SYNTHESIS AND CHARACTERIZATION OF Ag AND TiO_2 NANOPARTICLES AND THEIR ANTI-MICROBIAL ACTIVITIES

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Silver and Titanium dioxide nanoparticles (Ag & TiO_2 NPs) were synthesized by sonochemical and colloidal methods respectively. The formation of Ag and TiO_2 NPs were characterized by UV-Vis absorption spectroscopy, X-ray diffraction (XRD), Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM). The TEM images have confirmed that both Ag & TiO_2 NPs are regular spheres with the sizes in the range of 20-50 nm. The X-ray diffraction analysis of Ag-NPs showed that the face-centered cubic and TiO_2 NPs have body centered crystalline nature. In addition, antimicrobial activities against *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, and Klebsiella pneumonia* were studied and the results showed that Ag & TiO_2 NPs possess antimicrobial properties and Ag NPs have phenomenal antimicrobial activities than TiO_2 NPs.

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1. Introduction

Recently, metal nanoparticles (NPs) have attracted much attention due to their unique optical, mechanical, electronic, magnetic and chemical properties. Such special and unique properties of NPs are determined by their small sizes and large specific surface area. For these reasons, metallic nanoparticles have been used in many applications in different fields like electronics, catalysis and biomedical [1-2]. There are available a variety of synthesis methods like chemical reduction [3-4], ultrasonic irradiation [5], solvothermal [6], electrochemical [7], radiolysis [8], green synthesis [9], facile synthesis [10] and microwave plasma synthesis [11]. Silver treatment in clinical settings has a long history which goes back to ancient Romans. In 1920s, the US Food and Drug Administration approved colloidal Silver for wound treatment [12], as Silver has been known to have antibacterial properties since ancient times. Many new industries involve the production of antibacterial gels using Silver nanoparticles [13]. The antibacterial activities of Ag NPs are related to their size, with the smaller particles being more active when compared on the basis of equivalent Silver mass content [14]. A preliminary test showed that Silver nanoparticles are highly active against *Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa* and could act as a safer alternative to conventional antimicrobial and antibacterial agents [15]. Ag-NPs affect the protein level of phosphotyrosine and the membrane channel protein [16] and because of this reason, the membrane transport and signalling processes get blocked and finally the bacterial cells get killed [17]. This effect was size and dose dependent and was more pronounced against gram-negative bacteria than gram-positive bacteria [18].

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For many years, Silver has been known to exhibit antibacterial properties and this characteristic has been exploited in a wide variety of applications, such as catheters [19], textiles [20], water treatment, stainless steel materials [21], vascular grafts [22], dental materials [23] and human skin [24]. Also Ag NPs act like antimicrobial agents to reduce or prevent infections [25], and have potential antimicrobial activity against many pathogenic microorganisms [26]. Furthermore one report says nano-sized Silver–anionic clay formulations were tested as a new antimicrobial agent [27] and have effective antimicrobial activity towards *S. aureus* and *E. Coli*. In some cases it has been reported that TiO_2 NPs also have effective antimicrobial properties [28-30]. Nowadays, Ag & TiO_2 NPs have attracted attention about their antimicrobial activities. In this paper, we present the synthesis and characterization of Ag & TiO_2 NPs and compative studies of both materials for antimicrobial studies by using gram (+) and gram (-) bacteria.

2. Experimental

2.1. Materials

Silver nitrate, trisodium citrate, sodium borohydride, polyvinyl pyrrolidone, nutrient agar and Muller Hinton agar, $HClO_4$, Titanium tetra isopropoxide and polyvinyl alcohol were obtained from Merck Chemicals Ltd. All chemicals were used in the experimental analytic grade and used without further purification. All aqueous solutions were made using double distilled water (DDW) as a solvent throughout the reaction that DDW prepared by a water purification system in our laboratory.

2.2. Synthesis and Characterization of Ag & TiO_2 NPs

2.2.1. Synthesis of Ag NPs

A 250ml three necked round bottom flask was laid in the bath of a ultrasonic cleaner which has the ultrasonic frequency in the range 35-40 kHz and 50 watts. In this typical experiment, $AgNO_3$ (0.1M) was added to water (100ml) and polyvinyl pyrrolidone (5ml/0.025g) were added drop- wise simultaneously while continuously ultrasonicating. The mixture of purged by N_2 for 20 mins. to this solution lead to a colour change to greyish. The drop-wise addition of both solutions ($NaBH_4$ and PVP) caused immediate precipitation started to settle at the bottom of the flask. The obtained precipitate particles contained solution that was centrifuged at10000 rpm for 20 mins. and the precipitate washed three times with double distilled water to remove the free PVP, unreacted $AgNO_3$ and any other water soluble impurities. The precipitate was then dried in the oven at 80°C for 1hr.

2.2.2. Synthesis of TiO_2 NPs

TiO_2 nanoparticles were prepared by colloidal route. The colloidal TiO_2 was prepared from Titanium tetra isopropoxide by dissolution in $HClO_4$. The resulting solution was then bubbled with nitrogen for a few hours until a transparent solution was obtained. This TiO_2 sol was stabilised by mixing with 5 ml of 0.2 wt. % aqueous solution of polyvinyl alcohol.

2.3. Characterization

The synthesized NPs were confirmed by UV-vis spectroscopy by using water as the reference on a UV-2401 PC (SHIMADZU). The structure of NPs were characterised by x-ray diffraction (XRD) analysis using Cu-Kα radiation (λ=1.506A°) on a JPX-8030. The surface morphology of synthesized NPs were characterized by scanning electron microscopy (SEM) using E-sem-quanta-200-Fei-Netherland and the particle size of NPs were carried out by using transmission electron microscopy (TEM, Philips 200 model) at SAIF, IIT Mumbai.

2.4. Antimicrobial studies

The antibacterial activity was tested against gram-positive and gram-negative bacteria such as *Staphylococcus aureus, Staphylococcus epidermidis and Escherichia coli, Klebsiella pneumonia* respectively. These species were cultured in a Lysogeny broth (LB) called liquid nutrient broth medium with pH 7. The culture medium was incubated at 37 ºC and after 24 h the bacterial colony was counted. To examine the zone of growth inhibition, the agar-well diffusion method was applied. Aqueous dispersions of silver and TiO_2 NPs in two different concentrations (0.5 µg ml^{-1} and 10 µg ml^{-1}) were prepared. The prepared samples were introduced into the wells in petri dish containing four different bacterial species directly. Then these plates were incubated at 37 ºC for 24 h in a shaking incubator for well bacterial growth. After finishing the incubation period, the inhibition zone was monitored by the naked eye and compared with both Ag & TiO_2 NPs.

3. Results and discussion

UV-visible spectroscopy is one of the most widely used techniques for structural characterization of Silver nanoparticles. Formation and stability of Silver Nanoparticles in aqueous solutions are confirmed using the above spectral analysis. The absorption spectrum of the Silver nano particles dispersed in water and the spectral peak obtained in the visible range was at 410 nm (Fig.1). From the observed peak in fig.1, we clearly confirmed that Ag nanoparticles were present in our solution.

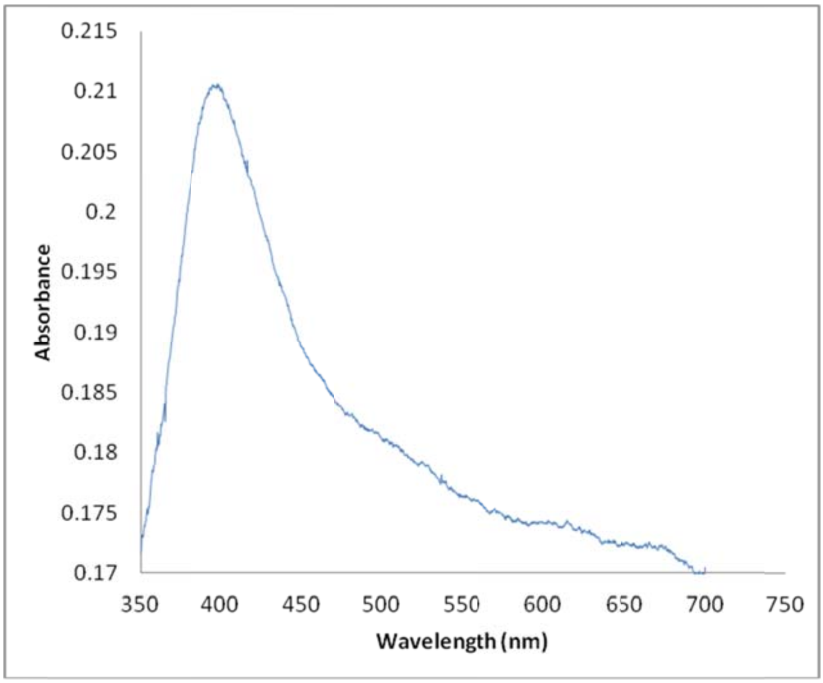

Fig.1. UV–Vis spectra of Ag NPs.

The crystallinity of the Ag & TiO_2 NPs were confirmed by the powder x-ray diffraction (XRD) patterns which are shown in Fig.2 and Fig.3 respectively. The corresponding ‘d’ values, intensity values and hkl values are given in Table 1 and Table 2. The observed data are in accordance with those of the JCPDS reported values of Nano-Ag (card No. 65-2871) and Nano-TiO2 (card No.89-4921).

Table-1 : X-ray data of silver nanoparticles.

Theoretical Data (card No. 65-2871)			Observed Data	
2θ	I/Io	hkl	2θ	I/Io
38.11	100	111	38.06	100
44.29	45	200	44.24	35
64.44	22	220	64.41	27
77.39	20	311	77.34	21

Table-2 : X-ray data of TiO2 nanoparticles.

Theoretical Data (card No.89-4921)			Observed Data	
2θ	I/Io	hkl	2θ	I/Io
25.35	100	101	25.42	100
48.14	41	200	48.01	41
55.18	29	211	55.11	19
62.81	21	201	62.52	22

The XRD data which were collected with Cu Kα radiation (λ =1.5418 A°) were attributed to the face-centered cubic (fcc) and body centered crystalline nature of the samples. The mean particle sizes can be calculated by the Scherrer's equation: $D = k\lambda/(\beta \cos\theta)$, $K = 0.89$, $\lambda = 0.15418$ nm, θ is the half-diffraction angle, β the full width half maximum (FWHM), D the diameter of crystalline particle. The average particle size obtained by Scherrer formula from XRD data is found to be about 45-50 nm. The XRD pattern thus clearly illustrates that Ag & TiO_2 NPs synthesized by the present ultrasonic and colloidal methods are crystalline in nature.

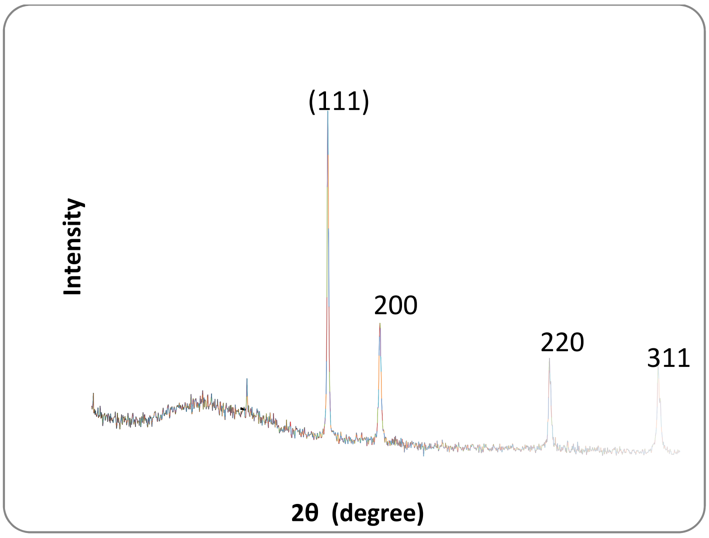

Fig. 2. XRD pattern of Ag NPs

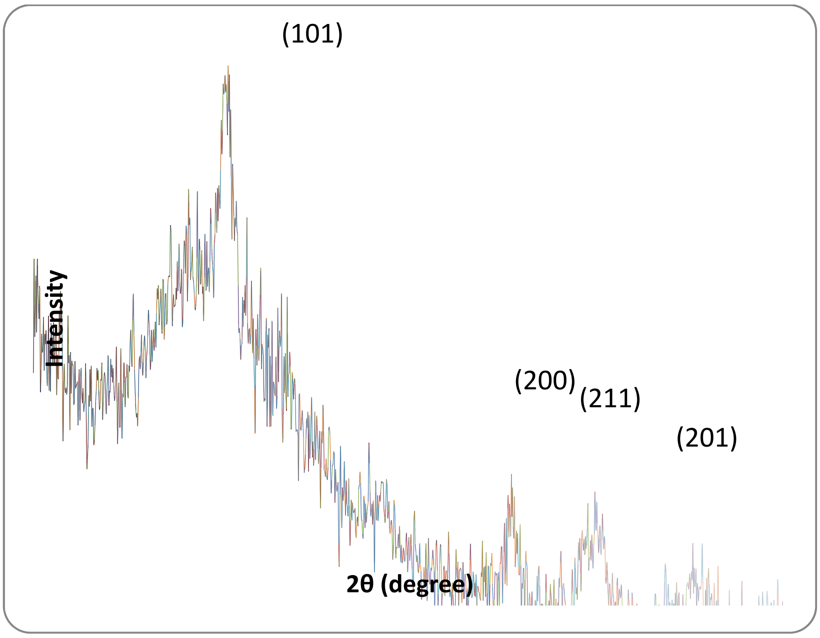

Fig. 3. XRD pattern of TiO_2 NPs

The surface morphology and size of the prepared dried sample was characterized by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The SEM micrographs in Fig. 4(A) and Fig. 4(B) have shown spherical Ag and TiO_2 NPs in the morphological structure. TEM characterization was used to obtain the information about the diameter, size distribution and aggregation of NPs. TEM images of Ag and TiO_2 NPs are shown in Fig. 5(A) & Fig. 5(B).

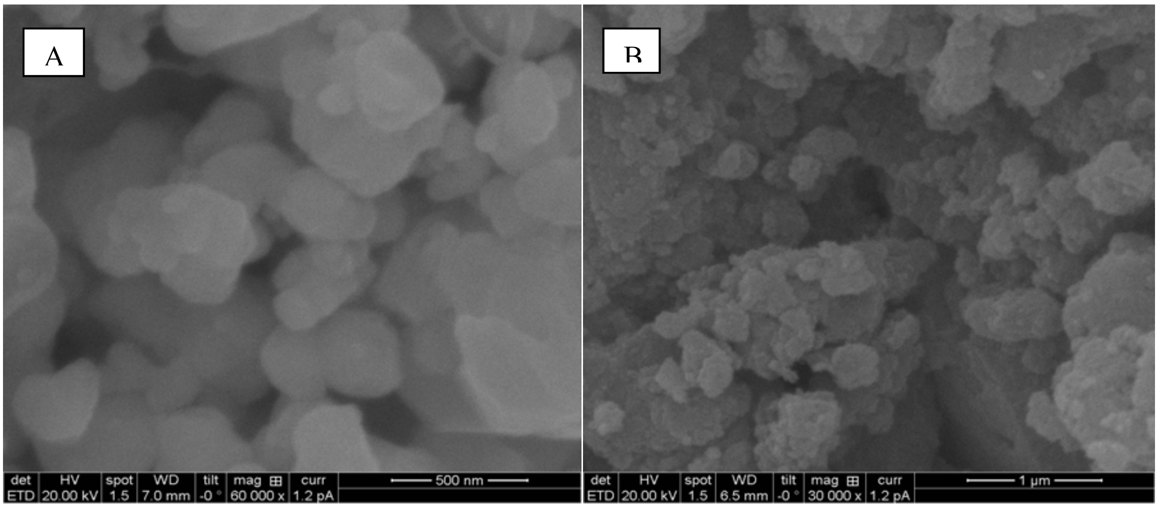

Fig.4. (A & B) SEM images of Ag and TiO_2 NPs

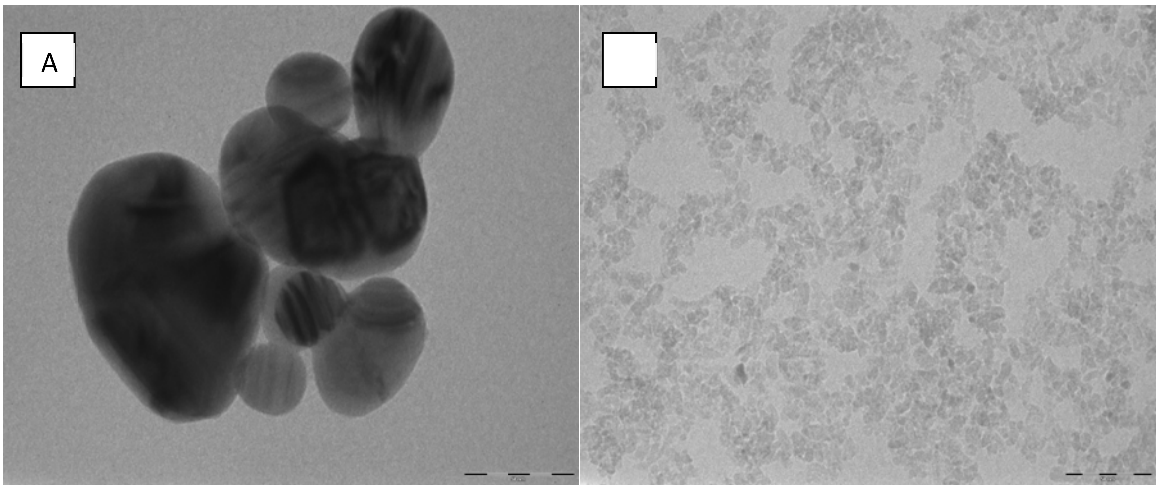

Fig. 5. (A & B) TEM images of Ag and TiO_2 NPs

To investigate the growth inhibition effect of synthesized Ag and TiO_2 NPs, they were tested against Gram-positive *Staphylococcus aureus, Staphylococcus epidermidis* and Gram-negative *Escherichia coli, Klebsiella pneumonia* by the minimum inhibitory concentration. Fig. 6A-D shows the observations of bacteria grown on agar plates after 24 h at two different concentrations of the Ag & TiO_2 NPs ranging from 0.5 and 1 µg ml^{-1}. It can be seen in Fig. 6A-D that, the control well (test samples without adding Ag & TiO_2 NPs) showed no inhibition zone. Whereas, in the remaining three wells the observed zone of inhibition area is large, as the addition of Ag NPs in *Staphylococcus aureus* and *Escherichia coli* has shown in Fig. 6A and Fig. 6B. For TiO_2 NPs applied in *Staphylococcus epidermidis* and *Klebsiella pneumonia* the zone of inhibition area is smaller and is shown in Fig. 6C and Fig. 6D.

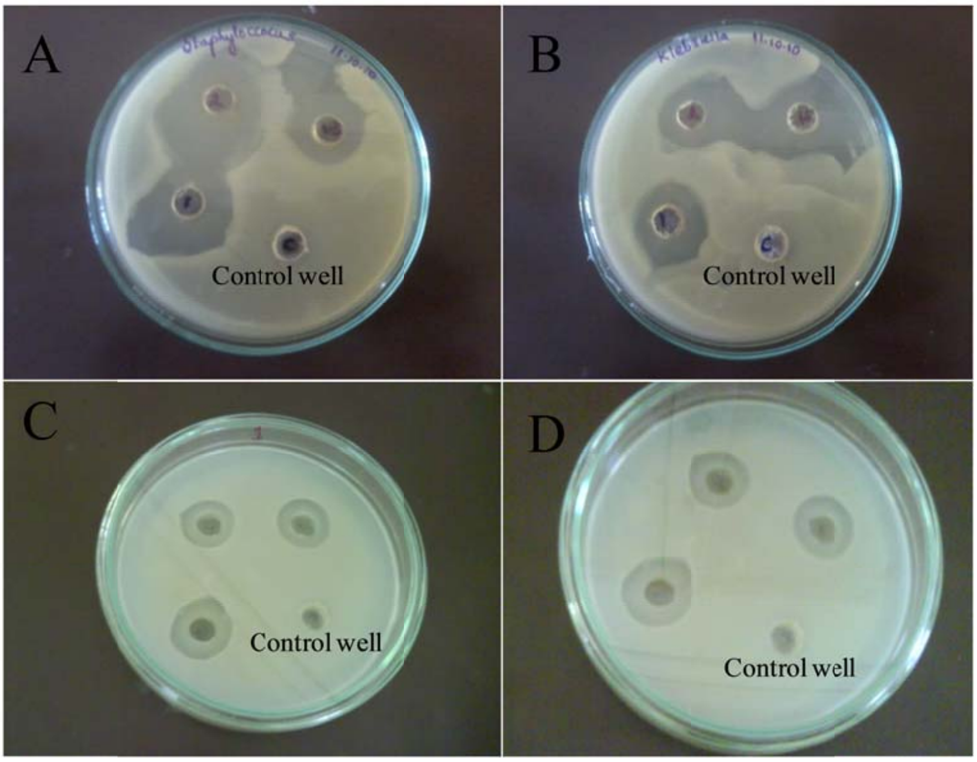

Fig. 6. (A-D) Representative images of agar plates containing antimicrobial activity of Ag NPs with Staphylococcus aureus & Escherichia coli (A & B) and TiO_2 NPs with Staphylococcus epidermidis & Klebsiella pneumonia (C & D) respectively.

4. Conclusion

In summary, simple ultrasonic and colloidal methods followed to prepare Ag and TiO_2 NPs. Even though both materials Ag and TiO_2 NPs have antimicrobial activities, when compared between them, the Ag NPs have much higher antimicrobial properties than TiO_2 NPs. These results suggest that Ag nanoparticles can be used as effective growth inhibitors in various microorganisms, making them applicable to diverse medical devices and antimicrobial control systems.

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