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One step synthesis and characterization of gold nanoparticles and their antibacterial activities against *E. coli* (ATCC 25922 strain)

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ABSTRACT : In this paper we report a simple one step microwave irradiation method for the synthesis of gold nanoparticles using Citric acid and CTAB as reducing agent. The expedition reaction is completed under microwave irradiation in short duration and can be applied to generation of spherical gold nanoparticles. The process was characterized using UV-VIS absorption spectroscopy which revealed the formation of gold nanoparticles with surface Plasmon absorption maxima at 590 and 560 nm for 40 and 70 seconds respectively. Morphology and average size of nanoparticles were estimated using AFM and TEM and thus spherical gold nanoparticles in the size range 1-22 nm are observed. Raman Spectra of the sample revealed peaks at 1197cm^{-1} and 1373 cm^{-1} which confirms the formation of gold nanoparticles. Antibacterial activity of gold nanoparticles as a function of particles concentration against gram negative bacterium *Escherichia coli* was carried out in solid growth media. Antibacterial properties of gold nanoparticles are attributed to their total surface area, as a larger surface to volume ratio of nanoparticles and it provides a more efficient means for enhanced antibacterial activity. Gold nanoparticles show high antimicrobial and antibacterial activity with zone of inhibition of about 22 mm.

Keywords : Gold nanoparticles, Antibacterial, *E. coli*, Electron microscopy, SPR**.**

INTRODUCTION

Nanoparticles seem a promising option when compared to the conventional materials used, with the range of applications that nanoparticles find in varied fields of engineering and science. Their uniqueness arises specifically from higher surface to volume ratio and increased percentage of atoms at the grain boundaries. They represent an important class of materials in the development of novel devices that can be used in various physical, biological, biomedical and pharmaceutical applications [1-4]. Gold nanoparticles have a wide range of applications in areas such as catalysis, medical, diagnostics, and biological imaging [5]. NPs have also served as precursors to form nanowires or nanorods [6]. The extraordinary properties of NPs largely depend on their particle size. Ease of chemical synthesis and less toxicity as compared to some other nanomaterials are advantages of gold nanoparticles. Although a number of synthesis of gold nanoparticles have been developed that allow for the preparation of nanoparticles of varying core dimensions and surface functionality [7-13], a significant challenge remains in developing strategies for the preparation of nanoparticles of high purity and that exhibit low polydispasity. In order to control the particle size and shape of nanoparticles various reductants, stabilizers, and solvents, etc have been utilized in nanoparticles preparation. The control of particle size and morphology by using stabilizers has been extensively studied [14-17]. Gold nanoparticles show strong light absorption in the visible region, this absorption results from nanoparticles' coherent oscillations of the free electrons on the particle surface, which is the surface Plasmon resonance. The surface Plasmon resonance of gold nanoparticles has broad application and has drawn great attention in recent years [18-19]. In this paper we report nanometer sized gold nanoparticles synthesized by simple and cost effective microwave heating process which showed antibacterial efficiency against *E. coli*. The antibacterial activity of silver nanoparticles against *E. coli* was reported earlier [20-21] and has demonstrated the encapsulation of silver nanoparticles in hype branches polymer exhibits antimicrobial activity.

The antibacterial efficiency of the gold nanoparticles was investigated by introducing the particles into a media containing *E. coli* and it was found that they exhibited antibacterial effect at low concentration; ultimately the interaction between bacteria and gold nanoparticles was studied.

EXPERIMENTAL DETAILS

Materials

All chemicals were used as received. Chloroauric acid ($HAuCl_4.3H_2O$), Citric acid, CTAB was purchased from CDH. Double-distilled deionised water was used for synthesis.

Characterization techniques

Ultraviolet-visible Spectroscopy (UV-VIS) was performed in (Schimadzu; Asia Pacific; UV 1700) Double Beam Spectrophotometer. The morphology, size and Composition

of nanoparticles were performed by means of transmission electron microscopy (TEM). A minute drop of nanoparticles solution was cast on to a gold – coated TEM grids using Jeol H-7500 Microscope, operating at 120 KeV.

AFM (Atomic Force microscopy) characterization of gold nanoparticles was obtained with (Veeco; Digital Instruments; Innova) in tapping mode under ambious conditions at room temperature, using a 5×5 μm^2 scanner and a silicon nitride tip attached to a cantilever with a spring constant of $0.9 Nm^{-1}$.

Raman spectra of as synthesized nanoparticles was recorded on a (Lab RAM; HR 800 JY) Raman Spectrophotometer. The samples were excited by a 488 nm Argon laser through a 50 × (NA = 0.75) objective resulting in a spot size of around 2¼ m in diameter. The Raman signal was collected with the same objective in a backscattering geometry. The polarization of the incident laser was changed using a half wave plate. Raman spectra were recorded for the gold nanoparticles synthesized for 40 secs as reference sample.

Synthesis of gold nanoparticles

In order to synthesize gold nanoparticles, 8ml-10ml of 12mM $HAuCl_4.3H_2O$, 0.2 gm of citric acid and 0.1 gm of CTAB were added to a beaker, the solution was stirred for 10 sec and then heated in a microwave oven (SAMSUNG; G273 V, max power 750 watt) at 100 watt power for 40 seconds. Color of the solution changes from light yellow to shining orange, indicating the formation of gold nanoparticles. Heating was continued for 30 seconds, a slight change in the color of the solution was observed. UV-VIS spectra of both the solutions were recorded in a 1cm quartz cuvett.

Antibacterial Activity Test

Antibacterial activities of gold nanoparticles were studied against Gram- negative bacteria. Standard strain of *Escherichia coli* (ATCC 25922 strain), was used for evaluating antibacterial property of Gold nanoparticles. Antibacterial activity of nanoparticles was evaluated using, standard agar well diffusion method in which gold nanoparticles solution were used. The bacterial strain used in test was grown on LB (Luria Bertani) Broth at 37ºC overnight up to a turbidity of 0.5 Mac Farland standard (10^8 CFU per ml) [22]. About 5mg/ml of this suspension was used to inoculate petridish filled with LB (Luria Bertani) agar. Wells (diameter = 6 mm) were punched in the agar plates and filled with different nanoparticles solutions [23], both the nanoparticles solutions were used for this purpose and the LB agar plates were incubated overnight at 37°C. Samples treated with nanoparticles were spread on nutrient agar plates and after incubation at 37°C for 24 hrs the number of CFU were counted.

RESULTS AND DISCUSSION

Gold nanoparticles were successfully synthesized using the microwave irradiation method; the shinning orange color of the solution indicates the formation of gold nanoparticles. The main advantages of microwave irradiation methods over conventional synthetic methods are increase in the kinetics of reaction by two orders of magnitude, rapid initial heating and generation of localized heat at reaction sites, thereby increasing the rate of reaction [24]. This rapid MW heating also provides uniform nucleation and growth conditions, leading to homogeneous nanomaterials with smaller sizes. Power dissipation is fairly uniform through out with “deep” inside-out heating of the polar solvents, which leads to a better crystallinity.

The Transmission electron microscopy (TEM) images of the synthesized gold nanoparticles are reported in Fig.1. which shows highly stable and nearly spherical nanoparticles with average diameter in the range from 1–22nm.

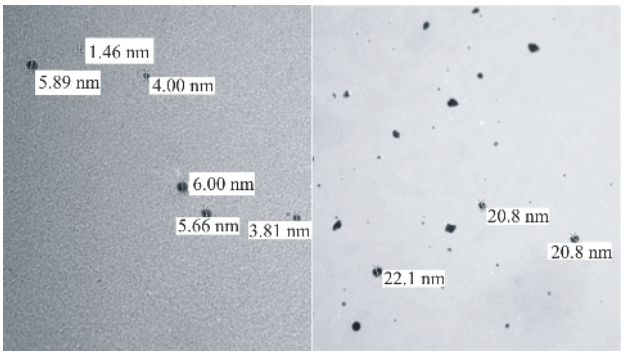

Fig.1. TEM micrographs of gold nanoparticles synthesized by microwave heating process (a) 40 secs. (b) 70 secs.

The UV-VIS absorption spectrum shown in Fig.2. of shining orange gold nanoparticles prepared by citric acid reduction shows strong surface Plasmon resonance peaks at 590 and 560 nm for 40 and 70 seconds respectively, indicating the presence of spherical gold nanoparticles.TEM imaging results are in well agreement with the UV-VIS spectrum of gold nanoparticles. The shape and position of surface Plasmon absorption depends on particles size, shape and the dielectric constant of the surrounding medium. There is a blue shift in the absorption peak to 560 nm and a smooth shoulder near 450 nm. A typical Raman spectrum of gold nanoparticles synthesized in microwave for 40 seconds is shown in Fig.3. The sensitivity of spectra obtained from noble metal nanoparticles strongly depends on the size and shape, especially the latter [25-31]. In the nineties of the twentieth century, Nie’s group demonstrated size-dependent SERS enhancement in single metal nanoparticles [32]. Suzuki and his co-workers also reported that gold nanoparticles films with different sizes generate different-intensity SERS signals [33].

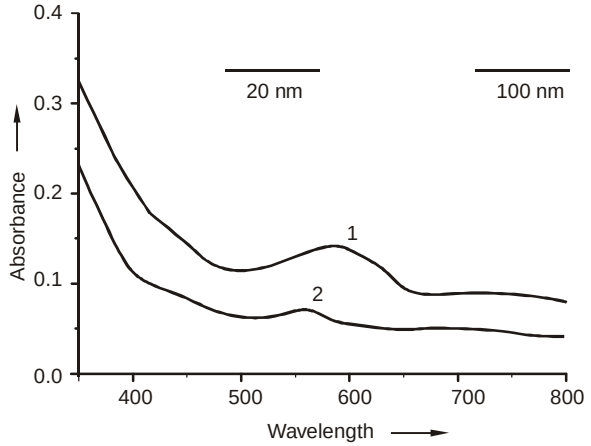

Fig.2. UV-VIS spectra of gold nanoparticles heated for (1) 40 secs. (2) 70 secs.

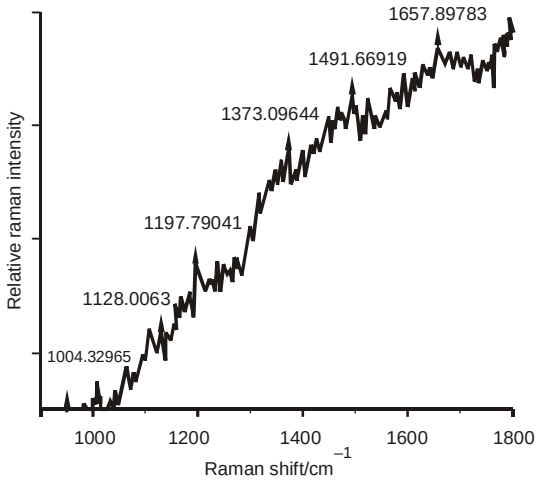

Fig.3. Raman spectra of gold nanoparticles heated for 40 secs.

Morphological characterization of gold nanoparticles by AFM from solution on to substrate surface should be credible approach to demonstrate the state of nanoparticles in solution, because the morphology of nanoparticles aggregates can be preserved during the process. The AFM image of gold nanoparticles immobilized on a glass substrate as shown in Fig.4. clearly reveals the semispherical morphology of nanoparticles aggregates.

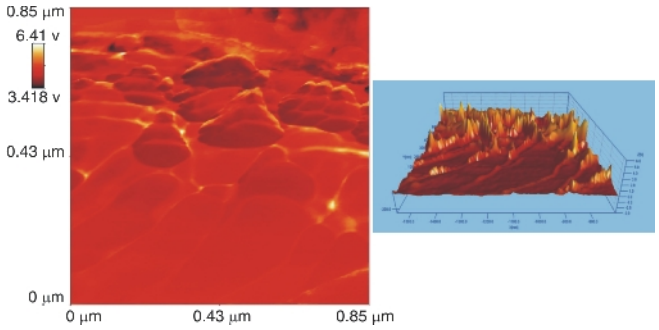

Fig.4(a). AFM image of gold nanoparticles synthesized by microwave heating process for 40 sec. **(b).** 3-D preview of AFM image of gold nanoparticles.

The size of the nanoparticles implies that it has a large surface area to come in contact with the bacterial cells and hence, it will have a higher percentage of interaction than bigger particles [34-37]. The nanoparticles smaller than 10nm interact with bacteria and produce electronic effects, which enhance the reactivity of nanoparticles. The antimicrobial efficacy of the nanoparticles depend on the shape of the nanoparticles also, this can be confirmed by studying the inhibition of bacterial growth by differentially shaped nanoparticles [36]. The zone of inhibition studied in the two gold nanoparticles solutions synthesized at 40 and 70 secs. were almost similar (22 mm) as shown in Fig.5(a) and 5(b) respectively. It is logical to state that the binding of gold nanoparticles to the bacteria depend on the surface area available for the interaction. Nanoparticles have large surface area available for interactions which enhances bactericidal effect than the large sized particles; hence they impart cytotoxicity to the micro organism [25].

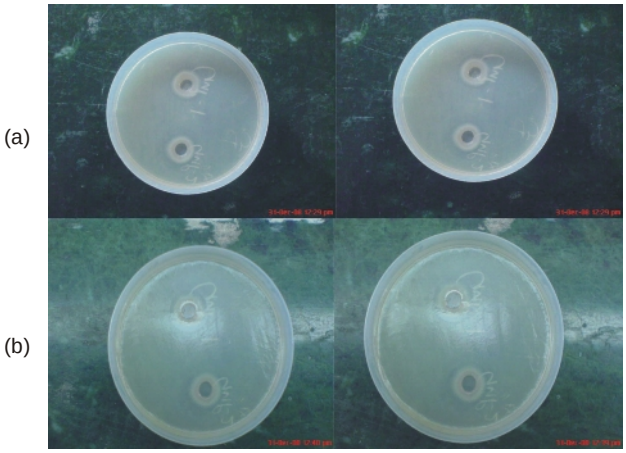

Fig.5(a). Zone of inhibition due to gold nanoparticles in *Escherichia coli.* at 40 secs. **(b)** Zone of inhibition due to gold nanoparticles in *Escherichia coli.* at 70 secs.

CONCLUSION

A novel efficient strategy for the preparation of gold nanoparticles in aqueous solution with citric acid as reducing agent is reported. Gold nanoparticles prepared are highly stable with average diameter of about 1-22 nm. Antibacterial activity of gold nanoparticles reveals that the zone of inhibition (22 mm) was almost similar in case of the two types of gold nanoparticles used in our study. This was a preliminary time bound study on the possible use of nanoparticles as an antibacterial agent. So far, we have done only in-vitro tests using few nanoparticles. It has been planned to include other types of nanoparticles and wider use of scanning probe microscopy. An in vivo study to look for possible side effects and toxicological impact on animals will also be done in the near future.

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