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Synthesis and Characterization of Silver Nanoparticles for Biological Applications

Peula Kumari Ponnaian[1], Rachel Oommen[2], Senthil Kumaran Chinna Kannaiyan[3], Santhanam Jayachandran[4], Muthukumarasamy Natarajan[5], Agilan Santhanam[6*]

[1]*Department of Physics, Chikkanna Government Arts College, Tirupur, TN, India.*
[2]*Department of Physics, Avinashilingam Deemed University for Women, Coimbatore, TN, India.*
[3,5,6*]*Department of Physics, Coimbatore Institute of Technology, Coimbatore, TN, India.*
[4]*Department of Veterinary Physiology, Veterinary College and Research Institute, Orathanadu, TN, India.*

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Abstract

Silver (Ag) nanoparticles have been synthesized by simple co-precipitation method. X-ray diffraction studies indicated the formation of Face centered cubic (FCC) Silver nanoparticles with grain size of 38 nm. Surface morphology of Silver (Ag) nanoparticles has been studied using scanning electron microscopy (SEM). Application of prepared silver nano particles for the treatment on the wounds of rabbits have been studied. It is inferred that both silver nanoparticle ointment and solution may be used as an alternate to antibiotics cream when antibiotic resistance is suspected.

Keywords: *Biological Study; Chemical method; Silver Nanoparticle; SEM; TEM.*

1. INTRODUCTION

Noble metal nanoparticles have been the focus of intense research in recent decade and it is motivated by the exceptional properties that a material gains when its size is reduced to nanoscale lengths which are extensively used in drug delivery, biosensors, bio imaging, antimicrobial activities, food preservation etc., (De *et al.* 2008). Among them Silver nanoparticles have attracted extensive research interest because of their unusual optical, electronic, and chemical properties which depend on their size, shape, composition, crystallinity, and structure (Jiang *et al.* 2005). They have been widely exploited for use as microelectronic materials (Hsu and Wu, 2007), antibacterial materials (Morones *et al.* 2005), catalytic materials and sensor materials (McFarland and VanDuyne, 2003) due to their unique properties. Nowadays, antimicrobial effects are intensively studied due to an enormously increasing bacterial resistance against excessively and repeatedly used classical antibiotics. Thus, day after day, the treatment of bacterial infections utilizing classical antibiotics is certainly becoming more serious global problem. As an evidence, let us mention the recent discovery of MDM-1 bacteria against which almost all known antibiotics are inefficacious. Since most of the researchers used efficacious antibiotics come from the 70th and 80th of the 20th century, it is certainly essential to develop new medical drugs for an effective fight with bacteria. Silver nanoparticles may be of promising help in this aspect as they effectively eliminate bacteria at relatively low concentrations of silver nanoparticles; concentrations that are not toxic for human cells. In addition, bacterial resistance against silver nanoparticles has not been documented so far (Robert *et al.* 2011).

Currently, many methods have been reported for the synthesis of Ag-NPs by using chemical, physical, photochemical and biological routes. Each method has its own advantages and disadvantages dealing with cost, scalability and particle size. In physical synthesis approach, the metallic NPs can be generally synthesized by evaporation-condensation, which could be carried out by using a tube furnace at atmospheric pressure. However, in the case of using a tube furnace at atmospheric pressure there are several drawbacks such as a large space of tube furnace, great consumption of energy for raising the environmental

***Agilan Santhanam** Tel. no: +919994909993*
e-mail: agilan@cit.edu.in

temperature around the source material and a lot of time for achieving thermal stability (Jung *et al.* 2006). It is not easy to have a large quantity of nanoparticles by using biological synthesis.

Among the existing methods, chemical method has been mostly used for production of Ag-NPs. Chemical methods provide an easy way to synthesize Ag-NPs in solution. Generally, the chemical synthesis process of the Ag-NPs in solution usually employs the following three main components: (i) metal precursors, (ii) reducing agents and (iii) stabilizing/capping agents. The formation of colloidal solutions from the reduction of silver salts involves two stages of nucleation and subsequent growth. It is also revealed that the size and the shape of synthesized Ag-NPs are strongly dependent on these stages. Furthermore, for the synthesis of mono dispersed Ag-NPs with uniform size distribution, all the nuclei are likely to have the same or similar size, and then they will have the same subsequent growth. The initial nucleation and the subsequent growth of initial nuclei can be controlled by adjusting the reaction parameters such as reaction temperature, pH, precursors, reducing agents (i.e. $NaBH_4$, ethylene glycol, glucose) and stabilizing agents (i.e. PVA, PVP, sodium oleate etc.)

In this work, Silver nanoparticle was synthesized by using the simple chemical method and characterized using XRD, Raman analysis, SEM with EDAX and TEM. The obtained results shows that silver nanoparticle with uniform size has been formed. There is a great need of finding new and alternate antibiotic agents for the wound healing treatment. Herein, we report the use of Silver nanoparticles for the antibiotic treatment and tabulated the effects of healing against the wounded rabbits. Here the as prepared silver nanoparticles accomplish the need and acts as a promising antibiotic medicine.

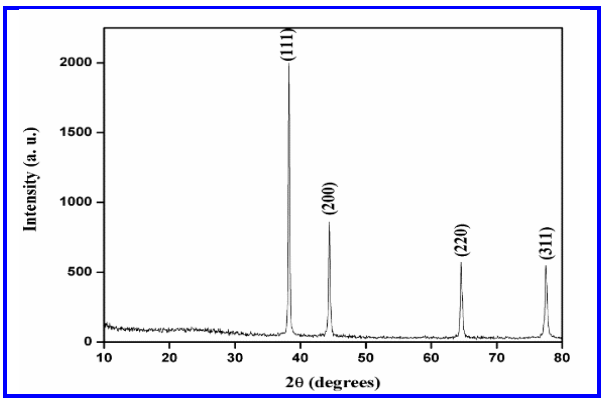

Fig. 2: X-ray diffraction pattern of Ag nanoparticles.

2. EXPERIMENTAL

As reported from our previous work (Peula Kumari Ponnaian *et al.* 2013), the flow chart shown below depicts the detailed preparation procedure. $AgNO_3$ and Na_2CO_3 were used as a precursor and the starch acting as a reductor agent. Silver Nitrate 0.05 M and 50 mg of starch were dissolved in aqueous medium, the obtained solution was stirred for one hour at room temperature. 0.025 M of sodium carbonate in aqueous solution is used as basic medium, and is added drop wise to the above solution. Thus, the $AgNO_3$ splits into positive silver ions (Ag^+) and negative oxide ion (NO^{3-}). Due to the splitting of ions the color of the solution changes to yellow which is unstable. This solution was stirred for 5 hours at room temperature and there is a complete transformation of Ag^+ ions with the indication of dark gray precipitates. The precipitate is separated by centrifugation process and other impurities are washed

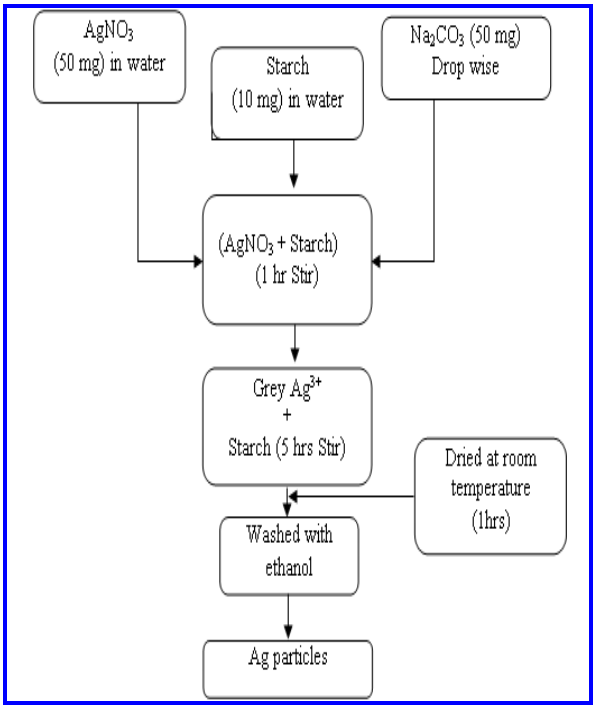

Fig. 1: Flow chart depicting the preparation of Ag nanoparticles.

X-ray diffraction studies have been carried out using PANalytical x-ray diffractometer, surface morphology of the samples has been studied using scanning electron microscope (JEOL JSMS 800-V). Transmission electron microscope (TEM) images of the prepared Ag have been recorded using a Philips TECNAI F20 microscope.

out by ethanol and the residues are dried at room temperature.

3. RESULTS & DISCUSSION

Fig. 2. Shows the X-ray diffraction patterns of Ag. The diffraction peaks at 2θ (degrees) values of 38.26°, 44.41°, 64.47° and 77.57° can be attributed to the (111), (200), (220) and (311) crystallographic planes respectively, of the face-centred cubic (FCC) structure of Ag nanocrystals (JCPDS).

No additional peaks belonging to other silver phase were observed, which indicates the good crystallinity and high purity of Ag nanoparticles.

The average size of Ag have been calculated using Debye Scherrer's equation,

$$D = \frac{K\lambda}{\beta \cos\theta}$$

Where, D is the grain size,
K is a constant taken to be 0.94
λ is the wavelength of the x-ray radiation, β is the full width at half maximum and
θ is the angle of diffraction.

Table 1. gives structural parameters of the prepared silver nano particles using X-ray diffraction technique.

Table 1. The lattice constant (a), d-spacing (d) and crystallite size (D) of the Ag samples.

Sample	D m	d m	a
Ag	38.189 x 10^{-9}	2.353 x 10^{-10}	4.0755

Fig. 3. shows a typical Raman spectrum of the as prepared silver (Ag) Nanoparticles.

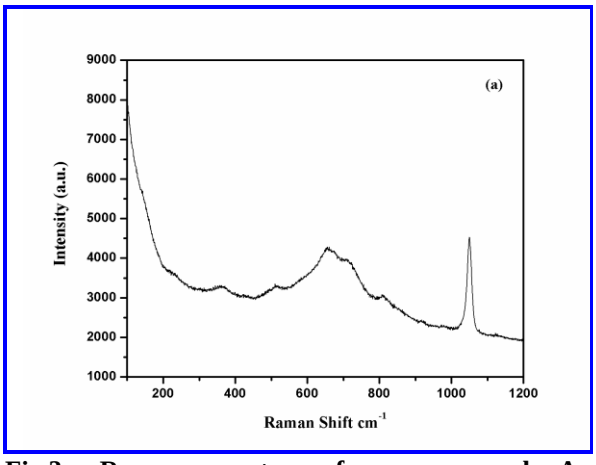

Fig.3: Raman spectra of as prepared Ag nanoparticles.

Raman spectroscopy is a powerful tool to investigate the structural properties of nanoparticles. The above obtained Raman spectra was similar to that of the spectrum of Ag nanoparticles reported by Jean-Christophe Valmalette *et al.* (2014).

Fig. 4. shows the scanning electron microscope (SEM) image of Silver nanoparticles. Morphology studies of the synthesized nanoparticles were carried out by using scanning electron microscopy (SEM).

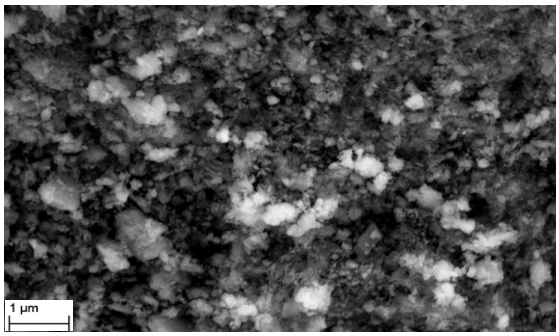

Fig. 4: SEM image of Silver nanoparticles.

Table 2. Wound size (in cm) treated with silver nitrate, silver nanoparticles and antibiotic in rabbits.

TREATMENT	Wound Size in cm					RATE OF HEALING
	DAY 1	DAY 3	DAY 5	DAY 7	DAY 9	
Control	1.7	1.5	1.1	0.7	0.3	5
Silver nitrate solution soaked gauze	1.6	1.4	1.0	0.5	Healed	4
Nanosilver solution soaked gauze	1.7	1.5	1.1	0.4	Healed	3
Nanosilver ointment	1.8	1.4	0.9	0.5	Healed	2
Commercial antibiotic ointment	1.7	1.2	0.8	0.3	Healed	1

The SEM image reveals that the sample has uniform grain distribution with well-connected grains. Fig. 5. shows the elemental composition pattern of silver nanoparticles.

EDAX spectra shows the strong peak corresponding to Ag atoms. Thus the analysis indicates that the obtained Ag nanoparticles are quite pure.

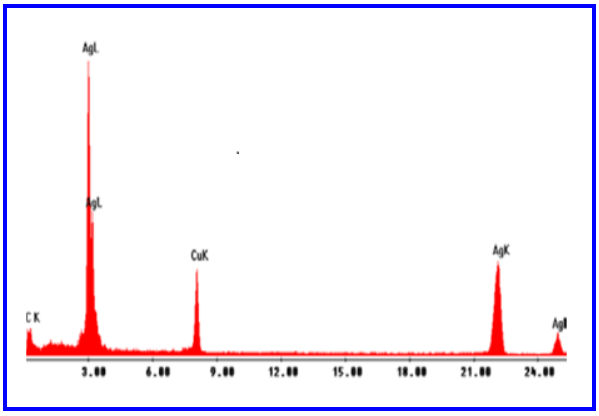

Fig.5: EDAX Spectra for Ag Nanoparticles

Fig. 6. Shows the TEM image of as-prepared silver nanoparticles.

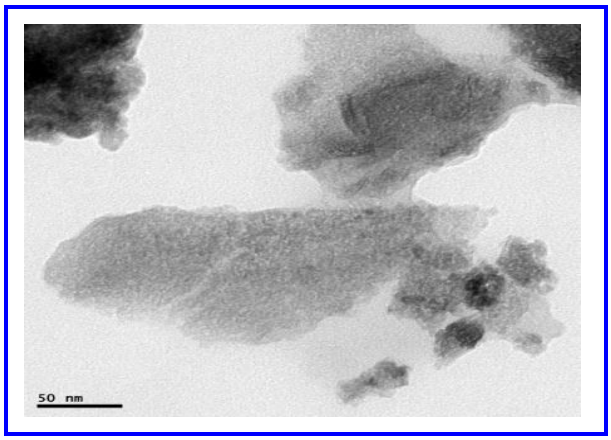

Fig. 6: TEM image of Silver nanoparticles.

Transmission Electron Microscope (TEM) shows the presence of closely packed nanoparticles and the nanoparticles have got agglomerated. The particle sizes of Ag are found to be 36 nm.

4. SILVER NANO PARTICLES FOR WOUND HEALING ACTIVITY

Preparation of wound dressing

The hospital grade sterilized cotton wound gauze was soaked overnight in the silver nanoparticles solution and dried under air in a sterile environment.

Preparation of ointment

10% silver nanoparticles loaded ointment was prepared for applying the wound area as an alternative to the antibiotic ointment.

4.1 Methods for Antibiotic Activity

Five numbers of 6 months old healthy rabbits (Soviet chinchilla) kept separately in cages.

Water and feed were offered ad libitum. Under local anesthesia (Lignocaine HCl, 2%), about 1.5 cm wound was created following standard surgical procedure wound was dressed with silver nanoparticles loaded gauzecommercial antibiotic ointment (gentamicin with betamethasone) silver nitrate solution soaked gauze ointment prepared with silver nanoparticles.Dressing was changed on alternate days. One rabbit was not dressed and left as control. Wound healing was assessed at alternate day interval for nine days healing of wound was recorded as reduction in size of the wound area.

5. RESULT & DISCUSSION FOR ANTIBIOTIC EFFECT

(i) Treatment of wound with silver nitrate solution soaked gauze resulted in 0.2, 0.4 and 0.5 cm reduction in wound area on day 3, 5 and 7 respectively. The wound was healed on 9th day.

(ii) Treatment of wound with silver nanoparticles loaded gauze resulted in 0.2, 0.4 and 0.7 cm reduction in wound area on day 3, 5 and 7 respectively and wound healing was observed on day 9.

(iii) Treatment of wound with ointment containing silver nanoparticles in rabbit yielded about reduction of 0.4, 0.5 and 0.5 cm in wound area on day 3, 5 and 7 respectively. The wound healing was observed on 9th day.

(iv) Treatment of wound with commercial antibiotic ointment resulted in 0.5, 0.4 and 0.5 cm reduction in wound area on day 3, 5 and 7 respectively and wound healing was observed on day 9.

(v) In the control animal as well, reduction in size of wound area by 0.2, 0.4 and 0.4 cm on day 3, 5 and 7 respectively observed. On day 9, there was 82% of the wound area is healed

(vi) Rate of wound healing by treatment with commercial antibiotic ointment was higher followed by ointment containing silver nanoparticles and

surgical gauze loaded with silver nanoparticles. The silver nitrate was also equally better.

(vii) It is inferred that both silver nanoparticle ointment and solution may be used as an alternate to antibiotics cream when antibiotic resistance is suspected.

The findings of this was supported by Rigo *et al.* (2013) who observed that the application of silver nanoparticles based dressings allows wound healing and recovery.

5. CONCLUSION

Ag nanoparticles have been prepared by a simple chemical method. X-ray diffraction pattern reveals that Ag nanoparticles exhibit face-centred cubic (FCC) structure and the average particle size of the nanoparticles is in the range of 38 nm. The TEM studies show that the average particle size of Ag nanoparticles is in the range around 36 nm. The prepared Ag nanoparticles have been used to study the antibiotic effect on five numbers of 6 months old healthy rabbits which was wounded about 1.5 cm and then we observed by following the standard surgical procedure to dress the wound with silver nanoparticles in order to reveals the rate of healing effect at different days by the as prepared silver nanoparticles.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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