International Journal of Engineering Technology Science and Research
IJETSR
www.ijetsr.com
ISSN 2394 – 3386
Volume 4, Issue 9
September 2017

# Spectral And Theoretical Studies On The Impact Of M(II) Complexes Of Amino Acid-Nucleobase Hybrid Ligand On BSA Binding: An Approach Towards New Metallodrugs

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## ABSTRACT
*Synthesis of new metallic complex of cobalt and zinc with amino acid-nucleobase hybrid ligand have been achieved by simple chemical reaction of metal salt with amino acid L-histidine and nucleobase adenine as ligands. The synthesized complexes were identified and characterized by elemental analysis, conductometric measurements, FT-IR, UV-visible, $^{1}H$ &$^{13}C$ NMR, mass spectroscopy and magnetic measurements. The interaction of the complex with BSA has been carried out using UV-visible titration and fluorescence spectroscopy. The reactivity toward BSA revealed that the quenching of BSA fluorescence by the two complexes are static quenching, and complex 1 exhibits a higher BSA-binding ability than the complex 2. The pharmacological activity of the ligand and the complexes was investigated by antioxidant activity, and they show promising effect against DPPH radical. The results of the molecular docking studies of thecomplexes reinforce all the above facts.*
***KEYWORDS:Co(II) complex; Zn(II) complex; Adenine; Histidine; BSA interaction; Molecular docking***

## 1. INTRODUCTION
Cancer remains the major cause of death in the world even though there is an effectivedevelopment in the novel anticancer drugs. The reason for this scenario is due to the drug resistanceor undesirable side effects of anticancer drugs. Metallo anticancer drug discovery remains as oneof the progressive areas of pharmaceutical research. Metal complexes which binds and cleavesBSA under physiological conditions are considered as potential candidates which can be used astherapeutic agents in medicinal applications and for genomic research [1-6]. Much attention has been focused on the development of metal complexes as bio-molecular mimics [7-11]. Transition metal complexes have also been used as therapeutic drugs [12-21] and as spectroscopic probes for proteins [22-27]. The review of articles by Sabine Van Rijit et al. illustrates notable progress in the field of bioinorganic chemistry to design innovative metal based drugs for antitumor and anticancer activity [21].
Serum albumins, the most abundant proteins in blood, have many important physiological functions. As a major depot and transport protein, the most important physiological role is capable of binding, transporting and delivering an extraordinarily diverse range of endogenous and exogenous compounds in the bloodstream to their target organs [14]. Significant interaction of any drug with a protein may result in the formation of a stable protein–drug complex, which has an important effect on the distribution, metabolism and the efficacy of drugs [15]. Therefore, the interaction between protein and drugs provides valuableinformation on the structural features that determine the therapeutic effectiveness of drugs and alsoto study the pharmacological response of drugs. It has become an important research field in life sciences, chemistry, and clinical medicine [20]. Rabindra Reddy *et al.*reported that the interaction of zinc and cobalt complexes with peptides like cysteinylglycine and histidylglycine and reported that they are possessing more affinity to BSA [8].
Although reports on the applications of adenine complexes of cobalt as well as histidine complexes of transition metal ions as drugs for some therapeutic applicationsare available [17], there is no report on the synergetic or combined effect of both the ligands adenine and histidine on therapeutic uses of the complexes. Inorganic complexes with the ligand as nucleic acids may have structural novelty as well as better biocompatibility. Therefore, the present study is aimed to investigate the synthesis of novel metal complexes with cobalt and zinc central metal ion. The work also reports (1) the binding efficacy of the complexes with

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IJETSR
www.ijetsr.com
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BSA (2) the pharmacological effect of the synthesized ligand and the complexes by investigating antioxidant and antimicrobial activity. Molecular docking studies of the ligand and the complexes with BSA was also performed to investigate their binding behavior.

## 2. EXPERIMENTAL

### 2.1. MATERIALS

Chemicals used in this work were of Analar grade and were used without further purification, however, the solvents used for the study were purified by following the standard procedure reported in literature [28]. Bovine Serum Albumin (BSA) was obtained from Sigma.

Elemental analysis data were obtained using a Euro Vector elemental analyzer. The molar conductivity of the complexes in DMSO solution ($10^{-3}$ M) was measured in a deep vision 601 model digital conductometer at room temperature. Room temperature magnetic susceptibility measurements were carried out on a Lakeshore VSM 7410 magnetic balance. Vibration spectra were recorded in a Perkin Elmer Spectrum RX I spectrophotometer using KBr disks. The NMR spectra of the ligand and $[Zn(L)_2Cl_2]$ complex was recorded on a Bruker AV-III FT NMR 500 MHz spectrometer operating at room temperature (RT). Molecular weights of the complexes were determined using JEOL GCMATE II GC-MS spectrometer. Perkin Elmer Lambda 35 UV–visible spectrophotometer and Hitachi F-2500 fluorescence spectrophotometer were used to record the electronic and fluorescence spectra, respectively.

### 2.2. SYNTHESIS OF THE LIGAND

For the synthesis of ligand (L) adenine (0.2 mM) was suspended in chloroform (15 ml) and neutralized with triethylamine. Ethanolic solution of L-histidine (0.2 mM) was added to the above solution and the resultant mixture was refluxed for 6 h. The solid product formed was filtered and washed with chloroform. The filtrate together with the washings were treated with citric acid solution (10%, 10 ml) and with water. The organic layer was separated. After drying over anhydrous $CaCl_2$, the solvent was removed by evaporation to obtain the ligand.

#### 2.2.1.CHARACTERISTIC FEATURES OF [L]

[Yield: 0.36 g, 58%], m.p.: 145°C, Analysis: Calculated for$C_{11}H_{12}N_8O$: C, 48.53; H, 4.44; N, 41.16; O, 5.88%. Found: C, 48.50; H, 4.42; N, 41.15; O, 5.85%. ESI-MS in DMSO: *m/z*272 $[C_{11}H_{12}N_8O]^+$. IR / $cm^{-1}$: 3412vs, 3105s, 2711m, 1608s, 1065w (vs, very strong; s, strong; m, medium; w, weak).UV-visible in DMSO [$\lambda_{max}$/nm (ε/ $M^{-1}$ $cm^{-1}$)]: 206 (40560) and 251 (32440). $^1$H NMR [DMSO-$d_6$, ppm]: δ 7.2 (7H), 8.1 (1H), 4.8 (2H), 4.0 (1H) and 3-3.5 (3H) ppm; $^{13}$C NMR [DMSO-$d_6$, ppm]: δ 123.4–135, 172, 122.4, 176.4-179.6, 51.3-64.6 ppm.

### 2.3.SYNTHESIS OF METAL COMPLEXES

To synthesize metal complexes, 2:1 molar mixture of ligand (L) (0.2 M) and metal chloride salt (0.1 M) were mixed in ethanol (15 mL). This mixture was then refluxed for 3 h while stirring constantly using a magnetic stirrer. The resultant product was washed with ethanol and then recrystallized. The solid product obtained was filtered, dried in vacuum and desiccated.

#### 2.3.1.COBALT(II) COMPLEX $[Co(L)_2Cl_2]$

[Yield: 0.67 g, 69%], m.p.: 215°C, Analysis: Calculated for$C_{22}H_{24}Cl_2N_{16}O_2Co$: C, 39.18; H, 3.59; Cl, 10.51; Co, 8.74; N, 33.23; O, 4.74%. Found: C, 39.17; H, 3.57; Cl, 10.50; Co, 8.71; N, 33.22; O, 4.71%. ESI-MS in DMSO: *m/z*674 $[C_{22}H_{24}Cl_2N_{16}O_2Co]^+$. IR / $cm^{-1}$: 3334br, 3172br, 2741w, 1601s, 1053m, 570w, 427m, (s, strong; m, medium; w, weak; br, broad).UV-visible in DMSO [$\lambda_{max}$/nm (ε/ $M^{-1}$ $cm^{-1}$)]: 268 (33154), 353 (21478) and 542 (9874). Conductivity in DMSO ($\Lambda_m$): 12.4 $\Omega^{-1}$ $cm^3$ $mol^{-1}$. $\mu_{eff}$:4.29 BM.

#### 2.3.2.ZINC(II) COMPLEX $[Zn(L)_2Cl_2]$

[Yield: 0.75 g, 71%], m.p.: 246°C, Analysis: Calculated for$C_{22}H_{24}Cl_2N_{16}O_2Zn$: C, 38.81; H, 3.55; Cl, 10.41; N, 32.92; O, 4.70; Zn, 9.60%. Found: C, 38.79; H, 3.53; Cl, 10.40; N, 32.91; O, 4.68; Zn, 9.57%. ESI-MS in DMSO: *m/z*680 $[C_{22}H_{24}Cl_2N_{16}O_2Zn]^+$. IR / $cm^{-1}$: 3373br, 2714w, 1638vs, 1115s, 617m, 435w, (vs, strong; s, strong; m, medium; w, weak; br, broad).UV-visible in DMSO [$\lambda_{max}$/nm (ε/ $M^{-1}$ $cm^{-1}$)]: 259 (30544) and 349 (19458). $^1$H NMR [DMSO-$d_6$, ppm]: δ 7.2 (7H), 7.6 (1H), 3.7 (2H), 4.0 (1H) and 3-3.5 (3H) ppm. $^{13}$C NMR [DMSO-$d_6$, ppm]: δ 123.4–135 (H), 168 (H), 119 (H), 176.4-179.6 (H) and 51.3-64.6 (H) ppm. Conductivity in DMSO ($\Lambda_m$): 14.8 $\Omega^{-1}$ $cm^3$ $mol^{-1}$. $\mu_{eff}$:Diamagnetic.

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ISSN 2394 – 3386
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### 2.4.BINDING STUDIES WITH BSA

The binding of complexes 1 and 2 with BSA was studied using fluorescence spectra recorded at a fixed excitation wavelength corresponding to BSA at 280 nm and monitoring the emission at 347 nm. The excitation and emission slit widths and scan rates were constantly maintained for all the experiments. Stock solution of BSA was prepared in 50 mM phosphate buffer (pH = 7.2) and stored in the dark at 4 °C for further use. Concentrated stock solutions of each test compound were prepared by dissolving them in DMF–phosphate buffer (5:95) and diluted with phosphate buffer to get required concentrations. 2.5 ml of BSA solutionwas titrated by successive additions of a $10^{-6}$ M stock solution of the complexes using a micropipette.To carry out the binding studies of metal complexes with BSA using absorption and fluorescence spectral titrations, the concentration of the BSA was maintained constant ($1 \times 10^6$ $M^{-1}$) while the concentration of the complexes increased from 0-6 × $10^3$ $M^{-1}$. By measuring the alterations in the LMCT absorption band upon increasing complex/ BSAconcentration, the apparent association constant $K_{app}$ was calculated. From the fluorescence spectral titrations, the intrinsic binding constant $K_b$ and Stern-Volmer constant $K_{sv}$ were calculated using appropriate equations. pH 8 was maintained using phosphate buffer throughout the absorption and fluorescence spectral studies.

### 2.5.BIOLOGICAL STUDIES

#### 2.5.1.ANTIOXIDANT STUDY

The free radical scavenging activities of the ligand and the complexes have been carried out by using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) inhibition assay [29]. The radical in the DPPH has a strong absorption maximum at 517 nm and the absorbance of DPPH decreases, when the radical reacts with the antioxidant. This assay is based on the monitoring the decrease in OD value of the intrinsic peak of DPPH at 517 nm on addition of different complex concentrations at different time points. The experiments have been performed by maintaining the DPPH concentration constant (2 mL, 25 µM) and varying the complex concentration from 0 to 300 µM and in each case the time duration has been varied from 0 to 30 min. The whole experiment has been performed in spectroscopic grade methanol solvent at 298 K. The radical scavenging activity has been measured by equation 1 [30].

% inhibition = $\{(A_{control} - A_{sample}) / A_{control}\}$ x 100 …….(1)

where, $A_{control}$is the absorbance of the control (pure DPPH solution) at 516 nm and $A_{sample}$is the absorbance of the test sample (DPPH solution with the test compound) at 517 nm and $A_{sample}$−blank is the absorbance of the sample solution (the test compound without DPPH) at 517 nm.

### 2.6.SOFTWARE DETAILS

AutoDock 4.2 using Lamarckian genetic algorithm for the prediction of binding affinity and searching of the optimum binding site together with the AutoDock Tools was employed to set up and perform blind docking calculations of the two complexes binding to BSA. The Structure of the complexes was sketched by CHEMSKETCH (http://www.acdlabs.com). The crystal structure of the BSA was downloaded from the protein data bank (http://www.rcsb.org./pdb). Receptor (BSA) and ‘‘the ligand’’ (complexes 1 and 2) files were prepared using AutoDock Tools. The heteroatoms including water molecules were deleted and polar hydrogen atoms and Kollman charges were added to the receptor molecule. All other bonds were allowed to be rotatable. In the docking analysis, the binding site was assigned across all of the BSA molecule, which was enclosed in a box with number of grid points in x × y × z directions, 60 × 60 × 60 and a grid spacing of 0.375 Å. Visualization of the docked pose has been done by using PyMOL.

## 3. RESULTS AND DISCUSSION

New amino acid-nucleobase hybridligand *viz.*, adenine and L–histidine and its Co(II) and Zn(II) complexes were synthesized and thoroughly characterized (Fig. 1). Both the ligand and the complexes are soluble only in DMF and DMSO.Molar conductance values of the complexes in DMF ($10^{-3}$ M solution at 25 °C) lying in the range of 12.4-14.8 $\Omega^{-1}$ $cm^3$ $mol^{-1}$. This support their non-electrolytic nature having the molar ratio of metal:ligand as 1:2 [31]. The presence of chloride (counter) ion is confirmed by Volhard’s test. The obtained elemental analysis data of the complexes were in good agreement with the calculated values and show that the complexes may have the stoichiometry of $[M(L)_2Cl_2]$, wherein the ligand acts as a bidentate ligand.

International Journal of Engineering Technology Science and Research
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ISSN 2394 – 3386
Volume 4, Issue 9
September 2017

### 3.1.IR SPECTRAL ANALYSIS

Vibration spectroscopy serves as an important tool to confirm the structure of the compounds. The ligand shows the characteristic C=O amide stretching peak at 1608 $cm^{-1}$, which confirms the formation of the ligand [32]. The ligand also exhibits a series of peaks at 3412 $cm^{-1}$ (N-H amide str), 3105 $cm^{-1}$ (C-H aromstr), 2711 $cm^{-1}$ (C-H str), (C=O amide str), 1065 $cm^{-1}$ (C-N str) which further supports the ligand formation. The frequency observed at ν 1140 $cm^{-1}$ due to ν(C2-N3) + (C4-N9) group confirms the non-involvement of N-9 nitrogen in acylation with histidine as there is no shift. At the same time, a large shift from ν 1232 to 1335 $cm^{-1}$ for ν(C8-H) + (N7-C8) group confirms the acylation of adenine at N-7 nitrogen [33]. In the spectrum of the Co(II) and Zn(II) complex, the peaks due to C=O amide stretch (1601-1115 $cm^{-1}$) and N-H amide stretch (3334-3373 $cm^{-1}$) was shifted to lower frequencies. This proves the coordination of caroboxylate C=O and amide N-H of the ligand with the metal [34]. The occurrence of bands at 570-617 $cm^{-1}$ (M-O) and 427-435 $cm^{-1}$ (M-N) provides evidence for the bonding of oxygen and nitrogen to the central metal ion. From the above discussion, the formation of ligand and the complexes were confirmed in which the ligand acts as a bidentate donor.

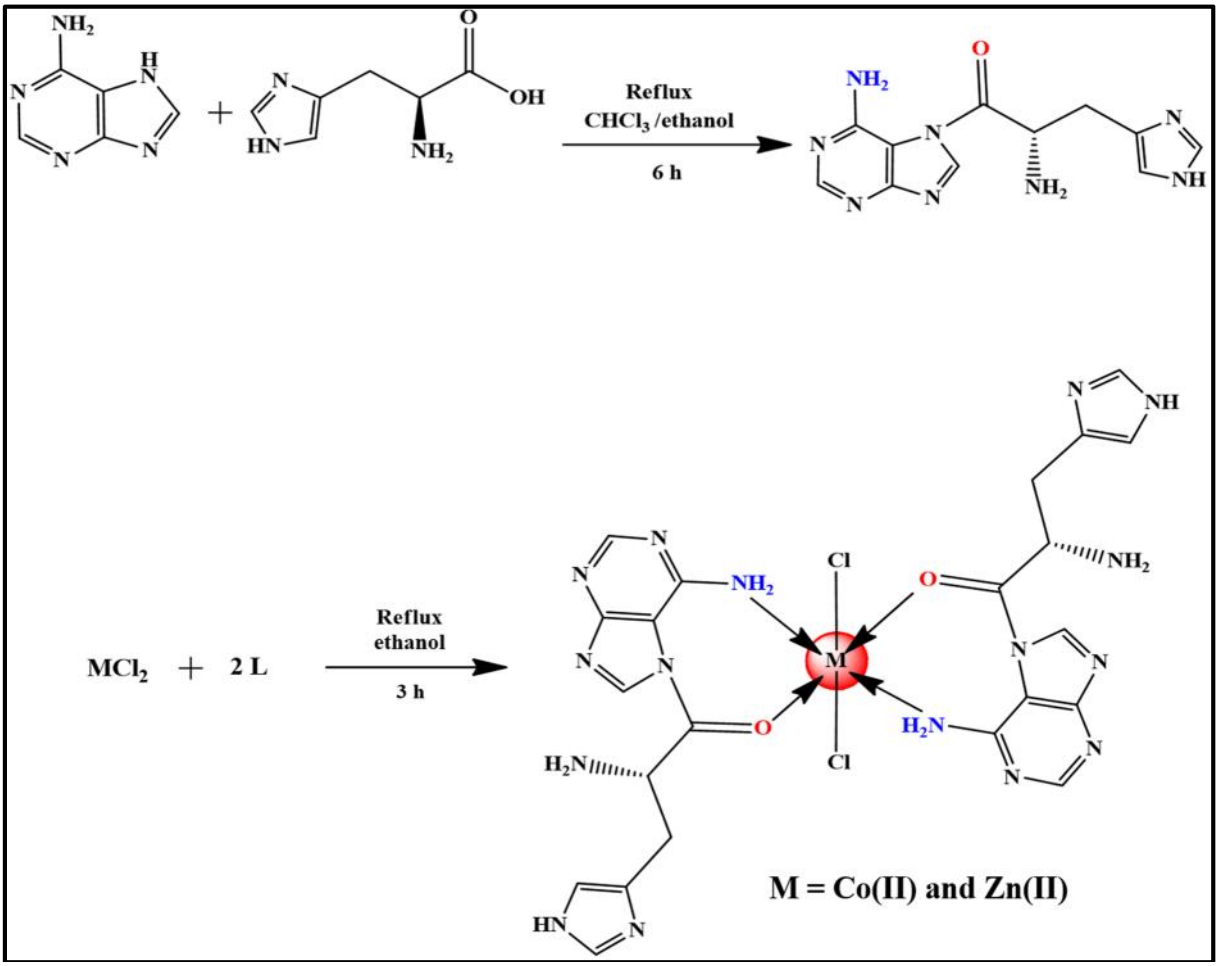


**Figure 1. Schematic diagram of synthesis of ligand (L), $[Co(L)_2Cl_2]$ and $[Zn(L)_2Cl_2]$ complex.**

### 3.2. ABSORPTION SPECTRA AND MAGNETIC SUSCEPTIBILITY MEASUREMENTS

The electronic absorption spectrum of the free ligand (L) was characterized by two absorption bands at 206 and 251 nm assigned to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$, respectively. Similarly, the spectra of the complexes display the characteristic $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transition bands, but due to the coordination of ligand with metal center they slightly shift to higher wavelength. However, the Co(II) complex exhibits a characteristic d-d transition band at 542 nm, which is assigned to $^3A_{2g} \rightarrow {}^3T_{1g}$ transition, suggesting an octahedral arrangement for the Co(II)

International Journal of Engineering Technology Science and Research
IJETSR
www.ijetsr.com
ISSN 2394 – 3386
Volume 4, Issue 9
September 2017

complex [35]. In addition, the CT band for Co(II) and Zn(II) complexes was located at 353 and 349 nm. In difference, Zn(II) complex does not exhibit any d-d band because of its completely filled $d^{10}$ transition. However, based on its elemental analysis and spectral data, an octahedral geometry is proposed for this complex also.

The magnetic moment of the Co(II) complex was measured at room temperature and represented in Fig. S3. The magnetic moment for complex was found to be 4.29 BM which suggests that the central metal ion is in a high spin configuration and is octahedral in geometry [36].

### 3.3. $^{1}$H AND $^{13}$C NMR SPECTROSCOPY

To further confirm the formation of the ligand and the complexes, the $^{1}$H and $^{13}$C NMR spectra of the ligand in the absence and presence of zinc ion were recorded at room temperature (25 °C) in DMSO-$d_6$with TMS as internal standard. TMS was used as an internal standard. The $^{1}$H NMR spectra of the ligand shows a peak at δ 8.1 ppm which can be assigned to CO-NH protons. This confirms the formation of the ligand by condensation of carboxylic -OH and –NHof adenine [15]. On complexation with zinc metal ion this peak shifted to lower value, which explains the coordination of the ligand to metal.

In the $^{13}$C NMR spectra of the ligand two peaks appeared at δ 172 and 122.4 ppm which is consigned to -CO-NH and -C-$NH_2$, respectively, supporting the formation of the ligand [15]. In the $^{13}$C NMR of the zinc complex these peaks were shifted to lower and higher value [(CO-NH) 168 and (C-$NH_2$) 119] due to the coordination with metal ion [15]. The peaks due to other protons remained unaltered which proves their non-involvement in coordination. Both the $^{1}$H and $^{13}$C NMR spectral studies confirms the formation of the ligand and its metal complexes.

### 3.4. ESI MASS SPECTROSCOPY

The formation of the ligand and the complexes and their proposed structure was further confirmed by the ESI mass spectra. The spectra of the ligand show its molecular ion peak at m/z 272 which is in good agreement with the formula weight. The spectrum also exhibited a series of peaks at m/z 253, 237, 168, 131, 120, 92, 63 which can be attributed to its further fragments. On the other hand, in the mass spectrum of Co(II) and Zn(II) complexes the molecular ion peaks observed at m/z 674 and 680, respectively, matches with their formula weight. In addition, the spectrum of both the complexes exhibit several peaks which can be assigned to their further fragments. It is worth mentioning that the results of mass spectrum are in agreement with micro analytical data and suggested formula.

### 3.5.BSA BINDING STUDIES

#### 3.5.1.ABSORPTION SPECTRAL STUDIES

UV absorption spectrum is a very simple and applicable method to explore the structural change and to know the complex formation in solution. BSA has an absorption peak at around 280 nm resulted from the aromatic amino acids(Trp, Tyr and Phe) [37]. This absorption peak was frequently used for monitoring theinteraction between BSA and compounds. Fig. 2 shows the UV absorption spectra of BSA in the presence of different concentrations of the two complexes. From this figure, BSA has two main absorption bands. One is located in the range of 220–240 nm, which is the skeleton absorption peak, and the other is at 278 nm, which is the absorption band of the aromatic amino acids (Trp, Tyr, and Phe). As can be seen, upon adding the two complexes, the BSA skeleton absorption intensity in the range of 220-240 nm is increased and red shifted. In addition, the maximum absorption at 278 nm is also enhanced with a slight blue shift (from 278 nm to 272 and 273 nm for complex 1 and 2, respectively). These results indicate that thepossible quenching mechanism of BSA by the compounds is a static quenchingprocess owing to the complex formation between compounds and BSA [38]. Theformation of ground state complex can be represented as below:

BSA + complex $\rightarrow$BSA-complex.

The $K_{app}$ value, which is the change in the intensity of absorption peak at 280 nm as a result of the formation of surface complex, is obtained according to the method reported by Benesi and Hildebrand [39]. A linear relationship between $1/A_{obs}$-$A_o$ and the reciprocal of complex concentration is viewed in Fig.2 based on the following equation.

$$\frac{1}{A_{obs}-A_o}=\frac{1}{A_c-A_o}+\frac{1}{K_{app}(A_c-A_o)[metal\ complex]} \qquad \ldots\ldots\ldots\ldots(2)$$

International Journal of Engineering Technology Science and Research
IJETSR
www.ijetsr.com
ISSN 2394 – 3386
Volume 4, Issue 9
September 2017

From the graphical representation, the slope which is equal to $1/K_{app}$ ($A_c$-$A_o$) and an intercept equal to $1/A_c$-$A_0$ were calculated. The value of apparent association constant determined is found to be $2.09 \times 10^5$ $M^{-1}$ and $2.67 \times 10^4 M^{-1}$ for the complexes 1 and 2, respectively.

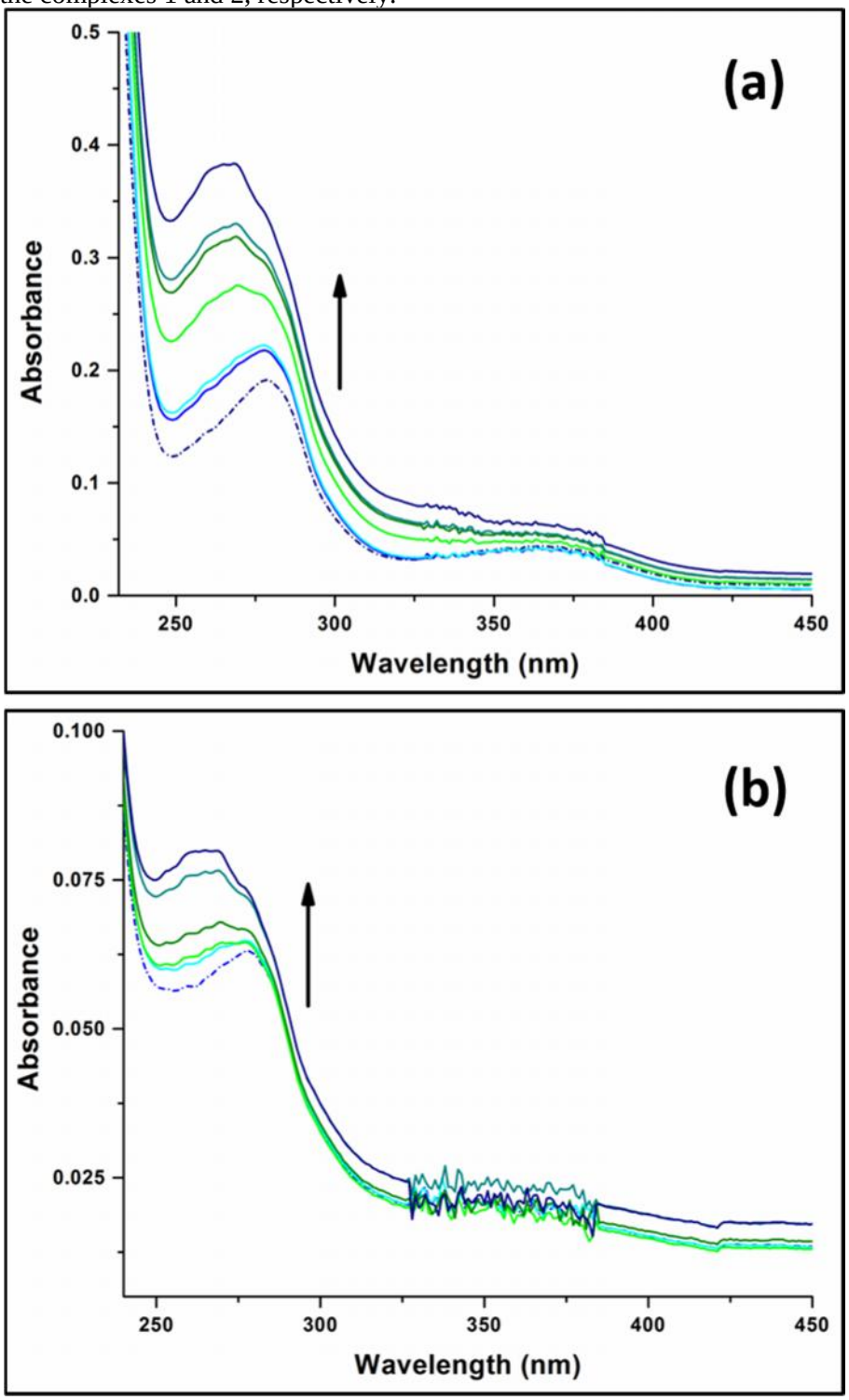


**Figure 2. Electronic absorption spectra of BSA upon addition of (a) $[Co(L)_2Cl_2]$ and (b) $[Zn(L)_2Cl_2]$, the arrow shows the absorbance changes upon increasing amounts of complex**

International Journal of Engineering Technology Science and Research
IJETSR
www.ijetsr.com
ISSN 2394 – 3386
Volume 4, Issue 9
September 2017

### 3.5.2.FLUORESCENCE SPECTRAL STUDIES

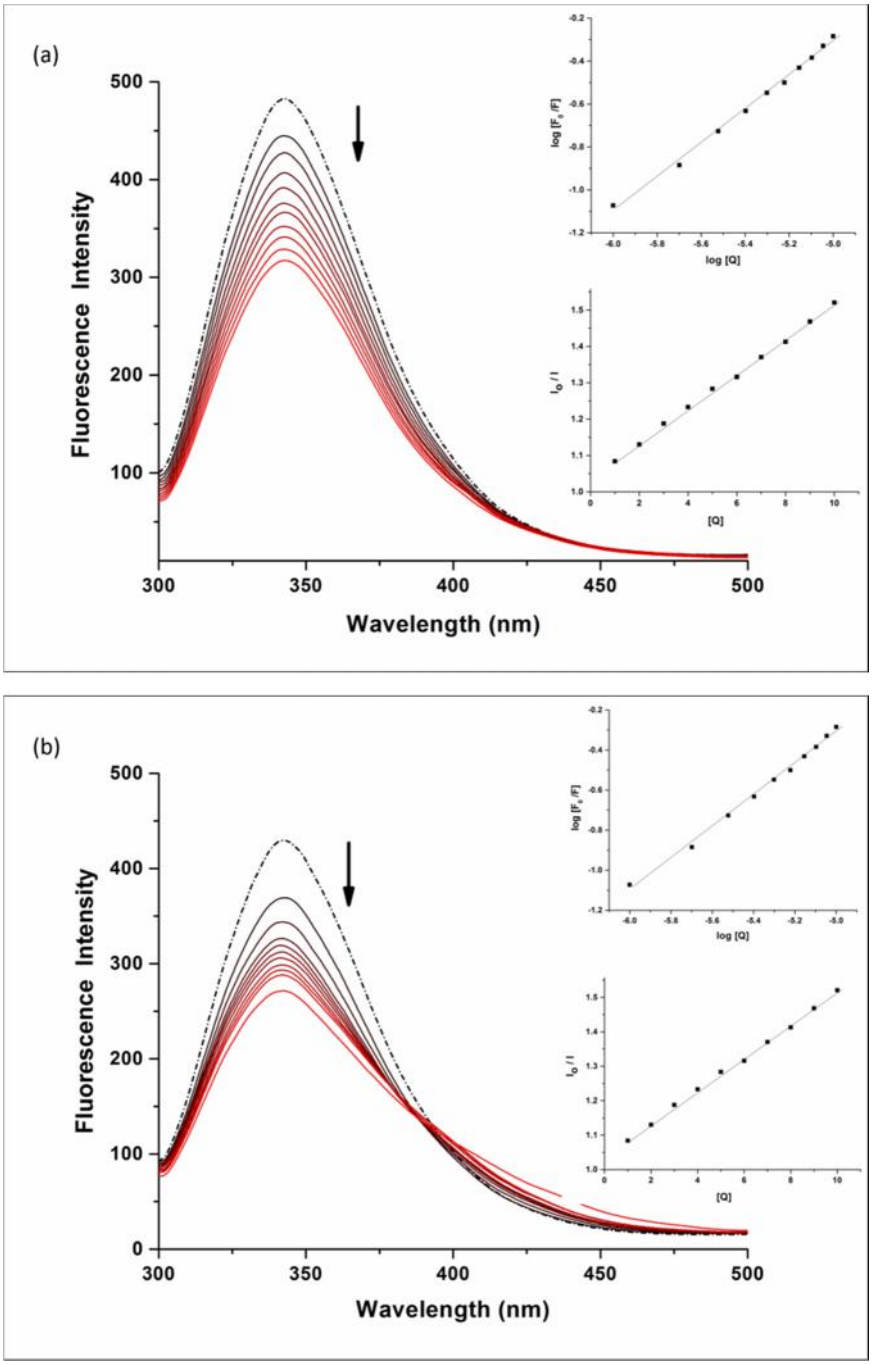


**Figure 3. Emission spectra of BSA in the presence of (a) $[Co(L)_2Cl_2]$ and (b) $[Zn(L)_2Cl_2]$ complex. The arrow shows the intensity changes upon increasing amounts of complex 1 and 2. Inset: Plot of $I_o$/ I Vs. [Q] and plot of log[$F_0$-F] Vs. log[Q].**

Fluorescence spectroscopy is an effective method to qualitative analysis of the binding of complexes to BSA. The fluorescence of a protein is due to intrinsic characteristics of its tyrosine and tryptophan residues. The relative ratio of fluorescence intensity of the three amino acid residues is 100:09:05. When BSA is excited, the fluorescence intensity at 280nm is observed because of tryptophan residues [40]. The interaction of BSA with the complex was studied and the significant changes in fluorescence were shown in Fig. 3. Addition of the complex solution to BSA showed a blue shift to 346 nm. It is mainly because of the fact that the active site in protein is buried in a hydrophobic environment. Similar observation was made by earlier studies [12, 15].

International Journal of Engineering Technology Science and Research
IJETSR
www.ijetsr.com
ISSN 2394 – 3386
Volume 4, Issue 9
September 2017

The quenching process of the complexes on BSA fluorescence can be analyzed by the Stern–Volmer equation [41]:

$I_0 / I = 1 + K_{sv}$ [Q]

where the $I_0$ and $I$ are the fluorescence intensities of fluorophore at 347 nm in the absence and presence of the complexes, respectively. [Q] is the concentration of quencher and $K_{sv}$ is the Stern–Volmer quenching constant. As shown in Fig. 3, the insets plot of $I_0/I$ versus [Q] exhibits a good linear relationship correlation coefficient $R_1$ = 0.871 and $R_2$ = 0.963. Also, the $K_{sv}$ value obtained from the slope of the linear are $1.72 \times 10^6$ $M^{-1}$ and $1.35 \times 10^4$ $M^{-1}$, respectively.

In general, fluorescence quenching usually occur by two different mechanisms which are classified as dynamic quenching and static quenching. Dynamic quenching refers to that the fluorophore and the quencher come into contact during the transient existence of the excited state and the static quenching refers to fluorophore–quencher form a ground state [42]. For static quenching interaction, the fluorescence intensity data can also be used to analyze the apparent binding constant ($K_b$) and the number of binding sites (n) for the complex and BSA system by the following equation [43]:

$$\log\left[\frac{I_o - I}{I}\right] = \log K + n \log[Q] \qquad \text{..... (3)}$$

where, $K_b$ is the equilibrium constant and $n$ is the number of binding sites per albumin that is calculated from the intercept and slope in Fig. 3 $\log((I_0 - I)/I)$ versus log[Q]. The values of $K_b$were obtained to be $1.44 \times 10^6$ $M^{-1}$ and $1.13\times 10^5$ $M^{-1}$, and $n$ were found to be 1.09 and 1.17, respectively. These values of $n$ are approximately equal to 1, suggest that there is only one binding site for these complexes on the BSA molecule. Since $K_b$ is more for complex 1 than that for complex 2, the former is more in a bound state with the fluorophore tryptophan (in BSA). Hence, the complex 1 has more chance of quenching the fluorophore of tryptophan and it is reflected in a slightly higher magnitude of $K_b$for the complex 1 than that of complex 2.

### 3.6.BIOLOGICAL STUDIES

#### 3.6.1.ANTIOXIDANT ACTIVITY

As the newly synthesized ligand and its transition metal complexes exhibit good DNA binding affinity, it is considered valuable to investigate their antioxidant activity. In the present study, total DPPH scavenging potential of the ligand (L) and the complexes at varying concentrations were measured and the results were represented in Fig. 4. Vitamin C was used as a reference compound.

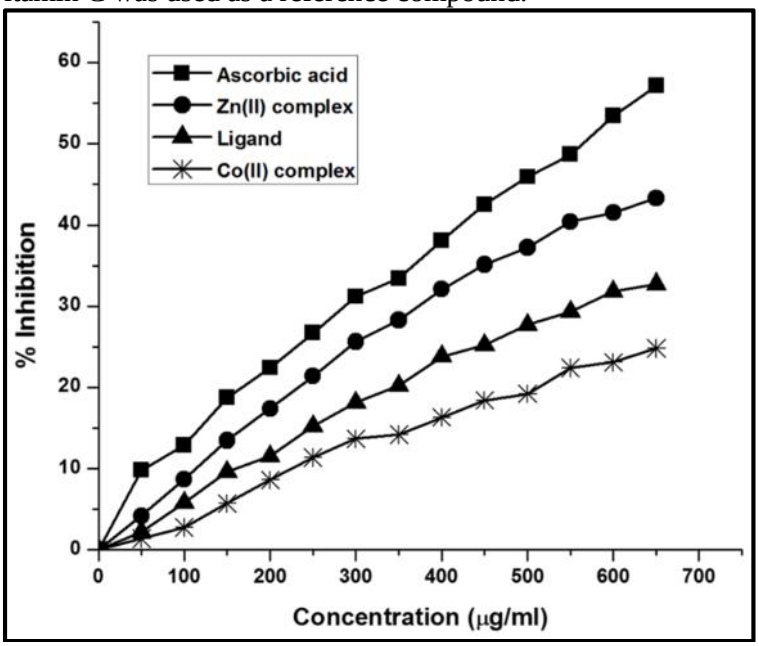


**Figure 4. DPPH radical scavenging activities of ascorbic acid, ligand (L), $[Co(L)_2Cl_2]$ and $[Zn(L)_2Cl_2]$ complex.**

International Journal of Engineering Technology Science and Research
IJETSR
www.ijetsr.com
ISSN 2394 – 3386
Volume 4, Issue 9
September 2017

It is obvious from the results that free radical scavenging activity of these compounds were concentration dependent. Among the compounds tested, Co(II) complex showed maximum radical scavenging activity (89%) that is comparable to that of vitamin C (standard drug). On the other hand, ligand (L) and Zn(II) complex exhibited moderate scavenging activity (57% and 69%) for DPPH radicals. The $IC_{50}$ value, i.e. the concentration of the compound required to reduce the initial absorption by 50%, was determined and it was found that the ligand (L) (45.12 µg/ml) and Zn(II) (38.45 µg/ml) have the highest $IC_{50}$ value, i.e. the lowest antioxidant activity, whereas Co(II) complex has the lowest $IC_{50}$ value (21.78 µg/ml) and the highest antioxidant activity, as shown in Fig. 4.

**3.7. Molecular docking studies**

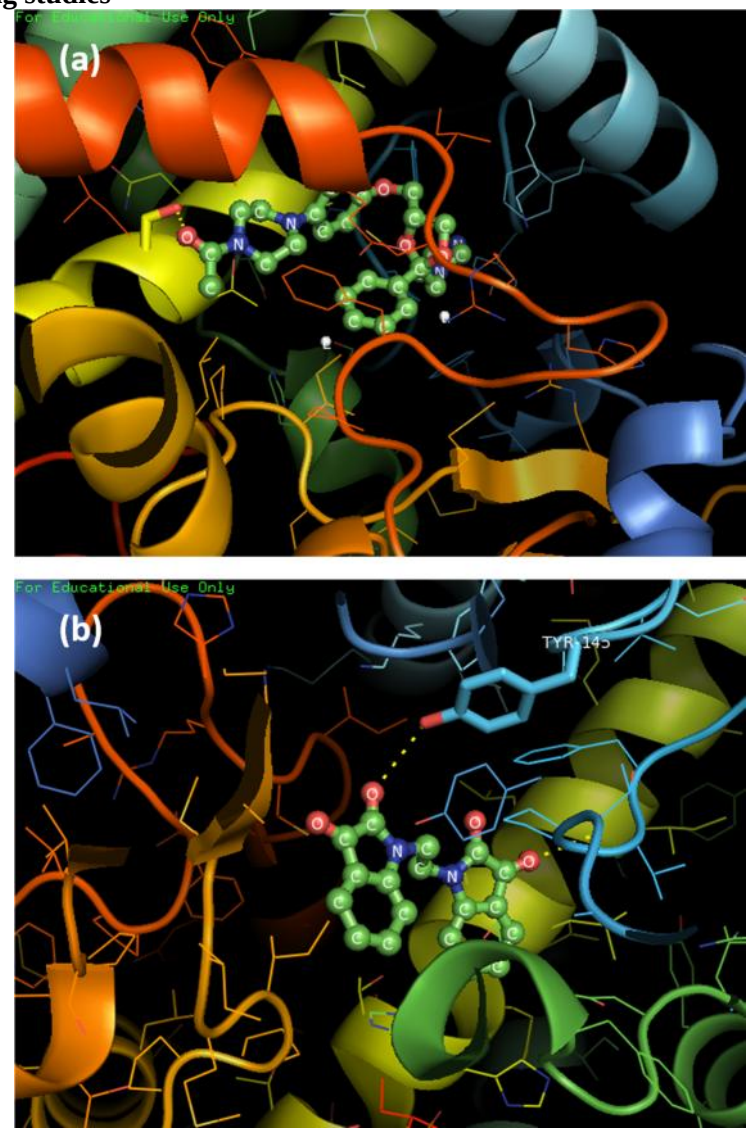


**Figure 5. Docking pose of (a) complex 1 and (b) complex 2 with BSA. The amino acid residues around the complex are shown in lines and the complex is represented with ball and stick model. The hydrogen bonds between HSA and resveratrol are shown as yellow dashed lines**

Molecular docking technique is an attractive platform to understand the drug-protein interactions for the rational drug design and discovery. For the purpose of obtaining insight into the preferred binding location and helping deepen the understanding of the complex-protein interaction, AutoDock was used to dock complexes 1 and 2 into protein BSA (Fig. 5). It has been found that two principal binding sites in BSA (PDB ID: 4F5S) molecule are in the proximity of Trp134 and Trp213, where Trp213 is located within a hydrophobic binding pocket, and Trp134 is located on the surface in the hydrophilic region of the molecule [44]. According

to the spectral results of BSA binding, both the two complexes may have only one binding site in BSA. Comparing the binding affinity of the two complexes, it can be seen that the complex 1 (-6.75 $KCalM^{-1}$) binds to BSA stronger than the complex 2 (-6.13 $KCalM^{-1}$), which is consistent with the spectral results. As for binding site selection, Trp134 is the most possible binding site for the complex 1, while for the complex 2, Trp213 is more favored. The present results suggest that the binding of the complex 1 to BSA is stronger than that of complex 2, which may be due to the coordinated counterion regulation.

## 4. CONCLUSION

In the present work, we have reported the synthesis of amino acid-nucleobase hybrid ligand and its Co(II) and Zn(II) complex. The synthesized ligand (L) and the complexes was thoroughly characterized by means of various physical, analytical and spectral techniques. The results suggested an octahedral geometry for both the complex having the general formula of $[M(L)_2Cl_2]$. According to the results arising from spectroscopic methods, we conclude that both complex 1 and 2 could quench the intrinsic fluorescence of BSA in a static quenching process. The binding constants ($K_b$) and the numbers of binding sites (n) obtained are $1.44 \times 10^6$ $M^{-1}$and 1.09 for complex 1 and $1.13 \times 10^5$ $M^{-1}$ and 1.17 for complex 2, respectively. DPPH free radical scavenging ability of the ligand and the complexes were investigated by antioxidant activity, which reveals that Co(II) complex exhibits higher scavenging ability than the ligand (L) and Zn(II) complex. The molecular modeling studies showed a binding mechanism, very close to the experimental result with a binding free energy of -6.75 and -6.13 $KCalM^{-1}$, respectively for complex 1 and complex 2. Both experimental and theoretical studies indicated that complexes 1 and 2 bind to the hydrophobic cavity of BSA. These results provide some significant clues for the clinical research and the pharmacological properties of the compounds under study.

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