

A Spectroscopy Approach for the Study of the Interaction of Oxovanadium(IV)-Salen Complexes with Proteins

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Abstract Oxovanadium(IV)-salen complexes bind with bovine serum albumin (BSA) and ovalbumin (OVA) strongly with binding constant in the range 10^4 – 10^7 M⁻¹ at physiological pH (7.4) confirmed using UV–visible absorption, fluorescence spectral and circular dichroism (CD) study. CD results show that the binding of oxovanadium(IV) complexes induces the conformational change with the loss of α -helicity in the proteins. Docking studies indicate that mode of binding of oxovanadium(IV)-salen complexes with proteins is hydrophobic in nature.

Keywords Oxovanadium(IV)-salen · BSA · Ovalbumin · Protein binding

Introduction

Serum albumins, the most abundant protein in circulatory system, play an important role in the binding, transportation and delivery of drug molecules and nutrients in the blood [1].

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Among the serum albumins, bovine serum albumin (BSA), with a molecular weight 68,000 g mol⁻¹ and wide range of physiological functions [2–5], binds and neutralizes endogenous and exogenous toxins by means of hydrogen bonding [6], hydrophobic, electrostatic and metal interaction [7]. BSA contains 583 amino acids in a single polypeptide chain, 17 disulfide bridges and one free –SH group, which can cause it to form a covalently linked dimer. BSA contains two tryptophan (Trp) residues, one located on the surface of the molecule (Trp-134) and the other at the bottom of hydrophobic cleft between domains I and III (Trp-212). It also has a high degree of α -helical content lying on the surface of hypothetical cylinder with an open channel running along the axis [8, 9]. BSA is known to exhibit a very high conformational adaptability to large variety of ligands [10, 11]. In general BSA has been used extensively in the past years, partly because of its high degree of homology with HSA and a similar structure of HSA with minor differences [12, 13].

Ovalbumin (OVA), the predominant protein in egg white, comprises approximately 54 % of the total protein and belongs to glycoprotein [14]. OVA is very similar in amino acid content to and excellent substitute for BSA. It is a typical globular protein with a diameter 5.5 nm [15], an isoelectric point (pI) ~4.9 [16] and a molecular weight ~45,000 g mol⁻¹ [17, 18]. This protein also carries a disulfide bond and a single polypeptide chain of 385 amino acid residues that folds into a globular conformation with a high secondary structure content (30.6 % α -helix and 31.4 % β -strand), and a flexible loop-helix-loop motif reactive center [18, 19]. Ovalbumin is widely used in the food industry for the emulsifying capability and the ability to form gels upon heating [20–22]. OVA is a key reference protein for immunization and biochemical studies.

The development of transition metal complexes that target and interact non-covalently with proteins is an emerging field that links inorganic chemistry with chemical and synthetic

biology [23–29]. When a protein is bound, a metal ion plays a structural role, particularly on protein folding, but can also determine the reactivity of a protein as part of redox or catalytic site [30]. Vanadium compounds show a wide variety of pharmaceutical properties but two important properties are their potential antidiabetic and antitumor activity [29, 31, 32]. Vanadium elicits a number of physiological responses e.g., the inhibition of phosphate- metabolizing enzymes such as phosphatases, ribonuclease, and ATPase, and its compounds show insulin-enhancing activity [33–40]. The VO^{2+} forms complexes with two high molecular mass components, human serum transferrin (hTF) [41, 42] and human serum albumin (HSA) [43]. Both hTF and HSA can bind VO^{2+} ion or remove it from an insulin-enhancing compound and transport it towards the target organs which seems to be the liver and muscles [44]. Vanadium(IV) often takes the form of the vanadyl cation (VO^{2+}), and as such can interact with proteins at cationic binding sites similar to divalent calcium, zinc or manganese [45]. Both V^{IV} and V^{V} also form a variety of coordination complexes that may interact with enzymes [46].

Due to these vanadium-biomolecule interactions, vanadium can be used to elicit desired responses at the organism level. The interaction between $\text{VO}(\text{acac})_2$ and serum albumin has been studied by isothermal calorimetry and fluorescence spectroscopy [47] and the above mentioned complex does not show any fluorescence emission spectrum. The cancer-targeting property of $\text{VO}(\text{acac})_2$ due to its intercellular interaction with glycolytic enzymes is reinforced by formation of macromolecular complexes with serum albumin [47]. The binding interactions of vanadyl complexes with serum proteins are not well understood, and studies have been carried out to investigate binding interactions of serum proteins with vanadate(VO_3^-) and vanadyl cations [48, 49]. EPR and ENDOR spectral results demonstrated that $\text{VO}(\text{acac})_2$ binds albumin with 1:1 stoichiometry [50].

Vanadyl cation also complexes with HSA at two binding sites with two affinity constants (strong and weak), while vanadate anion (VO_3^-) shows minor interaction with amino group [51]. Sasmal et al. [52] studied the interaction of three vanadyl complexes using heterocyclic bases as a ligand with BSA and reported that BSA fluorescence was quenched by the vanadyl complexes. Inorganic and organic chelates of VO^{2+} lead to adduct formation with BSA and possibly with other serum transport proteins [53]. Vanadyl cation (VO^{2+}) shows a major affinity for binding to nitrogen and oxygen donors of biomolecules [54, 55].

In the present study, BSA is selected as our protein model because of its long-standing interest in the protein community. We report the results on the interaction of oxovanadium(IV)-salen complexes with proteins BSA and OVA studied by spectroscopic techniques. The absorption titration was carried out by keeping the concentration of V(IV) complexes (2 μM) constant while varying the concentration of proteins (0 to

32 μM) and the fluorescence titration was performed by keeping the concentration of proteins constant while varying the concentration of oxovanadium(IV)-salen complexes.

Experimental

The ligands [56, 57] and oxovanadium(IV)-complexes [58–65] were synthesized by known procedures and characterized by UV-vis, MS, FTIR spectral studies and cyclic voltammetry measurements (Tables S1 and S2). Bovine serum albumin (BSA) and ovalbumin (OVA) were purchased from Ocimum Bioscience and Sigma-Aldrich respectively and used without any further purification.

Instrumentation and Methods

The stock solutions of BSA (1×10^{-4} M) and OVA (1×10^{-4} M) based on their molecular weight of 68,000 and 45,000 g mol^{-1} respectively were prepared in phosphate buffer solution at physiological pH (7.4) and no precipitation was detected under the experimental conditions. The stock solutions for complexes I to V and VIII (1×10^{-3} M) in acetonitrile (HPLC) and complexes VI and VII in dimethyl sulfoxide (DMSO) were prepared. All aqueous solutions were prepared in doubly distilled water for the experiment. The sample solutions were prepared freshly for each measurement.

Absorption spectra were recorded using JASCO 530 spectrophotometer and quartz cuvette of 1 cm was used. The absorbance titration was performed by keeping the concentration of V(IV) complexes (2 μM) constant while varying the concentration of proteins from 0 to 32 μM . The binding constant K_b , for the interaction of V(IV) complexes with proteins was determined by absorption spectroscopy by using the following Scatchard Eq. 1 [66].

$$\Delta A^{-1} = (\Delta \epsilon \cdot C_t)^{-1} + (K_b \cdot \Delta \epsilon \cdot C_t)^{-1} C_p^{-1} \quad (1)$$

Where ΔA is the absorbance difference in absence and presence of proteins, C_p is the concentration of proteins, C_t is the total concentration of the complex, and $\Delta \epsilon = \epsilon_b - \epsilon_f$ (ϵ_f and ϵ_b are the molar extinction coefficient of free complex and the totally bound complex). Thus, the double reciprocal plot of ΔA^{-1} vs C_p^{-1} , is linear and the binding constant (K_b) can be estimated from the ratio of the intercept to the slope.

The fluorescence measurements were recorded with JASCO FP-6300 at different concentration of vanadyl complexes from 0 to 100 μM keeping the concentration of proteins as constant (2 μM). The emission intensity of tryptophan residues of proteins at 340 nm (excitation wavelength 295 nm) was monitored using V(IV) complexes as quenchers with increasing concentration [66, 67]. The Stern-Volmer constant

(K_{SV}) and bimolecular quenching constant (k_q) values are determined from the Stern-Volmer equation (Eq. 2) [68].

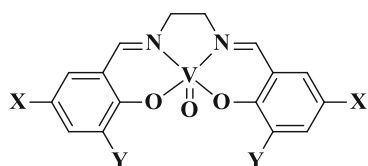
$$F_0/F = 1 + K_{SV}[Q] = 1 + k_q\tau_0[Q] \quad (2)$$

Here F_0 and F are the fluorescence intensity of proteins in the absence and presence of V(IV) complexes, $[Q]$ is the molar concentration of quencher (oxovanadium(IV) complexes) and τ_0 is the life-time of proteins. The life-time of BSA and OVA are 5.8 ns and 7.7 ns respectively. The plot of F_0/F vs $[Q]$ gives K_{SV} value as the slope and k_q , the quenching rate constant is calculated from obtained K_{SV} value. The K_{SV} value provides a direct measure of the quenching sensitivity. A linear Stern-Volmer plot is generally indicative of a single class of fluorophores, all equally accessible to the quencher. In many cases, the fluorophore can be quenched both by collision and by complex formation with same quencher.

Circular dichroism (CD) measurements were performed on a JASCO J810 spectropolarimeter at room temperature over the wavelength 200–260 nm. Parameters were set as follows: path length, 50 mm; resolution, 0.5 nm; scan speed, 50 nm min⁻¹; band width, 1 nm; response 1 s. Every spectrum was averaged two times. The concentration of BSA and OVA is 1×10^{-6} M and the concentration of vanadyl complexes is 1×10^{-6} M and 3×10^{-6} M.

Results and Discussion

The details of synthesis of salen ligands and oxovanadium(IV)-salen complexes and spectral characterization are given our previous reports [59, 61] but the spectral data are collected in the supporting information (Tables S1 and S2). The structure of the oxovanadium(IV)-salen complexes synthesized for the present study are shown in Chart 1. The green coloured complexes (I – V and VIII) are soluble in CH₃CN and orange coloured complexes (VI and VII) in DMSO. The absorption spectrum of complex I in CH₃CN is shown in Fig. S1 and the spectral data of oxovanadium(IV) complexes are collected in Table S2. The electronic absorption spectra of eight V(IV)



- | | |
|-------------------------------------|-----------------------------------|
| (I) X = H, Y = H; | (II) X = CH ₃ , Y = H; |
| (III) X = OCH ₃ , Y = H; | (IV) X = Cl, Y = H; |
| (V) X = Br, Y = H; | (VI) X = NO ₂ , Y = H; |
| (VII) X = Y = Cl; | (VIII) X = Y = <i>tert</i> -Bu |

Chart 1 Structure of oxovanadium(IV)-salen complexes

complexes (I – VIII) reveal strong absorption bands at 227–279 nm and less intense absorption bands at 352–384 nm. The absorption bands at 227–279 nm are assigned to ligand centered (LC) π - π^* transition and low energy absorption bands at 352–384 nm to spin allowed metal-to-ligand transfer (¹MLCT) transition from the V^{IV} d π -orbital to the π^* orbital of the ligand (d π V^{IV} \rightarrow π^* (diimine)). The absorption maximum of BSA is 278 nm but has emission at 340 nm when excited at 295 nm. The vanadium complexes do not have emission in the region 300–400 nm and thus the changes in emission intensity of BSA in the presence of varying concentration of vanadium complexes can be used to study the interaction between them.

Interaction of Oxovanadium(IV) Complexes with Proteins

The absorption maximum of BSA at 278 nm is characteristic absorption of tryptophan (Trp) and tyrosine (Tyr) residues in BSA [69] and the absorption spectrum of BSA alone is shown in the Fig. S2. The absorption spectrum of complex III has band at 274 nm due to ligand centred (LC) π - π^* transition close to BSA absorption. So the absorption titration is carried out with a change in the concentration of proteins while keeping the concentration of V(IV) complexes constant. The intensity of band at 384 nm is increased on the addition of BSA (Fig. 1) which indicates an interaction between V(IV) complex III and BSA. The binding constant (K_b) of complex III with BSA is calculated as 4.7×10^4 M⁻¹ from the change in the absorption spectral data using Scatchard equation [66].

Generally, the fluorescence of protein is caused by the intrinsic fluorescence of three molecules present in the

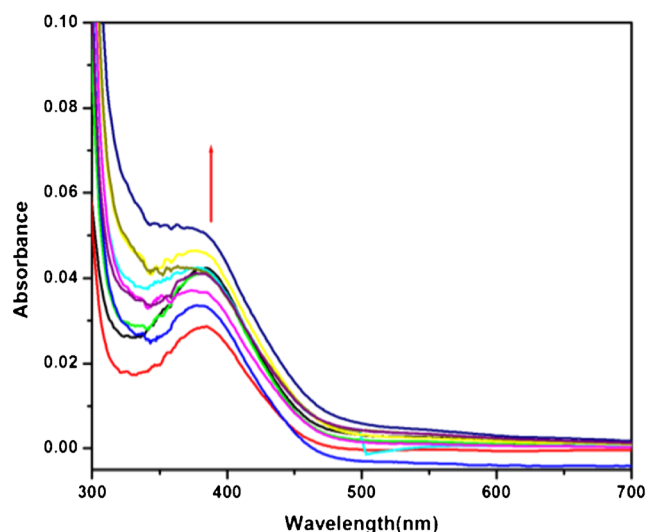


Fig. 1 UV-vis absorption spectra for the titration of III with BSA, [III] = 2×10^{-5} M, [BSA] = 0 – 32×10^{-6} M

protein, i.e., Trp, Tyr and phenylalanine (Phe) residues [70]. Actually, the intrinsic fluorescence of many proteins is mainly contributed by tryptophan alone because of very low quantum yield of Phe, and the fluorescence of Tyr is almost quenched via energy transfer to Trp if Tyr is ionized, near an amino or carboxyl group. In order to confirm the interaction between V(IV) complexes and proteins, fluorescence titration is carried by the keeping the concentration of protein constant while varying the concentration of V(IV) complexes. Before to record the change of fluorescence intensity of BSA in the presence of V(IV)-salen complexes we have checked the emission of oxovanadium(IV)-salen complexes. The excitation of complex I at 355 nm results in no emission. Thus the oxovanadium(IV)-salen complexes used in the present study are non-emissive. The emission intensity of proteins is found to be quenched gradually on increasing the concentration of V(IV) complex because of the changes in the secondary/tertiary structure of proteins in phosphate buffer medium affecting the orientation of the tryptophan residues of protein. The extent of quenching of the fluorescence intensity of proteins gives the measure of the extent of binding of the V(IV) complexes to proteins. The Stern-Volmer quenching constant (K_{SV}) values are obtained quantitatively by the plot of F_0/F vs $[Q]$ using the Stern-Volmer equation (Eq. 2). The interaction of vanadium(IV) complexes with proteins is found to produce the change on the emission profile (Figs. 2, 3 and S3). The fluorescence of BSA is quenched in the presence of vanadium(IV) complexes with a blue shift (≈ 10 nm) in the emission maximum. A similar result was reported on the interaction between flavonoids and BSA by Jin et al. [71].

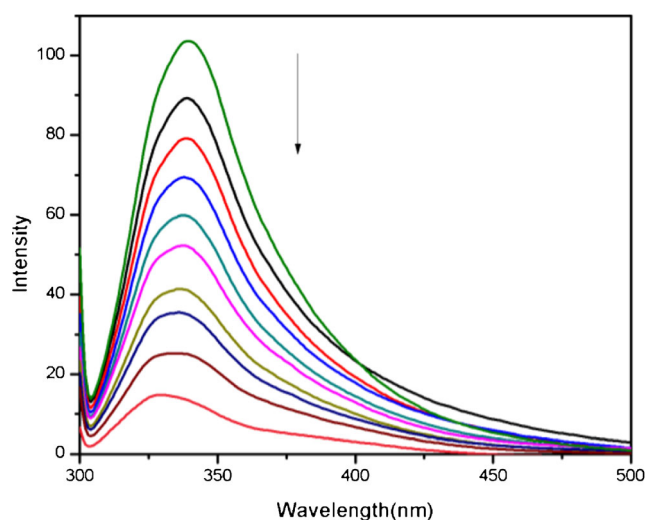


Fig. 2 Fluorescence quenching of BSA (2 μ M) in the presence of increasing concentration of complex I (0 \rightarrow 100) excitation maximum 295 nm

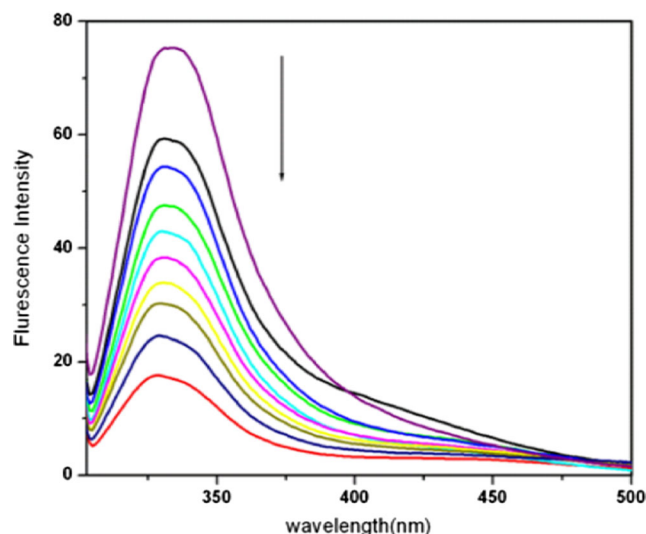


Fig. 3 Fluorescence quenching of OVA (2 μ M) in the presence of increasing concentration of complex I (0 \rightarrow 100) excitation maximum 295 nm

The values of K_{SV} and k_q , obtained from Stern-Volmer plots (Fig. S4) for quenching of proteins by vanadium(IV) complexes are summarized in Table 1. The high K_{SV} values (10^4 – 10^5 M^{-1}) and k_q values in the range 10^{12} – 10^{13} $M^{-1} s^{-1}$ indicate that the ground state interaction between V(IV) complexes and proteins leads to V(IV)–protein complex formation and thus the nature of quenching process is predominantly static rather than dynamic. Similarly for OVA the K_{SV} values are in the range from 10^3 to 10^5 M^{-1} and the bimolecular quenching constant (k_q) values are in the range from 10^{12} to 10^{13} $M^{-1} s^{-1}$. The fluorescence quenching of BSA and OVA with a blue shift in the emission maximum suggests that binding of V(IV) complexes is associated with change in the environment of at least one of the two Trp moieties in BSA and OVA.

Table 1 Values of Stern-Volmer constant (K_{SV}) and quenching constant (k_q) for the quenching of BSA (2 μ M) and OVA (2 μ M) using oxovanadium(IV)-salen complexes

Complex	BSA		OVA	
	K_{SV} (M^{-1})	k_q ($M^{-1} s^{-1}$)	K_{SV} (M^{-1})	k_q ($M^{-1} s^{-1}$)
I	3.2×10^4	5.5×10^{12}	4.3×10^4	5.6×10^{12}
II	1.6×10^4	2.9×10^{12}	1.9×10^5	2.5×10^{13}
III	2.4×10^4	4.1×10^{12}	2.0×10^4	2.7×10^{12}
IV	1.8×10^4	3.1×10^{12}	2.2×10^4	2.9×10^{12}
V	9.6×10^3	1.7×10^{12}	5.4×10^3	0.7×10^{12}
VI	1.6×10^5	2.7×10^{13}	2.7×10^4	3.5×10^{12}
VII	1.3×10^5	2.2×10^{13}	2.0×10^4	2.6×10^{12}
VIII	5.1×10^4	8.8×10^{12}	4.9×10^4	6.4×10^{12}

Binding Parameters

When a static quenching occurs, Eq. 3 [72] is used for the determination of the binding parameters between V(IV) complexes and proteins:

$$\log F_0 - F/F = \log K_b + n \log [Q] \tag{3}$$

where F_0 and F are the fluorescence intensities of BSA before and after addition of V(IV) complexes, K_b is the binding constant, reflecting the extent of binding of V(IV) complexes with proteins, n is the number of binding sites and $[Q]$ is the concentration of vanadium complexes. According to experimental results (Figs. 2, 3 and S3), the linear fitting plots of $\log F_0 - F/F$ versus $\log [Q]$ can be made in Fig. S5. Based on the plots the values of K_b and n are obtained and collected in Table 2.

According to the data shown in Table 2 the binding constant, K_b , values are in the range $1.0 \times 10^4 - 10^7 \text{ M}^{-1}$ for complexes I to VIII. This large binding constant values indicates that there is a strong interaction between proteins and V(IV) complexes. Binding constant, K_b value of complex III is in close agreement with the value calculated from the absorption spectral data (K_b) confirming the reliability of these values. The value of n is in the range 1.0–1.9 which indicates that in the binding process, the molar ratios of protein to V(IV) complex varies from 1:1 to 1:2 depending on the substituents in the salen moiety of V(IV) complex. From these observations it could be inferred that the oxovanadium(IV) complexes bind strongly with proteins.

The change of binding constant value with the change of substituent in the salen ligand deserves comment. The binding constant data collected in Table 2 indicate that the value is highly sensitive to the change of substituent in the salen ligand. The presence of electron-withdrawing group like $-\text{NO}_2$ enhances the binding constant value enormously from 10^6 to 10^7 M^{-1} but the electron donating group like $-\text{OMe}$ decreases

Table 2 Binding parameters (K_b and n) for the binding of oxovanadium(IV)-salen complexes with BSA and OVA

Protein →	BSA		OVA	
	K_b	n	K_b	n
I	4.1×10^6	1.5	8.6×10^6	1.6
II	2.3×10^5	1.1	9.1×10^6	1.4
III	1.4×10^4	1.0	9.3×10^4	1.2
IV	3.9×10^6	1.3	4.0×10^7	1.6
V	2.3×10^4	1.2	3.2×10^5	1.3
VI	1.2×10^7	1.9	7.4×10^6	1.5
VII	2.8×10^5	1.0	1.8×10^6	1.5
VIII	3.2×10^5	1.1	2.7×10^6	1.4

the binding constant value. These interesting results pointed out that binding efficiency of metal-salen complexes is susceptible to the structural changes of the ligand. Similar results have been reported on the binding of other metal-salen complexes with proteins [29].

Effect of V(IV) Complex on the Conformation of Protein

The absorption and emission titration experiments confirm the strong interaction between V(IV) complexes and proteins. It is important to examine how the structure of proteins is affected in the presence of V(IV) complexes. When probes bind to globular proteins, the intramolecular forces responsible for maintaining the secondary and tertiary structures can be altered, resulting in a conformational change of the protein [73]. Circular dichroism (CD) is a sensitive technique to monitor conformational changes of protein upon interaction with the small molecules. Both BSA and OVA have a high percentage of α -helical structure which shows characteristic strong double minima signals with two negative bands in the UV range at 208 and 222 nm (Figs. 4 and S6). Both bands are due to $n-\pi^*$ transition of the carbonyl group of peptide. The CD result is expressed as MRE (Mean Residue Ellipticity) in $\text{cm}^2 \text{ dmol}^{-1}$, which is defined in Eq. (4)

$$\text{MRE} = \text{Observed CD (mdeg)} / 10 \times n l C_p \tag{4}$$

Here C_p is the molar concentration of the proteins, n is the number of amino acid residues present in the proteins (583) and l is the path length (5 mm).

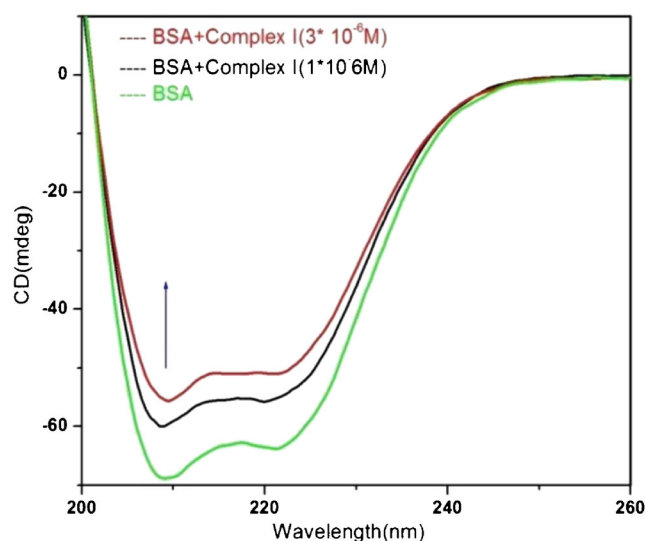


Fig. 4 CD spectra of BSA in the presence and absence of complex I. [BSA] = 1 μM , [I] = 1 and 3 μM

The helical content is calculated from the MRE values at 208 nm using the Eq. 5.

$$\alpha\text{-helix} = [(-\text{MRE}_{208}) - 4000 / (33000 - 4000)] \times 100\% \quad (5)$$

where MRE_{208} is the mean residue ellipticity in $\text{deg cm}^2 \text{d mol}^{-1}$, 4000 is the MRE of the β -form and random coil conformation cross at 208 nm, and 33,000 is the MRE value of a pure α -helical at 208 nm. The quantitative analysis of the CD data using Eq. (5) provides the α -helical content in the secondary structure of serum albumin. The α -value decreased on the addition of vanadium complexes with slight red shift, indicative of the loss of α -helicity. Upon addition of V(IV) (1 μM) to BSA (1 μM) the extent of α -helicity of the protein decreases from 66.7 to 53.5 % at 208 nm for complex **IV** (Table S3). Similarly for ovalbumin the α -helical content is 42.1 and 49.9 % at the wavelength 208 and 222 nm respectively (Fig. 5). The values are 17.7, 34.8, 27.2, 26.7, 29.2 and 15.6 % at 208 nm in the presence of V(IV) complexes **I**, **II**, **III**, **IV**, **V** and **VIII** respectively. The results suggest that the binding of oxovanadium(IV)-salen complexes with OVA induces substantial changes in the conformational structure of ovalbumin. The decrease in α -helicity is higher for BSA compared to OVA with V(IV) complexes. From these results the inference is that the binding efficiency of V(IV) complexes is more with BSA when compared with ovalbumin. It is apparent that the V(IV) complexes bind to the amino acid residues of the main polypeptide chain of BSA and destroy the H-bonding networks present in the amino acid residues of protein which may decrease the percentage of α -helicity of the protein [74, 75]. Therefore, we conclude that the effect of the V(IV) complexes on BSA and OVA causes a conformational change of the protein with loss of α -helicity.

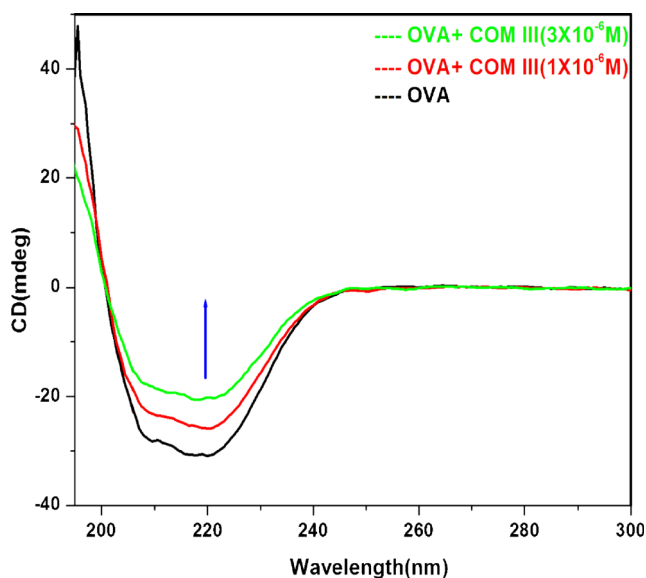


Fig. 5 CD spectra of OVA in the presence and absence of complex **III**. [OVA] = 1 μM , [**III**] = 1 and 3 μM

Docking Studies on the Binding of Oxovanadium(IV)-Salen Complex with BSA and OVA

Oxovanadium(IV)-salen complex has been docked into the structure of BSA in order to envisage a correlation between the experimental and docking results concerning the nature of the protein-ligand interaction. Of the two tryptophans in BSA, Trp-212 is located in a hydrophobic fold whereas Trp-134 is located on the surface of the molecule [74, 75]. Generally alkyl groups on Ala, Val, Leu and Ile interact with the ligand through hydrophobic interaction. In addition, benzene ring (aromatic rings) on the Phe and Tyr can stack together.

From the experimental results, it is clear that the vanadium(IV) complexes have effective binding with BSA. To get more knowledge on the binding efficiency and to know about the mode of binding we have done optimization and docking studies using Schrodinger 2012, 9.2 version software and protein data bank (pdb) files. The structure of the V(IV) complex was drawn using Hyperchem software and the docked structure was displayed by the Pymol. The crystal structure of HSA has been used because BSA has 80 % similar structure with HSA and downloaded HSA pdb file as model for BSA.

The oxovanadium(IV)-Schiff base complexes with polypyridyl derivatives show inhibition activities with protein tyrosine phosphatases (PTP1B) [76]. The competitive inhibition mode suggests that the mixed ligands of vanadium(IV) complexes could bind to the catalytic active site of PTP1B by competing with the substrate leading to enzyme inhibition. The putative mode of interaction of vanadium complex with PTP1B was modeled by using molecular docking techniques. The modeling studies suggest that vanadium(IV)-salen complex can be accommodated into the active site cleft of PTP1B and the phenolate oxygen of the complex is close to the sulfur atom of the active site cysteine residue (Cys215), at a distance

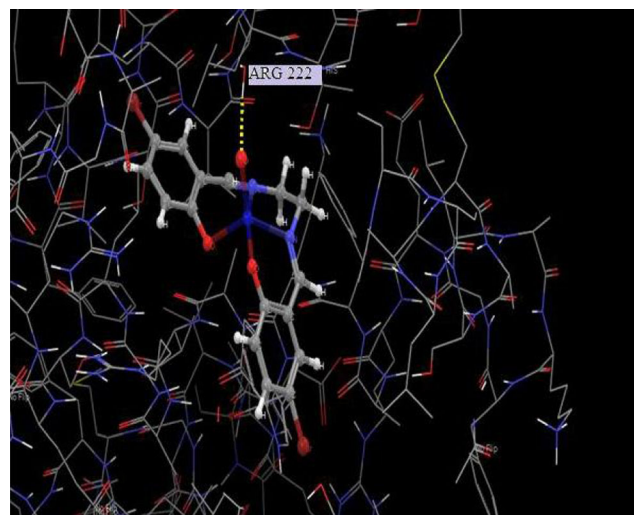
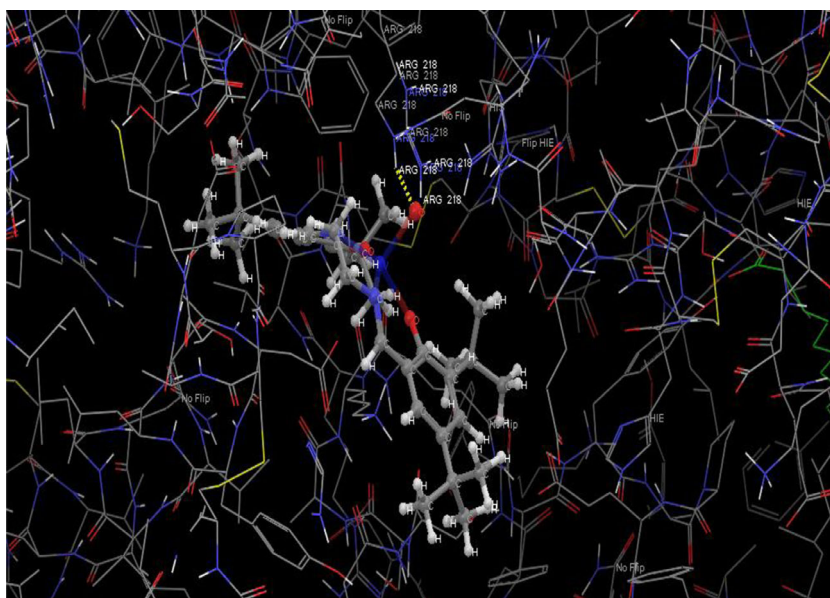


Fig. 6 Computational docking model of the binding of **III** to BSA

Fig. 7 Computational docking model of the binding of VIII to BSA



of 3.15 Å. Two hydrogen bonds may also formed between the vanadyl oxygen and the nitrogen of the Arg221 residue as well as the carboxyl oxygen of the complex and the nitrogen of the Gln266 residue in the enzyme [76, 77].

The docking pose of vanadium(IV)-salen complexes with BSA indicates that vanadyl oxygen of vanadium complexes **III** and **VIII** docks to BSA through hydrogen bond interaction with the amino acid residue (Arg 208) [76, 77] as shown in Figs. 6 and 7. The various residues are present within 5 Å distance from the probe [78] and the binding sites of amino acid residues as shown from the docked pose comprised of Arg 222, Ser 202 and Arg 218 (Fig. 7) [78, 79].

In the fields of computational chemistry and molecular modeling, scoring functions are fast approximate mathematical methods used to predict the strength of the non-covalent interaction (also referred to as binding affinity) between two molecules after they have been docked. Most commonly one of the molecules is a small organic compound such as a drug and the second is the drug's biological target such as a protein receptor [79]. From the docking model the docking score calculated for the interaction of V(IV)-salen complexes with the

proteins are tabulated in Table 3. The data collected in Table 3 indicate that the interaction between vanadium complexes and proteins is hydrophobic in nature.

Conclusion

The interaction of oxovanadium(IV)-salen complexes with proteins (BSA and OVA) have been investigated by using UV–vis absorption, fluorescence and circular dichroism (CD) spectral techniques. The fluorescence quenching results indicate that the V(IV) complexes bind strongly with BSA and OVA and the binding interaction is hydrophobic in nature. The CD spectral study indicates that the secondary structure of BSA and OVA is marginally changed in the presence of V(IV) complexes. The knowledge on the interaction of V(IV) complexes with proteins can be useful to realize the different biomedical applications (antidiabetic and antitumor activity) without loss of the structure of proteins. The molecular docking studies show that hydrophobic interaction exists between the V(IV) complexes and the proteins.

Table 3 Docking score of the interaction of oxovanadium(IV)-salen complexes with proteins

Complex	BSA	Ovalbumin
I	-4.386	-2.794
II	-5.038	-1.292
III	-4.216	-2.700
IV	-4.090	-2.935
V	-3.881	-0.953
VI	-4.228	-2.375
VII	-3.797	-1.358
VIII	-3.988	-1.895

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References

1. He XM, Carter DC (1992) Atomic structure and chemistry of human serum albumin. *Nature* 358:209–215

2. Brunaldi K, Huang N, Hamilton JA (2010) Interactions of very long-chain saturated fatty acids with serum albumin. *J Lipid Res* 51:120–131
3. Shim YY, Reaney MJT (2015) Kinetic interactions between cyclolinopeptides and immobilized human serum albumin by surface Plasmon resonance. *J Agric Food Chem* 63:1099–1106
4. Yamashita MM, Wesson L, Eisenman G, Eisenberg D (1990) Where metal ions bind in proteins. *Proc Natl Acad Sci U S A* 87:5648–5652
5. Bal W, Christodoulou J, Sadler PJ, Tucker A (1998) Multi-metal binding site of serum albumin. *J Inorg Biochem* 70:33–39
6. Rawel HM, Rohn S, Kruse HP, Kroll J (2002) Structural changes induced in bovine serum albumin by covalent attachment of chlorogenic acid. *Food Chem* 78:443–455
7. Zhang Y, Wilcox DE (2002) Thermodynamic and spectroscopic study of Cu(II) and Ni(II) binding to bovine serum albumin. *J Biol Inorg Chem* 7:327–337
8. Papadopoulou A, Green RJ, Frazier RA (2005) Interaction of flavonoids with bovine serum albumin: a fluorescence quenching study. *J Agric Food Chem* 53:158–163
9. Filenko A, Demchenko M, Mustafaeva Z, Osada Y, Mustafaev M (2001) Fluorescence study of Cu²⁺ – induced interaction between albumin and anionic polyelectrolytes. *Biomacromolecule* 2:270–277
10. Rosenoer VM, Oratz M, Rothschild MA (eds) (1977) Albumin structure, function and uses. Progman Press Inc., Oxford
11. Peters T (1985) Serum albumin. *Adv Protein Chem* 37:161–245
12. Zhou N, Liang Y-Z, Wang P (2007) 18β-glycyrrhetic acid interaction with bovine serum albumin. *J Photochem Photobiol A Chem* 185:271–276
13. Shang L, Jiang X, Dong S (2006) In vitro study on the binding of neutral red to bovine serum albumin by molecular spectroscopy. *J Photochem Photobiol A Chem* 184:93–97
14. Mine Y, Noutomi T, Haga N (1991) Emulsifying and structural properties of ovalbumin. *J Agric Food Chem* 39:443–446
15. Venturoli D, Rippe B (2005) Ficoll and dextran vs. globular proteins as probes for testing glomerular permselectivity: effects of molecular size, shape, charge, and deformability. *Am J Physiol Renal Physiol* 288:F605–F613
16. Cannan RK, Kibrick A, Palmer AH (1941) The amphoteric properties of egg albumin. *Ann N Y Acad Sci* 41:243–266
17. Hu HY, Du HN (2000) Alpha to beta structural transformation of ovalbumin: heat and pH effects. *J Protein Chem* 19:177–183
18. Nisbet AD, Saundry RH, Moir AJG, Fothergill LA, Fothergill JE (1981) The complete amino-acid sequence of hen ovalbumin. *Eur J Biochem* 115:335–345
19. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE (2000) The protein data bank. *Nucleic Acids Res* 28:235–242
20. de Groot J, de Jongh HHJ (2003) The presence of heat-stable conformers of ovalbumin affects properties of thermally formed aggregates. *Protein Eng* 16:1035–1040
21. Nakamura R, Ishamaru M (1981) Changes in the shape and surface hydrophobicity of ovalbumin during its transformation to s-ovalbumin. *Agric Biol Chem* 45:2775–2780
22. Arntfield SD, Murray ED, Ismond MAH (1991) Role of disulfide bonds in determining the rheological and microstructural properties of heat-induced protein networks from ovalbumin and vicilin. *J Agric Food Chem* 39:1378–1385
23. Lo KK-W, Louie M-W, Zhang KY (2010) Design of luminescent iridium(III) and rhenium(I) polypyridine complexes as *in vitro* and *in vivo* ion, molecular and biological probes. *Coord Chem Rev* 254:2603–2622
24. Meggers E (2009) Targeting proteins with metal complexes. *Chem Commun* 1001–1010
25. Maksimoska J, Feng L, Harms K, Yi C, Kissil J, Marmorstein R, Meggers E (2008) Targeting large kinase active site with rigid, bulky octahedral ruthenium complexes. *J Am Chem Soc* 130:15764–15765
26. Malcahy SP, Meggers E (2010) Organometallics as structural scaffolds for enzyme inhibitor design. *Top Organomet Chem* 32:141–153
27. Bhuvaneshwari J, Muthu Mareeswaran P, Shanmugasundaram S, Rajagopal S (2011) Protein binding studies of luminescent rhenium(I) diimine complexes. *Inorg Chim Acta* 375:205–212
28. Babu E, Muthu Mareeswaran P, Singaravadevel S, Bhuvaneshwari J, Rajagopal S (2014) A selective, long-lived deep-red emissive ruthenium(II) polypyridine complexes for the detection of BSA. *Spectrochim Acta A* 130:553–560
29. Gomathi Sankareswari V, Vinod D, Mahalakshmi A, Alamelu M, Kumaresan G, Ramaraj R, Rajagopal S (2014) Interaction of oxovanadium(IV)–salphen complexes with bovine serum albumin and their cytotoxicity against cancer. *Dalton Trans* 43:3260–3272
30. Liu C, Xu H (2002) The metal site as a template for the metalloprotein structure formation. *J Inorg Biochem* 88:77–86
31. Barrio DA, Etcheverry SB (2010) Potential use of vanadium compounds in therapeutics. *Curr Med Chem* 17:3632–3642
32. Balaji B, Somyajit K, Banik B, Nagaraju G, Chakravarty AR (2013) Photoactivated DNA cleavage and anticancer activity of oxovanadium(IV) complexes of curcumin. *Inorg Chim Acta* 400:142–150
33. Rehder D (1995) In: Sigel A, Sigel H (Eds) Metal ions in biological systems. Marcel Dekker, New York, 31:1–43
34. Crans DC, Smeey JJ, Gaidamauskas E, Yang L (2004) The chemistry and biochemistry of vanadium and the biological activities exerted by vanadium compounds. *Chem Rev* 104:849–902
35. Stankiewicz PJ, Tracey AS, Crans DC (1995) In: Sigel A, Sigel H (Eds) Metal ions in biological systems. Marcel Dekker, New York, 31:287–324
36. Pessoa JC, Tomaz I (2010) Transport of therapeutic vanadium and ruthenium complexes by blood plasma components. *Curr Med Chem* 17:3701–3738
37. Thompson KH, McNeill JH, Orvig C (1999) Vanadium compounds as insulin mimics. *Chem Rev* 99:2561–2572
38. Thompson KH, Orvig C (2001) Coordination chemistry of vanadium in metallopharmaceutical candidate compounds. *Coord Chem Rev* 219:1033–1053
39. Shechter Y, Goldwasser I, Mironchik M, Fridkin M, Gefel D (2003) Historic perspective and recent developments on the insulin-like actions of vanadium; toward developing vanadium-based drugs for diabetes. *Coord Chem Rev* 237:3–11
40. Kawabe K, Yoshikawa Y, Adachi Y, Sakurai H (2006) Possible mode of action for insulinomimetic activity of vanadyl(IV) compounds in adipocytes. *Life Sci* 78:2860–2866
41. Sun H, Cox MC, Li H, Sadler PJ (1997) Rationalisation of binding to transferrin: prediction of metal-protein stability constant. *Struct Bond* 88:71–102
42. Chasteen ND (1977) Human serotransferrin: structure and function. *Coord Chem Rev* 22:1–36
43. Chasteen ND, Grady JK, Holloway CE (1986) Characterization of the binding, kinetics, and redox stability of vanadium(IV) and vanadium(V) protein complexes in serum. *Inorg Chem* 25:2754–2760
44. Liboiron BD, Thompson KH, Hanson GR, Lam E, Aebischer N, Orvig C (2005) New insights into the interactions of serum proteins with bis(maltolato)oxovanadium(IV): transport and biotransformation of insulin-enhancing vanadium pharmaceuticals. *J Am Chem Soc* 127:5104–5115
45. Chasteen ND (1995) Vanadium-protein interactions. *Met Ions Biol Syst* 31:231–247

46. Rehder D (2015) The role of vanadium in biology. *Metallomics*. doi:10.1039/C4MT00304G
47. Mustafi D, Peng B, Foxley S, Makinen MW, Karczmar GS, Zamora M, Ejnik J, Martin H (2009) New vanadium-based magnetic resonance imaging probes: clinical potential for early detection of cancer. *J Biol Inorg Chem* 14:1187–1197
48. Heinemann G, Fichtl B, Mentler M, Vogt W (2002) Binding of vanadate to human albumin in infusion solutions, to proteins in human fresh frozen plasma, and to transferrin. *J Inorg Biochem* 90:38–42
49. Purcell M, Neault JF, Malonga H, Arakawa H, Tajmir-Riahi HA (2001) Interaction of human serum albumin with oxovanadium ions studied by FT-IR spectroscopy and gel and capillary electrophoresis. *Can J Chem* 79:1415–1421
50. Makinen MW, Brady MJ (2002) Structural origins of the insulin-mimetic activity of bis(acetylacetonato)oxovanadium(IV). *J Biol Chem* 277:12215–12220
51. Harford C, Sarkar B (1997) Amino terminal Cu(II)- and Ni(II)-binding (ATCUN) motif of proteins and peptides: metal binding, DNA cleavage, and other properties. *Acc Chem Res* 30:123–130
52. Sasmal PK, Saha S, Majumdar R, De S, Dighe RR, Chakaravarty AR (2010) Oxovanadium(IV) complexes of phenanthroline bases: the dipyrrophenazine complex as a near-IR photocytotoxic agent. *Dalton Trans* 39:2147–2158
53. Sakurai H, Fujii K, Fujimoto S, Fujisawa Y, Takechi K, Yasui H (1998) Structure-activity relationship of insulin-mimetic vanadyl complexes with VO(N₂O₂) coordination mode. *ACS Symp Ser* 711:344–352
54. Baran EJ (2000) Oxovanadium (IV) and oxovanadium(V) complexes relevant to biological systems. *J Inorg Biochem* 80:1–10
55. Nechay BR, Nanninga LB, Nechay PSE (1986) Vanadyl (IV) and vanadate (V) binding to selected endogenous phosphate, carboxyl, and amino ligands; calculations of cellular vanadium species distribution. *Arch Biochem Biophys* 251:128–138
56. Chen D, Martell AE (1987) Dioxygen affinities of synthetic cobalt Schiff base complexes. *Inorg Chem* 26:1026–1030
57. Thornback JR, Wilkinson G (1978) Schiff-base complexes of ruthenium(II). *J Chem Soc Dalton Trans* 110–115
58. Bonadies JA, Carrano CJ (1986) Vanadium phenolates as models for vanadium in biological systems. 1. Synthesis, spectroscopy, and electrochemistry of vanadium complexes of ethylenebis[(o-hydroxyphenyl)glycine] and its derivatives. *J Am Chem Soc* 108:4088–4095
59. Mathavan A, Ramdass A, Rajagopal S (2015) Kinetic study of the oxovanadium(IV)-salen catalyzed H₂O₂ oxidation of phenols. *Transit Met Chem* 40:355–362
60. Ando R, Mori S, Hayashi M, Yagyu T, Maeda M (2004) Structural characterization of pentadentate salen-type Schiff-base complexes of oxovanadium(IV) and their use in sulfide oxidation. *Inorg Chim Acta* 357:1177–1184
61. Mathavan A, Ramdass A, Ramachandran M, Rajagopal S (2015) Oxovanadium(IV)-salen ion catalyzed H₂O₂ oxidation of tertiary amines to N-oxides—critical role of acetate ion as external axial ligand. In *J Chem Kinet* 47:315–326
62. Smith KI, Borer LL, Olmsted MM (2003) Vanadium(IV) and Vanadium(V) complexes of salicylamidine ligands. *Inorg Chem* 42:7410–7415
63. Maurya MR, Kumar M, Kumar A, Pessoa JC (2008) Oxidation of *p*-chlorotoluene and cyclohexene catalysed by polymer-anchored oxovanadium(IV) and copper(II) complexes of amino acid derived tridentate ligands. *Dalton Trans* 4220–4232
64. Adeo P, Pessoa JC, Henriques RT, Kuznetsov ML, Avecilla F, Maurya MR, Kumar U, Correia I (2009) Synthesis, characterization, and application of vanadium – salen complexes in oxygen transfer reactions. *Inorg Chem* 48:3542–3561
65. Alsalam TA, Hadi JS, Al-Nasir EA, Abbo HS, Titinchi SJJ (2010) Hydroxylation of phenol catalyzed by oxovanadium (IV) of salen-type Schiff base complexes with hydrogen peroxide. *Catal Lett* 136:228–233
66. Lakowicz JR (2006) Principles of fluorescence spectroscopy, 3rd edn. Plenum Press, New York
67. Hu Y-J, Liu Y, Zhang L-X, Zhao R-M, Qu S-S (2005) Studies of interaction between colchicine and bovine serum albumin by fluorescence quenching method. *J Mol Struct* 750:174–178
68. Espenson JH (1995) Chemical kinetics and reaction mechanisms. McGraw-Hill, Inc, New York
69. Bhuvaneswari J, Fathima AK, Rajagopal S (2012) Rhenium(I)-based fluorescence resonance energy transfer probe for conformational changes of bovine serum albumin. *J Photochem Photobiol A Chem* 227:38–44
70. Wetlaufer DB (1962) In: Anfinsen CB, Bailey K, Anson ML, Edsall JT (eds) Advances in protein chemistry. Academic Press, New York, pp. 303–390
71. Shi X, Li X, Gui M, Zhou H, Yang R, Zhang H, Jin Y (2010) Studies on interaction between flavonoids and bovine serum albumin by spectral methods. *J Lumin* 130:637–644
72. Bourassa P, Kanakis CD, Tarantilis P, Pollissiou MG, Tajmir-Riahi HA (2010) Resveratrol, genistein, and curcumin bind bovine serum albumin. *J Phys Chem B* 114:3348–3354
73. Kragh-Hansen U (1981) Molecular aspects of ligand binding to serum albumin. *Pharmacol Rev* 33:17–53
74. Greenfield NJ, Fasman GD (1969) Computed circular dichroism spectra for the evaluation of protein conformation. *Biochemistry* 8:4108–4116
75. Peterman BF, Laidler KJ (1980) Study of reactivity of tryptophan residues in serum albumins and lysozyme by *N*-bromosuccinamide fluorescence quenching. *Arch Biochem Biophys* 199:158–164
76. Yuan C, Lu L, Wu Y, Liu Z, Guo M, Xing S, Fu X, Zhu M (2010) Synthesis, characterization, and protein tyrosine phosphatases inhibition activities of oxovanadium(IV) complexes with Schiff base and polypyridyl derivatives. *J Inorg Biochem* 104:978–986
77. Yuan C, Lu L, Gao X, Wu Y, Guo M, Li Y, Fu X, Zhu M (2009) Ternary oxovanadium(IV) complexes of ONO-donor Schiff base and polypyridyl derivatives as protein tyrosine phosphatase inhibitors: synthesis, characterization, and biological activities. *J Biol Inorg Chem* 14:841–851
78. Bardhan M, Chowdhury J, Ganguly T (2011) Investigations on the interactions of aurintricarboxylic acid with bovine serum albumin: steady state/time resolved spectroscopic and docking studies. *J Photochem Photobiol B Biol* 102:11–19
79. Bhattacharya B, Nakka S, Guruprasad L, Samanta A (2009) Interaction of bovine serum albumin with dipolar molecules: fluorescence and molecular docking studies. *J Phys Chem B* 113:2143–2150