Peptide assisted synthesis and functionalization of gold nanoparticles and their adsorption by chitosan particles in aqueous dispersion

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Abstract

We have reported a novel method of synthesis of gold nanoparticles (GNPs), using two different peptides, e.g. glutathione (GSH) and glycyl-glycine (GG), as reducing agents. The formation of GNPs was observed with the development of the surface plasmon resonance (SPR) peak in UV-visible spectrum. The nanoparticles phase has been investigated using powder x-ray diffraction (XRD) method and has been seen to be single phase. The as-synthesized GNPs were not fully covered by the used peptides as seen by the thermogravimetry analysis (TGA), and therefore, trisodium citrate (TSC) has been used further as a ‘filler’ agent for GNPs to become well dispersible in aqueous medium. The Fourier transform infrared (FTIR) spectroscopy method has confirmed the presence of peptides and TSC coatings on the nanoparticles’ surface. In comparison, the GNPs formed using GG have been observed to be more stable than those formed using GSH. The nanoparticle size was measured using XRD, dynamic light scattering (DLS) and transmission electron microscopy (TEM). These dispersions were further used to investigate the interaction between the GNPs and chitosan (CS) microparticles. The effects of this interaction were studied using UV-visible spectroscopy, DLS and FTIR. XRD and TEM showed that GNPs were uptaken by CS microparticles.

Keywords: glutathione, glycyl-glycine, gold nanoparticles, plasmon resonance, light scattering, chitosan

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1. Introduction

Biomolecules-assisted synthesis and stabilization of metal nanoparticles, in particular gold and silver nanoparticles, are having immense potential applications in widespread areas of research [1–3]. Apart from the availability of numerous methods for the formation of colloidal gold nanoparticles (GNPs) using conventional organic and inorganic reducing
agents [4–8], utilization of biological systems is inspired due to the capability of non-toxic, non-hazardous and environmental friendly green approaches [2]. There are still growing needs in synthesizing or conjugating GNPs using different peptides and other biologically important molecules as templates in order to build new hybrid structures with an ability to carry out multifarious tasks [9–18]. Cell penetrating peptides are being utilized to functionalize GNPs to improve internalization [19, 20]. The act of capping GNPs with numerous functional peptides marks it as a potential protein model demonstrating biosensing, bioimaging and enzymatic reactions [21, 22]. It has also been shown that gold nanocrystal orientations could be maneuvered using polypeptides [23]. In a nutshell, the importance of synthesizing biomolecules capped in organic nanostructures (in particular gold) had been proved for its viability towards bio-availability [24, 25].

Glutathione (GSH), a master antioxidant present in many living forms, consists of glutamic acid, cystine and glycine units. The presence of carboxyl functionality in GSH facilitates the coupling possibility with folic acid functionalized GNPs in treating and detecting tumor cells [26]. Important biomolecules like DNA and some proteins are being utilized as molecular linkers anchoring GNPs with respect to a certain motive of fabricating plasmonic nanostructures, colloidal networks which induce colloidal crystallization, etc, having in mind the beauty of surface plasmon resonance (SPR) sensitivity [25, 27–29]. Simple peptides like glycyglycine (GG) has been used as templates for the preparation of single-crystalline metal nanostructures [30].

Chitosan (CS), a second most abundant polymer, is a non-toxic and non-immunogenic, biodegradable natural polysaccharide that has the ability to act as a matrix to release the entrapped drugs [31] and also possesses the capability of acting as a reducing and stabilizing agent [32–34]. CS nanoparticles, even though they have numerous methodologies of synthesis (and the references therein [35]), can be easily achieved via ionic gelation [36] by utilizing a polyamionic molecule, namely sodium tri-polyphosphate (Na-TPP). CS nanoparticles have also proved to be a potential candidate in drug delivery and targeting applications [37]. CS nanoparticles along with embedded gold nanostructures, generated within the CS nanospheres with an external reducing agent [38], prove to be efficient multifunctional carrier vehicles [39]. Tying up the individual functionalities of two different systems into a single hybrid unit that is expected to stand apart from its parents could gain strength in performing simultaneous functions which can be utilized to advance biomedical applications. Generation of multifunctional hybrid units also gives us the responsibility of investigating their interactions within each other, thereby understanding the system in whole before any major implementation.

In the present work, we report a novel method for the synthesis of GNPs with the aid of GSH and GG. The reducing capability of the peptides and the stability of the GNPs were compared and discussed. The choice of the reducing peptides in the preparation of gold nanocolloidal solutions is due to the multidimensional applicability of the peptide coated GNPs and the effective roles of the peptide as such. Yang et al [30] discussed the formation of single crystalline silver nanoplatelets using GG as a template. Baruwati et al [40] used GSH to synthesis silver nanoparticles under microwave irradiation for producing spherical silver nanostructures of broad size distribution. Negishi et al [41] reported the investigations of GNPs capped with GSH. There are numerous reports on the formation of gold-polymer complexes and studies on the capping of GSH to GNPs [42–44]. The utilization of GSH and GG as reducing agents for the synthesis of GNPs has not been reported earlier for the formation of stable gold colloidal solution.

GNPs-entrapped CS microsphere has been studied in order to utilize it as an enhanced contrast flow tracer in dynamic x-ray imaging [45]. It has also been a focus of interest in developing advanced materials [45, 46].

2. Experimental details

GSH (GSH, $\geq 98.0\%$) and GG (GG, $\geq 99.0\%$) were purchased from Aldrich. Cholorauric acid (HAuCl$_4$, $\geq 97.0\%$), trisodium citrate (Na$_3$C$_6$H$_5$O$_7$, AR grade), and sodium tri-polyphosphate (Na-TPP, anhydrous) were purchased from Loba Chemie. Glacial acetic acid (99.99%), nitric acid and hydrochloric acid were purchased from Merck. CS had been gifted from the farm of Indian sea foods with a degree of deacetylation (DD) of 81%. To further purify, we dissolved this as-gifted CS powder in acetic acid and precipitated it with sodium hydroxide. The precipitate was centrifuged and dried for further use. Milli-Q water (18.2 MΩ) was utilized for sample preparations throughout the investigations.

2.1. Synthesis of GNPs

A novel one-pot method of synthesis of GNPs with the aid of GSH, a tripeptide and GG, a simple dipeptide, is reported. The synthesis procedure is briefly described. First, 1.78 mg of chlororauric acid (HAuCl$_4$) was dissolved in 20 ml milli-Q water and was sealed tightly with a small opening at the top of the round-bottomed flask. GSH and GG solutions of different concentrations, e.g. 0.15, 0.50 and 0.75 mM, were prepared and kept as stocks.

2.1.1. GSH as a template for GNP formation (GSH–Au)

HAuCl$_4$ solution was heated at 150 °C for about 2 min and then a pre-prepared cold GSH solution was added to it, in volume ratio HAuCl$_4$–GSH = 5 : 1. The mixture was homogenized by gentle shaking and then left unstirred at 150 °C for about 10 min. As the reaction progressed the solution underwent a series of color changes. It is to be noted here that the final solution appeared to be wine red, indicating the formation of GNPs [45]. The wine red colored GNP solution was kept at 4 °C in a dark environment to prevent coagulation.

2.1.2. GG as a template for GNP formation (GG–Au)

To the boiling GG solution pre-prepared HAuCl$_4$ solution was added immediately, in volume ratio HAuCl$_4$–GG = 1 : 5 and was left on the heating compartment unstirred for about 20 min. The solution underwent a single and spontaneous
color transformation, from achromatic appearance to pale wine red within 15 min, indicative of the formation of gold colloids [47]. The as-prepared GNP dispersion (wine red) was further mixed with 1% (w/v) of TSC aqueous solution (in 1:2 (v/v) ratio) to increase the stability of the colloidal dispersion. The citrated GNPs will be referred as CGNPs hereafter.

2.2. Preparation of CS microparticles

The formation of CS microparticles using Na-TPP is described elsewhere [35]. Briefly, 0.6 ml of 1% (w/v) Na-TPP was added to the 0.1% (w/v) of purified CS aqueous solution prepared using 1% of glacial acetic acid. To form CS microparticles, hereafter referred as CSM, the Na-TPP solution was added directly to the CS solution. The CSM dispersion was clear and no precipitation was observed. To study the interaction between CS and GNPs, solutions of CS and CGNP were mixed in 1:1 (v/v) ratio. This solution was further diluted in milli-Q water for experimental purposes.

2.3. Characterization techniques

A UV-visible spectrophotometer (Shimadzu UV 2450) equipped with quartz cuvette of path length 1 cm was used for performing optical absorption measurements. Dynamic light scattering (DLS) experiment was performed using a home-built setup [48, 49]. The incident vertically polarized beam of 1 mm diameter was from a 100 mW He-Ne laser source ($\lambda = 532$ nm) and was further focused on the sample using an optical plano-convex lens. The scattered beam from the sample is passed through a vertical polarizer and was detected by a photomultiplier tube (PMT) at 90° with respect to the incident beam direction. Cleaned cylindrical quartz cell of diameter 10 mm containing the dispersion was placed inside a glass cuvette consisting of index matching liquid (trans-decline, $\mu = 1.49$) fixed at the centre of the scattering plane. Scattering signal from PMT was amplified and digitized through several electronic circuits and fed to a 256 channel digital correlator (version 7132, Malvern, UK). The correlator card generated the relaxation curve used to determine the particle hydrodynamic diameter ($D_h$). The x-ray diffraction (XRD) patterns for dried CGNPs and CGNP-interacted CSM were obtained using a powder x-ray diffractometer, Bruker D8 Advance. Fourier transform infrared (FTIR) (Shimadzu IR Affinity 1) of 0.5 cm$^{-1}$ resolution was used to study the nanoparticle surface functionalization and the surface coating quantity was measured using a thermo-gravimetric analysis (TGA) TA instrument, model 2960 a DSC-TGA. The transmission electron microscopy (TEM) measurements were carried out.
Figure 2. (a) Absorbance spectrum of GNPs formed using GG template (inset) digital photographs of the colloidal solution before and after the reduction process; (b) absorbance spectrum showing coherence in the $\lambda_{\text{SPR}}$ position for both peptide (concentration 0.50 mM); (c) distribution of $\lambda_{\text{SPR}}$ for different molar concentrations of both peptides.

using a high resolution transmission electron microscope (HRTEM, model FEI Technai G230).

3. Results and discussion

The reductive activity of GSH \[50\] is formally represented in the following equation

\[
\text{GSH} \leftrightarrow \left( \frac{1}{2} \right) \text{GSSG} + e^- + H^+ ,
\] (1)

where GSSG denotes glutathione disulfide. In its thiolate form GSH is a potential chelating agent which can facilitate the redox reactions. The aqueous HAuCl$_4$ solution upon heating dissociates into gold chloride ions (Au(III)Cl$_3^-$) and HCl \[51, 52\] as follows:

\[
\text{HAuCl}_4 \xrightarrow{\Delta (150^\circ \text{C})} \text{Au(III)Cl}_3^- + \text{HCl}
\] (2)

The formation reaction of GNPs is shown in the following equation

\[
3\text{GSH} + \text{Au(III)Cl}_3^- \xrightarrow{\Delta (150^\circ \text{C})} \text{Au}^0 + \left( \frac{3}{2} \right) \text{GSSG} + 3\text{HCl},
\] (3)

where Au$^0$ is nucleated into metallic crystallites covered by a monolayer of GSSG adsorbate groups.

GSH acted as the source of electron to facilitate the reduction. Similarly, GG provides reduction of dissociated \( \text{Au(III)Cl}_3^- \) ions to spherical \( \text{Au}^0 \) colloidal particles with stabilization as in the following equation

\[
\text{GG} + \text{Au(III)Cl}_3^- \xrightarrow{\Delta (150^\circ \text{C})} (\text{GG} - \text{Au}^0) + \text{HCl}.
\] (4)

But reduction in the case of GG template may be initiated due to the amine functionality of the peptide since these peptides could facilitate intermolecular hydrogen bonding networks \[51\] \( \text{C} - \text{O} \cdots \text{H} - \text{N} \) with the neighboring molecules.

The formation of GNPs in the presence of 0.50 mM GSH was studied using the UV-Vis absorbance spectrum, where the development of the characteristic SPR absorption peak for gold was observed with time, as shown in figure 1(a). The increase in SPR peak intensity and shift in peak position with time are plotted in figure 1(b). The intensity variation with time could be fitted with the power law equation, \( A(t) \sim t^\alpha \), indicating the nucleation and growth behavior \[53\].

The curve showed saturation in less than 10 min, indicating the completion of reaction (formation of \( \text{Au}^0 \)) with the final color of the solution as wine red. All intermediate colors of the solution during the reaction process are also shown in figure 1(c). Even though the reaction time for the formation of GNPs using GG was comparable to the GSH system, the course of the reaction did not follow any successive color change rather than a spontaneous color.
Figure 3. FTIR spectra of (a) pure GSH, (b) as prepared GNP, and (c) CGNP. Modes due to N–H and S–H bond stretching are shown in (a). The absence of the S–H stretching mode in (b) indicates the formation of GSSG on GNP, (a) pure GG, (b) as prepared GNP using GG.

Figure 4. XRD pattern of dried as-prepared GNPs (a), GSH template and (b) GG template.

transformation, from colorless to light pink at the final stage of the reaction (shown as an inset in figure 2(a)). There was no considerable shift of the SPR peak position of GNPs formed using GSH and GG, with 0.50 mM peptide concentration, as in figure 2(b). It is also seen that the SPR position of the GG–Au is red shifted when compared with the GSH-Au colloids for concentration other than 0.50 mM, indicating that the utilization of GG yields higher particles sizes when compared with GSH, on other similar peptide concentrations (see figures S1 and S2 in supplementary data available from stacks.iop.org/ANSN/3/045010/mmedia). The distribution of the SPR peak positions (λ_{SPR}) for different peptide concentration is shown in figure 2(c).

It is also to be noted here that the addition of 0.15 or 0.75 mM GSH solution to HAuCl₄ solution showed SPR peak initially (see figure S3 in supplementary data available from stacks.iop.org/ANSN/3/045010/mmedia), but dropped in intensity suddenly indicating that other concentrations (i.e. 0.15 and 0.75 mM) of GSH were capable of initiating the reduction, but not appropriate for the formation of stable GNPs. GG–Au NPs solution showed longer stability than GSH–Au NPs. The colloidal gold solution formed using GSH was stable for about 3 days and afterwards started to agglomerate, which is conclusive from the observed red shift (see figure S3 in supplementary data). But GG acts as an effective stabilizer for GNPs when compared with

Figure 5. Typical autocorrelation function of as-prepared GNP dispersion; the solid line shows the monomodal fitting. The inset shows the size distribution of GNP with mean diameter around 18 nm.

GSH was stable for about 3 days and afterwards started to agglomerate, which is conclusive from the observed red shift (see figure S3 in supplementary data). But GG acts as an effective stabilizer for GNPs when compared with
GSH (see supplementary data). The longer stability of the GG–Au might be due to better coating of GG ligand for capping GNPs. By varying the concentration, GG was found to initiate the reduction but was not capable of stabilizing the gold colloids. Thus, the formed colloids instantaneously adsorbed on the walls of the reactions vessel leaving a dark black solution with some dark sediments, whereas by inverting the GSH volume in synthesizing GNPs it was found to be ineffective in initiating the process of reduction (see figure S4 in supplementary data available from stacks.iop.org/ANSN/3/045010/mmedia), the CGNP solution was stable for more than a month. The CGNP was preserved at 4°C and was used to study the interaction with CSM.

Powder XRD scan on dried GNP powder was obtained using both the peptides in the 2θ range between 30° and 70° with step size 0.05° and counting time per step 10 s. The corresponding pattern is shown in figure 3. The 100% peak of Au (111) appeared at around 38.19°, for GSH–Au powder and 38.17°, for GG–Au coated glass slide of 2θ which agrees with an earlier report [52, 54].

The crystalline size of nanoparticles was calculated after correcting the instrumental broadening effect to the full-width at half-maximum (FWHM) of (111) peak using the Scherrer formula [55]

$$d = \frac{0.94\lambda}{\beta \cos \theta_B},$$  (5)

where $\lambda$ is the x-ray beam wavelength (= 1.54 Å for Cu Kα), $\beta$ is the FWHM of the Bragg peak and $\theta_B$ is the peak position. The diameter $d$ was calculated to be around 15 nm for GSH–Au and 18 nm for GG–Au, which matches well with UV-visible result and is in close agreement with DLS and TEM results. This might suggest that the GNPs synthesized by us were single crystalline. The observed noise in the GG–Au sample could be due to the smaller quantity of the obtained gold colloidal solution which results in discrete coating on the substrate surface.
Figure 4 shows the FTIR spectra of GSH–Au and GG–Au: pure GSH (figure 4(a)), as-prepared GNPs (figure 4(b)), and citrate conjugated GNPs (figure 4(c)). The modes appearing at 3346, 3251, 3128 and 3026 cm\(^{-1}\) (curve a of figure 4) correspond to the N–H and that at 2525 cm\(^{-1}\) corresponds to the S–H stretching vibrations of pure GSH. Detailed description of different IR modes for GSH is given elsewhere [56]. The molecular structure of GSH is shown in the figure for better understanding. Reduction in intensity with broadening and shifting of 3346, 3251 and 2926 cm\(^{-1}\) modes in curve b indicates the hydrogen bonding interactions between nearby GSH molecule [57]. Disappearance of the stretching vibration of S–H in curve b indicates the formation of GSSG dimer through S–S bonding, as given by equation (3), which is anchored on to the GNP surface. Therefore, curve b shows the FTIR spectrum of GSSG alone [56] and no chemical bonding of GSSG with Au is evidenced. A cartoon of the corresponding GNP–GSSG conjugate is shown in the figure. Curve c shows the FTIR spectrum of citrate functionalized GNP–GSSG complex. Clearly, curve c broadly does not show any extra peak or peak shift indicating no chemical bonding of citrate with either GSSG or GNP. TSC is an amphiphilic molecule which gets ionized in aqueous solvent. The citrate chain, being the hydrophobic component, was hydrophobically anchored on the GNP surface. The corresponding cartoon of GNP–GSSG–citrate complex is shown in the figure. Similarly, the appearance of 3286, 1158, 3033 and 3067 cm\(^{-1}\) peaks [58] corresponds to the binding of N–H and symmetric and asymmetric stretching modes of NH\(_2\) as shown in figure 3(a). The reduction in the intensity and the broadening confirms the formation of C–O…H–N hydrogen bonding [51], figure 4(b) with the nearby molecules, which also could indicate that NH may be the active participant in the reduction process.

To evaluate the amount of GSH molecules on GNPs we performed TGA on dried GNP powder synthesized using both peptides. The weight loss between 125 and 200 °C (see figure S6 in supplementary data available from stacks.iop.org/ANSN/3/045010/mmedia) due to decomposition of GSH (decomposition temperature ∼192–195 °C) was measured to be approximately 9%. The calculated weight loss for GSH molecules sitting vertically on the surface of 18 nm nanoparticles is 11%. The difference may indicate that GNP surface was not completely covered by GSSG (see the cartoon in figure 2) and the nanoparticles tend to agglomerate after some time as observed in UV-visible study. On the other hand, citrates being smaller molecules diffused through gaps and anchored hydrophobically on the GNP surface as shown by the cartoon in figure 3 and the nanoparticle surface was completely covered. Thus, CGNP dispersion was more stable as mentioned earlier. Whereas the weight loss between 250–290 °C (see figure S7 in supplementary data available from stacks.iop.org/ANSN/3/045010/mmedia) is due to the decomposition of GG, decomposition temperature is ∼262–264 °C. The GG capping percentage on to the surface of the 4 nm GNPs was found to be 17%. The evidence of the formation of hydrogen bonding interaction with the neighboring molecules of GG and the increased capping density of GG on the surface of GNPs confirms that GG groups around the formed GNPs provide stabilization, sterically for a long period as compared with GSH. It could also be clearly noted from the effective concentration for the reduction of GNPs is large for GG when compared with GSH, which could also be an added advantage for the prolonged stability of the colloidal solution.

We measured the size of CGNP using DLS method. The DLS autocorrelation function, \(g^1(\tau)\), for monodispersed, spherical and non-interacting particles is given by

\[
g^1(\tau) = \int dt \, F(\Gamma) \exp(-\Gamma \tau),
\]

where \(\Gamma\) is the particle relaxation rate (s\(^{-1}\)) and is equal to \(q^2D\), \(q\) being the scattering wave vector (\(q = 4\pi n \sin(\theta/2)/\lambda\), \(n\) is the refractive index of the solvent, \(\theta\) is the scattering angle and \(\lambda\) is the wavelength of the incident laser beam) and \(D\) is the particles’ collective translational diffusion co-efficient, \(\tau\) is the delay time. \(F(\Gamma)\) gives the distribution function of \(\Gamma\), i.e. \(D\), which in turn gives the particle size distribution. The
Particle's hydrodynamic diameter \( d_h \) is calculated using the Stokes–Einstein relation \([59, 60]\)

\[
D_h = \frac{k_B T}{3\pi\eta d_h},
\]

where \( k_B \) is the Boltzmann constant, \( T \) is the solution temperature in Kelvin, \( \eta \) is the solvent viscosity at \( T \) and \( D_h \) is the particle's translational diffusion coefficient at zero solution concentration.

Figure 5 shows a typical autocorrelation function, \( g^1(\tau) \), obtained from GNPs dispersion (dots) and the solid line shows the fitting with the monomodal distribution function given in equation (1). The good fitting to experimental points indicates that nanoparticles were monodispersed and spherical. The inset of figure 5 shows the corresponding size distribution of GNPs with mean hydrodynamic diameter \( d_h \) around 18 nm, which correlates well with the size obtained using XRD. It is to be noted here that the simulated Mie plot for monodispersed spherical gold particles of 18 nm diameter has \( \lambda_{SPR} \) peak position at 523 nm, whereas we have observed the final SPR peak position at 530 nm (see figure S5 in supplementary data available from stacks.iop.org/ANSN/3/045010/mmedia). This difference could be due to the association of ligands (i.e. GSSG and GG) with GNPs. Alternately, the particle size for \( \lambda_{SPR} \sim 530 \text{ nm} \), calculated using an empirical relation reported by Haisn [61], was 16 and 25 nm for GNPs reduced using both peptides, which matches well with the particle size measured using DLS (see table S1 in supplementary data for nanoparticle size measured using different techniques, for both peptides available from stacks.iop.org/ANSN/3/045010/mmedia). As far as TEM image of the GSH-assisted as-prepared GNPs is concerned, the inset of figure 6 shows the corresponding histogram of particle size distribution. The particle size distribution seems to be narrow with maximum around 17.5 nm which is in agreement with the DLS result, but the TEM micrographs (figure 7) of GG–Au show the binary distribution of particles of size around 4 and 20 nm. This colloidal dispersion was found to be stable for a long period, as indicated by the absorbance spectrum (see supplementary data).

As mentioned earlier, GNP surface was further modified by TSC to increase dispersion stability and to facilitate favorable interaction between GNPs and CS. To study the interaction of GNPs with the CS particles, we mixed 100 \( \mu \text{L} \) of CSM dispersion (5\% v/v) and 100 \( \mu \text{L} \) of CGNP dispersion (0.25 mM, 5\% (v/v)) in 2000 \( \mu \text{L} \) of milli-Q water, so that the final ratio of CGNP to CS particles was 1:1. The solution was studied with time at 25°C using UV-Vis spectrum and DLS. Figure 8(a) shows the SPR peak (peak A) of CGNP at \( \lambda_{SPR} \sim 530 \text{ nm} \). Due to negative charges on CGNPs and positive charges on CSM, they interacted via Coulomb attraction, and as a result, CGNPs were uptaken by CSM particles. Consequently, the SPR peak intensity (peak B) was reduced, as indicated by the broken arrow; the gradual decrease of the peak intensity with time is shown in the inset of figure 8(a). The decrease of peak intensity with time is plotted in figure 8(b). The average hydrodynamic diameter \( D_h \) of pure CSM was measured using DLS to be around 1000 nm. After mixing CGNP and CSM in aqueous medium, the change in \( D_h \) with time was measured using DLS. It was observed that initially \( D_h \) decreased, and subsequently, increased with time. In the inset of figure 8(b) we have plotted the increase of \( D_h \) with time. By comparing both UV-visible and DLS results we could infer that the swelling (increase of \( D_h \)) started after adsorption of CGNP by CSM.

The CS micro-domains were initially compressed and subsequently swelled after adsorbing CGNPs. Simultaneously, an elastic stress could also develop in the bulk due to compression. The collapsed unstable structure will, therefore, try to relax to minimize its bulk energy. As a result the CSM swelled by reforming its network structure, accompanied by the diffusion of solvent (i.e. water) into it. The detail explanation of this compression and relaxation of CSM structure has been given in terms of energy equations in the supplementary information.

The FTIR vibrational spectra for the freeze dried CSMs and GNP adsorbed CSMs showed no difference.
Figure S8 in supplementary information available from stacks.iop.org/ANSN/3/045010/mmmedia) suggesting no change in network structure of CSM and no chemical bond formation between CGNP and CS. The broad peak between 3000 and 3800 cm\(^{-1}\) corresponds to O–H and N–H stretching vibrations, whereas peaks around 2867 cm\(^{-1}\) correspond to C–H stretching mode.

Figure 9(a) shows TEM image of CSM with entrapped CGNPs as black dots. Clearly, nanoparticles are uniformly distributed in the CSM. The entrapment of GNPs in CSM was also evidenced from the XRD pattern (see figure S9 in supplementary data available from stacks.iop.org/ANSN/3/045010/mmmedia). Figure 9(b) shows the HRTEM image of GNPs which clearly shows atomic planes (shown by arrow) due to (111) orientation with lattice spacing of 0.235 nm, the inset shows selected area diffraction (SAD) pattern showing amorphous background of CSM with three dots corresponding to three crystallographic directions (e.g., (111), (200) and (220)) in the GNPs as observed in XRD.

4. Conclusion

We have reported a novel synthesis of gGNPs using GSH and GG peptides. The reaction steps are explained. The reaction time was measured by monitoring the SPR peak of GNPs in UV-visible spectrum during the reaction. The nanoparticle size was found to be around 18 nm, for GSH–Au. The nanoparticles contain single gold phase. GG–Au colloidal suspension was found to be more stable than GSH–Au. The nanoparticle surfaces were further functionalized with TSC which increased the stability of the GNP dispersion and facilitated favorable interaction with CS in aqueous solution. GNPs diffused uniformly into the CSMs as evidenced from the TEM image.

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