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Novel Biological Approach for Biosynthesis of Anisotropic Gold Nanoparticles using *Aloe barbedensis*: Role of pH and temperature

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ABSTRACT

Biosynthesis of gold nanoparticles using Aloe barbedensis leaf extract was studied considering different parameter like pH (3, 4, 6, 8, 10), Temperature (4, 30, 60 and 100^{0}C) and concentration of aurochlorate solution. At lower pH and temperatures, formation of hexagonal and triangles of gold nanoparticles occurred, as viewed by TEM. The leaf extract was so efficient that change in color of reactant was noticed in < 2 minutes of interaction with aurochlorate solution at both low and high temperature. Formation of nanoparticles was characterized by UV-Vis Spectroscopy, XRD and TEM; which confirmed the synthesis of monodispersed gold nanoparticles. Gold nanoparticles synthesised using A.barbadensis was extremely stable as compared to chemically synthesized Gold nanoparticles synthesized using using citrate reduction (chemical) method.

Keywords: Biosynthesis, *Aloe barbedensis*, Gold nanoparticles.

INTRODUCTION

Owing to the small size and high surface – to - volume ratio, the nano-materials demonstrate unique mechanical, optical, electronic and magnetic properties. Nano-sized metals have gained importance only because of its changing structural and behavioral properties. Evolutionary confrontations and selection pressures over millions of years, faced by living organisms, have led to the emergence of skilled biological systems and molecules that are adapt nanomachines. It took a fraction of period for scientist to realize the cost effectiveness and high maintenance of biosynthetic route for synthesis of nano-metals.

Eukaryotes may use all these mechanisms, but the metal resistance is attributable to the intracellular compartmentation of toxic ions in complexes and/or within intracellular organelles. In yeasts and fungi a major proportion of accumulated Ca^{+2}, Mn^{+2}, Zn^{+2} are located in the vacuoles complexing with polyphosphates. The metal ion sequestration in eukaryotes occurs via three main molecules

i. Glutathione (GSH) [1]
ii. Phytochelatins [2, 3] and
iii. Cysteine rich metallothioneins [4].

Plants respond to heavy metal toxicity in a variety of different ways. Such responses include immobilization, exclusion, chelation and compartmentalization of the metal ions, and the expression of more general stress response mechanisms such as ethylene and stress proteins. These mechanisms have been reviewed comprehensively [5] for plants exposed to Cd, the heavy metal for which there have been arguably the greatest number and most wide-ranging studies over many decades. Organic acids and some amino acids, particularly histidine, also have roles in

the chelation of metal ions both within cells and in xylem sap [6,7]. Peptide ligands include the metallothioneins (MTs), small gene-encoded, Cys-rich polypeptides. Our current understanding of the functions and expression of MTs in plants, particularly Arabidopsis, have been reviewed elsewhere [6, 8]. In contrast, the Phytochelatins (PC) are enzymatically synthesized Cys-rich peptides. PC structure, biosynthesis, and function have been extensively reviewed [6, 9].

Recent advances in our understanding of aspects of PC biosynthesis and function are derived predominantly from molecular genetic approaches using model organisms**.** Gardea-Toresdey et.al [10] for the first time exploited Alfa Alfa plant as a factory for nano-metal synthesis by growing it in aurochlorate rich environment. Nucleation and growth of Au nanoparticles was confirmed by atomic resolution analysis. Neem (*Azadirachta indica*) has been used in extra-cellular synthesis of pure metallic silver and gold nanoparticles, and bimetallic Au/Ag nanoparticles [11]**.**

In this work, we are proposing the use of *Aloe barbedensis* for synthesis of anisotropic gold nanoparticles (GNP) with special emphasis on formation of nano-triangles in the solution. To best of our knowledge this is the first report on use of *A.barbadensis* for the synthesis of gold nano-triangles. The work also reveals involvement of capping proteins and hence the exceptional stability of the biogenic GNP over chemically synthesized GNPs.

Materials and Methods

Aloe barbedensis was procured from Pathare Nursery in Kalyan, Maharashtra. Gold aurochlorate was procured from sigma Aldrich, USA. The experiments were performed using double distilled water. The glassware was washed with aqua-regia to remove the traces of metal contaminant. The pipettes were pre-calibrated before using for the measurements. In order to record the temperature, local made thermocouple was used.

Preparation of aqueous extract of plants: Plant extract used for biosynthesis was prepared by macerating 10g thoroughly washed leaves of *Aloe barbadensis* in 50 ml of double distilled water under chilled conditions to retain the activity of the enzymes. The *Aloe barbadensis* extract was centrifuged at 5000 rpm for 10 mins to remove free cellular debris or compounds that interfere with gold nanoparticle synthesis. The resulting supernatant was redispersed in sterile distilled water and then filtered using 0.22 µm filters. This ultra filtered suspension was used for of nanoparticle.

Procedure for Biosynthesis of the Gold Nanoparticles (GNP): Clear extracts of *Aloe barbadensis* was used for the biosynthesis of GNPs. A stock solution of 50,000 ppm aurochlorate was prepared and diluted as per the pre-requisite of the experiment. The required amount of aurochlorate salt was added in a boiling solution of reaction vessel containing plant extract. In order to optimise the nanoparticle formation, the impacts of pH (4, 6,8,10 & inherent) on synthesis of GNPs were studied at low temperature (30°C) and high temperature (100°C). The parameters obtained from the above two experiments were kept constant to comprehend the impact of temperature and salt concentration on the optical as well as morphological features of GNPs.

Characterization of the Biosynthesized Gold Nanoparticles

UV- Vis Spectroscopy of the gold nanoparticles- The UV-Vis spectra of the GNPS formed were recorded using dual beam spectroscopy Lambda 25 Perkin Elmer, USA. High quality quartz cuvette (Perkin Elmer optics, USA) was used as a vessel to record the spectra.

Transmission electron micrographic Analysis-To elucidate the morphology of the GNPs biosynthesised using *Aloe barbedensis* plant extract high resolution transmission electron microscope (HRTEM), Carl Zeiss Micro imaging, GmbH, Germany, was used. Sample was ultrasonicated for 15 minutes and then coated on ultraclean carbon coated copper grid for analysis. The SAED pattern of the GNP indicates presence of crystalline GNPS as deciphered using the diffraction pattern using X-rays.

X-Ray diffraction studies (XRD)-To peep into the crystallinity and the lattice properties of the GNPs, XRD (P Analytical, Philips PW 1830, The Netherlands) operating at 40 kV and a current of 30 mA with Cu Kα radiation (λ = 1.5404 Å) was used. The colloidal suspension containing metal nanoparticles was dirtied on a small glass slab.

Fourier Transform Infra-Red Spectroscopy (FTIR): The *Aloe barbadensis* extract was centrifuged at 5000 rpm for 10 mins to remove free cellular debris or compounds that interfere with GNP synthesis. The resulting supernatant was redispersed in sterile distilled water and then filtered using 0.22 µm filter. This ultra filtered suspension was then used for nanoparticle synthesis.

RESULTS AND DISCUSSION

Impact of different pH on formation of GNP at 30 and 100^{0}C are presented in table -1, which shows that pH 6 has yielded the best results at both the tried temperatures. Therefore, further trials were done using pH 6 and a range of variable temperatures (Table-2). At same pH, the impact of gold salt concentration was also studied (Table-3)

Table – 1: Impact of pH and Temperature on the Biosynthesis of gold nanoparticles (GNP) using 100 ppm Aurochlorate of *Aloe barbedensis* leaf extract

pH	Temperatures	
	30^{0}C	**100^{0}C**
2	Change in color in 20 min Flat absorption spectrum XRD - Crystalline structure	Change in color in < 5 sec Sharp peak a 553 nm XRD Crystalline structure
4	Change in color in 20 min Intense UV-Vis peak at 536 nm XRD Crystalline structure	Change in color in < 5 sec Intense Peak at 553 nm XRD Crystalline structure
6	Change in color in 20 min Intense Peak at 537 nm XRD Crystalline structure	Change in color in < 5 sec Sharp peak at 554 nm XRD Crystalline structure
8	Change in color in 30 min Broad peak of medium intensity at 540 nm XRD Crystalline structure	Change in color in < 5 sec Sharp peak at 556 nm XRD Crystalline structure
10	Change in color in 30 min Sharp peak at 533 and a slight hump at 657 nm XRD Crystalline structure **TEM-Polydispersed anisotropic GNP**	Change in color in < 5 sec Sharp peak at 560 nm XRD Crystalline structure **TEM-Roughly spherical monodispersed GNP**
Inherent pH of plant extract 4.2	Change in color in 20 min Intense peak at 536 nm XRD Crystalline structure	Change in color in < 5 sec Sharp peak at 564 nm XRD Crystalline structure

Table – 2: Impact of different temperatures on the Biosynthesis of gold nanoparticles using 100 ppm Aurochlorate of *Aloe barbadensis* leaf extract at pH 6

Temperature	Observation		
	Visual	**UV-Vis Peak**	**XRD**
4 ºC	Change in colour in > 24 h	Flat absorption spectrum	Crystalline
RT (28 ± 2ºC)	Change in colour in < 30 min	Good peak at 541 nm	Crystalline
37 ºC	Change in colour within 20 min	Sharp peak at 542 nm	Crystalline
60 ºC	Change in colour in < 5 min	Sharp peak at 545 nm	Crystalline
100 ºC	Change in colour in < 5 sec	Sharp peak at 534 nm	Crystalline

Table 3: Impact of different concentrations of Aurochlorate on the Biosynthesis of gold nanoparticles at 100ºC using *Aloe barbadensis* leaf extract at pH 6

Concentrations	Observation	
	Visual	**UV-Vis Peak**
50 ppm	Change in colour in < 5 sec	Broad peak at 573 nm
100 ppm	Change in colour in < 5 sec	Good peak at 571 nm
150 ppm	Change in colour < 5 sec	Good peak at 568 nm
200 ppm	Change in colour in < 5 sec	Broad peak at 570 nm
250 ppm	Change in colour in < 5 sec	Broad peak at 569 nm

UV-Visible Spectroscopy of gold nanoparticles

As seen in figure 1, the SPR band resulting due the formation of GNP in the solution was found to be centred between 500 - 600nm. Such peaks in visible region can be attributed to the quantum mechanical effects when the size of the particles enters in the nano-regime. The phenomenon is called Surface Plasmon Resonance (SPR). The SPR exhibited by GNPs synthesised at pH 2 at 30°C exhibited poor optical property leading to a flat spectrum (Fig.1a). However, the optical response increased drastically when the GNPs were synthesised at pH 4. The sharp peak at 536nm indicates formation of monodisperse nanoparticles ranging from 20 - 30nm. At pH 4, the SPR band was same as that of pH 2. However, the area under the curve decreased at pH 6 indicating the uniformity in the size. At more alkaline pH value such as 8 and 10; there was red shift suggesting an increase in size and /or agglomeration. At pH 10, there was slight hump at 657 nm. The absorption in near infra red region explains the phenomenon of synthesis of non-spherical gold nanoparticles. Moreover, this can also be due to agglomeration of the nanoparticles under the influence of the pH.

The spectroscopic behaviour of the GNP was found to be altered when synthesised at higher temperature (100° C) using different pH values. In less than 5 seconds transformation of the colour from pale yellow to wine red and appearance of sharp peak at 553 nm in case of GNPs synthesized at pH 2 confirms the formation of monodispersed GNP. The result is in contrast with the GNPs synthesized at 30° C using at pH 2. This phenomenon may be due to increased activation energy as well as reducing power of the biological moieties at high temperature. With increase in pH (4 - 10) there was a red shift from 553 to 560 nm. This explains the gradual increment in the size of gold nanoparticles (Mirkin 1993). Moreover, nanoparticles formed at inherent pH (5.8) were highly stable at room temperature for more than six months (data not shown). This proves the efficiency of pH 2 as optimum parameter for the synthesis of GNPs using *Aloe barbedensis*.

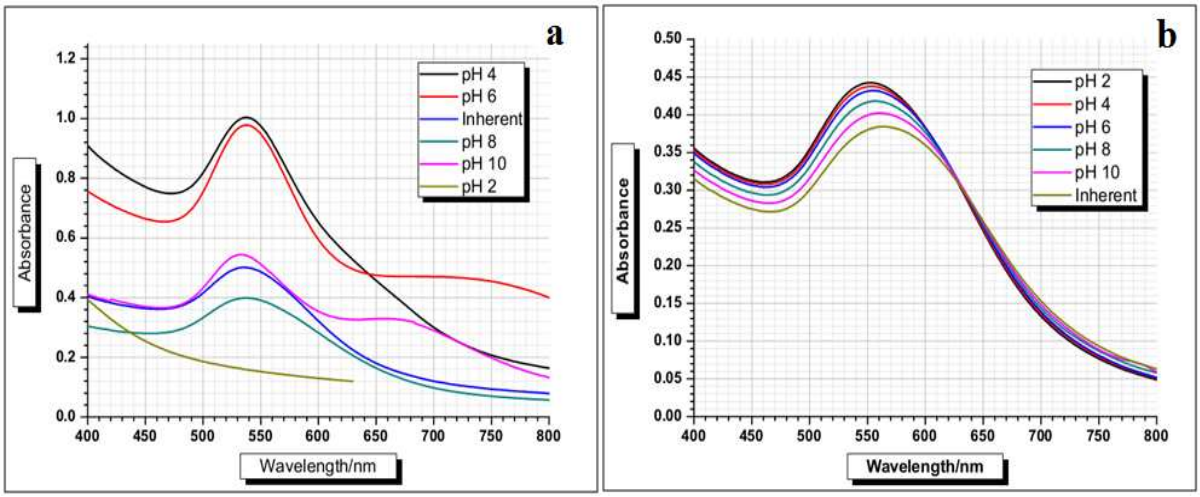

Figure-1: Impact of temperature and pH on bio-fabrication of gold nanoparticles using *A. barbedensis* as recorded by UV-Vis Spectroscopy (a) impact of different pH different pH at 30°C & (b) impact of different pH at 100°C

The impact of temperatures and concentration of the aurochlorate on GNP biosynthesis using inherent pH (5.8) is shown in figure 2a & b respectively. At 4° C, there was no synthesis of GNPs as indicated by flat absorption spectrum shown in figure 2a. At higher temperatures there was improvement in the optical response and a sharp peak was found at 534 nm. As per the previous work, size of the nanoparticles exhibiting SPR band at 534 nm are between 20 - 30 nm. Figure 2b depicts the impact of aurochlorate concentration.

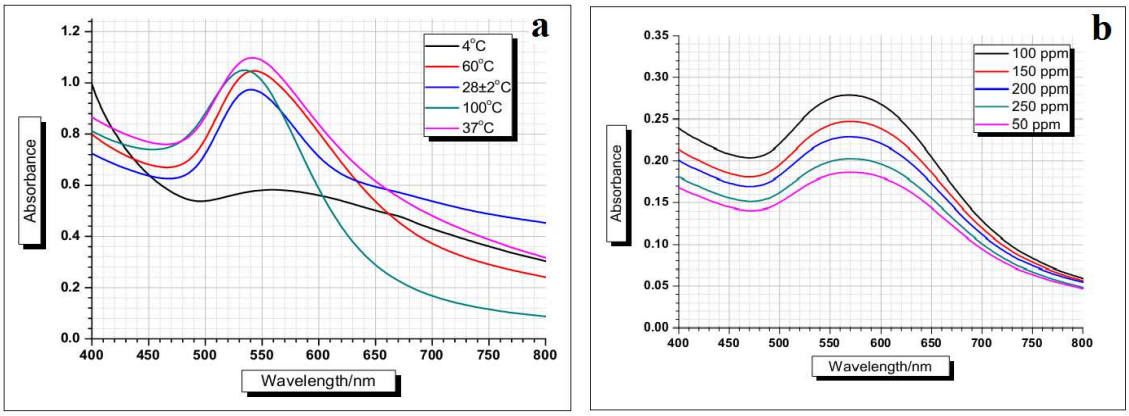

Figure- 2: UV-Vis spectra of gold nanoparticles biosynthesized at pH 6 using leaf extract of *A. Barbedensis* at (a) Different temperatures and (b) Different concentrations of aurochlorate.

The GNPs synthesized using 50 ppm aurochlorate exhibit low intensity and a hump at 573nm. The intensity gradually increased with increase in aurochlorate concentration. GNP synthesized using 100 ppm aurochlorate solution exhibited an intense peak at 534 nm, it could be due to the appropriate ratio of aurochlorate and reducing agent present in *Aloe barbedensis*.

Stability of Biogenic Vs chemically synthesized Gold nanoparticles

The most important concern in exploiting gold nanoparticles for theranaustics is there stability in solution particularly of high ionic strength. A comparative study of stability between chemically and biologically synthesised nanoparticles is shown in figure 3. The biogenic nanoparticles fabricated using *Aloe barbedensis* were found to have exceptional stability as displayed in figure 3. As shown in the figure 3 a, there was a red shift of 28.02

nm after addition of approximately 5 ml of 5M NaCl. In stark contrast to this, the shift in chemically synthesized nanoparticles using same parameters was found to be 130.67 nm after addition of merely 100µl of 5 M NaCl. This exceptional stability of biogenic nanoparticles can be attributed to protection of GNPs by intelligent capping proteins. Under optimal ionic strength of the solution these proteins avoid the columbic attraction between the nanoparticles by maintaining suitable surface potentials.

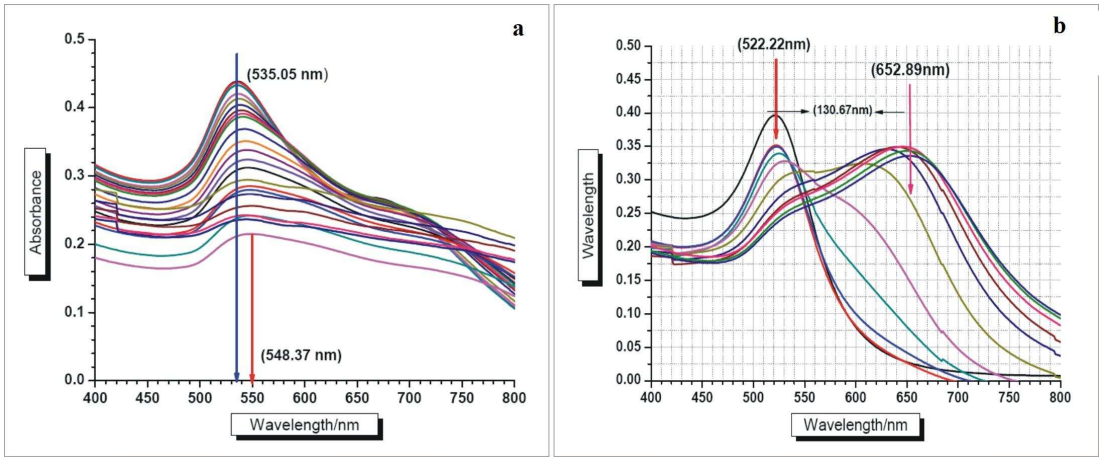

Figure3: UV-Visible spectra showing the stability of (a) Biogenic GNPs synthesized using *A. barbedensis* at pH 10, 100° C & 100 ppm aurochlorate solution (b) Chemically synthesized nanoparticles using the same parameters used for biogenic nanoparticles.

Transmission electron microscopic studies

TEM micrograph showed formation of GNPs in range of 10 to 60 nm in size. GNPs fabricated at 30° C at pH 10 were anisotropic eventually leading to the formation of triangular GNPs (Figure 4a), whereas those formed at 100° C at pH 10 were spherical in shape (Figure 4b). The mechanism for formation of such unique stable structures of GNPs by biological system is still in a nutshell. However, it can be speculated that this is due to the enzyme or capping protein (present in plant extract) assisted nucleation and growth at different facets (111, 200, and 220) of GNPs. The microscopic observation is in agreement with the UV-Vis spectroscopic studies.

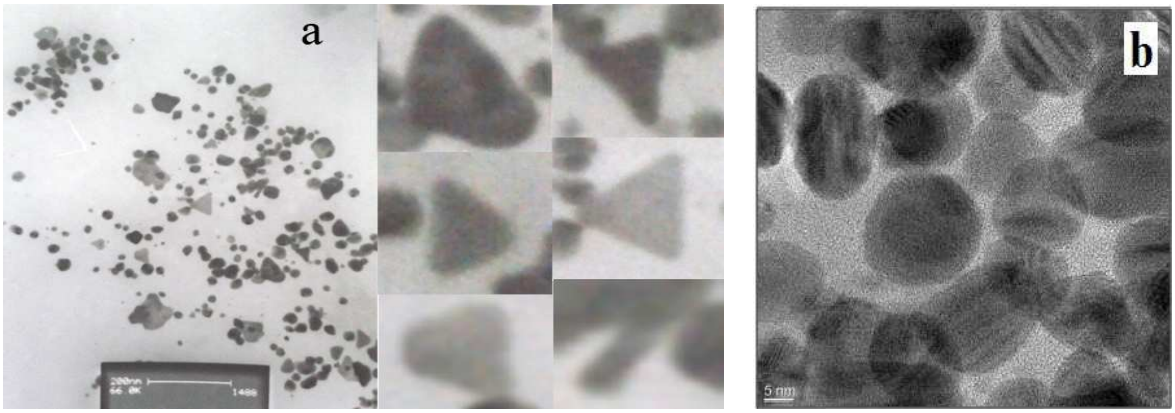

Figure-4: TEM image of gold nanoparticles synthesised using *Aloe barbedensis and* (a) 100ppm gold salt at pH 10 at 30 º C showing anisotropic structures (b) 100 ppm gold salt at pH 10 at 100 º C exhibiting spherical GNPs

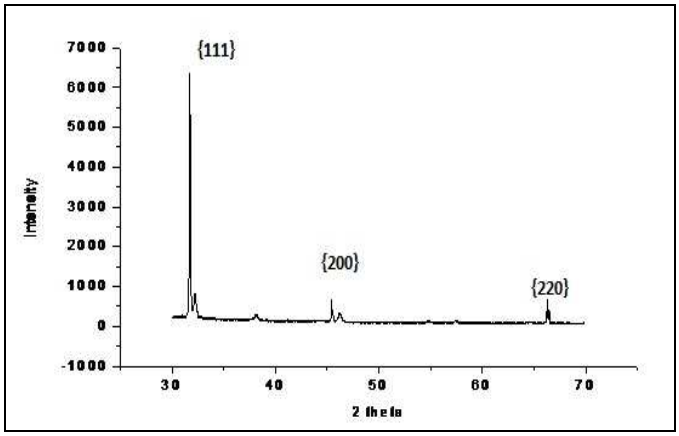

Figure- 5: XRD pattern of gold nanoparticles synthesized using leaf extract of Aloe barbadensis showing typical Bragg reflections for gold nanoparticles.

X-Ray Diffraction studies

The GNPs bio fabricated using *Aloe barbadensis* were found to be crystalline when studied using X-ray diffraction (figure 5). The samples were prepared by depositing colloidal gold solutions on glass substrate. The gold nanoparticles on a glass cube showed intense peaks at (111), (200), (220) and (311) Bragg reflections in the 2 θ range 30°-80 ° as shown in figure 5; this is in agreement with the previous data available on gold nanocrystals [12]. The 111 facet is extremely reactive due to high rate of electron transfer (ref). It must be mentioned here that XRD data of many samples were taken, but since they all showed similar results here only one typical XRD is presented.

The compounds present in *Aloe barbedensis* extract possess amino, sulphydryl, carboxyl as functional groups. At lower pH these functional groups attain positive charge due to very high proton concentrations. This indicates that the solution has got high reducing capacity but capping proteins are inactive at low pH thus leading to agglomeration of nanoparticles. At alkaline pH all the above functional groups possess very high reduction potential acting as a proficient nucleating agent. The capping proteins are also active binding 111 facets of the nanoparticles which are usually thermodynamically unstable. This leads to regulated growth of nanoparticle in 100 direction allowing synthesis of triangular nanostructures.

The *Aloe barbadensis* leaf extract posses important pharmaceutical components such as aloesone, aloesin, barbaloin, chrysophanol glycoside, aglycone and aloe-emodin which can act as reducing as well as capping agents.

Fourier Transform Infrared Red Spectroscopy

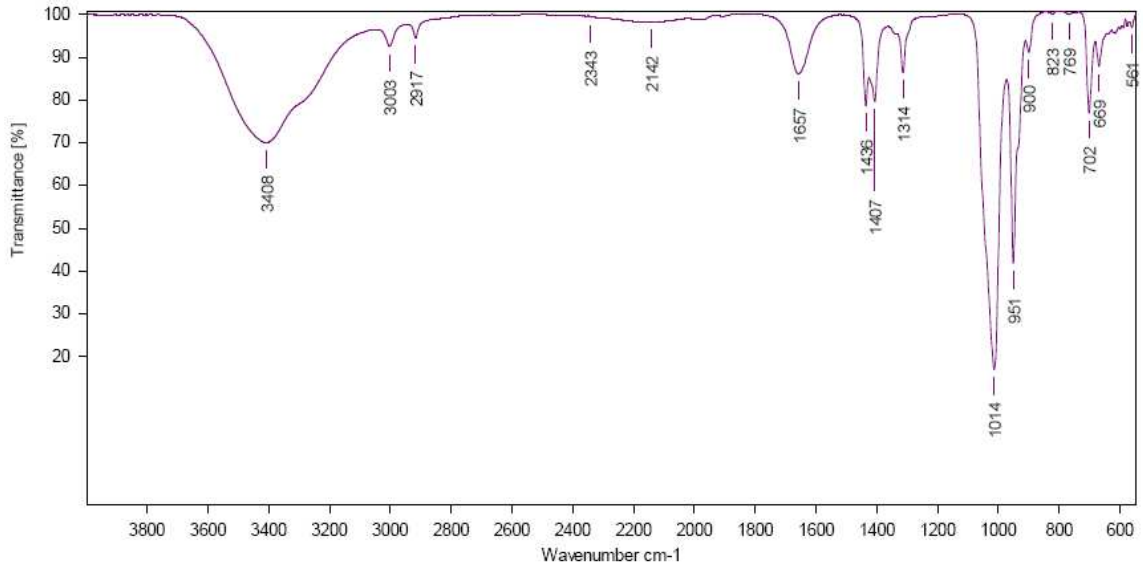

Fig. 6 - FTIR spectra of AuNP-encapped with proteins from Ultra-filtered plant extract

FTIR absorption spectra of ultra filtered extract after bio-reduction of aurochlorate offered important information about the functional groups involved. Some of the absorbance bands which are centered at 1657, 1436 and 1407 are associated with the bending vibration of NH_2, stretching vibration of CO and C=C respectively. Further, peak at 1657, 1436 and 1314 are considered to be amide I, II and III respectively. Hence, this corroborates the presence of proteins which are responsible for capping the GNPs. The absorbance band at 2142 and 829 cm^{-1} indicates the existence of isothiocyanates and stretching of S=O respectively, which desirably protects the surface of GNPs,leading to the decrement of the entropy. This decrease is due to the liberation of the solvation shell and exchange reactions due to the reaction of amide linkage with the thiol group. Further peaks at 951, 900, 823, 769, 702 cm^{-1} correspond to S=O stretching which confirms that the gold- thiol ligand is staunchly bound to Gold nanoparticles. The peak at 3408 cm-1 is found to be alcoholic or phenolic hydroxyl group while 2343 corresponds to C=C stretching.

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