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Salicylhydroxamic acid based azo dyes, synthesis, characterization, and properties

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Dedication

This study is dedicated from the bottom of my heart to my wonderful parents, who have given me so much, who gave me hope and strength when I wanted to give up, and who continue to be a source of moral, emotional, and financial support.

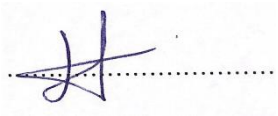
To my brothers and sisters who gave me their valuable advice.

Finally, to my loving husband, Melad, who has always been there for me and made sure I did everything I could to finish what I started.

Hala Alsheibat

Declaration

I certify that the thesis submitted for the degree of master is the result of my research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Signed: 

Hala Ramzi Mohammad Alsheibat

Date: 13/6/2023

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Abstract

The synthesis and characterization of a novel azo dye derived from naphthionic acid and salicylhydroxamic acid are presented in this research.

The synthesis process involves the diazo coupling reaction between naphthionic acid and salicylhydroxamic acid, forming the azo dye. The chemical structure and purity of the synthesized dye are confirmed using various characterization techniques, including ultraviolet visible spectroscopy (UV-Vis), infrared spectroscopy (IR), nuclear magnetic resonance (NMR), and high-performance liquid chromatography (HPLC). The properties of the newly synthesized azo dye, such as color, solubility, reactivity, and antimicrobial activity are thoroughly investigated.

The dye exhibits a unique color profile and solubility in polar solvents. The reactivity of the dye allows for potential modifications and derivatizations to enhance its versatility. This research provides valuable insights into the synthesis, characterization, and properties of the novel azo dye, offering prospects for its utilization in various applications.

The main result showed that the azo dye had an antifungal activity but three types of bacteria, Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa were resistant to the dye. Also, from the calculations, the pka was 3.9and the dye could be used as a chemical indicator.In addition, the prepared dye was investigated as an acid-base indicator for its effect in titrimetric analysis using acid-base titration techniques. In basic media, the color changed from pink to orange. The dye acted as an active acid-base indicator, and the color change occurred in the pH range (5-8).

Table of contents

List of Figures

List of tables

Abbreviations

SHAM	Salicylhydroxamic acid
UV-Vis	Ultraviolet Visible Spectrometery
IR	Infrared Spectroscopy
NMR	Nuclear Magnetic Resonance
HPLC	High Performance Liquid Chromatography
TLC	Thin Layer Chromatograp

Chapter One

1. Introduction

1.1 Background

Azo dyes have at least one nitrogen-nitrogen double bond (N=N) as shown in figure 1, however, many other structures can exist [1]. Monoazo dyes have only one (N=N) double bond, but diazo and triazo dyes have two and three (N=N) double bonds, respectively. The azo groups are often coupled to benzene and naphthalene rings, but can also be added to aromatic heterocycles such as chloroquine [2]. The side groups are required for the dye's color to be imparted. Most azo dyes are made by diazotizing a primary aromatic amine and then coupling it with one or more nucleophiles. Amino and hydroxyl groups are two of the most common coupling components [3].

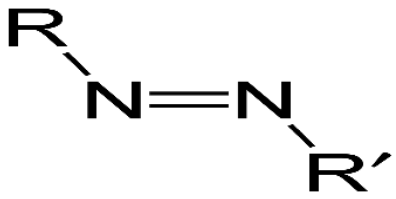


Figure 1. The general formula of azo dye

Azo dyes are synthesized through a process called diazo coupling, which involves the reaction of a diazonium salt with a coupling component. Here is a general overview of the preparation of azo dyes [4]:

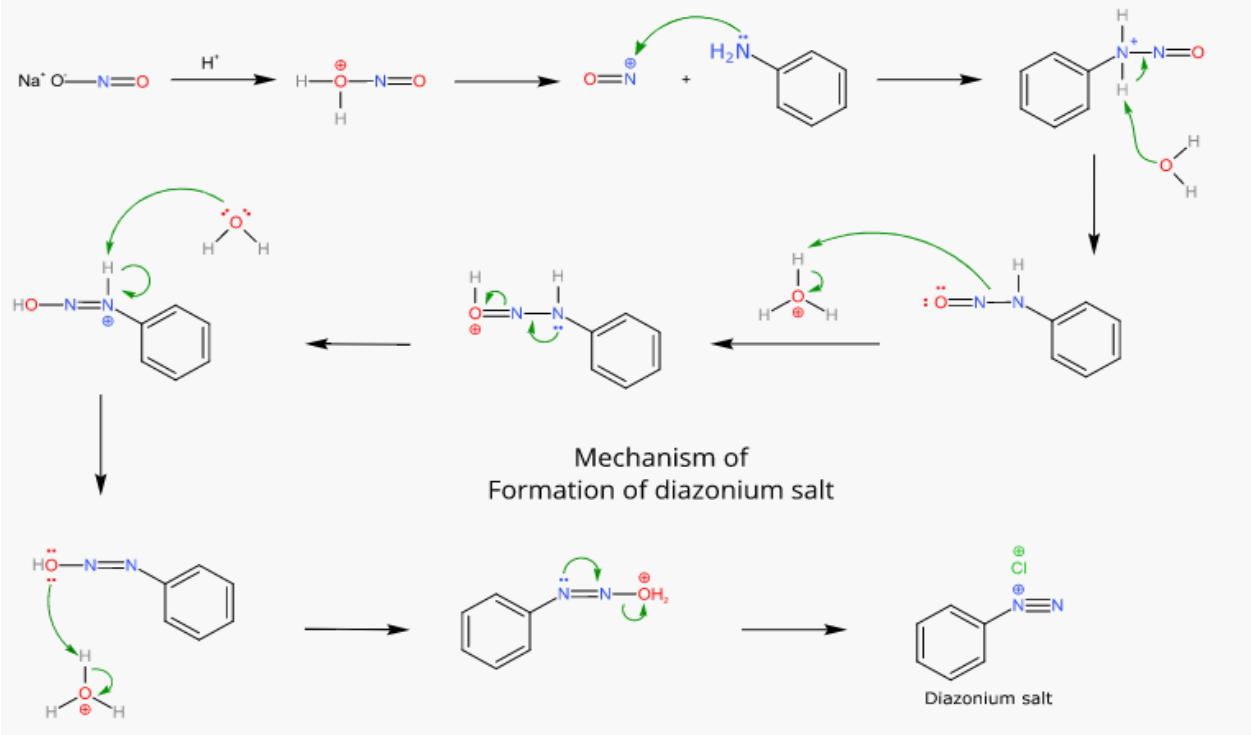
1- Diazotization: The first step involves the preparation of a diazonium salt from anaromatic amine. This is achieved by treating the amine with sodium nitrite ($NaNO_2$) in an acidic medium, typically using hydrochloric acid (HCl) or sulfuric acid (H_2SO_4). The reaction is carried out at low temperatures (around 0-5°C) to control the formation of the diazonium salt.

2- Coupling: After the diazonium salt is formed, it is quickly reacted with a suitable coupling component. The choice of coupling component depends on the desired color and properties of the azo dye. Common coupling components include phenols, aromatic amines, and naphthols. The reaction is typically carried out in an alkaline medium, such as sodium hydroxide (NaOH) or sodium carbonate (Na_2CO_3), at slightly elevated temperatures.

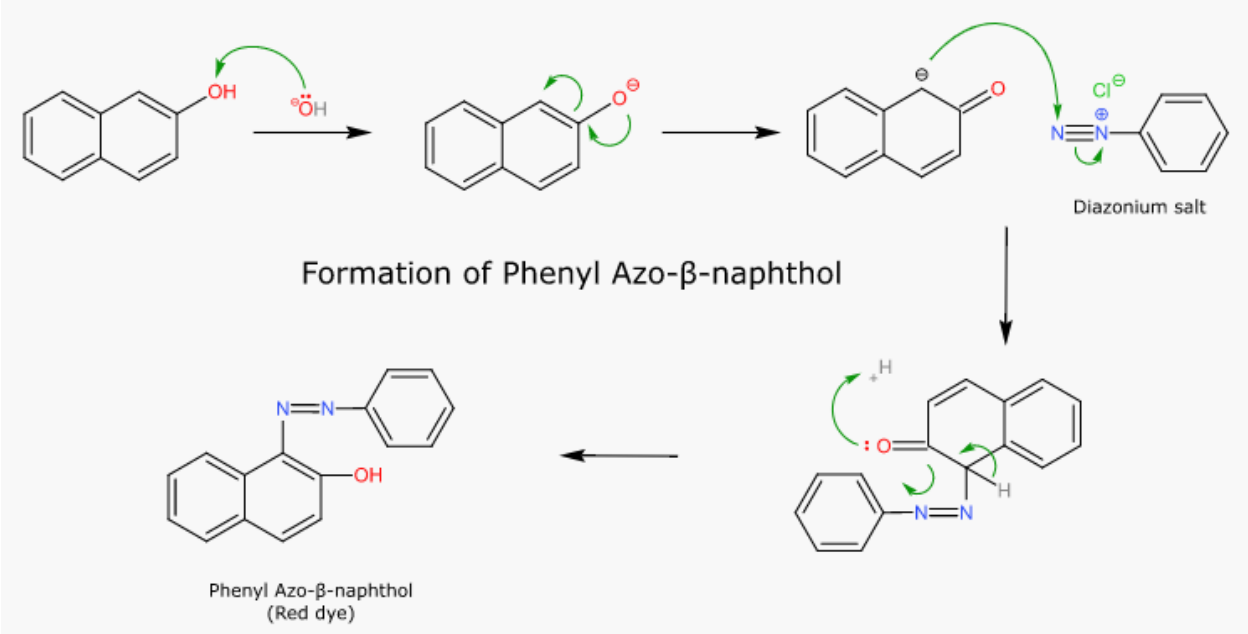
3- Isolation and Purification: Once the coupling reaction is complete, the azo dye is usually isolated by filtration or extraction. It may be necessary to purify the crude product through techniques such as recrystallization, column chromatography, or distillation to obtain a pure azo dye.

For example,the synthesis of phenyl Azo-βnaphtholin two steps [5]:

1- Diazonium salt preparation



2- Coupler preparation



- Classifications of azo dyes

Azo dyes, characterized by the presence of one or more azo (-N=N-) groups, are a diverse class of synthetic colorants. They are widely used in various industries, including textiles, printing, cosmetics, and plastics. Here are some of the main classes of azo dyes [6] with examples:

- Disperse Azo Dyes: These dyes are primarily used for coloring synthetic fibers, such as polyester and nylon [7]. They have low solubility in water and are typically applied from a dispersed form. Examples include Disperse Yellow 3 as shown in figure 2, Disperse Red 1, and Disperse Blue 1.

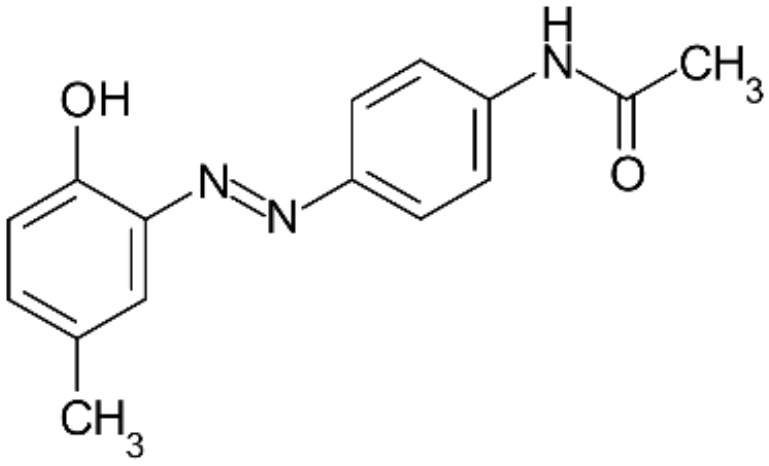


Figure 2. Chemical Structure of Disperse Yellow 3

- Acid Azo Dyes: Acid azo dyes have acidic functional groups, such as sulfonic acid or carboxylic acid groups, which make them soluble in water. They are commonly used for dyeing protein fibers (e.g., wool and silk) and as acid-base indicators [8]. Examples include Acid Red 1, Acid Yellow 23 as shown in figure 3, and Acid Orange 7.

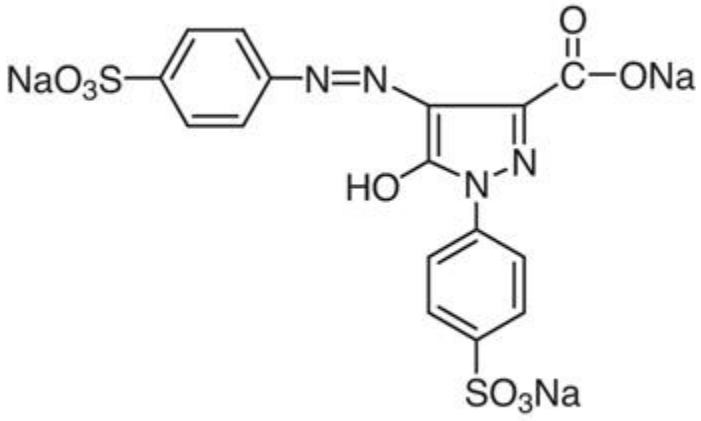


Figure 3. Chemical Structure of Acid Yellow 23

- Direct Azo Dyes: Direct azo dyes [9] are known for their strong affinity to various types of fibers without the need for a mordant. They are used for dyeing cellulosic fibers, such as cotton and rayon. Examples include Direct Red 81, Direct Blue 71, and Direct Yellow 86 as shown in figure 4.

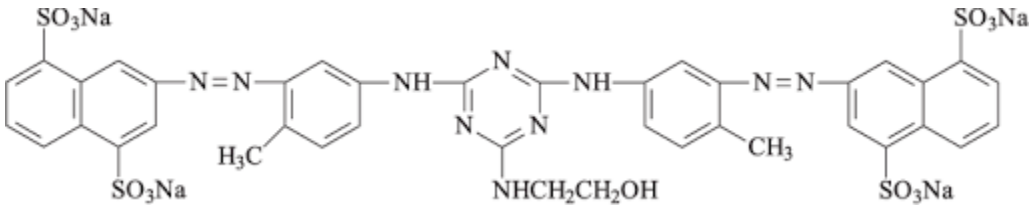


Figure 4. Chemical Structure of Direct Yellow 86

- Reactive Azo Dyes: Reactive azo dyes are a class of dyes that form covalent bonds with fibers through reactive groups, such as reactive chlorotriazine or vinyl sulfone [10]. They offer excellent wash fastness and are widely used for dyeing cellulosic fibers. Examples include Reactive Red 120, Reactive Orange 16, and Reactive Yellow 145 as shown in figure 5.

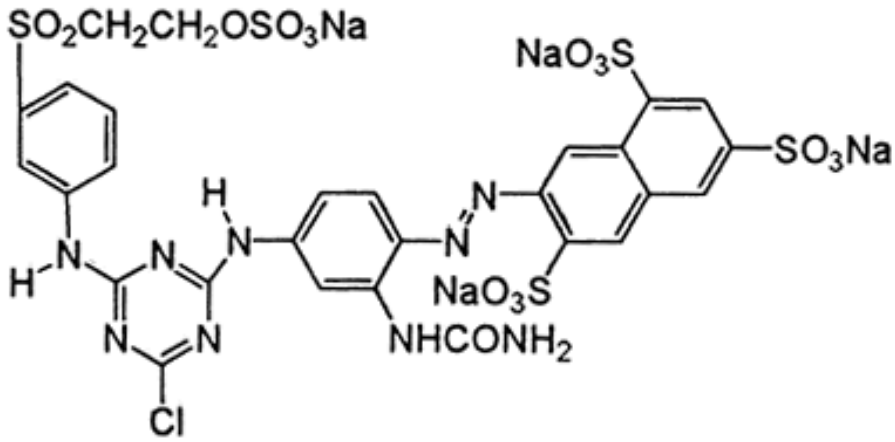


Figure 5. Chemical Structure of Reactive Yellow 145

- Basic Azo Dyes: Basic azo dyes are cationic dyes that have a positive charge on the chromophore. They are used for dyeing acrylic fibers, paper, and inkjet printing [11]. Examples include Basic Red 2, Basic Yellow 28 as shown in figure 6, and Basic Blue 9.

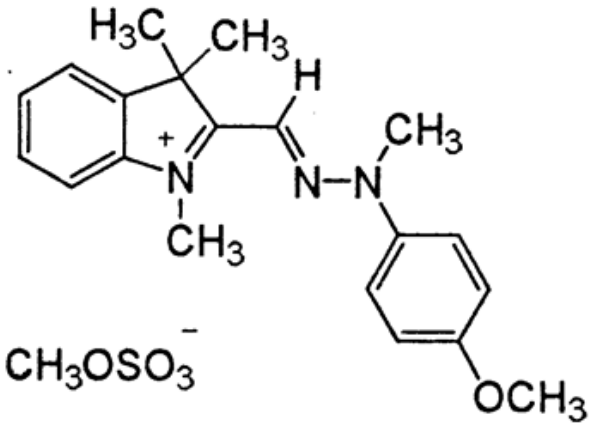


Figure 6. Chemical Structure of Basic Yellow 28

- Solvent Azo Dyes: Solvent azo dyes are soluble in organic solvents and are commonly used in inks, paints, and coloring plastics. Examples include Solvent Yellow 14 as shown in figure 7, Solvent Red 24, and Solvent Blue 35.

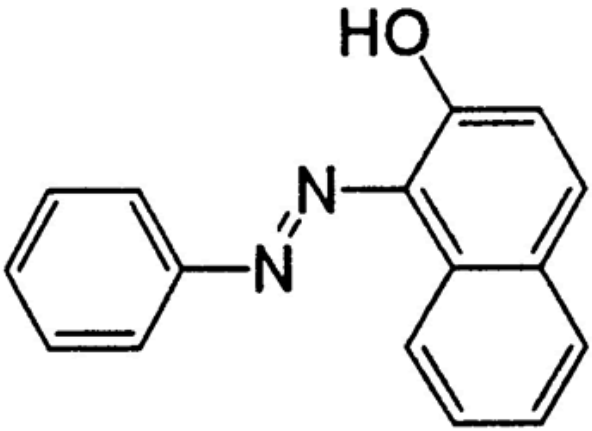


Figure 7. Chemical Structure of Solvent Yellow 14

- Food Azo Dyes: Azo dyes are also used as food colorants, where they add vibrant colors to various food and beverage products. However, their use is strictly regulated and subject to safety assessments [12]. Examples include Sunset Yellow (E110) as shown in figure 8, Allura Red (E129), and Tartrazine (E102).

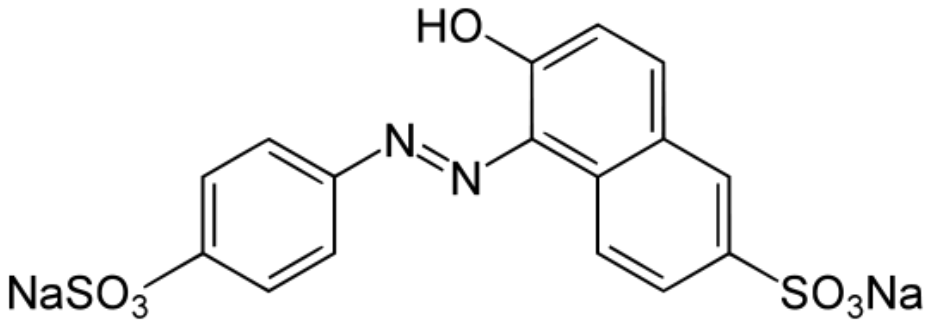


Figure 8. Chemical Structure of Sunset Yellow (E110)

It is important to note that while azo dyes offer a wide range of colors and applications, some azo dyes may have environmental and health concerns due to the presence of certain chemical groups or heavy metal residues. Therefore, it is important to use and dispose of them responsibly and to consider eco-friendly and safer alternatives in dye development and application.

Azo dyes have gained widespread interest for their usage in biological systems and as indicators in the complex potentiometric titrationof analytical chemistry [13,14]. Aromatic azo compounds, in particular, are utilized as acid-base indicators, as well as in biological strains and commercial colorants for apparel and polymers [15]. Color variations are induced by changes in the degree of electron delocalization. More delocalization changes the absorption maximum to longer wavelengths and causes the absorbed light to be redder, whereas less delocalization shifts the absorption max to shorter wavelengths [16].

As shown in figure 9 it is a common example of an azo dye. Nucleophiles are referred to as auxochromes when characterizing a dye molecule, whereas aromatic groups are referred to as chromophores.The dye molecule, when combined, is often referred to as a chromogen. Most azo dyes are made by diazotizing a primary aromatic amine and then coupling it with one or more nucleophiles. Amino- and hydroxy-groups are two common coupling components [17].

Azo dyes possess several key chemical properties that contribute to their vibrant coloration, solubility, stability, and reactivity. Here are some of the important chemical properties of azo dyes:

1- Azo Bond: Azo dyes are characterized by the presence of one or more azo (-N=N-) bonds in their chemical structure. The azo bond is responsible for the characteristic color of these dyes and provides them with chromophoric properties [18].
2- Chromophores: The presence of conjugated double bonds in azo dyes results in the absorption of visible light, leading to their vibrant colors. The chromophores in azo dyes contribute to their absorption and reflection of specific wavelengths, giving rise to a wide range of color possibilities [18].
3- Solubility: Azo dyes exhibit variable solubility properties depending on their structure and the functional groups present. Many azo dyes are soluble in polar solvents such as water, alcohol, and ketones. The solubility can be enhanced by incorporating suitable functional groups or by adjusting the pH of the solution [19].
4- Stability: Azo dyes generally possess good stability, especially under normal environmental conditions. They exhibit resistance to fading or degradation when exposed to light, heat, and certain chemicals. However, some azo dyes may undergo degradation or discoloration under extreme conditions or in the presence of specific agents [20].
5- Reactivity: Azo dyes exhibit chemical reactivity due to the presence of the azo (-N=N-) bond. This reactivity allows for further modifications and derivatization of the dye molecules, leading to the development of new dye variations with altered properties. Azo dyes can undergo reactions such as reduction, oxidation, coupling, and substitution, enabling the introduction of different functional groups and enhancing their versatility [21].
6- pH Sensitivity: The color of azo dyes can be influenced by the pH of the medium. Some azo dyes exhibit pH-dependent reversible color changes, making them useful as pH indicators or in applications where color alteration with pH variation is desired [22].

7- Sensitivity to Reducing Agents: Azo dyes are often susceptible to reduction by certain reducing agents. Reduction of the azo group leads to the cleavage of the azo bond and the formation of aromatic amines, resulting in a color change or bleaching effect [22].

There is a main property that azo dyes have, Tinctorial strength. It refers to the ability of a dye to impart color to a substrate at a given concentration. It is a measure of the dye's coloring power or intensity [23]. For azo dyes, tinctorial strength is influenced by several factors, including molecular structure, chromophoric groups, and the presence of auxochromes.

It is important to note that the specific chemical properties of azo dyes can vary depending on their molecular structure, substituents, and the presence of additional functional groups. These properties are crucial in determining the suitability of azo dyes for various applications in industries such as textiles, cosmetics, pharmaceuticals, and food products.

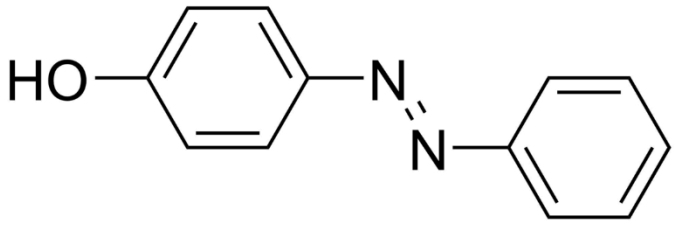


Figure 9. Example of an azo dye structure

1.2 **Salicylhydroxamic acid**

Salicylhydroxamic acid (SHAM) as shown in figure 10 is a popular ligand used in metallacrown production. The complex formation equilibrium is involved in M^{II}-SHAM systems; this is how SHAM could be employed as a metal indicator through the development of mixed-ligand complexes. Furthermore, as a ligand with potential oxygen and nitrogen donors, it has a broad variety of biological effects against bacteria [14].Naphthionic acid as shown in figure 11is one of many aminonaphthalenesulfonic

acids, which are naphthalene derivatives with both amine and sulfonic acid functional groups. Although commercial samples may appear gray, it is a white solid. It is employed in the manufacture of azo dyes like Rocceline (also known as Solid Red A), in which the amino group of the acid (in the form of a salt) is diazotized and then bonded with, in this case, naphthol. It is made by combining 1-aminonaphthalene and sulfuric acid.

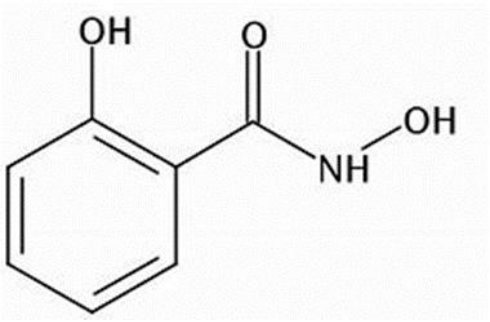


Figure 10. Chemical structure of Salicylhydroxamic acid

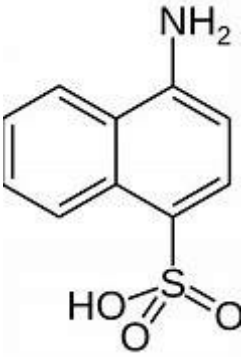


Figure 11. Chemical structure of Naphthionic acid

The emergence of drug resistance as well as unwanted side effects of certain antibiotics has prompted the quest for new antimicrobial agents with the goal of discovering new chemical structures that overcome the aforementioned disadvantages [25]. Hydroxamic acids are an interesting ligand with potential oxygen and nitrogen donors. They have received special attention not just because of the structural chemistry of their coordination modes, but also because of their usefulness in medical chemistry. These compounds have been utilized as medications, and they have been shown to have a variety of biological effects against bacteria, fungi, and certain types of cancers. As part

of our ongoing research, we are investigating the acid-base and complexation equilibria of a variety of biological oxygen and nitrogen donor medications with varying chelating properties [26].Theacid dissociation constant(pKa) value of an azo dye, offers several benefits in terms of its application and performance. Some of the key advantages of considering pKa in the context of azo dyes are:

1- pH Control: The pKa value provides information about the acid-base equilibrium of the dye molecule. By knowing the pKa, the pH range can be determined where the dye exists predominantly in its ionized or unionized form, using the Henderson-Hasselbalch equation:

$$pH = pKa + \log\frac{[A-]}{[HA]}$$

Thus, when the local pH equals the dye's pKa, the drug will beas shown in figure 12 50% ionized and 50% unionized (log 1 = 0). This allows for precise pH control during dyeing processes, ensuring optimal color development and stability [27].

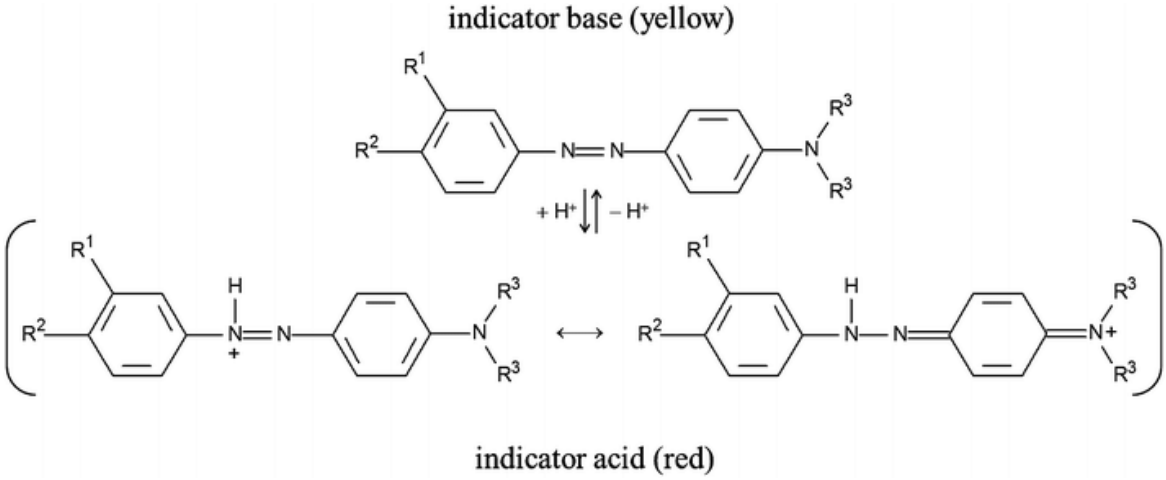


Figure 12. Structural changes of Azo dyes during protonation/deprotonation

2- Color Stability: Azo dyes can exhibit different colors depending on the pH of the surrounding environment. By understanding the pKa, it is possible to select the appropriate pH range to achieve the desired color and maintain its stability. pH-dependent color changes can be utilized to create color-shifting effects, enhance color intensity, or achieve specific color ranges in textiles, printing, and other applications.

1.3 Antimicrobial Activity

Botrytis cinerea belongs to the Sclerotiniaceae family of the Leotiomycetes class [28]. It is one of the most twenty-two important species of Botrytis [29]. Botrytis cinerea is a necrotrophic fungal plant pathogen that has been shown to be able to infect up to two hundred different plant species "dicot crop hosts", in addition to some monocots [30], it is the causal agent of the gray mould disease. Recently, it has been classified as the second most important phytopathogennrelative to itseffects onagroeconomics widespread and its significance as a pathogenic template. B. Cinerea is a typical pathogen of high risk [31].

Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa are three commonly studied bacterial species with significant medical and scientific importance. Each of these bacteria possesses distinct characteristics, habitats, and pathogenic potentials [32].

Escherichia coli, often abbreviated as E. coli, is a Gram-negative bacterium belonging to the Enterobacteriaceae family. It is a facultative anaerobe commonly found in the lower intestines of warm-blooded animals, including humans. While most strains of E. coli are harmless and even beneficial for digestion, certain pathogenic strains can cause severe gastrointestinal infections. These pathogenic variants produce toxins that can lead to symptoms such as diarrhea, abdominal pain, and fever. E. coli is a widely studied model organism in microbiology and genetics due to its ease of cultivation and rapid growth [33].

Staphylococcus aureus is a Gram-positive bacterium known for its ability to form characteristic clusters of cells resembling grape-like clusters under a microscope. It is a facultative anaerobe commonly found on the skin and mucous membranes of humans and animals. While S. aureus is a normal part of the human microbiota, it can also cause a wide range of infections, ranging from minor skin infections to life-threatening conditions such as pneumonia, bloodstream infections, and endocarditis. The bacterium is known for its ability to develop resistance to multiple antibiotics, making it a significant

concern in healthcare settings [34]. Methicillin-resistant Staphylococcus aureus is a particularly notable strain that is resistant to many commonly used antibiotics.

Pseudomonas aeruginosa is a Gram-negative bacterium known for its versatile metabolism and ability to adapt to various environments. It is an opportunistic pathogen commonly found in soil, water, and vegetation [35]. P. aeruginosa can cause infections in individuals with weakened immune systems or in those with chronic conditions such as cystic fibrosis. It is a leading cause of hospital-acquired infections, particularly in intensive care units. P. aeruginosa is known for its intrinsic resistance to many antibiotics and its ability to form biofilms, which contribute to its virulence and ability to persist in the host [36].

These bacteria have been extensively studied in various fields, including clinical microbiology, infectious diseases, and biotechnology. Understanding their physiology, virulence mechanisms, and antibiotic resistance is crucial for developing effective treatment strategies. Moreover, ongoing research aims to identify novel targets for antimicrobial therapy and develop new vaccines to combat infections caused by these bacteria.

1.4 Thesis objective

General Objective:

The main objective of this research was to synthesize and characterize the new azo dye.

Specific objectives:

1. Evaluate its antimicrobial activity.

2. Determine its pka.

Chapter Two

2. Literature Review

There are many papers in the literature on azo dyes, which currently account for most of the production volume of dye chemistry and whose importance may increase in the future. They are of critical importance in driving the dye and printing markets. These dyes are produced using a simple diazotization and coupling technique. To obtain the desired color properties, yield, and particle size of the dye for better dispersibility, many approaches and modifications are used [37].

For example, in this study, methyl orange as shown in figure 13, a water-soluble azo dye, is commonly used as a pH indicator in secondary and post-secondary chemistry education. In addition, azo compounds are commonly used in imaging applications such as textiles, paper, printing, and food. The current work focuses on the acid-base reaction, ammonium-diazonium tautomerism in acidic aqueous solutions using UV-Vis spectroscopy and exploring the chemical structures of methyl orange. The sites of H^+ attachment in methyl orange appear to be ambiguous [38].

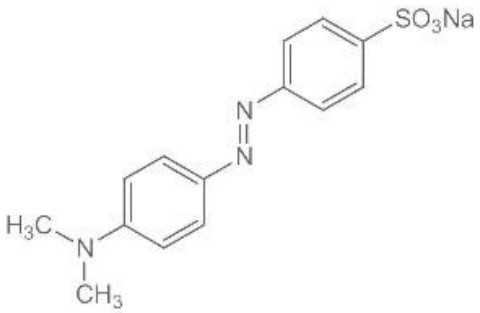


Figure 13. Chemical structure of Methyl Orange

Another example, Methyl Redas shown in figure 14 is not commonly used as indicator in complexometric titrations [39]. Complexometric titrations involve the formation of

stable complexes between metal ions and complexing agents (ligands) in a solution. The detection of the endpoint in complexometric titrations is typically done using metal ion indicators, such as Eriochrome Black T [40] or Murexide, which undergo a color change upon complexation with the metal ion being titrated.

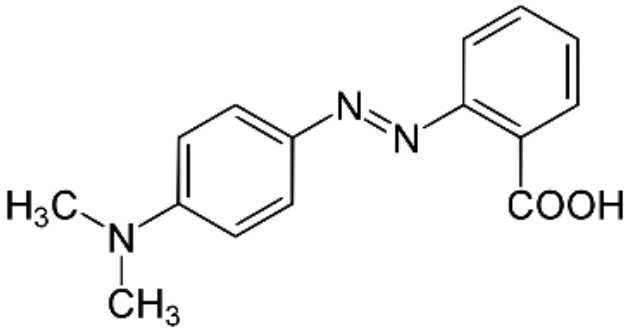


Figure 14. Chemical Structure of Methyl Red

However, Methyl Red finds extensive use as acid-base indicators in various acid-base titrations, such as strong acid-strong base and weak acid-strong base titrations [41]. In these types of titrations, the indicators undergo a distinct color change at a specific pH range, allowing for the determination of the endpoint and calculation of the unknown concentration of the analyte.

It is important to note that the choice of indicator in titrations depends on the specific acid-base system being studied, as different indicators have different pH ranges in which they exhibit the most distinct color changes. Methyl Red, for example, is well suited for titrations involving strong acids and weak bases, while Methyl Orange is commonly used in titrations of strong acids and strong bases [42].

In summary, while Methyl Red and Methyl Orange are not typically used as indicators in complexometric titrations, they are widely employed as acid-base indicators in various acid-base titrations. Their distinct color changes at specific pH ranges make them valuable tools for determining endpoints and assessing the concentration of analytes in acid-base systems.

There is a study about Eriochrome Black T [43] as shown in figure 15 is a commonly used complexation indicator in analytical chemistry. It belongs to the class of azo dyes

and is known for its ability to form stable complexes with metal ions, particularly with calcium, magnesium, and other transition metal ions. Its chemical structure consists of an azo group (-N=N-) and several functional groups that enable complexation with metal ions.

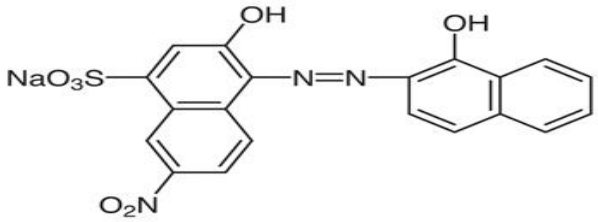


Figure 15. Chemical Strucure of Eriochrome Black T

As a complexation indicator, Eriochrome Black T undergoes a color change upon complex formation with metal ions. It exhibits a characteristic red color in its free form but forms a complex with metal ions, resulting in a shift to a blue color. This color change is used to indicate the presence or absence of metal ions in a solution.

The complexation reaction of Eriochrome Black T with metal ions is reversible, meaning the color change can be observed when the metal ion is added or removed from the solution. This property makes it suitable for various applications, including titrations, where it is used to determine the concentration of metal ions in a solution.

In titrations, Eriochrome Black T is often used as an indicator for complexometric titrations, which involve the formation of complexes between metal ions and a chelating agent. The indicator is added to the solution being titrated, and the color change indicates the endpoint of the titration when all the metal ions have reacted with the chelating agent.

Eriochrome Black T is particularly effective in complexometric titrations involving calciumand magnesium ionsas shown in figure 16. It forms stable complexes with these metal ions, allowing for accurate and precise determination of their concentrations in

various samples. The color change observed during the titration process provides a visual indication of the endpoint, facilitating the measurement of the metal ion concentration.

Overall, Eriochrome Black T is a versatile complexation indicator widely used in analytical chemistry, especially in complexometric titrations. Its ability to form colored complexes with metal ions and exhibit a distinct color change makes it a valuable tool for determining the presence and concentration of metal ions in solution.

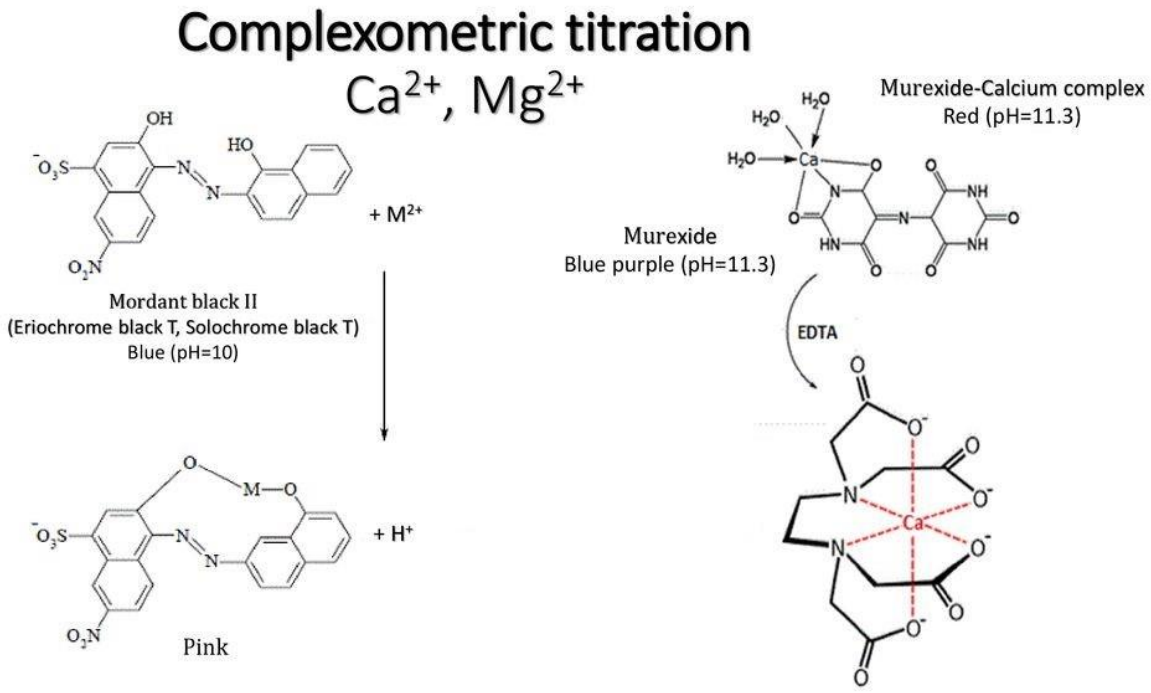


Figure 16. Complexometric Titration of Eriochrome Black T with Ca^{2+} and Mg^{2+}

In another study, it was shown that the chemical structure of an azo dye is represented by a basic skeleton, auxochrome groups, chromophoric groups, and solubilizing groups as shown in figure 17 [44]. The color of azo dyes is determined by the azo bonds and

the associated chromophores and auxochromes,

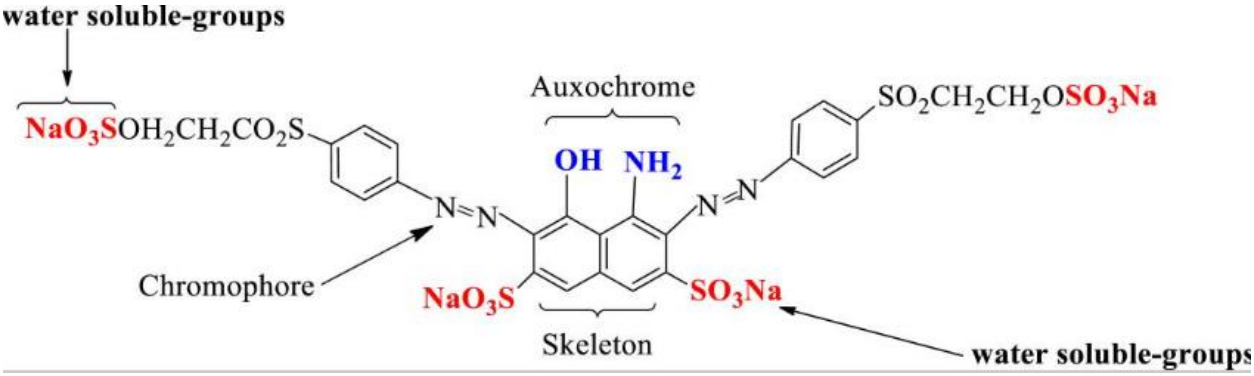


Figure 17. The basic skeleton of an azo dye

In another previous study, the ability of hydroxamic acids to form stable transition metal complexes was cited as the basis for their utility as analytical reagents for sensitive qualitative and quantitative analyses. In addition to the trace elements Mn, Fe, Co, V, and Cr, which are necessary for many types of life. Since hydroxamic acids are readily associated with carboxylic acids and hydroxylamines, both structurally and synthetically, they are used as drugs and have been reported to possess a wide range of biological activities against bacteria, fungi, and certain types of tumors [45].

In another study, salicylhydroxamic acid was shown to be formed by the interaction of hydroxylamine with methyl salicylate. The hydroxamic acids prepared were identified by their typical reactions with $FeCl_3$, melting points, nitrogen concentrations, and infrared spectra. These hydroxamic acids were evaluated for their antibacterial activity. Salicylhydroxamic acid showed no such activity against E. coli, Ps. aeruginosa, B. subtilis, or S. aureus. Salicylhydroxamic acid was investigated for its anti-inflammatory, analgesic, and antipyretic properties [46].

Chapter Three

3. Experimental

3.1 Materials

Pure standards of salicylhydroxamic acid, naphthionic acid, sodium nitrite ($NaNO_2$), sodium hydroxide 20% (NaOH), hydrochloric acid concentrated (HCl), urea 10%, ethanol 96%, ethyl acetate,potassium dihydrogen phosphate (KH_2PO_4), potassium hydrogen phthalate (KHP), distilled water (D.W.), methanol, chloroform, acetone, and water for analysis were of HPLC grade and purchased from Sigma Aldrich Company.

3.2 Instrumentation

3.2.1 High-Pressure Liquid Chromatography

High Pressure Liquid Chromatography system consists of an alliance 2695 HPLC, and a Waters Micromass® Masslynx ™ detector with a Photodiode array. Data acquisition and control were performed using Empower ™ software Analytes were separated on a 4.6 mm x150mm C18 XBridge ® column (5 µm particle size) in conjunction with a 4.6mmx20 µm XBridge ™ C18 guard column. A 0.45 µm microfilter (Acrodisc® GHP, Waters) was used.

3.2.2UV-Spectrophotometer

The concentrations of the samples were determined spectrophotometrically (UV spectrophotometer, Model: UV-1601, Shimadzu, Japan) by monitoring the absorbance at the λ_{max} for the dye.

3.2.3 pH meter

pH values were recorded on pH meter model HM-30G: TOA electronics ™ was used in this study to measure the pH value for each sample.

3.2.4 Infrared Spectroscopy (IR)

All infrared spectra were obtained from a KBr matrix (4000–400 cm-1) using a PerkinElmer Precisely, Spectrum 100, IR spectrometer.

3.2.5 Nuclear Magnetic Resonance NMR

Data were collected using Varian Unity Inova 500 MHz spectrometer equipped with a 5-mm switchable and data were processed using the VNMR software. For NMR, chemical

shifts are reported in parts per million (ppm, δ) downfield from tetramethylsilane. Spin multiplicities are described as s (singlet), brs (broad singlet), t (triplet), q (quartet), and m (multiplet).

3.2.6 Rotary Evaporator

Speed Control: 20-270 rpm, Digital bath for water or oil 20-180^{o}C, ±1^{o}C, Bath can also be used separately, Distillation glass set type G1 (Standard diagonal condenser).

3.3 Synthesis method

3.3.1 Azo dye preparation

- Preparation of diazonium salt According to [47]

Naphthionic acid (1 g) was dissolved in conc. HCl (1 g) precipitated the amine hydrochloride, and the resulting solution was added to a water-ice mixture. Over the amine hydrochloride, sodium nitrite solution was added dropwise. The system must be in an acid medium (pH 1.5-2) and the excess nitrite must be retained with iodine-starch paper until eliminated by the addition of 10% urea. The temperature must be maintained between -2 and 2 ^{o}C.

- Preparation of coupler

A certain amount of SHAM was suspended in at least 96% ethanol. A sodium hydroxide solution (20%) was prepared by dissolving a calculated amount of sodium hydroxide in water. This solution will be used to adjust the pH of the reaction mixture around 11, This was achieved by monitoring the solution with pH paper.

- Coupling reaction:

Add the diazonium salt gradually with stirring at a temperature of -2 to 2 °C (ice bath). Add continuously until a pH of 8.5 to 9 is reached. Allow to stand overnight at room temperature. It precipitated at pH 6.5 to 7, was filtered off, and dried at 80±0.5 °C. The resulting red powder was washed with hot water and dried. The reaction was monitored by TLC with eluent (chloroform). Subsequently, the final product was characterized by UV-vis, HPLC, NMR, and IR.

3.3.2 Determination of the Acid Dissociation Constant of the dye

To prepare the dye stock solution, 0.1 g of the dye was dissolved in a small amount of methanol and diluted with D.W. to 100 ml.

The following buffer solutions (Table 1) were prepared according to [48] for the dye by precisely mixing the following solutions in 7 (100 ml) volumetric flasks with burettes:

Approx. pH	KHP (0.1M)	KH_2PO_4(0.1M)	HCl (0.1M)	NaOH (0.1M)
2.5	50		38.8	
3	50		22.3	
4	50		0.1	
5	50			22.6
6		50		5.6
7		50		29.1
8		50		46.1

Table 1. Buffer solutions for dye

The pH meter was calibrated with a standard buffer whose pH value was within the pH range under investigation. The exact pH value was determined for each buffer solution. Using a pipette, exactly 1.0 ml of the indicator solution was added to 25 ml of each buffer. The "most acidic" solution was prepared by combining 1.0 ml of the indicator with 25 ml of 0.1 M HCl; the "most basic" solution was prepared by combining 1.0 ml of the indicator with 25 ml of 0.1 M NaOH. The visible absorption spectra of each solution were determined using 1-cm cuvettes. The maximum wavelengths were determined for the acidic and basic species. A note was taken of the wavelength of the isosbestic point as well as the temperature.

3.3.3 Metal complexes indicator at different pH

I-Cu(dye). $Cu(NO_3)_2$ (0.187g) was dissolved in minimal D.W. and then diluted in 100 ml volumetric flack to obtain 0.01M. 2.0 ml of the indicator from each solution with different

pH was added to 1.0 ml of metal. A note of any change of color or precipitate was taken.

II- Fe(dye).$7H_2O$. this was prepared as above using $FeSO_4$.7H2O (0.278g).

III- Fe(dye). Using $FeCl_3$ (0.1622g)

IV- Cr(dye). Using $Cr(NO_3)_3$ (0.238g)

V- Pb(dye). Using $Pb(NO_3)_2$ (0.331g)

3.3.4 Antimicrobial activity

- Antibacterial activity

The azo dye was tested for its antibacterial activity against the following bacteria: Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruguinosa. A precise amount of the dye was dissolved in a minimal amount of water, the entire surface of the selected agar was inoculated with bacteria, and sterile filter paper discs of uniform size and thickness containing known amounts of the azo dye were placed on the colonized agar. The plates were incubated at 37°C for 24 hours to check whether the discs were surrounded by clear or inhibitory zones.

- Antifungal activity

potatoes dextrose agar medium was used to prepare the medium 4.0 g of potatoes dextrose agarmedium with 0.1 g chloramphenicol was dissolved in 100 ml of water). Then placed on hot a plate with stirrer by using magnetic stirrer to homogenize and dissolve the main components; samples were autoclaved and allowed to cool down to 60˚C. 1.0 g of the test compound was added to 20 ml of growth media into a Petri plate (90 mm diameter). Fungal colonies (5 mm) were then added to the center of each Petri plate after cooling. Samples were incubated for 4 days at 25 ˚C. Fungal colonies diameters were then measured and then % of fungal inhibition was measured.

Chapter Four

4. Result and discussion

A new azo dye was synthesized, purified, and characterized by various spectroscopic and chromatographic techniques, also its antimicrobial activity and its acid base indicator properties was evaluated.

4.1 Synthesis of azo dye

The synthesis of an azo dye requires two organic compounds- a diazonium salt and a coupling component. The general synthesis of azo dyes is shown in figure 18:

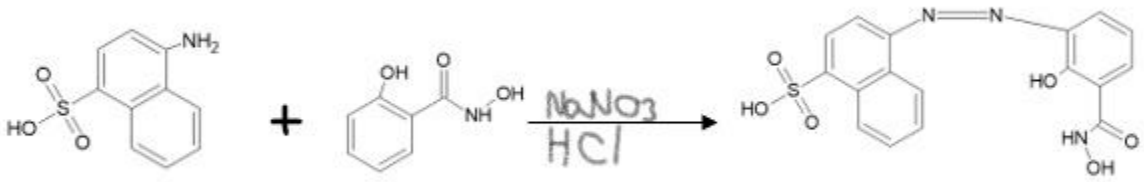


Figure 18. Synthesis of the azo dye

Through an electrophilic aromatic substitution mechanism, the diazonium salt reacts as an electrophile with an electron-rich coupling component, similar to a naphthol and naphthaline derivative. Unless that position is occupied, the aryl diazonium ion is directed to the para site by the hydroxyl group (such as naphthol).

4.2 Characterization

The new azo dye was characterized by HPLC, IR, NMR, and UV spectroscopy. IR spectrophotometers as IR which was used in the present study to confirm thepresence of functional groups as follows, distinguish the stretching vibration band of the azo group(N=N) at 1505 cm^{-1} as shown in (table 2) and as shown in figure 19, The asymmetry stretching vibration of S-O(SO3-H) group appearance at 1186 cm^{-1} position, while symmetry at 1050 cm^{-1} position. The NMR peaks correspond to 2 ppm (H_2C-CO-

N)6.02-7.65 ppm (HC=CH aromatic) as shown in figure20. As shown in figure21 HPLC spectra the synthesized azo dye was identified as a pure product with 98.36% purity.

Wave number (cm^{-1})	v S-O sym	v S-O asym	v C=C aromatic	v O-H phenolic	v C-N	v N=N
Azo dye	1050	1186	1586	3400	1271	1505

Table 2. Major stretching vibration of absorption bonds by IR spectroscopy

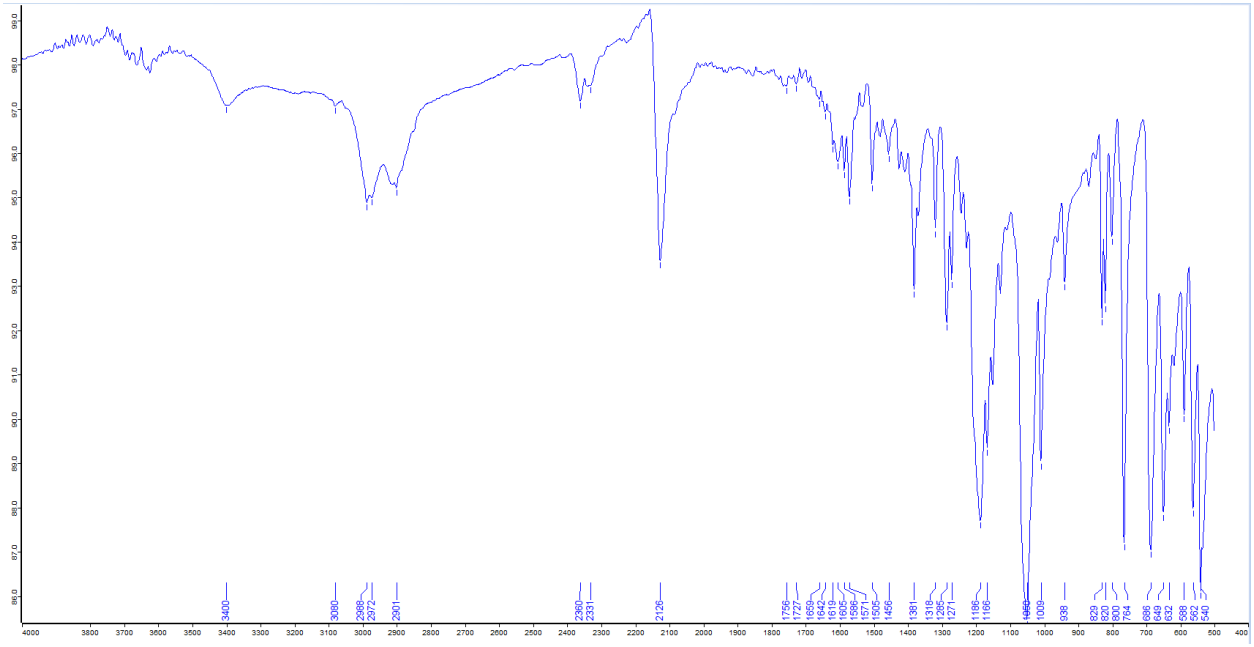


Figure 19. FTIR spectroscopy for azo dye compound

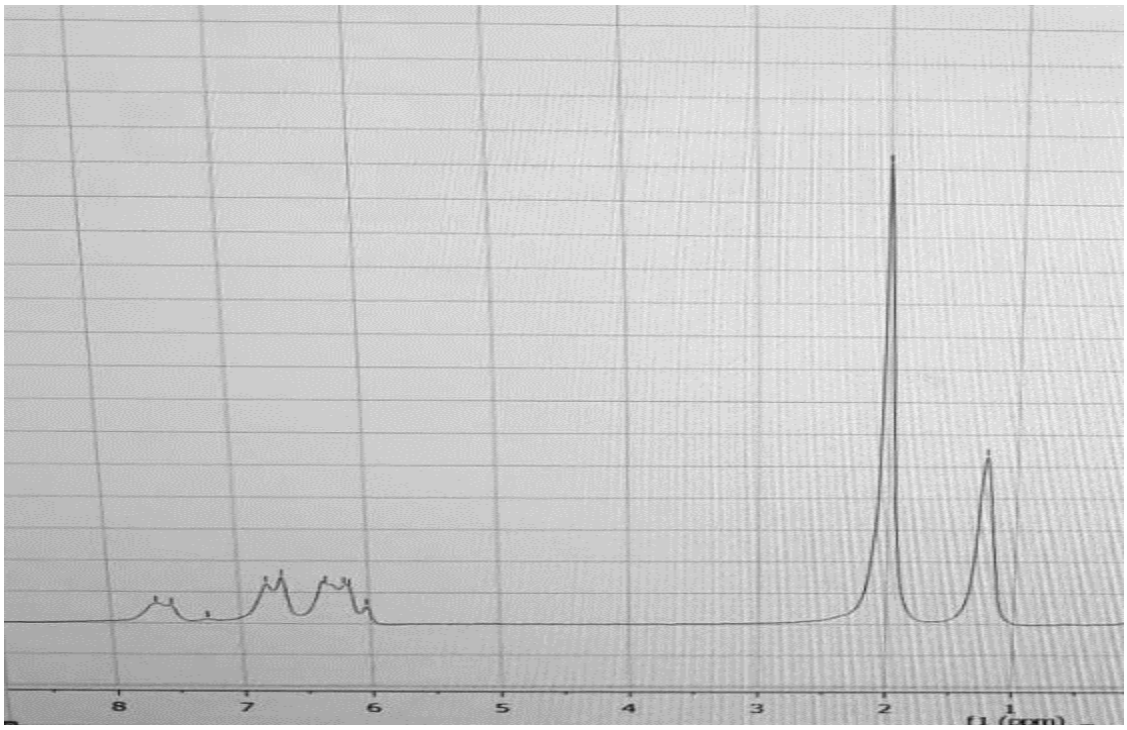


Figure20. NMR spectrum of azo dye

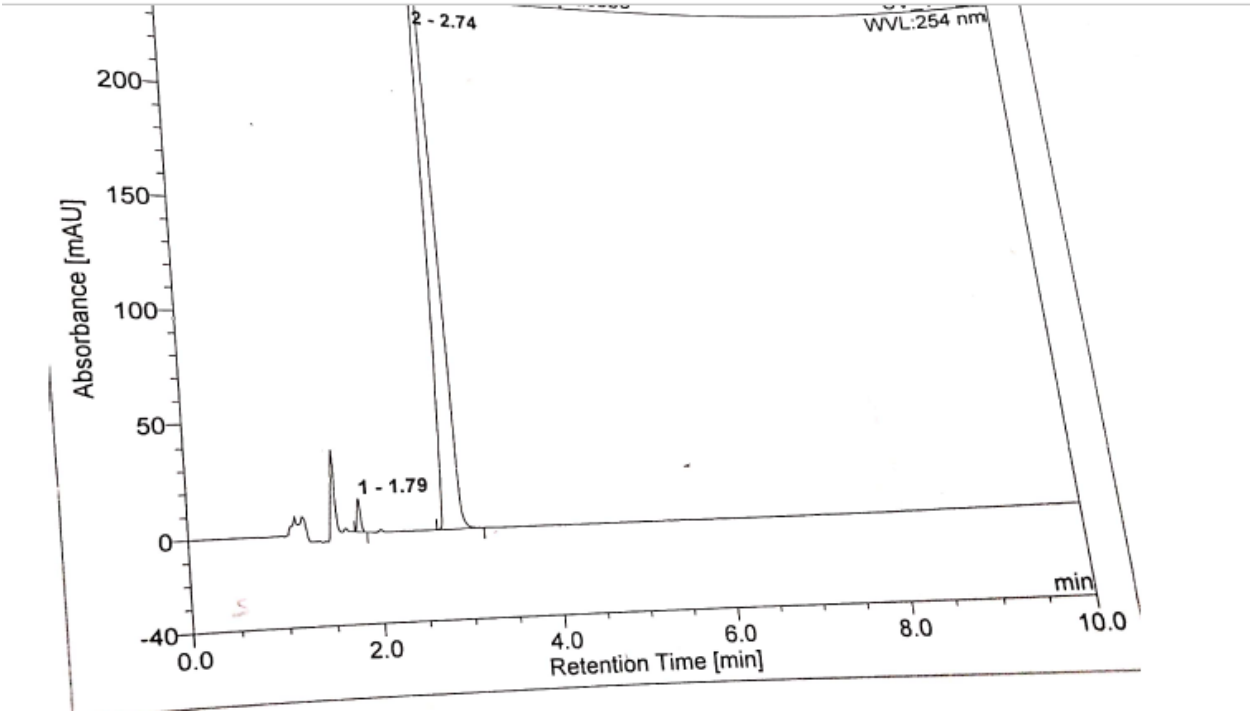


Figure21. HPLC chromatogram of azo dye

The previously described IR, NMR, and HPLC spectrum results verified that the intended azo dye was generated from the procedure.

4.3 Physicochemical properties

To investigate the absorption spectra of the prepared azo dyes, the electronic absorption spectra as shown in figure 22 were recorded in the range of 400-700 nm.The absorption spectra of the azo dye showed an absorption maximum of 510 nm.From the spectrum, the molar extinction coefficient at max (510 nm) is calculated using the Beer-Lambert law:

$$A = \varepsilon bC$$

From the plot ofas shown in figure 23 molar absorbtivity is 0.6221 $cm^{-1}mol^{-1}L$

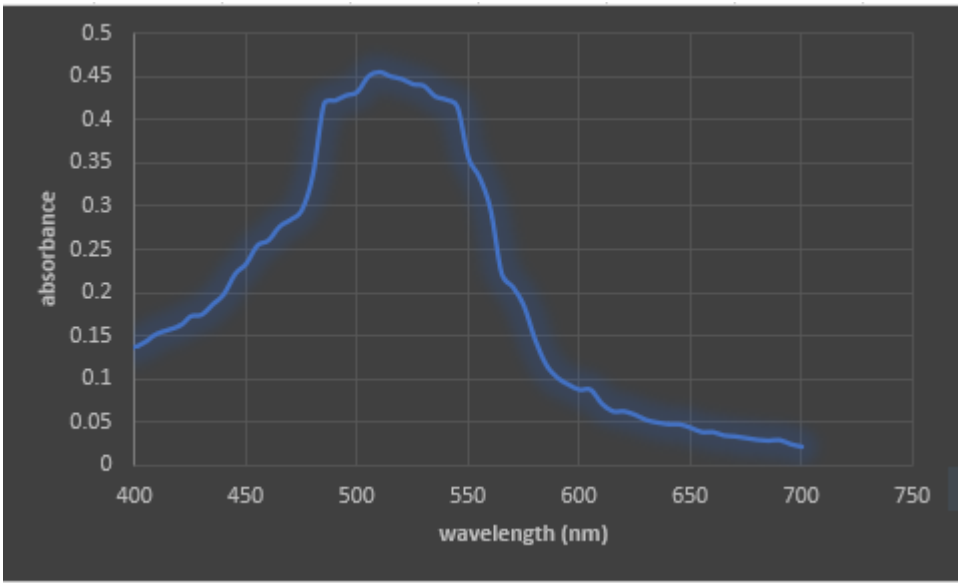


Figure 22. UV-Vis spectra of azo dye in water

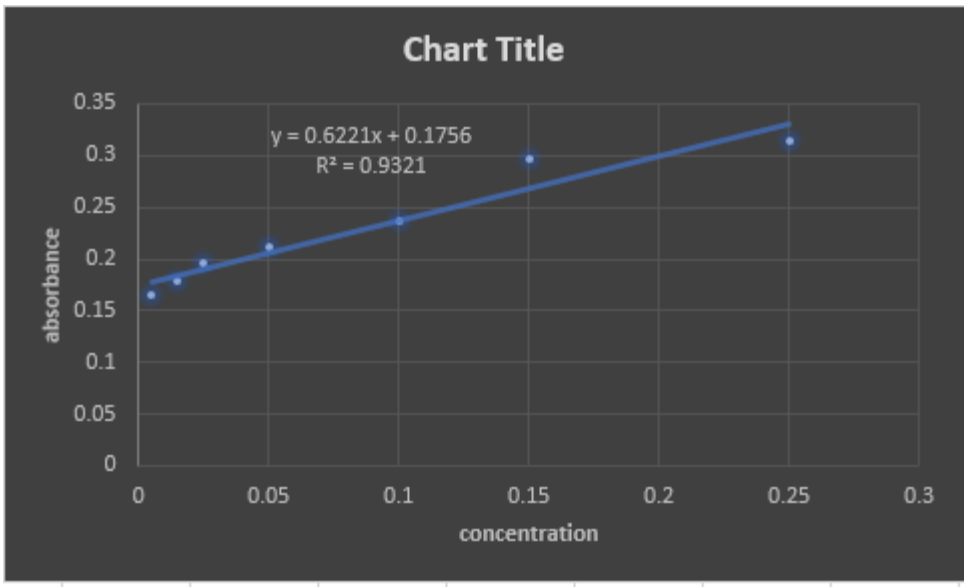


Figure 23. molar extinction coefficient

The dissociation constant was obtained for azo dye by measuring the absorbance of the indicator in buffer solutions of various pH. The wavelength used to calculate the dissociation constant should be the one with the greatest difference between the indicator's acid and basic forms as shown in figure24 460nm.

pKa = 3.978 was obtained from: (as shown in figure25)

$$pKa = pH + \log\frac{Ay - A}{A - Ax}$$

Were A_y absorbance of most basic, A_x absorbance of most acidic (Table 3)

Figure24. The isosbestic point

pH	Absorbance	log(Ay-A)\(A-Ax)
2.96	0.187	0.0164
3.29	0.190	-0.0328
3.66	0.191	-0.0492
5.16	0.196	-0.1321
6.05	0.210	-0.3837
7.01	0.212	-0.4241
7.74	0.236	-1.305

Table 3. Determination of pKa

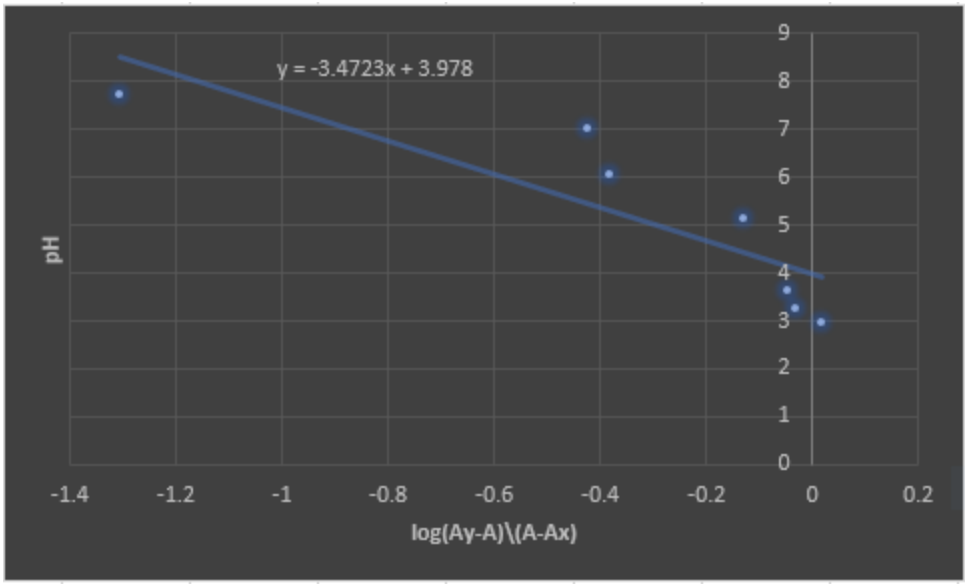


Figure25. pH against log (Ay-A)\(A-Ax)

4.4 Metal complexes of azo dye

The interaction of metal ions (Cu^{2+}, Fe^{2+}, Fe^{3+}, Cr^{3+}, Cr^{6+}, and Pb^{2+}) with the synthesized ligand (dye)was examined in solution at a pH range (2-8). Aqueous solutions were used. The colors of these combined solutions varied. InPb^{2+},Cu^{2+}, and Cr^{3+}cases, no calculations were done beyond the precipitation point, hence the hydroxyl species predicted to occur after this point could not be explored.

4.5 Antimicrobial activity

Surprisingly, the azo dye showed no antibacterial activity against any of the test bacteria organisms. However, Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruguinosa were resistant to the azo dye as shown in figure26.

Figure 26. Resistant of S. aureus,P.aeruginosa, and E.coli, respectively.

For Botrytis cinerea, after a 4-day period, the diameter of fungal colonies was assessed using the crossing method. The calculation for determining the inhibition rate percentage is as follows:

$$\text{Inhibition rate } \% = \left(\frac{dt}{dc}\right) \times 100\ \%$$

where dt represents the colony diameter of Botrytis cinerea and dc represents the colony diameter of the control.The result of Botrytis inhibition was (1.6/5.3) *100 = 30.1%.

Chapter Five

5. Conclusion and Recommendations

5.1 Conclusion

In conclusion, Salicylhydroxamic acid (SHAM) based azo dyes exhibit promising potential not only as vibrant colorants but also as compounds with antimicrobial activity.The synthesis of these dyes through the diazo coupling reaction between salicylhydroxamic acid and Naphthionic acid has been successfully achieved. Various characterization techniques, including UV-Vis spectroscopy, IR, NMR, and HPLC, have been utilized to confirm the chemical structure and purity of the synthesized dyes.

The properties of Salicylhydroxamic acid based azo dyes have been thoroughly investigated. These dyes exhibit vibrant colors and solubility in polar solvents. The presence of the azo (-N=N-) bond contributes to their chromophoric properties, enabling them to absorb and reflect specific wavelengths of light. Furthermore, the reactivity of these dyes allows for potential modifications and derivatizations, enhancing their versatility and expanding their application potential.

In addition to their coloration properties, Salicylhydroxamic acid based azo dyes have shown antimicrobial activity against Botrytis cinerea. The presence of functional groups, such as hydroxamic acid, in the dye structure contributes to their antimicrobial properties. The antimicrobial activity of these dyes opens up new possibilities for their application in medical textiles, wound dressings, and other healthcare-related products.

In addition to their coloration and antimicrobial properties, these dyes also possess acid-base indicator characteristics. Their pH sensitivity allows for reversible color changes in response to changes in acidity or alkalinity, making them useful in applications as acid-base indicators in laboratories, analytical chemistry, and other related fields.

Overall, the synthesis, characterization, and exploration of the properties of Salicylhydroxamic acid based azo dyes have provided valuable insights into their

potential applications. The combination of vibrant coloration, antimicrobial activity, and acid-base indicator properties makes them multifunctional compounds with diverse uses across various industries. Continued research and development in this field will contribute to the optimization and expansion of their applications, while also promoting their safe and sustainable utilization.

5.2 Recommendations

In reference to this work, the following recommendation would be outlined:

- This study needs more investigation of antimicrobial activity.
- Due to the lack of a sufficient amount of dye, we did not get the desired results of complexation studies. To confirm the results, we need to re-test, add different functional groups, and finally study their new properties.

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تصنيع ودراسة خصائص و مواصفات لأصباغ الآزو لـ (حمض ساليسل هيدروكسميك)

إعداد الطالبة: هالة رمزي الشعيبات

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الملخص:

يتم تقديم تحضير ودراسة خصائص ومواصفات صبغة آزو الجديدة المشتقة من حمض النفثيونيك وحمض الساليسيل هيدروكساميك في هذا البحث.

تتضمن عملية التحضير تفاعل اقتران الديازو بين حمض النفثيونيك وحمض الساليسيل هيدروكساميك ، مما يؤدي إلى تكوين صبغة الآزو.يتم تأكيد التركيب الكيميائي ونقاء الصبغة المركبة باستخدام تقنيات توصيف مختلفة ، بما في ذلك التحليل الطيفي للأشعة المرئية وفوق البنفسجية ، والتحليل الطيفي بالأشعة تحت الحمراء (IR) ، والرنين المغناطيسي النووي (NMR) ، والكروماتوجرافيا السائلة عالية الأداء (HPLC).تم فحص خصائص صبغة الآزو المُصنَّعة حديثًا ، مثل اللون ، والقابلية للذوبان ، والتفاعلية ، والنشاط المضاد للميكروبات.

تُظهر الصبغة مظهرًا فريدًا للون وقابلية للذوبان في المذيبات القطبية.تسمح تفاعلية الصبغة بإجراء تعديلات واشتقاقات محتملة لتعزيز تنوعها.يوفر هذا البحث رؤى قيمة حول تركيب وتوصيف وخصائص صبغة الآزو الجديدة ، مما يوفر آفاقًا لاستخدامها في مختلف التطبيقات.

أظهرت النتيجة الرئيسية أن صبغة آزو لها نشاط مضاد للفطريات ولكن ثلاثة أنواع من البكتيريا ، Escherichia coli و Staphylococcus aureus و Pseudomonas aeruginosa كانت مقاومة للصبغة.أيضًا ، من الحسابات ، كان pka 3.9 ويمكن استخدام الصبغة كمؤشر كيميائي.بالإضافة إلى ذلك ، تم فحص الصبغة المحضرة كمؤشر حمضي قاعدي لتأثيرها في تحليل المعايرة باستخدام تقنيات معايرة القاعدة الحمضية.في الوسائط الأساسية ، تغير اللون من اللون الوردي إلى البرتقالي.عملت الصبغة كمؤشر نشط للقاعدة الحمضية ، وحدث تغير اللون في نطاق الأس الهيدروجيني (8-5).