Spectroscopic studies on the interaction of amino acids with gold nanoparticles

Thesis
Submitted in partial fulfillment for the award of degree of

Master of Science in Chemistry

By

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CERTIFICATE

This is to certify that the dissertation entitled, **"Spectroscopic studies on the interaction of amino acids with gold nanoparticles"**, being submitted by **Ms. Arshdeep Kaur** in partial fulfilment of requirements for the award of the degree of **Masters of Science** in **Chemistry** and being submitted to the School of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, Patiala is a bonafide work carried out by her under my supervision. The work has reached the standard necessary for submission, and the contents of this dissertation have not been submitted to any other university or institute for the award of any degree or diploma.

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CANDIDATES' DECLARATION

I, hereby, declare that the work being presented in the dissertation entitled **"Spectroscopic studies on the interaction of amino acids with gold nanoparticles"** in partial fulfilment of the requirements for the award of the degree of **Masters of Science** in Chemistry and being submitted to School of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, Patiala is my own research work carried out during the period of January to July 2018 under the supervision of **Dr. Mily Bhattacharya**. I have not submitted the contents embodied in this dissertation for the award of any degree elsewhere.

Arshdeep Kaur
Arshdeep Kaur

Date: 30/June/18

It is certified that the above statement made by the student is correct to the best of my knowledge and belief.

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Arshdeep Kaur

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ABSTRACT

Gold nanoparticles are biocompatible and exhibit unique chemical, electrical as well as optical properties which vary in a size-dependent manner. They have generated an enormous interest due to their implications in various bioanalytical applications. For instance, they are used in the colorimetric determination of proteins, protein kinetics, and protein modifications. However, the stability of gold nanoparticles is an area of concern since they are extremely prone to aggregation depending upon the solution conditions such as pH, ionic strength, etc. Hence, the nanoparticles need to be surface-modified with biomolecules such as proteins for their utility in bioanalytics. Also, a detailed, systematic understanding of the gold-biomolecule interaction is required to ascertain the molecular basis of small molecule-induced aggregation. In this thesis, we have investigated the interaction of gold nanoparticles with amino acids, the building blocks of proteins, that were chosen based on their acidic or basic side-chains. In order to probe the effect of nanoparticle size during amino acid-induced aggregation, two different sizes (8 nm and 13 nm) of gold nanoparticles were synthesized by the Turkevich method and the amino acids were either used in isolation or as a combination with varying ratios. Aggregation kinetics of gold nanoparticles were monitored by changes in the absorption spectra and surface plasmon band. We observed that only cysteine induced aggregation of gold nanoparticles at pH 5 whereas all the remaining amino acids rendered the nanoparticles water-soluble and monodisperse. Varying the ratio of cysteine to that of lysine revealed that at higher cysteine concentrations, the extent of aggregation increased as a function of time. Additionally, both the extent and aggregation kinetics were faster for 13 nm gold nanoparticles compared to that for 8 nm.

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CHAPTER 1 INTRODUCTION

The field of nanotechnology has gained enormous popularity due to its vast opportunity of finding new materials with tunable properties and its wide implications in chemical and biomedical sciences. Nanotechnology is the recent field of development in science which is developing at a vast pace. The word 'Nano' is derived from a Greek word 'Nanos' which means dwarf or extremely small or tiny [1]. Nanotechnology is the science of the molecules which are less than 100 nm in size.

Nanoparticles are the particles with a size ranging between 1-100 nm. They are reactive in nature as compared to other molecules, due to their large surface area and exhibit unique chemical and physical properties [2]. Their optical properties depend on the size which produce different color due to the absorbance in the visible region [3]. Metal nanoparticles absorb the highest excitation band in the UV-Vis region. When a beam of light falls on the metal nanocrystals, the electrons in the conduction band start oscillating in the electric field which causes the electron cloud to disperse from its relative nuclei. As a result, the restoring columbic forces arise between the electron cloud and the nuclei which causes a collective oscillation of the electron cloud relative to the nuclear frame. This phenomenon is known as Surface Plasmon Resonance (SPR) [4]. The surface plasmon resonance band is affected by the density of the electron, electron mass, size and shape of the metal crystal. Nanoparticles are being widely used for synthesis of drugs, in electronic field, photodynamic therapy, sensors etc. Typically, there is a lot of interest in the utility of gold nanoparticles (GNP) because they are considered to be superior as compared to the other metal nanocrystals due to its biocompatibility and inertness [5]. Due to these properties they have many applications in biomedical and health sciences [6]. They can be used as a nanoscale transducers

for the detection of DNA and its properties [7]. In case of medical approach they have being used as a specific target and inducers of the cell death in cancer. They are used as a messenger for the delivery of drugs and other biomolecules.

Gold nanoparticles can also act as colorimetric sensors for the detection of various chemical system because of alteration in their surface plasmon resonance band [8]. GNP has intrinsically strong absorbance band and has a higher molar extinction coefficient of 10^8-10^{10} M^{-1} cm^{-1} in the visible region[9]. The solution of smaller gold nanoparticles is wine red in color in their dispersed state, whereas the solution of larger GNP or in their aggregated state appears to be purple or blue in color. The color change in the GNP solution is associated with the shift towards the longer wavelength i.e. red shift. A color change from wine red to purple can be observed even with the naked eyes. Normally the absorbance spectra of GNP is maximum at 435 - 524 nm, whereas when the colloidal solution of GNP forms aggregates, the SPR peak gets shifted towards the 612 – 623 nm [10]. Now a days, there is a lot of interest to study the interaction between the amino acid and the gold nanoparticles because gold nanoparticles are known to interact with biomolecules such as proteins, DNA etc [11]. In my thesis work, we have investigated the changes in optical properties of gold nanoparticles (GNP) in the presence of amino acids both in isolation as well as a mixture. There have been several reports on amino-acid and GNP interactions but reports on interaction between GNP and mixture of amino acids are extremely limited. This study is important because proteins are biological macromolecules that consist of a large number of different amino acids. In order to gain a better understanding of the binding of amino acids to GNP, we have used two different sizes of gold nanoparticles i.e. 8 nm and 13 nm. The gold nanoparticles were synthesized by Turkevich's method involving the citrate reduction that lead to spherical GNPs. Citrate acts as a reducing agent (+3→0) and capping agent which does not allow the gold

nanoparticles to form larger molecule [7]. Every particle of the gold nanoparticles is being electrostatically stabilized by the surface adsorption of the citrate ion which leads to an overall negative charge. This negative charge can be reduced by surface adsorption of other small molecules which results in the aggregation of the gold nanoparticles. Among the 20 amino acids, 6 amino acids were investigated namely, L-Lysine (Lys), L-Histidine (His), L-Cysteine (Cys), L-Methionine (Met), L-Aspartic acid (Asp), and L-Arginine (Arg). Besides the study of interactions of isolated amino acids with GNP, a mixture of Lys + Cys was also added to GNPs at different ratios i.e. 1:1, 1:3, 1:10 and 1:100. All these studies were carried out at pH 5 and the changes in optical properties of GNPs were monitored using various spectroscopic techniques such as UV-Vis spectroscopy, Fourier Transform Infrared spectroscopy (FTIR) and Scanning Electron Microscopy (SEM).

CHAPTER 2 LITERATURE REVIEW

Selvakannan *et al.* (2003) [12] had synthesized the gold nanoparticles by the reduction of chloroauric acid ($HAuCl_4$) with $NaBH_4$. They had used Lysine amino acid as capping agent. The solution was then allowed to stir for 12 hrs and the process if further carried out by ultracentrifugation to recover the pellet. The synthesized AuNPs were characterized using UV-Vis spectroscopy, TEM measurement, Thermo gravimetric Analysis (TGA) and ^{1}HNMR. Basu *et al.* (2007) [13] had reported the aggregation of gold nanoparticles on introducing the biomolecules, glutathione under acidic pH range. The AuNPs of different sizes were prepared by the reduction of $HAuCl_4$ with trisodium citrate. They had studied the effect of glutathione on the AuNP aggregation of various sizes by monitoring the changes in the optical properties. The aggregation was characterized by UV-Vis, FTIR, TEM, Raman and XRD. By the introduction of glutathione a new peak is observed which shows the red shift depending upon the nanoparticle size and the pH of the solution. Tomoaia *et al.* (2008) [4] had studied the interaction of the AuNP with L-cysteine. AuNP were prepared by citrate capping. They had investigated AuNP with different concentration and even the mixture ratio. The study was monitored by using UV-Vis spectra and Atomic Force Microscopy (AFM). Due to the high affinity of binding the cysteine with AuNP the interaction is quite strong. The binding of cysteine with the AuNP is with the thiol group present in cysteine amino acid. Different concentration of cysteine solutions were prepared i.e. 0.001 M and 0.0001 M. Addition of both the solution of cysteine leads to the change in color and a complete bathochromic shift is seen in the UV-Vis spectra indicating AuNP aggregation. From the *TEM* image AuNP with cysteine shows aggregation, where they are being linearly arranged by forming a complex network. Basu *et al.* (2008) [14] had studied the aggregation of gold nanoparticles of

varying size with the adsorption of 2-aminothiophenol (ATP). The interaction of ATP with AuNP was studied under the acidic pH range. The induced aggregation is being examined by UV-Vis spectroscopy, surface enhanced Raman spectroscopy, TEM, Zeta potential, and X-ray diffraction. The aggregation size, surface plasmon resonance band, inter-particle distance of the AuNPs was found to be dependent on the pH range and the capping agent. Yeh *et al*. (2012) [3] has discussed the properties, application and the synthesis of the gold nanoparticles. The gold nanoparticles were synthesized by the reduction of $HAuCl_4$ with citric acid in boiling water. Citrate act as both the reducing as well as the capping reagent. Further, using Frens method, spherical AuNP having the diameter size of 10-20 nm and even the larger size of particles were prepared which showed aggregation in the presence of thiolate ligands. This is because the Au has high affinity to bind with the sulfur, as the thiolate group is there in the solution the replacement of capping ligand (citrate) and the S-H starts binding with the AuNP. This process is followed by the non-covalent interactions, which further makes the bigger molecule of colloids. Zakaria *et al.* (2013) had demonstrated the interaction of gold nanoparticles with different amino acids and some small molecules at different pH range. The kinetics of small molecule-AuNP interactions were monitored using UV-Vis spectroscopy and Dynamic Light Scattering (DLS). To determine the aggregation, they introduced dual wavelength ratio i.e. 522nm/435nm. They suggested that if there is a decrease in the 522-435nm ratio, the AuNPs do not form aggregates whereas an increase in the 522-435 nm ratio determine the formation of AuNP aggregated colloids. Further, the aggregation was mostly observed in the low pH range (acidic pH) whereas no aggregation was observed at basic pH range. Zeta potential measurements suggested that the reduction in the charge over the carboxylic group indicate the formation of aggregates whereas an increase in the charge on the carboxylic group rendered the AuNPs to be well-dispersed in solution with higher stability.

The amino acids used were Alanine, Glutamate, Cysteine, Arginine, Histidine and Lysine. Cysteine was the only amino acid to form Au colloids at all pH range. Whereas other amino acids (Lysine, Histidine, Glutamate, Arginine and alanine) did not induce AuNP aggregation except only in low pH range i.e. at pH 3. Satnami *et al.* (2015) [15] had reported AuNP interaction with the cysteine's functional group (thiol; S-H) and glutathione. The samples were characterized by UV-Vis spectroscopy, FTIR and TEM. The AuNP interaction with thiol group and glutathione was found to be dependent on the concentration of ligand, and on the pH. The UV-Vis spectra showed a shift towards the longer wavelength, which indicates dipole-dipole interaction due to zwitterion formation and the adsorption-cum-interaction of these molecules with the AuNP surface were found to be sensitive to pH. Kainth *et al.*(2018) [16] had reported a quantitative detection of thiopurines using gold nanoparticles. They had synthesized gold nanoparticles of four different sizes. Characterization was carried out by FT-IR, TEM, DLS and UV-Vis spectroscopy. The SPR peak of the gold nanoparticles was observed at 521 nm gets shifted towards the longer wavelength i.e. at 700 nm upon addition of purine indicating formation of aggregates. This shows that the gold nanoparticles are sensitive towards the purine and sensitivity of AuNPs towards purines is higher at bigger size of the nanoparticles.

CHAPTER 3 MATERIALS AND METHODS

This chapter contains all the information on chemicals, glassware, labware, instruments and the methodologies that were used for carrying out the research work described in this thesis.

3.1 Materials

3.1.1 Chemicals used

Auric Chloride ($HAuCl_4$), Sodium Citrate Tribasic Dihydrate ($C_6H_5O_7.2H_2O.Na$), Sodium Borohydride ($NaBH_4$), Amino Acids (L-Lysine-HCl, L-Arginine, L-Alanine, L-Methionine, L-Cysteine, L-Histidine, and L-Aspartic Acid). All these chemicals were bought from Sigma-Aldrich. The aqueous solutions of all the chemicals were prepared in ultrapure Milli-Q water (Millipore).

3.1.2 Glassware and Labware required

Micropipettes (2-20 µL, 20-200 µL, 100-1000 µL; Eppendorf Research), micropipette tips, 3-necked round bottom flask (100 mL), glass condenser, oil bath, glass vials (25 mL), magnetic stir bars, microcentrifuge tubes (1.5 mL and 2 mL), reagent bottles, measuring cylinder, Quartz cuvettes (2.5 mL; Fig. 3.1), Pasteur pipettes, pH strips (from pH 1- pH 10), spatula, Kim-wipes.

Fig.3.1 UV-Vis Quartz cuvettes

3.2 Equipments and Instruments used

3.2.1 Magnetic stirrer with hot-plate

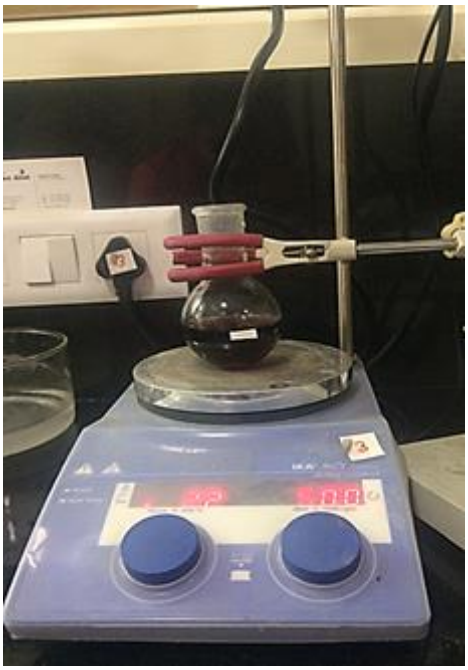

Fig 3.2 Magnetic Stirrer with Hot Plate

In this research work, a magnetic stirrer with hot-plate (IKA RCT Basic) was used (Fig. 3.2). It has a maximum speed of 1800 rpm and a maximum heating temperature is up to 310 ^{o}C. The magnetic stirrer is a laboratory device which is used to provide a magnetic field which allows the magnetic stir bar to rotate when immersed in the liquid. Additionally, it contains a hot plate that allows to heat the solution placed on it.

3.2.2. UV-Vis Spectrophotometer

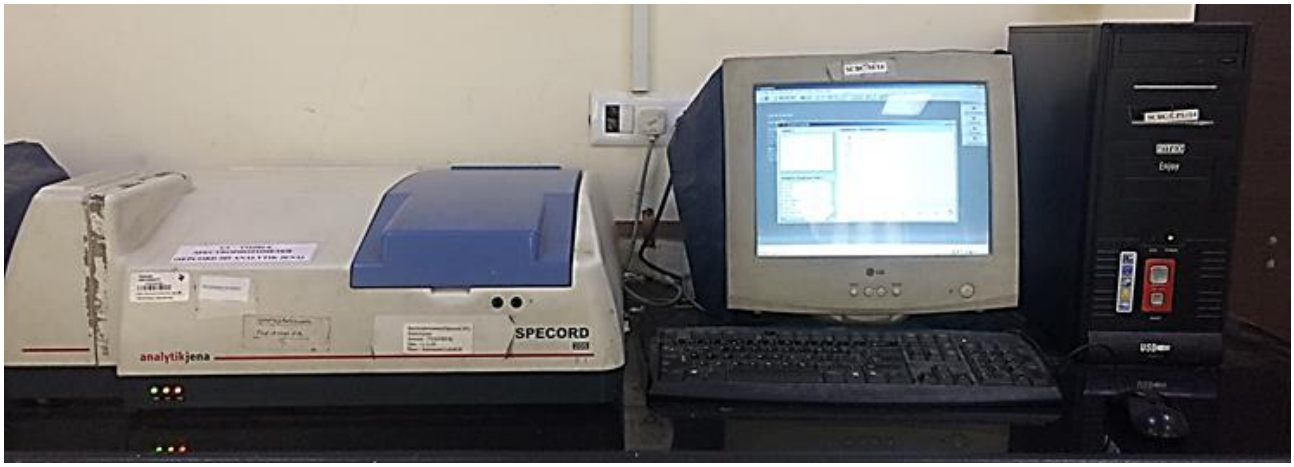

Fig 3.3 UV-Vis Spectrophotometer

The UV-Vis spectrophotometer used in this research work is of Specord 205 Analytik Jena (Fig. 3.3). The UV-Vis spectroscopy is the absorbance spectroscopy which falls in the ultraviolet-visible region. It corresponds to the absorption of light and allow transitions from one electronic energy level to another higher energy level. It is also known as the electronic spectroscopy. Range for the UV-Vis spectra is from 200 nm - 800 nm. The source of light used in UV region i.e. 200 nm - 400 nm is the deuterium lamp whereas for the Visible region i.e. 400 nm-800 nm is the tungsten lamp. When the UV-Vis radiation is incident on a particular compound, the molecules containing π-electrons and non- bonding electrons absorb the energy in the UV-Vis region which causes excitation of an electrons from its higher occupied molecular orbital (HOMO) to its lower unoccupied molecular orbital (LUMO). Four type of transitions can occur - π–π*, n–π*, σ–σ*, and n–σ* in order- σ–σ* > π–π*> n–σ* > n–π* [17].The law which is being followed during absorption of UV-Vis radiation is the Lambert-Beer's Law, which states that when a light falls on a substance

the light absorbed is directly proportional to the molar concentration of the sample and the path length.

$$\mathbf{A=\log_{10}(I_o/I)=\varepsilon cl}$$

Where, A – Absorbance, I_o – Intensity of incident light, I – Intensity of transmitted light

c – molar concentration of solute, l – path-length of the cuvette in cm, ε - molar absorptivity

Typically, in a double beam UV-Vis spectrophotometer the beam of light is being split into two. One of the beam is used for the reference and the other passes through the sample. The light falls on a sample in a form of dispersion by which the monochromator selects only one beam of a light. The beam of light contains a small region of electromagnetic spectrum. The intensity of a reference beam is taken to be 100% transmittance or 0 absorbance. Two detectors (photodiode) are present which detect the beam of both reference and sample one at a same time. The absorbance is plotted as a function of wavelength. Most of the samples measured in the UV-Vis are taken in the solution form, which is taken in a rectangular transparent cell known as the cuvettes that are generally made up of fused silica or quartz glass. They are transparent on opposite faces whereas opaque on remaining opposite faces. To record a spectrum, the transparent side of the cuvette, containing the sample solution, is placed in front of the lamp. This allows the light to get transmitted from the sample and is detected by the photodiode.

3.2.3. Fourier Transform Infrared (FT-IR) Spectrometer

Fig 3.4 Fourier Transform Infrared (FT-IR) Spectrometer

The FT-IR spectrometer used in this work is of Agilent Cary – 660 Series (Fig. 3.4). The range is from 400 cm^{-1} to 4000 cm^{-1}. It is used to identify the functional group present in the molecule and even one can detect the impurity present in a sample. Typically, molecular vibrations such as stretching and bending vibrations are measured/observed by FT-IR spectrometer. Stretching vibrations occur at higher frequencies than the bending vibrations. Stretching vibrations can occur as symmetric or asymmetric vibrations. Whereas, bending vibrations are classified as in-plane (horizontal) and out-of-plane (vertical) vibrations. In-plane vibrations are the bending and rocking and on the other hand out-of-plane vibrations are the wagging and twisting. FT-IR spectrometer consists of a radiation source, beam splitter, stationary mirror, movable mirror, sample cell and a detector. It uses Michelson's Interferometer, which is the generation of interference between two beams that gives an interferogram. A signal is being produced as a change of path-length between two beams. Movable mirror moves at a distance of 21 cm. From the radiation source the radiation falls on the beam splitter from which 50% of the radiation is transmitted to the stationary mirror

and 50% to the movable mirror. Again 50% is reflected from both movable and stationary mirror towards the beam splitter from which it is transmitted to the sample and reaches to the detector. When the distance between the stationary mirror and movable mirror to the beam splitter is same, constructive interference occurs. At this point maximum signal reaches to the detector. This condition is known as the zero path difference. Whereas, when the movable mirror move by ½ of the lambda, destructive interference occur and here minimum / no signal is detected by the detector. The signal falls on the detector which is described by a cosine wave. Each wavelength generates the cosine wave which add up and is detected by the detector and the interferogram is formed which is further converted into frequency domain by the Fourier transformation that results a plot of % Transmittance *vs* Wavenumber (cm^{-1}).

3.3 Methodologies

3.3.1 Preparation of stock solutions

3.3.1.1 Preparation of aqueous solution of Auric Chloride ($HAuCl_4$)

The stock solution of Auric Chloride was prepared in milli-Q water at a concentration of 0.01 M in 10 mL. Typically, 39 mg of powdered $HAuCl_4$ was weighed in an analytical balance and dissolved in 10 mL of water (milli-Q) which gave an amber colored solution. After the stock solution was prepared, the solution was stored in a reagent bottle, covering it with the aluminum foil and placed in dark at room temperature.

3.3.1.2 Preparation of aqueous solution of Sodium Citrate Tribasic Dihydrate ($C_6H_5O_7.2H_2O.Na$)

The stock solution of sodium citrate, required for the gold nanoparticle synthesis, was prepared freshly every time. Typically, 1% (w/v) citrate solution was prepared by adding 100 mg of powdered citrate into 10 mL of the milli-Q water at room temperature and was utilized quickly.

3.3.1.3 Preparation of HCl solution

Typically, a stock solution of HCl (0.5 M) was prepared in milli-Q water at room temperature. In order to use it for our required experiments, a sub-stock of HCl was prepared (0.01 M) for which a 200 µL aliquot was transferred into 10 mL of milli-Q water.

3.3.1.4 Preparation of aqueous solutions of Amino Acids

All the amino acid solutions (L-Lysine-HCl, L-Arginine, L-Alanine, L-Methionine, L-Cysteine, L-Histidine, and L-Aspartic Acid) were prepared in the milli-Q water with a stock concentration of 10^{-2} M in a total volume of 1 mL. The solutions were prepared in micro centrifuge tubes and stored in a refrigerator at 4 ^{o}C.

3.4 Synthesis of Gold Nanoparticles

Prior to the synthesis of gold nanoparticles, all the glassware were cleaned using freshly prepared Aqua Regia, washed thoroughly with copious amount of water and oven-dried. The colloidal gold nanoparticles (AuNP) were synthesized by Turkevich method (Fig. 3.5). Two different sizes

of AuNPs were prepared i.e. 8 nm and 13 nm. The make-up volume for the colloidal AuNP solution was 50 mL for all the reactions. Typically, to a 100 mL, dry round bottom flask, 1.25 mL of the gold solution was added into an appropriate volume of milli-Q water and a condenser was attached to the round bottom flask. The solution was allowed to boil at 100^{o}C and stirred at a speed of 450 rpm. After the auric chloride solution started refluxing, 2 mL of the freshly prepared trisodium citrate solution was added drop wise at an intermittent manner into the solution in the round bottom flask. The size of the AuNP is based on the ratio of the gold solution and citrate being added.

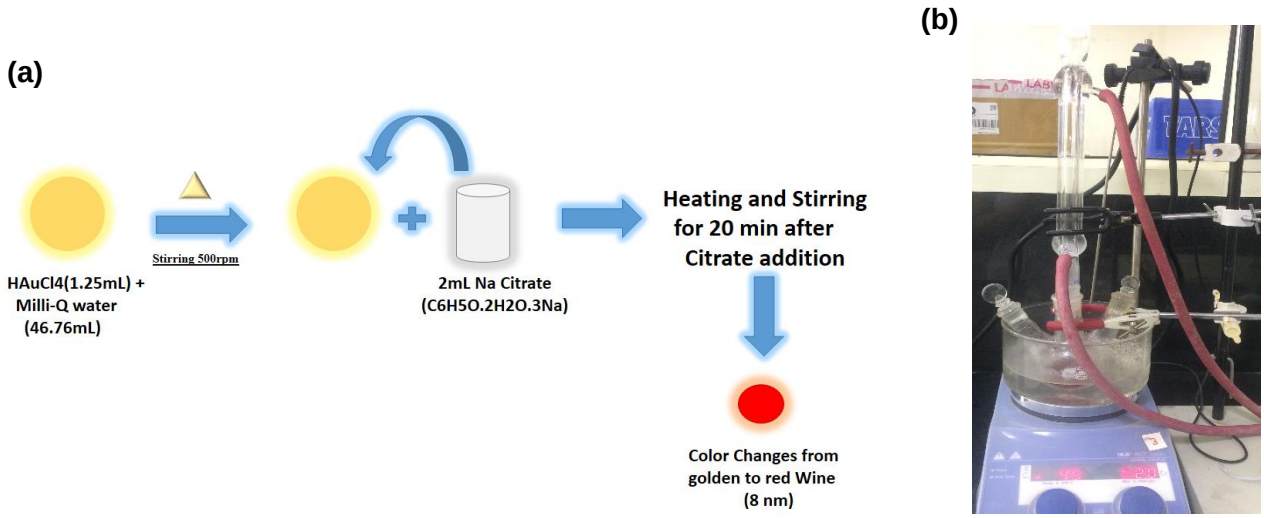

Fig 3.5 (a) Schematic diagram for the synthesis of AuNP by Turkevich method. (b) Wine-red color of AuNPs formed that was cooled down to room temperature.

For instance, addition of 2 mL of citrate led to the formation of 8 nm of colloidal AuNP (citrate-capped) whereas addition of a smaller volume i.e. 1.3 mL of citrate led to the formation of 13 nm of AuNPs (Fig. 3.6). It is well-known that citrate acts both as a reducing and capping reagent. *More the amount of the capping agent added, less will be the size of the AuNP.*Upon addition of citrate, the color of the solution changed to wine-red indicating the formation of AuNP. The

colloidal gold nanoparticles were further allowed to stir under reflux condition for another 15 min. The synthesized gold nanoparticles was then cooled to room temperature and transferred into a glass vial and stored at 4 ^{o}C in dark.

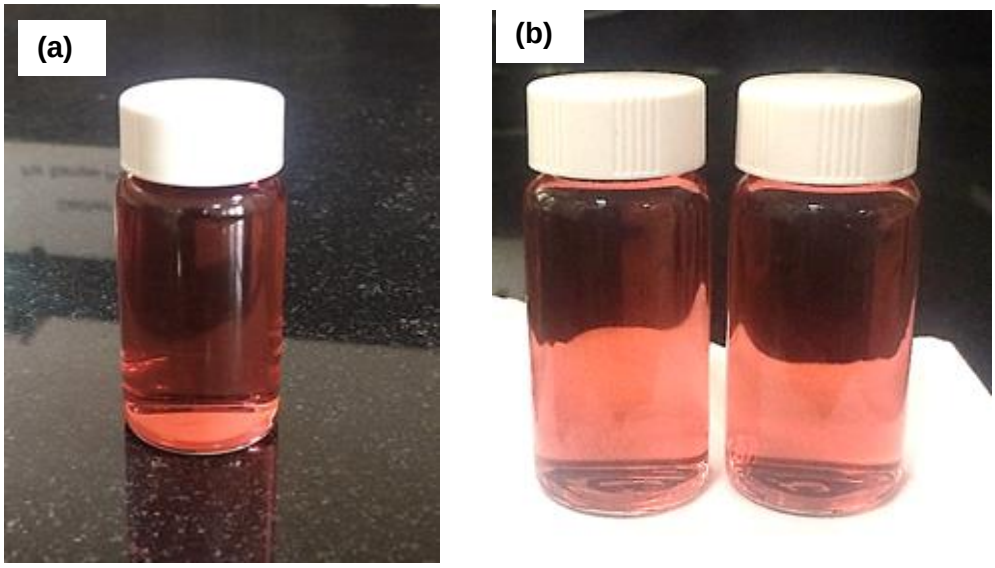

Fig 3.6 Gold nanoparticles synthesized by Turkevich method involving citrate reduction (a) 8 nm, (b) 13 nm.

3.5 Sample Preparation for UV-Vis Spectroscopy

3.5.1 For studying AuNP-Amino Acid (isolated) interactions

The interaction between AuNPs and isolated amino acids were studied at pH 5 by UV-Vis spectroscopy using a 10 x 10 mm quartz cuvette. Prior to performing the studies on interactions between AuNPs and amino acids, the AuNPs (both 8 nm and 13 nm) were characterized separately using UV-Vis spectroscopy. Typically, to the as-prepared AuNP colloidal solution, 10 mM HC lwas added drop wise and the pH of the solution was adjusted to 5 that was checked and confirmed by using pH strips. In order to ascertain whether AuNPs (8 nm and 13 nm) at pH 5 are stable for

longer duration, the UV-Vis spectra of the AuNPs were collected for at least 30 minutes. The AuNPs showed a characteristic absorption maximum at ~523 nm which remained constant as a function of time.

Now, in order to investigate AuNP-amino acid interaction, 3 mL of either 8 nm or 13 nm of colloidal gold solution was taken in a glass vial and stirred at a speed of 350 rpm at room temperature. The pH of the colloidal citrate-capped AuNPs was adjusted to pH 5 by drop wise addition of 10 mM HCl. Once the pH was set, 30 µL of the 10^{-2} M amino acid (mentioned in section 3.1.1) was added into colloidal AuNPs so that the final amino acid concentration was 10^{-4} M. The solution was quickly transferred into a 10 x 10 mm Quartz cuvette and the UV-Vis spectra were recorded as a function of time at an interval of 5 minutes till 3 hours and finally, a spectrum of the same solution was recorded after 24 hours. All the experiments were repeated 3-4 times at room temperature and the spectra were later plotted using Origin software.

3.5.2 For studying AuNP-Amino Acid (mixture) interactions

For this study, we added a mixture of L-Lysine (Lys) and L-Cysteine (Cys) in varying ratios (viz. Lys:Cys = 1:1, 1:3, 1:10 and 1:100) to either 8 nm or 13 nm AuNP colloidal solution. Briefly, 3 mL of either 8 nm or 13 nm of colloidal gold solution was taken in a glass vial and it was allowed to stir at a speed of 350 rpm at room temperature. The pH of the colloidal citrate-capped AuNPs was adjusted to pH 5 by drop wise addition of 10 mM HCl. Once the pH was set, at first 30 µL of 10^{-2} M Lys was added into colloidal AuNPs, and transferred into a 10 x 10 mm Quartz cuvette following which the UV-Vis spectrum was recorded for the AuNP + Lys mixture. Then, to the AuNP + Lys solution, required amount of Cys was added according to a respective ratio and the UV-Vis spectra were recorded thereafter as a function of time at an interval of 5 minutes till 3

hours and finally, a spectrum of the same solution was recorded after 24 hours. All the experiments were repeated 3-4 times at room temperature and all the spectral data were later plotted using Origin software.

3.6 Sample Preparation for FT-IR Spectroscopy

The FT-IR spectra of all the samples were collected in the Attenuated Total Reflectance (ATR) mode whereby the amino acids were in the solid form whereas the AuNP-amino acid mixture were in the solution form at pH 5. The solid amino acids were ground finely before the measurement. On the other hand, 30 µL of the amino acid (Lys and Cys) was added into 3 mL of AuNP solution at pH 5, and the mixture was allowed to incubate for 2 hours at room temperature. Following the incubation, the FT-IR spectra were collected.

CHAPTER 4 RESULTS & DISCUSSION

4.1. Characterization of gold nanoparticles and their complexes with amino acids using UV-Visible and FT-IR Spectroscopy

UV-visible spectroscopy was used to characterize the synthesized colloidal gold nanoparticles (AuNPs) as well as for the study of AuNPs-amino acids interactions. All the spectra were collected within the range of 200 nm - 800 nm at room temperature (See Materials and Methods). The isolated AuNPs and AuNP-amino acid mixture were also characterized by FT-IR spectroscopy and the results are described below.

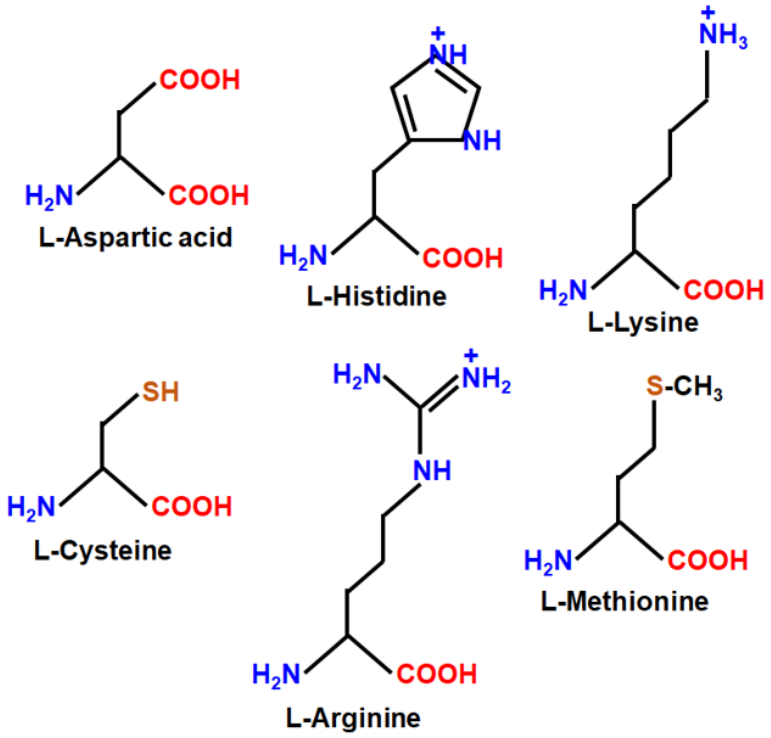

Figure 4.1. Structures of the amino acids used in the study

The amino acids selected for the interaction with the gold nanoparticles were L-Aspartic acid (Asp, D), L-Histidine (His, H), L-Lysine (Lys, K), L-Cysteine (Cys, C), L-Arginine (Arg, R), and L-Methionine (Met, M) (Fig. 4.1). The amino acids were selected on the basis of their side-chains that are either acidic or basic. These amino acids were used separately i.e. in isolation as well as a combination with varying ratios. On the other hand, AuNPs of two different sizes viz. 8 nm and 13 nm were synthesized by Turkevich method which were characterized by UV-Vis spectroscopy

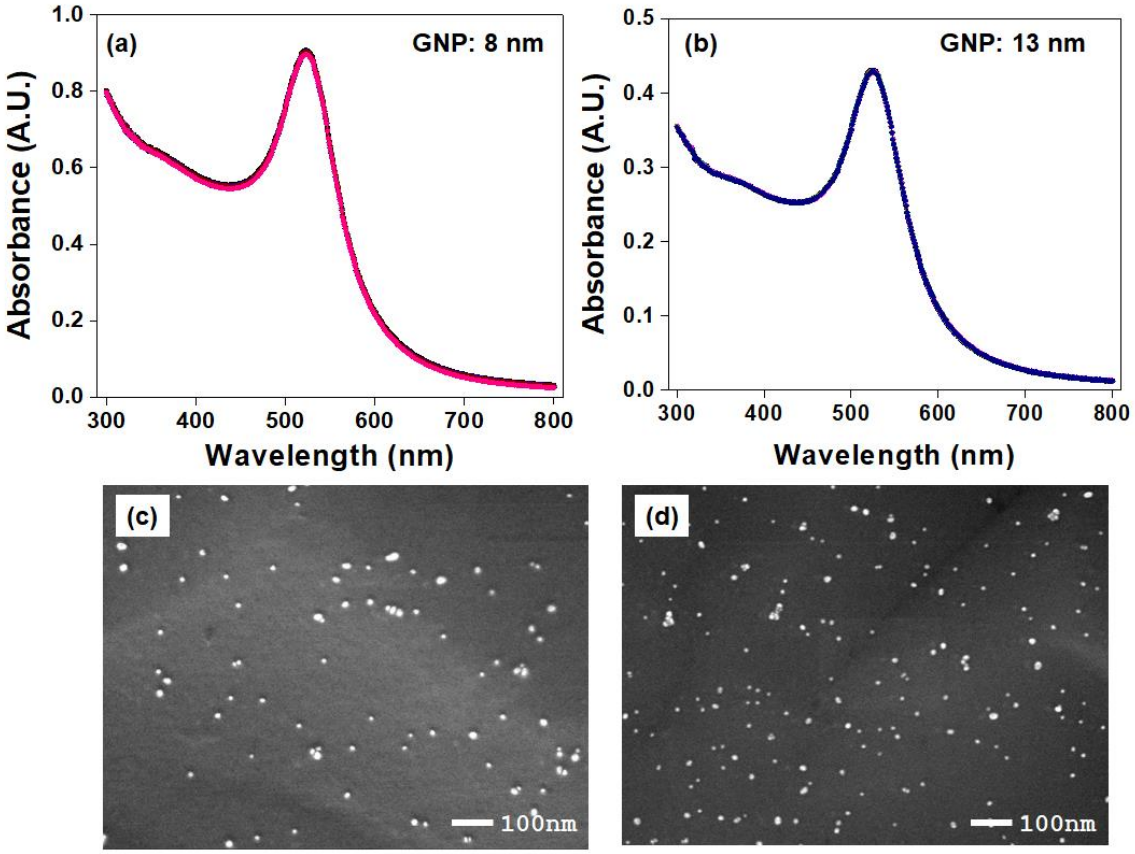

Figure 4.2. UV-Vis spectra of citrate-capped gold nanoparticles at pH 5 monitored for 30 minutes for two different sizes (a) 8 nm and (b) 13 nm. (c, d) SEM images of 8 nm and 13 nm gold nanoparticles, respectively.

(Fig. 4.2a, b) which showed a characteristic surface plasmon resonance (SPR) band at ~523 nm. The sizes of the AuNPs were further characterized by scanning electron microscopy (Fig. 4.2c, d). In order to ensure that the AuNPs remain stable at pH 5 at room temperature, the UV-Vis spectra of both 8 and 13 nm AuNPs were monitored for at least 30 minutes till 2 hours and the absorption maxima at ~523 nm did not show any change (Fig. 4.2a, b). Hence, it was concluded that the AuNPs are stable at pH 5.

4.1.1. Studies on interactions between 8 nm AuNP and isolated amino acids

The interactions of amino acids (in isolation) with 8 nm AuNP colloids were monitored as a function of time by UV-Visible spectroscopy and characterized by FT-IR spectroscopy. It was observed that upon addition of L-cysteine to the AuNP colloidal solution, the wine-red color turned purple after a while without any formation of visible aggregates (Fig. 4.3). On the other hand,

Fig 4.3. Glass vial containing aggregated AuNPs + Cysteine at pH5

when the remaining amino acids such as Lysine, Histidine, Arginine, Aspartic acid, and Methionine were added into the colloidal AuNP solution at pH 5, the nanoparticles remained water soluble and well-dispersed even after 24 hours and the color remained intact. In order to probe the shift in the absorption peak at ~523 nm as a result of AuNP aggregation, UV-Vis spectra of the AuNP-amino acid mixture were monitored in a time-dependent manner (Fig. 4.4).

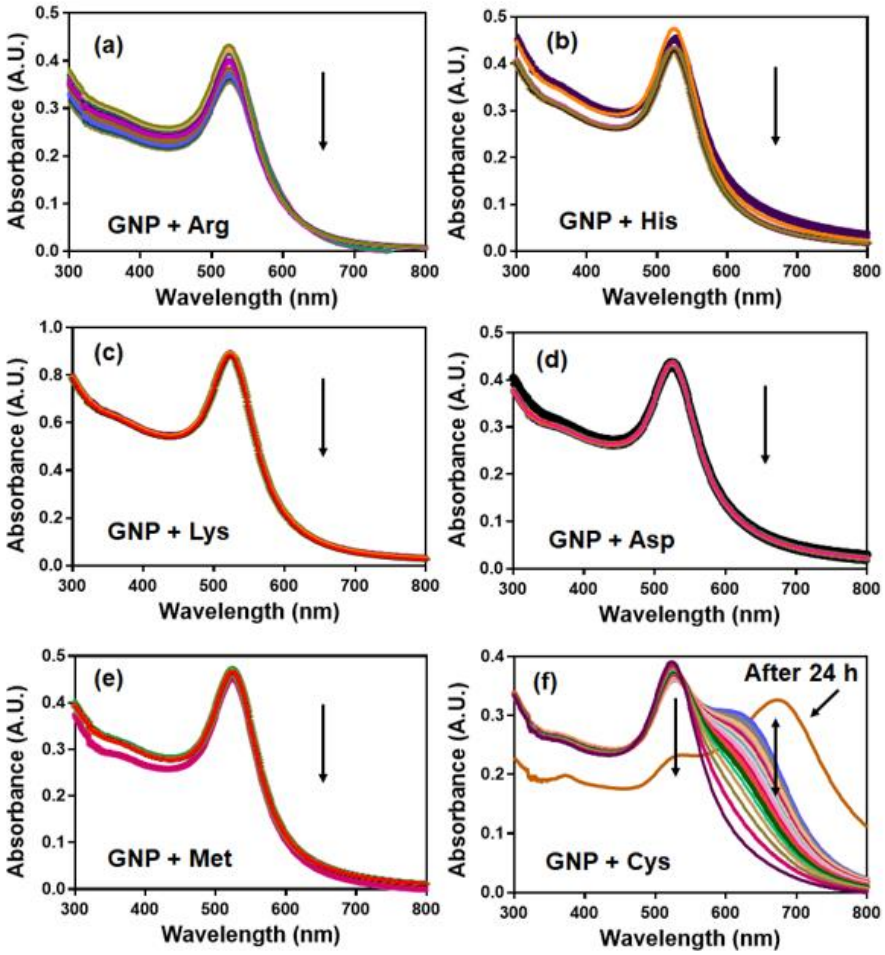

Figure 4.4. Time-dependent UV-Visible spectra of 8 nm AuNP-isolated amino acids recorded at room temperature. The downward black arrow indicates the time duration of 0 – 24 h for which the UV-Vis spectra were collected. For details, see Materials and Methods. The double-headed black arrow in (f) indicates increment of absorbance at wavelengths longer than 523 nm.

As expected, except cysteine, all the other amino acids did not induce any AuNP aggregation as there was no shift in the absorption maxima (Fig. 4.4a-e). Whereas, cysteine induced aggregation in gold nanoparticles as a result of which the surface plasmon peak shifted towards longer wavelength from 523 nm to ~650 nm. This is because cysteine contains a free thiol group (-S-H bond) which exhibits a stronger binding affinity to Au and replaces the citrate (COO^-) ions. This

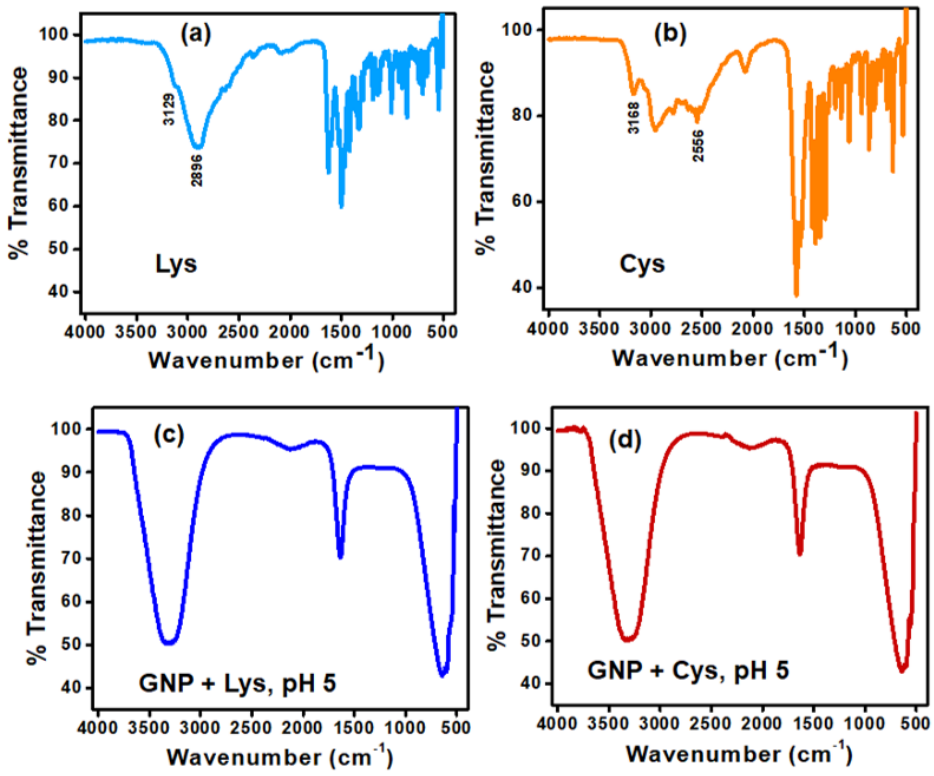

Figure 4.5. FTIR spectra of (a, b) Lysine and cysteine and (c, d) AuNP-lysine and AuNP-cysteine mixture, respectively at room temperature.

neutralizes the surface charge on AuNPs which in turn, facilitates gold nanoparticle aggregation. It was further observed that the surface plasmon resonance peak shifted towards 700 nm with a simultaneous drop in the absorbance after 24 hours of incubation. However, methionine did not induce any AuNP aggregation even after 24 hours (Fig. 4.4e) because the sulfur atom in

methionine is attached to a methyl group which is more hydrophobic and is less reactive than the free thiol group of cysteine [18]. Additionally, to check whether cysteine indeed interacts with gold nanoparticles, FT-IR spectra of the AuNP-amino acid mixture were collected at 400 - 4000 cm^{-1} (Fig. 4.5). Lysine contains an ammonium group as its side-chain which shows a stretching frequency of 3129 cm^{-1} and appears to be shifted in the presence of AuNPs (Fig. 4.5a, c) [19]. Whereas, cysteine contains a thiol group which appears at a stretching frequency of 2556 cm^{-1} that disappears when it is coordinated to the AuNP surface (Fig. 4.5b, d) [20].

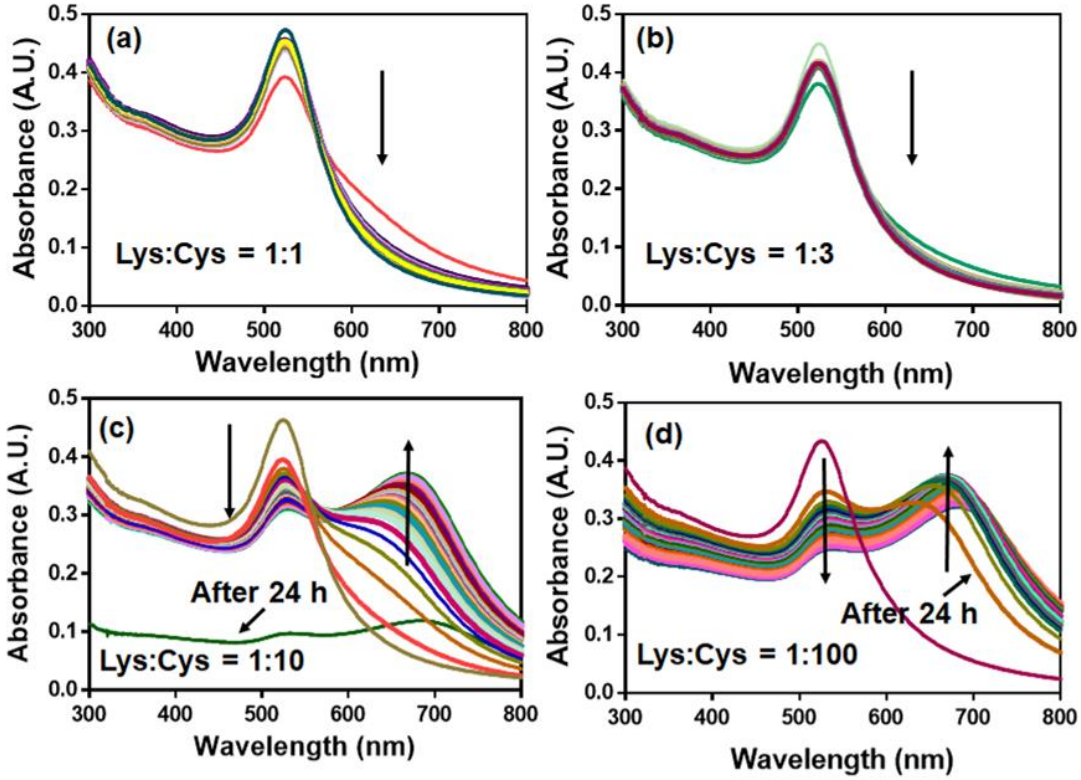

Figure 4.6. Time-dependent UV-Vis spectra of interaction between 8 nm AuNPs and a combination of two amino acids namely, lysine and cysteine in varying ratios viz. Lys:Cys at (a) 1:1, (b) 1:3, (c) 1:10, and (d) 1:100 at pH 5. The downward black arrow indicates the time duration whereas the upward black arrow indicates an increase in absorbance as well as a bathochromic shift in the SPR peak as a function of time.

4.1.2. Studies on interactions between 8 nm AuNP and combination of amino acids with varying ratio and side-chains

Next, in order to investigate how a combination of amino acids interacts with AuNPs, we chose a mixture of lysine and cysteine whereby the concentration of lysine was kept constant and that of the cysteine was varied. Since gold nanoparticles are known to interact with proteins that are composed of various amino acids, it is important to understand the interaction with an amino acid mixture at the molecular level. Lysine was chosen because it is found to be abundant in proteins and is present at the protein surface due to a polar, charged side-chain. Earlier, we have observed that isolated lysine does not induce any AuNP aggregation (Fig. 4.4c) whereas cysteine facilitates aggregation of AuNPs (Fig. 4.4f). The UV-Vis spectra for the mixture of lysine and

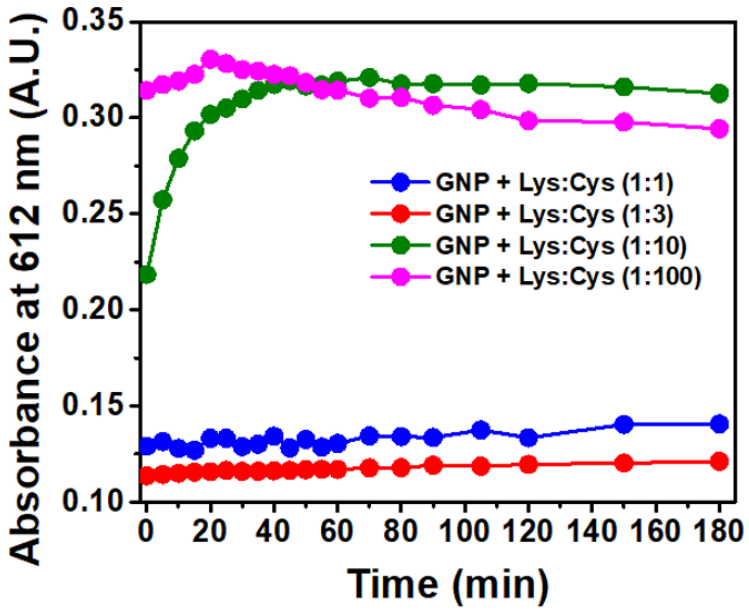

Figure 4.7. Change in absorbance at 612 nm as a function of time at different ratios of Lysine and Cysteine and 8 nm AuNP mixture monitored by UV-Vis spectroscopy at room temperature.

cysteine are shown in Fig. 4.6. It is observed that at lower cysteine concentrations, AuNPs remain monodisperse whereas at higher concentrations of cysteine, aggregations of AuNPs occurs with a concomitant red-shift of the SPR band. A plot of absorbance at a single wavelength namely 612 nm revealed that the increase in absorbance shows an exponential rise at Lys:Cys ratio of 1:10 whereas it appears to be apparently saturated at Lys:Cys ratio of 1:100 (Fig. 4.7). Additionally, the AuNP aggregation is induced when the pH of the gold colloids is adjusted to pH 5 whereas aggregation is not observed for as prepared AuNPs (Fig. 4.8) which suggests that pH also plays an important role in small molecule-induced AuNP aggregation.

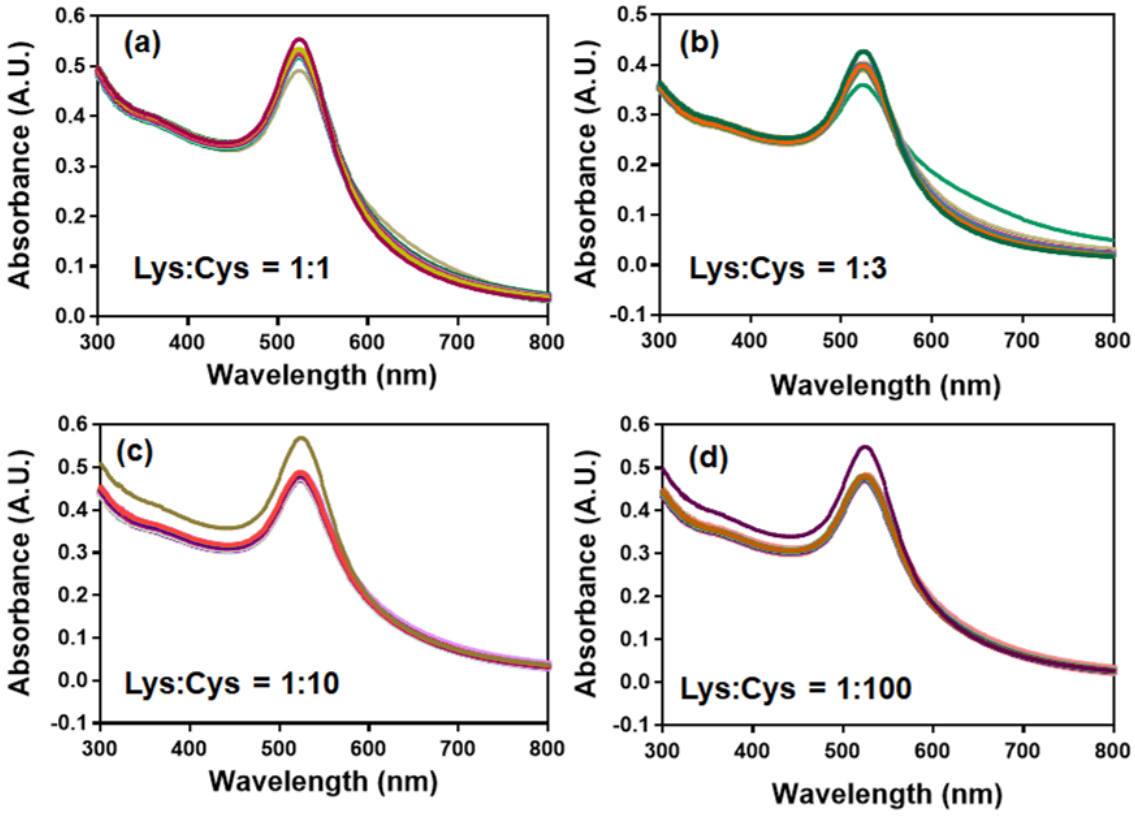

Figure 4.8. Time-dependent UV-Vis spectra of 8 nm AuNPs and a combination of two amino acids namely, lysine and cysteine in varying ratios viz. Lys:Cys at (a) 1:1, (b) 1:3, (c) 1:10, and (d) 1:100 for as-prepared, pH-unadjusted AuNPs.

4.1.3. Studies on interactions between 13 nm AuNP and isolated amino acids

Further, in order to investigate whether the size of gold nanoparticles plays any role on amino acid-induced aggregation, the studies on AuNP-isolated amino acids were carried out with 13 nm AuNPs at pH 5 by monitoring the changes in UV-Vis spectra in a time-dependent manner. Here also, we observed a similar trend (Fig. 4.9) as that observed with the 8 nm AuNPs. Except cysteine, all the other amino acids did not facilitate any aggregation of AuNPs.

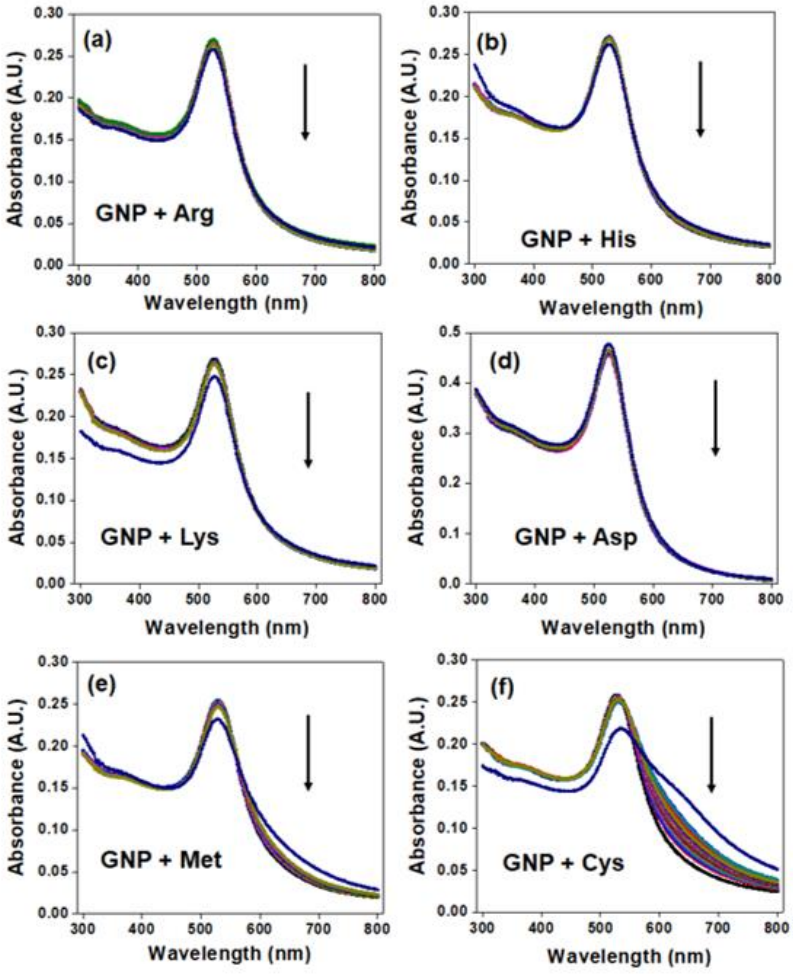

Figure 4.9. Time-dependent UV-Vis spectra of 13 nm AuNPs and isolated amino acid mixture collected at room temperature. The downward black arrow indicates the time duration of 0 – 24 h for which the UV-Vis spectra were collected. For details, see Materials and Methods.

4.1.4. Studies on interactions between 13 nm AuNP and combination of amino acids with varying ratio and side-chains

Here again, in order to probe how a combination of amino acids interacts with AuNPs, we chose a mixture of lysine and cysteine whereby the concentration of lysine was kept constant and that of the cysteine was varied (Fig. 4.10). It was observed that the AuNP aggregation kinetics was much faster for 13 nm AuNPs compared to that of 8 nm. Additionally, at 13 nm

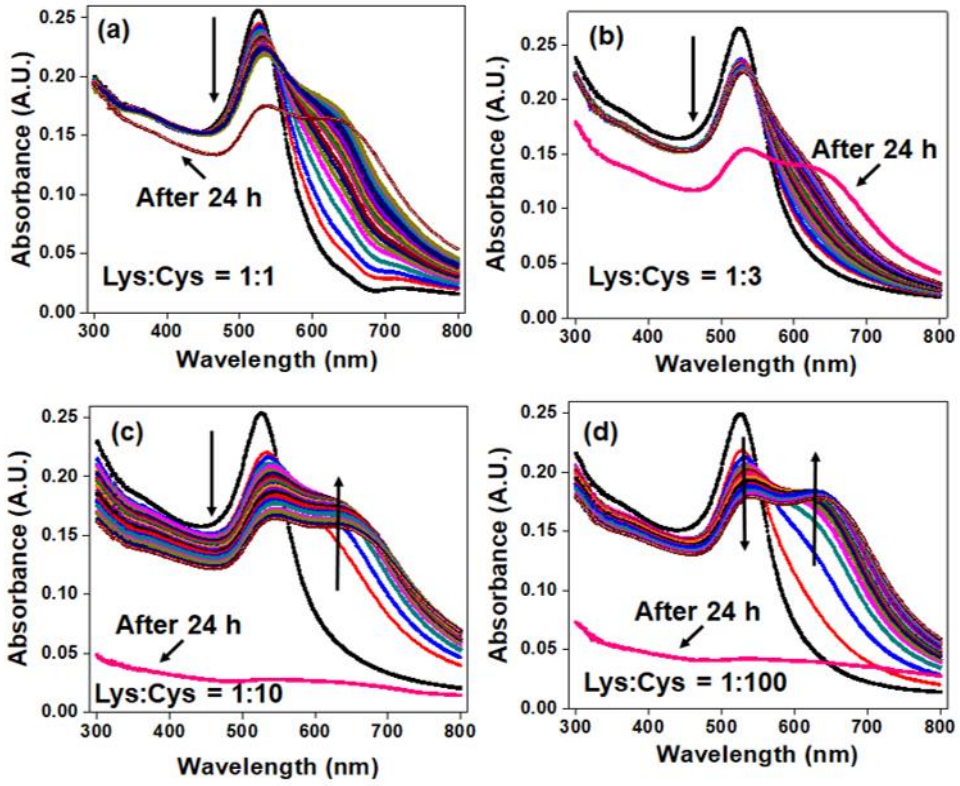

Figure 4.10. Time-dependent UV-Vis spectra of interaction between 13 nm AuNPs and a combination of two amino acids namely, lysine and cysteine in varying ratios viz. Lys:Cys at (a) 1:1, (b) 1:3, (c) 1:10, and (d) 1:100 at pH 5. The downward black arrow indicates the time duration whereas the upward black arrow indicates an increase in absorbance as well as a bathochromic shift in the SPR peak as a function of time.

AuNPs, the red-shift of the SPR band was more pronounced even at Lys:Cys ratios of 1:1 and 1:3 which was not well–defined in the case of 8 nm AuNPs (Fig. 4.10a, b). This could be due to the fact that higher size of AuNPs could accommodate more number of amino acids which can neutralize the surface negative charge more effectively and hence, may enhance AuNP aggregation. Here also, a plot of absorbance at a single wavelength namely, 612 nm revealed that the increase in absorbance shows an exponential rise at almost all the Lys:Cys ratios whereas the fastest aggregation kinetics is observed at the highest concentration of cysteine (Fig. 4.11). SEM images of these samples revealed the presence of heterogeneous morphologies such as nanospheres and nanocubes (Fig. 4.12).

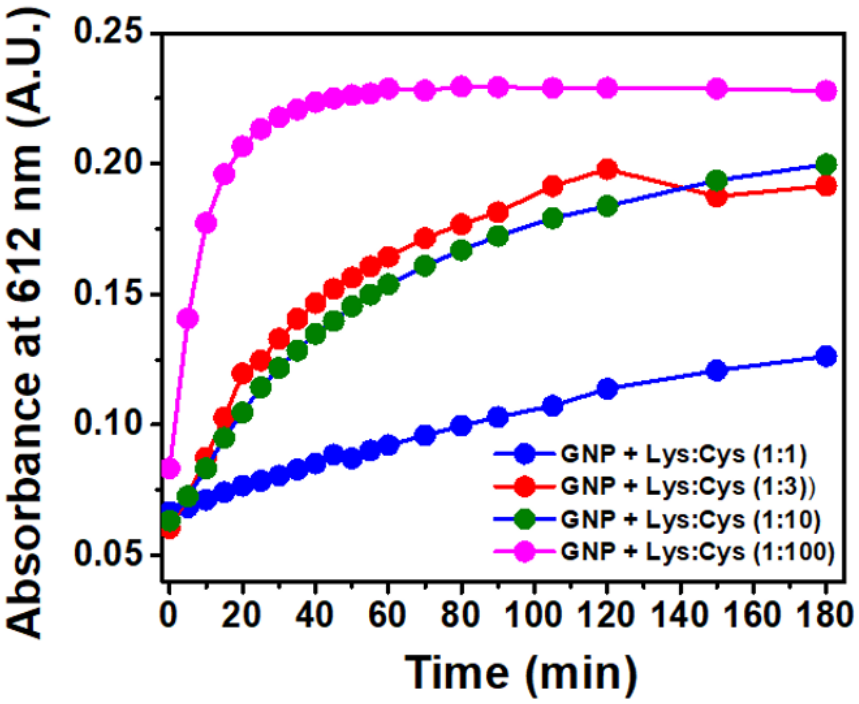

Figure 4.11. Change in absorbance at 612 nm as a function of time at different ratios of Lysine and Cysteine and 13 nm AuNP mixture monitored by UV-Vis spectroscopy at room temperature.

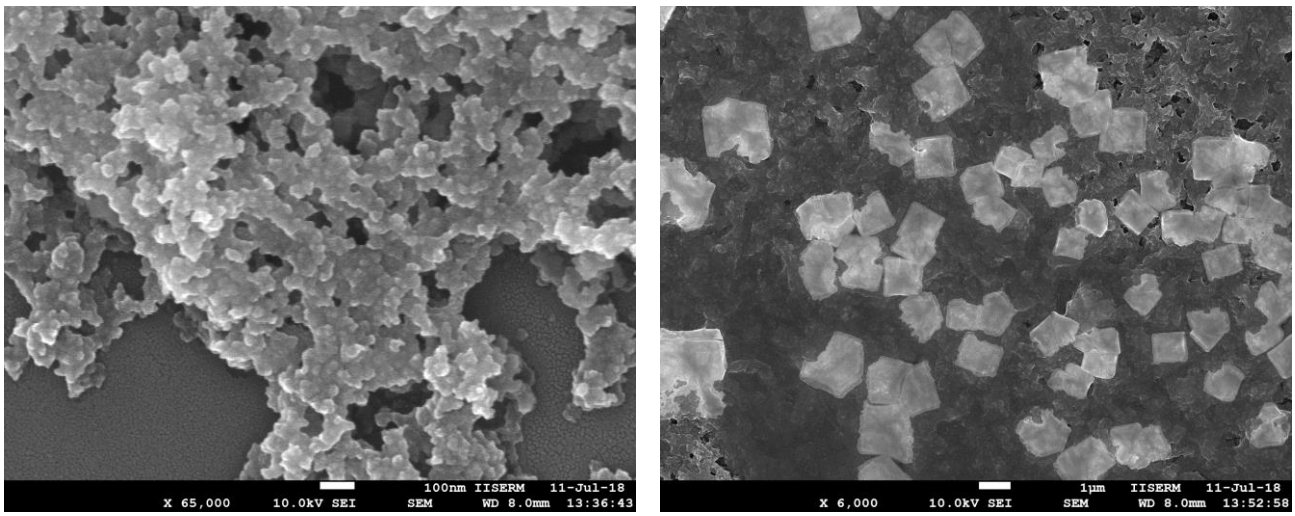

Figure 4.12. SEM images of the mixture of Lys:Cys with ratio 1:100, 13 nm of AuNP

CHAPTER 5 CONCLUSIONS

The present study describes our investigation on gold nanoparticle-amino acid interactions at pH 5 that were monitored using UV-Visible spectroscopy. We have synthesized gold nanoparticles of two different sizes viz. 8 nm and 13 nm using Turkevich citrate reduction method and the nanoparticles were characterized using various spectroscopic techniques. Our studies indicated that when isolated amino acids were used separately, only cysteine induced aggregation of both 8 nm and 13 nm gold nanoparticles due to Au-thiol interaction whereas all the other amino acids did not induce any aggregation even after 24 hours. When we used a mixture of lysine and cysteine of varying ratios i.e. Lys:Cys = 1:1, 1:3, 1:10, and 1.100, the aggregation kinetics increased progressively at higher cysteine concentrations. Additionally, the extent of aggregation was faster for 13 nm gold nanoparticles compared to that for 8 nm. We propose that the 13 nm gold nanoparticles will allow a greater number of amino acids to interact with the nanoparticles and lead to higher surface adsorption that would result in the reduction of surface negative charge which in turn, may facilitate aggregation. Moreover, it was observed that pH plays a major role in dictating the amino acid-induced aggregation of gold nanoparticles that exhibits diverse nanoscale morphologies.

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