In the previous chapters (i.e. Chapter III and IV), the focus was on the biomineralization of Gold (Au) nanoparticles (NPs) by using well characterized, water soluble proteins (Lysozyme, Cytochrome c and Bovine Serum Albumin) as weak reducing as well as capping/stabilizing agents in aqueous phase and then, to use protein-conjugated Au NPs with water insoluble zein protein to produce much robust biodegradable protein films for various industrial applications. This chapter includes a simple method to synthesize environmental friendly biodegradable starch films containing Au and cadmium sulfide (CdS) NPs with significantly improved mechanical properties than pure starch films for various industrial applications. Starch can easily be procured in comparison to zein protein and is better economically suited. Au NPs are synthesized in vitro by using starch as a weak reducing agent and simultaneously used them for the starch film formation. Starch complexed NPs are expected to influence the retrogradation as well as gelatinization of starch which in turn dramatically alters the mechanical properties of starch films. It helps us to understand that how Au NPs when synthesized in vitro along with starch affect the mechanical properties such as tensile strength and flexibility of starch films. Similarly, fluorescent starch films are produced by using fluorescence active CdS NPs. The synthesis of CdS NPs is not as simple as that of Au NPs, but the overall reaction can be conducted in the presence of glycerol which is used as plasticizer in the starch film formation.

5.1 Results

5.1.1 UV-Visible and fluorescence studies

Synthesis of Au NPs was carried out by reducing Au(III) into Au(0) using starch as a reducing agent and monitored simultaneously by the UV-Visible measurements. Figure 5.1a depicts a typical plot where no absorption by aqueous starch is observed in the visible region but as soon as Au nucleating centres are formed, a peak appears around 540 nm due to the SPR of Au NPs.\textsuperscript{160,162} It becomes more significant with the passage of time. Plot of intensity at 540 nm versus time (Figure 5.1b) shows an increase in the intensity with time as NPs grow in size before tending to a constant value (indicated by an arrow). This value decreases as the
amount of starch increases from 0.5 to 5 % in a linear fashion (Figure 5.1b, inset) which means that the reduction is proportional to the amount of the reducing agent.198,199 Another important feature of Figure 5.1a is the overall blue shift of about 20 nm in the absorbance of Au NPs and its variation with time is depicted in Figure 5.1c. For a reaction of 0.5% starch, at first it goes through a red shift about 40 nm which after attaining a maximum value (indicated by an arrow) reverts to a blue shift of 20 nm before tending to a constant value. This variation though is quite clear in the presence of 0.5 % starch and it diminishes at higher amounts. This is very much clear in case of 5 % starch which shows no red shift at all. In order to understand this

Figure 5.1 (a) UV-Visible scans of aqueous 1 % starch and 0.25 mM HAuCl\textsubscript{4} at 70 °C with time from 5 min (lowest scan) to 6 hours (upper most with dotted line). (b) Intensity at 540 nm of Au NPs versus time plots of reactions with 0.5, 1, 2, and 5 % starch and 0.25 mM HAuCl\textsubscript{4} at 70 °C. Inset shows a linear dependence of reduction time on the amount of starch. (c) Wavelength maximum of the absorbance of Au NPs versus time plots of reactions with 0.5, 1, 2, and 5 % starch and 0.25 mM HAuCl\textsubscript{4} at 70 °C. (d) UV-visible scans of aqueous 1 % starch and starch conjugated CdS NPs.
behavior further, we have divided 0.5 % starch curve into two parts; first part with red shift is related to the nucleation while the second part with blue shift can be related to the association of starch with NPs. Lowest amount of starch (i.e. 0.5 %) is expected to generate few nucleating centres which slowly grow under the autocatalytic effect to accommodate unreduced Au ions. This slow growth process is usually accompanied with a red shift because it leads to larger morphologies. In contrast, greater amount of starch (i.e. 5 %) converts maximum amount of Au ions into nucleating centres with relatively less possibility of further growth, hence no red shift is observed. Blue shift on the other hand is related to the screening of the SPR of Au NPs upon complexation with starch macromolecules once the nucleation is completed, and hence it happens in all cases with maximum effect for 5 % starch because as soon as the Au nucleating

![Photograph of Au NPs embedded starch film.](image1)

![Photograph of CdS NPs embedded starch film.](image2)

![Absorbance versus wavelength scans of starch films made with and without Au NPs.](image3)

![Similar plots of starch films made with and without CdS NPs.](image4)

**Figure 5.2** (a) Photograph of Au NPs embedded starch film. (b) Photograph of CdS NPs embedded starch film. (c) Absorbance versus wavelength scans of starch films made with and without Au NPs. Note absorbance around 540 nm due to the presence of Au NPs in the starch films. (d) Similar plots of starch films made with and without CdS NPs.
centres are created, they are complexed with much greater amount of starch (Figure 5.1c).

For CdS NPs, a simultaneous measurement of UV-visible spectrum at 150 °C was not possible, therefore the absorption of samples (as prepared at 150 °C) were carried out at room temperature (Figure 5.1d). A weak band close to 500 nm is observed in all cases due to the yellow color of the CdS NPs. Starch conjugated NPs suspensions from both reactions are directly converted into starch films (see experimental for details) which is usually pink or purple in color in the case of Au NPs (Figure 5.2a) while bright yellow for CdS NPs (Figure 5.2b). A uniform color of both films indicates that the NPs are evenly distributed throughout the starch film as

![Figure 5.3](image-url)

**Figure 5.3** (a) Plots of CdS NPs in suspension and embedded in starch films. Note a relatively weak emission of CdS NPs present in the starch films. (b) Steady state fluorescence of CdS NPs suspension. (c) Fluorescence lifetime decay of CdS NPs in starch films.
they were in the colloidal suspensions. The absorbance spectra of starch films with and without Au NPs are depicted in Figure 5.2c. Clear peaks due to SPR around 550 nm are due to the presence of embedded NPs which are absent in the respective control experiments. Likewise, CdS NPs also show similar absorption bands around 500 nm in their films (Figure 5.2d) which were previously demonstrated by their suspensions (Figure 5.1d).

Fluorescence spectra of CdS NPs suspension along with that of the films at excitation wavelength of 450 nm are shown in Figure 5.3a. NPs suspension gives clear emission around 510 nm whose intensity increases with the increase in the amount of CdS NPs (Figure 5.3b). Fluorescence emission of CdS NPs in starch films is suppressed with increase in the amount of starch and about 10 nm red shifted (Figure 5.3a). In addition, greater amounts are depicted in Figure 5.3c for CdS NPs films made with 1% starch. The lifetime decay of CdS NPs in films is fit to multiexponential function with average lifetime between 1 - 3 ns.

5.1.2 Color coordinates

Color coordinates (Figure 5.4a) further help us to characterize the color properties on the basis of isotropic nature of the films. A low value of L* in the presence of Au NPs of different concentrations can be attributed to the less bright or less transparent starch film in comparison to their absence and is obviously understood from the pink-purple color of the NPs incorporated in films. a* value indicates the presence of red color which is provided by the small NPs due to the SPR with absorbance around 540 nm in comparison to purple color of larger NPs with red-shifted absorbance. Since there is no red color in 2% starch control film, therefore a* values are only obtained for NPs incorporated films. Among them, a* is maximum for greater amount of Au NPs (i.e. 1 mM) which is obviously expected due to the presence of greater number of NPs. The b* value for this film indicates the presence of weak yellow color which might arise from the semi-crystalline nature of starch or to some extent due to unreacted Au(III). It is absent in films with 0.25 and 0.5 mM of Au NPs which means that almost all Au ions are converted into nucleating centres in these films. Similar behavior is observed for 1% starch films in the absence and presence of Au NPs (Figure 5.4b). In the case of CdS NPs starch films, the large b* values of color coordinates (Figure 5.4c) demonstrates the presence of strong yellow
color due to the presence of fluorescent CdS NPs while insignificant values of $a^*$ can be attributed to the absence of red color contrary to the Au NPs films.

**Figure 5.4** (a) and (b) Color coordinates of 2% and 1% starch films made without and with different concentrations of Au NPs, respectively. (c) Similar plots of 1% starch films made without and with different concentrations of CdS NPs.

**5.1.3 Microscopic and XRD studies**

TEM studies help us to understand the morphology of the Au and CdS NPs. Figure 5.5a,b shows the TEM images of purified Au NPs (see experimental) synthesized with 1 and 2% of starch, respectively, with corresponding EDS spectra indicating the emission due to Au (Figure 5.5c,d). NPs of polyhedral shapes with comparable sizes of $22 \pm 9$ and $25 \pm 12$ nm, respectively, are evident. Polyhedral shapes suggest that starch is though good stabilizing agent but is not a good shape
Figure 5.5 (a) and (b) TEM micrographs of Au NPs synthesized at 70 °C in the presence of 1 % and 2 % starch, respectively, along with HAuCl$_4$ = 0.25 mM. (c) and (d) represent corresponding EDS spectrum of TEM images (a) and (b).

directing agent. The latter property stems from an effective liquid-solid interfacial adsorption of a stabilizing agent, and that is not expected from starch due to its weak amphiphilic behavior. However, starch forms a good amorphous film with entrapped NPs in a relatively less purified sample (Figure 5.6). On the other hand, CdS NPs of about 20 nm exist in the form of small interconnected clusters of about 100 nm (Figure 5.7a). Close inspection (Figure 5.7b) suggests that each cluster is made up of 6 - 10 NPs. XRD patterns of Au NPs (Figure 5.8a) with and without starch films are sharp and hence indicate the crystalline nature of NPs with predominant growth at {111} crystal planes of fcc geometry of Au. Likewise, XRD
patterns of CdS NPs (Figure 5.8b) represent crystalline cubic structure with more prominent peaks for NPs in purified suspension rather than NPs in films. Peaks arising at 17° and 22° scattering angles belong to conjugated amorphous starch$^{206-208}$ and are more prominent for Au NPs films rather than CdS NPs films because Au NPs are synthesized in the presence of starch as a weak reducing agent whereas CdS NPs

Figure 5.6 TEM micrographs of less purified sample of Au NPs synthesized at 70°C in the presence of 2% starch along with HAuCl$_4$ = 0.25 mM.

Figure 5.7 (a) and (b) TEM micrographs of CdS NPs synthesized at 150°C in glycerol medium.
are prepared in the glycerol medium (see experimental). Due to the predominantly amorphous nature of starch films, it is difficult to quantify the effect of NPs on the crystalline arrangement of amylose during the film formation which usually exists in B-type polymorph. The presence of NPs (of ~ 12 - 24 nm) is expected to affect the overall dense double helical arrangement of starch made up of thin lamellar domains of about 4.5 nm thick by intercalating themselves. This effect is expected to be more
Figure 5.9 (a) and (b) AFM images of 1% starch film with and without Au NPs, respectively, showing the surface topography. (c) and (d) are the respective height images along with a surface plot (e). See details in the text.
prominent for Au rather than CdS NPs because Au NPs have been synthesized *in vitro*. We will correlate this aspect with mechanical properties later in the discussion section.

AFM studies further help us to understand the surface roughness of the starch films. Figure 5.9a shows the topography of NPs embedded starch film made with 1% starch. Panel b compares its control i.e. without NPs. Marked difference can be observed between the topography of two films. Topography of the film incorporating NPs (panel a) clearly shows the presence of peaks and valleys due to embedded

![AFM images](image_url)

**Figure 5.10** (a) and (b) AFM images of 2% starch film with and without Au NPs, respectively, showing the surface topography. (c) and (d) are the respective height images.
NPs whereas that of control (panel b) is relatively much smooth with no sign of peaks. Panels c and d show the respective height images of the films where one can see the presence of groups of embedded NPs in panel c whereas again panel d gives an almost flat surface. Panel e provides the three dimensional image with approximate height slightly more than 100 nm due to the embedded NPs, which is obviously expected in view of the thick coating of several layers of starch on NPs. Similar behavior is demonstrated by the film made with 2 % starch (Figure 5.10).

5.1.4 DSC studies

In view of the applications of present starch films in food and pharmaceutical industries as packaging materials, their thermal properties need to be evaluated. Each film gives two endothermic peaks both in the absence as well as in the presence of NPs (Figure 5.11). The first broad peak around 60 °C (Table 5.1) is considered to be due to the loss of entrapped water molecules in liquid crystalline phase of predominantly amylopectin region. The broad nature of the peak is related to slow release of the entrapped moisture with a steady increase in temperature and accompanied by a relatively little amount of weight loss (see corresponding dotted box in Figure 5.12) that increases with the amount of starch (i.e. 3, 8, and 15 %)
Table 5.1 Transition temperatures and endothermic enthalpies of starch films without and with Au and CdS NPs.

<table>
<thead>
<tr>
<th></th>
<th>Peak$_1$ T (°C)</th>
<th>ΔH$_1$ (J/g)</th>
<th>Peak$_2$ T (°C)</th>
<th>ΔH$_2$ (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 % starch</td>
<td>60.7</td>
<td>258.9</td>
<td>285</td>
<td>177.94</td>
</tr>
<tr>
<td>1 % starch</td>
<td>59.9</td>
<td>193.7</td>
<td>303</td>
<td>106.8</td>
</tr>
<tr>
<td>2 % starch</td>
<td>60.8</td>
<td>190.2</td>
<td>303</td>
<td>91.22</td>
</tr>
<tr>
<td>0.5 % starch + 1mM Au NPs</td>
<td>60.14</td>
<td>336.5</td>
<td>273</td>
<td>230.6</td>
</tr>
<tr>
<td>1 % starch + 1mM Au NPs</td>
<td>62.71</td>
<td>280.3</td>
<td>289</td>
<td>188.5</td>
</tr>
<tr>
<td>2 % starch + 1mM Au NPs</td>
<td>59.76</td>
<td>229.3</td>
<td>287</td>
<td>176.4</td>
</tr>
<tr>
<td>0.5 % starch + 1mM CdS NPs</td>
<td>60.7</td>
<td>352.8</td>
<td>279</td>
<td>58.60</td>
</tr>
<tr>
<td>1 % starch + 1mM CdS NPs</td>
<td>61.2</td>
<td>297.3</td>
<td>283</td>
<td>56.40</td>
</tr>
<tr>
<td>2 % starch + 1mM CdS NPs</td>
<td>70.6</td>
<td>179.4</td>
<td>267</td>
<td>24.17</td>
</tr>
</tbody>
</table>
weight loss for 0.5, 1, and 2 % starch, respectively, for the films without NPs). Likewise endothermic enthalpy (ΔH) decreases with the increase in the hydration in the order of 0.5% > 1% > 2% starch (Table 5.1). In the presence of NPs, all peaks are more prominent and again appearing close to 60 °C but with higher ΔH values (Table 5.1) with comparatively less weight loss in comparison to that in the absence of NPs. Stabilization of colloidal NPs in aqueous starch solution can be viewed in terms of DLVO theory\textsuperscript{211,212} where electrostatic interactions between the starch hydroxyl groups and the charged nanometallic surface plays a predominant role over the cohesive interactions due to van der Waals forces between the NPs. The former will replace previously associated water molecules in starch superhelix to provide necessary colloidal stability that in turn reduces the effective amount of entrapped moisture in comparison to that in the absence of NPs. Hence, the films thus produced in the presence of NPs contain less amount of moisture with relatively greater amount of crystallinity.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure512.png}
\caption{Weight loss plots of starch films made with 0.5, 1, and 2 % starch without and with Au NPs.}
\end{figure}

The second peak appearing at > 260 °C (Figure 5.11) is due to the melting of starch chains followed by the decomposition of glucose rings under the effect of thermal decomposition\textsuperscript{206,213} which is expected to release water most probably due to
condensation among the neighboring –OH groups of predominantly hydrophilic amylopectin and even the formation of C=C. That is why the corresponding overall transition temperature is much higher than the first peak, and even higher for 1 or 2 % in comparison to 0.5 % starch (Table 5.1). Likewise ΔH values are much lower than the corresponding first peak with the same trend. In the presence of NPs, we obtained slightly lower transition temperature in comparison to their absence with higher ΔH values. Although the ultimate weight loss (i.e. ~ 80 %) is more or less the same in the absence as well as in the presence of NPs, the major difference is in their slopes (corresponding dotted area in Figure 5.5b). For pure starch films, the slopes are relatively steeper than in the presence of NPs, suggesting a rapid decomposition of glucose rings in the absence of NPs. Complexation with glucose rings makes this process relatively slower. Similar behavior is observed for CdS NPs embedded starch films (Figure 5.13).

![Figure 5.13](image-url)

*Figure 5.13 Heat flow scans of starch films made with 0.5, 1, and 2 % starch with CdS NPs.*
5.1.5 Mechanical properties

Mechanical properties help us to understand the workability and applicability of starch film and are best understood in terms of tensile strength and strain at failure (flexibility). Figure 5.14a,b demonstrate both properties for 1 and 2 % starch in the absence and presence of Au NPs. Tensile strength decreases with increasing amounts of Au NPs while flexibility improves with 0.25 mM NPs but decreases thereafter. Decrease in the tensile strength can be correlated with a higher degree of crystallinity in the presence of NPs as evident from the thermal properties (Figures 5.11 and 5.12). However, increase in the flexibility with low amount of NPs i.e. 0.25 mM can be attributed to a decrease in the hydrogen bonding which is responsible for the superhelix arrangement among starch macromolecules. Greater hydrogen bonding generates stronger inter-helical interactions with less flexibility and vice versa. Low amount of NPs can be accommodated in the mesh of interconnected starch macromolecules (strands) without significantly disturbing their helical arrangement but a greater amount of NPs on the other hand disturbs the superhelix arrangement thereby decreases the flexibility.

![Figure 5.14](image-url)

Figure 5.14 (a) Tensile strength and (b) strain at failure plots of 1 and 2 % starch films embedded with Au NPs versus the amount of NPs.
Presence of CdS NPs dramatically improves the mechanical properties. A significant increase in the tensile strength (Figure 5.15a) and flexibility (Figure 5.15b) is observed in the CdS NPs incorporated starch films. Both tensile strength as well as flexibility increase with the increase in the amount of CdS NPs before tending to a constant value and are higher for 2% starch than 1%. This remarkable difference from that of Au NPs incorporated films might have arisen from a different mode of synthesis of CdS NPs. Synthesis of CdS NPs has been carried out in the presence of glycerol medium, thus glycerol stabilized starch coated CdS NPs help to provide greater stability and flexibility.

\[\text{Figure 5.15 (a) Tensile strength and (b) Strain at failure plots of 1 and 2 \% starch films embedded with CdS NPs versus the amount of NPs.}\]

5.2 Discussion

Present results demonstrate a systematic way of synthesizing NPs incorporated biodegradable starch films. We want to show that the presence of NPs makes a significant difference in the strength and flexibility of starch films for their industrial applications in comparison to the films simply made with only starch (without NPs). We are mainly looking for their versatile applications in the packaging and pharmaceutical industries where biodegradable starch films should provide required strength, moisture barrier, and be fluorescent active for easy detection. Our results
show that a systematic synthetic method as monitored by the UV-visible studies (Figure 5.1a-c) can be easily applied to produce starch stabilized Au NPs, which can simultaneously be incorporated into the starch films. Such starch films are UV-visible active due to the presence of Au NPs (Figure 5.2a,c). Similar films can also be produced by incorporating fluorescence active CdS NPs and for their synthesis a different route has to be followed in the glycerol medium. These films are highly fluorescent due to the quantum confinement effects of nanosized CdS NPs. Color coordinates studies further support the uniform distribution of CdS NPs throughout the starch film.

Both Au as well as CdS NPs are metallic in nature as evident from the XRD studies (Figure 5.8a,b) and their incorporation in the superheliix arrangement of starch (Figure 5.16) induces significant influence on the mechanical properties of the starch films. Au NPs though decrease the mechanical strength, their low amount increases the flexibility, while CdS NPs significantly increase the tensile strength as well as flexibility. Keeping in view of their almost similar dimensions, it all seems to be due to the different methods used in their synthesis. We believe that the stabilization of glycerol coated CdS NPs proves to be a better option rather than only starch coated Au NPs. Although glycerol has also been used as plasticizer in Au NPs incorporated starch films, the lower tensile strength of such films in comparison to that of CdS NPs suggest poor intercalation of glycerol molecules in the double helical arrangement of starch macromolecules. Therefore, NPs alone cannot increase the tensile strength as well as flexibility, it is the glycerol stabilized NPs which provide additional strength and flexibility.

In order to understand it further, we need to first understand the role of glycerol as plasticizer. Glycerol is a polyol with three hydroxyl groups. Addition of glycerol in starch reduces the direct hydrogen bonding between the different chains of starch macromolecules by incorporating among them. This increases the inter-helical distance among different chains and increases the flexibility. When such places are occupied by the glycerol stabilized NPs whose dimensions are no doubt much greater than glycerol molecules, distances between the helical chains are further increased which in turn contributes towards even greater flexibility (Figure 5.16). Since the Au NPs are synthesized in the absence of glycerol medium (in the presence of aqueous
Figure 5.16 A schematic representation showing glucose units joined together by glycosidic bonds to construct a superhelix layered structured interlocked by hydrogen bonding. Lower part of the diagram shows intercalated starch capped Au NPs and glycerol capped CdS NPs among different layers. See details in the text.
starch), therefore they are expected to be already coated with predominantly crystalline starch (Figure 5.16) because the reactions are conducted at 70 °C where substantial dehydration is expected. This leaves little possibility for glycerol molecules to intercalate effectively in the already predominantly crystalline starch coating unlike to that of CdS NPs which are synthesized only in the glycerol medium. Thus, NPs previously stabilized by glycerol prove to better candidates for inducing greater flexibility among starch superhelix chains in comparison to NPs already stabilized by predominantly crystalline starch. This seems to be the key step in increasing both tensile strength as well as flexibility of starch films. Furthermore, the nature of NP surface whether it is metallic or semiconductor does not matter. It only matters if NPs are properly coated and stabilized by glycerol that in turn produce films with better tensile strength and flexibility for various industrial applications.

5.3 Concluding Remarks

We have presented a simple method of synthesis of environmental friendly biodegradable starch film containing Au and CdS NPs with remarkable mechanical properties such as tensile strength and flexibility for various industrial applications. Results suggest that a uniform distribution of Au and CdS NPs practically improve their mechanical properties in comparison to starch films made without them.

Intercalation of NPs in the helical arrangement of starch reduces hydrogen bonding and increases the interhelical distances that in turn increases the flexibility. Glycerol capping of the NPs is considered to be the key factor in enhancing the tensile strength and flexibility of starch films due to its plasticizing nature. Nanoscale intercalation of the glycerol capped NPs significantly increases the flexibility by allowing glycerol to interact simultaneously with different helical chains and increasing interhelical distances. This effect is very much clear when glycerol stabilized NPs are used. These films can also be made UV-visible and fluorescent active if NPs which can absorb in these regions such as Au and CdS NPs.