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# Multispectroscopic and molecular modeling approach to investigate the interaction of zinc(II) complex containing amino alcohol ligand with biomacromolecule HSA

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**Abstract:** In this study, a zinc(II) complex containing amino alcohol ligand; [(cis-2-((2-((2-hydroxyethyl)aminoethylamino)benzene-1-ol) dibromo zinc(II)] or briefly [Zn(HAIP)$Br_2$]) has been synthesized and the interaction with Human Serum Albumin (HSA) studied using various spectroscopic techniques and molecular docking. The UV–vis absorption spectra of HSA decreased by adding the complex and therefore, suggesting that this complex led to a change in conformation of α-helical of protein. The fluorescence results showed that the HSA emission was quenched by zinc(II) complex through static quenching. In addition, the presence of hydrogen bond and van der Waals force was confirmed due to thermodynamic parameters. The molecular docking was used to simulate and predict the binding site of zinc(II) complex to albumin and to authenticate experimental results. There is a good agreement between the free binding energy of docking simulation and the binding constant of fluorescence experiments. These results very clearly authenticate that zinc complex–HSA docked model is in approximate correlation with our experimental results.

**Keywords:** Zinc complex; Amino alcohol ligand; Bio-macromolecule; Human Serum Albumin (HSA); Spectroscopic methods; Docking.

**Abbreviation:** (cis-2-((2-((2-hydroxyethyl)aminoethylamino)benzene-1-ol) dibromo zinc(II) ([Zn(HAIP)$Br_2$]), Human Serum Albumin (HSA), Circular Dichroism spectroscopy (CD), Ultra Violet Visible (UV-Vis).

## 1. Introduction

Metal ions play important roles in biological processes. Zinc is an essential trace element for humans, animals, plants, and microorganisms. Zinc is required for the function of over 300 enzymes and 1000 transcription factors and is stored and transferred in metallothioneins. The search for anticancer metal-based drugs alternative to platinum derivatives could not exclude zinc derivatives due to the importance of this metal for the correct functioning of the human body. Zinc like some other metals can bind to ligands and form complexes

with different properties. One group of these ligands are alkanolamines, which contain hydroxyl and amino functional groups [1]. Most of proteins have alcohols and amino groups simultaneously. β-amino alcohols can show various biological properties. Metal complexes contained β-amino alcohol can interact with macromolecules like HSA, which can be found in human blood. HSA is the most abundant protein in human blood plasma constitutes about half of serum protein and produced in the liver. It is soluble in water, and monomeric. HSA transports hormones, fatty acids, and other compounds.

HSA is a 66.5 kDa protein synthesized by the liver. It comprises 585 amino acids and has high cysteine content and a high proportion of negatively charged amino acids but no carbohydrate. HSA has three domains [2]. HSA is a 585-residue protein monomer containing three homologous helical domains each split into A and B subdomains and arranged to form a heart-shaped molecule [3]. Nanoparticles prepared by desolvation and subsequent crosslinking of HSA represent promising carriers for complex delivery [4].

Two metal complexes contained zinc and copper were studied by a research group [5]. The binding effect of these complexes to HSA showed a similar way as the antiproliferative activities of both complexes against cancer cell [6].

Lazar et al. Conducted studies on amino alcohols. Amino alcohols containing a primary amino group cycled with aldehydes are usually in mild conditions (for example at room temperature without any catalysts or additives). The ring closure of amino alcohols containing a primary or secondary amino group with formaldehyde also continues easily.

In this research, the interaction between a zinc(II) complex containing amino alcohol ligand with HSA has been investigated in details by employing a combination of spectroscopic methods and molecular docking simulation.

Cancer is the second leading cause of death in human societies. Therefore, the study and synthesis of new anticancer drugs has an important place in research. Assuming that biological metals such as zinc(II) may be less toxic, zinc(II) complexes could be introduced as new candidates with fewer side effects for cancer treatment. In this dissertation with this approach, the mechanism of interaction between zinc(II) complex containing amino alcohol ligand and vital biomolecule of the body, namely HSA have been investigated. For this purpose, experiments have been conducted and main effects and interactions have been investigated.

## *2. Materials and methods*

### *2.1. Apparatus*

The UV-Vis spectra were obtained using a Nordantec T80 UV–Visible spectrophotometer with a 1 cm quartz cell. Fluorescence measurements were performed using a JASCO (FP-6200) spectrofluorimeter with a quartz cuvette of 1 cm path length. Jenway 3510 pH meter was used for the measurement of pH values.

### *2.2. Materials*

HSA and warfarin were purchased from Sigma Co, Germany. Ibuprofen was purchased from Abidi pharmaceutical Co, Iran. $Na_2HPO_4$ and $NaH_2PO_4$ were purchased form Merk Co, Germany. The starting materials and solvents used without purification.

### *2.3 Sample preparation*

Stock solution of HSA was prepared in 0.1 M phosphate buffer of pH 7.4 and stored in the dark at 4 °C. Stock solution of warfarin ($1\times10^{-2}$ M, based on its molecular weight of 308.3) was prepared by dissolving 6.1 mg of warfarin in 2 ml water and stored 20 min. Stock solution of ibuprofen ($1\times10^{-2}$ M, based on its molecular weight of 206.3) was prepared by dissolving 4.1 mg of ibuprofen in 2 ml water and stored 20 min. Stock solution of the complex ($1\times10^{-3}$ M, based on its molecular weight of 454.46) were prepared by dissolving 0. 9 mg of complex in 2 ml of Phosphate buffer.

### *2.4 Synthesis of [Zn(HAIP)$Br_2$] complex*

[Zn(HAIP)$Br_2$] complex was synthesized as follows and the study was submitted before:

0.45 g (2 mmol) of ligand dissolved in 7 ml of methanol and added to a solution of 0.49 g (2 mmol) of zinc(II) bromide in 25 ml of methanol. The reaction mixture then was stirred for 6 h at 70 ° C. By slow evaporation of the solvent, yellow crystals suitable for X-ray crystallography were obtained. The properties of this crystals is the Yield of 22% (0.24 g) and melting point of 188°C. The elemental analysis is as follows:

Anal. Calc. for zinc(II) complex (%): C, 30.48; H, 3.72; N, 6.46, Found: C, 30.59; H, 3.85; N, 6.50. IR (KBr disc): 3501 (ν O−H), 3105 (ν N−H), 3015 (ν C−H)ar, 2951 (νas $CH_2$), 2903 (νs $CH_2$), 1630 (δs $NH_2$), 1544 (ν C=N), 1464 (ν C=C), 1446 (δas $CH_2$), 1403 (δs $CH_2$), 1248 (ν C−O), 1064 (ν C−N).

The chemical structure of mentioned complex has been illustrated in Fig.1.

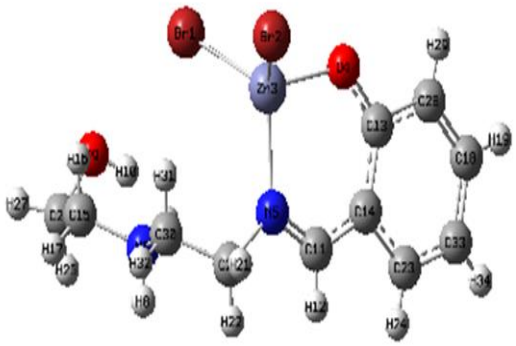

**Fig. 1. The chemical structure of [Zn(HAIP)$Br_2$] complex**

### *2.5 UV-Vis absorption measurements*

The absorbance measurements were performed by keeping the HSA concentration constant ($3 \times 10^{-6}$ M) while adding different concentrations of zinc(II) complex from $2.2\times10^{-5}$ to $7.1\times10^{-5}$ M. The spectra were recorded in the range of 250-350 nm.

### *2.6 Fluorescence measurements*

All fluorescence measurements were recorded with spectrofluorimeter equipped with quartz cells (1cm) and a thermostat bath. Fluorescence spectra were recorded from 310 to 430 nm. Fluorescence intensity of HSA in the absence and the presence of zinc(II) complex was measured by keeping the concentrations of HSA ($3 \times 10^{-6}$ M) constant while adding different concentrations of zinc(II) complex from $9.51 \times 10^{-7}$ M to $7.08\times 10^{-5}$ M at three different temperatures (293, 303 and 310 K). There was the following setting: 295 nm as excitation wavelength and emission observed between 310 and 430 nm.

### *2.7 Circular dichroism (CD) measurements*

Circular Dichroism (CD) measurements were recorded on spectropolarimeter (between 200 and 250 nm and cell length path was 1 cm) by keeping the concentration of HSA constant ($3 \times 10^{-6}$ M) while adding different concentrations of zinc(II) complex (ri = 0, 0.5). The complex concentration is $1.5 \times 10^{-6}$ M.

### *2.8 Molecular docking simulation*

The open-source Auto Dock Vina (version 1.1.2) with MGL tools 1.5.6 has conducted the docking calculation. The known crystal structure of HSA (PDB ID: 1AO6) was obtained from the Protein Data Bank. Receptor (HSA) and ligand (Zn complex) files were provided using Auto Dock Tools. The selected HSA model was enclosed in a box with the number of points in x, y and z dimensions of 34, 26 and 28 and center grid box of 17.84, -17.62 and 16.69 with a grid spacing of 1.00 Å. Lamarckian genetic algorithm was employed to perform docking calculations. All other parameters were default settings. Visualization of the docked pose has been carried out by using BIOVIA Discovery Studio Visualizer 2021.

## *3. Results and discussion*

### *3.1 UV-Vis absorption spectroscopy*

Spectroscopy techniques can be used to explore the structural changes of protein and to investigate protein–ligand complex formation [7]. The UV–vis absorption spectra of HSA decreased by adding zinc(II) complex (Fig. 2). Therefore, the UV–vis spectra of HSA in the presence of zinc(II) complex suggested that this complex led to a change in conformation of α-helical of protein. However, the prominent structure of protein remains α-helix. The UV–vis absorption spectra of HSA in the absence and presence of zonisamide (ZNS) [8], Cu(II) complex containing ranitidine drug and 1,10-phenanthroline ($C_{25}H_{40}N_8O_{14}SCu$) [9] and Cu-isothiosemicarbazonato complex [10] were similar to [Zn(HAIP)$Br_2$] and decreased by adding the complexes and drugs. This result indicated changing in protein conformation.

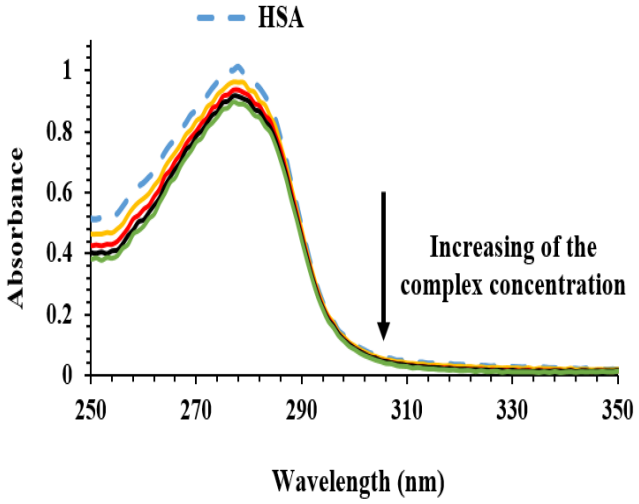


**Fig. 2. Absorption spectra of HSA in the absence and presence of increasing amounts of zinc(II) complex (from $2.2\times10^{-5}$ to $7.1\times10^{-5}$ M).**

### *3.2 Fluorescence studies*

Fluorescence quenching is the decrease of the quantum yield of fluorescence from a fluorophore induced by a variety of molecular interactions with a quencher molecule. Generally, the fluorescence of HSA comes from tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe) residues. A single tryptophan residue, Trp 214, located in the depth of subdomain IIA is largely responsible for the intrinsic fluorescence of HSA, because phenylalanine has a very low quantum yield and the

fluorescence of tyrosine is almost totally quenched if it is ionized or near to an amino group, a carboxyl group or a tryptophan [11]. The intrinsic fluorescence of HSA with increasing concentration of zinc(II) complex was recorded in the range of 310–430 nm. The addition of zinc(II) complex resulted in concentration-dependent quenching of Trp 214 fluorescence. Fluorescence spectroscopy has been widely used in the study of molecular interactions between small molecules and proteins owing to its unique sensitivity, selectivity, convenience, and the variety of parameters related with the interaction molecular that can be obtained; so in the current work, fluorescence measurements were carried out to investigate the binding mechanism of zinc(II) complex with HSA. We can get helpful information about albumin-zinc(II) complex interaction such as binding site, binding mode, binding mechanism, and binding constant by employing the fluorescence spectral studies [12]. The emission signals of albumin were noted before and after the addition of zinc(II) complex to clarify the effect of the complex on albumin fluorescence intensity. The enhancing concentration of zinc(II) complex quenched the intrinsic fluorescence of albumin through a concentration dependent manner, suggesting that zinc(II) complex interacted with albumin [13]. At different temperatures, by adding zinc(II) complex into HSA solution, the fluorescence intensity decreased (Fig. 3). Fluorescence quenching is because of any processes that decrease the fluorescence intensity of a sample. Fluorescence titration curve of HSA in the absence and presence of different concentrations of Pt $[Iso]_2$ complex ((cis−$[Pt(NH_2$-Isopentylamine$)_2$(Isopentylglycine)$]NO_3$) [14], Ru(II)-arene complex [15] and $[SnMe_2Cl_2(bu_2bpy)]$ [16] showed the same changes as [Zn(HAIP)Br2]. There are several mechanisms of quenching like dynamic quenching and static quenching.

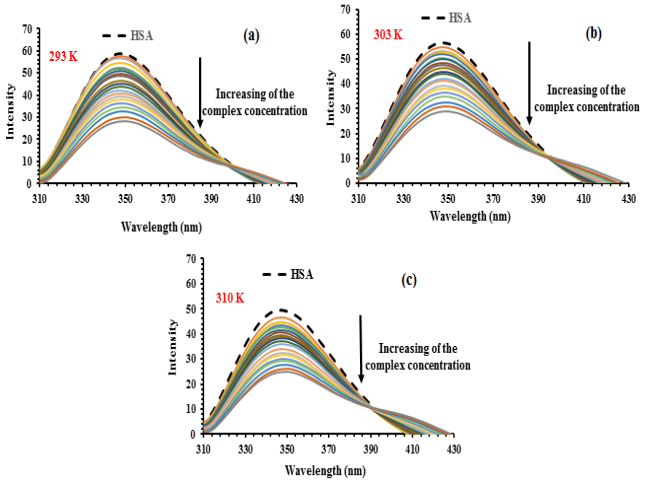


**Fig. 3. Fluorescence emission spectra of HSA ($3 \times 10^{-6}$ M) in the absence and presence of increasing amounts of zinc(II) complex from $9.51 \times 10^{-7}$ M to $7.08 \times 10^{-5}$ M at (a) 293 K, (b) 303 K and (c) 310 K.**

In this experiment, Stern–Volmer quenching equation (Eq. 1) was employed to analyze the fluorescence quenching data and to deliberate the quenching mechanism of zinc(II) complex with albumin.

$$F_0/F = 1 + K_q \tau_0 [Q] = 1 + K_{sv} [Q] \quad (1)$$

In this equation, $F_0$ and F is the emission intensity of albumin without and with zinc complex. $K_{sv}$ is the Stern–Volmer collisional quenching constant, $K_q$ is the quenching rate constant of albumin, [Q] is the concentration of zinc complex, and $\tau_0$ demonstrates the average lifetime of albumin without collisional. Fluorescence quenching may result from ground complex formation, energy transfer and dynamic quenching processes. Dynamic quenching refers to a process that the fluorophore and the quencher come into contact during the lifetime of the excited state, whereas static quenching refers to fluorophore–quencher complex formation [17]. One way to distinguish static quenching from dynamic quenching is to examine their differing dependence on temperature. Dynamic quenching depends upon diffusion: higher temperatures result in larger diffusion coefficients. As a result, the bimolecular quenching constants are expected to increase with temperature rising. In contrast, increased temperature is likely to result in decreasing stability of complex, and thus lower values of the static quenching constants. $K_{sv}$ and $K_q$ are obtained from the Stern–Volmer equation. The plot of $F_0/F$ versus [complex] has been used to calculate $K_{sv}$ (Fig. 4). It is seen that $K_{sv}$ and $k_q$ decrease as the temperature increases, indicating that the mechanism of the quenching may be a static quenching (Table 1). These two kinds of quenching mechanisms demonstrate some differences that can be distinguished experimentally, such as the change in the UV–vis spectra of the HSA. The dynamic quenching only affects the excited state of quenching molecule with no function on the absorption spectrum of quenching substances whereas a complex of HSA and the complex forms in static quenching, so there will be some changes in the UV–vis spectra of the HSA.

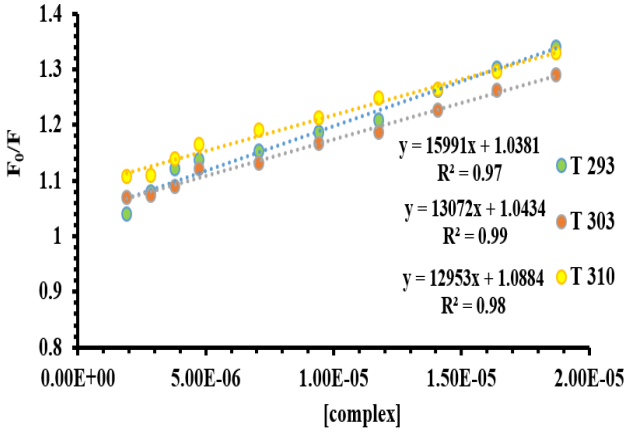


**Fig. 4. The plot of $F_0/F$ versus [complex] for calculating $K_{sv}$.**

**Table 1 Stern–Volmer static quenching constant of HSA-zinc(II) complex at different temperatures.**

| T (K) | $K_{sv}$ (L mol$^{-1}$) | $K_q$ (L mol$^{-1}$ s$^{-1}$) | $R^2$ |
|---|---|---|---|
| 293 | $1.59\times10^4$ | $1.59\times10^{12}$ | 0.97 |
| 303 | $1.30\times10^4$ | $1.30\times10^{12}$ | 0.99 |
| 310 | $1.29\times10^4$ | $1.29\times10^{12}$ | 0.98 |

The modified Stern-Volmer equation was utilized to evaluate the binding strength between zinc(II) complex and albumin and to compute Hill coefficient (n) of the zinc complex-albumin system [18]. For the static quenching interaction, if it is assumed that there are similar and independent binding sites in the biomolecule, the binding constant ($K_b$) and the Hill coefficient (n) can be determined according to the following equation:

$$\log(F_0 - F)/F = \log K_b + n \log [Q] \quad (2)$$

Where $K_b$ and n are the binding constant and Hill coefficient, respectively, which can be found from the double logarithm regression curve of $\log(F_0 - F)/F$ versus log[Q] (Fig. 5). In this equation n is the slope of binding curves. The results showed that the binding constant decreases with the increasing of temperature, which shows that the temperature has an effect on the binding between zinc(II) complex and HSA. Decreasing of $K_b$ indicated the reduction in stability of zinc complex-HSA complex. Furthermore, n is the Hill cooperativity coefficient to describe HSA binding sites cooperativity to put the zinc(II) complex in these sites, and $K_b$ (binding constant) utilizes HSA binding affinity of the zinc complex. The calculated $K_b$ and n are presented in Table 2. The downward trend of the Hill coefficient with temperature increasing, showing a negative cooperation effect.

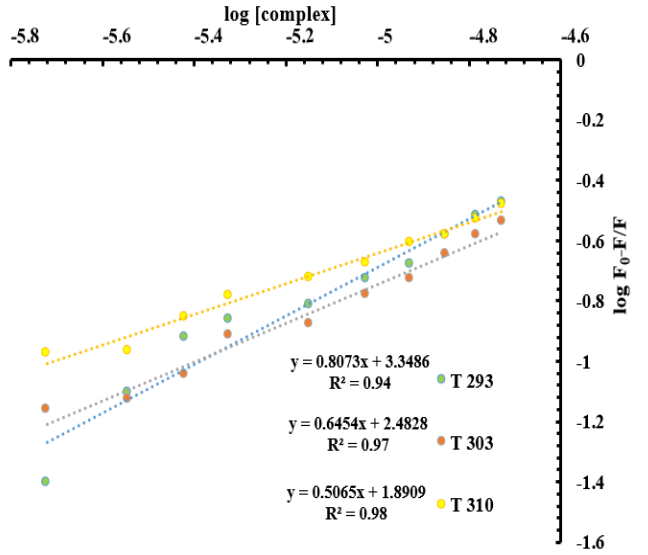


**Fig. 5. Plots for the interaction between the zinc(II) complex and HSA.**

**Table 2 Binding parameters of the interaction of zinc(II) complex with HSA.**

| T (K) | n | $K_b$(L mol$^{-1}$) | $R^2$ |
|---|---|---|---|
| 293 | 0.80 | $2.23\times10^3$ | 0.94 |
| 303 | 0.64 | $3.03\times10^2$ | 0.97 |
| 310 | 0.50 | $7.77\times10^1$ | 0.98 |

The van't Hoff equation (Eq. 3) has been employed to determine the enthalpy change ($\Delta H^0$), entropy change ($\Delta S^0$), and free energy ($\Delta G^0$) of this reaction.

$$\ln K_b = -\Delta H / RT + \Delta S / R \quad (3)$$

In addition, the Gibbs–Helmholtz equation (Eq. 4) has been used to evaluate the ΔH and ΔS at three temperatures:

$$\Delta G^0 = \Delta H^0 - T\,\Delta S^0 \quad (4)$$

According to the data of enthalpy change (ΔH) and entropy change (ΔS), the model of interaction between the complex and biomolecule can be concluded: (1) $\Delta H > 0$ and $\Delta S > 0$, hydrophobic forces; (2) $\Delta H < 0$ and $\Delta S < 0$, van der Waals interactions and hydrogen bonds; (3) $\Delta H < 0$ and $\Delta S > 0$, electrostatic interactions. The thermodynamic parameters of zinc complex-albumin interaction process were determined exper-

imentally to clarify the acting forces between zinc(II) complex and biological macromolecule (Fig. 6). Here, due to sign of enthalpy and entropy, we find that hydrogen bonding and van der Waals are the main forces. The thermodynamic parameter of $\Delta G^0$ can be calculated through the obtained binding constants according to Eq. 5 [19]. Where $K_b$ is the binding constant at the corresponding temperature, R is the gas constant, and T is the temperature. $\Delta G^0$ was calculated by thermodynamic equation that presented in Eq. 5.

$$\Delta G^0 = -RT \ln K_b \quad (5)$$

The negative value of $\Delta G^0$ shows that the binding process of zinc complex-albumin interaction is spontaneous. The computed value of $\Delta H^0$ is negative, displaying that an exothermic reaction occurred during the zinc complex-albumin interaction. In other words, the higher temperature, the weaker bound between albumin and zinc complex. This result also authenticated by the decreasing values of $K_b$ with enhance in temperature (Table 3).

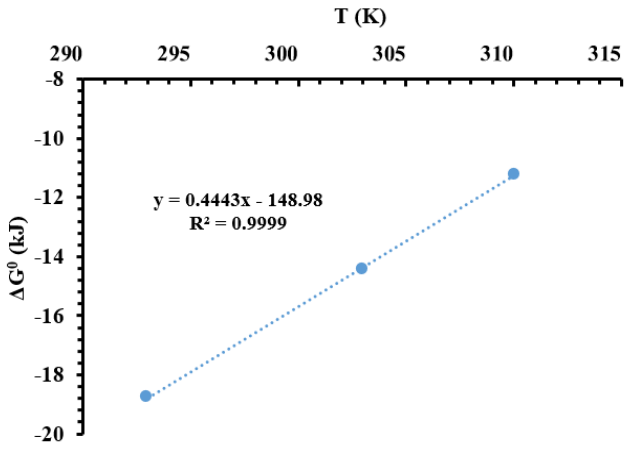


**Fig. 6. Van't Hoff plot of studies of zinc(II) complex to HSA.**

**Table 3 Thermodynamic parameters of the interaction of zinc(II) complex with HSA.**

| T (K) | ΔH (kJ mol$^{-1}$) | ΔS (J mol$^{-1}$K$^{-1}$) | ΔG$^0$(kJ mol$^{-1}$) |
|---|---|---|---|
| 293 | | | -18.78 |
| 303 | -148.98 | -444.3 | -14.40 |
| 310 | | | -11.22 |

### *3.3 Circular dichroism spectral studies*

Circular Dichroism (CD) of HSA ($3\times10^{-6}$ M) in the presence and absence of zinc(II) complex measured. All the spectra were recorded at room temperature. CD spectroscopy as a sensitive technique to monitor the secondary structural change of protein has been used to investigate the structural changes of HSA during the interaction with zinc(II) complex [20]. HSA displayed two negative bands at 208 and 222 nm, characteristic of α-helix structure in protein [21]. The binding of zinc(II) complex to HSA causes an increase in the ellipticity without a significant shift in the position of the bands, indicating that the binding of zinc(II) complex to HSA increases the stabilization of HSA (lack of change in α-helix structure of protein) and can act as a good drug delivery system illustrated in Fig. 7. CD spectra of HSA in the existence of different concentrations of $Fe_3O_4$@CaAl-LDH@L-Dopa (T = 298 K) [22] , [$SnMe_2Cl_2$($bu_2$bpy)] complex [16] and Cu(II) complex containing ranitidine drug and 1,10-phenanthroline ($Cu(NO_3)_2\cdot3H2O$) [9] showed similar changes.

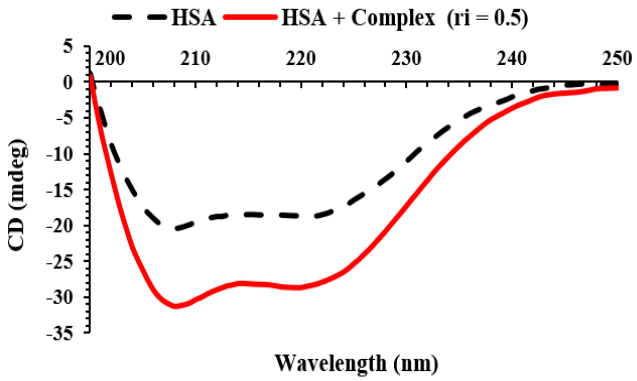


**Fig. 7. CD spectra of HSA in the absence and presence of zinc(II) complex in phosphate buffer at room temperature.**

### *3.4 Competitive binding studies with warfarin and ibuprofen*

Here, two site probes i.e., warfarin and ibuprofen employed for sites I and II, respectively. The zinc(II) complex was added to this solution to deliberate the binding constants of zinc complex–protein systems for sites I and II. For each experiment with different probes, the excitation wavelength and the range of wavelength remained unchanged [23]. Experiments were performed in order to establish the binding site in HSA for zinc(II) complex displacement. Crystallographic analyses have revealed that HSA consists of single polypeptide chain of 585 amino acid residues and comprises of three structurally homologous domains (I–III): I (1–195), II (196–383), III (384–585), that assemble to form a heart-shaped molecule, and each domain containing two subdomains called A and B. During the site marker competitive experiment, zinc(II) complex was gradually added to the solution of HSA and site markers. By adding warfarin, the maximum emission wavelength of HSA had a red shift (346 nm to 360 nm), and the fluorescence intensity was obviously lower than that of without warfarin. Then after adding zinc(II) complex

into above system, the fluorescence intensity of HSA–warfarin solution decreased gradually shown in Fig. 8(a), but the spectral change was much lower than that of without warfarin, showing that the binding of the zinc(II) complex was affected by adding warfarin (Table 4). On the contrary, in the present of ibuprofen, the fluorescence of the HSA–zinc(II) complex almost had no difference from that recorded without ibuprofen under same conditions, which indicated that site (II) marker did not prevent the binding of zinc(II) complex in its usual binding location which can be seen in Fig. 8(b). In addition, the binding constants of competitive experiments were calculated. In the presence of warfarin, the binding constant was significantly changed, while the influence in the presence of ibuprofen in the binding parameters was small. All the experiment results and analysis demonstrated that the binding of zinc(II) complex to HSA mainly located within site I (subdomain IIA) of HSA. Effects of the Chloroxine [13], $Fe_3O_4$@CaAl-LDH@L-Dopa [22] and platinum(II) complex containing antiviral drug ribavirin [23] on the fluorescence of the ibuprofen–HSA system and also the warfarin–HSA complex were similar to [Zn(HAIP)$Br_2$] changes.

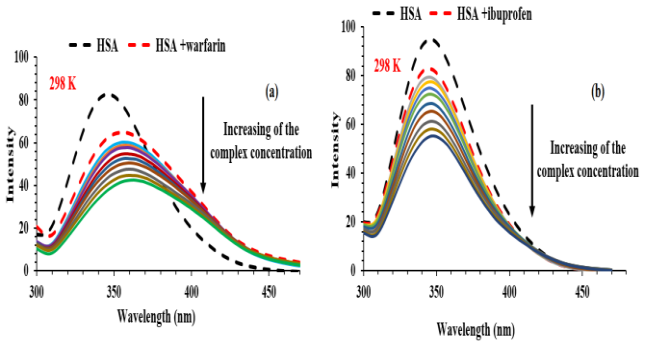


**Fig. 8. Effect of zinc(II) complex on the fluorescence of (a) warfarin-HSA complex ([warfarin] = $10^{-3}$) and (b) ibuprofen-HSA complex ([ibuprofen] = $10^{-2}$ M), in phosphate buffer, pH 7.40 and 298 K upon excitation at 295 nm, [HSA] = 3 ×$10^{-6}$ M and [zinc complex] from 0 to 6.67 ×$10^{-5}$ M.**

**Table 4 Binding constants of competitive experiments for the zinc complex–HSA system.**

| Compound | n | $K_b$(L/mol) | $R^2$ |
|---|---|---|---|
| HSA | 0.98 | 6.79×$10^3$ | 0.99 |
| HSA-ibuprofen | 0.94 | 3.89×$10^3$ | 0.98 |
| HSA-warfarin | 0.73 | 5.38×$10^2$ | 0.96 |

### *3.5 Molecular docking studies on the interaction mechanism of zinc(II) complex with HSA*

Docking results showed that the best docking energy result is picked up from the 20 minimum energy conformers from the 2000 runs. The run data for the conformers has been listed in Table 5. As can be seen, the zinc(II) complex is located within the binding pocket of site 1 (subdomains IIA) which is surrounded by Tyr150, Ser287, Lys195, Arg257, His288 and Ala291 (Figs. 9 and10). The interaction of zinc(II) complex with the HSA binding site residues was stabilized by hydrogen bonds with Tyr150, Ser287, Lys195 and Arg257. In this section, the molecular docking was employed to simulate and predict the binding site of zinc(II) complex to albumin and to authenticate experimental results. Among 20 conformers obtained from docking simulation, one conformer was taken with minimum binding energy conformer that is very close to the experimentally determined values. The following equation (Eq. 6) was employed to obtain the binding free energy change of binding, $\Delta G^0_{binding}$:

$$\Delta G^0_{binding} = -RTLnK_{binding} \quad (6)$$

The calculated free energy ($\Delta G^0$) of the zinc complex–HSA from the docking simulation is consistent with the experimental free energy of binding received from the fluorescence data (-4.48 kcal/mol). There is a good agreement between the free binding energy of docking simulation and the binding constant of fluorescence experiments. These results very clearly authenticate that zinc complex–HSA docked model is in approximate correlation with our experimental results. From docking results with optimal energy, it was found that zinc(II) complex inserted into HSA fragments and hydrogen bond plays main role in binding of zinc(II) complex to HSA. There is hydrogen bond between hydrogens of zinc(II) complex with HSA.

**Fig. 9. Scheme of HSA with zinc complex.**

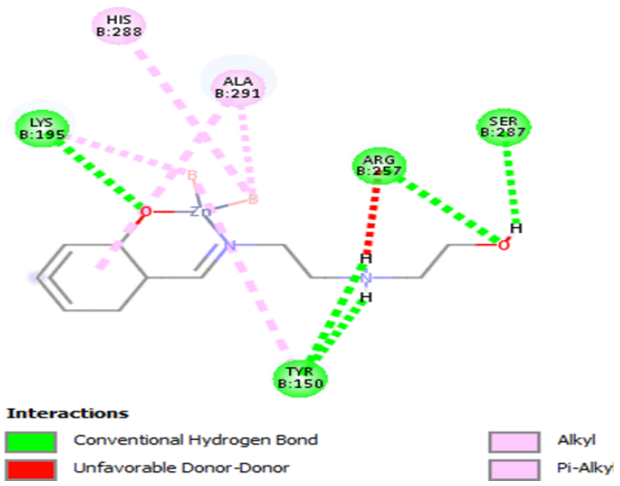


**Fig. 10. Docking scheme of HSA with zinc complex.**

**Table 5 Docking summary of HSA with zinc(II) complex by the Auto Dock program.**

| Mode | Affinity (kcal/mol) | Dist from rmsd l.b. | Best mode rmsd u.b. |
|---|---|---|---|
| 1 | -6.3 | 0 | 0 |
| 2 | -6.2 | 3.85 | 6.52 |
| 3 | -5.8 | 9.71 | 10.94 |
| 4 | -5.8 | 18.05 | 18.91 |
| 5 | -5.7 | 9.59 | 11.14 |
| 6 | -5.7 | 8.14 | 9.26 |
| 7 | -5.6 | 9.27 | 10.41 |
| 8 | -5.5 | 9.39 | 10.76 |
| 9 | -5.5 | 9.29 | 10.15 |
| 10 | -5.5 | 10.01 | 10.97 |
| 11 | -5.4 | 9.30 | 10.03 |
| 12 | -5.3 | 9.79 | 11.47 |
| 13 | -5.3 | 8.52 | 10.19 |
| 14 | -5.3 | 9.24 | 10.88 |
| 15 | -5.3 | 8.70 | 10.28 |
| 16 | -5.2 | 8.63 | 10.31 |
| 17 | -5.2 | 8.99 | 10.64 |
| 18 | -4.8 | 8.42 | 10.76 |
| 19 | -4.6 | 9.56 | 10.66 |
| 20 | -4.6 | 9.64 | 10.85 |

## *Conclusion*

The results of interaction between zinc(II) complex and HSA is as following:

I. UV–vis spectra indicated formation of a complex between HSA and zinc(II) complex and lack of change in α-helix structure of protein.

II. HSA fluorescence was quenched by zinc(II) complex through static quenching. The negative values of ΔH and ΔS indicated that hydrogen bond and van der Waals forces play major roles in the binding of the zinc(II) complex and HSA.

III. CD spectra indicated that the secondary structure of HSA is also predominantly α-helical even after binding to zinc(II) complex.

IV. Analysis of molecular probes (warfarin and ibuprofen) showed that the binding site located in Sudlow's site I.

V. The molecular docking simulation was employed to simulate and predict the binding site of zinc(II) complex to albumin and to authenticate experimental results.

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