Analytical study of some azo dyes and its medical applications

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ABSTACT

A new pharmaceutical-derived azo dye was prepared from salbutamol as an aromatic phenolic compound with sulfonamide, sulfadiazine and sulfathiazole, respectively. Dye characterization was described by Mass. , H1-NMR, I.R. and visible spectroscopy techniques. The electronic spectra of this azo dye have been studied in terms of proton harvesting and ionization. The other study was the effect of solvents with different polarities on the electronic spectra. The dye has many applications, such as its use as an indicator of a strong acid with a strong base and the use of the dye as an antibiotic. Some applications measured the biological activity of the prepared dyes against Escherichia Coli and Staphylococcus Aureus, and showed positive results.
Key Words: Azo dye Isopiestic points, Solvent effects , Indicator , Biological study

INTRODUCTION

Aromatic azo dyes are the largest group of organic dyes for their widespread applications in many areas of textile and medicine [1], Azo dyes are characterized by the presence of at least one (R1–N=N–R2) functional group. The azo group often helps to stabilize the dyes and form a conjugated system, which very often absorbs visible frequencies of light yielding colored compounds [2]. Many azo pigments are nontoxic, although some it has been reported that several bacteria and fungi are capable of catabolizing and mineralizing azo dyes [3] . Also, Azo Dyes participate in many biological reactions in the body as inhibitors of RNA and DNA of microbes for protein synthesis, as anti-cancers, and as antiseptics, because they form hydrogen bonds with microbes and cancer cells, which results in the destruction of these cells[4]. Azo compounds as reagents in analytical chemistry as indicators of pH [5].Sulfa compounds have been used widely for a long time in the treatment of diverse diseases [6]. In the medicinal uses, there are more than 30 drugs which are commercial used in markets, including antihypertensive, antibacterial,

antiprotozoal, antifungal, and anti-inflammatory [7]. One of the important clinically used sulfa drugs was called prontosil which is azo-sulfa drug that gave protective action against streptococci in animals. Prontosil has two effective features, and it was active in vivo, while it is ineffective in vitro. These features led to the fact that this drug was not the active [8,9]. Azo dyes have wide applications, these compounds have been used as cosmetics because of more stable than natural color's and used as pharmaceutical products [10]. According to stability, azo-dyes are stable in different ranges of pH, they do not vanish when exposed to oxygen or light, and some of them are soluble in water. However, azo dyes have not the ability to dissolve in fat or oily phases [11] Antioxidant compounds are species widely utilized against the free radicals which reduce oxidative stress in the cell. Natural and chemical sources may be containing these antioxidant compounds [12].

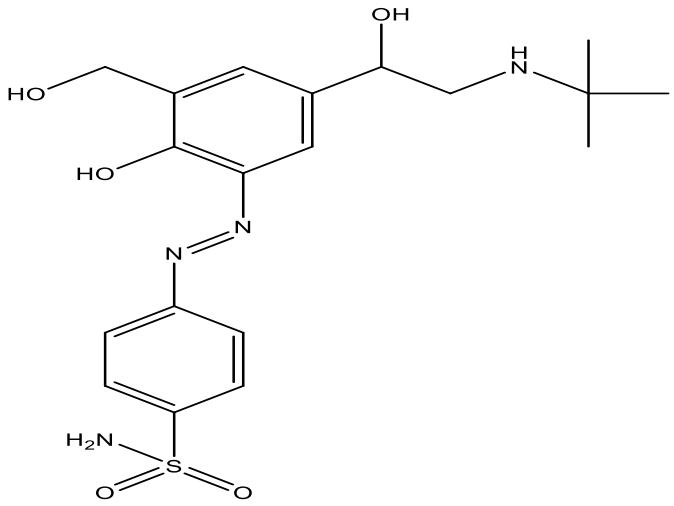
. Salbutamol was identified in pharmaceutical preparations and preparation of an azo-dye[13]. Hz1 diazotation reaction with Sulfonamide Azo dye Hz2 was also prepared from salbutamol with sulfadiazine in acidic medium. Also, a third azo dye Hz3 derived from salbutamol and sulfathiazole was prepared As for the reaction, the reaction was done in an acidic medium and at a temperature of less than 5 degrees Celsius[14]. Salbutamol is a medicinal compound used to relieve coughing and shortness of breath caused by some diseases, such as asthma. Chronic obstructive pulmonary disease. Bronchitis Likewise, sulfa drugs are used to treat a range of diseases caused by bacteria, and countless lives have been saved. It is one of the most important compounds used in medicine[15,16].

EXPERMINTAL

All the reagents and solvents were of reagent-grad quality. The progress of reaction was monitored by TLC using silica gel coated plate sand spots were visualized under UV radiation. Infrared spectra (in KBr pellets) were recorded on FT.IR-8400S shimadzu, Melting points were determined on melting point apparatus , Element analysis (Mass). The pH measurements were made with pH-Meter (H. Jurgons Co. Bremen, L.Puls Munchen15)**.** To calculate the ionization and protonation constants for hydroxyl and nitrogen groups, a series of acetate and universal buffer solutions were prepared with different pH values(2-12) [17].

Preparation of azo dye

The azo ligand PS with the structure depicted in figure 1 was prepared as described in the earlier methods for azo dyes[18].



(*E*)-4-((5-(2-(*tert*-butylamino)-1-hydroxyethyl)-2-hydroxy-3-(hydroxymethyl)phenyl)diazenyl)benzenesulfonamide

Fig.(1):HZ1 (E)-4-((5-(2-(tert-butylamino)-1-hydroxyethyl)-2-hydroxy-3-(hydroxymethyl)phenyl)diazenyl)benzenesulfonamide ($C_{19}H_{26}N_4O_5S$) (m.wt. 422)

-Solutions

***** 1 x 10^{-3}M stuck solution of azo dye Hz1

* Universal buffer solutions (pH 2-13) [19]

preparation

1- Dissolve 0.007 mol each of Sulfanilamide, Sulfadiazine, and Sulfathiazole, with a weight of 1.204 g, 1.752 g, and 1.02 for the dyes HZ1, HZ2, and HZ3, respectively, in (2.5 ml) of concentrated hydrochloric acid, and add 5 ml of distilled water, and place it in ice until the temperature reaches below 5 m 0.).

2- 2- Dissolve 0.55 gm of sodium nitrite in 5 ml of distilled water and place it in an ice bath.

3- Dizonium salt is prepared by adding the sodium nitrite solution prepared in step (2) in the form of drops to the cold solution prepared in step (1) with continuous stirring while maintaining the temperature below 5 °C.

4- Dissolve (1.675 g, 0.007 mol) of salbutamol sulfate in an amount of about 50 ml of sodium hydroxide solution (contains 2.1 g) and cool the solution below 5 °C in an ice bath

5- The solution prepared in step (3) is added slowly with continuous stirring to the solution prepared in step (4) and the mixture is left in an ice bath for 5 minutes and the resulting dye is left in the refrigerator for (24) hours, then the prepared dye is neutralized by adding

a dilute solution of hydrochloric acid To convert an azo dye from the salt form to the hydrogen form
6- The dye was filtered using a Buechner device, then the resulting dye was recrystallized using ethanol

-Procedures

*** For acid base**, the absorbance of of Hz1 dye solutions of concentration $6x10^{-5}$ M at different values of (2-12)pH buffer solutions ,were measured in range of 350-560 nm.

*** For solvent effect** , the absorbance of Hz1 dye solutions of concentration $4x10^{-5}$ M at different values solvents of different polarities ,were measured in range of 320-600nm.

RESULTS AND DISCUSSION

Chemical analysis

The azo dye Hz1 was characterized by important chemical analysis techniques like melting point (173C), IR and H^1NMR . From Figs.2 and 3 , the results collected in.

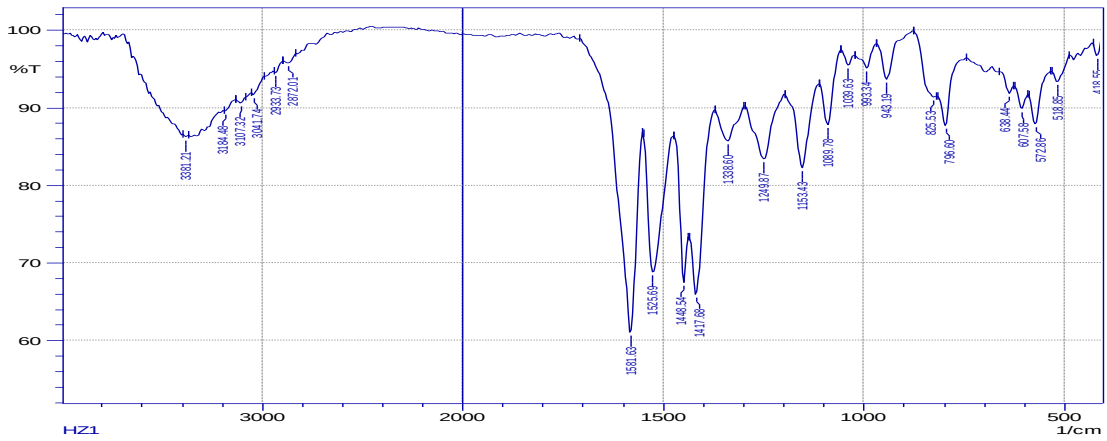


Fig.(2): IR-Spectrum of azo dye Hz1

Table (1) The results showed good agreement with chemicals structure in

(BEAM WAVENUMBER (CM-1 HZ1	package
3381m	Amplitude vibration of O-H and N-H bonds
1525s.sh	The amplitude vibration of the C=C bond
1448s.sh	Amplitude vibration of the bond N=N—
1153s	Bending vibration of the C-N bond
1338m 1153s	Amplitude vibration of the belt
3107w	Asymm.(O=S=O)
825s	O=S=O) Symmetric
1581	Amplitude vibration of the aromatic C-H bond
1249m	Bending vibration of the C-H bond

W Weak, m : middle , s : strong , sh : sharp:

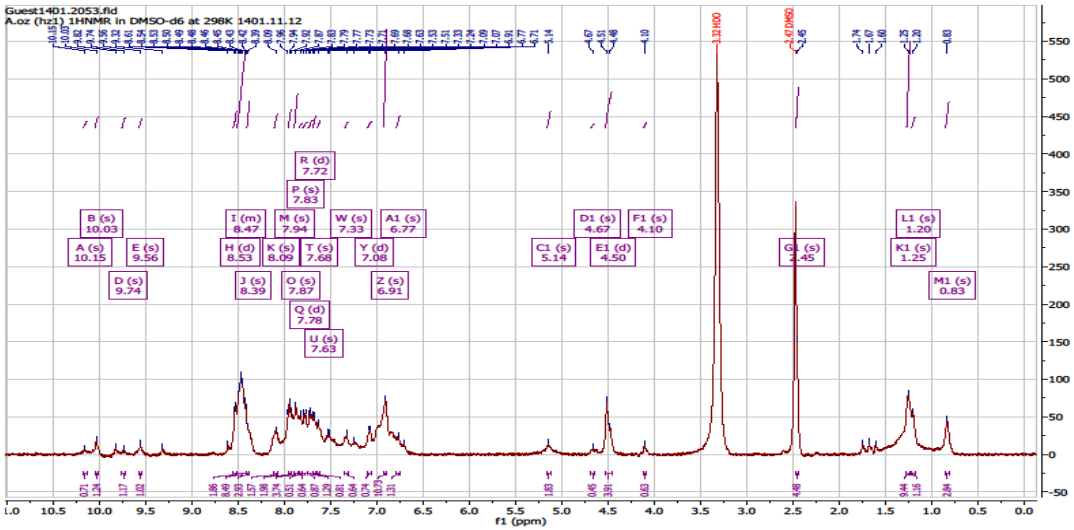


Fig.(3): NMR-Spectrum of azo dye Hz1

Table (2): Chemical analysis (IR and NMR) of azo dye HZ1

IR	**Wavenumber (cm^{-1})**					
	OH&NH	**C=O**	**N=N**	**C-N**	**C-O**	**C=C**
	3381.21w	1664.57s	1427.32s	1309.67	1219.01s	1579.70s
	Chemical shift (ppm)					
H^1NMR	**S,3H,OCH_3**	**S,H,HDO**	**S,6H,DMSO**	**m,7H,Ar-H**	**N-H**	**OH**
	1. 25	3.34	2.5	7.0-8.7	9.5	10.0

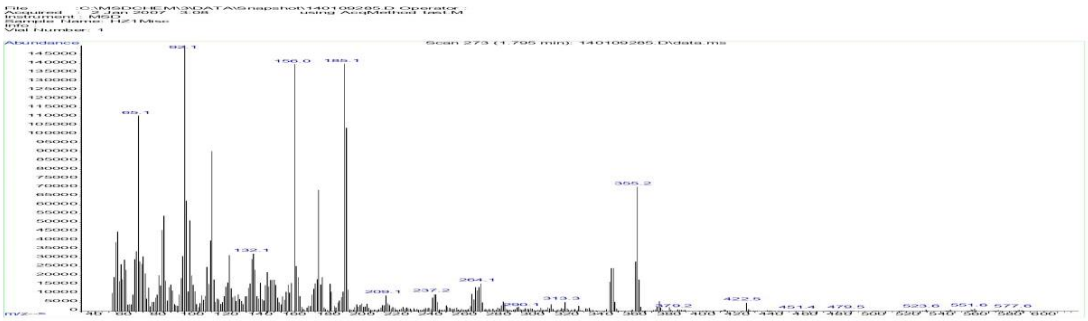


Fig (4): Mass spectrum of the HZ1 ligand Some of spectrophotometric studies of azo dye Hz1

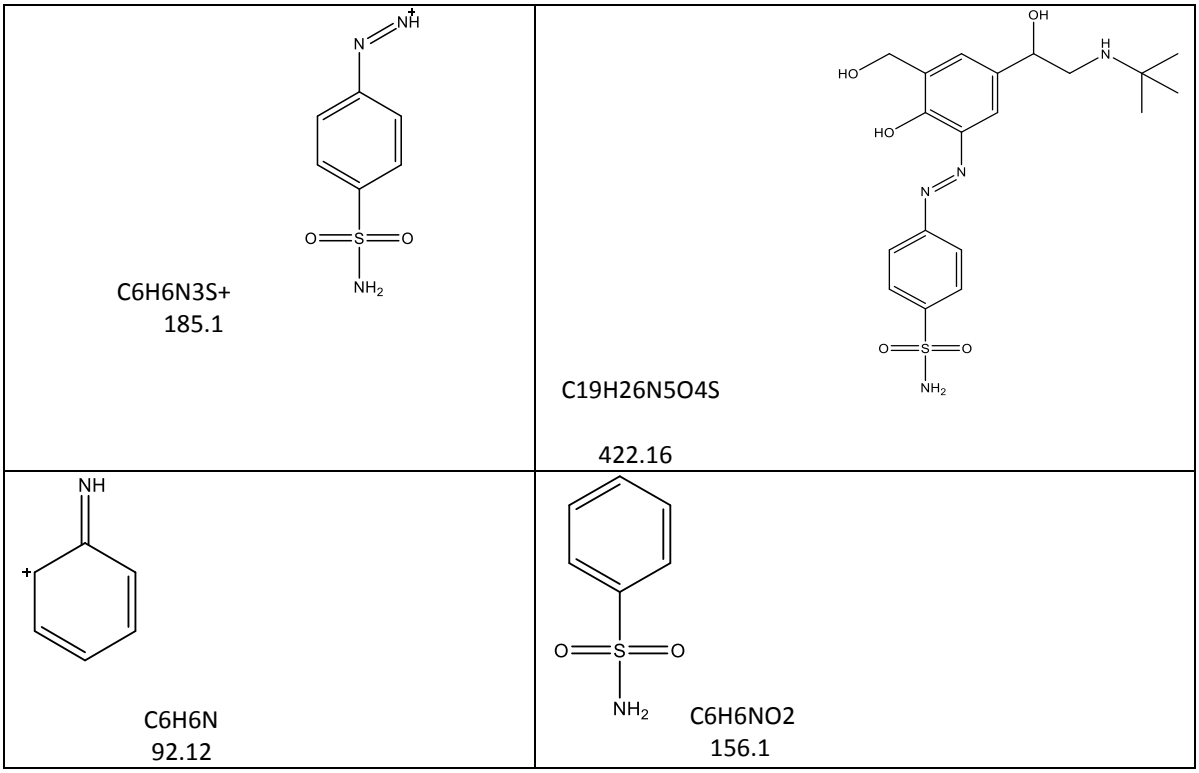


Fig (5): The most important fragmentation ions in the mass spectrum of the prepared ligands

1- Acid-Base properties and determination of protonation and ionization costants

The absorption spectra of 6 x10^{-5} M solution of dye in the range of wavelength (350- 560 nm) of varying pH values (2 – 12) were represented graphically (Fig. 6).

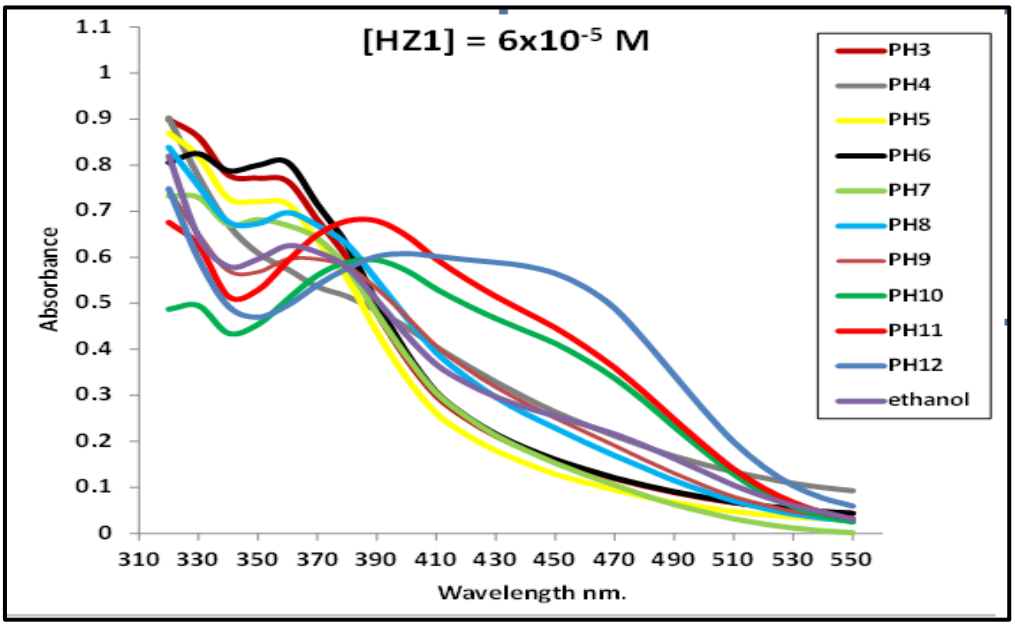


Fig.(6): electronic spectrum of dye Hz1 at different value of pH.

The spectrum characterized three bands, the first at 360 nm in the pH range (3-9) due to the protonation of dye. The bands (390) of high intense in pH range (8-12) . The spectrum also showed one isobestic point (is that point at wavelength having constant absorbance although changing in pH value medium) at 390 nm. The spectrum showed no and band at pH 2 value .

To determine the protonation and ionization constants of azo dye Hz1 , the wavelength 390 nm. is chosen as best wavelength (from Fig. 6) to draw a curve between pH values as x-axis with corresponding their absorbances (Fig. 7).

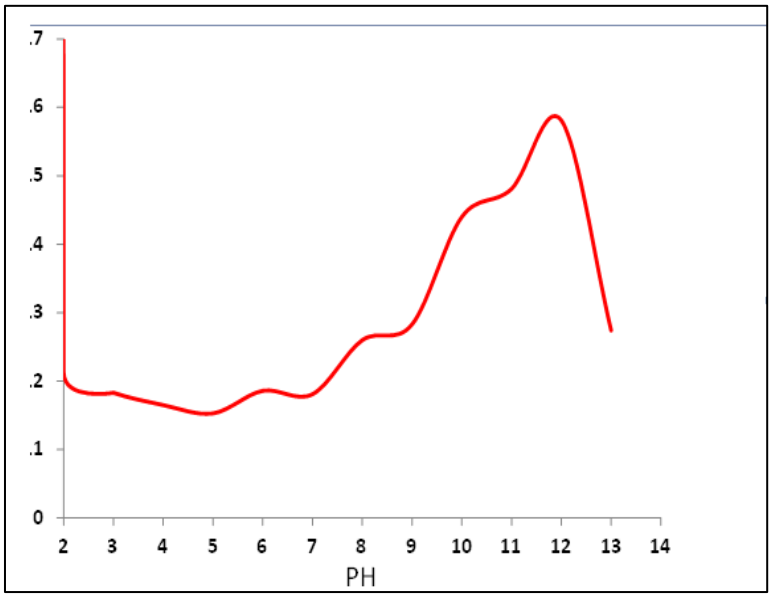


Fig.(7): Absorbance – pH curve of the azo dye Hz1

From (Fig.7) the ionization and protonation constants were calculated (Table 3) . From Absorbance–pH curve and by the aid of half-height method [25] , the pK values were obtained by this relation : pK = pH (at $A_{1/2}$) where ; $A_{1/2}$ = (A_L+ A_{min}.) / 2 and A_L and A_{min} are limiting and minimum absorbance's respectively.

Table (3): Protonation and ionization constants of azo dye Hz1

Dye	A_{min}	A_L	$A_{1/2}$	pK_{p1}	A_{min}	A_L	$A_{1/2}$	pK_{p2}	A_{min}	A_L	$A_{1/2}$	pK_{a2}	A_{min}	A_L	$A_{1/2}$	pK_{a2}
HZ1 at λ_{390}	0.16	0.27	0.22	3.4	0.12	0.16	0.14	5.6	0.14	0.24	0.19	7.5	0,24	0.43	0.34	9.5

If we reflect on the chemical composition of the dye and Table (3), we notice the presence of a common group, which is the hydroxyl of the dye of the compound salbutamol, and we also notice a convergence in the values of the ionization constant (pKa), evidence that it is the compound in which it connects (in the ortho site) via the azo group It has no significant effect on the pKa values. It is also noted that the dye group HZ1 has been

brittened

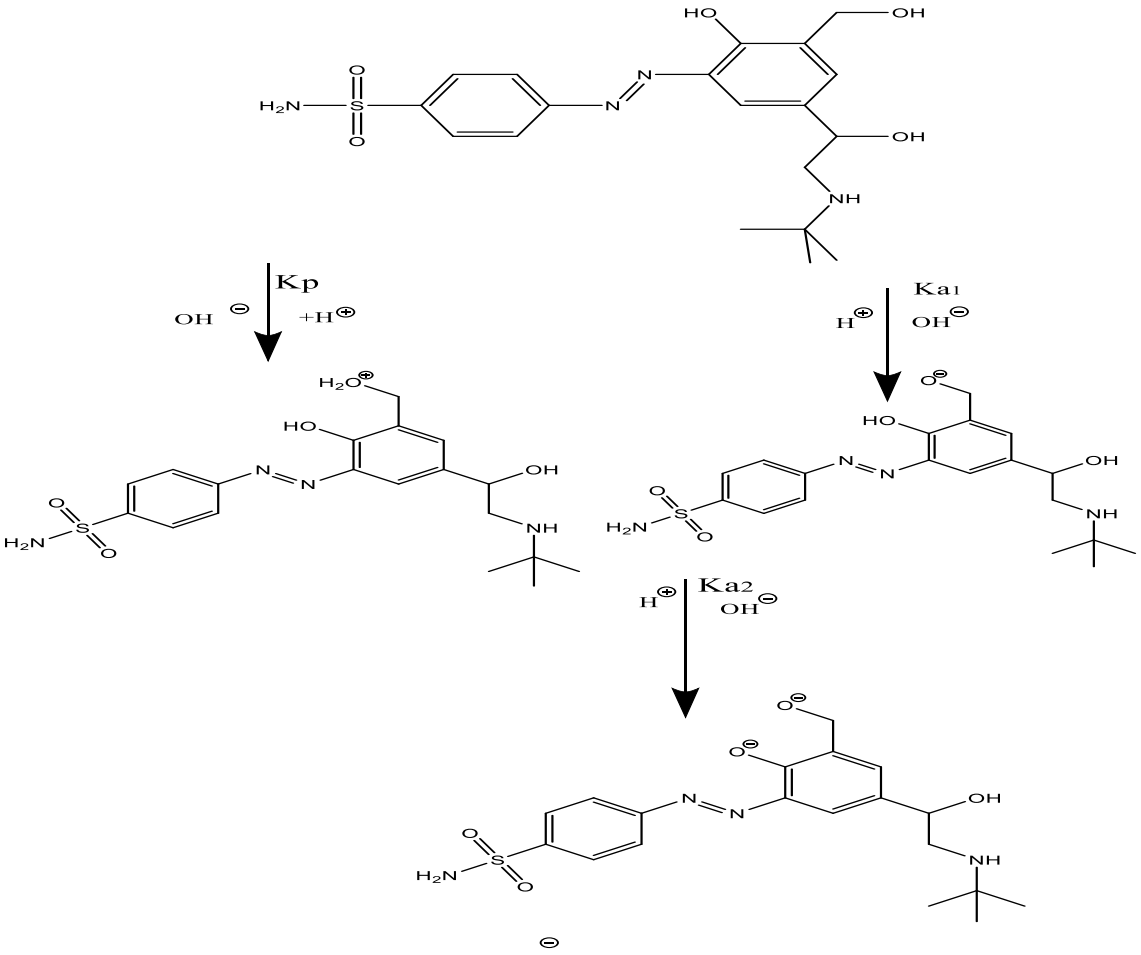


Fig.(8): Suggested mechanism of protonation and ionization of azo dye HZ1

2- The effect of solvents with different polarities:

Figure 7 shows the spectrum of azo dye Hz1 All the maximum peaks for all solvents for each dye range between (350-360) nanometers located within the linearity, and this means that the only effect is the dipole moment for it, except for the third dye, in which a red deviation occurs at a wavelength of 370 nanometers for the DMF solvent, and this is probably the result of hydrogen bonding between The solute and the solvent. Except for the third dye, where another effect is involved, which is hydrogen bonding.

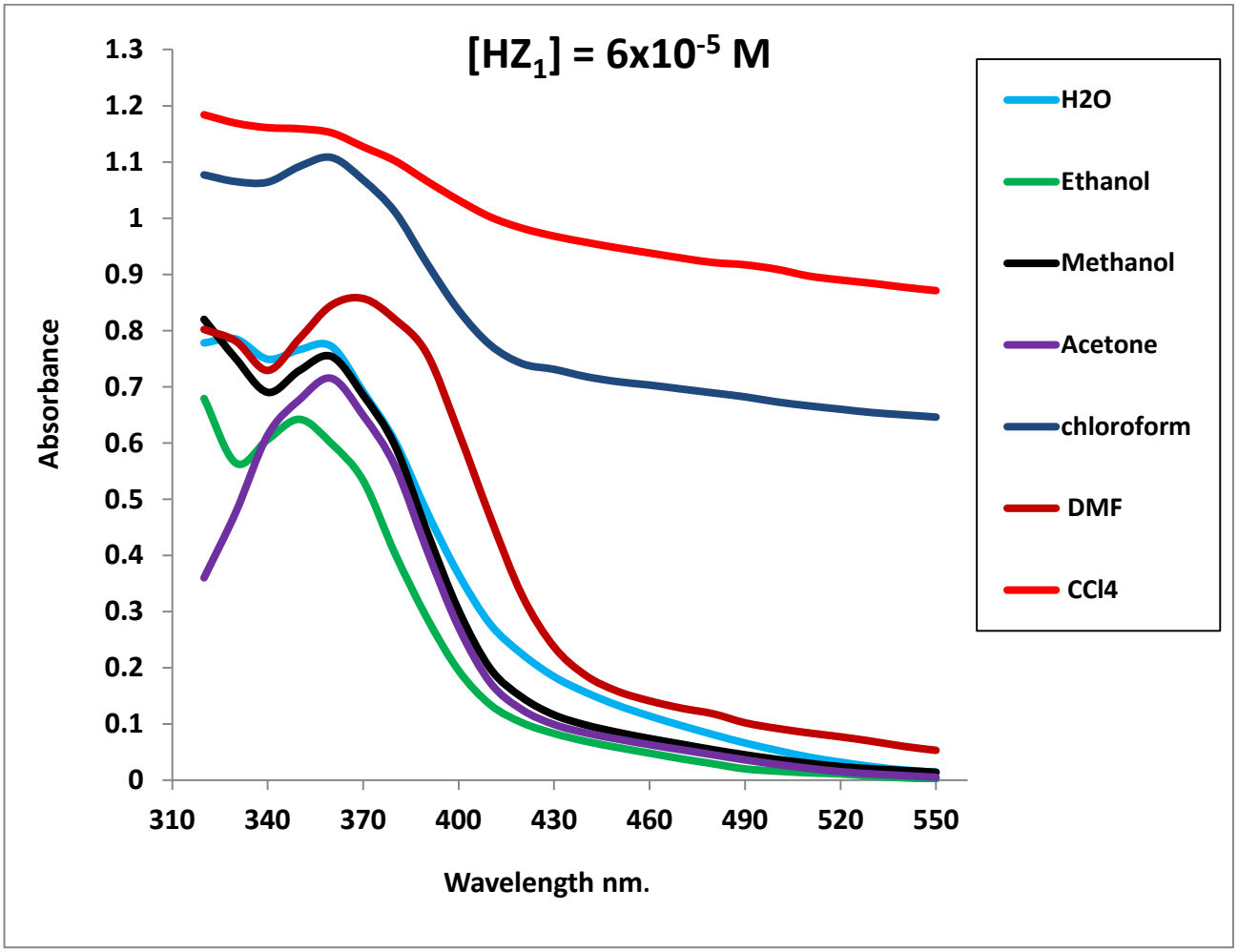


Fig.(9): Absorbance spectra of dye HZ1 at different solvents with different polarities

λ_{max} nm HZ_1	(D – 1) / (D + 1)	D	**solvents**	ت
360	0.909	21.00	Acetone	1
360	0.381	2.23	Carbon tetra chloride	2
360	0.650	4.72	Chloroform	3
370	0.947	36.71	DMF	4
350	0.940	32.70	Ethanol	5
360	0.920	24.55	Methanol	6
360	0.975	78.30	Water	7

Table (4) : values of dielectric function of solvents and maximum wavelength of dye HZ1 at different solvents

By plotting a curve between dielectric function (D-1)/(D+1) and λ_{max} of dye at different solvents (Fig. 10) , the linearity of all solvents which means the dielectric constant that affected except for the DMF gave deviation from the linearity because of hydrogen bonding between dye molecules (solute) and solvent molecules.

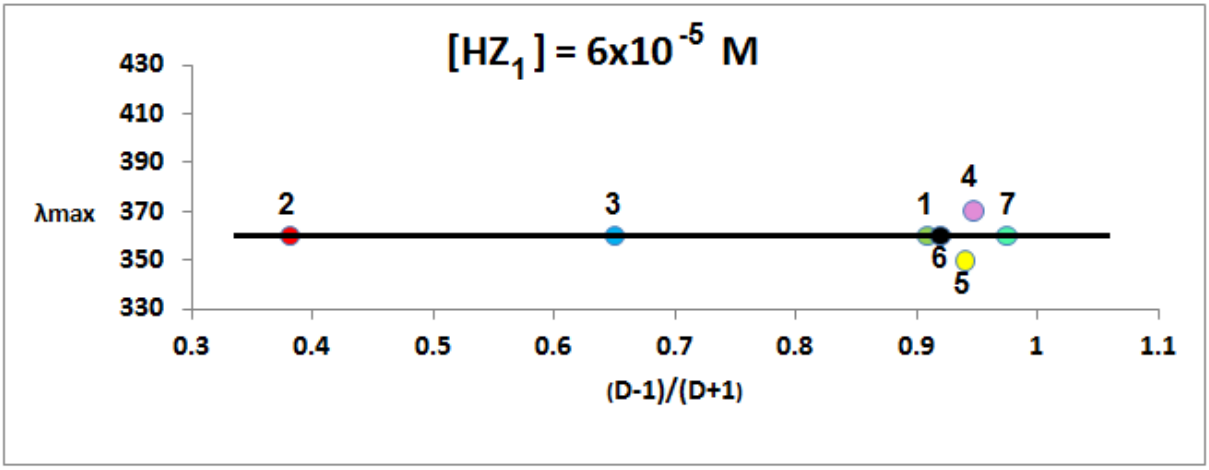


Fig.(10): between dielectric function (D-1)/(D+1) and λ_{max} of dye HZ1 at different solvents

Some applications of the azo dye HZ1

The effect of time was studied on the dye absorption spectrum (HZ1) with time periods .ranging from (1-1440) minutes. The results showed that the dye is stable up to 24 hours

This means that the dye is highly stable and it is better to start the measurements after 20-25 minutes to ensure that the complexes are fully formed. Table (3-7) shows the absorption values at the greatest wavelength of the dye (HZ1) and at increasing time periods:

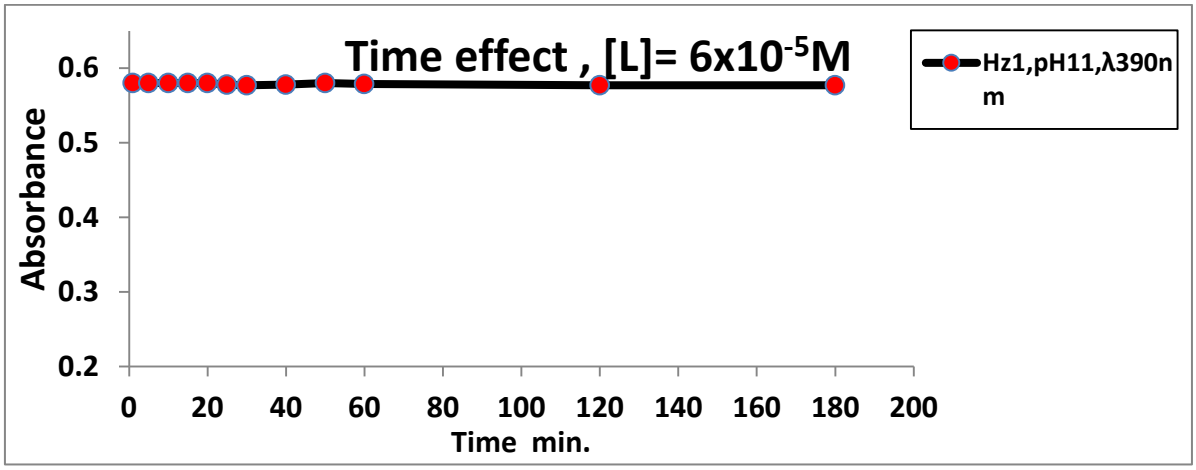


Fig.(11): Time effect for forming stable azo dye HZ1

Some applications of the azo dye HZ1

Biological study

Two types of bacteria were used for biological study due to their medical importance and :because they cause many diseases, the most important of which are

1-Escherichia coli

2- Staphylococcus Aureus

For the purpose of knowing the biological activity of the azo-dye (HZ1) against these two types of bacteria, solutions of these two dyes were prepared at a concentration of (5 mg/ml) in a solvent (DMSO), and the bacterial dishes were incubated after making holes in them with a corkscrew and placing (0.1 ml) of these solutions in each Puncture for a period of (24) hours at a temperature (1 ± 36 °C), after which it was extracted from the incubator. By measuring the diameter of the inhibition zone (15 mm), while the dye showed ineffectiveness (Hz1) and did not give any effect on Staphylococcus Aureuse .(bacteria

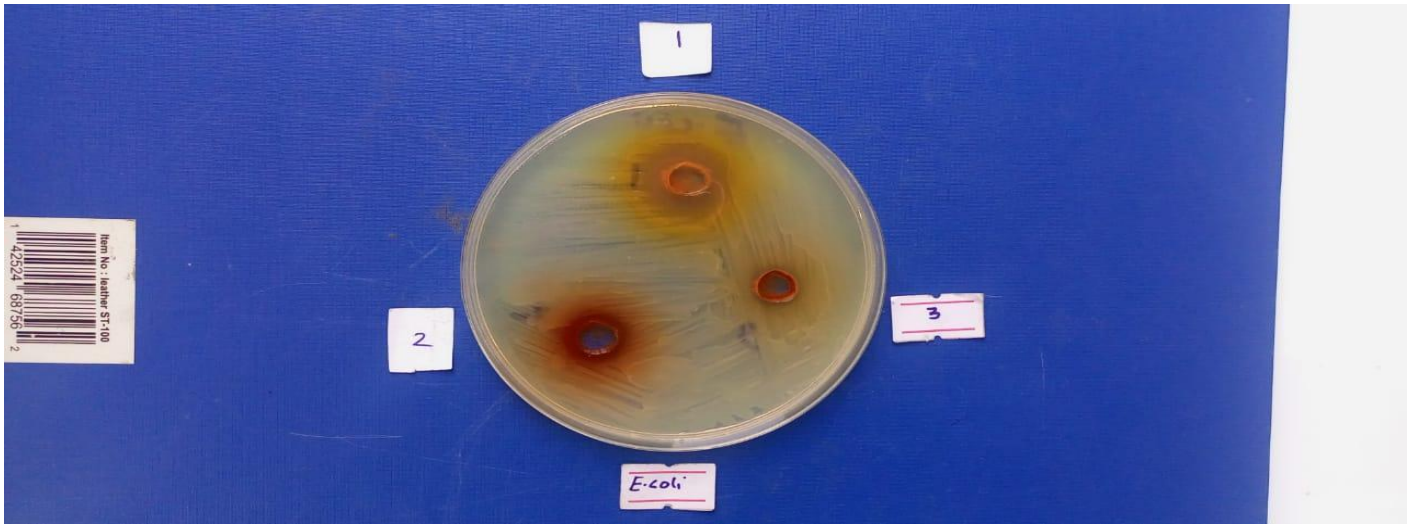


Figure (12) Effect of the two azo dyes (HZ1 and HZ2) on bacteria (Escherichia coli)

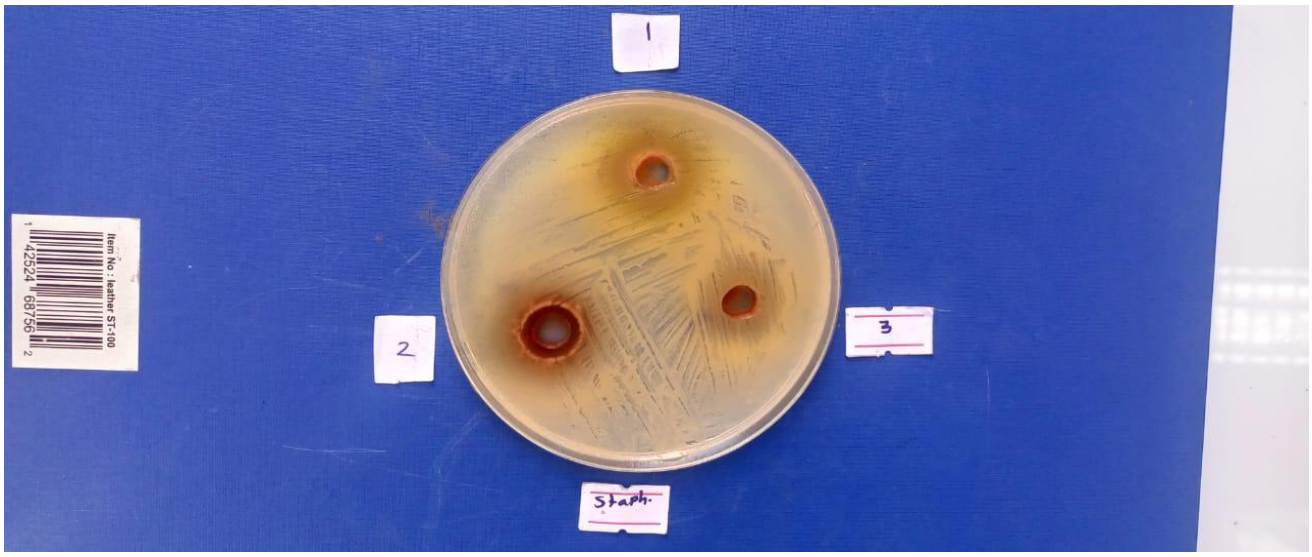


Figure (13) Effect of two azo dyes (HZ1 and HZ2) on Staphylococcus Aureuse bacteria

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