Chapter-2

Synthesis of biogenic gold nanoparticles using indole-3-carbinol and its characterization

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Abstract

Recent nanotechnology research offers eco-friendly and non-toxic procedures for the development of nanoparticles. The present investigation demonstrates the green synthesis and characterization of gold nanoparticles using a phenolic phyto-compound indole-3-carbinol. Newly synthesized gold nanoparticles (AuNPI3Cs) were characterized by involving DLS and Zeta potential measurement, UV–vis spectroscopy, FTIR, XRD, SEM, TEM, AFM study and NMR analysis. DLS measurement of AuNPI3Cs revealed that the size of AuNPI3Cs is 6.858 nm and Zeta potential is -18.8 mv. UV-vis spectrum of synthesized gold nanoparticles showed a peak around 500 nm. The crystalline nature of the particles was confirmed from X-ray diffractometer analysis. Fourier transform infrared spectroscopy determined the involvement of reductive groups on the surface of nanoparticles. Transmission electron microscopy (TEM) micrograph analysis of the gold nanoparticles indicated that they were well dispersed and ranged in sizes 2-4 nm. The method describes a cheap and easily available biosynthesized material.

2.1. Introduction

Nanotechnology encompasses a combination of biology, chemical and physical sciences that generates nano-sized particles having particular functions (Mariselvam et al., 2014; Kumar et al., 2014). Recently, nanoparticles have drawn astounding consideration because of their unique properties on numerous fields such as electronic, optical, medical, sensor and catalytic application (Mohanpuria et al., 2008). Metal nanoparticles have gained very much attention due to their easy synthesis as well as their size, shape, distribution (Burda et al., 2005). Noble metal nanoparticles like silver, gold have been usually abused because of their unique electronic, mechanical, optical, chemical properties (Nalawade et al., 2014).

2.1.1. Gold nanoparticles

Michael Faraday, in 1857, firstly synthesized the pure sample of gold colloid and revealed that the color of gold colloids was dependent on its size. The most important properties of synthesized AuNPs are their plasmonic properties and surface enhanced Raman-scattering effects.

2.1.2. Green synthesis of metal nanoparticles

To synthesize metal nanoparticles, different chemical methods are usually involved to produce well-defined nanoparticles with manageable shapes and sizes (Yuqing et al., 2009). The chemical methods involve toxic treatments, such as inert gas condensation, pyrolysis (Rao et al., 2004), hydrothermal and solvo-thermal synthesis (Moreno-Alvarez et al., 2010). The growth of metal nanoparticles using chemical methods are toxic due to the use of toxic reducing agents and these are also relatively expensive.

For the synthesis of nanoparticles, there has been an increase in the development of eco-friendly methods which do not require the toxic chemicals. Green synthesis of nanoparticles have developed as an important division of nanotechnology in the last decade, particularly for noble metals such as gold, silver.

The synthesis of nano particles using various parts of the plants are gaining more interest (Raghunandan et al., 2010; Thakkar et al., 2010). The methods involve the use of plant phytochemicals such as terpenoids (Thakkar et al., 2010), flavonoids (Kumar and Yadav, 2009), phenol derivatives (Jacob et al., 2011), plant enzymes and their derivatives, di-hydric phenols (Jha et al., 2009) as reductants. The synthesis of nanoparticles using plant extracts or phytocompounds is more advantageous over microbial route such as simple and user-friendly process, economical and less reaction time.

The bioreduction of gold nanoparticles from chloroaurate ions due to their surface plasmon resonance (SPR) has been accomplished using different plants such as tamarind (Kumar and Yadav, 2009), *Helianthus annus*, *Basella alba*, *Cinnamomum camphora* (Narayanan and Sakthivel, 2008), *Capsicum annuum*, *Avena sativa*, *Azardirachta indica* (Armendariz et al., 2004) and *Pelargonium graveolens* (Sharma et al., 2007) have been reported.

This chapter is designed to focus on the synthesis of biogenic nanoparticles using indole-3-carbinol and its characterization.

The characterization of biogenic gold nanoparticles is vital prior to the assessment of their biological activity. For the basic analysis of structure of any nanoparticles X-ray diffraction (XRD) study is important, although other techniques including Fourier transform infrared (FTIR), UV–Vis, spectroscopy, scanning and transmission electron microscopy (SEM and TEM)), dynamic light scattering (DLS), nuclear magnetic resonance (NMR), atomic force microscopy are complementary.

2.2Materials and methods

2.2.1 Chemicals and reagents

Indole-3-carbinol was purchased from Sigma Aldrich. For reagent preparation analytical grade hydro-chloroauric acid ($HAuCl_4$), sodium borohydrade (NaHB4), tri sodium citrate, and potassium bromide (KBr) were procured from Merck India, Ltd., Mumbai, India.

2.2.2 Solution preparation of indole-3-carbinol

For the biogenic synthesis, 0.05 mg ml^{-1} of indole-3-carbinol was suspended in Millipore water.

2.2.3 Synthesis of gold nanoparticles (AuNPs) by chemical method

First 3.94ml of hydro-chloroauric acid ($HAuCl_4$) was diluted ten times, then 1ml 1% trisodium citrate ($Na_3C_6H_5O_7$) solution was added. The liquid mixture was continuously stirred and sodium borohydrate ($NaBH_4$ solution- 0.02mol/l in ice cold water) was diluted ten times by ice cold water. Then it was added drop by drop into the ice cold mixture. The color of the mixture solution immediately turned into pink. The solution was vigorously stirred for another 15 min. The color of the solution finally tuned wine-red. The samples were stored in brown bottle and kept in cool environment at 4°C (Jingyue and Bernd, 2015).

2.2.4 Synthesis of biogenic gold nanoparticles using indole-3-carbinol

Firstly, for the synthesis of bioengineered gold nanoparticles from indole-3-carbinol (I3C), 0.05 mg ml^{-1} I3C was suspended in triple distilled water and then treated an aqueous solution of 0.5 mM hydro-chloroauric acid ($HAuCl_4$) for 10-15 min at room temperature with the help of magnetic stirrer. Then filtration was done through a 0.2 mm filter (PALL Life sciences). For purification, obtained AuNPI3Cs were centrifuged at 10,000 rpm for 10 min at 4°C followed by re-dispersion of the pellet in Milli-Q water and was stored at 4°C for use. Thereafter, the purified suspension or powder was used for characterization of AuNPI3Cs and other experimentations.

2.2.5 Characterization of AuNPI3Cs

2.2.5.1 Dynamic light scattering (DLS) and surface zeta potential measurement

Zetasizer Nano ZS instrument (Malvern Instruments, U.K.) was used to measure dynamic light scattering (DLS) and zeta potential of AuNPI3Cs at 25 ± 1 °C constant temperature.

The solutions were filtered through syringe filter (0.2µm) for DLS and zeta measurements. To measure the particle size distribution of AuNPI3Cs, the measurements were done triplicate and the equilibration time was set to 60 s. The polydispersity index (PDI) was determined to detect the distribution of particle size. In both experiments the pH value of AuNPI3Cs was fixed (Sahu et al., 2010).

2.2.5.2 UV-vis spectral analysis

The production and stabilization of the reduced gold nanoparticles was monitored by UV-vis spectrophotometric analysis which is one of the most important techniques to identify the formation and stability of the gold nanoparticles in aqueous solution. UV-vis spectrophotometer (UV-1800 Shimadzu, Japan) was used to determine the UV-Vis spectra of the solution at the spectral range of 200−850 nm (Sanpui et al., 2011). Origin8 software was used for the plotting of the recorded spectra.

2.2.5.3 X-ray diffraction (XRD) analysis

The crystallinity of the synthesized gold nanoparticles was determined by X-ray diffraction (XRD) analysis. To confirm the synthesis of AuNPI3Cs, X-ray diffractometer (Model PW 1710) was used to analyse XRD pattern. The interaction of X-ray radiation with crystalline sample was predicted by Bragg’s law. The phase identification for AuNPI3Cs reported in this study was made by matching the peak positions of XRD patterns with the patterns of the JCPDS (Joint Committee on Powder Diffraction Standards) database (Anandalakshmi et al., 2016).

2.2.5.4 Fourier transforms infrared spectroscopy (FTIR)

The dried samples of AuNPI3Cs were examined for the detection of biomolecules by FTIR analysis. Perkin Elmer Spectrum Express Version 1.03.00 FTIR spectrometer was used to record the spectra at 4000 cm^{-1} to 400 cm^{-1} wavelength range.

Biogenic gold nanoparticles (AuNPI3Cs) as well as indole-3-carbinol were investigated with a resolution of 4cm^{-1} and accuracy of ±0.01cm^{-1} .The spectra of the samples were assessed after setting the reference spectrum of potassium bromate (KBr) pellet for accuracy (Ghosh et al., 2011).

2.2.5.5 High resolution transmission electron microscopic (HR-TEM) study

The size and structure of AuNPI3Cs were evaluated by CM12 PHILIPS high-resolution transmission electron microscope operating at an accelerating voltage of 200 kV. Briefly, synthesized AuNPI3Cs was sonicated until it formed a homogeneous suspension. A drop of AuNPI3Cs suspension was placed on the TEM grid and stand for 2 min, removing the excess solution with blotting paper, and allowing the grid dry and was observed using transmission electron microscope (Papis et al., 2009).

2.2.5.6 Scanning electron microscopic (SEM) study

Surface morphology of newly formed gold nanoparticles was determined using scanning electron microscope. By scanning electron microscopy (CAMSCAN-2 JEOL, Japan), the shape and morphology of AuNPI3Cs was determined at an accelerated voltage of 15 kV (Papis et al., 2009).

For SEM analysis thin films of AuNPI3Cs were prepared on a carbon coated copper grid. Samples were placed in sample stub using adhesive carbon tape and then the samples were placed under the microscope and observed.

2.2.5.7 Energy dispersive X-ray analysis (EDX)

To confirm the presence of gold in synthesized AuNPI3Cs particles, energy dispersive X-ray analysis (OXFORD) study was performed for 19 s at an acceleration voltage of 20 kV (Kumar et al., 2006).

2.2.5.8 Atomic force microscopy (AFM) study

AFM imaging was performed using 5500 Atomic Force Microscope (N9410S) and contact mode imaging was performed using silicon-wafer. Topography, forward and reverse sensor and z-piezo images were collected simultaneously. The processing and analysis of images was carried out using WSxM software ver. 4.0. (Rao et al., 2007).

2.2.5.9 NMR study

1H and 2D NMR spectra were recorded using a Bruker Ascend^{TM} 400 equipped with a 5-mm triple-resonance inverse Z gradient probe. All diffusion measurements were made by using the stimulated echo pulse sequence with bipolar gradient pulses. Liquid NMR samples were prepared in chloroform and the experiment was done at 25°C. The solid-state cross-polarization magic angle spinning NMR measurements were performed on a 400- MHz BrukerAvance III spectrometer and equipped with a MAS probe head and a 4-mm ZrO_2 rotor. The spectra were recorded with a proton 90° pulse length of 4 ms, contact time of 2 ms, repetition delay of 4 s, and 8 kHz MAS rotation rate (Khatuna et al., 2017).

2.3. Results

2.3.1 Dynamic light scattering and zeta potential measurement

Dynamic light scattering study revealed the average size of AuNPI3Cs as 6.858 nm. Its polydispersity index was 0.285 (Figure 2.1) and the corresponding zeta potential was -18.8 mV.

The AuNPI3Cs are well distributed with respect to stability.

2.3.2 UV-Vis spectral analysis

After mixing of indole-3-carbinol with aqueous solution of hydro-chloroauric acid, the solution turned from colorless into light violet color. Colour change indicated that chloro-aurate ions were reduced into gold nanoparticles and the color change basically occurs due to the surface plasmon resonance (SPR) phenomenon. Free electrons of metal nanoparticles give the SPR absorbtion band due to the vibration of electrons and bands of AuNPI3Cs were detected around 498 nm (Figure 2.2).

2.3.3 X-ray diffraction analysis

The crystalline structure of AuNPI3Cs was determined using the analytical technique of X-ray diffraction. Four distinct intense diffraction peaks of AuNPI3Cs at scattering angles (2θ) of 31.78°, 38.08°, 44.63°, 64.11°, 77.2° which corresponds to the lattice planes (100), (111), (200), (220) and (311) respectively (Figure 2.3).

2.3.4 FTIR analysis

FTIR measurements evaluated the identification of the biomolecules which are responsible for capping and stabilization of the synthesized metal nanoparticles. FTIR analysis of indole-3-

carbinol are shown in figure 2.4A. The peaks of indole-3-carbinol are detected at 1454.22, 1663.52, 2870.62, 3382.98 cm^{-1}. In AuNPI3Cs, the peaks are shifted to 1457.73, 1694.88, 2835.35, 3405.74 cm^{-1} respectively (Figure 2.4B).

This suggests that the phenolic phytochemicals present in indole-3-carbinol is involved in reducing and stabilizing the gold nanoparticles.

2.3.5 Transmission electron microscopy and scanning electron microscopy analysis

The TEM images (Figure 2.5A) confirm the formation of gold nanoparticles and it has been observed that the newly formed nanoparticles are spherical. The size distribution of gold nanoparticles diameter is 2-4 nm. Figure 2.5B shows the SEM images of the newly synthesized gold nanoparticles. It was observed that the surface morphology of gold nanoparticles synthesized from indole-3-carbinol clearly indicates that they are same shapes and well dispersed. Scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) studies revealed the spherical nature of particles synthesized from gold metal (Figure 2.6).

2.3.6 AFM study

Sphere-like morphologies for AuNPI3Cs was observed in AFM micrographs with the size ranges from 2-6 nm. A 3D rendering type of the surface topography of is presented in Figure 2.7.

2.3.7 NMR analysis

The peaks at 7-8 ppm were due to aromatic group that is present in indole-3-carbinol. The peaks at 7-8 ppm of AuNPI3Cs were slightly stretched (Figure 2.8). Appearance of the peculiar signals at 4 ppm was due to the formation of amide bond through reaction between indole-3-carbinol and chloroauric acid as shown in Figure 2.8. Appearance of the other at 1-2 ppm was due to the formation of aliphatic stretching.

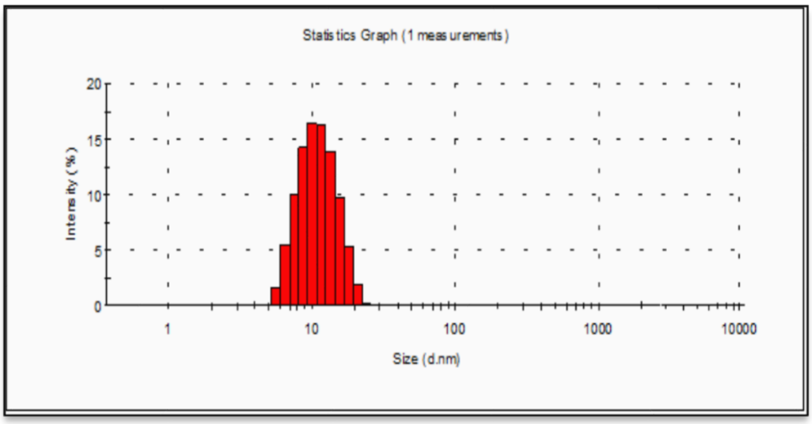

Figure 2.1: Size distribution analysis of synthesized biogenic gold nanoparticles (AuNPI3Cs)

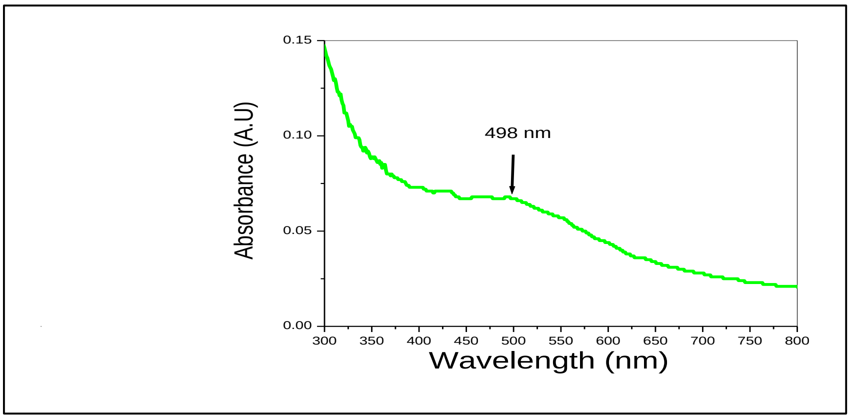

Figure 2.2: UV-vis spectra of synthesized biogenic gold nanoparticles (AuNPI3Cs) at 15min.

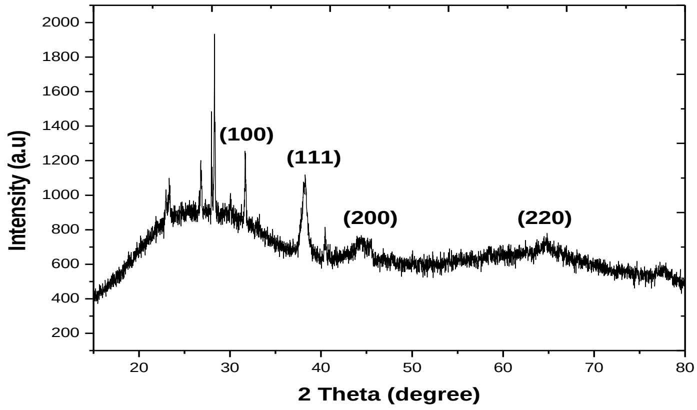

Figure 2.3: XRD pattern of biogenic AuNPI3Cs showing the facets of crystalline gold.

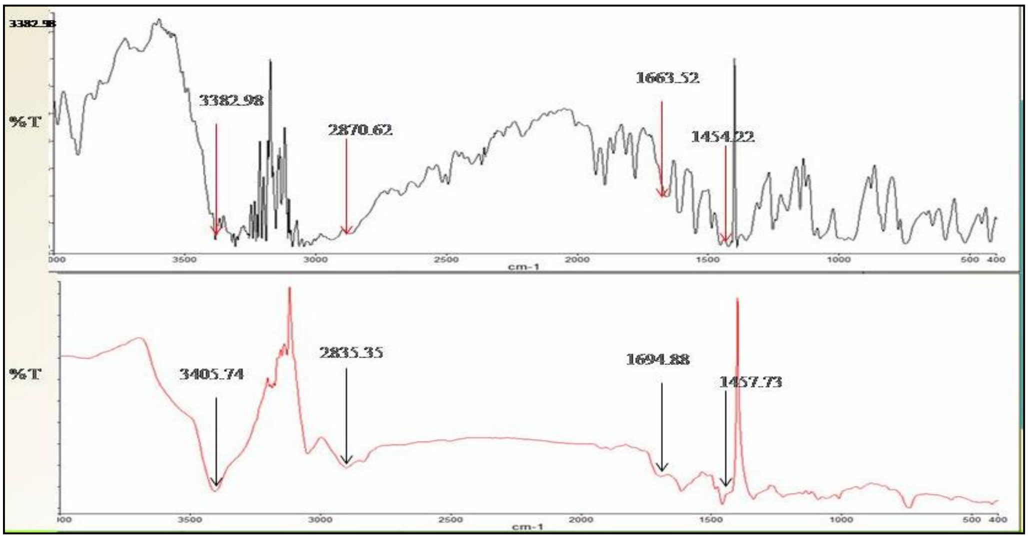

Figure 2.4: FTIR spectrum of indole-3-carbinol and synthesized gold particles (AuNPI3Cs)

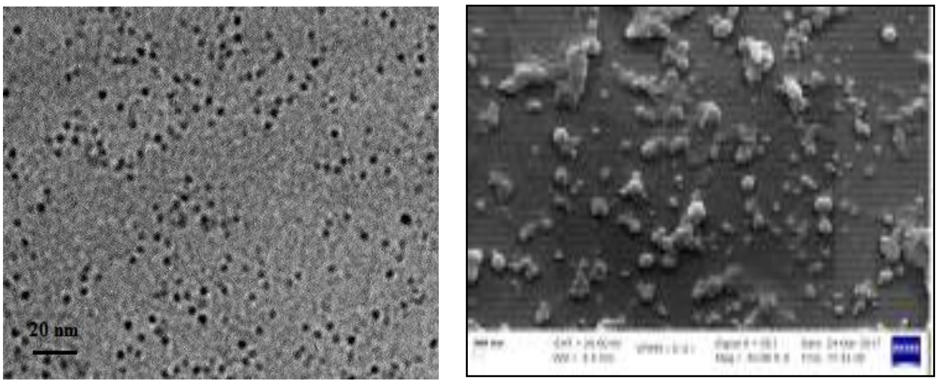

Figure 2.5A HRTEM study of biogenic gold nanoparticles (AuNPI3Cs); (B): SEM image of AuNPI3Cs;

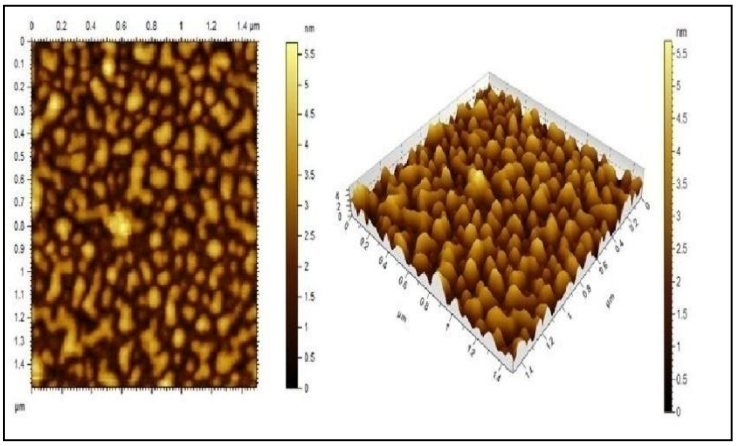

Figure 2.7: Atomic force microscopy of synthesized gold particles (AuNPI3Cs)

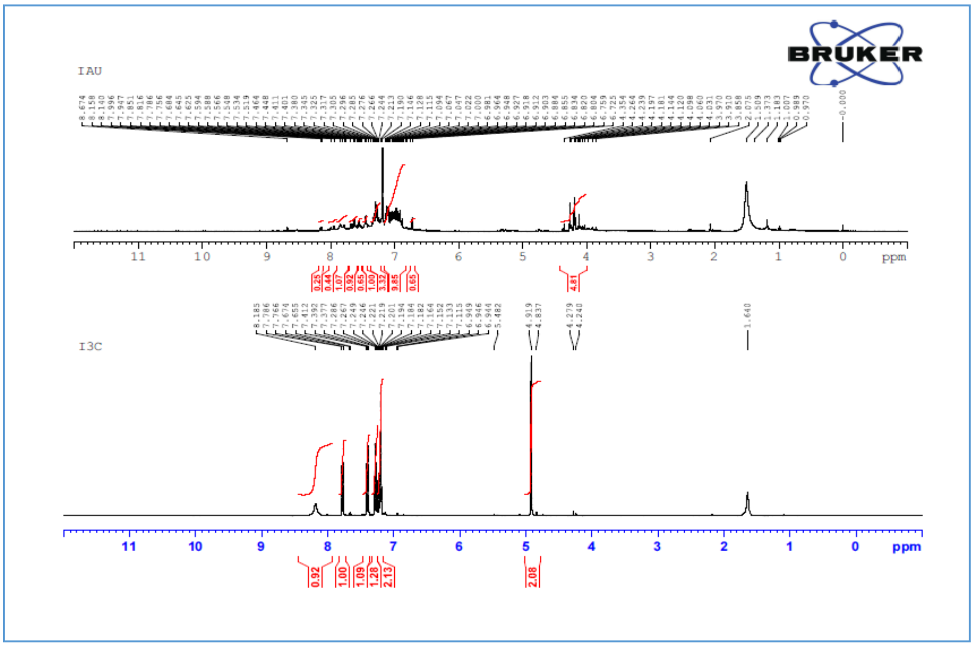

Figure 2.8: NMR analysis of indole-3-carbinol and synthesized gold particles (AuNPI3Cs)

2.4 Discussion:

Design and improvement of nanoparticles with physico-chemical features is a corner stone in nano-science. This chapter discloses the bioreduction property of gold ions into gold nanoparticles using indole-3-carbinol, phenolic phytomedicine.

The size distribution of AuNPI3Cs was examined by dynamic light scattering technique (Figure 2.1). Histogram of particle size distribution reveals that AuNPI3Cs are polydispersed in nature, with hydrodynamic diameter of 6.858 nm with a polydispersity index (PDI) of 0.285.The corresponding zeta potential of AuNPI3Cs is -18.8 mV. The high negative potential reveals long term stability and high dispersity of AuNPI3Cs due to negative-negative repulsion (Mukherjee et al., 2014).

Figure 2.2 shows the UV-vis absorption spectra of AuNPI3Cs at 498 nm. I3C reduce hydro-chloroauric acid (HAuCl4) to form AuNPI3Cs which show highly stable uniform spherical morphology that are identified from the peaks found around 500 nm. It was confirmed from UV-Vis spectroscopy study.

The peak originates from the excitation of electrons of AuNPI3Cs induced by the electromagnetic field (Liz and Tailoring, 2006). To prove the formation of crystalline structure of AuNPI3Cs, X-ray diffraction (XRD) is an appreciable research tool. In this method, monochromatic X-ray beam is projected on the nanomaterials at theta angel (Sharma et al., 2012).

Figure 2.3 shows the XRD patterns of air-dried synthesized AuNPI3Cs. A number of Bragg reflections can be seen with 2q values of 31.78°, 38.08°, 44.63°, 64.11°, 77.2° corresponding to the lattice planes, which may be indexed to (100), (111), (200), (220) and (311) facets of gold, respectively. It indicates that AuNPI3Cs are crystalline in nature, and the peaks well matched with the standard JCPDS (Jt. Com. Powder Diffraction Stds) cards of the gold metal (JCPDS, 1991).

Fourier transform infrared (FTIR) was measured to identify the possible biomolecules responsible for the reduction of gold ions and capping of the biosynthesized gold nanoparticles. The FTIR spectra of indole-3-carbinol and synthesized AuNPI3Cs are shown in Figure 2.4.

The peak at 3382.98 cm^{-1} represents N–H stretching vibrations of the peptide linkages (Schmitt and Flemming, 1998); 2870.62 cm^{-1} (Brown et al., 1999) corresponds to symmetric and anti-symmetric vibration bands of CH2 and CH3 groups of amino acid side chains. The peak at 1663.52 cm-1 (Seelenbinder et al., 1999) corresponds to the stretching C=O and bending. The peak at 1454.22 cm^{-1} (Kamnev et al., 2002) indicates the bending of CH2 modes. The shifts in the peak positions from 3382.98, 2870.62, 1663.52, and 1454.22 cm-1 to 3405.74, 2835.35, 1694.88 and 1457.73 cm^{-1}, respectively, in synthesized AuNPI3Cs indicate that indole-3-carbinol is involved in reduction and stabilization of the gold nanoparticles.

Transmission electron microscopic (TEM) study reveals the size of the particles and the morphology of biogenic AuNPI3Cs. From TEM study it was revealed that AuNPI3Cs are spherical in nature having mean diameter of 2-4 nm. It was also found that there was no aggregation of particles (Figure 2.5A). The observed size of the nanoparticles was approximately lower than the hydrodynamic diameter obtained from DLS experiment. A little difference was seen obtained from TEM and DLS measurement. TEM study actually represents the actual diameter of the nanoparticles as it is measured at the dry state (Gao et al., 2008).

SEM study showed that AuNPI3Cs are spherical in nature (Figure 2.5B). Gold nanoparticles generally showed (Figure 2.6) typical optical absorption peak approximately at 3 KeV due to surface plasmon resonance. EDX study showed that gold was the major constituent element as shown in figure 2.6.

Sphere-like morphologies for AuNPI3Cs was observed in AFM micrographs with the size ranges from 2-6 nm. A 3D rendering type of the surface topography of is presented in Figure 2.7. Using AFM analysis, individual particles can be resolved and unlike other microscopy techniques, the AFM offers visualization and analysis in three dimensions (Rao et al., 2007).

1H NMR proved reduction of hydro-chloroauric acid to synthesized gold nanoparticles. The peaks at 7-8 ppm were due to aromatic group that is present in indole-3-carbinol. The peaks at 7-8 ppm of AuNPI3Cs were slightly stretched (Figure 2.8). Appearance of the peculiar signals at 4 ppm was due to the formation of amide bond through reaction between indole-3-carbinol and chloroauric acid as shown in Figure 2.8. Appearance of the other at 1-2 ppm was due to the formation of aliphatic stretching. In this study, 1H solution NMR spectroscopy was used to investigate the interface of the functional groups of AuNPI3Cs. Via 1D-1 H and DOSY NMR spectra, we were able to highlight that indole-3-carbinol is responsible for AuNPI3Cs formation.

2.5 Conclusion

In conclusion, we have investigated the use of indole-3-carbinol as a reducing and stabilizing agent for the synthesis of biogenic AuNPI3Cs. The synthesis of gold nanoparticles was confirmed by color change of liquid medium from white to dark purple and it exhibited its maximum absorbance at 498 nm which played a prominent role in the reduction of hydro-chloroauric acid to gold nanoparticles. The crystalline nature of nanoparticles was confirmed by the X-ray diffractometer. The Fourier transform infrared spectroscopy showed the binding capability of functional groups of indole-3-carbinol with gold nanoparticles. The UV-vis, TEM and DLS results show that as synthesized AuNPI3Cs are spherical in shape with an average size ranging from 2-7 nm. The advantages of phytomedicine used for synthesis are very decisive because it is cost effective, protects human health and environment.