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Green Synthesis and Characterization of Gold Nanoparticles using *Mucuna monosperma*

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ABSTRACT

The utility of plant-based phytochemicals in general synthesis and engineering of nano-phytomedicine is the association between plant science and nanotechnology that gives intrinsically green approach to nanotechnology referred as green nanotechnology. The present work aims at green synthesis of gold nanoparticles from different concentrations of *Mucuna monosperma* seed extract and its characterization by methods like scanning electron microscopy (SEM), UV-Visible spectrophotometer and Fourier transform infra red (FTIR) spectroscopy. The gold nanoparticles using 4% seed extract showed good stability over other concentrations. Analytical tools like FTIR, UV-Visible spectrophotometer and SEM helped understand the surface properties of the gold nanoparticles. The synthesized gold nanoparticles show spherical shape and also possibilities of L-DOPA from plant coating on it. This opens door to a lot of applications such as a major application in novel drug delivery system (NDDS).

1. Introduction

Nanoscience and nanotechnology are the study and use of extremely small things and can be utilized over the various science fields, for example, chemistry, biology, physics, material science, and engineering [1]. Though modern technology demands the development of nanotechnologies in multidisciplinary science, including the production of nanoparticles (NPs), it dates back from the Before-Christ era [2]. Nanoparticles can be synthesised using multiple methods like physical, chemical or biological, also known as green synthesis. Green synthesis includes use of microorganisms like fungi, yeast (eukaryotes) or bacteria, actinomycetes (prokaryotes); use of plant extracts or enzymes or use of templates like DNA, membranes, viruses and diatoms. Several of the current processes of nanoparticle generation uses poisonous chemical either as reducing agent for metals or as stabilizing agents to stop agglomeration of the nanoparticles. In spite of the fact that chemical and physical strategies may effectively create unadulterated, well characterized nanoparticles, these are very costly and possibly unsafe to the environment. As an alternative to poisonous and costly physical strategies for synthesis of, using microorganisms, plants and algae will aid a lot. Also, the poisonous quality of the by-product would be very less as compared to other synthetic methods [3]. The utility of plant-based phytochemicals in general synthesis and engineering of Nano-phytomedicine is the association between plant science and nanotechnology that gives intrinsically green approach to nanotechnology referred as green nanotechnology [4]. Phytochemicals show synergistic effect in the reduction of gold salt into its nanophytomedicine [5].

Au^{+3} ions (in gold solution) reduced to Au^{+1} and further Au^0 by reducing agents in the plant extract. Finally many Au^0 atoms assembled via nucleation process to form gold nanoparticles. Depending on the phytochemicals present in plant extract it support to form nanoparticles, which is charachterized by change in colour. Applications of nanotechnology have allowed overcoming the challenges and technical boundaries related to the insolubility, bioavailability, steadiness and delivery of bio-actives from foods/nutraceuticals. The most predominant strategy for the formation of monodisperse circular gold nanoparticles was pioneered by Turkevich et. al. in 1951 and afterward refined by Frens et. al. in 1973 [6-9]. This strategy uses the chemical reduction of gold salts such as hydrogen tetrachloroaurate ($HAuCl_4$) utilizing a reducing agent. Besides, the gold surface offers an interesting opportunity to conjugate ligands such as oligonucleotides, proteins, and antibodies containing useful groups such as thiols, mercaptans, phosphines, and amines, which illustrates a strong affinity for gold surface [10].

Mucuna monosperma is a large climbing shrub from family Fabaceae. Literature survey revealed that the seeds of *Mucuna monosperma* possess various pharmacological properties like astringent, cardiotonic, restorative, expectorant and used in the treatment of asthma, cough and tongue infection [11]. It is a rich source of L-DOPA, which is a non essential amino acid and widely used in the treatment of Parkinson's disease.

2. Experimental Methods

2.1 Developing Herbal Gold Nanoparticle using M. Monosperma Seed Extract

2.1.1 Preparation of M. monosperma Seed Extract [12]

Powdered seed was dissolved in methanol:water (50:50) (v/v). This solution was allowed to stand for 24 hrs. The resulting solution was centrifuged at 3000 rpm for 5 min and supernatant was collected and used further.

2.1.2 Preparation of Different Concentration of Seed Extract

Mucuna monosperma seed extract was prepared at various % concentrations ranging from 10-100%, at intervals of 10%. Later 1-10% range at intervals of 1% was prepared.

2.1.3 Preparation of $HAuCl_4$ Solution

Chloroauric acid ($HAuCl_4$) salt crystals were obtained from Synthman Chem Industry, GIDC, Rajkot, Gujarat, India. 1 mM solution of $HAuCl_4$ was prepared as described by Subramanian and Sabesan [13].

2.1.4 Preparation of Herbal-Gold Nanoparticles

10 mL of 1 mM $HAuCl_4$ was added to 10 mL of prepared different concentrations of seed extract (1%-100%) and subjected to heat in a water bath at 55 °C for 15 mins. The indication of gold nanoparticles formation identified by the colour change from pale yellow to purple [13].

2.2 Characterization of Gold Nanoparticles

The UV-visible spectra of synthesized gold nanoparticles were measured through a range of 200-900 nm using Shimadzu UV-1800

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spectrophotometer to check to the surface modification of the gold nanoparticles.

The Fourier transform infrared (FTIR) spectroscopy was carried out to identify the biomolecules for the synthesis of gold nanoparticles and a possible overlap on them. The characterization was done by Bruker, Germany; Model 3000 Hyperion Microscope with Vertex 80 FTIR System at Sophisticated Analytical Instrument Facility, IIT Bombay, Powai.

Samples for Field Emission Gun - Scanning Electron Microscopic (FEG-SEM) analysis was prepared by drop coating Au nanoparticles solutions onto carbon-coated copper SEM grids. The films on the SEM grids were allowed to stand for 2 min following which the extra solution was removed using a blotting paper and the grid is allowed to dry, prior to the measurement. FEG-SEM measurements were performed by JEOL JSM-7600F instrument operated at an accelerating voltage at 0.1 to 30 kv at Sophisticated Analytical Instrument Facility, IIT Bombay, Powai. SEM provides detailed high-resolution images of the sample by bombarding a focussed electron beam across the surface and detecting secondary or backscattered electron signal. An energy dispersive X-ray spectroscopy (EDAX) is also used to provide elemental identification and quantitative compositional information.

3. Results and Discussion

3.1 Wet Lab Results of Gold Nanoparticles

The initial yellow color of $HAuCl_4$ solution, when added the seed extract and kept at 55 °C, changed in sequence to colorless, dark blue, and finally purple. At this final state the solution was then cooled untill it reach the room temperature.

Various concentration of *Mucuna monosperma* seed extract which were used for nanoparticle synthesis showed various colour solution and showed agglomerization in the range of 10-100% as shown in Fig. 1.

Fig. 1 Gradation of colour - formation of gold nanoparticles from 10%-100% at intervals of 10% seed extracts

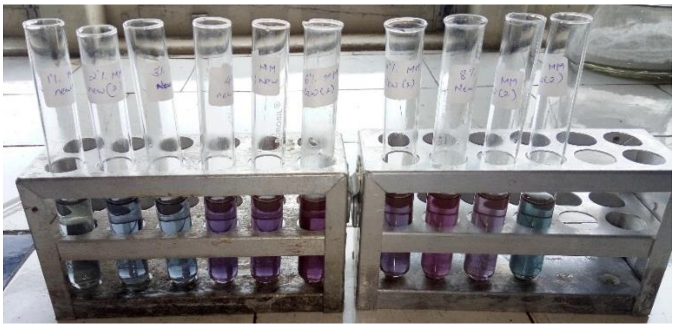

Fig. 2 Gradation of colour - formation of gold nanoparticles from 1%-10% at intervals of 1% seed extracts

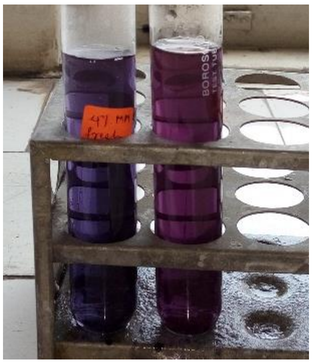

Fig. 3 Formation of gold nanoparticles from 4% and 5% seed extracts

As seen in Fig. 2, on further dilution, range of 1-10% seed extract used for synthesis of gold nanoparticles showed a good and stable colour change indicating the preparation of gold nanoparticles. Out of all the concentrations used, gold nanoparticles made from 4%, 5% and 6% *Mucuna monosperma* seed extracts showed deep purple colour indicating formation of gold nanoparticles as seen in Fig. 3. Gold nanoparticles made by 6% seed extract showed agglomerization in 1-2 hrs.

3.2 UV-Visible Spectroscopy of Gold Nanoparticles

Gold nanoparticles show a particular optical property, otherwise known as, localized surface plasmon resonance (LSPR), that is, the collective oscillation of electrons in the conduction band of gold nanoparticles in reverberation with a particular wavelength of incident light. The gold nanoparticles made with various percent concentration of *Mucuna monosperma* seed extract when analysed by UV-Visible spectroscopy showed a single peak in the range of 540-570 nm indicating the formation of nanoparticles. 5% and 4% seed extract solutions show distinct Gaussian shaped peaks at 552 nm and 556 nm respectively indicating formation of more monodispered nanoparticles as compared to other concentrations which indicate flat type of peaks as shown in Figs. 4 and 5.

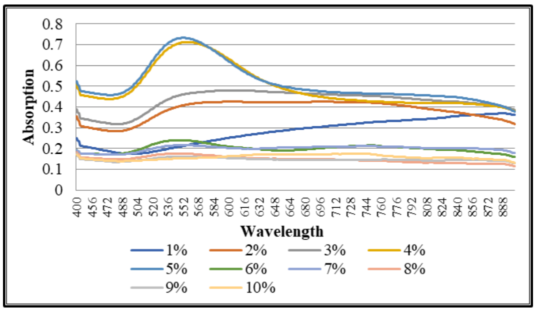

Fig. 4 UV-Vis absorption spectra of gold nanoparticles made by various concentration of *M. monopserma* extract

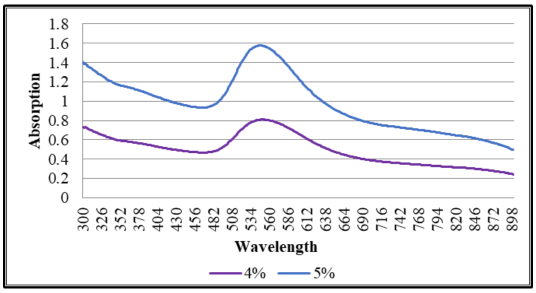

Fig. 5 UV-Vis absorption spectra of gold nanoparticles made by 4% and 5% *M. monosperma* extract

Fig. 6 Agglomerization of gold nanoparticles made by 5% seed extract

The gold nanoparticles made with 5% *M. monosperma* extract were not stable for more than 7 days and showed agglomeration as seen in Fig. 6. Also, the absorption spectra of gold nanoparticles made from 5% seed extract displayed dampening of absorption spectra, whereas nanoparticles made from 4% seed extract retains its peak as seen in Fig. 7, showing better stability. Also, when compared freshly prepared nanoparticles and 7-day old samples, nanoparticles showed similar peak as seen in Fig. 8, indicating the stability of monodispersed and spherical gold nanoparticles. Hence, gold nanoparticles synthesized by 4% extract were considered for further characterization.

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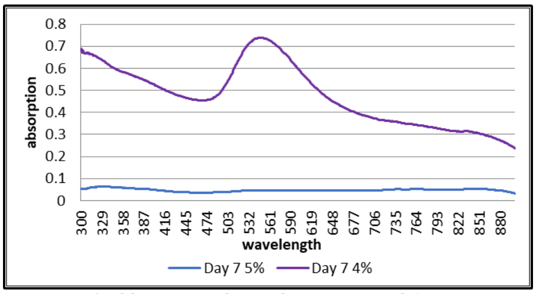

Fig. 7 Comparison of gold nanoparticles made using 4% and 5% *M. monosperma* seed extract after 7 days

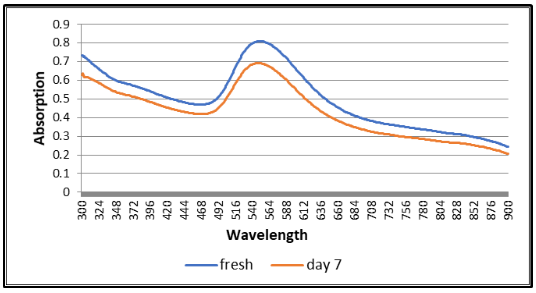

Fig. 8 Comparison of freshly prepared and 7 day old nanoparticles of 4% *M. monosperma* seed extract

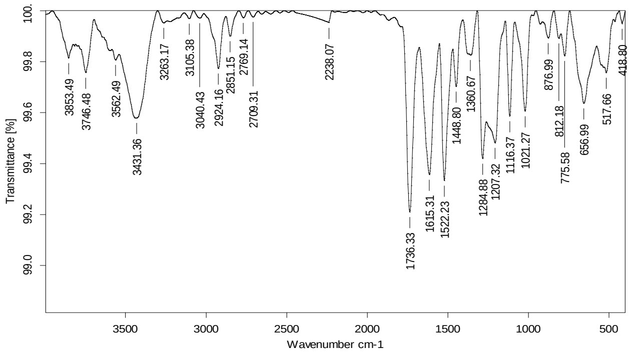

Fig. 9 FTIR spectrum of standard L-DOPA

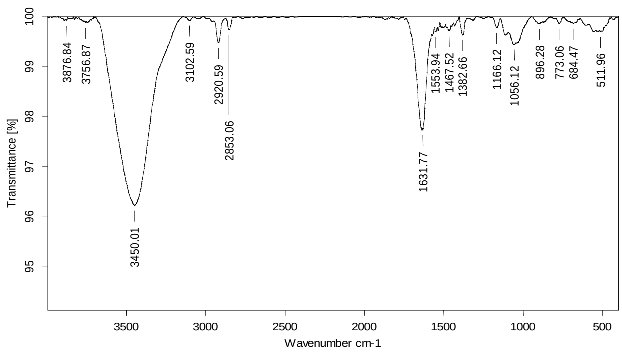

Fig. 10 FTIR spectrum of gold nanoparticles synthesised by using 4% seed extract

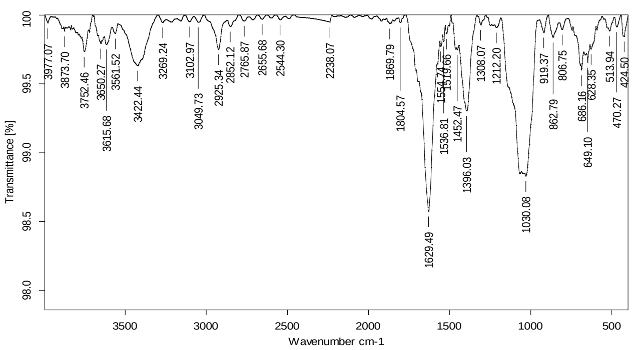

Fig. 11 FTIR spectrum of *M. Monosperma* seed extract

3.3 FTIR Analysis of Gold Nanoparticles

Surface modification of gold nanoparticles with L-DOPA from seed extract was verified by comparing the FTIR spectra of gold nanoparticles, seed extract and standard L-DOPA. The following readings will help us understand the chemical composition of the gold nanoparticles. Table 2 and Figs. 9-11 helps us understand, various peaks in the L-DOPA standard and its corresponding peak in the seed extract and gold nanoparticles.

Peak between 2220-2260 cm^{-1} indicates presence of cyanide indicating presence of toxic substances. As per the FTIR spectrum, this peak is present in the standard L-DOPA and also in the seed extract, but it is absent in the gold nanoparticles. This gives the gold nanoparticles made an uper hand or advantage over the traditional seed extract or the synthetic L-DOPA and indicates the absence of toxic compounds from the gold nanoparticles synthesized [14].

Presence of aromatic ring and primary alcohol indicates that at L-DOPA from plants to have successfully adsorbed with the gold nanoparticles. The small change in the frequency could be because the hydroxyl groups of L-DOPA support cross-link reactions between L-DOPA from the seed extract and gold nanoparticles. This study shows that the aromatic ring system of L-DOPA functions as a surface anchor to the gold nanoparticles.

Table 2 FTIR results of syntheised gold nanoparticles, seed extract and std. L-DOPA

Peak No.	L-DOPA Std. (cm^{-1})	*M. monosperma* seed extract (cm^{-1})	Gold nanopaticles (cm^{-1})	Functional group
1.	3431.36	3422.44	3450.01	Primary amines
2.	3105.38	3102.97	3102.59	=CH and $=CH_2$
3.	2924.16	2925.34	2920.59	Carboxylic acid
4.	2851.15	2852.12	2853.06	CH stretching
5.	1615.31	1629.49	1631.77	C=C bond
6.	1360.67	1396.03	1382.66	aromatic ring
7.	1021.27	1030.08	1056.12	Primary alcohol
8.	656.99	686.16	684.47	Weak O-H bonding out of the plane

3.4 SEM and EDAX Analysis

SEM consolidates the benefits of a significantly easier example arrangement and higher depth-of-field imaging therefore giving a semi-3D data on the morphology and cell structure. SEM, nonetheless, offers a high spatial resolution and great depth of field. The gold nanoparticles made by 4% *M. monosperma* extract under scanning electron microscope showed the presence of clustered nanoparticles as shown in Fig. 12. When zoomed in a selected area, mostly spherical in shape, nanoparticles of size ranging from 10-50 nm were found as shown in Fig. 13.

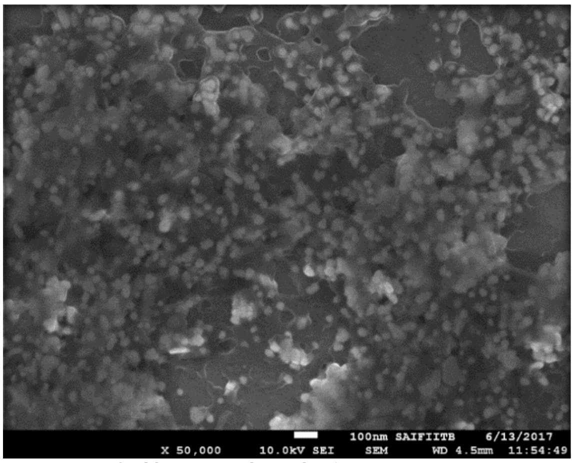

Fig. 12 SEM image of gold nanoparticles under 50000x

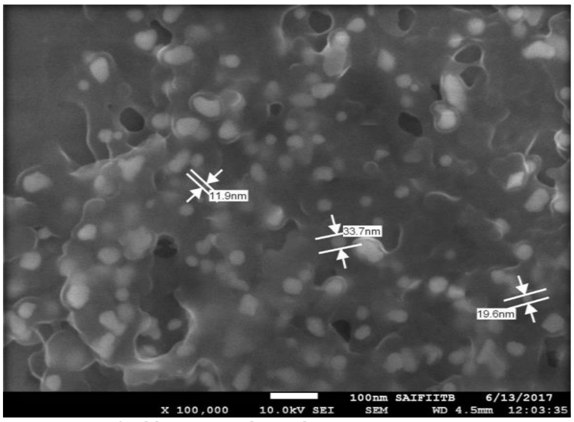

Fig. 13 SEM image of gold nanoparticles under 100000x

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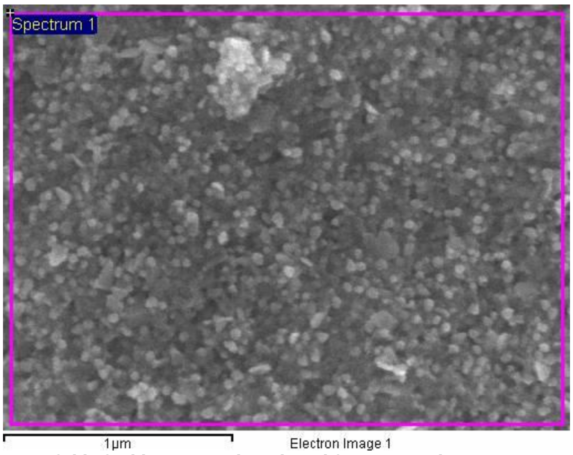

Fig. 14 SEM field of gold nanoparticles selected for EDAX analysis

Table 3 Composition of elements in the elemental analysis by EDS

Element	Weight%	Atomic%
C	14.97	58.86
N	4.57	15.42
O	1.96	5.78
Cl	1.02	1.35
Au	77.48	18.58

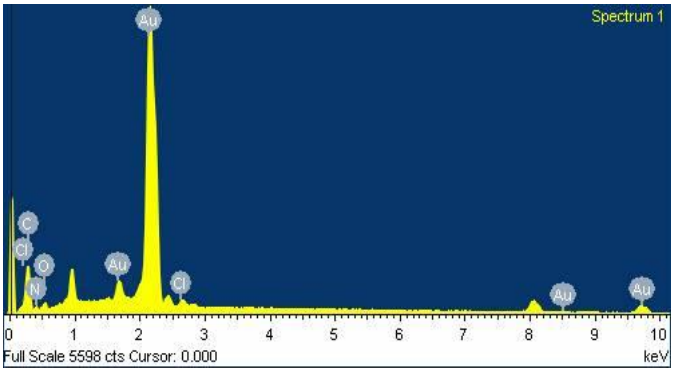

Fig. 15 EDS spectrum of gold nanoparticles synthesised by using 4% *M. monosperma* seed extract

EDAX provides supportive information as it gives elemental analysis of a sample. It depends on interaction of electrons and matter, where trademark X-beams are created, among different process. The EDAX analysis also showed the presence of elements like C, N, O other than Au (Figs. 14 and 15). Considering the use of $HAuCl_4$ and seed extract to prepare gold nanoparticles, the presence of elements other than Au as seen in Table 3 could possibly be due to the coating of L-DOPA from *M. monosperma* extract on nanoparticles.

4. Conclusion

The synthesis of gold nanoparticles using a seed extract was successfully achieved. Using *M. monosperma* seed extract, gold nanoparticles were successfully synthesised. The gold nanoparticles using 4% *M. monosperma* seed extract showed good stability over other concentrations. Analytical tools like FTIR, UV-Visible spectrophotometer and Scanning Electron microscope with EDAX helped to understand the surface chemistry of the gold nanoparticles. The gold nanoparticles synthesised showed spherical shape and also possibilities of L-DOPA coating on it. This could opens door to a lot of applications. One major application could be novel drug delivery system (NDDS).

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