



## Using 5-(4-aminoantipyrineazo)-8-hydroxyquinoline as Complexometry Reagent with Copper (II) and Zinc (II) Ions and its Biological Activity

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### Abstract

The complexes of azo dye 5-(4-aminoantipyrineazo)-8-hydroxyquinoline (L) with copper (II) and zinc (II) ions were synthesized. Characterization of these azo dyes complexes have been done on the basis of elemental analysis and IR techniques. The work involves a study of optimum conditions (time, pH, sequence of addition and temperature effects) for forming the complexes. The spectra of the complexes have been studied for a range of concentrations which Lambert – Beer's law were obeyed with sensitivity of the spectrophotometric method in terms of molar absorptivity  $1.6 \times 10^4$  and  $1.2 \times 10^4 \text{ l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$  for zinc and copper azo dyes complexes respectively. The stoichiometry of the complexes has been found to be 1:2 (metal : ligand). The overall stability constants were determined by the corresponding solutions method. The interferences effects of the foreign ions were studied. The biological activity of two complexes with two type of Bacteria (*Clostridium botulinum* and *Escherichia Coli*) were also studied.

**Keywords:** Azo dye complexes; Solvent effects; Stability constant; Biological activity



## Council for Innovative Research

Peer Review Research Publishing System

**Journal:** Journal of Advances in Chemistry

Vol. 11, No. 1

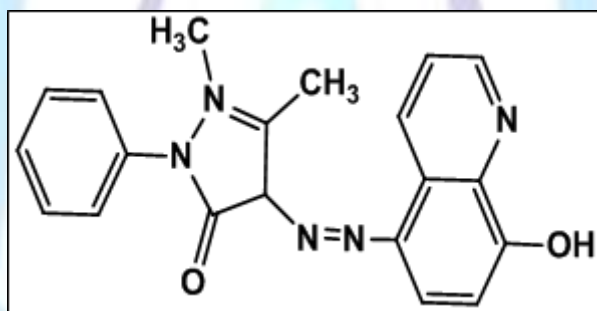
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## Introduction

Azo compounds are a very important class of chemical compounds receiving attention in scientific research. They are highly colored and have been used as dyes and pigments for a long time. Furthermore, they have been studied widely because of their excellent thermal and optical properties in applications [1]. Azo derivatives complex combinations have been widely used as dyes for synthetic polyamide supports and as pigments [2]. Azo derivatives and their metal complexes are very important pigments for synthetic leather and vinyl polymers and used as analytical reagent of many metals ions [3]. On the other hand, azo compounds are known to be involved in a number of biological reactions, such as inhibition of DNA, RNA, and protein synthesis, nitrogen fixation [4,5]. Furthermore metal-azo complex dyes are used in the recording layer of DVD-R ( Digital Versatile Disc-Recordable) discs . In comparison with the dyes themselves, metal-azo dyes are more light stable, allow for easier control of the wavelength by selection of the appropriate substituent groups and have good thermal stability [6,7]. The systematic name of 4-aminoantipyrene is 4-amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one or 4-amino-phenazone which is widely used in medicine and pharmaceutical fields [8]. The antimicrobial activity of the 4-aminoantipyrene and its complexes (  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$  ) have been extensively studied on microorganisms such as Staphylococcus aureus, Klebsiella pneumonia, Bacillus subtilis, Escherichia coli, Citrobacter ferundii and Salmonella typhi , most of the complexes have higher activities than that of the free ligand [9] . The azo dyes compounds have been used as antiseptic and antibiotic [10] and also used as drugs , like 3-phenylazopyridine-2,6-diyl diamine[ 11] and salicylic azo sulfopyridine [12]. The sulfa azo dyes derived from 1-hydroxy-2-naphthoic acid were prepared as antibiotic and complexing reagents [ 13,14] . A azo compound, 8-quinolinol-5-azoantipyrene, was studied as an inhibitor for the corrosion of mild steel in 1M HCl [15]. The 7-arylazo-8-hydroxyquinoline is a good reagent for forming a stable complexes with dioxouranium (VI) [ 16] and Cu(II) [17] . Zinc (II) ions were determined spectrophotometrically with 7-(4-Nitro phenylazo )-8-hydroxyquinoline-5-sulfonic acid ) [ 18] .

The present work involves the use of azo dye 5-(4-aminoantipyreneazo)-8-hydroxyquinoline ( which was prepared previously in part I (Scheme 1 )) [19] for the formation of complexes with copper (II) and zinc (II) ions under optimum conditions (time , pH , sequence of addition and temperature effects ) . Study the ability of these complexes as biological reagents for two types of Bacteria ( Clostridium botulinum and Escherichia Coli).



Scheme 1

## Experimental

Double distilled water and 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 K Br pellets) were recorded on IR-8400S shimadzu , Melting points were determined on melting point apparatus , Element analysis (C.H.N.) were carried out by Perkin element 2400 clement analysis and UV/V is absorption spectra studies of the dyes were recorded using Perkin Elmer Lampda EZ 210 UV/V Spectrophotometer. The pH measurements were made with pH-Meter (H. Jurgons Co. Bremen,L. Puls Munchen15). The Cu(II) and Zn(II) ions were determined by atomic absorption spectrophotometer U.K., Pg. instruments AA500 .

## Solutions

- A stock solution of ( $1 \times 10^{-3}$  M) of azo dye L were prepared by dissolving an accurately weighed amount of the compounds in the ethanol and more dilute solution were obtained by accurate dilution.
- A stock solution of ( $1 \times 10^{-3}$  M) of each  $\text{Cu}(\text{NO}_3)_2 \cdot 3 \text{H}_2\text{O}$  and  $\text{Zn}(\text{NO}_3)_2 \cdot 6 \text{H}_2\text{O}$  were prepared by dissolving the accurate weights in required volume of distilled water , and more dilute solution were obtained by accurate dilution.
- Acetate (pH<sub>1-2</sub>) and Universal ( pH<sub>2-12</sub> ) and buffer solutions [20] were prepared .

## Synthesis of the solid complexes

Azodyes complexes of Cu(II) and Zn(II) were prepared in a similar manner as described [21]. Thus, (0.004 mol) of each metal ions was added to a hot solution of the dyes (0.008 mol) in ethanol (70 ml) and the resulting mixture was refluxed for 1 h. A dark violet precipitates separated, which were collected by filtration, and recrystallized from mixture of ethanol : chloroform (1:3 v/v) .



## Results and Discussion

The analytical and physical data of the L-Cu<sup>2+</sup> and L-Zn<sup>2+</sup> complexes are presented in Table 1. Elemental analysis and continues variation method of the complexes indicate the stoichiometry to be 1: 2 (metal: ligand).

Table 1: Analytical and physical data of complexes.

Comp	Molecular Formula ( M.wt.)	Color	M.P °C	Elemental Analysis Found (Calc.)				Cond. S cm <sup>2</sup> mol <sup>-1</sup>
				%C	%H	%N	M%	
L-Cu <sup>2+</sup>	C <sub>40</sub> H <sub>32</sub> N <sub>10</sub> O <sub>4</sub> Cu ( 779.5)	Violet	260	61.33 (61.58)	3.09 (4.11)	18.01 ( 17.96)	8.16 (8.14)	15.6
L-Zn <sup>2+</sup>	C <sub>44</sub> H <sub>42</sub> N <sub>10</sub> O <sub>6</sub> Zn (871.4)	Violet	285	61.43 (60.59)	4.98 (4.82)	16.44 (16.07)	7.62 (7.51)	18.02

The IR has proven to be, in this particular case, a suitable technique to give enough information to elucidate the way of bonding of the ligands. Thus a detailed interpretation of IR spectra of these and the effect of binding of metal ions on the vibration frequencies of the free dye ( Table 2).

Table -2: The I.R spectral data of ( KBr disk )

Comp.	$\nu(\text{OH})$	$\nu(\text{N}=\text{N})$	$\nu(\text{C}=\text{O})$	$\nu(\text{C}=\text{N})$	$\nu(\text{M}-\text{O})$	$\nu(\text{M}-\text{N})$
L	3402	1470	1660	1620	--	-
L-Cu <sup>2+</sup>	3990	1470	1670	1590	510	410
L-Zn <sup>2+</sup>	3988	1470	1665	1595	495	425

The IR spectrum of the ligand (Fig.(1)) exhibited broad band at (3402 cm<sup>-1</sup>) was assigned to the stretching vibration of  $\nu(\text{OH})$  of the carboxyl and phenol[22,]. Strong band in the ligand spectrum was observed at (1660 cm<sup>-1</sup>) ascribed to the  $\nu(\text{C}=\text{O})$  for the carboxyl group. Significant change in the position to higher frequency was also observed on complexation with metal ion. The strong band in the free ligand spectrum at (1604 cm<sup>-1</sup>) due to  $\nu(\text{CO})$  asymmetric vibration, significant change in the intensity and in position to lower frequency was observed on complexation with metal ion.

The IR spectra showed that values of  $\nu(\text{N}=\text{N})$  stretching frequency (1501 cm<sup>-1</sup>) in dye LR1R and its synthesized complexes and (1504 cm<sup>-1</sup>) in dye LR2R and its synthesized complexes remain practically unchanged indicating that  $\nu(\text{N}=\text{N})$  group have no part in coordination whereas the carbonyl group  $\nu(\text{C}=\text{O})$  stretching frequency (1668 cm<sup>-1</sup>) in dye LR2R have unchanged values as well, indicating that carbonyl group is not taking part in coordination. However, the high changing in the shape and the position of  $\nu(\text{O}-\text{H})$  stretching and  $\nu(\text{O}-\text{H})$  in plane bending and  $\nu(\text{O}-\text{H})$  out of plane bending and changing in the position of  $\nu(\text{C}=\text{N})$  stretching in pyridyl group as compared to spectra of free ligands indicate that (O-H group and N atom) in 8-hydroxyquinoline are involved in coordination between azo-dye (LR1R) with their synthesized complexes. In addition, in the complexes (, LR1R-Cu; L<sub>1</sub>-Zn) we obtained two new bands in every complex and they are assigned to M-N stretching , M-O stretching frequency the IR spectra of the Zinc (II) and Copper (II) complexes indicate that phenolic OH has deprotonated during complexation. Band due to  $\nu(\text{O}-\text{H})$  becomes further broadened and increased in intensity in the complexes indicating non participation of phenolic oxygen in complexation. The broadening of  $\nu(\text{O}-\text{H})$  vibrations may be due to the overlapping with absorption due to lattice water coordinated water [22]

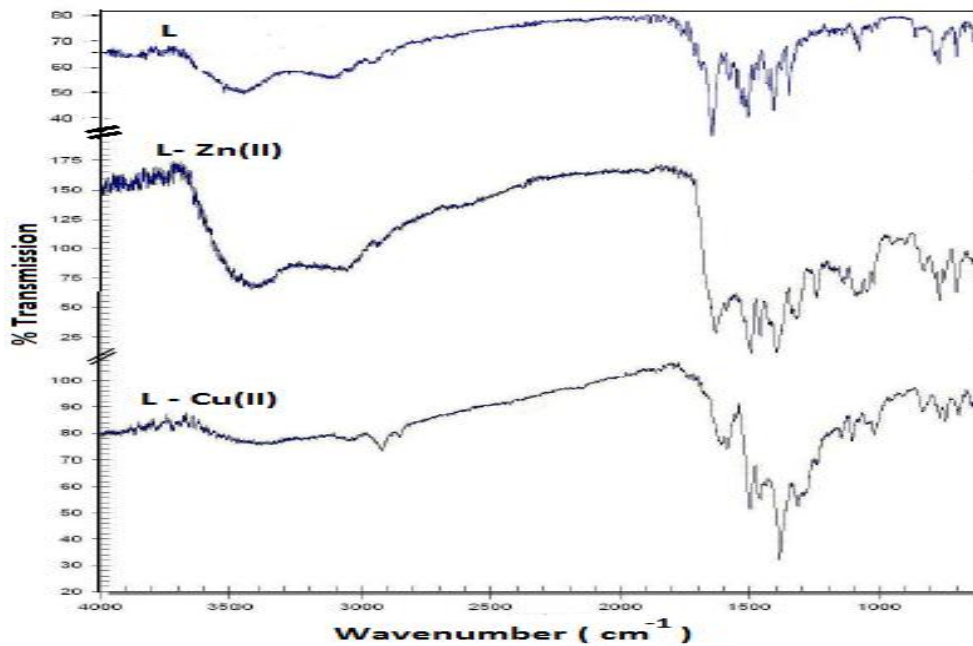


Fig-1: IR spectra of  $L_1$  and its complexes

### Complex formation studies

The complexes of  $Cu^{2+}$  and  $Zn^{2+}$  ions with L solutions were always performed. These complexes were identified optimum conditions for the composition of these complexes (time, pH, temperature and sequence of addition effects).

#### *pH* – effect

The absorption spectra of  $L-Cu^{2+}$  and  $L-Zn^{2+}$  were studied at pH values range (0.65 – 12) (Fig. 2), by using acetate and universal buffer solutions in the range of wavelength 250 – 600 nm. It was found the pH 12 is the suitable value for giving highest absorbance due to the formation of basic form (anionic form) which gives essayer condition for attraction with cations.

The interaction of  $Cu^{2+}$  and  $Zn^{2+}$  ions with the dye (L) manifest itself in the absorption spectra by the appearance of a peak in the range (510 and 480 nm) for  $L-Cu^{2+}$  and  $L-Zn^{2+}$  complexes respectively. A great red shift in the visible region was detected in the complex solutions spectra with respect to that of the free dye ( $\lambda_{max} = 390$  nm.). The shift in the ( $\lambda_{max}$ ) gave a good indication for complex formation. (Fig.-3).



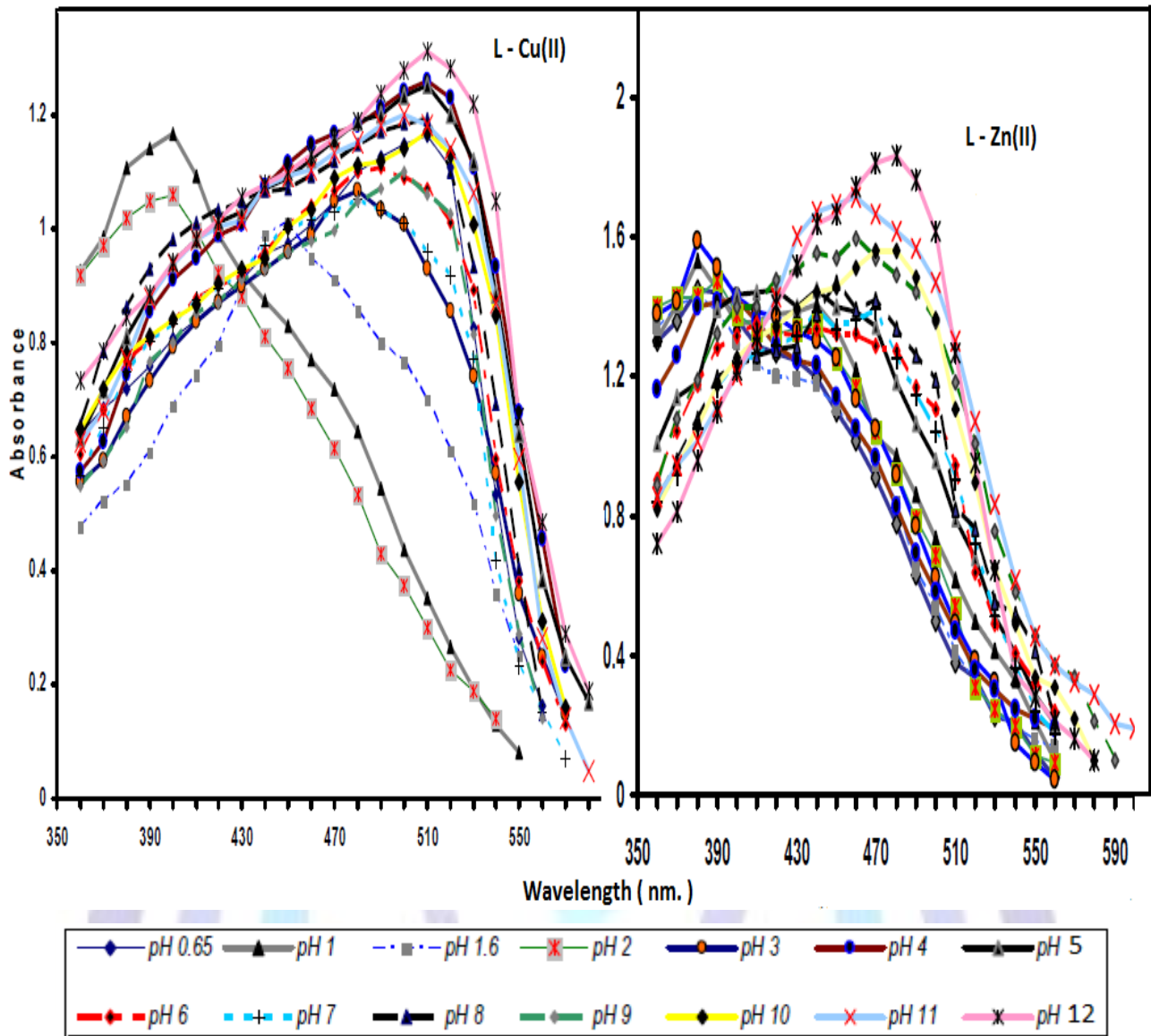


Fig.-2: Absorption spectra of L-Cu<sup>2+</sup> & L-Zn<sup>2+</sup> complexes at different pH values, [Cu<sup>2+</sup>]=[Zn<sup>2+</sup>] = [L] = 8x10<sup>-5</sup> M

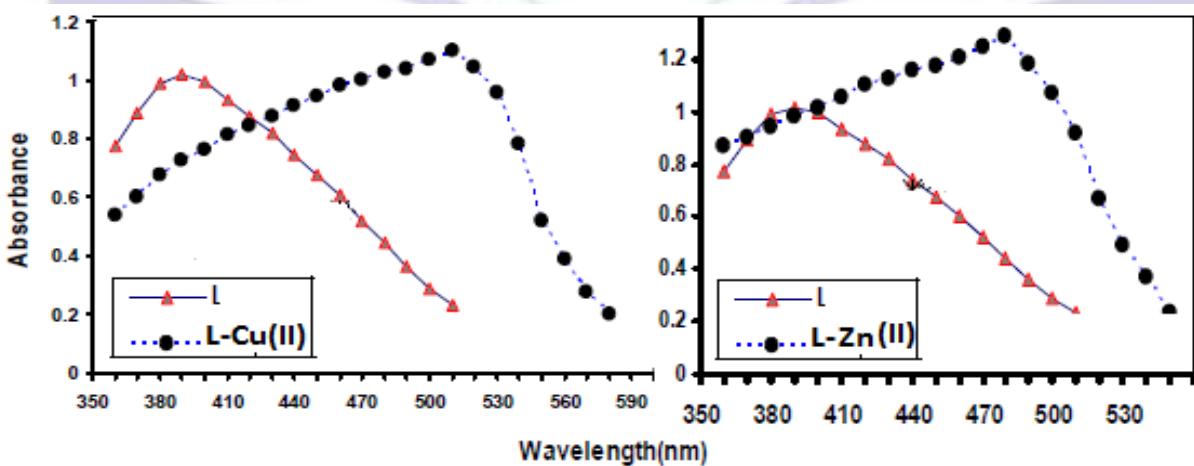


Fig.-3 : Absorption spectra of dye L and its complexes at different pH 12, [Cu<sup>2+</sup>] = [Zn<sup>2+</sup>] = 8x10<sup>-5</sup> M, [L] = 1.6 x 10<sup>-4</sup> M



### Time effect

It was found from Fig. (4) the absorbencies are constant from 15 min up to 48 hrs. that means the complex is of high stability .

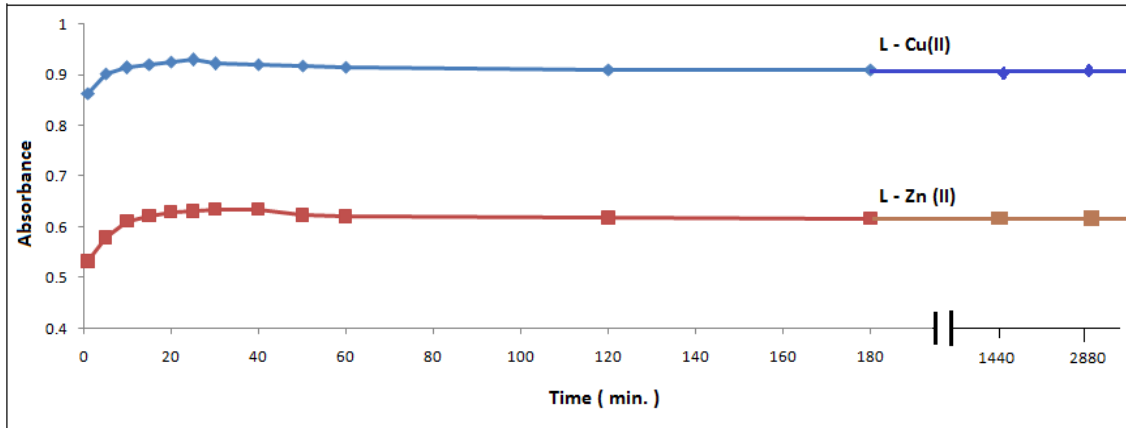


Fig.-4 : Effect of time on the absorbance of azo dyes complexes at  $\lambda_{max}$  of each complex  
 $[Cu^{2+}] = [Zn^{2+}] = [L] = 8 \times 10^{-5} M$

### Sequence of additions

It was found from Table – 3, that the sequence (Dye + pH<sub>12</sub> + M<sup>2+</sup>) having heighest absorbance value of the azo dyes complexes . This attributed to the effect of alkaline medium of buffer solution ( anionic form ) , which makes the dye suitable for accepting metal ions .

Table-3: Sequence of addition of reagents ( [ L ] = [ Cu<sup>2+</sup> ] = [ Zn<sup>2+</sup> ] = 8 x 10<sup>-3</sup> M

	Sequence of addition	Absorbance at $\lambda_{max}$	
		L – Cu <sup>2+</sup>	L – Zn <sup>2+</sup>
1	Dye + M <sup>2+</sup> + pH <sub>12</sub>	0.732	1.003
2	Dye + pH <sub>12</sub> + M <sup>2+</sup>	0.821	1.105
3	M <sup>2+</sup> + pH <sub>12</sub> + Dye	0.791	1.033

### Temperature effect

Fig- 5 shows the effect of temperature range ( 10 – 90 C<sup>o</sup> ) on the absorbance of each complex . It was found that, the complexes be stable up to 40 C<sup>o</sup> ( i.e. very slightly change in absorbance ) .Up this temperature (40 C<sup>o</sup> ) the complexes are unstable may be for decomposition of complexes .

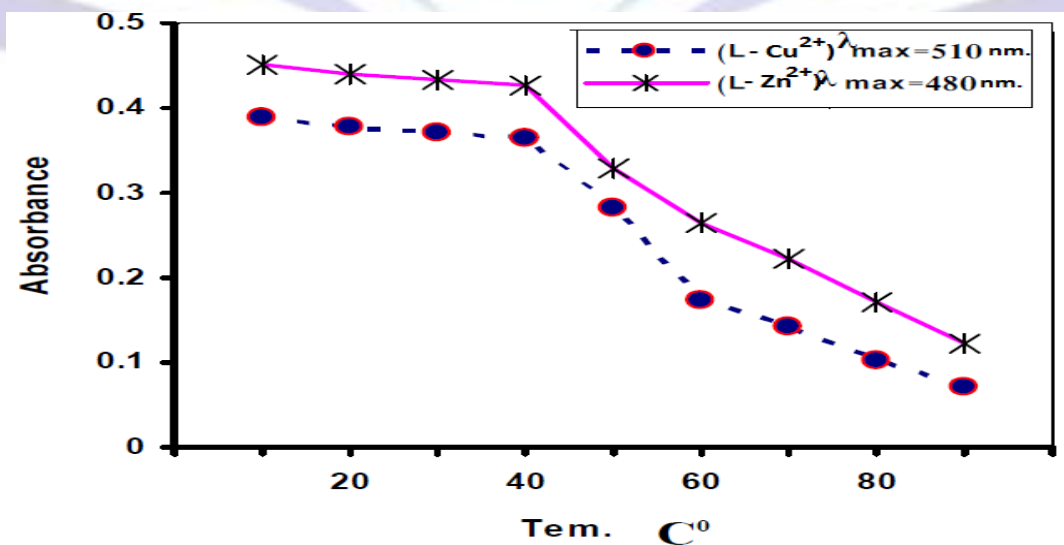


Fig. – 5: Temperature effect on the absorbance of azo dyes complexes  $[Cu^{2+}] = [Zn^{2+}] = [L] = 8 \times 10^{-5} M$



### The composition of the complexes (stoichiometry)

The composition of the complexes formed has been established by continues variation and mole ratio methods [23]. From Figs.-6 & 7 , it was found that the ( $M^{2+}$  : azo dye) equal to (1 : 2) .

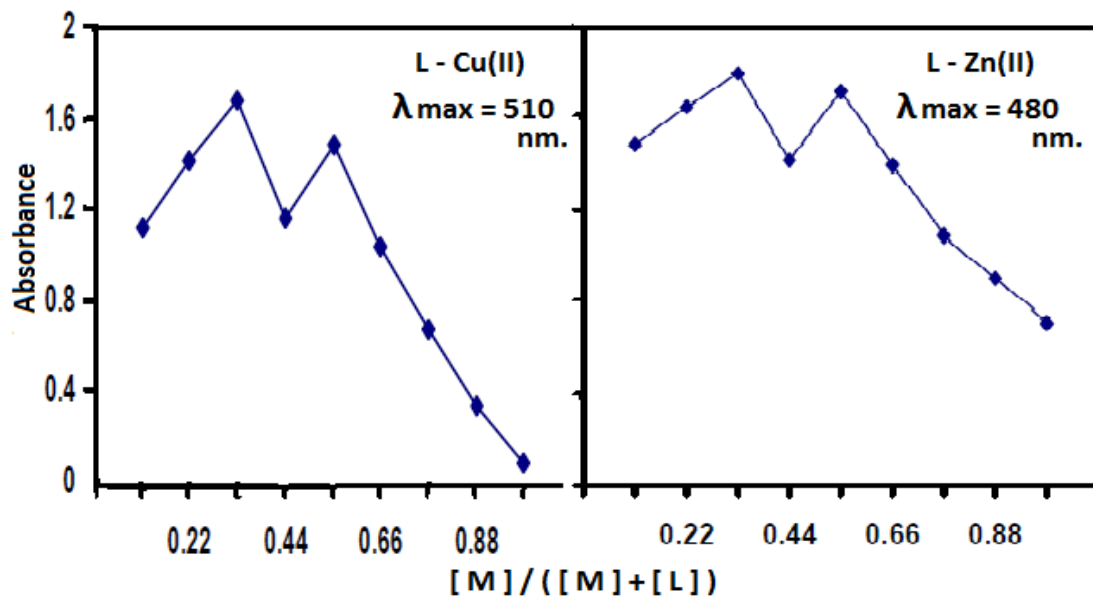


Fig.-6: Continues variation method for azo dyes complexes

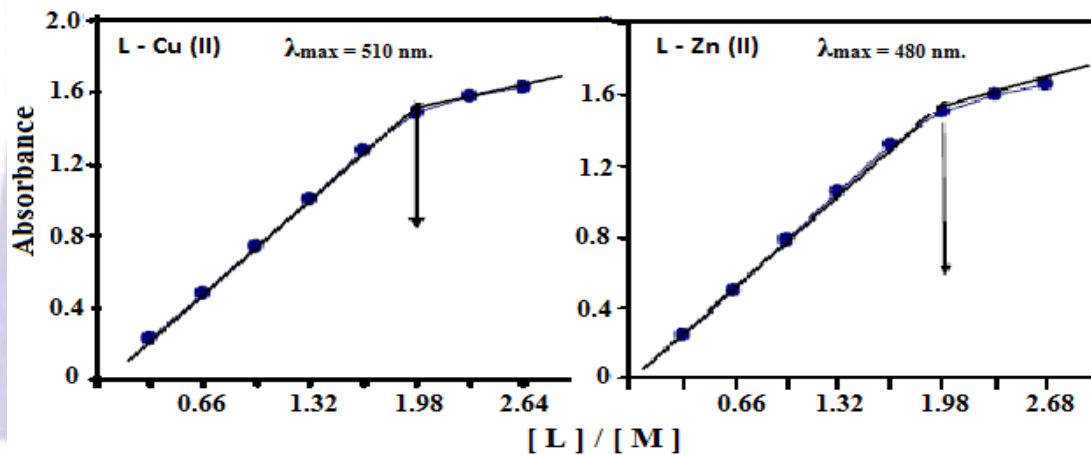


Fig.-7: Mole ratio method for azo dyes complexes

### Beer's law and sensitivity

To get better results for beer's law , the optimum blank composition technique [ 23 ] was applied ( i.e. using unreacted dye as blank solution from knowledgement of stoichiometry of complexes )

The calibration curve ( Fig.- 8 ) shows that Beer's law is obeyed up to concentration range  $8.87$  and  $7.80 \mu\text{g ml}^{-1}$  for Cu(II) and Zn(II) in  $L\text{-Cu}^{2+}$  and  $L\text{-Zn}^{2+}$  complexes respectively . Table ( 4 ) shows the data obtained , that represented by the molar absorptivity coefficient ( $\epsilon$ ,  $L\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$ ) and sensitive index ( $S$ ,  $\mu\text{g}\cdot\text{cm}^{-2}$ ), and the detection limit ( DL  $\mu\text{g}\cdot\text{ml}$  ) of the complexes. With high precision that represented by the results of standard deviation (S.D), and high linearity of Beer's law which represent by the correlation coefficient ( $r$ ) which is nearly to unity. From a table, it was found the method of high precision and high sensitivity.



Table –4: Some results obtained from Beer's law

Complex	$\epsilon \times 10^4$ l/mol.cm	S.D	S $\mu\text{g/ml}$	r	Beer's law limit ppm
L-Cu(II)	1.2	0.010	0.0053	0.9857	0.131 – 8.87
L-Zn(II)	1.6	0.012	0.0042	0.9459	0.189 – 7.80

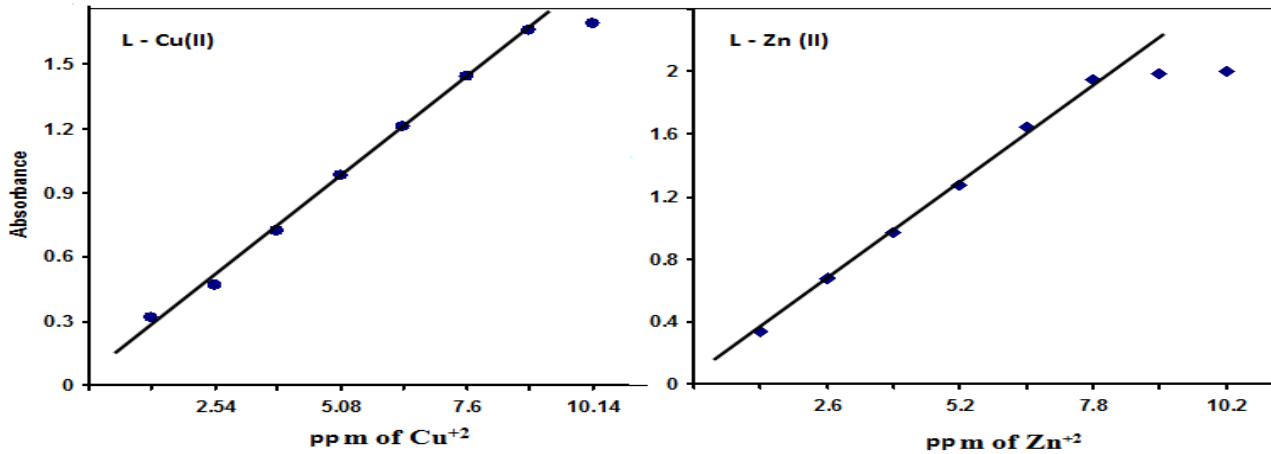


Fig-8: The calibration curves of L-Cu<sup>2+</sup> and L-Zn<sup>2+</sup> complexes at  $\lambda_{\text{max}}$

### Interference effect of foreign ions

It was so important to study the interference the effect of foreign ions in different ranges of concentration . From Table- 5 , it was found that ions ( Cl<sup>-</sup>,Pb<sup>+2</sup>,Bi<sup>+3</sup>,Cd<sup>+2</sup>,Na<sup>+</sup>,Ca<sup>+2</sup>,Ni<sup>+2</sup>,Mg<sup>+2</sup> , Co<sup>+2</sup> , VO<sub>3</sub><sup>5+</sup>, tartaric acid , ascorbic acid , citric acid , and sodium thiosulphate ) did not effected on the absorbance (that means not interfered ) of L- Cu<sup>2+</sup> complex in all ranges of concentrations . While the other ions ( Ag<sup>+</sup> , Al<sup>+3</sup>,Fe<sup>+2</sup>,Fe<sup>+3</sup> , Cr<sup>+3</sup>,Zn<sup>+2</sup> and EDTA ) were done.

In case of L- Zn<sup>2+</sup> complex , the ions (Cl<sup>-</sup>,Pb<sup>+2</sup>,Bi<sup>+3</sup>,Cd<sup>+2</sup>,Na<sup>+</sup>,Ca<sup>+2</sup>,Ni<sup>+2</sup>, Mg<sup>+2</sup> VO<sub>3</sub><sup>5+</sup>, tartaric acid , ascorbic acid , citric acid , and sodium thiosulphate) were not interfered in all ranges of concentrations . while the other ions ( Ag<sup>+</sup> , Al<sup>+3</sup>,Fe<sup>+2</sup>,Fe<sup>+3</sup> , Cr<sup>+3</sup>,Co<sup>+2</sup> , Cu<sup>+2</sup> and EDTA ) were interfered in all ranges of concentrations ,except for ions (Al<sup>+3</sup>,Fe<sup>+2</sup>,Fe<sup>+3</sup> and EDTA ) were interfered only in 5-fold and 10 – fold concentrations .

Table – 5: The absorbance of complexes in presence of foreign ions ( [Zn<sup>2+</sup>]=[Cu<sup>2+</sup>]=[L] =8x10<sup>-5</sup> M

Interfering ions	Absorbance of L-Cu(II) ,510 nm.			Absorbance of L-Zn(II) , 480 nm.		
	1 - fold	5 - fold	10 - fold	1 - fold	5 - fold	10 - fold
Ag <sup>+</sup>	0.921	0.9431	0.974	1.311	1.295	1.354
Al <sup>+3</sup>	0.941	0.955	0.975	1.245	1.277	1.301
Co <sup>+2</sup>	0.741	0.752	0.778	1.301	1.311	1.335
Cr <sup>+3</sup>	0.902	0.919	0.945	0.940	0.972	1.012
Fe <sup>+3</sup>	1.012	1.093	1.145	1.245	1.301	1.348
Fe <sup>+2</sup>	0.941	0.978	1.081	1.241	1.281	1.309
Mg <sup>+2</sup>	0.830	0.805	0.805	1.121	1.142	1.131
Ni <sup>+2</sup>	0.819	0.824	0.851	1.109	1.108	1.107
Ca <sup>+2</sup>	0.807	0.813	0.810	1.101	1.107	1.105
Na <sup>+</sup>	0.801	0.801	0.803	1.103	1.109	1.107





Cl <sup>-</sup>	0.820	0.822	0.830	1.104	1.045	1.047
Cd <sup>+2</sup>	0.740	0.790	0.780	1.045	1.055	1.043
Bi <sup>+3</sup>	0.801	0.780	0.790	1.100	1.051	1.030
Pb <sup>+2</sup>	0.817	0.810	0.809	1.101	1.097	1.109
VO <sub>3</sub> <sup>5+</sup>	0.811	0.815	0.822	1.109	1.151	1.110
EDTA	1.013	1.071	1.100	1.230	1.291	1.310
Sodium thiosulphate	0.815	0.811	0.819	1.101	1.107	1.108
Tartaric acid	0.812	0.813	0.821	1.103	1.105	1.102
Citric acid	0.817	0.815	0.812	1.101	1.108	1.109
Ascorbic acid	0.809	0.811	0.823	1.102	1.105	1.103
Zn <sup>+2</sup>	0.981	1.1	1.18	-	-	-
Cu <sup>+2</sup>	-	-	-	1.32	1.37	1.47

The absorbance of L - Cu (II) complex in absence of foreign ions = 0.820

The absorbance of L - Zn (II) complex in absence of foreign ions = 1.105

### Masking of interfering ions

If each of the following interfering ion (Ag<sup>+</sup>, Al<sup>+3</sup>, Cr<sup>+3</sup>, Zn<sup>+2</sup>, Fe<sup>+2</sup> and Fe<sup>+3</sup>) is present separately with Cu<sup>2+</sup> or Zn<sup>2+</sup> ion, 0.01 mole of the following masking agents ( sodium thiosulphate, tartaric acid , ascorbic acid , citric acid , sodium thiosulphate and sodium thiosulphate) respectively . In case of EDTA, 0.15 mmole of (VO<sub>3</sub>)<sup>+5</sup> was added.

It was found that the absorbance of L-Cu<sup>2+</sup> and L-Zn<sup>2+</sup> complexes were affected by  $\pm 0.009$  and  $\pm 0.007$  respectively.

### The stability of the complexes

The stability constants (formation constant) of complexes Cu<sup>2+</sup> and Zn<sup>2+</sup> ions with L were determined by using Corresponding solutions method [25] , which depends on aid of half- value method [26]. This method required two series of solutions of total metal ion concentration C<sub>1M</sub> = 8 x 10<sup>-4</sup> M ( concentrated series ) and C<sub>2M</sub> = 4 x 10<sup>-4</sup> M (diluted series ) , where C<sub>1M</sub> > C<sub>2M</sub> and varying ligand concentrations C<sub>1L</sub> (0.2 – 2 x 10<sup>-4</sup>) and C<sub>2L</sub> (0.1 – 1.2 x 10<sup>-4</sup> M) . Then the absorbance of diluted series was multiplied by the factor (C<sub>1M</sub> / C<sub>2M</sub> which equal 2) . The corresponding solutions are those which have the same absorbance at different ligand ( dye ) concentration. From the absorbance - C<sub>L</sub> plots ( Fig.-9) , many pairs of C<sub>1L</sub> and C<sub>2L</sub> consequently,  $\bar{n}$  (complex formation function) and [L] (free ligand concentration) can be determined where :

$$\bar{n} = (C_{1L} - C_{2L}) / (C_{1M} - C_{2M}) \quad \text{and} \quad [L] = (C_{1M} C_{2L} - C_{2M} C_{1L}) / (C_{1M} - C_{2M})$$

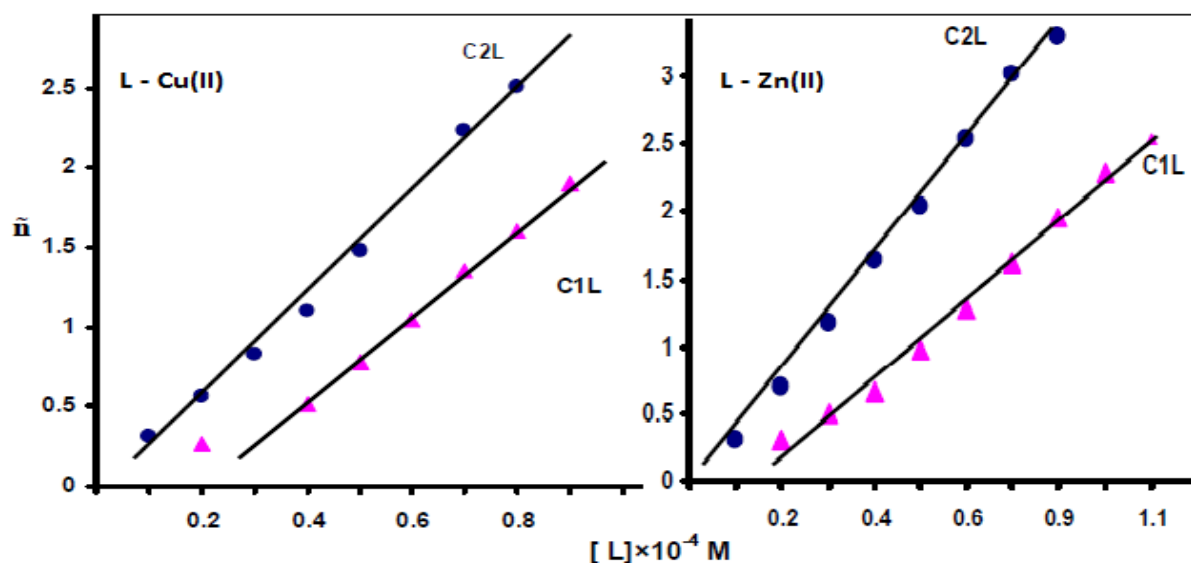


Fig.-9 : Absorbance – C<sub>L</sub> plot of complexes Cu<sup>2+</sup> and Zn<sup>2+</sup> ions with L at  $\lambda_{max}$  of each of them

Then  $\bar{n} - pL$  was plotted (Fig.-10) and by using the half value method, the  $\log \beta_1$  and  $\log \beta_2$  are obtained (Table -6). When  $\bar{n} = 0.5$  gives  $\log \beta_1$  ( $\log K_1$ ) and  $\bar{n} = 1.5$  gives  $\log \beta_2$  ( $\log K_1 + \log K_2$ )

(Where  $K$  and  $\beta$  are step stability and overall stability constants).

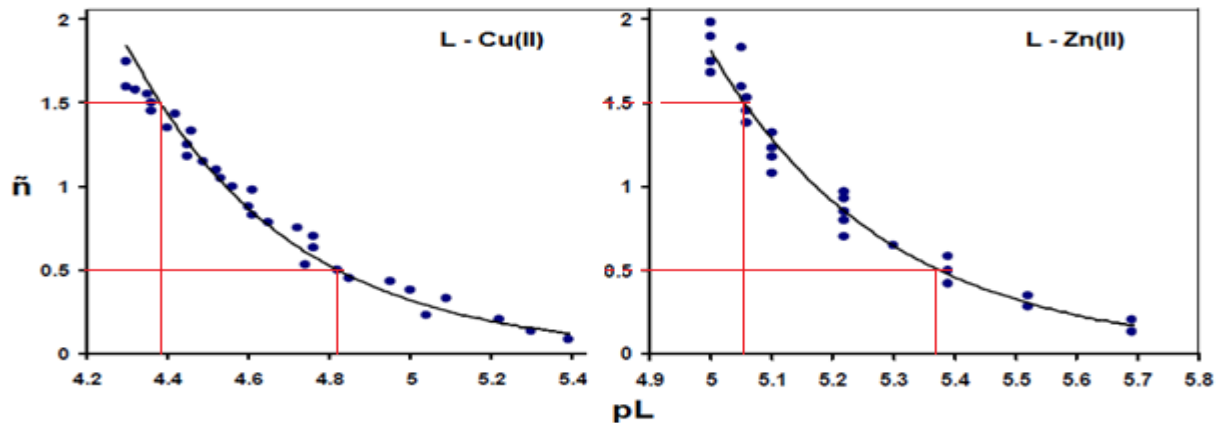


Fig.-10:  $\bar{n} - pL$  curves for complexes  $Cu^{2+}$  and  $Zn^{2+}$  ions with L

Table -6: Log  $K$  and Log  $\beta$  values for complexes of  $Cu^{2+}$  and  $Zn^{2+}$  ions with L complexes

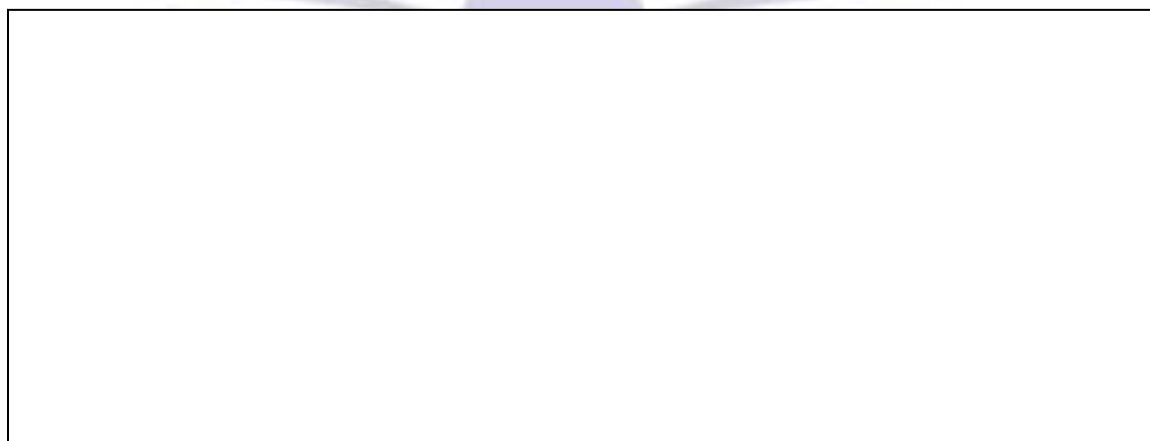
Log $K$ and Log $\beta$ Values	Complex	
	L - Cu(II)	L - Zn(II)
Log $K_1 = \log \beta_1$	4.38	5.06
Log $K_2$	4.88	5.37
Log $\beta_2$	9.26	10.43

It was found from Table -6, the sequence of azo dyes complexes stability are  $L-Zn(II) > L - Cu(II)$  and also found that  $\log K_1$  is nearly equal to the  $\log K_2$ , that means the azo dyes complex reaction is spontaneous and happened in one step.

### Structural interpretation

From the C.H.N. analysis, Atomic analysis for Cu and Zn, IR technique and conductivity measurements, the structure of complexes  $L-Zn(II)$  and  $L - Cu(II)$  were suggested. The  $Zn^{2+}$  and  $Cu^{2+}$  ions have been coordinated through phenolic O atom and N atom of pyridine of 8-hydroxyquinoline of the azo dye L by forming square planer structure with respect to  $Cu^{2+}$  ions and octahedral structure with respect to  $Zn^{2+}$  ions ( in case of  $L-Zn$  complex two molecules of ethanol have been coordinated with metal ion ). Consequently, the proposed general structures are shown in

Schemes 2.



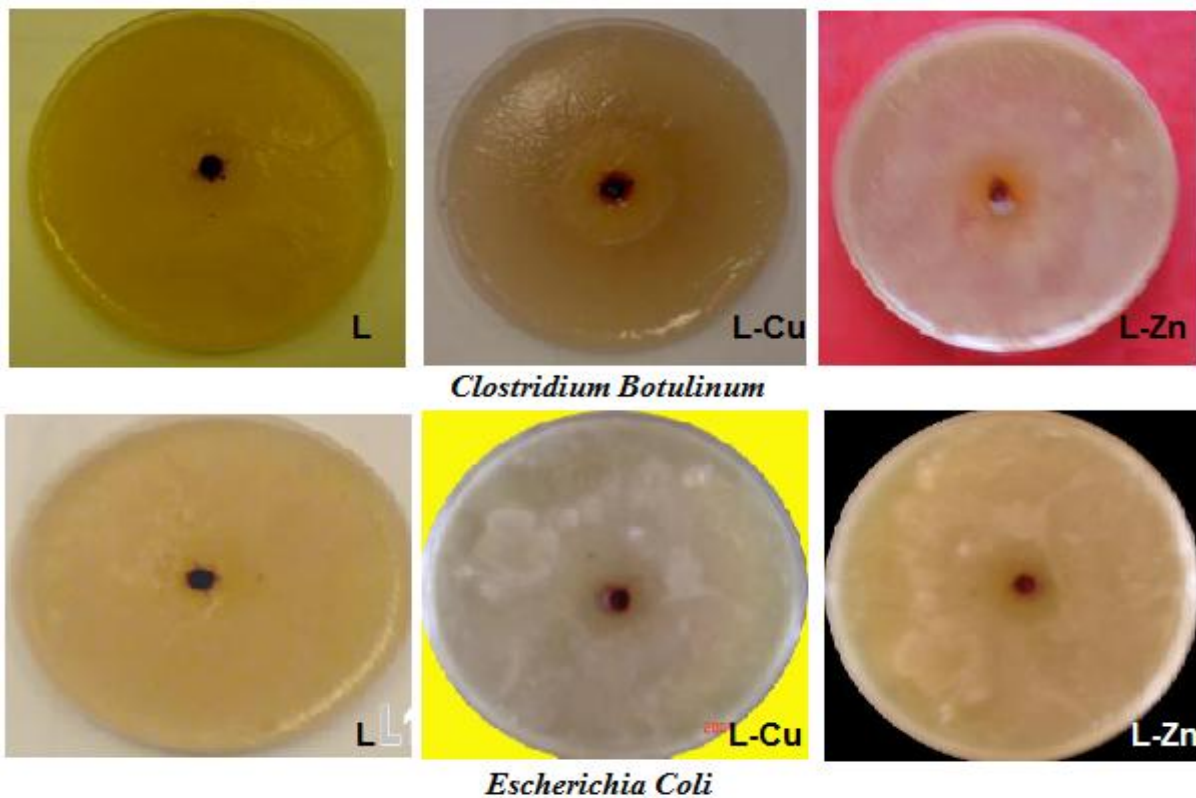
Scheme 2

### Biological activity of the complexes

Table -7 and Fig. (11) show the comparison of biological activities of the dye ( L ) and its complexes with  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$  ions on two types of bacteria (*Escherichia Coli* and *Clostridium Botulinum*). It was found that the dye and its complexes having only biological activity on *Clostridium Botulinum*, while no effect on *Escherichia Coli*, this because may be thickness of cell's wall slightly hard ( high lipidely ) that resists the chemical substances to enter the cell. It was also found that quince of biological activity of free dye, L- Cu(II) complex and L- Zn(II) complex as; L – Cu(II) > L – Zn(II) > Dye ( L ) in two concentrations 1 and 5  $\text{mg.ml}^{-1}$ .

**Table – 7 : The biological activity of free dye and its complexes on *Escherichia Coli* and *Clostridium Botulinum* with diameter (in cm) of effected zone**

Compound	Concentration $\text{mg.ml}^{-1}$	<i>Clostridium Botulinum</i> ( cm )	<i>Escherichia Coli</i> ( cm)
Dye ( L )	1	1.5	0
L-Cu(II)	1	2.9	0
L-Zn(II)	1	2.2	0
Dye ( L )	5	2	0
L-Cu(II)	5	3.5	0
L-Zn(II)	5	2.8	0



**Fig.-11 : The effect of Dye and its complexes on *Escherichia Coli* and *Clostridium Botulinu* in case of concentration of 1  $\text{mg.ml}^{-1}$**



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