Bovine Serum Albumin (BSA) conjugated gold nanoparticles (Au NPs) have been directly synthesized by using BSA as a weak reducing as well as capping/stabilizing agent with HAuCl$_4$ in aqueous phase at 40, 60 and 80 °C. The physical state of BSA that is folded or unfolded, plays a governing role in configuring the overall shape and size of Au NPs. The unfolded gel form of BSA is the most ideal candidate to study the protein film formation. It has been observed that an appropriate balance between the temperature and concentration parameters of a biomineralization process is required to achieve an appropriate protein film formation. The soft film is used with water insoluble zein protein to produce much robust biodegradable protein films suitable for various food and pharmaceutical applications.

4.1 Results

4.1.1 UV-Visible studies

4.1.1.1 Temperature effect

Each reaction (as mentioned in the experimental section) is monitored simultaneously by UV-Visible measurements over a period of six hours. Figure 4.1a,b shows typical scans of a reaction with Au/BSA mole ratio = 167 at 40 and 80 °C, respectively. Peak close to 220 nm belongs to AuCl$_4^-$ ions while that at 295 nm is due to aromatic tryptophan residues of BSA. Colloidal Au NPs usually show absorbance due to SPR around 520 nm. At 40 °C (Figure 4.1a), peaks at 220 nm and 295 nm are quite prominent and their intensity decreases with time whereas no peak is visible around 520 nm. Normalized intensity plots (Figure 4.1a, inset) indicate that the absorbance due to tryptophan diminishes within 90 minutes of the reaction whereas that for AuCl$_4^-$ ions takes about 250 minutes. The AuCl$_4^-$ ions peak takes more than double the time to disappear and demonstrates the weak reducing behaviour of BSA. Same reaction at 80 °C (Figure 4.1b) produces a prominent peak at 550 nm due to Au NPs whose intensity increases with time. Note a much broader tryptophan peak at 295 nm, which vanishes as the peak at 550 nm achieves maximum intensity and reaction equilibrates. The disappearance of tryptophan peak can be related to the simultaneous adsorption of BSA on the freshly produced NPs. As more and more NPs are produced by simultaneous conversion of AuCl$_4^-$ ions into the nucleating centres, more BSA is associated with the NP surface. Such an adsorption leads to a partial unfolding of BSA due to the breaking of disulfide bridges in view of the covalent interactions of
Figure 4.1 (a) UV-Visible scans of a reaction with \([\text{Au/BSA}] = 167\) at 40 °C at different time intervals over a time span of six hours. Prominent peaks at 220 nm and 295 nm belong to \(\text{AuCl}_4^-\) ions and BSA, respectively. The intensity of both peaks decreases from top to bottom with time. Relative to these peaks, the absorbance due to SPR of Au NPs close to 520 nm is insignificant. Inset shows plots of normalized intensity versus time for the three peaks (see details in text). (b) The same reaction carried out at 80 °C now shows prominent peaks at 550 nm due to SPR of Au NPs.

cysteine residues with NP surface\(^{63}\) and causes conformational changes.\(^{40,159,179}\) Tryptophan has been found to be deep embedded in the hydrophobic environment thus produced with the result of which its absorbance decreases.\(^{63}\) The progress of a reaction can be studied by plotting the intensity of 550 nm peak versus reaction time (Figure 4.2) because it can be related to the number density of Au NPs.\(^{162,180}\) Figure 4.2 indicates that a negligible amount of NPs is produced at 40 °C, but the amount increases at 60 °C, and becomes maximum at 80 °C. At 80 °C, as soon as the reaction is placed in the thermostat bath, the intensity of this peak increases exponentially and reaction equilibrates within an hour. Obviously, low temperature of the reaction (“i.e.” 40 °C) provides much weaker reducing ability of BSA that increases as the temperature increases to 60 °C and becomes maximum at 80 °C. At 80 °C, it is further accelerated due to the unfolding of BSA which provides even better reducing ability due to a breakdown of maximum number of disulfide bridges. Thus, two factors that is temperature and unfolding of BSA, simultaneously control the reduction of Au (III)
into Au (0) and can be best studied by monitoring the reaction with respect to temperature.

![Graph showing intensity at 550 nm versus time at different temperatures](image)

**Figure 4.2** Plots of intensity (at 550 nm) versus time for the reactions carried out at 40, 60, and 80 °C, demonstrating the effect of temperature on the synthesis of Au NPs.

### 4.1.1.2 Denaturation of BSA

A systematic change in the reaction temperature from 20 to 70 °C not only indicates the formation of NPs but also simultaneously demonstrates the denaturation of BSA (see UV-Visible scans with temperature in Figure 4.3a). A variation in the intensity of Au NPs peak at 550 nm for the reactions at different Au/BSA mole ratios is illustrated in Figure 4.3b. It is quite similar in all cases but much different from that of Figure 4.2. Note that Figure 4.2 represents the intensity variation with time at a constant temperature where BSA exists in a particular physical state. But this is not the case with Figure 4.3b, where reaction temperature increases successively from 20 to 70 °C which causes a complete change in the BSA structure from native to unfolded state. Since BSA is a reducing agent and its physical state significantly influences the reduction process, therefore the variation in 550 nm peak intensity should reflect the BSA denaturation behaviour. Thus, Figure 4.3b can be explained in terms of a phase diagram with distinct regions. Region I refers to a temperature...
Figure 4.3 (a) UV-Visible scans of a reaction with Au/BSA mole ratio = 68 from 20 to 70 °C. (b) Reactions of different mole ratios carried out with temperature from 20 to 70 °C showing the denaturation process of BSA.

variation from 20 - 42 °C with a little change in the intensity because no significant amount of NPs is produced in this region as observed in Figure 4.2 (at 40 °C) though intensity values for mole ratio 68 are slightly higher than those of the lower mole ratios due to a greater amount of Au salt. This region represents predominantly the native state of BSA where BSA exists in the globular form with most of its disulfide bridges intact. It is to be noted that BSA consists of 17 disulfide bridges, therefore breaking of a few disulfide bridges due to its association with few small Au NPs will not significantly influence the tertiary structure of BSA and hence the 550 nm peak intensity does not show any marked change within this region.

In region II from 42 - 52 °C, marked increase in the intensity is observed for the mole ratio of 68 again because of the greater amount of Au, but the variation is not so significant for the lower mole ratios except a slight discontinuity around 42 °C suggesting some visible conformational changes have taken place in the BSA tertiary structure. Region II represents the temperature range where reversible conformational changes are taking place in the BSA structure but a dramatic increase in the intensity especially with mole ratio 68 suggests much more than this. In fact, no reversible changes are expected in the presence of Au NPs because the partially unfolded...
domains will speed up the rate of the reduction process which will in turn produce greater number of nucleating centres. This will allow greater amount of BSA to be adsorbed on the NPs surface leading to a partial breakdown of the disulfide bridges. Freshly exposed cysteine residues further participate in the reaction and the reaction turns into an autocatalytic process. This effect will be minimum with lower mole ratios. Further increase in the temperature under such circumstances leads us to region III from 52 - 58 °C, where most dramatic and identical changes are taking place for all the mole ratios. This temperature range brings irreversible changes to BSA tertiary structure by breaking all disulfide bridges and unfolding of $\alpha$-helices. The reduction process thus passes through a maximum at 55 °C when all cysteine residues fully participate in the reaction. Thereafter, system relaxes at 58 °C and progresses towards $\beta$-aggregation and gel formation in region IV. Interestingly, region IV indicates that intensity continues to rise for the mole ratio 68 whereas it almost vanishes for 34 and 17. It means that the gel phase thus created at lower mole ratios, where the amount of BSA is sufficiently higher than that of Au salt, encloses all NPs in such a way that their SPR completely screens. Concentration effect will make this point more clear.

4.1.1.3 BSA Concentration effect

Increase in the amount of BSA at fixed HAuCl$_4$ (Figure 4.4a) especially at 80 °C (as shown in region IV of Figure 4.3b) reduces the absorbance of Au NPs due to entrapment of NPs by the gel phase. The origin of this behaviour can be explained on the basis of simultaneous measurement of reaction pH (Figure 4.4c,d). pH remains fairly constant and acidic after a slight initial fall within 15 minutes in each reaction. Acidic pH is generated by the dissociation of HAuCl$_4$ to release H$^+$ ions, which in

\[
\text{HOOC} \equiv \text{NH}_3^+ + \text{HAuCl}_4
\]

\[
\text{HOOC} \equiv \text{NH}_3^+ \text{AuCl}_4^- + \text{H}^+
\] (4.1)
turn allows electrostatic interactions between R-NH$_3^+$ groups of BSA and AuCl$_4^-$ ions (equation 4.1). Figure 4.4b demonstrates the variation in pH with Au/BSA mole ratio at 40 and 80 °C. In both cases, pH decreases with a steep slope up to Au/BSA = 350 and then tends to level off. The pH values at 80 °C are lower than at 40 °C. It means that greater dissociation of HAuCl$_4$ takes place at 80 °C and is obviously understood from the greater unfolding of BSA at 80 °C rather than at 40 °C because more aqueous exposed amino acids will attract more AuCl$_4^-$ ions. Thus, high temperature, high BSA contents, and low pH facilitate the reduction and simultaneous entrapment of Au NPs in unfolded BSA. That is why practically no SPR is observed for Au/BSA = 17 and

**Figure 4.4** (a) Plots of intensity (at 550 nm) versus time for the reactions carried out at 80 °C indicating the effect of BSA concentration on the synthesis of Au NPs. (b) A variation in the reaction pH at different mole ratios. (c) A variation in the reaction pH with time for different Au/BSA mole ratios at 80 °C. (d) Similar plot at 40°C.
34 in comparison to 68 especially in region IV of Figure 4.3b, and is further explained by the following mechanism.

A low pH than isoelectric point, pH = 4.7, is primarily responsible for the denaturation of BSA$^{182,183}$ and is expected to enhance its reducing ability with more pronounced effect at 80 °C rather than at 40 °C. This is all related to the fact that albumins consist of a high content of cysteine and the charged amino acids with 17 disulfide bridges. The disulfide bridges are located almost exclusively between helical segments. None of the disulphide bonds is accessible to solvent in the pH range 5-7, but becomes progressively available as pH falls below the isoelectric point. As unfolding progresses due to low pH or high temperature as of 80 °C, more reducing amino acids like cysteine expose to aqueous phase and hence participate in the reduction of $\text{AuCl}_4^-$ ions in much greater extent. The denatured BSA thus produced, instantaneously adsorbs on the NP surface$^{42,184,185}$ due to covalent interactions of cysteine residues with Au surface.$^{63}$ Such assemblies then act as seeds to further interact with BSA conjugated NPs or free BSA either in the unfolded or partial folded states$^{186,187}$ leading to the formation of large aggregated assemblies. Under such

![Figure 4.5](image)

**Figure 4.5** Plots of the amount of BSA associated with NPs (BSAc) for the reactions carried out at different temperatures based on Bradford method.
circumstances, the reaction temperature decides if the conjugated BSA should exit in form of a sol or gel state.\textsuperscript{188,189} If the reaction temperature lies in region I of Figure 4.3b then obviously BSA conjugated NPs will exist in the form of sol, and if it is in region IV, it will exist in the form of gel. Thus, Au NPs present in sol show more SPR than those embedded deep in the gel phase. Furthermore, protein analysis based on the Bradford method allows us to quantitatively evaluate the amount of BSA associated with NPs (BSAc) (Figure 4.5). It is lowest at 40 °C where BSA is considered to be mainly in its folded state, whereas maximum at 80 °C where BSA acquires a complete unfolded form which shows that the latter form has maximum affinity for Au NPs. The gel phase thus created also acts as a soft-template\textsuperscript{35-37} and provides a kind of semi-solid support for the growing nucleating centres. It separates out from the aqueous phase in the form of small black flakes upon aging. Figure 4.6 shows a picture of such fragments deposited on a glass cover slip with a marked difference from a drop cast of a sol.

\textbf{Figure 4.6} A picture of spin coated Au NPs and black flakes deposited on a glass cover slip
The above results not only indicate the facilitation of the reduction process at 80 °C and at high BSA contents, the latter conditions are best suited for a soft protein film formation with entrapped Au NPs. Kinetic parameters evaluated from the similar plots following BSA absorption at 295 nm of Figure 4.1a indicate that reduction process primarily depends on the amount of BSA and facilitates at 80 °C (rate constant = $1.03 \times 10^{-5}$ s$^{-1}$) rather at 40 °C (rate constant = $6.18 \times 10^{-5}$ s$^{-1}$). With respect to the amount of BSA, the rate constant (“i.e.” 4.82, 4.41, and $4.31 \times 10^{-5}$ s$^{-1}$) at Au/BSA = 17, 34, and 167, respectively, decreases with the decrease in the amount of BSA. It means that the greater amount of BSA in its unfolded state is required for the facilitation of the reduction process which allows maximum number of reducing amino acids to come in contact with AuCl$_4^-$ ions to initiate the reduction. Hence, unless BSA is not in its unfolded state, appropriate reduction cannot be achieved even if we have greater amount of AuCl$_4^-$ in the solution.

4.1.2 Microscopic studies

4.1.2.1 BSA concentration effect

SEM and TEM studies not only help us to evaluate the shape and size of Au NPs but also provide direct evidence of the presence of NPs in the gel phase. Figure 4.7a,b shows SEM and TEM micrographs, respectively, of denatured BSA bearing Au NPs of 7.5±3.5 nm of a sample prepared with Au/BSA mole ratio of 167 at 80 °C. Bright and dark field SEM (Figure 4.7c,d) further confirm the presence of BSA as a gel support for the NPs. EDS analysis (Figure 4.7e) performed on a large NP (shown in dotted circle in Figure 4.7c) shows emission only due to Au, while XRD patterns (Figure 4.7f) can be indexed to fcc geometry of the bulk Au. Surface topography of a BSA film with NPs can be best studied with the help of AFM measurements in contact mode. Figure 4.7g,h shows height images of adsorbed NPs as bright spots whereas Figure 4.7i demonstrates the surface topography. Line analysis (Figure 4.7h) computes the average size of NPs 32.1±10.6 nm which is much larger than 7.5±3.5 nm determined from the TEM analysis (Figure 4.7b, inset) because it is quite difficult to differentiate between the actual NP boundary and BSA coating.
Figure 4.7 (a) FESEM and (b) TEM micrographs of Au NPs as bright spots on unfolded BSA as soft-template of a sample with Au/BSA mole ratio 167 synthesized at 80 °C. Inset shows the size distribution histogram for TEM image. (c) and (d), show bright field and dark field images, respectively, differentiating between Au NPs and unfolded BSA. (e) and (f), EDS spectrum and XRD patterns of Au NPs, respectively. (g) AFM height image of unfolded BSA as soft-template bearing Au NPs. (h) Close up image showing the line analysis of various Au NPs. (i) Topography of the BSA film bearing Au NPs showing peaks and valleys. (j) TEM micrograph of large plate like NPs of a sample with Au/BSA mole ratio 17 prepared at 80 °C.
On the other hand, no typical BSA film with NPs is observed for the same sample prepared at 40 °C (Figure 4.8a) due to the presence of predominantly globular nature of BSA. Instead, deformed shapes with larger size are obtained, which were indeed expected at 40 than 80 °C due to a weaker capping ability and reducing potential of globular BSA than that of an unfolded BSA at 80 °C (see corresponding intensity plots in Figure 4.2). A stronger reducing potential produces many folds of higher number of nucleating centres\textsuperscript{190} than a weaker reducing potential, and hence relatively less number of Au\textsuperscript{0} atoms are accommodated per centre in comparison to a fewer nucleating centres\textsuperscript{191} against a constant amount of HAuCl\textsubscript{4}. That is why the former produces many small NPs (Figure 4.7a,b) than latter.

![Figure 4.8 TEM micrographs of a sample with (a) Au/BSA mole ratio 167 synthesized at 40 °C (b) Au/BSA mole ratio 17 synthesized at 80 °C.](image)

Increase in the amount of BSA (from Au/BSA mole ratio of 167 to 17) at 80 °C produces even much aggregated BSA with entrapped NPs (Figure 4.8b) as indicated by Figure 4.3b in its region IV where practically no SPR is observed for mole ratio 17. But it also produces much larger well defined plate like geometries (Figure 4.7j) as observed previously by other authors\textsuperscript{38}, demonstrating a clear correlation between Figure 4.3b and 4.4a. As nucleation is a time dependent process
(in the presence of weak reducing agent like BSA), therefore such morphologies evolve with time (see a slow increase in the intensity of Au NPs absorbance in six hours in Figure 4.4a for Au/BSA mole ratio 17).

4.1.2.2 Gold salt concentration effect

Figure 4.9a shows a SEM image of a sample prepared with Au/BSA mole ratio of 334 at 80 °C. A fine interconnected tree like arrangement of NPs is evident but without the presence of any BSA gel support contrary to the sample made with a mole ratio of 167 (Figure 4.7a). Close up image (Figure 4.9a, inset) indicates the presence of fused NPs in the form of small nanowires (NWs). AFM image provides even better picture (Figure 4.9b) where tree like branches are formed due to an oriented attachment (Figure 4.9c) of large NPs (194±38.6 nm, see line analysis) with clear facets (Figure 4.9d). It is usually explained on the basis of Ostwald ripening process\(^{192-194}\) where poorly capped NPs undergo inter-particle fusions to produce bigger geometries. Further increase in the amount of Au salt from 334 to 668 mole ratio converts the NPs with facets into even much larger triangular plate like morphologies (Figure 4.9e) of 217±158 nm (size distribution histogram, Figure 4.9e, inset) and smaller icosahedral NPs (Figure 4.9f) of 21±8 nm (Figure 4.9f, inset). It demonstrates that a systematic increase in the amount of HAuCl\(_4\) in the following order of mole ratios 167 (Figure 4.7a) > 334 (Figure 4.9a) > 668 (Figure 4.9e,f) dramatically change the shape and size of NPs as well as their association with BSA. In fact, the appearance of triangular plates bound with \{111\} facets is due to a preferential adsorption of unfolded BSA on \{111\} crystal planes\(^{33}\) that prevents any crystal growth along \{111\} directions as the amount of HAuCl\(_4\) increases. It first converts the small polyhedral NPs (Figure 4.7a) into large NPs with clear facets (Figure 4.9d), which ultimately acquire plate like geometries (Figure 4.9e) as growth proceeds on \{110\} crystal planes as depicted in Figure 4.9g. Thus, increasing size and acquiring plate like geometries in fact facilitates the interfacial adsorption of unfolded BSA\(^{195}\) rather than the film formation. Hence, low amounts of HAuCl\(_4\) with small NPs favours their adsorption on the BSA film while high amounts result in the formation of large plate like geometries.
Figure 4.9 (a) FESEM image showing tree like branches of Au NWs formed through oriented attachment of Au NPs of sample with Au/BSA mole ratio 334 prepared at 80 °C. Inset, close up of (a). (b) Low resolution AFM height image of this sample showing the presence of tree like long NWs of Au NPs similar to those shown in FESEM image (a). (c) Close up image of NWs shows that they are made through the oriented attachment of prism like Au NPs. (d) AFM height image of independent NPs with clear facets. (e) and (f) represent TEM images of a sample with Au/BSA mole ratio of 668 prepared at 80 °C. Inset shows the size distribution histogram for TEM image. (g) A schematic representation of the evolution of NPs morphology with the increase in the amount of gold salt.
4.1.3 Protein film and its properties

From the above results, one thing is clear that BSA conjugated NPs with Au/BSA mole ratio = 167 exist in the form of a clear protein film whereas further increase in this ratio only increases the size with plate like morphologies of NPs without any clear film formation. This aqueous insoluble film precipitates out on aging in the form of small flakes (Figure 4.6) which could be having limited applications. However, it can be converted into a large robust continuous biodegradable sheet by combining it with water insoluble zein protein (see experimental). Figure 4.10a,b shows such protein films made from samples of mole ratios 167 and 668, respectively. Visually both films do not show any practical difference but color coordinates and mechanical properties demonstrate a significant difference between the two because the first one is made from a sample whose images are shown in Figure 4.7a,b while the second one is made from the sample of Figure 4.9e,f.

Color coordinates help us to determine the isotropic nature of the protein film. A variation in L*-, a*- and b*- values (see experimental) of zein films without NPs (“i.e.” zein + BSA), and with BSA conjugated Au NPs (of mole ratios 167, 334, and 668) are shown in Figure 4.10c. A film containing bioconjugated NPs of mole ratio 167 has lower L*- values and almost equal a*-value to a film without NPs. A lower L*-value indicates that the film is less bright or more dark and is attributed to a uniform distribution of small NPs throughout the film. But further increase in the mole ratio (to 334 and 668) increases L* due to an uneven distribution of large NPs arranged in NWs (Figure 4.9a-c) or plate like morphologies (Figure 4.9e). The variation in b* is considered to be entirely related to the protein structure because it refers to the blue and yellow parts of the electromagnetic spectrum. The positive b* value is due to the light yellow color of the protein film which represents the dried state of unfolded self aggregated protein. The presence of NPs is expected to decreases b* value due to the incorporation of NPs in the cohesive arrangement of unfolded protein.

Figure 4.10d,e shows the variation in the tensile strength and strain at failure, respectively, for the above mentioned films. Interestingly, film made with Au/BSA
Figure 4.10 Photos of biodegradable protein films made with BSA conjugated NPs with Au/BSA mole ratios of (a) 167 and (b) 668. See experimental or the composition of different components. (c) Histogram of color coordinates for the films made with different mole ratios. (d) and (e) Histograms of tensile strength and strain at failure versus different mole ratios, respectively. See details in the text.
mole ratio of 167 (or with a sample of Figure 4.7a,b) shows a large increase in both properties in comparison to that in the absence of NPs (“i.e.” zein + BSA), but further increase in the mole ratio to 334 and 668 regularly decreases these properties. Generally, addition of platisizer decreases the tensile strength and increases strain at failure (also known as elongation), which makes the film more flexible and less strong than in the absence of platisizer. However, in our case, mole ratio 167 produces a remarkable stronger film with even better flexibility than in the absence of BSA conjugated NPs, which is of course not maintained with mole ratios 334 and 668 because these samples do not contain BSA conjugated NPs in the form of a self assembled film (Figure 4.9).

4.2 Discussion

A collective comparison of all results indicates that a robust biodegradable protein film with better mechanical properties can only be obtained when self assembled bioconjugated NPs are used along with zein (Figure 4.11). Our results show that it happens with Au/BSA mole ratio of 167 where one can find the formation of self assembled NPs in the form of soft film following the seeding method (Figure 4.11a-c) as discussed in the results section. Simple calculations suggest that there are about 3.4 sites available on a single BSA macromolecule per AuCl₄⁻ ion if BSA is considered to be in a fully unfolded state which is usually not the case because even at 80 °C some of the hydrophobic domains are away from the aqueous phase. This perception is based on the fact that we do not observe any independent Au NPs in this sample (Figure 4.7a,b). But on the contrary, as the mole ratio increases to 334 or 668, there are plenty of independent NPs which exit as NWs (Figure 4.9a-c) or large plate like geometries (Figure 4.9e,f). Likewise, neither a decrease in the mole ratio from 167 to 17 helps us to achieve the film formation (Figure 4.7j) because an excessive amount of BSA promotes its self aggregation without any clear film formation (Figure 4.11). Moreover, it is not only the bioconjugated NPs which initiate the seeding for self aggregation but desorption of denatured protein from the NP surface also triggers the seeding with free protein to promote the protein - protein aggregation. Thus, an appropriate balance between the amounts of HAuCl₄ and BSA is required to obtain bioconjugated NPs which entirely exist in the form of self assembled soft films (Figure 4.11).
The following mechanism explains that how self assembled NPs promote the mechanical properties of zein film rather than large nanoplates. In fact, the seeding process which is responsible for the synthesis of self assembled NPs is also expected to work with zein. Zein is highly hydrophobic protein, therefore it will have preferential hydrophobic interactions with self assembled NPs because self assembled NPs are also predominantly hydrophobic due to the charge neutralization in the course of BSA adsorption on the NP surface. This is not the case with NPs exist in the form of NWs or large plates. Therefore, an appropriate BSA coating around NP in its self assembled state (Figure 4.11c) favours the BSA - zein interactions for better assimilation of self assembled NPs in zein film (Figure 4.11d) rather than the interactions between poorly capped nanoplates and zein protein (only {111} crystal planes are mainly occupied by unfolded BSA, Figure 4.9g). In addition, lateral
alignment of unfolded BSA with zein (Figure 4.11d) in producing a liquid crystalline arrangement also matters a lot in deciding the cohesive strength of the resulting protein film. Again, such kind of association is predominantly due to hydrophobic interactions between the non-polar domains of both proteins, and re-establishment of hydrogen bonding between the respective charged amino acids. Colloidal self assembled NPs in the form of soft films thus provide better orientations for unfolded zein to interact rather than independent plate like morphologies to produce strong and flexible protein films (Figure 4.11e).

4.3 Concluding remarks

The results conclude that one can synthesize bioconjugated nanomaterials by following a simple reduction of metal ions by biomolecules. These biomaterials can then be used for biodegradable protein film formation. We have carried out this study by following a direct reduction of Au ions into NPs by BSA and have further used them along with zein protein to form biodegradable protein films. The reduction of Au ions into atoms depends on various factors which include the concentration, pH, temperature, and the physical state of BSA. All factors significantly change the shape, structure, and morphology of BSA conjugated NPs which are the governing criteria for a versatile biodegradable protein film formation with better tensile strength and flexibility. We have found that a narrow Au/BSA mole ratio of 167 only produces small NPs adsorbed on the unfolded BSA in the form of a soft protein film in colloidal state. Any decrease or increase in this mole ratio produces large plate like triangular shapes of Au NPs with no sign of soft protein film formation or much aggregated BSA entrapping NPs, respectively. We have used these different morphologies of BSA conjugated NPs along with zein to produce more robust biodegradable protein films appropriate for various applications. Zein film produced with BSA conjugated NPs (especially in their colloidal state of soft film) has been found to have better tensile strength and flexibility than the one produced with large plate like NPs. Thus, the present results conclude that an appropriate biodegradable film with better strength and flexibility can be achieved by choosing appropriate morphologies of bioconjugated nanomaterials in their self aggregated state.