SUMMARY

Now-a-days, people are becoming more and more interested in materials with recognition properties toward biological macromolecules, such as protein and nucleic acids. A nanometer (nm) scale has significant relevance to the biological systems, where many proteins are in the nm size. Accurately designed nanostructure can also be incorporated into the biological systems. The synthesis of nanocrystals (NCs) of metals and metal alloys by biological molecules is known as biomineralization and thus the nanomaterials obtained are called bioconjugated nanomaterials. Bioconjugated nanomaterials have become the focus of intensive research due to their applications in drug delivery, biological labeling, luminescence tagging, etc. Noble metal nanomaterials have been frequently used for such applications while semiconducting chalcogenides have been used for optical filters, optical recording materials, thermoelectric cooling materials, sensors, solar cells, superionic materials, and laser materials, etc. Synthesis and characterization of such materials still associated with inherent complexities due to the complex nature of biomolecules which are usually employed as weak reducing/stabilizing/capping agents in order to generate suitable bioconjugated nanomaterials with appropriate applications. We have carried out a comprehensive and systematic work to synthesize bioconjugated nanomaterials by using various biomolecules and tried to evaluate their role in controlling the nucleation and shape directing properties of noble metal or chalcogenide materials. These materials have been characterized to arrive at an appropriate mechanism and further used in the synthesis of environmental friendly biodegradable protein film for their industrial applications especially in the food and pharmaceutical industries. The work has been accomplished through the following objectives.

1. Synthesis and characterization of metal chalcogenides and noble metal nanocrystals by using various biomolecules as capping or stabilizing agents.
2. Evaluation and influence of molecular and structural factors of different kinds of biomolecules on the shape directed synthesis of biomaterials at nanoscale.
3. Use of bioconjugated nanomaterials for the synthesis of biodegradable protein films for their industrial applications.
Following four chapters summarize the entire work:

1. **Chalcogenide Bioconjugated Nano and Microcrystals.**
2. **Self Assembly of Bioconjugated Gold Nanoparticles.**
3. **Bioconjugated Hybrid Nanomaterials.**
4. **Synthesis of Gold Nanoparticles by using zein protein and the formation of biodegradable protein films.**

1. **Chalcogenide Bioconjugated Nano and Microcrystals**

   Bovine serum albumin (BSA) a well characterized and well studied water soluble protein has been used as a shape directing agent to produce semiconductor

\[\text{Figure 1. Schematic phase diagram showing different morphologies of PbSe NCs with respect to the concentration of BSA.}\]
nanomaterials of appropriate shape and size. Aqueous phase synthesis of lead selenide (PbSe) and cadmium selenide (CdSe) bioconjugated chalcogenide nanoparticles (NPs) was carried out at 85 °C by using BSA as capping/stabilizing agent in the concentration range from 1-10 × 10⁻⁴ g/mL. Selenium (Se) rods are obtained as reaction by-product both in the reactions of PbSe and CdSe, but their overall morphologies remain same in both reactions though they become quite well-defined at higher amounts of BSA used. An increase in the amount of BSA converts the polyhedral morphologies of PbSe into thread like bundles of micro-rods (MRs) which ultimately end up in cubic faced MRs (Figure 1). This is not observed in the case of CdSe. From low to high amounts of BSA used, CdSe NPs (≈ 2 - 3 nm) always exist in large scattered groups. FESEM images do not show any significant difference between their overall morphologies with respect to the amount of BSA used. A systematic protein assay with respect to reaction time has been carried out to determine the amount of BSA associated with nanomaterials on the basis of Bradford method. An increase in the amount of conjugated BSA with respect to time simply indicates its enhanced association with the NP surface to obtain well defined geometry. Apart from this, EDX and XRD measurements were also noted and discussed in context with overall preview of all shapes of PbSe, CdSe and Se NC/MR obtained. The shape directing ability of BSA has been explained on the basis of its selective interfacial adsorption at liquid-solid (PbSe) interfaces. A correlation between the conversion of polyhedral geometries into long micro-rods, and that with the amount of BSA, suggests its favourable adsorption at {100} crystal planes of cubic PbSe geometry which shifts the overall growth at {111} planes in order to produce micro-rods. This is probably not happening in the case of CdSe NPs with wurtzite (hexagonal) geometries because no significant change is observed in their morphology within the range of BSA used. Still, BSA proved to be an excellent stabilizing agent for colloidal CdSe NCs. Thus, BSA a low molecular weight protein is a good capping/stabilizing agent for semi-conducting nanomaterials and can be easily used in an aqueous phase to synthesize nano-biomaterials.

2. Self Assembly of Bioconjugated Gold Nanoparticles

Metal NPs is another important category of nanomaterials with several applications in nanotechnology. Keeping in view of the relatively stable nature of gold
Figure 2. (a) A chain like arrangement of 1-palmitoyl-2-oleoyl-sn-glycero-3-[phospho-rac-(1-glycerol)] (sodium salt) (POPG) capped Au NPs synthesized by using 0.125 mL of Au seed solution. Block arrows indicate the junction between the NPs presumably created by the fused POPG bilayers of adjoining NPs. (b) Chain like arrangement of Au NPs synthesized in the presence of POPG by using 0.25 mL of Au seed solution; and (c) and (d) chains of dendrite shaped large Au NPs synthesized in the presence of POPG by allowing 1 hour time interval between the two steps of the seed-growth method.
(Au) and silver (Ag), we have synthesized and characterized their NPs by using various biomolecules with monomeric as well as polymeric nature such as DNA, chitosan, phospholipids, and BSA following seed-mediated approach at room temperature. All reactions produced roughly spherical geometries of NPs with size range from 20 to 100 nm. We also observed a very peculiar phenomenon where NPs always arranged in a typical pearl-necklace type arrangement except in the presence of BSA (Figure 2). This behaviour can be attributed to the biomolecule-bimolecule interactions which arise due to lipid bilayer fusion, where lipid molecules have been used as the capping/stabilizing agents, or protein-protein interactions, where proteins have been used as the capping/stabilizing agents. This unique behaviour is usually not present when such NPs are produced in the presence of non-biomolecules such as alcohols and other low molecular weight water soluble polymers. Fluorescence spectroscopy was used to identify the interactions between biomolecules and Au NPs in aqueous colloidal solutions where growing nucleating centres are instantaneously capped and stabilized by the biomolecules. Ionic lipids have been found to be the best stabilizing agents due to their ionic nature in comparison to other biomolecules such as DNA, chitosan, and BSA. Thus, a careful selection of biomolecules can provide us a simple and inexpensive method to fabricate wave guides or photonic devices for applications in bio-medical imaging.

3. Bioconjugated Hybrid Nanomaterials

Bioconjugated hybrid nanomaterials is another important class of nanomaterials with extraordinary properties because they are the combination of entirely two different kinds of materials such as semiconducting chacogenides and noble metals. Synthesis and characterization of such materials is therefore an important aspect of materials chemistry. We have used BSA conjugated Se nanorods (NRs) to selectively grow Au/Ag NPs on them. Particularly, conjugated BSA to Se NCs act as hot spots where gold or silver ions are electrostatically attracted and then reduced to generate nucleating centres, the growth of which ultimately lead to the formation of hybrid nanomaterials. Conjugated denatured BSA not only controls the crystal growth but also stabilizes the colloidal crystals by forming an electric double layer at water-solid interface. The results present a simple synthesis of semiconductor-noble metal hybrid nanomaterials by using a low molecular weight protein, BSA, as hot spots. It was carried out by a simple reduction of Au/Ag salt by ascorbic acid in
the presence of fixed amount of BSA capped Se NRs. This allowed Au/Ag nuclei to attach preferentially on the hot spots for further growth. In addition, cystein residues of unfolded BSA were also expected to take part in the reduction process which further facilitated the synthesis of hybrid NCs (Figure 3). The hybrid NCs thus obtained were characterized by SEM, TEM, and EDX analysis while their synthesis was monitored simultaneously by UV-visible absorbance due to the surface plasmon resonance of Au and Ag NPs. In both cases, a gradual decrease in the absorbance of Au/Ag NPs with respect to reaction time was observed which indicated a diminishing number density of such particles in colloidal aqueous phase. SEM and TEM analyses then explained the presence of Au NPs in self assembled ball shaped aggregates and their selective adsorption on Se NRs, whereas no self aggregated balls of Ag NPs were observed and they always grew on the Se NRs. Thus, in order to generate a suitable hybrid nanomaterials, it is important to selectively grow one kind of materials on the other through surface driven process.

**Figure 3.** Proposed mechanism of the formation of Au/Ag nucleating centres specifically on the hot spots created by the adsorption of the capping BSA. (a) Cysteine residues interact electrostatically with Au/Ag ions (b) and thereby reduce them to respective centres (c) initiating the formation of hybrid Se-Au/Ag NCs.
4. Synthesis of Gold Nanoparticles by using zein protein and the formation of biodegradable protein films

With dramatic rise in the use of plastic for packaging, sheets, munches in our day-to-day life, their recycling as well as disposing have put serious concerns due to environmental pollution. Incineration of a huge amount of plastic waste produces cancer causing dioxins while land filling waste will take several thousands of years to decompose. Therefore, an effective reliable substitution of plastic with affordable biodegradable materials is an urgent need to keep environmental pollution in control. Economically suitable biodegradable protein films with comparable tensile strength may completely replace plastic sheets or munches. Zein protein films may be the replacement of plastic films at industrial scale due to their low price and readily availability, but require substantial improvement. Zein is a hydrophobic protein and is

![Figure 4](image)

*Figure 4.* a, b, c, and d are the photos of biodegradable protein films made with only zein, samples of 0.2% zein and [HAuCl$_4$] = 0.25 mM, 0.4% zein with [HAuCl$_4$] = 0.25 mM, and 0.6% zein with [HAuCl$_4$] = 0.25 mM respectively.
practically insoluble in water in the absence of the surfactant, therefore aqueous surfactant solution has been used first to dissolve zein in aqueous phase, then aqueous soluble zein is used to produce Au NPs. Zein conjugated Au NPs thus obtained have been used with alcoholic zein solution to produce biodegradable zein protein films for their industrial applications. We have carried out this study by following a direct reduction of gold ions into NPs by zein and have further used them along with zein protein to form biodegradable protein films (Figure 4). The reduction of gold ions into nucleating centres depends on various factors which include the concentration, temperature, and physical state of zein. All factors significantly change the shape, structure, and morphology of bioconjugated NPs which are the governing criteria for a versatile biodegradable protein film formation with better tensile strength and flexibility. We have used these different morphologies of bioconjugated NPs along with zein to produce robust biodegradable protein films appropriate for various applications. Zein film produced with (0.2% concentration of zein) bioconjugated NPs has been found to have better tensile strength and flexibility than the one produced with higher concentration (0.4% and 0.6%) of zein. Therefore, such biodegradable protein films can very well replace the plastic packaging materials if economical concerns are properly addressed.