Synthesis and Characterization of Silver Nanoparticles for Biological Applications

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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 nanoparticles 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 (Balan et al. 2007; Jiang, 2005). They have been widely exploited for use as microelectronic materials (Hsu and Wu, 2007), antibacterial materials (Morones et al. 2005), catalytic materials (Shiraishi and Toshima 1999) 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 by day, the treatment of bacterial infections utilizing classical antibiotics is certainly becoming more serious global problem. As 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 (Prucek 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 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 (Tran et al. 2013).

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 (Dang et al. 2012). 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. NaBH₄, ethylene glycol, glucose) and stabilizing agents (i.e. PVA, PVP, sodium oleate etc.) (Patil et al. 2012).

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.

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.

2. EXPERIMENTAL

The flow chart shown in Fig.1, depicts the detailed preparation procedure. AgNO₃ and Na₂CO₃ were used as a precursor and the starch acting as a reductor agent. 50 mg of Silver Nitrate (0.05 M) and 10 mg of starch were dissolved in aqueous medium; the obtained solution was stirred for one hour at room temperature. Sodium carbonate (0.025 M) in aqueous solution is used as basic medium, and is added drop wise to the above solution. The colour of the solution was changed into yellow, indicating the formation of silver particles. The solution was stirred for 5 hrs at room temperature and the colour changed to grey. Then the precipitate was allowed to settle down. After 5 hrs, the suspension was centrifuged and washed with water and ethanol several times. The sample was then suspended in ethanol. After centrifugation, the sample was dried at room temperature. The dried sample was displayed in the Petri dish for further use.

![Fig. 1: Flow chart depicting the preparation of Ag nanoparticles.](image.png)

3. RESULTS & DISCUSSION

Fig.2. Shows the X-ray diffraction patterns of Ag. The diffraction peaks at 20 (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 Card No. 89-3722).

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

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
\( \lambda \) is the wavelength of the x-ray radiation,
\( \beta \) is the full width at half maximum and
\( \theta \) is the angle of diffraction.

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>D m (nm)</th>
<th>d m (Å)</th>
<th>a (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag</td>
<td>38.18</td>
<td>2.353 x 10^{-10}</td>
<td>4.0755</td>
</tr>
</tbody>
</table>

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

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).

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.

Raman spectroscopy is a powerful tool to investigate the structural properties of nanoparticles. The below shown Raman spectra was similar to that of the spectrum of Ag nanoparticles reported by Jean-Christophe Valmalette et al. (2014).
EDAX spectra shows the strong peak corresponding to Ag atoms. Thus the analysis indicates that the obtained Ag nanoparticles are quite pure.

Fig. 6. Shows the TEM image of as-prepared 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 gauze commerical 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.

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

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Wound Size in cm</th>
<th>RATE OF HEALING</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Silver nitrate solution soaked gauze</td>
<td>1.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Nanosilver solution soaked gauze</td>
<td>1.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Nanosilver ointment</td>
<td>1.8</td>
<td>0.5</td>
</tr>
<tr>
<td>Commercial antibiotic ointment</td>
<td>1.7</td>
<td>0.3</td>
</tr>
</tbody>
</table>

4.2 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 antibacterial effect on five numbers of 6 months old healthy rabbits which were 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.

REFERENCES


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