SUMMARY

Level of gelsolin in plasma has been related to more than 20 diseased cases, so the present need is an accurate quantitative estimation method, because gelsolin has been also marked as prognostic health marker (Peddada, Sagar et al. 2012). Biosensors are the newest technology with ultrasensitive detection, which mostly utilizes NPs. There are few reports which showed that colloidal gold particles can interact with plasma proteins and it has been shown that binding profile of these proteins on the NPs surface contributing to the particle's longevity in bloodstream, which could be of therapeutic value (Dobrovolskaia, Patri et al. 2009; Lacerda, Park et al. 2009). Another interesting observation was that gold particles were able to sequester gelsolin in human blood samples and when injected back to the injured patient the recovery rate was improved. Thus, it could be said that gold particles were able to indirectly enrich gelsolin in blood samples (Schneider 2011).

Additionally, biofunctionalization studies have revolutionized the field of nanoscience combining biology with the material science, indicated by several instances of successful utilization of biomaterial conjugated NPs. However, the cumbersome and multistep processes involved in the biofunctionalization have made it less fascinating. Very recently, nanotoxicity studies have concerned the applications of the nanoparticle in different field such as cosmetics, food and biomedical. Therefore, more eco-friendly methods utilizing the non-toxic and biomaterials based methodologies have been preferred to avoid long term side effects. With the similar prospective, initially different microbes were screened for metal NPs synthesis, as microbes are known to digest metal, using a method called biomineralization. Several years of understanding the process of NPs formation, resulted that majorly the protein content seemed to be the sole component, which makes the microbes and plant capable of forming nanoparticle (Malhotra, Dolma et al. 2013). With this speculation, several researchers screened the easily available protein such as BSA to form NPs (Zhang, Swift et al. 2007). Primarily, the proteins were assisted with reducing agent in the reaction mixture to form NPs, but

later on after optimizing several physical factors, it was found that protein can also act as both reducing and stabilizing agent, provided favourable conditions (Zhang, Swift et al. 2007). In very recent years, several other proteins have also been reported to form silver and gold nanoparticle (Yang, Li et al. 2007; Eby, Schaeublin et al. 2009; Bakshi, Kaur et al. 2010; Xavier, Chaudhari et al. 2010; Chanana, Correa-Duarte et al. 2011; Le Guével, Daum et al. 2011; Wei, Wang et al. 2011; Shilo, Berenstein et al. 2015). Based on these observations, the objectives of this thesis was framed to understand the formation of purified gelsolin and its minimal version assisted nanoparticle formation which could in future be used for devising a method of gelsolin estimation.

Preliminary results confirmed that gelsolin is capable of forming both Ag and AuNPs in 3 to 5 days of incubation time at reaction temperature of 37 °C. Both reactions gave the first confirmation, by change in colour from colourless to yellowish and pinkish-purple in case of both Ag and AuNPs, respectively. Further the reaction mixture were characterized using UV-visible spectrophotometer, where the coloured colloidal solution gave peaks at 420 nm and 550 nm, which are the characteristic peaks of Ag and AuNPs, respectively. Furthermore to confirm the NPs in terms of its size and shape, both the silver and gold nanoparticle were characterized using transmission electron microscopy (TEM). AgNPs were mostly circular in shape and in the size range of 30-60 nm and AuNPs were mostly square or rectangular with size range of 50-110 nm.

Although the finding of gelsolin being capable of forming both Ag and AuNPs was very fascinating, but longer time of incubation compared to the already reported protein based metal NPs synthesis methodologies subjected for requirement of further optimization of the reaction conditions. Different factors such as concentration of gelsolin, Ag^{+} or Au^{3+} ions to gelsolin molar ratios, incubation time, temperature and electric current were considered for screening condition for nanoparticle synthesis. In addition, gelsolin is a well known Ca^{2+} ions and pH sensitive protein and it undergoes large scale shape changes further resulting into activation of this protein from its closed inactive protein form. So, in order to understand the relation of its F-actin

depolymerization activity with the function of its nanoparticle formation, we also studied nanoparticle formation under different pH and calcium ranges. Molar ratios of Ag^{+} ions to gelsolin confirmed that increase in concentration of gelsolin, resulted into increase in NPs formation yield and kinetics; however in case of AuNPs, it is reverse and it form stable NPs at certain lower concentration of protein. The most stable concentration of Ag^{+} or Au^{3+} ions to gelsolin for both Ag and AuNPs formation, confirmed in terms of the NPs yield and colloidal stability was 50:1 and 300:1, respectively. Incubation time of the reaction mixture to form stabilized Ag or Au NPs confirmed that it starts forming at 20 or 40 hours, and finally saturates at 40 or 60 hours of incubation, respectively. Under the influence of different pH buffers, AgNPs formation kinetics and yield increases with increase in pH from 5 to 9, while stable AuNPs formation is favoured under pH range of 6 to 8. Further, in case of calcium titration in the reaction mixture, there was no significant change in NPs formation rate kinetics as well as its yield. Both pH and calcium titration experiments confirmed that gelsolin's function of filamentous actin depolymerization, is completely different with its newly discovered function of nanoparticle formation. Furthermore, the minimal versions of gelsolin such as N-terminal half (G1-G3), C-terminal half (G4-G6),1-161, 28-161 and 56-161, all designed to study the function of each portion of gelsolin, were also screened for the formation of both Ag and Au NPs. We speculated that screening all the minimal versions may help in understanding the specific part of gelsolin that is the most functional in the process of nanoparticle formation. If there is any sequence specificity that contributes to nanoparticle formation, there is whole repertoire of gelsolin like families which may perform the similar function of silver and gold nanoparticle formation. The result confirmed that all the minimal versions were capable of forming both Ag and AuNPs. Although, in the beginning of reaction the rate kinetics was slightly different in case of all the minimal versions, but towards end, the yield and kinetics both were comparable with no significant change in kinetics or yield of nanoparticle. This confirmed that there is no sequence specificity; it is majorly the presence of amino acids that may be responsible

for the formation of both Ag and AuNPs, like in case of other reported proteins. Ca^{2+} ions and pH activated minimal versions of gelsolin perform different functions as a function of the portion of full length gelsolin; however, when the same condition was used for metal nanoparticle formation, the results were not comparable. This again confirmed that gelsolin(s) function of filamentous actin depolymerization is completely different from its function of metal nanoparticle formation. Temperature was also considered as another factor, where temperature range in the 30 °C to 40 °C showed comparable rate kinetics, while temperature below 30 °C and above 40 °C led to slow down in the rate kinetics. Therefore lower temperature was preferred for more stability of the protein while forming nanoparticle. All theses factor contributed to yield of nanoparticle formed, however they did not contributed largely to the rate kinetics which is the main concern, because protein solution kept at 30 °C for 3 to 5 days may affect its activity or functionality that may result in nanoparticle stabilized with non-functional protein.

After the close observation of the conditions that contribute for gelsolin based NPs formation and its properties, it was found that light, heat and their inter-conversion was explained in case of thermally triggered classical citrate based nanoparticle formation (Turkevich, Stevenson et al. 1951). Here the heat energy captured during the process of nanoparticle formation is transformed in the form of light. We speculated, can light provided during the process may assist in nanoparticle formation which will further stored as heat energy in the nanoparticle. Interestingly, very recent reports of sunlight induced citrate based nanoparticle also strengthened our speculation of role of light in the process of nanoparticle formation (Luo 2007; Chien, Huang et al. 2011; Kim, Lavin et al. 2011; Baffou and Quidant 2014; Kim, Twaddle et al. 2014; Tang, Sun et al. 2015).

Surprisingly, when the reaction mixture was exposed to 200W light (Philips), the colour of AgNPs reaction mixture was turned into a yellowish coloured solution, whereas its negative control sample without the protein was colourless within 2 hours of exposure time. This observation clearly suggested that light plays a significant role in accelerating the process of gelsolin based AgNPs formation. However, in case in AuNPs reaction

mixture only after 10 hours of exposure time, the reaction started changing its colour (colourless to light pinkish). This reaction was continued to 3 days, where the reaction seems to be saturated with intense pink colour appearance as compared to reaction kept in absence where light pinkish colour starts appearing. This observation of colour change rate in both Ag and Au NPs reaction mixture in presence and absence of light indicated that light accelerates the process of both Ag and Au NPs formation using gelsolin. Further, when the reaction mixture was exposed to different power of light (25 W to 200 W), confirmed that apart from role of light, power of light also contributes to efficiency of gelsolin to get excited and donate electrons to the Ag^{+} or Au^{3+} ions thus forming metal NPs. To study the role of specific wavelength of light source on the process of gelsolin based Ag and Au NPs formation, different coloured broad range band pass filters (blue, green, yellow and red) were used for obtaining different wavelength incident light on the reaction admixture. Ag and Au NPs formation using gelsolin and 28-161, respectively in presence of different wavelengths of light illustrates that blue light gave the maximum yield of NPs as compared to green and yellow light whereas, red light completely failed to trigger NPs formation independent of exposure or reaction time. This confirmed that gelsolin based Ag and Au NPs formation are specifically triggered by 200 W blue light having wavelength range of 400-450 nm.

Blue light significantly accelerated the process of gelsolin based Ag and AuNPs formation. To confirm, the role of blue light in other biomolecules based NPs formation as a general phenomenon, different range of molecules including proteins, peptides, small molecules, oligonucleotides where screened for Ag and Au NPs formation under similar conditions. The results obtained in case of both presence and absence of blue light, further confirmed that role of blue light in accelerating the process of NPs formation is not specific to gelsolin(s) only; rather it is a general factor that can be applied to all the biomolecules based NPs formation. Further, amplifying the intensity of blue light, when blue laser was subjected on the reaction mixture, it resulted into NPs formation in seconds of exposure time, again specifically indicating the role of blue light.

Summary and Conclusions

Finally after the formation of stabilized Ag and Au NPs, it is important in terms of biological molecules that the biomaterial is functional at the post formation. To confirm the presence of biomaterial on surface of NPs and retaining its native like functionality, ELISA was performed using the antibodies and aptamer specific to each protein. The ELISA results confirmed that proteins (gelsolin, insulin and PDZ-ZO1) were present on the surface of NPs indicated by their reactivity against their antibodies (anti-gelsolin and anti-His antibodies). Further, the presence of gelsolin molecules on the surface of NPs was again confirmed using a strong gelsolin binding aptamer. Furthermore, when the same aptamer was used to form NPs, they were also able to be captured using gelsolin. Finally, anti-His antibodies based NPs were also able to be detected using gelsolin, which is also a His-tagged protein with much higher sensitivity. Thus, all these results confirmed that biomaterial based Ag and Au NPs formed using the present methodology resulted into a functionally active biomaterial stabilized or coated nanoparticles. This unique methodology of one step process of obtaining a functional biomaterial capped NPs has been already filed for patent (0188NF2014). Finally, with the concern to toxicology of the present methodology based nanoparticle formation, the synthesized Ag and Au NPs were also tested for cell viability in three different cell lines after their treatment in different doses. The result confirmed that biomaterial capped NPs were comparably non-toxic to all the cell lines.

Overall, research work compiled in the form of this thesis has successfully developed a generalized new methodology of blue light triggered biomaterial based Ag and Au NPs formation in a single step process. Finally, the biomaterial coated nanoparticles are also functional in terms of their binding to their respective natural binding partners and are additionally non-toxic to different cell lines.