Research Article

Microwave Assistance for High Yielding Monodispersed *A. indica* Gold Nanoparticles for Therapeutic Applications

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ABSTRACT

Gold nanoparticles (GNP), in recent years have gained special impetus in different fields of biomedical sciences such as targeted drug delivery, DNA labelling, sensors etc. As the morphologies and size of nanoparticles determine their properties and application; developing an easy, reproducible and eco-friendly synthesis method which can yield nanoparticles of uniform size and shape distribution along with high yield and exceptional biocompatibility becomes a priority. In this quest to synthesize GNPs with narrow size distribution, exceptional biocompatibility and ease of functionalization, we synthesized GNP in an eco-friendly manner from leaf extract of *Azadirachta indica,* commonly called as neem. However, achieving a considerable amount of monodispersivity and yield with conventional heating method was the bottle neck of our research. This study is therefore a comprehensive effort to develop biogenic GNPs by microwave assistance in the view to improve their yield and monodispersivity. GNPs synthesized by conventional hot plate heating and microwave assistance were reviewed for their size distribution and yield by UV-Visible spectroscopy, Nanoparticle tracking analysis and Transmission electron microscopy. The Full Width Half maxima (FWHM) were also calculated to attain a mathematical background to our spectral data to understand the size distribution of the GNPs. As against the traditional heating method, microwave heating method showed uniform heating profile increasing the monodispersivity and yield of GNPs. Microwave method thus provides better control on the shape and size distribution of gold nanoparticles and makes itself a suitable method for industrial synthesis of biogenic GNPs intended for therapeutic applications.

Keywords: *Azadirachta indica,* gold nanoparticles, Microwave, Nanoparticle Tracking Analysis (NTA), Transmission Electron Microscopy (TEM), Full Width Half Maxima (FWHM).

INTRODUCTION

Nanotechnology is the study and manipulation of materials at atomic and sub-atomic level.[1] Materials at this level do not follow Newton's physical laws but they are rather governed by quantum mechanical principles. Thus they possess different physical and chemical properties with respect to their macroscale counterparts. With these novel properties of nanoparticles, there is an increasing optimism that nanoscience can be applied to medicine field to bring out significant advancement in diagnosis and treatment of various diseases. Over the past decade, metallic nanoparticles are used in different fields of biomedical sciences such as chemical and biological sensors, DNA labelling, targeted drug delivery, cosmetics, coatings and packaging etc. [2,3,4]

Among metal nanoparticles, gold nanoparticles are the most ventured nanostructures in biological studies. Gold nanoparticles (GNPs) are favourable for their facile synthesis and ease of functionalization. The unique chemical and physical properties of GNPs provide versatility in delivery methods and tunability of surface properties.[5] However, for successful application of these nanoparticles, development of an easier, reproducible, less time consuming and eco-friendly process is necessary which can yield nanoparticles of uniform size and shape distribution along with high yield and exceptional biocompatibility. Up to now, various chemical methods have been developed for synthesis of GNPs. However, chemical protocols dedicated to synthesize GNPs exhibit two cardinal issues for GNPs to be used in drug delivery and other bio-analytical applications:

1. Lack of considerable monodispersivity in size and stability under physiological conditions.
2. Inherent toxicity towards biological cells.

To synthesize GNPs with narrow size distribution, exceptional biocompatibility and ease of functionalization, we tried to synthesize GNP in an eco-friendly approach from leaf extract of *Azadirachta indica,* commonly called as neem. The GNPs synthesized by this method showed noteworthy biocompatibility and provided natural polypeptide linker chemistry that was explored to cargo anti-retroviral drugs for HIV treatment.[6] In the conventional heating method used for GNPs synthesis, we were unable to achieve a considerable monodispersivity in size of GNPs. As the size and shape of the nanoparticles dictate their properties intended for various biomedical applications. It becomes very important that the nano-materials formed should possess the same physical and chemical properties. This study is thus a comprehensive research effort to tune the morphologies and size of GNP by varying the reaction conditions (heating method in this study). This is the first report of synthesis of biogenic GNPs by microwave assistance, in which we have particularly focused on improving the yield and monodispersivity of biocompatible nanoparticles synthesized by eco-friendly plant mediated method using fresh leaf extract of *A.indica*.

MATERIALS AND METHODS

Reagents

Chloroauric acid required for synthesis of gold nanoparticles (GNP) was purchased from Sigma Aldrich, USA. All experiments were performed in nano-pure water (18 MΩ). Glassware for nanoparticles synthesis were washed prior starting the experiments with Aqua regia ($HCl: HNO_3$) to remove traces of metal contaminant.

Ethical Approval

All the procedures were conducted in accordance with guidelines approved by the Institutional Ethics Committee (Ethics Committee Approval No. HITRT/IEC/12/2011 dated 24th January 2011).

Synthesis of GNP (*A.indica GNP*)

A. indica plant leaf extract was used for synthesis of gold nanoparticles. For preparing the plant extract the leaves were crushed in distilled water, centrifuged and the supernatant was filtered using Whatman filter paper to obtain a clear solution. The extract was stored at 4°C till further use. A stock solution of 50,000 ppm chloroauric acid ($HAuCl_4$) was prepared and diluted as per pre-requisite for the experiment.

The nature (shape, size and monodispersity) of the nanoparticles is known to vary with reaction conditions such as temperature, pH of the reaction solution and the stoichiometric ratio of metal salt solution and the reducing agent. In this study, we therefore optimized all the parameters for synthesis of GNP and focused only on variation in heating techniques for analyzing the change in the yield and mono-dispersivity of the nanoparticles. For 100 ppm of Chloroauric acid, diluted neem extract(1:50) at inherent pH (5.7) was added and heatedin aqueous solution at 100°C for optimum color change and synthesis of GNPs to occur. However, for attaining the required temperature we followed two different techniques: (a) Conventional thermal heating using a hot plate and (b) Microwave irradiation employing a microwave oven.

Characterization of GNPs

Surface Plasmon Resonance

Metals when reduced to nanoscale level, exhibit a unique property of resonance which is shown by their vibrating electrons confined onto their small surface.[7] This phenomenon of Surface Plasmon resonance (SPR) gives us a preliminary understanding about the size, shape and anisotropy of nanoparticles and can be analyzed by scanning the sample in the entire visible range (400nm–800nm) by spectroscopy. Spectroscopic measurements to calculate the SPR of the samples were thus executed using a dual beam Varian Cary® UV-Vis spectrophotometer, keeping deionised water as a reference blank.

Calculating the Full Width Half Maxima (FWHM)

The typical bell shaped Gaussian curve shown by the GNPs in a visible spectrum can be explored to analyze the size distribution and monodispersivity of the nanoparticles synthesized. Size distribution and thus the degree of monodispersivity is determined by calculating the Full width Half Maxima (FWHM), which is the difference between the two extreme values of the independent variable at which the dependent variable is equal to half of its maximum value.

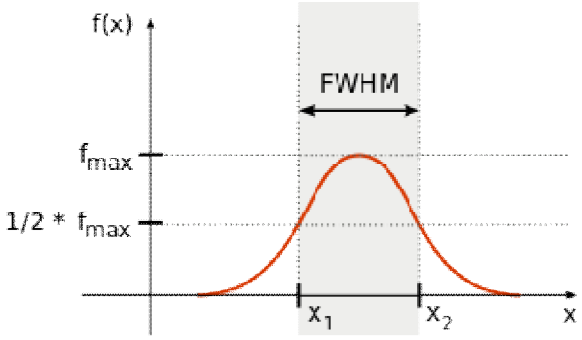

Figure 1: Full Width Half Maxima of the Gaussian Curve highlighting the area under the curve (adapted from Wikipedia).

For GNPs the FWHM is calculated by multiplying the difference between the wavelength of maximum absorption i.e. λmax (i.e. the SPR) and the forward wavelength at half the maximum absorption i.e. 1/2 λmax (x_2 of figure 1) by 2 and given by following equation:

$$FWHM = [\lambda max - 1/2\ \lambda max]*2 \qquad ....(1)$$

Nanoparticle tracking analysis (NTA)

Monodispersivity in size and concentration of GNPs synthesized were calculated by correlating the particle size with the relative intensity of the nanoparticles using a NANOSIGHT's nanoparticles tracking analysis software, version 2.3 build 0027.

Transmission Electron microscopy (TEM)

Further more sophisticated analysis to obtain deeper intricacies and confirmation of these preliminary observations of nanoparticles was done by Transmission Electron Microscopy (TEM)(Zeiss Microimaging GmbH, Germany). For sample preparation, 2-3 drops of the colloidal gold solution were dispensed onto a carbon-coated 200-mesh copper grid and dried under ambient condition before examination.

Elemental mapping

Finally confirmation of the presence of elemental gold in the nanoparticles formed was executed by Energy dispersive analysis of X-ray (EDAX) (Zeiss Microimaging GmbH, Germany).

RESULT AND DISCUSSION

Formation of *A.indica* GNP from Chloroauric acid was seen as a transition in colour of the solution from light greenish yellow to wine red colour. However, the time required for colour change in microwave assisted heating was less than a minute as compared to the conventional hot plate heating which took around 2 to 5 minutes for a significant colour change to occur.

The Microwave assisted heating is based on two mechanism of the conversion of the electromagnetic radiation into heat energy, namely dipolar rotation and ionic conduction.[8] In our case, the synthesis probably proceeded by ionic conduction heating mechanism; wherein the dissolved ionic charged particles oscillate back and forth under the electric influence of microwave irradiation which resulted in conversion of radiation energy into thermal energy necessary for nanoparticle synthesis. The microwave heating method thus indicates rapid synthesis of GNP as compared to conventional heating.

Visible spectral analysis (figure 2) of these colloidal sols of gold showed an intense peak (SPR) in the range between 500nm – 600nm. This 'SPR' is a unique property of metallic nanoparticles and majorly dependent upon the size distribution and shape of the nanoparticles. According to Beer's law, the absorbance of the sample is directly proportional to the path length and concentration of the suspension. From the figure 2a, we can thus conclude that the sol synthesized by microwave method has a higher concentration of GNP as compared to the suspension synthesized by conventional heating.

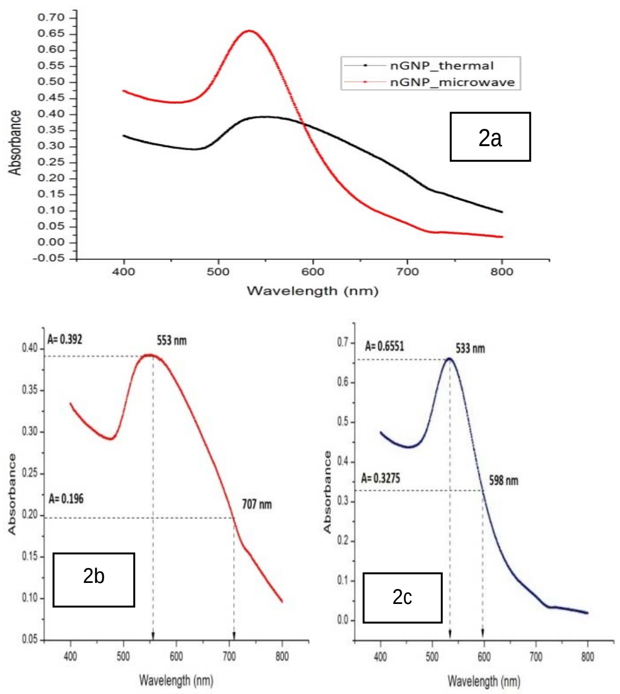

Figure 2: (a) Absorption spectrum of *A.indica* GNPs synthesized by two different heating techniques; i.e. microwave and conventional hot plate heating, (b) and (c) Area under the curve of GNPs synthesized by conventional and microwave heating respectively along with calculated wavelengths for FWHM.

Area under the curve (AUC) of these GNPs was further analyzed to comprehend the size distribution of the nanoparticles. Understanding the size distribution was executed by calculating the FWHM' of both set of GNPs using equation 1. Table 1, highlights the FWHM of GNPs synthesized by both types of heating method.

Table 1: FWHM of GNPs synthesized by both conventional and microwave heating methods.

Heating Method	ʌmax, absorption	1/2 ʌmax, absorption	FWHM
Conventional heating	553nm, 0.392	707nm, 0.196	308
Microwave heating	533nm, 0.655	598nm, 0.327	130

AUC' and FWHM' are directly proportional to the size distribution of the nanoparticles. Thus more the AUC' and FWHM'; greater is the size distribution of the nanoparticles synthesized and vice –versa. From the spectral data and the FWHM calculated, it can be very well intrapolated that the monodispersivity of GNPs is seen more favourable in microwave assisted heating; rather than conventional heating method. Microwave assistance provided faster and more uniform heating profile than conventional heating and resulted in fine monodispersed nanoparticles.

Further clarification on both the heating methods and the change in properties of GNP related to them was obtained by investigating the GNPs through NANOSIGHT's Nanoparticle Tracking Analysis (NTA), version 2.3build 0027. Based on a laser illuminated microscopic technique, NTA analysis tracks the Brownian motion of nanoparticles in real-time by a CCD camera, each particle being simultaneously but separately visualised and tracked by a dedicated particle tracking image analysis programme. The dot plot from figure 3a suggests that the GNPs synthesized by conventional heating have a broad size distribution between 10 to 80 nm. The histogram of GNP by conventional heating (figure 3c) was also found to be small with broad AUC'; highlighting polydispersivity in size and less yield of the nanoparticles. GNPs synthesized through microwave assistance however, showed a narrower dot plot (figure 3b) with size distribution between 5 to 30 nm along with a sharp and intense histogram peak (figure 3d).

The concentration of GNP by microwave technique wasalso found to be more (9.17E8 particles/ml) as compared to GNPs synthesized by conventional heating (7.60E8 particles/ml). NTA analysis therefore, goes in accordance with the spectral data and confirms that microwave provides GNPs with a narrow size distribution and high yield as compared to conventional heating method.

TEM images further confirmed that microwave method support synthesis of smaller and uniform sized nanoparticles than conventional heating method. Microwave synthesis provided high monodispersed character with particle size distribution lying between 5nm – 15nm (figure 4b) as against 10nm – 40nm for conventional heating method (figure 4a) respectively.

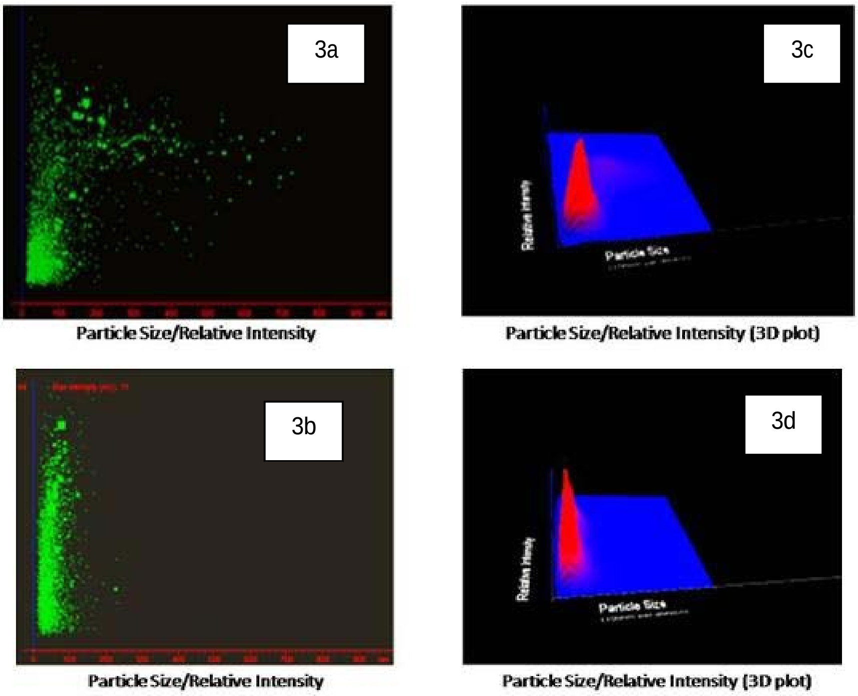

Figure3:(a) and (b) represents the dot plot of particle size vs. relative intensity of GNPs synthesized by conventional and microwave heating methods respectively, whereas (c) and (d) describes the 3D histogram of particle size vs. relative intensity of GNPs synthesized by conventional and microwave heating methods respectively.

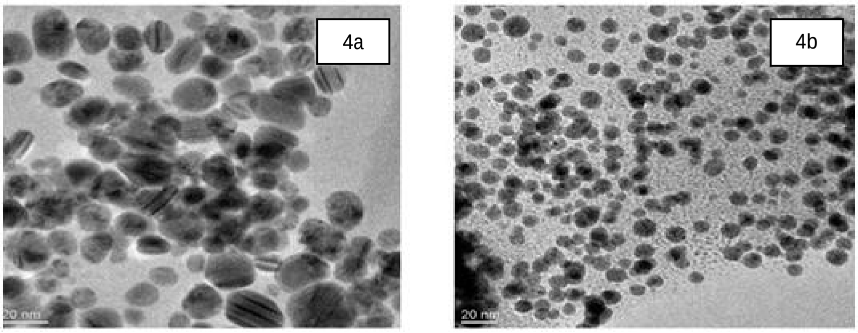

Figure 4: (a) and (b) TEM images of *A.indica* GNP synthesized by conventional and microwave heating method respectively.

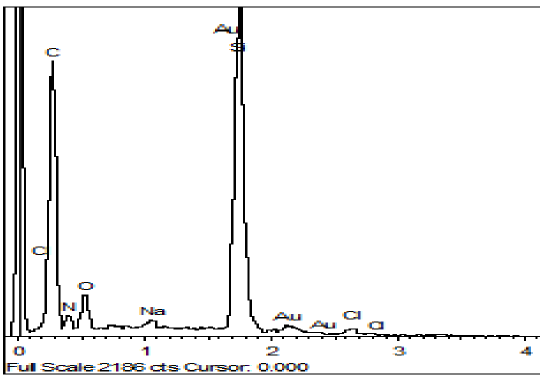

Figure 5: Elemental mapping executed by energy dispersive spectroscopy (EDS) to find presence of metallic gold in nanoparticles synthesized.

Elemental mapping on scanning electron microscope mode enlightened that the biogenic gold nanoparticles employing neem extract was elemental gold (Figure.5).

CONCLUSION

Microwave heating was found to increase the kinetics of the reaction rate and thus resulted in rapid synthesis of GNPs. As against the traditional heating method, microwave heating method showed uniform heating profile increasing the monodispersivity and yield of GNPs. Microwave method thus provides better control on the shape and size distribution of gold nanoparticles and makes itself a suitable method for industrial synthesis of biogenic GNPs intended for therapeutic applications.

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