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Preparation and Characterization of Some New Azo Dyes and Study of their Stability and Bacterial and Laser Activity

Hatem Abdel Karim Ibrahim, Diana A. Shaker

Chemistry Department \ College of Education \ Samarra University \ Iraq

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Annotation: The research included the preparation of some azo dyes by reacting the diazonium salt prepared from the reaction of 5methyl-1,3,4-thiadiazole-2-amine with hydrochloric acid and sodium nitrite and then reacting the salt with the coupling solution (betanaphthol, alpha-naphthol, 4-hydroxy-3methoxybenzaldehyde, 2-aminophenol, 4chloroaniline). The structures of the prepared compounds were confirmed by physical and spectroscopic methods such as infrared spectrum and proton nuclear magnetic resonance spectrum (1H-NMR). The stability of the dye was tested by dyeing three pieces of cloth, wool and cotton, then washing them with soap and water and comparing them afterwards. The effectiveness of the bacterial dyes was tested against two types of Gram-positive and Gram-negative bacteria. Then some of the compounds were with a helium-neon laser for four periods, then the physical properties after that.

Keywords: Azo dyes, Biological activity, laser.

1. Introduction

An azo group consists of two nitrogen atoms linked by a double bond [-N=N-] and attached to an aromatic or aliphatic carbon atom, and its complexity depends on the number and nature of the oxidized dye groups present within it. Azo dyes may contain one azo group [called a monoazo dye] or more than two azo groups (called a monoazo group). Triazo dyes [1]. There are several ways to classify azo dyes, which are classified according to their chemical structure, each of which

has a color index classification, abbreviated as (CI-Number), which represents the chemical properties of the dye, and the nominal system (CI-Name), which represents the use of the dye, if it contains groups such as (COOH, OH-, -SO₃H), or if it contains groups such as [COOH, OH-, -SO₃H], it can also be classified as acidic dyes based on the oxygen color groups present in it. In the case of groups such as (-NRH, -NH2 -NR₂), which are basic, if the dye contains both groups, its classification depends on the number of groups and their strength [2,3]. According to the IUPAC system, azo dyes are called diamide derivatives HN=NH [4], which contain two aryl groups and are the most stable, while the -N=N- group is called an azo group [5]. Another classification depends on the methods used to apply these dyes on an industrial scale, with dyes classified as dispersed, acidic, basic, or reactive [6]. The main factor in displaying color is the presence of unsaturated groups in the molecule, and just as oxidized dye groups are important for increasing the intensity of the color, they impart acidic or basic properties to the dye molecule, thus increasing its ability to contact the material to be dyed [7]. Azo compounds are of great importance in life, as they have been used in various fields of science, technology, and medicine and are considered reagents in the field of organic synthesis [8]. As for medicines, they are also of great importance, as they are used as important antibacterials [9], such as bacteria that cause chronic intestinal diseases [10], in addition to the discovery of many effective antibacterial drugs [11], and they are also used to stain tissues [12].

2. Experimental:

2.1. Material: All chemicals used in this work were purchased from Fluka, Aldrich, and BDH and used without further purification.

2.2. Preparation of oxazepine derivatives [HA1-HA5) [13]

Azo dyes are mainly prepared by two steps to form diazonium salts:

Step 1: Preparation of diazonium salt:

1. Dissolve (0.5 g) of (5-methyl-1,3,4-thiadiazol-2-amine) in a conical flask (2.5 ml of 37%) hydrochloric acid solution (10 ml distilled water + 10 ml hydrochloric acid), ice bath, temperature 0-5 °C, with continuous stirring.

2. In a second conical flask, dissolve NaNO₂ (0.3 g) in as little as possible of distilled water (4 ml) and add it to the solution in the first flask at temperature 0-5 °C, then add it gradually in drops with stirring until a color change occurs, indicating the formation of the diazonium salt.

Step 2/Preparation of coupling solution:

Dissolve (0.004 mol) reagent in a minimum amount of sodium hydroxide (10 mL, 10%) at 0-5 °C. After complete dissolution by continuous stirring, add the diazonium salt prepared in step 1, stir for 1 h, and use TLC to confirm the completion of the reaction. Pour the product onto crushed ice, filter, dry the precipitate, and recrystallize with ethanol. As in Table 1

Comp No	Ar	Molecular Formula/ M.Wt g/mol	M.p. °C	Yield %	Color
HA ₁	ОН	$C_{13}H_{10}N_4OS$	123-121	80	Yellow
HA ₂	ОН	$C_{13}H_{10}N_4OS$	129-127	75	Dark Yellow
HA ₃	CHO OCH ₃	$C_{11}H_{10}N_4O_3S$	117-113	78	Brown
HA4	OH NH ₂	C9H9N5OS	133-131	71	Red

HA ₅	NH ₂	C ₉ H ₈ ClN ₅ S	122-120	68	Orange
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2.3. Color stability test of azo dye derivatives (HA1-HA5):

Azo dye derivatives (HA1-HA5) were used in the dyeing process. (0.1 g) of azo dye was dissolved in (20 ml) of 1,4-dioxane solvent, and the resulting solution was used to dye equal weights (150 mg) of cotton, wool, and cloth. These materials were placed in a beaker containing the dye (completely submerged). Stir (25 minutes) and place the beaker in the oven (hot air) at (100°C) until dry [14,15].

2.4. Biological activity study.

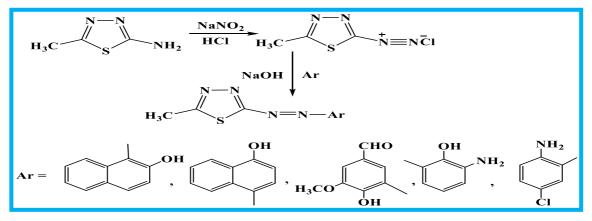
In this investigation, two harmful bacteria were used: Escherichia coli, which is Gram-negative, and Staphylococcus aureus, which is Gram-positive. These bacteria were extracted from the Department of Life Sciences' labs at the College of Education for Pure Sciences, and Multer Hinton Agar was employed as the growth medium [16-18]. Chemical solutions of (HA₁, HA₂, HA₃, HA₄, and HA₅) were produced in concentrations of 0.01–0.001–0.0001 mg/ml using the solvent Dimethyl Sulfoxide (DMSO) to test and determine the minimum inhibitory concentration (MIC). Using the diffusion method in the nutritional medium Mueller-Hinton agar, the sensitivity test for the bacterial isolates utilized in the study was conducted [19-21]. After the medium was created and autoclave sterilized, it was divided into plates and allowed to solidify. Four tiny pits were then formed on each plate. After that, it was incubated for 24 hours at 37 °C. The next day, the data were perused to demonstrate the derivatives sensitivity derivatives employed, which are contingent upon the diameter of the inhibition seen in the dishes around the holes utilized, as the diameter increases, The term "inhibition" refers to rise in the produced compounds' biological activity as measured by the diameter of inhibition [22-24].

2.5 Measurement of laser efficiency of some compounds

The laser activity of some of the prepared compounds (HA1, HA4, HA5) was measured using He-Ne laser (visible laser). Each compound was irradiated for four time periods (15, 30, 45, 60) seconds and its physical properties were studied again[25].

3. Results and discussion

Azo dyes are prepared by reacting equimolar amounts of 5-methyl-1,3,4-thiadiazol-2-amine with sodium nitrite and hydrochloric acid in the presence of distilled water to form diazonium salts and reacting with one of the coupling solutions (β -naphthol, α -naphthol, 4-hydroxy-3-methoxy benzaldehyde, 2-aminophenol, 4-chloro-aniline) to produce azo dyes in the presence of sodium hydroxide as a solvent, as shown in the following formula:



Scheme (1): Route of prepared compounds [HA1-HA5)

3.1. Characterization of azo dye derivatives (HA1-HA5)

When studying the infrared spectrum of azo compounds (HA1-HA5), it was noted that the (NH2) band disappeared in 5-methyl-4,3,1-thiadiazole-2-amine, and the appearance of an absorption band (3388-3271) cm-1 due (OH), a band in (3070-3035) cm-1 due (Ar-CH), two bands in the range (2942-2920 & 2917-2816) cm-1 due aliphatic (CH), a band in (1631-1600) cm-1 due (C=N) thiadiazole, a band in (1462-1438) cm-1 due (N=N). two bands in (1577, 1494) cm-1 is due (C=C), a band in (763-744) cm-1 due (C-S) bond in the thiadiazole ring [26,27], as shown in Table 2

				IR (KBr) cm ⁻¹							
Com p. No.	Ar	νO H	vC- H Aro m	vC- H Alip h	vC= N	vC= C Aro m	vN= N	vC- S	Others		
HA ₁	OH OH	328 6	3047	2920 2843	1600	1539 1512	1462	744			
HA ₂	OH	329 6	3039	2942 2917	1603	1526 1485	1456	755			
HA3	CHO OH OH	327 1	3051	2920 2816	1631	1581 1516	1458	771	ν (C-O) 1384 ν (C=O) 1701		
HA ₄	OH NH ₂	338 8	3035	2923 2875	1620	1575 1483	1460	748	v (NH ₂) <i>asy</i> . (3245) <i>sym</i> . (3226)		
HA ₅	NH ₂		3070	2931 2874	1608	1550 1489	1438	763	v (NH ₂) <i>asy</i> . (3329) <i>sym</i> . (3259)		

Table (2): Infrared absorption results (cm-1) for azo compounds (HA1-HA5).

When studying the 1H-NMR spectrum of the compound [HA1] using the solvent (DMSO-d6), a signal was observed at (9.88) ppm for (OH), a multiple signal at (7.29-8.30) ppm for the aromatic rings, a signal at (2.12) ppm for (CH₃), and a signal at (2.51) ppm attributed to the solvent (DMSO-d6), as in Figure (5).

When studying the 1H-NMR spectrum of the compound [HA4] using the solvent (DMSO-d6), it was observed that signals appeared at (6.93-7.77) ppm for the aromatic rings, a signal at (5.19) ppm for (NH₂), a signal at (2.43) ppm for (CH₃), and a signal at (2.53) ppm for the solvent (DMSO-d6), as in Figure (6).

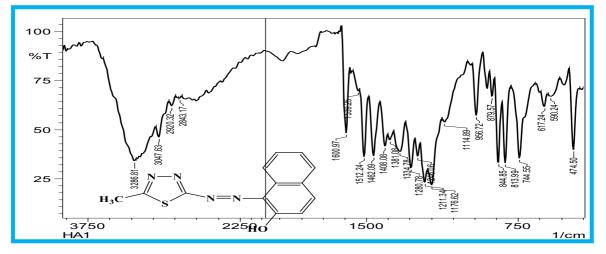


Figure (1): FT-IR spectrum of the compound (HA1).

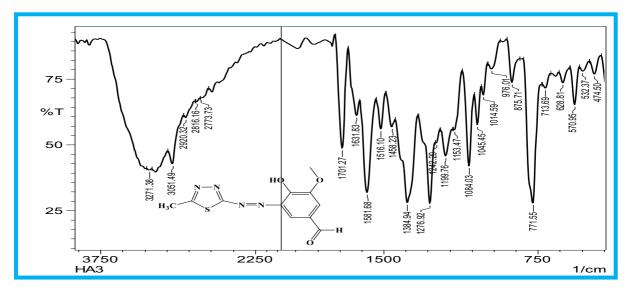


Figure (2): FT-IR spectrum of the compound (HA3).

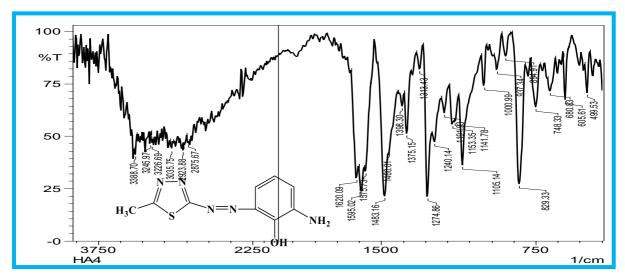


Figure (3): FT-IR spectrum of the compound (HA4).

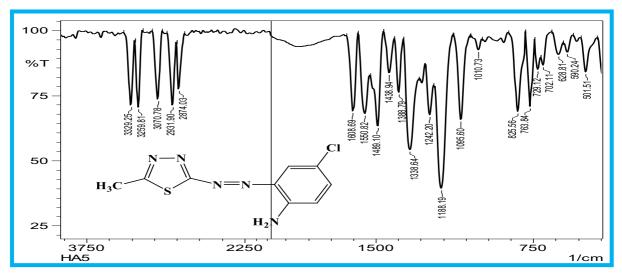


Figure (4): FT-IR spectrum of the compound (HA5).

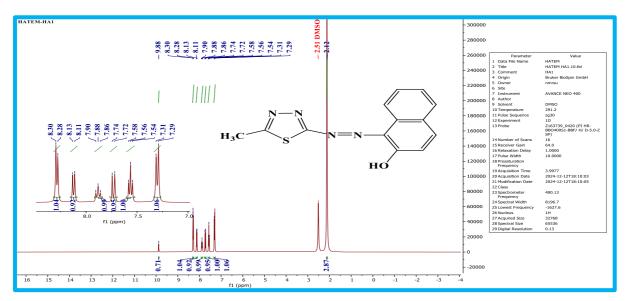


Figure (5): 1H-NMR spectrum of the compound (HA1).

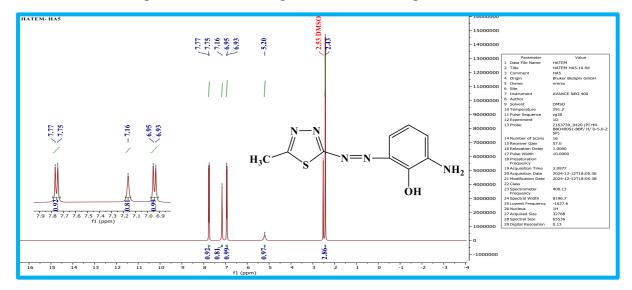


Figure (6): 1H-NMR spectrum of the compound (HA4).

3.2. Study of the color stability of azo dye derivatives (HA1-HA5)

The prepared azo dye compounds (HA1-HA5) were tested during the dyeing process for three materials: cotton, wool, and wood. According to the part of the dyeing process, the results showed strong and clear stability after washing with water and liquid soap. The stability of these compounds is attributed to their high polarization ability and their containing two azo groups (N=N)[28], as shown in Table (3).

	Cot	tton	W	ool	Cloth		
Comp. No.	After washing with water	After washing with soap	After washing with water	After washing with soap	After washing with water	After washing with soap	
HA ₁	+++	+++	+++	+++	+++	+++	
HA ₂	++	+++	++	+++	+++	+++	
HA ₃	+	++	++	++	++	+++	
HA ₄	++	++	++	+	++	+++	

Table (3): Color stability of prepared azo dyes (HA1-HA5)

	HA ₅	+++	++	+++	+++	++	-
* +++ =	= high stab	ility, $++ = m$	edium stabil	ity, + = poor	stability =	no stabilit	v

3.3. Evaluation of the Biological Activity of Prepared Compounds

Different biological activities are shown by compounds having non-homogeneous rings against both Gram-positive and Gram-negative bacteria. In this dissertation, the biological activity of the synthesized compounds was evaluated against *Staphylococcus aureus* and *Escherichia coli*, two medically relevant bacteria that cause various illnesses. Moreover, these bacteria exhibit different antibiotic resistance patterns [29-31]. The agar well diffusion approach assessed the biological activity of generated medicines by measuring the inhibitory zone width [32-34]. The findings indicate that the compounds generated have varying degrees of ability to inhibit the growth of both Gram-positive and Gram-negative bacteria. The compounds demonstrated significant inhibitory action against Escherichia coli and exceptional inhibitory effects against Staphylococcus aureus [35, 36]. Table 4 indicates that the concentration and inhibition relationship was dose-dependent, with higher percentages of inhibition seen at 0.01 mg/mL[37-39].

Comp. No.	Esch	erichia	a coil	Staphylococcus aureus			
Conc.	0.00	0.0	0.01	0.00	0.00	0.01	
mg/ml	01	01		01	l		
HA_1	8	10	12	NIZ	5	10	
HA ₂	5	5	10	4	8	12	
HA ₃	5	5	15	5	10	15	
HA ₄	NIZ	10	10	5	12	12	
HA ₅	5	5	5	5	5	10	
Amoxicillin	20	22	28	20	28	33	

Table(4): Biological effectiveness of prepared compounds and control treatments [inhibition in mm].

3.4. Results of measuring the laser activity of some prepared compounds

The study showed that during the periods (15, 30, 45) seconds, there was no change in the physical properties of the chemical compounds, as they maintained their structural form and physical properties without being affected by the laser beams. However, when exposed for (60 seconds), changes in the physical properties were observed, represented by a significant decrease in the melting points of all the studied compounds and a slight change in the colors. This is attributed to a breakage in some bonds within the compounds [40,41].

Comp	15 S		30 S		45 S		60 S	
No.	Color	M.P (⁰ C)	Color	M.P (⁰ C)	Color	M.P (⁰ C)	Color	M.P (⁰ C)
HA ₁	Yellow	121- 123	Yellow	121- 123	Yellow	121- 123	Light Yellow	105-107
HA ₄	Red	131- 133	Red	131- 133	Red	131- 133	Orange	122-124
HA ₅	Orange	122- 120	Orange	122- 120	Orange	122- 120	Yellow	137-139

 Table (5): Results of measuring the laser activity of some prepared compounds.

4. Conclusions

The azo dyes showed purity through physical measurements such as melting point and spectroscopic measurements such as infrared spectrum and proton nuclear magnetic resonance spectrum. The product percentage was good. The prepared compounds showed high stability

towards washing conditions and upon laser irradiation. In addition, they showed effectiveness against the two types of bacteria used, where the effectiveness varied from one compound to another.

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