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Investigation of binding of bovine serum albumin with metallic nanoparticles

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ABSTRACT

Study of interactions of bovine serum albumins (BSA) with different metallic nanoparticles (NPs) receives great attention in the recent years due to their key impact in the biomedical field. Interaction can be studied using several methods but due to its high sensitivity and simplicity fluorescence spectroscopy is the most useful one. Detailed characteristics about the interactions of NPs with serum albumin can be derived from the information accumulated by the fluorescence quenching studies. The present review underlies on the interaction of various metallic NPs with BSA. This review helps in understanding the features of NPs essential for their affinity for BSA.

Keywords: Serum albumins, Spectroscopy, Fluorescence quenching, Interaction, Nanoparticles.

INTRODUCTION

Study on the interactions of drug/nanomaterials with biomolecules like protein, DNA receives great interest in the field of chemistry, life science and clinical medicine for decades. The nature and the magnitude of these interactions influence the biosafety, delivery rate, pharmacological response, therapeutic efficacy and the design of drugs. So, the studies on these interactions help in understanding the structural features essential for the bio-affinity of drugs/nanomaterials toward the pharmacological activity [1–4]. Nanomaterials can show a strong effect on the structural and functional properties of proteins and thereby gradually an interest has been increasing to understand fundamental effects of NPs for interaction with proteins.

Optical techniques like absorption spectroscopy, circular dichroism, ellipsometry, differential light scattering, Raman spectroscopy, and fluorescence spectroscopy are useful tools for studying the drug/nanoparticles–protein interactions in vitro due to their exceptional sensitivity, speed, theoretical foundations, and simplicity [4–7]. Among the several optical techniques, a large amount of information is acquired about the structural fluctuations and the microenvironment surrounding the fluorescent labels of proteins from the measurements and analyses of fluorescence spectra, fluorescence lifetime, fluorescence polarization, etc. Therefore, fluorescence spectroscopy plays a crucial role in the study of interactions between the drug molecule/NPs and the serum albumins. In particular, fluorescence quenching studies are widely utilized for revealing the accessibility of drug/NPs to the fluorophore moiety in a protein, which in turn helps us to understand the nature and the underlying mechanism of NPs–protein interactions [8]. So, Fluorescence spectroscopy is a powerful tool for the study of the reactivity of chemical and biological systems since it allows non-intrusive measurements of substances in low concentration under physiological conditions.

Serum albumins are the most abundant soluble protein in the systemic circulation comprising 52-60 % in plasma. Serum albumin are synthesized by the parenchymal cells of the liver and exported as a non-glycosylated protein.

They are capable of bind reversibly with a large variety of relatively insoluble endogenous and exogenous ligands. The principal function of serum albumin is to transport metabolites such as nutrients, hormones, fatty acids and a variety of pharmaceuticals. Among serum albumins, human serum albumin (HSA) and BSA is extensively studied due to their significance in the pharmacology. Both HSA and BSA display approximately 80 % sequence homology and a repeating pattern of disulfides. The molecular weights are nearly same for BSA and HSA 66 kDa and 66.5 kDa respectively. The tertiary structures of HSA and BSA are also alike, show 76 % similarity [9]. Crystal structure analyses have revealed that HSA contains 585 amino acid residues with 17 tyrosyl residues and only one tryptophan (Trp) located at position 214 along the chain (subdomain IIA); whereas, BSA contains 582 amino acid residues with 20 tyrosyl residues and two tryptophan's located at positions 134 and 212 and Trp-134 at the surface of the molecule [9-19]. The chemical microenvironment of Trp-212 in BSA is similar to that of Trp-214 in HSA. Absorption peak maxima of serum albumins are around 280 nm. The intrinsic fluorescence of serum albumins appears at 340 nm when excited at 280 nm which is originating from the three aromatic L-amino acid tryptophan, tyrosine, and phenylalanine residues. Now, the intrinsic fluorescence of serum albumins is mainly contributed by the tryptophan and tyrosine residues because of the low fluorescence quantum efficiency of phenylalanine. The intrinsic fluorescence characteristics are very sensitive to the microenvironment of the fluorescent residues or changes in the local surroundings of serum albumins, such as conformational transition, biomolecular binding and denaturation [20]. In major work, BSA has been selected as protein model because of its medicinal importance, low cost, ready availability, and unusual ligand-binding properties.

Herein we review the recent literature about the interactions of BSA with various metallic NPs studied using fluorescence spectroscopy. This review does not seek to provide an absolute review of all articles published on NPs–BSA interactions, rather it describes use of fluorescence quenching studies involving the interactions between BSA and metallic NPs and emerging research in this area.

EXPERIMENTAL SECTION

2.1. Fluorescence quenching studies

Study of interaction of various molecules with serum albumins may be easily deduced using fluorescence spectroscopy as intrinsically fluorescent serum albumins are very sensitive to local changes in the polarity, conformation and/or exposure to the solvent. The interaction will decrease or increase fluorescence intensity. Fluorescence quenching may result from several processes such as excited state reactions, molecular rearrangements, energy transfer, ground-state complex formation or collisional interactions [8]. The interacting molecule can quenches the intrinsic fluorescence of BSA with or without any shift (red- or blue-shift) in the emission peak maxima (λ_{max}). If the interacting molecule quenches the fluorescence of serum albumin without changing the spectral maximum, the hydrophobicity and polarity in the microenvironment of the fluorophore (tryptophan or tyrosine) is not altered. When the interacted molecule quenches the fluorescence with a blue-shift in the spectral maximum, it suggests a decrease in the polarity or an increase in the hydrophobicity of the microenvironment surrounding the fluorophore site; whereas a red-shift suggests increase in the polarity or decrease in the hydrophobicity of the microenvironment [21].

The fluorescence quenching for interacted molecule quencher and protein can be analyzed by the Stern-Volmer equation (Eq. 1) [22].

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

Where, F_0 and F represent the fluorescence intensities in absence and presence of quencher, K_q is the bimolecular quenching rate constant, K_{SV} is the Stern Volmer constant, τ_o is the average lifetime of the molecule without quencher and [Q] is the concentration of the quencher. Equation 1 is used to calculate K_{SV} by the plot of F_0/F vs [Q].

The fluorescence quenching can either be dynamic or static. Dynamic quenching refers to a process where the fluorophore and the quencher interact during the excited-state lifetime of the fluorophore; whereas, static quenching refers to the formation of the fluorophore-quencher complex in the ground state. Static quenching can easily be separated from dynamic quenching by examining their temperature dependence, or by the lifetime measurements [8, 23-24].

When bioactive substances bind independently to a set of equivalent sites on serum albumin, the equilibrium between free and bound molecules is given by following equation-

$$log\frac{F_0 - F}{F} = logK + n\log[Q] \quad (2)$$

The number of binding sites (n) and the binding constant (K) between nanoparticle and BSA have been calculated using the above Eq. 2. A plot of log $[(F_0-F)/F]$ vs log [Q] forms a straight line, whose slope equals to n (the number of binding sites of NP on BSA) and the length of intercept on Y-axis equals to log K. The binding constant for the interaction of BSA with various metallic NPs is summarized in Table 1. If the value of the binding constant K is in the range $1\text{-}15\times10^4\ M^{-1}$, then the binding affinity is moderate [25].

The force of interaction between bioactive substances and biomolecules may include electrostatic interactions, multiple hydrogen bonds, weak van der Waals interactions, and hydrophobic interactions. The nature of interaction force can be measured using the signs and magnitudes of thermodynamic parameters such as enthalpy change (ΔH), free energy change (ΔG) and entropy change (ΔS). These three parameters can be computed using the following Van't Hoff equations (Eq. 3 and Eq. 4).

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

$$\Delta G = \Delta H - T\Delta S = -RT\ln K \quad (4)$$

According to the views of Ross and Subramanian [26], when ΔH>0 and ΔS>0: indication of hydrophobic forces; ΔH<0 and ΔS<0: indication of van der Waals interactions and hydrogen bonds; ΔH≈0 and ΔS>0: indication of electrostatic interactions. The thermodynamic parameters such as ΔH, ΔG, and ΔS for the interaction of BSA with various metallic NPs are summarized in Table 1.

Forster resonance energy transfer (FRET) [27] is a sensitive method for the detection of interactions between bioactive substances and serum albumin. FRET efficiency can be used to evaluate the distance between the bound bioactive substances and the fluorophore present in serum albumins [8, 28]. According to FRET, the transfer of energy, this occurs through the direct electrodynamic interaction between the primarily excited molecules and their neighbors [29]. The Forster theory points out that the energy transfer efficiency E, in addition to its dependence on the distance between the acceptor and the donor, depends upon the critical energy transfer distance, R_0. Hence the efficiency of energy transfer for a single donor-single acceptor system is expressed by the following equation-

$$E = 1 - \frac{F}{F_0} = R_0^6 / (R_0^6 + r^6) \quad (5)$$

Where, F and F_0 are the fluorescence intensities of BSA in presence and absence of NP, r is the distance between acceptor and donor and R_0 is the critical distance when the transfer efficiency is 50 %, which can be calculated by following equation-

$$R_0^6 = 8.8\times10^{-25}k^2N^{-4}\phi J \quad (6)$$

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$$R_0^6 = 8.8\times10^{-25}k^2N^{-4}\phi J \quad (7)$$

Where, k^2 is the spatial orientation factor between the emission dipole of the donor and the absorption dipole of the acceptor. The dipole orientation factor, k^2, is the least certain parameter to be used in the calculation of the critical transfer distance. Although theoretically k^2 can range from 0 to 4, the extreme values require very rigid orientations. If both the donor and acceptor are tumbling rapidly and free to assume any orientation, k^2 equals 2/3. If only the donor is free to rotate, k^2 can vary from 1/3 to 4/3 [30]. In eq. 7, N is the refractive index of the medium, ϕ is the fluorescence quantum yield of the donor and J is the overlap integral of the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor given by the following equation-

$$J = \sum F(\lambda)\varepsilon(\lambda)\lambda^4\Delta\lambda / \sum F(\lambda)\Delta\lambda \quad (8)$$

Where, F (λ) is the fluorescence intensity of the fluorescent donor at wavelength λ and is dimensionless; ε (λ) is the molar absorption coefficient of the acceptor at wavelength λ. It has been reported for BSA that $k^2 = 2/3$, $\phi = 0.15$ and N = 1.336 [31]. Using the above three Eqs. (6)-(8), the donor-to-acceptor distance, r, can be calculated. If $r < 7$ nm [32-33] and $0.5R_0 < r < 1.5R_0$ [34], the probability of energy transfer from serum albumins to bioactive substances is high.

Synchronous fluorescence spectroscopy introduced by Lloyd [35-36], which involves the simultaneous scanning of excitation and the fluorescence monochromators of a fluorimeter, while maintaining a fixed wavelength difference ($\Delta\lambda$) between them, is a simple and effective means to measure the fluorescence quenching and the possible shift of the maximum emission wavelength (λmax) relative to the alteration of the polarity around the chromophore at physiological conditions. When $\Delta\lambda$ is stabilized at 15 nm or 60 nm, synchronous fluorescence offers the characteristics of tyrosine residues or tryptophan residues in the serum albumins [37]. In the synchronous fluorescence spectra, the fluorescence intensity decreases with or without any shift in the emission maximum. A decrease in fluorescence intensity without any shift indicates that the microenvironment around that particular residue is not disturbed. Red-shift is indicative of an increase in the hydrophilicity around the fluorophore in BSA. Blue-shift should be due to an increase in the hydrophobicity around the fluorophore moiety [38-39].

RESULTS AND DISCUSSION

3.1. Binding capability of bovine serum albumins with various metallic nanoparticles

During the past decade, a lot of attention has been focused on recognizing the interactions of NPs with biomolecules [40]. The size-dependent optical properties of various metallic NPs establishing NPs promising for various advanced biomedical applications from diagnosis to therapy [41]. Therefore, the evaluation of the interactions between NPs and biomolecules such as BSA becomes remarkable. Table 1 represents the list of binding constants and thermodynamic parameters for various metallic NPs-BSA interactions discussed in this present manuscript.

Metal NPs are of great importance due to their high surface area and a high fraction of surface atoms. In particular, GNPs are employed in many fields like bio-sensing, catalysis, electronics, enzyme electrodes, super conductors, and cancer therapy [42-47]. Furthermore the biocompatibility and stability of GNPs make them excellent candidates for in vivo phototherapy of cancer [48], the sensitive detection of HIV-1 in plasma [49], cell imaging due to the sensitive detection of Adenosine triphosphate (ATP) in live cells [50]. Upon simple conjugation of GNPs onto therapeutically inactive monovalent small organic molecules, they can be converted into highly active drugs that effectively inhibit HIV-1 fusion to human T cells [51]. Due to the above vast biomedical applications, the study on interaction of GNPs with BSA becomes vital.

The binding of serum albumins with GNPs synthesized using sonochemical reduction method were investigated using optical techniques by Naveenraj et al. [52]. The effect of these GNPs, synthesized by different methods, on the fluorescence spectra of serum albumins show gradual decrease in the fluorescence intensity of serum albumins without any changes to their fluorescence spectral shape and maximum, which indicates the formation of non-fluorescent ground state complexes. Also, the sonochemically synthesized NPs show uniform size and shape, which is evident from high resolution transmission electron micrographs. In the binding affinity study conducted by Pramanik et al. [53] using GNPs having diameters of 8, 10, 16, 25, 34, 41, 47, 55 and 70 nm, which are synthesized by varying the [Au] to citrate ratio, the Stern–Volmer constant, K_{SV} increases with decrease in the size of GNPs. In other words, the fluorescence quenching is more efficient in the case of smaller GNPs, which suggests that smaller particles will have more binding interaction due to the large surface area. Wangoo et al. [54] described interaction of

GNPs with BSA. The obtained K value was 3.16×10^{11} Mol^{-1}. The conformational change in BSA at its native form after conjugation with GNPs confirmed that protein undergoes a more flexible conformational state on the boundary surface of GNPs after bio-conjugation. The CD studies further showed a decrease in the α-helical content after conjugation. The results confirmed that the change in conformation was larger at higher concentrations of GNPs. Shi et al. [55] also studied interactions between GNPs and BSA. The formation of GNPs-BSA conjugates led to a red shift in the intrinsic fluorescence emission of BSA. BSA bound to the GNPs with high affinity (binding constant K = 7.59×10^{8} L/mol), and the intrinsic fluorescence of BSA was quenched by the GNPs in accordance with the static quenching mechanism. Both fluorescence spectroscopy and ATR-FTIR showed that GNPs induced conformational changes in BSA, which resulted in it becoming less compact and increased the polarity of the microenvironment around the tryptophan residue Trp-212. Gao et al. [56] also reported interactions of colloidal GNPs and serum albumins, including BSA and HSA, were studied by fluorescence and absorption spectrometry. The obtained K value was 1.12×10^{7} $LMol^{-1}$. Study of interaction between BSA protein and the surface of GNPs of different shapes (nanospheres and nanorods) by using localized surface plasmon resonance (LSPR) spectroscopy, fluorescence spectroscopy and surface-enhanced Raman scattering (SERS) was reported by Iosin et al. [57]. They found that the binding constant of BSA to spherical GNPs is higher than in the case of nanorod-like GNPs. The Raman and SERS data confirm the molecular specificity of conjugation and inform about possible conformational changes that BSA undergoes at the surface of GNPs.

Among the metallic NPs, silver nanoparticles (SNPs) have received large attention because of their wide range of applications. Silver nanoparticles have been used since ancient time. Silver nanoparticles have a wide range of applications in different aspects such as in non-linear optics, spectrally selective coating for solar energy absorption, bio-labeling, intercalation materials for electrical batteries, as optical receptors, catalyst in chemical reactions [58]. Due to their antimicrobial activity, SNPs are proposed to be used to treat burn wounds, as coatings in surgical masks, in implantable devices [59-61] etc. Silver nanoparticles have been used as biosensors to detect *E.coli* in apple and milk juice [62]. They have been used in conjunction with 9-aminoacridine an antitumor drug to study the antiproliferation effect on HeLa cells [63]. They are also known to exhibit antiplatelet properties in vivo which is useful in the treatment of thrombotic disorders. Anticoagulant and thrombolytic therapies for thrombotic disorders are usually associated with serious bleeding complications, and hence SNPs serve as potential antiplatelet/antithrombolytic agents as they do not confer any lytic effects on platelets [64]. So, considering the useful application of SNPs the study of interaction of BSA-SNPs becomes very important.

Recently the interaction of SNP with BSA using fluorescence spectroscopy was studied by Mariam et al. [65]. The SNP quench the fluorescence of BSA with a blue-shift in their emission maximum. The non-linearity of Stern–Volmer plots indicates that both static and dynamic quenching is involved. Further, 'n' value obtained from the modified Stern–Volmer plot and the blue-shift in the synchronous fluorescence spectra ($\Delta\lambda$ = 60 nm) indicate that SNP lower the polarity or increase the hydrophobicity of the microenvironment of the tryptophan residues in BSA. Shen et al. [66] also studied interaction between serum albumins and SNP. The studies with the surface plasmon bands indicate that the electrostatic and hydrophilic interactions are the major forces between serum albumins and SNP; the number of adsorbed monolayer serum albumin molecules to a SNP with the size of 60 nm is about 6.7×10^{5}. The far-UV CD spectra provide the evidence that the secondary structure of adsorbed serum albumins adopt a looser and more extended conformation, in which the content of α-helix decreases, whereas the content of β-sheet, turn and unordered coil increases. Interaction of biosynthesized SNP with BSA using spectroscopy was also investigated recently [67]. Biosynthesized SNP has a strong ability to quench the intrinsic fluorescence of BSA by dynamic quenching mechanisms. The obtained binding constant was 10.23×10^{3} $LMol^{-1}$. The binding constant for the system of chemically synthesized SNP and BSA at different temperatures were quite high compare to biosynthesized SNP. The interaction is driven by both hydrophobic and electrostatic interactions. Synchronous fluorescence spectra indicate the small change in the microenvironment of tryptophan and tyrosine residues. The binding distance r for this interaction indicated that the energy transfer occurs between BSA and biosynthesized SNP with high probability, and also indicated that the fluorescence quenching of BSA was a non-radiative transfer process. Ali et al. [68] also described interaction of chemically synthesized SNP with BSA. The quenching of BSA fluorescence takes place with 1:1 complex formation between the albumin and NPs. Hydrophobic forces play significant role in the conformational changes during the binding process and BSA gets partially unfolded in presence of Ag-PVTNPs. The quenching process was found to be static with a binding distance of 2.84 nm between the BSA and NPs.

Titanium dioxide nanoparticle (TiO_2NPs) is used extensively in paint, pigment, food, medicine and pharmaceuticals. More than 70 % of the total produced TiO_2NPs is utilized as pigments owing to high brightness, large refractive index and resistance to discoloration [69-70]. It reflects UV light more strongly than the natural bulk material of same composition thus, vastly applied in sunscreen and personal care products. In some products the amount of TiO_2NPs is even more than 10 % by weight [71-72]. Owing to the practical application of TiO_2NPs the study of interaction of BSA-TiO_2NPs is vital.

The binding abilities of TiO_2NPs and Ag doped TiO_2NPs with serum albumins were studied using optical techniques by Kathiravan et al. [73-74]. They inferred that both TiO_2 and Ag-TiO_2NPs quench the fluorescence without any shift in the emission maxima. Lifetime measurements confirm that both nanoparticles follow the static quenching mechanism. In the synchronous fluorescence spectra of serum albumins at $\Delta\lambda$ = 60 nm, there is no shift in the emission wavelength, which confirms the absence of binding site near the tryptophan residue. In the synchronous fluorescence spectra ($\Delta\lambda$ = 15 nm) of serum albumins, Ag-TiO_2 addition showed a red-shift, which suggests that the binding site is near the tyrosine region and the environment is more polar (or less hydrophobic) [75] and more exposed to the solvent molecules [76]. On the other hand, pure TiO_2NPs show a blue-shift, which suggests that the binding site is also near tyrosine region but it is in less polar (or more hydrophobic) environment and less exposed to the solvent molecules. Gao et al. [77] described interactions between TiO_2NPs and BSA. They showed the mechanism of fluorescence quenching was static quenching with non-radiative energy transfer. SDS-PAGE revealed that the structure of BSA was not obviously destroyed upon binding with TiO_2NPs in different systems. Hydrogen bond and van der Waals interaction were deduced, on the basis of the thermodynamic parameters, to be the major driving forces.

Cadmium sulphide is well studied materials [78-79] as their well-established relationship between the optical absorption and their size. Cadmium sulfide could be used as bioorganic detector of proteins [80] or DNA [81-82]. With a good surface modification we can obtain, with cadmium sulfide, core/shell NPs [83-84], which have enhanced luminescence properties. Cadmium sulphide is an important semiconductor and has many optoelectronic applications including solar cells, photodiodes, light emitting diodes, nonlinear optics and heterogeneous photo catalysis. The two ions Cd^{2+} and S^{2-} are component parts of quantum dots which are harmful to human body. Study of interaction between BSA and CdSNP is also very important owing to the applications of CdSNP in the field of biology and medicine.

Studies of interaction of CdSNP with BSA are investigated by Jhonsi et al. [85] and Ghali [86]. BSA molecules were adsorbed on the surface of colloidal CdS through the capping agent. The apparent association constant (K_{app} = 2.54×10^2 M^{-1}) and degree of association has been calculated (α = 1.12) from absorption studies. The binding constant from fluorescence quenching method (6.6×10^2 M^{-1}) matches well with that determined from the absorption spectral changes. Static quenching mechanism and conformational changes on BSA molecules were confirmed by time resolved and synchronous fluorescence measurements respectively. Starch-capped, thioglycerol-capped, or uncapped CdSNP interact with BSA by the formation of ground state complex. Starch-capped CdSNP quench the fluorescence of BSA with blue-shift in the emission maximum; whereas, thioglycerol capped CdSNP quench the fluorescence of BSA with red-shift.

Among the various types of NPs that have been developed, nanostructured metal oxides have recently aroused much interest in biomedical applications. ZnO, having wide band gap (3.37 eV), piezoelectric, and pyroelectric properties [87-88], has attracted much attention for potential range of applications in optics, optoelectronics, sensors, and actuators. ZnONPs have also good biocompatibility and chemical stability, with ability to be nontoxic in vitro and in vivo [89]. In addition, ZnO is biomimetic and exhibits high electron transfer property [90-91], a property for potential applications in biosensors. The antimicrobial potential of ZnO has also been well explored in the past [92]. Study of interaction between BSA and ZnONPs is also very important owing to the applications of ZnONPs in the various fields.

Recently Bhogale et al. [93] reported the interaction studies of ZnONP nanoparticles with BSA. The obtained results confirmed that the ZnONPs quench the fluorophore of BSA by forming ground state complex in the solution. The obtained binding constant was 1.51×10^{13} $LMol^{-1}$. They showed that BSA-ZnONPs binding takes place spontaneously involving hydrogen bond and van der Waals forces. Synchronous fluorescence spectra permitted us to establish that the microenvironment close to both the tyrosine and tryptophan residues of BSA is perturbed. In addition, the hydrophobicity of both residues increases in the presence of ZnONPs. Finally, from the resonance light

scattering and circular dichroism spectra we inferred on the formation of aggregates of BSA-ZnONPs to induce slight conformational modification in BSA.

Table1: Binding constant and thermodynamic parameters for BSA-nanoparticles interaction using fluorescence spectroscopy

Sl. No.	System	K (M^{-1})	ΔH ($kJmol^{-1}$)	ΔS ($Jmol^{-1}K^{-1}$)	ΔG($kJmol^{-1}$)
1.	BSA-SNP	1.71×10^{10} $LMol^{-1}$	37.71	396.8	-58.75
2.	BSA-SNP	16.20×10^{-5}	-4.59	-333	91.67
3.	BSA-SNP	10.23×10^{3} $LMol^{-1}$	-20.78	5.98	-22.54
4.	BSA-SNP	4.45×10^{3} $LMol^{-1}$	-	-	21.66
5.	BSA-Cu_2ONC	3.23×10^{4} M^{-1}	-63.39	-126.45	-25.72
6.	BSA-CdSNP	6.6×10^{2} Mol^{-1}	-	-	-
7.	BSA- GNPs	3.71×10^{4} $LMol^{-1}$	-	-	-
8.	BSA- GNPs	2.57×10^{9}	-	-	-
9.	BSA- GNPs	3.16×10^{11} Mol^{-1}	-	-	-
10.	BSA- GNPs	2.07×10^{14} Mol^{-1}	-	-	-
11.	BSA- GNPs	7.59×10^{8} $Lmol^{-1}$	-	-	-
12.	BSA- GNPs	1.12×10^{7} $Lmol^{-1}$	536.45	1950.25	-44.73
13.	BSA- GNPs	2.34×10^{11}	-	-	-
14.	BSA-ZnONP	1.51×10^{13} $LMol^{-1}$	-100.85	-242	-32.40
15.	BSA-CuNPs	5.01×10^{6} M^{-1}	62.3	344.5	-36.56
16.	BSA-TiO_2NPs	-	-105.5	-285.6	-20.37
17.	BSA-AgTiO_2NPs	3.71×10^{5} M^{-1}	-	-	-

Copper is an essential trace element for the proper functioning of organs and metabolic processes, and vital for all living organism including human beings. Various forms of copper have been used for medical purpose throughout the history of mankind [94]. Antioxidant and antimicrobial are the prime properties leading to pharmacological development of copper-based drugs to destabilize tumor and cancer cells [95-96]. Biocidal properties of copper such as antibacterial, antifungal, molluscicidal, nematocidal, antiviral, etc. have been known since ancient times [97]. Investigation of interaction between BSA and CuNPs is also very important owing to the applications of CdSNP in the various fields.

Recently Bhogale et al. [98] reported the interaction studies of CuNPs with BSA. Fluorescence quenching results suggest that CuNPs interact with BSA molecule through static mechanism, as inferred from the quenching of BSA fluorophore. The obtained binding constant was 5.01×10^{6} M^{-1}. BSA-CuNPs interaction occurs through spontaneous binding process involving hydrophobic forces and mainly entropy driven. The presence of a BSA–CuNPs ground state complex was confirmed by resonance light scattering, absorption of BSA, and fluorescence polarization spectra. In addition, by the analysis of the synchronous fluorescence spectra, we clarified that the interaction between CuNPs and BSA involve changes in the microenvironment of tryptophan rather than that of tyrosine residues. The α-helicity of the BSA decreases due to its interaction with CuNPs as indicated by circular dichroism and Raman spectra. An efficient energy transfer from excited state of BSA fluorophore to the CuNPs is favored by their close proximity (8.5 nm). Ju et al. [99] described interaction between cuprous oxide nano cube (Cu_2ONC) and BSA using spectroscopy. UV-Vis and circular dichroism results showed that the addition of Cu_2O changed the secondary structure of BSA and led to a decrease in α-helix. The nature of quenching was static quenching and the binding constant (K) was 3.23, 1.91, and 1.20×10^{4} M^{-1} at 298, 304, and 310 K, respectively, and the number of binding sites was 1.05. Hydrogen bonds and van der Waals forces played a major role in stabilizing the BSA–Cu_2ONC complex.

CONCLUSION

A critical need in the field of nanotechnology is the study of the in vitro binding interactions of various NPs with biomolecules such as bovine serum albumins. Fluorescence spectroscopy is a powerful tool to accomplish this need. In this review, the fluorescence quenching studies involving the interaction of metallic NPs with BSA are discussed. The results reviewed here represent the dependence of the size and stabilizing agent of metallic NPs on their interactions with BSA.

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