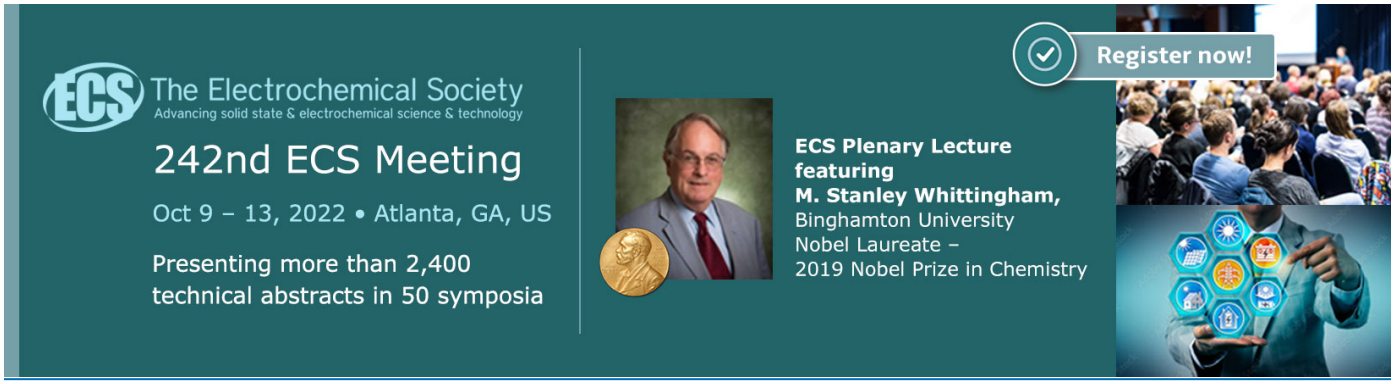
Preparation, Characterization and Analytical Studies of novel Azo dyes and Diazo dyes



Preparation, Characterization and Analytical Studies of novel Azo dyes and Diazo dyes

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Abstract. The amino {4-hydroxy-3-[(*E*)-{4-[(5-methylisoxazol-3-yl) sulfamoyl] phenyl} diazenyl] phenyl} acetic acid (1) and the amino {4-hydroxy-3,5-*bis*[(*E*)-{4-[(5-methylisoxazol-3-yl) sulfamoyl] phenyl} diazenyl] phenyl} acetic acid (2) were Prepared. The resulting azo and diazo dyes were then characterized using m.p., IR, UV-visible, mass spectrum and ^{1}H-NMR spectrum. Analytical studies were carried on the azo dye (1) and the diazo dye (2). The best solubility of (1) and (2) was in methanol and ethyl acetate respectively, with no deviation from the linear relationship in each dye, which is due to the fact that the effect of the dielectric constant is the main factor that can control the shift of the absorption beaks. Though, the pH effects of (1) and (2) in a range of buffer solution were showed one and two isopiestic points respectively. Calculation of the ionization (pK_a) and the protonation (pK_p) constants by using the half height method was associated to the nitrogen atom and the OH-groups respectively. The pK_{p1}, pK_{a1} and pK_{a2} were also intended. But, the value of the pK_{p2} was absent in the azo dye (1) and seems to be equal to 2.5 in the diazo dye (2). These results were indicated the suggested ionization scheme in acidic and basic medium of each dye. Furthermore, the dyes with different concentrations have harmless, nontoxic and no haemolysis effects.

1. Introduction

Scientific research are regarding the azo dyes [1] [2] [3] [4], besides expended a great rank in chemical analysis. the powerfully coloured dyes, depending on their exact molecule structure were created as very significant compounds as pigments for an extensive period[5]. The structural features in the organic compounds, that typically display colour are C=C, N=O, N=N, aromatic rings, C=O and NO_2. However, the azo (–N=N–) and nitroso (–N=O) groups can confer colour, while the other groups actually dose in convinced conditions[6]. one or more azo groups, that are linked to SP_2 hybridized carbon atoms also founded on same dyes [7]. dyes can hold an extra active group able to express chelatic coordinational complexes with metal ions. Essentially reactive dyes, that reported for their pharmaceutical significance as antidiabetic[8], antineoplastic[9], antibacterial [10], and anticancer agent[11]. Analytical studies were carried on some diazo dyes [12]. The solvents and the pH effects of these dyes displayed high solubility in ethanol and distil water, and appropriate values, (pH= 12) in each dye were established. Three isopiestic points were gained in these dyes owing to envision their pK_a and pK_b constants. But, the pK_{a1} was disappeared. Theoretical studies were also carried on the structure of each synthetic dye in order to explain the relation between the structure and their properties. The structure of each synthetic diazo dye was accepted by means of the internal coordinate mechanics (ICM), the conformational analysis and the molecular mechanics (MM2) studies [12]. Furthermore, the best minimization of these dyes was attended successfully.

2. Experimental section

The melting points of azo and diazo dyes were attended using Buchi B190K. The IR spectrum was carried out on aFT-IR-8400S.Fourier Transform Infrared Spectrophotometer Shimadzu (Japan) by using a KBr disc in the range (600 – 4000) cm^{-1}. The UV-Visible spectrum was done using ethanol (1 x 10^{-4} M). The IR, UV-Visible spectrophotometer and melting point completed by Chemistry Department–Education College of pure science– Basrah University, Iraq. Correct mass spectrum and the ^{1}H NMR were measured in Tehran University.

2.1 Preparation of amino {4-hydroxy-3-[(E)-{4-[(5-methylisoxazol-3-yl) sulfamoyl] phenyl} diazenyl] phenyl} acetic acid (1=L_3 in spectrum figures)

The azo dye was prepared using each of sulfamethoxazole (0.005 mol., 1.266 g), with (1.75 mL) conc. HCl in separate beaker followed by add 10 mL of distilled water. Then, the solution of $NaNO_2$ was prepared by dissolving (0.468 g) in (5 mL) of distilled water, the $NaNO_2$ solution was then added to the 1st beaker. The resulting diazonium salt was then added to 4-hydroxyphenylglycine (HPG) (0.005 mol., 0.836 g) in 25% sodium hydroxide solution. The resulting crude was recrystallized in ethanol and hexane to yield (63%) from the titled dye; m.p.: (177-179) ^{o}C This showed δ_H (500 MHz, DMSO): 2.32 (3H, CH_3);4.66 (H, CH); 6.20 (1H, CH, isoxazole); 7.01-8.07(7H, Ar-H); 9.41(1H, OH); 10.56 (1H, NH). υ :(3223.05, 3157.05, 1433.11, 1506.41, 1465.90, 1168.86, 1334.74, 1168.86, 3091.89, 2993.52, 2889.37, 1614.42) cm^{-1}; λ_{max}: 360 nm and 390 nm.

2.2. Preparation of amino {4-hydroxy-3,5-bis[(E)-{4-[(5-methylisoxazol-3-yl) sulfamoyl] phenyl} diazenyl] phenyl} acetic acid (2=L_4 in spectrum figures)

The diazo dye was prepared using sulfamethoxazole (0.005 mol., 1.266 g), with (1.75 mL) conc. HCl in separate beaker followed by add 10 mL of distilled water. Then, the $NaNO_2$ were prepared by dissolving 0.468 g in 5 mL of distilled water, the $NaNO_2$ solution was then added and the resulting diazonium salt then was added to azo dye (1) (0.005 mol., 3.478 g) in (25%) sodium hydroxide solution. The resulting crude was recrystallized in ethanol and hexane to yield (62%) from diazo dye; m.p: (232-233) ^{o}C; This showed δ_H (500 MHz, DMSO): 2.33 (6H, $2CH_3$);4.62 (1H, CH); 6.21 (2H, 2CH, isoxazole); 7.01-8.23(10H, Ar-H); 9.42(1H, OH); 10.58 (2H, 2NH);11.63(1H, OH). υ :(3442.94, 3161.33, 1494.83, 1494.83, 1465.90, 1168.86, 1334.74, 1168.86, 3091.89, 2985.81, 2893.22, 1616.35) cm^{-1}; λ_{max}: 340 nm and 380 nm.

2.3 The pH effect

The solutions of each azo and diazo dye was prepared by dissolving weights (0.0215 g) and (0.0347 g) respectively in (50 mL) of ethanol to give the stock solution (1 x 10^{-3} M) from each. Then, the (0.5 mL) of each dye was took from their stock solution, (1 x 10^{-3} M) and diluted with (5 mL) using range of buffer solutions, (2-12), to give (1x10^{-4} M) concentration from each in each buffer solution.

2.4 The Solution of the azo and diazo dye in ethanol

The stock solution of each azo dye and diazo dye was prepared by dissolving weights (0.0215 g) and (0.0347 g) respectively in (50 mL) of ethanol to give the resulting concentration (1 x 10^{-3} M) from each dye. Then, the (0.5 mL) of each dye was took from their stock solution, (1 x 10^{-3} M) and diluted with (5 mL) of ethanol, to give (1x10^{-4} M) concentration.

2.5 The solvent effect

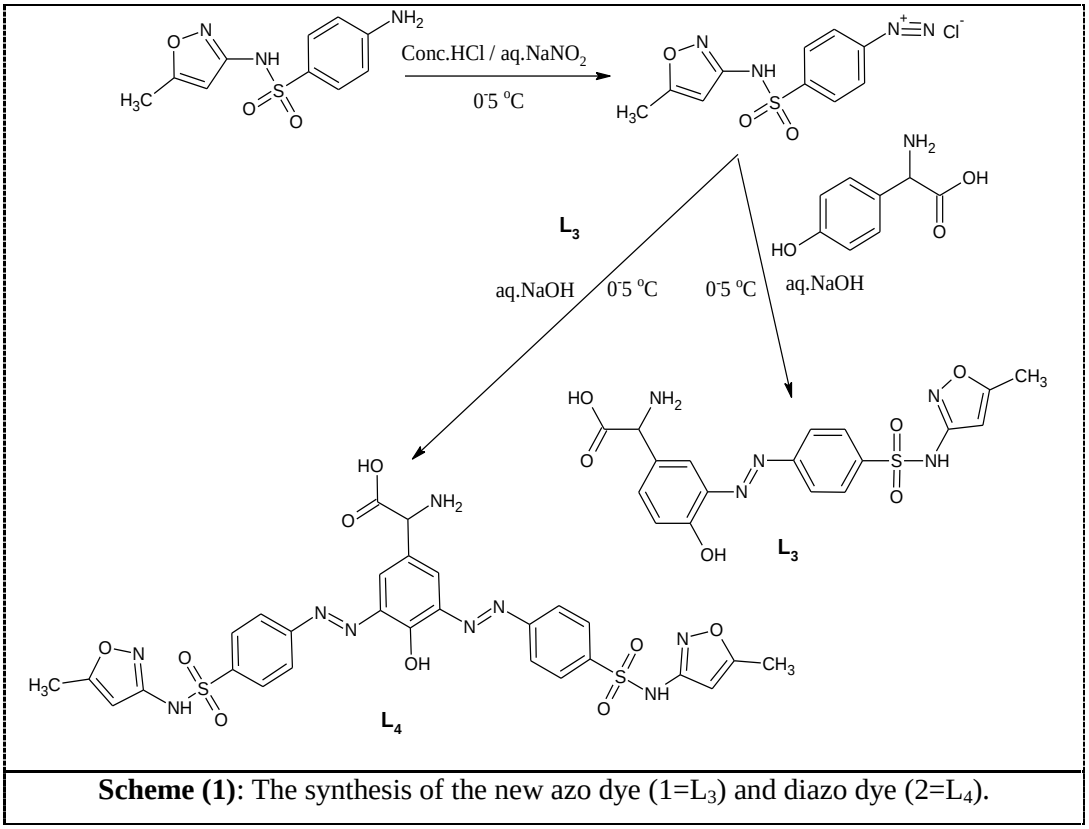
The stock solution of each azo dye and diazo dye was prepared by dissolving weights (0.0215 g) and (0.0347 g) respectively in (50 mL) of solvents, (ethanol, methanol, water, DMSO, DMF, chloroform and ethyl acetate) to give the resulting concentration (1 x 10^{-3} M) from each dye in each solvent. Then, the (0.5 mL) of each dye was took from their stock solution, (1 x 10^{-3} M) and diluted with (5 mL) of each solvent, to give (1x10^{-4} M) concentration.

2.6 The toxic effect of azo and diazo dyes in the haemolytic red blood cells

A stock solution (200 mg / mL) was prepared, then a series of diluted (1000, 500, 250, 125,62.25) $\mu g/mL$ solutions were setup individually (0.8 mL) with red blood cells (0.2 mL) in Eppendorf tubes. Two tubes then equipped. At that point, Ringer solution (0.8 mL) was added to the first tube as a negative control, then the tap water used as a positive control in the second tube. Followed by adding 0.2 mL of red blood cells to each tube. These tubes kept for 3 hours at 37ºC in a special incubator, and the results recorded.

3. Result and Discussion

The azo dye (1) and the diazo dye, (2) were prepared as seen in Scheme (1) below.



Scheme (1): The synthesis of the new azo dye (1=L_3) and diazo dye (2=L_4).

The synthetic dyes, (1) and (2) were derived from the amoxicillin drug using with optimize stoichiometry and conditions of the reactions. Then, the azo and diazo dyes were characterized by IR spectrum, mass spectrum, ^{1}H NMR spectrum and the UV-visible spectrum. The IR spectrum of the (1) and (2), (Figures 1 and 2) were showed the stretching vibration of the ν (O-H) and (N-H) groups in the regions 3223.05 cm^{-1} and 3442.94 cm^{-1} respectively. But, the (N-H) groups of NH_2 were in the regions 3157.05 cm^{-1} and 3161.33 cm^{-1} respectively. the stretching vibration band of ν (N=N) was looked at 1465.90 cm^{-1} and 1465.90 cm^{-1} respectively [2][3]. Additional bands considered as skeletal vibrations, the (C=C) seems at 1506.41 cm^{-1} and 1494.83 cm^{-1} respectively[2][3]. The (C=O) and the aromatic CH bands were appeared in the regions (1614.42 and 3092.83) cm^{-1} and (1616.35 and 3091.89) cm^{-1} respectively [3]. Though, the C-N bands were appeared in the regions1168.86 cm^{-1} and 1168.86 cm^{-1} respectively. The (O=S=O) asymmetric w. and symmetric s. bands were appeared in the regions (1334.74 and 1168.86) cm^{-1} and (1334.74 and 1168.86) cm^{-1} respectively.

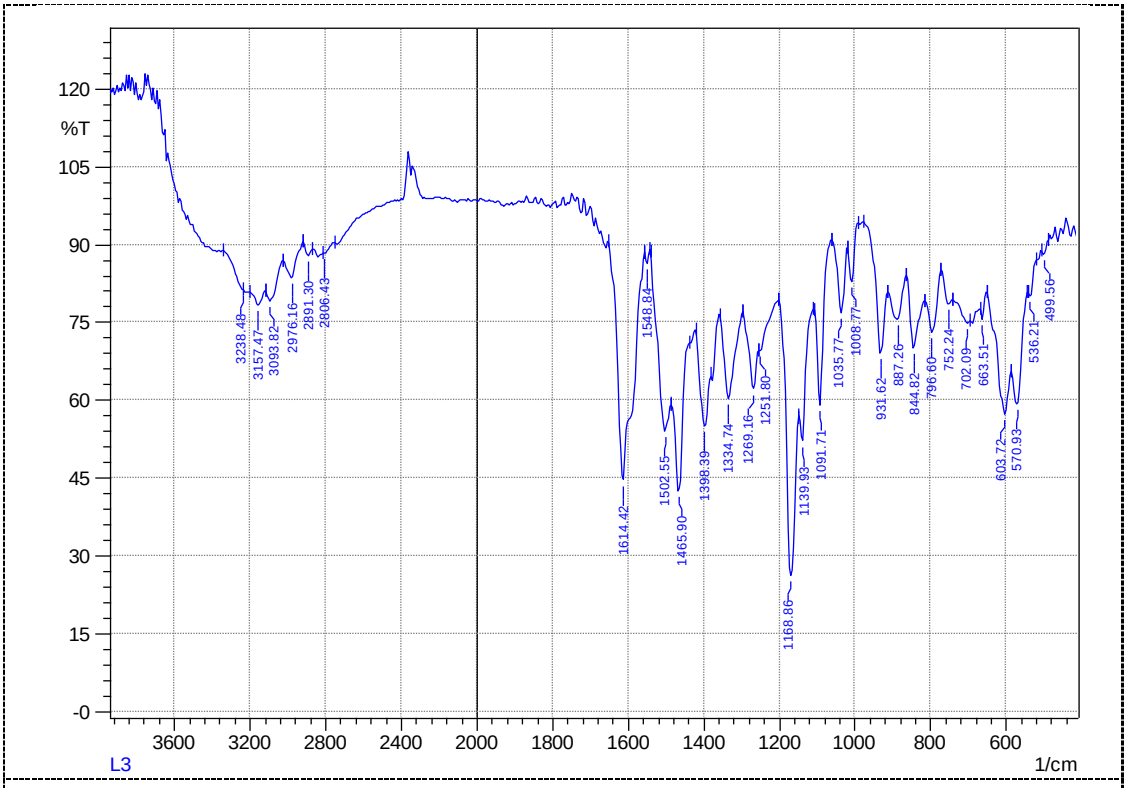


Figure 1. FT-IR spectrum (1=L_3).

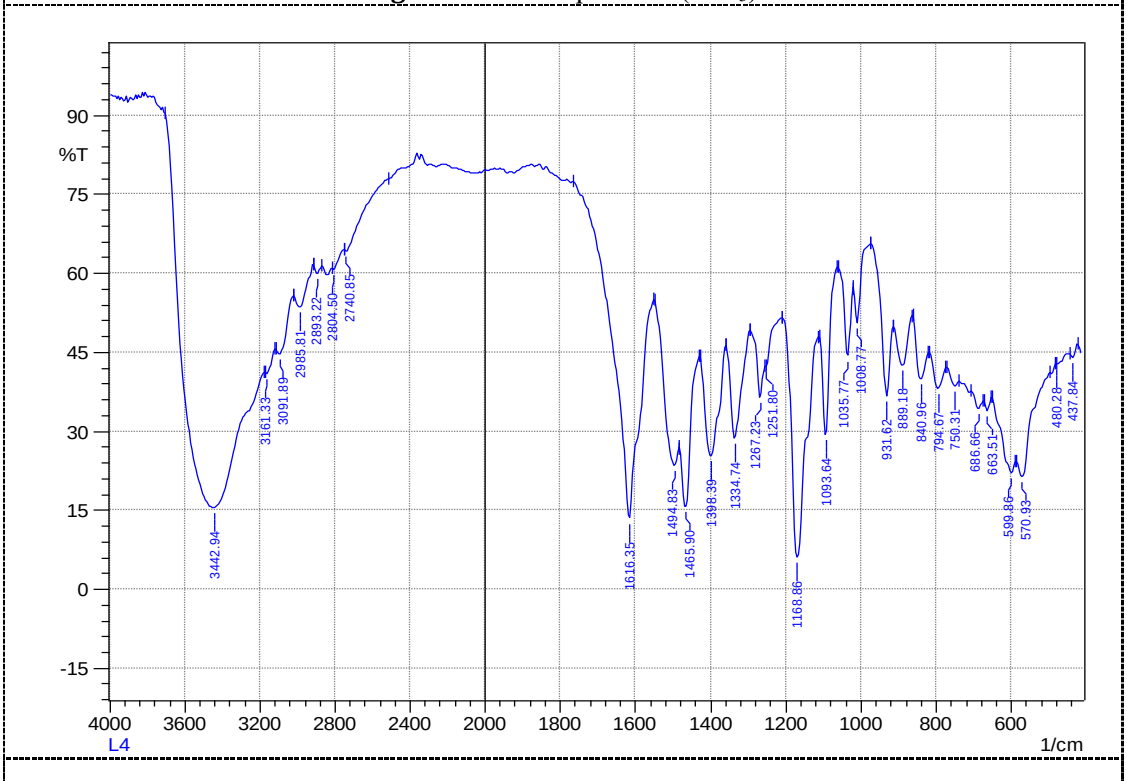


Figure 2. FT-IR spectrum (2=L_4).

Therefore, the mass spectrum was showed that the peak of (1) and (2) at *m/z* were equal to 431 and 695 respectively as seen in figures (3) and (4).

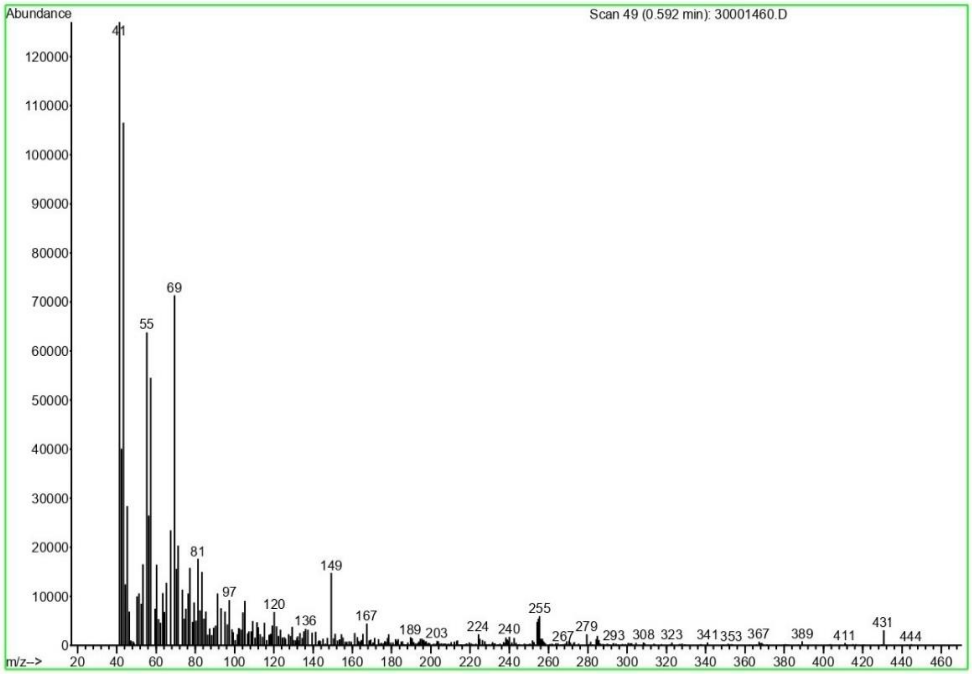


Figure 3. The mass spectrum of the azo dyes (1=L_3).

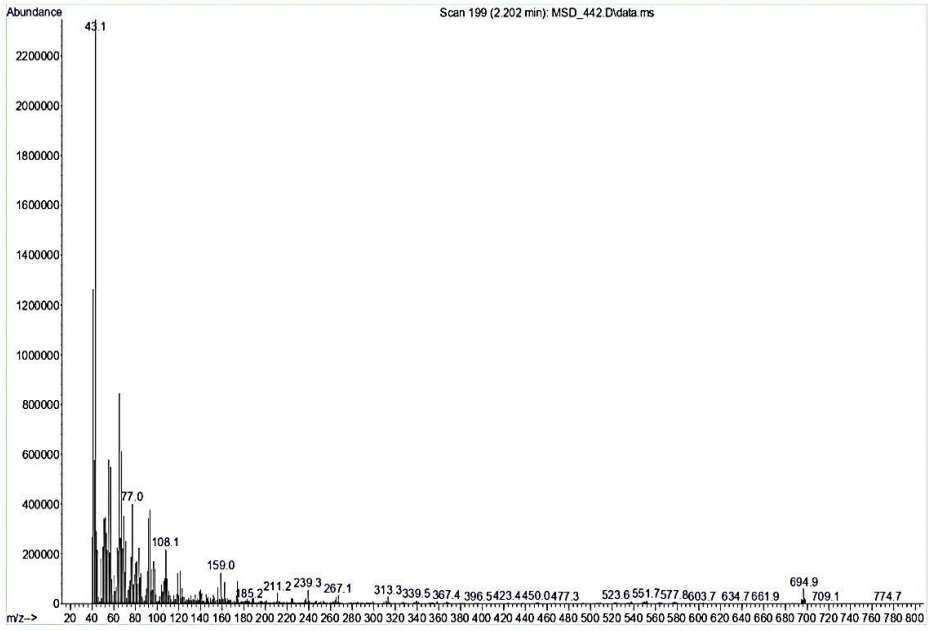


Figure 4. The mass spectrum of the diazo dyes (2=L_4).

Add to which, the ^{1}H NMR spectrum was confirmed the formation of the synthetic azo dyes (1) and diazo dye (2), (figures 5 and 6).

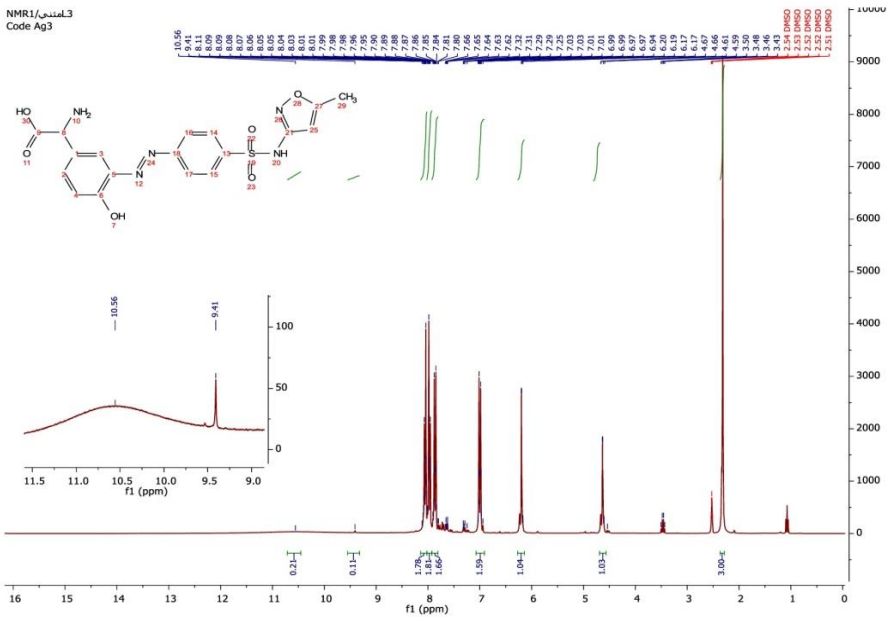


Figure 5. The ^{1}H NMR spectrum of the azo dyes (1=L_3).

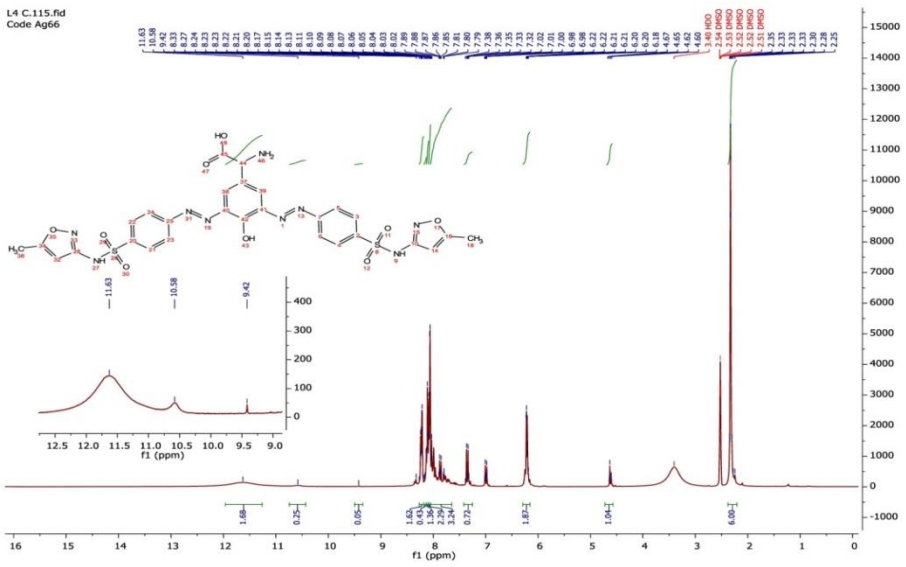


Figure 6. The ^{1}H NMR spectrum of the azo dyes (2=L_4).

The UV-visible spectrum was documented at the range (320-520) nm in ethanol for each synthetic dye. The absorption spectrum of synthetic azo dye (1) and diazo dye (2) were showed bands at (360 nm and 410 nm) and (330 nm and 380 nm) related to (π -π*) and (n-π*) respectively. Analytical studies were also curried on (1) and (2), first of all, the solvent effect of (1) and (2) were intended, (Figures 1 and 2) using set of different solvents. The results displayed that the best solubility of (1) in methanol and (2) in ethyl acetate.

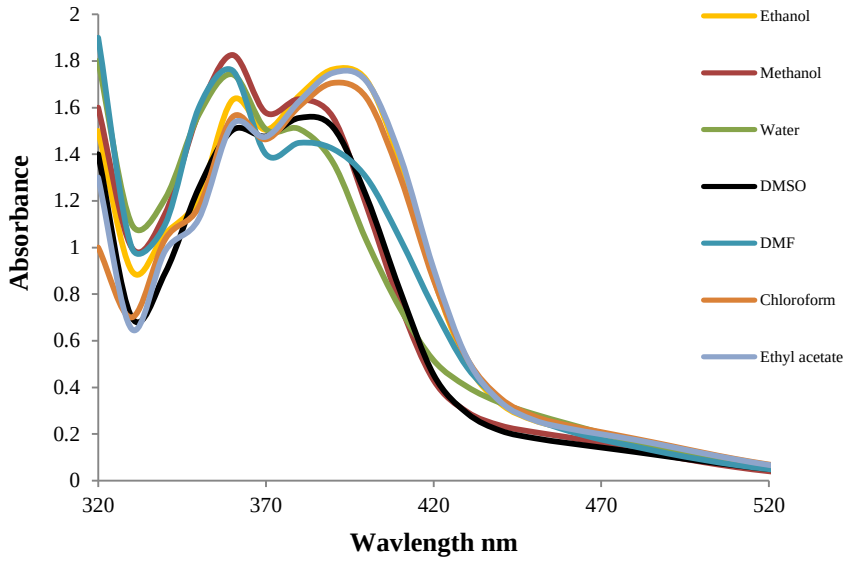


Figure 7. The UV-vis spectrum of the novel azo dyes (1=L_3) in a range of solvents.

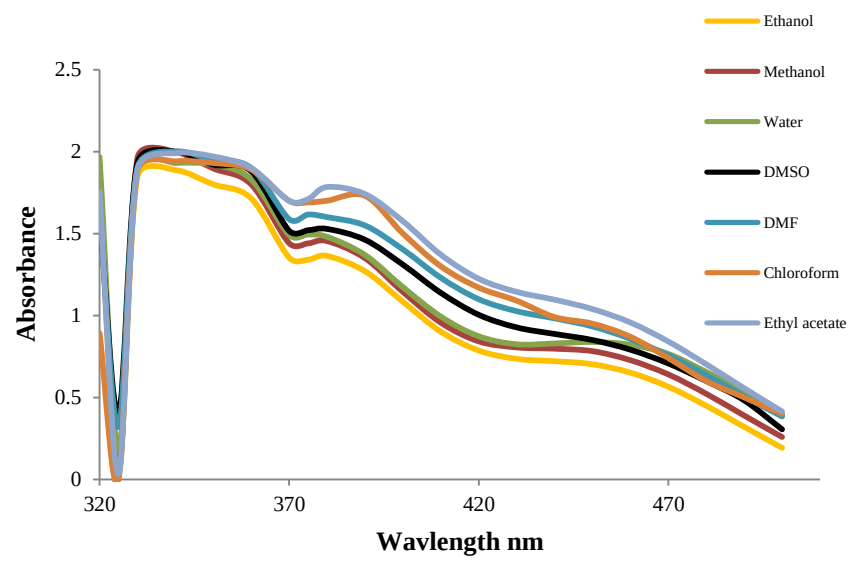


Figure 8. The UV-vis spectrum of the novel azo dyes (2=L_4) in a range of solvents.

Each of azo dye (1) and diazo dye (2) was gave different values of λ_{max}, (Table 1) attributed to π -π* transition of the azo group and indicates the absence of the hydrazone formula within the studied region.

Table 1. The UV-visible spectrum of the azo dye (1) and the diazo dye (2).

Solvent	(1)		(2)	
	λ_{max}(nm)	$\varepsilon_{max}(\times 10^{-4})$	λ_{max}(nm)	$\varepsilon_{max}(\times 10^{-4})$
Methanol	390	1.76	380	1.36
Ethanol	380	1.64	380	1.45
Water	380	1.51	375	1.49
DMSO	380	1.56	380	1.53
DMF	380	1.45	375	1.62
Chloroform	390	1.71	390	1.73
Ethyl acetate	390	1.75	380	1.78

The table shows the λ_{max}(nm) in the absorption spectrum for each dye. The azo dye (1) was displayed a slight red shift towards a higher wavelength using methanol and ethyl acetate in contrast with the diazo dye (2). These results indicated that the synthetic dyes were affected by the solubility and dielectric constant (D), which can be expressed in relation to Gati and Szalay [15] as follows:

$$\Delta\tilde{V}=\left[(a-b)\left(n^2-1/2n^2+1\right)\right]+b\left(D-1/D+1\right)$$

The F(D) and Φ(D) were also calculated, (Table 2) which gave a linear relationship when the dielectric constant is the only effect controlling the beak shift.

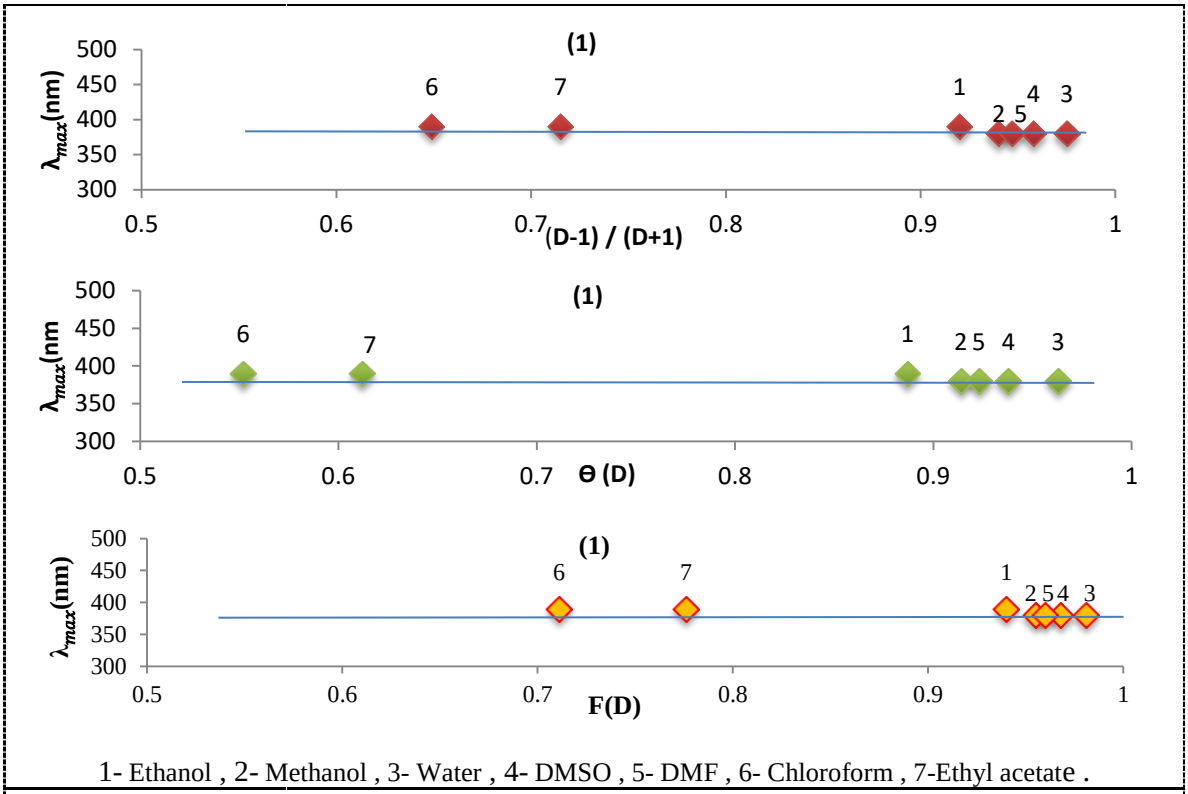
$$F(D)=2\left(D-1\right)/\left(2D+1\right)$$

$$\Phi(D)=(D-1)/(D+2)$$

Table 2. The values of the solvents of the dipole moment constants with the maximum wavelengths of the synthetic dyes**.**

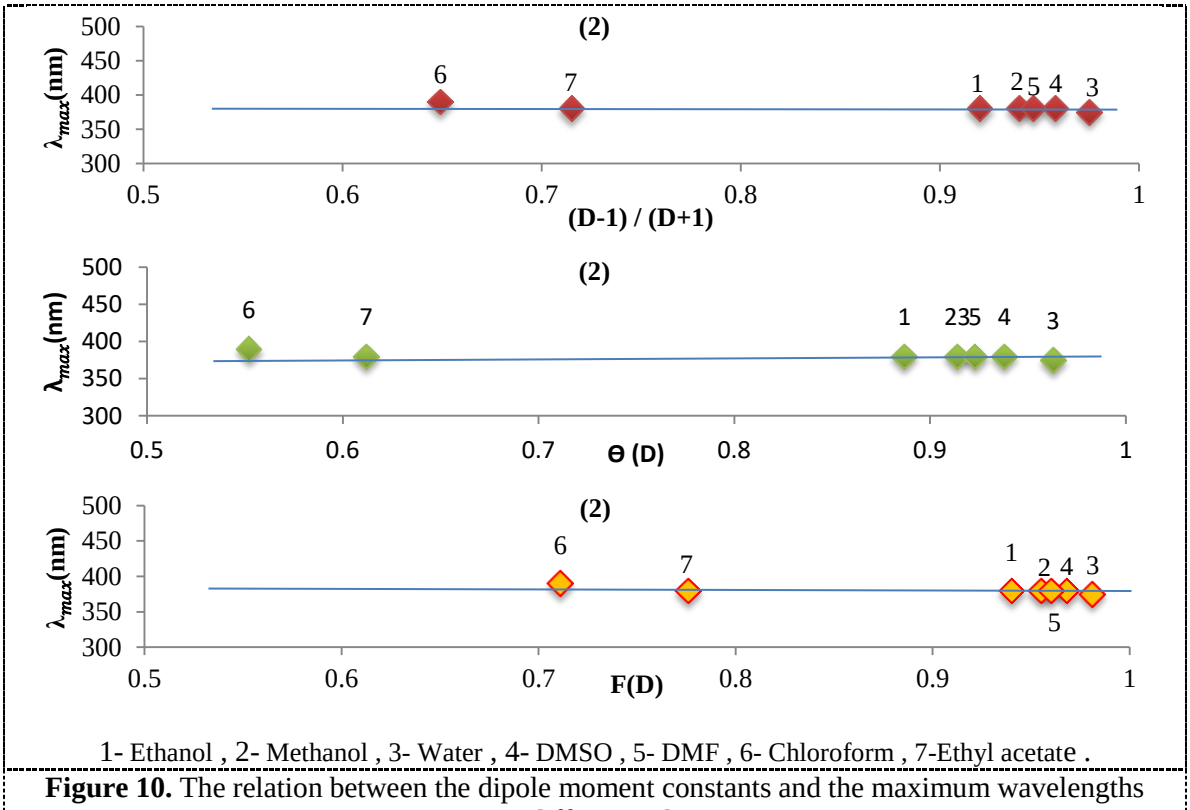
The Solvent	D	(D-1)/(D+1)	D(Θ)	F(D)
Ethanol	24.55	0.92	0.887	0.94
Methanol	32.6	0.94	0.914	0.955
Water	78.3	0.975	0.963	0.981
DMSO	46.7	0.958	0.938	0.968
DMF	36.71	0.947	0.923	0.96
Chloroform	4.7	0.649	0.552	0.711
Ethyl acetate	6.02	0.715	0.612	0.776

The results, (figures 9 and 10) also indicated, that there is no deviation from the linear relationship, which is due to the fact that the effect of the dielectric constant can control the shift of the absorption beaks.



1- Ethanol , 2- Methanol , 3- Water , 4- DMSO , 5- DMF , 6- Chloroform , 7-Ethyl acetate .

Figure 9. The relation between the dipole moment constants and the maximum wavelengths using different solvent.



1- Ethanol , 2- Methanol , 3- Water , 4- DMSO , 5- DMF , 6- Chloroform , 7-Ethyl acetate .

Figure 10. The relation between the dipole moment constants and the maximum wavelengths using different solvent.

Further, the pH effect in the range of λ (320-620) nm was also studied for each dye (1) and (2) in pH (1-12) using 1×10^{-4} M concentration as seen in figures (11) and (12) below.

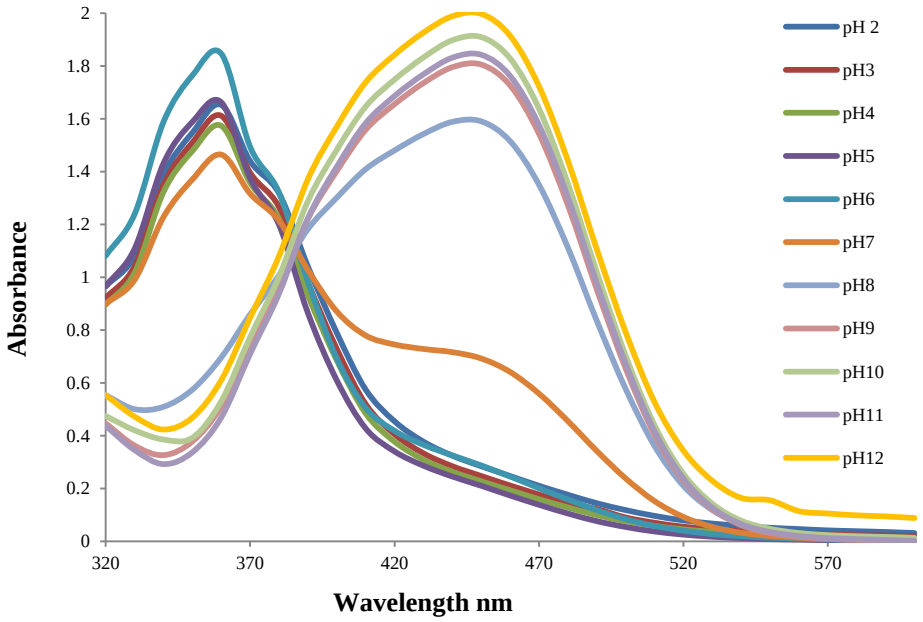


Figure 11. The pH effect in the novel azo dyes (1=L_3).

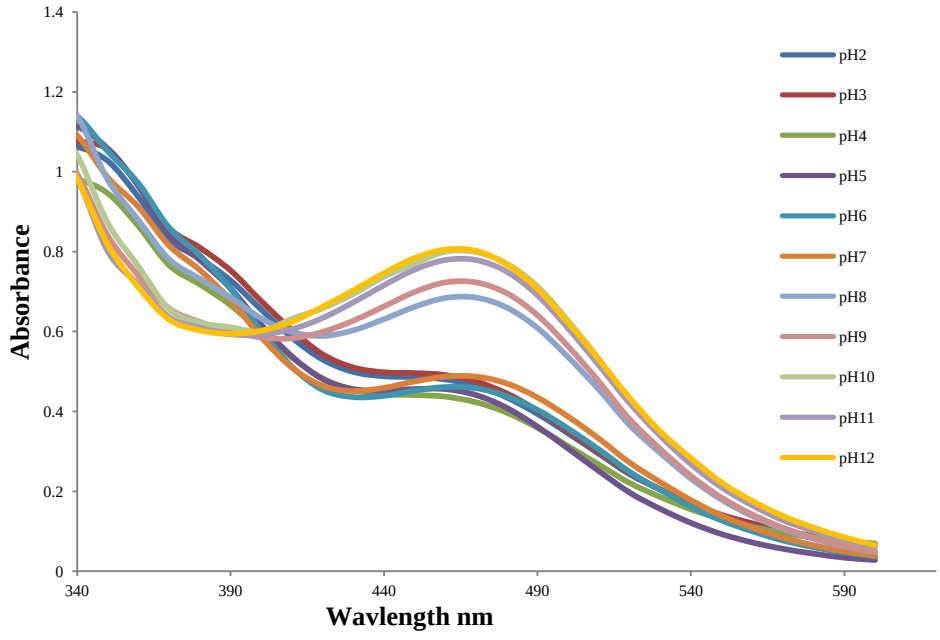


Figure 12. The pH effect in the novel azo dyes (2=L_4).

The results revealed suitable pH values at pH12 in each dye with one and two isopiestic points as seen in Figures (11 and 12) above respectively. Due to calculate the pK_a and pK_b in each of (1) and (2) applying half height method [16], (Figures13 and 14).

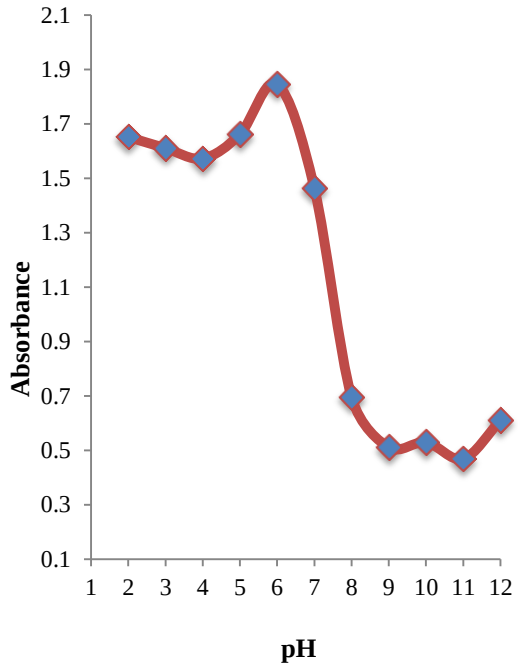


Figure 13. Absorbance-pH effect of (1=L_3) at λ_{max} 360 nm.

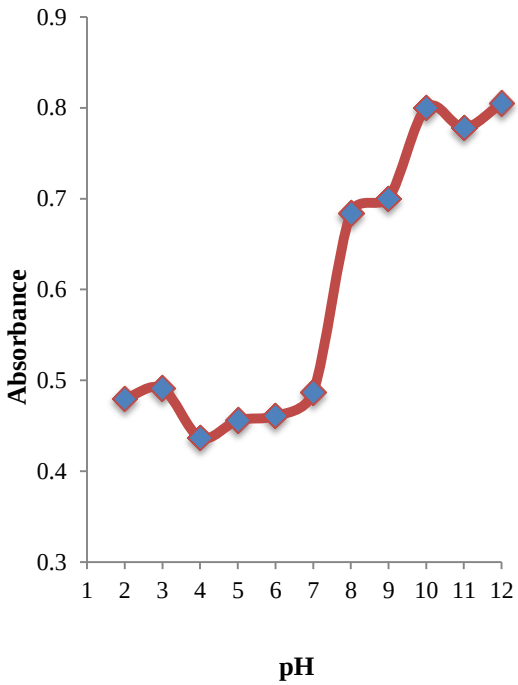


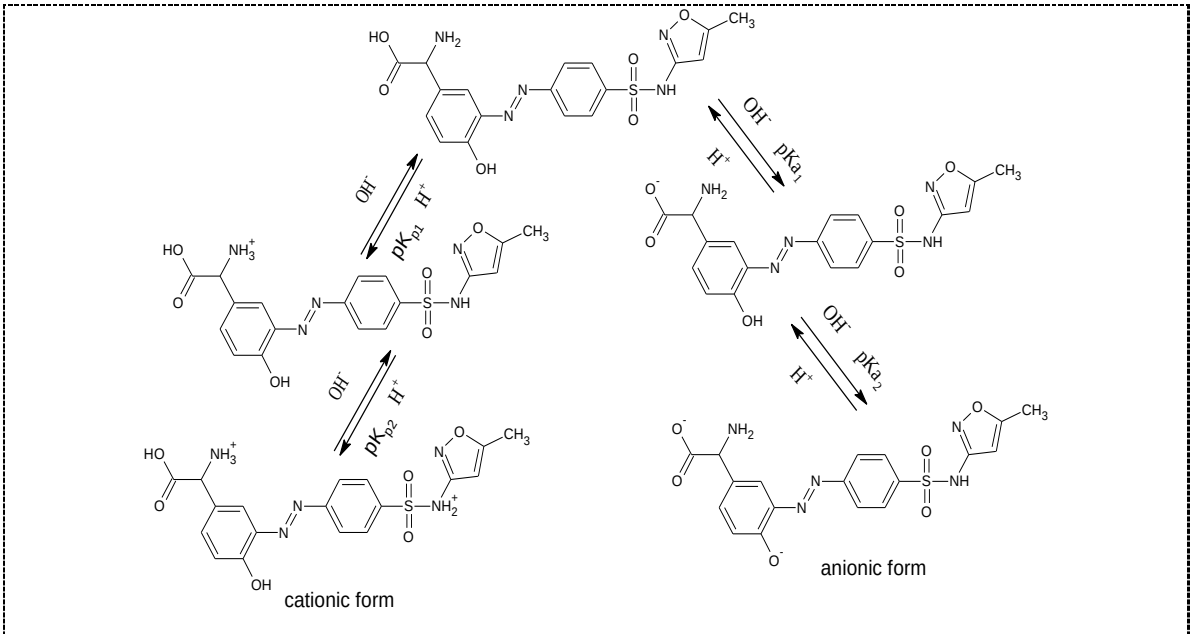
Figure 14. Absorbance-pH effect of (2=L_4) at λ_{max} 460 nm.

The results obtained from the absorbance-pH curve in figures above presented in table (3) below.

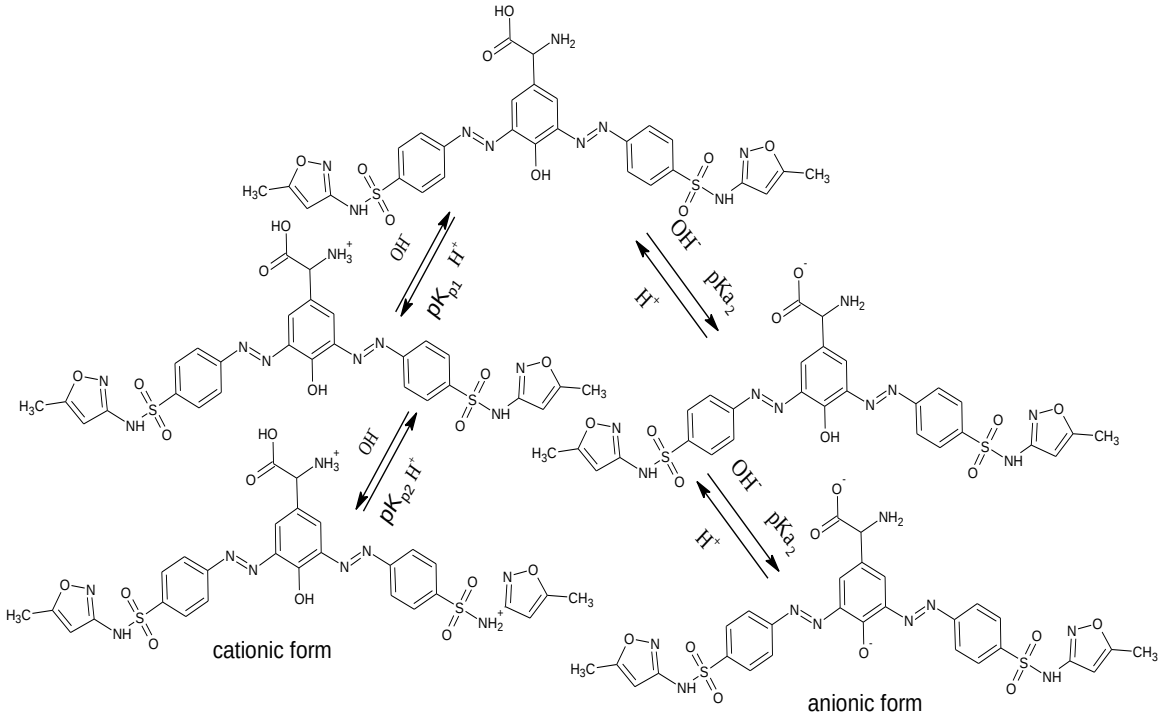
Table 3. define the ionization and protonation constants of (1) and (2).

The synthetic dye	$\lambda_{max\ (nm)}$	$A_{1/2}$	pK_{p1}	$A_{1/2}$	pK_{p2}	$A_{1/2}$	pK_{a1}	$A_{1/2}$	pK_{a2}
Azo dye (1)	360	1.709	5	….	….	0.521	9.5	0.539	11.5
Diazo dye (2)	460	0.449	5	0.485	2.5	0.75	9.5	0.791	11.5

The following equilibrium schemes, (2 and 3) displays the suggested ionization of azo and diazo dye respectively in acidic and basic medium.

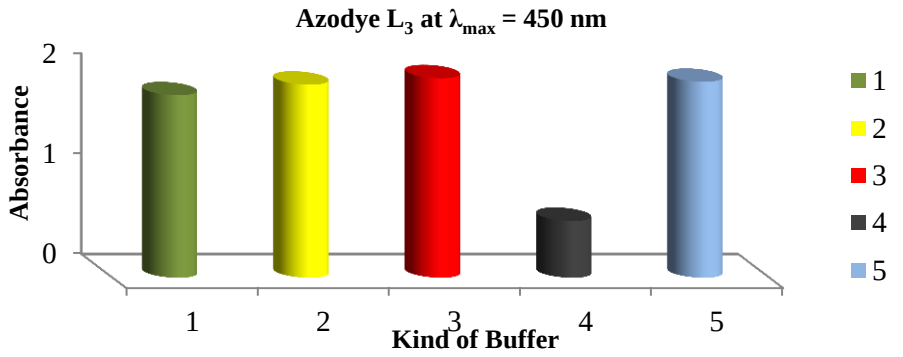


Scheme 2. Optional ionization of (1) in acidic and basic medium.



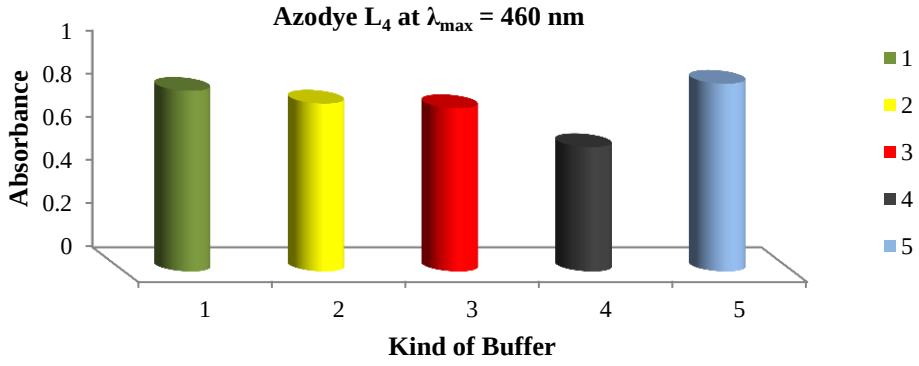
Scheme 3. Recommended ionization of (2) in different media.

Different buffer solutions effect each dye, (Figures 15 and 16).



1 = Universal, 2 = KCl + NaOH, 3 = $Na_2HPO_4.2H_2O$ + NaOH, 4 = Hexamine, 5 = NaOH + $Na_2B_4O_7.10H_2O$

Figure 15. The effect of different buffer solutions in azo dye (1).



1 = Universal, 2 = KCl + NaOH, 3 = $Na_2HPO_4.2H_2O$ + NaOH, 4 = Hexamine, 5 = NaOH + $Na_2B_4O_7.10H_2O$

Figure 16. The effect of different buffer solutions in diazo dye (2).

Furthermore, the results were displayed effect of different buffer solutions on (1) and (2), (Tables 4).

Table 4. The effect of different buffer solutions on azo dye (1) and diazo dye (2).

No.	Buffer Solutions	The absorption at λ_{max}	
		L_3 (450)nm	L_4 (460)nm
1	Universal	1.82	0.834
2	KCl + NaOH	1.926	0.775
3	$Na_2HPO_4.2H_2O$ + NaOH	1.99	0.754
4	Hexamine	0.567	0.574
5	NaOH + $Na_2B_4O_7.10H_2O$	1.965	0.867

The method [14] applied for toxity measurement using haemolytic red blood cells in vitro. The results were showed that all prepared compounds harmless and didn't display haemolysis effect in the cells, using different concentrations.

4. Conclusion

This study focused on the synthesis, characterization and analytical studies of novel synthetic azo and diazo dyes. These dyes were prepared cheaply because the starting materials are obtainable and most of the chemistry is prepared at or below room temperature. Moreover, the synthetic dyes gained good colour and revealed non-toxic properties.

5. References

[1] Kirkan, B. and Gup, R. "Synthesis of New Azo Dyes and Copper (II) Complexes Derived from Barbituric Acid and 4-Aminobenzoylhydrazone", *Turk. J. Chem*., 2008, 32, 9-17.
[2] H. Majeed, Synthesis, Characterization and study of the spectral and electronic properties of a New Azo Dyes Compounds, *J. Thi-Qar Sci*, 2013, 4, 91 – 101.
[3] Ali H., H. Majeed, A. Hussain, Synthesis, Analytical and Theoretical studies of (Z)-4amino-3-hydroxy-2-((4-(N-(5-methyl isoxazol-3yl) sulfamoyl) phenyl) diazenyl) naphthalene-1-Sulfonic Acid, *Journal of Natural Sciences Research*, 2017, 7, 81 – 88. 16.
[4] Ali H., H. Majeed, I. Al-Asadi, A. Abdulredha, A. Hussain, Structures effect of two azo dyes associated with their antimicrobial activity, *Journal of Chemical, Biological and Physical Sciences*,2018, 8, 171-185.
[5] Otutu, J. O., " Synthesis and application of azo dyes derived from 2-amino-1, 3,4-thiadiazole-2-thiol on polyester fibre", *J. IJRRAS*, 2013, 15, 292 – 296.
[6] Fayadh, R. H. F., Ali, A. A. and Al –Jabri, F. M., Synthesis and Identification Symmetrically Azo Dyes Derived from Sulfa Compounds and Spectrophotometric study of Nickel (II) Complexes with Prepared Dyes, *International Journal of Engineering and Technical Research* (IJETR), 2015, 3, 25-28.
[7] Zollinger, H., "Color chemistry; synthesis, properties and Application of organic Dyes and Pigments" ,*VCH*,1991.
[8] Garg H. G. and Praksh C., "Preparation of 4-arylazo-3,5-disubstituted-(2H)-1,2,6-thiadiazine1, 1-dioxides," *Journal Medicinal Chemistry*,1972, 15,.435–439.
[9] Child R. G., Wilkinson R. G., and Tomcu-Fucik A., "Effect of substrate orientation of the adhesion of polymer joints," *Journal of Chemical Society*, 1977, 87, 6031–6038.
[10] Ali H., Majeed H., Al-Asadi I., Abdulredha A. and Hussain A., Structures effect of two azo dyes associated with their antimicrobial activity, *Journal of Chemical, Biological and Physical Sciences* 2018, 8, 171-185.
[11] Farghaly T. A. and Abdallah Z. A., "Synthesis, azo-hydrazone tautomerism and antitumor screening of *N*-(3-ethoxycarbonyl4,5,6,7-tetrahydro-benzo[b]thien-2-yl)-2-arylhydrazono-3-oxobutanamide derivatives," *Archieve for Organic Chemistry*, 2008, 17, 295–305.
[12] Ali H., Majeed H., Hussain A. and Mohsin R., Journal of Global Pharma Technology Analytical and Theoretical Studies of Some Diazo Dyes, *Journal of Global Pharma Technology*, 2019 11, 353-362.
[13] Xian-guo H. and Ursula M., Determination of hemolysis against fresh sheep RBCs, *J. Ethnopharmacol*, 1994, 43, 173-177.
[14] Ali, A. A., Fahad, T. A., & Al-muhsin, A. A. (2020, November). Preparation and Spectroanalytical Studies of Two New Azo Dyes Based on Luminol. In *IOP Conference Series: Materials Science and Engineering* (Vol. 928, No. 5, p. 052007). IOP Publishing.
[15] Issa RM, Zewail AH (1971) J. Chem. U.A.R., (Egypt J. Chem.), 14, 461