GREEN SYNTHESIS OF SILVER NANOPARTICLES USING CHITOSAN AS REDUCING AGENT

Tin Moe Swe[1], Khin Aye May[2], KhinThan Yee[3]

Abstract

The development of eco-friendly process for the synthesis of nanoparticle is one of the main steps in the area of nanotechnology research. In this study, the synthesis of silver nanoparticles was conducted from silver nitrate ($AgNO_3$) by using chitosan solution as reducing agent. Silver nanoparticles (Ag nanoparticles) were prepared by adding different volumes of silver nanoparticles (1mL,2mL,3mL,4mL) of 0.01M $AgNO_3$ solution to various concentrations of chitosan solution (1% and 0.5% w/v). To avoid the chemical toxicity, biosynthesis (green synthesis) of metal nanoparticles is proposed as a cost-effective and environmental friendly alternative. The existence of nanoparticles in colloidal solution was confirmed by Tyndall effect. The synthesized silver nanoparticles were characterized by UV-visible spectroscopy, FT IR, SEM, XRD, EDXRF and determined their antimicrobial activities. The maximum absorption of prepared silver nanoparticles using chitosan solution as reducing agent were observed at the wavelengths of near 390 nm indicating the presence of Ag nanoparticles in colloidal solution. According to antimicrobial activity tests, Ag nanoparticles prepared using 1% (w/v) chitosan solution as reducing agent were found to be more active than 0.5%(w/v) chitosan solution. Among them, the Ag-nanoparticle prepared from 4mLof 0.01 M $AgNO_3$ in 1% w/v of chitosan solution exhibit the highest activity on *P.aeruginosa*. From the SEM micrograph of this sample, the prepared Ag nanoparticles had spherical shape of various size. XRD analysis of Ag nanoparticles showed the amorphous form. The functional groups of Ag nanoparticles in this sample was identified by FT IR analysis which indicates the presence of Ag-O stretching and deformation. The relative abundance of silver in the prepared sample was investigated by EDXRF.

Keywords: chitosan, silver nanoparticles, antimicrobial activity, Tyndall effect

[1.] 3PhD, Candidate, Department of Chemistry, University of Yangon
[2.] Associate Professor, Department of Chemistry, Dagon University
[3.] Lecturer, Department of Chemistry, University of Yangon

Introduction

In recent years, noble metal nanoparticles have been the subject of focused research due to their unique electronic, optical, mechanical, magnetic and chemical properties that are significantly different from those of bulk counterpart. Nowadays, silver nanoparticles has gain tremendous popularity in the world in the field of sensors because of outstanding significant optical, electronic and chemical properties (Kumar and Rani, 2013). Silver nanoparticles exhibit new optical properties, which are observed neither in molecules nor in bulk metals. One example is presence of absorption band in visible light region. This band appears due to the surfaceplasmon-oscillation modes of conduction electrons which are coupled through the surface to external electromagnetic fields(Hussain*et al*., 2011). The green synthesis is a concept that is introduced to define the method used in synthesis, which is favored over solvent medium. This is because it is environmentally friendly and contains a reducing agent that is benign to the environment. Besides, it also utilizes a non-toxic stabilizer in forming Ag nanoparticles. Chitosan, a polysaccharide biopolymer, is a product of deacetylation of chitin, which is the second most abundant natural polymer in the world after cellulose. The biocompatibility and antibacterial properties of chitosan and its being an environmentally friendly polyelectrolyte makes it attractive in academic research (Mansor *et al.,* 2011).

Silver exhibits the highest electrical and thermal conductivities among all the metals for centuries. People have used silver for its antibacterial qualities. Nanoparticles usually have better or different qualities than the bulk material of the same element. In the case of silver nanoparticles the antibacterial effect is greatly enhanced and because of their tiny size (Link*et al*., 1999). Nanoparticles have immense surface area relative to volume. Therefore minuscule amounts of silver nanoparticles can lend antimicrobial effects to hundreds of square meters of its hostmaterial. Nanomaterials are the leading requirement of the rapidly developing field of nanomedicine and bionanotechnology (Mandal and Sastry, 2014).

Nanoparticles are being utilized as therapeutic tools in infections, against microbes thus understanding the properties of nanoparticles and their effect on microbes is essential to clinical application (Khan *et al*., 2014).

Among noble metal nanoparticles, silver nanoparticles have received considerable attention owing to their attractive physicochemical properties. Silver nanoparticles exhibit distinct optical activities that have found wide use in electronics, catalysis and in sensing based applications (Matti *et al*., 2012). Moreover, it displays antimicrobial activity against a broad spectrum of bacteria and fungi and thus finds use as a biocide and also in the preparation of bactericidal nanomaterials for wound dressings and surgical purposes. Silver nanoparticles are nontoxic to human in low concentrations. The silver-nanoparticles can inactivate proteins, blocking respiration and electron transfer (Ramos *et al*., 2010).

Silver nanocrystals, mostly hydrosols are one of the most attractive inorganic material not only because of its tremendous applications in photography, catalysis**,** biosensor, biomolecular detection, diagnostics, and particularly antimicrobial activities but also because of its environmentally benign nature**.** Synthesis of different morphologies of advanced silver nanomaterials (nanotubes, nanowires, nano cubes, nanorods, and nanosheets) has been the subject of a large number of researchers in many laboratories (Shah *et al*.,2012). Silver, a naturally occurring element, is non-toxic, hypoallergenic, does not accumulate in the body to cause harm and is considered safe for the environment. Many manufactured goods like washing machines, air conditioners and refrigerators are using linings of silver nanoparticles for their antimicrobial qualities. Sportswear, toys and baby articles, food storage containers, HEPA filters, laundry detergent etc. are made with silver nanoparticles. The medical field also is using products with silver nanoparticles, such as heart valves & other implants, medical face masks, wound dressings and bandages (Nagaonkar and Rai, 2015). Nanomaterials are the leading in the field of nanomedicine, bionanotechnology and in that respect nanotoxicology research is gaining great importance. Silver exhibits the strong toxicity in various chemical forms to a wide range of microorganism is very well known and silver nanoparticles have recently been shown to be a promising antimicrobial material. Analysis of bacterial growth showed that the toxicity of silver nanospheres is higher than that of gold nanospheres. In addition, noresearch has discovered any bacteria able to develop immunity to silver as they often do with antibiotics (Zhang*et al*., 2007). Therefore, eco-friendly or green chemistry and non-toxic biological

methods have also been widely considered for synthesis of silver nanoparticles.

Aim

To synthesize silver nanoparticles by green synthesis and apply on burn wound healing.

Materials and Methods

Materials

Commercial chitosan sample from shrimp shell was purchased from Asian Technology Groups Co., Ltd., Local Industry,Yangon, Myanmar. All other chemicals used were of analytical reagent grade. In all investigations, the recommended standard methods and techniques involving both conventional and modern methods were provided.

Preparation of Chitosan Solutions (1% and 0.5%)

Chitosan (1g) was dissolved in 1%(v/v) acetic acid solution. It was stirred until the chitosan was completely dissolved. Then the solution (1 % w/v) was filtered and stored for further use and analyses. The above solution was diluted with acetic acid solution to form 0.5 % (w/v) chitosan solution.

Synthesis of Silver Nanoparticles (Ag nanoparticles)

Silver nanoparticles (Ag nanoparticles) were prepared using chitosan solutions (1% and 0.5% w/v) as reducing agents as well as stabilizing agents. 100 mL of 1% and 0.5% (w/v) chitosan solution was heated to 80 ± 3 ºC. The collagen when heated at 80±3 ºC denatures chitosan which acts as reducing/stabilizing reagent. 1,2,3,4 mL each of the $AgNO_3$ solution (0.01M) was added rapidly at a stirring rate of 3000 rpm. A colour changesto pale yellow was observed due to the complex formation between chitosan and Ag ion. The reaction was carried out under dark conditions and the contents were subjected to vigorous stirring at 80±3 ºC to ensure the complete formation of chitosan capped silver nanoparticles. The prepared (Ag nanoparticles were denoted as samples

1,2,3,4 when using 1,2,3,4 mL of 0.01 M $AgNO_3$ solution respectively in 1% (w/v) chitosan solution. Samples 5,6,7 and 8 were prepared by addition of 1,2,3,4 mL each of 0.01M $AgNO_3$ solution to 0.5% w/v of chitosan solution.

Characterization of Prepared Ag nanoparticles

The prepared Ag nanoparticles(Samples1-8) were characterized by UV-vis sprectroscopy, XRD analysis and determined their antimicrobial properties.

Results and Discussion

Tyndall Effect

Tyndall effects on Ag nanoparticles are shown in Figures 1 and 2. It was found that the laser light passes through the solutions due to the presence of nanoparticles.

Sample 1 Sample 2 Sample 3 Sample 4

Figure 1: Tyndall effect on Ag nanoparticles (Samples 1, 2, 3, 4)

Sample 5 Sample 6 Sample 7 Sample 8

Figure 2: Tyndall effect on Ag nanoparticles (Samples 5, 6, 7, 8)

Analysis of Silver Nanoparticles by UV-vis Spectroscopy

The synthesized nanoparticles were confirmed using UV-vis spectroscopy as depicted in Figures3 to 10.The maximum absorption wavelengths of Ag nanoparticleswere found to be near 390nm (Table 1) which confirmed that the presence of nanoparticles in colloidal solutions were Ag nanoparticles with the size of 1-5nm.

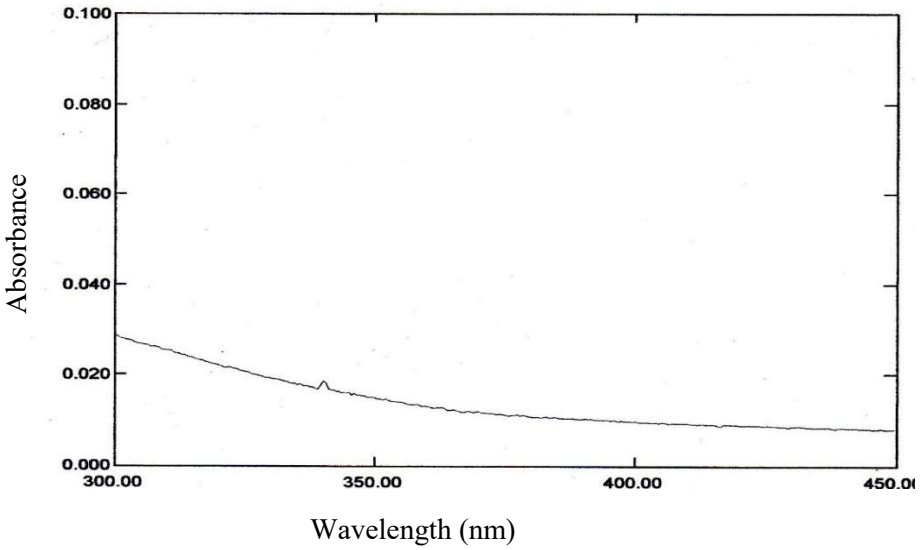


Figure 3: UV-vis spectrum of silver nanoparticles in colloidal solution (Sample 1)

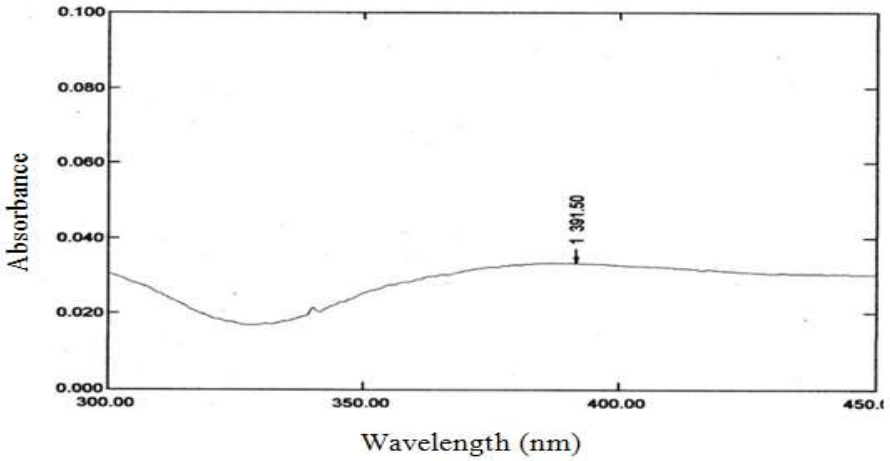
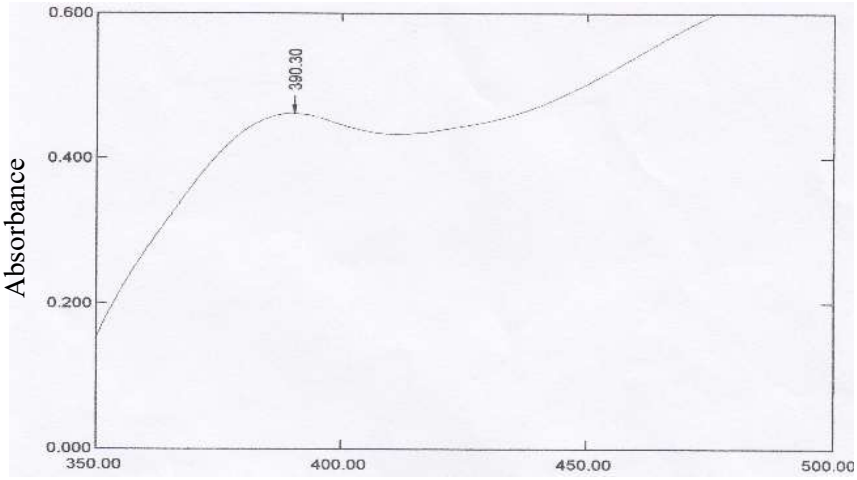


Figure 4: UV-vis spectrum of silver nanoparticles in colloidal solution (Sample 2)

Wavelength (nm)

Figure 5: UV-vis spectrum of silver nanoparticles in colloidal solution (Sample 3)



Wavelength (nm)

Figure 6: UV-vis spectrum of silver nanoparticles in colloidal solution (Sample 4)

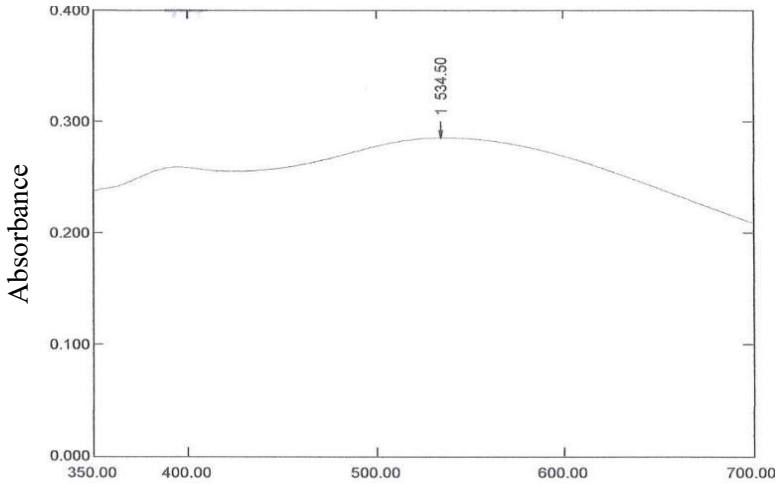


Figure 7 : UV-vis spectrum of silver nanoparticles in colloidal solution (Sample 5)

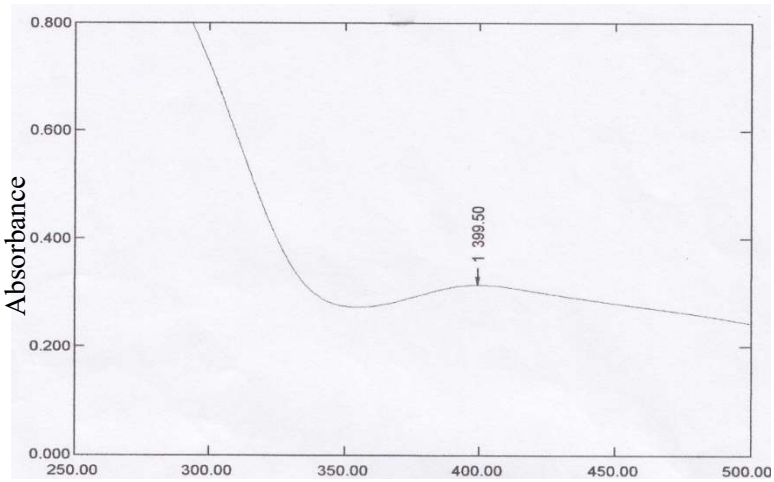


Figure 8: UV-vis spectrum of silver nanoparticles in colloidal solution (Sample 6)

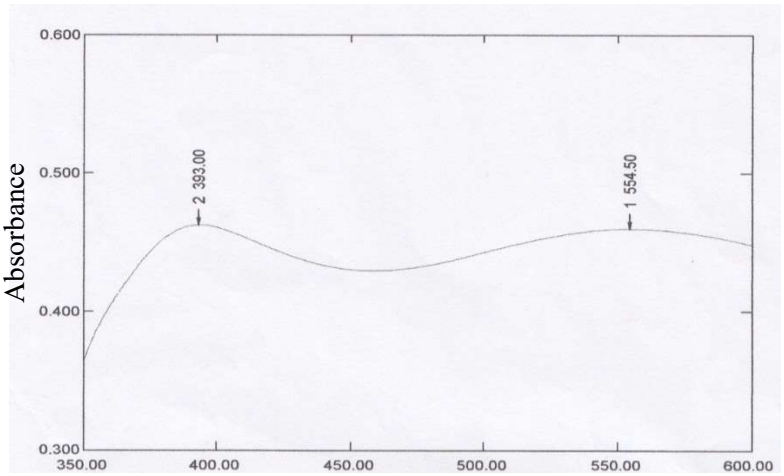


Figure 9: UV-vis spectrum of silver nanoparticles in colloidal solution (Sample 7)

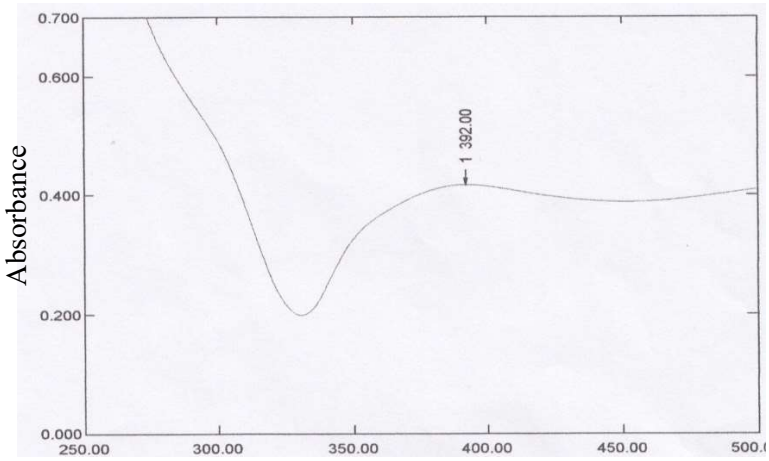


Figure 10 : UV-vis spectrum of silver nanoparticles in colloidal solution (Sample 8)

Table 1: Wavelength of Maximum Absorption of Ag Nanoparticles

Ag nanoparticles Sample	Observed wavelengths of maximum absorption (nm)
1	-
2	391
3	392
4	**390**
5	390,534
6	399
7	399,554
8	392

X-ray Diffraction Analysis

X-ray diffractograms of synthesized Ag nanoparticlesare shown in Figures11 to 18. All of the XRD sprectra show the amorphous nature of prepared silvernanoparticles.

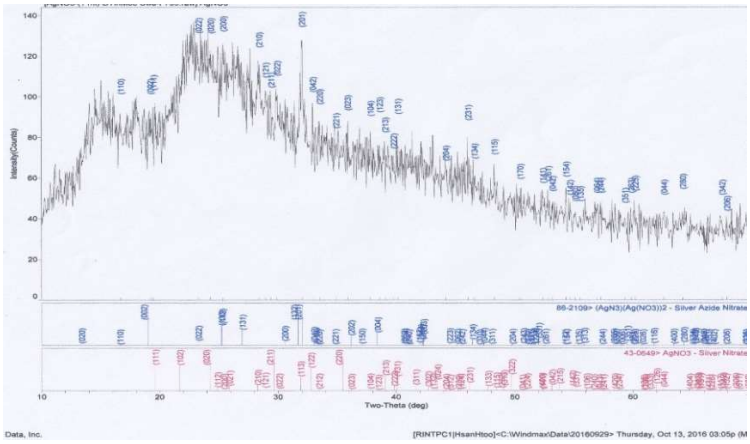


Figure 11 : X-ray diffractogram of silver nanoparticles (Sample 1)

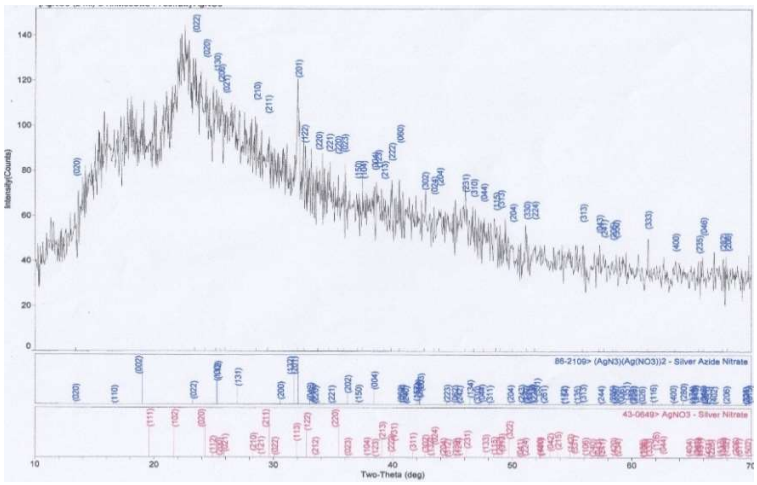


Figure 12: X-ray diffractogram of silver nanoparticles (Sample 2)

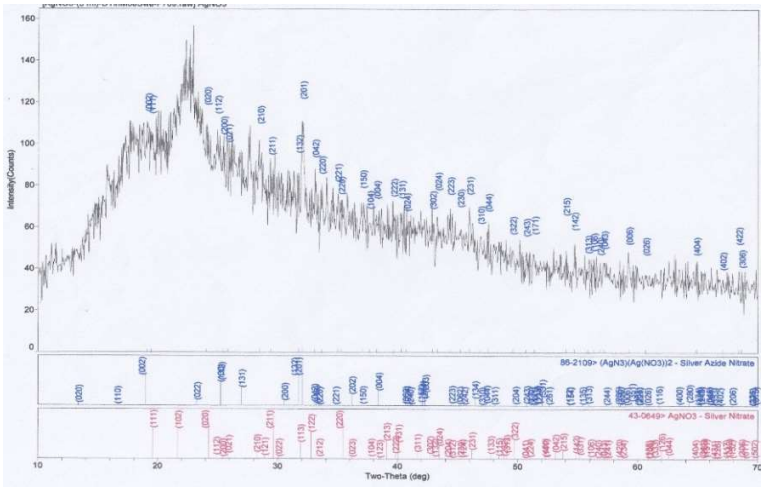


Figure 13 : X-ray diffractogram of silver nanoparticles (Sample 3)

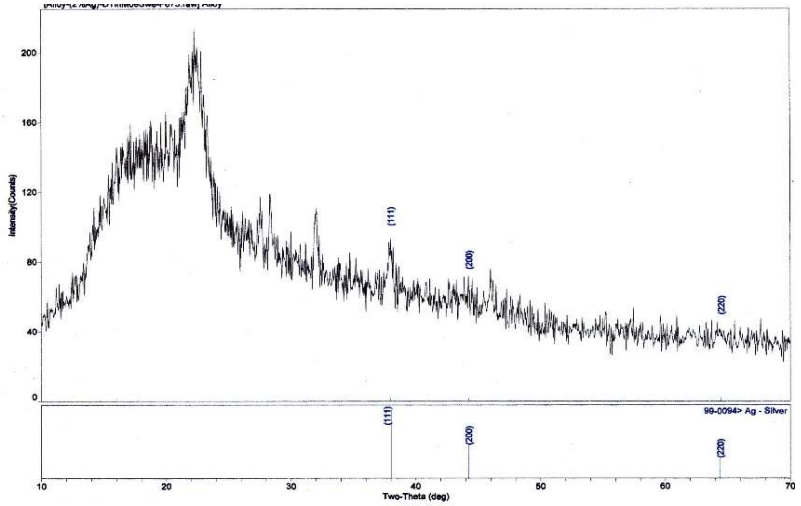


Figure 14: X-ray diffractogram of silver nanoparticles (Sample 4)

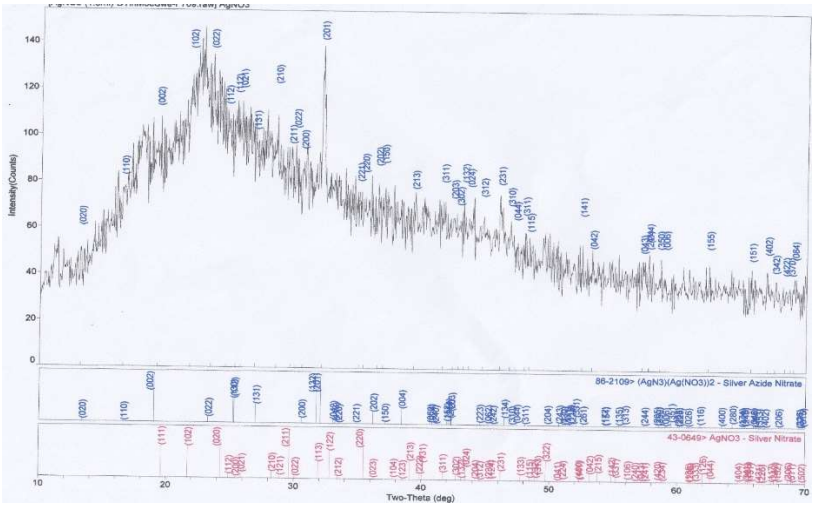


Figure 15: X-ray diffractogram of silver nanoparticles (Sample 5)

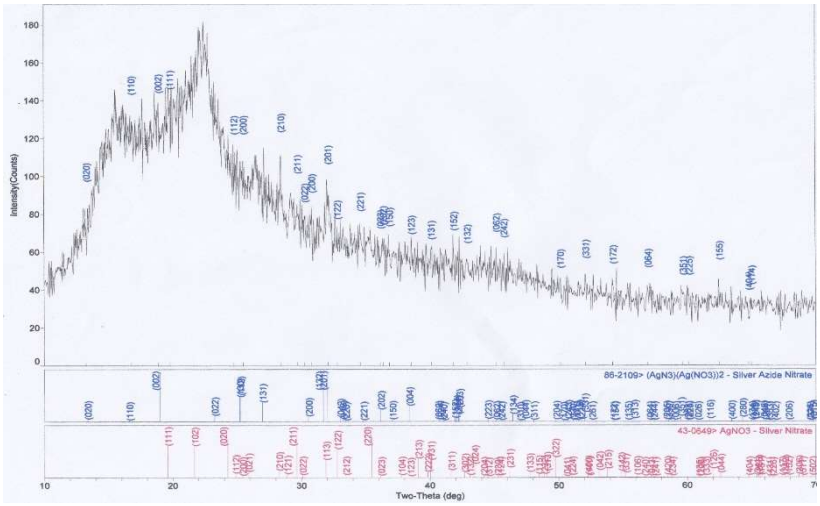


Figure 16: X-ray diffractogram of silver nanoparticles (Sample 6)

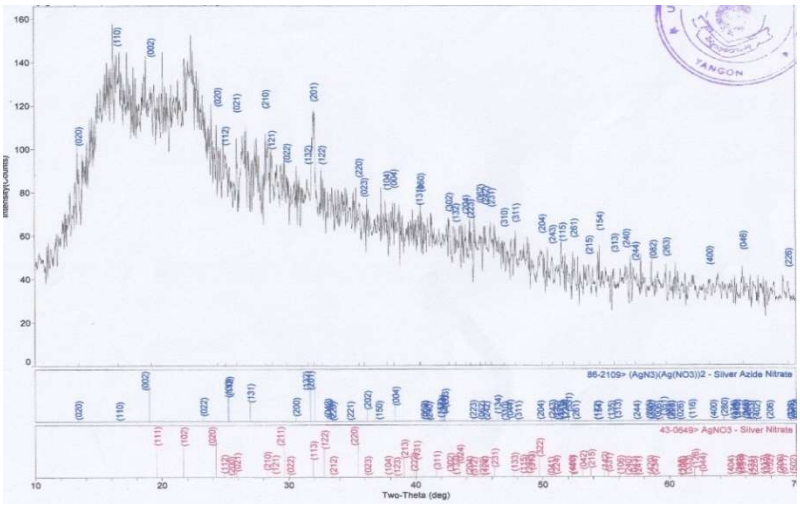


Figure 17: X-ray diffractogram of silver nanoparticles (Sample7)

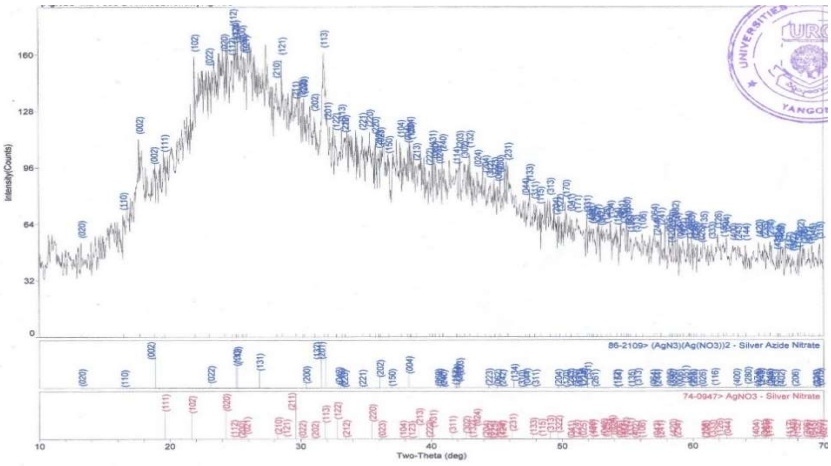


Figure 18: X-ray diffractogram of silver nanoparticles (Sample 8)

Antimicrobial Activities of Silver Nanoparticles

The antimicrobial activities of Ag nanoparticlestested against 6 microorganisms are shown in Figures19 and 20 and Tables 2 and 3.. According to the results, although chitosan itself show the avtivity on microorganisms, the presence of silver nanoparticles enhance the activitysignificantly.Samples5,6,7,8 were less active than samples 1,2,3,4 except *S. aureus.* So 1% (w/v) chitosan was chosen for the preparation of Ag nanoparticles. Among samples 1 to 4, the most active sample 4 was chosen for the preparation of Au-Ag bimetallic nanoparticles based on UV-vis spectroscopic analyses and antimicrobial activities.

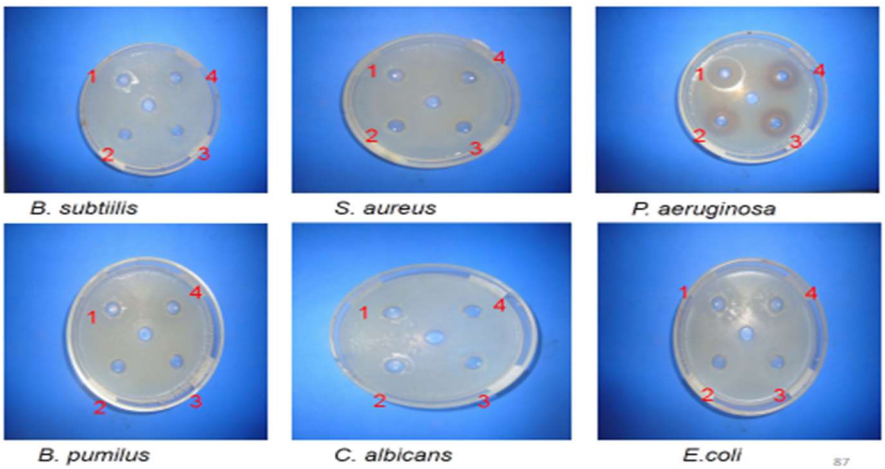


Figure 19: Antimicrobial activities of Ag nanoparticles (Samples 1,2,3 and 4)

Table 2: Antimicrobial Activities of the Prepared Silver Nanoparticles (Samples 1-4) against Six Microorganisms

Sample No	Inhibition Zone Diameter (mm)					
	B. subtilis	*S. aureus*	*P. aeruginosa*	*B. pumilus*	*C. albicans*	*E. coli*
1	13 (+)	-	30 (+++)	26 (+++)	12 (+)	30 (+++)
2	13 (+)	-	25 (+++)	25 (+++)	13 (+)	30 (+++)
3	13 (+)	-	24 (+++)	25 (+++)	12 (+)	30 (+++)
4	11 (+)	-	26 (+++)	29 (+++)	11 (++)	30 (+++)
1%Chitosan	-	-	-	-	-	-

Agar well – 10 mm

10 mm ~ 14 mm (+)

15 mm ~ 19 mm (++)

20 mm above (+++)

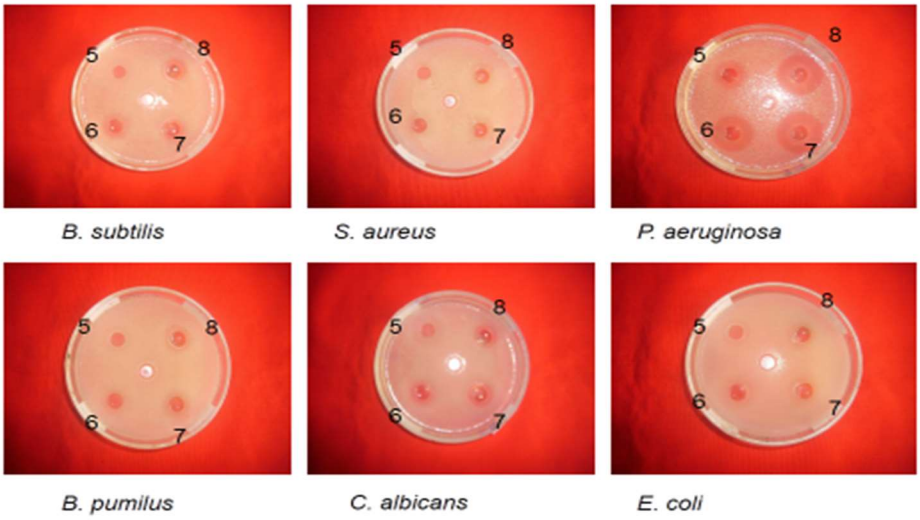


Figure 20: Antimicrobial activities of Ag nanoparticles (Samples 5,6,7,8)

Table 3: Antimicrobial Activities of the Prepared Silver Nanoparticles (Samples 5-8) against Six Microorganisms

Sample No	Inhibition Zone Diameter (mm)					
	B. subtilis	*S. aureus*	*P. aeruginosa*	*B. pumilus*	*C. albicans*	*E. coli*
5	13 (+)	12 (+)	15 (++)	12 (+)	13 (+)	13 (+)
6	15 (++)	11 (+)	21 (++)	13 (+)	15 (+)	15 (++)
7	17 (++)	12 (+)	24 (+++)	13 (+)	12 (+)	15 (++)
8	17 (++)	12 (+)	25 (+++)	13 (+)	11 (++)	13 (+)
0.5% Chitosan	-	-	-	-	-	-

Agar well - 10mm

10mm ~ 14mm (+)

15mm ~ 19mm (++)

20mm above (+++)

Scanning Electron Microscopic (SEM) Analysis

Structural and surface morphology of silver nanoparticles (Sample 4)was analyzed by SEM analysis. The micrograph is presented in Figure 21. The various spherical shape of silver nanoparticles were observed.

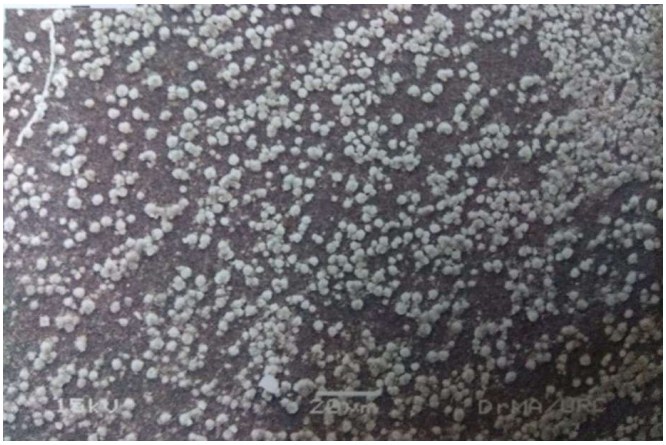


Figure 21: SEM micrograph of silver nanoparticles (Sample 4)

Fourier Transform Infrared Spectrpscopy (FT IR)

The synthesized silver nanoparticles (Sample 4) was characterized by FT IR spectroscopy. The FT IR sprectra of Ag nanoparticleswas shown in Figure 22.Infraed studies was carried out in order to ascertain the purity and nature of the metal nanoparticles. Metals generally give absorption band in finger print region i.e. below 1000cm^{-1} arising from inter-atomic vibrations. The peak observed at 3448cm^{-1} are may be due to O-H stretching. The peaks at 1397 and 650 cm^{-1} are corresponding to Ag-O stretching and deformation, respectively. The observed wave numbers of prepared Ag nanoparticles (Sample 4) were accordance with the literature values (Mansor *et al.,* 2011).

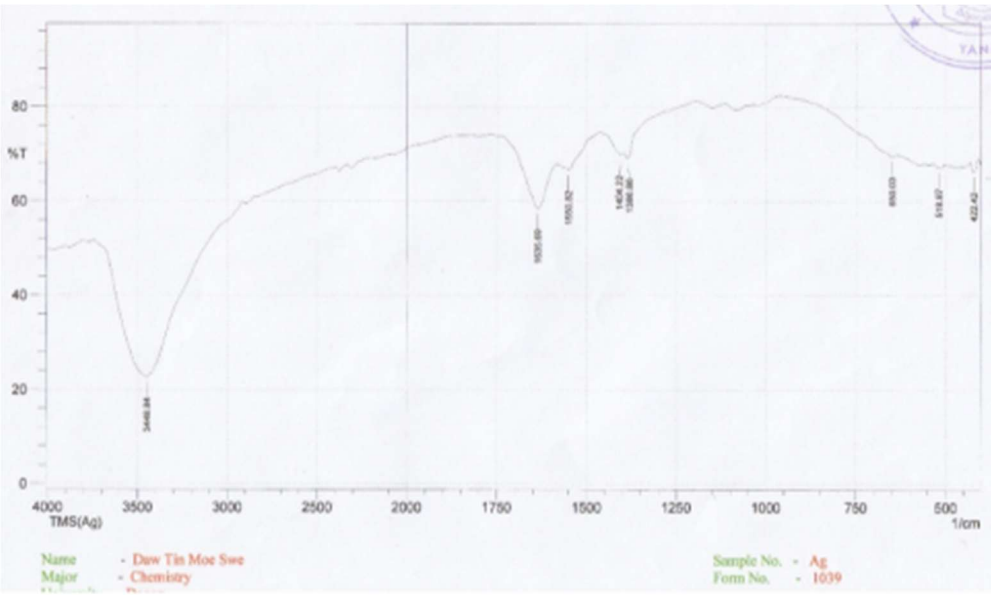


Figure 22: FT IR spectrum of silver nanoparticles (Sample 4)

Table 4: FT IR Spectral Assignment for Silver Nanoparticles (Sample 4)

Observed wavenumber (cm^{-1})	**Literature wavenumber (cm^{-1})**	**Band assignment**
3448	3200-3600	-OH stretching
1397*	~ 1400	Ag-O stretching
650	~ 650	Ag-O deformation

* Mansor *et al,* 2011

Energy Disperse X-ray Florescence (EDXRF)

EDX RF spectrum confirms the presence of silver (%) in sample 4. (Figure 23)

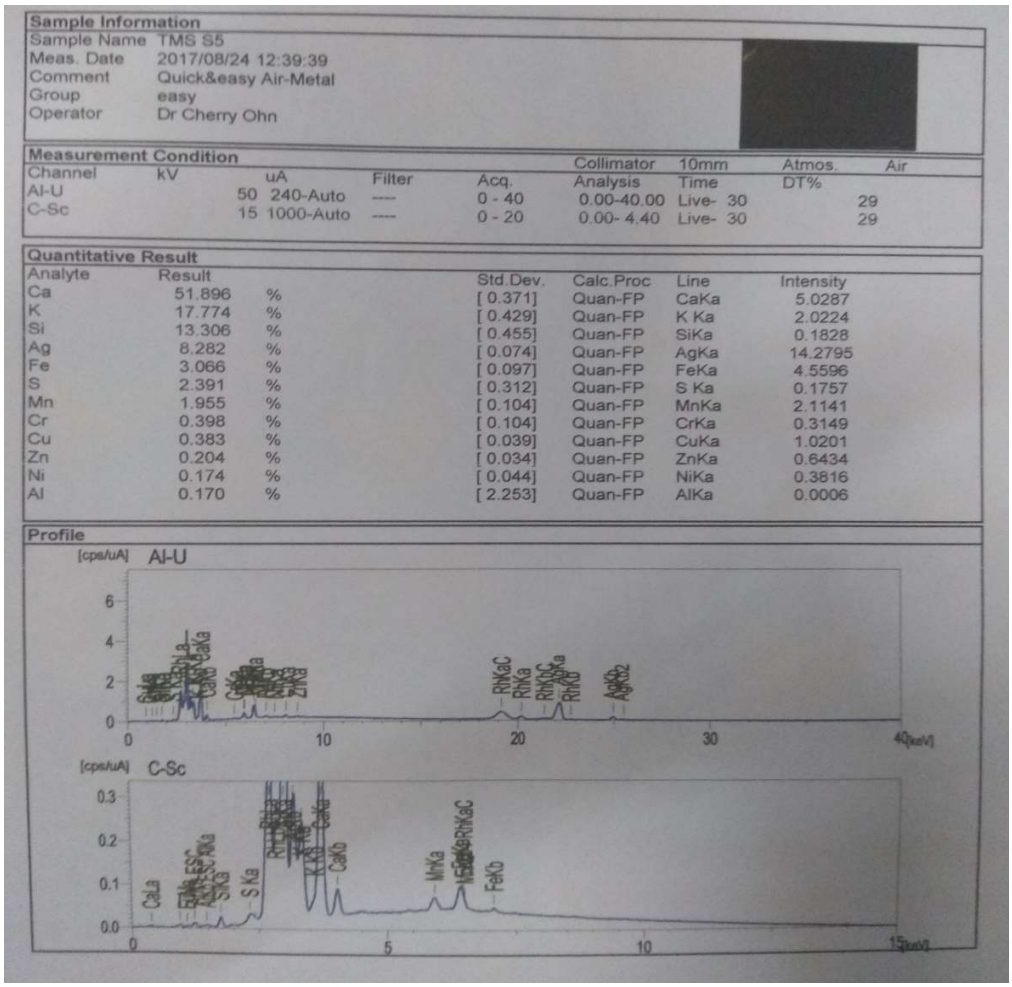


Figure 23: EDXRF spectrum of silver nanoparticles (Sample 4)

Conclusion

From the overall assessment of the present work, the following inferences can be deduced. Pale yellow colour of Ag nanoparticles was successfully synthesized. The prepared particles which were in the nano range was confirmed by Tyndall scattering. The laser light passes through the solution due to the presence of nanoparticle. The prepared particles which were in the wavelength of nano range was confirmed by UV-visible spectroscopy. The maximum absorption wavelengths of Ag nanoparticles were found near 390nm. All of the X-ray diffractogram shows the amorphorus nature. According to the results of antimicrobial activities of silver nanoparticles using 1% w/v chitosan solution as reducing agent it showed high activities for five tested microorganisms except *S. aureus*. Based on UV-vis and antimicrobial activities of samples 1 to 4, the maximum mixing ratio of 4 mL and 0.01M $AgNO_3$ in 1% w/v chitosan was chosen for further studies. The EDXRF results of sample 4 was found to be 8.282% of Ag. From the SEM micrograph of sample 4, the prepared Ag nanoparticles had spherical shape with various sizes. According to the FT IR spectrum of sample 4, three obvious infrared bands were observed at 3448cm^{-1}, 1397 cm^{-1} and 650cm^{-1}due to the presence of O-H stretching, Ag-O stretching and Ag-O deformation, respectively.

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