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Spectroscopic characterization of the interactions of bovine serum albumin with medicinally important metal ions: platinum (IV), iridium (III) and iron (II)

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Serum albumin protein plays a key role in the transportation and distribution of bioactive species including metal ions and metal-based drugs and, therefore, the nature of their binding could provide important insight for the development of new drugs. In the present investigation, binding interactions of bovine serum albumin (BSA) with three biologically important metal ions: Pt^{4+}, Ir^{3+} and Fe^{2+} were screened using easy-to-use and cost-effective Fourier-Transform Infrared (FT-IR) and Ultraviolet-Visible (UV-Vis) spectroscopic techniques. Prior to the screening, the protein and metal ions were allowed to interact at physiological pH (7.4) and the spectral changes were monitored upon interaction. In FT-IR spectrum, the position of amide I band (C=O stretching) was shifted from 1652 cm^{-1} in case of free BSA to 1659, 1657 and 1656 cm^{-1} in BSA-Pt^{4+}, BSA-Ir^{3+} and BSA-Fe^{2+} complexes, respectively. This spectral shifting was due to the binding of metal ions to N and O atoms of BSA peptide bonds. The interaction was further demonstrated by a remarkable reduction in spectral intensities of amide I and II bands. Secondary protein structure analysis revealed conformational changes characterized by a substantial decrease in α-helix (11.29–27.41%) accompanied by an increase in β-sheet and β-antiparallel contents. The absorption of BSA at a constant concentration at 280 nm was successively reduced as the concentration of Pt^{4+} and Ir^{3+} ions increased. On the other hand, the absorption of BSA-Fe^{2+} complex successively increased with the increase in the concentration of Fe^{2+} in the test solution. The binding constants for BSA-Pt^{4+}, BSA-Ir^{3+} and BSA-Fe^{2+} complexes were calculated to be 1.55×10^4, 5.67×10^4 and 3.78×10^4 M^{-1}, respectively. The results revealed that the three metal ions showed binding affinities with the BSA protein in the order: $Ir^{3+}>Fe^{2+}>Pt^{4+}$.

Keywords: bovine serum albumin, binding interaction, metal ions, FT-IR spectroscopy, UV-Vis spectroscopy

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Abbreviations: ACE, Affinity Capillary Electrophoresis; BSA, Bovine Serum Albumin; DNA, Deoxyribonucleic Acid; FT-IR, Fourier-Transform Infrared; HSA, Human Serum Albumin; NMR, Nuclear Magnetic Resonance; NO, Nitric oxide; Trp, Ttryptophan; UV-Vis, Ultraviolet-Visible.

INTRODUCTION

Serum albumin is the most abundant and one of the most extensively investigated circulatory plasma protein owing to its multifunctional characteristics and remarkable capacity to bind a variety of ligands. In the biological system, it is responsible for transportation and distribution of a large number of metal ions, therapeutic agents, metabolites, nutrients and other compounds and, therefore, displays several biochemical and pharmaceutical applications (de Wolf & Brett, 2000). Among different serum albumins, bovine serum albumin (BSA) shows the highest resemblance to human serum albumin (HSA) as the only differences concern the surface of the molecules. The binding characteristics of both albumins are similar; therefore, BSA is widely chosen as an appropriate model protein in most of the drug-protein and metal ion-protein interaction studies (Majorek *et al.*, 2012**;** Urquiza *et al.*, 2012; Xiang *et al.*, 2010). BSA is a relatively large heart-shaped globular protein (molecular weight 66.4 kDa), consisting of 583 amino acid residues in a single chain cross-linked with seventeen cysteine residues (Fig. 1). The binding sites for endogenous and exogenous ligands including drugs are generally present in subdomains IA and IIA of BSA molecule; these sites are also known as Sudlow's site I and II, respectively (Tayeh *et al.*, 2009).

Some metal ions are important because of their crucial role in maintaining the normal physiological functions of the biological system, and they are also used to treat and

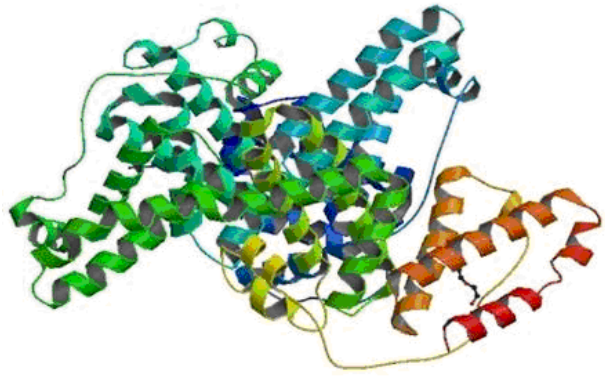

Figure 1. Bovine serum albumin (3D structure) (Downloaded from protein data bank; ID: 4F5S (www.rcsb.org))

diagnose a variety of diseases. Binding of the metal ions to biomolecules including proteins, serves various physiological and medicinal purposes, such as transportation of bioactive metal ions as well as metal-based drugs to their sites of action through metal ion-serum albumin interaction (Alhazmi, 2019). Metal ions are complexed to develop metal-based drugs, which have been extensively studied as potential therapeutic agents for several diseases. Currently, a large number of metal-based therapeutic agents are used for the treatment and diagnosis of various diseases. In addition, a range of metal complexes is in the clinical trial phase of drug development demonstrating a significant therapeutic potential for the management of several health issues (Boros *et al*., 2020; Bruijnincx & Sadler, 2008).

It is evident that the studies of serum protein-metal ion interactions are important to understand the pharmacology and pharmacokinetic profile of bioactive metal ions as well as metal-based drugs. Moreover, binding to metal ions changes the conformation of the protein molecule and may lead to protein unfolding and even aggregation if exposed to higher concentrations and can cause neurodegenerative diseases. The extent and type of binding of metal ions to albumin and other important body proteins are important to characterize in order to minimize the exposure and therefore the untoward effects of metal-based compounds on the body. The protein-metal ion interactions have been characterized using a number of modern analytical techniques including fluorescence spectroscopy, FT-IR spectroscopy, UV-Vis spectrophotometry, circular dichroism spectroscopy, X-ray crystallography, NMR spectroscopy, mass spectrometry, capillary electrophoresis and affinity chromatography (Alhazmi *et al*., 2017; Samari *et al*., 2012; Xu *et al*., 2008; Belatik *et al*., 2012; Grasso and Spoto 2013). FT-IR spectroscopy and UV-Vis spectrophotometry have several advantages over the other techniques because they offer simple, fast, accurate and cost-effective methods for studying protein-metal ion interactions. In UV-Vis spectrophotometry, measurement of UV-absorption of protein before and after the interaction with the metal ion provides an insight to their complexation behavior. Moreover, the absorption spectrum is also helpful to understand the structural changes in protein after ligand interaction (Kragh-Hansen, 1981; Xu *et al*., 2013). FT-IR spectroscopy provides information about the structural dynamics and ligand interactions of biomolecules such as proteins, nucleic acids, enzymes etc. Characteristic functional groups present in the structures of biomolecules possess vibrational fingerprints at specific frequencies of IR light allowing the determination of structure and composition of these functional groups by examining the position and intensity of the spectral bands in FT-IR. Amide I ($C{=}O_{stretching}$) and amide II ($NH_{bending}$) are the characteristic bands present in the FT-IR spectrum of BSA protein (Korkmaz *et al*., 2012; D'Souza *et al*., 2008; Jackson and Mantsch 1996) (Fig. 2), the position and intensities of which can be measured before and after interaction with metal ions to assess the nature, type and extent of binding.

Herein, the present study was focused on the investigation of binding interactions of three medicinally and physiologically important metal ions (Pt^{4+}, Ir^{3+} and Fe^{2+}) with BSA at physiological pH using FT-IR spectroscopy and UV-Vis spectrophotometry. With the help of UV-Vis spectrophotometry, conformational changes in the BSA molecule were identified and measured. The binding constant was determined using the absorption spectrum of the metal ion-BSA complex by taking fixed concentration of protein and increasing amounts of metal ions. FT-IR spectrum was used to evaluate the alteration of characteristic amide bands of BSA upon interaction. Moreover, a possible modification in the secondary structure of BSA (amide I band) was studied using the curve-fitting method.

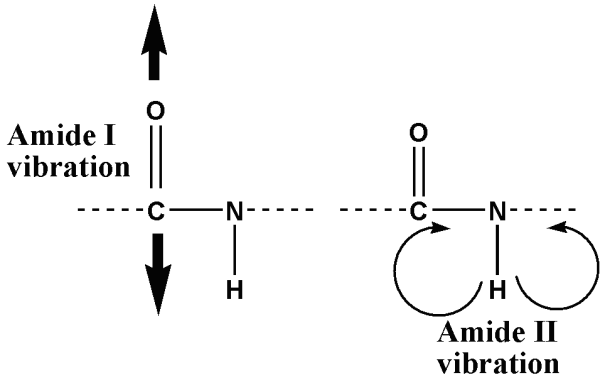

Figure 2. Amide I and Amide II band vibrations in the infrared spectrum of BSA protein

MATERIALS AND METHODS

Chemicals and instruments

Bovine Serum Albumin (BSA, 99%), platinum (IV) chloride ($PtCl_4$), iridium (III) chloride hydrate (H_2Cl_3IrO), iron (II) chloride ($FeCl_2$), Tris powder, CH_3COOH were purchased from Sigma Aldrich, Steinheim, Germany and were used without further purification. The double-distilled deionized ultrapure water was produced in our lab. FT-IR spectrometer (Nicolet iS10, Thermo Scientific, USA) and double beam UV-Visible Spectrophotometer (Shimadzu, Japan) were used to perform FT-IR and UV-Vis spectroscopic measurements, respectively.

Preparation of the solutions

Tris buffer (20 mM) solution was prepared by dissolving 2.42 g of Tris base in 200 mL ultrapure water and adjusting the pH to 7.4 using acetic acid. The final volume was adjusted to 1000 mL using ultrapure water. Protein (BSA) solution (0.5 mM) was prepared by dissolving 1.65 g of BSA powder in 50 mL of the described Tris buffer. Stock solutions of metal ions (1 mM) were prepared by dissolving appropriate quantities of metal salts in 50 mL Tris buffer.

FT-IR spectroscopic measurements

Methodology. The protein (BSA) and metal ions (Ir^{3+}, Fe^{2+} and Pt^{4+}) solutions were appropriately mixed to obtain target protein (0.25 mM) and metal ion (0.25 and 0.5 mM) concentrations. The complexes of metal ions and the protein were prepared separately by incubating the above mixtures at room temperature for 2 h. FT-IR spectra of hydrated film samples of BSA protein alone and metal ion-protein complexes were obtained in the range of 4000–400 cm^{-1} at 4 cm^{-1} resolution and 100 scans. Difference spectrum was generated by subtracting the spectrum of pure BSA from the spectrum of metal ion-protein complexes (Dousseau *et al*., 1989).

Protein conformation analysis. Upon metal ion-protein complex formation, spectral shifting and intensity variations of amide I (1700–1600 cm^{-1}), amide II (1550 cm^{-1}) and amide A bands (3500 cm^{-1}) were analyzed. Alterations in the secondary structure of the protein were also studied using the previously reported method (Byler *et al.*, 1986). Six significant peaks of amide I band, associated with the BSA secondary structure were deconvoluted by the curve fitting method using OriginPro 2019b software (Byler *et al.*, 1986). Using Gaussian functions, the characteristic peaks of α-helix, random coil, β-sheet, β-turn and β-antiparallel at 1660–1650, 1648–1638, 1637–1614, 1678–1670 and 1691–1680 cm^{-1}, respectively, were set and their corresponding areas were calculated. These areas were summed up to get the total area of amide I band. Percentages of amide I components were calculated by dividing the corresponding peak area by the total area (Ahmed *et al.*, 1995).

UV-Vis spectroscopy measurements

Methodology. The UV-Vis spectroscopic analyses were performed to investigate the complexation of BSA with tested metal ions using the method described previously (Zhong *et al.*, 2004; Stephanos *et al.*, 1996) with a slight modification. The 0.5 mM BSA and 1 mM metal ion solutions (Ir^{3+}, Fe^{2+} and Pt^{4+}) were prepared separately by dissolving accurately measured quantity of BSA and metal salts in 20 mM Tris buffer solution pH 7.4. The metal stock solutions were further diluted using the same Tris buffer to achieve working concentrations of 8, 16, 24, 32, 40, 48, 56 and 64 μM, whereas, the BSA solution was diluted to a concentration of 24 μM. The protein and metal ion solutions were mixed in equal proportions (1:1) by stirring at room temperature in order to obtain different metal ion concentrations of 0, 4, 8, 12, 16, 20, 24, 28, 32 μM and the BSA concentration of 12 μM in the final solution. The above mixtures were vortexed and incubated at 25±2°C for 2 h. After the incubation, absorption spectra were recorded for the BSA-metal ion complexes as well as for BSA alone at 280 nm wavelength (λ_{max}) using double beam UV-Visible spectrophotometer. The spectra of complexes were corrected by subtracting the spectra of metal ions to avoid any interference resulting from the absorption of un-reacted metal ions at the measured wavelength.

Determination of Binding Constants. Binding constant (K) was determined using absorbance data for BSA before and post-complexation with metal ions using a previously reported method (Zhong *et al.*, 2004; Stephanos *et al.*, 1996). If we assume only one type of interaction exists between metal ions and BSA in the aqueous solution, equations 1 and 2 can be established:

$$BSA + Metal \Leftrightarrow BSA:Metal \quad (1)$$

$$K = \frac{[BSA : Metal]}{[BSA]\,[Metal]} \quad (2)$$

where: K is the binding equilibrium constant for metal ion:BSA complexes.

Considering [BSA:Metal] = C_B

$$K = \frac{C_B}{(C_{BSA} - C_B)(C_{Metal} - C_B)} \quad (3)$$

Where: C_{BSA} and C_{Metal} are the analytical concentrations of BSA and metal ions in aqueous solutions, respectively.

As per the Beer-Lambert law:

$$C_{BSA} = \frac{A_0}{\varepsilon_{BSA} . \lambda} \quad (4)$$

$$C_B = \frac{(A_0 - A)}{\varepsilon_B . \lambda} \quad (5)$$

Where: A_0 and A are the absorbances of BSA in the absence and presence of metal ions, respectively, at 280 nm wavelength. ε_{BSA} and ε_B are the molar extinction coefficients of BSA and the bound metal ions, respectively, whereas, λ is the path length and was assumed to be 1 cm.

Now, if we substitute the values of C_{BSA} and C_B from equations (4) and (5) to equation (3), it can be deduced to:

$$\frac{A_0}{A_0 - A} = \frac{\varepsilon_{BSA}}{\varepsilon_B} + \frac{\varepsilon_{BSA}}{\varepsilon_B . K} \times \frac{1}{C_{Metal}} \quad (6)$$

Using this equation, a linear double reciprocal plot was obtained with $\frac{1}{C_{Metal}}$ on X-axis, and $\frac{1}{A_0 - A}$ on Y-axis. The binding constant (K) was calculated as the ratio of the intercept to the slope.

RESULTS AND DISCUSSION

FT-IR analysis

Upon interaction of metal ions (0.5 mM) with BSA protein (0.25 mM), a notable reduction in intensities of

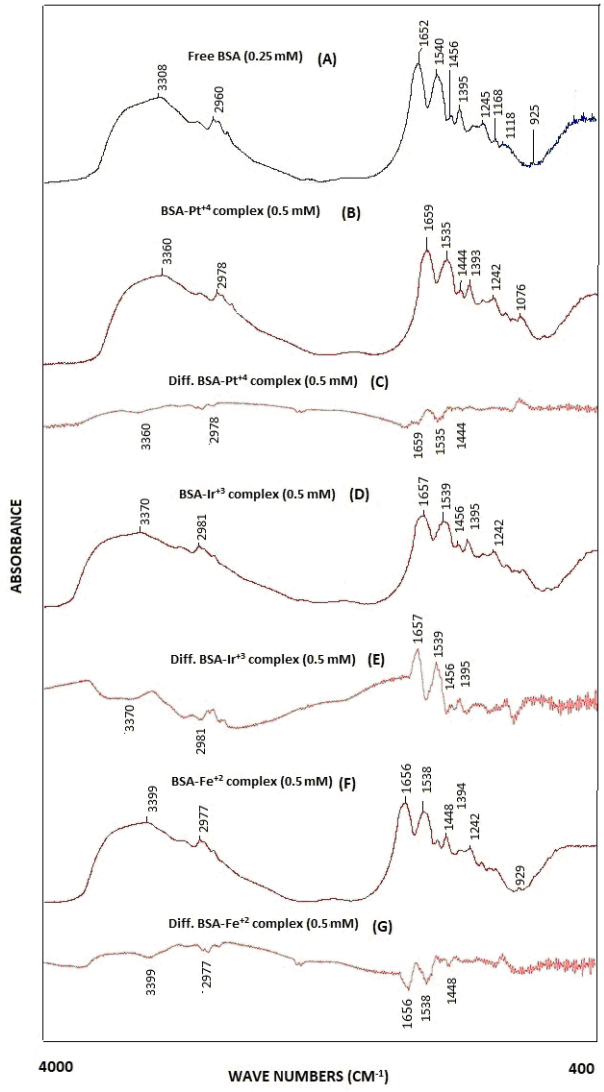

Figure 3. FT-IR spectra of (A) free BSA (0.25 mM) (B) Pt(IV)-BSA complex (0.5 mM) (C) Diff. Pt(IV)-BSA complex (0.5 mM) (D) Ir(III)-BSA complex (0.5 mM) (E) Diff. Ir(III)-BSA complex (0.5 mM) (F) Fe(II)-BSA complex (0.5 mM) (G) Diff. Fe(II)-BSA complex (0.5 mM)

Table 1. Secondary structure analysis of BSA protein and its Pt^{4+}, Ir^{3+}, and Fe^{2+} complexes at physiological pH

Amide I Components (cm^{-1})	Free BSA (%)*	Ir^{+3}-BSA complex (%)**	Fe^{+2}-BSA complex (%)**	Pt^{+4}-BSA complex (%)**
α-helix (± 4) 1660–1650	62	45	48	55
β-sheet (± 2) 1637–1614	14	19	18	16
Random coil (± 2) 1648–1638	5	14	11	11
β-turn (± 2) 1678–1670	16	11	10	12
β-antiparallel (±1) 1691–1680	3	11	13	6

*Percentage secondary structure of free BSA (0.25 mM); **Percentage secondary structure of Ir^{3+}- BSA, Fe^{2+}-BSA and Pt^{4+}-BSA complexes (0.5 mM)

amide I and II bands of Ir^{3+}-BSA and Fe^{2+}-BSA complex was observed, whereas, a minor change was found in case of Pt^{4+}-BSA complex (Fig. 3B, 3D, 3F). In the difference spectra of Pt^{4+}-BSA complex, amide I (1659 cm^{-1}; negative) and amide II (1535 cm^{-1}; negative) bands were present (Fig. 3C). In Ir^{3+}-BSA and Fe^{2+}-BSA complexes, amide I bands were observed at 1657 cm^{-1} (positive) and 1656 cm^{-1} (positive) while amide II bands were present at 1539 cm^{-1} (positive) and 1538 cm^{-1} (positive) (Fig. 3E, 3G). The loss of intensity might be due to the reduction in α-helix component in the protein structure. Additionally, amide I band shifted from 1652 cm^{-1} in case of free BSA to 1659, 1656 and 1657 cm^{-1} for Pt^{4+}-BSA, Fe^{2+}-BSA and Ir^{3+}-BSA complexes, respectively. Shifting of the amide bands might have happened due to the binding of metal ions to C-*N* and C-*O* groups of BSA protein. All the selected metal ions showed a considerable effect on carbonyl stretching vibration. However, $C\text{-}N_{stretching}$ and $C\text{-}N_{bending}$ vibrations were affected weakly by Ir^{+3} and Fe^{+2} ions.

The secondary structure quantitative analysis was also performed using the curve fitting method (Belatik *et al.*, 2012) and the amide I band of free BSA was deconvoluted using the Origin software. It showed the presence of α-helix (1652 cm^{-1}; 62%), β-turn (1671 cm^{-1}; 16%), β-sheet (1617, 1630 cm^{-1}; 14%), random coil (1639 cm^{-1}; 5%) and β-antiparallel (1684 cm^{-1}; 3%) as main components of the protein structure. In secondary structure analysis of complexes, a marked reduction in the α-helix content (62% in case of free BSA to 55, 48 and 45% for Pt^{4+}-BSA, Fe^{2+}-BSA and Ir^{3+}-BSA complexes, respectively) was observed with a rise in β-sheet component (14% in case of free BSA to 16, 18 and 19% for Pt^{4+}-BSA, Fe^{2+}-BSA and Ir^{3+}-BSA complexes, respectively) (Table 1; Fig. 4A–D). The percentage change in the α-helical content was also calculated for the three complexes and was found to be –27.41, –22.58 and –11.29% for Ir^{3+}, Fe^{2+} and Pt^{4+} metal ion complexes, respectively, (Table 2) indicating that the interaction of Ir^{3+} ion with BSA was the strongest followed by Fe^{2+} and Pt^{4+}. These findings support the "hard and soft acids and bases (HSAB) theory" of metal-ligands interaction since a strong interaction of hard metal ion (Ir^{3+}) with C=O ligand of BSA protein was observed. The decrease in intensity of the bands was because of the reduction in α-helix portion of amide I band of the protein at the tested metal ions concentration.

UV-Vis Spectroscopy

The effect of increasing concentration of metal ions (Pt^{4+}, Ir^{3+}, and Fe^{2+}) on the structural variations of BSA protein was studied (Fig. 5A–C) using UV-Vis spectroscopy. The absorption spectra for Ir^{3+} and Pt^{4+} complexes with BSA (Fig. 5A, 5C) showed that the peak intensities of BSA at 280 nm decreased upon complexation with Pt^{4+} and Ir^{3+} ions as the concentrations of metal ions increased from 4 to 32 μM. The observed hypochromism effect with a minor shift in the wavelength indicated the interaction of Pt^{4+} and Ir^{3+} metal ions with the hydrophobic region of the protein. It thus revealed the conformational change in BSA (Buranaprapuk *et al.*, 2000) upon interaction. The hypochromism effect also indicated the increased polarity and decreased hydrophobicity around the tryptophan residue (Varlan *et al.*, 2010). The binding constants for BSA-Pt^{4+} and BSA-Ir^{3+} adducts were calculated to be 1.55×10^4 M^{-1} and 5.67×10^4 M^{-1}, respectively (Fig. 6A, 6C). On the other hand, the intensities of absorption peaks of BSA increased upon the addition of increasing concentration of Fe^{+2} ion from 4 to 32 μM (Fig. 5B). The hyperchromic effect was observed in this case which indicated that the Fe^{2+} ion interacted with the exposed tryptophan residue on the protein surface through electrostatic forces (Maciel *et al.*, 2013).. The binding constant for BSA-Fe^{2+} complex was determined to be 3.78×10^4 M^{-1} (Fig. 6B).

Binding constant or the association constant of a ligand is a measure of its interaction with the protein and denotes the speed at which the ligand-protein complex forms. Usually, the binding constant of a ligand should be sufficiently high so that a considerable quantity of ligand gets distributed throughout the body, at the same time it should be low enough so that the ligand can be released once it interacts with the binding sites. An ideal range of binding constant is thought to be 10^4 – 10^6 M^{-1} (Rajendiran *et al.*, 2007) and the ligands having binding constants in this range are believed to be ideal candidates to be used as drugs. The binding constants of metal ions in this study revealed that these metal ions efficiently bind to BSA protein and are distributed effectively in the biological system.

Table 2. Comparative evaluation of FT-IR and UV-Vis spectroscopy results and the findings of ACE for metal ions-protein interaction.

Metal Ions	$\Delta R/R_f \pm cnf$ values in ACE* (Alhazmi, 2015)	% change in α-helix (Present study)	Binding constant (M^{-1}) (Present study)
Ir^{3+}	–0.2511±0.0132	–27.41	5.67×10^4
Fe^{2+}	–0.1290±0.0100	–22.58	3.78×10^4
Pt^{4+}	–0.0335±0.0105	–11.29	2.8×10^4

*ΔR, Difference in mobility ratios; R_f, mobility ratio of BSA alone; *Cnf*, confidence interval

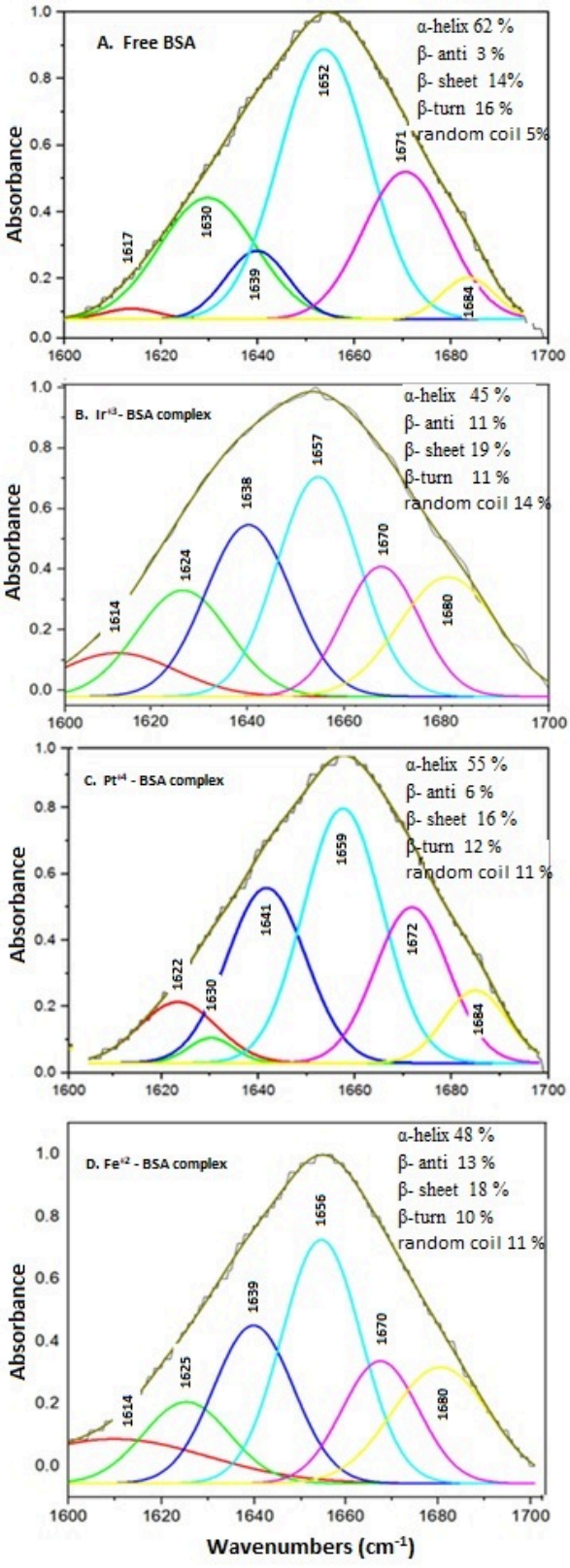

Figure 4. Curve-fitted spectra of amide I band at 1700–1600 cm^{-1} (R^2=0.99) for (A) free BSA (B) Ir^{+3}-BSA (C) Pt^{+4}-BSA (D) Fe^{+2}-BSA complexes

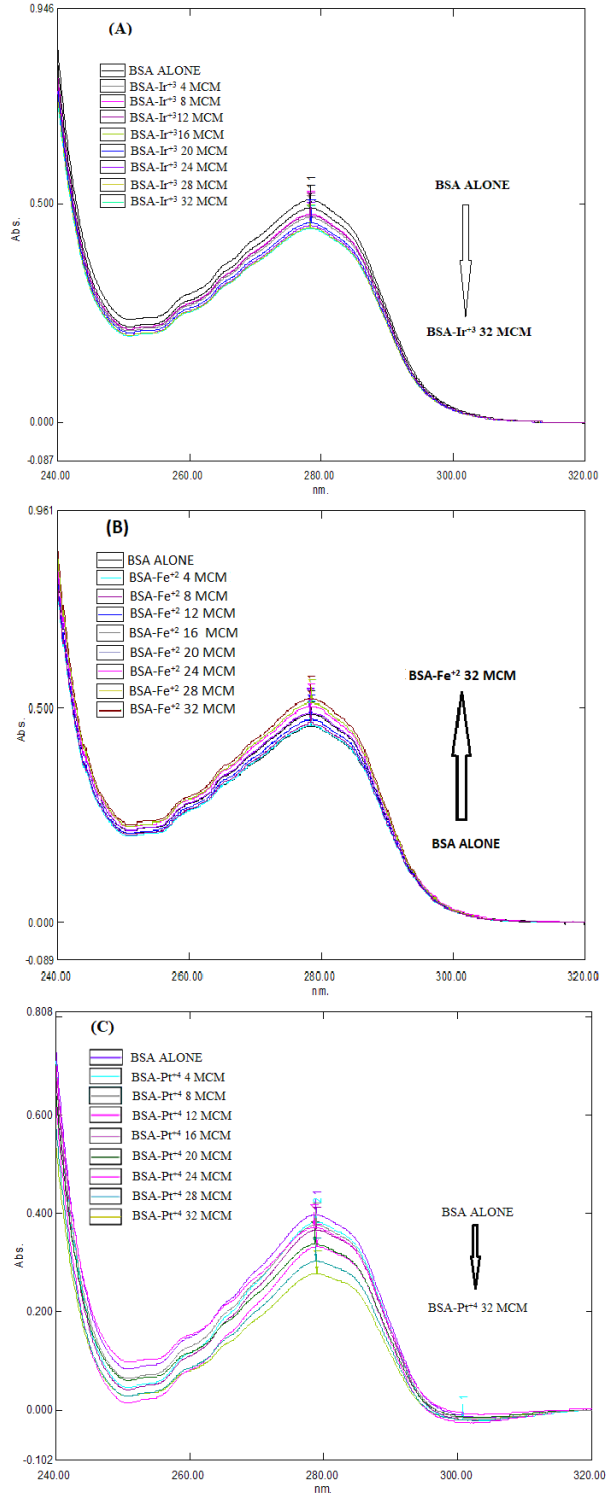

Figure 5. Spectral overlay of increasing concentration of (A) Ir^{+3} ion (B) Fe^{+2} ion, and (C) Pt^{+4} ion on UV absorption of BSA; C_{BSA}=12 μM; C_{metal}=0, 4, 8, 12, 16, 20, 24, 28 and 32 μM; Tris buffer (pH 7.4)

Previously, we studied the interactions of several biologically and medicinally important metal ions with important biological proteins using affinity capillary electrophoresis (ACE) (Alhazmi *et al.*, 2015). The results obtained in the present study were compared to the results of our previous study on these metal ions in order to establish the accuracy of the employed spectroscopic techniques. The percentage variation in the α-helical component obtained from FT-IR spec-

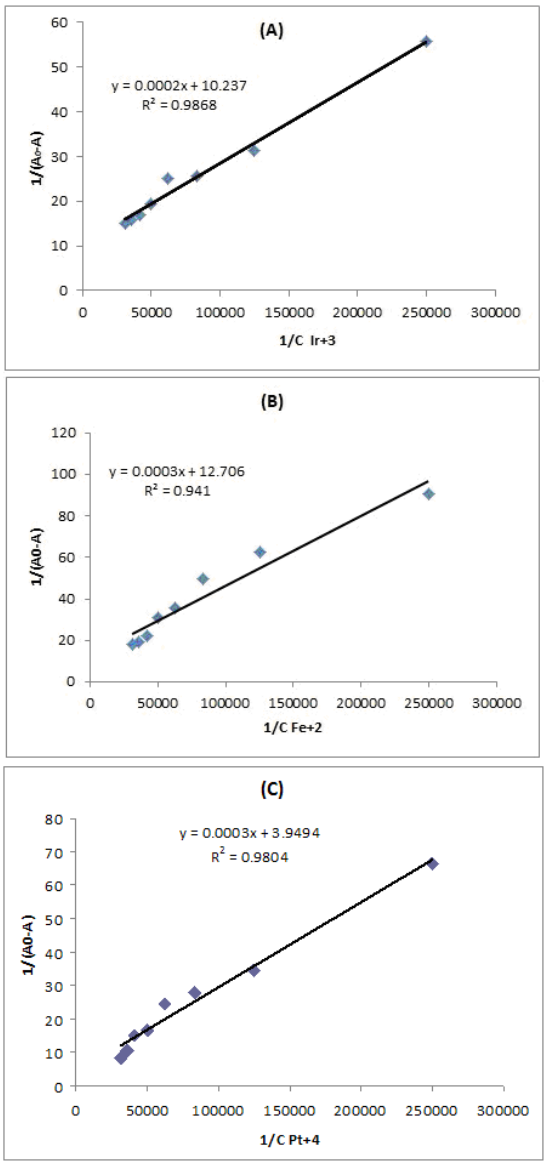

Figure 6. The plot of *1/(A_0-A) versus 1/C_{metal}*; A_0 and *A*=absorbances of BSA alone and BSA-metal complexes, respectively; C_{metal}=Ir^{3+}, Fe^{2+}, Pt^{4+} concentrations. C_{BSA}=12 μM; C_{metal}=0, 4, 8, 12, 16, 20, 24, 28 and 32 μM; Buffer: Tris (pH 7.4); Binding constant: (A) K_{Ir3+}=5.67×10⁴ M^{-1}, (B) K_{Fe2+}=3.78×10⁴ M^{-1} and (C) K_{Pt4+}=1.55×10⁴ M^{-1}

troscopy and the binding constants of metal ions calculated using UV-Vis spectroscopy were compared to the mobility ratio of these metal ions measured using ACE (Table 2). Interestingly, similar findings were obtained using all the techniques, and Ir^{3+} ions showed the strongest binding to BSA followed by Fe^{2+} and Pt^{4+} ions. The comparable results revealed that all three methods could successfully be used to analyze metal ions-protein interactions with adequate accuracy.

Studying protein-metal ion interaction is of prime importance in the development of metal-based drugs as the affinity of these drugs towards numerous biomolecules such as proteins and nucleic acids would determine their potential to be used as therapeutic agents against various diseases. Metal-based agents, especially the coordination compounds, exhibit adjustable ligand kinetics, flexible geometries and promising redox activities to be used against microbial infections (Alyar *et al.*, 2012; Asadi *et al.*, 2014; Bellu *et al.*, 2005), tumors (Xin Zhang & Lippard, 2003; Bakhtiar & Ochiai, 1999), and as radiopharmaceuticals (Guo & Sadler, 1999). Previously, a number of mononuclear and polynuclear divalent metal ion complexes such as Cu^{2+}, Zn^{2+}, Ni^{2+}, Pt^{2+} and Co^{2+} with BSA protein were studied (Sathyadevi *et al.*, 2012; Gharagozlou & Boghaei, 2008; Krishnamoorthy *et al.*, 2011; Sathyadevi *et al.*, 2011; Samari *et al.*, 2012). The specificity of the binding interactions between metal ions as ligands and proteins is affected by the planarity of the ligands (Sathyadevi *et al.*, 2011) and it was suggested that the metal ions bind to the Trp134 residue present on the protein surface which is the most accessible one (Xue *et al.*, 2012). The displacement studies revealed that metal ions preferentially bind to the subdomain IIA present in the site-I of the BSA protein (Asadi *et al.*, 2014; Samari *et al.*, 2012).

Extensive ligand exchange is one of the key features of the metal-based drugs which are responsible for the interaction with essential biomolecules in the body system including proteins, enzymes and nucleic acids. Examples of such drugs are Pt^{2+}-based anticancer drugs: cisplatin, carboplatin and oxaliplatin, Au^{+}-compound auranofin, an anti-arthritic agent and several others. Metal ions covalently bind to the biomolecules such as DNA, proteins and enzymes which might inhibit their function leading to cell death through apoptosis, necrosis etc. The serious adverse effects encountered by patients receiving metal-based chemotherapeutic agents are due to lack of selectivity; as the metal-based agents not only bind to their target present in the cancerous cells, they also bind covalently to the biomolecules of the normal cells. For instance, DNA in the cancerous cells is the main target for cisplatin, however, it can interact with proteins as well (Han Ang & Dyson, 2006; Messori & Merlino, 2016).

It was observed that the interaction between metal ions and BSA results in the disruption of disulfide bonds of the protein leading to perturbation of its secondary structure. The α-helix conformation is partially lost leading to unfolding of the protein (Samari *et al.*, 2012) and there is also a change in polarity around the tryptophan (Trp) residue (Ehteshami *et al.*, 2013) due to the molecular interactions including rearrangements, energy transfer, collision quenching processes etc. (Samari *et al.*, 2012; Ehteshami *et al.*, 2013; Jalali *et al.*, 2014). Due to the partial unfolding or rearrangement of the protein's structure, the distance between the amino acid residues (Trp) changes leading to the reduction in the energy transfer and collision quenching between the neighboring amino acid residues. This would change the polarity of the microenvironment surrounding the amino acid residues to which they are exposed. The ligand exchange kinetics of the metal ion is greatly affected by its oxidation state and, therefore, it might be more active (or more reactive) in one oxidation state, while less active (less reactive, even may be inactive) in another state. Due to this difference, there exists an intrinsic mechanism of activation, permitting the administration of less active (hence less toxic) species, which upon activation by oxidation or reduction elicit their activity. This approach has been used to administer Pt(IV)- and Ru(III)-based compounds, which are bioactivated via reduction to Pt(II) and Ru(II) species, respectively, possessing enhanced cytotoxic properties (Graf & Lippard, 2012; Gibson,

2016; Kenny & Marmion, 2019). In some of the metal-based drugs, the metal ion is used as a carrier that helps to deliver and ultimately releases the biologically active molecules (ligands) to their sites of action.

Among such drugs, NO-releasing metal complexes have been studied extensively due to several important therapeutic effects of nitric oxide; for instance, regulation of the function of the cardiovascular system as an inhibitor of platelet aggregation and vasodilatation in addition to immune-defense and apoptosis (Lewandowska *et al*., 2011). Apart from gases, metal ions are also used to deliver other biologically active molecules to their site of action. This approach has been used previously to deliver curcumin that exhibits a wide range of pharmacological activities including antioxidant, anti-inflammatory and anti-tumor. However, the use of curcumin is associated with solubility issues, light sensitivity, high rate of metabolism, rapid clearance and lower bioavailability. All these problems can be overcome by complexing curcumin with certain essential and non-essential metal ions before administration (Renfrew *et al*., 2013; Renfrew 2014; Caruso *et al*., 2012).

Physiologically, serum albumin is involved in the transportation and handling of 45% of Mg^{2+} and Ca^{2+} ions and hence, the plasma levels of biologically active or ionized forms of these metals are controlled (Majorek *et al*, 2012). The binding sites for Mg^{2+} and Ca^{2+} are coordinated by aspartic and glutamic acids side chains present in domain I (Majorek *et al*., 2012; Jalali *et al*., 2014; Xu *et al*., 2013). During metal ion-protein binding, the amino acid residues of the protein function as negatively charged (electron-rich) moiety and cationic metal ions provide electron-deficient site. Consequently, the binding interaction is a result of the attraction between the two species. In addition to that, charge-accepting capacity, valency and atomic radius also regulate the interactions of metal ions with appropriate binding sites of proteins (Dudev *et al*., 2014; Sarkar, 1987). Protein-metal ion binding may influence the structural stability of the protein. In BSA molecule, the metal ion interaction results in disruption of the disulfide bonds, alteration of the secondary structure and unfolding through a significant loss in α-helix conformation. Furthermore, protein-metal ion binding may change the overall polarity and facilitate the exposure of tryptophan residue, resulting in molecular re-arrangement and collision quenching (Samari *et al*., 2012; Ehteshami *et al*., 2013; Jalali *et al*., 2014).

CONCLUSION

Nowadays, metal complexes have been extensively explored as potential therapeutic agents for the development of new antifungal, antiviral, antibacterial and anticancer drugs. Metal-based drugs are well accepted in chemotherapy owing to their less reported resistance and promising therapeutic potentials. In drug design of metal-based agents, it is crucial to evaluate their ability to bind the serum albumin protein because it plays an essential role in transportation, distribution, metabolism and excretion of these drugs. By considering this, interactions of BSA with some medicinally important metal ions (Pt^{4+}, Ir^{3+} and Fe^{2+}) were successfully studied at physiological pH using two simple and cost-effective spectroscopic techniques. Significant variations in the intensity of amide I band of BSA were observed upon interaction with metal ions which were utilized to measure the extent of their interaction. Shifting of amide A, amide I and amide II bands in FT-IR spectra were also observed supporting the interaction of these metal ions with the binding sites of BSA protein. Significant reduction in the α-helical content and its conversion to β-antiparallel and β-sheet proved the alteration and partial unfolding of the secondary structure of BSA protein. As the subdomain I of BSA protein containing the Trp134 residue is more resistant to denaturation than the subdomain II, this partial unfolding would result in the exposure of subdomain IIA in the site I, where the Trp213 residue is present, making it more exposed to the polar environment. The UV-Vis spectroscopy results revealed that the metal ions interacted with the protein surface through electrostatic forces and bound to the hydrophobic aromatic amino acid residues of the protein changing its conformation. The calculated binding constants showed that the selected metal ions bind efficiently to BSA protein. The experimental results of UV-Vis and FT-IR spectroscopic studies showed that the tested metal ions interacted with the BSA protein in the order: $Ir^{3+}>Fe^{2+}>Pt^{4+}$. These results could be helpful to understand the binding dynamics of the selected metal ions with BSA *in vivo* and the transportation and biotransformation of BSA-metal ion complexes inside the body.

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Declarations of interest

The authors report no disclosures of interest.

Authors' contribution

All authors contributed equally to this work.

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