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Preparation and Spectroanalytical Studies of Two New Azo Compounds Derived from the Drug Methyl-4-Amino Benzoate

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Abstract . Two new azo compounds were prepared from the drug methyl-4-amino benzoate with pyridoxine hydrochloride (B6) (A_1) and with ventolin (salbutamol sulphate) (A_2). The characterization of azo compounds have been described by I.R., mass spectra and visible spectroscopic techniques. At various pH values (2-12) , the electronic spectra of these azo compounds were investigated in terms of acid-base properties ,which includes establishing isobestic points and determination of protonation and ionization constants. The effect of solvents of different polarities on the electronic spectra was the subject of the other research. The azo compound (A_2) has been used in a variety of applications for nitrite determination.

INTRODUCTION

Azo compounds are commonly used as dyes because of their numerous uses in fields such as textile fabrics, material coloring, biomedical research, lasers and organic synthesis [1-3]. A variety of biological applications for azo compounds have been published, including antineoplastics, antidiabetics, antiseptics, anti-inflammatory, and other useful chemotherapeutic agents [4-7]. Since most azo compounds have acid-base properties and have fixed isobestic points (which reflect the number of equilibriums in such an azo compound), they are used as acid-base indicators [8] and some can also be used as metallochromic indicators. Some of them have applications as antipyretic reagents, and corrosion inhibitors. They were found to be effective reagents for metal ion extraction and spectrophotometric determination [9]. The effect of solvents of different polarities on the electronic spectra were also investigated [10]. spectrophotometric method has been investigated for determination of microgram amount of pyridoxine hydrochloride .The method is based on the oxidation of 2,4-dinitrophenylhydrazine to produce diazotized2,4-dinitroaniline which will be coupled with pyridoxine hydrochloride (vitamin B6) in basic medium to form a stable colored product with maximum absorption at 522 nm. [11] A spectrophotometric method has been described for the determination of pyridoxine hydrochloride (vitamin B6) in pure and pharmaceutical preparations. The method is based on coupling reaction of vitamin B6 with diazotized p-nitroaniline in alkaline solution with maximum absorption at 480 nm.[12]. The ventoline (salbutamol sulphate) was also used as reagent for forming azo compounds like reaction with 2-chloro-4-nitroaniline to form colored dye at maxima 463 nm. [13] . The ventoline forming colored azo compounds with several compounds , like with 2,4-dinitroaniline reagent in an alkaline medium at 558 nm. [14] and with ρ-bromoaniline reagent in alkaline medium of Triton X-100 at 441 nm. [15] . There are several methods for determination of nitrite by using of coupling method and forming colored azo compound, like ventolin and p-bromoaniline at 442 nm.[16] and 2-Amino-6-ethoxybenzothiazole and 4-Chloro-3,5-dimethylphenol at 510nm.[17]. This work involves the synthesis and characterization of two new azo compounds, A_1 (methyl (E)-4-((5-hydroxy-3-(hydroxymethyl)-4-methoxy-6-methylpyridin-2-yl)dia- zenyl)benzoate) and A_2 (methyl (E)-4-((5-(2-(tert-butylamino)-1-hydroxyethyl)-2-hydroxy-3-(hydroxymethyl)phenyl)diazenyl)benzoate). Acid-base properties of A_1 and A_2 at different pH values (2-12) were studies ,.The azo compound A_2 was used to determine nitrite spectrophotometrically.

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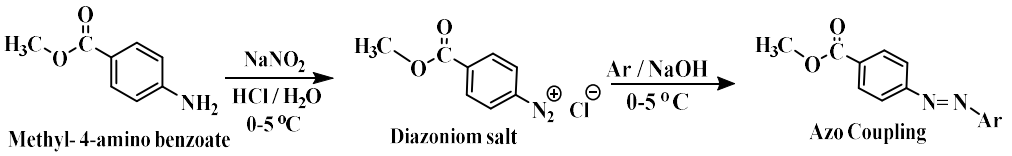
EXPERIMENTAL

Materials and Method

Aldrich and Merck obtained all the reagents and solvents . Infrared spectra were recorded as KBr discs using a SHIMADZU FT-IR-8400S. Melting points of compounds were established by a thermo. Scientific (9100). Visible spectra measured by JENWAY 6305 Spectrophotometer. Mass spectra were recorded by EI Technique using Agilent Technologies . The pH measurements were made with pH-Meter (H. Jurgons Co. Bremen,L.Puls Munchen15).

Preparation of Compound Azo Compounds

The two azo compounds were prepared by recommended procedure for preparing the azo compounds [10] by taking 0.006 mole of each methyl-4-amino benzoate and pyridoxine hydrochloride or ventolin in 1.8 % w/v. NaOH . By the aid of IR and mass spectra, the suggested molecular structures of azo compounds were shown in Scheme 1.



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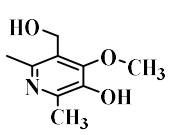
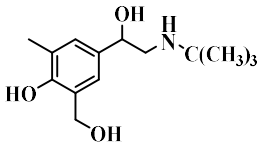


A_1

,



A_2

SCHEME 1. Preparation of azo compounds A_1 and A_2.

A_1 = methyl (E)-4-((5-hydroxy-3-(hydroxymethyl)-4-methoxy-6-methylpyridin-2- yl) diazenyl) benzoate
A_2= methyl (E)-4-((5-(2-(tert-butylamino)-1-hydroxyethyl)-2-hydroxy-3-(hydroxymethyl) phenyl) diazenyl) benzoate.

Solutions

* 1 x 10^{-3}M of each of azo compounds A_1 and A_2
* Universal and acetate buffer solutions (pH 2-12) [18,19]
* Buffer solutions of pH 12 (universal, hexamine, NaOH + Sodium Tetra Borate, KCl + NaOH, Na_2HPO_4. $2H_2O$ + NaOH) [19].

Procedure

Acid– base Properties at Different pH Values

A series of buffer solutions were prepared with different pH values (2-12) for total azo compound concentration $0.8x10^{-4}$ M (for A_1 and A_2) via using universal buffer. The absorbance of these solutions (by using the pH value as blank solution) were recorded at range of (320-530 nm.).

Solvent Effect of Different Polarities

A series of solution of azo compound at total concentration $0.8x10^{-4}$ M (for A_1 and A_2) were completed to the mark with, Ethanol, Methanol, Water, Acetone, DMSO, THF, dichloromethane, 1,4-Dioxane and n-Hexane, the absorbance of these solutions were recorded at range of (330–530 nm.) using the solvent as blank solution.

RESULTS AND DISCUSSION

The azo compounds are stable in air and soluble in DMF, DMSO, methanol, ethanol, and acetone at room temperature. Table 1 summarizes the physical properties of azo compounds (A_1 and A_2), with good results was obtained.

TABLE 1. Physical properties for novel azo compounds.

Comp.	Molecular formula	Molecular weight	m.p ºC	Yield (%)	Color
A_1	$C_{16}H_{17}N_3O_5$	331.33	142-144	86	**orange**
A_2	$C_{21}H_{27}N_3O_5$	401.46	224-227	81	**yellow**

IR Spectra

The most important IR absorption bands corresponding to the azo compounds (Fig. 1&2) are presented in table 2. The observed band in the range (1450.47,1429.25) cm^{-1} are due to ν (N=N) group of the azo compounds (A_1 and A_2 respectively). The sharp band in the range (1604.77,1595.13) cm^{-1} are due to ν (C=C) respectively [20].

TABLE 2. Selected infrared data of A_1 and A_2.

Comp.	ν(O-H) cm^{-1}	ν(C=O) cm^{-1}	ν(C=C) cm^{-1}	ν(N=N) cm^{-1}	ν(C-N) cm^{-1}	ν(C-O) cm^{-1}
A_1	3333.34br	1695.43br	1604.77m	1450.47br	1311.59w	1242.16br
A_2	3333.33 br	1685.79s	1595.13s	1429.25m	1284.59m	1251.80w

s = Strong , m = Medium , w = Weak , br = Broad

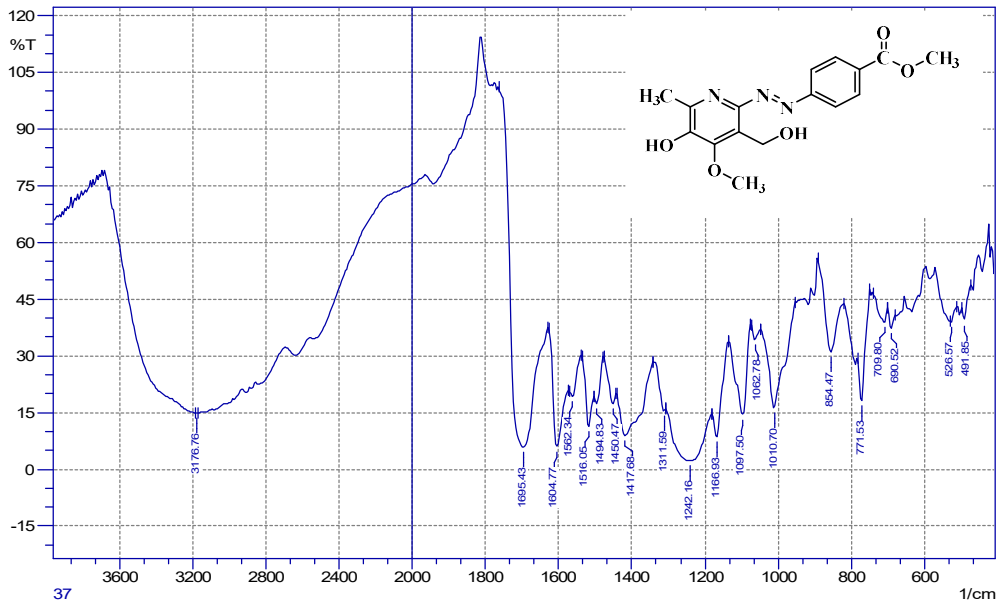


FIGURE 1. IR spectrum of A_1.

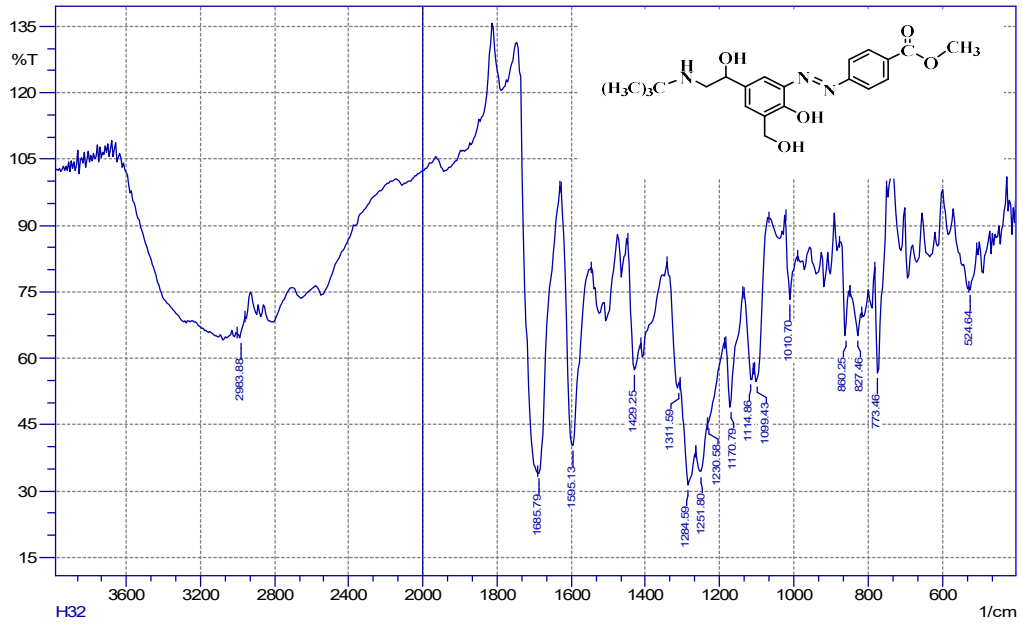


FIGURE 2. IR spectrum of A_2.

Mass Spectrum

The structural characterization of organic molecules relies heavily on mass spectrometry.[21] .The EI-MS of the azo compounds at 70 eV. The mass spectra of azo compounds at m/z = 331.1 for A_1 and m/z = 401.1 for A_2, which correspond to $[C_{16}H_{17}N_3O_5]^{+\cdot}$ and $[C_{21}H_{27}N_3O_5]^{+\cdot}$ species, respectively, revealed molecular ion peaks. Base peaks could be seen at m/z =137.1 and 41.1, as seen in (Fig. 3&4).

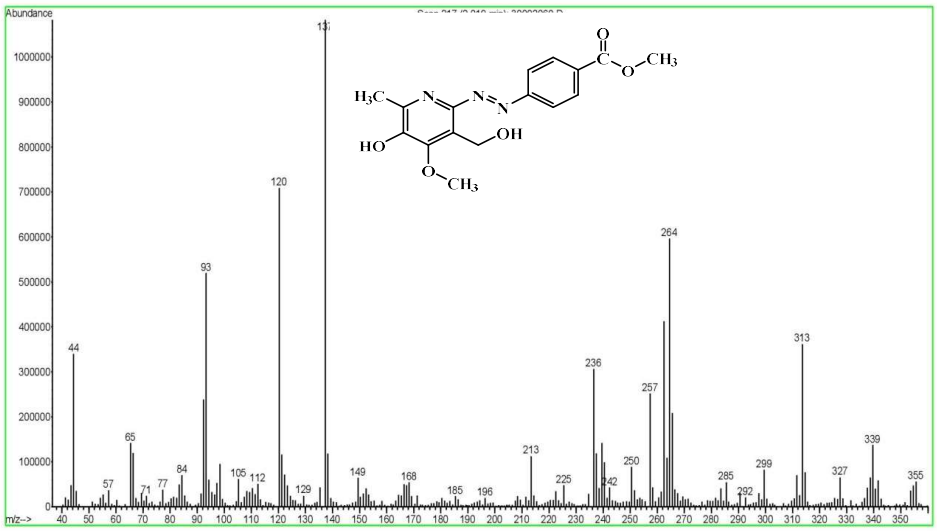


FIGURE 3. Mass spectra of A_1.

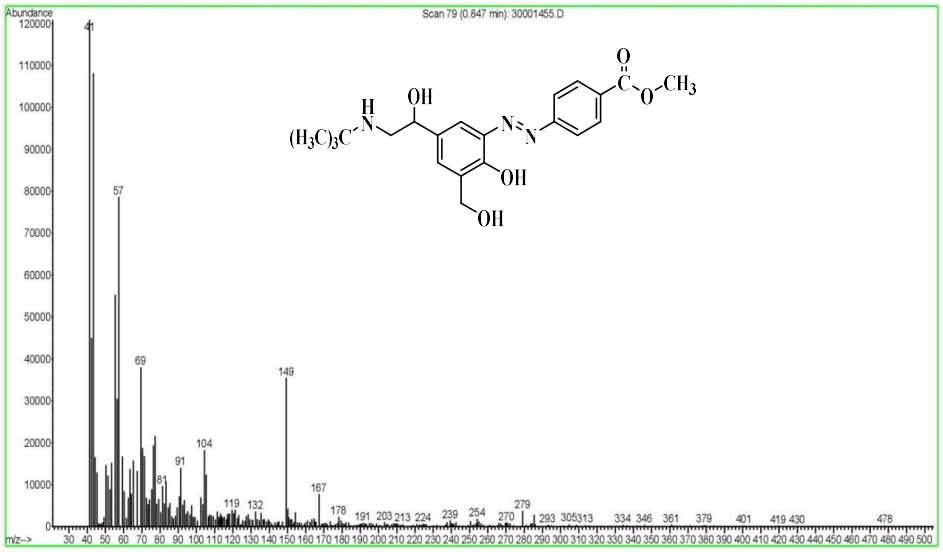


FIGURE 4. Mass spectra of A_2.

Acid-Base Properties

A series of universal buffer solutions were prepared at different pH values (2-12) for each azo compound to study the effects of acidity and basicity of universal buffer solutions on the azo compounds and to measure the ionization and protonation constants. At (320-530 nm.), the absorption spectra of a $0.8x10^{-4}$ M solution of A_1 azo compound at various pH values (2-12) is graphically depicted (Fig. 5&6). The spectra characterized by two maximal bands the first at (360-380 nm.) in pH range (2-8) which represents protanated form (cation form). The second high intense band at wavelength range (420-450 nm.) at pH value range (9-12), which represent basic form (anionic form). From the figure it was found there is two isobestic point at (400,440 nm.) and (380,410 nm.) In case of azo compounds (A_1 and A_2) respectively.

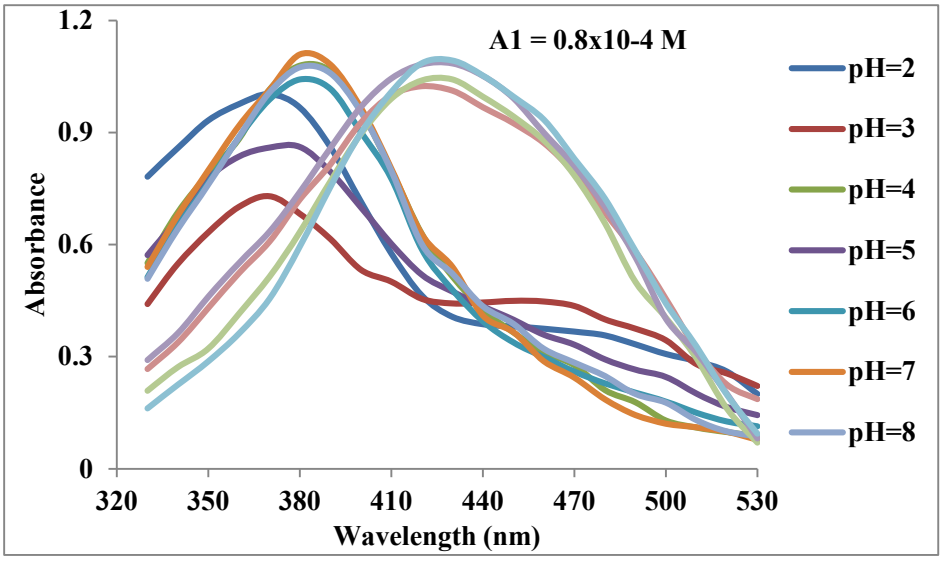


FIGURE 5. Absorption spectra of azo compound A_1 at different pH values.

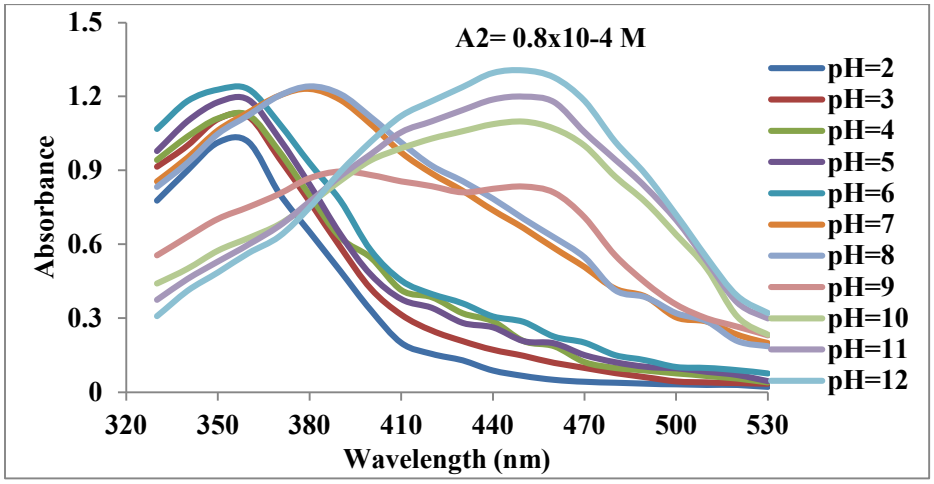


FIGURE 6. Absorption spectra of azo compound A_2 at different pH values.

The absorbance – pH curves of the azo compounds A_1 and A_2 were plotted to determine the ionization and protonation constants of the azo compounds A_1 and A_2 at certain wavelengths (430 nm.) and (440 nm.), respectively (Figs. 5&6). (Fig. 7). The ionization and protonation constants were calculated (Table 3). From Absorbance-pH curve and by the aid of half-height method [10], 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.

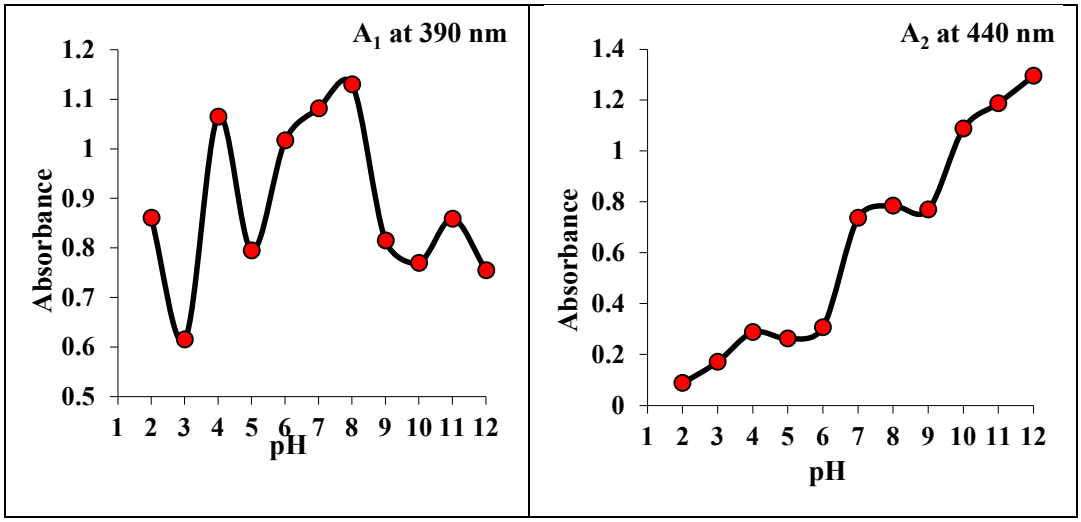


FIGURE 7. The Absorbance – pH curves of Azo compounds A_1 and A_2.

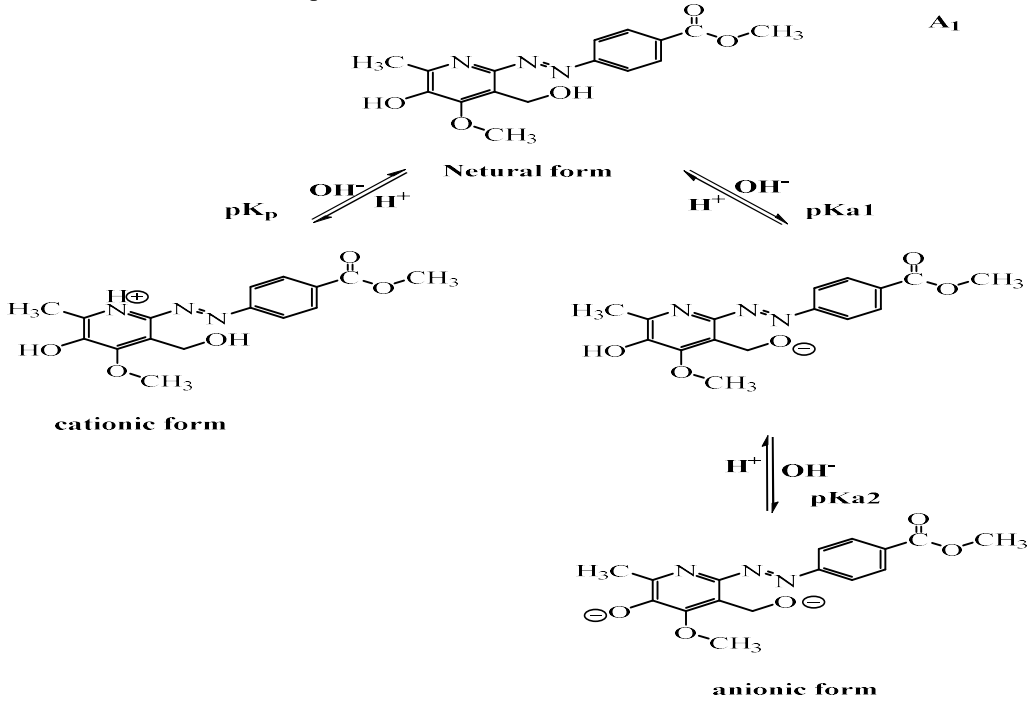
The protonation (pKp) and ionization (pKa) constants were measured using the Absorbance – pH curve (Table 3).

TABLE 3. The protonation and ionization constants of azo compounds.

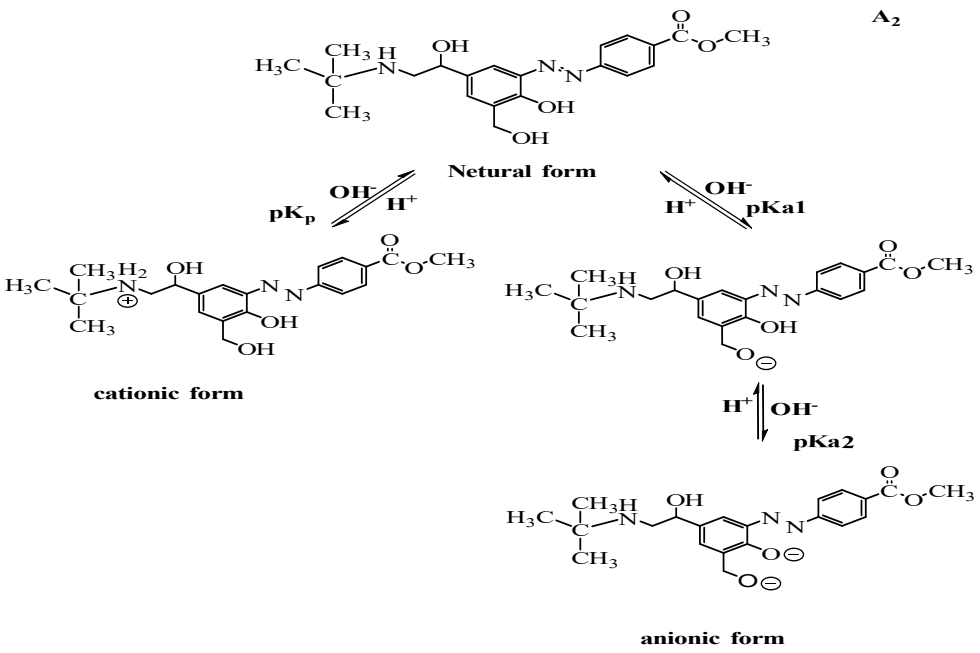
Azo comp. A_1 at λ = 390 nm				Azo comp. A_2 at λ = 440 nm			
A_m	A_L	$A_{1/2}$	pK	A_m	A_L	$A_{1/2}$	pK
0.615	1.065	0.443	3.5 (p)	0.088	0.288	0.188	3 (p)
0.795	1.130	0.505	6.51 (a1)	0.263	0.785	0.448	6.51 (a1)
0.770	0.859	1.042	10.5 (a2)	1.088	-------	-------	> 11 (a2)

p = protonation constant of H for nitrogen atom of (pyridoxine & ventolin) molecule, a1 = The first ionization constant of H for hydroxyl group in alcohol, a2 = The second ionization constant of H for hydroxyl group in molecule phenol molecule.

The suggested chemical mechanism of protonation and ionization was shown in Schemes 2&3 .



SCHEME 2. Suggested mechanism of protonation and ionization of azo compound A_1.



SCHEME 3. Suggested mechanism of protonation and ionization of azo compound A_2.

Kind of Buffer Solution of pH 12

To fine the effect of kind of pH12, several solution of buffer that gives pH 12 like (1= Universal, 2= Hexamine, 3= NaOH + Sodium Tetra Borate, 4= KCl + NaOH, 5= Na_2HPO_4. $2H_2O$ + NaOH). The best kind which gives highest absorbance were Hexamine Buffer solution with respect of azo compound A_1 and Universal Buffer with respect to azo compound A_2 (Fig. 8).

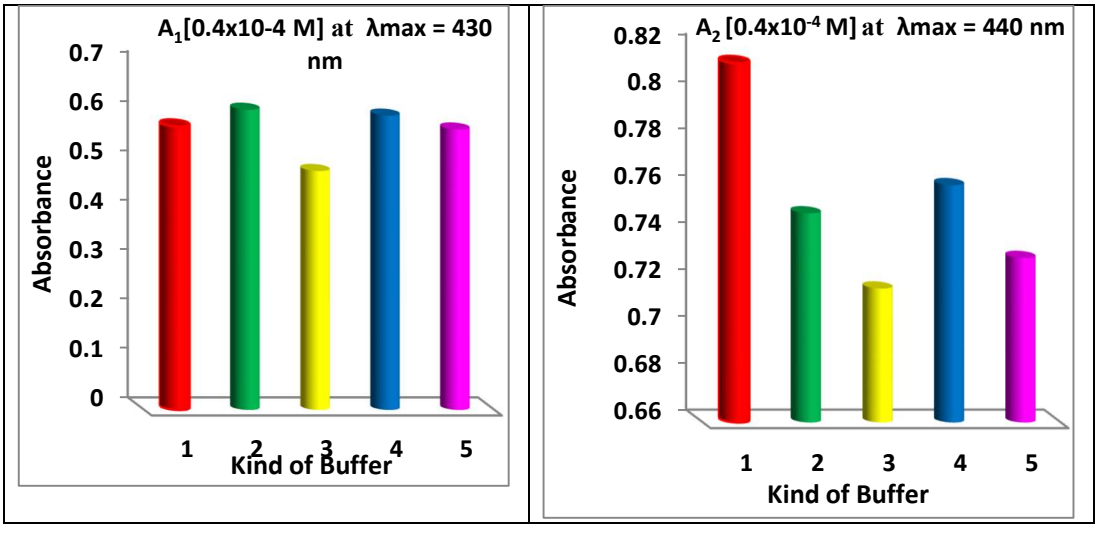


FIGURE 8. Effect kind of buffer solution of Azo compounds A_1 and A_2.

Effect of Solvents of Different Polarities

(Fig. 9&10) displays the spectra of azo compounds A_1 and A_2 in various solvents of different polarities (Ethanol, Methanol, Water, Acetone, DMSO, THF, dichloromethane, 1,4-Dioxane and n-Hexane) with a maximum wavelength of (340-530 nm.). From (Fig. 9), the compound A_1 having only tow band in all solvents. There is a slight blue shift at (460-470 nm.) with solvents (Ethanol, Methanol, Water and Acetone) compared with non polar solvent (DMSO) at (480 nm.) In case of azo compound A_2 (Fig.10) it was found observed blue shift at (350 nm.) with other solvents compared with non polar solvent (DMSO) at (360 nm.). The compound A_2 having only one band in all solvents. The absorption spectra of different solvents are affected by the solvent's salvation and/or dielectric effects. Table 4 displays the results.

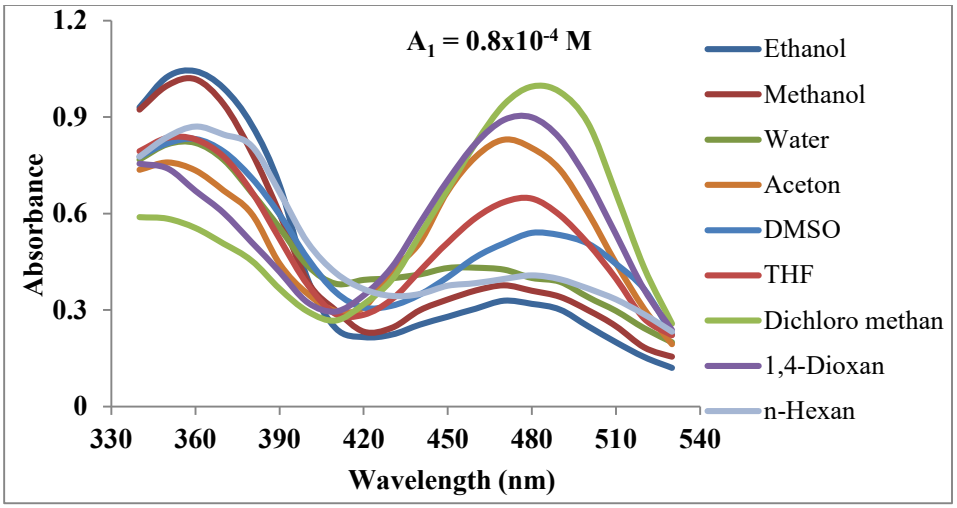


FIGURE 9. Electronic spectra of azo compound A_1 at different solvents.

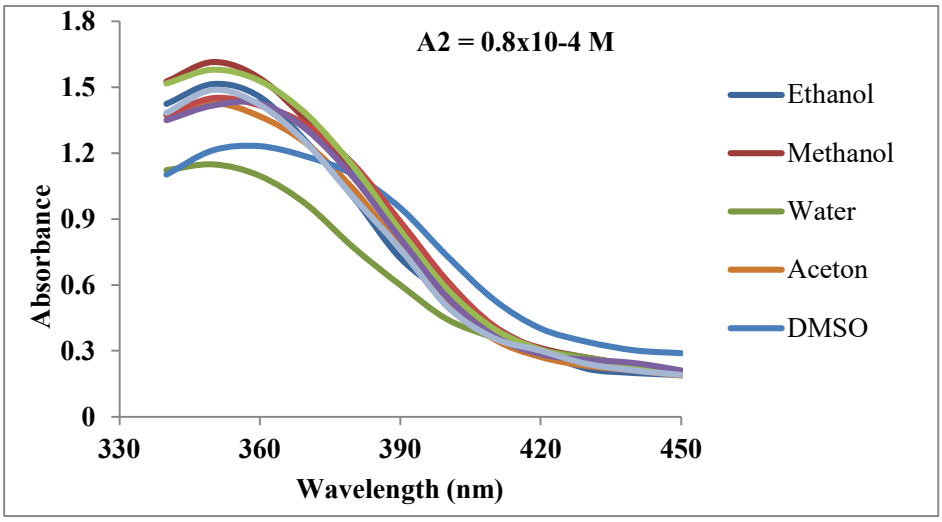


FIGURE 10. Electronic spectra of azo compound A_2 at different solvents

Table 4 and the plot of the maximum vs. dielectric function of solvents from Gati and Szalay equation indicate a linearity relationship, indicating that the dielectric constant is the most important factor affecting salvation.

TABLE 4. Solvent effect on spectra of A_1 and A_2.

Symbol No.	Solvent	D [19]	(D-1)/(D+1)	λ_{max} nm	
				A_1	**A_2**
1	Ethanol	24.30	0.921	360s,470w	350s
2	Methanol	33.60	0.956	360s,470w	350s
3	Water	78.30	0.980	360s,460w	350s
4	Acetone	20.60	0.929	360m,470s	350s
5	DMSO	46.67	0.968	360s,480m	360s
6	THF	7.58	0.686	360s,480m	350s
7	Dichloromethane	9.10	0.844	360w,480s	350s
8	1,4-Dioxane	2.30	0.464	360m,480s	360s
9	n-hexane	1.89	0.372	360s,480w	350s

s = Strong , m = Medium , w = Weak

The plot of (D-1)/(D+1) against the λmax of azo compound A_1 and A_2 gives more or less high linear relation with solvents of moderate polarities (Fig. 11).

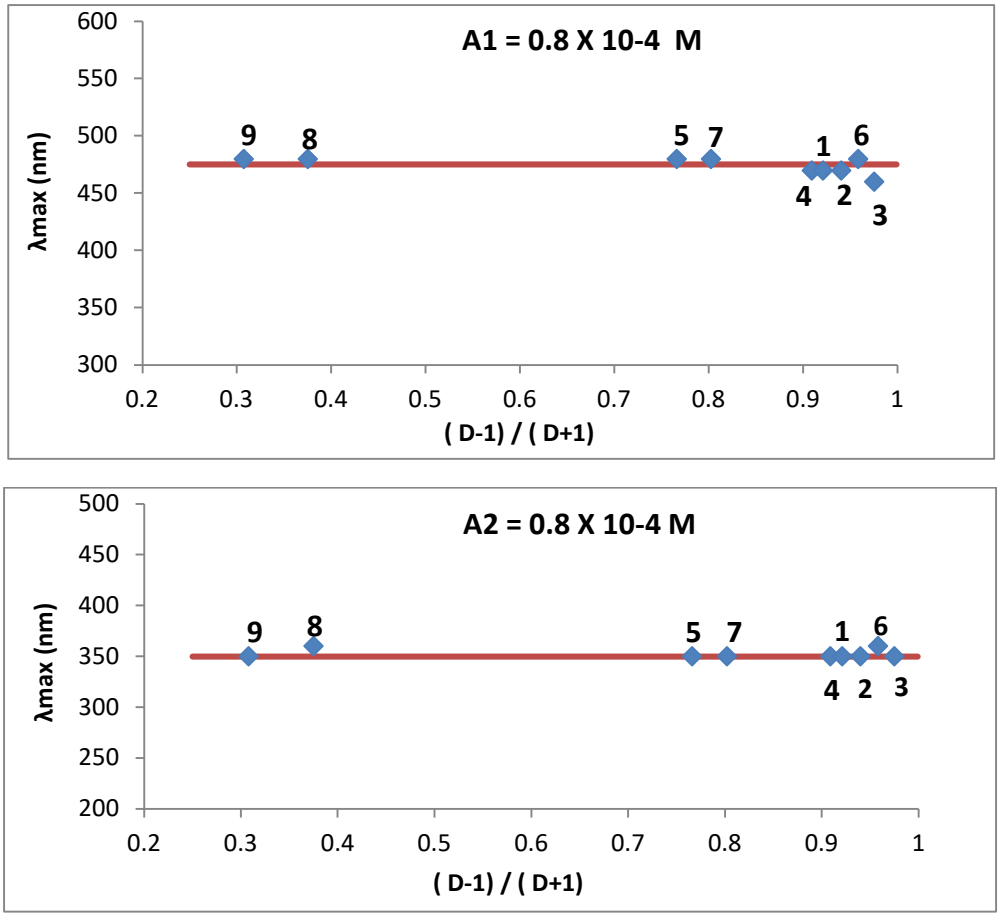


FIGURE 11. The relation between λmax and (D-1)/(D+1) for azo compounds A_1 and A_2.

Determination of Nitrite

A curve of absorbance and wavelength was plotted to determine the maximum wavelength of azo compound (A_2) that produced (Fig. 12).

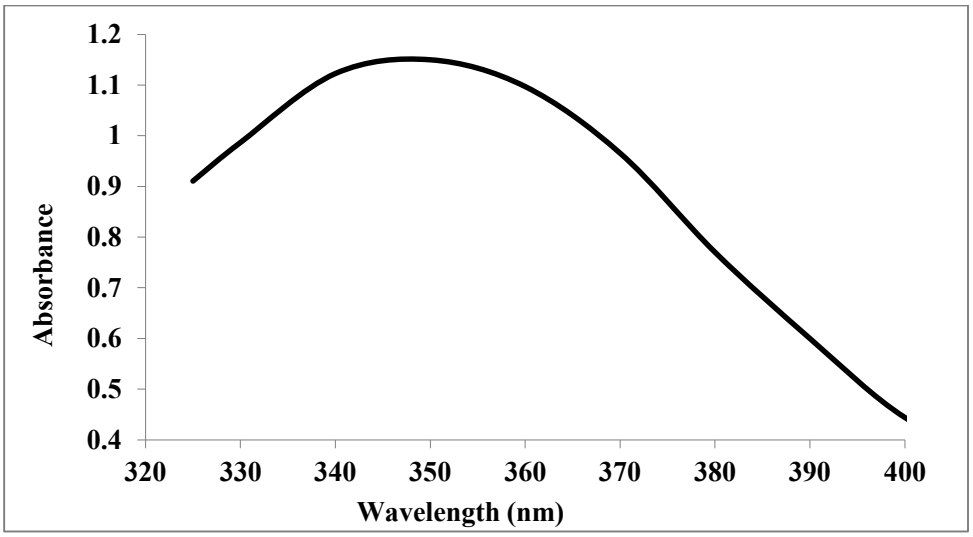


FIGURE 12. Absorption curve of azo compound $A_2 = 0.8x10^{-6}$ M and $[NO_2] = 3.94 \times 10^{-4}$ M.

The sensitivity of the system and the limits of Beer's law were determined by using a calibration curve (Beer's law) to measure precession (Fig.13).

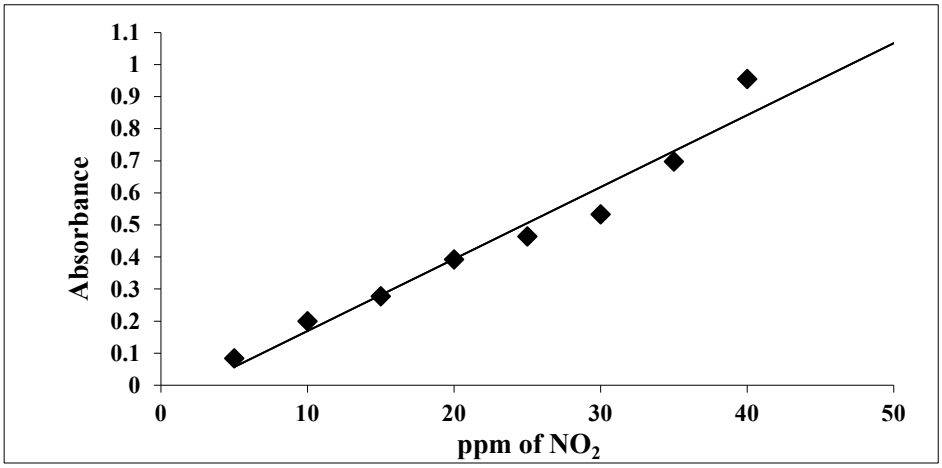


FIGURE 13. Calibration curve for determination nitrite.

TABLE 5. Beer's law of the azo compound A_2 .

ε x10^4 l.mol^{-1}.cm^{-1}	a	S µg.cm^{-2}	R^2	S.D.	D.L x10^{-5}	Beer's law up to ppm	λmax nm
0.817	0.018	0.0556	0.969	0.0067	0.754	35	350

Where ε , a , S , R^2 ,S.D. and DL are molar absorptivity coefficient , specific absorptivity , Sandel sensitivity , standard deviation and detected limit respectively .

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