Bull. Pharm. Sci., Assiut University, Vol. 48, Issue 1, 2025, pp. 319-333.

Bulletin of Pharmaceutical Sciences
Assiut University
Website: http://bpsa.journals.ekb.eg/

PREPARATION, CHARACTERIZATION, APPLICATIONS AND ANALYTICAL STUDIES OF SOME NEW AZO DYES DERIVED FROM SULFANILAMIDE

Alaa A. AL-muhsin[1 *] and A. A. Ali [2]

[1]*Basrah University, College of Education for Pure Science, Department of Chemistry, Basrah, Iraq*
[2]*Al-Kunooz University College, Department Pharmacy, Basrah, Iraq*

Background: *Azo compounds are a class of organic molecules characterized by the presence of a double bond between two nitrogen atoms and contain one or more azo functional groups.* ***Aim of study:*** *This study involves the preparation of two new azo dyes from Sulfanilamide with 8-hydroxyquinoline (L_1)[4-((8-hydroxyquinolin-5-yl) diazenyl) benzenesulfonamide] and Vanillic acid (L_2)[4-hydroxy-3-methoxy-5-((4-sulfamoylphenyl) diazenyl)benzoic acid]. C.H.N., ^{1}H-NMR, FT-IR, and visible spectroscopic techniques have described the characterization of dyes.* ***Results:*** *The electronic spectra of these azo dyes were studied in terms of acid-base properties at different pH values (2-12), which includes establishing isobestic points and determination of protonation and ionization constants. The impact of various polarity solvents on the electronic spectra was the subject of the other investigation. The biological effectiveness were also studied with two types of bacteria (Escherichia Coli and Staphylococcus Aureus) and two types of fungi (Candida albicans and Aspergillus niger). The Azo compounds solutions (L_1 and L_2) were used to determine the nitrite ion by forming the two dyes and a study of the precision and accuracy was investigated. The ability to use the two dyes as acid-base indicators*

Keywords: *Azo Dye, Sulfanilamide, Determination of nitrite, Escherichia Coli*

INTRODUCTION

A double bond between two nitrogen atoms and the presence of one or more azo functional groups define the class of organic molecules known as azo compounds[1-3]. Because of these compounds' distinct qualities and varied compositions, which make them valuable in a variety of industries like textiles, medicine, and industrial preparations, they have become increasingly important[4]. It is of great importance in the pharmaceutical industry because it plays an important and effective role in the biological system[5]. At different pH values[2-12], the electronic spectra of azo dyes can be studied in terms of acid–basic properties, this includes Figuring out the protonation and ionization constants with determination of the isopesteic points; the sensitivity and linearity of Beer's law of azo dyes at pH 7 was also investigated; the Standard Deviation (S.D) and Detection Limit (D.L) were also computed[6].

One can investigate the effects of various polarity solvents, their spectra revealed a major absorption package that was suggested for the (π-π*) transition in the iso group using various polar solvents; a linear relationship between the dielectric constant and great wavelengths was discovered, indicating that the bi-polar torque is the primary factor controlling the band shift[7].

Can be studied of the azo dye solutions and titration indications of acid-base spectroscopy (strong acid-strong base, weak acid-strong base), based on the curves and diagnostics of equivalence points, it was found to be within acceptable error[8]. *Staphylococcus*

Received : 24/8/2024 & Accepted : 1/12/2024

*Corresponding author: Alaa A. AL-muhsin, E-mail: mustafafadil837@gmail.com

aureus and *Escherichia coli* are two species of bacteria that can be used to test the biological efficacy of azo dyes, as can *Candida albicans*, the three azo dyes were demonstrated to be biologically active by the results[9]. Researcher Asaad and his group conducted a study that included the preparation of the azo compound (HZ_1) derived from the reaction of sulfanilamide with salbutamol, the prepared compound was characterized by FT-IR and Mass techniques, and the maximum wavelength was determined at (440 nm)[10]. A recent study by Radhi and her group included the preparation of the azo compound (IPDHQ) resulting from the reaction of 8-hydroxyquinoline with 4-iodoaniline, the azo compound was characterized by FT-IR, ^{1}HNMR, UV–Vis, spectrophotometry, C.H.N, and Mass Spectroscopy, the maximum wavelength was determined at (284 nm) and its melting point was (111℃)[11].

The present work includes the preparation of two azo compounds from sulfanilamide with 8-hydroxyquinoline (L_1) and vanillic acid (L_2), which were characterized 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 of the visible spectra of these two dyes with a group of solvents of different polarities. As well as estimating nitrite ion and 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 compounds (L_1 and L_2) as indicators.

MATERIAL AND METHODS

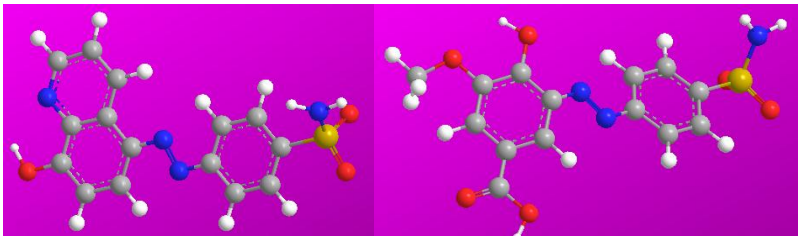
All of the solvents and reagents belonged to the reagent-grade class. All chemicals are of a superior quality [Sulphanilamide 99%(BDH),8-Hydroxy Quinoline 99%(Merck), Vanillic Acid99%(Merck)]. Spots were evident under UV light when the TLC technique was employed to track the reaction's development on 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 JENWAY 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 Manufactured by Thermo Fisher and on the 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.

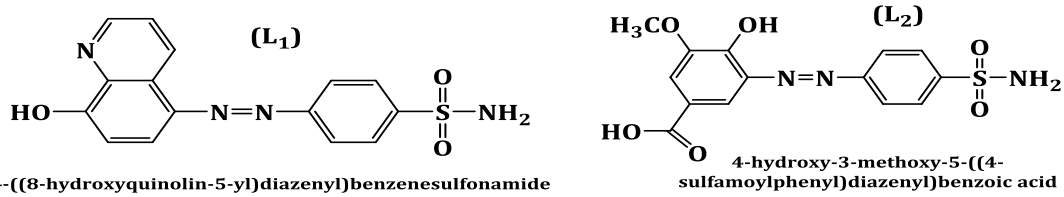
Preparations of the two azo dyes

For the Preparation of Azo dyes, 2.1 ml of strong HCl was used to dissolve (0.006 mol) (1.0332 g) of Sulfanilamide, and (10 ml) of deionized water was added thereafter. To create diazonium salt, add drops at a time, stirring a solution of (0.456 g) sodium nitrite in (10 ml) deionized water to a temperature of (5 ^{o}C). The aforementioned diazonium salt was mixed with an alkaline solution of (0.006 mol) (0.8709 or 1.0088g) of (8-hydroxyquinoline or vanillic acid) in (1.8% w/v. NaOH). By adding diluted HCl, the produced dye can be changed from sodium salt form to hydrogen form. After filtering the resulting dye precipitate, purify the dyes by recrystallization from ethanol. Creating azo dyes by drying in a (50°C) oven. ^{1}HNMR, CHN, and IR were used to help determine the chemical structures of azo compounds were suggested as shown in **Scheme 1.**

Solutions

* 1×10^{-3} M of L_1 and L_2 azo dyes each
* Universal buffer solution (pH 2-12)[12]
*0.1 M solutions of acetic acid, sodium hydroxide, sodium carbonate, and hydrochloric acid, each calibrated using the suggested technique[13]
*355.6 ppm stock solution of nitrite (corresponding to 533.4 mg/L of sodium nitrite), then different concentrations of nitrite were prepared in the range of (5 – 50 ppm).





Scheme 1: Azo dyes (L_1 and L_2)**.**

Procedure

***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 serious of (1.2×10^{-4} M) of each two dyes L_1 and L_2, were measured at pH values (2-12) in wavelength range (320-650 nm) using pH buffer solution as blank solution.

***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, Deionized Water] were used. Using the solvent as a blank solution, the absorbance of ($1.2x10^{-4}$ M) of each of the two dyes was measured at a wavelength range of (320–650 nm).

***Acid – Base titration**

0.1 M, 0.1 M and 0.1 M solutions of NaOH, HCl and HAc, were prepared and standardized by recommended methods[13], and found to be 0.0987 N, 0.0975 N and 0.1001N respectively. The absorbance were measured at λ_{max} for each dye (L_{410} and L_{390}) by titrations of sodium hydroxide with each HCl and HAc. The absorbance were measured at λ_{max} for each dye, when by addition volume of sodium hydroxide .

Determination of nitrite ion

The aromatic amine + (1% HCl v/v) to get final concentration of 0.0067 M was in ice (less than 5°C). Then pour the phenolic compound (1% NaOH w/v), with final concentration 0.0067 M (in ice also). Drop by drop with slowly, add a serious of deferent amounts of sodium nitrite solution on above mixture, to form azo dye. Measure the absorbance of the dye at λ_{max} of that dye.

RESULTS AND DISCUSSION

Identification of azo dye L1 and L2

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 illustrated from **Table 1**, dyes are in good agreement with a proposed molecular formula that, C.H.N Analysis [Carbon, Hydrogen, Nitrogen].

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	$C_{15}H_{12}N_4O_3S$	295	Brown	54.81 (46.31)	3.65 (3.28)	17.05 (13.93)
L_2	$C_{14}H_{13}N_3O_6S$	233	Orange	47.81 (45.25)	3.70 (3.74)	11.95 (10.54)

FT-IR Analysis [Fourier-transform infrared spectroscopy]

Using infrared spectroscopy, the two produced azo dyes (L_1 and L_2) were identified within a 400–4000 cm^{-1} range. **Fig. 1 and 2** depict the two dyes' infrared spectra, while **Table (2)** lists the dyes' most significant frequencies. The bands in the range (3302 and 3319.5cm^{-1}) and (1475.5 and 1470) are due to ν (O-H) and azo group (N=N) for dyes 1 and 2 respectively.

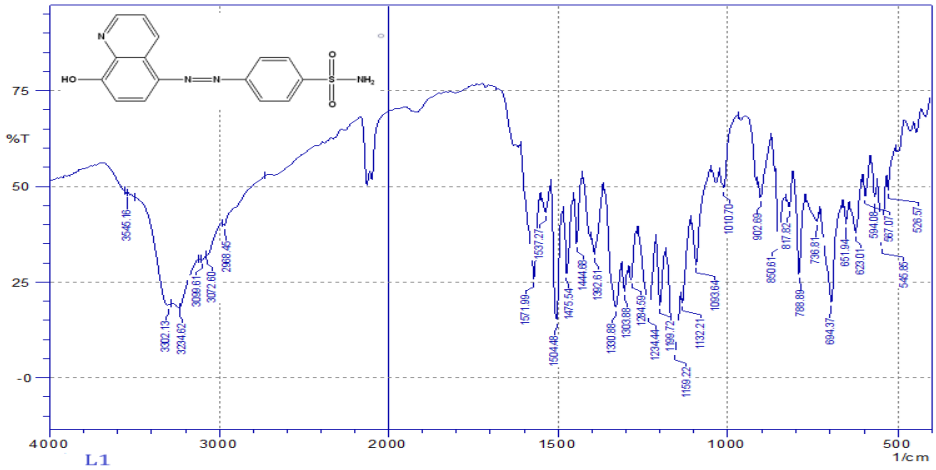


Fig. 1: F.T. infrared spectroscopy of L_1.

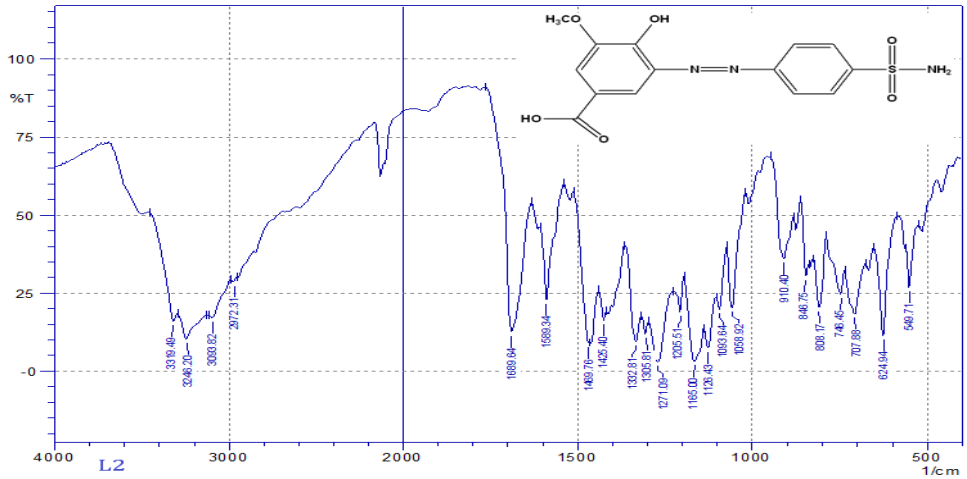


Fig. 2 : F.T. infrared spectroscopy of L_2.

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	3302.13 M	3234.62 m	3099.61 W	---	---	1571.99 s	1475.54 S	1330.88 s	1234.44 s	1159.22 s
L_2	3319.49 M	3246.20 m	3093.82 M	2972.31 S	1689.64 S	1589.34 s	1469.76 S	1332.81 s	1271.09 s	1165.00 S
w = weak, s = Strong, m = Medium										

NMR spectra of azo dye compounds

The azo dyes' ^{1}H-NMR spectra **(Fig. 3 and 4)** were obtained using dimethyl sulfoxide as the solvent. The aromatic ring signal at (7-8.5 ppm), and the OH group signal appears at (9.3 ppm) for azo compounds (L_1 and L_2) while the methoxy group signal appears at (3.904 ppm). The result is shown in **Table (3).**

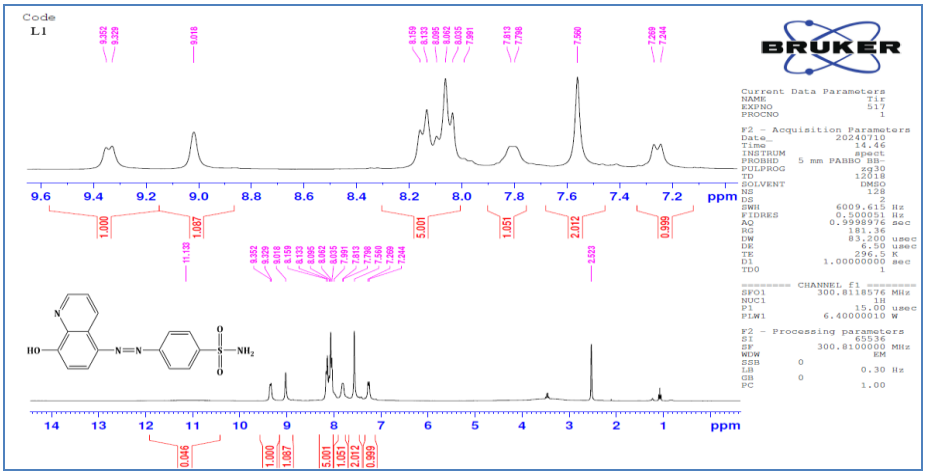


Fig. 3: proton nuclear magnetic resonance (^{1}HNMR) spectrum of the azo (L_1).

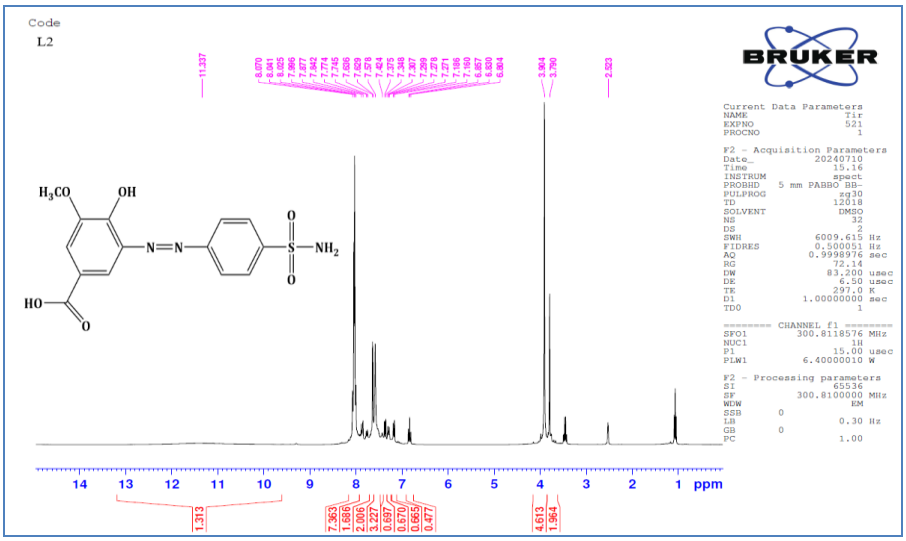


Fig. 4 : proton nuclear magnetic resonance (^{1}HNMR) spectrum of the azo (L_2).

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	2.523(s,6H, DMSO), 3.4(s, H, HOD),9.329 (s, 2H, NH_2) 9.018 (s, H, O-H), 7.244-8.159(m, 9H, Ar-H)
L_2	2.523(s,6H, DMSO), 3.4(s, H, HOD),6.804 (s, 2H, NH_2) 11.337 (s, H, O-H), 7.160-8.070(m, 6H, Ar-H),3.790 (s,3H, OCH_3)

Acid-base properties with different pH values

To compute the ionization and protonation constants and investigate the impact of the azo dyes' acidity and basicity using universal buffer solutions with pH values ranging from (2-12). Different pH values (2–12) were used to generate a range of dye solutions. The absorption spectra of (1.2×10^{-4} M) solution of each L_1 and L_2 dyes, were represented graphically at wavelength range (320-650nm) **(Fig. 5 and Fig. 6)**. In case of L_1, the spectra

were characterized by two maximal bands the first at wavelength range (450-470 nm) of pH range (2-6) which represents protonated form (cation form). The second at wavelength range (460-500 nm) of pH value range (7-12), which represent basic form (ionic form). From the Fig. ure it was found three isobestic points at (420,450 and 580 nm). From **(Fig. 5)** the highest absorbance was at pH range (9-11). In case of L_2 dye **(Fig. 6)**, the spectra are characterized by two maximal bands the first at (390-400 nm.) in the pH range (2-6), which represents protonated form and the second at the wavelength range (400-490 nm) at pH value range (7-12), for basic form. The spectra shows two isobestic points at (400 and 410 nm), the highest absorbances were at pH range (10-12).

To determine the ionization and protonation constants of the azo dyes (L_1 and L_2), at certain wavelengths (450 and 360 nm) azo dyes (L_1 and L_2) respectively from **(Fig. 5 & 6)**, the absorbance – pH curves was plotted **(Fig. 7)**. With the use of the half-height approach[14], the ionization and protonation constants were computed **(Table 4)**. The pK values were determined using the equation :

pK = pH (at A1/2), where A1/2 = (AL+ Amin)/2

AL and Amin represent the limiting and minimum absorbance, respectively.

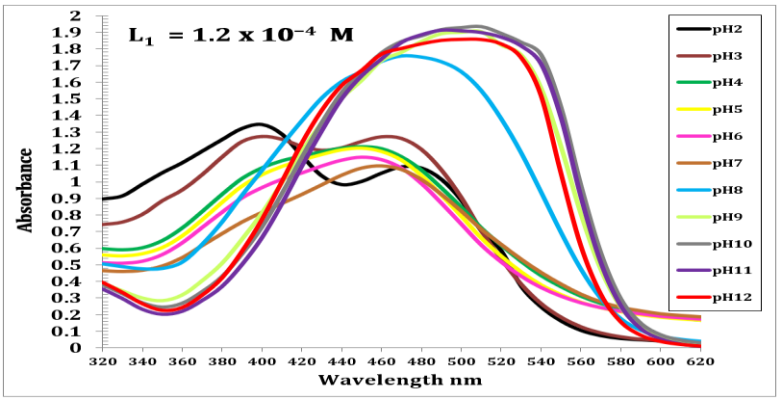


Fig. 5: Visible absorption spectra of the azo dye (L_1) **.**

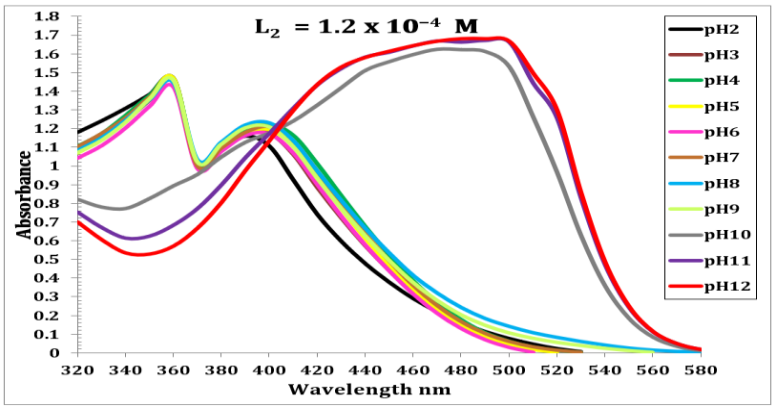


Fig. 6: Visible absorption spectra of the azo dye (L_2) **.**

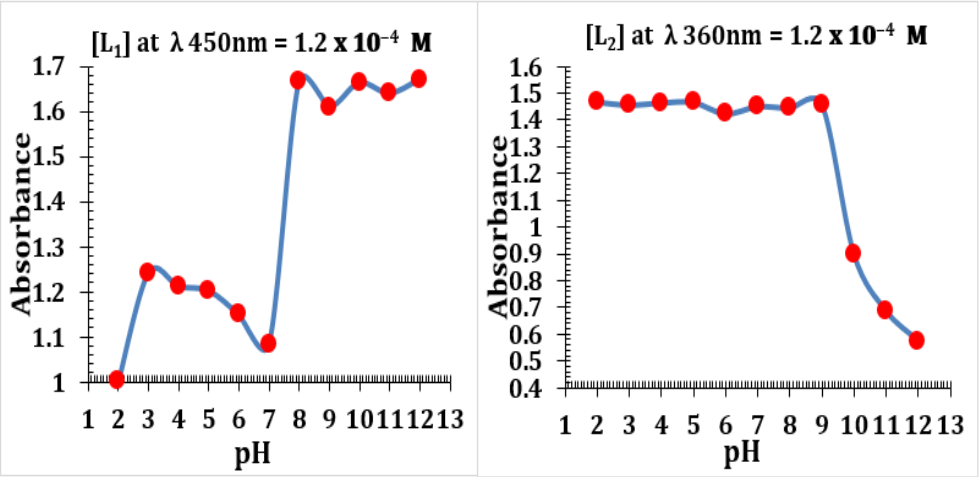


Fig. 7 : The azo compounds' absorbance-pH curves (L_1 and L_2).

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

Azo dye L_1 at λ = 450 nm				Azo dye L_2 at λ = 360 nm			
A_{min}	A_L	$A_{1/2}$	Pk	A_{min}	A_L	$A_{1/2}$	*pK
1.00	1.24	1.12	2.40 (p)	1.42	01.46	1.44	5.50 (p)
1.08	1.66	1.37	7.50 (a1)	0.68	1.45	1.06	9.50 (a)
1.60	1.66	1.63	9.50 (a2)				

pKp is the protonation constant for the oxygen atom of an 8-Hydroxyquinoline molecule.
pKa_1 is the ionization constant for the hydroxyl group of 8-Hydroxyquinoline.
pKa_2 is the ionization constant for the amino group of sulfanilamide.
*pK_p is the protonation constant for the oxygen atom of the Vanillic acid molecule.
* pKa is the ionization constant for the main group of sulfanilamide.

Solvents effect

From **Fig. 8** for the azo compound (L_1), more than one peak were found **(Table 5)** for all solvents except for the solvents DMF, Water, and Methanol, which have one peak. The first peak is found at (360 nm) and the second peak at (420 nm). A red shift is observed for the solvent DMSO at (430 nm) and Methanol at (440 nm). It is also observed that there is a blue shift (at 410nm) for the following solvents (Benzene, DCM, Ethanol, Chloroform and Ethyl Acetate) with respect of the azo dye (L_2), also more than one peak is found for all solvents except for the solvent (DCM) which has one peak. The first peak appears at 360 nm, whereas the solvents (DMF and DMSO) at 430 nm and water at 400 nm show the second peak at 390 nm with a significant red shift. Blue shift is observed for the solvent (DCM) at (370 nm). Solvents' dielectric and/or salvation effects affect the absorption spectra in different solvents. To verify whether a shift in salvation energy or pure dielectric is the source of the band shift (Δv), the solvent and λ max functions of azo dyes (L_1) and (L_2) were demonstrated.

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)**.

With solvents of moderate polarity, the plot of (D-1)/(D+1) versus the λ max of azo dye (L_1) yields a relatively high linear relation **(Fig. 10)**. This indicates that the primary factor controlling the band shift in such solvents is the medium's dielectric constant. All solvents in azo dye (L_2) exhibit the same linearity effect, with the exception of DMF and DMSO, which exhibit non-linearity. This phenomenon could be attributed to hydrogen bonding between the solvent and solute **(Fig. 11)**.

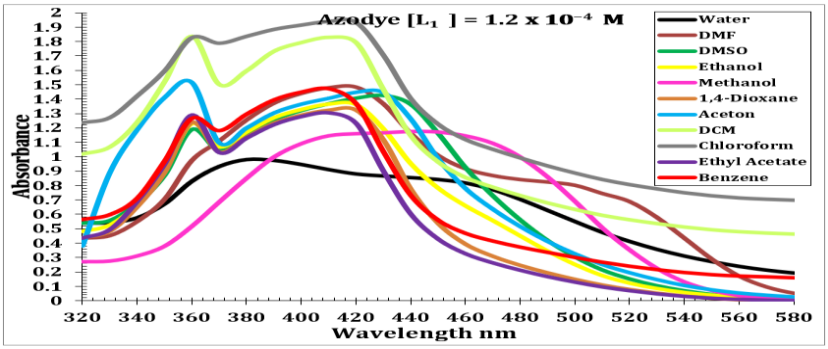


Fig. 8: Visible absorption spectra of the azo compound (L_1) in solvents of different polarity.

Table 5: Solvents' dielectric function (D-1 / D+1) 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	360s, 410m	360s, 390m
2	Aceton	20.60	0.907	360s, 420m	360s, 390m
3	Ethanol	24.30	0.921	360s, 410m	360s, 390m
4	Methanol	32.70	0.940	440m	360s, 390m
5	DMSO	47.00	0.958	360s, 430m	360s, 430m
6	DMF	36.71	0.946	420m	360s, 430m
7	1,4-Dioxane	2.20	0.375	360s, 420m	360s, 390m
8	DCM	9.10	0.802	360s, 410m	370m
9	Ethyl Acetate	6.02	0.715	360s, 410m	360s, 390m
10	Benzene	2.28	0.390	360s, 410m	360s,390m
11	Water	78.40	0.975	380m	360s,400m
W = Weak, m = Medium, s = Strong					

Table 6: The λ max and ε max azo dye values (L_1 and L_2) at various solvent concentrations.

Solvent Symbol	Dye	Solvent	$\pi \rightarrow \pi^*$		$\pi \rightarrow \pi^*$ (azo)	
			λ max nm	ε_{max} x10^4 l.mol^{-1}.cm^{-1}	λ max nm	ε_{max} x10^4 l.mol^{-1}.cm^{-1}
1	L_1	Chloroform	360	1.52	410	1.61
2		Aceton	360	1.26	420	1.20
3		Ethanol	360	1.02	410	1.13
4		Methanol	---	---	440	0.98
5		DMSO	360	0.99	430	1.19
6		DMF	---	---	420	1.23
7		1,4-Dioxane	360	1.03	420	1.10
8		DCM	360	1.53	410	1.52
9		Ethyl Acetate	360	1.07	410	1.08
10		Benzene	360	1.05	410	1.22
11		Water	380	0.81	---	---
1	L_2	Chloroform	360	1.47	390	1.52
2		Aceton	360	1.22	390	0.95
3		Ethanol	360	1.25	390	0.97
4		Methanol	360	1.25	390	0.97
5		DMSO	360	0.82	430	1.16
6		DMF	360	1.00	430	1.15
7		1,4-Dioxane	360	1.23	390	0.95
8		DCM	370	1.58	---	---
9		Ethyl Acetate	360	1.24	390	0.94
10		Benzene	360	1.44	390	1.21
11		Water	360	1.22	400	0.99

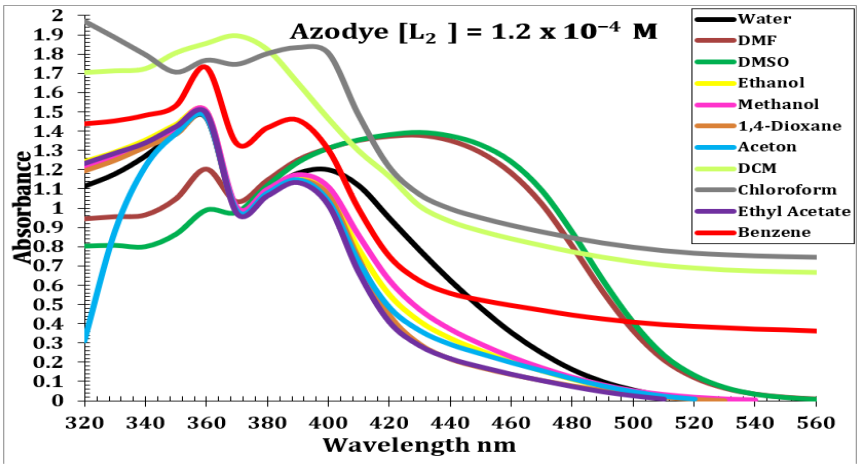
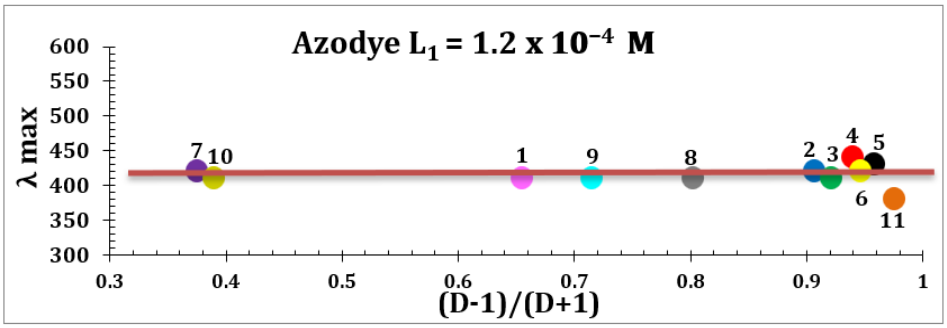
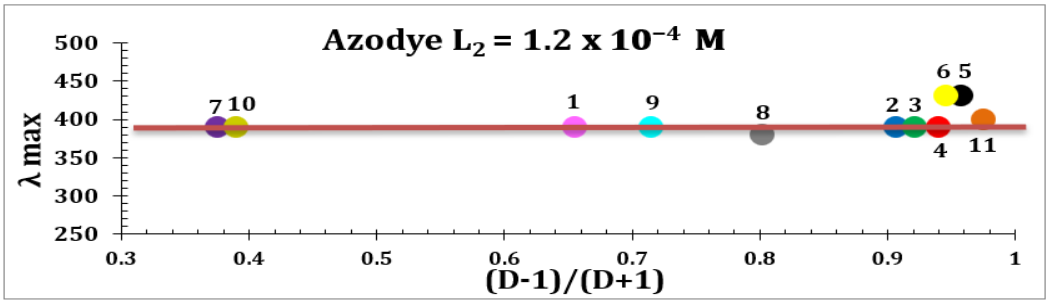


Fig. 9: Visible absorption spectra of the azo compound (L_2) in solvents of different polarity.



1	2	3	4	5	6	7	8	9	10	11
Chloroform	Aceton	Ethanol	Methanol	DMSO	DMF	1,4 Dioxane	DCM	Ethyl Acetate	Benzene	Water

Fig. 10: The relationship between the maximum wavelengths of the azo compound (L_1) and the function of the dielectric constant of solvents of different polarities.



1	2	3	4	5	6	7	8	9	10	11
Chloroform	Aceton	Ethanol	Methanol	DMSO	DMF	1,4 Dioxane	DCM	Ethyl Acetate	Benzene	Water

Fig. 11: The relationship between the maximum wavelengths of the azo compound (L_2) and the function of the dielectric constant of solvents of different polarities.

Spectrophotometric titration for the determination of sodium hydroxide using (L_1 and L_2) as indicators. After the hydrochloric acid, acetic acid, and sodium hydroxide were titrated volumetrically and visually using the approved methods [13]. The azo can be used in spectrophotometrically analysis because they have different colors in basic and acidic environments **(Fig. 12)**. Sodium hydroxide was spectrophotometrically titrated with both hydrochloric acid and acetic acid using the prepared azo (L_1 and L_2) as indicators as in

Fig. (13and 14), where the end points are detected. Several concentrations of the prepared azo dyes (L_1 and L_2) were used and the relative error for each concentration was found **(Table 7)** For L_1 , it was found that all concentrations of the compound are suitable, especially at concentration of [5×10^{-4} M] with relative error 0.6 and jump in absorbance per 0.1 ml of titrant (ΔA/0.1ml) equal to 0.17 by using HCl ,while for using acetic acid at concentration [3×10^{-4} M] has the highest jump (ΔA=0.16 and R.E = -0.16%). For L_2 the results are; concentration range [4-5 $\times10^{-5}$ M], ΔA/0.1ml = 0.5 and R.E. = 0.5-0.6% that is with HCl. But by using acetic acid in the concentration range [2.5-3 x 10-5 M], ΔA/0.1ml = 0.25-0.36 and R.E. = 1.5-1.8%.

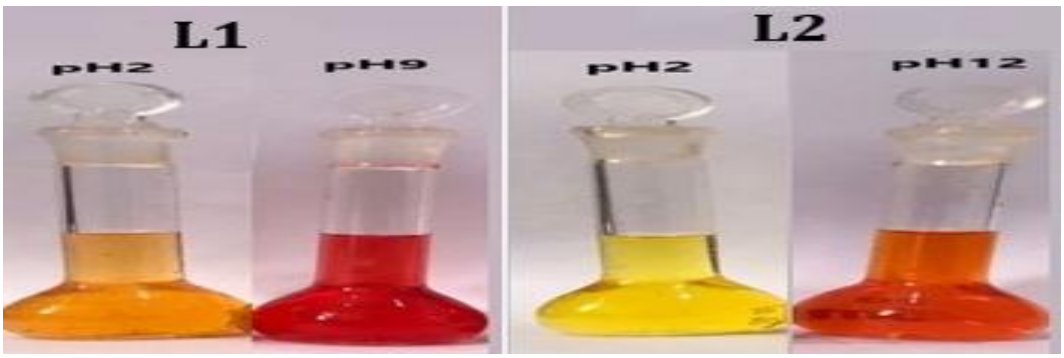


Fig. 12: Colors of solutions of azo compounds (L_1 and L_2) at different pH levels.

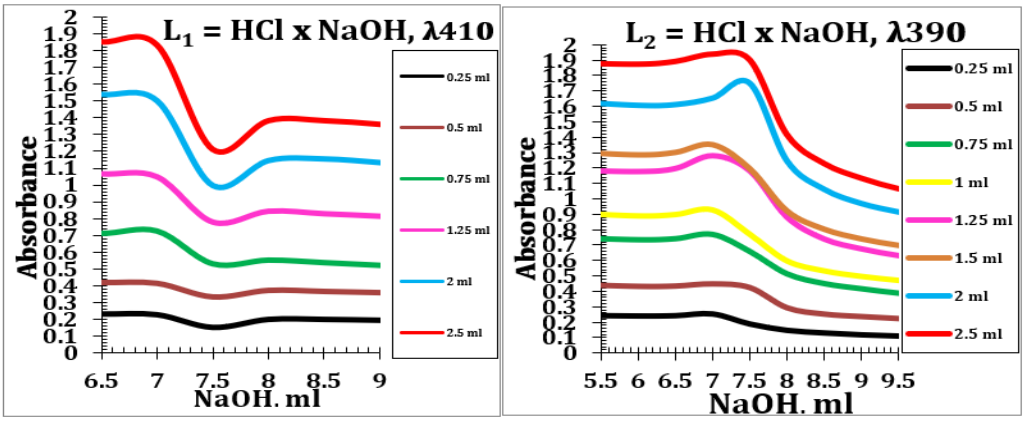


Fig. 13 : Spectrophotometric titration (HCl x NaOH) for L_1 and L_2.

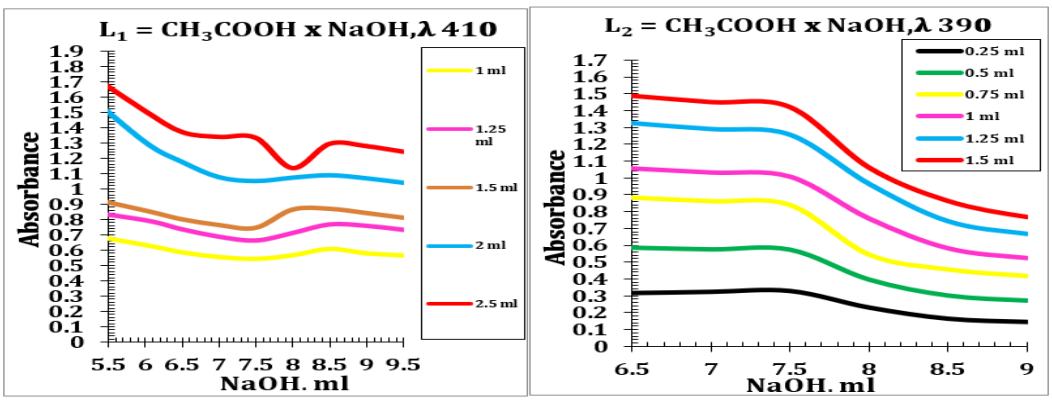


Fig. 14: Spectrophotometric titration (HAc x NaOH) for L_1 and L_2.

Table 7: Values of the concentrations of the prepared azo compounds (L_1 and L_2) used as indicators and the concentrations of sodium hydroxide and the relative error**.**

Titration of HCl × NaOH using L_1				
[L] x 10^{-4} M	[NaOH]M Taken	[NaOH]M found	Relative error %	Jump (ΔA/0.5 ml NaOH)
0.5	0.0987	0.1007	2.02	0.048
2	0.0987	0.1005	1.82	0.202
2.5	0.0987	0.0997	1.01	0.065
3	0.0987	0.1003	1.62	0.208
4	0.0987	0.0994	0.70	0.149
5	0.0987	0.0993	0.60	0.171
Titration of CH_3COOH× NaOH using L_1				
2	0.0987	0.0977	-1.01	0.041
2.5	0.0987	0.0979	-0.81	0.056
3	0.0987	0.1004	1.72	0.005
4	0.0987	0.0991	0.40	0.016
5	0.0987	0.0974	-1.31	0.158
Titration of HCl × NaOH using L_2				
0.5	0.0987	0.1005	1.82	0.041
1	0.0987	0.0984	-0.30	0.041
1.5	0.0987	0.0998	1.11	0.147
2	0.0987	0.0989	0.20	0.173
2.5	0.0987	0.0992	0.50	0.301
3	0.0987	0.10000	1.31	0.274
4	0.0987	0.0993	0.60	0.502
5	0.0987	0.0982	-0.50	0.483
Titration of CH_3COOH× NaOH using L_2				
0.5	0.0987	0.0992	0.50	0.098
1	0.0987	0.1006	1.92	0.178
1.5	0.0987	0.1004	1.72	0.295
2	0.0987	0.1001	0.01	0.249
2.5	0.0987	0.1005	1.82	0.292
3	0.0987	0.1002	1.51	0.359

Spectrophotometric determination of nitrite
Time effect for forming azo dyes

It was found from **Fig.15**, the absorbances of azo dyes L_1 and L_2 are nearly constant for a period of time (1 – 30 min) at maximum wavelengths 410 and 360 nm respectively. That means the two azodye are stable.

Beer's law

Nitrite was determined by adding serious quantity of nitrite to the aromatic amine and HCl mixture, forming diazonium salts of the dyes (L_1 and L_2). The absorption of the resulting solution was measured at the maximum wavelength (390 and 410 nm) for azo compounds (L_1 and L_2) respectively. The beer's law were plotted **(Fig. 16)**. The precision and accuracy data abstracted in **table 8**.

The high value of the molar absorption coefficient of the azodyes ligands obtained from Beer's law indicates the high sensitivity. Also, the correlation coefficient values close to one indicate the linearity of Beer's law. It was also noted that the standard deviation values are very low, which indicates its high conformity.

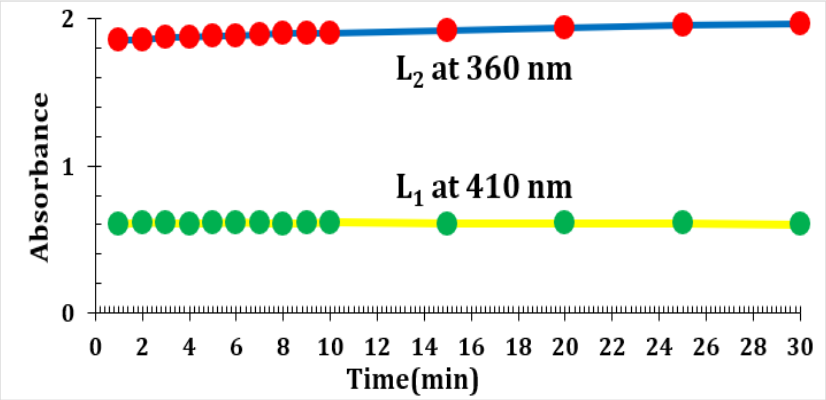


Fig. 15: The effect of time on the formation of azo azo dyes (L_1 and L_2).

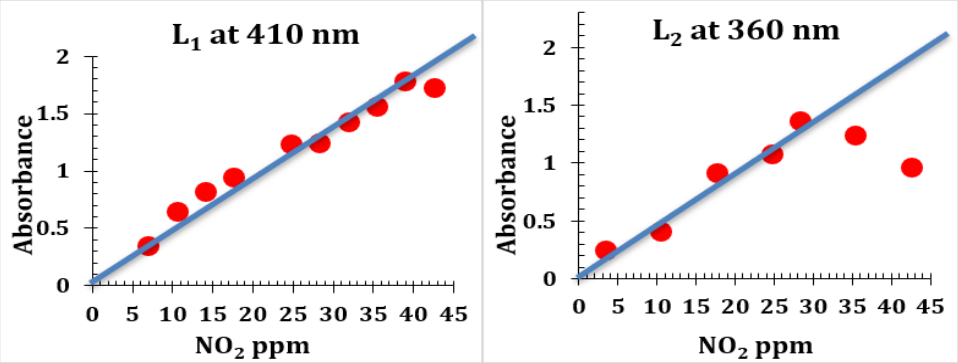


Fig. 16: Beer's law of nitrite for forming azo dyes L_1 and L_2.

Table 8: Values of Beer's law detection limit, standard deviation, specific absorbance, Sandel sensitivity, and correlation coefficient.

Dye Symbol	Limit of Beer' s law ppm	DL x10^{-5} µg.ml^{-1}	S. D	a ml.g^{-1}.cm^{-1}	S µg.cm^{-2}	ε x10^4 L.mol^{-1}.cm^{-1}	R^2
L_1	0-39.116	0.10	0.0079	0.0075	0.133	0.247	0.9733
L_2	0-28.448	0.09	0.0091	0.0067	0.149	0.237	0.9683

Antibacterial Activity

The picture in **Fig. (17 and 18)** and **table (9)** showed the clear effect of the azo compound (L_1) in inhibiting the growth of bacteria (*Staphylococcus aureus*) by measuring the diameter of the inhibition zone (40 mm). It also showed inhibition of bacterial growth. (Escherichia coli) by measuring the diameter of the inhibition zone (25 mm) and also showed inhibition of the growth of the fungus (*Candida albicans*) by measuring the diameter of the inhibition zone (mm 45) and also showed inhibition of the growth of the fungus (*Aspergillus Niger*) by measuring the diameter of the inhibition zone (mm 50). As for the azo compound (L_2), there is a clear effect in inhibiting the growth of bacteria (*Staphylococcus aureus*) by measuring the diameter of the inhibition zone (15 mm) and it did not give any effect on bacteria (*Escherichia coli*) and the fungi *Candida Albicans* and *Aspergillus Niger*.

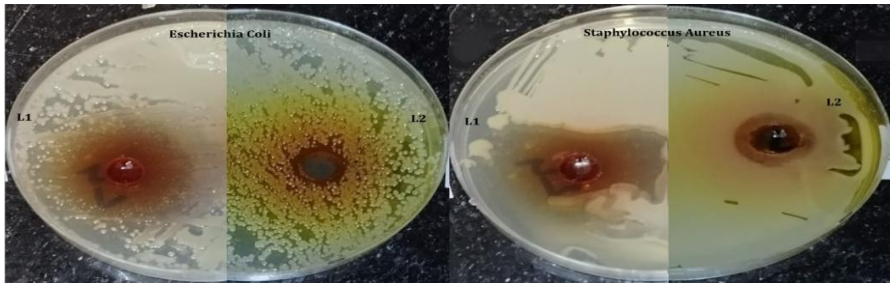


Fig. 17: The effect of the two azo dyes (L_1 and L_2) on *Escherichia coli* and *Staphylococcus aureus* bacteria.

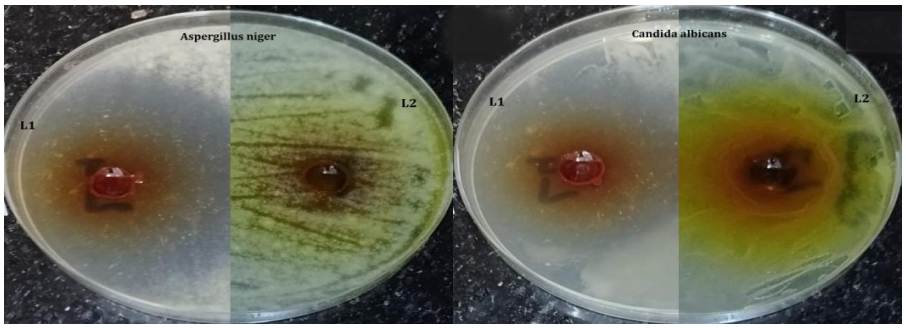


Fig. 18 : The effect of the two azo dyes (L_1 and L_2) on the fungi *Candida albicans* and *Aspergillus niger*.

Table 9 : 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	40	25	45	50
L_2	15	Zreo	Zreo	Zreo

Conclusions

Two new azodyes prepared from pharmaceutical substances, the first Sulfanilamide with 8-hydroxyquinoline (L_1) and the second from Vanillic acid (L_2) , they were characterized by C.H.N., ^{1}H-NMR, FT-IR, and visible spectroscopic techniques. The azodyes were studied in terms of acid-base properties at different pH values (2-12) and solvents effect of different polarities. Some of different applications were carried out like, biological effectiveness, determination of nitrite and as pH - indicators.

REFERENCES

1. R. Kılınçarslan, E. Erdem and H. Kocaokutgen, "Synthesis and spectral characterization of some new azo dyes and their metal complexes", ***Trans Metal Chem***, 32(1), 102–106 (2017).
2. N. Abbas, S.A.S. Tirmizi, G. Saeed, *et al.*, "Chromium (III) complexes of azo dye ligands: Synthesis, characterization, DNA binding and application studies", ***Inorga and Nano-Metal Chem***, 48(1), 57–66 (2018).
3. O. I. Lipskikh, E. I. Korotkova, Ye. P. Khristunova *et al.*, "Sensors for voltammetric determination of food azo dyes - A critical review", ***Electrochim Acta***, 260, 974–985 (2018).
4. S. M. Kadhim and S. M. Mahdi, "Preparation and Characterization of New (Halogenated Azo-Schiff) Ligands with Some of their Transition Metal Ions

Complexes", ***Iraqi J of Sci***, 63, 3283–3299 (2022).
5. N. M. Aljamali, "Review in Azo Compounds and its Biological Activity", ***Biochem & Analy Biochem***, 04(02), 1-4 (2015).
6. A. Ali, T. A. Fahad, A. A. Al-muhsin, "Preparation and Spectroanalytical Studies of Two New Azo Dyes Based on Luminol", ***IOP Confer Ser***, 928, 052007–052007 (2020).
7. A. A. Al-Muhsin, T. A. Fahad and A. A. Ali, "Preparation and characterization azo dyes derived from 4- hydroxycoumarin and studying their analytical Applications", ***J of Phy: Confer Ser***, 1999(1), 012010-012025 (2021).
8. H. T. Thuiny, T. A. Fahad and A. A. Ali, "Preparation, analytical Studies and application of a New Azodye Derived from Pharmaceutical Materials (Procaine Hydrochloride & Salicylic acid", ***J of Phy: Confer Ser***, 2063(1), 012024–012024 (2021) .
9. H. M. Ali, A. S. Abdulredha, A. A. Hussain, *et al.*, "Synthesis and Biological Activity of Three Novel Azo Dyes", ***J of Nat Sci Res***, 8(22), 7–12 (2018).
10. A. A. Ali, H. A. Ali, "Analytical study of some azo dyes and its medical applications", ***J of Kufa for Chem Sci***, 3(2), 16–30 (2024).
11. E. R. Radhi, F. J. Ali and K. J. Ali, "Synthesis and characterization of a new azo quinoline ligand and its metal complexes with spectrophotometric determination and biological efficacy study of its Hg(II) complex", ***Bull of the Chem Soc of Ethio***, 37(6), 1423–1433 (2023).
12. J.A. Dean, "Lange's Handbook of Chemistry", 15th ed., ***McGraw-Hill, Inc.***, NewYork, Scientific Research Publishing, Scirp.org, 1999.
13. A.I.Voge, "Text Book of Practicl Organic Chemistry", 4th ed., ***Longman***, NewYork, London, 1975, p.723-724.
14. T.A. Fahad, A. A. Ali and A. H. Baty, "Synthesis, Characterization and Analytical Studies of Some New Azodyes Driven From O-Vanillin", ***World J of Pharma Res***, 8(2), 707-715 (2019).

Bull. Pharm. Sci., Assiut University, Vol. 48, Issue 1, 2025, pp. 319-333.

نشرة العلوم الصيدليـــــة
جامعة أسيوط

BULL. PHARM. SCI. Assiut Univ.

تحضير وتشخيص وتطبيقات ودراسات تحليلية لبعض الأصباغ الآزوية الجديدة

علاء عدنان المحسن[1*] – اسعد عبود علي[2]

[1] جامعة البصرة، كلية التربية للعلوم الصرفة، قسم الكيمياء، البصرة، العراق

[2] كلية الكنوز الجامعة، قسم الصيدلة، البصرة، العراق

الخلفية: المركبات الآزوية هي صنف من الجزيئات العضوية تتميز بوجود آصرة مزدوجة بين ذرتين من النتروجين وتحتوي على مجموعة وظيفية آزوية واحدة أو أكثر.

الهدف من الدراسة: تتضمن هذه الدراسة تحضير صبغتين آزويتين جديدتين من السلفانيلاميد مع ٨-هيدروكسي كوينولين (L_1) [٤- ((٨-هيدروكسي كوينولين-٥-يل) ديازينيل) بنزين سلفوناميد] وحامض الفانيليك (L_2) [حامض ٤-هيدروكسي-٣-ميثوكسي-٥- ((٤-سلفاموبل فينيل) ديازينيل) بنزويك]. وقد شخصت بتقنيات التحليل الطيفي C.H.N. و^{1}H-NMR و FT-IR والتحليل الطيفي المرئي لتشخيص الاصباغ.

النتائج: تمت دراسة الأطياف الإلكترونية لهذه الاصباغ الآزوية من حيث الخصائص الحامضية والقاعدية عند قيم pH مختلفة (٢-١٢)، والتي تتضمن تحديد نقاط الايزوبستية وتحديد ثوابت البرتنة والتأين. كان تأثير المذيبات ذات القطبية المختلفة على الأطياف الإلكترونية موضوع البحث الآخر، كما تمت دراسة الفعالية البيولوجية مع نوعين من البكتيريا *Escherichia Coli* و *Staphylococcus Aureus* ونوعين من الفطريات *Candida albicans* و*Aspergillus niger* . تم استخدام محاليل مركبات الآزو (L_1 وL_2) لتحديد أيون النتريت من خلال تكوين الصبغتين وتم دراسة الدقة والضبط وتم التحقق من إمكانية استخدام الصبغتين كدلائل حامضية - قاعدية.