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Synthesis and Physicochemical Characterisation of Two Azo Dyes Derived from Aspirin and Paracetamol

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Azo compounds are a class of organic molecules characterised by the presence of a double bond between two nitrogen atoms (azo functional group). Two new azo dyes are prepared using sulfanilamide with paracetamol(L_1) and luminol with aspirin (L_2). The elemental analysis (C, H, N), FTIR, ^{1}HNMR and visible spectroscopic techniques have been conducted for the characterisation of new dyes. The electronic spectra of these dyes were studied in terms of acid-base properties at different pH values 2-12 by using a universal buffer solution, which includes establishing isobestic points and determination of protonation and ionisation constants. The impact of various polarity solvents on the electronic spectra. To know the biological effectiveness of the prepared azo compounds against bacteria and fungi, the biological effectiveness was studied with two types of bacteria (*Escherichia cole* and *Staphylococcus aureus*) and two types of fungi (*Candida albicans* and *Aspergillus niger*). The Azo compound solution (L1) was investigated as acid-base indicator. Biological effectiveness in terms of what, as staining dyes, their effectiveness as pH indicator. It means, with these dyes at least, one titration must be carried out to compare with established indicators to see its effectiveness, such as less concentration of new azo dyes will be shown to be more effective than conventional dyes used as indicators.

Keywords: azo dye; solvent effects, acid–base titration; isobestic points; acid-base properties; luminol

I. INTRODUCTION

Diazonium salts are among the most important and famous compounds in the preparation of azo compounds, they are very effective and unstable compounds. Due to their instability, they are rarely isolated, so they are used immediately after preparation (Jing Hui *et al.*, 2017). The presence of the azo moiety (–N=N–) coupled with two aromatic or heteroaromatic frameworks in their structure characterises these substances. Owing to their unique physical-chemical characteristics and biological functions, they have found versatile applications in the food, pharmaceutical, cosmetic, colouring, and material industries, as well as analytical research. Azo compounds are well known for their therapeutic and are thought to have uses in the areas of antidiabetic, antimicrobial, antifungal, relaxing, antineoplastic, and anticancer applications (Patil & Nehete, 2015; Kareem & Salman, 2017). Research on azo dyes with intriguing spectrophotometric and physical characteristics has been ongoing, azo dyes are a very significant class of weak acids or weak bases that have garnered the interest of numerous researchers recently. They include heterocyclic moieties. extensively utilised in a wide range of real-world applications, including colourants, sensors, indicators, photochromic materials, and non-linear optics (Fayadh *et al.*, 2015). The majority of industries that use azo compounds extensively include textile dyeing, biological research, sophisticated organic synthesis applications, and high-tech industries like lasers (Gayathri & Ramalingam, 2008). Because the majority of azo dyes have fixed isobestic points, which indicate the number of equilibria in an azo dye, they are utilised as indicators of

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acid-base conditions (Ali, 2014). There is a study of two novel azo dyes are prepared from luminol using 2-vanillin [5-(luminolazo)-2-vanillin] and 4-hydroxycoumarin [3-(luminolazo)-4-hydroxycoumarin]. Two spectral studies are involved in the work: the first examines the acid-base characteristics of various pH values, and the second looks at the solvent effect of various polarity (Ali *et al.*, 2020). Sulfanilamide is an antibacterial of the sulfonamide class, the use of the powder has reduced infection rates and contributed to a significant reduction in mortality rates (Aftab *et al.*, 2013). Spectral study of the effect of the acid function on the thermodynamics of the formation of two azo dyes prepared from the reaction of paracetamol with both meta-aminophenol and para-aminobenzoic acid in acidic, neutral and basic media at a temperature of 288K, the optimum conditions for each prepared azo dye were studied, as well as the optimum molar ratios of its components (Hashim & Mahmoud, 2023). Everall azo compounds have been synthesised using the simple azo reaction pathway, the synthesised compounds contain the pharmacological moiety of aspirin, which exhibits excellent antimicrobial activity, the structures of all compounds have been confirmed by ^{1}HNMR and FT-IR (Pathan & Borul, 2008).

The present work includes the preparation of two new azo compounds from sulfanilamide with Paracetamol (L_1) and Luminol with Aspirin (L_2), which were characterised by C.H.N., ^{1}H-NMR, FT-IR, and visible spectroscopic techniques. Also, two studies include the first study of the effect of pH on the electronic absorption spectra and the second study with a group of solvents of different polarities. As well as the biological activity of two types of bacteria and fungi. Sodium hydroxide was titrated with both hydrochloric acid and acetic acid spectrophotometrically using the prepared azo compound (L_1) as an indicator.

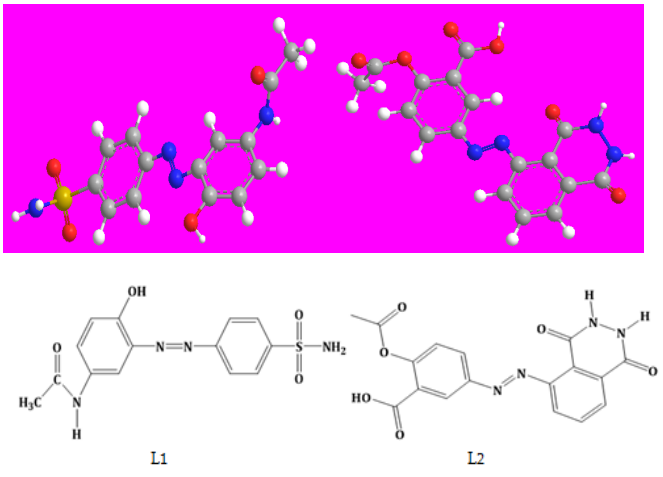
II. MATERIALS AND METHODS

A. Chemicals and Materials

All of the solvents and reagents belonged to the reagent-grade class. All chemicals are of a superior quality. Spots were evident under UV light when the TLC technique was employed to track the reaction's development on a silica gel-coated plate. The pH was measured using a drying oven (KARL KO/Germany), Heidolph MR Series Magnetic Stirrer-Hotplates, and a pH-Meter (H. Jurgons Co. Bremen, L. Puls Munchen15). The 6305 Spectrophotometer was used to record the UV/V absorption spectra of the dyes. Element analysis (C.H.N) was carried out by Perkin element 2400 element analysis, melting points were determined on the melting point apparatus. Shimadzu FT.IR-8400S, In KBr pellets, infrared spectra were captured. A range of pH values 2–12 for universal buffer solutions were created. The ^{1}HNMR spectra of the ligands were determined in DMSO, internal standard TMS.

B. Preparation

For the preparation of Azo dyes, (2.1 ml) of strong HCl was used to dissolve (0.006 mole of each sulfanilamide or luminol; 1.0332 or 1.0629 g, respectively) with (10 ml) of deionised water. To create diazonium salt, add drop by drop with stirring a solution of (0.456 g) sodium nitrite in (10 ml) deionised water to a temperature of (below than 5^{o}C). The aforementioned diazonium salt was mixed with an alkaline solution of (0.006 mol) (0.9070 or 1.0809g) of (paracetamol or aspirin) in (1.8% w/v. NaOH). Add diluted HCl, to convert the sodium salt form to hydrogen form. After filtering the resulting dye precipitate, purify the dyes by recrystallisation from ethanol. Creating azo dyes by drying in a (50°C) oven. ^{1}HNMR, C.H.N, and IR were used to help determine the chemical structures of azo compounds were suggested as shown in scheme -1.



Scheme 1. Azo dyes (L_1 and L_2)

C. Solutions

* 1×10^{-3} M of L_1 and L_2 azo dyes each.

* Universal buffer solution (pH 2-12) (Dean, 1999).

* 0.1 M solutions of acetic acid, sodium hydroxide, sodium carbonate, and hydrochloric acid, each calibrated using the recommended technique (Vokel, 1975).

D. Procedure

1. Acid-base properties at different pH values

To study the effect of pH values on azo compounds (L_1 and L_2), the universal buffer solutions were prepared in the range of 2-12. The absorbance of a series of 2.4×10^{-4} and 0.8×10^{-4} M of each two dyes L_1 and L_2, respectively, were measured at pH values 2-12 in wavelength range 320-600 nm using pH buffer solution as blank solution.

2. Solvent effect of different polarities

To demonstrate the impact of various polarity solvents (Chloroform, Dimethylformamide (DMF), Dichloromethane (DCM), Benzene, Dimethyl sulphoxide (DMSO), Ethanol, Methanol, 1,4-Dioxane, Acetone, Ethyl Acetate, Deionised Water) were used. The absorbances of 0.76×10^{-4} and 1.8×10^{-4} M of each two dyes L_1 and L_2, respectively, were measured at a wavelength range of 320–560 nm, using the solvent as a blank solution.

3. Acid–Base titration

0.1 M, 0.1 M and 0.1 M solutions of NaOH, HCl and HAc, were prepared and standardised by recommended methods (Vokel, 1975), and found to be 0.0987 N, 0.0975 N and 0.1001 N, respectively. The absorbances were measured at λ_{max} for dye (L_1, 420nm) by titrations of sodium hydroxide with each HCl and HAc, by adding different volumes of sodium hydroxide.

III. RESULT AND DISCUSSION

A. Identification of Azo Dye L1 and L2

1. Elemental analysis

The physical characteristics and analytical information of the prepared azo dyes (L_1 and L_2) are listed in Table 1. It was found the results in good agreement with a proposed molecular formula.

Table 1. M.p and elemental analysis of novel azo dyes L_1 and L_2

Dye Symbol	Molecular formula	m.p (°C)	Color	C % Cal. (found)	H % Cal. (found)	N% Cal. (found)
L_1	$C_{14}H_{14}N_4O_4S$	355	Black	50.24 (46.31)	4.18 (3.53)	16.74 (15.47)
L_2	$C_{17}H_{12}N_4O_6$	290	Orange	55.38 (51.29)	3.25 (3.53)	15.20 (16.76)

2. FT-IR Analysis

By using infrared spectroscopy, the two produced azo dyes (L_1 and L_2) were identified within a 400–4000 cm^{-1} range. Figures 1 and 2 depict the two dyes' infrared spectra, while Table 2 lists the dyes most significant frequencies, the bands in the range 3400 and 347.58 cm^{-1} and 1429.25 and 1444.68 are due to ν (O-H) and azo group (N=N) for dyes L_1 and L_2, respectively.

Table 2. L_1 and L_2 selected infrared data

Dye Symbol	The Wave Number of the Beam (cm^{-1})									
	(O-H) Stretching	(N-H) Stretching	(C-H) Aromatic	(C-H) Aliphatic	(C=O) Stretching	(C=C) Stretching	(N=N) Stretching	(C-O) Stretching	(C-N) Bending	(O-H) Bending
L_1	3400.00 W	3304.06 M	3082.25 W	2950.00 W	1643.35 M	1618.28 M	1429.25 S	1338.60 S	1274.95 S	1166.93 S
L_2	3458.37 M	3174.83 M	3037.89 M	2895.15 M	1643.35 S	1598.99 S	1438.90 S	1338.60 M	1251.80 S	1166.93 S

W = weak, S = Strong, M = Medium

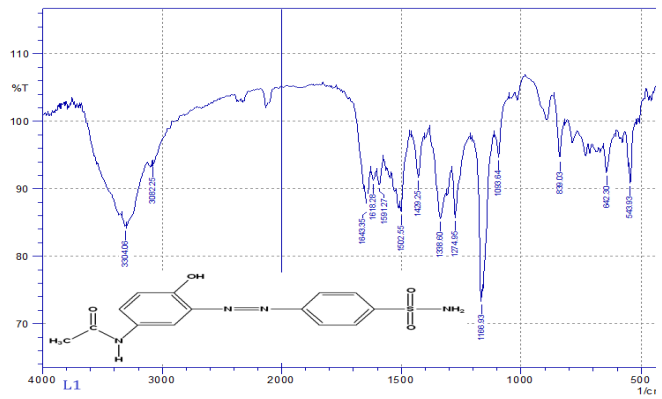


Figure 1. F.T. infrared spectroscopy of L_1

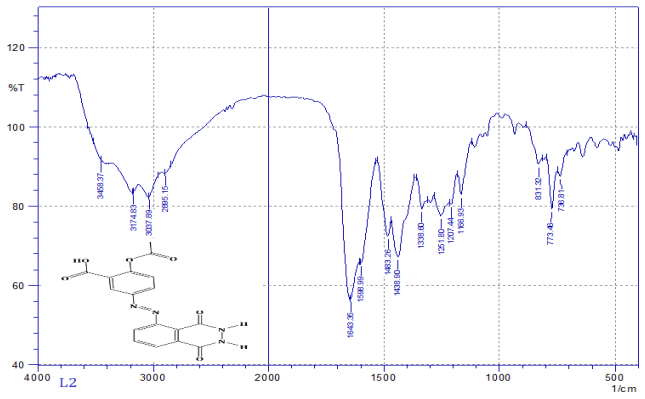


Figure 2. F.T. infrared spectroscopy of L_2

3. *^{1}HNMR spectra of azo dye compounds*

The azo dyes ^{1}H-NMR spectra (Figures 3 and 4) were obtained using dimethyl sulfoxide as the solvent. The DMSO signal appears at 2.5 ppm, the HOD signal at 3.4 ppm, the aromatic ring signal at 6.8-8.5 ppm, and the OH group signal appears at 10.21 ppm for azo compound (L_1) and 12.19 ppm for azo compound (L_2). The result is shown in Table 3.

Table 3. Chemical shifts of the proton nuclear magnetic resonance (^{1}HNMR) spectra of the azo compounds L_1 and L_2 in dimethyl sulfoxide (DMSO) solvent

Compound Symbol	Chemical Shift (ppm)
L_1	9.80 (s, 2H, NH_2), 10.21 (s, H, N-H), 10.67 (d, H, OH), 6.67-8.14 (m, 7H, Ar-H), 2.04 (s, 3H, CH_3)
L_2	11.66 (s, 2H, N-H), 6.86-8.62 (m, 6H, Ar-H), 1.20 (s,3H, CH_3), 12.19 (s, H, O-H),

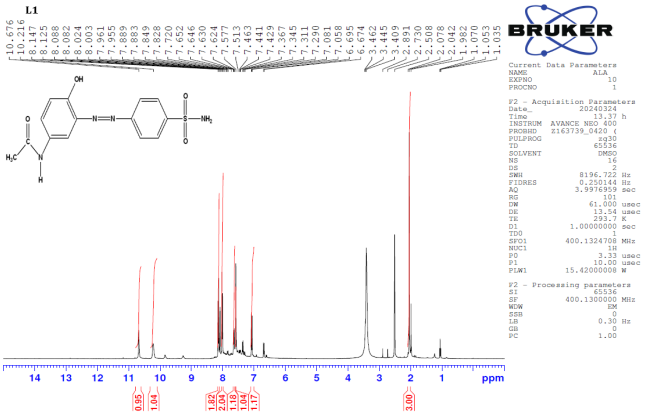


Figure 3. proton nuclear magnetic resonance (^{1}HNMR) spectrum of the azo (L_1)

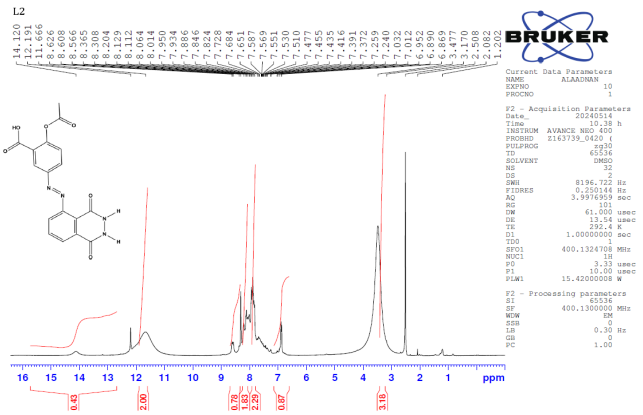


Figure 4. proton nuclear magnetic resonance (^{1}HNMR) spectrum of the azo (L_2)

B. Acid-Base Properties with Different pH Values

To compute the ionisation and protonation constants and investigate the impact of the azo dyes acidity, the universal buffer solutions with pH values ranging from 2-12 is used. The absorption spectra of 2.4×10^{-4} or 0.8×10^{-4} M solutions of each L_1 and L_2 dyes, were represented graphically at wavelength range 320-650nm (Figures 5 and 6). In case of L_1, the spectra were characterised by three maximal bands the first at wavelength 390 nm of pH range 2-8. The second and third at wavelength range 420 and 490 nm, respectively, of pH value range 9-12, which represent basic

form (ionic form). From the figures, it was found two isobestic points at 400 and 410 nm, with the highest absorbance was at pH range 9-11. In case of L_2 dye (Figure 6), the spectra are characterised by three maximal bands the first at 360-380 nm in the pH range 2-6, which represents the protonated form and the second at the wavelength range 340 and 369 nm at pH value range 7-12 for basic form. The spectra show one isobestic points at 410 nm, with highest absorbances were at pH 12.

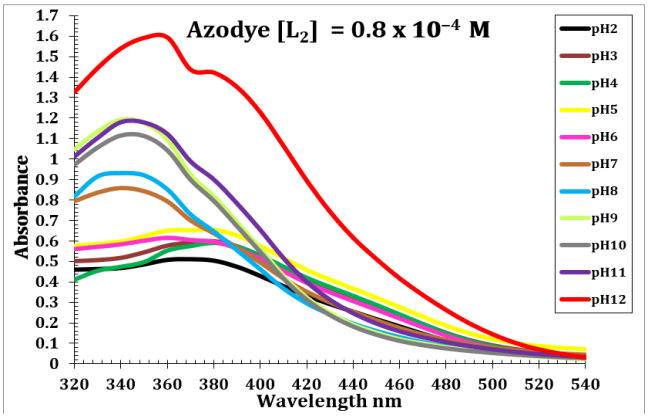


Figure 6. Visible absorption spectra of the azo dye (L_2)

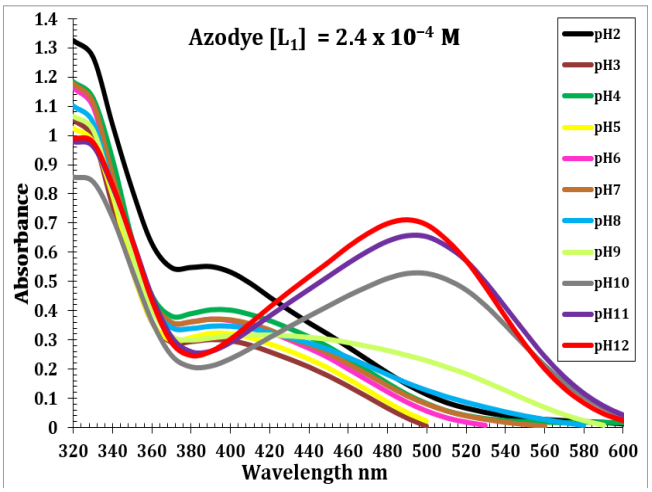


Figure 5. Visible absorption spectra of the azo dye (L_1)

To determine the ionisation and protonation constants of the azo dyes (L_1 and L_2), the wavelengths 490 and 350 nm were chosen for azo dyes (L_1 and L_2), respectively. From Figures 5 and 6, the absorbance–pH curves were plotted (Figure 7). Using the half-height approach (Fahad *et al.*, 2013), the ionisation and protonation constants were calculated (Table 4). The pK values were determined using the equation:

$$pK = pH \text{ (at } A_{1/2}), \text{ where } A_{1/2} = (A_L + A_{min}) / 2$$

A_L and A_{min} represent the limiting and minimum absorbance, respectively.

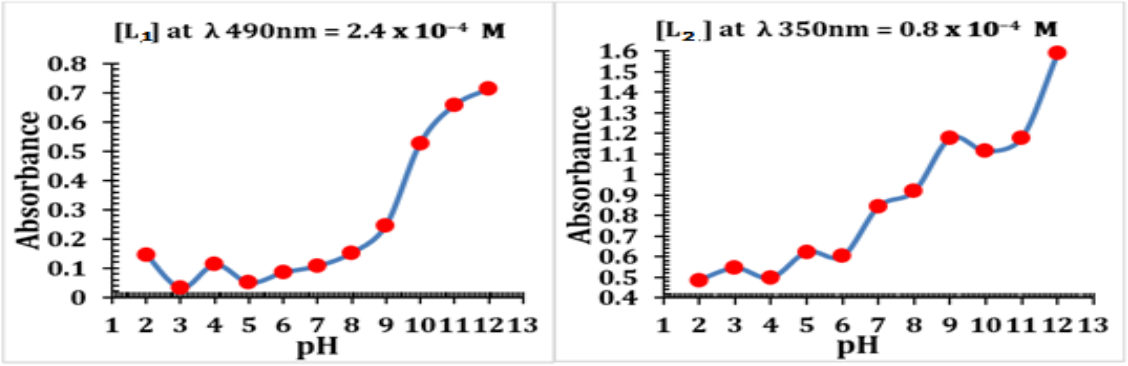


Figure 7. The azo compounds' absorbance-pH curves (L_1 and L_2)

Table 4. The azo compounds' ionisation and protonation constants (L_1 and L_2)

Azo dye L_1 at λ = 490 nm				Azo dye L_2 at λ = 350 nm			
A_{min}	A_L	$A_{1/2}$	pK	A_{min}	A_L	$A_{1/2}$	pK
0.03	0.11	0.07	3.50 (pKp_1)	0.49	0.62	0.55	4.50 (pK_{p1})
0.05	0.10	0.07	6.00 (pKp_2)	0.60	0.91	0.75	6.60 (pK_{a1})
0.15	0.52	0.33	9.40 (pKa)	0.84	1.18	1.01	8.30 (pK_{a2})
				1.11	1.59	1.35	11.50 (pK_{a3})

For L_1: pKa is ionisation of OH group of paracetamol, pK_{p1} and pK_{p2} are protonation constants of NH_2 group of sulfonamide and NH group of paracetamol, respectively. For L_2: pK_{p1} is the protonation of the OH group of aspirin, pKa1, pKa2, and pKa3 are the ionisation constants of the OH group of aspirin, the OH group of enoform of C=O luminol and the OH group of the second enoform of C=O, respectively.

C. Solvents Effect

From Figure 8 for the azo compound (L_1), it was found more than one peak as in Table 5 for the following solvents (Aceton, DMSO, DMF, 1,4-Dioxane and Ethyl Acetate), while the rest of the solvents (chloroform, ethanol, methanol, DCM, benzene and water) having one peak. The absorbances were measured at a range of 320 – 540 nm using the solvent as a blank solution. From Figure 8, it was found that the chloroform gives the highest. In case (L_2), Figure 9, it is noted that all solvents give one band with the highest absorbance by using chloroform also, there is observed red shift with the polar DMF solvent at 450 nm compared with non-polar solvent 1.4-dipxane (380 nm). To verify whether a shift in salvation energy or pure dielectric is the source of the band shift (Δv), the solvent's λ max plot vs dielectric functions (D-1 / D+1) of azo dyes (L_1) and (L_2) were demonstrated (Figures 10 and 11).

Table 5. Dielectric function of different solvents and their λ max

Solvent Symbol	Solvent	D	(D-1/ D+1)	λ max nm	
				L_1	L_2
1	Chloroform	4.8	0.655	430m	380m
2	Aceton	20.60	0.907	330s, 430m	390m
3	Ethanol	24.30	0.921	420m	390m
4	Methanol	32.70	0.940	420m	390m
5	DMSO	47.00	0.958	330m, 430m	-----
6	DMF	36.71	0.946	330m, 430m	450m
7	1,4-Dioxane	2.20	0.375	330m, 430m	380m
8	DCM	9.10	0.802	430w	370m
9	Ethyl Acetate	6.02	0.715	330m, 430m	390m
10	Benzene	2.28	0.390	430w	380m
11	Water	78.40	0.975	390m	390m

w = Weak, m = Medium, s = Strong

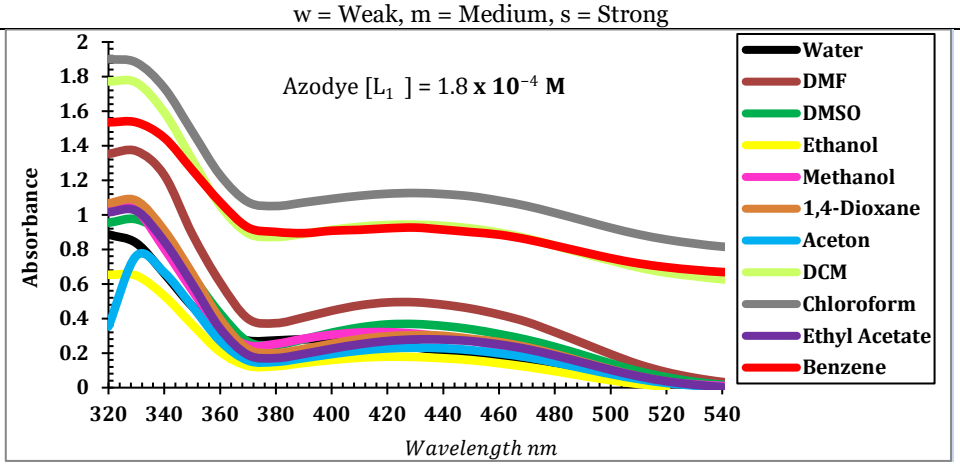


Figure 8. Visible absorption spectra of the azo compound (L_1) in solvents of different polarity

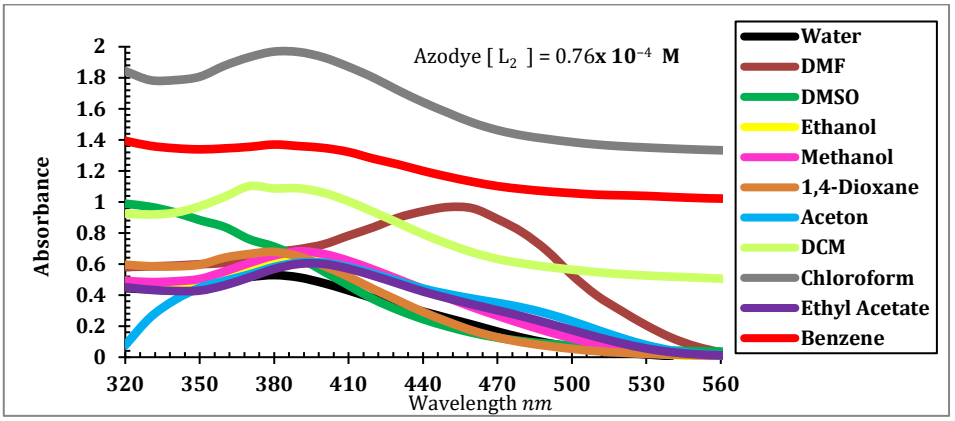


Figure 9. Visible absorption spectra of the azo compound (L_2) in solvents of different polarity

The molar absorption coefficients and maximum wavelengths of the azo dyes (L_1 and L_2) at various solvent polarities are displayed in Table 6.

Table 6. The λ_{max} and ε max of azo dyes (L_1 and L_2) at various solvents used

Solvent Symbol	Dye	Solvent	π→π*		π→π* (azo)	
			λ_{max} nm	ε_{max} x10⁴ l.mol⁻¹.cm⁻¹	λ_{max} nm	ε_{max} x10⁴ l.mol⁻¹.cm⁻¹
1	L_1	Chloroform	---	---	430	0.62
2		Aceton	330	0.42	430	0.19
3		Ethanol	---	---	420	0.10
4		Methanol	---	---	420	0.18
5		DMSO	330	0.54	430	0.20
6		DMF	330	0.76	430	0.27
7		1,4-Dioxane	330	0.60	430	0.17
8		DCM	---	---	430	0.52
9		Ethyl Acetate	330	0.57	430	0.15
10		Benzene	---	---	430	0.51
11		Water	390	0.15	---	---
1	L2	Chloroform	380	2.59	--	---
2		Aceton	390	0.80	--	---
3		Ethanol	390	0.86	--	---
4		Methanol	390	0.89	--	---
5		DMSO	---	---	--	---
6		DMF	---	--	450	1.27
7		1,4-Dioxane	380	0.89	--	---
8		DCM	370	1.45	--	---
9		Ethyl Acetate	390	0.79	--	---
10		Benzene	380	1.80	--	---
11		Water	390	0.69	--	---

Figures 10 and 11 for the azo compound ($L_1$and L_2) show the presence of linearity for all solvents used, due to the dipole moment, while it was shown a deviation from linearity for the solvent (Water and DMF) respective of L_1 and L_2, perhaps due to the hydrogen bonding between the solvent and the solute.

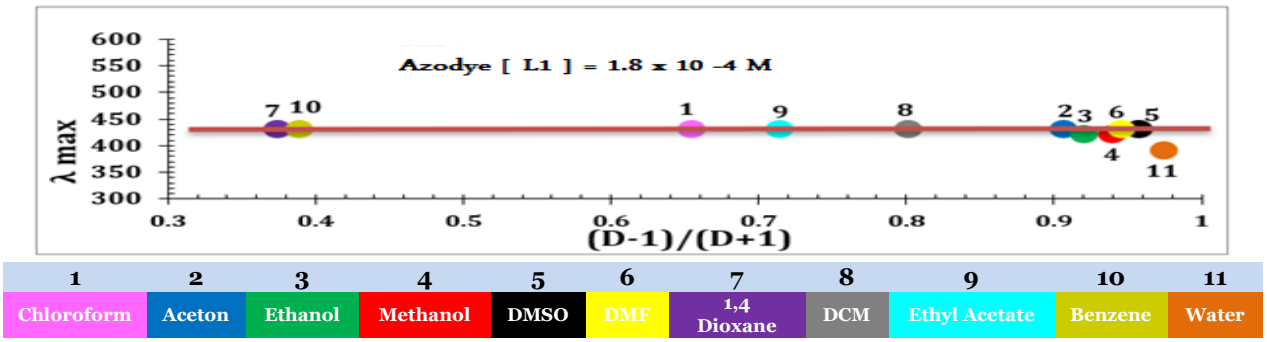


Figure 10. The relationship between λ max of the azo compound (L_1) and the function of the dielectric constant

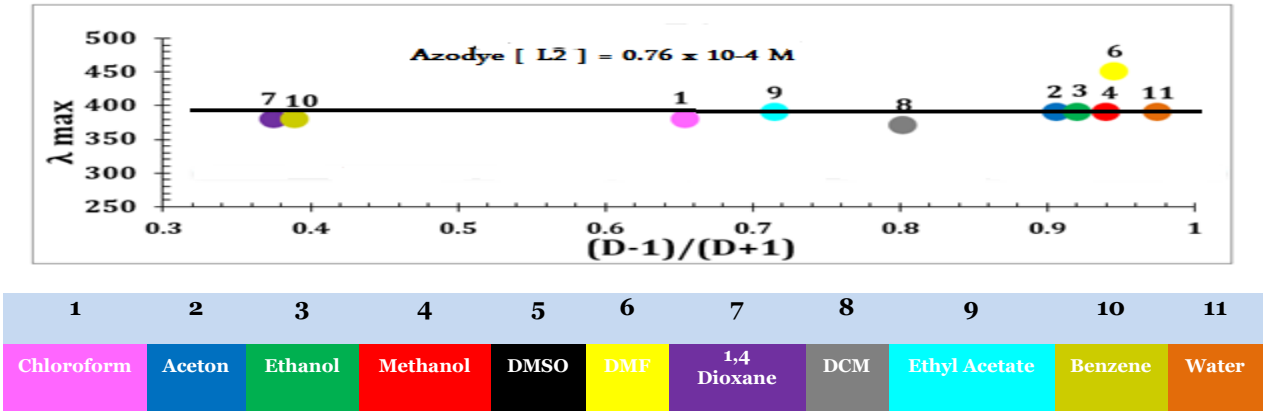


Figure 11. The relationship between λ max of the azo compound (L_2) and the function of the dielectric constant

D. Spectrophotometric Titration for the Determination of Sodium Hydroxide using (L_1) as Indicator

After the concentration of hydrochloric acid, acetic acid, and sodium hydroxide was titrated volumetrically by the recommended method (Vokel, 1975). The azo compound L_1 can be used as an indicator by spectrophotometric analysis through the change in its colour in acidic and basic media Figure 12. Sodium hydroxide was spectrophotometrically titrated with both hydrochloric acid and acetic acid using the azo (L_1) as indicators as in Figure 13, where the end points are detected. Several concentrations of the azo dye were used and the relative error for each concentration was calculated (Table 7). It was found from Table 7, all concentrations of the are suitable except for the concentration (21×10^{-4} M), and it is also noted that the azo compound with a concentration 15×10^{-4} M is the best one because of the jump (ΔA/ 0.1ml) and relative error. The titration of acetic acid with sodium hydroxide, all concentrations of the compound are suitable, and it is also noted that the azo compound with a concentration 9×10^{-4} M is the best.

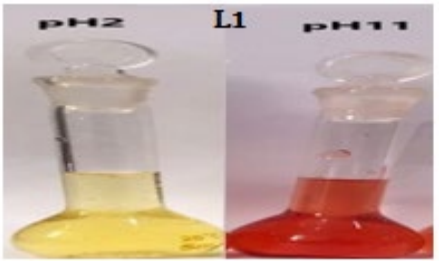


Figure 12. Colours of the solution of azo compound (L_1) at pH 2 and 1

Table 7. The relative error and jump values at different concentrations of azo compound (L_1)

[L] x 10^{-4} M	[NaOH]M Taken	[NaOH]M found	Relative error %	Jump (ΔA/0.5 ml NaOH)
Titration of HCl × NaOH using L_1				
3	0.0987	0.1000	1.31	0.034
6	0.0987	0.1026	0.20	0.039
9	0.0987	0.1002	1.51	0.037
12	0.0987	0.0970	-1.72	0.020
15	0.0987	0.0972	-1.51	0.092
18	0.0987	0.0979	-0.81	0.057
21	0.0987	0.0951	-3.64	0.023
Titration of CH_3COOH× NaOH using L_1				
3	0.0987	0.0991	0.40	0.031
6	0.0987	0.0969	-1.82	0.043
9	0.0987	0.0995	0.81	0.066
12	0.0987	0.0972	-1.51	0.016
15	0.0987	0.0962	-2.53	0.153

E. Antibacterial Activity

The picture in Figures 14 and 15 and results in Table 8 show the clear effect of the azo compound (L_1 and L_2) in inhibiting the growth of bacteria and fungus: *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger* and *Candida albicans*.

Table 8. Biological activity of the prepared azo compounds ($L_1$and L_2) with bacteria and fungi

Dye Symbol	Inhibition zone (mm)			
	Staphylococcus aureus	***Escherichia coli***	***Candida albicans***	***Aspergillus niger***
L_1	15	Zero	15	15
L_2	20	20	20	15

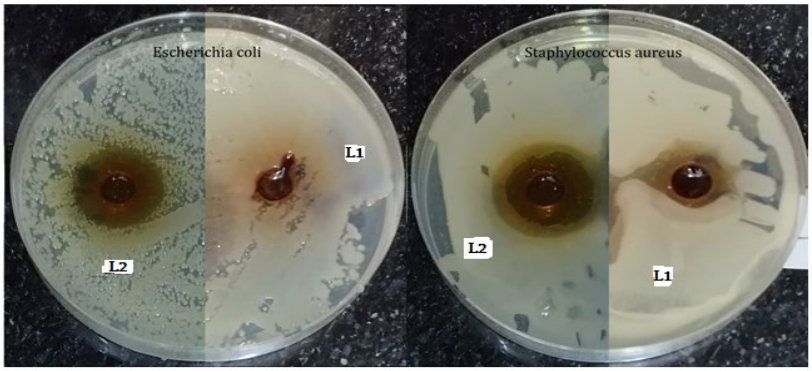


Figure 14. The effect of the two azo dyes (L_1 and L_2) on *Escherichia coli* and *Staphylococcus aureus* bacteria

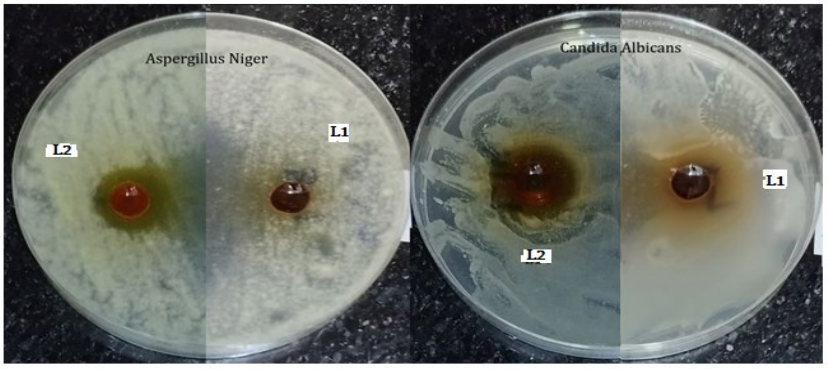


Figure 15. The effect of the two azo dyes (L_1 and L_2) on the fungi *Candida albicans* and *Aspergillus niger*

IV. CONCLUSION

Preparation of new L_1-L_2 azo compounds and their identification using modern analytical techniques. The results of the elemental analysis of azo compounds showed that the theoretical values of the proposed formulas are close to the practical values, which means that the proposed chemical structures of the compounds are correct. The visible spectra of L_1-L_2 azo compounds were studied with a group of solvents of different polarities to show the extent of the effect of these solvents in terms of polarity on the displacement of these compounds in a range of wavelengths 320-650 nm. Their spectra showed a main absorption band attributed to the transition ($\pi \rightarrow \pi^*$) in the azo group using solvents of different polarity, and a linear relationship was found between the maximum wavelengths and the dielectric constants, which means that the dipole moment is what controls the displacement of the peaks. The possibility of using the azo compound L_1 as an indicator in the calibration process (strong acid-strong base and weak acid-strong base) instead of phenolphthalein and methyl orange because they have different colours in the acidic and basic environments,

and the results were spectrally close by calculating the relative error and providing high accuracy and precision. It is concluded that the closeness of the concentrations of sodium hydroxide obtained from the two methods (volumetric method and spectroscopic method) means the possibility of using these azo compounds as indicators in calibration (strong acid - strong base). The azo compounds showed an effect on the bacteria (*Staphylococcus aureus*) as well as the effect of the azo compound L_2 on the bacteria (*Escherichia coli*). The effect of the azo compounds L_1-L_2 on the fungus (*Candida albicans*) as well as the effect of the azo compounds on the fungus (*Aspergillus niger*).

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