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PREPARATION, DIAGNOSIS AND MEASUREMENT OF THE BIOLOGICAL ACTIVITY OF AZO COMPOUNDS

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Abstract:

The research work was divided into three parts:

The first part: synthesis of azo compounds (A_1), by the reaction of two amines (methyl 2-aminobenzoate) as a coupling reaction with phenolic compounds (pyridoxine hydrochloride, ortho-vanillin, para-vanillin, and kojic acid) to forming azo compounds. The synthesized compounds were characterized by UV-visible spectra, FT-IR spectra, mass spectra and ^{1}H NMR spectra.

Also, a solid complex of copper (II) was prepared with two azo compounds (A_1), and it was identified by infrared spectroscopy and measurements of the percentage of copper (II) using flame atomic absorption spectroscopy, as well as molar conductivity and thermal analysis

Second part : It included a spectroscopic study of azo compounds (A_1)in the visible region with a range of wavelengths(520)nm, as their maximum wavelengths were determined, which are (520)nm for compounds (A_1).

The visible rays spectra of these compound were studied with a group of different polar solvents (n-hexane, 1,4-dioxane, chloroform, acetone, ethanol, methanol, dimethyl sulfoxide and deionized water) in a range of wavelength(550)nm. And the dielectric constant, which means that the dielectric constant controls the deviation of the peaks beams.

The effect of pH was studied in the electronic absorption spectra of azo compounds (A_1) in the visible region, in a range of wavelengths (530)nm, using different buffer solutions with different pH values (2-12). The ionization and ionization constants were calculated using the mid-height method, and the isopstic points and the proposed ionization and ionization mechanics were determined.

It also included a spectral study of the copper (II) complex with the azo compound (A_1), and it was noted that the complex was colored (red) after the color of the azo compound concerned was orange. The effect of pH was studied in a range of buffer solutions (2-12), and the optimal conditions for the formation of this complex were determined, including determining the greatest wavelength, and it was (520)nm in a solution of acidity (pH 10) for its complex with copper (II). In addition to the effect of the succession of addition necessary for the subsequent spectroscopic study, through which the proportions of the molecular structures of the complexes formed were determined based on the method of continuous changes, as the results showed that the complex is formed in a ratio of (1:2) (ligand:metal).

The study also dealt with the applicability of Beer's law and the sensitivity of the spectral method for azo compound (A_1) with copper (II). So the value of the molar absorption coefficient (ε) was (2.48×10^4) l.mol.$^{1-}$cm^{-1}, the specific absorptivity coefficient (a) was (0.004)mg.g^{-1}.cm^{-1}, the Sandel sensitivity (S) was (0.263)µg.cm^{-2}, the standard deviation (S.D) values were (0.0004), and the detection limit (DL) was (0.034) µg.ml^{-1}.

The interference effect of some important ions (K^+, Na^+, Ag^+, Ti^+, Ni^{+2}, Co^{+2}, Mg^{+2}, Mn^{+2}, Cd^{+2}, Cu^{+2}, Hg^{+2}, Ba^{+2}, Pb^{+2}, Fe^{+2}, VO^{+2}, Cr^{+3}, Al^{+3}, Bi^{+3}, Cl^-, NO^{-3}, SO_4^{-2}, and PO_4^{-3}) that could interfere and affect the complex adsorption value were studied. Copper (II) with an azo compound (A_1). It was noted that there is a difference in the intensity of their interference, as the ions (VO^{+2} and Cr^{+3}) affect the absorption values of the copper (II) complex with the azo compound (A_1).

Keywords: Azo compounds, biological activity

1- INTRODUCTION

Humans have been interested in dyes since ancient times, when nature was the only source of dyes. The Egyptians and Indians used indigo, which is considered the most important and oldest dye, and its use dates back to 3000 BC. It is extracted from the indigo plant and is a water-insoluble dye. Indigo paste must be treated with a diluted alkaline substance before dyeing. The chemical reaction makes the paste appear white and produces a substance that is easily soluble in water. After dyeing cotton or wool, it is taken out of the dye bath and exposed to the air for oxidation to obtain a dark blue color that cannot be easily removed with water (1).

2.1 AZO COMPOUNDS

The term azo compounds or dyes refers to a large class of industrial organic dyes that contain nitrogen in the form of azo (−N=N−) groups within their molecular structure, in the general formula R)−N=N−R). These dyes vary in color, But in general it is not very stable.

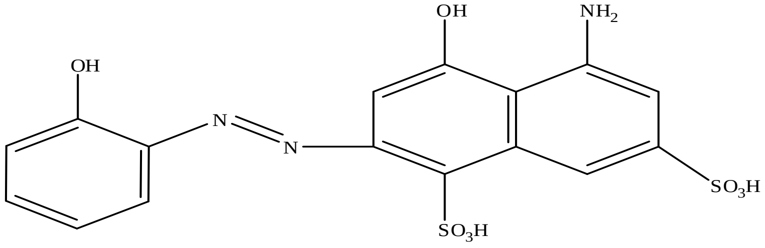
Figure: Azo dyes

The reason for these colors is the number of double bonds and the process of continuous conjugated double bonds between the bonds in the compound. The most accessible azo dyes in terms of application are those classified as direct: they are soluble in water and are absorbed from the solution in the cotton wool. The chemical dye was The first direct one is Congo red, which was discovered in 1884 AD, and which was later replaced mainly by acid- and fade-resistant dyes. Other azo dyes contain chemical groups that bind to metal ions. Among the many metal salts used with these dyes is chromium. And copper are common. Often metal ions are also bound to the fibers which improve the resistance of the dye to washing. There are also some disperse dyes, which also belong to some anthraquinone dyes and azo compounds. The latter are insoluble in water, but can be suspended in water with soap, and in This condition is absorbed from the suspension by cellulose acetate fibers (6,5).

Classification of azo compounds

Depending on the nature of the rings attached to either end of the bridge azo group

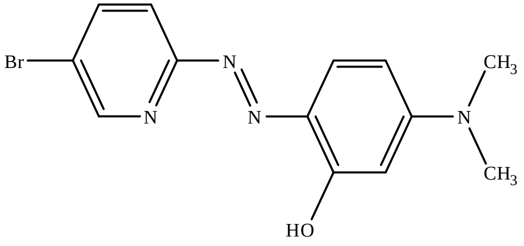
Homocyclic: In this title, the interlocking azo group is linked between 2 unified rings that do not contain a hybrid atom, such as (S, N, O). These rings are linked to an acid on one or both of the rings, an example of which is the compound (10).



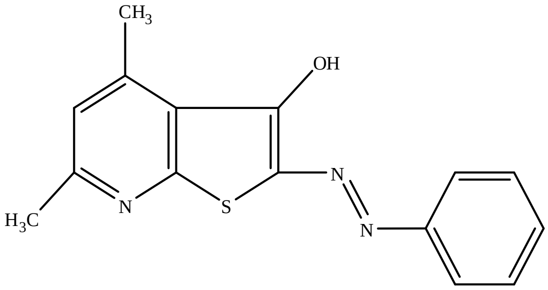
(*E*)-5-amino-4-hydroxy-2-((2-hydroxyphenyl)diazenyl)naphthalene-1,7-disulfonic acid

Heterocyclic: This type of compound is of great importance in many fields because it contains in its composition heterocyclic rings, which often contain hybrid atoms such as nitrogen, oxygen, or sulfur, and other compounds such as imidazole, thiazole, pyrimidine, and pyridine. These compounds were, as Described below, it is of particular interest to researchers in the fields of analytical chemistry, inorganic chemistry, and biochemistry. Heterocyclic compounds are characterized by the presence of nitrogen atoms in the heterocyclic ring, which is believed to be the process of isomerization with metal ions and the formation of complexes. According to the cross-linked azo group, in which the nitrogen atom takes the ortho position, non-covalent electron duplexing on the nitrogen atom allows the formation of stable complexes due to Formation of chelating rings (11).

In general, pentagonal or hexagonal rings are considered the most stable complexes (12). The presence of some protonating aggregates (including acidic protons) on the aromatic ring increases the chelate bond between the azo compound and the metal ion, which increases the stability of the compound formed by forming a five- or six-ring chelate. Below are some synthetic formulas For some heterocyclic azo compounds (13).



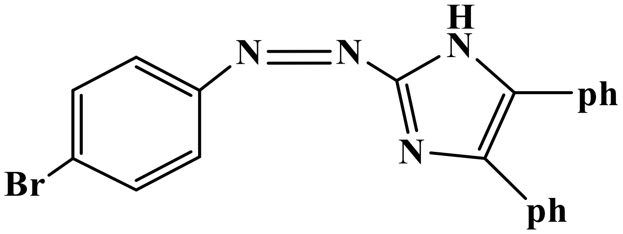
(*E*)-2-((5-bromopyridin-2-yl)diazenyl)-5-(dimethylamino)phenol



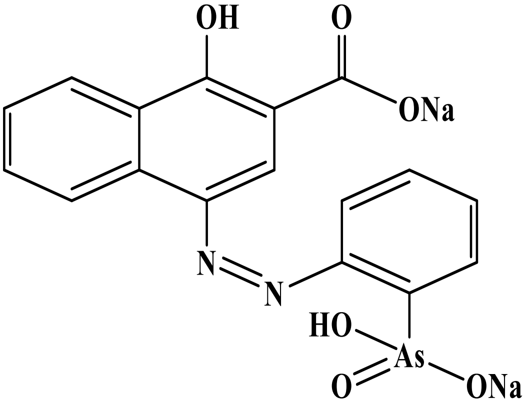
(*E*)-4,6-dimethyl-2-(phenyldiazenyl)thieno[2,3-*b*]pyridin-3-ol

Applications of azo compounds

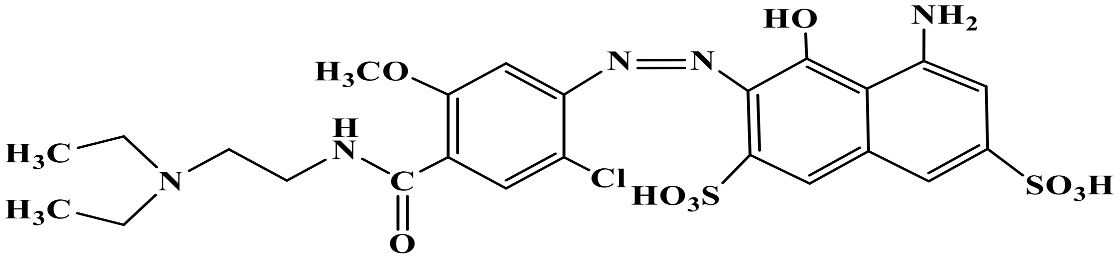
It has many uses due to its stability and speed of interaction with metal ions, especially in the field of analytical chemistry in the quantitative and qualitative estimation of metal ions (14). An analytical study to estimate microgram quantities of the lanthanum (III) ion showed the use of the pharmaceutical azo compound 2-[(4-Bromophenyl) azo]-4,5-diphenylimidazol at a maximum wavelength of 508 nm and a molar absorption coefficient of 60,000 lmol-1.cm-1 and limits Beer's law applies between (0.2-8) micrograms. ml-1 and has the following structure (15)



A study by researcher Hadi (16) showed the use of the azo compound sodium (Z)-1-hydroxy-4-((2-(hydroxyxidoarsoryl)phenyl)diazenyl)-2-naphthoate in determining the ions of iron (III) and chromium (III) at wavelength. The maximum is 410 nm and the molar absorption coefficient is (31,700 and 27,300) lmol-1.cm-1 for the two ions, respectively, and has the following structure.



Researcher Nuha (17) estimated the silver ion at the highest wavelength of 560 nm within the limits of application of Beer's law (9-0) µg.ml-1, and a molar absorption coefficient of 22000 l.mol-1.cm-1 using the pharmaceutical azo compound (Z) - 5-amino-3-((2-chloro-4-((2-diethylamino)- ethyl) carbamoyl)-5-methoxyphenyl)diazenyl)-4-hydroxynaphthalene-2,7-disulfonic acid has the following composition



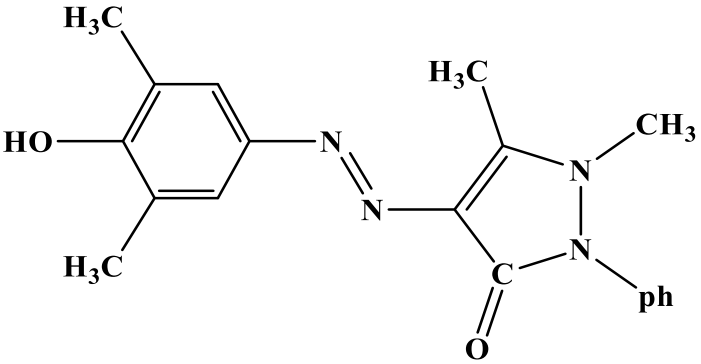
Determination of copper (II) ion

Copper is the ninth element of the first transition series and the oldest and most abundant element in nature, in small quantities in the form of a free metal, but its compounds are mostly in the form of widespread sulfides (18). Copper is distinguished by its wide uses. For example, it is used in the manufacture of household appliances and in roofing ships, and copper(II) oxide is used to color glass, sunglasses, and porcelain (19). It is also used for electrical purposes, in water tanks and pipes, and is also used as a pesticide for some agricultural pests and as an auxiliary agent (20).

Its complexes with amino acids are also used for cancer patients treated with radiation as radiation protective substances (21) and for wound healing (22).

Copper has oxidation states (I, II, and III). The oxidation state (II) of copper is one of its most important states, as most copper (I) compounds are quickly oxidized to copper (II) (23). The copper ion (I) has the coordination numbers (2, 3, 4, and 6), and the coordination number (4) is the most common, as the metal atom is surrounded by four ligands forming a tetrahedral shape (24). As for the coordination numbers (2 and 3), they are less common. For copper (I), the electronic arrangement of the copper (I) ion is [Ar] 3d10, where the d orbitals are completely saturated with electrons, so these compounds are magnetic and colorless (25). The appearance of some colors for a number of these complexes can be attributed to ligand absorption bands or charge transfer bands (26). As for the electronic arrangement of the copper (II) ion, it is [Ar] 3d9, and for this reason the value of the spin magnetic moment for the copper ion is only B.M (1.73)μeff = but due to the presence of spin-orbital coupling, this value is observed to be higher. This The increase occurs in copper (II) complexes that contain weak ionic or covalent bonds between the copper (II) ion and the ligand, as the value of the magnetic moment at room temperature is estimated at B.M (1.98–2.2) μeff =, while copper complexes (II) Containing strong covalent bonds, the value of its magnetic moment is estimated at 1.82-1.72) B.M) μeff (27).

There are a large number of methods for determining the copper (II) ion, especially spectroscopic methods, primarily in azo compounds. The azo compound 4-[(4-antipyrene) azo]-2,6-dimethyl phenol was used to estimate copper (II) spectroscopically with a molar absorption coefficient of 1630. l.mol-1.cm-1 at the maximum wavelength of 508 nm and the formula is as follows (28)



The effect of solvents on the visible absorption spectra of azo compounds

Solvatochromism is the ability of the chemical composition of a substance to change color as a result of a change in the polarity of the solvent. This change could be negative (Negative Solvatochromism) as a result of the blue shift by increasing the polarity of the solvent, or positive (Positive Solvatochromism) as a result of the red shift. As for the signal, it depends on the difference in the dual moment between the stable and excited states of the chromophore, as shown in Figure

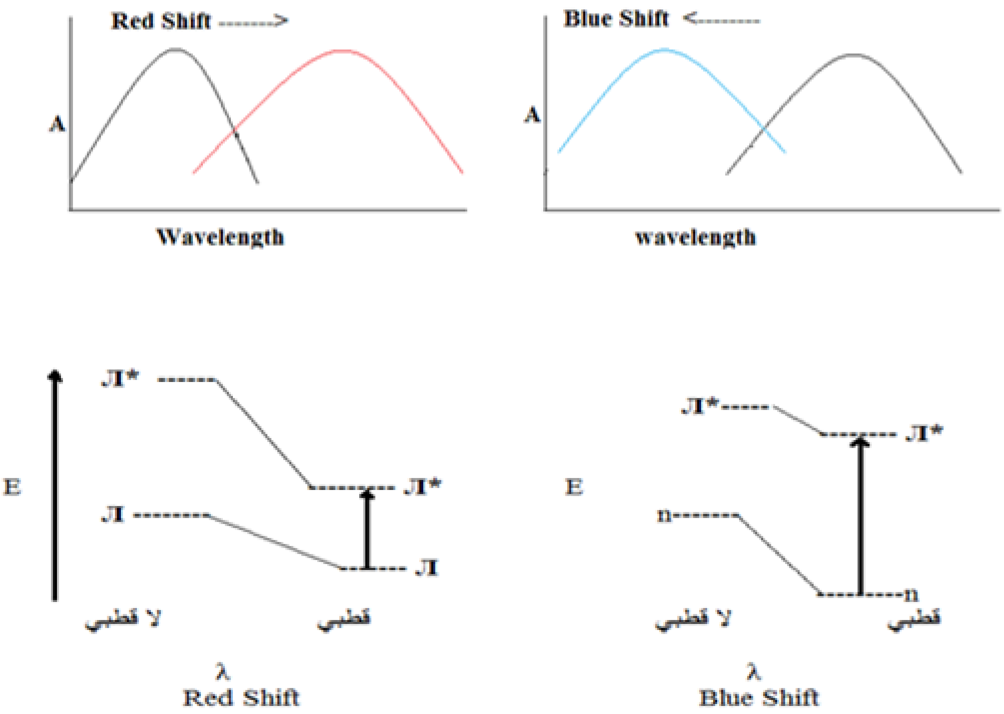


Figure: Red Shift and Blue Shift

It is noted that the effect of the polarity of the solvent causes the shift, as the presence of a polar solvent causes a shift towards the wavelength with the highest value, which is called the red shift due to the decrease in energy (п· п*), or towards the wavelength with the lowest value, which is called the blue shift. Because of the increase in energy (пп· *), it also provides us with useful information about the nature of the mutual influence between solute and solvent (29).

A spectroscopic study by researcher Asaad and his group (30) showed the effect of the solvent on the electronic absorption spectra in the visible region with a range of wavelengths (340-600) nanometers using solvents of different polarity on the azo compound 3-(luminol azo)-4-hydroxycoumarin, which was characterized by the appearance of two peaks with longer lengths. Maximum wavelength (425 nm for the solvent 1,4-dioxane and 430 nm for the solvents acetone, dimethylformamide, dichloromethane, dimethyl sulfoxide, ethanol, methanol, cyclohexanol, and deionized water) and has the following composition

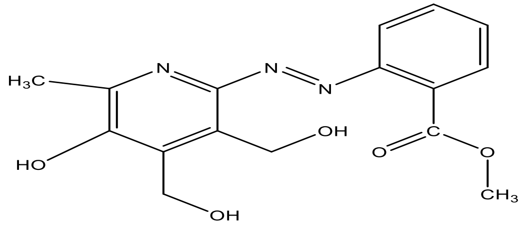
2-Preparation methods

Preparation of azo compounds

Azo compounds (A4-A1) derived from the aromatic amine (2-aminobenzoate methyl) were prepared by the approved method (31) and according to the following steps:

1. Dissolve (0.006 mol) equivalent to (0.9070) grams of (2-aminobenzoate methyl) in (2.1) ml of concentrated hydrochloric acid with the addition of (5) ml of deionized water and place it in an ice bath until it reaches a temperature of less than 5 m.o.
2. Dissolve (0.456) grams of sodium nitrite in (5) ml of deionized water and place it in an ice bath as well.
3. Prepare diazonium salt by slowly adding the sodium nitrite solution prepared in Step No. (2) in droplets to the cold solution prepared in Step No. (1) with continuous stirring while maintaining the temperature below 5°C.
4. I dissolve (0.006 mol) equivalent to (1.2338, 0.9129, 1.9129, and 0.8527) grams of each of (pyridoxine hydrochloride, 4-acetaminophenol, ortho-vanillic acid, and kojic acid), respectively, in (50) ml of ion-free water and add 1.8)) One gm of sodium hydroxide is dissolved in (10) ml of deionized water and cooled to below 5°C in an ice bath.
5. Add the diazonium solution from step No. (3) slowly with continuous stirring to the solution prepared in step No. (4) and leave the mixture for 24 hours to complete sedimentation in the refrigerator.
6. The solution is neutralized by adding dilute hydrochloric acid and making the surroundings slightly acidic to convert the azo compound from the sodium salt form to the hydrogen form by adjusting the pH between (7-6).
7. The precipitate is filtered, left to dry, then recrystallized with hot ethanol and n-hexane

Table shows the structure, nomenclature, and some physical properties of the azo compounds (A1)

color And outcome %	Composition and nomenclature Molecular formula and molecular weight		m.p	Code
orange 84	$C_{16}H_{17}N_3O_5$ 331.33	 methyl (E)-2-((5-hydroxy-3,4-bis(hydroxymethyl)-6-methylpyridin-2-	216-218	**A_1**

Preparation of the solid complex of copper (II) with azo compound A1

The azo complex was prepared with a molar ratio of 2:1 (metal: ligand) in the light of prior analyzes by the following general method (93):

In a circular flask with a capacity of (100) ml, mix (0.001) mole of copper (II) nitrate trihydrate, equivalent to (0.2416) g, dissolved in (20) ml of absolute ethanol to (0.002) mole with the two azo compounds (A1), equivalent to 0.6627). 1 gm dissolved in (30) ml absolute ethanol and the mixture was returned with continuous stirring for (1-3) hours. The mixture was then cooled, then the formed crystals were filtered and washed with water, hot ethanol, and ether to get rid of the remaining unreacted parts. Table 3-2 shows the molecular formula and some physical properties of the complex.

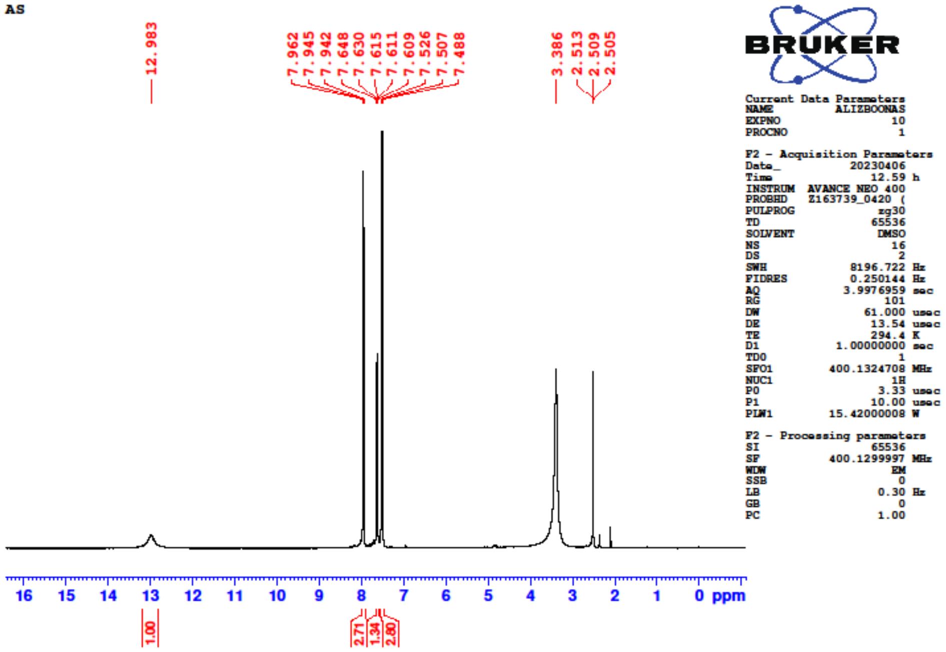
Table: Some physical properties and formula of the copper (II) complex with the azo compound (A1)

outcome %	color	m.p	Molecular weight	Complex formula
61	أحمر	Up 300	814.19	$[Cu(A_1)_2].5H_2O$

3-DISCUSSION AND RESULTS

1H NMR spectrum of the azo compound A1

The 1H NMR spectrum of compound A1 is characterized by the appearance of a single signal due to the protonation of the phenolic O-H group with a chemical shift of 12.98 ppm)δ. Multiple signals appear in the spectrum with a chemical shift in the range of 7.96-7.48 ppm (δ) dating back to the protons of the aromatic ring (108). We notice that the protons of the (CH3) group attached to the aromatic ring (Ar-CH3) appear as a single signal with a chemical shift of ppm(3.38)δ. Either the single signal due to the protons of the (CH3) group in the aliphatic chain appears in the form of a single signal with a chemical shift ppm(2.51)δ. As shown in the figure



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FIDRES            0.250144 Hz
AQ               3.9976959 sec
RG                     101
DW                  61.000 usec
DE                   13.54 usec
TE                   294.4 K
D1              1.00000000 sec
TD0                      1
SFO1           400.1324708 MHz
NUC1                    1H
P0                    3.33 usec
P1                   10.00 usec
PLW1           15.42000008 W

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PC                    1.00
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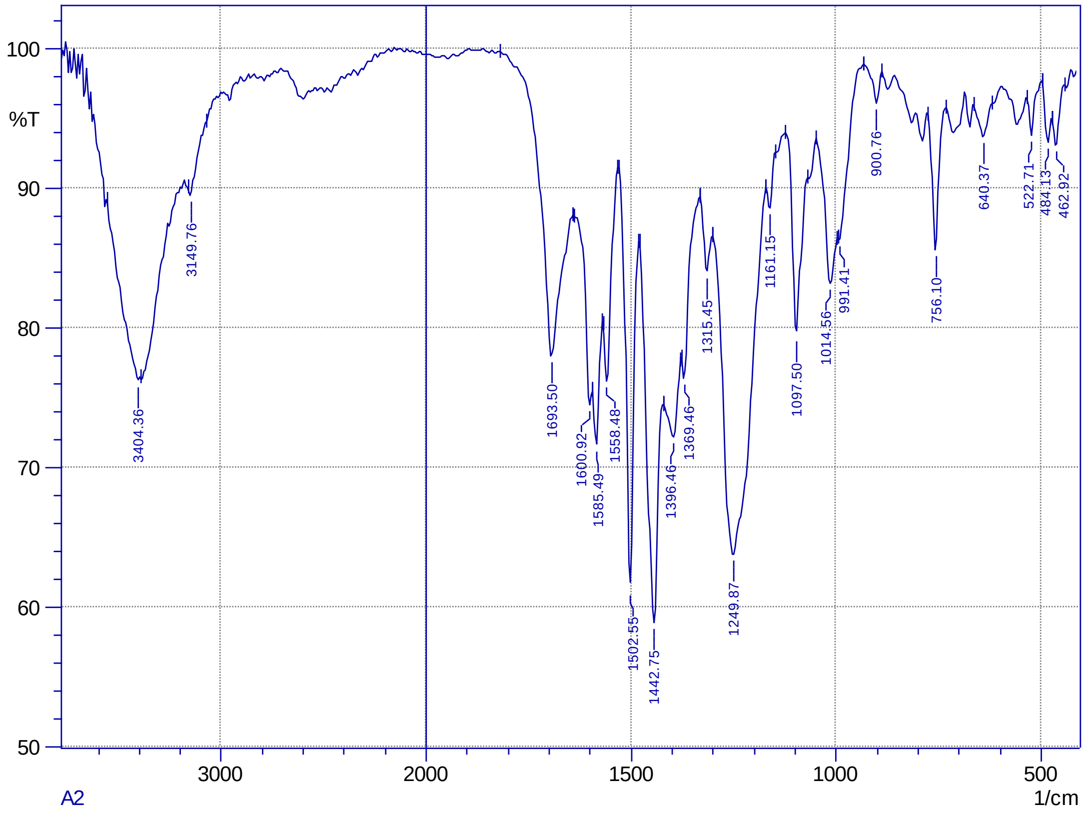
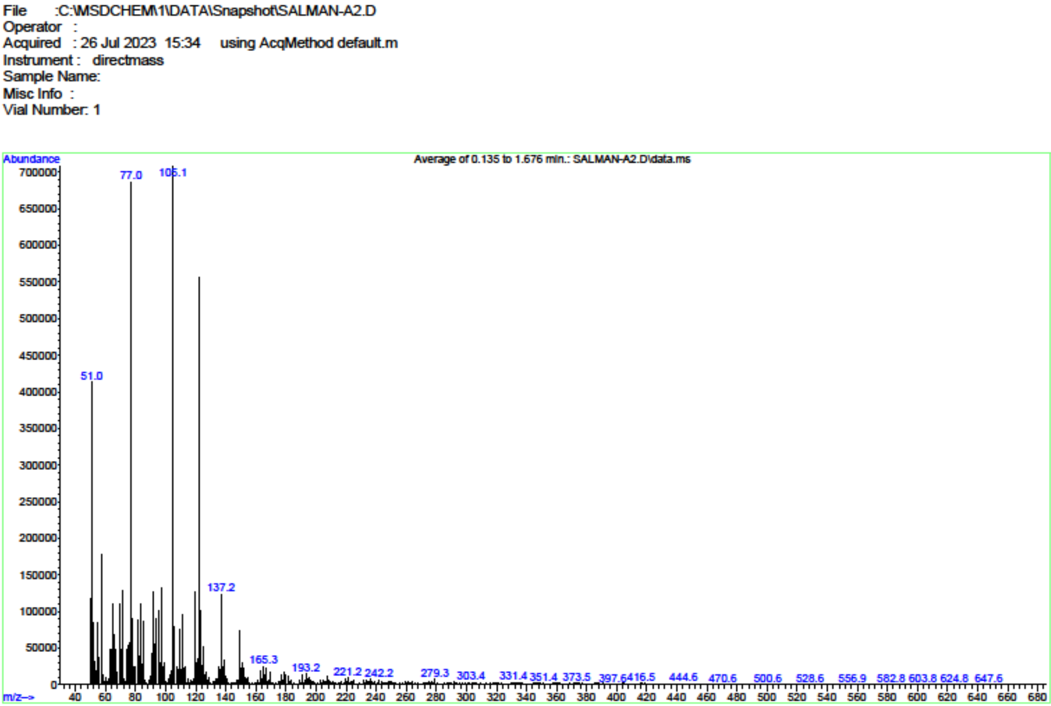
Infrared spectrum of a compoun



Mass spectrum of a compoundA1



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Sample Name:
Misc Info  :
Vial Number: 1
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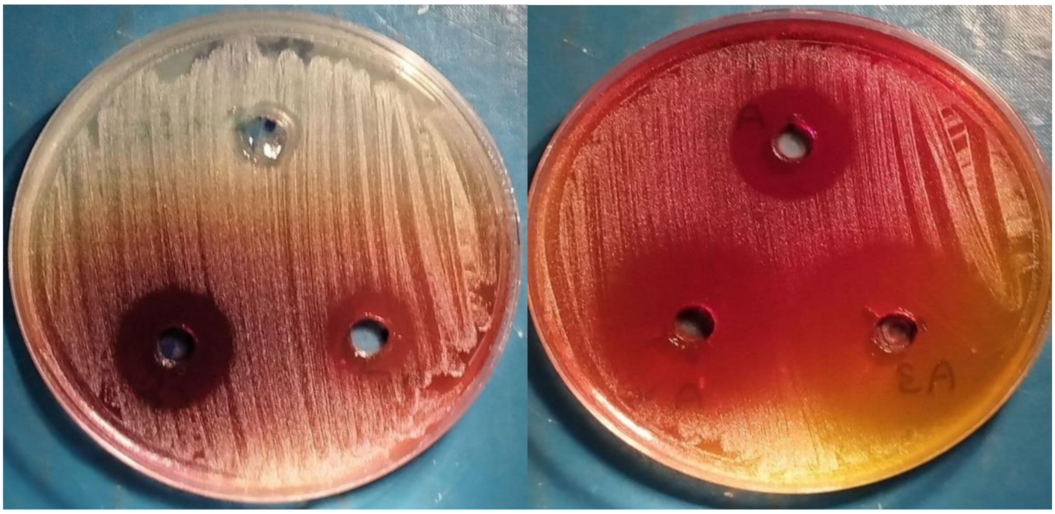
Biological activity of a compoundA1

The biological activity of the azo compound (A1) and the copper (II) complex with the azo compound (A1) was studied against two types of bacteria (Staphylococcus Aureuse) and (Escherichia Coli). Figure (44-3) shows the clear effect of the compounds in inhibiting the growth of Staphylococcus aureus bacteria, as well as their effect on Escherichia coli bacteria, respectively, as these compounds also showed a clear effect in inhibiting their growth.The effect of the azo compounds was measured by measuring the diameter of the inhibition zone, which ranged between (20-10) mm.

The resistance of a type of bacteria to some chemicals may result from the presence of a thick envelope surrounding the cell because it contains a high percentage of fats, or the occurrence of a mutation in a specific bacterial gene, which leads to the production of an enzyme that works to resist these bacteria to the chemical used (33).

Damping diameter(mm)		compound	
Escherichia Coli).	Staphylococcus Aureuse		
10	20	A_1	
10	20	$[Cu(A_1)_2].5H_2O$	



bacteria (Staphylococcus Aureuse) and (Escherichia Coli). Figure (44-3)
complex copper and compound A1

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