

Green route synthesis of stable isotropic gold nanoparticles using leaf extract of *Curcuma longa* and their characterization

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ABSTRACT

*In this work, the facile green route synthesis of ultra-stable, spherical Gold nanoparticles (GNPs) has been demonstrated by the simple green biosynthesis method reducing Chloroauric acid (HAuCl₄). This investigation described; use of farm-fresh leaves of *Curcuma longa* plant for green biosynthesis of extremely stable isotropic and monodisperse GNPs. The stability of the GNPs was found to be up to 10.27 M, when tested using 5M NaCl solution. The Flocculation Parameter (FP) value was found to be max up to 0.92 P. The size of the GNPs found to be ranging from 10 nm to 30 nm with average size of 20 nm. The best parameters for the synthesis of GNPs were; fifty folds diluted leaf extract, 100°C; at pH 6.68 of leaf extract, and 100 ppm HAuCl₄. The GNPs were characterized and investigated by ultraviolet-visible (U.V.-Visible) spectrophotometry, Field Emission Gun Scanning Electron Microscopy (FEG-SEM); Energy Dispersive Analysis of X-rays (EDAX).*

Keywords: *Curcuma longa*, isotropic; monodisperse, Flocculation Parameter (FP) value; Field Emission Gun; Scanning Electron Microscopy (FEG-SEM), Energy Dispersive Analysis of X-rays (EDAX).

INTRODUCTION

Different chemical and physical methods have used to synthesize nanoparticles with different chemical compositions, sizes, and shapes. However, with the development of these new methods, the concern for environmental contaminations is also increasing as the uses of chemical procedures in the synthesis of nanomaterials generate a large amount of hazardous byproducts. Thus “green chemistry” concept that uses biological organisms such as microorganisms, which includes different species of bacteria, actinomycetes; algae, fungi; yeast, and plant biomass or plant extracts. It is a safe alternative to conventional chemical and physical methods for the production of nanomaterials by reducing salts of metals to corresponding nano-metals. [1] Chloroauric acid when exposed to aqueous extracts of the plant; it has resulted in the intracellular as well as the extracellular formation of metal nanoparticles. The rate of formation of nanoparticles and the size of the nanoparticles, to an extent, manipulated by controlling parameters such as pH, temperature, and substrate dilution ratios.

The biological production of metal nanoparticle involves the reduction of metals by the enzymes and active phytochemicals in the leaf extract. This results in synthesis of metal nanoparticles from plant material of different shape and size, which provides an environmentally friendly method to produce invaluable materials as biosynthesis eliminates the need to use harsh and toxic chemicals.

GNPs are under consideration of an important area of research. Increasing awareness towards “green chemistry” and other biological processes has led to a desire to develop an eco-friendly approach for the synthesis of nanoparticles.

Since the synthesis of nanoparticles of different chemical compositions, sizes; shapes, and controlled dispersity are significant aspect of nanotechnology; new cost-effective procedures are under development status. Researchers also envisage that the Citric acid, Ascorbic acids, flavonoids, reductases, terpenoids and dehydrogenases and extracellular electron shuttlers; play a significant role in Nanomaterial Synthesis. [2]

To the best of my knowledge, I am presenting a first report on the use of aqueous extract of *Curcuma longa* leaf for the synthesis of GNPs. The stability of the respective GNPs is studied and calculated using a physical quantity called "Flocculation Parameter" (FP) originally given by Weisbecker et al. [3] *Curcuma longa* is locally famous in India as Haldi; and it belongs to the tropical variety of family Zingiberaceae. It contains an active flavonoid Curcumin and Cardiac glycosides, Phenols; etc. as other phytochemical. [4]

MATERIALS AND METHODS

Biological Material:

Farm-fresh leaves of adult *Curcuma longa* plant, commonly known as Turmeric leaves were procured from insecticides, pesticides or chemical fertilizer free plant resource for green biosynthesis of GNPs.

Chemicals and Glassware's:

Chloroauric acid was procured from Sigma Aldrich, USA. NaCl and 1N HCl and 1N NaOH solutions were procured from HiMedia Laboratories Pvt. Limited, India. The experiments were performed using double distilled water (18MΩ) in Erlenmeyer Conical Flask. For storage of GNPs; Small Glass Bottles, with caps were used. The glassware's were washed with aqua regia to remove the traces of metal contaminants.

Instruments:

For recording the temperature, thermocouple was used, whereas for measuring pH; pH meter was used. For performing various essential procedures during intermediate steps Pre-calibrated instruments were used.

Preparation of Plant Extract:

To prepare the extract, leaves of adult *Curcuma longa* plant having a length of about 1 foot were cut by using a pre-cleaned pair of scissors. From 100 grams of leaf crude extract was prepared in 1:1 (wt. /v) ratio (i.e. 100 grams of leaves were crushed in 100 ml of distilled water) using mortar and pestle. The paste was then filtered using muslin cloth. The filtered extract was further centrifuged at 5000 RPM for 8 minutes to get the clear extract. The supernatant was used as reducing agent for synthesis of Gold nanoparticles. In order to retain the activity of the enzymes like Nitrate Reductase and other factors such as phytochemicals, which are flavonoids, the extract was made in the ice box. The fresh extract was prepared and used every time for the synthesis of GNPs.

Dilution of standard 50,000 ppm $H AuCl_4$ to form 50, 100; 150, 200; and 250 ppm working Gold stock:

For making various $H AuCl_4$ dilution's following formula is used:

Calculation:

x = Required Volume
 R = Required Concentration
 T = Total Volume
 G = Given Concentration

$$\therefore x = \frac{R \times T}{G} \quad (1)$$

Procedure for Biosynthesis of the Gold Nanoparticles:

Clear extracts of adult *Curcuma longa* plant leaves were used for the biosynthesis of GNPs. A stock solution of 50,000 ppm $H AuCl_4$ was prepared and diluted as per the pre-requisite of the experiment to form 50, 100; 150, 200; and 250 ppm working Gold stock solutions. Due to, most significantly narrow and best peak was observed at 538 nm; because of which the most influential concentration of $H AuCl_4$ was found to be at 100 ppm. Hence, for all the experiments, 100 ppm concentration was used. In order to optimize the nanoparticle formation, the impacts of various dilutions (1:5, 1:10; 1:50, 1:100; 1:500, and 1:1000), temperature (4°C, 30±2°C (R.T.), 60°C and 100°C) and various pH ranges (inherent (6.68), 2; 4, 6; 8, and 10) on synthesis of GNPs were studied. All the pH values

(inherent (6.68), 2; 4, 6; 8, and 10 were adjusted using 1N NaOH and 1N acetic acid to avoid chloride induced flocculation [5] of the GNPs. The parameters obtained from the above first two experiments were kept constant to comprehend the impact of pH on the optical as well as morphological features of GNPs.

COMPUTATIONAL PART

GNPs synthesized at inherent pH (6.68) value were selected for performing the computational part. For mathematical calculations, Windows 7 computer with Microsoft Office 2010 was used. For calculation of stability and Flocculation Parameter (FP) value; instruments like dual beam spectroscopy Lambda 25, Perkin Elmer, USA, was used whereas for characterization of the Biosynthesised GNPs; Field Emission Gun Scanning Electron Microscopy (FEG-SEM), and Energy Dispersive Analysis of X-rays (EDAX) were used.

Stability and Flocculation Parameter:

GNPs synthesized at inherent pH (6.68) value were selected for performing a stability test after optimization of parameters like dilution ratio, temperature and pH. 3 ml of the GNPs synthesized at respective above parameters was taken in an ultra-clean quartz cuvette, and initial spectrum was recorded between 400 and 900 nm. 5M NaCl salt was added in a gradually increasing manner (10 μ l, 50 μ l; 100 μ l, 500 μ l; and so on, increasing values) to the same cuvette, and U.V.-Visible spectrum was recorded to see the effect of salt in the form of red or blue shift of the maximum absorbance peak in the spectrum. This procedure was repeated until the peak became flat or stable. Flocculation parameters were calculated by measuring the integrated absorbance between 529 and 532 nm in case of GNPs. [6] Equation (2) was used to calculate the integrated absorbance:

$$P = \int_{529}^{532} I_{Abs} \lambda (dx) \quad [5] \quad (2)$$

Characterization of the Biosynthesised Gold Nanoparticles:

U.V.-Visible Spectroscopy of the Gold nanoparticles: -

The U.V.-Visible spectra of the GNPs formed were recorded using High-quality quartz cuvette (as a vessel) and dual beam spectroscopy Lambda 25, Perkin Elmer, USA. The cuvette containing GNPs solution was placed directly inside the spectrophotometer machine, and the electromagnetic wave absorption readings took by adjusting the reading range between 400-900 nm.

Scanning electron micrographic Analysis and EDAX: -

To elucidate the morphology of the GNPs biosynthesized using *Curcuma longa* leaf extract; Field Emission Gun Scanning electron microscope (FEG-SEM), Carl Zeiss Micro imaging; GmbH, Germany; operating at 10 KV was used. The small glass bottle containing GNPs solutions was mixed well, and with the help of a plastic tip auto pipette; 5-10 μ l of solutions were poured on the small square shaped acetone ultra-cleaned silicon wafer chips. The samples were allowed to air dry completely, and then with the help of forceps the silicon wafer chips were picked-up and kept inside the FEG-SEM for the analysis of GNPs coated on the surface.

RESULTS AND DISCUSSION

Impact of leaf extract dilution ratios on inherent pH (6.68); with different temperatures on the biosynthesis of GNPs using 100 ppm $HAuCl_4$ presented in table - 1, which showed that 1:50 dilution yielded the best results at both the tried temperatures. Therefore, further trials were done to detect the impact of temperatures at inherent pH (6.68) with 1:50 leaf extract dilution on the biosynthesis of GNPs using 100 ppm $HAuCl_4$ in table - 2, which showed that 1:50 dilution yielded the best results at 100 $^{\circ}$ C temperature. In the next step, impact of various pH at 100 $^{\circ}$ C temperature; with 1:50 leaf extract dilution on the biosynthesis of GNPs using 100 ppm $HAuCl_4$ showed in table - 3. In conclusion; at the constant 1:50 dilution and 100 $^{\circ}$ C temperature; inherent pH (6.68) yielded the best results. At inherent pH (6.68), the impact of $HAuCl_4$ concentration was also studied (tabular and graphical data not shown). For checking the salt stability and flocculation parameter (FP) of GNPs using 5M NaCl; sample made with 1:50 dilution of a leaf extract at 100 $^{\circ}$ C temperature having an inherent pH (6.68) is selected. The sample showed salt tolerating stability up to 10.27 Molar with FP value of 0.92 P.

Visual Observation:

GNPs formation using the plant leaf extract was confined due to the change of color from colorless to wine red, pink and blue color depending on the size of the nanoparticles. [7] GNPs get formed by the reduction of Au^{+3} to Au^0 .

This is due to the fabrication of GNPs with the molecular assistance of biological reducing agents present in the plant extract. [2] The intensity for the appearance of the color varied drastically with the dilution ratio, temperatures and pH. The time taken for the reduction of Gold ions to Gold nanoparticles was also found to be dependent on the dilution ratio, temperature of the reactants, pH as well as the concentration of $HAuCl_4$.

In table - 1, 2, and 3; the ruby-red color accounts for the smallest nanoparticle [8], whereas; the blue and a purple colors indicated the increment in size and hence peculiar interactions with light.

In table – 1; it was also observed that; faster synthesis of GNPs in the solution \propto Dilution of reducing species and availability of substrate to the enzyme (up to certain extent).

In table – 2; it was also observed that; Faster synthesis of GNPs in the solution \propto Increase in temperature (up to a certain extent).

In table – 3; it was also observed that; faster synthesis of GNPs in the solution $1 / \propto$ Increase or decrease in the pH (up to certain extent).

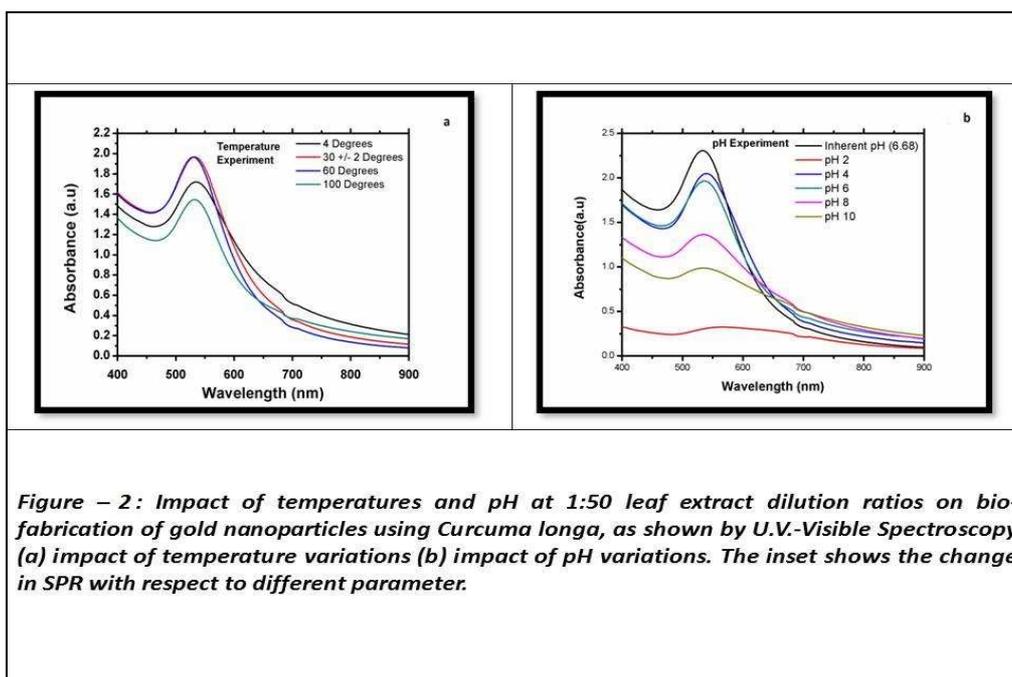
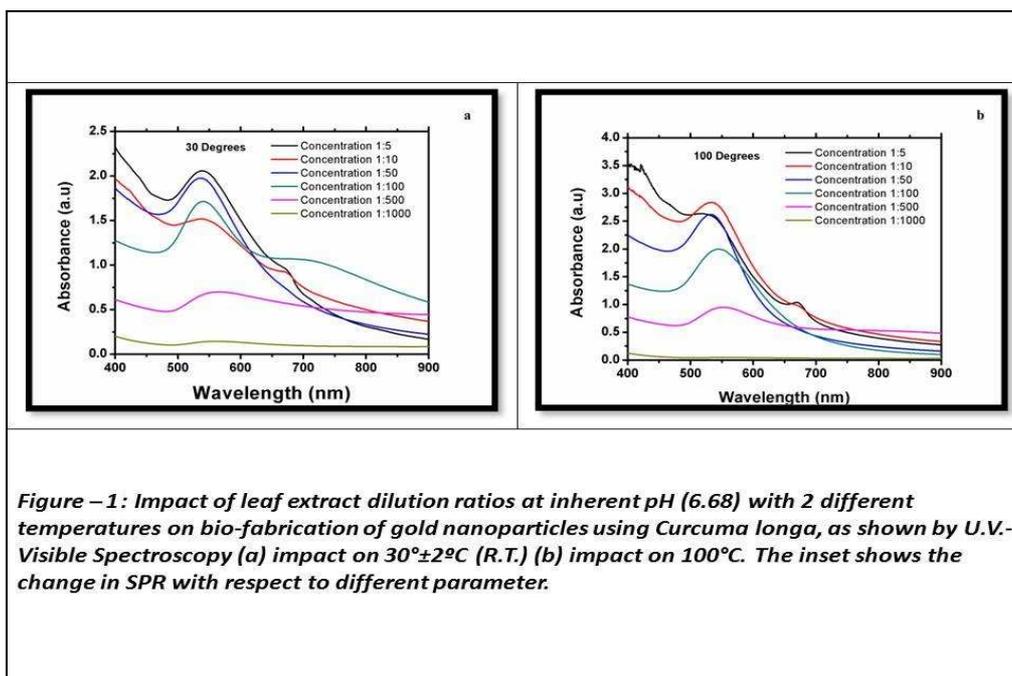
With the results obtained from above three different parameters affecting the synthesis of GNPs experiments; the 1:50 *Curcuma longa* leaf extract dilution ratio at 100°C with inherent pH (6.68) was selected. GNPs salt stability and Flocculation Parameter (FP) using 5M NaCl was checked, to see the effect of salt in the form of the red or blue shift of the maximum absorbance peak in the U.V.-Visible spectrum.

Characterization of GNPs with the U.V.-Visible dual beam spectrophotometer:

SPR property is exploited for determining the qualitative and quantitative analysis of the presence of isotropic or anisotropic nanoparticles in the solution. Singular, narrow peak (TSPR); it indicates monodispersed nanoparticles and singular wide peak indicates polydispersity. Dual peak (TSPR and LSPR); it dictates polydispersity as well as the presence of anisotropic nanoparticles. Usually, the maximum absorption occurs in the range of 400 nm to 1000 nm i.e. from a visible range to NIR-range.

U.V.-Visible Spectroscopic analysis:

As shown in U.V.-Visible spectra (figure - 1 (a, b), figure - 2 (a, b); and figure - 3 (a)); the SPR bands centered between 500-600 nm confirm the formation of GNPs in the solution. The presence of the sharp peak near to 520-530 nm range; it indicates the formation of good quality isotropic and monodispersed GNPs in the solution. The appearance of the peak is due to the size dependent quantum-mechanical phenomenon called SPR. This effect becomes influential when the De-Broglie wavelength of the valence electrons becomes equal to or less than the size of the particle (less than 50 nm). [7] Formation of the range of colors and the SPR in GNPs are due to the confinement of electrons; when the size of the metal particles enters in the nano-scale (10-100 nm). The free mobile electrons are captured and locked in GNPs and show a characteristic collective coherent oscillation of Plasmon Resonance giving rise to Surface Plasmon Resonance (SPR).



In table – 1, Part - A; at 30±2°C, the SPR band of the GNPs synthesized at inherent pH with a range of dilution ratios were found to be centered between 537 to 565 nm. The most influential dilution affecting the synthesis of GNPs was found to be 1:50. This can be speculated because of a sharp peak centered at 537 nm. As per the studies, the size of the nanoparticles exhibiting a peak at this wavelength is between 30-40 nm. There was a minor red shift in the SPR of GNPs synthesized at inherent dilution 1:5 and 1:10 (from 537 nm to 538 nm); with sharp (1:5), and broad, and a minor peak (1:10) respectively. Red shift is also observed at 1:100 dilution showing dual peaks; first sharp at 540 and another weak peak at 668 nm; which was appeared in the near infra-red region as shown in table - 1. The appearance of dual peaks at 532 and 670 nm may be due to reference [10]. In 1:500; broad and minor peak at

565 nm with a red shift of 28 nm is observed. Dilution ratio 1:1000 with no peak, nearly flat absorption spectra was found to be almost failed in the formation of GNPs. Due to the combined effect of agglomeration and the orientation of the nanoparticles in the solution; there was a red shift observed at dilution ratios 1:5, 1:10; 1:100, and 1:500 (table - 1).

Table - 1: Impact of leaf extract dilution ratios at inherent pH (6.68) with different temperatures on the Biosynthesis of GNPs using 100 ppm HAuCl₄:
Part - A

Observations for dilution ratio variation at 30°C						
Sr. No.	Dilution Ratio (1/1)	Temperature (°C)	Visual Observation	U.V.-Visible Peak	Inference of U.V.-Visible Peak	Color of GNPs
1.	1:5	30±2°C (R.T.)	Change in color in < 24 hours	Sharp peak at 538 nm	Formation of isotropic GNPs in the solution.	
2.	1:10	30±2°C (R.T.)	Change in color in < 24 hours	Broad and minor peak at 538 nm	Presence of large GNPs in the solution. GNPs solution at this dilution was found to be unstable.	
3.	1:50	30±2°C (R.T.)	Change in color in < 24 hours	Sharp peak at 537 nm	Formation of monodisperse GNPs in the solution.	
4.	1:100	30±2°C (R.T.)	Change in color in < 24 hours	Dual peak, Sharp at 540 and weak at 668 nm	Formation of anisotropic nanoparticles, agglomeration of the nanoparticles or the combined effect of both the phenomenon.	
5.	1:500	30±2°C (R.T.)	Change in color in < 24 hours	Broad and minor peak at 565 nm	Formation of large spherical as well as non spherical GNPs in the solution	
6.	1:1000	30±2°C (R.T.)	Change in color in < 24 hours	No peak, nearly flat absorption spectra	Formation hardly few GNPs or absence of GNPs in the solution.	

In table - 1, Part - B; at 100°C, the SPR band of the GNPs synthesized at inherent pH with a range of dilution ratios; were found to be centered between 517 to 553 nm. The most influential dilution affecting the synthesis of GNPs was found to be 1:50. This can be speculated because of a sharp peak centered at 534 nm. As per the studies, the size of the nanoparticles exhibiting a peak at this wavelength is between 10-40 nm. There was a blue shift in the SPR of GNPs synthesized at inherent pH; 1:5 dilution with 17 nm. Blue shift indicates the decrease in the diameter of the GNPs as well as its stabilization in the solution. Blue shift in this case can be either due to a reduction in the size of GNPs and/or multiple coatings of surface protecting agents on GNPs. It also indicates stabilization of the GNPs in the solution due to the favourable thermodynamic interaction of capping proteins on the surface of GNPs. Dilution 1:10 showed a moderately sharp peak at 534 nm. Red shift is also observed at 1:100 dilution; showing sharp peak at

546 with 12 nm red shifts. In 1:500; minor and broad peak at 553 nm with a red shift of 19 nm is observed. Dilution ratio 1:1000; with no peak, nearly flat absorption spectra was found to be failing in the formation of GNPs.

Table - 1: Impact of leaf extract dilution ratios at inherent pH (6.68) with different temperatures on the Biosynthesis of GNPs using 100 ppm HAuCl₄:
Part - B

<i>Observations for dilution ratio variation at 100°C</i>						
Sr. No.	Dilution Ratio (1/l)	Temperature (°C)	Visual Observation	U.V.-Visible Peak	Inference of U.V.-Visible Peak	Color of GNPs
1.	1:5	100°C	Change in color in < 5 seconds	Minor and insignificant peak at 517 nm	Formation of least quantity of anisotropic GNPs in the solution.	
2.	1:10	100°C	Change in color in < 5 seconds	Moderate and sharp peak at 534 nm	Formation of significant quantity of isotropic GNPs in the solution.	
3.	1:50	100°C	Change in color in < 5 seconds	Sharp peak at 534 nm	Formation of very large quantity of isotropic GNPs in the solution.	
4.	1:100	100°C	Change in color in < 5 seconds	Sharp peak at 546 nm	Formation of very large quantity of isotropic GNPs in the solution.	
5.	1:500	100°C	Change in color in < 5 seconds	Minor and broad peak at 553 nm	Formation of medium quantity of anisotropic GNPs in the solution.	
6.	1:1000	100°C	Change in color in < 5 seconds	No peak, nearly flat absorption spectra	Absence of GNPs in the solution.	

In table - 2, at 1:50 dilution ratio; the SPR band of the GNPs synthesized at inherent pH with a range of temperature variations were found to be centered between 530 to 535 nm. The most influential temperature influencing the ultra fast, and most stable synthesis of GNPs; were found to be at 100°C. This can be speculated because of a sharp peak centered at 533 nm. As per the studies, the size of the nanoparticles exhibiting a peak at this wavelength is between 10-30 nm. There was a minor red shift in the SPR of GNPs synthesized at inherent pH; 4°C temperature with 2 nm. At 30±2°C temperature; minor blue shift of 1 nm was observed. Further blue shift of 3 nm with a sharp peak at 530 nm was observed at 60°C temperature; indicating reduction in size of GNPs.

Table – 2: Impact of temperatures at inherent pH (6.68) with 1:50 leaf extract dilution on the Biosynthesis of GNPs using 100 ppm HAuCl₄

<i>Observations for temperature variation</i>						
Sr. No.	Dilution Ratio (l/l)	Temperature (°C)	Visual Observation	U.V.-Visible Peak	Inference of U.V.-Visible Peak	Color of GNPs
1.	1:50	4°C	Change in color in > 24 hours	Sharp peak at 535 nm	Formation of large quantity of isotropic GNPs in the solution.	
2.	1:50	30±2°C (R.T.)	Change in color in < 24 hours	Sharp peak at 532 nm	Formation of very large quantity of isotropic GNPs in the solution.	
3.	1:50	60°C	Change in color in < 5 minutes	Sharp peak at 530 nm	Formation of very large quantity of isotropic GNPs in the solution.	
4.	1:50	100°C	Change in color in < 5 seconds	Sharp peak at 533 nm	Formation of large quantity of isotropic GNPs in the solution.	

In table - 3, at 1:50 dilution, the SPR band of the GNPs synthesized at 100°C with range of pH variations were found to be centered between 534 to 567 nm. The most influential pH affecting the synthesis of GNPs was found to be the inherent at 6.68. This can be speculated because of a sharp peak centered at 534 nm. As per the studies, the size of the nanoparticles exhibiting a peak at this wavelength is between 10-30 nm. There was a major red shift in the SPR of GNPs synthesized at pH 2 with 33 nm. A minor, insignificant and nearly flat peak approximately at 567 nm was observed with pH 2; which may be due to excess protonation which resulted in change of charge of the capping proteins. GNPs at pH 4 showed a sharp peak at 540 nm with 6 nm red shifts. At pH 6; a sharp peak at 536 nm with minor 2 nm red shift was observed. Moderate peak at 534 nm is shown by pH 8. At pH 10, broad and minor peak at 535 nm, indicating formation of low quantity of anisotropic GNPs in the solution with minor 1nm red shift.

Table – 3: Impact of various pH values at 100°C temperature with 1:50 leaf extract dilution on the Biosynthesis of GNPs using 100 ppm HAuCl₄

<i>Observations for pH variation</i>							
Sr. No.	pH	Dilution Ratio (1/1)	Temperature (°C)	Visual Observation	U.V.-Visible Peak	Inference of U.V.-Visible Peak	Color of GNPs
1.	Inherent pH (6.68)	1:50	100°C	Change in color in < 5 seconds	Sharp peak at 534 nm	Formation of large quantity of isotropic GNPs in the solution.	
2.	2.00	1:50	100°C	Change in color in < 5 seconds	Minor, insignificant and nearly flat peak at 567 nm	Formation of least quantity of anisotropic GNPs in the solution.	
3.	4.00	1:50	100°C	Change in color in < 5 seconds	Sharp peak at 540 nm	Formation of very large quantity of isotropic GNPs in the solution.	
4.	6.00	1:50	100°C	Change in color in < 5 seconds	Sharp peak at 536 nm	Formation of very large quantity of isotropic GNPs in the solution.	
5.	8.00	1:50	100°C	Change in color in < 5 seconds	Moderate peak at 534 nm	Formation of medium quantity of isotropic GNPs in the solution.	
6.	10.00	1:50	100°C	Change in color in < 5 seconds	Broad and minor peak at 535 nm	Formation of low quantity of anisotropic GNPs in the solution.	

Stability of biogenic versus chemically synthesized GNPs:

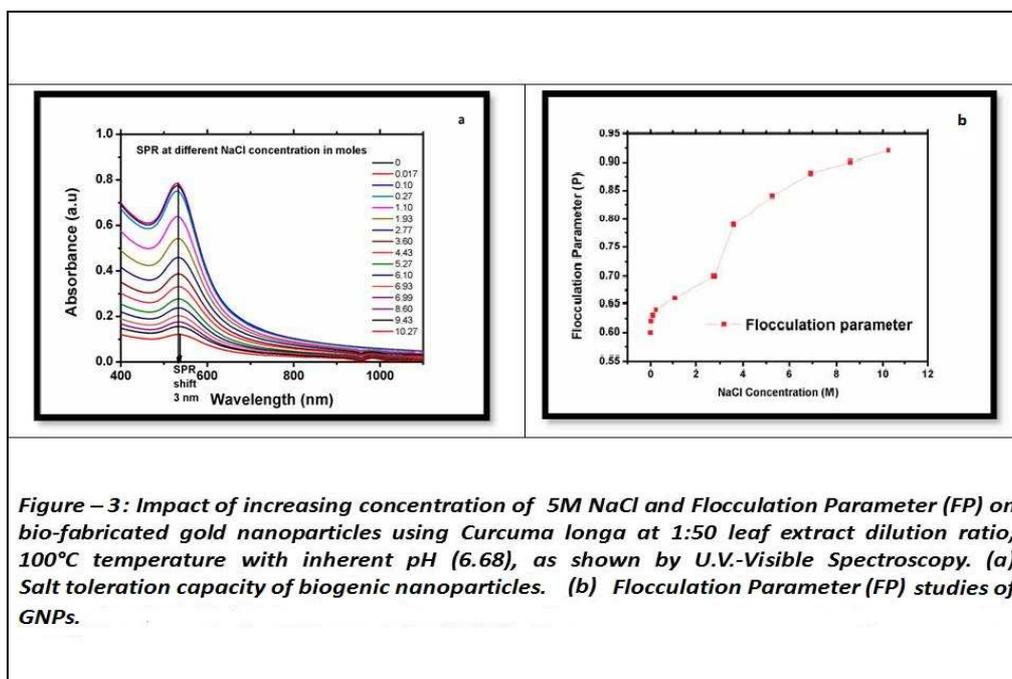
For solving the problem of agglomeration of GNPs in solution; particularly while their suspension in high salt concentration for clinical trial such as drug delivery, the stability of biological nanoparticles was tested against very high salt concentration. As shown in the figure - 3 (a), there was a red shift of 3 nm after addition of 6.160 ml (6160 µl) of 5M NaCl. The stability of the GNPs synthesized at 1:50 leaf extract dilution, at 100°C with inherent pH (6.68); was reluctant for coagulation even after the addition of 6160 µl of 5M NaCl. Additions after 6160 µl of 5M NaCl; GNPs were found to lose their capacity to tolerate increasing quantity of 5M NaCl concentration and no electromagnetic wave absorption peak; nearly flat absorption spectra was observed. Therefore; the 5 M salt stability, absorption results were showing up to 6160 µl. 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 100 µl of 5 M NaCl. This exceptional stability of biogenic nanoparticles can be attributed to the protection of GNPs by intelligent capping proteins. Under the optimal ionic strength of the solution; these proteins avoid the coulombic attraction between the nanoparticles by maintaining suitable surface potentials. [2]

Stability of the GNPs and Flocculation Parameter at 1:50 dilution, 100°C with inherent pH 6.68 Value:

Stability of the GNPs was studied by adding multiples of 500 μl of 5M NaCl; with respect to increasing concentration of Sodium chloride salt, and recording the spectra after each addition of salt solution. The purpose behind this was to study the change in the spectral properties due to flocculation induced by increasing salt concentration. Under the influence of salt, nanoparticles start forming clusters and hence shift in the peak towards longer wavelengths (529-532 nm in case of GNPs) results. [11] This can be seen in the form of red shift. Physical relation between the origin of the red shift and aggregation of the metal nanoparticles was given originally by Quinten and Kreibitz, 1986. [12] According to their studies, when the distance between flocculating spheres is smaller than the radius of the spheres, the resonance shifts to longer wavelengths. [5]

As the red shift value increases; stability of the particle decreases at that salt concentration. Figure - 3 (a) and figure - 3 (b) shows the spectral properties of GNPs; after the addition of the salt at inherent pH 6.68. At that pH; initial minor blue shift was observed with 1 nm after addition of total 160 μl of 5M NaCl. The initial minor red shift was observed to be 1 nm; with a sharp peak at 531 nm after addition of total 660 μl of 5M NaCl. Further; additions of 5M NaCl lead to increase in red shift maximum up to 535 nm with a continuous decrease in peak intensity. Maximum low quality peak was observed after addition of 4160 μl of 5M NaCl. Moreover, the continuous decreasing minor peak was observed from addition of 4160 μl to 6160 μl . Increasing red shift value of 535 nm was observed up to addition of 5660 μl of NaCl solution, with a minor peak at 535 nm. After addition of 6160 μl of 5 NaCl; the minor blue shift with 2 nm was observed. Maximum minor hump at 535 nm after addition of 6160 μl of 5M NaCl; indicates agglomeration of GNPs in the solution. Further increasing salt concentration additions after 6160 μl ; showed complete absence of peak so Flocculation Parameter was studied up to 6160 μl . The calculated FP value was found to be max up to 0.92 P after the last addition of 6160 μl of 5M NaCl.

The most important terminology to display the stability of the nanoparticles is a flocculation parameter (FP) which was originally used by Wiesbecker et al. [3] and it was partially modified by Sastry et al. [6]. FP of GNPs with respect to increasing 5M NaCl concentration is shown in figure - 3 (b). It can be clearly seen that; the inherent pH value 6.68, FP was found to be increasing with respect to increasing salt concentration; indicating the agglomeration of GNPs. This finding is also supported by a minor red shift seen at this pH value (figure - 3 (b)). Moreover, presence of turbidity in the GNPs (data not shown) shows agglomeration as evidence to above finding. Blue shift in this case indicated the agglomeration or an increase in the multiple coatings of NaCl on the surface of nanoparticles, which may finally result in the agglomeration. At inference; it can be stated that increase in FP value is $1 / \propto$ increased stability of GNPs in the solution. [13] and [14].



Nitrate Reductase Activity:

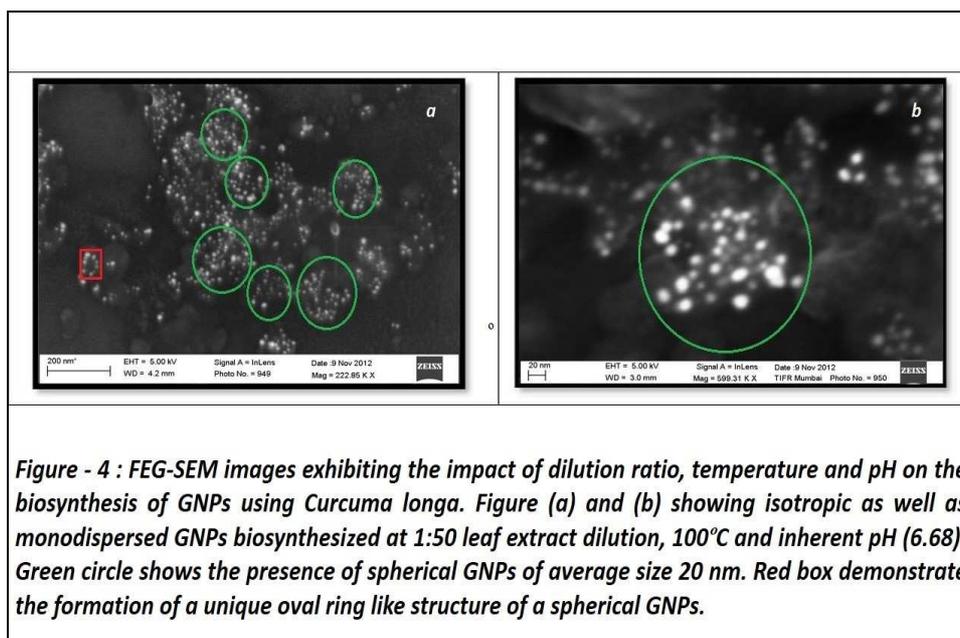
The Nitrate Reductase activity involved in catalyzing the nanoparticle biosynthesis was studied by Vega and Cárdenas method (data not shown). Nitrate Reductases help in the NADH dependent extracellular reduction of Au^{+3} to Au^0 ; thus leading to the formation of GNPs. Nitrate Reductases are assumed to be the most efficient NADH-dependent enzyme, which act as a nucleating as well as capping agent for the Gold nanoparticle biosynthesis. Nitrate Reductase activity in reduction of HAuCl_4 , and later formation, stabilization of GNPs; done by capping proteins and Curcumin, which acting as an active flavonoid. In further steps; Nitrate Reductase enzyme acts as a capping agent, thus ensuring the complete formation of thermodynamically stable nanoparticles. [15] (Tabular and graphical data not shown).

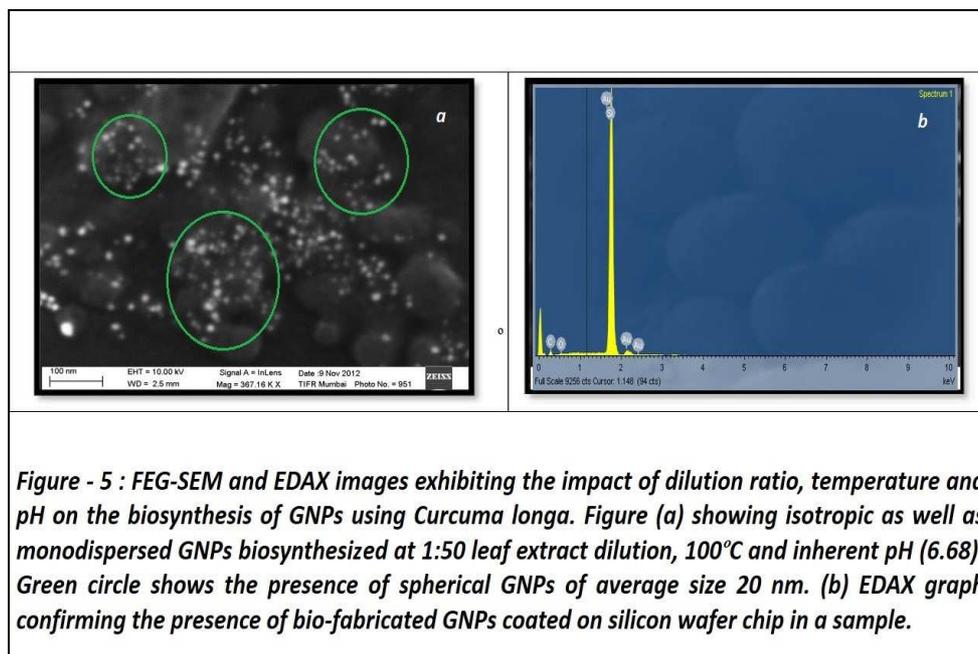
Electron microscopic studies:

The morphology of GNPs was studied using field emission gun scanning electron microscope (FEG-SEM). FEG-SEM image of the GNPs bio-fabricated at 1:50 plant leaf extract dilution, 100°C , and inherent pH (6.68), exhibit the presence of extremely small GNPs (figure - 4 (a, b), and Figure - 5 (a)). The size of the nanoparticles was found to be ranging between 10 to 30 nm; having an average size of 20 nm. Inherent pH, like earlier results, was observed to be the most favorable condition for the synthesis of isotropic, monodisperse, and stable nanoparticles. At 1:50 plant leaf extract dilution, 100°C and inherent pH (6.68); the GNPs with unique morphological features were observed. In figure - 4 (a, b), and figure - 5 (a) aggregation of isotropic GNPs having a spherical shape in green rings is shown. Moreover, the typical oval-shaped ring of GNPs was observed and covered in the red box. This may be: An intermediate step in the formation of non-spherical nanoparticles. Due to less potential at edges, Gold nano-spheres must have aligned themselves in such typical morphology. This also helps in the overall thermodynamic stability of the structure. The mechanism for the formation of such unique structures by the biological system is still in a nutshell. However, it can be speculated that this is due to the enzyme or capping protein assisted nucleation and growth at different facets of GNPs. [9]

EDAX graphical studies:

In graphical figure - 5 (b); each peak is indicating the presence of specific material from the sample. The Au (Gold), Si (Silicon); C (Carbon), O (Oxygen) materials showed their characteristic excitation peaks in the sample region. In the present analysis; spherical nanoparticle was selected randomly under FEG-SEM and X-rays were emitted from the source. The X-rays came in contact with nanoparticle; gave one large and many small peaks in EDAX graph. The larger and smaller peaks indicated the presence of Au (Gold) in the sample. The large peak also indicated the Si (Silicon) below the Au (Gold); which confirmed that the coating of the GNPs solution on the surface of thin Silicon wafer chip.

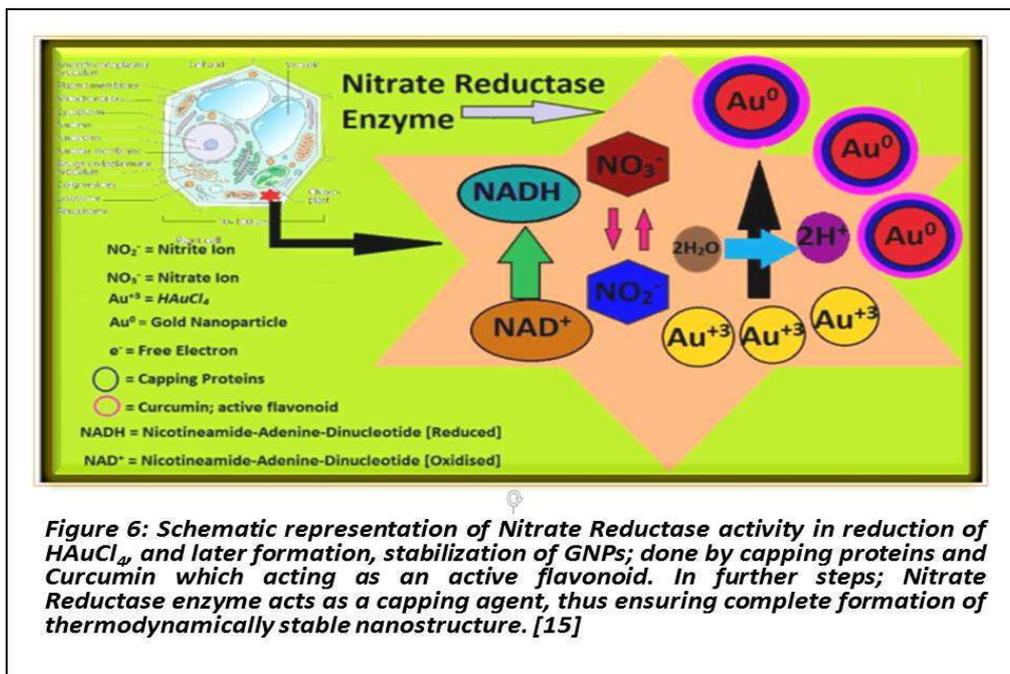




CONCLUSION

In conclusion; the reducing potential of the *Curcuma longa* leaf extract was exploited to synthesize stable, isotropic, and monodispersed GNPs. *Curcuma longa* can be used as a potential alternative to available chemical methods used for commercial production of GNPs due to its exceptionally high reducing potential; which in turn was because of, presence of, the high amount of Curcumin as an active flavonoid. It took less than 5 seconds at 100°C for the reduction of GNPs which made it one of the most powerful plant materials reported until a date. Green chemistry is an emerging field and green route synthesis being an eco-friendly process is non-hazardous. GNPs synthesized were temperature stable, pH stable, and NaCl stable so can be used for a plethora of applications in the field of Bioimaging, optics; drug delivery, and textile industry.

*In the future, there will be a huge potential for research using plant sources to synthesize GNPs; and which can be safely used as therapeutic carriers in treatment of various diseases, and also in allied areas. The clear colloidal suspension of GNPs was stable for more than six months at 4°C. Presence of an active flavonoid Curcumin content in the leaves of *Curcuma longa*; has a large advantage over other plants as it highly reduced HAuCl_4 to form isotropic and monodispersed GNPs, and the average particle size was 20 nm which was extremely small, and this made the nanoparticles favourable for a wide-spread application; with increased salt stability, good flocculation parameter, and extremely higher shelf life.*



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