

## Synthesis , Spectroscopic and Antibacterial Studies of Zinc(II) Complexes Derived from Salicylaldehyde, Leucylalanine and Glycylglycine

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### Abstract:

Two Schiff base ligands  $L_1$  and  $L_2$  have been obtained by condensation of salicylaldehyde respectively with leucylalanine and glycylglycine then their complexes with Zn(II) were prepared and characterized by elemental analyses , conductivity measurement , IR and UV-Vis .The molar conductance measurement indicated that the Zn(II) complexes are 1:1 non-electrolytes. The IR data demonstrated that the tetradentate binding of the ligands  $L_1$  and  $L_2$  . The in vitro biological screening effect of the investigated compounds have been tested against the bacterial species *Staphylococcus aureus*, *Escherichia coli* , *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa* by the disc diffusion method . A comparative study of inhibition values of the Schiff base ligands and their complexes indicated that the complexes exhibit higher antimicrobial activity than the free ligands . Zinc ions are proven to be essential for the growth-inhibitor effect. The extent of inhibition appeared to be strongly dependent on the initial cell density and on the growth medium .

**Key words:** Dipeptide , Schiff base, Antibacterial, Zn(II ) complexes

### Introduction:

The coordination chemistry of amino acid Schiff base is of considerable interest due to their biological importance [1,2]. However, little attention has been paid to systems in which the Schiff bases derived from simple peptides. A vanadium complex  $VO(\text{sal-glygly})(\text{H}_2\text{O})_n(\text{sal-glygly} = \text{N-salicylidene-glycylglycine})$ ;  $n=1.5-3.0$  has been isolated from relatively concentrated solutions containing oxovanadium (IV) , glycylglycine and salicylaldehyde [3]. Synthesis , crystal structure and magnetic studies of cis-configuration copper (II) – M(II) (M = Ba , Ca ) complexes of the sal – glygly Schiff base have been determined [4] . Sallam and coworkers have been prepared and characterized the uranyl complexes of Schiff base obtained by

condensing glygly with hydroxybenzaldehyde [5]. Recently , two copper (II) tripeptide Schiff base complexes:  $[\text{Mg}(\text{H}_2\text{O})][\text{CuL}]2.3.5\text{H}_2\text{O}$  and  $[\text{Cd}(\text{H}_2\text{O})_4(\text{CuL})_2].3.5\text{H}_2\text{O}$  ( $\text{H}_3\text{L} = \text{N-sal-glygly}$ ) have been synthesized and structurally characterized [6]. Also, Binuclear copper, nickel and cobalt of the Schiff bases have been prepared by condensation of glycylglycine with acetylacetone, benzoylacetone, dibenzoylmethane and thenoyltrifluoroacetone [7]. Many metals complexes have powerful antimicrobial activities and are already common day-to-day use in medicinal field such as silver bandages for treatment of burns , zinc antiseptic creams , bismuth drugs for the treatment of ulcers and metal clusters

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as anti-HIV drugs . The potential for further development of metal-based drugs and treatment as an antimicrobial agent [8,9] is enormous and also of great importance with the evolution of drug-resistant bacteria and threats from a range of viral diseases . The discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology for the control of infectious diseases. Metal-based drugs represent a novel group of anti fungal agents with potential applications for the control of fungal infection . This inspires synthetic chemists to search for new metal complexes for bioactive compounds and zinc in particular has attracted the researchers . The field of macrocyclic chemistry of metals is developing very rapidly because of its application and importance in the area of coordination chemistry [10] .The finding that the tetrahedral zinc complexes in its cavities generally represent the optimal , least strained structures among various zinc polyhedra may explain why four-coordinate zinc is chosen to play a structural role in zinc fingers and enzymes. Recently, Nair and coworkers have been synthesized and characterized some Schiff base metal complexes and their *in vitro* antimicrobial activities have been investigated [11,12]. In the present paper , I report the result on the synthesis, characterization and antimicrobial activities of Zn(II) complexes of Schiff base ligands L1 and L2 derived from salicylaldehyde and leucylalanine and glycylglycine respectively.

## Materials and Methods :

### Reagents and apparatus.

The dipeptide, leucylalanine and glycylglycine has been purchased from Fluka (LTD) and used without further

purification .Salicylaldehyde have been obtained from Fluka and Zn(II) acetate was obtained from Merck (LTD) .All other reagent and solvent have been purchased from commercial sources and were of analytical grade. Solvent have been purified and dried by standard methods. Elemental analysis was done using a Perkin-Elmer elemental analyzer .IR spectra have been recorded in KBr disc on a (8300) (FT-IR) Shimadzu spectrophotometer in the range 4000-400  $\text{cm}^{-1}$  region. The electronic spectra have been recorded on a Shimadzu,160,using a quartz cell of (1.0) cm length spectrophotometer .Molar conductance of the complexes has been measured in DMSO ( $10^{-3}\text{M}$ ) solution using a coronation digital conductivity meter.

### *In vitro* antimicrobial activity.

Antibacterial activity of the ligands and their complexes have been tested against the bacterial species *Staphylococcus aureus*, *Escherichia coil*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa* by Kirby bauer Disc diffusion method [13]. *Amikacin*, *Ofloxacin* and *Ciprofloxacin* have been used as the standard antibacterial agent .The test organisms were grown on nutrient agar medium in petri plates. The compounds have been prepared in DMSO and soaked in filter paper disc of 5mm diameter and 1mm thickness .The disc were placed on the previously seeded plates and incubated at 37 C° and the diameter inhibition zone [14] around each disc has been measured after 24 h for bacteria .

**Synthesis of the Dipeptide Schiff base Potassium Salt.** A solution containing (1.01 gm, 5mmol) of leucylalanine and (0.66gm,5 mmol) of glycylglycine in 20 ml of water was added to 15 mL of an ethanol solution containing (0.36 gm ,5 mmol) of KOH. The resulting solution was

stirred on a water bath at 25 C° for half an hour and then filtered . The filtrate was added dropwise to 20 mL of an ethanol solution of Salicylaldehyde (0.61 gm , 5 mmol ) with stirring on a water bath at 25 C° for one hour . The

volume of the solution was reduced by distillation to 5 mL and then ethanol (10 mL) was added . The yellowish precipitate formed was separated by filtration . The precipitate was recrystallized from methanol . [15]

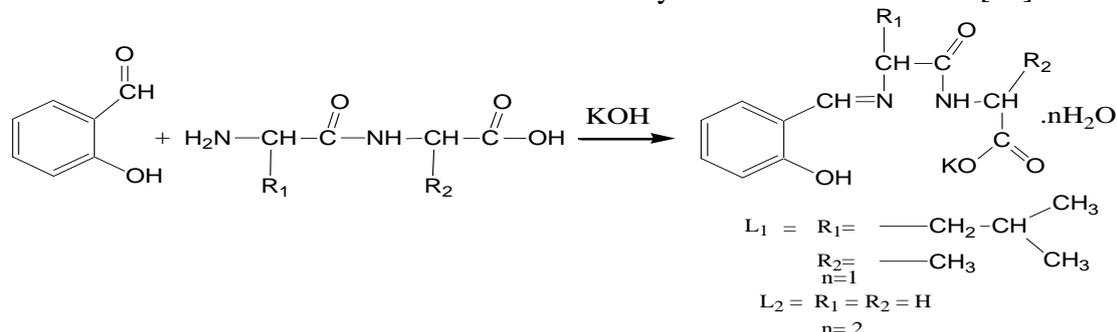


Fig.1 Synthesis and structures of ligands

**Synthesis of Zn(II ) Schiff base complexes.** The Zinc acetate (1.09 gm , 5mmol) was dissolved in 20 mL of water . The solution was filtered and added dropwise into 25 mL of an ethanol solution of the Schiff base ligands (1.81 and 1.55 gm .respectively ,5mmol). The reaction

mixture was stirred at 25 C° on water bath for 3 h. The resulting precipitate has been filtered , washed with ethanol and diethyl ether ,then recrystallized from methanol , and dried in vacuum desiccator .[15] The analytical data are summarized in Table 1.

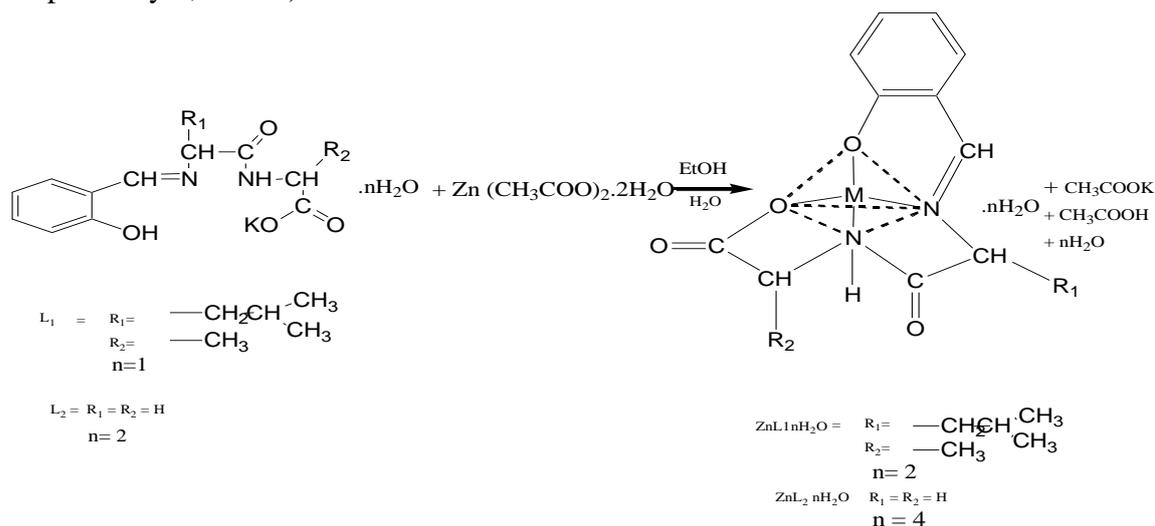


Fig.2 Synthesis and proposed structure for ZnL<sub>1</sub> and ZnL<sub>2</sub> Schiffbase complexes

### Results and Discussion:

Zn(II) complexes are stable at room temperature, insoluble in water but soluble in DMF and DMSO. The physical properties and analytical data of the ligands and their complexes are given in Table 1. Elemental analysis

data of the complexes are in good agreement with theoretical values. The analytical data (Table 1) indicate that the metal to ligand ratio is 1:1 in all the complexes systems and it can be represented as [ZnL<sub>1</sub>]. nH<sub>2</sub>O and [ZnL<sub>2</sub>]. nH<sub>2</sub>O, where L<sub>1</sub> and L<sub>2</sub> are

Schiff base ligands obtained by the condensation of salicylaldehyde respectively with leucylalanine and glycylglycine. The values of the conductivities of the Zn(II) complexes

are in the  $(7.2 - 12.5) \Omega^{-1} \text{mol}^{-1} \text{cm}^2$  range, indicating that the proposed complexes are 1:1 non-electrolytes [16].

**Table 1. Physical and analytical data of the Schiff base ligands and their complexes**

Compound	Formula	Mol. Wt. gm/mol	M.p (C°)	Yield (%)	Conductivity $\text{Ohm}^{-1} \text{cm}^2 \text{mol}^{-1}$	Found (calc.) (%)			
						C	H	N	M
KHL <sub>1</sub> .H <sub>2</sub> O	C <sub>16</sub> H <sub>23</sub> KN <sub>2</sub> O <sub>5</sub>	362.46	134	35	–	53.09 (53.02)	6.31 (6.40)	8.01 (7.73)	–
KHL <sub>2</sub> .2H <sub>2</sub> O	C <sub>11</sub> H <sub>15</sub> KN <sub>2</sub> O <sub>6</sub>	310.06	120	38	–	42.79 (42.57)	4.97 (4.87)	9.43 (9.03)	–
ZnL <sub>1</sub> .2H <sub>2</sub> O	C <sub>16</sub> H <sub>24</sub> N <sub>2</sub> O <sub>6</sub> Zn	405.78	> 265	68	7.2	47.44 (47.36)	5.88 (5.96)	7.29 (6.90)	16.24 (16.12)
ZnL <sub>2</sub> .4H <sub>2</sub> O	C <sub>11</sub> H <sub>18</sub> N <sub>2</sub> O <sub>8</sub> Zn	371.68	> 250	65	12.5	35.40 (35.55)	4.53 (4.88)	7.66 (7.54)	17.44 (17.60)

**IR spectra.** The Schiff base ligands L<sub>1</sub> and L<sub>2</sub> show (C=N) azomethine bands observed at 1618 cm<sup>-1</sup> and 1615 cm<sup>-1</sup> respectively. On complexation, this band was shifted to 1610 cm<sup>-1</sup> and 1600 cm<sup>-1</sup> regions [17] due to the coordination of azomethine nitrogen to the Zn (II) ion. In the spectra of Schiff base ligands, the peptide bands were observed at 1521 cm<sup>-1</sup> and 1518 cm<sup>-1</sup>. On complexation, this band was shifted to 1525 cm<sup>-1</sup> and 1525 cm<sup>-1</sup> region, indicating the linkage between metal ion and the peptide nitrogen atoms. The asymmetric carboxyl stretching  $\nu_{\text{asy}}(\text{COO}^-)$  was shifted to higher and lower frequency in the 1600 cm<sup>-1</sup> and 1560 cm<sup>-1</sup> range and the symmetric carboxyl stretching  $\nu_{\text{sym}}(\text{COO}^-)$  was shifted to higher and lower frequency in the 1520 cm<sup>-1</sup> and 1440 range, indicating the linkage between the metal ion and carboxylato oxygen atom. The asymmetric and

symmetric stretching vibration of the carboxylato group in the complexes shows the separation value ( $\Delta\nu$ ) greater than 150 cm<sup>-1</sup>. This indicates monodentate binding of carboxylato group in Zinc (II) complexes. Furthermore, the presence of coordinated and lattice water molecules appeared respectively at 3375 and 3380 cm<sup>-1</sup> in ZnL<sub>1</sub> and ZnL<sub>2</sub> complexes may be attributed to O-H stretching vibration. The appearance of two bands at 510 and 520 cm<sup>-1</sup> corresponds to  $\nu$  (M-O) and the bands at 430 and 425 cm<sup>-1</sup> corresponds to  $\nu$  (M-N) stretching vibrations respectively (see Fig 3). Thus, the IR spectral data indicate that ZnL<sub>1</sub> and ZnL<sub>2</sub> complexes tetradentate binding through azomethine nitrogen, amide nitrogen, phenolic oxygen and carboxylato oxygen atoms. The IR data are summarized in Table 2

**Table.2. Important IR Absorption Bands (cm<sup>-1</sup>) of the Schiff base ligands and their complexes**

Compound	$\nu_{\text{OH}}$	$\nu_{\text{C=N}}$	$\nu_{\text{asCOO}^-}$	$\nu(\text{CONH})$	$\nu_{\text{symCOO}^-}$	$\nu_{\text{C-N}}$	$\nu_{\text{C-O}}$	$\delta_{\text{COO}^-}$	$\nu_{\text{M-O}}$	$\nu_{\text{M-N}}$
KHL <sub>1</sub> .H <sub>2</sub> O	3380 s,br	1618 s	1598 s	1521 s	1460 m	1398 s	1240 w	765 s	-	-
KHL <sub>2</sub> .2H <sub>2</sub> O	3385 s,br	1615 s	1600 m	1518 s	1450 m	1395 s	1245 w	765 s	-	-
ZnL <sub>1</sub> .2H <sub>2</sub> O	3375 s,br	1610 s	1600 s	1525 m	1520 m	1390 m	1160 s	755 s	430 w	510 w
ZnL <sub>2</sub> .4H <sub>2</sub> O	3380 s,br	1600 s	1560 s	1523 m	1442 s	1380 s	1242 m	768 m	425 w	520 w

s= strong, br= broad, m= medium, w= weak

**UV/Vis. Spectra.** The Schiff base ligands L1 and L2 show the absorption bands at 291 nm and 280 nm, which is assigned to  $\pi$ - $\pi^*$  transition of the C=N chromophore. On complexation, this band was shifted to lower wavelength region 285 nm and 275nm, suggesting the coordination of azomethine nitrogen with Zn(II) ion [18](see Fig 4). Zn(II) complex does not exhibit d-d electronic transition due to the completely filled (d) orbital. Four coordinate Zn(II) complexes would have tetrahedral geometry (Fig.2).

**Antimicrobial activity.** The *in vitro* biological screening effect of the investigated compounds were tested against some bacterial species (isolated from patients) by the disc diffusion method. The results of the antibacterial activities were given in Table 2. The results show that both the

Schiff base ligands have moderate activity in the antibacterial species. Against all organisms, ZnL<sub>1</sub> and ZnL<sub>2</sub> complexes were found to be highly active in the bacterial species of *S. aureus*, *E. coil* and *P. aeruginosa*. However, ZnL<sub>1</sub> has moderate activity in the species of *K. penunaniae*. Moreover, the results point out that in *P. vulgaris*, ZnL<sub>1</sub> complex is less active and ZnL<sub>2</sub> is moderately active. Again, the comparison of the above results with *Amikacin*, *Ofloxacin* and *Ciprofloxacin* antibacterial standards demonstrates that *S. aureus*, *K. penunaniae*, *P. vulgaris* and *P. aeruginosa* are moderately active. However, the standards *Amikacin* and *Ciprofloxacin* show higher activity in the *E. coil* species, while *Ofloxacin* is less active in the *P. aeruginosa* species. Table 3.

**Table 3. Antibacterial activity of Schiff base ligands and their complexes**

Compound	Gram positive bacteria	Gram negative bacteria			
	<i>S. aureus</i>	<i>K. penunaniae</i>	<i>P. vulgaris</i>	<i>E. coil</i>	<i>P. aeruginosa</i>
KHL <sub>1</sub> .H <sub>2</sub> O	++	++	++	++	++
KHL <sub>2</sub> .2H <sub>2</sub> O	++	++	++	++	++
ZnL <sub>1</sub> .2H <sub>2</sub> O	+++	++	+	+++	+++
ZnL <sub>2</sub> .4H <sub>2</sub> O	+++	+++	++	+++	+++
Amikacin <sup>a</sup>	++	++	++	+++	++
Ofloxacin <sup>a</sup>	++	++	++	++	+
Ciprofloxacin <sup>a</sup>	++	++	++	+++	++

Inhibition values = 0.1-0.5 cm beyond control = + (less active); inhibition values = 0.6-1.0 cm beyond control = ++ (moderate active); inhibition values = 1.1-1.5 cm beyond control = +++ (highly active). <sup>a</sup>Standards.

**Mode of action.** Although the exact mechanism hasn't been understood biochemically, mode of action of antimicrobials may involve various target in microorganisms.

(i) Interference with the cell wall synthesis, damage as a result of which cell permeability may be altered (or) they may disorganize the lipoprotein leading to the cell death.

(ii) Deactivate various cellular enzymes, which play a vital role in different metabolic pathways of these microorganisms.

(iii) Formation of a hydrogen bond through the azomethine group with the active center of cell constituents, resulting in interference with the normal process.

**Effect of azomethine (C=N) group.**

The mode of action of the compounds may involve formation of a hydrogen bond through azomethine group (C=N) with the active centers of cell constituents[19] resulting in interferences with the normal process.

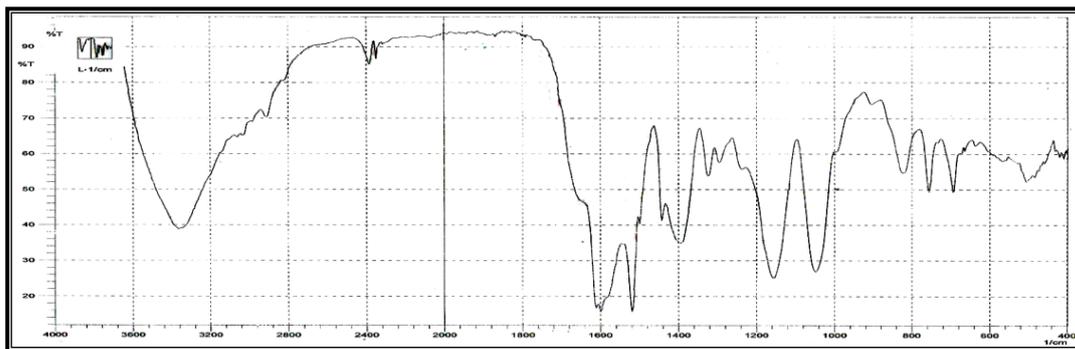
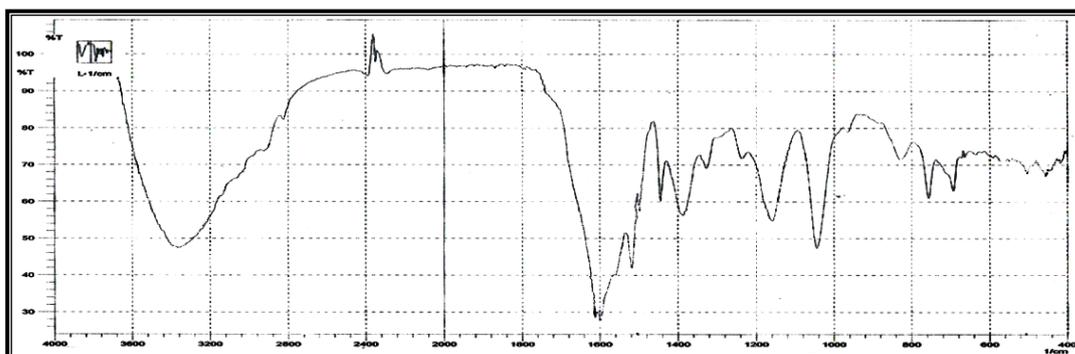
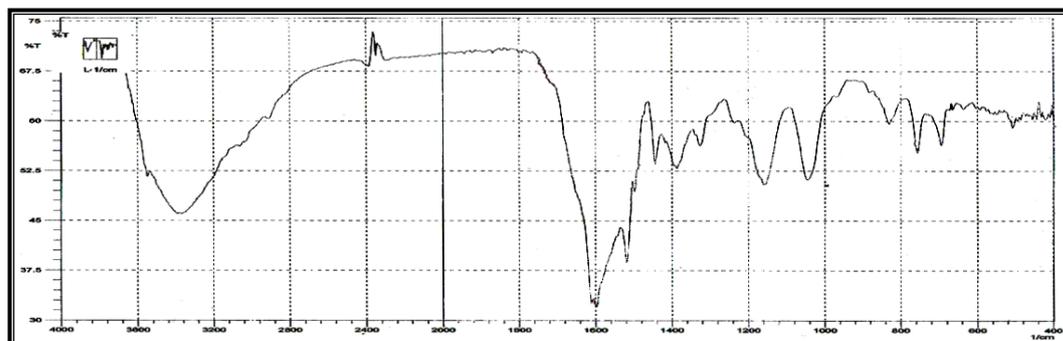
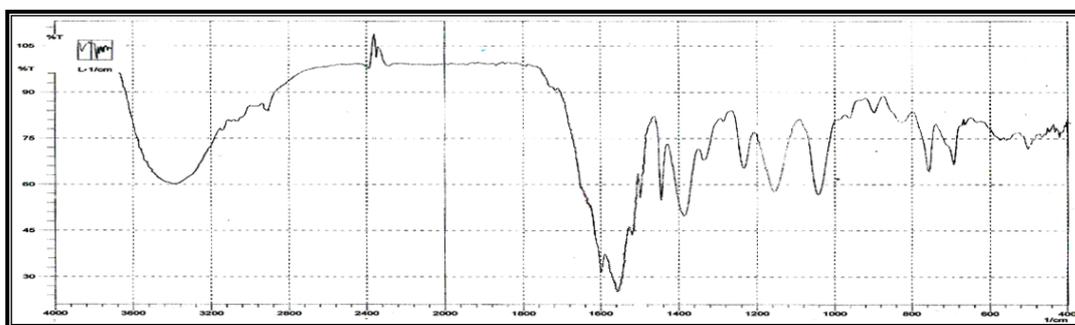
IR spectra of ligand L<sub>1</sub>IR spectra of ligand L<sub>2</sub>IR spectra of ZnL<sub>1</sub>·2H<sub>2</sub>OIR spectra of ZnL<sub>2</sub>·4H<sub>2</sub>O

Fig. 3 IR spectra for the prepared compounds

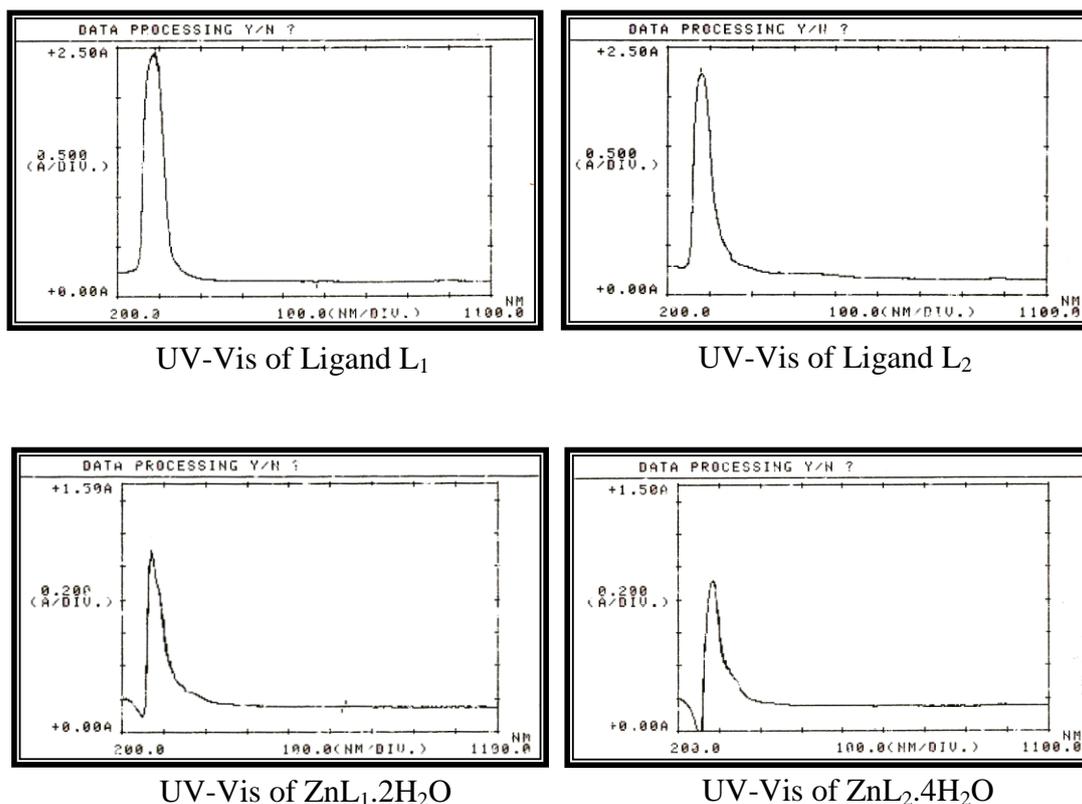


Fig. 4 UV-Vis spectra for the prepared compounds

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## تحضير ودراسة طيفية وبكتيرية لمعدّات الزنك الثنائية والمشتقة من السالسليدهايد و ليوسيل-الانين و كلايسيل- كلايسين

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### الخلاصة:

تم الحصول على ليكاندين لقواعد شف ( $L_1, L_2$ ) بواسطة تكثيف السالسليدهايد على التوالي مع ليوسيل-الانين وكلايسيل- كلايسين ومن ثم تحضير معدّاتهما مع ايون الزنك الثنائي وقد تم تشخيص هذه المركبات باستخدام التحليل الدقيق للعناصر والتوصيلية المولارية. كذلك تم استخدام التقنيات الطيفية مثل الأشعة ما تحت الحمراء والأشعة فوق البنفسجية – المرئية . بينت قياسات التوصيلية المولارية ان معدّات الزنك الثنائية هي بنسبة 1:1 وغير الكتروليتية . كذلك بينت الأشعة ما تحت الحمراء الارتباط الرباعي للليكاندين ( $L_1, L_2$ ). وقد درست الفعالية الحياتية المضادة للبكتريا للمركبات المحضرة على مجموعة من البكتريا وباستخدام طريقة (Disc diffusion). وقد تم إجراء دراسة مقارنة لقيم التثبيط للليكاندات قواعد شف ومعدّاتها وبينت الدراسة أن معدّات قواعد شف أظهرت فعالية حياتية عالية ضد البكتريا على عكس ليكاندات قواعد شف الحرة . ايون الزنك الثنائي ضروري للتأثير على عملية نمو التثبيط . وان التوسع في عملية التثبيط تظهر بقوة اعتمادا على كثافة الخلية الأولية وعلى نمو الوسط.