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New Zinc (II) mixed ligand complexes with Schiff bases and N,N′- donor ligands: Synthesis, characterization and antimicrobial studies

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Abstract
Present paper focuses on the study of synthesis of metal complexes of zinc with schiff base (sal-leu or sal-ala), where sal= salicyladehyde, leu= leucine and ala= alanine, as primary ligands and 1,10-phenanthroline or 2,2-bipyridyl as secondary ligands. The synthesized complexes have been characterized by using UV-Vis, IR and CHN. The UV-Vis spectroscopic techniques have been further used to study the metal-protein binding by investigating the interaction of synthesized complexes with bovine serum albumin. The antimicrobial activities of these complexes have been tested against Arthrobacter Citrus and Aspergillus Fumigates. It has been observed that the complexes show better antimicrobial activity than ligand alone.

Keywords: Schiff base ligands, spectral studies, BSA interaction, antimicrobial activity

1. Introduction
The discovery of pencillin (antibiotic) by Alexander Flemming leads modern science and technology to great heights for the treatment and cure of infectious diseases [1]. Millions of people are still losing their lives due to these infectious diseases as these pathogens are developing resistance against these agents. Schiff base metal complexes have been attracted the attention of chemists all over the globe because of their structural versatility, easier formation and their stability under various oxidative and reductive conditions [2]. Mixed ligand complexes of transition metals containing aldehyde group are of great interest because after binding with metal ion, the aldehyde group undergoes various transformations and gets activated [3, 4]. Therefore they pose numerous applications in biological, analytical, agricultural, pharmacological and clinical areas therefore these are extensively used against various infectious diseases as chemotherapeutic agents [5-11].
Literature survey has shown that zinc complexes are very effective in the treatment of athlete foot, ringworm, dry skin, atopic dermatitis, vitiligo, UVB - induced sunburn, anaphylactic shocks etc. [5]. Therefore it is widely used in creams, lotions, shampoo, wipes, pad, ointments etc.
Salicylic acid, derived from Latin word *'Salix'* implies willow tree which is an anti-inflammatory and a mild comedolytic agent. Complexes of salicyladehyde with zinc are of rising interest as drugs made from these complexes have been shown to be more persuasive and enviable as compared to ligands themselves. Salicyladehyde is a versatile ligand due to the presence of two donor sites. Moreover inter and intra molecular hydrogen bonding is also possible through hydroxyl group of salicyladehyde. It is indicated from earlier reports that pharmacological activities of these complexes can be further enhanced by the presence of secondary nitrogen donor ligands like 1,10-phenanthroline and 2,2-bipyridyl [12-13].
Human body is composed of twenty percent of proteins and amino acids form the building block of all proteins. Amino acids form major components of all the cells, tissues and bones. Since they contain both carboxylic and amino group, so the amalgamation of these functional groups permit amino acids to act as effective polydentate ligands for metal-amino acid chelating ligands [14-15]. They play crucial role in neurotransmission, biosynthesis, removal of metabolic waste etc.
Keeping in view the above observations, present work focuses on the synthesis and characterization of complexes of zinc with schiff base (derived from salicyladehyde and

amines (Leucine or Alanine) as primary ligand and 1,10-phenanthroline or 2,2-bipyridyl as secondary ligand. These complexes are then screened against number of pathogenic bacteria and fungi. All the complexes show better antimicrobial activity then the ligand. This is in further enhancement of the unremitting interest in deployment of the mixed ligand complexes of transition metals to cure various human ailments, against the raising problems of infectious problems caused by multi-drug resistant microorganisms, across the globe.

2. Materials and Method

2.1 Materials

Salicyladehyde, leucine, alanine, 1,10-phenanthroline, 2,2-bipyridyl, tris buffer, zinc(II)chloride, potassium hydroxide were purchased from Loba chemie, India and BSA was obtained from SDFCL, India. Tris buffer solution was prepared using double distilled water.

2.2 Physical Measurements

UV-Vis absorption spectra were recorded on a Shimadzu UV2600 (200-800 nm). IR spectra were obtained on a Nicolet Shimadzu FT-IR spectrometer in the range of 4000-400 cm^{-1} using KBr pellets. Melting points were determined in an open capillary tube and were uncorrected. The experiments were carried out at room temperature. The antimicrobial properties were measured by disc diffusion method.

2.3 Synthesis of ligands and metal complexes

The method for the synthesis of the complexes is as follows [16-18]:

2.3.1 Synthesis of [sal-leu Schiff base]

L-Leucine (5 mmol, 0.65 g) and potassium hydroxide (5 mmol, 0.28 g) were dissolved in hot methanol (50 ml) and were stirred for 2 h. A solution of salicylaldehyde (5 mmol, 0.5 ml) in 25 ml of methanol was added drop wise to above solution. The mixture was then stirred at 323 K for 2 h. Then the resultant solution was filtered and the filtrate was held at room temperature overnight. A light yellow colored precipitates of [sal-leu Schiff base] was appeared with yield 87%.

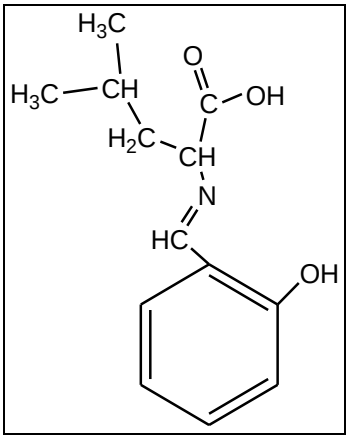


Fig 1: Structure of sal-leu (sal-leu= Salicylideneleucine) Schiff base ligand

2.3.2. Synthesis of [Zn(sal-leu)(bipy)H_2O] complex

L-Leucine (5 mmol, 0.65 g) and potassium hydroxide (5 mmol, 0.28 g) were dissolved in hot methanol (50 ml) and were stirred for 2 h. A solution of salicylaldehyde (5 mmol, 0.5 ml) in 25 ml of methanol was added drop wise to above solution. The mixture was then stirred at 323 K for 2 h. A solution of zinc chloride (5 mmol, 1.61 g) in 10 ml water was added drop wise to the above solution and stirred continuously for 2 h. Finally, a methanol solution of 2,2′-bipyridyl (5 mmol, 0.78 g) in 20 ml methanol was added drop wise and stirred for 2 h. Then the resultant solution was filtered and the filtrate was held at room temperature for 3 days, light yellow colored precipitates appeared with yield 65%.

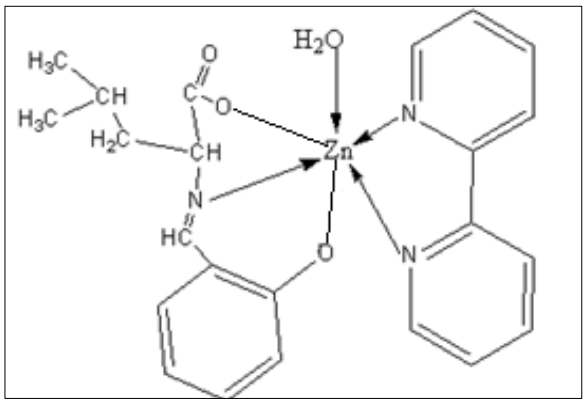


Fig 2: Structure of [Zn(sal-leu)(bipy)H_2O]complex

2.3.3 Synthesis of [Zn(sal-leu)(phen)H_2O]

L-Leucine (5 mmol, 0.78 g) and potassium hydroxide (5 mmol, 0.28 g) were dissolved in hot methanol (50 ml) and were stirred for 2 h. A solution of salicylaldehyde (5 mmol, 0.5 ml) in 25 ml of methanol was added drop wise to above solution. The mixture was then stirred at 323 K for 2 h. A solution of zinc chloride (5 mmol, 1.61g) in 10 ml water was added drop wise to the above solution and stirred continuously for 2 h. Finally, a methanolic solution of 1,10-phenanthroline (5 mmol, 0.99 g) in 20 ml methanol was added dropwise and stirred for 2 h. Then the resultant solution was filtered and the filtrate was held at room temperature for 7 days, light yellow colored precipitates appeared yield 62%.

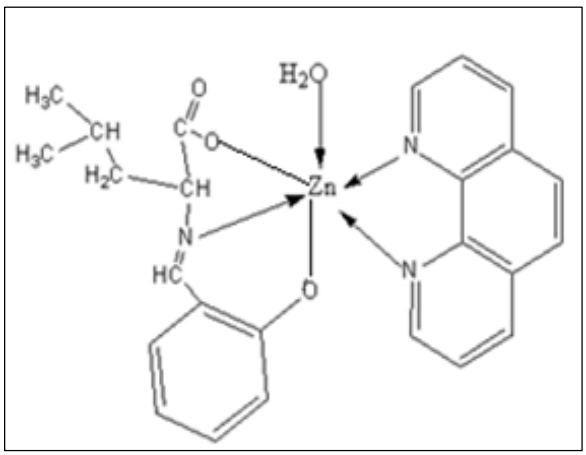


Fig 3: Structure of **[**Zn(sal-leu)(phen)H_2O] complex

2.3.4 Synthesis of [sal-ala Schiff base] complex

Alanine (5 mmol, 0.48 g) and potassium hydroxide (5 mmol, 0.28 g) were dissolved in hot methanol (50 ml) and were stirred for 2 h. A solution of salicylaldehyde (5 mmol, 0.5 ml) in 25 ml of methanol was added drop wise to above solution. The mixture was then stirred at 323 K for 2 h. Then the resultant solution was filtered and the filtrate was held at room temperature for 7 days, light yellow colored precipitates appeared with yield 82%.

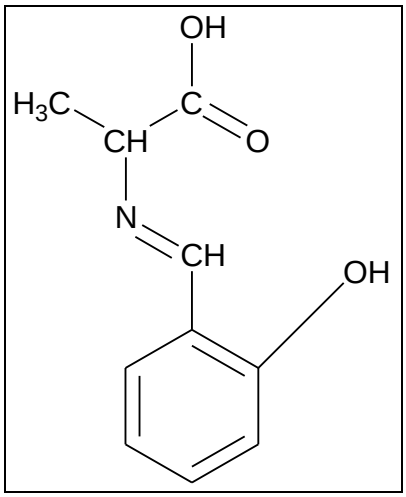


Fig 4: Structure of sal-ala (sal-ala = salicylidenealanine) Schiff base ligand

2.3.5 Synthesis of [Zn(sal-ala)(bipy)H2O] complex

Alanine (5 mmol, 0.48 g) and potassium hydroxide (5 mmol, 0.28 g) were dissolved in hot methanol (50 ml) and were stirred for 2 h. A solution of salicylaldehyde (5 mmol, 0.5 ml) in 25 ml of methanol was added drop wise to above solution. The mixture was then stirred at 323 K for 2 h. A solution of zinc chloride (5 mmol, 1.61 g) in 10ml water was added drop wise to the above solution and stirred continuously for 2 h. Finally, a methanolic solution of 2,2′-bipyridyl (5 mmol, 0.78 g) in 20ml methanol was added drop wise and stirred for 2 h. Then the resultant solution was filtered and the filtrate was held at room temperature for 3 days, brown colored precipitates appeared with yield 72%.

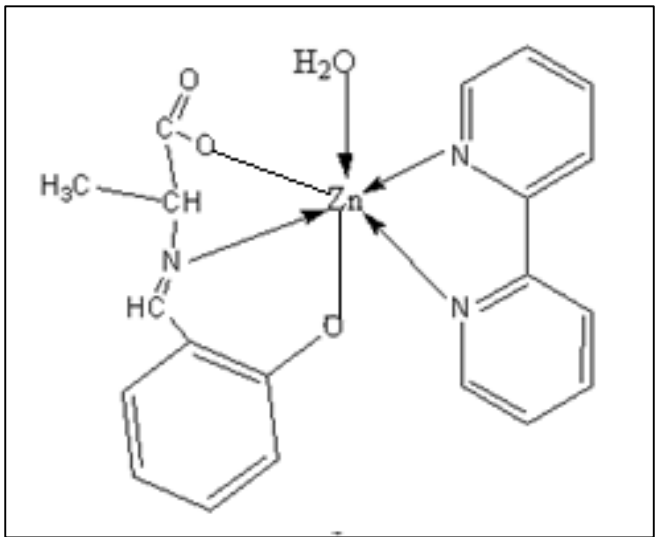


Fig 5: Structure of [Zn(sal-ala)(bipy)H2O] complex

2.3.6 Synthesis of [Zn(sal-ala)(phen)H2O]

Alanine (5 mmol, 0.48 g) and potassium hydroxide (5 mmol, 0.28 g) were dissolved in hot methanol (50 ml) and were stirred for 2 h. A solution of salicylaldehyde (5 mmol, 0.5 ml) in 25 ml of methanol was added drop wise to above solution. he mixture was then stirred at 323 K for 2 h. A solution of zinc chloride (5 mmol, 1.61g) in 10ml water was added drop wise to the above solution and stirred continuously for 2 h. Finally, a methanolic solution of 1, 10-phenanthroline (5 mmol, 0.99 g) in 20ml methanol was added drop wise and stirred for 2 h. Then the resultant solution was filtered and the filtrate was held at room temperature for 2 days, light yellow colored precipitates appeared with yield 64%.

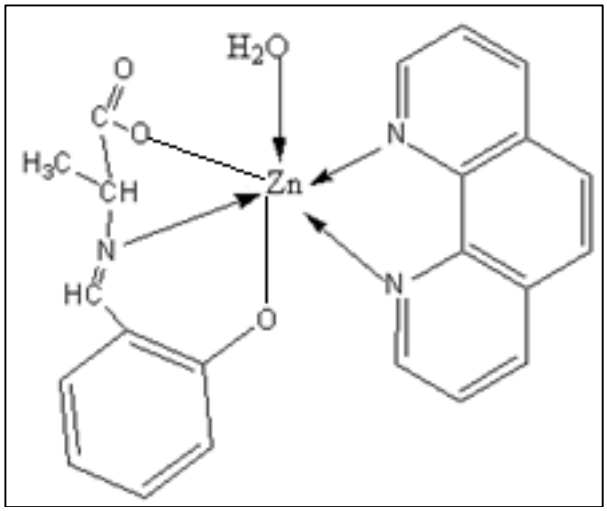


Fig 6: Structure of [Zn(sal-ala)(phen)H2O] complex

3. Results and Discussion

3.1 UV-Vis, IR spectra and Elemental analysis

All obtained complexes exhibit similar electronic absorption spectra, which indicate that the central ions and ligands are coordinated in a similar mode. The comparison between the ligand spectra (sal-leu or sal-ala) with the UV-Vis spectrum of different complexes of Zn shows the complexation of metal with the ligand as there are shifting in positions of absorption bands as presented in Table 1. The absorptions in the range 250-390 nm at low concentrations in methanol indicates shifting in n to π* and π to π* transitions of complex as compared to UV spectra of ligand, which confirm binding of 2,2′-bipyridyl and 1,10-phenonthroline with metal centers Zn(II). Zinc did not show the d-d transition as it is d^{10} system.

The significant regions of IR spectra of all complexes are very similar and the following observations are of interest (Table 1 and 2). The significant peak of $\nu_{C=N}$ at 1600-1650 cm^{-1} showed the formation of schiff base ligand (>C=N-). From the IR spectrum of the complexes, the absorption band at 3200-3300 cm^{-1} is due to the absorption of H_2O group stretching vibration indicating the attachment of water molecule at sixth coordination site of metal ion. The absorption bands at 680-1100 cm^{-1} are due to the vibration of 3-phenonthroline ring, C-H stretching frequency. The absorption bands at 1530-1600 cm^{-1} are due to skeleton vibrations of pyridyl ring of 2,2-bypyridyl. The absorption bands at 1400-1550 cm^{-1} are due to -COO- asymmetric and symmetric vibrations frequency. The characteristic absorption bands 450-750 cm^{-1} suggesting coordination (M-N and M-O bonds) of both nitrogen and oxygen atoms to metal center in complex. Thus from evaluation of I.R. spectra we will able to say that complex has formed [19-21].

Table 1: Physical, Analytical, UV-Vis and IR spectra of mixed ligands metal complexes.

S. No.	Complexes	Chemical Formula	Color of Complex	M.P. (℃)	Elemental analysis as % Found (% Cal.)				
					C	H	N	O	Zn
1	[Sal-leu Schiff base]	$C_{13}H_{17}NO_3$	Golden yellow	215	65.78 (66.36)	8.21 (7.28)	4.80 (5.95)	21.12 (20.40)	-
2	[Zn(sal-leu)(bipy)H_2O]	$C_{23}H_{25}N_3O_4Zn$	Pale yellow	280	58.12 (58.42)	6.57 (5.33)	7.80 (8.89)	13.85 (13.53)	12.55 (13.83)
3	[Zn(sal-leu)(phen)H_2O]	$C_{25}H_{25}N_3O_4Zn$	Rust yellow	210	59.65 (60.43)	5.52 (5.07)	8.02 (8.46)	13.07 (12.88)	11.58 (13.16)
4	[Sal-ala Schiff base]	$C_{10}H_{11}NO_3$	Pale yellow	286	61.50 (62.17)	5.95 (5.74)	7.02 (7.25)	24.61 (24.84)	-
5	[Zn(sal-ala)(bipy)H_2O]	$C_{20}H_{19}N_3O_4Zn$	Pale yellow	230	56.15 (56.96)	2.40 (2.39)	10.01 (9.96)	14.98 (15.18)	14.95 (15.51)
6	[Zn(sal-ala)(phen)H_2O]	$C_{22}H_{19}N_3O_4Zn$	Pale yellow	245	57.83 (58.10)	4.68 (4.21)	9.65 (9.24)	14.00 (14.07)	14.05 (14.38)

Table 2: Selected UV and IR frequencies of the mixed ligand complexes.

S. No.	Complexes	UV transitions λ max (nm)		IR Spectra (cm^{-1})		
		n to π^*	π to π^*	$\nu_{(C=N)}$	$\nu_{(M-N)}$	$\nu_{(M-O)}$
1	[Sal-Leu Schiff base]	382	256, 330	_	_	_
2	[Zn(sal-leu)(bipy)H_2O]	358	273,235	1634	536	760
3	[Zn(sal-leu)(phen)H_2O]	359	234,274,280	1625	403	507
4	[Sal-Ala Schiff base]	380	239,310	_	_	_
5	[Zn(sal-ala)(bipy)H_2O]	235,291	1634	416	538	
6	[Zn(sal-ala)(phen)H_2O]	267,222	1625	425	537	

3.2 Bovine Serum Albumin Binding with Complexes

The metal protein binding studies have been done with the help of UV-Vis absorption spectroscopy (Fig. 7 & 8). 0.1 M tris buffer solution of pH 7.4 is used for preparing the solution of the complex and BSA. The spectra were observed after titrating incremental amount of BSA solution of 500 µM to the fixed volume of metal complex (10 µM). The intensity of bands increases with increase in concentration of BSA. The ground-state association constant (Ka) was calculated using the Benesi-Hildebrand equation. The binding constants were determined from the BSA-complex titration graphs by plotting $1/(A-A_0)$ versus 1/[BSA] concentrations, where A_0 is the initial absorption band for free complex and A is the absorption band for different complex-BSA concentrations. The value of binding constants comes out to be around 9.18 - 24.77 mM^{-1}. It has been assumed that the interaction between the ligand L and the substrate S is 1:1; for this reason a single complex S_L (1:1) is formed.

The relationship between the observed absorbance change per centimeter and the system variables and parameters is as follow.

$$\frac{\Delta A}{b} = \frac{S_t K_{11} \Delta \varepsilon_{11} [L]}{1 + K_{11}[L]} \quad (1)$$

Where $\Delta A = A - A_o$,
S_t is total concentration of substrate
$\Delta\varepsilon_{11} = \varepsilon_{11} - \varepsilon_S - \varepsilon_L$
And ε_{11} is the molar absorptivity of the complex
ε_s is the molar absorptivity of the substrate
ε_L is the molar absorptivity of the ligand

From the mass balance expression $S_t = [S] + [SL]$, we get $[S] = S_t / (1 + K_{11}[L])$.
Where [S] is the concentration of the uncomplexed substrate
[L] is the concentration of the uncomplexed ligand
[SL] is the concentration of the complex

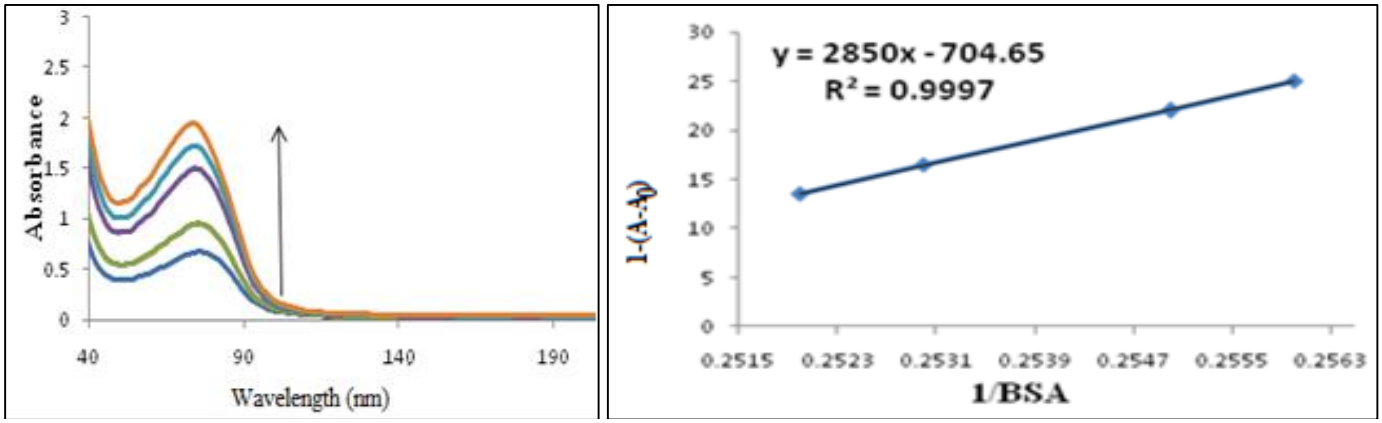
Eq. (1) is the binding isotherm, which shows the hyperbolic dependence on free ligand concentration. The double-reciprocal form of plotting the rectangular hyperbola $1/y = f/d \times 1/x + e/d$ is based on the linearization of Eq. (1) according to the following equation

$$\frac{b}{\Delta A} = \frac{1}{S_t \varepsilon_{11}} + \frac{1}{S_t K_{11} \Delta \varepsilon_{11} [L]} \quad (2)$$

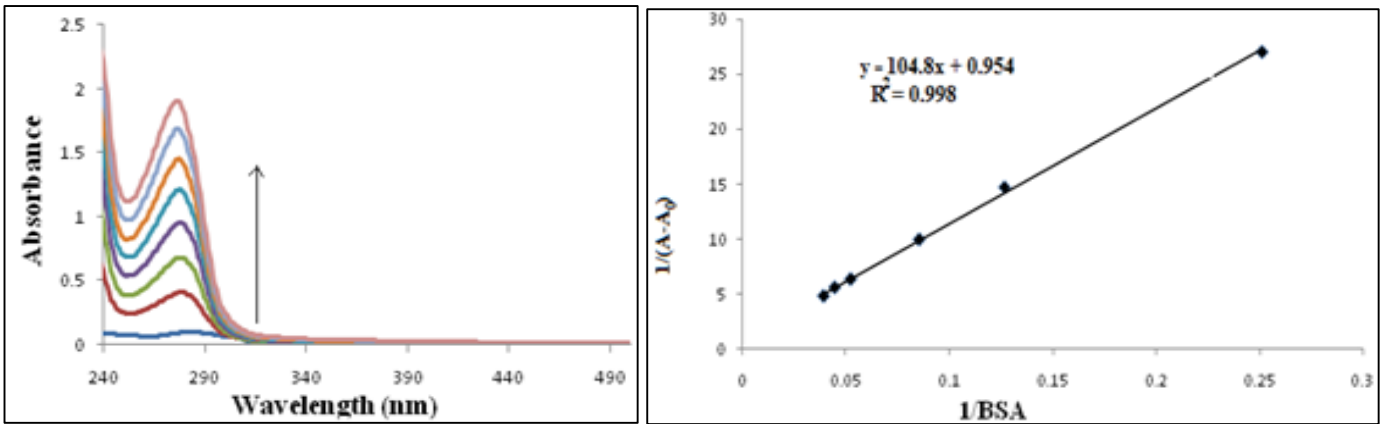
Thus, the double reciprocal plot of $1/\Delta A$ versus 1/[L] is linear and the binding constant can be estimated from the following equation [22-23].

$$K_{11} = \frac{Intercept}{Slope}$$

The UV-visible spectra observed after titrating 10 µM of complex with increasing mol equivalents of complex showed that initially up to around 2.5 µM complexes concentration BSA-complex adduct showed increasing absorption. While on further increase in complex concentration BSA-complex adduct band disappears which indicate saturation at BSA binding site. From these BSA-complex titration binding constant were also determined and presented in Table 3. Binding constants indicate high-affinity at the binding site of bovine serum albumin with the complex corresponds to the complex binding area in subdomain IIIA. This itself indicate that BSA can be successfully work as good transport biological material for these mixed ligand complexes of zinc.

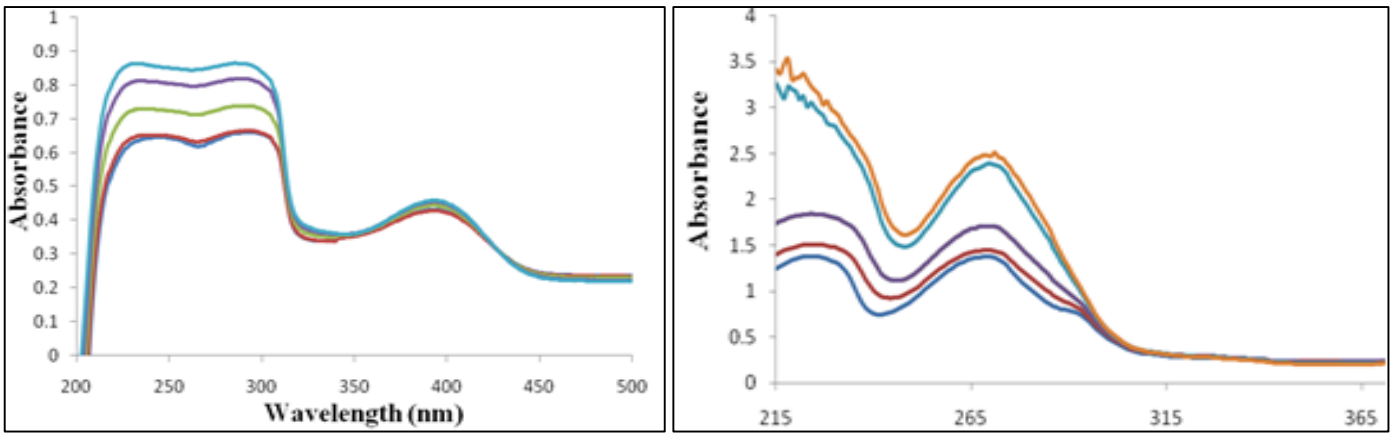


7 (a)



7 (b)

Fig 7: Absorption spectra complex solution (10 µM, 2000 µL) in the presence (0, 5, 10, 13, 15, 17.5 µL of 500 µM) concentration of BSA 7(a) of the [Zn(Sal-Leu)(bipy)H_2O] 7(b) [Zn(Sal-Ala)(bipy)H_2O] complex solution with BSA respectively.



8(a) 8 (b)

Fig 8: Absorption spectra complex solution (10 µM, 2000 µL) in the presence (0, 5, 10, 13, 15, 17.5 µL of 500 µM) concentration of BSA 8(a) of the [Zn(Sal-Leu)(phen)H_2O] 8(b) [Zn(Sal-Ala)(phen)H_2O] complex solution with BSA respectively.

Table 3: Binding constant of the mixed ligand complexes with BSA calculated from U.V. absorption titration studies

S. No.	Name of the mixed ligand complexes	Binding Constant (mM^{-1})
1	[Zn(Sal-Leu)(bipy)H_2O]	24.77
2	[Zn(Sal-Ala)(bipy)H_2O]	9.18
3	[Zn(Sal-Leu)(phen)H_2O]	35.53
4	[Zn(Sal-Ala)(phen)H_2O]	15.62

4. Antimicrobial screening of all the complexes

Coordination complexes of zinc metals play crucial role in biological study, many metal complexes now been extensively studied for their antimicrobial properties and comprehensive investigations in the field of metal complexes have been reported [4-8, 10, 16, 25, 27, 28]. The complexes were screened in vitro for its microbial activity against certain pathogenic bacterial and fungal species using disc diffusion method [11, 24-25]. The minimum inhibitory concentrations (MIC) of complexes/products were investigated against Arthrobacter citrus (bacteria) and Aspergillus fumigatus (fungus) seeded in tubes with nutrient broth (NB). The

Nutrient Agar medium (NB) was used to culture bacteria, Arthrobacter citrus and Potato Dextrose Agar medium (PDA) was used to culture fungus, Aspergillus fumigates. In the laminar chamber the autoclaved homogenous suspensions of NB and PDA was poured into petri dishes and allow for solidification. After cooling as well as solidification of the NB and PDA in petri plates the paper disc of different concentration 2000 ppm, to 500 ppm of the investigated compound applied using a sterilized forceps and then these concentrations were further diluted to check the lowest inhibitory concentration as given in the Table 4. After incubation for 48h in an incubator at 37°C and 28°C for bacteria and fungi, respectively, the inhibition zone diameters were measured and expressed in mm. Filter discs impregnated with 10 mm^3 solvent (water/methanol) were used as negative control. The percentage of inhibition was calculated by using the formula: % inhibition = [((A-B)/A)*100]; here A = inhibition (in mm) by solvent (water) and B = inhibition (in mm) by compound at above mentioned concentrations. The complexes exhibit significant activity against fungus and bacteria. In our biological experiments using zinc complexes, we observed considerable antifungal and anti bacterial activity against Aspergillus fermigatus, Arthrobacter citrus.

Table 4: Antimicrobial activities of [Zn(Sal-Leu)(phen/bipy)H_2O] and [Zn(Sal-Ala)(phen/bipy)H_2O] complexes.

S. No.	Name of the mixed ligand complexes	Concentration (ppm)	Diameter of zone of inhibition (mm)	
			Arthrobacter citrus	Aspergillus fumigatus
1	[Zn(Sal-Leu)(bipy)H_2O]	1500	21	24
2	[Zn(Sal-Ala)(bipy)H_2O]	1100	15	18
3	[Zn(Sal-Leu)(phen)H_2O]	1200	17	20
4	[Zn(Sal-Ala)(phen)H_2O]	1000	19	21

5. Conclusion

In the present paper, zinc complexes with schiff base (Sal-Leu or Sal-Ala) as primary ligand and 1,10-phenanthroline or 2,2′-bipyridyl as secondary ligand have been reported. The newly synthesized complexes are of the general formula $[ZnL_1L_2X]$ where L_1= Sal-Leu or Sal-Ala, L_2= 1,10-phenanthroline or 2,2′-bipyridyl and X = H_2O. The mode of binding between the metal and the ligand is determined with the help of UV-Vis, IR and CHN studies. The UV-Vis titration techniques have been used to study the interaction of the prepared complexes with bovine serum albumins, which indicate that the complexes bind moderately BSA. The antimicrobial studies have been reported against Arthrobacter citrus and Aspergillus fumigates. The complexes show better antimicrobial activity as compared to the ligands.

6. Acknowledgement

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