Synthesis, characterization and analytical studies of two new shighff bases

Ali Kamil Mohsin[1],
Ali95ahmeedali@gmail.com
1,2 Department of Chemistry, College of Education for pure Sciences, University of Basrah, Iraq

Hanan M. Ali[2]
1,2 Department of Chemistry, College of Education for pure Sciences, University of Basrah, Iraq

ABSTRACT

Schiff bases, 4-((4-(dimethylamino) benzylidene) amino) benzenesulfonamide (Sb_1) and 4-((2-hydroxybenzylidene) amino) benzenesulfonamide (Sb_2) were synthesised. and then characterized using FTIR, UV-visible, ^{1}HNMR and mass spectrum. Analytical studies were showed that the best solubility of (Sb_1) and (Sb_2) were in DMSO and ethanol respectively. Though, the suitable pH values of (Sb_1) and (Sb_2) were found to be in the pH12, and three isopiestic points were gained in the spectrum of each base. The protonation constants pKb of nitrogen atom and the ionization constants pKa of SO_2-group and OH-group were attended for (Sb_1) and (Sb_2) respectively. These results indicated the suggested ionization scheme in acidic and basic media for each base. Further, the (Sb1) and (Sb2). Further, the biological activity of each base was tested against three different bacteria, (*Staphylococcus Aureus, Escherichia Coli and Pseudomonas Aeruginosa*). The results showed that each of (Sb_1) and (Sb_2) was shows reasonable activity against *Escherichia Coli*. But, the (Sb_1) did not have any affect towered *Staphylococcus Aureus* and *Pseudomonas Aeruginosa* in contrast with (Sb_2), which was showed similarity in its activity toward *Staphylococcus Aureus* and *Pseudomonas Aeruginosa*.

Keywords: Schiff base, Analytical studies, Buffer Solutions, Ionization Constant, Protonation Constant

1. Introduction

Schiff bases have been extensively studied as they possess many interesting features, including photochromic and thermochromic properties, proton transfer tautomeric equilibria, biological and pharmacological activities, as well as suitability for analytical applications.[1] Owing to synthetic flexibility and simple preparation procedure these compounds have received a great attention as appropriate ligands for coordination and determination of various metal ions. Schiff bases are attractive as analytical reagents because they enable simple and inexpensive determinations of various organic and inorganic substances.[2] In general, there are two principal ways of their analytical application: first, determination of organic compounds bearing an amino or an active carbonyl group by the formation of coloured (chromophore-containing), fluorescent or insoluble Schiff bases, and secondly, the determination of various metal ions, as well as amino and carbonyl compounds, using complex formation reactions. The analytical methods based on complex formation are used more frequently. Owing to the relatively simple preparation procedures of Schiff bases, it is possible to obtain ligands of different design and characteristics by selecting appropriate reactants. A phosphate buffer was found to significantly promote tetra-methylpyrazine (TMP) formation in an acetoin (3-hydroxy-2-butanone)/ammonium hydroxide system.[3] The effect of the phosphate ion on TMP formation was additive in the range of 0.05-0.2 M. The change in pH value of the system reveals that a proton-coupled redox type of reaction occurred during TMP formation. Phosphate serves both as proton donor and acceptor to facilitate proton transfer during the Schiff base formation between ammonia and 3-hydroxy-2-butanone. Protic solvents, methanol, and ethanol, were found to attract the water released from the system. The combination of a phosphate buffer and protic solvent led to the completion of TMP formation. The TMP formation mechanism in a phosphate buffer (pH 7.2) is proposed. The possibility of using a Schiff base as an acid-base indicator.[4] This surprising phenomenon can be considered as an interest due to the fact that Schiff bases are usually unstable in solutions and definitely undergo hydrolysis. It was found that such a specific observation depends merely upon the chemical structure and type of the substitute of amine that reacts with aldehyde to give the Schiff base. The latter reagent 4[(4-dimethylamino-benzylidene)-amino]- benzene sulfonamide was synthesized from the condensation of sulfanilamide with *p*-dimethylaminobenzaldehyde. The reagent solution shows a reproducible change in its color due to the addition of acid and base. A UV-Vis spectroscopic characterization and acid-base equilibrium study of the reagent for its possible use as an indicator were investigated. The results show that the reagent is an amphoteric which possesses four ionization constants Ka1, Ka2, Kb1 and Kb2 of weak dibasic and diacidic properties. The value of pKa2 (9.80) is parallel to the observed transition interval pH 9.5 (yellow) and pH 10.5 (colorless), which is considered to be the indicator exponents pKi. It was concluded that the benzyl sulfonamide group plays a key role in the stability of the reagent towards hydrolysis and also for indicator characteristics through breaking the conjugation.[4]Further, the 2,5-dimethyl-4-((4-nitrobenzylidene) amino) phenol (1), *N,N'*-([1,1'-biphenyl]- 4,4'-diyl)bis(1-(2,4-dimethoxyphenyl)methanimine (2) and 4,4'-(([1,1'-biphenyl]-4,4'-diylbis(azaneylylidene))*bis* (methaneylylidene))*bis*(2-methoxyphenol) (3) were synthesized,[5] and were used as anti-breast cancer cells type MCF-7. The results of this study were showed high inhibition activities of prepared Schiff bases.

2. Experimental section

2.1 Synthesis of the two novel shighff bases

Each of 4-(dimethylamino) benzaldehyde (861gm, 0.005mole) and 2-hydroxybenzaldehyde (0. 611gm, 0.005 mole) were stirred in separated round bottom flask and heated in each solution mixture of 4-aminobenzenesulfonamide (0. 746gm, 0.005 mole), that dissolved in, 25 mL from each of absolute ethanol and 3 drops of acetic acid was add to each with reflux. After 5 hours each mixture of (Sb_1) and (Sb_2) was filtrated and each precipitate recrystallized using ethanol and hexane to result a yellow and orange shighff bases, (1.15 g, 85%) and (1.20 g, 80%) respectively. m.p.: (233-235) ^oC and (212-215) ^oC. This showed δH (500 MHz, DMSO): 2.5 (s,6H, DMSO), 3.3 (s, H, HOD), 2.2,2.4 (s, 6H, CH3), 6.5-8.5 (s, 6H, Ar-H), 8.7 (s,1H, CH=N), 9.04 (s, H, OH), (Sb_1); 2.5 (s,6H, DMSO), 3.3 (s, H, HOD), 3.8 (s, 12H, OCH3), 6.5 - 8 (m, 14H, Ar-H), 8.7 (s, 2H, CH=N), (Sb_2).

2.2 Solution of two novel shighff bases in ethanol

The solutions (50 mL) of each of (Sb_1) and (Sb_2) in ($1*10^{-3}$ M) concentration.

2.3 The solvent effect

The solution of each of (Sb_1) and (Sb_2) was prepared by dissolving of each in different solvents, (ethanol, methanol, DMSO and chloroform) to give ($1x10^{-4}$ M) concentration.

2.4 The *pH effect*

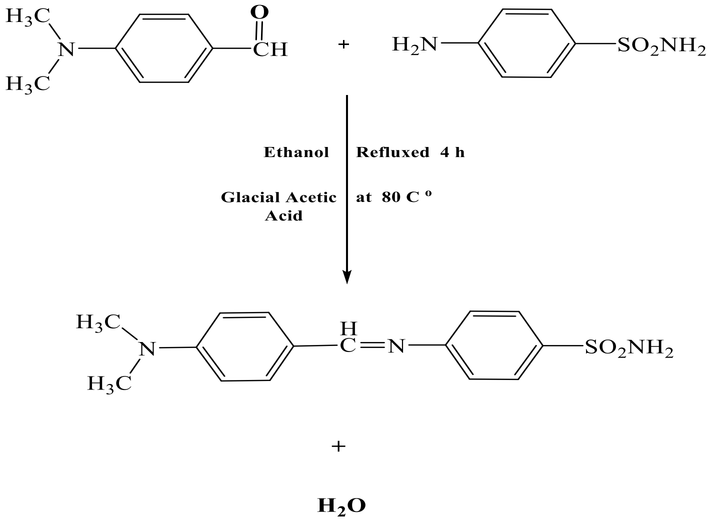
The solution of each of (Sb_1) and (Sb_2) was prepared by dissolving in a range of different buffer solutions, (2-12) to give ($1x10^{-4}$ M) concentration from each.

2.5 Cellular toxicity

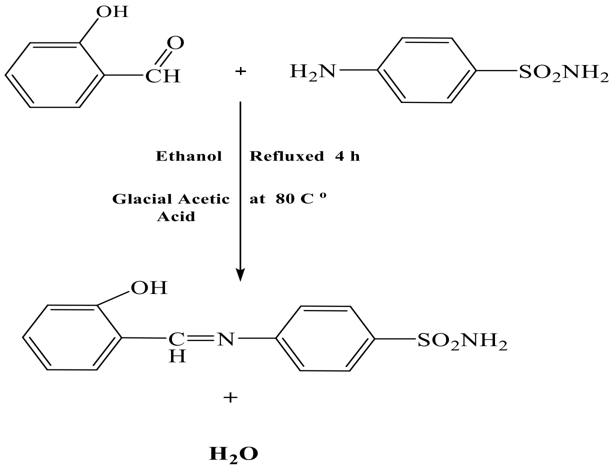
The Xian-guo and Ursola method, was applied to measure the toxity of (Sb_1) and (Sb_2), using haemolytic red blood cells as following: A stock solution of 200 mg / mL was prepared and followed by preparing a series of diluted (1000, 500, 250, 100,50 "µg" /"mL") solutions. Then 0.8 mL of each diluted solution was added to Eppendorf tubes. Followed by adding 0.2 mL of red blood cells to each tube and equipped. Thus, In the first tube, 0.8 mL of Ringer solution was added as a negative control, but the tap water was added to second tube as a positive control. Then 0.2 mL of red blood cells was added to each tube. The results were recorded after the incubation for 3 h. in a special incubator and the variations in these solutions were checked.

4.Result and Discussion

The 4-((4-(dimethylamino) benzylidene) amino) benzenesulfonamide (Sb_1) and 4-((2-hydroxybenzylidene) amino) benzenesulfonamide (Sb_2) were synthesised, (scheme 1 and 2).



Scheme (1): Synthesis of shighff base (L1).



Scheme (1): Synthesis of shighff base (L2)

The synthetic shighff bases (Sb_1) and (Sb_2) were prepared and then were characterized by IR spectrum, mass spectrum, ^1H NMR spectrum and the UV-visible spectrum. The IR spectrum of the (Sb_1) and (Sb_2), (Figures 1 and 2). The results were showed, that the stretching vibration of the ν (O-H) group in (Sb_2) was appeared in the regions 3342 cm^{-1}. But, the ν (CH=N) stretching vibration band of (Sb_1) and (Sb_2) were appeared in the region (1600-1650 cm^{-1}). The infrared spectra of the prepared Schiff bases also showed the disappearance of the carbonyl group bands belonging to the aldehyde in the region (1700-1780 cm^{-1}), the amine group band in the region (3000-3500 cm^{-1}), and the C-H group band for the aldehyde in the region (2700-2880 cm^{-1}), to give clear evidence of the formation of Schiff rules.

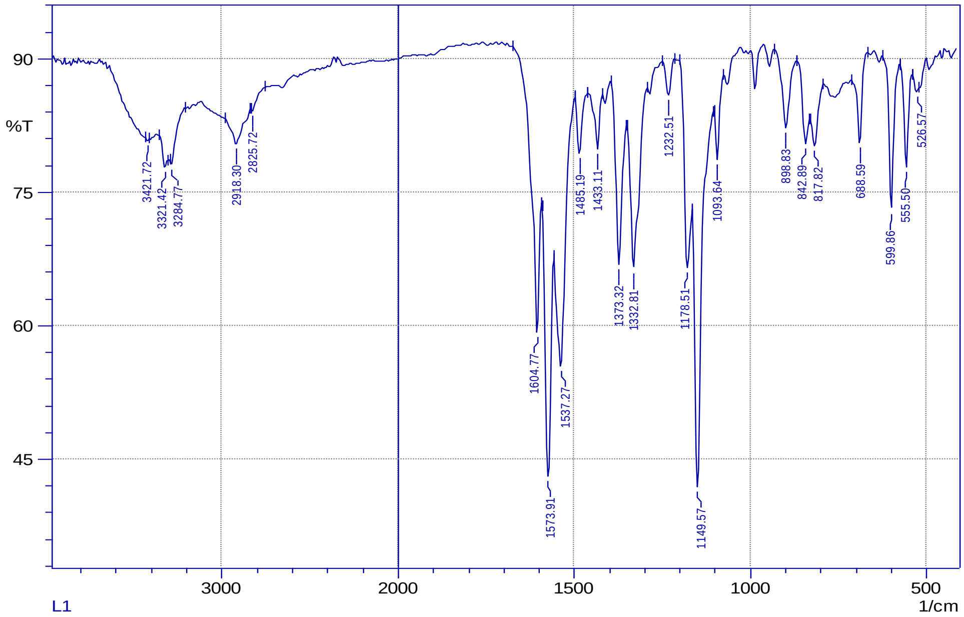


Figure 1. FT-IR spectrum of (Sb1).

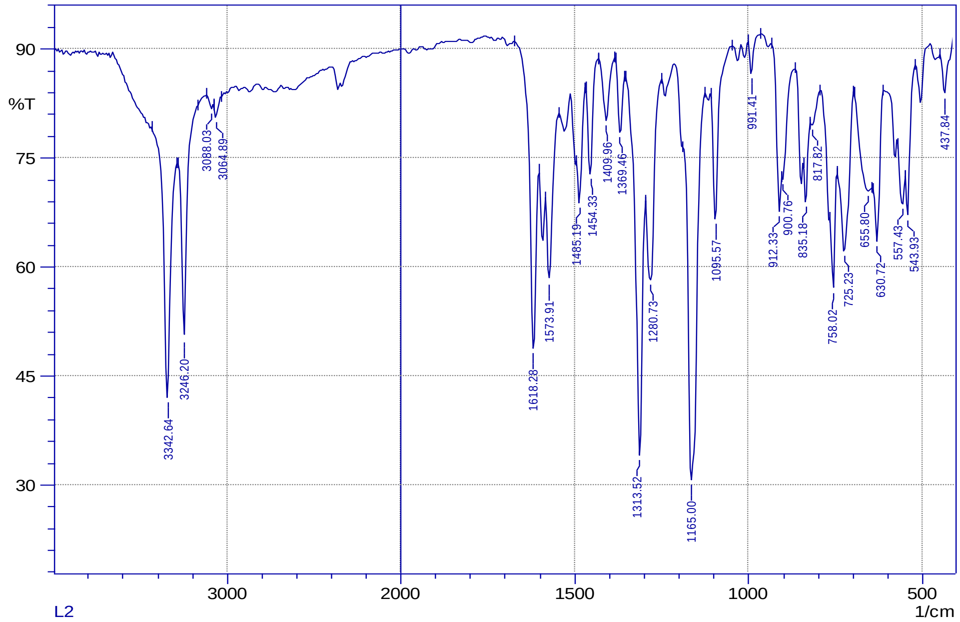


Figure 2. FT-IR spectrum of (Sb2)

The mass spectra of the prepared Schiff bases (Sb1) and (Sb2) were showed that the peak of at *m/z* equal to 303.1 and 276.1 respectively, (Figures 3 and 4).

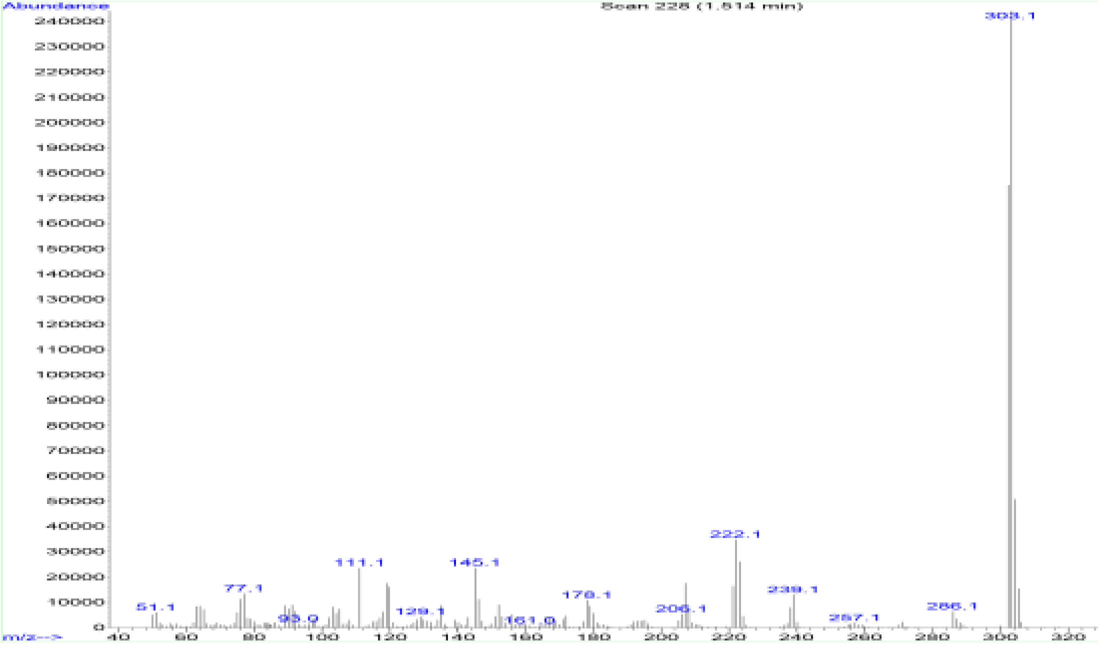


Figure 3. The mass spectrum of (Sb_1).

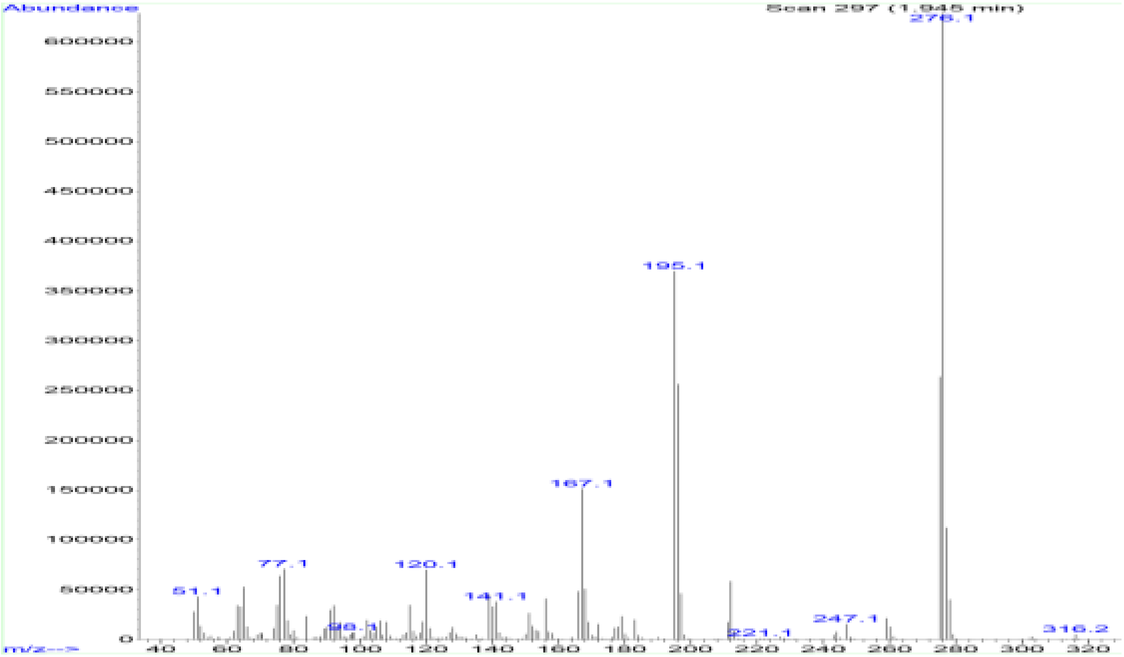


Figure 4. The mass spectrum of (Sb_2).

Add to which, the ^1H NMR spectrum was confirmed the formation of each of (Sb_1) and (Sb_2), (Figures 5 and 6).

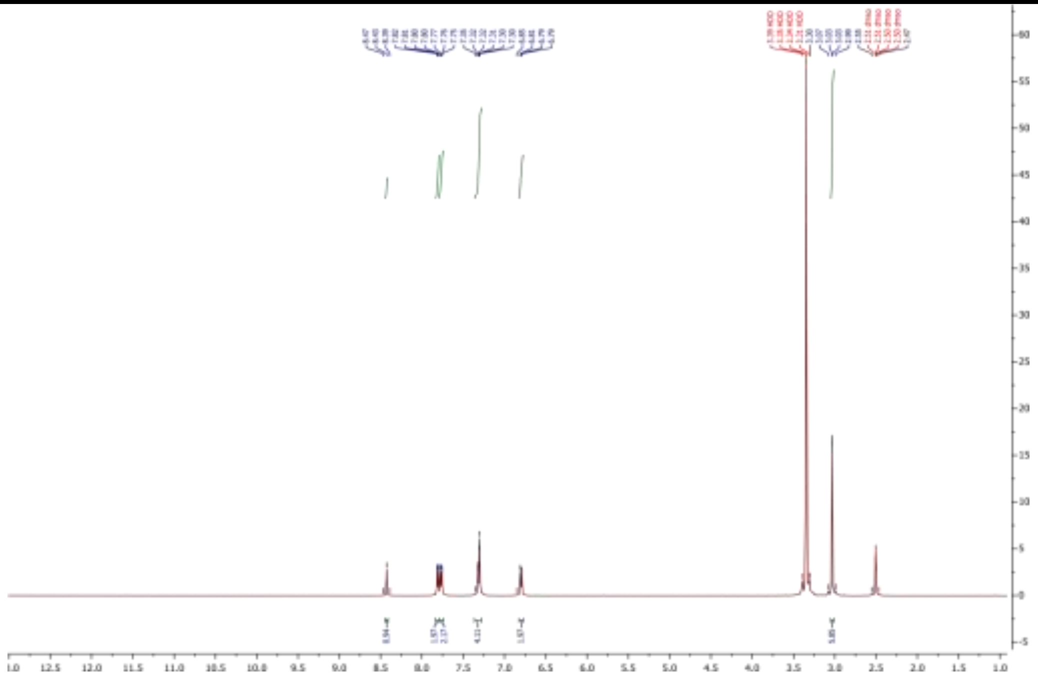


Figure 5. The ^{1}H NMR spectrum of (Sb_1).

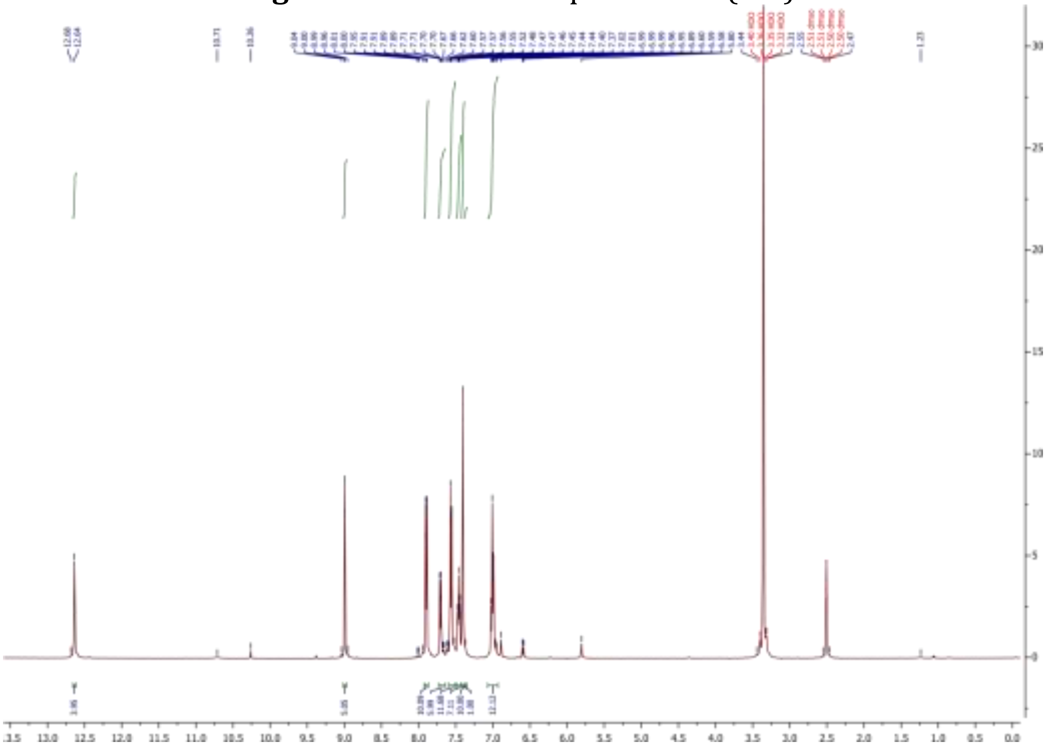


Figure 6. The ^{1}H NMR spectrum of (Sb_2).

The UV-visible spectrum was documented at the range (250-500) nm in ethanol for each of the synthetic Shighff bases, (Figures 7 and 8). The absorption spectrum of (Sb_1) and (Sb_2) were showed bands at (270 nm and 350 nm); (290 nm and 330 nm)) respectively related to (π -π*).

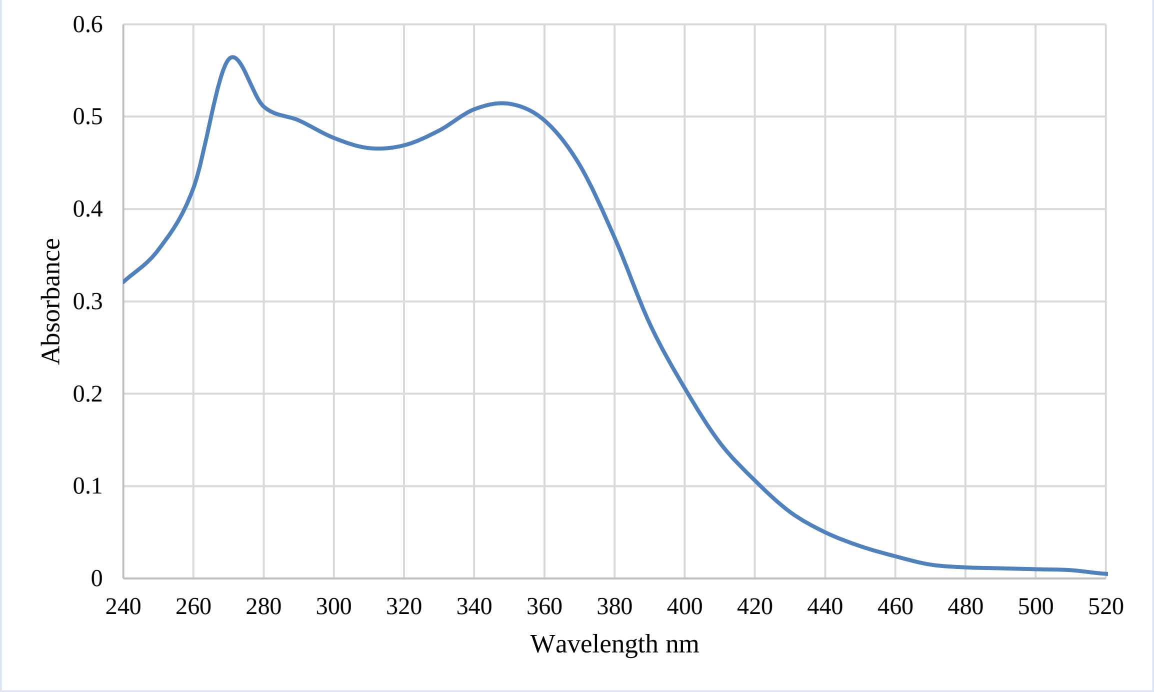


Figure 7. The UV-visible spectrum of (Sb_1).

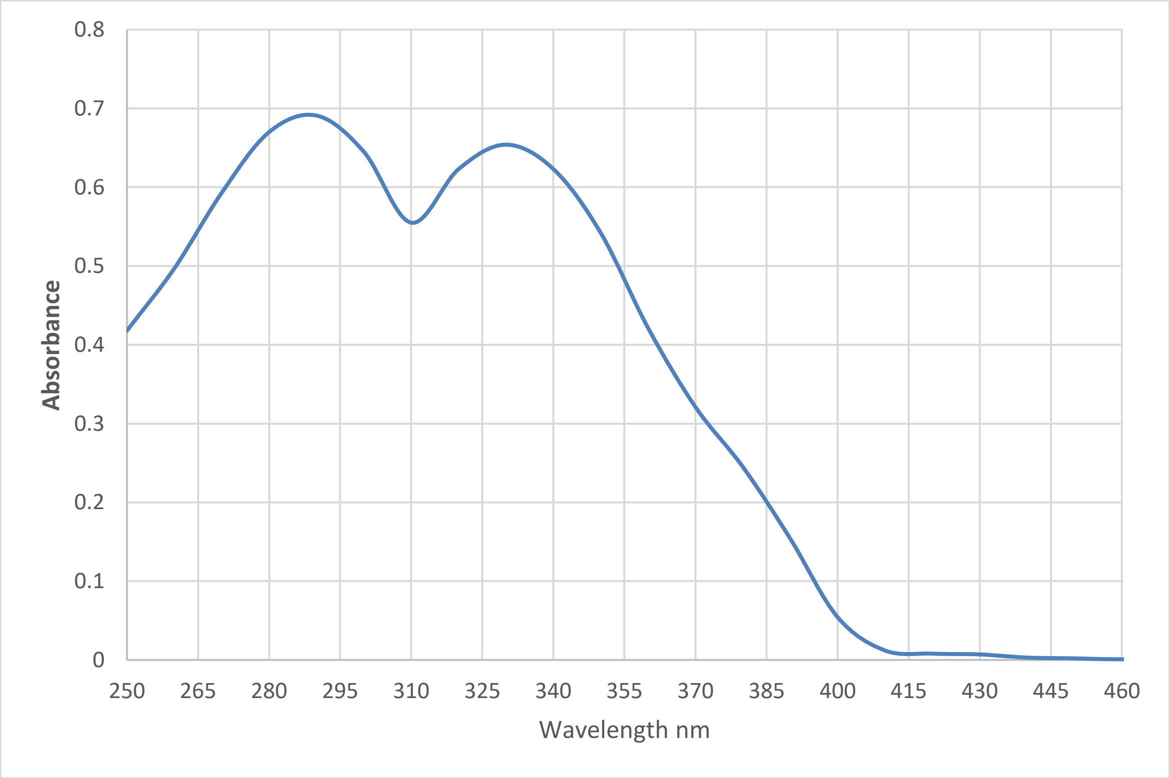


Figure 8. The UV-visible spectrum of (Sb_2).

Analytical studies were also curried on (Sb_1) and (Sb_2), the solvent effect of each studied, (Figures 9 and 10) using set of different solvents, (chloroform, methanol, ethanol and DMSO). The results were showed that the best solubility of (Sb_1) was in DMSO, but the best solubility of shighff bases (Sb_2) was in ethanol.

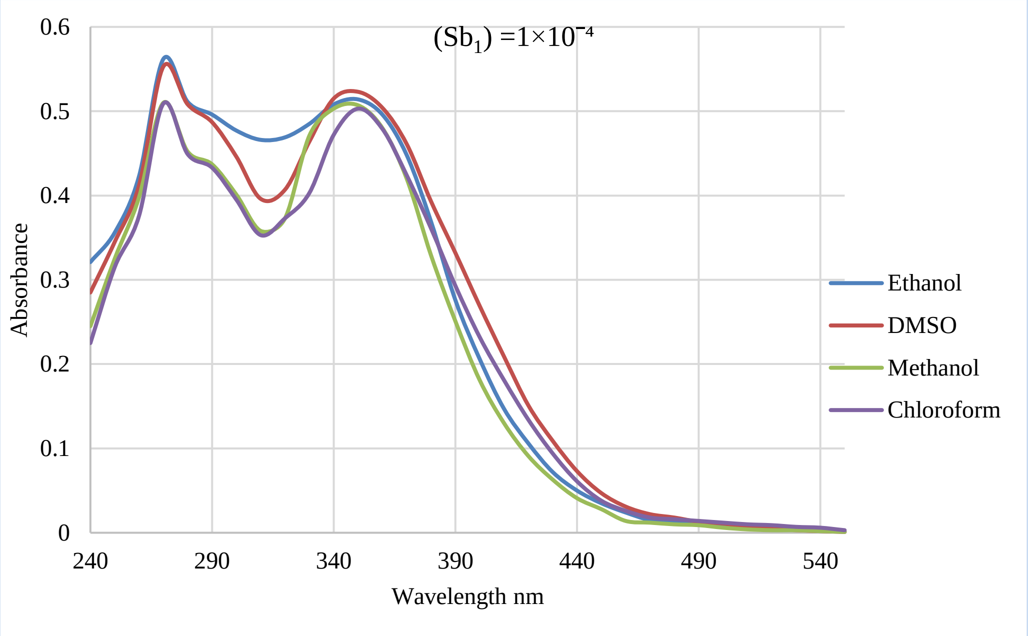


Figure 9. The solvent effect of (Sb_1).

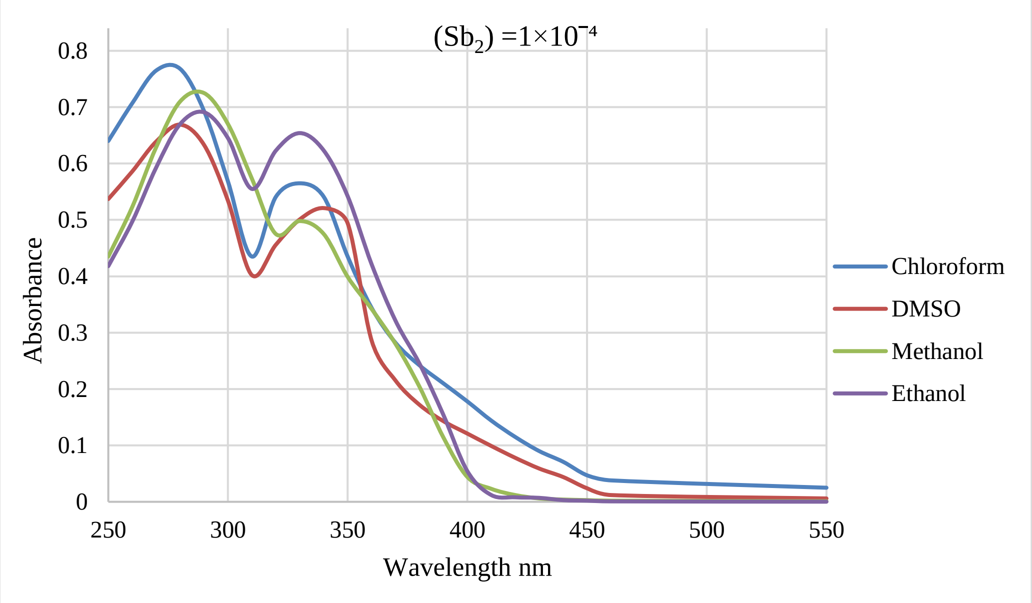


Figure 10. The solvent effect of (Sb_2).

Each of (Sb_1) and (Sb_2) gave different values of λ_{max} using different solvents, (Table 1) attributed to (π - π^*) transitions, which related to the aromatic system and (-HC=N-) group.

Table 1. The UV-visible spectrum of (Sb_1) and (Sb_2)

No.	Base	Solvents	λmax (nm)	εmax x10^4 l.mol^{-1}.cm^{-1}
1		Chloroform	350	0.503
2		DMSO	350	0.523

3	(Sb_1)	Ethanol	350	0.514
4		Methanol	350	0.507
1	(Sb_2)	Chloroform	340	0.565
2		DMSO	330	0.521
3		Ethanol	330	0.654
4		Methanol	330	0.498

The table above displays the type of the solvents effect in (Sb_1) and (Sb_2), which were showed two and three absorption peeks respectively, (250-500) nm contributed to the different electronic transitions using different solvents. These results were indicated that the shighff bases were affected by the solvation and the dielectric constant (D), which can have expressed by the relation of Gati and Szalay as below:

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

Table 2. The values of the solvents of the dipole moment constants with the maximum wavelengths of the (Sb_1) and (Sb_2)

The Solvent	D	(D-1)/(D+1)	λ_{max} nm	
			(Sb_1)	(Sb_2)
Chloroform	9.10	0.802	350	330
DMSO	47.00	0.958	350	340
Ethanol	24.30	0.921	350	330
Methanol	32.70	0.940	350	330

The results, (Figures 11 and 12) were indicated, that there is no deviation from the linear relationship, 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 with a slight deviation from the linearity, which effected by the hydrogen bond between the base and the solvent.

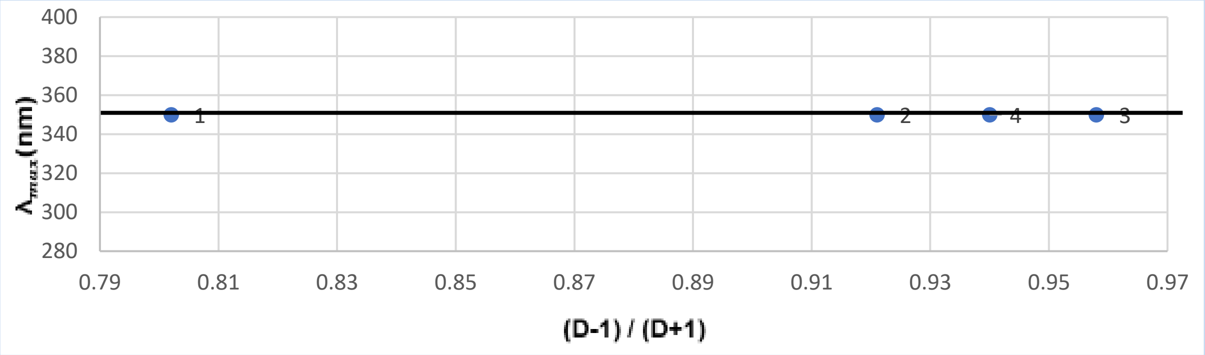


Figure 11. Relation between (D) and λ max(nm) in diverse solvents of (Sb_1).

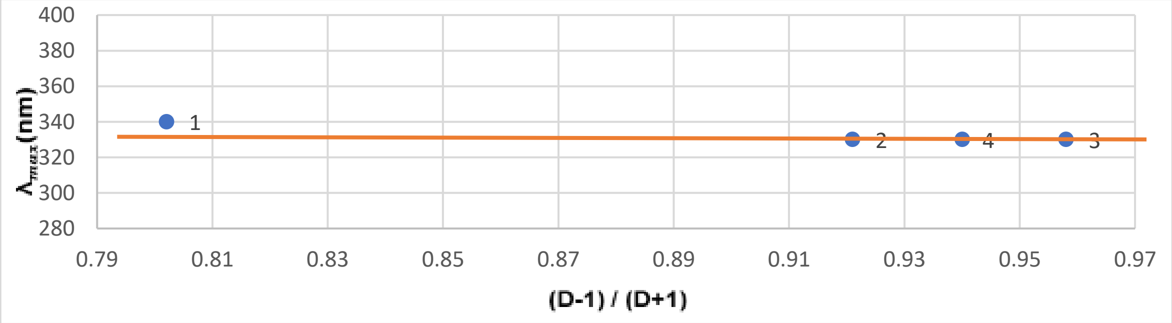


Figure 12. Relation between (D) and ⅄ max(nm) in diverse solvents of (Sb_2).

Further, the pH effect in the range of λ (250-500) nm was also studied for each Schiff base (Sb_1) and (Sb_2) in a range of buffer solution at pH (1-12) using ($1*10^{-4}$ M) as seen in figures (13) and (14) below.

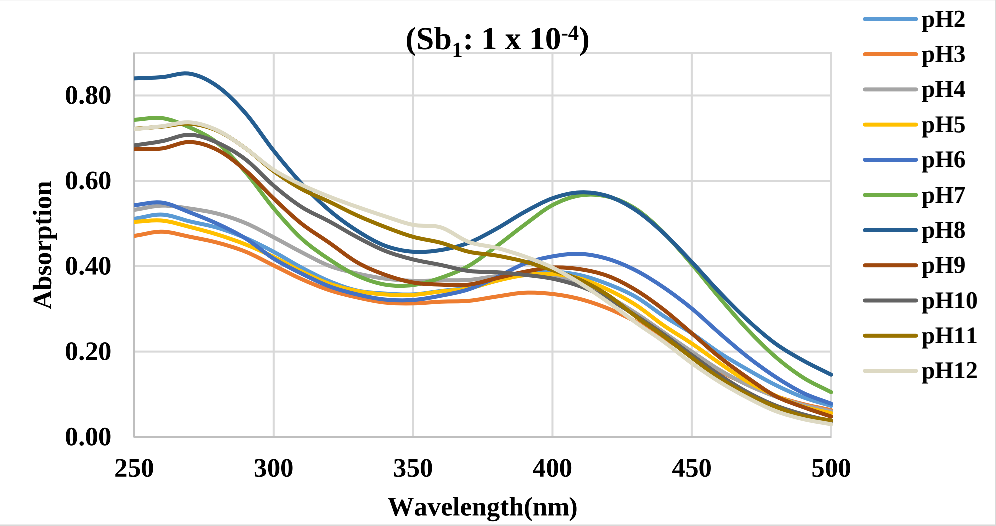


Figure 13. The pH effect in the (Sb_1) using a range of buffer solution.

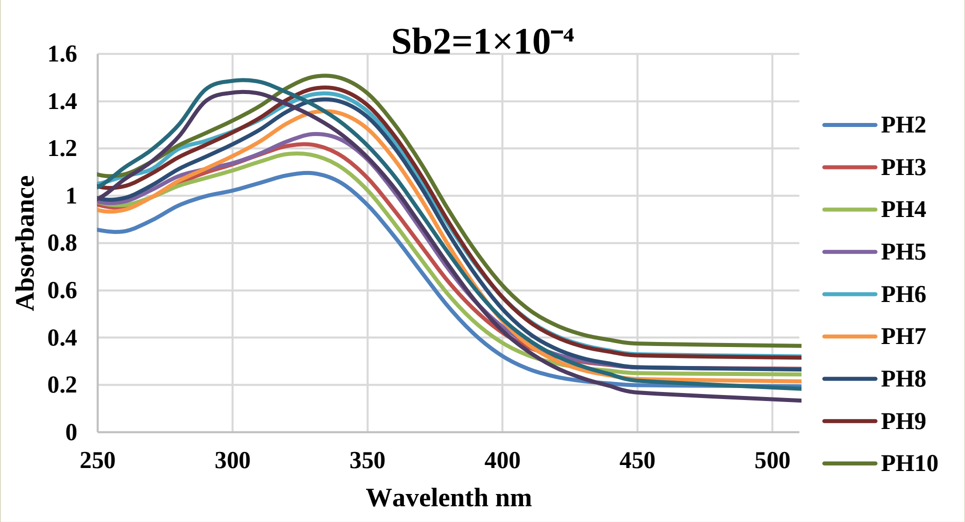


Figure 14. The pH effect in the (Sb_2) using a range of buffer solution.

The results were showed that the suitable pH values of (Sb_1) and (Sb_2) were found to be in the pH12. Three isopiestic points were gained in Figures (13) and (14) at (310 nm, 380 nm and 400 nm) and (280 nm, 320 nm and 410 nm). Therefore, the pKa of sulfonyl group and hydroxyl group and the pKb of the nitrogen atom in the (Sb_1) and (Sb_2) respectively were calculated by applying the half height method. From this method the pK values were attended using equations (1) and (2) below. This method was depending on the fact that the limiting absorption each of (Sb_1) and (Sb_2) represents complete conversion of one form to other. Since pK is equal to pH at which the two forms exist in equivalent amount, then the pH corresponding to half the height of the absorbance, the pH curve is equal to pK.

pK= pH (at A1/2) .... (1)

$A1/2=\frac{A_l+A_{min}}{2}$ .... (2)

The pK at (A1/2) of (Sb_1) and (Sb_2) were intended from the absorbance-pH curve as realized in Figure (15) and (16) below.

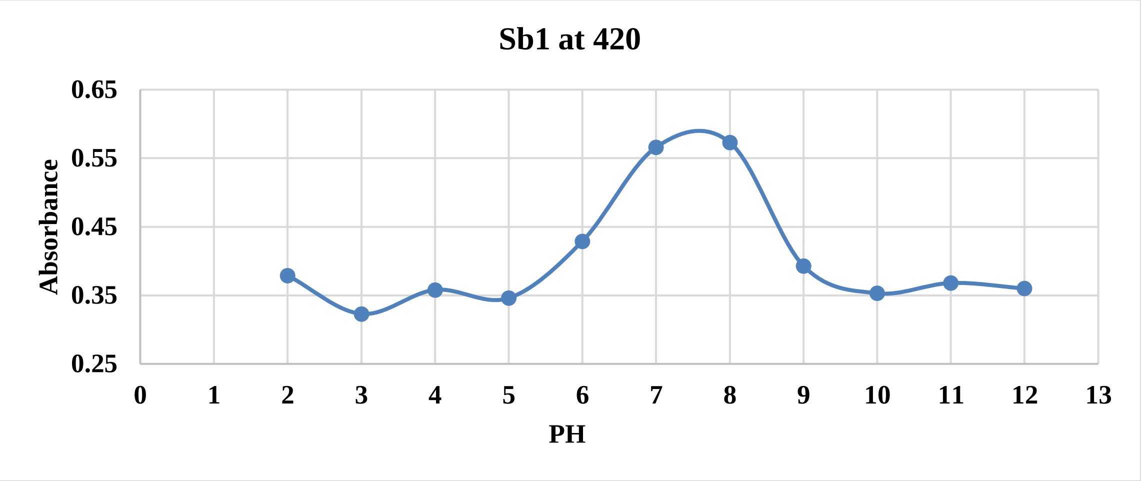


Figure 15. Absorbance-pH effect of (Sb_1) at λ_{max} 420 nm.

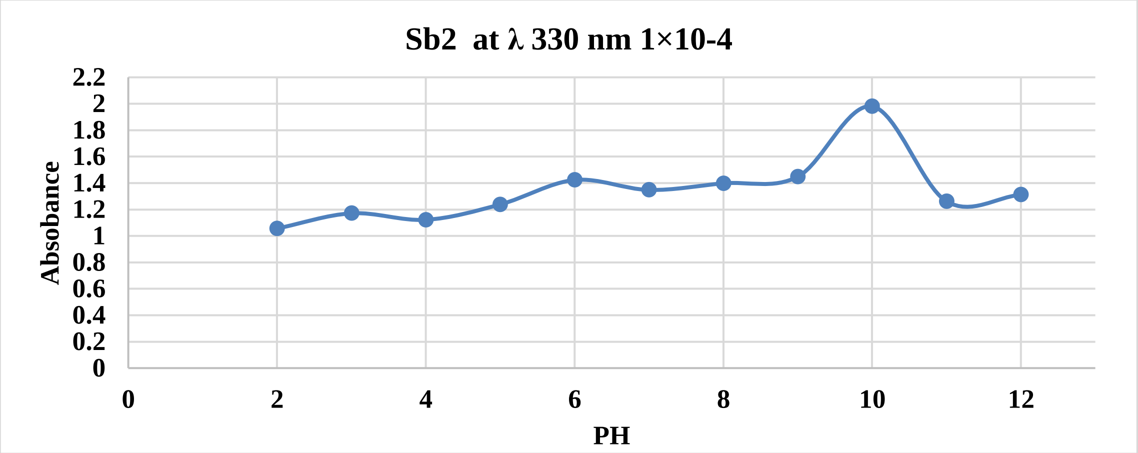


Figure 16. Absorbance-pH effect of (Sb_2) at λ_{max} 330 nm.

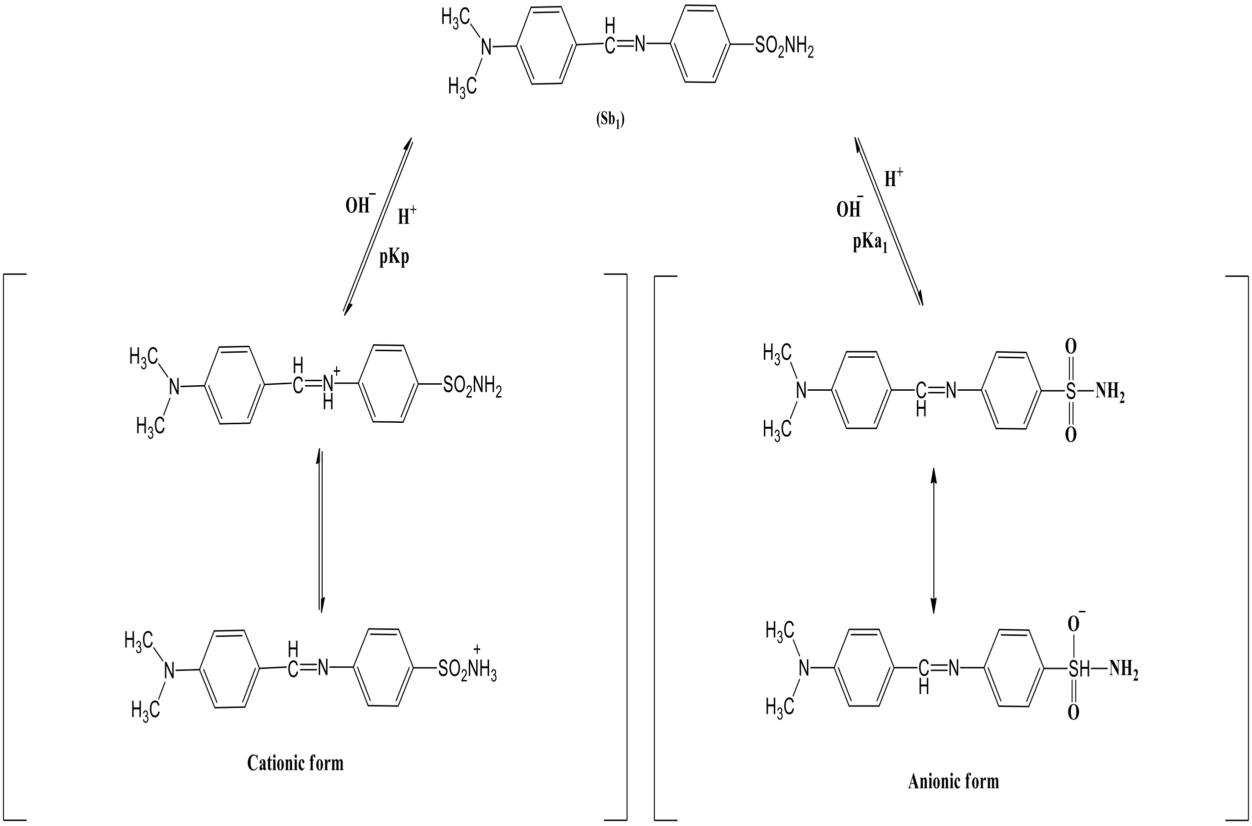
The results obtained from the absorbance-pH curve in figures above are given in table (3) below

Table 3. Spectrophotometric determination of ionization and protonation constants of (Sb_1) and (Sb_2).

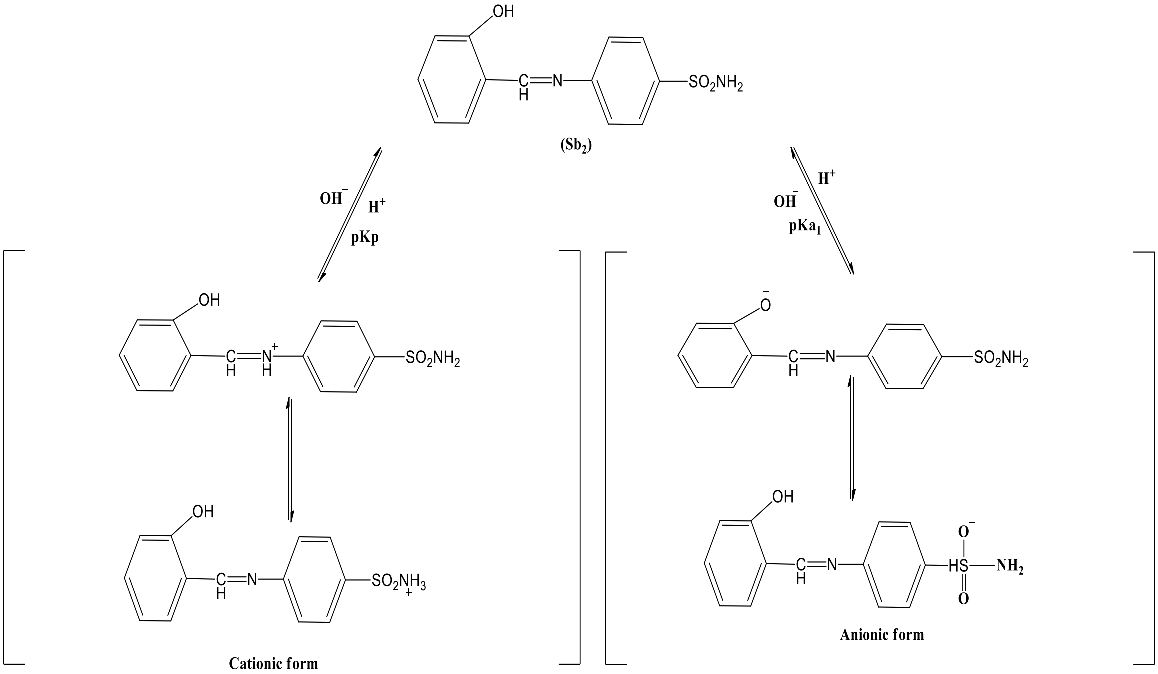
Shef	λ_{max}	A ½	PKp1	A ½	PKp2	A ½	Pka
Sb_1	420	0.340	3.4	0.381	7.26	0.360	10.5
Sb_2	330	1.272	…	1.689	9.3	…..	….

pK_p= Protonation of the nitrogen atom and pK_a= Ionization of the SO_2 and OH-groups.

The absorption spectra of (Sb_1) and (Sb_2) in the varying pH values are explained in the Schemes (2 and 3) below. The results were indicated the existence of the following equilibrium schemes of which displays the suggested ionization of each of (Sb_1) and (Sb_2) in acidic and basic media.



Scheme 2. The suggested ionization of (Sb_1) in the acidic and basic media.



Scheme 3. The suggested ionization of (Sb_2) in the acidic and basic media.

Furthermore, the biological activity of (Sb_1) and (Sb_2) against *Staphylococcus Aureus, Escherichia Coli* and *Pseudomonas Aeruginosa* were tested, (Figures 17 and 18).

Figure 17. The biological activity of Sb_1 with three different bacteria.

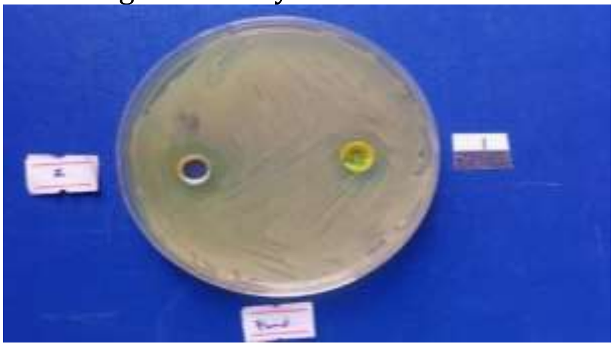


Figure 18. The biological activity of Sb_2 with three different bacteria.

Each of (Sb_1) and (Sb_2) was shows reasonable activity against *Escherichia Coli*. But, the (Sb_1) did not have any affect in the *Staphylococcus Aureus* and *Pseudomonas Aeruginosa* in contrast with (Sb_2), which gave equal activity towered *Staphylococcus Aureus* and *Pseudomonas Aeruginosa,* (Tables 2 and 3).

ID.	INHIBITION ZONE (MM)		
	Escherichia coli	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
(Sb_1)	30	...	...

Table 2. The biological activity of (Sb_1).

ID.	INHIBITION ZONE (MM)		
	Escherichia coli	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
(Sb_2)	24	15	15

Table 3. The biological activity of (Sb_2).

Tables (2) and (4) above illustrates, that the reactivity of each of (Sb_1) and (Sb_2) were realistic against *Escherichia Coli*, (Figure 19).

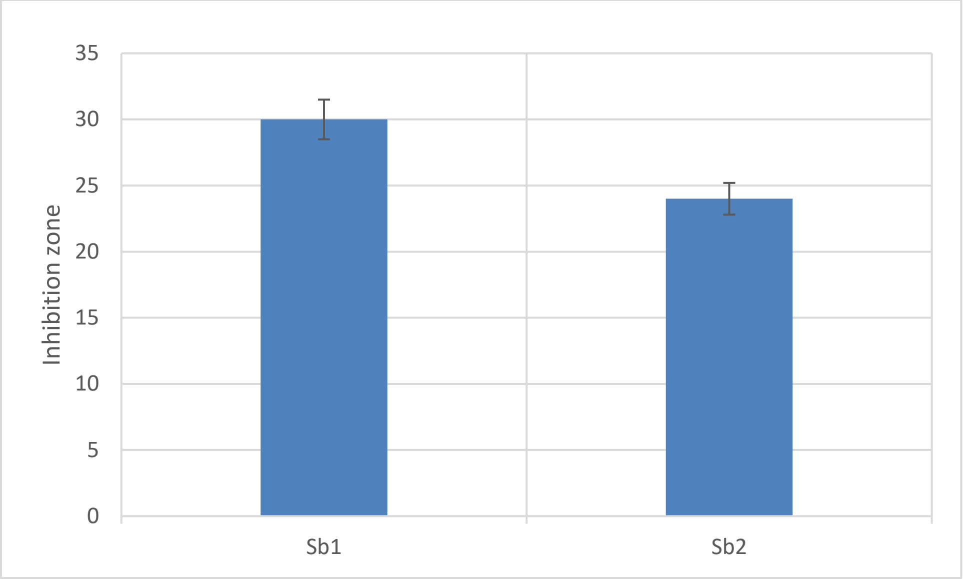


Figure 19. The biological activity of (Sb1) and (Sb2) against *Escherichia Coli*.

Figure (19) above shows, that the biological activity of each of (Sb1) and (Sb2) were variable towered *Escherichia Coli*. in depend on its chemical structure.

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