, creating vibration modes, relating to molecule bond nature. FTIR spectrum measures wavenumbers (4000-600 cm−1), directly related to energy and frequency.

### 3.4.2.1 FTIR Instrumentation

An infrared light source, an interferometer, a sample compartment, a detector, an amplifier, and a computer make up the FTIR spectrometer system. The sample is exposed to radiation from the light source, which is amplified and converted into digital signals. The interferogram is transformed into a spectrum using the fast Fourier transform algorithm. The primary component of the spectrometer is the Michelson interferometer, which consists of a moveable mirror, a fixed mirror, and a beam splitter. A sample signal's interferogram is gathered, the Fourier transform is used, and the spectrum is shown. Fourier transform infrared spectra is the name of this method (FTIR).

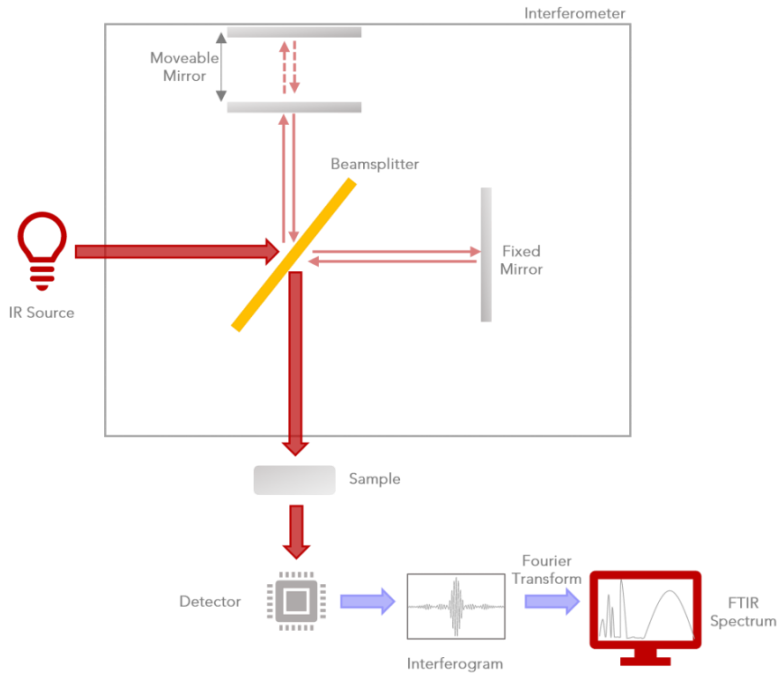



**Fig10. FTIR Instrumentation**[26].

### 3.4.3 Nuclear Magnetic Resonance Spectroscopy (NMR)

In a strong constant magnetic field, a weak oscillating magnetic field can disturb nuclei, causing the nucleus to produce an electromagnetic signal at its own frequency. The term nuclear magnetic resonance, or NMR, refers to this phenomenon. Nuclear magnetic resonance (NMR) is a powerful tool in modern science that can solve complex structures and interactions in heterogeneous samples. It has great potential for environmental research.

When utilising nuclear magnetic resonance (NMR) spectroscopy, a specific nucleus—typically the proton—is targeted by the spectrometer. The simplest method is the continuous wave (CW) method, which involves radio frequency radiation broadcasting, spinning a sample within

a glass tube, and monitoring the radiation released and absorbed. By changing the magnetic field or the radiation's frequency, the NMR spectrum can be obtained.

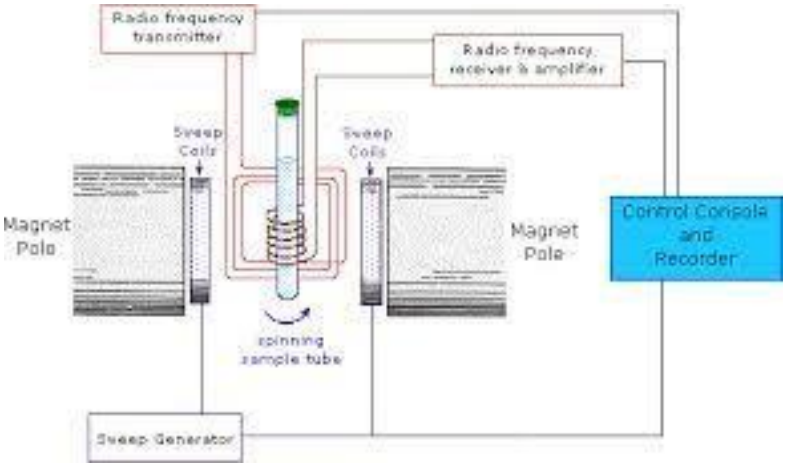



**Fig11. NMR Instrumentation**[27]**.**

### 3.5 UV spectral analysis

- UV visible absorption spectra were recorded using the following solvents: Acetone, Chloroform and Dichloromethane.
- $10^{-5}$M solutions of the samples in each solvent was prepared and 3.0ml of the solutions were used for recording the spectra.
- The spectra were recorded using Shimadzu UV 1800 double beam spectrophotometer.

### 3.6 DFT calculations

DFT calculations was performed using Gaussian 09 software package for windows.

- The calculations were done at B3LYP/6-31G(d,p) level
- The calculations in the solution phase were carried out using CPCM model which is part of the Gaussian software package.
- Using output from calculations we can
- visualize HOMO and LUMO orbitals.
- Calculate the HOMO-LUMO gap from the energies of HOMO and LUMO orbital.
- Calculate the excitation energy ($\lambda_{max}$) using TDDFT method.
- DFT is a quantum mechanical method used to investigate the electronic structure of molecules.
- Hohenberg and Kohn's two theorems serve as its foundation:

(1) any ground state property of a molecule is functional of the electron density

(2) any trial electron density function will give energy higher than the true ground state energy.

- DFT calculations are based on Kohn-Sham approach.
- The accuracy of DFT methods is comparable to high level *ab initio* methods but it uses less computer time.

In *ab initio* methods the total energy of an 'n' electron system is a function of 3n coordinates but in DFT calculations electron density is a function of only 3 coordinates.

### 3.7 Computational Chemistry

Computational chemistry techniques can be used for the investigation of reaction mechanisms, total energies of ground and excited state, transition states, etc. with the help of Schrodinger equation.

Using output from calculations we can

- visualize HOMO and LUMO orbitals.
- Calculate the HOMO-LUMO gap from the energies of HOMO and LUMO orbital.
- Calculate the excitation energy ($\lambda_{max}$) using TDDFT method.
- The results usually complement the experimental data.
- *ab inito* and DFT methods are highly accurate methods available in computational software's. Approximate methods are molecular mechanics and semi-empirical methods.
- There are free software and paid software.
- Free software - Games
- Paid software – Gaussian, spartan

### 3.7.1 Gaussian software

- Gaussian is first commercially available software for calculating molecular properties of all kinds of organic molecules and metal complexes, and currently, widely used for quantum –chemical calculations.
- Initially released in 1970 as Gaussian 70 and the current version is Gaussian 16, but the results described in this thesis are generated using Gaussian 09.
- Gaussian software incorporates a wide range of theoretical methods for calculations such as molecular mechanics, semi-empirical methods, Hartree-Fock, MPn (Mollar-Plesset perturbation theory of order n=2, 4), DFT methods.
- This software can be used to determine local minima of molecules, chemical reactivity, energies of molecules and transition states, intrinsic reaction co-ordinates of chemical transformations, IR, UV, and NMR spectra of molecules.

## 3.8 Determination of Antibacterial activity

### 3.8.1 methodology

#### 3.8.1.1 Preparation of nutrient media

Nutrient broth was prepared by dissolving 1.3 gm of nutrient broth in 100 ml distilled water. Five millilitres of nutritional broth were placed inside test tubes, which were then autoclave-sterilized.

Nutrient agar media was prepared by mixing 1.3gm of nutrient broth and 2gm of agar agar in 100 ml distilled water. The media was autoclaved and 20 ml each poured into sterile petri plates under aseptic conditions.

### 3.8.1.2 Preparation of microbial cultures

The test organisms Escherichia coli (E. coli) and Staphylococcus aureus were inoculated into 5 ml of sterilized nutrient broth and kept for overnight incubation at 37°C.

### 3.8.2 Well diffusion method

Cotton swabs that had been sterilised were used to create a lawn culture of every bacteria. After being sterilised, a swab was dipped into the bacterial suspension and moved from top to bottom, covering every possible area. To cover the entire plate with bacteria, the plate was rotated 90 degrees, and the same process was carried out again. After the lawn was ready, sterile well cutters were used to cut 6 mm diameter wells into agar plates. After labelling the wells, 20µL of sample (name the samples) was added to the appropriate wells. The samples' (give name) antibacterial activity was contrasted with that of the common antibiotics on hand. For twenty-four hours, this plate was incubated at 37°C. Using a standard ruler, the radius of each zone was calculated in centimetres. No colonies will grow if the substance is effective against bacteria at a particular concentration. This zone of inhibition serves as a gauge for the compound's efficacy; the greater the clear space surrounding the well, the more potent the compound.

### 3.8.3 Killing and disposing

After the experiment, the bacteria are destroyed by autoclaving the plates for 20 min. All the

glassware used for the experiment were also autoclaved to remove any bacteria if present.

## 3.9 Characterization

### 3.9.1 Ultraviolet-Visible spectroscopy

In ultraviolet-visible spectroscopy, light from the UV area is absorbed by the molecule, which causes the electrons to be excited from their ground state to a higher energy state. The chemical compounds' apparent colour is directly influenced by the absorption or reflectance within the visible range. Acetone was used to record UV visible absorption spectra. 10.0 ml of the prepared 10-5M solutions of the samples in solvent were used to record the spectra. Shimadzu UV 1800 double beam spectrophotometer was used to record the spectra.

### 3.9.2 FT-IR Spectroscopy

A type of vibrational spectroscopy is Fourier transform infrared spectroscopy. IR spectrophotometer was used to record IR spectra. The IR portion of the electromagnetic spectrum is the subject of infrared spectroscopy. The types of functional groups that are present in the ligands can be determined from their IR spectra.

### 3.9.3 Nmr spectroscopy

A typical nuclear magnetic resonance spectroscopy method is also used for the effective characterization of compounds by dissolving in acetone.

Significant peaks get with different chemical shift values are obtained which is useful to determine the structure of azo compound from their NMR spectra.

### 3.10 Molecular Docking

The three dimensional structure of the azo compounds and antibacterial drug for relative study were obtained by DFT calculations at B3LYP/6-31-G (d,p) level. The protein molecules were downloaded from RCSB protein data bank. Know drug molecule were downloaded from Pub chem. Docking of known drug molecule (phenazopyridine) and optimized azo compounds, with protein (3twd) were performed using Auto dock Vina. Gaussian 09 software package became used for DFT calculation and TD-DFT calculations. DFT calculations gives geometry optimization and IR spectrum calculation.TD- DFT calculations gives UV-visible spectrum. The calculations were done at B3LYP/6-31G(d,p) level. The calculations in the solution phase was carried out using CPCM model which is part of the Gaussian software package. HOMO and LUMO for all the molecules are identified. Gauss View 5 cowers finished the use of HPv185e workstation computer equipped with Intel 7 core processor and 24 GB RAM, Microsoft Windows as the operating system.

# Chapter 4

## Results and discussion

### 4.1 Mechanism for the synthesis of NAA ,NAR and NAY

Azo dyes are synthesized in a two step reaction process. In the reaction process aromatic diazonium ion are prepared from aniline derivatives. In the second step diazonium salt is coupling reaction with an aromatic compound. The precipitate formed, dried and recrystallized. The compounds were characterized by UV-Visible, infrared and Nuclear Resonance Spectroscopic methods. Solvatochromic effect of the compounds were studied by experimental and DFT calculations, since HOMO and LUMO orbitals of the compounds are located at two different ends of the same molecule.The general mechanism of synthesis of azo dyes are given below.

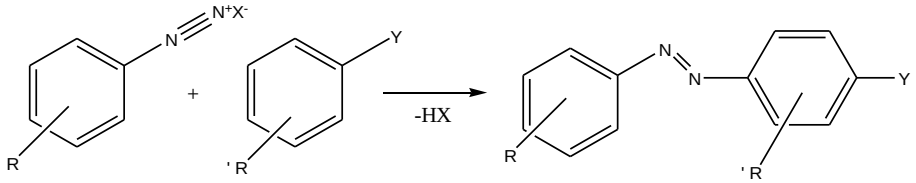



**Fig.12 General mechanism of synthesis of azo dyes.**

( R - $NO_2$, 'R - OH, COOH and Naphthalene and Y - OH)

## 4.2 Characterization of compounds NAA, NAR and NAY

### 4.2.1 UV-Visible Spectroscopy

The UV-Vis spectrum are present in the range of 200-500 nm. For azo compounds, a sharp peak is obtained in the range of 350-500. In the UV spectrum of synthesized azo dye are found out that over same range of 350-500 nm to the absorption of azo compounds. This peak is confirmed the formation of azo dyes.

**Compound 1 –NAA in Acetone**

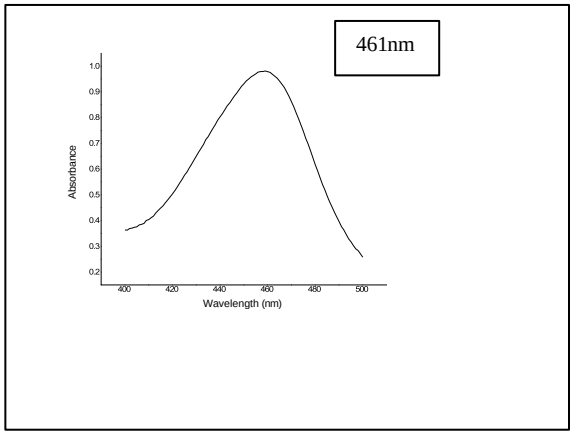



**Fig13.UV absorptionmaxima of NAA**

### 4.2.2 FTIR Analysis

The peak at 3376cm$^{-1}$ show the OH-stretching frequency of hydrogen bonded phenolic group. The peak at 1500cm$^{-1}$ shows the presence of azo group. The peak at 1316cm$^{-1}$ and 748cm$^{-1}$ shows the stretching N=O stretching frequency of aromatic nitro compound.

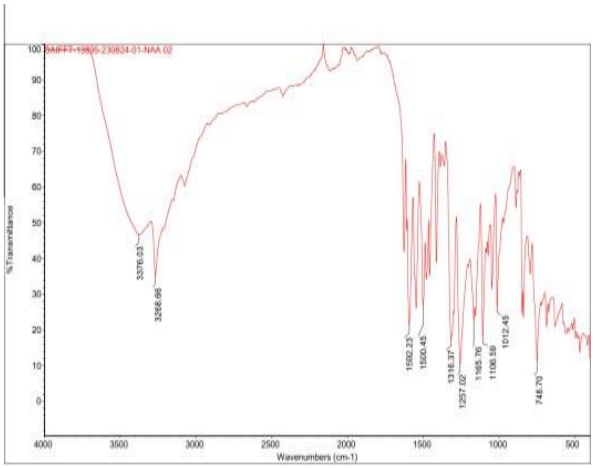



**Fig14. IR Spectrum of NAA**

### 4.2.3 NMR Analysis

The peak at 6.8-6.5ppm shows the aryl protons of NAA. The peak at 5.2ppm shows the presence of phenolic hydroxyl group. The peak at 8.3-7.2ppm shows the aryl protons of p-nitroaniline.

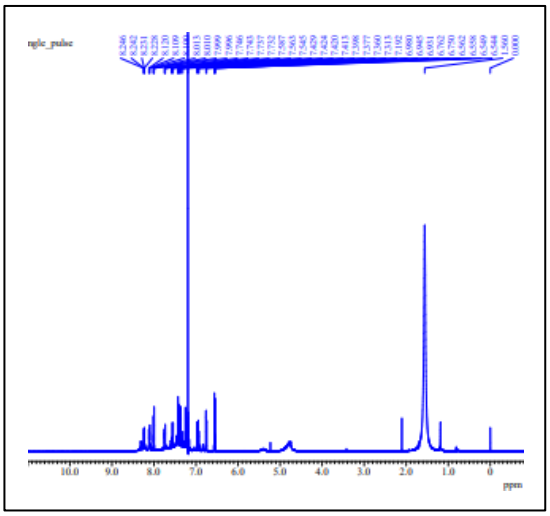



**Fig15. NMR Spectrum of NAA**

### 4.2.4 Compound 2 –NAR in Acetone

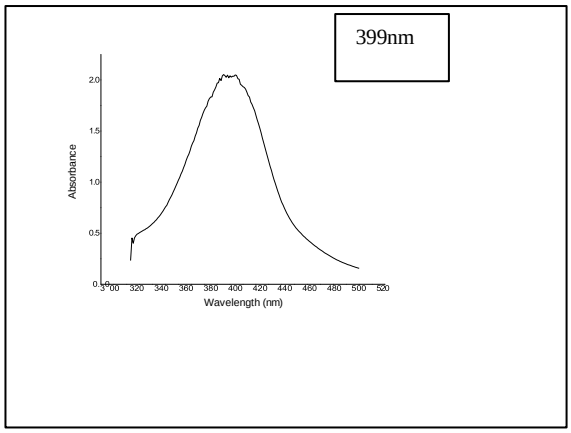



**Fig16.UV absorption maxima of NAR**

### 4.2.5 FTIR Analysis

The peak at 3366cm$^{-1}$ show the OH stretching frequency of hydrogen bonded phenolic group.The peak at 1444cm$^{-1}$ show the presence of azo group. The peak at 1329cm$^{-1}$ and 728cm$^{-1}$ show the N=O stretching frequency of aromatic nitro compound.

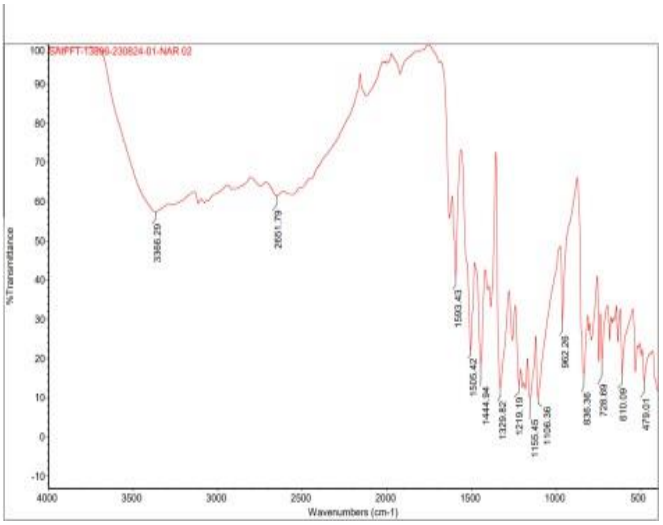



**Fig17. IR Spectrum of NAR**

### 4.2.6 NMR Analysis

The peak at 6.3-6.6ppm show the aryl protons of NAR. The peak at 4.6ppm show the phenolic hydroxyl group. The peak at 8.4-7.0ppm show the aryl protons of p-nitroaniline.

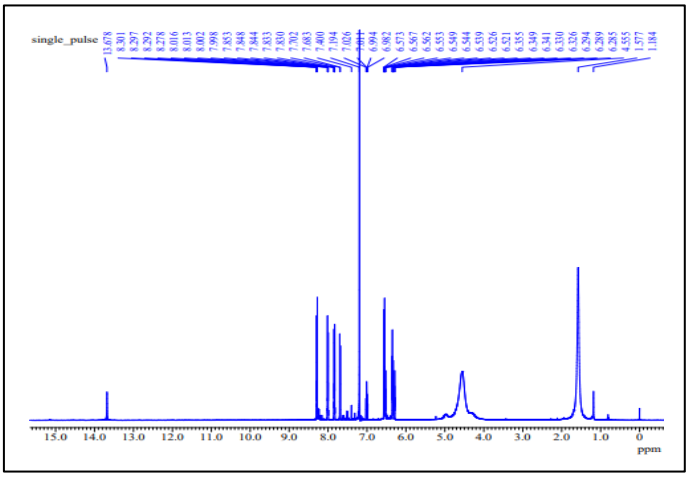



**Fig18. NMR Spectrum of NAR**

### 4.2.7 Compound 3–NAY in Acetone

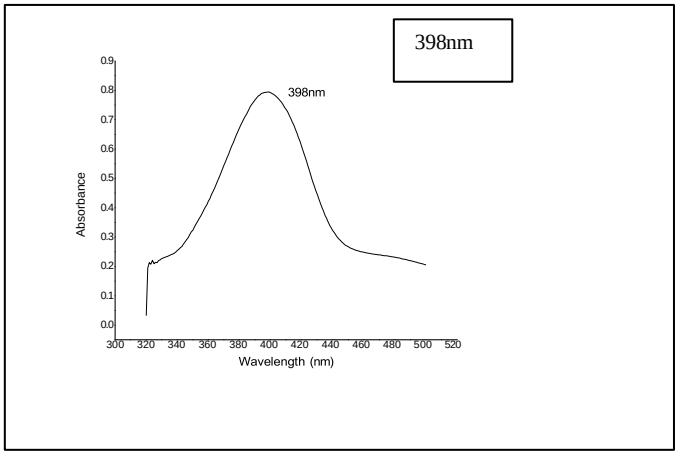



**Fig19.UV absorption maxima of NAY**

### 4.2.8 FTIR Analysis

The peak at 3461cm$^{-1}$ show the OH stretching frequency of hydrogen bonded phenolic group.The peak at1471cm$^{-1}$ show the presence of azo group. The peak at 1346cm$^{-1}$ and 753cm$^{-1}$ shows presence of aromatic nitro compound.

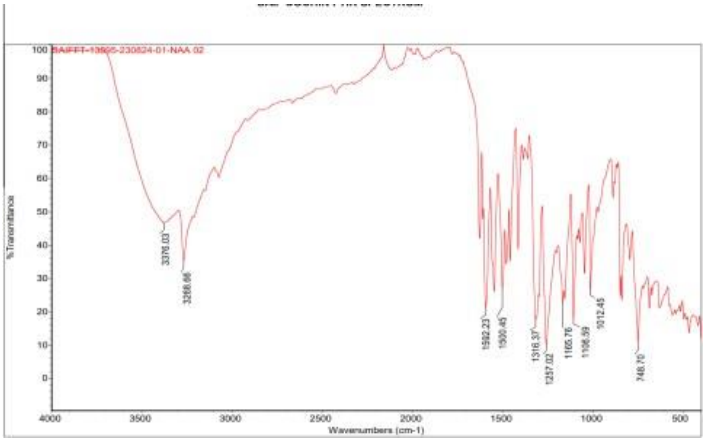



**Fig20. IR Spectrum of NAY**

### 4.2.9 NMR Analysis

The peak at 6.9-6.6ppm show the aryl protons of NAY. The peak at 8.3-7.6ppm show the aryl protons of p-nitroaniline. The peak at 3.2ppm show the phenolic hydroxyl group.

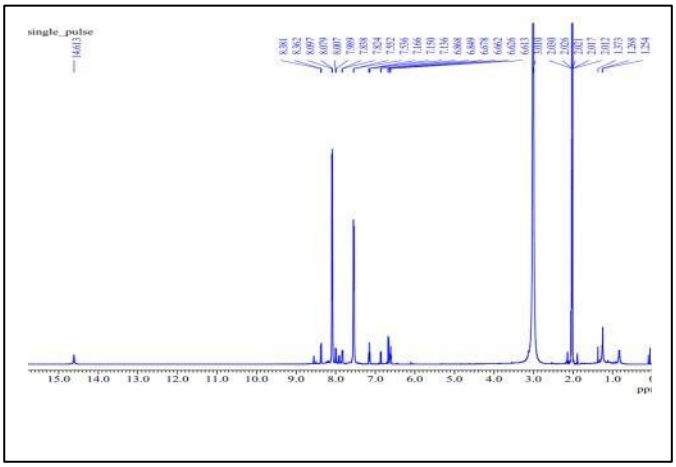


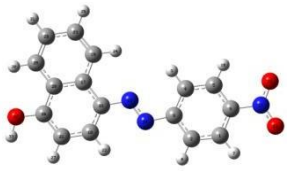
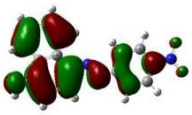
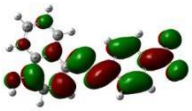
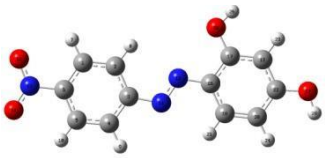
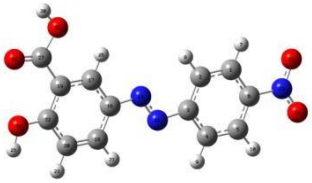
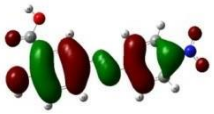
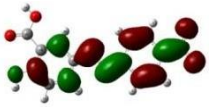

**Fig.21 NMR Spectrum of NAY**

## 4.3. Solvent Effect on UV-Visible Absorption Spectra-Solvatochromism

### 4.3.1 DFT Calculations

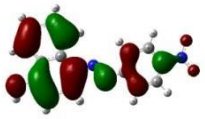
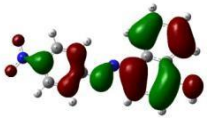
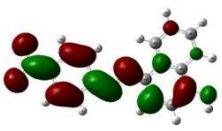
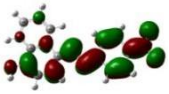
The geometry optimization of the compounds NAA, NAR and NAP were performed by DFT calculations. The ground state optimize structure and HOMO and LUMO orbitals of the selected NAA, NAR and NAP in the absence of solvent are given in **Table 1**. The HOMO and LUMO orbitals cover different regions of the molecules. Re-optimization of the compounds in various solvents show similar arrangement for the two orbitals and show that the electronic transitions occur from the HOMO to the LUMO orbital. Hence the absorption band in the UV spectrum corresponds to a CT transition. The charge transfer compounds show solvatochromism.

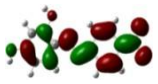
**Table 1. DFT Optimized structure, HOMO and LUMO orbitals of Compounds 1, 2 & 3 without solvent**

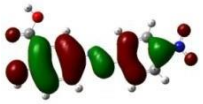
| Compound | HOMO | LUMO |
|---|---|---|
| NAA  |  -0.2135 |  -0.1073 |
| NAR  |  -0.22 |  -0.096 |
| NAY  |  -0.2396 |  -0.1119 |

The HOMO and LUMO orbitals of NAA, NAR and NAY in different solvents with increasing solvent polarity such as Acetone, Dichloromethane and Chloroform, are given in Table 1,Table 2,Table 3 respectively.

**Table 2. HOMO and LUMO orbitals of compound NAA**

| Solvents | HOMO | LUMO | HOMO-LUMO gap in eV | $\lambda_{max}$ (calculated) nm |
|---|---|---|---|---|
| Acetone |  |  | 2.69 | 460.44 |
| Dichloromethane |  |  | 2.73 | 454.44 |
| Chloroform |  |  | 2.74 | 454.34 |

| **Table 3 HOMO and LUMO orbitals of compound NAR** | | | | |
|---|---|---|---|---|
| Solvents | HOMO | LUMO | HOMO-LUMO gap in eV | $\lambda_{max}$ (calculated) nm |
| Acetone |  |  | 3.04 | 407 |
| Dichloromethane |  |  | 3.06 | 405 |
| Chloroform |  |  | 3.09 | 401 |

| **Table 4.HOMO and LUMO orbitals of compound NAY** | | | | |
|---|---|---|---|---|
| Solvents | HOMO | LUMO | HOMO-LUMO gap in eV | $\lambda_{max}$ (calculated) nm |
| Acetone |  |  | 3.31 | 375.07 |
| Dichloromethane |  |  | 3.32 | 373.35 |
| Chloroform |  |  | 3.34 | 370.84 |

The HOMO-LUMO gap is calculated by subtracting HOMO orbital energy from the energy of LUMO orbital and it is decreases with increases in solvent polarity. The absorption maxima values are calculated for NAA, NAR and NAP in different solvents and it is increases with increase in solvent polarity. To compare the calculated absorption maxima values

with the measured value, UV-Visible spectra of three azo compounds were measured in same solvents of different polarities. Both compounds show shortest wavelength for absorption maximum in non-polar solvent chloroform and largest absorption maximum is observed in polar solvent Acetone. With increasing solvent polarity, the absorption maxima of these compounds show a positive solvatochromic effect.

### 4.3.2 Solvatochromism

The charge transfer compounds shows solvatochromism. To study the properties of solvatochromism, the compounds are dissolve in different solvents according to increasing order of polarity by UV Analysis.

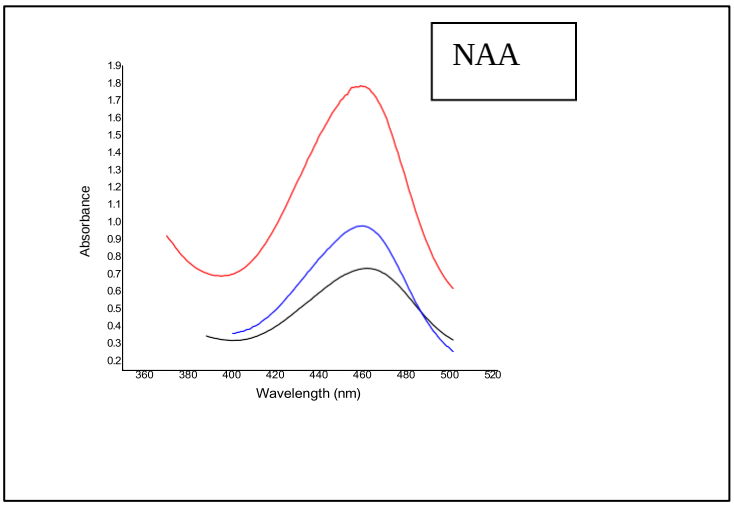



**Fig 22. UV-Visible absorption spectra of NAA in Acetone, Dichloromethane and Chloroform.**

| **Solvent** | **$\lambda_{max}$** |
|---|---|
| Acetone | 460.44 |
| Dichloromethane | 454.44 |
| Chloroform | 454.34 |

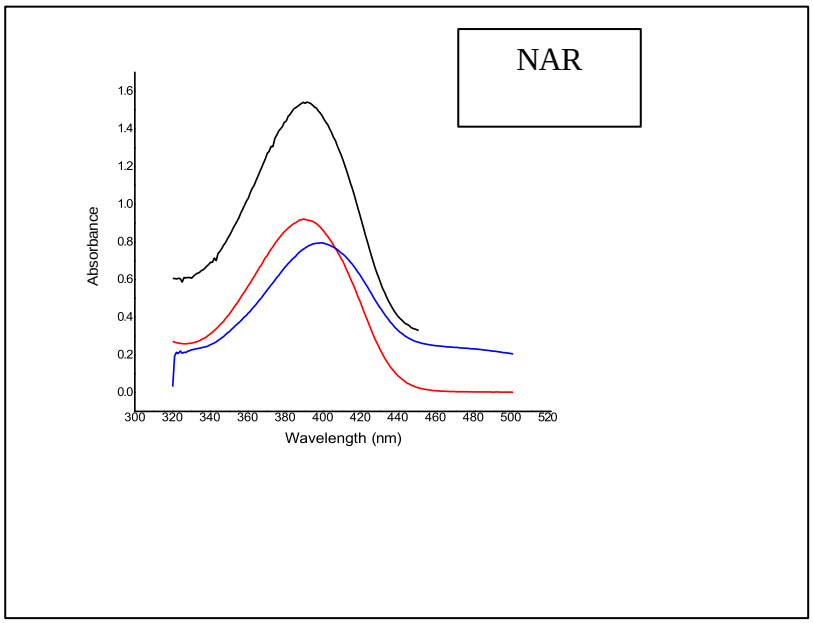



**Fig 23. UV-Visible absorption spectra of NAR in Acetone, Dichloromethane and Chloroform.**

| **Solvent** | **$\lambda_{max}$** |
|---|---|
| Acetone | 407 |
| Dichloromethane | 405 |
| Chloroform | 401 |

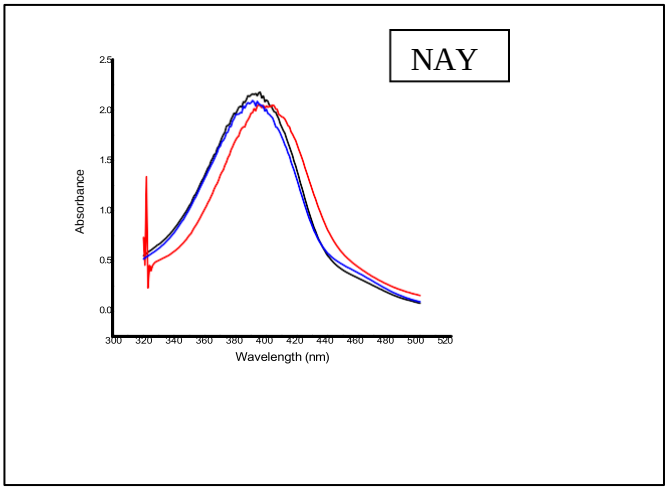



**Fig 24. UV-Visible absorption spectra of NAYin Acetone, Dichloromethane and Chloroform.**

| **Solvent** | **$\lambda_{max}$** |
|---|---|
| Acetone | 375.07 |
| Dichloromethane | 373.35 |
| Chloroform | 370.84 |

The positive solvatochromic shifts are observed in both calculation and measurement, Table 5.

**Table 5.Calculated and measured absorption maxima value of NAA, NAR and NAP in different solvents**

| **Solvent** | **NAA** | | | **NAR** | | | **NAY** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **λmax** | **Calc. λmax** | **HLG eV** | **λmax** | **Calc. λmax** | **HLG eV** | **λmax** | **Calc. λmax** | **HLG Ev** |
| Acetone | 461 | 460.44 | 2.69 | 399 | 407 | 3.04 | 398 | 375.07 | 3.31 |
| Dichloromethane | 460 | 454.44 | 2.73 | 396 | 405 | 3.06 | 391 | 373.35 | 3.32 |
| Chloroform | 458 | 454.34 | 2.74 | 391 | 401 | 3.09 | 389 | 370.84 | 3.34 |

### 4.4. Antibacterial activity of NAA, NAR and NAY

The bioassay results for antibacterial activity of the NAA,NAR and NAY are presented in the Table 6 respectively. Both the dyes are show different antibacterial against the tested organism. Azo dyes show the highest anti bacterial activity against staphylococcus aureus. Azo dyes shows no antibacterial activity against E.coli.

| **Table 6.Antibacterial activity of azo dyes** | | | | |
|---|---|---|---|---|
| **Name of the organism** | **Zone of inhibition in centimeter** | | | **Antibiotic Nalidixic acid** |
| | **NAA** | **NAR** | **NAY** | |
| E-coli | No Activity | No Activity | No Activity | 2.2 cm |
| S.aureus | 0.7cm | 0.9cm | 1cm | 2 cm |

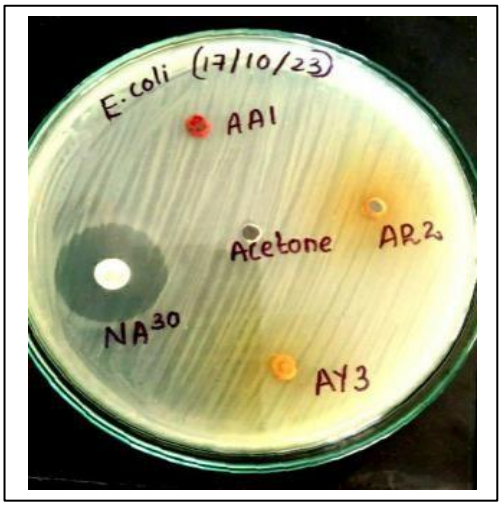



**Fig 25. Antibacterial activity against E.coli.**


**Fig 26. Antibacterial activity against staphylococcus aureus.**

## 4.5. Molecular docking studies

### 4.5.1 Antibacterial activity

In the molecular docking we use phenazopyridine as a drug and 3twd as a protein.Phenazopyridine show antibacterial activity against 3twd protein.

Binding affinity of phenazopyridine-3twd interaction found by molecular docking.Then the binding affinity of 3twd-azo dyes interactions by docking is calculated.The binding affinity of above interactions with standard is compared and predicted the antibacterial activity of azo dyes.

Azo dyes which show higher numerical binding affinity than phenazopyridine has a better antibacterial activity than phenazopyridine.

Docking of phenazopyridine with 3twd is shown in Fig.27

**Binding Energy = -7.9kcal/mol**

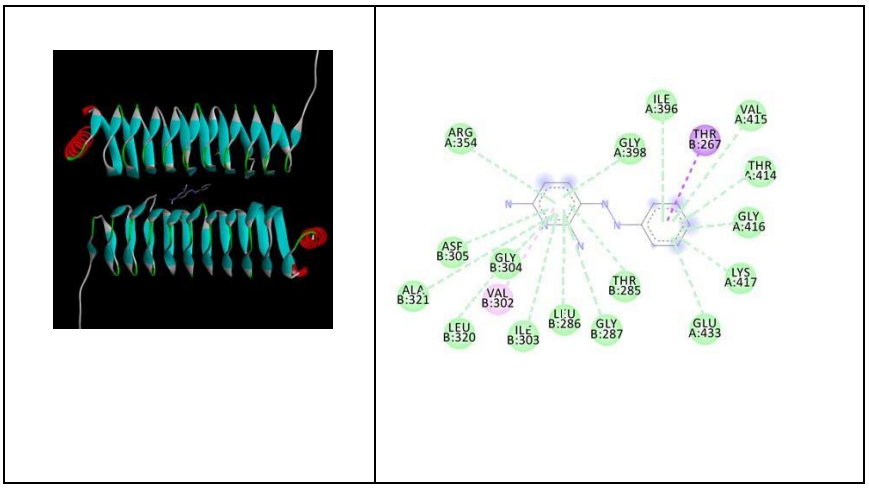



**Fig.27 Phenazopyridine-3twd interaction**

Docking of NAA with 3twd is show Fig.28

**Binding Energy = -7.6kcal/mol**

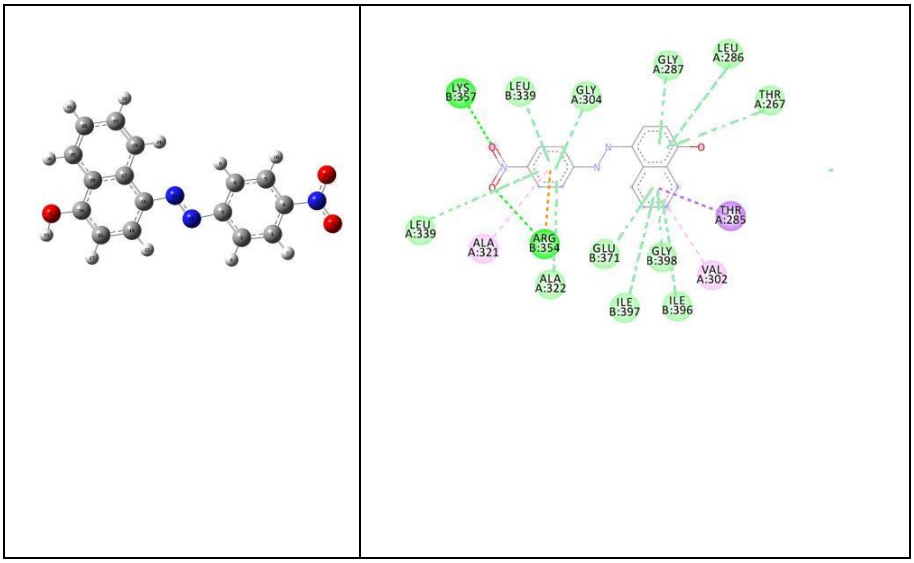



**Fig.28 NAA-3twd interaction**

Docking of NAR with 3twd is show in Fig 29

**Binding Energy = -6.5kcal/mol**

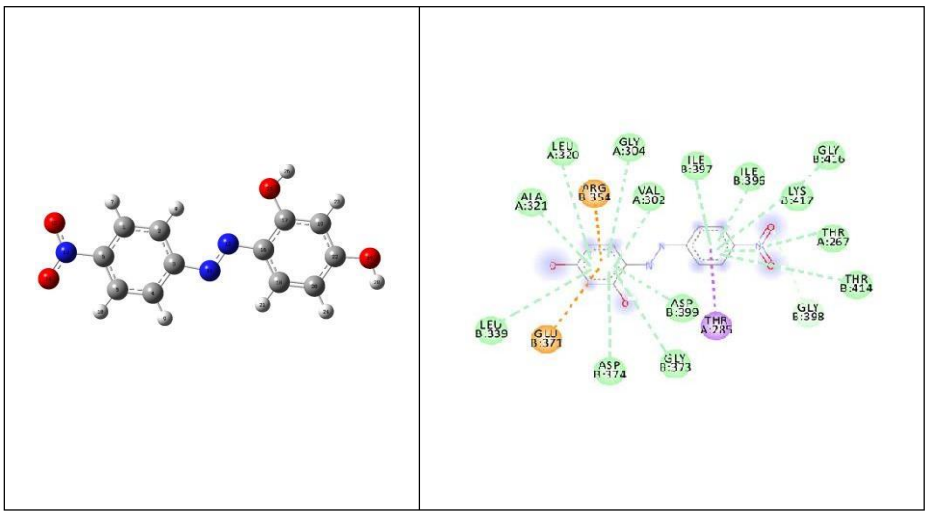



**Fig.29 NAR-3twd interaction**

Docking of NAY with 3twd is show in Fig 30

**Binding Energy = -9.6kcal/mol**

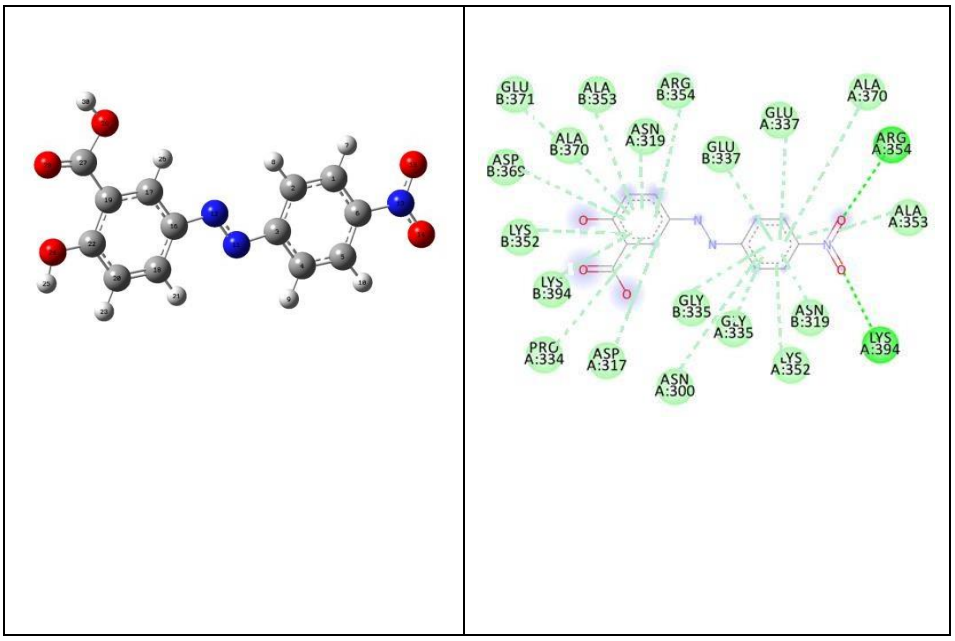



**Fig.30 NAY-3twd interaction**

Anti bacterial activity of azo dyes is shown in table 7

**Table 7.Binding energy of azo dyes.**

| Compound | Binding energy(K cal/mol) |
|---|---|
| NAA | -7.6 |
| NAR | -6.5 |
| NAY | -9.6 |

# Chapter 5

## Conclusions

Three para-Nitro aniline azo derivatives we synthesized to study the effect of solvatochromism. Ther are NAA, NAR, NAY. NAA synthesized by the reaction between para nitro aniline and 1-naphthol. NAR by reaction between para nitro aniline and resorcinol. NAY by the reaction between para nitro aniline and salicylic acid.To study the effects of solvatochromism of these compounds we dissolved them in 3 different solvents acetone, dichloromethane, chloroform .These 3 solvents have different polarity. By observing the UV spectral analysis of 3 compounds in 3 different solvents we can observe a bathochromic shift. i.e. the wavelength at which absorbance peak shows increases with increase in polarity. Acetone is highly polar among the solvents so it shows a greater absorbance peak at greater wavelength. By DFT calculation methods as well we analyzed the shift in wavelength in these solvents, there also we observed a similar bathochromic shift in wavelength.

One of the applications that we done is to compare the Antibacterial Activity of 3 compounds that we synthesized. It is done by both experimental and calculation methods. By the experimental methods we used well diffusion method. We checked the Antibacterial Activity of 3 compounds against E.coli and S. aureus. Our samples showed Antibacterial Activity against S. aureus and no activity against E. coli. NAY shows highest Antibacterial Activity. To determine the antibacterial

activity by calculation methods we used Auto dock, vina, conf software's. We compared the Antibacterial Activity of phenazopyridine against an antibacterial drug ,3twd and obtain a binding energy of -7.9 kcal/mol. We compared the binging energy of our 3 compounds NAA, NAR, NAY with 3twd. Among them NAY shoes highest Antibacterial Activity of -9.6 kcal/mol. So by both experimental and calculated methods we come in to the conclusion that NAY has the highest Antibacterial Activity.

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