Ministry of Higher Education
and Scientific Research

Journal of Kufa for Chemical Science

A refereed

Research Journal Chemical Science

Vol.2 No.7 Year 2021
ISSN 2077-2351

Address:
Iraq-Najaf-University of Kufa
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Using Ethyl-4-Amino Benzoate to prepare and analytical studies of Two New Azo Compounds

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الملخص

استخدم الأمين الدوائي أثيل-4-أمينو بنزويت (البنزوكايين) لتحضير مركبي آزو جديدين مع البيردوكسين هيدروكلوريد (A_6) (B6) و الفنتولين (كبريتات السالبوتامول) (A_7). تم تشخيص مركبي الآزو بواسطة الأشعة تحت الحمراء وطيف الكتلة وتقنيات التحليل الطيفي المرئي. تم دراسة الأطياف الإلكترونية لمركبي الآزو من حيث الخصائص الحامضية- القاعدية، والنقاط الايزوبستية، وتحديد ثوابت البرتنة والتأين بقيم الأس الهيدروجيني المختلفة (2-12). وتضمن البحث دراسة تأثير المذيبات ذات الأقطاب المختلفة على الأطياف الإلكترونية. أيضا استخدم مركب الآزو (A_7) لتقدير النتريت.

Abstract

The amin drug ethyl-4-amino benzoate (benzocaine) was used for preparation two new azo compounds, with pyridoxine hydrochloride (B6) (A_6) and with ventolin (salbutamol sulphate) (A_7). The two azo compounds were characterized by I.R., mass spectra and visible spectroscopic techniques. the electronic spectra of these azo compounds were investigated in terms of acid-base properties ,which includes establishing isobestic points, determination of protonation and ionization constants at various pH values (2-12). The effect of solvents of different polarities on the electronic spectra was the subject of the other research. The azo compound (A_7) has been also to determination of nitrite **.**

Keywords: Azo Compound, Isobestic Points, Acid-Base Properties , Solvent Effects.

INTRODUCTION

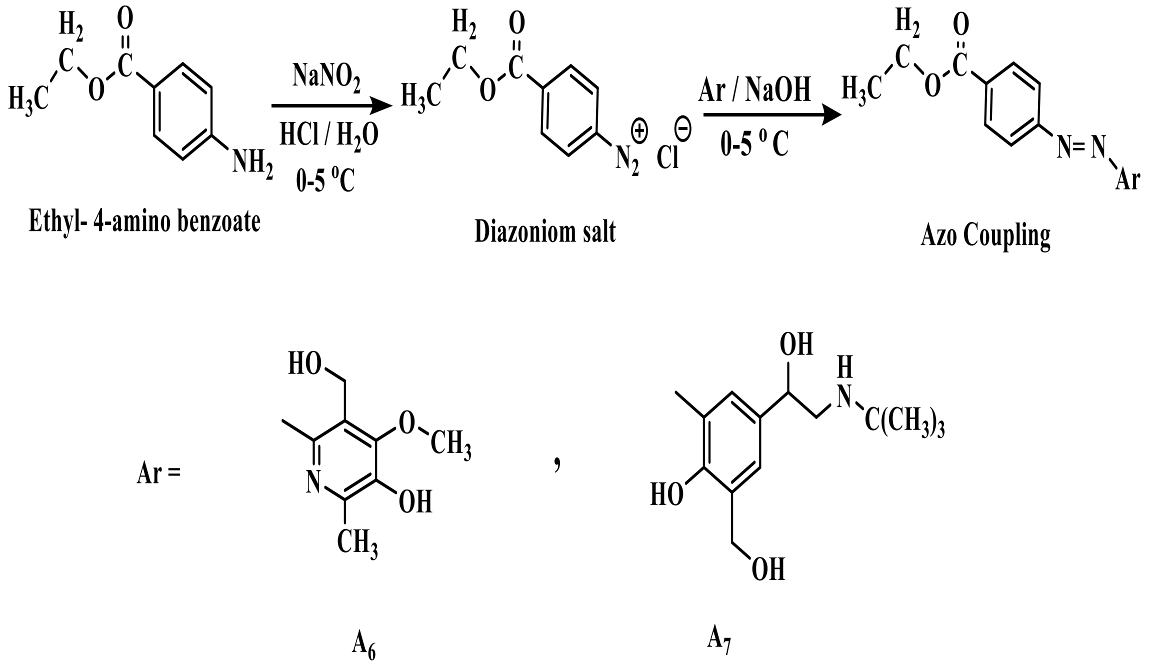
Azo colorants are the most important class of synthetic dyes representing (60-80%) of all organic colorants used widely in substrates such as textile fibers, leather, plastic, papers, hair, mineral oils, waxes, food stuffs and cosmetics [1]. The azo compounds of heterocyclic compounds are regarded highly active towards most of the metal ions. They have great importance in chemical analysis because these compounds contain more than one active group, which is able to formulate chelating coordination complexes with metal ions distinguished by their color [2-4]. Azo compounds are one important organic compounds in many fields such as pharmaceuticals and cosmetics [5,6]. Some of these azo compounds have been used as drugs because they have in inhibitory of bacteria [7], germicidal colon and bacteria causing chronic disease [8,9]. A variety of biological applications for azo compounds have been, including antineoplastics, antidiabetics, antiseptics, anti-inflammatory, and other useful chemotherapeutic agents [10,11]. Since most azo compounds have acid-base properties and have fixed isobestic points, they are used as acid-base indicators [12] The effect of solvents of different polarities on the electronic spectra were also investigated [13]. In previous work, the Ethyl-4-Amino Benzoate used as reagent for forming azo compounds with pyridoxine hydrochloride and ventolin [14]. Spectrophotometric methods have been investigated for determination pyridoxine hydrochloride (vitamin B6) with 2,4-dinitrophenylhydrazine to produce diazotized 2,4-dinitroaniline at 522 nm. [15] and with p-nitro aniline at 480 nm. [16]. The ventolin (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. [17] and with 2,4-dinitroaniline at 558 nm. [18] and with ρ-bromoaniline reagent in alkaline medium of Triton X-100 at 441 nm. [19]. 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. [20], 2-Amino-6-ethoxybenzothiazole and 4-Chloro-3,5-dimethylphenol at 510 nm. [12]. The present work involves the synthesis and characterization of two new azo compounds A_6 and A_7. Acid-base properties and solvent effect of A_6 and A_7 were studied. The azo compound A_7 was used to determine nitrite spectrophotometrically.

MATERILS AND METHODS

The solvents used of high purity from Aldrich and Merck companies. 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 azo compounds

The two azo compounds were prepared by recommended procedure [13], by taking 0.006 mole of each ethyl-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.



Scheme -1: Preparation of azo compounds A_6 and A_7.

A_6 = ethyl (E)-4-((5-hydroxy-3-(hydroxymethyl)-4-methoxy-6-methylpyridin-2-yl)diazenyl) benzoate.

A_7 = ethyl (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_6 and A_7
* Universal and solution (pH 2-12) [21].
* Buffer solutions of pH 10 (universal, hexamine, NaOH+ Sodium Tetra Borate, H_3BO_3 + NaOH, NH_4OH + NH_4Cl) [21].
* Buffer solutions of pH 12 (universal, hexamine, NaOH + Sodium Tetra Borate, KCl + NaOH, Na_2HPO_4. $2H_2O$ + NaOH) [21].

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_6 and A_7) via using universal buffer. The absorbance of these solutions (by using the pH value as blank solution) were recorded at range of (330-530 nm.).

Solvent effect of different polarities

A series of solutions of azo compounds A_6 and A_7 of total concentration 0.8x10^{-4} M were done in Ethanol, Methanol, Water, Acetone, DMSO, THF, Dichloromethane, 1,4-Dioxane and n-Hexane. Then the absorbance of these solutions were recorded at range of (340–540 nm.) using the solvent as blank solution.

Determination of nitrite

A series of solutions of azo compound A_7 of total concentration 0.8x10^{-6} M and [NO_2] = 3.94x10^{-4} M were prepared and the absorbance of these solutions were recorded at range of (330–400 nm.) for establish the λ_{max}. The beer's law done at λ_{max} (355 nm.).

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_6 and A_7), with good results was obtained.

Table 1: Physical properties for novel azo compounds .

Azo Comp.	Molecular formula	Molecular weight	m.p °C	Yield (%)	Color
A_6	$C_{17}H_{19}N_3O_5$	345.36	162-164	84	orange
A_7	$C_{22}H_{29}N_3O_5$	415.49	143-146	78	yellow

IR spectra

The most important IR absorption bands corresponding to the azo compounds A_6 and A_7 (Fig. 1&2) respectively are presented in table 2. The observed bands in the range (1458.18,1463.97) cm^{-1} are due to ν (N=N) group of the azo compounds (A_6 and A_7 respectively). The sharp band in the range (1645.28,1600.92)cm^{-1} are due to ν (C=C) respectively [22].

Table 2: Selected infrared data of A_6 and A_7 .

Azo 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_6	3500.80 br	1707.00 s	1645.28 w	1458.18 m	1282.66 s	1234.44 m
A_7	3333.33 w	1689.64 s	1600.92 s	1463.97 w	1278.81 s	1253.73 w

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

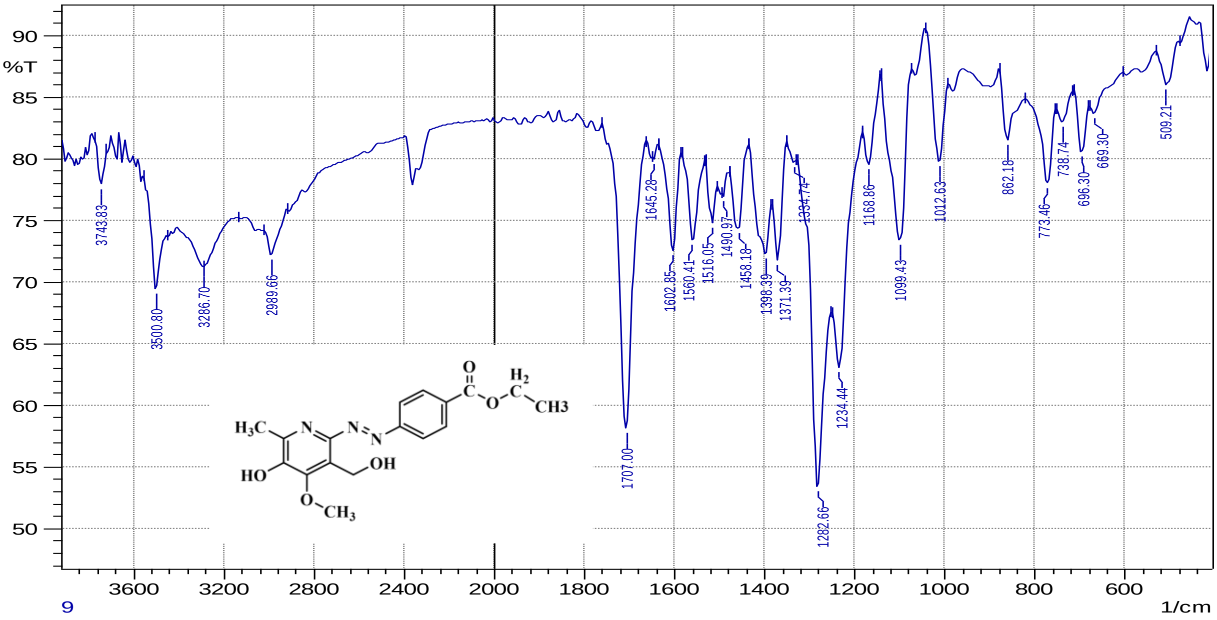


Fig. 1: IR spectrum of A_6.

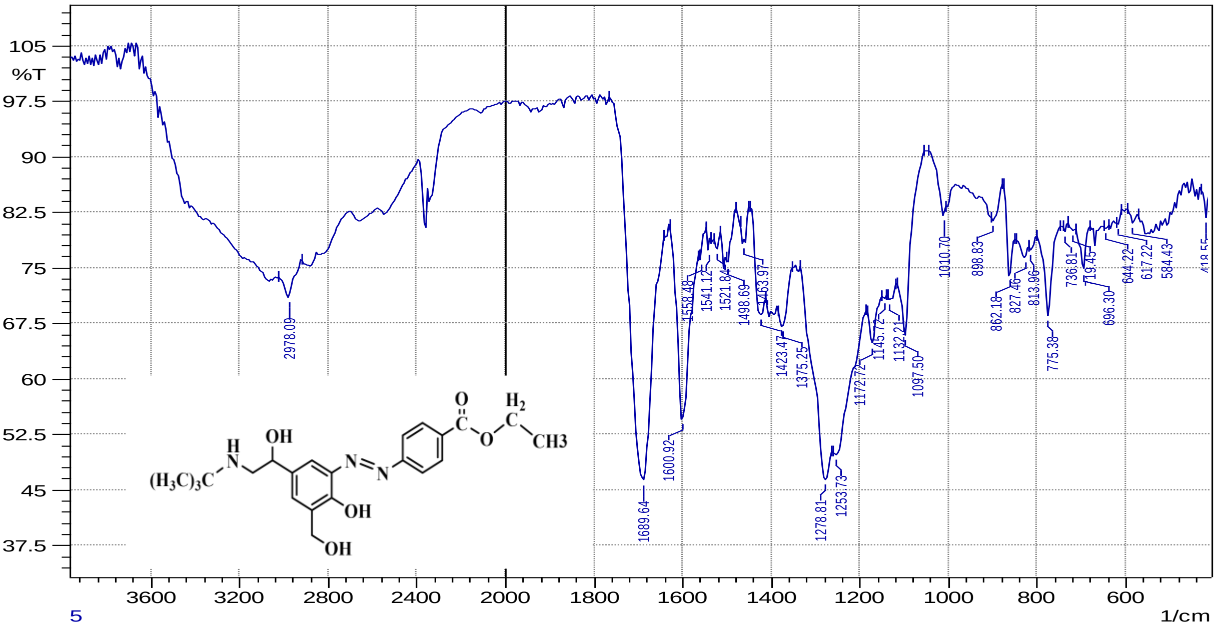


Fig. 2: IR spectrum of A_7.

Mass spectra

The structural characterization of organic molecules relies heavily on mass spectrometry [23]. The EI-MS of the azo compounds at 70 eV. The mass spectra of azo compounds at m/z = 345.1 for A_6 and m/z = 415.1 for A_7, which correspond to $[C_{17}H_{19}N_3O_5]^{\cdot+}$ and $[C_{22}H_{29}N_3O_5]^{\cdot+}$ species, respectively, revealed molecular ion peaks. Base peaks could be seen at m/z =120.1 and 41.1, as seen in (Fig. 3&4).

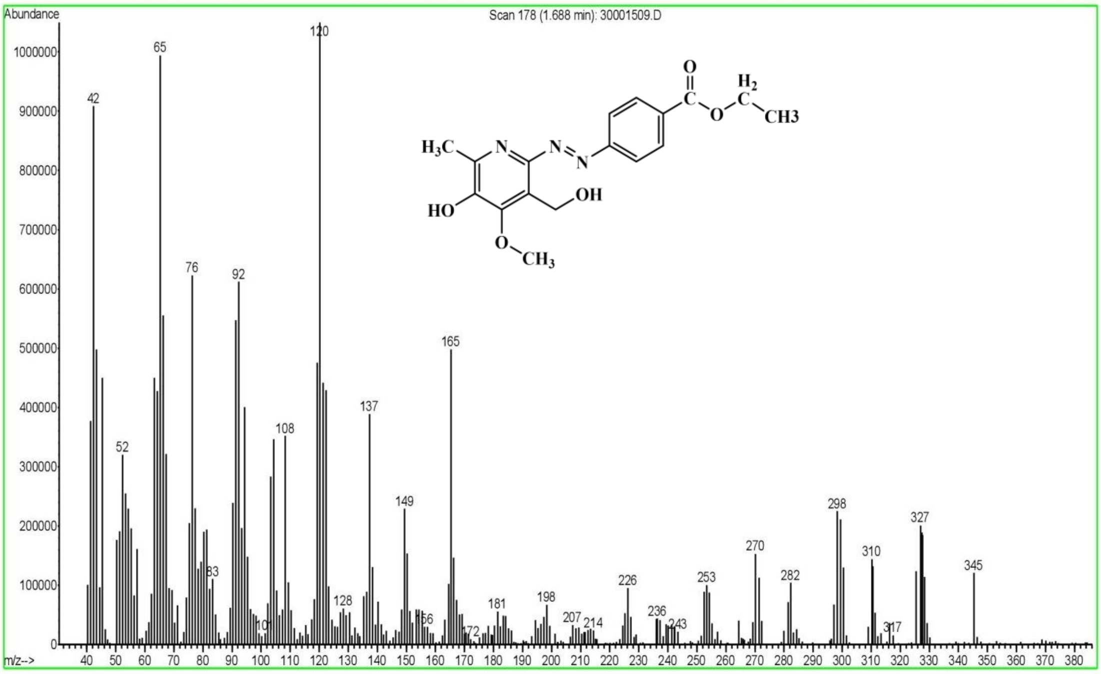


Fig. 3: Mass spectra of A_6.

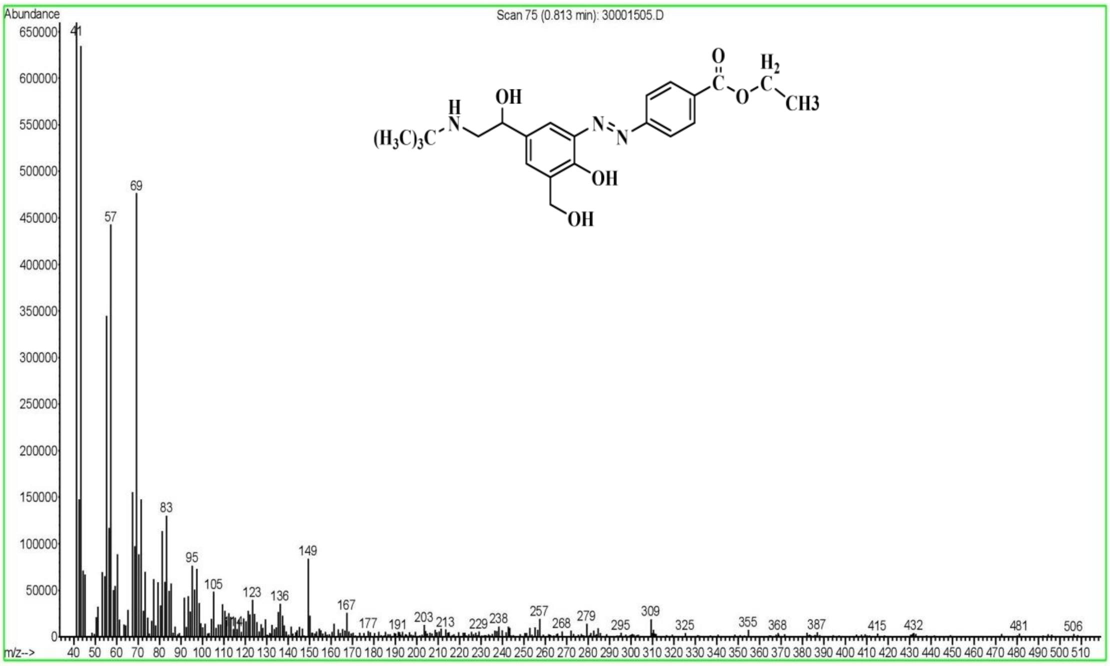


Fig. 4: Mass spectra of A_7.

Acid-Base properties

A series solutions of compound A_6 and A_7 were prepared using universal buffer solutions of different pH values (2-12). To study the effect of pH values on azo compound A_6 (Fig. 5), the spectrum shows intense bands (λ_{max} of 435 nm.) in alkaline medium at pH range (9-12) due to the ionization of azo compound (anionic form) forming solid which is soluble in aqueous medium than acidic form. There are other bands in the pH range (5-8) of λ_{max} 390 nm. while at acidic medium due to the protonation of azo compound (cationic form) at pH rang (2-4), there are two max bands at 365 nm. and 480 nm.. The spectra show three isobestic points (385, 405 and 440 nm.). The highest absorbance band observed at pH 10.

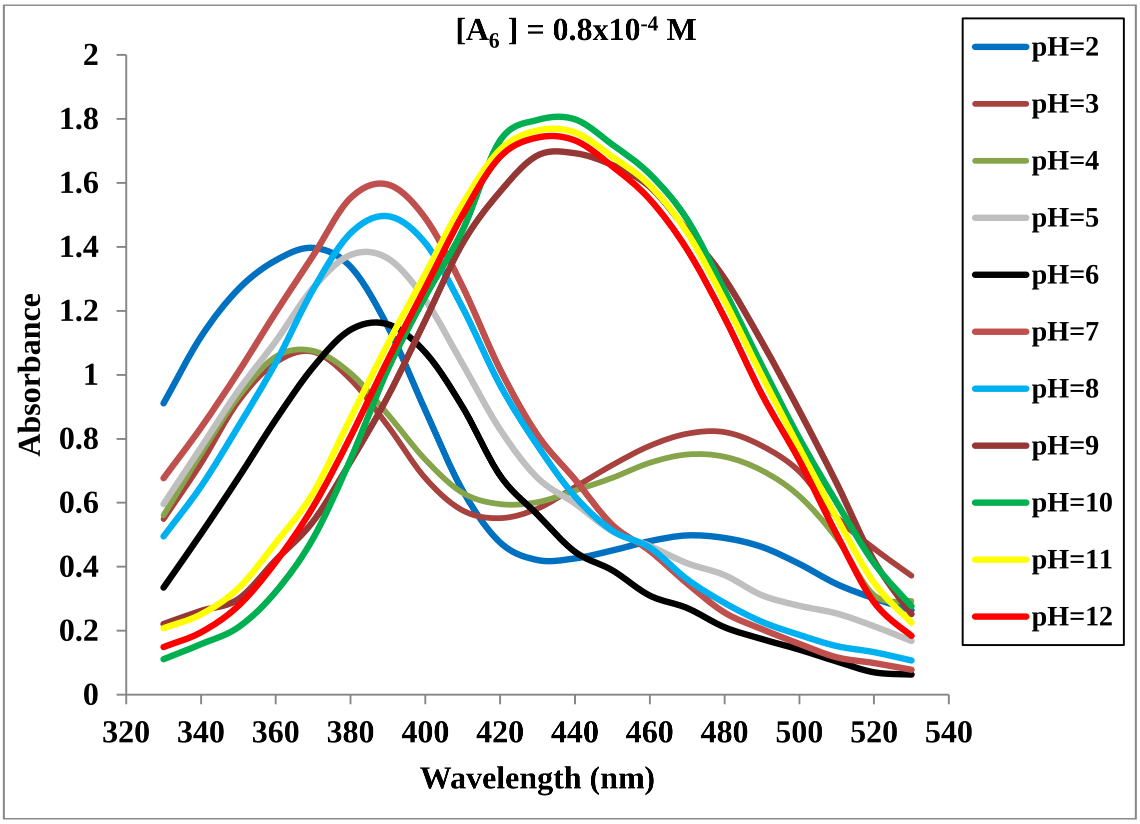


Fig. 5: Absorption spectra of azo compound A_6 at different pH values

In case of azo compound A_7, the Fig. 6 shows bands in alkaline medium at pH range (10 -12) with λ_{max} of 460 nm. and bands in the pH range (8-9) of λ_{max} 390 nm., 370 nm. at pH 7. While at acidic medium of pH rang (2-6) bands at 360 nm. (due to less solubility of hydrogen form). The spectra show two isobestic points 370 nm. and 410 nm.. The highest absorbance band observed at pH 12 .

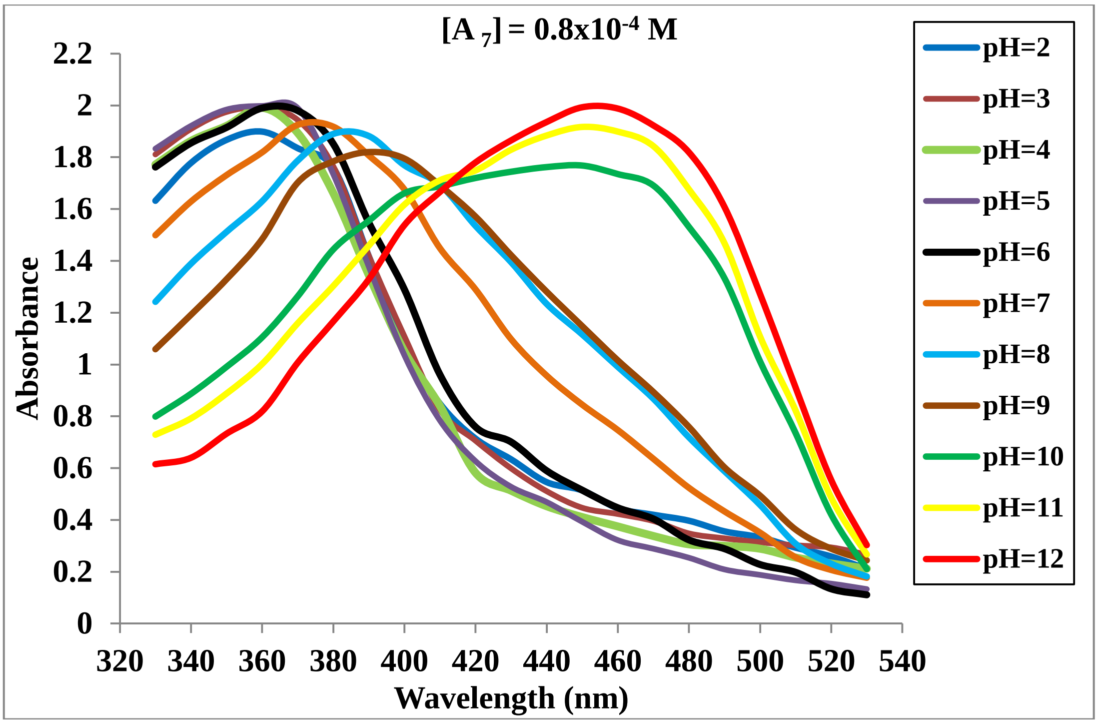


Fig. 6: Absorption spectra of azo compound A_7 at different pH values.

From the absorbance – pH curves of the azo compounds A_6 and A_7 were plotted to determine the ionization and protonation constants of the azo compounds A_6 and A_7 at certain wavelengths 430 nm. and 450 nm., respectively (Fig. 7). The ionization and protonation constants were calculated (Table 3). By the aid of half-height method ,the pK values were obtained by this relation
pK = pH (at $A_{1/2}$) where ; $A_{1/2} = (A_L + A_{min.}) / 2$

A_L and A_{min} are limiting and minimum absorbencies respectively. Then the protonation (pKp) and ionization (pKa) constants were measured using the Absorbance – pH curve (Table 3).

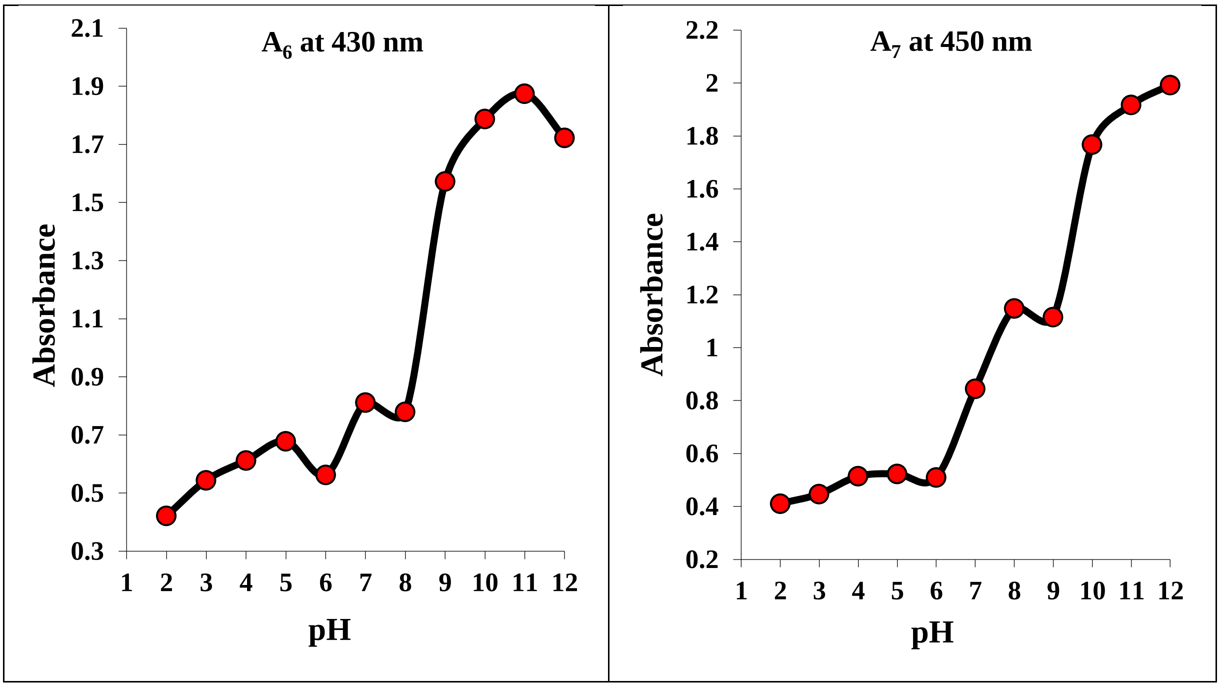


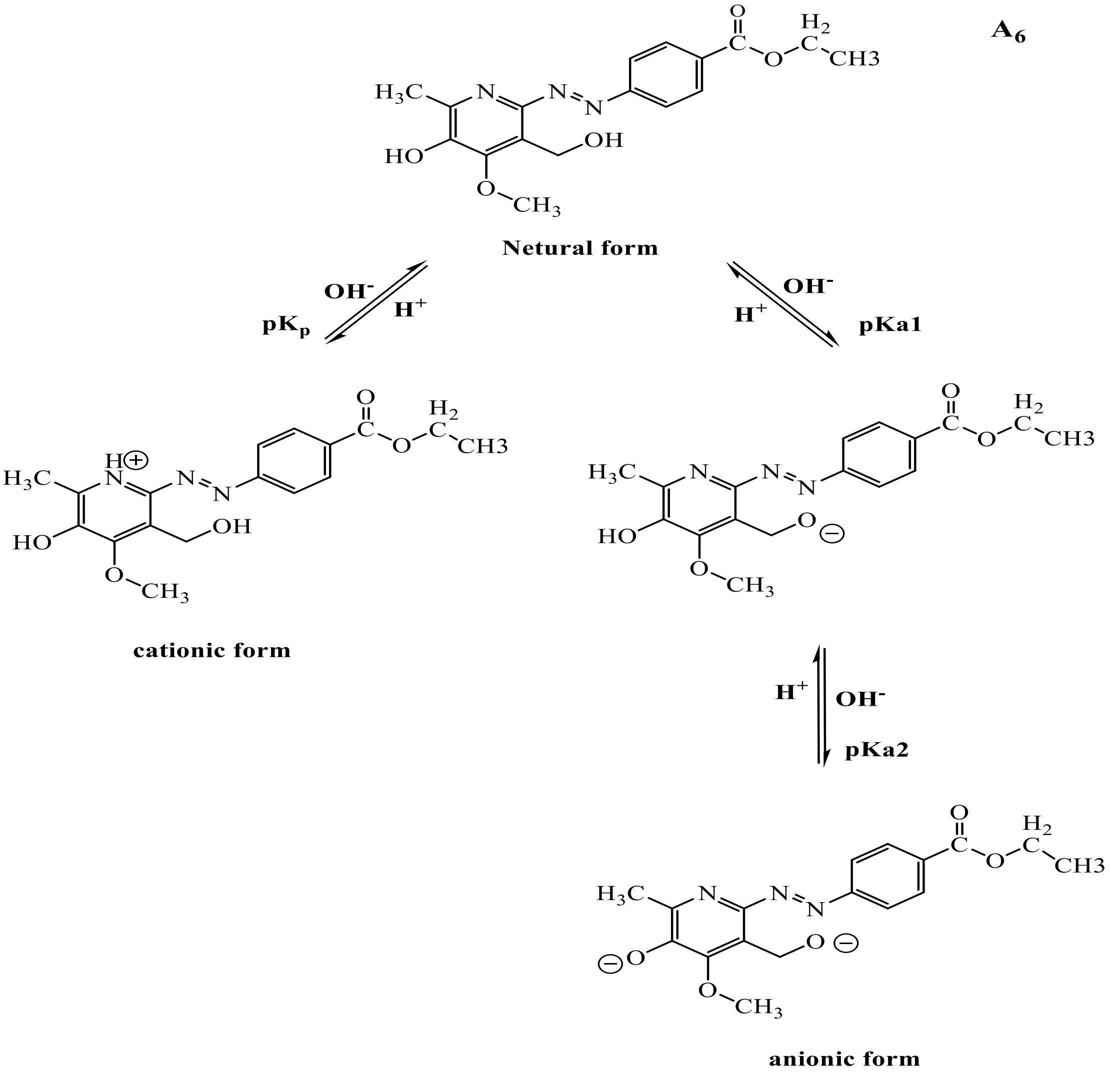
Fig. 7: The Absorbance–pH curves of Azo compounds A_6 and A_7.

Table. 3: The protonation and ionization constants of azo compounds A_6 and A_7.

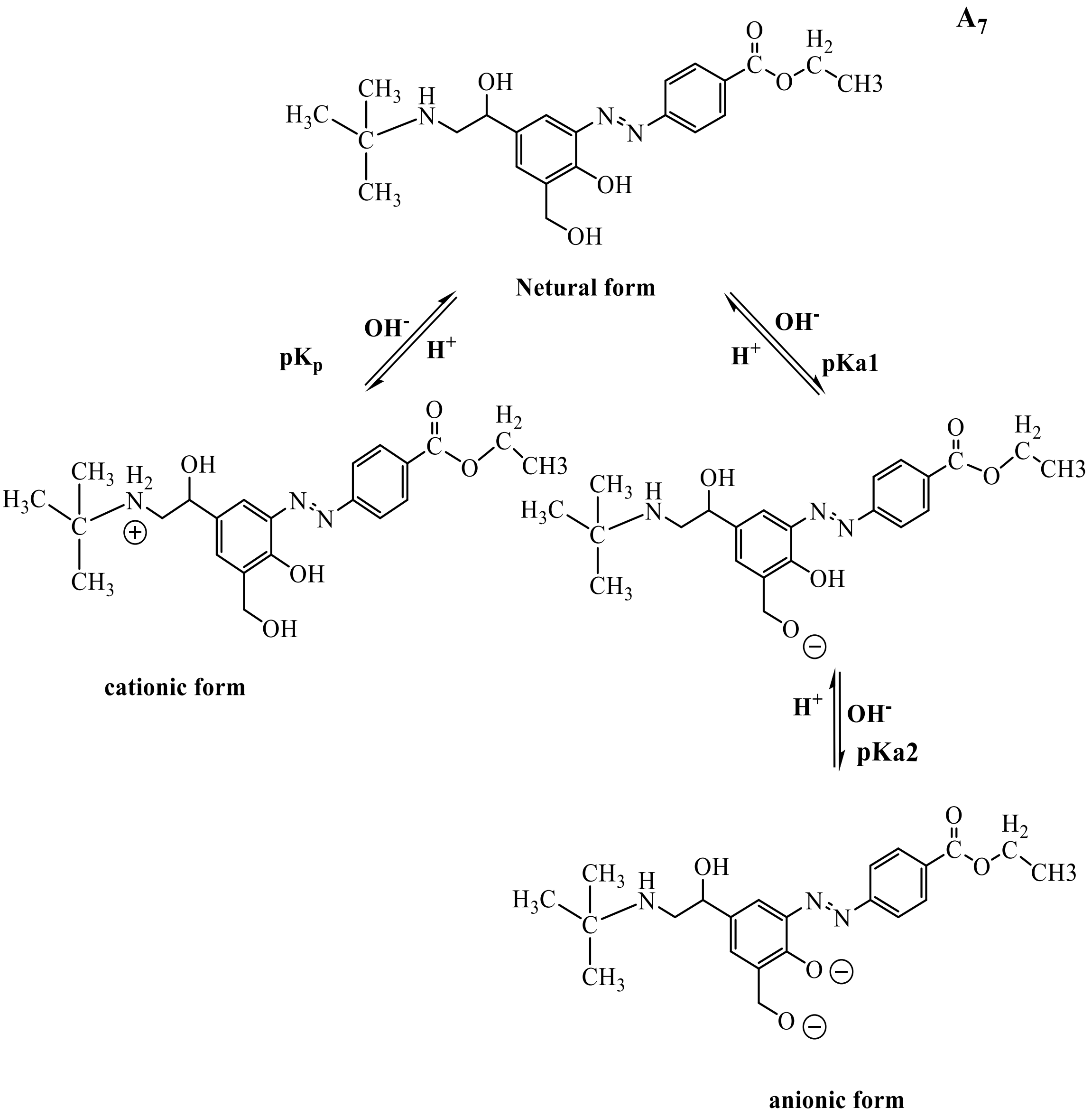
Azo comp. A_6 at $\lambda = 430$ nm				Azo comp. A_7 at $\lambda = 450$ nm			
A_m	A_L	$A_{1/2}$	pK	A_m	A_L	$A_{1/2}$	pK
0.421	0.678	0.550	3.5 (p)	0.411	0.523	0.467	3.5 (p)
0.563	0.812	0.688	6.51 (a1)	0.510	1.148	0.829	7 (a1)
0.779	1.874	1.327	9.5 (a2)	1.116	-------	-------	> 11 (a2)

p = protonation constant of H for nitrogen atom of (pyridoxine & ventolin) molecules, a1 = the first ionization constant of H for hydroxyl group in alcohol, a2 = The second ionization constant of H for hydroxyl group in 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_6.



Scheme -3: Suggested mechanism of protonation and ionization of azo compound A_7.

Kind of buffer solution

To fine the effect of kind of buffer solutions to get highest absorbance of azo compounds A_6 and A_7 (pH10 & pH12 respectively), several buffer solutions were prepared that gives pH 10 and pH12. For pH 10 (for A_6) the buffer used are (1= Universal, 2= Hexamine, 3= NaOH + Sodium Tetra borate, 4= H_3BO_3 + NaOH, 5= NH_4OH + NH_4Cl) . For pH 12 (for A_7) the buffer used are (1= Universal, 2= Hexamine, 3= NaOH + Sodium Tetra Borate, 4= KCl + NaOH, 5= $Na_2HPO_4.2H_2O$+ NaOH). It was found from Fig.7. The best kind buffer solution of pH 10 and pH 12 are (NH_4OH+ NH_4Cl) and universal buffer solutions respectively.

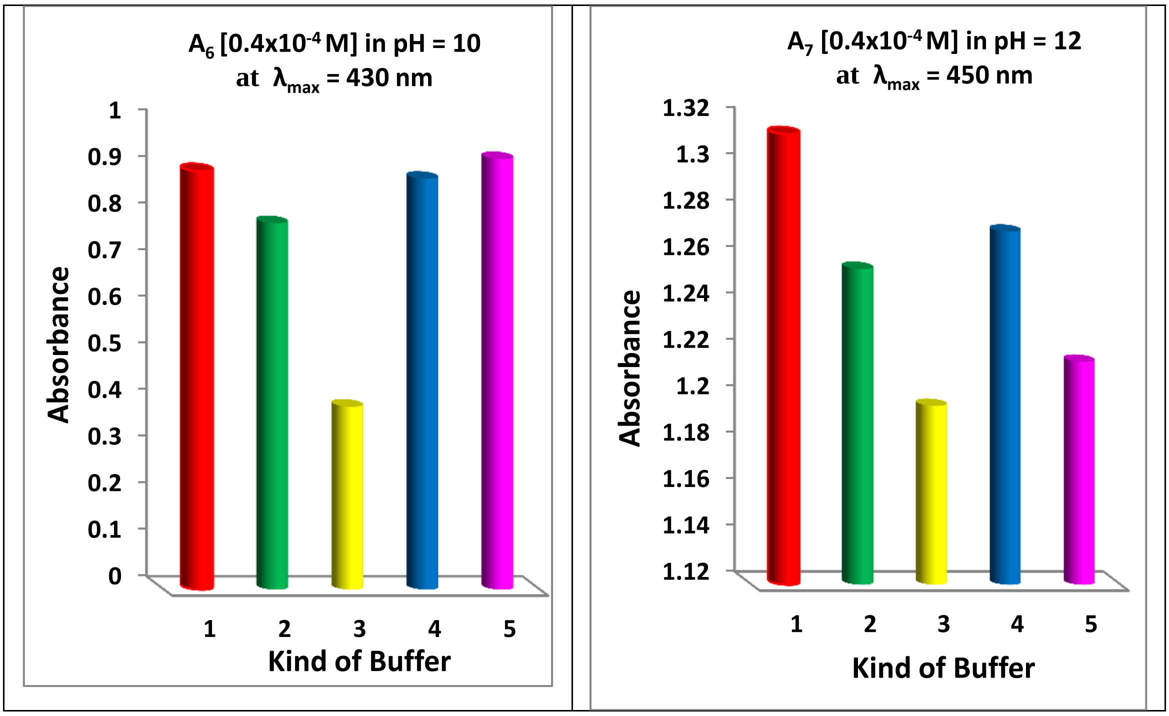


Fig. 7 : Effect kind of buffer solution on Azo compounds A_6 and A_7.

Effect of solvents of different polarities

(Figs. 8&9) displays the spectra of azo compounds A_6 and A_7 in various solvents of different polarities (Ethanol, Methanol, Water, Acetone, DMSO, THF, dichloromethane, 1,4-Dioxane and n-Hexane). From (Fig. 8), the compound A_6 having only two bands at all solvents used at 360 nm. and 480 nm. The intensities of solvents were changed from less absorbance (n-hexane which is consider as non polar solvent) to the highest one (Dichloromethane). In case of azo compound A_7 (Fig. 9), it was found one band observed of all solvents in the range of absorbance wavelength (355-365 nm.) except for DMSO with red shift deviation at 375 nm. because of high dielectric constant and may be forming hydrogen bonding. The intensities of solvents were changed from less absorbance (n-hexane) to the highest one (Dichloromethane) (same as in A_7).

The absorption spectra of different solvents are affected by the solvent's solvation and/or dielectric effects (Table 4).

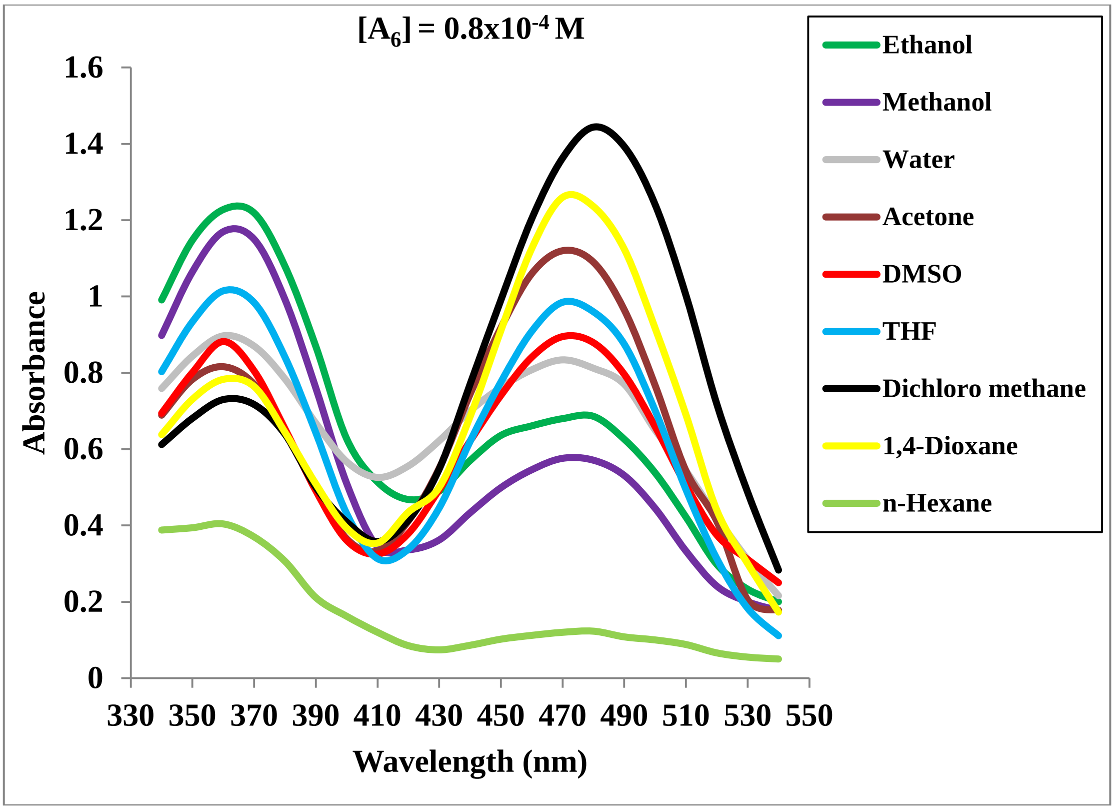


Fig. 8: Electronic spectra of azo compound A_6 at different solvents.

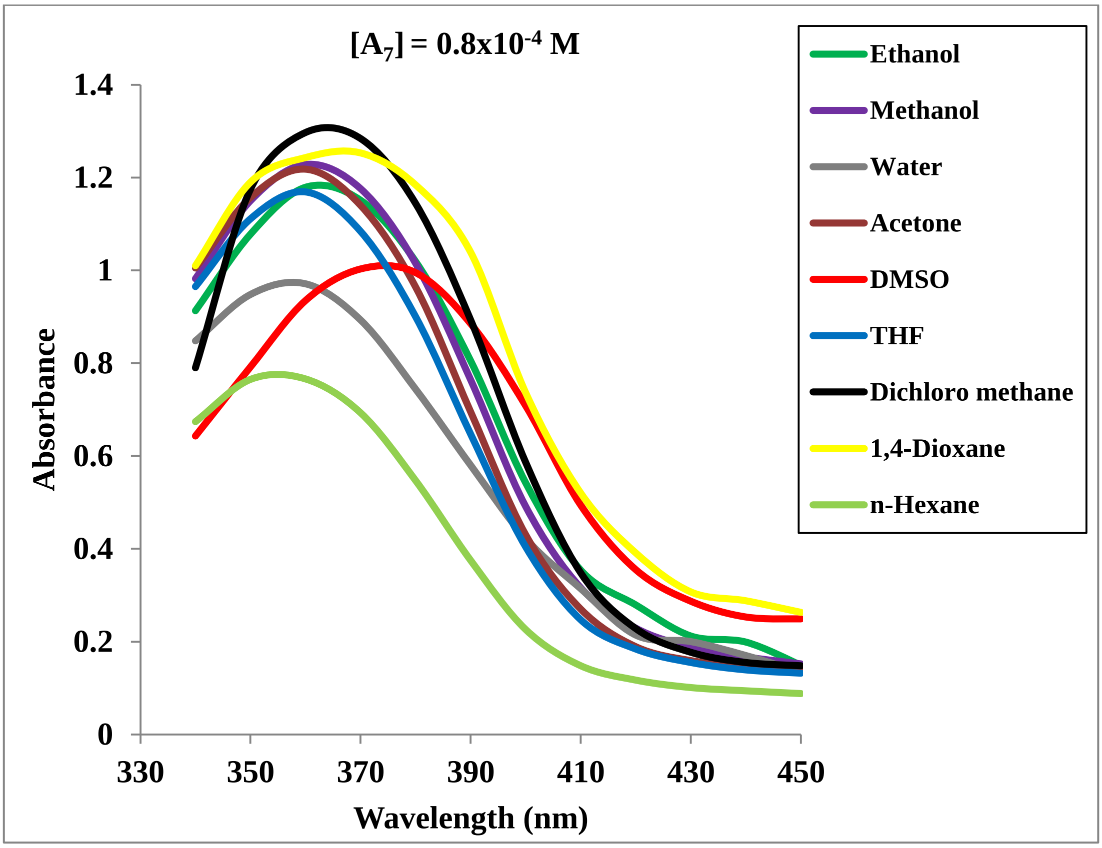


Fig. 9: Electronic spectra of azo compound A_7 at different solvents.

Table 4: Solvent effect on spectra of azo compounds A_6 and A_7.

Symbol No.	Solvent	D [21]	(D-1)/(D+1)	λ_{max} nm	
				A_6	A_7
1	Ethanol	24.30	0.921	360s,480w	365s
2	Methanol	33.60	0.956	360s,470m	360s
3	Water	78.30	0.980	360s,470m	360s
4	Acetone	20.60	0.929	360m,470s	360s
5	DMSO	46.67	0.968	360m,470m	375s
6	THF	7.58	0.686	360m,470m	360s
7	Dichloromethane	9.10	0.844	360m,480s	365s
8	1,4-Dioxane	2.30	0.464	360m,470s	370s
9	n-hexane	1.89	0.372	360w,470w	355m

s = Strong, m = Medium, w = Weak

The plot of dielectric function (D-1)/(D+1) against the λ_{max} of azo compounds A_6 and A_7 (Figs. 10&11), give more or less high linear relation with solvents of moderate polarities. A linearity relationship, indicating that the dielectric constant is the most important factor affecting solvation.

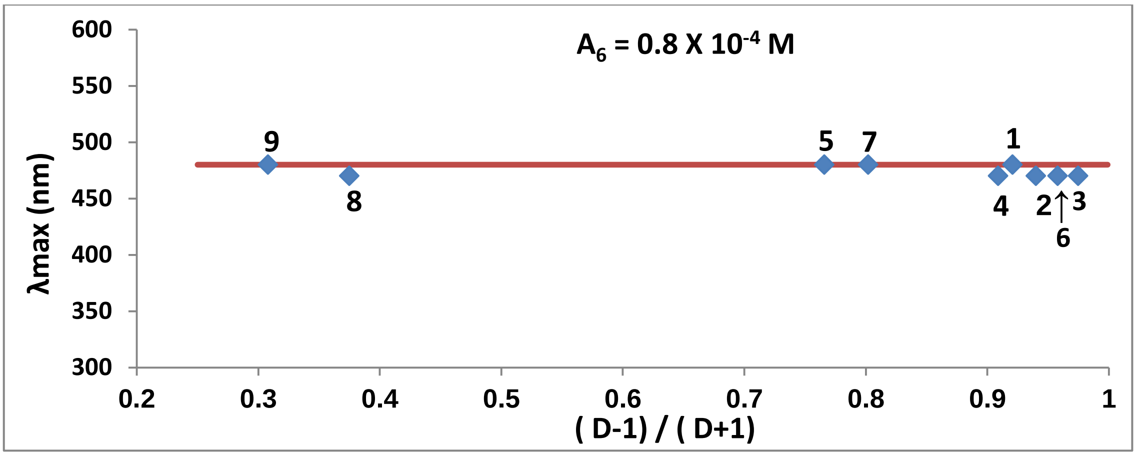


Fig. 10: The relation between λ_{max} and (D-1)/(D+1) for azo compounds A_6.

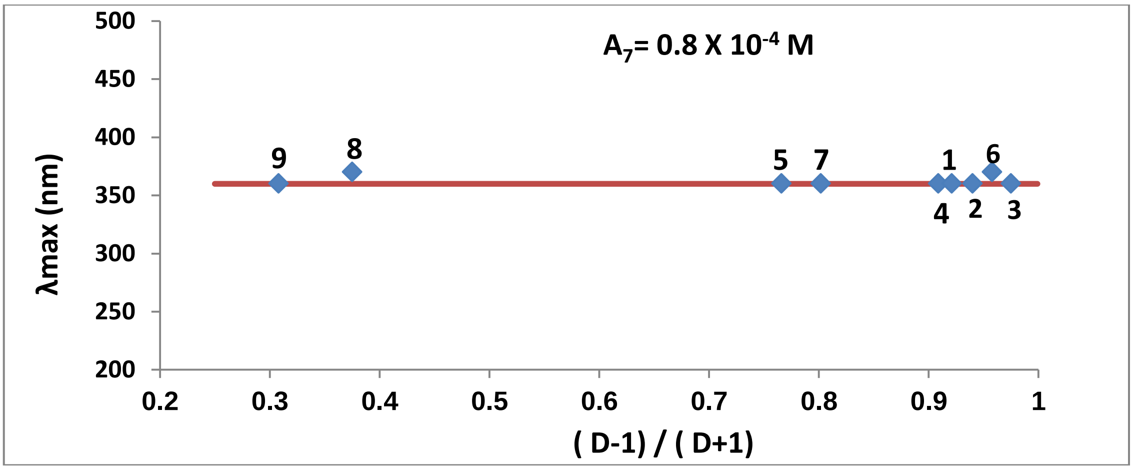


Fig. 11: The relation between λ_{max} and (D-1)/(D+1) for azo compounds A_7.

Determination of nitrite

A curve of absorbance with wavelength was plotted to determine the maximum wavelength of azo compound (A_7) that produced (Fig. 12) to determine the λ_{max}.

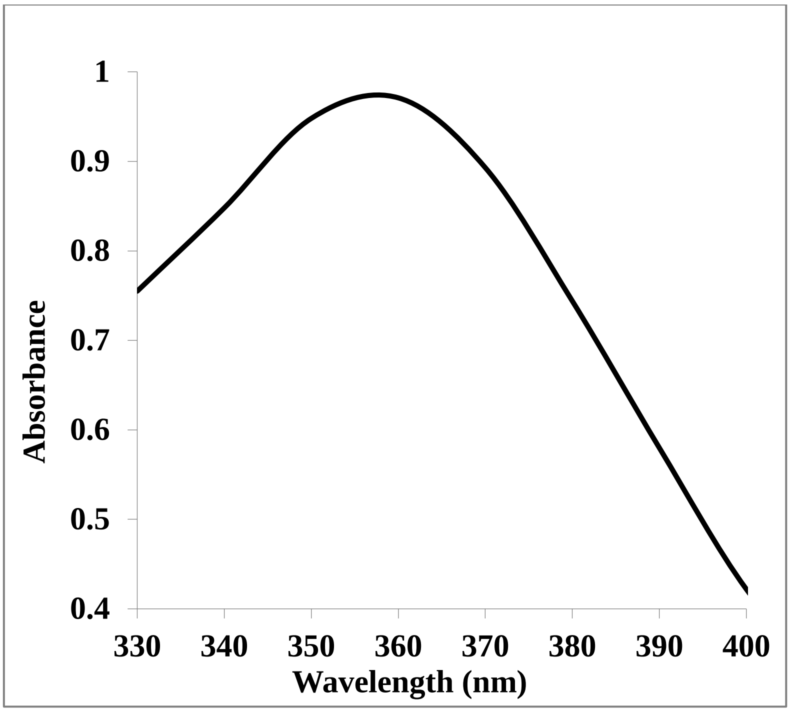


Fig. 12: Absorption curve of azo compound [A_7] = $0.8x10^{-6}$ M and [NO_2] = $3.94\ x10^{-4}$ M

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

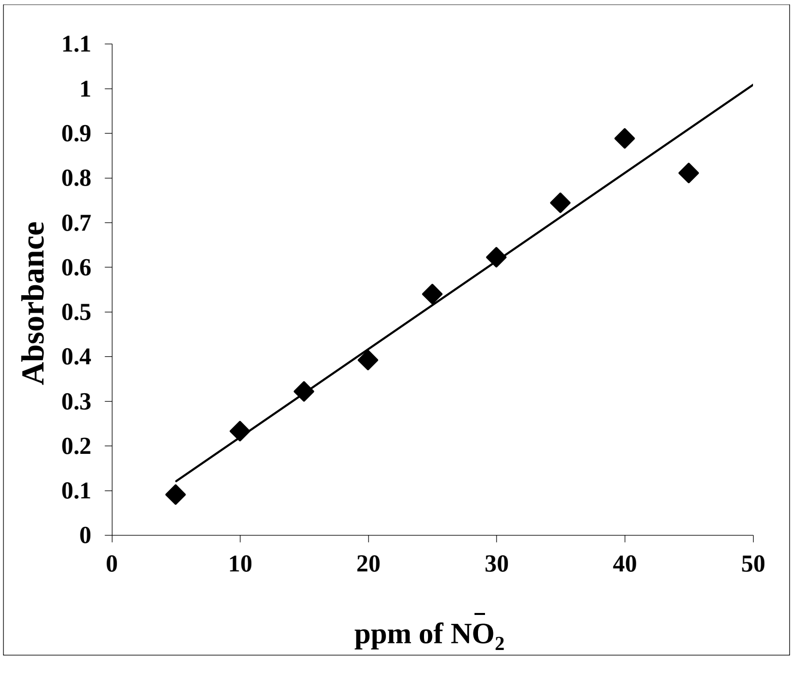


Fig. 13 :Calibration curve for determination nitrite at 355 nm.

Table 5: Beer's law of the azo compound A_7 .

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**
0.954	0.021	0.0476	0.969	0.0111	1.071	40	355

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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