1.1 General Introduction

Nanoscience\textsuperscript{1-3} is the emerging science of nanoscale materials. A major output of this activity is in the development of new materials: nanoparticles with the smallest dimensions ranging from few nanometers to less than 100 nm. There are two approaches for synthesis of nanomaterials and the fabrication of nanostructures (Figure 1.1): 1) ‘top-down’ and 2) ‘bottom-up’.\textsuperscript{4} In the ‘top-down’ approach, it all begins from a bulk piece of material, which is then gradually or step-by-step removed to form objects in the nanometer-size. In the ‘bottom up’ approach\textsuperscript{5}, instead of starting with large materials and chipping it away to reveal small bits of it, it all begins from atoms and molecules that get rearranged and assembled to larger nanostructures. Bottom up routes are more often used for preparing most of the nanomaterials due to the ability to generate uniform size, shape and distribution.

\textbf{Figure 1.1 Flow diagram of top-down and bottom-up approaches.}

Synthesis of functional inorganic materials by organisms (Figure 1.2) is a unique natural process where biomolecules are directly involved in their synthesis and this process is known as biomineralization. Biomineralization\textsuperscript{6-17} is a fast developing area to synthesize biofunctional nanomaterials by directly using biomolecules in their shape control synthesis\textsuperscript{18-20} with several biological applications.\textsuperscript{21-26} Biomolecules
such as amino acids and phospholipids also act as fine capping/stabilizing agents\textsuperscript{27-30} to achieve appropriate geometries. They occupy low energy crystal planes selectively\textsuperscript{18-20} and hence reduce their participation in overall nucleation process that directs the overall crystal growth at uncapped or poorly capped high energy crystal planes to achieve desired morphologies. Low molecular weight water soluble globular proteins are less surface active than fibrous proteins due to their relatively less interfacial adsorption.\textsuperscript{31,32} On the other hand, unfolded proteins are always more surface active than folded ones.\textsuperscript{33,34} Unfolded protein with its large surface area can also provide soft-template effects\textsuperscript{35-37} for growing nucleating centres. Use of simple and low molecular weight proteins such as Lysozyme (Lys), Cytochrome c (Cyc,c) and Bovine Serum Albumin (BSA) sometimes makes it easier to follow and understand their involvement in the bioconjugate materials. The conjugation of protein with metal nanoparticles (NPs) not only affords stabilization to the system but also introduces biocompatible functionalities into these NPs for further biological interaction or coupling. Proteins (Lys, Cyc,c, BSA) initiate\textsuperscript{38-41} the biomineralization of metal NPs simply by following the reduction of metal. Once atoms are produced, they undergo nucleation process which is simultaneously controlled by the capping/stabilizing behaviour of protein. Here, the physical state of protein that is

\textbf{Figure 1.2} Magnetite crystals in magnetotactic bacteria. These are tiny compasses that guide these organisms to move vertically in river bed sludge. MV indicates empty vesicles.
folded or unfolded, plays a governing role in configuring the overall shape and size of NPs.

BSA is composed of 580 amino acid residues with 17 inter-chain disulfide bonds.\textsuperscript{42-44} The overall shape of BSA is oblate and it consists of three domains I, II, and III, which are divided into nine loops. Each domain is made up of a sequence of large-small-large loops forming a triplet. BSA remains in native state up to 40 °C, between 40 - 50 °C, the reversible changes in its conformation occur whereas they become irreversible between 50 - 60 °C. Above 60 °C, unfolding of BSA with \( \beta \)-aggregation begins which ultimately leads to a gel formation around 70 °C\textsuperscript{44} (Figure 1.3). The unfolded gel form of BSA is expected to act as a soft template and is the most ideal candidate to study the protein film formation in its soft state. Thus, temperature is the main contributing factor to achieve the gel state, but it also speeds up the reduction and nucleation of NPs. Therefore, an appropriate balance between

\textbf{Figure 1.3} Native and denatured states of BSA under the influence of temperature.
the temperature and concentration parameters of a biomineralization process is required to achieve an appropriate protein film formation.

Lys contains 129 amino acids arranged in a single polypeptide chain with average formula weight of 14,300 and is freely available in plant and animal tissues. It is also abundant in egg white, human tears, saliva, and endocrine glands, and is highly surface active. The surface activity is primarily related to its hydrophobic nature which is mainly contributed by the presence of four disulfide bridges to produce an overall closed structure (Figure 1.4). Lys, stored as a dry lyophilized or crystalline powder at 2 - 8 °C, is stable for years. Solutions at pH 4-5 are stable for several weeks refrigerated and for days at ambient temperatures. It is composed of a predominantly α-helical part (α-domain) and a part with predominantly β-sheet structure (β-domain). As the name implies, it is an enzyme (biological catalyst). Its substrate has a specific sequence in the bacterial cell wall of Micrococcus, a potential invading organism of eggs.

Cyc,c, on the other hand, consists of a single polypeptide chain of 104 amino acid residues that are covalently attached to a heme group with average molecular
weight of 12,400. The active heme center consists of a porphyrin ring where four pyrrole nitrogens are coordinated to the central Fe atom forming a square planar complex (Figure 1.5). The iron center switches between the ferric (Fe³⁺) and the ferrous (Fe²⁺) state, thus acting as a one-electron carrier. Because of the outer electronic structure of Fe³⁺, it can exist in a high spin or a low spin state. In solution, the iron of cyc c exists in the low spin state at pH between 2 and 12. The heme center is surrounded by tightly packed hydrophobic side chains and an outer covering of hydrophilic side groups. Both Lys and Cyc,c are known for providing capping and stabilization to colloidal particles due to their interfacial activities, that lead to their conformational changes upon adsorption on the water-solid interfaces.

In the past decade, starch has been shown as a good host for many guests of inorganic NPs, such as gold, silver and iron oxides to form inclusion complexes. Starch is a highly organized mixture of two carbohydrate polymers, amylose and amylopectin, which are synthesized by plant enzymes and simultaneously packed into dense water-insoluble granules. Starch is the major energy reserve for plants. It is located mainly in the seeds, roots or tubers, stem pith, and fruits. The basic building block is a glycosyl monomer, measuring 0.3 nm. The ratio between amylose and amylopectin varies depending on the starch source. In normal starches, amylose constitutes about 15-30% of total starch. Waxy starches have
approximately 0-5% amylose, whereas the amylose contents of high amylase starches are in the range of 35-70%.

Amylose is defined as a linear molecule of (1→4) linked α-D-glucopyranosyl units, but it is today well established that some molecules are slightly branched by (1→6)-α-linkages (Figure 1.6). The molecular weight of amylose range from $1 \times 10^5$ to $1 \times 10^6$. The degree of polymerization (DP) of amylose has been shown to vary from 690 to 6340 with around 9-20 branch points equivalent to 3-11 chains per molecule. Each chain contains approximately 200-700 glucose residues equivalent to a molecular weight of 32,400-113,400. Amylose forms helical complexes with iodine, fatty acids and monoglycerides. The location of amylose in a starch granule is still in dispute. The possible locations are as follows: (1) amorphous growth ring, (2) amorphous lamellae, or (3) interspersed or co-crystallized with amylopectin molecules.

Amylopectin is the major component of all starches with a weight average molecular weight of the order $10^7-10^9$. Amylopectin (Figure 1.7) is composed of linear chains of (1→4) linked α-D-glucopyranosyl units connected through (1→6)-α-linkages (5-6%). The DP is typically within the range 9600-15,900 but comprises three major species with DP 13,400-26,500, 4400-8400 and 700-2100. In common with amylose, the molecular size, shape, structure and polydispersity of the molecule

Figure 1.6 Structure of amylose.
varies with botanical origin. Unlike amylose, there is great additional variation with respect to the unit chain lengths (CLs) and branching patterns. Amylopectin unit chains are relatively short compared to amylose molecules. The currently accepted amylopectin structure involves short amylopectin chains forming double helices and associating into clusters. These clusters pack together to form a structure which consists of alternating crystalline and amorphous lamellae. They are typically, 18-25 units long on average although the range is extended (19-31), if high-amylose starches are also included. The individual chains can be specifically classified in terms of their CLs and consequently position within starch granules. Hizukuri has classified the amylopectin unit chains as A, B and C. The A chains are the shortest (DP 6-12) and are linked by a single (1→6)-α-linkage to the amylopectin molecule. B chains are classified into B₁, B₂, B₃ and B₄ depending on their length and the number of clusters they span. The A and B₁ are the most exterior chains and form double helices within the native granules, which are packed into lamellae crystallites. The B₂-B₄ chains act as connecting chains in the amylopectin molecule. Typical CLs for A, B₁-B₄ chains for different starches (after debranching with isoamylase) are 12-16, 20-24, 42-48, 69-75 and 101-119, respectively. The ratio of A- to B-chains depends on the starch source and is typically of the order of 1:1 to 2:1 on a molar basis or 0.5:1 to 1:1 on a weight basis.

Figure 1.7 Structure of amylopectin.
Starch, when heated in the presence of excess water, undergoes an order-disorder phase transition called gelatinization over a temperature range characteristic of the starch source. The above phase transition is associated with the diffusion of water into the granule, water uptake by the amorphous background region, hydration and radial swelling of the starch granules, loss of birefringence, uptake of heat, loss of crystalline order, uncoiling and dissociation of double helices and amylose leaching.\textsuperscript{73,85-87} The gelatinization parameters of starches were determined using differential scanning calorimetry (DSC). On cooling, the starch chains (amylose and amyllopectin) in the gelatinized paste interact, leading to the formation of a more ordered structure. These molecular interactions are collectively termed retrogradation. Retrogradation is accompanied by increase in the degree of crystallinity and gel firmness, exudation of water (syneresis). During Retrogradation, amylose forms double helical association of 40-70 glucose units.\textsuperscript{88,89} Whereas amyllopectin crystallization occurs by association of outermost short branches (DP=15).\textsuperscript{90} The retrograded starch contains both crystalline and amorphous regions. Retrogradation properties of starch have been investigated by DSC, rheological measurements, Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy and X-ray diffraction (XRD).\textsuperscript{91} Starch presents an interesting dynamic supramolecular associations facilitated by inter- and intra-molecular hydrogen bonding resulting helical structure, which can act as nanoreactors for the growth of NPs.\textsuperscript{92}

Over the past two decades, synthetic polymer materials have been widely used in every field of human activity. These polymers, i.e., polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS), and polycarbonate (PC), are usually petroleum based and are regarded as non-degradable.\textsuperscript{93} These plastics are used as packaging material in almost every product we buy, most of the foods and drinks we consume. This is because they provide excellent protection for the product, are cheap to manufacture and are durable. However, they are not biodegradable due to their very large polymer molecules and stable carbon-hydrogen bond, both of which make its decomposition by organisms rather difficult. The non-biodegradable and non-renewable nature of these plastics is of major environmental concern. Because of the increased awareness in preserving the natural environment, efforts have been made to develop biodegradable plastics that
are made from renewable resources, such as plants, which are compostable. Such ‘green’ changes in packaging will improve sustainability and reduce the adverse impact plastic packaging places on the environment because of their biodegradability. Consequently, there is a growing interest in developing biodegradable polymers made from renewable and natural polymers such as cellulose, starch, and proteins to replace synthetic non-degradable materials. Of these materials, starch, the natural polymer, is an inexpensive and readily available resource, which is often used as filler for the replacement of petroleum derived synthetic polymers to reduce environmental pollution. However, starch itself has poor processability, dimensional stability and mechanical properties for its end products. Therefore, native starch cannot be used directly as packaging material. In its native granular form starch has limited applications. To fully realise the starch biopolymer’s functionality, granule disruption and sometimes chemical modification are necessary. To produce biodegradable packaging, starches are first gelatinized. This process transforms the starch from a crystalline granular into a rubbery paste with no structure at all. During gelatinisation several changes occur to the starch-water system and the loss of crystallinity is the most important regarding the formation of films. Pure starch films are very brittle as a result of the strong cohesive energy density of the polymers. Starch films are usually modified by the addition of plasticizers. Polyols (glycerol, sorbitol and polyethylene glycol) are commonly used as plasticizers. These additives decrease the intermolecular attraction between adjacent polymeric chains, resulting in film flexibility and decrease in film strength.

Starch films and coatings have been used for various food and pharmaceutical applications. Films prepared from starch are isotropic, odourless, tasteless, colorless, non-toxic and biodegradable. Edible films and coatings can be prepared from native and modified starch. Starch coatings are nutritious, safe and economic and have been used in the storage and marketing of foods. Their physical characteristics, chemical resistance and mechanical properties are similar to plastic films. Fanta and coworkers applied starch coatings to polyethylene films by immersing in starch solutions to impart hydrophilic properties. Palviainen et al. and Krogars et al.
have also reported the use of native starch films and coatings for the preparation of tablets and pellets for the drug release.

Such biodegradable starch films can be made more robust by incorporating the nanomaterials for their better industrial applications mostly in the case of food and pharmaceutical packaging where greater tensile strength as well as flexibility are required. By incorporating nanomaterials such as Gold (Au) NPs, we can make such films UV-Visible active due to the surface plasmon resonance (SPR) of Au NPs as well as more strong and flexible. Likewise, it is also possible to incorporate fluorescent Cadmium Sulphide (CdS) NPs to make such film fluorescent active for their easy detection. Previously, biodegradable nanocomposite polymers have been prepared using organic-inorganic nanoclay or silicate with improved mechanical properties, and reduced water vapour permeability. Likewise, we can make biodegradable protein films by using freely available corn protein commonly known as zein protein. Pure zein films have little use due to their highly brittle and least flexible nature. Therefore, appropriate blending is to be done by incorporating conventional plasticizers such as glycerol, polyethylene glycol, fatty acids, and monoglycerides to explore their best applications. Though, plasticizers play a significant role in achieving the flexibility, they also cause a decrease in tensile strength. Appropriate use of the Au NPs in the synthesis of zein protein films provide them additional tensile strength as well as flexibility. This can be easily done though the biomineralization process where nanomaterials are synthesized in vitro by using the biomolecules such as proteins or starch.

1.2 Review of literature

The concept of ‘biomineralization’ was introduced by Lowenstam and Weiner and signifies mineralization processes that proceed and progress in close association with organic molecules or matrices. The awareness that the formation of minerals could be guided by organic molecules contributed notably to the understanding of the formation of inorganic skeletons by and in living organisms. Bio-conjugated NPs have become the focus of intensive research due to their applications in drug delivery, biological labeling, luminescence tagging, etc. Synthesis of Au NPs has received considerable attention because of their potential
applications in the field of biology, electronics, transport and information technology. One of the most common proteins investigated for Au NPs synthesis has been BSA. BSA is a strong candidate for the synthesis of Au NPs because it contains a large number of cysteine, tyrosine, and charged residues and has a known propensity to bind to gold ion complexes. BSA is also attractive for metal NPs synthesis research, as the protein is readily available and relatively inexpensive. Because of its popularity among biomimetic metallization researchers, BSA-based research provides interesting examples of the variety of methods and results that may be obtained with the use of a single protein in the production of metallic NPs. Burt and coworkers were the first to explore the use of BSA in Au NPs synthesis. In this initial study, NaBH₄ was utilized to reduce Au ions in the presence of BSA and yielded well-dispersed NPs less than 2 nm in size. Utilizing a variety of spectroscopic characterization techniques, Burt et al. determined that BSA was conjugated, most likely through its cysteine residues, to the surface of the Au NPs. In a separate study, larger Au nanospheres (7.7 ± 0.9 nm in diameter under pH 7 reaction conditions) were obtained when Au ions were reduced in the presence of BSA with UV irradiation. Singh and coworkers utilized the surfactant like properties of BSA to produce Au ion charged foams of BSA. These Au ion charged foams were reduced with hydrazine hydrate vapors, producing irregularly shaped Au NPs <100 nm in size. NPs composed of gold-silver alloys were also reported to be produced with this BSA foam and reduction method. Xie et al. have recently utilized the intrinsic reducing and shape-directing capabilities of BSA to produce Au nanoprisms and nanopolygons in high yields (up to 80%) under acidic conditions at 37 °C. These authors attributed the appearance of Au nanoplatelets to the slow reaction kinetics imposed by the mild reducing power of BSA and a “surface wrapping” model of NPs formation, where shells of new material sweep across the faces of the growing nanostructures. Au nanoplatelet yield and size could be adjusted through the manipulation of reaction temperature, pH, and addition of silver ions.

Yang et al. have carried out the synthesis of Lys monolayer-stabilized Au NPs in aqueous medium by chemical reduction of HAuCl₄ with NaBH₄ in the presence of Lys and these Au NPs were characterized by UV-Visible spectroscopy, Transmission electron microscopy (TEM), Atomic force microscopy (AFM) and X-
ray photoelectron spectroscopy (XPS). The results showed that the monodisperse Au NPs are well-coated directly with Lys. On the basis of their excellent colloidal stability, controlled self-assemble ability and biocompatible surface, the Lys monolayer-stabilized Au NPs hold great promise for being used in nanoscience and biomedical applications. Verma et al.\textsuperscript{127} have studied that a single protein spacer (Lys) can be utilized for modular and efficient self-assembly of NPs into controlled composites. The self-assembly process can be directed to feature large interparticle spacing, varied morphology and tunable collective optical response in the ensembles. With the use of this methodology, the interparticle spacing can be segregated from the collective optical response in the biomaterial, thereby demonstrating a unique level of control in the self-assembly process. This work displayed one of the potential benefits in using proteins for directing the self-assembly of NPs and can be extended toward assembly of Au NPs with bigger core sizes or the self-assembly of magnetic NPs for tuning of collective optical or magnetic response in the biomaterials.

Jiang et al.\textsuperscript{128} have studied the effect of colloidal Au size on the conformational changes of the adsorbed Cyc,c by Circular Dichroism, UV-Visible and FTIR spectroscopic techniques. The results showed that there are different conformational changes for Cyc,c adsorbed on Au NPs with different sizes due to the different interactions between Cyc,c and Au NPs. Jensen et al.\textsuperscript{129} have synthesized stoichiometric Cyc,c-Au NP hybrid systems to improve the efficiency of long-range charge transfer in molecular bioelectronics. The systems were investigated in homogeneous solution and at liquid/solid interface. Conjugation of Cyc,c resulted in a small but consistent broadening of the NP plasmon band. This phenomenon can be explained in terms of long-range electronic interactions between the Au NPs and the protein molecule.

Among several biomolecules, starch is one of the most abundant naturally occurring polysaccharide (polymer) with biocompatibility and biodegradation on earth. The knowledge on the structure of starch has been recently advanced.\textsuperscript{130} The description of starch is well-known as polymer structure containing mostly linear amylose and branched amylopectin. The spectroscopic and microscopic methods are now well advanced for probing the branching pattern and the branch length of starch polymers.\textsuperscript{131-134} The structure of starch is packed in semicrystalline granules
containing concentric growth rings. Amylose and amylopectin are arranged radically and aligned perpendicularly to the growth rings and to the granule surface. Starch granules can be transformed into other forms such as starch paste, starch gel, and retrograded starch depending on the cooking conditions.\textsuperscript{72,135-137} The starch transformation provides diverse structures and properties for numerous applications. Several environmental friendly approaches have been shown by using starch and polysaccharides as templates for NPs synthesis.\textsuperscript{138-142} The concept of green chemistry used in the synthesis of NPs was first reported by \textit{Wallen and coworkers} in which the synthesis of silver nanoparticles (Ag NPs) was carried out and stabilized by soluble starch and using β-D-glucose as a nontoxic reducing agent.\textsuperscript{65,141}

\textit{Chairam et al.}\textsuperscript{143} developed a green route to synthesize size- and shape-controlled Ag and Au NPs using mung bean starch which also acts as a stabilizing template. This approach is very simple, safe and easily accessible. This offers an opportunity to design new fine structures and utilize mung bean starch for the large-scale synthesis of size- and shape-controlled Ag and Au NPs for chemical and biological applications. \textit{Hussain et al.}\textsuperscript{144} prepared Au NPs by the reduction of chloroaurate anions solution with hydrazine in the aqueous starch and ethylene glycol at room temperature, at atmospheric pressure, and without any additional flow of inert gas. The shape and stability of NPs was evaluated by high resolution transmission electron microscopy (HRTEM) and UV-visible spectroscopy. Starch capped Au NPs can be dried and stored as powder that can be reutilized by simply redissolving them in water or any other polar solvent to give stable colloidal solution of Au NPs. The use of soluble starch as capping agents to prepare Au NPs in aqueous solutions is attractive because organic solvents are not used and the corresponding pollutants are absent, moreover resultant NPs are biologically compatible. The synthesis method reported in this work is helpful for providing an economic route for the large-scale production of highly stable and biologically compatible monodispersed Au NPs.

Since the petroleum resources are limited and the increasing use of non-biodegradable polymers has caused serious environmental problems. Therefore, biodegradable polymer materials have been attracting an increasing attention since the 1970s.\textsuperscript{145-147} \textit{Gao et al.}\textsuperscript{148} examined the effects of plasticization of a kafirin (highly homologous with the maize storage protein zein) film by glycerol in the absence of
water by a combination of spectroscopic (NMR and infrared), rheological, and calorimetric methods. The results suggested that at low glycerol levels the glycerol was absorbed onto and possibly into the protein. Increasing the level of glycerol increased the motion of the protein and changed the protein conformation. There were corresponding changes in the mechanical properties of protein films. At 40% (w/w) of glycerol, two glass transition temperatures were observed, one of which corresponded to the glass transition temperature of pure glycerol. This result indicated that at this level of plasticizer there were sufficient glycerol/glycerol interactions occurring to allow a separate glass formation process for glycerol. Singh et al.\textsuperscript{113} have carried out film casting by dissolving zein (15% w/v) in aqueous ethanol (90% v/v) with and without glycerol (30% on zein wt basis) as plasticizer. For casting zein-iodine films, iodine (3% w/w) was dissolved in aqueous ethanol (90% v/v). Zein films were characterized by secondary structure (determined by infrared spectroscopy) and dielectric and mechanical properties.

Tang et al.\textsuperscript{149} studied the effect of nano-SiO$_2$ on the structure and properties of starch film. The addition of nano-sized SiO$_2$ particles improved the physical properties, i.e. mechanical properties and elongation at break, and water resistance of starch/PVA blended polymers. The FTIR and XPS analysis indicated that the intermolecular hydrogen bond was formed in nano-SiO$_2$ and starch/PVA, and the strong chemical bond C—O—Si was also formed in nano-SiO$_2$/starch/PVA hybrid materials. The scanning electron microscopy (SEM) analysis predicted that there was good miscibility between nano-SiO$_2$ and components in the blends. The nano-SiO$_2$ and starch/PVA blends also formed a network structure to prevent the water molecules from dissolving the film, which greatly increased the water resistance and mechanical properties of the film. Yoon et al.\textsuperscript{93} synthesized the starch/PVA composite films from corn starch, PVA, and nano-sized poly(methyl methacrylate-co-acrylamide) (PMMA-co-AAm) using a casting method. They investigated the effect of nano-sized PMMA-co-AAm on physical properties such as tensile strength, elongation at break, degree of swelling, solubility, water vapor absorption, and biodegradation. The results showed that tensile strength and degree of swelling increased with increasing PMMA-co-AM NPs contents, whereas elongation at break, solubility, and water vapor
absorption decreased. Thus, the physical properties of each film were improved by adding PMMA-co-AM NPs.

In view of all these studies, we have carried out the synthesis of bioconjugated Au NPs by directly using simple, water-soluble, well-characterized important proteins such as BSA, Lys and Cyc, c, as well as starch as weak reducing and stabilizing agents under different experimental conditions. Biomaterials synthesized directly by using biomolecules are far more chemically pure and easy to characterize than by using other reducing and stabilizing agents in the presence of biomolecules because both reduction as well as stabilization can also be provided by biomolecules with amphipathic properties. Thus, an appropriate biomolecule such as a water soluble protein or starch initiates the biomineralization of Au NPs simply by following the reduction of Au(III) into Au(0) and simultaneously stabilizes the colloidal NPs produced from a nucleation process. In this way reactions have to be carried out in an aqueous phase while keeping environment and green chemistry concerns in mind. The bio-conjugated NPs thus produced through biomineralization of gold salt by biomolecules can be used in the biodegradable film formation to achieve better mechanical properties. The results have been generalized on the basis of various factors which include the concentration, pH, temperature, and the physical state of a biomolecule which are the governing criteria for a versatile biodegradable film formation with better tensile strength and flexibility.

1.3 Objectives

- **Biomineralization of gold nanoparticles by following a simple reduction of Au(III) into Au(0) in vitro by using water soluble proteins.**
- **Use of bioconjugated nanoparticles to produce biodegradable zein protein films to achieve better tensile strength and flexibility.**
- **Exploring biomineralization by using starch instead of proteins to produce nanoparticles incorporated starch films with better tensile strength and flexibility for vast industrial applications.**