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Research Article

Bottom-up Approach for the Preparation of Capped Silver Nanoparticles and their Antibacterial Activity

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Abstract

Aims and Objective: Silver nanoparticles (AgNPs) are the tiny particles of silver from 1 to 100 nm in size. The current study describe the preparation of capped AgNPs based on trisodium citrate (TSC) bottom up approach. AgNPs are majorly applied in medicine for diagnostic applications to therapeutic, apart from its antimicrobial activity.

Research Methods: Turkevich method has been used to prepare silver precursor i.e. silver salt ($AgNO_3$). The ions of silver are reduced by reducing agent for their non-valent metal atom for this research, Trisodium Citrate ($Na_3C_6H_5O_7$) was used as the reducing agent in this study. However, capping agent used was Adipic acid (AA). Characteristic of the AgNPs/AA were analyzed by UV-Vis spectrophotometer and FT-IR for the analysis of functional groups.

Results and Conclusion: The AgNPs are colloidal, stable, uniform and polydisperse in nature. Their size is less 30 nm. These silver nanoparticles were checked against six antibiotics (Erythromycin, Gemifloxacin, Ciprofloxacin, Sparfloxacin, Azithromycin, Ofloxacin) for Gram-positive (*Enterobacteriaceae, Escherichia coli, Staphylococcus aureus*) and Gram-negative bacteria, (*pseudomonas aeruginosa, salmonella typhi, klebsiella pneumoniae*) ATCC 8885, ATCC 2881, ATCC 8868, ATCC 9353 by disc susceptibility test. The best resistance was shown against Azithromycin for *K. pneumoniae.*

Significant Impact: Bacterial resistance is increasing with the adaptation of microbes regularly. Current medical strategies are unable to address related to antibacterial and anti-inflammation diseases. These nano-particles may play some role in this quest.

Keywords: Silver Nanoparticles; Bottom-up Approach; Adipic Acid; Azithromycin; *K. pneumoniae*

Abbreviations

ATCC: American Tissue Culture Collection; AgNPs: Silver Nanoparticles; H-AgNPs: Capped Silver Nanoparticles; AA: Adipic Acid

Introduction

Bottom-up approach, although often referred in nanotechnology, is not a newer concept., After the industrial revolution this approach has been used in industries for the manufacturing and synthesizing of new molecules [1]. Bottom-up methods may generate nanoparticles by building them from the molecules in solution. It is attained by controlled precipitation and evaporation [2]. These processes can occur in the bulk solution or in droplets [2]. Bottom-up approach has been used and prove its important role in the fabrication and processing of nano structures, the advantage of bottom-up is the cost, scalability and in general better uniformity of the product.

Mechanism of action of capped AgNPs against antimicrobial properties and pharmacokinetics studies

There are various proposed mechanisms of antimicrobial properties of silver nanoparticles, however the exact mechanism not well understood. AgNPs cause structural changes like permeability of cell membrane that is lethal to cell. Another mechanism of action is formation of free radicals by AgNPs that trigger the cell death. The free radicals usually damage the cell membrane as they interact with bacteria by making it porous, causing death of the cell [3]. AgNPs and silver ions show efficient antimicrobial property due to their large surface area, as compare to other salts and metals [4]. It is also proposed that mainly silver ions get in contact with thiol groups of many essential enzymes in bacteria and inactivate them [3]. Silver is generally considered as soft acid, and it is a basic chemistry phenomenon of an acid to react with soft base. The sulfur and phosphorus are potential soft bases in the cells to react with nanoparticles and causing cell mortality [5]. DNA is mainly comprised of sulfur and phosphorus, the interaction of these sulfur and phosphorus with nanoparticles can be challenging for DNA synthesis and multiplication of bacteria. The peptide substrates of tyrosine residues get dephosphorylate by nanoparticles and inhibit signal transduction inhibition and thus hindered the growth of microbes [3].

Pharmacokinetics of AgNPs are being studied to evaluate the accumulation of drug in the target tissues and distribution in non-target tissues for the unnecessary toxicity. It should be considered for safe and efficacious biomedical applications of NPs [6]. The absorption of nanoparticles via oral and tropical administration of silver nanoparticles are studied by fewer researcher found less bioavailable traces based on faecal excretions mostly absorbed in GI tract [7,8]. Similar to all drugs, liver is in frontline and primary organ for silver distribution other than spleen and kidneys. It has been observed that females have two-fold increase of silver accumulation as compare to males [9]. The metabolism can be suggested as forming Ag–GSH (Glutathione) polymer complexes reacting with GSH and thiol complexes with silver and excretion is reported less in urine than faeces depending upon the size of Ag particles, it can take from 12h to several weeks in mice [7].

Materials and Methods

Turkevich method was used to prepare silver precursor i.e. silver salt ($AgNO_3$). The ions of silver are reduced by reducing agent for their non-valent metal atom for this research, Trisodium Citrate ($Na_3C_6H_5O_7$) was used as the reducing agent in this study. However, capping agent used was Adipic acid.

For capping, in a10 mL reaction flask 1 mL of Tri Sodium Citrate, mixture of NaOH (0.5 ml) and Adipic acid (7 mL) was taken and heated to 70^0C was added drop wise under vigorous stirring on magnetic stirrer, for 10 minutes. 1 mL (10 %, silver nitrate solution) was added dropwise to the reaction flask. The reaction mixture was kept stirred for 15 minutes till the color turned to pale yellow. The change of color was observed from colorless to bright yellow.

$$4Ag^{+} + Na_3C_6H_5O_7 + 2H_2O \xrightarrow{(COOH(CH_2)_4COOH)} 4Ag^{0}(COOH(CH_2)_4COOH) + C_6H_8O_7 + 3Na^{+} + H^{+} + O_2$$

The characteristic of the AgNPs/AA were analysed by UV-Vis spectrophotometer (Model # UV-1800 Parma Spec., Shimadzu, Japan), to detect maximum the absorbance (λ_{max}), FT-IR, (Shimadzu IR-Prestige-21) for the analysis of functional groups and antimicrobial susceptibility test carried out by Disk susceptibility technique.

Six antibiotics namely (Erythromycin, Gemifloxacin, Ciprofloxacin, Sparfloxacin, Azithromycin, Ofloxacin), against Gram positive (*Enterobacteriaceae, Escherichia coli, Staphylococcus aureus*) and Gram negative bacteria, (*Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumoniae*), ATCC 8885, ATCC 2881, ATCC 8868, ATCC 9353 discs loaded with the synthesized nanoparticles were placed in sterile Muller-Hinton agar plate with bacterial inoculums. Antibiotics, Nanoparticles and silver nitrates paper discs were prepared by soaking them in 100 ppm solution of respective chemicals, followed by drying. The dishes were then incubated at 36°C ± 1°C, for 24 hours. Three replicate trials were performed against each bacterial strain for each concentration. Zones of inhibition were carefully measured using digimatic calipers in millimeters (Mitutoyo Rochester, NewYork). 0.5 McFarland standard (1.0 x 10^8 CFU/ml) bacterial strains were used for the control study.

Results and Discussion

The different ratio of $AgNO_3$ and TSC were tested and the best molar concentration has been selected. The different combinations of $AgNO_3$ with adipic acid were optimized with stirring time of nanoparticles are observed as, 5,10,15 mins stirring time for

Figure 1: SPR and Optical images of synthesized and capped AgNPs/AA with different concentrations 1a, AgNO3 (0.5 mM) conc. 1b, AgNO3 (0.75mM) conc. 1c, AgNO3 (1mM) conc. 1d. pH conc.

reaction, 0.25 mM, 2.12 mM, 4 mM Adipic Acid with 0.5mM $AgNO_3$ (Figure 1a), 5,10,15 mins stirring time for reaction, 0.25 mM, 2.12 mM, 4 mM Adipic Acid with 0.75 mM $AgNO_3$ (Figure 1b) and 5,10,15 mins stirring time for reaction, 0.25 mM, 2.12 mM, 4 mM Adipic Acid with 1 mM $AgNO_3$ (Figure 1c). The temperature and pH was optimized and found to be 70°C for the nanoparticle formation (also provide the optimization data if the author did such optimization studies) (Figure 1d). The change of color was observed yellow after the synthesis of particles at 400 nm.

Six antibiotics were tested ofloxacin, sparfloxacin, ciprofloxacin, gemifloxacin, azithromycin and erythromycin, the mean has been calculated and presented in figures. Among them Azithromycin has shown the best resutls (Figure 2). It is also a potential antibiotic used to treat many different types of infections caused by bacteria. AgNPs/AA with azithromycin showed high antibacterial activity against *K. pneumoniae*, more than other organisms (Figure 3 and 4).

Figure 2: Six antibiotics results with H-AgNPs X-axis antibiotics Y-axis antibiotics conc.

Figure 3: Antibacterial results with azithromycin X-axis zone of inhibition. Y-axis Bacterial organisms.

Figure 4: Proposed mechanism for the AgNPs action on bacteria.

Discussion

H-AgNPs has the potential of antibacterial and anti-inflammatory activities. The current study explores it by synthesizing the H-AgNPs by bottom up approach method and susceptibility test shows its high activity against *K. pneumoniae*. The surveyed literature indicates that colloidal sliver nano particle sizes between 2 -8 nm can be highly effective to suppress bacterial infection. There is well established scientific research on the antibacterial and antiviral properties of colloidal silver particles [10]. AgNPs can physically interact with the cell surface of various bacteria. In gram-negative bacteria, the adhesion and accumulation of AgNPs to the bacterial surface shows as particle size decreases, the surface area-to-volume ratio greatly increases. Smaller nanoparticles (<30 nm) seem to have a higher ability to penetrate bacteria mostly gram negative. The zeta potential along with and size of nanoparticles is a fundamental parameter for controlling the antimicrobial activity. The reduced size and positive zeta potential nanoparticles are more effective for this mechanism. The probable mechanism for the AgNPs/AA antibacterial activity is due to chemical bonding between AgNPs and antibiotic. The antibiotics having active functional groups, like amine groups and hydroxyl groups interact with large surface area of the AgNPs by chelation [11].

Silver ions released from nanoparticles, have the penetrating tendency and as less effective alone but in combination with nanoparticles penetrance enhances produce reactive oxygen species (ROS) being source DNA damage and fatal for cell [12] (Figure 4). The widespread utilization of nanoparticles can lead to severe health and environmental hazards in future.

Conclusions

These nanoparticles can be utilized in various application, AgNPs are utilized in industries like electronics and health care [5]. The antibacterial activity has been checked and it was found to be resistance by Azithromycin. The usage must be perceptive and shrewd. It should be well thoughtful to take advantage of it rather produce damage. The limitation to this study was the precise controlled production of nanoparticles for that the parameter are to be monitored meticulously. Another limitation is the nanotoxicity of these particles to be utilized for the therapeutic applications. Aggregation of AgNPs is another limitation to address.

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Conflict of Interest

No conflict of interest.

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