Research Article

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Investigation of bovine serum albumin aggregation upon exposure to silver(I) and copper(II) metal ions using Zetasizer

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Abstract: Depending upon the metal coordination capacity and the binding sites of proteins, interaction between metal and proteins leads to a number of changes in the protein molecule which may include the change in conformation, unfolding, overall charge, and aggregation in some cases. In this study, Cu(II) and Ag(I) metal ions were selected to investigate aggregation of bovine serum albumin (BSA) molecule upon interaction by measuring the size and charge of the aggregates using nano-Zetasizer instrument. Two concentrations of metal ions were made to interact with a specific concentration of BSA and the size and zeta potential of BSA aggregates were measured from 0 min upto 18 h. The Cu(II) and Ag(I) metal ions showed almost similar behavior in inducing the BSA aggregation and the intensity of peak corresponding to the normal-sized protein decreased with time, whereas the peak corresponding to the protein aggregate increased. However, the effect on zeta potential of the aggregates was observed to be different with both metal ions. The aggregation of protein due to interaction of different metal ions is important to study as it gives

insight to the pathogenesis of many neurological disorders and would result in developing effective ways to limit their exposure.

Keywords: copper, silver, metal ions, BSA, aggregation, Zetasizer

1 Introduction

Copper (Cu) and Silver (Ag) are closely resembled coinage metals grouped together in group 11 and are being used for centuries as antimicrobial agents in healthcare systems and agriculture. Various copper- and silver-based agents are utilized increasingly nowadays, leading to increased exposure of these metal ions and therefore their toxicity. The underlying mechanisms of toxicity of these agents include protein aggregation, which could ultimately affect various important biological processes. In bacterial cell, these ions are shown to bind to important molecules, leading to disruption of their function [1]. Exposure to these metal ions often occurs via skin contact and inside the human body; these metal ions bind to various macromolecules including proteins inducing structural changes and aggregation which may result in health hazards.

These metal ions upon reaching the systemic circulation interact with plasma proteins leading to aggregation of proteins and increase in the coefficient of friction, thereby influencing the biointerface reactions. Studying effects of these metal ions on the protein adsorption and aggregation is vital in order to understand the untoward reactions and to design safer compounds. The protein-metal conjugates may lead to biocorrosion and have toxic effects on biological systems including neurodegenerative diseases [2]. Previously, Co(II) and Cr(III) metal ions were shown to aggregate albumin protein in phosphate buffer saline, leading to increase in the coefficient of friction in artificial joint prosthesis [3]. Another study

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revealed formation of amyotrophic lateral sclerosis due to interaction of trace metal ions with the albumin protein forming protein aggregates [4].

Several methods are reported in literature for the measurement of protein aggregation by metal ions including light scattering techniques, electrophoretic mobility, fluorescence spectroscopy, ultrafiltration, atomic absorption spectroscopy circular dichroism [2], etc. These methods either include costly instruments or expertise to perform the experiments as these techniques are much sophisticated. This study was aimed to utilize the Zetasizer instrument to measure the size of protein molecule before and after interaction with different concentrations of metal ions at different time intervals. To the best of our knowledge, no such study was conducted previously to measure the protein aggregation using the Zetasizer instrument. As we know, the size of bovine serum albumin (BSA) molecule remains around 10–13 nm in the native form and increases in case of aggregation. As the aggregates are formed, several protein molecules attach together forming polymeric protein molecules. Measuring the size of protein molecule after interaction with metal ions would give insight of the extent and type of aggregation and the metal-concentration at which the protein aggregation occurs can be determined. This method would provide preliminary information of the concentration of metal ions causing protein aggregation and will be useful in limiting the usage of metal-based compounds avoiding toxicity.

2 Materials and methods

2.1 Chemicals and instruments

Bovine Serum Albumin (BSA, 99%), CuCl_2 , and AgNO_3 were purchased from Sigma Aldrich (Steinheim, Germany). The ultrapure bidistilled water was produced in our lab. Zetasizer Nano Range (Malvern Panalytical, Ltd, Malvern, UK) was used to measure the nanosize and zeta potential of samples.

2.2 Preparation of solutions

2.2.1 Preparation of tris buffers

Two sets of tris buffers (20 mM, pH 7.4) were prepared for the study. For each set, accurately weighed (2.42 g) tris base was dissolved in 200 mL of ultrapure deionized

double distilled water. For the preparation of tris HCl buffer, pH 7.4 was maintained using dilute hydrochloric acid and the volume was adjusted to 1,000 mL using ultrapure water. The other buffer, tris acetate, was prepared by adjusting the pH 7.4 using acetic acid and the final volume was maintained to 1,000 mL using ultrapure double distilled water. Tris HCl buffer was utilized to make solutions of Cu(II) metal ions, whereas the tris acetate buffer was used for Ag(I) metal as the later is precipitated with the Cl^- ions of HCl [5].

2.2.2 Preparation of BSA solution

The protein (BSA) solution (10 μM) was prepared by accurately weighing 33 mg of BSA powder in a 50 mL volumetric flask and making up the volume using the prepared tris HCl buffer (pH 7.4) for studying Cu(II) ions and in tris acetate buffer (pH 7.4) for Ag(I) ions.

2.2.3 Preparation of metal solutions

Each metal salt was dissolved in the corresponding tris buffers to give stock solution of 1 mM which were diluted appropriately to obtain working solutions of concentrations 40 and 160 μM .

2.3 BSA-metal ion interaction

The BSA protein and each metal ion solution were added in equal proportions to obtain 1:4 and 1:16 concentrations, which were mixed properly and transferred to Zetasizer cells. The nanosize and zeta potential of the mixture were measured at 0 min immediately after mixing and the cells were incubated at 37°C with slow shaking and readings at different time intervals of 30, 60, 120, 180, 240, 300, 1,020, and 1,080 min were taken.

2.4 Measurement of nanosize and zeta potential using Zetasizer

The cells were placed in the Zetasizer instrument and the peaks of different molecular size (d.nm) with their relative intensities were obtained using the Malvern Zetasizer software version 7.01. Subsequently, zeta potential (mV) of all the samples were also measured using the software at different time intervals.

Ethical approval: The conducted research does not include the use of human or animals.

3 Results and discussion

3.1 Interaction of BSA with Cu(II) ion

Two concentrations of Cu(II) ion solutions, 40 and 160 μM , were mixed with 10 μM BSA solution in tris HCl buffer to give a concentration ratio of 1:4 and 1:16, respectively, which were incubated at 37°C and the size and zeta potential of samples were measured at 0, 30, 60, 120, 180, 240, 300, 1,020, and 1,080 min time interval. The BSA blank solution was also analyzed to check the size and zeta potential of native BSA protein in the corresponding buffer. Table 1 summarizes the size (nm), peak intensity (%), and zeta potentials (mV) measured for all samples at different time points.

The size of BSA protein at 10 μM concentration was measured in the tris HCl buffer and was found to be of 13.18 nm (87.5%) with a zeta potential of -15.5 mV, which showed that the BSA molecules were present in the normal size corresponding to the native protein (peak 1) and were segregated in the solution. Few agglomerates of BSA protein were also present which was evident by another peak (peak 2) corresponding to the size 348.3 nm of intensity 9.7%. When the prepared BSA solution was mixed with Cu(II) solution in a ratio of 1:4, the change in the intensities of both peaks was observed immediately after mixing the solutions. The intensity of peak 1 corresponding to the normal-sized protein decreased markedly from 87.5 to 62.7% and the peak 2 corresponding to the

protein aggregate showed an increase in intensity from 9.7 to 25.5%.

The zeta potential was also observed to be increased slightly at 0 min from -15.5 to -17.3 mV (more negative). Interestingly, this aggregation kept on increasing more or less with time as the intensity of peak 1 decreased and that of peak 2 increased (Figure 1). There was a sharp increase in the intensity of peak 2, and therefore, a decrease in peak 1 after 60 min, showing fast aggregation at this time. The increase in aggregation which was evident from the increase in the intensity of peak 2 and decrease in the intensity of peak 1 kept on increasing with time and became almost constant after 17 h. This showed that the time of exposure to the metal ions had marked effect on the aggregation of proteins and the accumulation of metal ions in the body for longer time would have more deleterious effects. The zeta potential of the BSA protein also changed with time as the charge became more negative after interaction with Cu(II) ions, showing that the Cu(II) ions after interacting with the amino acids present in BSA interacted with the surrounding anions increasing the negative charge of the protein. The negative charge kept on increasing as more and more Cl^- ions coordinated with the Cu(II) metal ions and became almost constant after some time, showing saturation of the surface.

Similarly, the interaction of BSA with higher concentration of Cu(II) was also studied to see the effects of concentration of metal ions on the aggregation of protein.

Table 1: Interaction of BSA with different concentrations of Cu(II)

S. no.	Sample	Conc./ratio	Time (min)	Peak 1 size (nm)	Peak 1 intensity (%)	Peak 2 size (nm)	Peak 2 intensity (%)	Zeta potential (mV)
1	BSA (Blank)	10 μM	0	13.18	87.5	348.3	9.7	-15.5
2	BSA + Cu(II)	1:4	0	12.88	62.7	367.2	25.5	-17.3
			30	12.89	72.2	185.2	28.3	-17.6
			60	11.94	55.9	219.1	40.6	-16.4
			120	11.66	30.3	281.8	64.7	-19.1
			180	8.26	8.8	292.4	78.5	-22.6
			240	9.92	8.4	260.7	81.4	-24.9
			300	10.22	7.8	187.8	83.0	-23.6
			1,020	9.61	5.0	192.7	91.5	-23.9
			1,080	15.01	4.8	210.7	92.0	-23.2
3	BSA + Cu(II)	1:16	0	11.70	93.6	243.0	6.4	-10.7
			30	10.14	61.4	141.5	34.6	-18.2
			60	10.05	55.9	145.4	38.8	-20.7
			120	8.84	62.1	127.4	40.8	-18.9
			180	9.86	53.2	158.0	42.1	-18.6
			240	10.23	52.2	142.0	44.4	-20.8
			300	10.74	49	144.6	47.0	-21.3
			1,020	9.88	32.4	257.0	51.3	-20.7
			1,080	9.96	32.8	173.8	64.5	-20.5

Figure 1: Graph showing the interaction of BSA protein with Cu(II) metal ion at 1:4 concentration ratio. An increase in the intensity of peak 2 corresponding to the BSA protein aggregate and decrease in the intensity of peak 1 corresponding to normal-sized native protein from 0 to 1,080 min (18 h) were observed.

Figure 2: Graph showing the interaction of BSA protein with Cu(II) metal ion at 1:16 ratio. An increase in the intensity of peak 2 corresponding to the BSA protein aggregate and decrease in the intensity of peak 1 corresponding to normal-sized native protein from 0 to 1,080 min (18 h) were observed.

BSA solution was mixed with 16 folds higher Cu(II) solution in a ratio of 1:16 and the size and zeta potentials of BSA molecules were measured. When the Cu(II) solution at 160 μM concentration was added to the BSA solution, immediately after the addition (at 0 min), interestingly an increase in the intensity of peak 1 and decrease in the intensity of peak 2 were observed (Figure 2). There was also a marked decrease (from -15.5 to -10.7 mV) in the zeta potential. This was attributed to the disaggregation of agglomerated protein molecules upon addition of higher concentration of Cu(II) metal ions. This decrease in the zeta potential then led to the re-attraction of BSA molecules and formation of aggregates. This was evident from the data obtained for the sample measured at 30 min showing decrease in the intensity of peak 1 because of the decrease in the concentration of normal-sized protein and increase in the concentration of aggregates. The zeta potential also increased to -18.2 mV, showing complexation of Cu(II) ions with the BSA protein and further coordination with surrounding anions. This behavior was consistent thereafter with increasing time as the intensity of peak corresponding to native size of protein kept on decreasing consistently with an increase in the intensity of peak 2, showing time-dependent increase in aggregation at this concentration.

To study the effect of metal-complexation on the zeta potential of the BSA protein with time, the overlay graph of native-sized BSA protein together with the Cu(II)-BSA complex at 30 and 180 min was studied (Figure 3) at 1:16 concentration. As evident from the figure, the zeta potential

curve was shifted to the left hand side (becoming more negative) upon interaction with Cu(II), which further shifted to the more negative side with time. Interestingly, the intensity or the total counts of peak increased upon complexation as well as with time, showing increased concentration of charged particles with time. Cu(II), being a divalent metal ion, has more capacity to coordinate with the negatively charged BSA molecules forming more and more aggregates with time. It also has the capacity to bind with the surrounding anions present in the solution which further increased the negative charge.

As far as the effect of concentration on the size and availability of protein aggregate is concerned, an interesting behavior was observed when the concentration of Cu(II) metal ion was increased to 1:16. The size of aggregate in case of 1:4 as evident from the size distribution of peak 2 was found to be in the range of 185.2–367.2 nm, whereas the same was 127.4–257.0 nm, in case of 1:16 concentration ratio (Figure 4). It showed that the size of aggregate was comparatively smaller in case of higher concentration ratio. Similarly, the availability of aggregate which was evident from the intensity of peak 2 was found to be lesser in case of higher concentration as it increased to 64.5% at 18 h, whereas the intensity of peak 2 at 18 h in case of 1:4 ratio was 92% (Figure 5). It showed the presence of higher concentration of aggregates with faster aggregation at lower concentration of metal ions than the higher one. The concentration of normal-sized protein in case of 1:4 concentration was observed to be 4.8% at the end of 18 h, whereas 32.8% normal-sized

Figure 3: Overlay graph of zeta potentials obtained for blank BSA and Cu(II)-BSA complex at 30 and 180 min. The peaks can be seen shifted to the more negative side with an increase in intensity with time.

Figure 4: Comparison of peak 2 (aggregate) size at different Cu(II) metal ion concentrations. Increased aggregate size was observed at lower metal ion concentration (1:4) as opposed to the higher one (1:16).

Figure 5: Comparison of peak 2 (aggregate) intensity at different Cu(II) metal ion concentrations. The peak 2 intensity, thereby the availability of aggregate, was found to be higher along with faster aggregation in case of lower metal ion concentration (1:4) as compared to the higher one (1:16).

protein was still available in case of 1:16 concentration at this time. The higher metal ion concentration saturated the binding sites of the BSA protein and the exposed amino acids coordinated with the excess metal ions keeping the exterior surface of protein hydrophilic, slowing down the formation of protein aggregate and aggregates of smaller size were formed.

3.2 Interaction of BSA with Ag(I) ion

Similarly, the effects of Ag(I) metal ions on the aggregation of BSA protein were also studied at different concentrations

at various time points. Ag(I) ion solutions were prepared at concentrations of 40 and 160 μM and were mixed with 10 μM BSA solution to achieve the concentration ratio of 1:4 and 1:16, respectively, which were incubated at 37°C and the size and zeta potential of samples were measured at 0, 30, 60, 120, 180, 240, 300, 1,020, and 1,080 min time interval. The average molecular size and zeta potentials measured for all BSA-Ag(I) samples are summarized in Table 2.

The BSA blank sample prepared in tris acetate buffer was also analyzed to measure the size and zeta potential.

Interestingly, the size of BSA protein in tris acetate buffer was larger than that present in the tris HCl buffer and it was observed to be 38.19 nm with 100% intensity showing only single size distribution in the solution. The zeta potential of the sample was measured to be -26.6 mV, which was again higher than that obtained in case of tris HCl buffer showing greater repulsive forces between BSA molecules and that is why no agglomerates were obtained as opposed to the tris HCl buffer. Initially, BSA solution was mixed with Ag(I) metal ion solution in 1:4 ratio and the average size and zeta potential were measured at different time intervals. The aggregation observed due to Ag(I) ions was observed to be more or less similar to the Cu(II) ions at 1:4 concentration and the formation of aggregates was observed immediately after mixing the two solutions at 0 min. Appearance of peak 2 of size 205.5 nm with 57.9% intensity was observed showing sudden aggregation of BSA molecules (Figure 6).

Importantly, the zeta potential in case of Ag(I) did not change considerably, showing that the Ag(I) ions did not coordinate with the surrounding ions and the total charge of the protein molecule remained unchanged. The peak 1 after addition of the metal ion solution changed considerably as the size also reduced to 11.01 nm and the intensity reduced greatly to 18.1%. This reduction in the intensity of peak 1 was consistent with time as it kept on decreasing and became 7.2% after 18 h of interaction. Similarly, the intensity of peak 2 corresponding to the

aggregate increased with time and was observed to be 91.8% at the end of 18 h, showing time-dependent aggregation of BSA molecules in presence of Ag(I) metal ions (Figure 6). The zeta potential again did not change much and remained around -28 mV at all times.

When compared with the Cu(II) metal ions at this concentration, the aggregation in case of Ag(I) was observed to be faster owing to the appearance (from 0%) of peak 2 of high intensity (57.9%) immediately after the addition of metal ion solution. In contrary, Cu(II) ion showed lesser increase in the peak 2 intensity from 9.7 to 25.5% immediately after the addition of metal ions.

Similar behavior was observed when the protein and Ag(I) metal ion solutions were mixed in a ratio of 1:16. It showed appearance of peak 2 immediately after the addition of metal ion solution at 0 min, although the intensity of peak 2 was lesser than that obtained in case of 1:4. A distinct peak 2 of size 232.6 nm corresponding to the BSA aggregate was observed with an intensity of 31.7%. When the two solutions were incubated for longer time, the intensity of peak 1 decreased consistently with time showing the decrease in the concentration of normal-sized protein with an increase in the intensity of peak 2 showing increased concentration of aggregates (Figure 7). The zeta potential values in case of higher concentration at different time points were observed to be decreased from the zeta potential of blank BSA protein, which showed the increased attraction between BSA molecules upon interaction with the Ag(I)

Table 2: Interaction of BSA with different concentrations of Ag(I)

S. no.	Sample	Conc./ratio	Time (min)	Peak 1 size (nm)	Peak 1 intensity (%)	Peak 2 size (nm)	Peak 2 intensity (%)	Zeta potential (mV)
1	BSA (Blank)	10 μ M	0	38.19	100.0	—	—	-26.6
2	BSA + Ag(I)	1:4	0	11.01	18.1	205.5	57.9	-25.1
			30	10.17	19.6	179.0	77.8	-28.4
			60	11.52	21.3	180.5	76.1	-28.5
			120	10.22	16.5	186.2	78.6	-28.2
			180	9.63	15.8	190.3	80.6	-28.3
			240	9.95	11.0	159.0	83.7	-28.2
			300	8.39	8.7	224.1	84.6	-28.8
			1,020	9.87	8.1	213.8	86.5	-25.3
			1,080	9.09	7.2	204.2	91.8	-28.1
3	BSA + Ag(I)	1:16	0	11.3	68.3	232.6	31.7	-23.3
			30	9.85	61.0	262.9	32.0	-20.9
			60	10.22	60.8	224.3	33.33	-19.9
			120	9.89	58.4	122.5	35.2	-20.4
			180	10.22	54.7	203.3	37.5	-15.7
			240	10.05	52.5	213.5	38.5	-17.7
			300	10.19	50.9	202.1	40.0	-19.9
			1,020	10.87	37.8	273.5	57.2	-18.4
			1,080	9.73	36.0	250.8	57.8	-20.0

Figure 6: Graph showing the interaction of BSA protein with Ag(I) metal ion at 1:4 ratio. An increase in the intensity of peak 2 corresponding to the BSA protein aggregate and decrease in the intensity of peak 1 corresponding to normal-sized native protein from 0 to 1,080 min (18 h) were observed.

metal ions. This could be the reason for increased aggregate size at higher metal ion concentration. Also, Ag(I) is known to be reduced to nanoparticles by BSA protein which acts as a reducing agent. These nanoparticles have reduced zeta potential which, when combined with BSA protein, reduces zeta potential of the protein [6–8].

The effect of complexation with the Ag(I) metal ion on the zeta potential of BSA protein with time was also studied using the overlay graph of blank BSA with Ag(I) + BSA at 30 and 180 min (Figure 8). Interestingly, opposite effect of what was seen in case of Cu(II) was observed in case of Ag(I) ions as the zeta potential of BSA molecule tends to decrease or become less negative upon complexation with Ag(I) ions which further decreased and shifted to the right hand side or less negative (towards zero) side with time. Also, the intensity or the total counts of the corresponding peaks decreased in case of complex and further decreased with increase in time. It showed that the concentration of charged particles decreased with time. This further supported the reason that more and more Ag(I) ions got reduced by BSA with time and the Ag(I) complexed with BSA could not coordinate with other anions and the concentration of charged particles decreased with time.

To study the effect of concentration on the peak size and intensity of BSA aggregates, the data obtained for the concentration 1:4 and 1:16 were compared and a slightly different behavior was observed as compared to the Cu(II) metal ions. The size distribution of peak 2 which was corresponding to the BSA aggregates was

Figure 7: Graph showing the interaction of BSA protein with Ag(I) metal ion at 1:16 ratio. An increase in the intensity of peak 2 corresponding to the BSA protein aggregate and decrease in the intensity of peak 1 corresponding to normal-sized native protein from 0 to 1,080 min (18 h) were observed.

measured to be in the range of 159.0–224.1 nm in case of 1:4 concentration, whereas it was in the range 122.5–273.5 nm for 1:16 concentration. At most of the time points (except two), the size of peak 2 was greater for higher concentration than that of the lower one in contrast to the behavior seen in Cu(II) ions (Figure 9). This showed that increasing the concentration of Ag(I) metal ions led to an increase in the size of the aggregates. However, the effect of concentration on the intensity of peak 2 was similar to that observed for Cu(II) ions as increasing the concentration decreased the rate of aggregation and reduced intensities were observed at all time points (Figure 10). The highest intensity observed at 18 h in case of 1:4 concentration was 91.8%, whereas it was merely 57.8% for higher concentration at the same time point. There was 7.2% normal-sized native protein remaining at the end of 18 h for concentration ratio 1:4 as compared to 36% in case of 1:16 showing greater aggregation at lower concentration with time.

A number of methods have been developed to analyze the interactions of metal ions/metallodrugs with serum albumin protein including fluorescence and 3D fluorescence spectroscopy, UV-visible spectroscopy, Fourier transform infrared spectroscopy (FT-IR), matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS), and quantum dot-assisted laser desorption/ionization-mass spectrometry (QALDI-MS) [9]. Previously, our research group has also been involved in investigating the binding interaction of various metal ions and drugs with serum proteins such as human serum albumin (HSA) and BSA using cost-effective

Figure 8: Overlay graph of zeta potentials obtained for blank BSA and Ag(I)-BSA complex at 30 and 180 min. The peaks can be seen shifted to the less negative side with a decrease in intensity with time.

and efficient techniques [5,10–13]. These techniques revealed that the exogenous and endogenous ligands including drugs/metallodrugs generally bind to the binding sites present in the subdomains IA and IIA of the albumin protein which are also known as Sudlow's site I and II, respectively. Metal ions are known to covalently bind to the macromolecules including proteins, DNA, and enzymes present in our body, which might have inhibitory effects on their functioning leading to apoptosis, necrosis, and cell death. Interaction of metal ions with serum proteins leads to the disruption of disulfide bonds present in the protein leading to change in its secondary structure. The

α -helix component of the secondary structure of protein is partially lost upon interaction with metal ions, leading to partial unfolding of the protein [14].

The polarity around the tryptophan residue present at the binding site of albumin protein changes due to these interactions [15,16] as the distance between the amino acid residues changes. Various types of interactions between metal ions and the serum protein include rearrangements, energy transfer, and collision quenching processes [14–16]. Previously, several divalent metal ions complexes were prepared using Cu(II), Zn(II), Ni(II), Co(II), and Pt(II) metal ions and their interaction with BSA

Figure 9: Comparison of peak 2 (aggregate) size at different Ag(I) metal ion concentrations. Increased aggregate size was observed at lower metal ion concentration (1:4) as compared to the higher one (1:16).

Figure 10: Comparison of peak 2 (aggregate) intensity at different Ag(I) metal ion concentrations. The peak 2 intensity, thereby the availability of aggregate, was found to be higher along with faster aggregation in case of lower metal ion concentration (1:4) as compared to the higher one (1:16).

protein was studied [17–19]. It was found that the specificity of binding interactions between the BSA protein and the metal ions as ligands was affected by the planarity of ligands. It was observed that these metal ions bind specifically to the Trp134 residue present on the surface of the protein being the most accessible residue for the metal ions. Metal ions bind preferentially to the sub-domain IIA present in the Sudlow's site I of the BSA protein [20].

Studying the aggregation of protein due to interaction with different metal ions is important to determine as several evidences are there which indicate their role in the pathogenesis of a number of diseases. For instance, the formation of fibrils by β -amyloid peptide, which is the major component of plaque in brain, is accelerated by these metal ions owing to the formation of oligomeric aggregates of these proteins increasing its toxicity [21,22]. Similarly, neurodementia disease is characterized by the abnormal deposition of protein aggregates in brain due to the exposure of metal ions leading to the neurological disorder [23,24]. These metal ions, therefore, have important consequences on the aggregation and/or unfolding of the protein and many studies are being devoted to determine the effects of these metal ions on peptide and protein aggregation and elucidating the mechanisms involved [25–28]. The results obtained indicated that the aggregation and unfolding process largely depends on the types and nature of proteins and metal ions and their respective concentrations.

Metal ions can act as promoters or inhibitors of the protein aggregation process depending upon the protein/metal ion ratio and the binding mechanism of metal ions [29–32]. Moreover, the presence of metal ions can significantly affect the conformation of proteins and therefore the aggregation which may ultimately lead to the formation of protein gels [33–35]. Importantly, the protein aggregation process is supposed to be lesser in case of monovalent ions than the divalent metal ions as they can form bridges and provide electrostatic attractions between the negatively charged species of surrounding protein molecules [36,37].

The repulsive forces between the charged protein molecules are decreased or lost which results in the proximity of protein molecules and the formation of non-covalent interactions of low energy [33]. The metal ions and proteins, however, interact with specific bonding forces and the binding sites present on the protein molecule are characterized previously [38–40]. The binding mode of metal ions is an important consideration in studying the formation of metal-protein complex as a metal ion can bind to the protein in two specific ways, intermolecular

and intramolecular. In case of intramolecular binding, the geometry around the metal ions is in such a way that the atoms which participate in the coordination with metal ions are all present in the same protein molecule. Therefore, the intermolecular interactions are not favored; however, the coordination with metal ions can change the secondary structure of the protein. The amino acid residues present in the protein molecule including Tyrosine (Tyr) and Tryptophan (Trp) also show a change in the microenvironments of their side chains due to these interactions. On the other hand, intermolecular binding involves the formation of intermolecular bridges between the metal ion and protein molecules and the aggregation process depends upon the coordination capacity of the metal ions.

4 Conclusion

The Zetasizer instrument successfully measured the size and zeta potential of the BSA protein molecules before and after interaction with different concentrations of metal ions. The Zetasizer instrument has many advantages over other techniques as it is a very simple and cost-effective technique which does not require sample preparation steps and expertise to perform the experiment. The instrument was sensitive in detecting the size and zeta potential of the protein molecules and gave a preliminary idea about the metal-concentration and time at which the protein aggregation could take place in biological systems. Both Cu(II) and Ag(I) metal ions led to the aggregation of BSA protein at different concentrations at different time points which were evident by the increase in the size of protein. Metal ions at lower concentrations exposed for longer time were observed to increase the aggregation as compared to the metal ions at higher concentration. As protein aggregation is known to be the major reason for a number of neurological disorders, measuring the concentration of metal ions which could cause the protein aggregation would help us in getting limited exposure to these metal ions.

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