Synthesis and Comparative Assessment of Biocompatibility and Antibacterial Potential of AgNPs and AgNCs

By

Muhammad Irfan

(NUST2019000000319161)

Supervisor

Dr. Rumeza Hanif

Atta-ur-Rahman School of Applied Biosciences National University of Sciences and Technology

Islamabad, Pakistan

2022

Synthesis and Comparative Assessment of Biocompatibility and Antibacterial Potential of AgNPs and AgNCs

By

Muhammad Irfan

(NUST2019000000319161)

A thesis submitted in partial fulfillment of the requirement for the degree of Master of Sciences

In

Healthcare Biotechnology

Supervisor

Dr. Rumeza Hanif

Thesis Supervisor's Signature: _____

Atta-ur-Rahman School of Applied Biosciences National University of Sciences and Technology

Islamabad, Pakistan

2022

THESIS ACCEPTANCE CERTIFICATE

Certified that the contents and form of thesis entitled **"Synthesis and comparative assessment of biocompatibility and antibacterial potential of AgNPs and AgNCs"** submitted by **Muhammad Irfan (Registration No. NUST 00000319161)** have been found satisfactory for the requirement of the degree.

Supervisor: _____

Dr. Rumeza Hanif

ASAB, NUST

Head of the Department: _____

Dr. Sobia Manzoor

ASAB, NUST

Principal: _____

Dr. Hussnain A. Janjua

ASAB, NUST

Dated: _____

CERTIFICATE FOR PLAGIARISM

It is certified that MS thesis titled **"Synthesis, and Comparative assessment of biocompatibility and antibacterial potential of AgNPs and AgNCs.** of **Muhammad Irfan, Reg No. 00000319161** has been examined by me. I undertake that,

1. Thesis has significant new work/knowledge as compares to already elsewhere. No sentence, table, equation, diagram, paragraph or section has copied verbatim from previous work except when except when placed under quotation marks and duly referenced.
2. The work presented is original and own work of author i.e. there is no plagiarism. No idea, results or works of others have been presented as author's own work.

There is no fabrication of data or results such that the research is not accurately represented in the records. The thesis has been checked using Turnitin, a copy of the originality report attached and focused within the limits as per HEC plagiarism policy and instruction based from time to time

_____

(Supervisor)
Dr. Rumeza Hanif
Associate Professor
ASAB, NUST

DECLARATION

I, Muhammad Irfan, declare that all work presented in this thesis titled **"Synthesis and comparative assessment of biocompatibility and antibacterial potential of AgNPs and AgNCs"** is the result of my own work. The work has not been presented elsewhere for assessment. The work herein was carried out while I was a postgraduate student at Atta-ur-Rahman School of Applied Biosciences (ASAB), NUST under the supervision of Dr. Rumeza Hanif.

_____

Muhammad Irfan

DEDICATION

Dedicated to
My beloved parents and my lovely sisters!

ACKNOWLEDGEMENT

In the name of Allah, the Most Gracious and Merciful, all praises to Allah for the strength and blessing in completing this thesis. First and foremost, I want to thank my supervisor, Dr. Rumeza Hanif, for her tremendous guidance, unwavering support, and patience throughout this project. Her vast expertise and wealth of experience have inspired me throughout my academic research and daily life. My GEC members Dr, Maria Shabbir, Dr, Saira Justin, and Dr, Amin Faheem for their help and guidance. I would like to thank Prof. Dr, Hasnain Janjua, Principal of Atta ur Rahman School of Applied Biosciences (ASAB), and Dr, Sobia Manzoor, HoD Healthcare Biotechnology, for providing the opportunity to carry out research work in a professional environment and with great facilities.

Sincere thanks to my lab fellows specially Aleena Haqqi, Hafsa Athar, Khushbukhat Khan, Sidra Anwar, and my best friend Sakina for their helpful comments and contributions throughout the study. A heartfelt thanks to all my friends for their unending drive, kindness, and moral support during my studies. Last but not least, I am eternally grateful to my dear parents, as well as all of my family members, for their unending love, prayers, and encouragement.

Muhammad Irfan

Abstract

The appearance of multi-drug resistant (MDR) bacteria has become an alarming health issue. The diseases caused by MDR bacteria are increasing at an exponential rate causing the death of millions of people worldwide. About 700,000 people die due to illnesses related to antimicrobial resistance and this figure is expected to rise up to 10 million by 2050. Moreover, the development of new antibiotics is a time consuming and expensive process. Nanoparticles on the other hand provide an alternative to antibiotics which can effectively kill bacteria without inducing resistance in them. A significant number of studies has been performed to investigate the antimicrobial properties and mechanisms of metal and metal oxide nanoparticles including iron, zinc, silver, gold and lead etc. Metallic nanoparticles especially silver nanoparticles are potent antibacterial agents and have been widely used in a number of applications. In the present study silver nanoparticles of both spherical (AgNPs) and cubical shaped (AgNCs) have been synthesized using a simple polyol synthesis approach. The NPs synthesized were subjected to characterization with UV-VIS , SEM, and XRD which confirmed their successful synthesis, their crystalline structure, aspect ratio and shapes. Moreover, the functional groups attached to both types of NPs were found using FTIR analysis. The cytotoxicity of as synthesized AgNPs and AgNCs was evaluated with MTT assay which revealed a dose dependant cytotoxicity and a comparatively high biocompatibility of AgNCs than AgNPs on MCF-7 and MDA-MB-231 cell lines. The DPPH assay showed a higher antioxidant activity of AgNPs as compared to AgNCs. Both the NPs were found to be compatible for use in blood showing negligible haemolytic activities. The antibacterial activity of both the NPs were also evaluated using disk diffusion assay. The AgNPs showed an overall high inhibition zone on all the MDR strains as compared to AgNCs. The AgNCs synthesized in this study can also be used as templates for the synthesis of gold nanocages for cancer diagnosis.

Table of Contents

List Of Figures

List Of Abbreviations

AgNPs	Silver nanoparticles (Spheres)
AgNCs	Silver nanocubes
NP	Nanoparticles
MDR	Multi Drug Resistant
MRSA	Methicillin Resistant Staphylococcus aureus
VRE	Vancomycin-resistant Enterococci
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
DPPH	2,2-diphenyl-1-picrylhydrazyl
PVP	Polyvinylpyrrolidone
SEM	Scanning Electron Microscope
UV-Vis	Ultraviolet-visible
XRD	X-ray Diffraction
UV	Ultraviolet
FTIR	Fourier transform infrared
$AgNO_3$	Silver Nitrate
Na_2S	Sodium sulphide
EG	Ethylene glycol

Chapter 1

INTRODUCTION

Nanoparticles are minute particles that range in size from 1 to 100 nanometers. Nanoparticles are the fundamental particles of nanotechnology which have been used in a variety of applications in different fields including agriculture, cosmetics, pharmaceutical industries, water purification and biomedicine owing to the advantages they provide as compared to their bulk counterparts. These unique physicochemical properties are due to the small size of these nanoparticles (Ray & Bandyopadhyay, 2021). In the last 30 years the field of biomedicine have been revolutionized by the integration of nanotechnology with biology and medicine. In biomedicine nanotechnology have been used in different applications such as early detection, and treatment of a variety of diseases. Nanoparticles can be used for the targeted delivery of a number of chemicals including drugs, and therapeutic agents for disease treatment and also imaging substances for diagnostic purposes. They can also be used to deliver different biological molecules such as DNA. RNA, antigens and antibodies to their target site (El-Sayed, Kamel, & Research, 2020). Other biomedical applications of nanomaterials include the use of nanoparticles as antiviral agents, and their use against drug-resistant bacteria, as well as in vaccine production.

Nanoparticles can be broadly categorized into three major groups: Organic NPs, inorganic NPs and carbon-based NPs. The inorganic nanoparticles are further divided into metal and metal-oxide nanoparticles (Ealia & Saravanakumar, 2017). The metallic nanoparticles are mostly synthesized by both constructive and destructive approaches. One of the main methods used for synthesizing metallic nanoparticles include the chemical reduction of metal precursors such as chloroauric acid and silver nitrate to synthesize silver and gold-based nanoparticles. Some of the common metals that are used to synthesize metal-based nanoparticles include Iron, silver, cobalt, copper,

aluminium etc (Dong et al., 2020; García-Barrasa, López-de-Luzuriaga, & Monge, 2011). Metal-based nanoparticles have been used in a number of biomedical applications such as a vehicle for drug delivery (Martinho, Damgé, Reis, & nanobiotechnology, 2011), gene and protein delivery (Joshi, Bhumkar, Joshi, Pokharkar, & Sastry, 2006; Riley & Vermerris, 2017), and as a contrast agent for imaging and diagnostics (Cormode, Naha, Fayad, & imaging, 2014). Moreover, metallic nanoparticles are also used as antiviral, antifungal and antibacterial agents against a number of multi-drug resistant bacteria (de Kraker, Stewardson, & Harbarth, 2016; Sintubin, Verstraete, Boon, & Bioengineering, 2012).

Among metallic nanoparticles the silver-based nanoparticles are the most studied nanostructures due to their fascinating physicochemical properties suitable for a number of different applications in the field of biomedicine. Different approaches have been implied for the synthesis of silver-base nanoparticles of different sizes and morphologies. These approaches include physical methods (Brobbey et al., 2017; He, Ren, & Chen, 2017), chemical (Han, Yu, Kim, & Im, 2017; Khatoon, Rao, Mohan, Ramanaviciene, & Ramanavicius, 2017), and green synthesis (Dutta et al., 2017; Singh et al., 2017). Silver nanoparticles have been used as effective antimicrobial agents for a long time in different fields from healthcare (Nedelcu et al., 2014) to food packaging (Fortunati, Peltzer, Armentano, Jiménez, & Kenny, 2013) and textile coating (Pannerselvam et al., 2017). The overuse of antibiotics for bacterial infections has led to the emergence of many drug-resistant bacteria. In the present time it has become very difficult to develop novel antibiotics. The development of new antibiotics is an expensive and time-consuming process requiring of research to investigate the efficacy of antimicrobial agents. On the other hand, the MDR bacteria associated illnesses are increasing at a high rate resulting in the deaths of millions of people globally (Betts, Hornsey, & La Ragione, 2018). Unlike antibiotics the metallic nanostructures especially silver nanoparticles

can help fight the bacteria without causing resistance (Natan & Banin, 2017). The market of AgNPs has been increasing rapidly over the past 15 years, and it has been estimated that about 500 tons of AgNPs are synthesized in a year through different methods to meet the demands of different industries (Yaqoob, Umar, & Ibrahim, 2020). Out of the total nanoparticles-based products in the market about 55.4% are the products incorporated with AgNPs (Vance et al., 2015). Owing to their growing market and extensive use in a variety of products, the investigation of their biocompatibility and mechanism of their action has become a matter of interest (Ferdous & Nemmar, 2020). One of the mechanisms by which AgNPs kill microorganisms specially bacteria is by releasing Ag^{+} ions which binds with the cell walls of the bacteria disturbing the permeability of membrane and also inactivate key enzymes leading to the death of microorganisms (Hamad, Khashan, Hadi, Polymers, & Materials, 2020).

The use of AgNPs as a potent antibacterial agent is well studied and documented however, the investigation of the effects of size and shape of AgNPs on their antibacterial properties is still the topic of debate. A number of studies have reported that certain shapes of AgNPs including prism shaped silver nanoparticles, nanotriangles and silver nanoplates shows increased antimicrobial activity (Raza et al., 2016). A study was performed to investigate the comparison of antibacterial potential of triangular AgNPs with truncated edges and spherical AgNPs which showed that the triangular AgNPs shows higher bactericidal activity than that of spherical and rod shaped AgNPs (Pal, Tak, Song, & microbiology, 2007). In another study Dong et al found that in the comparison of spherical AgNPs the triangular nanoparisms of silver having sharp edges showed enhanced antibacterial activity (Van Dong, Ha, Binh, & Kasbohm, 2012). Similarly, silver nanoplates were found to have high antibacterial activity than rod and sphere shaped AgNPs (Sadeghi et al., 2012). The facets of these anisotropic shaped AgNPs was found to be responsible for their increased

bactericidal potential due to the high atom-density (Morones et al., 2005). Contrary to the above-mentioned studies a number of other studies also showed high antibacterial activities of spherical AgNPs (Agnihotri, Mukherji, & Mukherji, 2014; Torres et al., 2013). The argument is that Spherical AgNPs are smaller in size and thus provide maximum site for reactivity due to its high surface to volume ratio thus leading to high bactericidal activities. Therefore, it is of great importance to study the influence of geometrical shape, size and surface charge of AgNPs on their antibacterial potential (Albanese, Tang, & Chan, 2012).

The major aim of the current study was to investigate the influence of shape and size of silver nanoparticles against MDR bacterial strains. The two different shapes of silver nanoparticles synthesized in the present study was silver nanocubes with sharp edges and spherical silver nanoparticles. A polyol chemical route was used for the synthesis of both AgNPs and AgNCs. The antibacterial properties of both the nanoparticles were investigated and compared using disk diffusion assay. The zones of inhibition for all the MDR bacterial strains were measured and analysed in the light of current literature.

Aims and Objectives

1. Synthesis of AgNPs, AgNCs and optimization of their protocols.
2. Characterization of AgNPs and AgNCs using UV-VIS, SEM, FTIR and XRD.
3. To evaluate and compare the biocompatibility of AgNPs and AgNCs on human cell lines.
4. To investigate the comparison of antibacterial potential of AgNPs and AgNCs against MDR strains of bacteria.
5. To evaluate the comparative antioxidant and hemolytic activities of both AgNPS and AgNCs.

Chapter 2

Literature Review

2.1. Nanotechnology

The word ‘’nano’’ is derived from a Greek word which means ‘’Dwarf’’. The field of Nanotechnology deals with the matter at the atomic, molecular or macromolecular scale within the size range of 1-100 nanometers. Another definition of nanotechnology is the science and engineering of synthesis, designing, and characterization of materials at the nanoscale (Kaur, Singh, & Kumar, 2012; Silva, 2004). Usually, the matter exhibits unusual properties as its size decreases and the surface area increases. Nanotechnology uses two main strategies for the synthesis of such nanomaterials such as top-down and bottom-up approaches. The top-down approach includes the reduction in the size of larger structures to smaller ones and vice versa (Abid et al., 2021). Taking the advantage of these approaches and the unique properties of materials at the nanoscale, scientists have designed devices by manipulating the shape and size of different materials with a vast range of applications in a number of fields such as electronics, medicine, computing, space science, etc (Subedi, 2014). The applications of nanotechnology specially in the field of medicine require the designing of materials and devices to interact with cells and tissues with high specificity, allowing the integration of nanotechnology and biological systems (Silva, 2004).

2.2. Nanoparticles

Nanoparticles are small particles that range in size from 1 to 100 nanometers. They are formed of carbon, metal, metal oxides, or organic substances (Hasan, 2015). In other words, a nanoparticle is a minute entity that functions as a single unit and serves as a bridge atomic structure and their bulk counterparts. Nanoparticles have resulted in increased performance compared to their bulk

counterparts owing to their unique and tunable physicochemical properties such as increased surface area, difference in melting point, thermal and electrical conductance, light absorption, and scattering properties (Jeevanandam, Barhoum, Chan, Dufresne, & Danquah, 2018). Nanoparticles vary in size, shape, and dimensions which are crucial for specific applications. Different shapes of nanoparticles can be produced such as spherical nanoparticles, nanorods, cylindrical, nanocubes, nanowires, hollow core-shell, spiral, and flat nanoparticles, etc (Khan, Saeed, & Khan, 2019) give more references and this reference is not original.

2.3. Classification of Nanoparticles

Nanoparticles are generally categorized into three groups:

i. Organic nanoparticles
ii. Inorganic nanoparticles
iii. Carbon-based nanoparticles

2.3.1. Organic Nanoparticles

Organic nanoparticles are solid nanomaterials made of organic compounds such as lipids, with a diameter from 10nm-1 μm (Drexler, 1981). They are also known as polymeric nanoparticles. These nanoparticles are comparatively less toxic, environment friendly and biodegradable. Organic nanoparticles include liposomes, dendrimers, micelles ferritin, etc. Some of the organic nanoparticles including liposomes and micelles are also called nanocapsules because of their hollow interior. Due to their biocompatibility, and the ability to entrap or adsorb drugs efficiently, they are an excellent choice for drug delivery (Ealia & Saravanakumar, 2017) give original reference. Organic nanoparticles are synthesized through different methods such as emulsification-based methods, nano-precipitation, and methods based on the drying process (R. Kumar & Lal, 2014).

2.3.2. Inorganic NPs

The inorganic nanoparticles generally include:

a) Metallic nanoparticles

b) Metal oxide nanoparticles

2.3.2.1. Metallic NPs

Metallic nanoparticles as the name indicates are synthesized from metal precursors through different methods. Some of the common metals that are used in the synthesis of metal-based nanoparticles include gold, silver, zinc, cobalt, copper, iron and lead, etc. Metal nanoparticles possess unique properties such as small size which gives these NPs a high surface area and high surface charge density (Salavati-Niasari, Davar, & Mir, 2008) give more references here. Both top-down and bottom-up approaches are used for the synthesis of these nanoparticles. One of the main challenges in the synthesis of metal-based nanoparticles through chemical methods is agglomeration which causes a decrease in the reactivity of nanoparticles. To avoid this agglomeration, metal nanoparticles are usually coated with stabilizers and polymers. Metal nanoparticles especially that of noble metals are of great interest for biomedical applications owing to their comparatively low cytotoxicity and enhanced thermal stability. The noble metal nanoparticles are used in a number of biomedical applications including bioimaging, biosensing, biomedical diagnosis, and drug delivery (Azharuddin et al., 2019) this is not the original reference. Metal nanoparticles also act as potent antimicrobial agents and have been used in food packaging, wound healing, and wastewater treatment (Sánchez-López et al., 2020).

2.3.2.2. Metal oxide NPs

Metal oxide NPs are synthesized in order to modify the properties of their respective metal nanoparticles. Oxides of different metals such as Fe_2O_3, ZnO, CuO, TiO_2 MgO and $Al2O_3$ are used

for the synthesis of such nanoparticles. Based on dimensions, metal oxides are categorized into zero, one, two and three-dimensional nanoparticles. They are highly stable and easy to synthesize and can be engineered into specific shapes and sizes for different biomedical applications (Nikolova & Chavali, 2020). They can be incorporated into hydrophilic and hydrophobic systems and can be effectively functionalized with different molecules because of their negative surface charge which makes them promising materials for various biomedical applications. They are used in a number of biomedical applications such as imaging, immunoassays, tissue repair, as antibacterial agents etc. Iron-oxide nanoparticles are used in different imaging modalities such as magnetic resonance imaging (MRI) as a contrast and has a potential to be used in drug and gene delivery. Furthermore, they are also an interesting candidates for magnetic hyperthermia for killing cancer cells (Campos, Pinto, Oliveira, Mattos, & Dutra, 2015).

2.3.2.3. Carbon-based NPs

Carbon-based nanoparticles include carbon nanotubes, fullerene. graphene and its derivatives such as nanodiamonds and carbon-based quantum dots. These nanoparticles have some unique properties such as fluorescence, increased photostability and tunable narrow emission spectrum which makes them interesting targets for using in bioimaging and diagnosis. Some other properties of carbon-based nanoparticles include high surface area, aqueous stability and interaction with cells and tissues which make them desirable. Moreover, their properties can be further optimized by functionalizing their surfaces with specific functional groups. The biofunctionalization of these nanoparticles reduce clearance and increase their retention time inside the body thus enhancing their efficacy. Carbon-based nanomaterials are used in a diverse range of biomedical applications such as bioimaging, biosensing, drug delivery and fluorescence labelling (Konios, Stylianakis, Stratakis, & Kymakis, 2014; Patel, Singh, & Kim, 2019). Few recent studies have also showed

their anticancer activities, as they are found to stimulate reactive oxygen species (ROS) when enter the cell which causes DNA damage and eventually kill the cell (Schinwald, Murphy, Jones, MacNee, & Donaldson, 2012).

2.3.3 Silver NPs

Silver nanoparticles (AgNPs) represent a class of nanoparticles that range in size from 1 to 100 nm. There has been increasing interest in studying silver nanoparticles, owing to their unique physical, and chemical properties (A. Kumar, Vemula, Ajayan, & John, 2008; Sun et al., 2016; Syafiuddin, Salim, Beng Hong Kueh, Hadibarata, & Nur, 2017). AgNPs possess interesting properties in terms of surface plasmon resonance, heat and electrical resistance due to which they are extensively investigated for different applications such as antimicrobial agents in wound dressing, wastewater treatment and food packaging, moreover they have also been used in biosensing and anticancer agents (Hembram et al., 2018; Kalantari et al., 2020; C. Zhang, Hu, Li, & Gajaraj, 2016).

The significant antimicrobial activities of AgNPs have increased the development of a variety of AgNPs based products such as food packaging, antiseptic sprays, and bandages etc. This antimicrobial activity of AgNPs depends upon the size, shape and different coatings of AgNPs. The shape of AgNPs is a very important property which can significantly influence their other physiochemical properties. Some of the most frequently used silver nanostructures are spherical nanoparticles, nanowires, nanorods, nanoplates and nanocubes (Rycenga et al., 2011). Therefore, it is important to synthesize AgNPs with controlled structural and physicochemical properties to enhance their biomedical applications (Wei et al., 2015). Furthermore, research have showed that

the surface charge is also an important consideration for AgNPs which can influence the interaction of AgNPs with living systems (Powers, Badireddy, Ryde, Seidler, & Slotkin, 2011).

Most of the optical properties of AgNPs are due to a phenomenon called Localized Surface Plasmon Resonance (LSPR) which is caused by their interaction with the light. The interaction of light with the surface of AgNPs causes coherent oscillation of free electrons. This coherent oscillation of free electrons causes radioactive decay with scattering of light, it can also result in nonradioactive decay which leads to the conversion of light energy into heat. Both of these mechanisms have been exploited to use in bioimaging and therapeutics (Austin, Mackey, Dreaden, & El-Sayed, 2014). The LSPR also depends upon the size and shape of AgNPs, moreover the dielectric environment and the ability of AgNPs to interact with each other. Thus the LSPR of AgNPs can be tuned in the near infra-red (NIR) region by controlling these parameters to use these nanoparticles in applications like photothermal therapies (Braun et al., 2014; T. Huang & Xu, 2010; Mahmoud & El-Sayed, 2013).

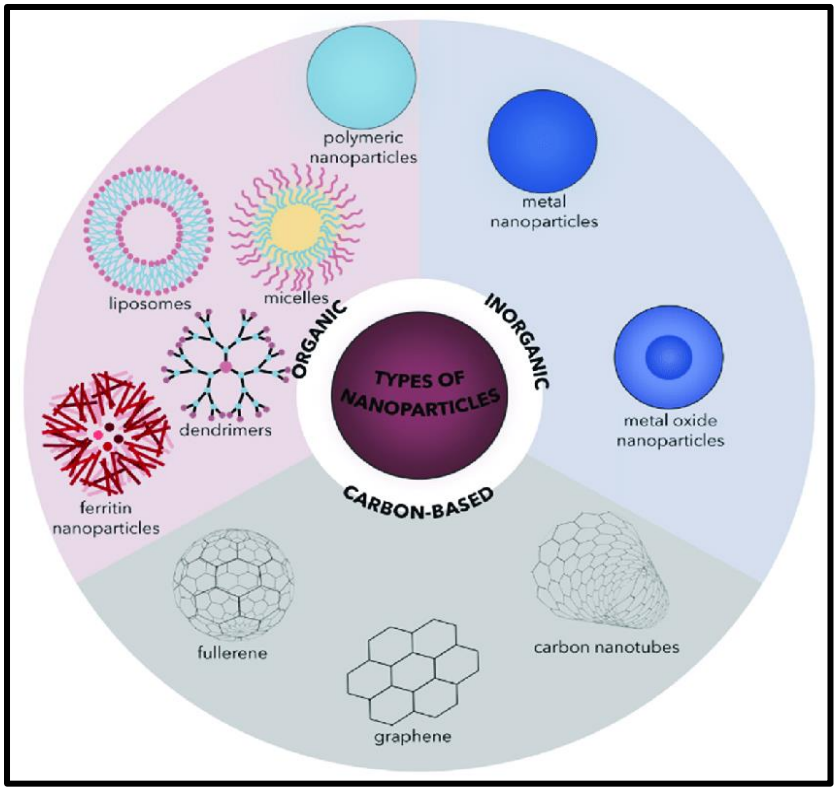

Figure 2. 1: Different types of NPs (Spirescu et al. 2021)

2.3.3.1 Methods for synthesis of silver nanoparticles

Currently there are different physical, chemical, and biological methods used to synthesize AgNPs with different sizes and shapes.

These methods are briefly discussed below:

2.3.3.1.1 Chemical methods

The chemical route of synthesizing AgNPs of different size and shapes is the most widely used method which provide an easy way to prepare AgNPs. The chemical synthesis of AgNPs generally require three components: 1) Metal precursor 2) reducing agent and 3) capping agent. The synthesis of AgNPs starts with the nucleation followed by the growth. These stages are very important as they determine the specific size and shape of AgNPs.

Spherical AgNPs having tunable size have been prepared through a simple polyol method. The injection rate of the precursor and the temperature of the reaction are the most important parameters in this method which facilitate the synthesis of monodispersed AgNPs with uniform size. To synthesize AgNPs of 17 ± 2 nm in size the reaction temperature is kept at 100 ◦C while the injection rate of the Ag precursor is kept at 2.5 ml/s (Kim, Jeong, & Moon, 2006).

To synthesize silver nanocubes the silver nitrate is used as metal precursor which is reduced in ethylene glycol acting both as solvent and reducing agent in the presence of polyvinylpyrrolidone (PVP) (Kim et al., 2006). The most critical parameter for synthesis of nanocubes with uniform size and shape is the molar ratio of PVP and silver nitrate. Moreover, the features of nanocubes can also be tuned by controlling other parameters such as temperature and stirring rate (Siekkinen, McLellan, Chen, & Xia, 2006).

Monodispersed AgNPs have also been synthesized in oleylamine-liquid paraffin system. The formation of AgNPS completes in three stages: growth of AgNPs, incubation, and Ostwald ripening. The chemicals used in this method include metal precursor silver nitrate, oleylamine and liquid paraffin. The paraffin has a high boiling point of 300 ◦C which makes it an interesting candidate to be used in reactions involving a range of temperatures to control the size and shape of AgNPs. Similar to the polyol synthesis, this method is also dependent on the ratio of oleylamine and silver nitrate (Chen et al., 2007).

2.3.3.1.2. Physical synthesis

The physical synthesis of AgNPs generally uses evaporation-condensation method in a tube furnace method at atmospheric pressure. This method has few drawbacks including large space of tube furnace, and increased energy consumption. Therefore, various other methods of physical synthesis of AgNPs have been developed.

AgNPs were successfully synthesized in powder form using thermal decomposition. A reaction of $AgNO_3$ with sodium oleate at a temperature of 290 ◦C results in a formation of Ag1+–oleate complex which was decomposed to prepare AgNPs. The average size of AgNPs obtained through this method was 9.5 nm indicating a narrow size distribution of AgNPs (Lee & Kang, 2004).

Another method of synthesizing AgNPS using physical routes was reported by Jung et al (Jung, Oh, Noh, Ji, & Kim, 2006). A small ceramic heater was used for the evaporation of source material to prepare AgNPs. It was found that the temperature of surface of the ceramic heater was directly related with geometric standard deviation and the total concentration of AgNPs. It was found to be a stable method of synthesizing AgNPs with no or less agglomeration as the temperature of the heater surface does not change with time.

An unconventional approach was developed by Siegel et al (Siegel et al., 2012) to physically prepare silver and gold nanoparticles having average diameter of 3.5 nm. A direct metal sputtering into the liquid medium was used for the synthesis of Ag and Au NPs. This method provides an alternate route to the laborious chemical synthesis approaches

The electrical discharge machining system was used by Tien et al (Tseng, Lee, Liao, Chen, & Lin, 2013) for the rapid and efficient synthesis of AgNPs without using any surfactant. This system consists of two electrodes (cathode and anode). The silver rods (99.9%) were used as electrodes dipped in deionized water. It was found that AgNPs were synthesized by arc discharge method in the absence of surfactant, moreover, both metallic AgNPs and ionic silver was present in the suspension. The consumption rate of silver rod was 100 mg/min which resulted in the synthesis of AgNPs with 10nm in size and silver ions at a concentration of about 11ppm to 19ppm. In conclusion the physical method uses physical energies including heat, ac current, and arc discharge to prepare AgNPs with narrow size distribution and large quantities in a single process.

2.3.3.1.3. Biological methods

The synthesis of nanoparticles by chemical methods is not economical and eco-friendly that is why there is an increasing need of developing new and safe methods. Unlike chemical synthesis which require a reducing agent and a stabilizer, in the biological method of AgNPs synthesis these chemicals come from a living source such as bacteria, plants, fungi, and algae.

Bacteria is one of the most commonly used living system for the synthesis of AgNPs. A fecile and fast biosynthesis of AgNPs was reported using Enterobacteria including *Klebsiella pneumonia*, *Escherichia coli*, and *Enterobacter cloacae* to reduce Ag^+ ions (Shahverdi, Minaeian, Shahverdi, Jamalifar, & Nohi, 2007). This method is a very fast route of synthesizing AgNPs as the nanoparticles are obtained within 5 minutes of the interaction of Ag^+ with bacterial cells. It was found that an enzyme called nitroductase catalaze the reduction of Ag^+ ions.

A simple and economical biological synthesis of AgNPs was reported in which *Shewanella oneidensis* was used (Suresh et al., 2010). This bacterium is a metal reducing strain which was seeded with $AgNO_3$ solution. Small monodispersed spherical AgNPs were obtained with average size of 4 nm. The AgNPs obtained through this method were found to be hydrophilic with larger surface area. This method requires less energy and is economical with high reproducibility as compared to chemical synthesis methods.

A fungus, *Fusarium oxysporum*, was used to synthesize AgNPs with size ranging from 5 to 50 nm (Ahmad et al., 2003). It was found that no agglomeration was observed even after 30 days of the synthesis, as the nanoparticles were stabilized with proteins for long term. Similarly, in another study, *Trichoderma viride*, was used for the extracellular synthesis of AgNPs in the presence of $AgNO_3$ solution. The AgNPs obtained through this method were found to have variable shapes as both spherical and rod shaped NPs were observed (Tran & Le, 2013).

2.3.3.2 Applications of AgNPs

Owing to their interesting physiochemical properties AgNPs have been widely used in a number of applications in different areas including healthcare, food industry, environment and biomedicine (Burdușel et al., 2018). AgNPs have attracted increased interest in the biomedicine field due to their unique properties and intrinsic toxicity towards microbial agents. They have been extensively used as a highly potent antimicrobial agent showing activity against bacteria (Franci et al., 2015), fungi (Panáček et al., 2009), and viruses (Galdiero et al., 2011). Besides their antimicrobial activities AgNPs have also been used as anti-inflammatory and anticancer agents. They have been found to induce apoptosis and sensitize cancer cells. Nano silver also possess synergistic activities with different anticancer agents thus enhancing the effect of therapeutic drugs. It has been found that AgNPs in combination with gemcitabine enhanced the cytotoxicity and induced apoptosis in A2780 cells (Yuan, Peng, & Gurunathan, 2017). AgNPs damages mitochondria and release reactive oxygen species leading to oxidative stress that causes DNA damage in different cell types (Hembram et al., 2018). The small size and sharp plasmon resonance make them an interesting candidate to be used as a contrast agents in different imaging modalities to study diseases like inflammation, and cancers, etc (Caro, Castillo, Klippstein, Pozo, & Zaderenko, 2010). The surface of AgNPs can be loaded with therapeutic agents and specific ligand molecules for targeted drug delivery in a number of diseases (Prasher et al., 2020). The functionalized AgNPs possesses interesting plasmonic properties such as localized surface plasmon resonance (LSPR) which can be exploited for biological sensing to detect biomarkers using immunoassays (Lee & Kang, 2004).

2.3.3.3. Antibiotic resistance and silver nanoparticles

The discovery of antibiotics is one of the most important discoveries in the field of medical sciences, but this therapeutic accomplishment is jeopardized by the emergence of resistance of

common pathogenic bacteria against these antibacterial agents. The antibiotic resistance is a major public health concerns which affects millions of people all over the world (Organization, 2014). The diseases caused by multi-drug resistant (MDR) bacteria have high mortality in comparison with those caused by bacteria that are susceptible. The economic burden of MDR infections in USA only is estimated to be 20 billion dollars (Sydnor & Perl, 2011). Although the antibiotic resistance is a naturally occurring phenomenon, but the inappropriate and excessive use of antibiotic agents have boosted this process. There are several ways by which bacteria acquire resistance to antibiotics. These include bacteria making their cell wall impermeable for the drugs to pass, the overexpression of efflux pumps by bacteria to expel out multiple drugs that have entered inside. Bacteria also induce mutations in its DNA specifically of the proteins that are target of a particular drug, thus decreasing the susceptibility. Moreover, bacteria also release certain enzymes that degrade drugs prior their interaction with targets (Blair, Webber, Baylay, Ogbolu, & Piddock, 2015).

AgNPs possesses intrinsic antibacterial properties acting as alternate antibacterial due to their high surface area and other surface properties. Moreover, AgNPs have the potential to overcome resistance of major antibiotic resistant bacteria as they have shown bactericidal activity to a number of multi-drug resistant bacterial strains (Lara, Ayala-Núnez, Ixtepan Turrent, & Rodríguez Padilla, 2010; Rai, Deshmukh, Ingle, & Gade, 2012; X.-F. Zhang, Liu, Shen, & Gurunathan, 2016). The antibacterial effects of AgNPS have been extensively studied in a variety of applications yet the mechanism by which the silver kill bacteria is not completely understood. The bactericidal effects of AgNPs can be mainly classified into four main mechanisms: (1) The attachment of AgNPs on the cell membrane and induction of structural changes in the bacterial membrane (Feng et al., 2000) (2) Penetration of AgNPs inside the bacteria and disabling the

bacterial intracellular structures such as ribosomes, mitochondria, and vacuoles. Moreover, the silver ions also interact with the thiols groups of some important bacterial enzymes thus disabling these enzyme (Gupta, Maynes, & Silver, 1998; Matsumura, Yoshikata, Kunisaki, & Tsuchido, 2003). (3) The formation of free radicals and Reactive Oxygen Species (ROS) by AgNPs which causes cellular toxicity, and (4) Signalling pathways modulation by AgNPs. Some studies propose that the loss of ability of DNA to replicate after the treatment with silver ions is responsible for the potent bactericidal effects of AgNPs (Dakal, Kumar, Majumdar, & Yadav, 2016). Evidence shows that AgNPs also help in inhibition of microbes by modulating immune responses of human cells (Tian et al., 2007).

AgNPs have been effectively used as bactericidal agents different MDR gram positive and gram negative bacteria (Lara et al., 2010). The AgNPs against multi-drug-resistant (MDR) bacteria have been used both alone and in combination with other anti-infective agents (Namasivayam, Ganesh, & Avimanyu, 2011; Nanda & Saravanan, 2009). Both AgNPs and antibiotics are used in a combination for enhanced antibacterial activities. AgNPs in combination with different antibiotic agents shows synergistic effects against different Gram-positive and Gram-negative bacterial strains (De Souza, Mehta, & Leavitt, 2006; Fayaz et al., 2010; Siegel et al., 2012). Rajawat and Qureshi investigated the synergistic effects of AgNPs in combination with antibiotics against a Gram-negative bacterium, *S. typhi,* and showed that the cell wall lysis activity of ampicillin was enhanced in the presence of AgNPs (Rajawat & Qureshi, 2012).

Chapter 3

Methodology

The research is mainly focused on synthesis, characterization and biomedical applications of AgNPs and AgNCs. The research was carried out in Cancer Biology Lab, Atta-ur-Rahman School of Applied Biosciences (ASAB), National University of Science and Technology (NUST), Islamabad. The materials and methods used in the study are given below:

3.1. Synthesis of AgNPs

For the synthesis of spherical silver nanoparticles, the protocol of Vazquez-Muñoz et al (Vazquez-Muñoz, Arellano-Jimenez, Lopez, & Lopez-Ribot, 2019) was followed with slight modifications. All the stock solutions were prepared in distilled water. In the first step a solution of $AgNO_3$ (30 ml, 15mM) was taken in a 200 ml beaker and heated on a stirring hot plate at a temperature of 80 °C with vigorous stirring. After the first step PVP solution (5 ml, 30mM) was added to the $AgNO_3$ solution at the same stirring rate. Immediately after the PVP addition 300 µl of $NaBH_4$ was added drop by drop. The colorless solution turned into brownish or greyish color. The reaction was further proceeded for another 10 min at the same temperature and stirring rate. After 10 min the reaction was quenched by transferring the synthesized AgNPs to a falcon tube and letting it cool at room temperature. The synthesized AgNPs were stored at 4 °C. A step by step process of synthesis of AgNPs is depicted in Fig 3.1.

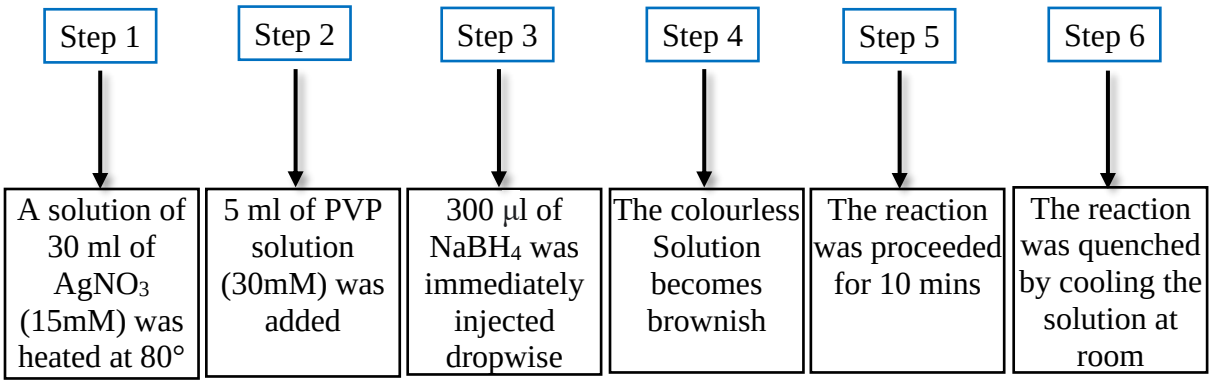

Figure 3. 1: Stepwise process of synthesis of spherical silver nanoparticles (AgNPs)

3.2. Synthesis of Silver nanocubes

All the glassware and magnetic stir bars were cleaned with aqua regia and distilled water a day before synthesis of silver nanocubes. The cleaned glassware was allowed to dry overnight in incubator and taken out just before use. For the synthesis of silver nanocubes 4 reactions was performed simultaneously with varying concentration of Na_2S. The Na_2S concentration was different in each vial to compare the results.

For nanocubes all the stock solutions were prepared in EG. To synthesize silver nanocubes, 35 ml of anhydrous ethylene glycol was taken in a round bottom flask (100 ml) and a clean egg-shaped magnetic stir bar was added to the flask. The flask was immersed in silicone oil bath using a custom-made holder on top of hotplate. The oil bath was set at a temperature of 155 °C. The ethylene glycol was heated for 1 hour with continues stirring at 500-600 rpm. After a uniform temperature have been achieved, a solution of 400 mg PVP dissolved in 5 ml of EG was added using a pipette. After five minutes, 400 µl of EG solution of Na_2S (3mM) was injected to the flask. After this the reaction was proceeded for another 5-6 minutes and then 2.5 ml of EG solution of $AgNO_3$ (282mM) was added dropwise. The addition of $AgNO_3$ turned the solution black, then the

color slowly changed into yellowish, followed by opaque ochre green with clear silver plating on the walls of the flask. At this stage the reaction was quenched by placing the flask in ice bath. The nanocubes were tuned by changing the concentration of sulphide ions and temperature. A step by step process of synthesis of AgNCs is depicted in Fig 3.2.

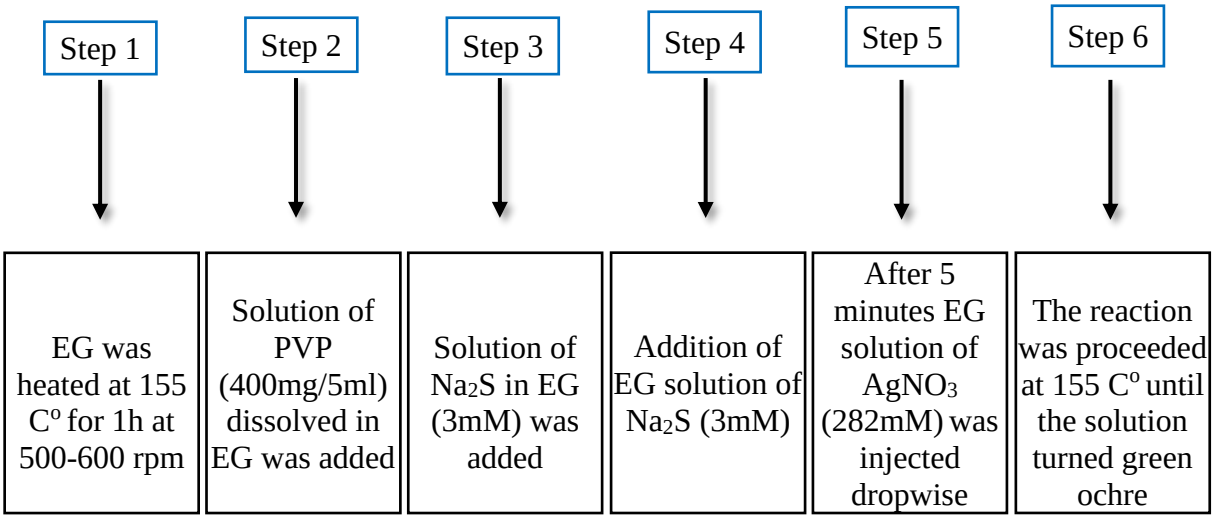

Figure 3. 2: Stepwise process of synthesis of spherical silver nanocubes (AgNCs)

3.2.1. Purification of silver nanocubes

The obtained solution of nanocubes was diluted by adding acetone (1:1 by volume) and then centrifugated at 12000 rpm for 25 minutes. The supernatant was discarded, and the pallet was dissolved in ethanol through sonication. This washing step was repeated a total of 6 times to completely wash off the excess and unreacted EG and PVP. The silver nanocubes were further washed three times with deionized water and finally stored in ultrapure water.

3.3. Characterization

Before starting biomedical applications the AgNPs and AgNCs were subjected to characterization using UV/VIS spectroscopy, SEM, XRD and FTIR.

3.3.1. UV/VIS Spectroscopy

The freshly prepared AgNPS and AgNCs were primarily characterized with UV/Vis spectroscopy to measure their optical properties. The UV/Vis spectrophotometer model AE-S90-2D (A & E Lab, UK) was used to obtain the absorption peak for both AgNPs and AgNCs. Both the nanoparticles have a charecteristic absorption spectra and absorb light at a specific wavelength.The wavelength range was set from 300 to 800 nm. For the UV/VIS spectroscopy the cuvette was filled with sample (AgNPs & AgNCs) and then loaded in the spectrophotometer. Distilled water was used as used as blank for baseline correction. The spectral data obtained was analyzed with UV/VIS spectral analysis software and plotted on graph.

3.3.2. Scanning Electron Microscopy (SEM)

After obtaining the absorption spectra of both the nanoparticles, the Scanning Electron Microscopy was performed using SEM (Jeol JSM-6490LA, Japan). The SEM was used to evaluate the size, surface morphology and chemical composition of both AgNPs and AgNCs. The SEM scans the surface of the sample using high energy electrons and give information about the composition and morphology.

Sample Preparation

For preparation of sample for the SEM the AgNPs and AgNCs were first diluted in distilled water and then subjected to bath sonication for 1h at 37^{o}C. The sonication of nanoparticles is important as it causes the deagglomeration and even distribution of the nanoparticles and thus improve the resolution . After this a glass slide of about 1 cm^2 was cut and a drop of sonicated sample was placed on it using micropipette. The drop-casted sample was then allowed to dry under lamp for 30 minutes. The dried sample on glass slide was then subjected to gold-palladium sputter coating

using sputtering coating machine (Jeol JFC-1500, Japan) to make the surface of NPs conductive for electron microscopy. The voltage was set to 10KV and the magnification was set to 80000X.

3.3.3. Fourier Transform Infrared Spectroscopy (FTIR)

For the identification of various functional groups attached on the surface of AgNPs and AgNCs FTIR was performed using Perkin-Elmer Spectrum-100-Spectrophotometer (United States). The FTIR analyze the sample by measuring its absorption of light in the Infra-red (IR) region. The Scanning Wavelength was set in the range of 400-4000 cm^{-1} while the spectral resolution was set to 4 cm^{-1}.

Sample preparation

The sample nanoparticles (AgNPs & AgNCs) were used in dried form. The dried nanoparticles were mixed with KBr and then pressed to form a pellet of KBr and nanoparticles using a hydraulic press. The KBr acts as a carrier for the nanoparticles. It is transparent in the Infra-red region and thus no interference in the absorption is caused by it. The pellet was the subjected to FTIR for which the wavelength was set in the range of 400-4000 cm^{-1}. The obtained spectra was plotted taking Wavenumber (cm^{-1}) on X-axis and percentage transmittance on Y-axis. The FTIR spectral peaks were then manually compared with standard functional group charts.

3.3.4. X-ray Diffraction Analysis (XRD)

The X-ray Difraction is one of the most common technique used to characterize nanoparticles. It provides information about the crystalline structure and grain size of nanoparticles. The XRD analysis for AgNPs and AgNCs was performed using D8 ADVANCE with DAVINCI design (BRUKER, Germany).

Sample preparation

For the preparation of sample for XRD analysis the glass slides was cut and the sample (AgNPs & AgNCs) was drop-casted on it. The sample was then allowed to dry. This step was performed 3-4 times to obtain a thicker layer of nanoparticles on the glass slide. The dried drop-casted sample was then subjected to XRD and the spectra was recorded. The wavelength of X-ray radiation was set to $\lambda = 1.58$ Å.

3.4. Cytotoxicity of AgNPs and AgNCs

The cyctotoxicity analysis of AgNPs and AgNCs was carried out using MTT 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide) assay. The MTT assay was performed to evaluate the cytotoxicity of both the nanoparticles on MCF-7 and MDA-MB-231 cell lines.

3.4.1. Cell culturing

The MCF-7 and MDA-MB-231 cells were cultured in RPMI-1640 (Sigma-Aldrich, USA) supplemented with 10% Fetal Bovine Serum (FBS) (Sigma Aldrich) and 1% streptomycin/penicillin. The cells were incubated at 37 ^{o}C and 5% CO_2 after the addition of media.

3.4.2. Sub-culturing

Both the cell lines were grown in 250 cm^2 cell culture flasks and ttheir confluency was measured under microscope. The cells were splitted at 80% confluency. The splitting started with removing the media and washin the cells with 0.01M autoclaved Phosphate Buffer Saline (PBS) (Sigma Aldrich, USA). After the washing the PBS was removed and 2 ml of Trypsin EDTA solution was added to the cells to detach them from the walls of the flask as both of the cells lines were adherent. This was followed by incubation of the cells at 37 ^{o}C for 5 minutes. The detachment of cells were confirmed by checking the flasks under microscope. The cells were then entrifuged at 13000 rpm

for 3-4 minutes. The supernatant was discarded and the pellet of cells was resuspended in RPMI-1640 at 200 rpm for 2 minutes.The cells were then seeded in 250 cm^2 flask at 1 x 105 cells/ml density and incubated for at 37 ºC and 5% CO_2.

3.4.3. MTT Assay

The MTT assay is a cell viability assay that is used to investigate the biocompatibility of nanoparticles and other drugs on different cell lines. This assay measure the mitochondrial integrity of the cells. The live cells maintain their mitochondrial integrity and break the MTT down to formazan dye with the help of a mitochondrial enzyme succinate dehydrogenase. Before the assay a solution of MTT in PBS (Sigma Aldrich, USA) was made (5 mg/ml) which was then filtered, sterilized and stored at 4ºC. The assay was performed in 96 well plate with flat bottom wells. Each well was filled with 1.92 x 10^4 cells, and the plate was then incubated for 24 hours.After then incubation the different concentrations of both AgNPs and AgNCs were added to the wells and further incubated at 37 ºC for 24 – 72 hours. After the completion of this step, 15 µl of the prepared solution of MTT dye (5mg/ml) was added in each well and then incubated again for 3 hours at 37 ºC. Followed by the incubation, 150 µl of DMSO was added to the wells as a solubilizing agent after the MTT was removed without disturbing the formazon crystals. The plate was the nincubated at room temperature for few minutes and the content of each well was mixed by pioetting up and down to dissolve the purple crystals. The plate was wrapped completely in aluminium foil to prevent it from light. The absorbance of recorded at 550 nm using spectrophotometer. The assay was performed in three replicates and the average value was calculated. The statistical analysis of the obtained results was done using statistical software Graphad Prism.

3.4.4. Calculation of % survival and inhibition

For the evaluation of percentage survival and inhibition the following formula was used:

$$\textbf{Percent Cell Survival} = \frac{(\text{Sample absorbance} - \text{Blank absorbance})}{(\text{Control absorbance} - \text{Blamk absorbance})}\ X\ 100$$

% Cell Inhibition = 100 - % cell survival

3.5 Haemolysis Assay

The Haemolysis assay was performed to evaluate the biocompatibility of AgNPs and AgNCs on red blood cells (RBC's). The assay was started by taking fresh blood (3ml) in vacutainer supplemented with ethylene diamine tetra acetic acid (EDTA) to avoid blood coagulation. The blood sample was centrifuged at 6000 rpm for 10 min at room temperature to collect RBCs only. The collected RBCs were washed 5 times with 3ml of isotonic PBS and then suspended in 20ml of ice-cold PBS. The different concentrations (25, 50, 75. 100, 125 ug/ml) of both AgNPs and AgNCs were prepared concentrations for evaluating their haemolytic activity. The dilutions of NPs (20ul) were incubated with diluted blood cells suspension (180ul) at 37°C for 30mins with constant agitation. PBS was taken as negative control while 0.1% Triton X-100 was taken as the positive control. Followed by centrifugation of samples at 1500rpm, the supernatant received was diluted with isotonic PBS in 9:1 in the 96-well microtiter plate. The optical density of microtiter plate was measured at 550nm. The schematic illustration of haemolysis assay is given in Fig 3.3. The percentage haemolysis was calculated through the following formula:

$$\textbf{Percent Haemolysis} = \frac{(\text{Sample OD} - \text{Negative control OD})}{(\text{Positive control OD} - \text{Negative control OD})}\ X\ 100$$

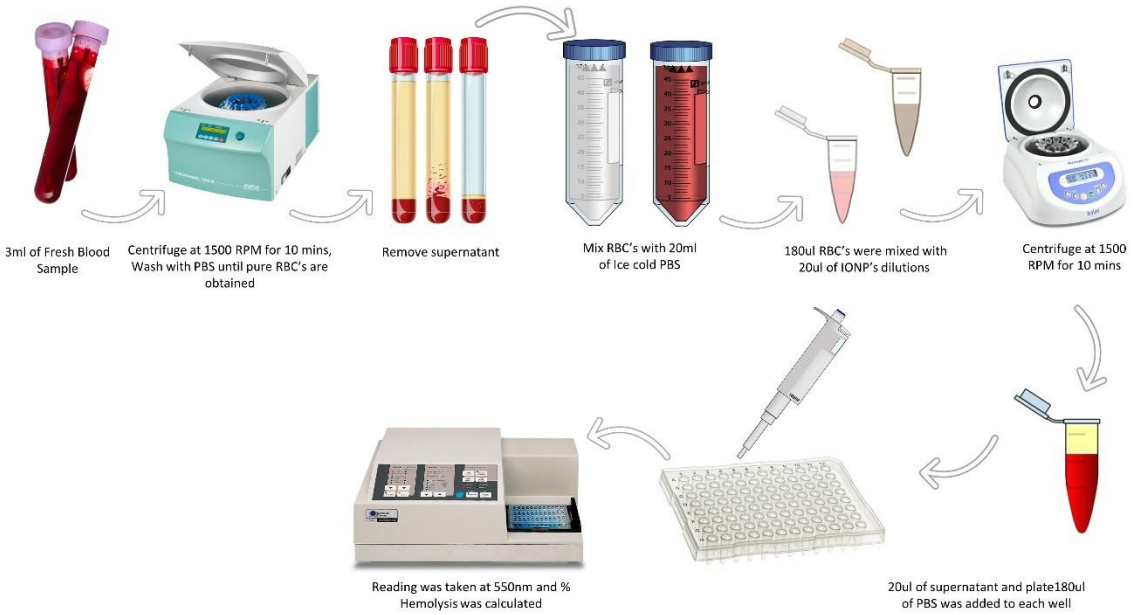

Figure 3. 3: Schematic illustration of Haemolysis assay

3.6. Antioxidant activity

The DPPH assay is a free-radical scavenging assay which is commonly used to determine the antioxidant activity of variety of compounds. The assay is based on the reduction of DPPH solution in methanol in the presence of an antioxidant. The DPPH solution in methanol shows absorption at 517nm. The absorption decreases as the DPPH gets reduced and its color changes from deep violet to yellowish color. The degree of reduction is directly related to the decolorization of alcoholic solution of DPPH. This method was used to determine the antioxidant activity of spherical silver nanoparticles and silver nanocubes in comparison with ascorbic acid which is a known antioxidant.

3.6.1. DPPH Assay

To perform DPPH assay 3.9mg of fresh DPPH (Sigma Aldrich, USA) was dissolved in 100 ml of methanol in a flask. The flask was covered in aluminium foil and stored in dark till use to prevent it from light. The same concentrations (100, 200, 400, 600, 800, 1000 ug/ml) of AgNPs, AgNCs and ascorbic acid were made in labelled eppendorf tubes. Similarly, DPPH solution (1ml) was also taken in eppendorf tubes. After that 1 ml of each concentration of the sample (AgNPs, AgNCs) and standard (Ascorbic acid) was taken and mixed with 1ml of DPPH solution in another set of eppendorf tubes. All the tubes were covered in aluminium foil. The tubes were then vortexed vigorously to mix the solutions and then incubated at room temperature for 30 minutes. After the incubation the absorption was measured at 517 nm. The methanol was used as a blank in this assay. The formula used to find the percentage scavenging activity of both the samples and ascorbic acid is given below:

$$\%\ \textbf{Scavenging Activity} = \frac{(\text{Control absorbance} - \text{Test sample absorbance}}{\text{Control absorbance}}\ \text{X}\ 100$$

3.7. Antibacterial activity of AgNPs and AgNCs

To evaluate and compare the antibacterial potential of both AgNPs and AgNCs, the antibacterial disk diffusion assay was performed.

3.7.1 Bacterial Strains

This study included a total of 4 MDR bacterial strains (Methicillin Resistant Staphylococcus aureus (MRSA), Ecoli, and Vancomycin Resistant Enterococcus).

3.7.2. Antibiotic Resistance Profile of MDR strains

An antibiotic susceptibility test was performed for all the strains with 12 different antibiotics. Kirby-Bauer antibiotic assay on Muller-Hinton Agar (MHA) was used for the determination of antibiotic resistance profiles of all the strains. The strains were streaked from glycerol stocks which were stored at -80°C onto nutrient agar plates followed by incubation at 37°C for 24 hours. After that the revived strains were inoculated on MHA plates by taking colonies from agar plates and suspending it in saline water. After vertexing the bacterial suspension was compared with 0.5 McFarland standard. The antibiotic discs (Oxoid, USA) were placed on MHA plates upon which the bacterial isolates were previously spread plated. The plates were incubated at 37^{o}C for 24 hours and then zones of inhibition measured.

3.7.3. Preparation of Culture Media

The Nutrient Agar (NA) was prepared for reviving the glycerol stored bacterial culture and the MHA was prepared for the Antibacterial susceptibility testing. For the preparation of NA media 28g of NA was dissolved in 1000ml of distilled water while for MHA 38g of MHA was dissolved in 1000 ml of distilled water. Both the media were sterilized by autoclaving at 121°C and 15psi. The autoclaved media were then poured into sterilized petri plates (20ml/plate) in Laminar flow hood and allowed to solidify at room temperature. All the petri plates were sealed and incubated at 37°C for 24h for sterility testing.

3.7.4. Preparation of Disks

Before the preparation of disks four different concentrations (10, 20, 40, and 60 μg/ml) of both AgNPs and AgNCs were made. The disks were prepared by cutting filter paper and then impregnating it with nanoparticles. The same was done for antibiotic disks. The disc with only distilled water was used as negative control while the standard antibiotic disk was used as positive control.

3.7.5. Disk Diffusion Assay

Before performing disk diffusion assay the subcultures of all the bacterial strains were prepared the previous day on nutrient agar plates. The disk diffusion assay was started by picking 4 -5 isolated colonies using sterile loop and dissolving them in 1.5 ml of autoclaved saline water. This suspension was vortexed, and the turbidity was adjusted to 0.5 Mcfarland standard which corresponds to 1.5×10^8 CFU/ml. A sterile swab was dipped in bacterial suspension and MHA plates were inoculated by streaking the swab on MHA surface to evenly distribute the bacteria. The plates were then allowed to dry for about 5 minutes. The antibiotic, nanoparticles and antibiotics + nanoparticles impregnated disks were placed on the media using forceps. The plates were then covered and placed inverted in incubator at 37 ^{o}C for 18-24 hours. After 24 hours the inhibition zones were calculated. The assay was performed in triplicates and the antibacterial potential of the nanoparticles and antibiotics was determined by taking the average sizes of inhibition zones in mm.

Chapter 4

RESULTS

4.1. Characterization

4.1.1. Visual detection and Confirmation of AgNPs and AgNCs

For the synthesis of AgNPs the $AgNO_3$ solution was taken in a beaker on a hot plate, after which the PVP solution was added to the beaker maintaining vigorous stirring. At the end $NaBH_4$ was added dropwise and a color change was observed. The colorless solution was converted to a brownish or yellowish solution as shown in Fig 4.1(b). The color of the solution depends on the concentration of $NaBH_4$. The appearance of yellowish color shows the formation of spherical nanoparticles. The AgNCs were synthesized using a modified polyol synthesis. The synthesis starts with heating EG at 155 °C with continuous stirring, after which a solution of PVP in EG was added followed by the addition of trace amount of Na_2S and then addition of EG solution of $AgNO_3$. The dropwise addition of $AgNO_3$ turned the solution to yellowish color followed by opaque ochre green color with the appearance of silver platting on the walls of the flask. The green ochre color as shown in Fig 4.1(a) confirms the synthesis of silver nanocubes with sharp edges. The reaction was quenched at this stage as further proceeding the reaction results in the rounding of corners of the cubes.

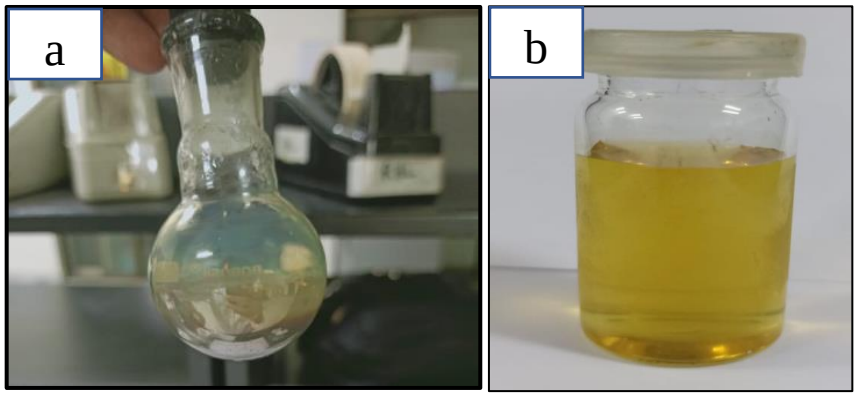

Figure 4. 1: Confirmation of color of as synthesized (a) AgNCs and (b) AgNPs

4.1.2. Optical Properties of AgNPs and AgNCs

To confirm the synthesis and determine the optical properties of both AgNPs and AgNCs UV/VIs Spectroscopy was used. Every type of nanoparticles possess a characteristic absorption spectra i,e absorb light at a specific wavelength, which confirms their synthesis. The spectral data obtained was compared with the reported spectra of AgNPs and AgNCs from literature. The AgNPs showed maximum absorbance at wavelength $\lambda = 401$ nm which is a typical wavelength for spherical silver nanoparticles. The UV-Vis spectrum of AgNCs showed two major peaks one at 450 nm and other at 320 nm which are characteristics of cube shaped geometry. The increase in the diameter of the AgNCs leads to shifting towards longer wavelengths. The optical properties of AgNCs are very different from that of AgNPs of the same size. Owing to their cubical geometry and sharp corners and edges the AgNCs possesses additional plasmonic modes occurring at distinct wavelengths in the UV-VIS spectrum. The UV/Vis spectra of AgNPs and AgNCs is given in Fig 4.2.

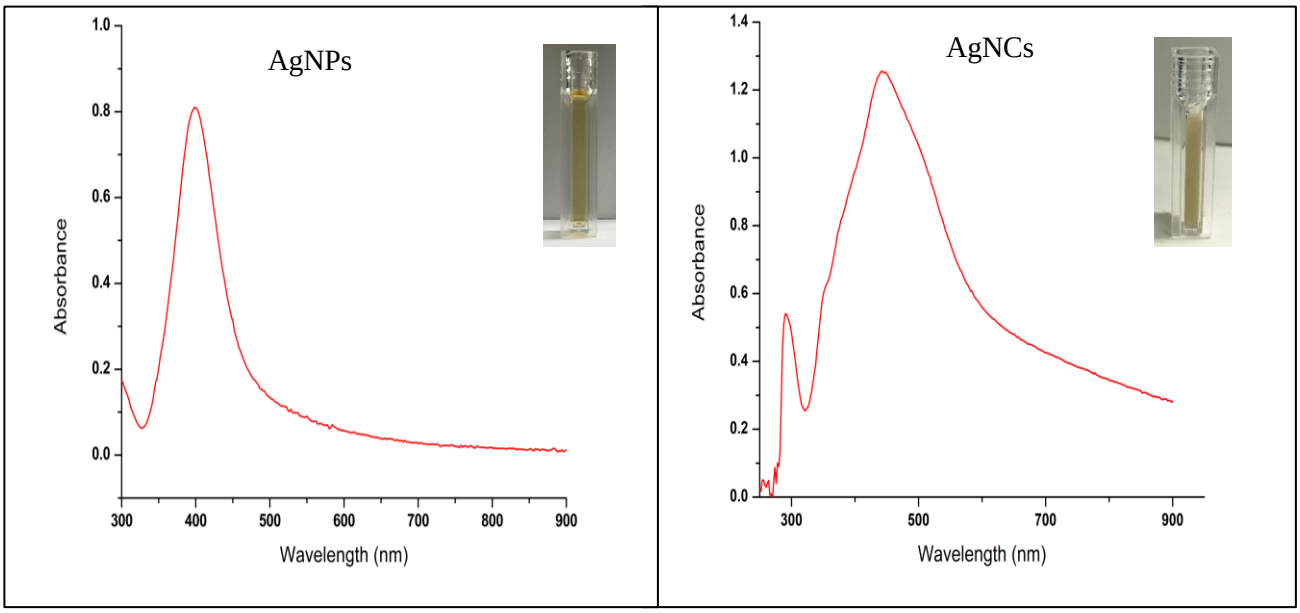

Figure 4. 2: UV-Vis spectra of AgNPs and AgNCs

4.1.2. Scanning Electron Microscopy

The confirmation of morphology and size of AgNPs and AgNCs was done using Scanning Electron Microscopy. The SEM images of AgNPs confirmed the formation of nanoparticles of spherical shape. Moreover, the size of AgNPs was found to be in nanometer range. The sonication prior to the SEM analysis resulted in increased resolution and even distribution of AgNPs which is evident from the SEM images. Similarly, the SEM images of AgNCs confirm the formation of monodispersed silver nanocubes with sharp edges and corners. The Fig 4.3 shows Scanning electron micrograph of AgNPs at 30,000X and 50,000X, While the Fig 4.4 represent the Scanning electron micrograph of AgNCs at 50,000x, 100,000 and 200,000X.

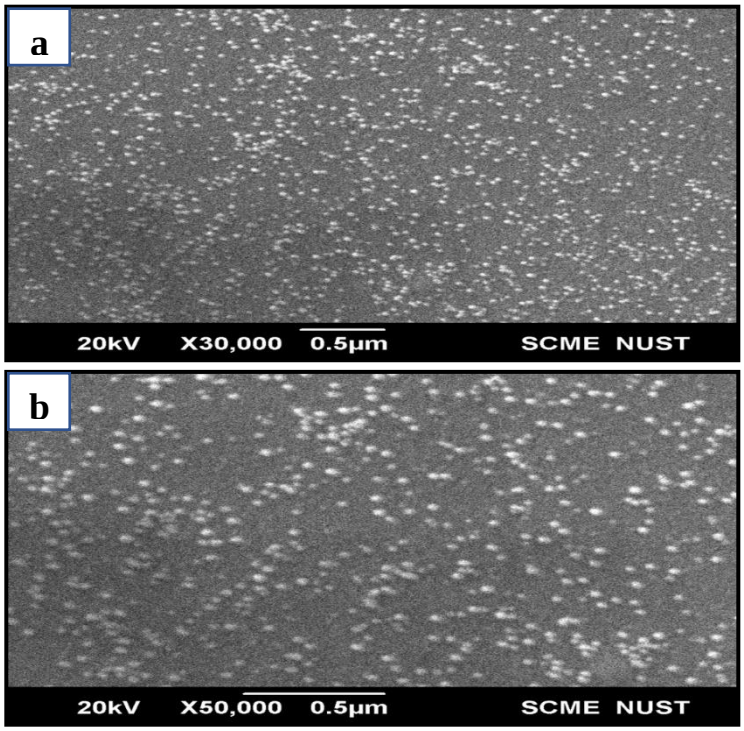

Figure 4. 3: Scanning Electron Microscope (SEM) image of Spherical silver nanoparticles (AgNPs) at (a) 30,000X and (b) 50,000X

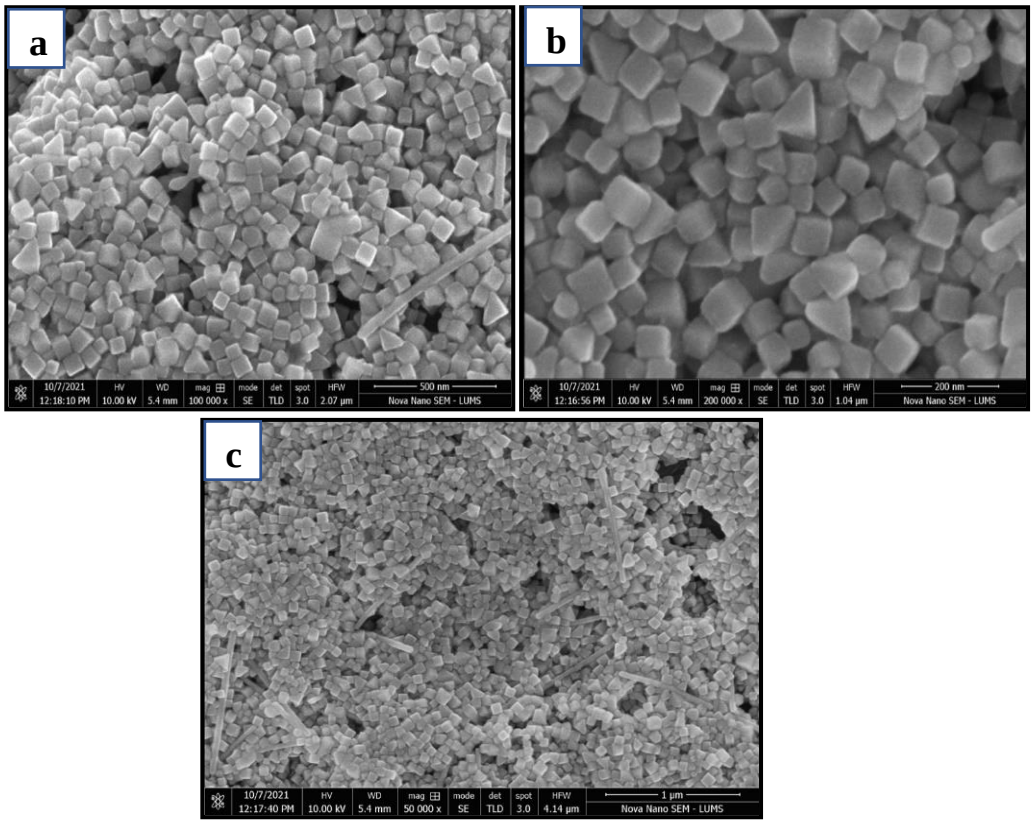

Figure 4. 4: Scanning Electron Microscope (SEM) image of silver nanocubes (AgNCs) at (a) 50,000X (b) 100,000X and (c) 200,000X

4.1.3. XRD Analysis

The AgNPs and AgNCs were also characterized using XRD to determine the crystalline structure of as synthesized nanoparticles. The Fig 4.5 and 4.6 shows the typical XRD pattern of AgNPs and AgNCs respectively. The crystallinity of nanoparticles is evident from sharp XRD peaks. For XRD the diffracted intensities were recorded from 20° to 80°. The XRD results of as prepared AgNCs showed three strong Bragg reflections at 38.12°., 44.2°, 64.7°, and 77.6° corresponding to (111), (200), and (220) planes of Ag crystals respectively. The peak positions are typical of face-centered silver nanocubes. It has been observed that PVP selectively binds to (100) facet of Ag seed allowing the (111) facet to grow longitudinally which is evident from the XRD spectra of AgNCs.

The XRD of AgNPs showed peaks at 38°, 44°, 64°, and 74° which corresponds to (111), (200), (220), (311) planes of AgNPs.

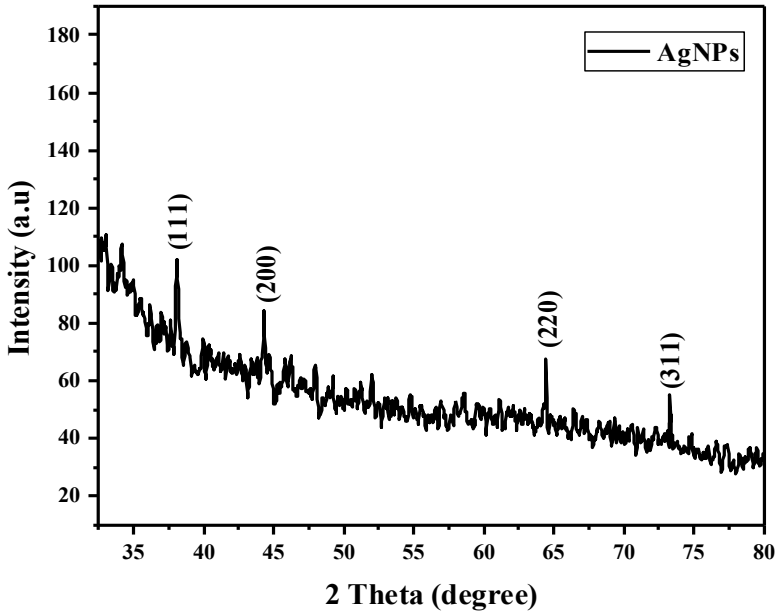

Figure 4. 5: X-ray Diffraction (XRD) pattern of silver nanoparticles (AgNPs)

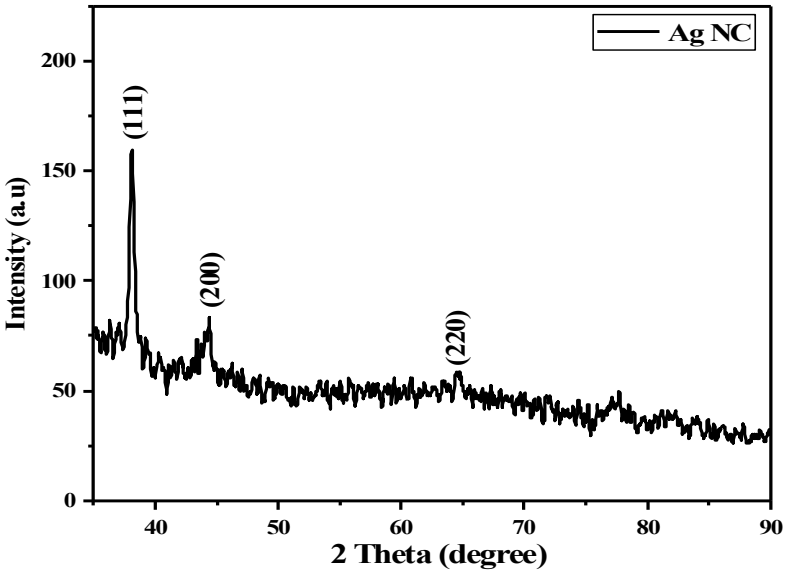

Figure 4. 6: X-ray Diffraction (XRD) pattern of Silver nanocubes (AgNCs)

4.1.4. FT-IR Analysis

The AgNPs and AgNCs were subjected to FT-IR analysis for the determination of different functional groups attached on the nanoparticles. Every functional group has its own characteristics peak. The FT-IR spectrum of AgNPs showed strong peaks at 3435, 2075, 1634 and 714 cm^{-1}. The peak at position 3426 cm^{-1} is assigned to N-H stretching of primary amines. The sharp peak at 1634 cm^{-1} corresponds to stretching vibrations of carbonyl group C=O. The peak at 706 cm^{-1} corresponds to stretching vibration of pyrrolidone ring. The FTIR peak at position 2070 cm^{-1} corresponds to C–H asymmetric stretches of alkene group. Similarly, the AgNCs also showed similar peaks at position 3440, 2072, 1645 and 710 corresponding to N-H, C=O, and stretching of pyrrolidone ring respectively. The Fig 4.7 and 4.8 shows the FTIR spectra of AgNPs and AgNCs respectively.

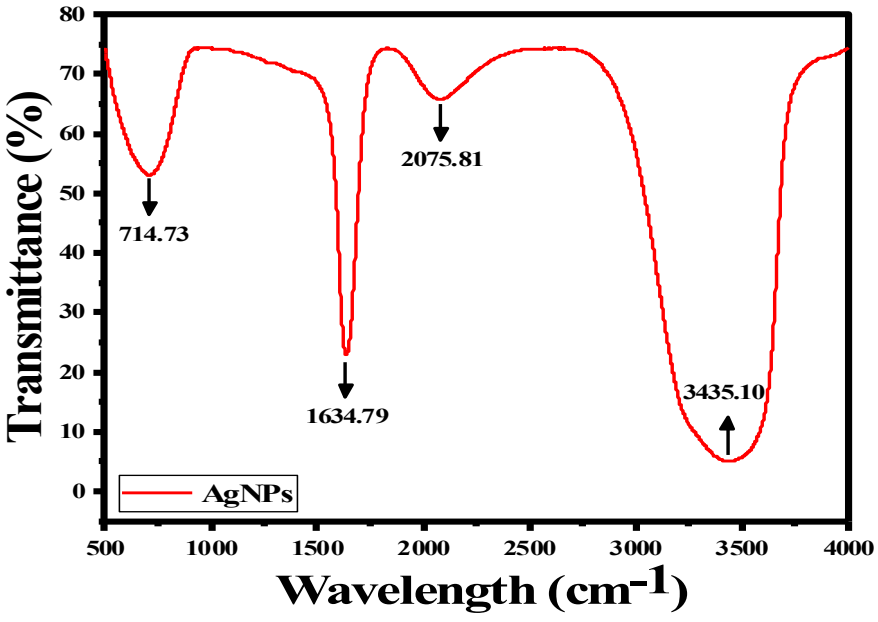

Figure 4. 7: FTIR spectra of silver nanoparticles (AgNPs)

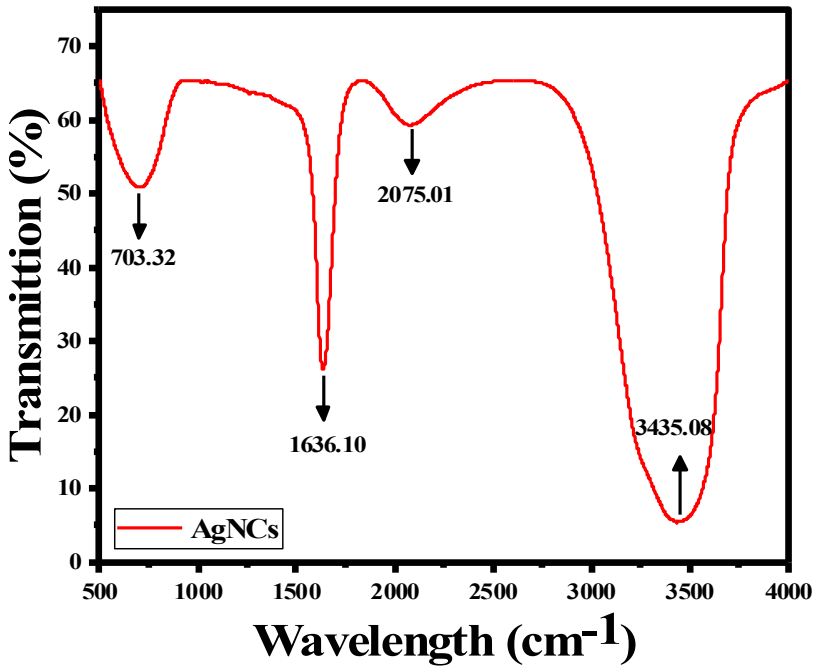

Figure 4. 8: FTIR spectra of Silver nanocubes (AgNCs)

4.1.5. Cytotoxicity Analysis of AgNPs and AgNCs

The biocompatibility of AgNPs and AgNCs was evaluated using MTT assay on Human breast epithelial estrogen receptor positive cell lines (MCF-7) and triple negative breast cancer cells (MDA-MB-231). Both of the cell lines were exposed to different concentration of AgNPs and AgNCs (0.625, 1.25, 2.5, 5, 10 µg/ml) The results showed a dose dependant cytotoxicity of AgNPs and AgNCs on both the cell lines. The Increase in the concentration of nanoparticles was found to be inversely related to biocompatibility. The comparison of biocompatibility of AgNPs and AgNCs on MDA-MB cell lines revealed that the AgNPs are less biocompatible than that of AgNCs, as the AgNPs resulted in less percentage viability of MDA-MB-231 cell lines. At the same concentration (0.625 µg/ml) the AgNPs exposed MDA-MB cell lines showed 58.7% viability

while AgNC treated showed 65.23%. Similarly, the biocompatibility of AgNCs in MCF-7 cell lines were also higher than that of AgNPs. At the same concentration (0.625 µh/ml) the AgNPs treated MCF-7 cells showed 61.9% while AgNCs treated cells showed 67.5% viability. The results of MTT assay on MDA-MB and MCF-7 are given in Figure 4.9 and 4.10 respectively.

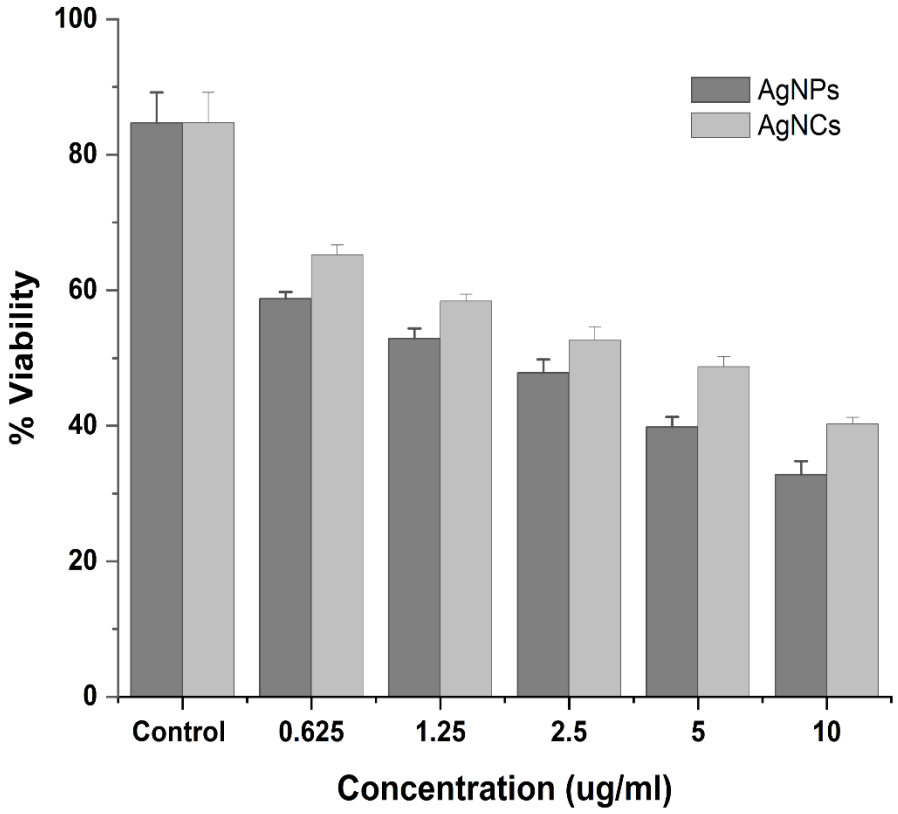

Figure 4. 9: Comparison of cytotoxicity of different concentration of AgNPs and AgNCs on MDA-MB-231 cell lines

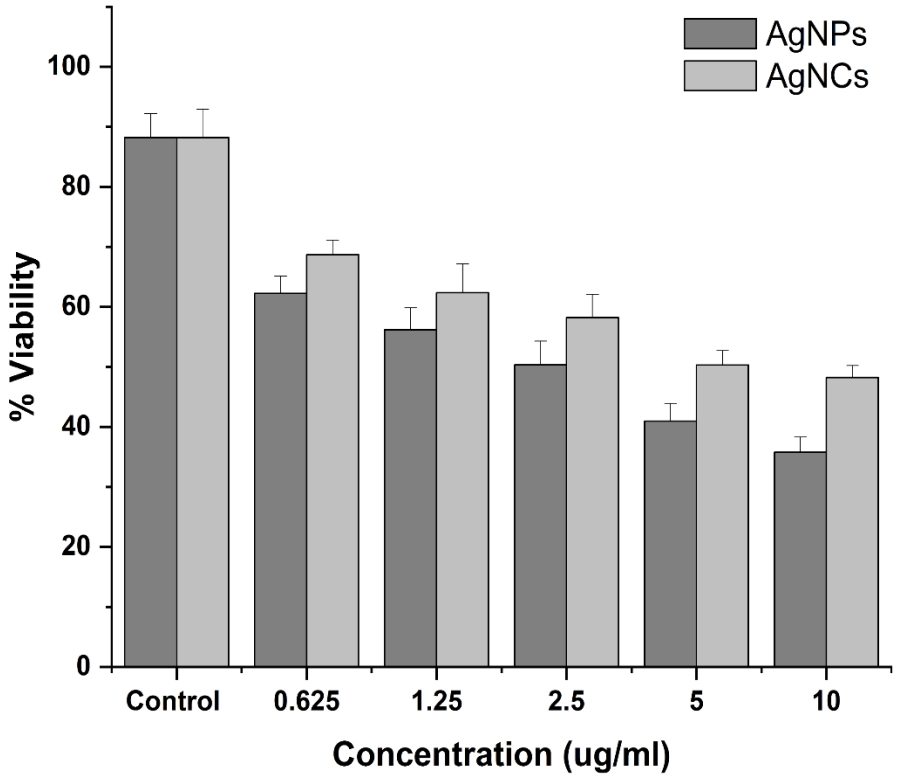

Figure 4. 10: Comparison of cytotoxicity of different concentration of AgNPs and AgNCs on MCF-7 cell lines

4.1.6. Haemolytic Activity

Haemolysis assay was performed to evaluate and compare the ability of AgNPs and AgNCs to cause the lysis of RBC's by damaging its cell membrane to release haemoglobin. The haemolysis assay of was conducted at 6 different dilutions (25, 50, 75, 100, 125, and 150 µg/ml) of NPs. The percentage haemolysis activity was concentration dependent for both AgNPs and AgNCs. The highest percentage haemolysis activity of AgNPs was measured to be 1.2% at 125µg/ml while the AgNCs showed comparatively less hemolytic activity of 0.91% at the same concentration. The Fig 4.11 and 4.12 shows the result of haemolysis of AgNPs and AgNCs respectively.

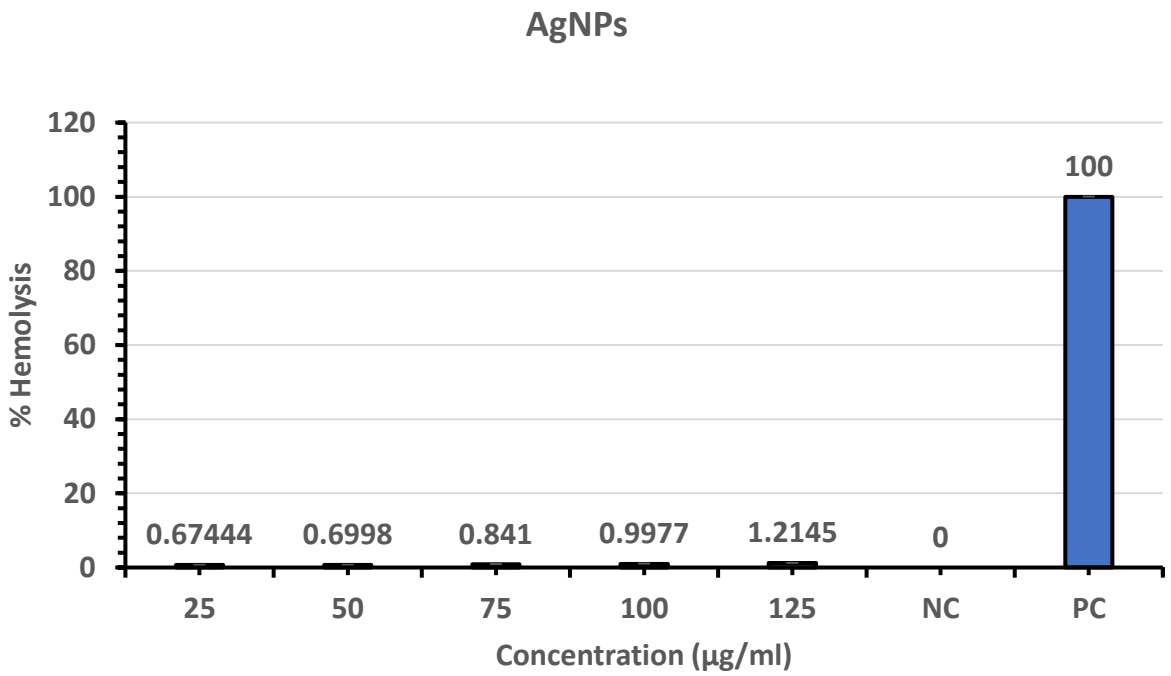

Figure 4. 11: Determination of haemolytic activity of spherical silver nanoparticles (AgNPs)

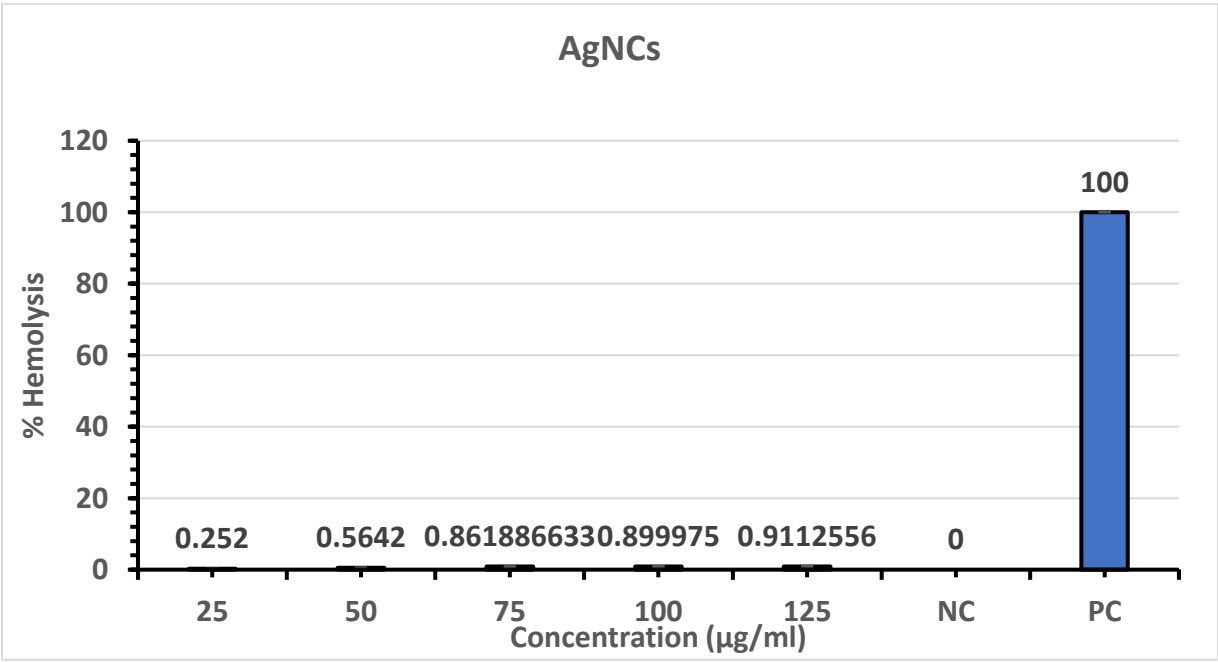

Figure 4. 12: Determination of haemolytic activity of silver nanocubes (AgNCs)

4.1.7. Antibacterial Activity of AgNPs and AgNCs

4.1.7.1 Antimicrobial Resistance Profiles of Bacterial Strains

The antibiotic resistance profiles of all the MDR strains (*Acinetobacter baumanii, Methicillin Resistant Staphylococcus aureus* (MRSA), *Ecoli, and Vancomycin Resistant Enterococcus*) were measured using Kirby-Bauer antibiotic assay on Muller-Hinton Agar (MHA). The standard antibiotic discs were placed on the MHA plates inoculated with MDR bacteria and the zones of inhibition were measured. The *Acinetobacter baumannii* bacteria was found to be resistant to all the major antibiotics tested. The MRSA strain showed resistance to Ciprofloxacin, Doxycycline, Penicillin, Erythromycin, Azithromycin and Cefoxitin. Similarly, the MDR *Ecoli* was found to be resistant to Ciprofloxacin, Ampicillin, Doxycyclin, Cefipime, Ceftriazone, Amoxicillin, Cefuroxime, and Cefazoline. The Vancomycin Resistant Enterococcus showed resistance to all the antibiotics except Linezolid.

Ecoli	
Drug	**Antimicrobial Sensitivity**
Gentamicin	S
Amikacin	S
Ciprofloxacin	R
Imipenem	S
Piperacillin	S
Ampicillin	R
Doxycyclin	R
Cefipime	R
Co-triamoxazole	S
Ceftriaxone	R
Amoxicillin	R
Cefuroxime	R
Cefazoline	R

Vancomycin Resistant Enterococcus	
Drug	**Antimicrobial Sensitivity**
Ciprofloxacin	R
Ampicillin	R
Pinicillin	R
Ampicillin	R
Tetracycline	R
Nitrofurantoin	R
Linezolid	S
Fosfomycin	R
Vancomycin	R
Teicoplannin	R
Norfloxacin	R

Figure 4. 13: Antimicrobial resistance profile of *E coli* and VRE

Acinetobacter baumannii	
Drug	**Antimicrobial Sensitivity**
Gentamicin	R
Amikacin	R
Ciprofloxacin	R
Imipenem	R
Piperacillin	R
Cefipime	R
Levofloxacin	R
Cefotaxime	R
Ceftriaxone	R
Doxycycline	R
Cotrimoxazole	R

Methicillin Resistant Staphylococcus aureus (MRSA)	
Drug	**Antimicrobial Sensitivity**
Gentamicin	S
Amikacin	S
Ciprofloxacin	R
Chloramphenicol	S
Doxycycline	R
Co-triamoxazole	S
Penicillin	R
Erythromycin	R
Clindamycin	S
Linezolid	S
Azithromycin	R
Cefoxitin	R
Ceftaroline	S

Figure 4. 14: Antimicrobial resistance profile of *Acinetobacter baumannii* and MRSA

4.1.7.2. Agar Disc Diffusion Assay

The antibacterial activity of AgNPs and AgNCs was investigated against 4 MDR bacterial strains (*Acinetobactor baumanii, Methicillin Resistant Staphylococcus aureus* (MRSA), *Ecoli,* and *Vancomycin Resistant Enterococcus*) and normal strains of bacteria (*Bacillus Subtilis, Pseudomonas aeruginosa*). The disc diffusion approach was used to evaluate the antibacterial potential of both types of silver nanoparticles on these strains. The assay was performed in triplicates and the zones of inhibition were measured around the discs. Both AgNPs and AgNCs showed a dose dependant antibacterial activity on all bacterial strains. The Fig 4.15 and 4.16shows

the antibacterial activity of AgNPs and AgNCs respectively against all the strains. The highest zones of inhibition were observed against Vancomycin Resistant Enterococcus (VRE).

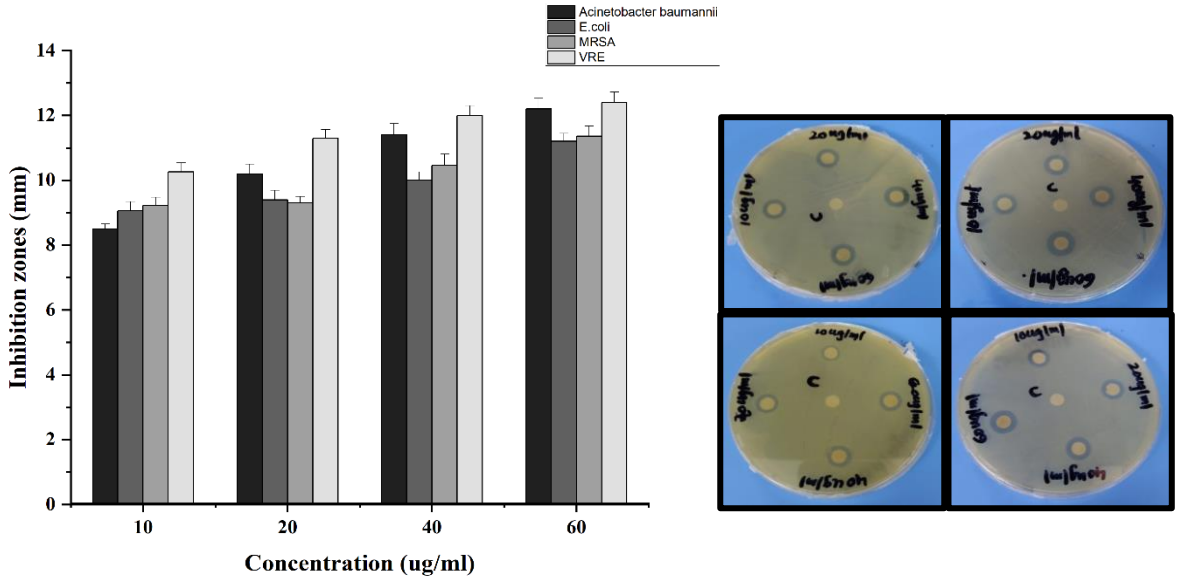

Figure 4. 15: Antibacterial activity of silver nanoparticles (AgNPs) MDR bacterial strains

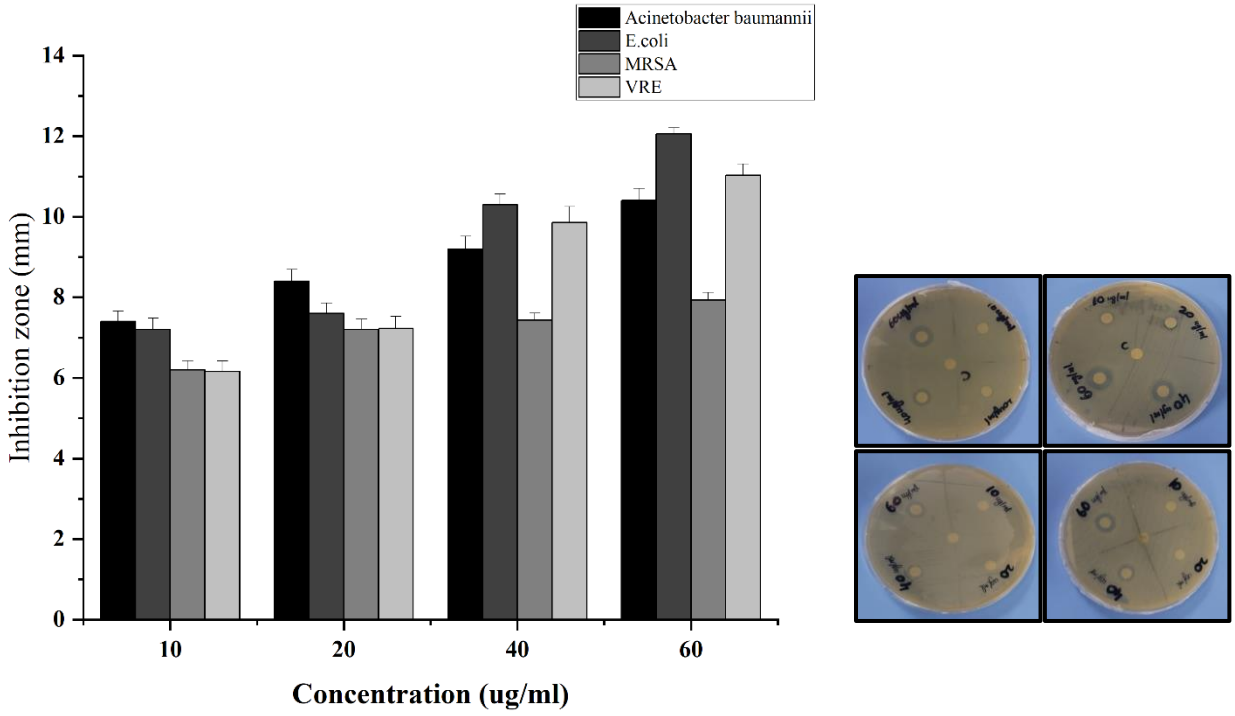

Figure 4. 16: Antibacterial activity of silver nanocubes (AgNCs) against normal and MDR bacterial strains

4.1.8. Antioxidant Activity of AgNPs and AgNCs

The free radical Scavenging activity of AgNPs and AgNCs was evaluated using DPPH assay. The scavenging activity of nanoparticles and positive control (Ascorbic acid) resulted in the reduction of DPPH and conversion of its purple violet color to yellowish color. The percentage free radical scavenging activity of AgNPs, AgNCs and ascorbic acid was calculated using the following formula:

$$\% \textbf{ Scavenging Activity} = \frac{\text{Control absorbance} - \text{Test sample absorbance}}{\text{Control absorbance}} \text{ X } 100$$

The percentage scavenging activity of nanoparticles was compared with that of ascorbic acid.

The results of DPPH assay revealed that both AgNPs and AgNCs possess antioxidant activity. However, the AgNPs showed higher scavenging activity than that of AgNCs in comparison with Ascorbic acid which showed the highest activity. At a 100 ug/ml the AgNCs showed 16% while AgNPs showed 53% scavenging activity. The ascorbic acid at the same concentration showed 91% scavenging activity.

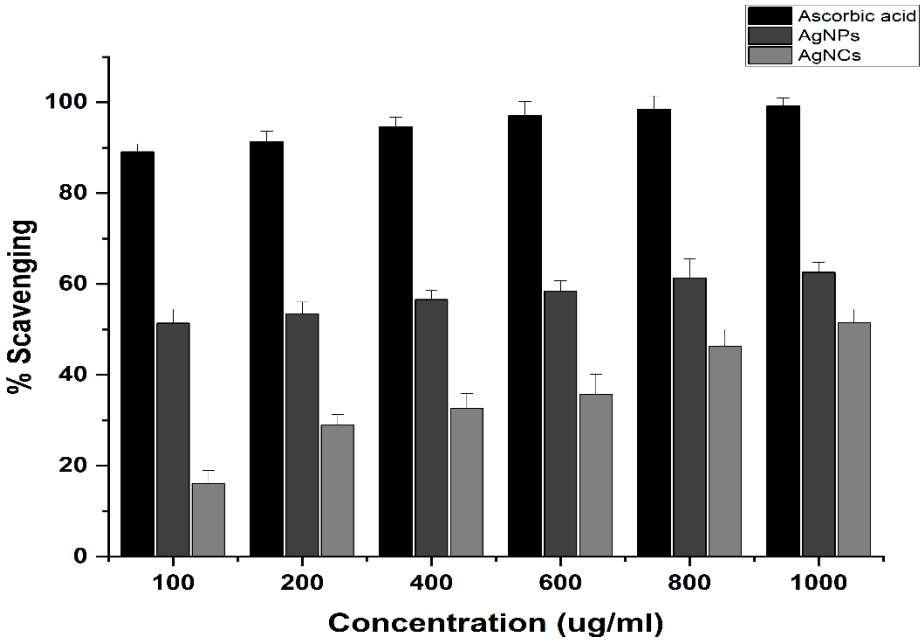

Figure 4.17: % radical scavenging activity of a of AgNPs, AgNCs and vitamin C

Chapter 5

DISCUSSION

The nanotechnology has gained much attention in the last decade due to its diverse applications. The most important development in the field of nanotechnology is the production of nanoparticles of different compositions, sizes and shapes. Generally, nanoparticles are minute particles in the range of 1-100 nm. The metallic nanoparticles provide unique physicochemical properties due to which they offer a variety of interesting applications in the field of biomedicine. These nanoparticles can be synthesized with tunable aspect ratios and shapes (Kogan et al., 2007).

The silver nanoparticles have been used in a variety of applications for decades, owing to their ease of synthesis and unique physicochemical properties. Silver nanoparticles of different shapes including spherical, nanocubes, nanorods etc can be easily synthesized using simple polyol synthesis (Khodashenas & Ghorbani, 2019) The polyol synthesis provides an easy and rapid platform for the synthesis of silver nanoparticles of varying shapes and aspect ratios. In the present study silver nanoparticles of both spherical and cubical shapes have been synthesized using the PVP mediated polyol synthesis.

The AgNPs and AgNCs synthesized in the present study were primarily characterized with UV-VIS spectroscopy. The AgNPs showed a maximum absorbance at 401 nm while AgNCs showed two major peaks one at 450 nm and other at 320 nm which is in accordance with other reported studies (Fahmy et al., 2019; Gamboa, Rojas, Martínez, & Vega-Baudrit, 2019; Pandey et al., 2014; Peng, Song, Yang, Guo, & Yao, 2017; Siekkinen et al., 2006; Vazquez-Muñoz et al., 2019).

The crystalline structure is one the most important properties of nanoparticles. The crystallinity of both AgNPs and AgNCs was measured using XRD. The AgNCs showed strong Bragg reflections at 38.12°., 44.2°, 64.7°, and 77.6° corresponding to (111), (200), and (220) planes of Ag crystals respectively. These peak positions are characteristics of cubical silver nanoparticles. Studies show that the PVP selectively binds to (100) facet (lowest energy facet) of the Ag seed and thus grow more slowly than that of (111) facets leading to the longitudinal growth of (111) facets causing the evolution from spherical shaped seeds to silver nanocubes (Guo, Xing, & Yang, 2015; Murshid & Kitaev, 2014; Xia, Zeng, Zhang, Moran, & Xia, 2012). Similarly, the XRD of AgNPs showed peaks at 38°, 44°, 64°, and 74° which corresponds to (111), (200), (220), (311) planes of AgNPs, as previously reported (Kim et al., 2006; Shameli et al., 2012).

After assessing the crystallinity, the presence of different functional groups on the surface of synthesized AgNPs and AgNCs were confirmed through FTIR analysis. The peaks at 3426 and 3440 for AgNPs and AgNCs respectively corresponds to stretching vibrations of N-H bond in amine group. The FTIR analysis also revealed a sharp FTIR peak at position 1632 and 1645 cm^{-1} for AgNPs and AgNCs which corresponds to C=O of carbonyl group of PVP which shows the bonding of PVP on NPs to prevent them from agglomeration (Farouk, El-Molla, Salib, Soliman, & Shaalan, 2020). The peak at 706 cm^{-1} corresponds to stretching vibration of pyrrolidone ring. The FTIR peak at position 2070 cm−1 corresponds to C–H asymmetric stretches of alkene group. Similarly, the AgNCs also showed similar peaks at position 3440, 2072, 1645 and 710 corresponding to N-H, C=O, and stretching of pyrrolidone ring respectively. These peak positions are in accordance with previous studies (Bryaskova, Pencheva, Nikolov, & Kantardjiev, 2011; Farouk et al., 2020).A sharp absorption peak at position 1638 cm^{-1} corresponds to stretching of

carbonyl group of PVP, this indicates the capping of AgNPs with PVP which prevents NPs from aggregation and agglomeration.

The evaluation of biocompatibility of nanoparticles is very critical before using them effectively for biomedical applications. Generally, MTT assay is used in order to measure the cytotoxicity of the synthesized nanoparticles on human cells. The cytotoxicity of AgNPs and AgNCs synthesized in the present study was evaluated using MTT assay on MCF-7 and MDA-MB-231 cell lines. Both of the NPs showed a dose dependant cytotoxicity on human cell lines. At the same concentration (0.625 µg/ml) the AgNPs exposed MDA-MB cell lines showed 58.7% viability while AgNC treated showed 65.23%. Similarly, the biocompatibility of AgNCs in MCF-7 cell lines were also higher than that of AgNPs. At the same concentration (0.625 µh/ml) the AgNPs treated MCF-7 cells showed 61.9% while AgNCs treated cells showed 67.5% viability. The AgNCs showed less toxicity as compared to AgNPs which is due to their larger size and small surface area. The silver nanoparticles show toxicity on human cells in a size dependent manner. Different studies on the effect of shape on the toxicity of silver NPs have revealed that AgNPs with smaller size shows more toxicity due to its larger surface area and surface reactivity (Carlson et al., 2008; Liu et al., 2010).Moreover, the shape and morphology of human cells is different than that of bacterial cells due to which the NPs showed more toxicity towards bacterial cells than that of human cells (Agrawal et al., 2021).The shape of AgNPs is also an important property which can influence the cytotoxicity towards mammalian cells. The shape of NPs influences the cellular uptake of a particular cell. Moreover, the degree to which at which the NPs are taken up by the cells depends upon the size, shape and type of cell (AshaRani, Low Kah Mun, Hande, & Valiyaveettil, 2009; Jiang et al., 2013; Sahu et al., 2014). To assess the compatibility of as synthesized NPs with erythrocytes haemolysis assay was performed. The assay involved treatment of silver NPs at 5

different concentrations (25, 50, 75, 100, 125 ug/ml) with blood and measuring the release of haemoglobin to evaluate the haemolytic activity of NPs. The results showed that both AgNPs and AgNCs demonstrate a low haemolytic activity at all the concentrations similar to other studies (Golubeva et al., 2010; H. Huang et al., 2016).

The DPPH assay is one of the most frequently used method to determine the antioxidant activities of a compound. The DPPH is a stable free radical which is purple in color which upon reduction changes to yellow color. The degree of disappearance of color of DPPH is directly proportional to the concentration of antioxidant used. Silver nanoparticles have been studied to have excellent antioxidant capacity (Bedlovičová, Strapáč, Baláž, & Salayová, 2020). The Results of DPPH assay revealed the antioxidant potential of both AgNPs and AgNCs. However, the AgNPs showed higher scavenging activity than that of AgNCs in comparison with Ascorbic acid which showed the highest activity. At a 100 ug/ml the AgNCs showed 16% while AgNPs showed 53% scavenging activity. The ascorbic acid at the same concentration showed 91% scavenging activity. The antioxidant activity of both type of NPs was less in comparison with the standard ascorbic acid. These findings were similar with other studies involving the evaluation of antioxidant properties of silver NPs (Keshari, Srivastava, Singh, Yadav, & Nath, 2020; Sreelekha, George, Shyam, Sajina, & Mathew, 2021).The evaluation of antioxidant potential of AgNCs and their comparison with silver nanoparticles has not been reported before.

To determine and compare the antibacterial activity of both AgNPs and AgNCs the disk diffusion assay was used on MHA plates (Ruparelia, Chatterjee, Duttagupta, & Mukherji, 2008). Both the AgNPs and AgNCs in four different concentrations (20, 40, 40, and 60 ug/ml) were tested against 4 MDR bacterial strains including *Acinetobacter baumanii*, *Methicillin Resistant Staphylococcus*

aureus (MRSA), *Ecoli, and Vancomycin Resistant Enterococcus*. It was found that both AgNPs and AgNCs exhibit antibacterial activities by appearance of inhibition zones in the range of 6-12mm. At the concentration of 60 ug/ml the AgNPs showed highest activity against Vancomycin resistant enterococcus making inhibition zone of 12.7 mm. Similarly, at the same concentration the AgNCs showed the highest concentration against Ecoli with inhibition zone of 12 mm and lowest activity against MRSA with inhibition zone of 7.9 mm. Overall the AgNPs were found to have more potent antibacterial activity as compared to AgNCs against all bacterial strains. Previous studies have showed a higher antibacterial activity of anisotropic silver nanoparticles due to the presence of (H. Huang et al.) facets which have higher energy and thus high reactivity as compared to {111} facets. (Duval, Gouyau, & Lamouroux, 2019).This high bactericidal activity of spherical silver nanoparticles in the present study can be attributed to their small size and large surface area which results in closer contact of AgNPs with bacterial cells. Moreover, the small sized silver nanoparticles are more susceptible to the release of Ag ions (Shanmuganathan et al., 2018).The AgNCs on the other hand have low surface area due to their larger size which limits their antibacterial potential (Martínez-Castañon, Nino-Martinez, Martinez-Gutierrez, Martinez-Mendoza, & Ruiz, 2008; Wang, Huang, & Pirestani, 2003).There are many factors which can affect the antibacterial activities of silver nanoparticles and more studies are required to completely investigate their antibacterial potential.

SUMMARY & FUTURE PERSPECTIVE

The cube shaped nanoparticles are the less explored silver nanoparticles. In the current study silver nanoparticles of spherical and cubical shape were synthesized and compared for their compatibility against human cell lines and their antibacterial potential against MDR strains. Both the NPs showed antibacterial activities but also showed cytotoxicity against human cell lines. The AgNPs showed highest antibacterial activities in comparison with AgNCs but were also found to be less compatible owing to their comparatively smaller size and high surface to volume ratio. Further studies need to be performed for the enhancement of biocompatibility of silver nanoparticles by coating them with different polymers to obtained safe nanoparticles with high antibacterial activities against MDR bacteria.

REFERENCES

Abid, N., Khan, A. M., Shujait, S., Chaudhary, K., Ikram, M., Imran, M., . . . Maqbool, M. (2021). Synthesis of nanomaterials using various top-down and bottom-up approaches, influencing factors, advantages, and disadvantages: A review. *Advances in colloid and interface science*, 102597.

Agnihotri, S., Mukherji, S., & Mukherji, S. J. R. A. (2014). Size-controlled silver nanoparticles synthesized over the range 5–100 nm using the same protocol and their antibacterial efficacy. *4*(8), 3974-3983.

Agrawal, N., Mishra, P., Ranjan, R., Awasthi, P., Srivastava, A., Prasad, D., & Kohli, E. (2021). Nano-cubes over nano-spheres: shape dependent study of silver nanomaterial for biological applications. *Bulletin of Materials Science, 44*(3), 1-9.

Ahmad, A., Mukherjee, P., Senapati, S., Mandal, D., Khan, M. I., Kumar, R., & Sastry, M. (2003). Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium oxysporum. *Colloids and surfaces B: Biointerfaces, 28*(4), 313-318.

Albanese, A., Tang, P. S., & Chan, W. C. J. A. r. o. b. e. (2012). The effect of nanoparticle size, shape, and surface chemistry on biological systems. *14*(1), 1-16.

AshaRani, P., Low Kah Mun, G., Hande, M. P., & Valiyaveettil, S. (2009). Cytotoxicity and genotoxicity of silver nanoparticles in human cells. *ACS nano, 3*(2), 279-290.

Austin, L. A., Mackey, M. A., Dreaden, E. C., & El-Sayed, M. A. (2014). The optical, photothermal, and facile surface chemical properties of gold and silver nanoparticles in biodiagnostics, therapy, and drug delivery. *Archives of toxicology, 88*(7), 1391-1417.

Azharuddin, M., Zhu, G. H., Das, D., Ozgur, E., Uzun, L., Turner, A. P., & Patra, H. K. (2019). A repertoire of biomedical applications of noble metal nanoparticles. *Chemical Communications, 55*(49), 6964-6996.

Bedlovičová, Z., Strapáč, I., Baláž, M., & Salayová, A. (2020). A brief overview on antioxidant activity determination of silver nanoparticles. *Molecules, 25*(14), 3191.

Betts, J. W., Hornsey, M., & La Ragione, R. M. (2018). Novel antibacterials: alternatives to traditional antibiotics *Advances in microbial physiology* (Vol. 73, pp. 123-169): Elsevier.

Blair, J., Webber, M. A., Baylay, A. J., Ogbolu, D. O., & Piddock, L. J. (2015). Molecular mechanisms of antibiotic resistance. *Nature reviews microbiology, 13*(1), 42-51.

Braun, G. B., Friman, T., Pang, H.-B., Pallaoro, A., De Mendoza, T. H., Willmore, A.-M. A., . . . Sugahara, K. N. (2014). Etchable plasmonic nanoparticle probes to image and quantify cellular internalization. *Nature materials, 13*(9), 904-911.

Brobbey, K. J., Haapanen, J., Gunell, M., Mäkelä, J. M., Eerola, E., Toivakka, M., & Saarinen, J. J. J. A. S. S. (2017). One-step flame synthesis of silver nanoparticles for roll-to-roll production of antibacterial paper. *420*, 558-565.

Bryaskova, R., Pencheva, D., Nikolov, S., & Kantardjiev, T. (2011). Synthesis and comparative study on the antimicrobial activity of hybrid materials based on silver nanoparticles (AgNps) stabilized by polyvinylpyrrolidone (PVP). *Journal of chemical biology, 4*(4), 185-191.

Burdușel, A.-C., Gherasim, O., Grumezescu, A. M., Mogoantă, L., Ficai, A., & Andronescu, E. (2018). Biomedical applications of silver nanoparticles: an up-to-date overview. *Nanomaterials, 8*(9), 681.

Campos, E. A., Pinto, D. V. B. S., Oliveira, J. I. S. d., Mattos, E. d. C., & Dutra, R. d. C. L. (2015). Synthesis, characterization and applications of iron oxide nanoparticles-a short review. *Journal of Aerospace Technology and Management, 7*, 267-276.

Carlson, C., Hussain, S. M., Schrand, A. M., K. Braydich-Stolle, L., Hess, K. L., Jones, R. L., & Schlager, J. J. (2008). Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species. *The journal of physical chemistry B, 112*(43), 13608-13619.

Caro, C., Castillo, P. M., Klippstein, R., Pozo, D., & Zaderenko, A. P. (2010). Silver nanoparticles: sensing and imaging applications. *Silver nanoparticles, 95*, 201-224.

Chen, M., Feng, Y.-G., Wang, X., Li, T.-C., Zhang, J.-Y., & Qian, D.-J. (2007). Silver nanoparticles capped by oleylamine: formation, growth, and self-organization. *Langmuir, 23*(10), 5296-5304.

Cormode, D. P., Naha, P. C., Fayad, Z. A. J. C. m., & imaging, m. (2014). Nanoparticle contrast agents for computed tomography: a focus on micelles. *9*(1), 37-52.

Dakal, T. C., Kumar, A., Majumdar, R. S., & Yadav, V. (2016). Mechanistic Basis of Antimicrobial Actions of Silver Nanoparticles. *Frontiers in Microbiology, 7*. doi: 10.3389/fmicb.2016.01831

de Kraker, M. E., Stewardson, A. J., & Harbarth, S. J. P. m. (2016). Will 10 million people die a year due to antimicrobial resistance by 2050? *, 13*(11), e1002184.

De Souza, A., Mehta, D., & Leavitt, R. (2006). Bactericidal activity of combinations of Silver–Water Dispersion TM with 19 antibiotics against seven microbial strains. *Current Science*, 926-929.

Dong, J., Carpinone, P. L., Pyrgiotakis, G., Demokritou, P., Moudgil, B. M. J. K. P., & Journal, P. (2020). Synthesis of precision gold nanoparticles using Turkevich method. *37*, 224-232.

Drexler, K. E. (1981). Molecular engineering: An approach to the development of general capabilities for molecular manipulation. *Proceedings of the National Academy of Sciences, 78*(9), 5275-5278.

Dutta, P. P., Bordoloi, M., Gogoi, K., Roy, S., Narzary, B., Bhattacharyya, D. R., . . . Pharmacotherapy. (2017). Antimalarial silver and gold nanoparticles: Green synthesis, characterization and in vitro study. *91*, 567-580.

Duval, R. E., Gouyau, J., & Lamouroux, E. (2019). Limitations of recent studies dealing with the antibacterial properties of silver nanoparticles: Fact and opinion. *Nanomaterials, 9*(12), 1775.

Ealia, S. A. M., & Saravanakumar, M. (2017). *A review on the classification, characterisation, synthesis of nanoparticles and their application.* Paper presented at the IOP conference series: materials science and engineering.

El-Sayed, A., Kamel, M. J. E. S., & Research, P. (2020). Advances in nanomedical applications: diagnostic, therapeutic, immunization, and vaccine production. *27*(16), 19200-19213.

Fahmy, H. M., Mosleh, A. M., Abd Elghany, A., Shams-Eldin, E., Serea, E. S. A., Ali, S. A., & Shalan, A. E. J. R. a. (2019). Coated silver nanoparticles: Synthesis, cytotoxicity, and optical properties. *9*(35), 20118-20136.

Farouk, M. M., El-Molla, A., Salib, F. A., Soliman, Y. A., & Shaalan, M. (2020). The role of silver nanoparticles in a treatment approach for multidrug-resistant Salmonella species isolates. *International journal of nanomedicine, 15*, 6993.

Fayaz, A. M., Balaji, K., Girilal, M., Yadav, R., Kalaichelvan, P. T., & Venketesan, R. (2010). Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: a study against gram-positive and gram-negative bacteria. *Nanomedicine: Nanotechnology, Biology and Medicine, 6*(1), 103-109.

Feng, Q. L., Wu, J., Chen, G. Q., Cui, F., Kim, T., & Kim, J. (2000). A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. *Journal of biomedical materials research, 52*(4), 662-668.

Ferdous, Z., & Nemmar, A. J. I. J. o. M. S. (2020). Health impact of silver nanoparticles: a review of the biodistribution and toxicity following various routes of exposure. *21*(7), 2375.

Fortunati, E., Peltzer, M., Armentano, I., Jiménez, A., & Kenny, J. M. J. J. o. F. E. (2013). Combined effects of cellulose nanocrystals and silver nanoparticles on the barrier and migration properties of PLA nano-biocomposites. *118*(1), 117-124.

Franci, G., Falanga, A., Galdiero, S., Palomba, L., Rai, M., Morelli, G., & Galdiero, M. (2015). Silver nanoparticles as potential antibacterial agents. *Molecules, 20*(5), 8856-8874.

Galdiero, S., Falanga, A., Vitiello, M., Cantisani, M., Marra, V., & Galdiero, M. (2011). Silver nanoparticles as potential antiviral agents. *Molecules, 16*(10), 8894-8918.

Gamboa, S. M., Rojas, E., Martínez, V., & Vega-Baudrit, J. J. I. J. B. B. (2019). Synthesis and characterization of silver nanoparticles and their application as an antibacterial agent. *5*, 166-173.

García-Barrasa, J., López-de-Luzuriaga, J. M., & Monge, M. J. C. E. j. o. c. (2011). Silver nanoparticles: synthesis through chemical methods in solution and biomedical applications. *9*(1), 7-19.

Golubeva, O. Y., Shamova, O., Orlov, D., Pazina, T. Y., Boldina, A., & Kokryakov, V. (2010). Study of antimicrobial and hemolytic activities of silver nanoparticles prepared by chemical reduction. *Glass Physics and Chemistry, 36*(5), 628-634.

Guo, H., Xing, G., & Yang, Z. (2015). *The Controllable Synthesis of Silver Nanowires by Using the Polyol Method with Trace Agent.* Paper presented at the 2015 International Symposium on Material, Energy and Environment Engineering.

Gupta, A., Maynes, M., & Silver, S. (1998). Effects of halides on plasmid-mediated silver resistance in Escherichia coli. *Applied and environmental microbiology, 64*(12), 5042-5045.

Hamad, A., Khashan, K. S., Hadi, A. J. J. o. I., Polymers, O., & Materials. (2020). Silver nanoparticles and silver ions as potential antibacterial agents. *30*(12), 4811-4828.

Han, H. J., Yu, T., Kim, W.-S., & Im, S. H. J. J. o. C. G. (2017). Highly reproducible polyol synthesis for silver nanocubes. *469*, 48-53.

Hasan, S. (2015). A review on nanoparticles: their synthesis and types. *Res. J. Recent Sci, 2277*, 2502.

He, R., Ren, F., & Chen, F. J. M. L. (2017). Embedded silver nanoparticles in KTP crystal produced by ion implantation. *193*, 158-160.

Hembram, K. C., Kumar, R., Kandha, L., Parhi, P. K., Kundu, C. N., & Bindhani, B. K. (2018). Therapeutic prospective of plant-induced silver nanoparticles: application as antimicrobial and anticancer agent. *Artificial cells, nanomedicine, and biotechnology, 46*(sup3), S38-S51.

Huang, H., Lai, W., Cui, M., Liang, L., Lin, Y., Fang, Q., . . . Xie, L. (2016). An evaluation of blood compatibility of silver nanoparticles. *Scientific reports, 6*(1), 1-15.

Huang, T., & Xu, X.-H. N. (2010). Synthesis and characterization of tunable rainbow colored colloidal silver nanoparticles using single-nanoparticle plasmonic microscopy and spectroscopy. *Journal of materials chemistry, 20*(44), 9867-9876.

Jeevanandam, J., Barhoum, A., Chan, Y. S., Dufresne, A., & Danquah, M. K. (2018). Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. *Beilstein journal of nanotechnology, 9*(1), 1050-1074.

Jiang, X., Foldbjerg, R., Miclaus, T., Wang, L., Singh, R., Hayashi, Y., . . . Beer, C. (2013). Multi-platform genotoxicity analysis of silver nanoparticles in the model cell line CHO-K1. *Toxicology letters, 222*(1), 55-63.

Joshi, H. M., Bhumkar, D. R., Joshi, K., Pokharkar, V., & Sastry, M. J. L. (2006). Gold nanoparticles as carriers for efficient transmucosal insulin delivery. *22*(1), 300-305.

Jung, J. H., Oh, H. C., Noh, H. S., Ji, J. H., & Kim, S. S. (2006). Metal nanoparticle generation using a small ceramic heater with a local heating area. *Journal of aerosol science, 37*(12), 1662-1670.

Kalantari, K., Mostafavi, E., Afifi, A. M., Izadiyan, Z., Jahangirian, H., Rafiee-Moghaddam, R., & Webster, T. J. (2020). Wound dressings functionalized with silver nanoparticles: promises and pitfalls. *Nanoscale, 12*(4), 2268-2291.

Kaur, G., Singh, T., & Kumar, A. (2012). Nanotechnology: A review. *IJEAR, 2*, 50-53.

Keshari, A. K., Srivastava, R., Singh, P., Yadav, V. B., & Nath, G. (2020). Antioxidant and antibacterial activity of silver nanoparticles synthesized by Cestrum nocturnum. *Journal of Ayurveda and integrative medicine, 11*(1), 37-44.

Khan, I., Saeed, K., & Khan, I. (2019). Nanoparticles: Properties, applications and toxicities. *Arabian journal of chemistry, 12*(7), 908-931.

Khatoon, U. T., Rao, G. N., Mohan, K. M., Ramanaviciene, A., & Ramanavicius, A. J. V. (2017). Antibacterial and antifungal activity of silver nanospheres synthesized by tri-sodium citrate assisted chemical approach. *146*, 259-265.

Khodashenas, B., & Ghorbani, H. R. (2019). Synthesis of silver nanoparticles with different shapes. *Arabian Journal of Chemistry, 12*(8), 1823-1838.

Kim, D., Jeong, S., & Moon, J. (2006). Synthesis of silver nanoparticles using the polyol process and the influence of precursor injection. *Nanotechnology, 17*(16), 4019.

Kogan, M. J., Olmedo, I., Hosta, L., R Guerrero, A., Cruz, L. J., & Albericio, F. (2007). Peptides and metallic nanoparticles for biomedical applications.

Konios, D., Stylianakis, M. M., Stratakis, E., & Kymakis, E. (2014). Dispersion behaviour of graphene oxide and reduced graphene oxide. *Journal of colloid and interface science, 430*, 108-112.

Kumar, A., Vemula, P. K., Ajayan, P. M., & John, G. (2008). Silver-nanoparticle-embedded antimicrobial paints based on vegetable oil. *Nature materials, 7*(3), 236-241.

Kumar, R., & Lal, S. (2014). Synthesis of organic nanoparticles and their applications in drug delivery and food nanotechnology: a review. J Nanomater Mol Nanotechnol 3: 4. *of, 11*, 2.

Lara, H. H., Ayala-Núnez, N. V., Ixtepan Turrent, L. d. C., & Rodríguez Padilla, C. (2010). Bactericidal effect of silver nanoparticles against multidrug-resistant bacteria. *World Journal of Microbiology and Biotechnology, 26*(4), 615-621.

Lee, D. K., & Kang, Y. S. (2004). Synthesis of silver nanocrystallites by a new thermal decomposition method and their characterization. *Etri Journal, 26*(3), 252-256.

Liu, W., Wu, Y., Wang, C., Li, H. C., Wang, T., Liao, C. Y., . . . Jiang, G. B. (2010). Impact of silver nanoparticles on human cells: effect of particle size. *Nanotoxicology, 4*(3), 319-330.

Mahmoud, M. A., & El-Sayed, M. A. (2013). Different plasmon sensing behavior of silver and gold nanorods. *The Journal of Physical Chemistry Letters, 4*(9), 1541-1545.

Martínez-Castañon, G.-A., Nino-Martinez, N., Martinez-Gutierrez, F., Martinez-Mendoza, J., & Ruiz, F. (2008). Synthesis and antibacterial activity of silver nanoparticles with different sizes. *Journal of nanoparticle research, 10*(8), 1343-1348.

Martinho, N., Damgé, C., Reis, C. P. J. J. o. b., & nanobiotechnology. (2011). Recent advances in drug delivery systems. *2*(05), 510.

Matsumura, Y., Yoshikata, K., Kunisaki, S.-i., & Tsuchido, T. (2003). Mode of bactericidal action of silver zeolite and its comparison with that of silver nitrate. *Applied and environmental microbiology, 69*(7), 4278-4281.

Morones, J. R., Elechiguerra, J. L., Camacho, A., Holt, K., Kouri, J. B., Ramírez, J. T., & Yacaman, M. J. J. N. (2005). The bactericidal effect of silver nanoparticles. *16*(10), 2346.

Murshid, N., & Kitaev, V. J. C. c. (2014). Role of poly (vinylpyrrolidone)(PVP) and other sterically protecting polymers in selective stabilization of {111} and {100} facets in pentagonally twinned silver nanoparticles. *50*(10), 1247-1249.

Namasivayam, S., Ganesh, S., & Avimanyu, B. (2011). Evaluation of anti-bacterial activity of silver nanoparticles synthesized from Candida glabrata and Fusarium oxysporum. *Int J Med Res, 1*(3), 131-136.

Nanda, A., & Saravanan, M. (2009). Biosynthesis of silver nanoparticles from Staphylococcus aureus and its antimicrobial activity against MRSA and MRSE. *Nanomedicine: Nanotechnology, Biology and Medicine, 5*(4), 452-456.

Natan, M., & Banin, E. J. F. m. r. (2017). From nano to micro: using nanotechnology to combat microorganisms and their multidrug resistance. *41*(3), 302-322.

Nedelcu, I.-A., Ficai, A., Sonmez, M., Ficai, D., Oprea, O., & Andronescu, E. J. C. O. C. (2014). Silver based materials for biomedical applications. *18*(2), 173-184.

Nikolova, M. P., & Chavali, M. S. (2020). Metal oxide nanoparticles as biomedical materials. *Biomimetics, 5*(2), 27.

Organization, W. H. (2014). *Antimicrobial resistance: global report on surveillance*: World Health Organization.

Pal, S., Tak, Y. K., Song, J. M. J. A., & microbiology, e. (2007). Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli. *73*(6), 1712-1720.

Panáček, A., Kolář, M., Večeřová, R., Prucek, R., Soukupova, J., Kryštof, V., . . . Kvítek, L. (2009). Antifungal activity of silver nanoparticles against Candida spp. *Biomaterials, 30*(31), 6333-6340.

Pandey, J. K., Swarnkar, R., Soumya, K., Dwivedi, P., Singh, M. K., Sundaram, S., . . . biotechnology. (2014). Silver nanoparticles synthesized by pulsed laser ablation: as a potent antibacterial agent for human enteropathogenic gram-positive and gram-negative bacterial strains. *174*(3), 1021-1031.

Pannerselvam, B., Jothinathan, M. K. D., Rajenderan, M., Perumal, P., Thangavelu, K. P., Kim, H. J., . . . Rangarajulu, S. K. J. E. j. o. p. s. (2017). An in vitro study on the burn wound healing activity of cotton fabrics incorporated with phytosynthesized silver nanoparticles in male Wistar albino rats. *100*, 187-196.

Patel, K. D., Singh, R. K., & Kim, H.-W. (2019). Carbon-based nanomaterials as an emerging platform for theranostics. *Materials Horizons, 6*(3), 434-469.
Peng, Y., Song, C., Yang, C., Guo, Q., & Yao, M. J. I. j. o. n. (2017). Low molecular weight chitosan-coated silver nanoparticles are effective for the treatment of MRSA-infected wounds. *12*, 295.
Powers, C. M., Badireddy, A. R., Ryde, I. T., Seidler, F. J., & Slotkin, T. A. (2011). Silver nanoparticles compromise neurodevelopment in PC12 cells: critical contributions of silver ion, particle size, coating, and composition. *Environmental health perspectives, 119*(1), 37-44.
Prasher, P., Sharma, M., Mudila, H., Gupta, G., Sharma, A. K., Kumar, D., . . . Chellappan, D. K. (2020). Emerging trends in clinical implications of bio-conjugated silver nanoparticles in drug delivery. *Colloid and Interface Science Communications, 35*, 100244.
Rai, M. K., Deshmukh, S., Ingle, A., & Gade, A. (2012). Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria. *Journal of applied microbiology, 112*(5), 841-852.
Rajawat, S., & Qureshi, M. S. (2012). Comparative study on bactericidal effect of silver nanoparticles, synthesized using green technology, in combination with antibiotics on Salmonella typhi. *J. Biomater. Nanobiotechnol, 3*(4), 480.
Ray, S. S., & Bandyopadhyay, J. J. N. R. (2021). Nanotechnology-enabled biomedical engineering: Current trends, future scopes, and perspectives. *10*(1), 728-743.
Raza, M. A., Kanwal, Z., Rauf, A., Sabri, A. N., Riaz, S., & Naseem, S. J. N. (2016). Size-and shape-dependent antibacterial studies of silver nanoparticles synthesized by wet chemical routes. *6*(4), 74.
Riley, M. K., & Vermerris, W. J. N. (2017). Recent advances in nanomaterials for gene delivery—a review. *7*(5), 94.
Ruparelia, J. P., Chatterjee, A. K., Duttagupta, S. P., & Mukherji, S. (2008). Strain specificity in antimicrobial activity of silver and copper nanoparticles. *Acta biomaterialia, 4*(3), 707-716.
Rycenga, M., Cobley, C. M., Zeng, J., Li, W., Moran, C. H., Zhang, Q., . . . Xia, Y. (2011). Controlling the synthesis and assembly of silver nanostructures for plasmonic applications. *Chemical reviews, 111*(6), 3669-3712.
Sadeghi, B., Garmaroudi, F. S., Hashemi, M., Nezhad, H., Nasrollahi, A., Ardalan, S., & Ardalan, S. J. A. P. T. (2012). Comparison of the anti-bacterial activity on the nanosilver shapes: nanoparticles, nanorods and nanoplates. *23*(1), 22-26.
Sahu, S. C., Zheng, J., Graham, L., Chen, L., Ihrie, J., Yourick, J. J., & Sprando, R. L. (2014). Comparative cytotoxicity of nanosilver in human liver HepG2 and colon Caco2 cells in culture. *Journal of Applied Toxicology, 34*(11), 1155-1166.
Salavati-Niasari, M., Davar, F., & Mir, N. (2008). Synthesis and characterization of metallic copper nanoparticles via thermal decomposition. *Polyhedron, 27*(17), 3514-3518.
Sánchez-López, E., Gomes, D., Esteruelas, G., Bonilla, L., Lopez-Machado, A. L., Galindo, R., . . . Camins, A. (2020). Metal-based nanoparticles as antimicrobial agents: an overview. *Nanomaterials, 10*(2), 292.
Schinwald, A., Murphy, F. A., Jones, A., MacNee, W., & Donaldson, K. (2012). Graphene-based nanoplatelets: a new risk to the respiratory system as a consequence of their unusual aerodynamic properties. *ACS nano, 6*(1), 736-746.

Shahverdi, A. R., Minaeian, S., Shahverdi, H. R., Jamalifar, H., & Nohi, A.-A. (2007). Rapid synthesis of silver nanoparticles using culture supernatants of Enterobacteria: a novel biological approach. *Process Biochemistry, 42*(5), 919-923.

Shameli, K., Ahmad, M. B., Zamanian, A., Sangpour, P., Shabanzadeh, P., Abdollahi, Y., & Zargar, M. (2012). Green biosynthesis of silver nanoparticles using Curcuma longa tuber powder. *International journal of nanomedicine, 7*, 5603.

Shanmuganathan, R., MubarakAli, D., Prabakar, D., Muthukumar, H., Thajuddin, N., Kumar, S. S., & Pugazhendhi, A. (2018). An enhancement of antimicrobial efficacy of biogenic and ceftriaxone-conjugated silver nanoparticles: green approach. *Environmental Science and Pollution Research, 25*(11), 10362-10370.

Siegel, J., Kvítek, O., Ulbrich, P., Kolská, Z., Slepička, P., & Švorčík, V. (2012). Progressive approach for metal nanoparticle synthesis. *Materials Letters, 89*, 47-50.

Siekkinen, A. R., McLellan, J. M., Chen, J., & Xia, Y. (2006). Rapid synthesis of small silver nanocubes by mediating polyol reduction with a trace amount of sodium sulfide or sodium hydrosulfide. *Chemical physics letters, 432*(4-6), 491-496.

Silva, G. A. (2004). Introduction to nanotechnology and its applications to medicine. *Surgical neurology, 61*(3), 216-220.

Singh, T., Jyoti, K., Patnaik, A., Singh, A., Chauhan, R., Chandel, S. J. J. o. G. E., & Biotechnology. (2017). Biosynthesis, characterization and antibacterial activity of silver nanoparticles using an endophytic fungal supernatant of Raphanus sativus. *15*(1), 31-39.

Sintubin, L., Verstraete, W., Boon, N. J. B., & Bioengineering. (2012). Biologically produced nanosilver: current state and future perspectives. *109*(10), 2422-2436.

Sreelekha, E., George, B., Shyam, A., Sajina, N., & Mathew, B. (2021). A comparative study on the synthesis, characterization, and antioxidant activity of green and chemically synthesized silver nanoparticles. *BioNanoScience, 11*(2), 489-496.

Subedi, S. K. (2014). An introduction to nanotechnology and its implications. *Himalayan Physics, 5*, 78-81.

Sun, X., Shi, J., Zou, X., Wang, C., Yang, Y., & Zhang, H. (2016). Silver nanoparticles interact with the cell membrane and increase endothelial permeability by promoting VE-cadherin internalization. *Journal of Hazardous Materials, 317*, 570-578.

Suresh, A. K., Pelletier, D. A., Wang, W., Moon, J.-W., Gu, B., Mortensen, N. P., . . . Doktycz, M. J. (2010). Silver nanocrystallites: biofabrication using Shewanella oneidensis, and an evaluation of their comparative toxicity on gram-negative and gram-positive bacteria. *Environmental science & technology, 44*(13), 5210-5215.

Syafiuddin, A., Salim, M. R., Beng Hong Kueh, A., Hadibarata, T., & Nur, H. (2017). A review of silver nanoparticles: research trends, global consumption, synthesis, properties, and future challenges. *Journal of the Chinese Chemical Society, 64*(7), 732-756.

Sydnor, E. R., & Perl, T. M. (2011). Hospital epidemiology and infection control in acute-care settings. *Clin Microbiol Rev, 24*(1), 141-173. doi: 10.1128/cmr.00027-10

Tian, J., Wong, K. K., Ho, C. M., Lok, C. N., Yu, W. Y., Che, C. M., . . . Tam, P. K. (2007). Topical delivery of silver nanoparticles promotes wound healing. *ChemMedChem: Chemistry Enabling Drug Discovery, 2*(1), 129-136.

Torres, L., Gmez-Quintero, T., Padron, G., Santana, F., Hernandez, J., Castano, V. J. S. F., California: IADR/AADR/CADR General Session, & Exhibition. (2013). Silver nanoprisms and nanospheres for prosthetic biomaterials.

Tran, Q. H., & Le, A.-T. (2013). Silver nanoparticles: synthesis, properties, toxicology, applications and perspectives. *Advances in natural sciences: nanoscience and nanotechnology, 4*(3), 033001.

Tseng, K.-H., Lee, H.-L., Liao, C.-Y., Chen, K.-C., & Lin, H.-S. (2013). Rapid and efficient synthesis of silver nanofluid using electrical discharge machining. *Journal of Nanomaterials, 2013*.

Van Dong, P., Ha, C. H., Binh, L. T., & Kasbohm, J. J. I. N. L. (2012). Chemical synthesis and antibacterial activity of novel-shaped silver nanoparticles. *2*(1), 1-9.

Vance, M. E., Kuiken, T., Vejerano, E. P., McGinnis, S. P., Hochella Jr, M. F., Rejeski, D., & Hull, M. S. J. B. j. o. n. (2015). Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory. *6*(1), 1769-1780.

Vazquez-Muñoz, R., Arellano-Jimenez, M. J., Lopez, F. D., & Lopez-Ribot, J. L. (2019). Protocol optimization for a fast, simple and economical chemical reduction synthesis of antimicrobial silver nanoparticles in non-specialized facilities. *BMC research notes, 12*(1), 1-6.

Wang, J., Huang, C., & Pirestani, D. (2003). Interactions of silver with wastewater constituents. *Water research, 37*(18), 4444-4452.

Wei, L., Lu, J., Xu, H., Patel, A., Chen, Z.-S., & Chen, G. (2015). Silver nanoparticles: synthesis, properties, and therapeutic applications. *Drug discovery today, 20*(5), 595-601.

Xia, X., Zeng, J., Zhang, Q., Moran, C. H., & Xia, Y. J. T. J. o. P. C. C. (2012). Recent developments in shape-controlled synthesis of silver nanocrystals. *116*(41), 21647-21656.

Yaqoob, A. A., Umar, K., & Ibrahim, M. N. M. J. A. N. (2020). Silver nanoparticles: various methods of synthesis, size affecting factors and their potential applications–a review. *10*(5), 1369-1378.

Yuan, Y.-G., Peng, Q.-L., & Gurunathan, S. (2017). Silver nanoparticles enhance the apoptotic potential of gemcitabine in human ovarian cancer cells: combination therapy for effective cancer treatment. *International journal of nanomedicine, 12*, 6487.

Zhang, C., Hu, Z., Li, P., & Gajaraj, S. (2016). Governing factors affecting the impacts of silver nanoparticles on wastewater treatment. *Science of the Total Environment, 572*, 852-873.

Zhang, X.-F., Liu, Z.-G., Shen, W., & Gurunathan, S. (2016). Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches. *International journal of molecular sciences, 17*(9), 1534.