Gold Nanorods as Carriers for Anti-Cancer Drug

Huzaifa Tahir

Registration # 00000274706

MS Healthcare Biotechnology

Supervised by: **Dr. Rumeza Hanif**

Department of Healthcare Biotechnology,

Atta-ur-Rahman School of Applied Biosciences (ASAB)

National University of Sciences & Technology (NUST),

Islamabad, Pakistan.

Huzaifa Tahir

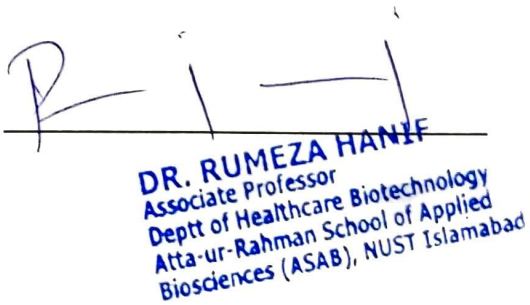
Registration # 00000274706

A thesis submitted in partial fulfilment of the requirements for the degree of MS Healthcare Biotechnology

Supervisor:

Dr. Rumeza Hanif

Thesis Supervisor's Signature:



Department of Healthcare Biotechnology,

Atta-ur-Rahman School of Applied Biosciences (ASAB)

National University of Sciences & Technology (NUST),

Islamabad, Pakistan.

It is certified that the contents and form of MS thesis entitled "**Gold Nanorods as Carriers for Anti-Cancer Drug**" submitted by **Huzaifa Tahir** (Registration no. **00000274706**), of Atta-ur-Rahman School of Applied Biosciences (ASAB) has been verified by undersigned, found complete in all respects as per National University of Science and Technology (NUST) Statues/Regulations/MS policy, is free of plagiarism, errors and mistakes and is accepted as partial fulfilment for award of MS degree.

Supervisor: _____

DR. RUMEZA HANIF
Associate Professor
Deptt of Healthcare Biotechnology
Atta-ur-Rahman School of Applied Biosciences (ASAB), NUST Islamabad

Dr. Rumeza Hanif

ASAB, NUST

Head of the Department: _____

Dr. Touqeer Ahmed
Head of Department (HoD)
Deptt of Healthcare Biotechnology
Atta-ur-Rahman School of Applied Biosciences (ASAB), NUST Islamabad

Dr. Touqeer Ahmed

ASAB, NUST

Principal: _____

Principal
Atta ur Rahman School of
Applied Biosciences (ASAB)
NUST Islamabad

Dr. Hussnain A. Janjua

ASAB, NUST

Dated: _____

FORM TH-4

National University of Sciences & Technology

MS THESIS WORK

We hereby recommend that the dissertation prepared under our supervision by: (Student Name & Regn No.) Huzaifa Tahir & 00000274706 **Titled**:

Gold Nanorods as Carriers for Anti-Cancer Drug be accepted in partial fulfillment of the requirements for the award of MS Healthcare Biotechnology degree with (B+ grade).

Examination Committee Members

1. Name: Dr. Faheem Amin Signature: _____

2. Name: Dr. Maria Shabir Signature: _____

3. Name: Dr. Salik Javed Kakar Signature: _____

Supervisor's name: Dr. Rumeza Hanif Signature: _____

Date: 28-07-21

DR. RUMEZA HANIF
Associate Professor
Deptt of Healthcare Biotechnology
Atta-ur-Rahman School of Applied Biosciences (ASAB), NUST Islamabad

Dr. Touqeer Ahmed
Head of Department (HoD)
Deptt of Healthcare Biotechnology
Atta-ur-Rahman School of Applied Biosciences (ASAB), NUST Islamabad

Date: 28/7/2021

Head of Department

COUNTERSINGED

Principal
Atta ur Rahman School of Applied Biosciences (ASAB)
NUST Islamabad

Date: _____

Dean/Principal

DECLARATION

I, Huzaifa Tahir, declare that all work presented in this thesis is the result of my own work. Where information has been derived from other sources, I confirm that this has been mentioned in the thesis. The work here in was carried out while I was postgraduate student at Atta-ur-Rahman school of Applied Biosciences NUST under the supervision of Dr. Rumeza Hanif.

Huzaifa Tahir

CERTIFICATE FOR PLAGIARISM

It is to confirm that MS thesis entitled "**Gold Nanorods as Carriers for Anti-Cancer Drug**" of **Mr. Huzaifa Tahir** Reg No. **00000274706** 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.

DR. RUMEZA HANIF
Associate Professor
Dept. of Healthcare Biotechnology
Atta-ur-Rahman School of Applied Biosciences (ASAB), NUST Islamabad

(Supervisor)

Dr. Rumeza Hanif

Associate Professor

ASAB, NUST

Dedicated to My Parents

ACKNOWLEDGEMENT

All grace and gratitude for the One Who doesn't leave us for the blink of an eye, the One Who listens to all our whims and woes and walks all roads with us; to The Almighty, The Creator, the Exalted.

I would like to express my gratitude to Rector NUST, esteemed Principal ASAB, **Dr. Hussnain Ahmed Janjua**, and HoD Healthcare Biotechnology, **Dr. Touqeer Ahmed**, for providing me an opportunity to complete my degree of MS Healthcare Biotechnology at ASAB.

I consider myself lucky to have a supervisor as accomplished as **Dr. Rumeza Hanif**. Other than my academic mentor, she is a constant source of information. Her door was always open whenever I ran into a trouble or had a question. This research would not have been completed without her unabated support, enthusiasm, patience and immense knowledge.

I would express my sincere gratitude to my GEC members **Dr. Maria Shabbir**, **Dr. Salik Javed Kakar**, and my external examiner **Dr. Faheem Amin** from School of Natural Sciences (SNS), NUST for their insightful comments and encouragement, which helped me widen my research from various perspectives.

I would like to acknowledge efforts and continuous moral support of my friends throughout my research work. Being a hostelite, friends become your family and your support system and I would like to mention my family away from home who were always there for me; Mudassir Khan, Dawood Amjad, Abu Bakar, Abdullah, Usama, Zomia, Maham and Anam. I would also like to thank and acknowledge the support of my lab senior Mishal Amjad who was always there to guide me. My lab-mates Noor-ul-ain, Humeira and Sidra were always so supportive and helpful and I would like to extend my gratitude towards them as well.

Finally, I express my sincere love and gratitude to the most important people of my life; my parents and my sisters for providing me with unfailing support, continuous encouragement, love and prayers throughout my life.

Huzaifa Tahir

Contents

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AuNRs	Gold Nanorods
AuNPs	Gold Nanoparticles
3D	Three Dimensional
Nm	Nanometer
μm	Micrometer
NPs	Nanoparticles
SPR	Surface Plasmon Resonance
Au	Gold
$HAuCl_4$	Chloroauric acid
UV/Vis	Ultraviolet/Visible
FTIR	Fourier Transformed Infrared Spectroscopy
XRD	X-Ray Diffraction
SEM	Scanning Electron Microscopy
DPPH	2,2-diphenyl-1-picrylhydrazyl hydrate
MTT	3-(4,5-Dimethylthiazole-2yl)-2,5-Diphenyltetrazolium Bromide
UV	Ultraviolet
XPS	X-Ray Photoelectron Spectroscopy
CTAB	Cetyltrimethylammonium bromide
Mg	Milligram
mM	Millimolar
ml	Millilitre
MRI	Magnetic Resonance Imaging
KBr	Potassium Bromide
DNA	Deoxyribonucleic Acid
FBS	Fetal Bovine Serum
PBS	Phosphate Buffered Saline

Rpm	Revolutions per minute
DMSO	Dimethylsulfoxide
DI	De-ionized water
AgNO3	Silver Nitrate
Al_2O_3	Aluminum Oxide
CeO_2	Cerium Oxide
Fe_2O_3	Iron Oxide
Fe_3O_4	Magnetite
SiO_2	Silicon Oxide
4-OHT	4-Hydroxytamoxifen
Hif	Hypoxia-inducible factor
MCF-7	Michigan Cancer Foundation-7
ER	Estrogen Receptor
PEG	Polyethylene Glycol
NaBH4	Sodium Borohydride
RT-PCR	Real-time polymerase chain reaction
RPMI	Roswell Park Memorial Institute

Abstract

Cancer is the second leading cause of death all over the world due to its high incidence, uncontrolled cell growth and recurrence/relapse. Traditionally, tumor resection and chemotherapy is of prime interest while treating cancer but they result in non-specific drug accumulation, systemic damage and resistance to these therapies. Among a variety of nanomaterials, metallic nanoparticle such as gold nanoparticles have gained much importance in past few years due to their applications in cancer theranostics and pharmaceutical industries. Gold Nanorods have gained importance recently due to their shape and unique optical and anisotropic properties. In this study, Seed-mediated approach was used for their synthesis. These nanorods were further verified by UV/Vis spectroscopy, X-Ray Diffraction patterns and Fourier Transformed Infrared spectroscopy. Morphology and size were measured by microscopic analysis such as Scanning Electron Microscopy. Antimicrobial activity of gold nanorods was performed against strains of *Escherichia coli*, *Staphylococcus aureus and Klebsiella pnuemoniae*. The synthesized nanomaterials were identified as rod shaped with a size of around 50-100nm. FTIR spectra also indicated the presence of hydroxyl (OH), aliphatic groups, amide, stretching inorganic carbonate (-C=O), and alkyl halide functional groups on the AuNRs surface The activity of Gold Nanorods against 3 bacterial strains have shown promising results. DPPH inhibition assay revealed scavenging ability of nanorods compared with ascorbic acids at different concentrations. The *in vitro* evaluation of 4-Hydroxytamoxifen (4-OHT) and Hif 1-alpha inhibitor conjugation with Gold nanorods was carried out on MCF-7 breast cancer cell line through MTT assay. The therapeutic activity of drugs conjugated with AuNRs was slightly enhanced. These results provide rationale for further progress of AuNRs-assisted combination chemotherapy.

Chapter 1

Introduction

Nanotechnology refers to the field of advanced technology which deals with the particles of size less than 100nm (McNeil, 2005; Poole Jr & Owens, 2003). With advent of nanotechnology, new era of research began with wide range of applications in engineering, cosmetic, textile, hardware and mechanical industries (Buluş, Bulus, & Yakuphanoglu, 2020; Dikshit et al., 2019; Hameed et al., 2019; Krifa & Prichard, 2020; Ye et al., 2018). It is now being developed in biomedical applications including drug delivery, biosensors, medical equipment and bioimaging (Altintas, 2017; Corsi et al., 2018; Hu et al., 2018; Yao et al., 2018). Nanotechnologies are also widely being explored in environmental applications and to combat effects of climate change (Corsi et al., 2018).

Nanomaterials are one of the most advanced materials used in present technologies (Lu & Astruc, 2020). Nanomaterials can be organic, inorganic or composite based (Khan, 2020). Nanoparticles are widely known to show unique physical and chemical properties depending on their size such as, optical, thermodynamic, magnetic, and electrochemical properties (Khan et al., 2019; Nikzamir et al.. 2021; Zhou et al., 2021). Organic nanoparticles have their structure similar to biological membranes and possess wide range of advantages such as biocompatibility and easier delivery methods through liposomes, dendrimers, micelles and by use of protein/peptide-based carriers (Y. Shi et al., 2017; Zhou et al., 2021). Inorganic nanoparticles however are very important owing to their magnetic, fluorescent, optical and electronic properties. They include ceramic magnetic quantum dots, polystyrene and metallic nanoparticles having central core of inorganic substances that give characteristic properties to particular structure (Pugazhendhi et al., 2018; G. Yang et al., 2019).

Inorganic nanoparticles includes both metallic and non-metallic nanoparticles that are being widely used and both of them have their respective advantages (Choudhary et al., 2020; Choudhury & Goswami, 2017; Patil & Kim, 2018). However, in biological systems metallic nanoparticles are widely used due to their potential applications in theranostics such as development of efficient drug delivery system and non-invasive imaging which gives them clear advantage over conventional pharmaceutical agents (Dayem et al., 2017; Cheetham et al., 2020; Huang et al., 2020). Once administered in biological systems these

nanoparticles can be used to facilitate bio-imaging using variety to techniques such as X-ray, computed tomography, bone scan, magnetic resonance imaging and positron emission tomography (McNamara & Tofail, 2017; Mousavi et al., 2020). Commonly used metallic nanoparticles are gold (Au), iron (II, III) oxides (Fe2O3, Fe3O4, FeO), nickel (Ni), silver (Ag), gadolinium (Gd), titanium dioxide (TiO2), platinum (Pt) and zinc oxide (ZnO) (Ghaffari & Dolatabadi, 2019). Metallic NPs are preferred in various fields. They are easily synthesized, have large surface area to volume ratio, and other remarkable properties such as enhanced permeability, higher stability, wide range of chemical modification and retention of solutes, that make them promising materials in wide range of fields (N'Konou & Torchio, 2019; Sardoiwala et al., 2018). Not only these metals used have advantages but they can also be conjugated with several chemical and biological functional groups such as drugs, enzymes, receptors, ligands and antibodies, hence imparting wide window of modification that can be explored in biotechnology (Evans et al., 2018; Oliveira et al., 2019; Ovais et al., 2018) . Properties of nanoparticles are greatly affected by size, shape and nature of particles.

Of all the metallic nanoparticles employed in biological research, AuNPs, which are also referred to as colloidal gold, are of prime importance due to their unique magnetic, optical and catalytic properties (Giljohann et al., 2010; Sperling et al., 2008). AuNPs can be classified into 3 categories based on thier dimensions; one dimensional including nanotubes nanorods and nanowires, two dimensional including nanoplates, pentagon, nanospheres and three dimensional branched AuNPs including nanostars, nanodumbles and nanopods (Jin et al., 2001; Kim et al., 2013; Sardar & Shumaker-Parry, 2008). Properties of AuNPs are tunable specially their optical properties which plays huge part in their biomedical applications such as, imaging, biosensing and therapeutics (Saha et al., 2012; Sardar et al., 2009; Sperling et al., 2008). Recently, researchers have focused on developing chemical, physical and biological methods for the synthesis of AuNPs including variety of morphologies and sizes. Physical methods includes gamma or microwave irradiation, thermolytic process, photochemical process, laser ablation or sonication (Crespo et al., 2014; Nakamoto et al., 2005). Chemical methods for synthesis are commonly used that involve various chemical reactions by reducing agents. This method is more controlled and gives desirable product properties (Kuo & Huang, 2005; Ziegler & Eychmuller, 2011). Lastly there is a biological method that involves living organisms as a source such as plants,

fungi bacteria or viruses (Gericke & Pinches, 2006). These three methods have variable efficiency in producing different shapes and sizes of AuNPs.

Stability, biocompatibility and selectivity are most important factors while choosing metal of interest, method for synthesis and desired shape. Of all the shapes, AuNRs have acquired great attention due to strong narrow absorbance band. This is linked to their longitudinal surface plasmon resonance (LSPR) mode. Typically, molar extinction coefficients of AuNRs is larger than other morphologies, that enables them to give higher light absorption hence letting greater heat generation i.e., greater photothermal effect. AuNRs can also exhibit as a suitable contrast in photo acoustic imaging (Deb et al., 2019; Kah et al., 2015; Pérez-Juste et al., 2005). Wide range of sizes of AuNRs are used particularly in cancer treatment and there light scattering properties have made them an attractive candidate to be used as biosensors (Hlapisi et al., 2019; Shams et al., 2019).

Thermodynamic and kinetic controls are required for AuNRs synthesis; therefore, a lot of parameters should be considered. Similar to AuNPs, AuNRs can also be synthesized by physical, biological and chemical ways (Alekseeva et al., 2006; Gole & Murphy, 2004). Of all the available methods chemical methods are reproducible, economical and can be scaled up easily (Scarabelli et al., 2015). There are four main methods to synthesize AuNRs in wet chemistry, template method, electrochemical method, seeded method and seedless method.

The first is the template method and this method is based on gold deposition into template porous membranes that can be made up of alumina or polycarbonate. This is one of the initial methods used to make AuNRs. Once Au was deposited on nanomembranes by electrochemical plating, nanorods could be obtained in the presence of any polymeric stabilizer through selective dissolution. In the end, rods can be dispersed in solvent by means of agitation or sonication. AuNRs obtained by this method will have same diameter as that of template. Similar techniques can also be used to synthesize other nanomaterials by altering shape and size of nanomembrane template. This method is practically acceptable for initial studies but yield remain a limiting factor that discourage researchers for use of template method (Gao et al., 2011; Meng et al., 2019).

Low yield of AuNRs was overcome by new method for synthesis such as electrochemical method. The synthesis involves two electrode type electrochemical cell (Saldan et al.,

2018). A sacrificial anode gold plate is used. Different chemicals are used and then electric current is applied. This method synthesizes rods within reverse micelles in organic solved systems. This method also utilizes silver ions however the role of silver ions is not understood. It is believed that, the release rate of ions affect length of AuNRs at cathode (Niidome & Niidome, 2007). Sometimes template based electrochemical methods are also used that also shows promising results (Kim et al., 2010).

Two of the most common methods used for the synthesis of AuNRs are seed-mediated and seedless method. One of the most promising, old, well-studied and successful methods of forming nanorods is seeded method for AuNRs with face-centered cubic (FCC) metals such as silver, gold, platinum, etc. (John et al., 2013). This method is concerned with separate preparation of metal seeds which are then added to the growth solution with metal precursor. At the same time ascorbic acid, a weak reducing agent, is also added that will reduce metal ions (Nikoobakht & El-Sayed, 2003). The exact mechanism for how we receive nanorods from this method is unknown. As growth solution and seed are mixed together seed starts their growth habits to form nanorods. Although it is a simple process but slight change in chemical condition can have great impact on yield and aspect ratio of AuNRs (Sachdeva et al., 2020). Increase in concentration of gold salt leads to reduced aspect ratio with less length that is reduced stability, moreover increase in temperature and increase in seed concentration can also lead to similar effects. Hence maintaining aspect ratio is very important to have optimized size and desirable properties of AuNRs (Jana et al., 2001). Similarly, it was observed that addition of Silver nitrate increases yield but decreases aspect ratio. Concentration of surfactant such as CTAB is also very important as changing its concentration can alter the morphology (Smith & Korgel, 2008). Optimizing its concentration can also give more control over tunable properties of AuNRs (Li et al., 2018).

The limitation of seeded method was its reproducibility mostly because of two steps; therefore, seedless method makes the synthesis one pot protocol for synthesis of AuNRs. Wide range of substrates can be employed as reductants in seedless methods (Liopo et al., 2015; Requejo et al., 2018). Seedless protocols have been widely improved with every new research different factor are identified that directly affect synthesis, morphology, and optimization of AuNRs. NaBH4 and sodium oleate are one of the limiting reactants (Samal

et al., 2010) but modification of concentrations of CTAB gives advantage to control morphology in earlier stages of synthesis of AuNRs (An et al., 2017; Roach et al., 2018).

Optimization of protocol for synthesis of AuNRs is the major challenge. Once synthesized and characterized, they can be used in wide range of therapies including cancer, diabetes and autoimmune diseases (Ali et al., 2017; Liang et al., 2018; Mao et al.,, 2020; Wang et al., 2020). Scientist have been exploring the properties of these nanorods and how manipulation of size, shape and surface functionalities can affect their medicinal and biological applications (Pérez-Juste et al., 2005). They have extraordinarily high surface area to volume ratio consequently, broad window is accessible for surface alterations that might lead to change in surface electronic characteristics (Jiang & Pinchuk, 2015). As AuNRs are considered to have anisotropic shape i.e., they have two absorption bands, one as a result of longitudinal absorption while other as a result of transverse absorption. As the length of rods increases, plasmon frequency also increases this means that they show absorption peaks over wide range of wavelengths simply by change in length of rods (Bala et al., 2019; Wei & Mulvaney, 2014).

Metals are known to have different properties at nanoscale as compared to bulk counterparts (Pokropivny et al., 2007). Major focus of research community designing AuNPs is cytotoxicity (Buzea et al., 2007). Gold salt in bulk have been successfully used for treatment of rheumatoid arthritis (Shah, 2021) and are considered safe however at nanoscale with change in properties, toxicity is also increased. Cytotoxicity of cancer cells and cell viability can be measured by method of transcriptional and translational assay (MTT) (Guo et al., 2021). Researches showed that gold itself was not cytotoxic but the components for synthesis of AuNRs, such as CTAB, imparts cytotoxic affect to nanoparticles as it is known to disrupt cell membranes (Han et al., 2021; Lu et al., 2021). However, this can be reduced by coating AuNRs with polyethylene glycol. These AuNRs get entry into the cells through endocytosis which is receptor mediated (Fang et al., 2021). Different functional groups (e.g. amides) can be utilized to enhance the stability of these AuNRs. These additional coatings can also facilitate conjugation with different proteins, drugs or antibodies on nanorods surface (Centi et al., 2014; Shi et al., 2021; Soulé et al., 2011).

After cardiovascular illnesses, cancer is the greatest cause of mortality worldwide (Zaorsky et al., 2017). Traditional treatment and diagnostic options are obsolete now and possess

wide range of side effects (Devlin et al., 2017). This increases the concern among research community to explore new ways to combat this life-threatening disease. With the progresses being made in nanotechnology there is a hope that nanotechnology when associated with cancer theranostic can do wonders (Chen et al., 2021). In specific AuNRs have been proved by multiple researchers in applications such as sensing, drug delivery, tracking and imaging and photothermal therapeutics. Sensing of biological species is done by particle aggregation of AuNRs that can lead to development of highly sensitive and specific system based on nanotechnology-based sensing (Yan et al., 2021). Moreover, AuNRs are capable of changing their dielectrics and their interparticle spacing that make them best candidate for biosensing (Khan & Luo, 2021). The high specificity and sensitivity of AuNRs make them good reporters that can play effective role in developing multiplex assays (Amri et al., 2021; Zhang et al., 2021). Nanotechnology has played great role in another biological application i.e., tracking and imaging. AuNRs are intensely being investigated are contrast for imaging by use of dark field microscopy, when coupled with selective photothermal therapy by low energy lasers on cancer cells can be proposed as effective therapeutic strategy (Sudri et al., 2021). Evidence provided by light scattering confirms that AuNRs selectively binds with cancerous cell (Xu et al., 2021; Xu et al., 2021; Yan et al., 2021).

There have been tremendous discoveries in drug discovery in recent years, however, effective vehicle to transfer these drugs to target organ is a major challenge faced by researchers. AuNRs posed to be a promising vehicle to carry not only anti-cancerous drugs but also drugs for other ailments (Guo et al., 2021; Jin et al., 2021). The primary target for nanoparticle-based drug delivery has been cancer due to failure of previously employed methods to transfer drugs. Nanoparticles not only enhance the permeation of drug but also increase the retention in cancerous cells as compared to normal tissue hence known as passive targeting (Mahmoud et al., 2021). AuNRs have an extra advantage over other shapes of nanoparticles in having high surface area to volume ratio so even after loading drugs molecules on surface some room is still left for attachment of other recognition molecules such as antibodies, thus enhancing the specificity (Jin et al., 2021; Shan et al., 2021). These nanorods are known to support photothermal effects, Hence, drug release can be enhanced by dissipation of light energy as heat (Shan et al., 2021; Sudri et al., 2021). Binding of drugs with nanocarrier is of prime importance as strong binding will lead to

slow release and week binding will lead to premature drug release and targeted drug delivery would not be possible.

AuNRs are widely considered non-toxic (Jakhmola et al., 2021). Several in vitro biochemical assays can be used to evaluate toxicity such as, genetic toxicity assays, oxidative stress assays, and general viability assays. The dose-dependent toxicity of nanoparticles on cell cultures can be determined in viability assays among which the colorimetric (MTT) assay is one of the most commonly employed method which is primarily based upon the mitochondrial metabolic activity to reduce the MTT dye (Kiss et al., 2021). With the advancement of technology several kit-based oxidative assays are now available commercially that can determine the level of the reactive oxygen species (ROS), and techniques such as DNA-micro array and real-time polymerase chain reaction (RT-PCR) can be used for evaluation of effects of these AuNRs at the DNA level.

Due to flexible properties, AuNRs can be promising alternative in several biomedical applications. However, the research regarding them is in infancy stage further validation is required specially in vitro as photothermal properties of these particles although very attractive but can lead to damage of not only tissues but also alter the morphology of nanoparticles (Yang et al., 2019). Once optimized, AuNRs can act as 'gold standard' vehicle in diagnostics as well as therapeutics.

1.1 Problem Statement and Objectives

Commercially available chemotherapeutic drugs have poor bioavailability, high-dose requirements, adverse systemic damage, low therapeutic indices, non-specific targeting and development of multiple drug resistance. Nanoparticle-based drug delivery can provide distinct benefits over traditional medication therefore AuNRs can be exploited to determine their importance as a potential drug delivery system.

1.1.1 Objectives

The objectives of the research are as follows:

1. The synthesis of AuNRs.

2. The optimization of the parameters for the synthesis of AuNRs.

3. The characterization of the synthesized AuNRs using techniques like UV/Vis, XRD, FTIR and SEM.

4. The demonstration of the role of AuNRs in efficient drug-delivery systems

5. Determination of antioxidant ability of AuNRs by 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) assay.

6. Determination of the anti-bacterial activity of AuNRs against 3 different strains of bacteria.

Chapter 2

Literature Review

2.1 Nanotechnology and Nanoparticles

Scientists started using nanotechnology in applied research a couple of decades ago and it has been used in different fields of research since then (Daniel & Astruc, 2004). This concept of Nano systems and nanotechnology was properly introduced to the science world in a Lecture which was called 'There's plenty of room in the bottom' by Richard P Feynman in 1959 (Feynman, 1960). After this revelation, a great number of studies and revolutionary developments in physics, chemistry and biology started happening across the globe and nanotechnology became a hot topic. Nanotechnology basically acts as a theoretical and experimental field for applied sciences and technology. It is involved in manipulating matter at atomic and molecular level and the production of various functional systems. The term 'nanotechnology' was firstly used by Norio Taniguchi to give this field extra precision and a proper dimension (Hulla et al., 2015).

Different types of materials are produced and dealt with in the nanotechnology. Nanoparticles are basically a class of materials that are 0-100nm in size where at least one dimension of the nanoparticles should be less than 100nm (Laurent et al., 2010). These can have one, two or three dimensional shape. Researchers started more work on these nanoparticles after they found out that transition of micro-particles to nanoparticles can affect the physicochemical properties of substances. There are two major factors in this change in physicochemical properties. Increase in surface area to volume ratio is the first one while the size of the particle that moves to a realm where the quantum effects predominate is the second factor.

Nanotechnology has applications in wide range of industries. Nanotechnology products in huge numbers can already be found in the market and research is going on in full flow to widen the products even in the global markets of agriculture, non-fuel commodities and minerals (Barker *et al.*, 2009). Products of everyday use contain nanoscale materials. Few examples are: the presence of nanoscale zinc oxide and titanium oxide in sunscreens that reflects ultraviolet (UV) to avoid sunburns. Nanomaterials have been used to manufacture

batteries to provide more power, efficacy and dissipate less heat. Nanotechnology also finds its application in the manufacturing of devices, sports goods, vehicles and cosmetics.

Nanoparticles although so tiny, but can't be considered as simple molecules. They have uniqueness in their size, structure, composition and shape. Therefore, they are composed of three layers:

- A layer which is functionalized with surfactants, polymers, metal ions and various small molecules designated as the surface layer
- A layer which is different from the core of nanoparticles called the shell layer
- The most important portion of the nanoparticle which is almost nanoparticle itself, the core (Huo *et al*., 1994)

2.2 Properties of Nanoparticles

Nanoparticles have unique and important physical and chemical characteristics due to their nano-size (1-100nm) and are therefore termed as multifunctional constructs (Dionysiou, 2004). These nanoscale particles open new horizons in the field of therapy and diagnosis due to their range of dimesions which is similar to that of nucleic acids, membrane receptors and antibodies amongst other biomolecules. Along with these biomedical properties, nanoparticles' property of surface to volume ratio makes them even more valuable in the commercial sectors such as medicine, cosmetics, manufacturing and electronics (Sanvicens *et al*., 2008).

2.2.1 Size and Surface functionality

The feature of surface to volume ratio is very important in the capabilities of nanoparticles (Bruce et al., 2008). The behaviour of nanoparticles in the environment and *in vivo* can be described by their size and surface characteristics. Surface charge and hydrophobicity are very important in this regard. Other features such as their reactivity and solubility can be altered by their interaction with the environment (Nowack & Bucheli, 2007). In controlling the interaction of nanoparticles with various biological, chemical and physical systems, their size and surface functionality plays a vital role. For example, the impact of the manufactured nanoparticles in the environmental sector can be altered with different choice of coating material (Lee *et al*., 2010). In the biomedical sector, this is very important as

well. Controlling the surface properties of nanoparticles can help in increasing their efficacy in imaging and therapeutics (Kim *et al*., 2013).

The core of a nano-biomaterial is a major constituent of a nanoparticle. This core can be composed of inorganic or polymeric biomolecules and can act as an appropriate surface for molecular assembly. A membrane or layer may have surrounded it as well making it appear in the form of a nano-vesicle (Bruce et al., 2008). As for shapes, there are many which can be imagined e.g. cylindrical, spherical or plate-like (Teo, et al., 2010). If the particle has to pass through a porous structure of a membrane, the factor of shape and size can be crucial there too. When material characteristics are regulated by the quantum sized properties, the size and shape functionalities come in critical there as well (Salata, 2004).

The core of nanoparticle can be protected using various monolayers of inert materials such as silica. The surface of the nanoparticle can also be protected by the process of adsorption. Many organic materials have this ability to get adsorbed or chemisorbed on their surface (Huo *et al*., 1994). The purpose of a biocompatible material is also served by this layer. The addition of a layer of linker molecules to nanoparticles can also improve their functionalization. Both ends of this linker molecule contain reactive groups. (Salata, 2004). One group aids in its attachment to the nanoparticle, while the other aids in attaching the nanoparticle to a variety of compounds such as fluorophores, antibodies or biocompatibles depending on the function they are required for (Katz *et al.*, 2004).

2.2.2 Geometrical Structure

Nanoparticles can appear in different shapes and geometrical forms as well despite having same composition. They may have spherical, triangular, rod-shaped or cubic. Different banding patterns are provided by their different shapes. On the absorbance profile, the spherical nanoparticles show only one peak, while other shapes may form more than one peak (Mie, 1908). Due to this reason, usually spherical particles are used as they have increased uptake than those having rod-shape (Chithrani et al., 2006). Different charged polarization edges are present on differently shaped or edged nanoparticles along with corner atoms and these provide different reactivity to the particles. Different optical technologies and conductors are also developed by these (Kelly et al., 2003; Panda & Deepa, 2011).

2.2.3 Plasmon Resonance

Metallic nanoparticles show significantly different physicochemical properties than the physicochemical properties of bulk metals (Eustis & El-Sayed, 2006). The specific applications of nanoparticles determine their characterization techniques. Plasmon resonance is designated as oscillations of excited free electrons in metal.

The ultimate characteristic and importance of a nanoparticle is held by its optical property. For instance, a characteristic wine red color is exhibited by a 20nm AuNPs, yellowish grey by a Silver nanoparticle and black by the Platinum and Palladium nanoparticles (Horikoshi et al., 2013).

The Plasmon resonance optical spectrum of various nanoparticles is related to size and shape which is determined by high resolution microscopy (usually TEM). Specific geometrical shapes of nanoparticles generate various spectral responses. Heating can be used to change the particle morphology due to which the individual particle spectrum is formed which then supplies clear means to modify spectral responses to a suitable optical wavelength. The developed colloidal preparations help in the formation of a homogenous population of similar colors and shapes of the nanoparticles (Mock et al., 2002).

2.3 Classification of Nanoparticles

A variety of characteristics can be used to classify nanoparticles. That is why, based on their shape, size, chemical makeup, and physical features, they are divided into several types. Nanoparticles possess various forms of shapes and structures. They can possess shapes like spheres, rods, tubes, cages or may have irregular shape. They can also exist as agglomerates or in fused state. Following are the significant categories of nanoparticles.

2.3.1 Carbon based Nanoparticles

Carbon based nanoparticles are an important and wide group of nanomaterials. These include carbon nanodiamonds, nanotubes, grapheme, fullerenes, nanocones, nanofibres, nanoohms and their functionalized states as well (Paradise & Goswami, 2007). Carbon nanotubes and fullerenes are two important classes of these NPs. In sample preparation, a large number of these carbon-based NPs are known as sorbent substances. Carbon based NPs can also form non-covalent bonds like hydrogen bonds, electrostatic forces, hydrophobic or wan der waal's interactions with other organic molecules due to their

characteristic structures. They can also be used as adsorbent materials due to their hollow nanosized structures and covalent structures (K. Scida *et al.*, 2011; Zhang, *et al*., 2013).

Carbon nanotubes that are formed of coaxial graphite sheets are known to be related to fullerenes. These structures are also known to be excellent heat conductors due to their effective strength and electrical properties. They also possess great metallic and semiconductor behaviour. These are also very favourable for use in the drug delivery system (S. Polizu, *et al., 2006*).

2.3.2 Magnetic Nanoparticles

Mostly, these magnetic nanoparticles are in the form of spherical nanocrystals which range in size from 10-20nm. Fe^{2+} and Fe^{3} form the core of these crystals which is enclosed by PEG or dextran molecule (Park *et al*., 2007). These nanoparticles have proven to be very favourable in the bioassays for labelling biomolecules and MRI contrast agents due to their magnetic characteristics. They are also great agents for drug delivery, diagnostics and therapy due to their surface functionalization (A.H. Lu, *et al.*, 2007).

2.3.3 Organic and Inorganic Nanoparticles

These nanoparticles can have two or more dimensions and range in size from one to one hundred nm. They have distinctive thermodynamic, magnetic, catalytic, chemical, electrochemical and physical properties (Dionysiou, 2004). All of these characteristics are determined by the nanoparticles' size, shape, and chemical functionalization. Organic polymers are used to prepare organic nanoparticles, whereas, inorganic nanoparticles are synthesized from inorganic elements. Organic nanoparticles mostly do not show any sort of toxicity and they are also biodegradable. Few of these organic NPs have a hollow core which means they have a little space free at the center and so they are also designated as nanocapsules (Kumari *et al*., 2010). Just like magnetic nanoparticles, they also have exceptional capability of holding and binding drugs thus making those efficient agents for drug delivery systems. Examples of organic nanoparticles are liposomes, dendrimers, polymeric micelles and carbon nanomaterials.

Just like inorganic compounds, inorganic nanoparticles are also considered the ones which don’t possess carbon. Common examples of inorganic nanoparticles are metal oxides, quantum dots, polystyrene and metallic nanoparticles (Kumari *et al*., 2010).

2.3.4 Metal oxide based Nanoparticles

These kind of nanoparticles have just been recently introduced and developed and have soon started to attract the researchers. This interest in them has aroused to use them in the development of biosensors as immobilizing matrices (Bang & Suslick, 2010).

Nanostructured oxides of the metals like titanium, iron, tin, zinc, cerium, zirconium and magnesium are known to exhibit significant non-toxic, functionally bio-compatible, catalytic and non-morphological activities (Solanki *et al*., 2011).

Enhanced characteristics of biosensing and improved electron transfer are also carried out with the help of these metal oxide NPs because of the fact that these materials provide favourable microenvironments for biomolecules immobilization. The characteristics of metallic nanoparticles can also be modified by these metal oxide nanomaterials. The example of it can be seen when iron nanoparticles are instantly oxidized to Fe_2O_3 in the presence of oxygen which helps in enhancing its activity in comparison to other iron nanoparticles

2.3.5 Metallic nanoparticles

Metallic nanoparticles are the most studied and explored nanoparticles due to their unique physical and chemical characteristics that differ from those of their bulk components. This is because of their tiny size and high surface to volume ratio. As more and more research is being carried out on these NPs and there potential in various disciples has started to unveil, they have become a focus of interest for matter of concentrated research in various disciplines (Wang & Z. L, 2013). Nanoparticles with a larger surface-to-volume ratio are also more reactive. For instance, Gold when in bulk form is considered to be an inert substance but in the nanoscale, its reactivity with surrounding compounds increases and it acts a suitable catalyst (Brust & kiely, 2002). Their optical properties are also another important point of their significance because the electrons in the NPs are not as mobile due to their smaller size and this causes the nanoparticles to react with light in a different way than do their bulk state materials (Nath & Chilkoti, 2002). High efficiency of light scattering and absorbance is displayed by the nanoparticles (Au and Ag) which make them exhibit the property of plasmonic resonance.

Several new advancements have been made in the researches associated with the metallic nanoparticles that they may now be conjugated with drugs, antibodies, and ligands, among

other functional groups. This has paved the way to various applications in biotechnology that include drug delivery, gene delivery, magnetic separation and diagnostic imaging etc. Over the last decade or two, a number of imaging modalities such as ultrasound, MRI, CT scan, PET and optical imaging have been introduced which assist in imaging the diseased condition (Fayaz *et al*., 2010). All these imaging tools have their own category of technical functionality and also require a contrast material with unique physiochemical properties. These metallic nanoparticles such as Au, Ag and magnetic nanoparticles serve as these contrast agents. In addition to these nanoparticles, different nanocages and nanoshells have also been developed to aid in diagnostic imaging and cancer therapy (Mody *et al*., 2010).

2.4 Gold Nanoparticles (AuNPs)

AuNPs are the suspensions of nanoscale particles that are formed from gold and are also referred as colloidal gold. In the Roman era, this colloidal gold was used for decorative purposes where it was used as staining agents (Giljohann *et al.*, 2010). Different properties of suspensions of this colloidal gold from the bulk material were observed by Micheal Faraday in 1850s and this resulted in the evolution of modern AuNPs (Peter *et al*., 2007). The colloidal solution of gold either possesses an intense red or a dull yellowish color. These unique optical features are a result of their fascinating interactions with light. The free electrons in AuNPs oscillate in line with the metal lattice when exposed to an oscillating electromagnetic field. Therefore, in an aqueous solution, gold nanospheres with size around 10nm display strong absorption which is maximum around 520nm.

The size and morphology affect the properties of the colloidal AuNPs. For example, when the shape of the nanoparticles in the colloidal solution is changed, the colour they exhibit also changes. As we can see in the AuNRs, due to their rod-like shape they exhibit dual resonance. The plasmon oscillation along the long axis creates one, and the nanorod short axis creates the other. When the rods' aspect ratio is changed, the long-axis LSPR wavelength position moves from red visible to NIR, increasing the oscillation intensity. Rod-like particles display both the longitudinal and transverse absorption peaks (Sharma *et al*., 2009). Due to these distinctive optical properties, AuNPs are studied and researched for various purposes including a range of applications in electronics, therapeutics, diagnostics and material science. Due, to this reason, highly reliable and efficient synthesis methods are required.

AuNPs that are less than 100nm can be synthesized in the form of various geometric shapes such as nanorods, nanocages, nanospheres and nanoshells. They are a great option for use in biosensing as they have exceptional detection techniques like optic absorption, electrical conductivity and fluorescence (Medintz, *et al.*, 2005).

In terms of synthesis processes, they may be synthesized in a variety of ways, including chemical and physical approaches (Nikoobakht & El-Sayed, 2003). Microwave irradiation, photochemical process, γ radiation and sonochemical methods are among the physical methods of synthesis. The most common and prevalent method for their synthesis is the chemical method where gold salts, such as tetra choloroauric acid ($HAuCl_4$) also called hydrogen tetrachloroaurate, are reduced and citrate is used as the reducing agent. This method was introduced in 1951 by *Turkevich et al.* and then in 1973 it was reformed by *Frens et al.* Using this approach, monodispersed spherical AuNPs were produced. Their synthesis method can be controlled to produce various shapes of the AuNPs.

2.5 Gold Nanorods (AuNRs)

AuNRs are the nanoparticles of gold with rod-like shape and they have specifically attracted the interest of the biomedical scientists and physicists due to their small size effect, macroscopic quantum tunnelling effect, unique optical properties, surface effect and quantum size effect (Xu et al., 2021). All these properties rely on their morphology and have been used widely in therapeutics, catalysis and diagnostics such as bioimaging and in biosensors.

AuNRs range in size from a few nanometers to a few hundred nanometers. They are kind of capsule-like AuNPs and they are known to have more peculiar optical properties than spherical AuNPs. This is due to their localised surface plasmon resonance effect (LSPR) (LSPR). Due to the anisotropy in their structure, there exists a different kind of polarisation of electrons in almost all the directions of AuNRs. Individually, AuNRs have separate electron collective vibrations in both the diameter and long axis directions (Ma et al., 2021). When electrons resonate with light in the 510-530nm region along the rod's long axis, this is known as transverse surface plasmon resonance absorption (TSPR). The vibration is caused by the rod scattering incident light along its long axis. This scattering, which ranges from visible to near-infrared, is referred to be longitudinal LSPR. When the synthesis is modulated, the longitudinal LSPR peaks of AuNRs with varying aspect ratios move from

the visible to the near-infrared region. Furthermore, the longitudinal LSPR's oscillator strength is greater, allowing it to play a significant role in the optical characteristics of AuNRs (Yin et al., 2020). That is why AuNRs are referred to be tuneable.

Biological tissues are known to be relatively transparent in the near-infrared range. Many promising possibilities for both photothermal and photodynamic cancer therapy may be found in this region. Because of its capacity to increase the surface Raman scattering of adsorbed molecules, AuNRs may also be used as a Raman probe substrate (Yang et al., 2018). Despite all these exceptional applications, there are still some concerns over AuNRs in their synthesis methods, their toxicity or biocompatibility.

2.5.1 Synthesis of AuNRs

A number of synthesis methods have been established for AuNPs and AuNRs. Some of the synthesis methods that are typically used for AuNRs synthesis are as follows:

1. Seed-mediated growth method
2. Electrochemical synthesis
3. Lithographic methods
4. Photochemical reduction
5. Template-assisted growth method

Seed mediated growth method is discussed in detail here.

2.5.1.1 Seed-Mediated Growth Method

This growth method is a frequently used chemical synthesis method for colloidal AuNRs (Chang & Murphy, 2018). It is popular because of its high yield and quality along with the simplicity of the procedure. Also, there is flexibility for structural methods and ease of particle size controlling in this method. In 1989, according to a report by Wiesner and Wokaun, anisometric gold colloids were formed by the addition of gold nuclei to chloroauric acid growth solutions. HAuCl4 was reduced with phosphorous to form the nuclei and then with the help of H_2O_2, the growth of AuNRs was initiated.

In 2001, Jana et al. established the current notion of seed-mediated growth by creating colloidal AuNRs via a procedure that involved adding citrate-capped tiny gold nanospheres to a bulk chloroauric acid growth solution (Mallick et al., 2001). This growth solution was created by reducing HAuCl4 with ascorbic acid in the presence of silver ions and CTAB

(cetyltrimethylammonium bromide) as a surfactant. Ascorbic acid can convert gold ions to gold atoms in the presence of metal nanoparticles that catalyse the reduction. The same group of researchers expanded on this technology by developing a three-step approach for synthesis of long rods in the absence of silver nitrate up to a 25 aspect ratio in the absence of silver nitrate. First-stage nanorods are employed as seeds in this process for second growth. The ones that were generated in the second growth stage are subsequently used in the third development stage. This procedure also produces a huge number of spheres, which is a major disadvantage because it needs a lot of centrifugation to separate the rods from the spheres. It was also discovered that adding nitric acid during the third seeding aided the development of AuNRs with a high aspect ratio, resulting in an increase in yield.

In 2003, Nikoobakht and El-Sayed introduced two changes to the previous technique. The first was the substitution of a CTAB stabiliser for sodium citrate, and the second was the inclusion of silver ions to adjust the aspect ratio of AuNRs. This approach begins with the preparation of a gold seed solution by reducing chloroauric acid with ice-cold sodium borohydride in the presence of CTAB. The seed solution is then mixed with the Au+ stock solution, which is made by reducing chloroauric acid with ascorbic acid in the presence of the surfactant (CTAB). Before adding the seed solution, silver nitrate is added to the solution to adjust the aspect ratio and assist rod production. With an aspect ratio ranging from 1.5 to 4.5, this approach yields a very high yield, i.e. 99 percent of the nanorods. Separating spheres in this procedure does not need extreme centrifugation. Overgrowth can be halted by using sodium sulphide.

BDAC (benzyldimethyhexadecylammonium chloride) is a co-surfactant used to develop nanorods with greater aspect ratios in the original growing fluid. This binary surfactant system creates AuNRs with aspect ratios up to 10 by varying the silver contents.

2.6 Characterization Techniques

Following the synthesis of AuNRs, to gain an insight in the physical behaviour, morphological features and reactivity of nanorods, characterization is the most crucial step. Various techniques are available to characterize specific nanomaterials such as UV/Vis

spectroscopy, scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier transformed infrared (FTIR) spectroscopy (Khan *et al*., 2017).

2.6.1 Visual Color Change and UV/Vis analysis

One of the most principal phases of characterizing nanorods is their indication by the change of color of their solution (Daniel & Astruc, 2004). Firstly, in the seed solution, CTAB and chloroauric acid combined give a clear yellow color and then after the addition of sodium borohydride appears dull brownish after incubation. Now, the growth solution appears yellowish after the addition of gold salt, CTAB and silver nitrate. Then, right after addition of ascorbic acid, the solution turns colorless. Then after the addition of seed solution, the solution starts turning to purple to reddish depending on their aspect ratio and size. This phenomenon of color change lies on the principle of surface plasmon resonance exhibited by AuNRs as their size increases.

Due to higher electron oscillations on the surface of the AuNRs, the change in optical properties also plays a major role in the change of color.

2.6.2 Structural Characterization

Structural characterization is used to examine the properties of nanoparticles such as composition and nanomaterial bonding. Zieta sieze analysing, Energy-dispersive X-ray spectroscopy (EDS) and X-ray Diffraction (XRD) are the most common techniques used to get an understanding of the specific nanomaterial. To study the phase of the nanomaterial and to analyse the crystalline structure, XRD is mostly used. By analysing it with Debye Scherer equation, it also gives a vague idea about the size for nanoparticles of single or multi phases. EDS analyses the elemental composition of nanomaterials (Veith et al., 2002). This technique not only gives an idea about the intuition of the elements in the sample, but also their relative abundance.

Another technique X-ray photoelectron spectroscopy (XPS) can be used to determine the occurrence of bonding between elements in a nanoparticle and their elemental ratio (Chen *et al*., 2011).. Simple spectroscopic value is the factor in which the principal of this technique depends and this technique also provides an in-depth knowledge of the variation of composition of any specific nanoparticle. Along the X-axis in XPS, lies the bonding energy of electrons, while number of electrons is depicted on Y-axis.

The nanomaterial vibrations are characterized by Fourier Transform Infrared Spectroscopy (FTIR) and Raman spectroscopies. Among the techniques or practices that provide elemental analysis of nanoparticles, these two are the most established. Exclusive molecules that can be used as capping agents and which can support the stabilization of AuNPs can be identified through FTIR. Other methods such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM), and dynamic light scattering (DLS) are commonly employed to acquire information regarding the size of nanoparticles. All of these can tell the size and analyze them, but Zeta Potential has the ability to predict and analyze the size of extremely small nanoparticles (Bhardwaj *et al*., 2009).

2.6.3 Morphological Characterization

As previously stated, the size and shape of nanoparticles are the two most essential elements that impact their varied properties. Transmission electron microscopy (TEM), Scanning electron microscopy (SEM), and Polarized Optical Microscopy are common techniques for analysing morphological features (POM). SEM is based on the principal of electron scanning and it provides all the basic information about nanoparticles. TEM also has principal based on electron transmittance (Williams & Carter, 1996). Through this technique, all the information about nanoparticles can be collected with high to low magnification. It is also reported that if a nanomaterial has multiple or several layers, TEM can also analyze that such as quadrupolar hollow spherical structure of Co_3O_4 nanoparticles.

2.7 Applications of AuNRs

AuNPs have been used in various forms especially as additives for aesthetic purposes but during the last decade, there have been researches in both nanoparticles and nanorods and they have gained increased popularity among researchers as tools for bioimaging, biological sensing, therapeutics and drug delivery. They also show various antimicrobial and antioxidant properties.

2.7.1 Sensing

Because of the interaction between their size, shape, and surroundings as a consequence of changes in dielectric and interparticle spacing, AuNRs are appropriate materials for sensors. Yu and Irudayaraj performed a multiplex biosensor test in which they monitored

variations in the plasmon bands caused by slight changes in the immediate environment to detect varied responses of AuNRs towards particular targets. The CTAB bilayer of AuNRs with varied aspect ratios was substituted with 11-mercaptoundecanoic acid, which then binds to human, mouse, or rabbit antibodies. Different red shifts were identified in the nanorod plasmon bands, and the binding of three recognition agents to their specific ligands was also examined (Stone et al., 2011). Mathematical models were employed to determine these parameters and binding events. AuNRs were shown to have high specifity and sensitivity, as well as a dynamic response in the range of 10-9-10-6. For a stronger probe-target affinity, the detection limit can be lowered to femtomolar levels. Huang et al. took use of the ability to track refractive index changes caused by molecular interactions in AuNRs' near proximity.

2.7.2 Imaging

Isotropic gold nanospheres' one of the earliest application was their use as contrast agents for imaging in both bright and darkfield imaging systems. After that, other structures of AuNPs which include nanorods have been used for similar purpose. Darkfield microscopy occurs when steady-state white light is blocked, allowing only dispersed light to be detected (Stone et al., 2011). Single AuNRs may be seen as a single point of scattered light under darkfield imaging due to a particular resolution of roughly 200 nm, because scattered light propagates as a cone. Darkfield photography allows researchers to track nanorod movement "visually" as their surroundings change.

2.7.3 Photothermal Therapeutics

Several papers have defined gold nanostructures as therapeutic agents that use laser-induced photothermal heating to selectively kill malignant cancer cells while leaving healthy cells unchanged (Stone et al., 2011). The success of photothermal therapy is determined on the strong electromagnetic fields associated with the metal surface, which convert the energy from absorbed photons into heat, causing permanent cell damage. Photothermal therapy is regarded to be less harmful to human tissues than typical chemotherapies when it comes to cancer treatment. For medical photothermal therapy, nanorods whose absorption band maximums can be tuned to this wavelength regime are advantageous since the NIR area of the electromagnetic spectrum has increased tissue transparency.

2.7.4 Drug Delivery

With technological advances, many new traditions for treating difficult diseases have emerged, and nanotechnology has identified new features in disease diagnosis and treatment (Takahashi et al., 2005). Since nanoparticles can be made in a variety of sizes and morphologies, they have a lot of potential as a drug delivery vehicle in different cells. Antibodies and antitumor agents are the most commonly used drug delivery vehicles. The surface of AuNPs, in particular, provides a robust medium for advanced modifications such as conjugating functional molecules to form a specific monolayer that is associated with improved dispersion in organic media, extended stability, and the addition of targeted drugs or probes (Stone et al., 2011).

These changes to AuNPs allowed them to be targeted at cancerous sites in a passive or active mechanistic manner. Tumors are coated with antitumor drugs using the improved permeability and retention (EPR) effect in a passive method of targeting. Active targeting drugs or peptides, on the other hand, are conjugated with AuNPs for tumour detection and targeting. AuNPs' interaction with various functional groups is another feature; these functionalized AuNPs have been identified as active gene delivery vehicles. PEGylated AuNPs, for example, have been identified as an effective transfection mechanism, and the rate of gene expression was increased by about 100 times when DNA was conjugated with PEGylated AuNPs versus naked DNA. These transgenes were said to be stable in the bloodstream.

In recent years, AuNRs have gained popularity as drug delivery vehicles. In one process, near-IR laser pulses are exposed to DNA–AuNR complexes, resulting in DNA release due to photo-induced morphological changes in the nanorod.

2.7.5 Antimicrobial Activity

Because of their simple mode of synthesis, high efficiency of transfection, and customization of their surface by conjugating several molecules, AuNPs, among all metallic nanoparticles, have a wide range of applications in every possible aspect (Majdalawieh *et al*., 2014). While AuNPs do not have the same degree of antimicrobial activity as silver nanoparticles, they do have some antibacterial activity. AuNPs have been identified as a unique alternative to high-dose antibiotic administration, and as a result, they

have been found to be very effective against a variety of infectious diseases, including antibiotic-resistant infections.

The toxicity of nanoparticles administered, their surface modifications, fundamental properties, and the bacterial species being studied all influence the antimicrobial activity of AuNPs. Smaller AuNPs are known to do more damage to bacterial cells by entering them and eventually killing them (Bindhu & Umadevi, 2014).

2.7.6 Antioxidant Activity

Researchers demonstrated that multiple nanoparticles serve as a potential antioxidant and free radical scavenger, thanks to the widespread use of nanotechnology in the prevention and treatment of infectious diseases (Elswaifi et al., 2009). A compound's or substance's anti-oxidant potential aids in the defence of cells from oxidative stress induced by the presence of free radicals. The ability of a substance to inhibit oxidation of molecules is generally due to its ability to cause the inhibition of an overall origination phase of an oxidation chain reaction by the creation of natural stable radicals that are not very reactive. A large number of oxidants with a high reductive potential for removing free radicals have been reported (Gordon, 1990). These materials have the potential to reduce and minimise a variety of syndromes, including heart disease and atherosclerosis, which is caused by the oxidation of very low density lipoproteins (VLDL). Several pathogenic agents have been documented to retain their pathogenicity by being able to produce free radicals, which then cause cell damage. For example, the toxin pyocyanin produced by Pseudomonas aeruginosa is known to cause cell damage and compromise the immune system by producing reactive oxygen species (ROS). Nanoparticles as antioxidants provide unique opportunities to combat pathogen virulence and their ability to form biofilms, both of which are linked to elevated oxygen levels and ROS development (Raghupathi et al., 2011).

There are several methods for determining a substance's antioxidant potential, but the DPPH assay has been shown to be a well-described synthetic solid radical for evaluating biosynthesized AuNPs (Kumar *et al*., 2015). The capacity of AuNPs to reduce DPPH by accepting hydrogen and electron was calculated spectrophotometerically due to a shift in colour from deep purple to yellow. In a comparison analysis, silver nanoparticles were found to have higher inhibition than AuNPs, which can be due to the fact that silver is a strong oxidant and loses electrons easily (Elswaifi *et al*., 2009).

Chapter 3

Materials and Methods

The research is based on the chemical synthesis, characterization and applications of Gold Nanorods. The research was carried out in the Cancer Biology Lab of Atta-ur-Rahman School of Applied Biosciences (ASAB), National University of Science and Technology (NUST), Islamabad. The materials and methods used in this research are as follows:

3.1 Synthesis of Gold Nanorods

Gold nanorods were synthesized using the chemical synthesis method. There are two main methods in chemical synthesis named as seed-mediated method and seedless method. Seed-method was adapted for this research (Chang & Murphy, 2018) and the chemicals used in this methods are mentioned in Table 1.

Table 1 List of chemicals used in the synthesis of gold nanorods

Chemicals	Concentration
Tetrachloroauric acid ($HAuCl_4$)	0.01M
Cetyl trimethylammonium bromide (CTAB)	0.1M
Sodium Borohydride ($NaBH_4$)	0.01M
Silver Nitrate ($AgNO_3$)	0.01M
Ascorbic Acid	0.1M
Hydrochloric acid (HCl)	1%

Firstly, the seed solution was prepared by taking 125µl of 0.01M tetra-chloroauric acid ($HAuCl_4$) solution in a test tube. 5ml of 0.1M Cetyl trimethylammonium bromide (CTAB) solution was added to this solution. The color of the solution changed to yellow. Then 300µl of freshly prepared ice-cold 0.01M sodium borohydride ($NaBH_4$) solution was added to it as it degrades after 15 minutes of preparation. The solution was then vigorously stirred for

10 minutes keeping the test tube uncapped so that gases may release. This solution was kept for two hours at 37°C and the color changed was observed after two hours which was brown. After the preparation of seed solution, growth solution was prepared. 10ml of CTAB solution was taken in a falcon tube. Then50µl of 0.01M Silver nitrate ($AgNO_3$) was added in it followed by addition of 500µl of HAuCl4 solution in it. This resulted in a clear yellow colour. After that, 55µl of 0.1M Ascorbic acid solution was added to it which changed the solution's colour to colourless. 12µl of the seed solution previously prepared was then added to the growth solution. The pH of the solution was adjusted to slight acidic (approx. 6) using minute quantities of HCl. The solution was incubated at 37°C overnight. After the preparation of this solution, it was then centrifuged at 13000rpm for 10 minutes and then supernatant was discarded to get rid of excessive surfactant. The pellet was dispersed in distilled water and was centrifuged again at 13000rpm for ten minutes to again redisperse the pellet in the distilled water.

3.2 Characterization of Gold Nanorods

3.2.1 UV/Vis Spectrophotometry

The ultraviolet-visible spectrophotometer of model AE-S90-2D (A and E Lab, UK) was used to confirm the synthesis of AuNRs because these particles interfere with light at a certain wavelength, resulting in unique optical properties. The synthesis of AuNRs was confirmed by measuring the absorbance spectra of reaction mixture aliquots in the 300-800nm range. The spectra were collected using a 200µl test volume and a quartz cuvette with a 1cm path length. For spectrophotometer's base line correction, distilled water was used as a blank. The data was registered, stored, and analysed using UV/Vis spectral analysis tools. For comparison, the numerical data was plotted on graphs.

3.2.2 Scanning Electron Microscopy

An instrument of model JSM-6490A (JEOL company, Tokyo, Japan), was used to determine mean particle size, uniformity, and morphology of nanoparticles using SEM analysis. For each sample, the microscope range's accelerating voltage was set to 10kV. In glass vials, 10µl of colloidal gold nanoparticle solution was combined with 1ml of distilled water. These glass vials were sonicated for 2 hours at 37°C. A drop of this homogenised sample was put on 1 cm^2 cut glass slides and dried for fifteen minutes in the oven. To make these samples conductive, they were sputter coated with gold palladium using a Sputter

coater model no. JFC-1500 at 18-20 mA, 240V to a thickness of 15nm, then used for SEM analysis. For the characterization of AuNRs, images were taken at various magnifications and resolutions (10000X, 15000X, 43000X; 0.5µm, 1µm 2µm).

3.2.3 Fourier Transform Infrared (FTIR) Spectroscopy

The Spectrum-100 spectrometer (Perkin-Elmer, USA) was used to determine the functional groups present on the AuNRs. Due to its hygroscopic properties, KBr was combined with air-dried samples to eliminate any remaining water molecules. The sample and KBr pellets were formed using a hydraulic press. Infrared waves were used to scan these pellets, which were scanned at a resolution of $4cm^{-1}$ and a length of $4000\text{-}350cm^{-1}$. The obtained spectra were plotted as wave number (cm-1) vs. transmittance (percent) on the X-axis. The manual peak picking method was used to compare the peaks obtained from these spectra to standard functional group charts.

3.2.4 X-Ray Diffraction (XRD)

XRD analysis was performed on an air-dried sample of AuNRs using an X-ray generation system, model D8 Advance with DAVINCI design (BRUKER, Germany). A spectrum was recorded after the sample was loaded onto the instrument's glass plate. The instrument was optimised at 40kV and 30mA with Cu-Kα radiation. Samples were scanned at 2θ range of 20-80O at 6O/min with an increment of 0.02O interval. The average crystallite diameter was calculated using the Debye-Scherrer equation: $D = 0.9\lambda/\beta\cos\theta$, where D (nm) represents the size, λ (nm) represents the wavelength of Cu-K radiation, β ($\lambda = 1.58$) represents the full width at half maximum (FWHM), and θ (radians) represents half of the Bragg angle.

3.2.5 Antibacterial Activity

One of the key applications of AuNRs is antibacterial activity. Three pathogenic bacterial isolates were used in a disc diffusion assay to evaluate this activity.

3.2.5.1 Collection of Bacterial Isolates

Identified strains of *Escherichia coli, Klebsiella* and *Staphylococcus Aurues* were collected from a Microbiology Lab, ASAB. All these strains were grown on Luria Broth Media (Sigma Aldrich, Germany) overnight to get new and fresh cultures. Then, these isolates were further streaked on Nutrient Agar (Sigma Aldrich, Germany) the next day to get fresh

and isolated colonies. After that Muller Hinton Agar (Sigma Aldrich, Germany) was prepared as it is usually used for anti-bacterial assay. It was poured and its plates were prepared and kept.

After that, loop full bacterial cultures were taken and were mixed with saline in 1.5 ml eppendorfs. This solution of all the cultures mixed in saline was then compared with Mcfarland solution and the turbidity of all the solution was same as that of Mcfarland solution so this implied that they could be used for swabbing. The MHA plates were then swabbed with the colonies of all the three bacterial cultures taken (*Escherichia coli, Klebsiella* and *Staphylococcus Aurues*).

3.2.5.2 Preparation of Gold Nanorods Concentrations

In order to disperse the AuNRs pellets, 700µl of deionized water was used. These suspensions were used for the evaluation of antibacterial activity.

3.2.5.2.1 Well Diffusion Method

Antibacterial activity of AuNRs was evaluated through Kirby-Bauer disc diffusion method *(Kirby et al., 1956)*. This experiment was performed in laminar flow hood after application of UV light for 15 minutes to avoid contamination. Nutrient agar petri plates were prepared for this activity. 4 wells were made on each plate of each bacterium. One well was a negative control and the other three had different concentrations of Gold Nanorods (35µl, 45µl and 55µl). A positive control plate which contained antibiotic discs was taken against each bacterium. All these plates were then incubated at 24°C overnight and the zones of inhibition were seen and analysed the next day.

The wells in each plate were made using a sterilized pipette tip (1000µl). Also, all the activity was performed in triplicates.

3.2.6 Antioxidant Activity of Gold Nanorods

These newly synthesised and fully characterised AuNRs were then tested using the 2, 2-di-phenyl-2-picryl hydrazyl hydrate (DPPH) assay to see how effective they were at free radical scavenging activity. This approach is the most effective and commonly used for assessing the antioxidant activity of a variety of materials.

A modified protocol was followed in this study (Wang *et al*. 2008). This assay is established on the ability of antioxidants to donate an electron and hydrogen. DPPH is known to be a calorimetric material and has the potential to interact with the antioxidants in a way that results in their reduction. A change in color of DPPH from deep purple to yellow is used as an indication and is observed by spectral analysis of UV/Vis spectrophotometer at 517nm. For the analysis of antioxidant ability of AuNRs, fresh 1mM DPPH solution was prepared in methanol and different AuNR concentrations were also prepared (50µg/ml, 100µg/ml, 250µg/ml and 500µg/ml) and were added to the solution. Control samples were untreated DPPH solution in methanol whereas, test samples were AuNRs treated with methanolic DPPH solution. The formula used to assess the free radical scavenging ability is:

% scavenging radical = [Absorbance of Control – Absorbance of Test Sample/ Absorbance of Control] X 100

This experimentation was completed in triplicates and acquired results were plotted on graph to compare antioxidant property of AuNRs with Ascorbic acid which has the highest antioxidant ability at different concentrations.

This reaction mixture was kept in dark at 37^{O}C for 30 minutes prior to measuring their absorbance at 517 nm.

3.2.7 In *vitro* evaluation of Drug conjugated Gold Nanorods

3.2.7.1 Chemicals Preparation

Various chemicals were prepared as working solutions from stock for use during the experiment in this study. To conjugate drugs with nanoparticles, the drugs had to be diluted from stock, and a linker working solution had to be prepared as well.

3.2.7.1.1 Polyethylene Glycol (PEG)

For coating drugs over nanorods, polyethylene glycol was used as a linker. To make a 10% PEG linker, 1g of PEG 6000 (Sigma Aldrich, USA) was dissolved in 10ml of deionized water and stirred continuously with a magnetic stirrer until fully dissolved.

3.2.7.1.2 Drugs Stock Solution

Two drugs HIF 1 alpha inhibitor (Chem Cruz, USA) and (Z)-4-Hydroxytamoxifen (Sigma Aldrich, USA) were used, and 1% ethanol was added to each drug to achieve the final concentration required for testing purposes. Each drug was prepared at a concentration of 50g/ml using a different protocol. This concentration was obtained by dissolving 5mg of HIF 1 alpha inhibitor in 100ml of 1% ethanol. Similarly, 1g of (Z)-4-Hydroxytamoxifen was dissolved in 200ml of 1 percent ethanol to make a 500 g/ml drug stock.

Table 2 Preparation of Drugs Solutions

Drug	**Drugs Stock Solution**		
	Drug Amount	1% Ethanol	Final Concentration
HIF 1 alpha inhibitor	5mg	100ml	50µg/ml
(Z)-4-Hydroxytamoxifen	1g	200ml	50 µg/ml

3.2.7.1.3 Complete RPMI preparation

Roswell Park Memorial Institute (RPMI) 1640 media (Fischer Scientific, UK) contains more glutathione (a reducing agent) and vitamins than Dulbecco's modified media and is used to grow a variety of cell lines. 10% foetal bovine serum (FBS) (Fischer Scientific, UK) was added to RPMI 1640 to provide growth factors for cellular differentiation and proliferation, while 1% Penicillium-streptomycin (Pen-strep) (Fischer Scientific, UK) was added to avoid bacterial contamination (prophylaxis). The cell line that was used in this study was MCF-7 cell line.

3.2.7.1.4 MTT Preparation

The yellow dye 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) (Sigma Aldrich, USA) is used to assess cell metabolic activity (including cell viability and cytotoxicity). Since it is a light-sensitive dye, it was stored at -20oC after being wrapped in aluminium foil to shield it from light. Since it is carcinogenic, it should be treated with caution.

MTT (5mg) was added per 1ml of Phosphate Buffered Saline (PBS) (Fischer Scientific, UK) to prepare the dye as a working solution to determine cell viability.

3.2.7.2 Gold Nanorods conjugation with Drugs

In this study, two drugs HIF-1 alpha inhibitor and (Z)-4-Hydroxytamoxifen (4-OHT) were conjugated with AuNRs synthesized using seed mediated chemical synthesis. The protocol of drug conjugation was as follows:

- Equal proportions of Nanorod solution and PEG solution (synthesis of PEG solution explained in the previous section) were first subjected to vortexing for 5 minutes and then incubated for 24 hours and its UV/Vis analysis was done
- The nanorod-PEG solution was then mixed with drug solution (synthesis of drug solution synthesis also explained in the table above) and were vortexed for 5 minutes and incubated for 24 hours. It's UV/Vis analysis was also done

Table 3 Properties of chemicals used for drug conjugation with their quantities and company

Chemicals Required for Drug Conjugation					
1- Anti-cancerous Drugs					
Drug		**Quantity**	**Condition**	**Form**	**Company**
Hypoxia Inducable factor-1 (Alpha) Inhibitor		1mg	-20°C	Solution	ChemCruz
(z) 4-Hydroxytaoxifen		5mg	4°C	Solution	ChemCruz
2- Polyethylene Glycol (PEG)					
Substance Quantity	**Density**	**Condition**	**Form**	**pH**	**Company**
1kg Polyethylene Glycol (PEG) 6000	1.5gm/cm^3	20°C	Solution	5-7	Sigma Aldrich

Table 4 Concentrations of AuNRs and drugs

Concentration of Gold Nanorods	
AuNR	**Concnetration**
Solution of AuNRs	50µg/ml
Concentration of Drugs	
Drug	**Concentration**
Hypoxia Inducable factor-1 (Alpha) Inhibitor	50µg/ml
(z) 4-Hydroxytaoxifen	50µg/ml

Table 5 Concentrations of Hif-1α inhibitor and 4-OHT conjugated AuNPs

Concentration of Drug Conjugated AunNRs	
Names	**Concentration**
Hypoxia Inducable factor-1 (Alpha) Inhibitor conjugated AuNRs	19, 28 and 43µg/ml
4-OHT conjugated AuNRs	23, 33 and 46µg/ml

3.2.7.3 Drug conjugated Gold Nanoparticles cellular viability evaluation

MCF-7 cells were cultured in complete RPMI-1640 (Sigma Aldrich, USA) and incubated at 37 °C in 5% CO2 at 37 °C. The cells were incubated at 37 ° C in a 5% CO2 atmosphere. Phosphate-buffered saline (PBS) (Sigma Aldrich, USA) was used to wash cell lines before each test. After centrifugation, cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) solution (Sigma Aldrich, USA) and resuspended in full RPMI at 2000 r.p.m for 2 minutes.

3.2.7.4 Cell Counting

The cells that were obtained after centrifugation from flask contain confluent cell lines counted using haemocytometer. Cells counted from 4 wells named as A, B, C, D. A=108, B=95, C=89, D=92 Cell counting was performed using (Creghton University, 2012; Maria Fuentes, 2020) formula given below:

$$\boldsymbol{Cell\ count\ average} = (\boldsymbol{number\ of\ cells} \div \mathbf{4}) \times \mathbf{2} \times \mathbf{10^4}$$

$$\boldsymbol{or}$$

$$\boldsymbol{Media\ required\ for\ plating} = (\boldsymbol{number\ of\ squares\ counted} \times \mathbf{1} \times \mathbf{10^4} \boldsymbol{cells\ ml}) \div \boldsymbol{Total\ counted\ cells}$$

The total cells per ml counted was $192\times10^4 = 1.92\times10^6$ /ml

Three dilutions each of Hif-1α inhibitor and 4-OHT conjugated AuNRs were needed. Also, three dilutions for AuNRs and three dilutions of AuNRs+PEG were also needed to see if there was any effect of these on the cell line. The media and number of cells were counted accordingly. Each well must have 10,000 cells and one plate contains 96 wells. Thus calculation was done accordingly and 20ml of media was added to cells containing media and after homogenization and vortexing used to fill the wells of plate carefully.

3.2.7.5 Dilution Plating

On the next day, dilutions were prepared from 2.5µg/ml, 5µg/ml, 10 µg/ml for Hif-1α inhibitor conjugated AuNRs and 4-OHT conjugated AuNRs. Also same dilutions were prepared for solutions of AuNRs and AuNRs+PEG. Hif-1α inhibitor and 4-OHT positive control and negative control were also labelled. All the dilutions were performed in three replicates, and then 200µl of each dilution was added on the plate accordingly. Afterwards, plate was covered without disturbing wells and wrapped with aluminium foil. Subsequently, the plates were labelled with cell line, date and supervisor name kept in incubator at 37 °C temperature under 5% CO_2 for 24 hours.

3.2.7.6 MTT Assay

The MTT assay is the most common form of cell line viability assay. MTT (3-(4,5 dimethyl-2-thiazolyl)-2,5,5-diphenyl-2H-tetrazolium bromide) is a cell proliferation dye that is used in vivo. The use of tetrazolium salt to determine the metabolic function of cells from microbial to mammalian was typical in cell biology. The methodology used included

many steps. Firstly, viability of cells was observed under microscope after 12, 24 and 48 hours and then was replaced in incubator. 15-20µl of MTT was added in each well of the plate. It was then incubated at 37°C for 3 hours. Purple-colored farmazan crystals appeared in wells. Media containing MTT was cleared by slightly tilting the plate to avoid damaging farmazan crystals. 150µl of Dimethyl sulfoxide was then added in each well. Optical density (OD) was then checked under spectrophotometer at 550nm.

Chapter 4

Results

4.1 Initial Confirmation of AuNRs Synthesis on the Basis of Color Change

As the reaction proceeded, the color change was the most initial and the easiest way to detect or confirm the synthesis of Gold Nanorods. As mentioned in the methodology section, the seed-mediated method was adapted for the synthesis of Gold Nanorods. So, the first color change was visible after the formation of the seed solution. As per previous studies, the color came out to be dull brown.

Figure 1 Figure depicting the colour change of the seed solution to dull-brown proving the formation of gold seeds in the process of synthesis of gold nanorods

Similarly, the color changed during the gold nanorod after the addition of ascorbic acid. The bright yellow color of the gold salt changed to colorless.

After the addition of gold seed and incubation for 24 hours, the gold nanorod solution developed a colour depending on the aspect ratio. The colour can vary from blue to purple

to slight green to red. These aspect ratios can be obtained using different concentrations of gold salt and silver nitrate.

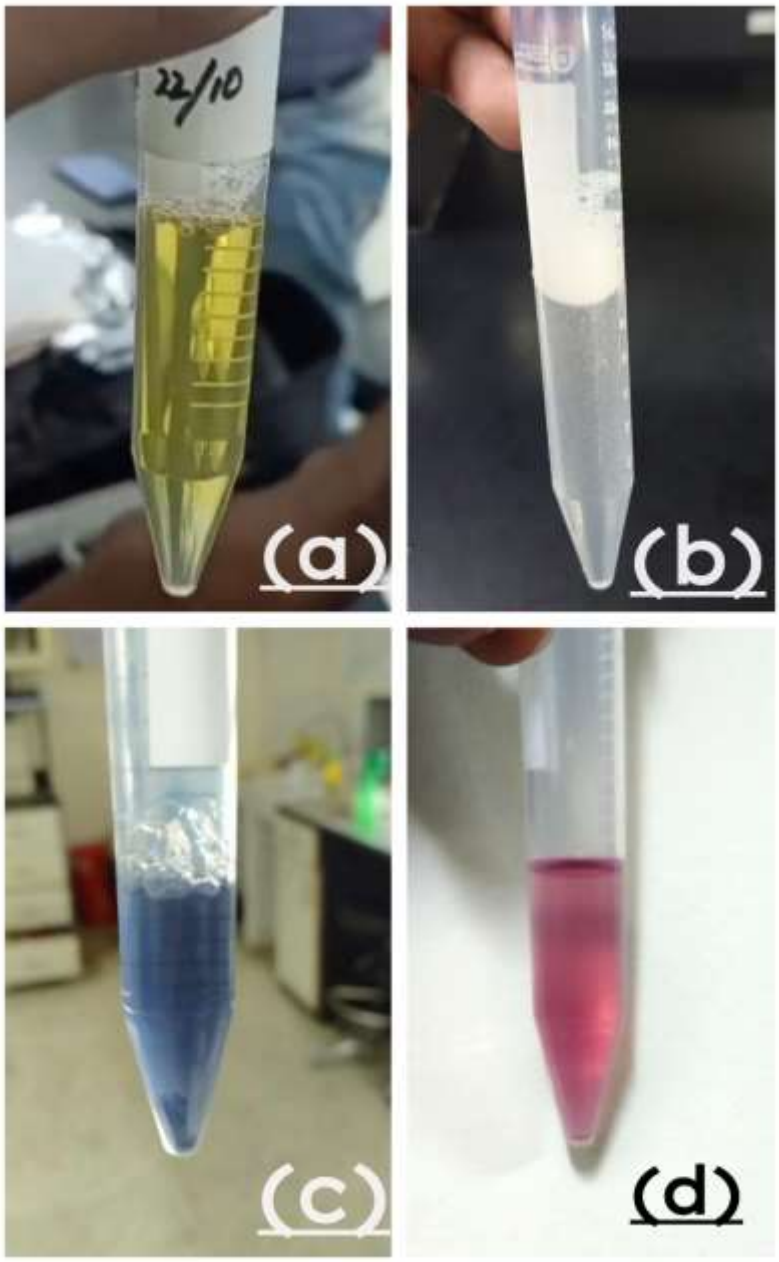


Figure 2 Figure depicting the pattern of colour change through the growth solution during the process of formation of gold nanorods (a) shows the bright yellow coloured solution before the addition of ascorbic acid (b) shows the colorless solution formed after ascorbic acid reduced the gold present in the solution (c) and (d) show the colors of gold nanorods of different aspect ratios. The aspect ratio depends on the concentrations of Silver nitrate/ Silver ions added to solution

Figure 3 shows the nanorod solutions obtained after the gowth solution after 24 hours was centrifuged and then dispersed in water so as to eliminate any surfactant in the solution

The last photo was taken after centrifuging different nanorods solutions obtained by using different gold salt concentrations.

4.2 Confirmation by UV/Vis Spectral Analysis

The synthesis of AuNRs was further confirmed by Ultra-Violet/Visible (UV/Vis) spectrophotometer. Two absorbance peaks usually lie in the case of AuNRs. At a lower wavelength, there was the transverse peak that came around 530-550nm and the other one was the longitudinal that came at a higher wavelength from 650-800nm depending upon the aspect ratio of the nanorods. Figure shows the UV/Vis spectra of the nanorod solution showing both the transverse and the longitudinal peaks around 540 nm and 700nm respectively which confirmed the synthesis of AuNRs in the reaction mixture. The spectral analysis between 200-300 nm was not analysed as certain proteins show absorbance between these regions and contribute in making noise.

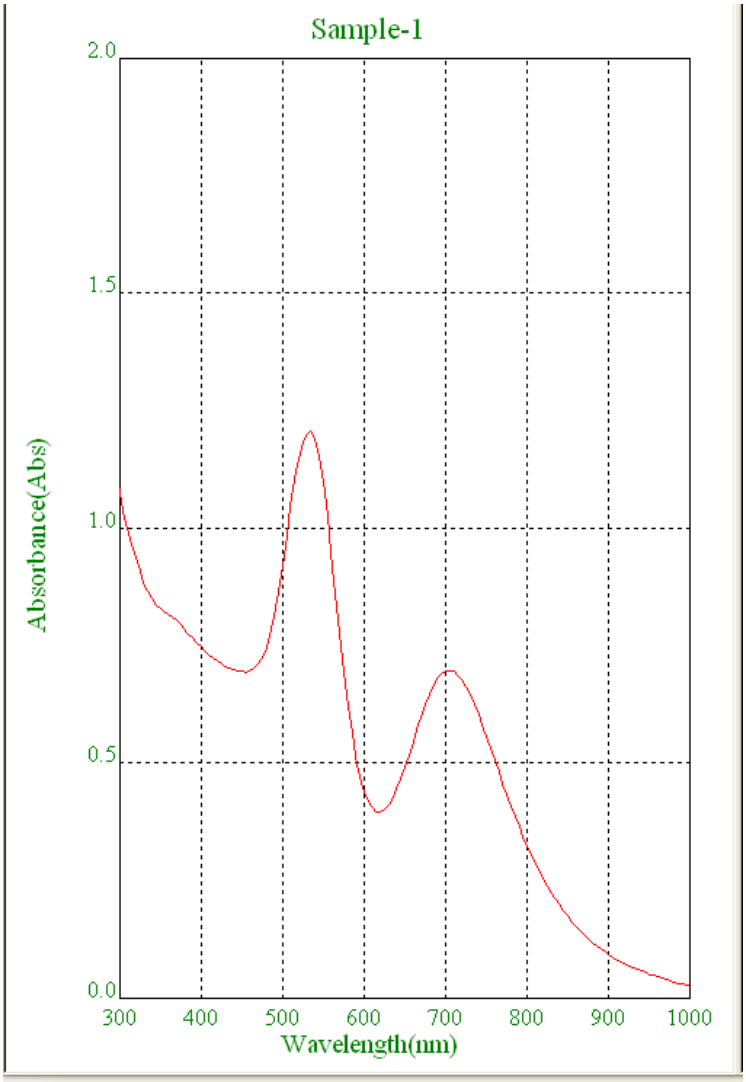


Figure 4 Graph of UV/Vis analysis illustrates the absorption spectra of gold nanorods solution. First peak around 530nm is the transverse peak and the second peak is around the 700nm mark which is the longitudnal peak

When the gold naorods were conjugated with PEG, the UV/Vis analysis was pefomed again to check the stability of the nanorod-ligand nanosystem and both the peaks were seen there too referring that the nanosystem was a stable one.

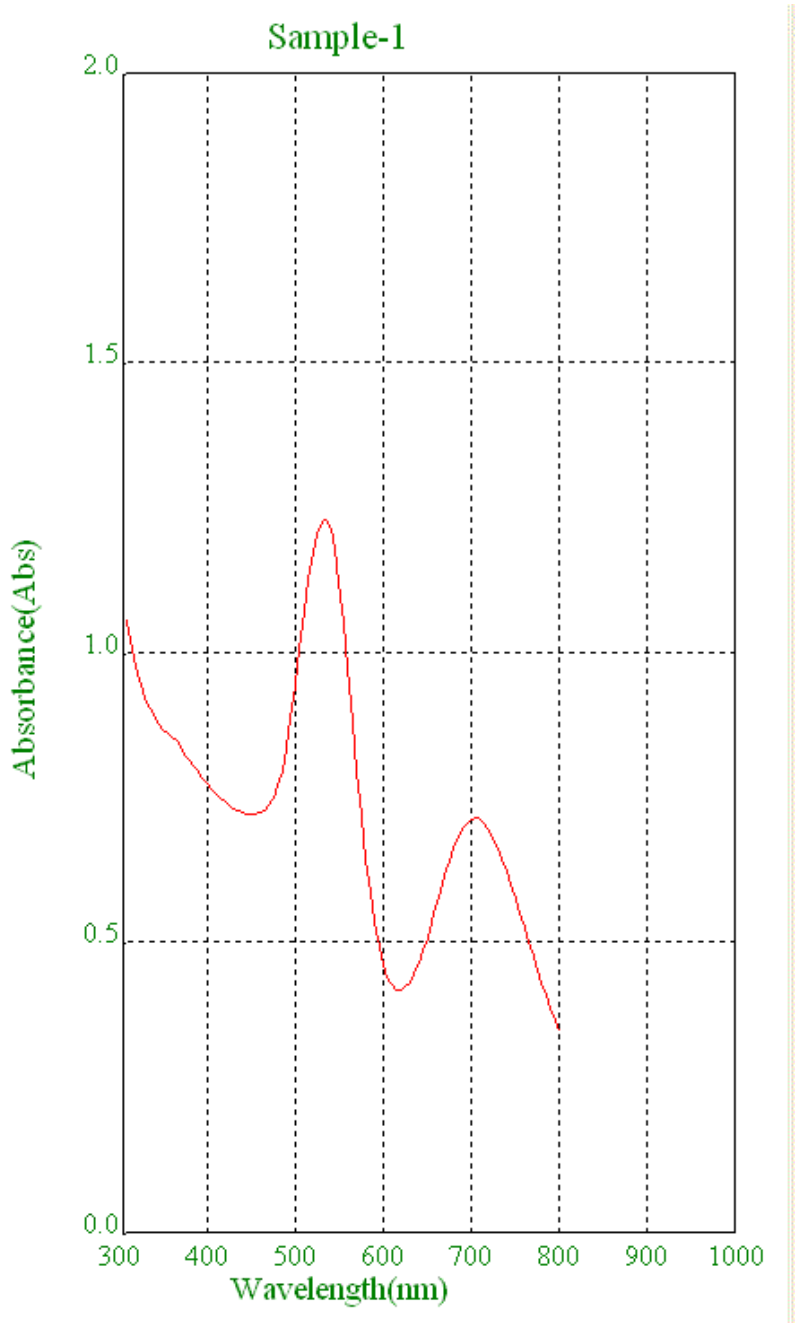


Figure 5 Figure illustrates the stability of the nanoparticle-ligand nanosystem (Nanorods-PEG). Both the peaks i.e. transverse and longitudinal peaks can be seen in the graph showing the absorption spectra of gold nanorods-PEG nanosystem solution

4.3 X-Ray Diffraction (XRD) Analysis

In order to confirm the crystalline structure of AuNRs, XRD analysis was performed on D8 Advance (Bruker, Germany). The instrument was operated at 40 kV and 30 mA with Cu-Kα radiation (λ = 1.54 Å). It required the sample to be in solid state, thus AuNRs crystals were used for this purpose. The intensity of diffracted X-rays was recorded from 0^O to 80^O. It was deduced from the analysis that the shape identified was face centred cube with the lattice parameter of a=b =4.0796 and c=4.083. The diffraction pattern of the

chemically synthesized AuNRs is presented in the Figure 6. AuNRs displayed gold peaks at around 38.06℃, 47.16℃, 64.54℃ and 77.28℃ and their peaks can be indexed to the (111), (200), (220) and (311) respectively, as indicated in the Figure.

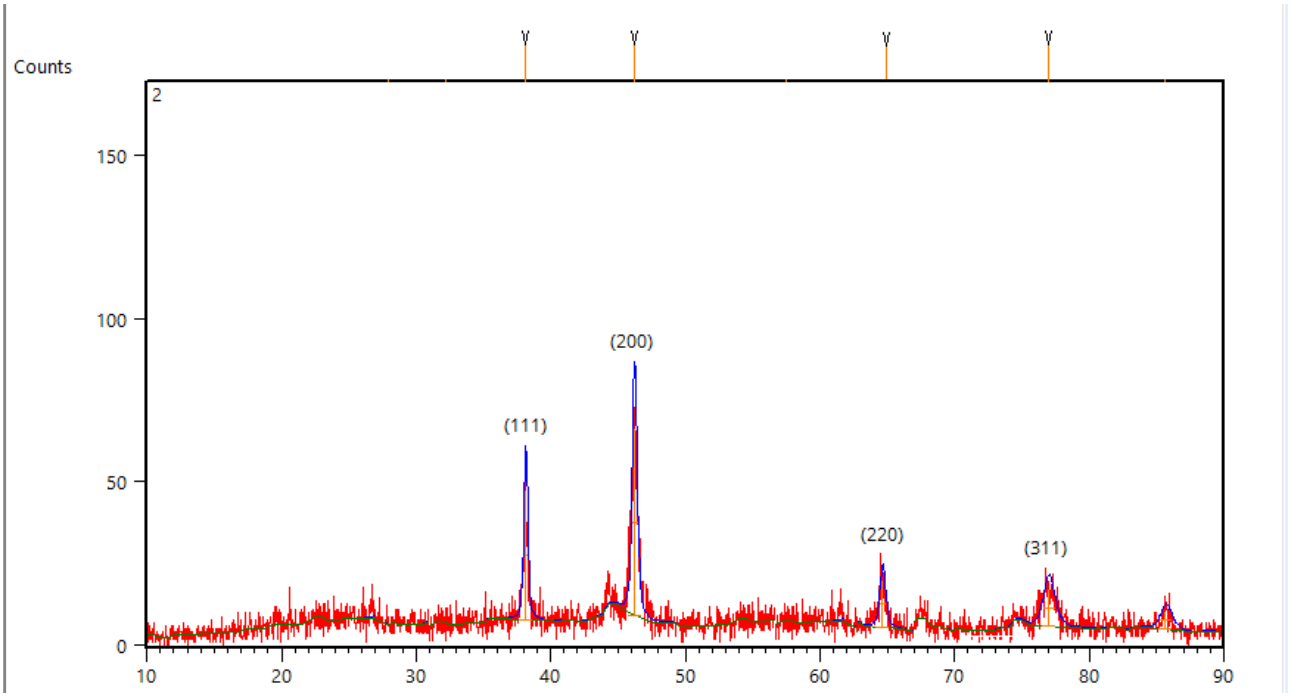


Figure 6 Figure indicates the XRD spectrum of the gold nanorod solution. AuNRs displayed gold peaks at around 38.06℃, 47.16℃, 64.54℃ and 77.28℃ and their peaks can be indexed to the (111), (200), (220) and (311) respectively

4.4 Fourier Transformed Infrared (FTIR) Analysis

FTIR analysis of AuNRs was used to determine potential of the components in the sample to form functional groups. Several functional groups also act as stabilizing and capping agents for nanoparticles. AuNRs were subjected to FTIR spectral analysis and graph is depicted in Figure 7. AuNPs are known to exhibit additional bands as compared to gold salt. The bands shown at 3435.19, 2923.42/2853.25, 1643.85, 1488.45, 950.39 and 719 cm^{-1} were due to the stretching vibrations of hydroxyl (O H), aliphatic groups, amide, stretching inorganic carbonate (-C=O), alcohol (C O) groups and alkyl halides, respectively.

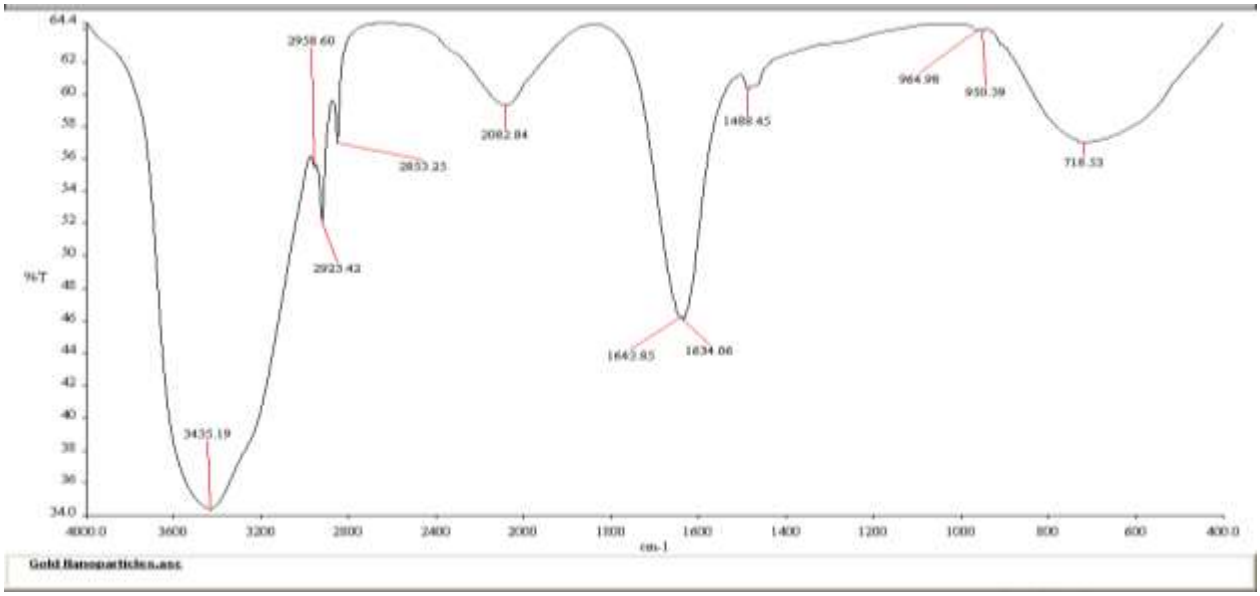


Figure 7 FTIR spectrum of gold nanorod solution. The bands shown at 3435.19, 2923.42/2853.25, 1643.85, 1488.45, 950.39 and 719 cm-1 are due to the stretching vibrations of hydroxyl (O H), aliphatic groups, amide, stretching inorganic carbonate (-C=O), alcohol (C O) groups and alkyl halides

Table 6 Details of bands shown in Fourier Transform infrared spectroscopy

Origin	**Group frequency wavenumber (cm^{-1})**	**Assignment**
O-H (stretch, H-bonded)	3435 (Sharp)	Hydroxyl group, H-bonded OH stretch
CH and CH_2	2923 (sharp)	Aliphatic group
C-O amide I band	1643 (sharp)	Amide
-C=O	1488 (sharp)	Stretching inorganic carbonate
C O	950 (sharp)	Alcohol
C-Cl	719 (broad)	Alkyl Halides

4.5 Scanning Electron Microscopic (SEM) Analysis

In order to determine the characteristic particle size and morphology of AuNRs, Scanning Electron Microscopic Analysis was done at 15,000X and 43,000X magnifications. The images clearly presented that the newly synthesized nanorparticles possess rod shape and

their distribution was uniform throughout the aqueous colloidal solution. According to SEM analysis, the average particle size of Gold Nanorods was recorded as 100nm. Due to the delay between sonication process and SEM analysis, agglomerates of nanorods were synthesized at some points.

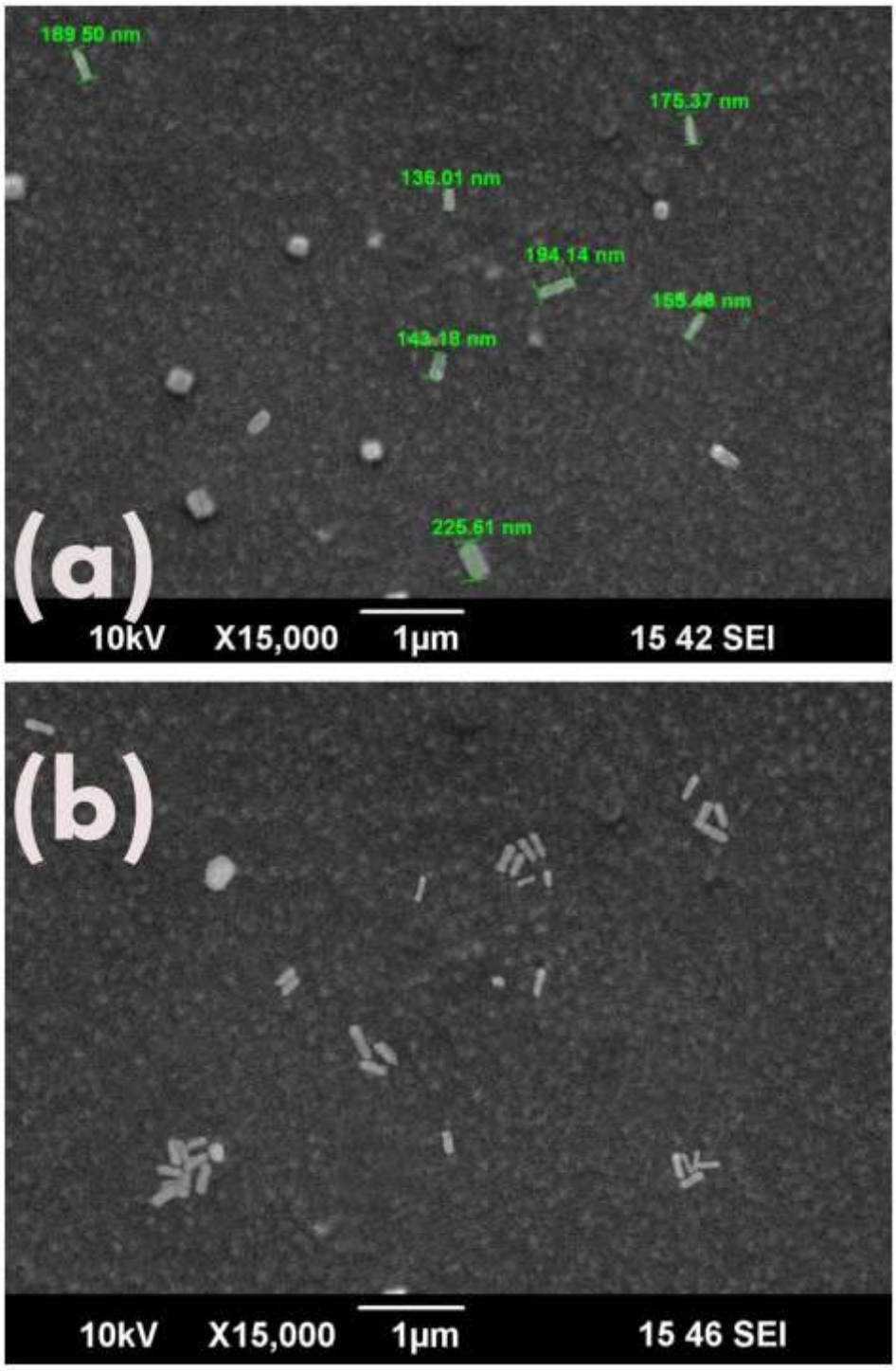


Figure 8 SEM Micrographs illustrating the size and morphology of the Gold nanorods formed through the chemical synthesis. (a) shows the labelled sizes of the nanorods. (b) also shows the morphology of the nanorods. Some can be seen in clusters whie others are single

4.6 Antibacterial activity of AuNRs

In order to observe the antibacterial activity of AuNRs, 3 pathogenic strains of bacteria i.e., *Escherichia coli, Klebsiella pneumonia* and *Staphylcoccus aureus* were collected from hospital and were subjected to well diffusion assay by maintaining several concentrations of AuNRs solution in the wells. Well diffusion assay using AuNRs indicated dose dependent bactericidal activity against these strains as they displayed zone of inhibition of increasing diameter with increase in the concentrations of AuNRs as shown in the figure 9. AuNRs were observed to be effective against 2 strains of bacteria but didn't show any considerable zone against *K. pneumoniae*. It was also seen that there was no zone against *K. pneumonia* even in the positive control. Maximum diameter of zone of inhibition was recorded as approximately 25mm signifying the efficacy of AuNRs against pathogenic bacterial strains. No zone of inhibition was observed around the wells which were treated with deionized water only, that act as a negative control, thus, it can be deduced that the wells containing different concentrations of AuNRs gave the zones of inhibition depicting antibacterial activity.

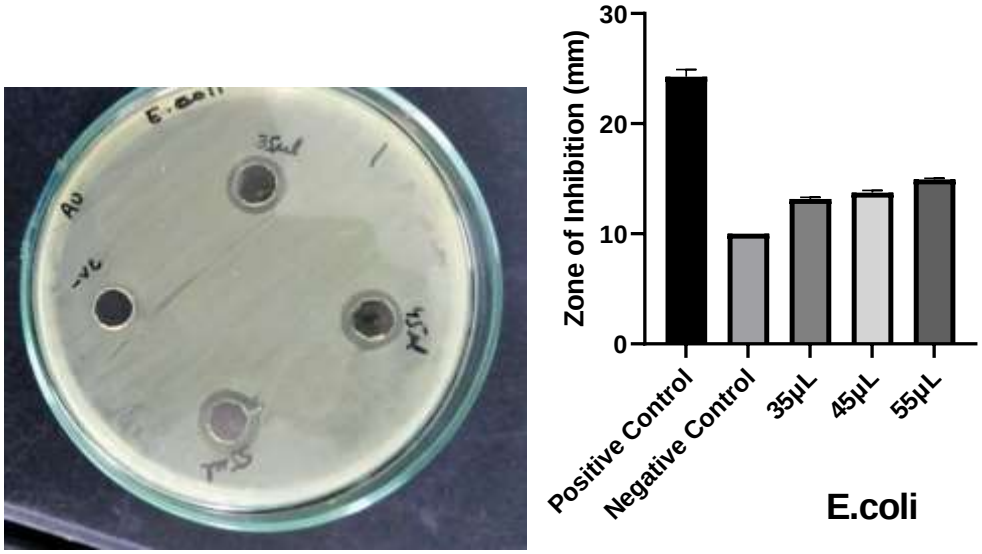
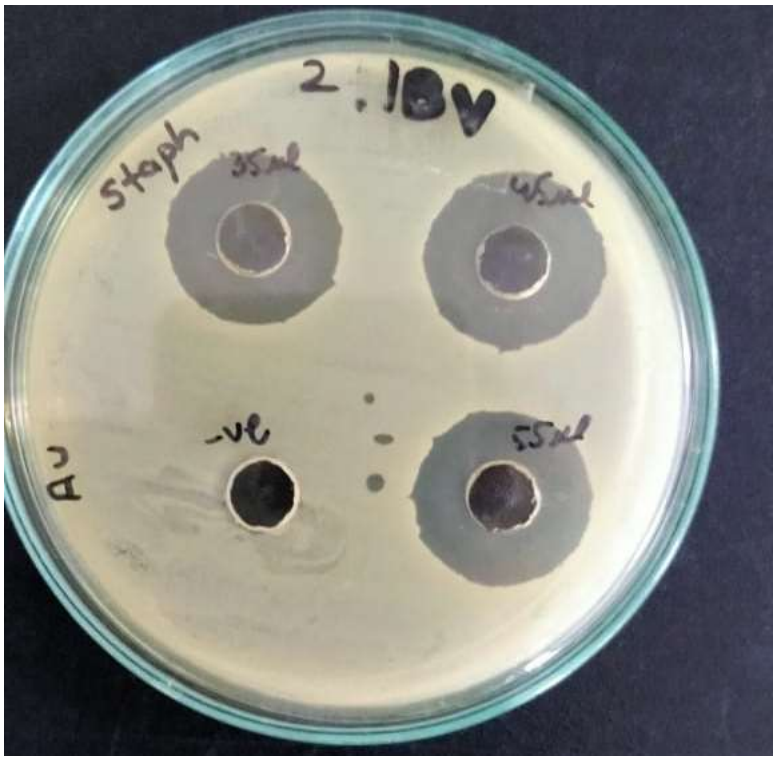


Figure 9 shows the picture of the plate that has *E. coli* culture treated with 3 different concentrations of gold nanorod solution and a negative control which was treated with deionized water. It can be seen that there is no zone around the negative control and clear zones of inhibition around the wells treated with gold nanorods solution. Also the zone size increases with increase in concentration. The graph represents the increase in antibacterial activity with increase in the concentration of nanorod solution. Thus, the nanorod solution showed a slight anti-bacterial activity against E. coli



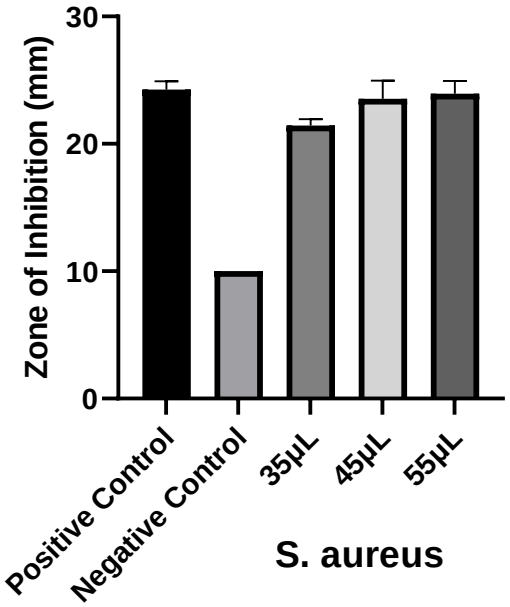


Figure 10 shows the picture of the plate that has *Staphylococcus aureus* culture treated with 3 different concentrations of gold nanorod solution and a negative control which was treated with deionized water. It can be seen that there is no zone around the negative control and clear zones of inhibition around the wells treated with gold nanorods solution. The zones are quite bigger than that of *E.*coli. Also the zone size increases with increase in concentration The graph represents the increase in antibacterial activity with increase in the concentration of nanorod solution

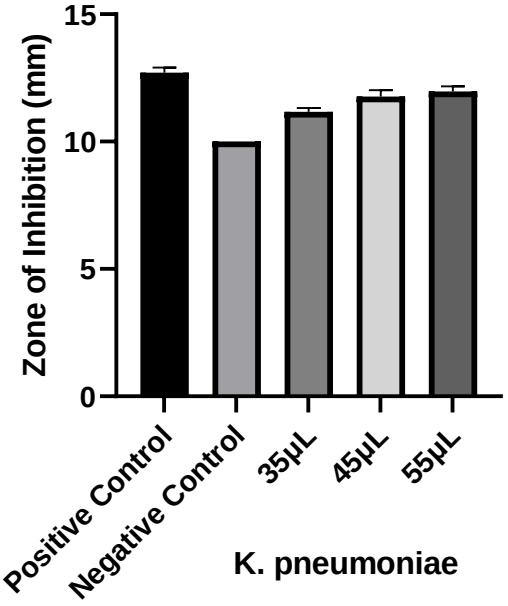


Figure 11shows a picture of a plate that is a *K. pnuemonia* culture and it can be seen that the nanoparticles solution has shown a very little or no antibacterial activity in all the concentrations. The graph represents a very slight increase in antibacterial activity with increase in the concentration of nanorod solution. The graph also suggests that there is a very little antibacterial activity of the gold nanorods solution on this bacterial culture.

4.7 Free Radical Scavenging Ability of AuNRs

The potential of chemically synthesised AuNRs to scavenge free radicals was investigated using the DPPH assay. As the concentration of AuNRs was increased, the assay revealed that the AuNRs' scavenging capacity improved, as shown in Figure 12. The percent inhibition of DPPH was assessed and compared to that of ascorbic acid, which was used as

a positive control. Ascorbic acid is an antioxidant with an antioxidant scavenging potential of 86.35 percent at 500µg/ml. Though, as shown in Figure 12, the maximum inhibition percentage of AuNPs was 53.5 percent at the same concentration as Ascorbic acid. These results suggest that the scavenging abilities of AuNRs differ substantially from those of Ascorbic acid and that AuNRs have substantial antioxidant activity but are not equivalent to natural antioxidants. AuNRs may also be used as free radical scavengers to further examine their potential in medical science.

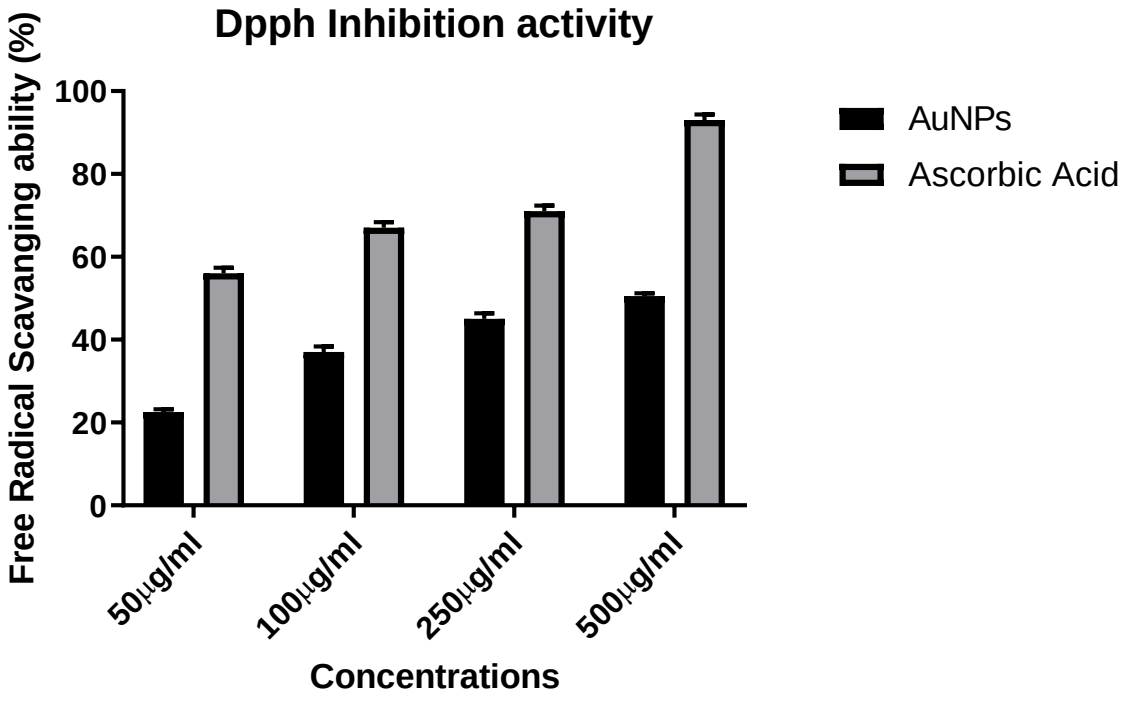


Figure 12 The graph shows the pattern of anti-oxidant activity of gold nanorods solution compared to that of ascorbic acid. These results suggest that the scavenging abilities of AuNRs differ substantially from those of Ascorbic acid and that AuNRs have substantial antioxidant activity but are not equivalent to natural antioxidants.

4.8 Cytotoxicity analysis and *in vitro* evaluation of Drug conjugated gold nanorods

The cytoxicity and *in viro* analysis of drug conjugated gold nanorods was done. Breast cancer cell line MCF7 was used for this study. As for the cytotoxicity analysis, there was no cytotoxicity detected on part of the simple gold nanorod solution. Also, there was no cytotoxicity detected by a pegylated gold nanorod solution. Both these results can be seen in the graphs.

Now talking about the drug conjugated gold nanorods, there were two drugs used; HIF-1 α and 4-OHT. As theMCF-7 cells are ER and PR+ and 4-OHT is the down-regulator of ER, so it showed considerable cell death of the MCF-7 cells. HIF-1 α also worked on the MCF-7 cells but not as well as did the 4-OHT. Both drugs, on the other hand, when conjugated with gold nanorods gave a slight efficient cytotoxicity as compared to when they were administered alone. This implies that there is a slightly better activity of the drug when conjugated with gold nanorods.

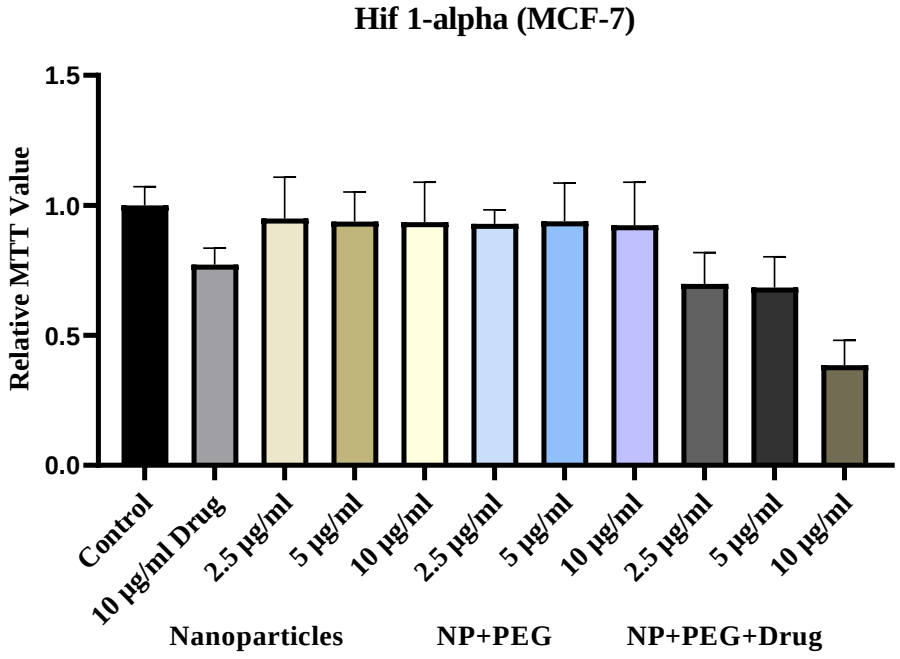


Figure 13 The graph illustrates the in vitro analysis of AuNRs conjugated Hif-1 alpha inhibitor drug. The drug has shown cell death on this cell line but it is not as efficient as 4-OHT because 4-OHT, being an ER down-regulator, specifically targets MCF-7 cell line. The nanoparticles here did not kill the cells which means they are not cytotoxic. Similarly, NP+PEG solution has also shown zero cytotoxicity. When conjugated with PEGylated AuNRs, the drug showed similar action at lower concentrations of AuNRs but at higher concentration, the AuNRs conjugated drug worked even better than when the drug was administered alone

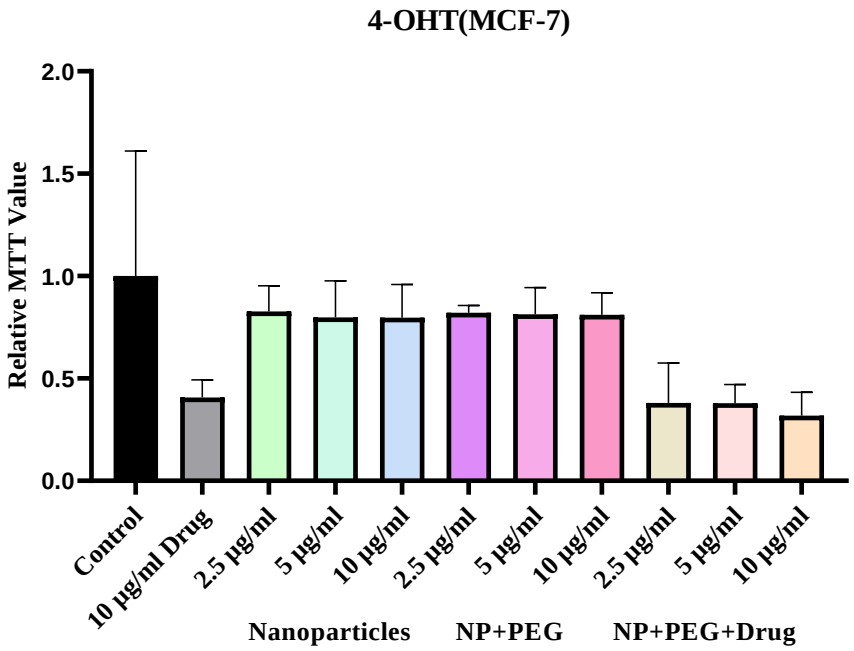


Figure 14 The graph illustrates the in vitro analysis of AuNRs conjugated 4-OHT drug. The graph shows that the drug has shown significant cell death on this cell line as 4-OHT4-OHT, being an ER down-regulator, specifically targets MCF-7 cell line. The nanoparticles did not kill the cancer cells and thus are not cytotoxic. Similarly, NP+PEG solution has also shown near to zero or zero cytotoxicity. When conjugated with PEGylated AuNRs, the drug showed similar action at lower concentrations of NPs but at higher concentration, the AuNRs conjugated drug worked even better than when the drug was administered alone

Chapter 5

Discussion

As an integrative branch of science, nanotechnology has its applications in almost all fields but their importance is remarkable in the field of medicine also known as nanomedicine. It is an emerging branch of nanotechnology and has expanded due to its crucial role in diagnosis and therapeutics (Sandhiya et al., 2009). It provides alternate ways to develop drugs and therapies through targeted approach that prevents the side effects caused by conventional methods of treatment and has opened ways for high quality research.

Nanoparticles have gained enormous attention due to their exceptional characteristics that allow them to perform their role in the field of biotechnology for targeted delivery of drugs, probes for disease detection, drug discovery and tissue engineering. Among metallic nanoparticles, Gold nanoparticles have been extensively studied and used in biotechnology due to their ease of synthesis and significant electronic, magnetic, catalytic and optical properties (Murphy et al., 2005). AuNPs can be excited by light which allows them to be used as contrast agents in diagnostic phototherapeutic applications such as photo-thermal therapy, Raman spectroscopy and light scattering imaging. Furthermore, they can be manipulated by changing their size, adjusting their electronic and optical properties, aggregation and surface functionality, thus allowing them to be used in various branches of science. Gold Nanorods, among other shapes, have been recently considered especially in this regard. AuNRs, due to their significant anisotropic properties and tunable aspect ratio are being studied. Their small size effect and tunneling effect and their unique optical properties are the ones that attract the scientists' interest. All these properties rely on their morphology and have been widely used in therapeutics, catalysis and diagnosis (Meng et al., 2019).

Many techniques regarding synthesis of AuNRs have been introduced till now but for this research, chemical method of synthesis was used and seed-mediated approach was selected (Chang & Murphy, 2018). This is a method which is very simple, synthesizes rods of small size and has a high yield. Also, this method was selected as it was easy to execute. Another reason to select this method was that it synthesizes the nanorods in a matter of 24 hours and as we had to optimize the method in our conditions, it was a suitable method. After carrying

out the method, the colloidal AuNRs were characterized to confirm their synthesis, their shape, size and other information.

Initial confirmation of AuNRs synthesis was done by observing color change throughout the process. As it has been reported that the seed solution attains the color of dull-brown after its incubation period (Sharma et al., 2009), the color was observed and it was dull-brown. During the synthesis of growth solution, the bright yellow color of the gold salt solution was changed to colorless as it was reduced due to the addition of ascorbic acid. After the addition of seed solution to growth solution, the color changed to blue after 24 hours. All these color changes have been previously indicated (Gole & Murphy, 2004). These results clearly indicated the formation of AuNRs but it was also confirmed using other characterization techniques.

Synthesis was further verified by UV/Vis spectral analysis that normally shows characteristic AuNRs peaks at 540nm and around 650-900nm (Scarabelli et al., 2015). One of these is a longitudinal peak and the other one is the transverse peak. In our study, both of these peaks were observed when the UV/Vis analysis of the colloidal AuNRs sample was carried out as shown in figure 4. Also, the stability of ligand-nanoparticle nanosystem was analyzed after the addition of PEG to the solution. The UV/Vis analysis after the addition showed the same results indicating that PEG has not affected the nanorods.

Crystallinity of AuNRs was confirmed by their XRD profile analysis. Figure 6 represents XRD diffractogram of AuNRs. Four peaks were indexed to (111), (200), (220) and (311) planes according to Bragg's equation. Sharpest peak was measured at 38.06^{O} that corresponds to (111) plane. Similar peaks were observed in a study with colloidal gold AuNRs (Long et al., 2009).

FTIR spectrum of the AuNRs provide information about presence of functional groups in the solution. AuNPs are known to exhibit additional bands as compared to gold salt. The bands shown at 3435.19, 2923.42/2853.25, 1643.85, 1488.45, 950.39 and 719 cm^{-1} were due to the stretching vibrations of hydroxyl (O H), aliphatic groups, amide, stretching inorganic carbonate (-C=O), alcohol (C O) groups and alkyl halides, respectively. These could e compared with functional groups of gold nanoparticles synthesized through different sources (Barros et al., 2016).

AuNRs were further analyzed by SEM. Figure 8 showed AuNRs to be rod-shaped and ranging in nanometric size. Images of SEM analysis revealed that AuNRs are rod-like in shape and did not lose their shape even after purification and disruption of cells. All of these characterization techniques helped firstly in the optimization of the process of synthesis and secondly to confirm the synthesis of AuNRs.

Bactericidal property of AuNRs was studied against various bacteria to provide an alternative to the use of antibiotics due to the emerging problem of antibiotic resistane. In this study, antibacterial potential of AuNRs was evaluated against *E. coli, S. aureus and K. pnuemoniae*. Different concentration of AuNRs were used. Increase in concentration of AuNRs increased its bactericidal property. Chmielewska et al., explained the bactericidal properties of various shapes of gold nanomaterials.

Free radical scavenging activity of AuNRs was evaluated by DPPH assay. AuNRs can reduce DPPH and it can be observed by measuring absorbance value at 517nm using UV/Vis spectroscopy. Results of this assay showed that free radical scavenging ability of AuNRs increase with increase in concentration of AuNRs. These results also showed that AuNPs possess significant radical scavenging ability but it cannot be compared with natural antioxidants. Previosuly, antioxidant abilities of gold nanoparticles have been reported as well (Medhe et al., 2014).

In breast cancer, usually chemotherapy lasts a long time and has certain very painful side-effects. In order to test the nanoparticles as drug-carrying vehicles, several studies have been going on. AuNRs have also been used and conjugated with doxorubicin against breast cancer cell lines (Nima et al., 2017). In our study, 4-OHT and Hif-1α inhibitor were used as drugs and AuNRs were conjugated with them through a linker PEG. The MTT assay showed that the drugs conjugated with AuNRs had a slightly better rate of cell killing than that of drug alone and hence AuNRs had some positive results as drug-delivery vehicles.

Conclusion and Future Perspectives

Current study was focused on the synthesis of AuNRs and their applications. Various physicochemical parameters such as pH, temperature, concentration and incubation time were optimized for the synthesis. It was found that a very slightly acidic pH and a temperature of 37 degrees Celsius was ideal for the synthesis of AuNRs. Also the size and shape of AuNRs also depend on the concentrations of CTAB and AgNO3 used in the seed-mediated synthesis. The ideal incubation time of the growth solution was found to be 16-24 hours.

As for the applications, it was found at that AuNRs have decent bactericidal properties and in future they can be tested further to be utilized to deal with problem of antibiotic resistance. They can also be considered as drug-delivery vehicles for antibiotic drugs as well. Their action against other microbes is also not clear yet and can be studied in future.

The drug-delivery with AuNRs is the brightest prospect for the future. These AuNRs can be tested as drug-delivery vehicles on mice models as well and then if positive results keep coming, this research can even make its way to trials on other models or even humans. Researches can also be conducted regarding the exact targeted delivery of drugs. Anticancer medications must be delivered to the site of tumor with as little loss of volume and biological activity as possible while in circulation. When the medicine reaches the tumor location, it should be possible to achieve targeted tumor cell death without harming healthy cells, as well as a controlled release mechanism for the active version of the medication. To address the hazards associated with current cancer treatment methods, these strategies can be fulfilled by customized nanostructures.

References

- Daniel, M. C., & Astruc, D. (2004). Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. *Chemical reviews*, *104*(1), 293-346.
- Feynman, R. P. (1960). There's plenty of room at the bottom. *California Institute of Technology, Engineering and Science magazine*.
- Laurent, S., Forge, D., Port, M., Roch, A., Robic, C., Vander Elst, L., & Muller, R. N. (2008). Magnetic iron oxide nanoparticles: synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications. *Chemical reviews*, *108*(6), 2064-2110.
- Huo, Q., Margolese, D. I., Ciesla, U., Demuth, D. G., Feng, P., Gier, T. E., ... & Chmelka, B. F. (1994). Organization of organic molecules with inorganic molecular species into nanocomposite biphase arrays. *Chemistry of Materials*, *6*(8), 1176-1191.
- Barker, T. F., Fatehi, L., Lesnick, M. T., Mealey, T. J., & Raimond, R. R. (2009). Nanotechnology and the Poor: Opportunities. In *Nanotechnology & society* (pp. 243-263). Springer, Dordrecht.
- Dionysiou, D. D. (2004). Environmental applications and implications of nanotechnology and nanomaterials. *Journal of Environmental Engineering*, *130*(7), 723-724.
- Sanvicens, N., & Marco, M. P. (2008). Multifunctional nanoparticles–properties and prospects for their use in human medicine. *Trends in biotechnology*, *26*(8), 425-433.
- Bruce, P. G., Scrosati, B., & Tarascon, J. M. (2008). Nanomaterials for rechargeable lithium batteries. *Angewandte Chemie International Edition*, *47*(16), 2930-2946.
- Nowack, B., & Bucheli, T. D. (2007). Occurrence, behavior and effects of nanoparticles in the environment. *Environmental pollution*, *150*(1), 5-22.
- Lee, J., Mahendra, S., & Alvarez, P. J. (2010). Nanomaterials in the construction industry: a review of their applications and environmental health and safety considerations. *ACS nano*, *4*(7), 3580-3590.

- Kim, S. T., Saha, K., Kim, C., & Rotello, V. M. (2013). The role of surface functionality in determining nanoparticle cytotoxicity. *Accounts of chemical research*, *46*(3), 681-691.
- Teo, B. M., Suh, S. K., Hatton, T. A., Ashokkumar, M., & Grieser, F. (2010). Sonochemical synthesis of magnetic Janus nanoparticles. *Langmuir*, *27*(1), 30-33.
- Salata, O. V. (2004). Applications of nanoparticles in biology and medicine. *Journal of nanobiotechnology*, *2*(1), 3.
- Katz, E., & Willner, I. (2004). Integrated nanoparticle–biomolecule hybrid systems: synthesis, properties, and applications. *Angewandte Chemie International Edition*, *43*(45), 6042-6108.
- Mie, G. (1908). Articles on the optical characteristics of turbid tubes, especially colloidal metal solutions. *Ann. Phys*, *25*(3), 377-445.
- Chithrani, B. D., Ghazani, A. A., & Chan, W. C. (2006). Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. *Nano letters*, *6*(4), 662-668.
- Panda, T., & Deepa, K. (2011). Biosynthesis of gold nanoparticles. *Journal of nanoscience and nanotechnology*, *11*(12), 10279-10294.
- Kelly, K. L., Coronado, E., Zhao, L. L., & Schatz, G. C. (2003). The optical properties of metal nanoparticles: the influence of size, shape, and dielectric environment: ACS Publications.
- Eustis, S., & El-Sayed, M. A. (2006). Why AuNPs are more precious than pretty gold: noble metal surface plasmon resonance and its enhancement of the radiative and nonradiative properties of nanocrystals of different shapes. *Chemical society reviews*, *35*(3), 209-217.
- Horikoshi, S. A. T. O. S. H. I., & Serpone, N. I. C. K. (2013). Introduction to nanoparticles. *Microwaves in Nanoparticle Synthesis: Fundamentals and Applications*, 1-24.
- Mock, J. J., Barbic, M., Smith, D. R., Schultz, D. A., & Schultz, S. (2002). Shape effects in plasmon resonance of individual colloidal silver nanoparticles. *The Journal of Chemical Physics*, *116*(15), 6755-6759.
- Paradise, M., & Goswami, T. (2007). Carbon nanotubes–production and industrial applications. *Materials & design*, *28*(5), 1477-1489.

- Zhang, B. T., Zheng, X., Li, H. F., & Lin, J. M. (2013). Application of carbon-based nanomaterials in sample preparation: a review. *Analytica chimica acta*, *784*, 1-17.
- K. Scida, P.W. Stege, G. Haby, G.A. Messina, C.D. García
- S. Polizu, *et al.*Applications of carbon nanotubes-based biomaterials in biomedical nanotechnology
- Park, J., Joo, J., Kwon, S. G., Jang, Y., & Hyeon, T. (2007). Synthesis of monodisperse spherical nanocrystals. *Angewandte Chemie International Edition*, *46*(25), 4630-4660.
- Lu, A. H., Salabas, E. E., & Schüth, F. (2007). Magnetic nanoparticles: synthesis, protection, functionalization, and application. *Angewandte Chemie International Edition*, *46*(8), 1222-1244.
- Kumari, A., Yadav, S. K., & Yadav, S. C. (2010). Biodegradable polymeric nanoparticles based drug delivery systems. *Colloids and Surfaces B: Biointerfaces*, *75*(1), 1-18.
- Bang, J. H., & Suslick, K. S. (2010). Applications of ultrasound to the synthesis of nanostructured materials. *Advanced materials*, *22*(10), 1039-1059.
- Solanki, P. R., Kaushik, A., Agrawal, V. V., & Malhotra, B. D. (2011). Nanostructured metal oxide-based biosensors. *NPG Asia Materials*, *3*(1), 17.
- Wang, Z. L. (Ed.). (2013). *Nanowires and nanobelts: materials, properties and devices. Volume 1: Metal and Semiconductor Nanowires*. Springer Science & Business Media.
- Nath, N., & Chilkoti, A. (2002). A colorimetric gold nanoparticle sensor to interrogate biomolecular interactions in real time on a surface. *Analytical chemistry*, *74*(3), 504-509.
- Mody, V. V., Siwale, R., Singh, A., & Mody, H. R. (2010). Introduction to metallic nanoparticles. *Journal of Pharmacy and Bioallied Sciences*, *2*(4), 282.
- Giljohann DA, Seferos DS, Daniel WL, Massich MD, Patel PC, Mirkin CA. AuNPs for biology and medicine. Angew Chem Int Ed Engl. 2010;49:3280–94.
- Peter PE, Meurig JT. Fein verteiltes Gold - Faradays Beitrag zu den heutigen Nanowissenschaften13. Angewandte Chemie. 2007;119:5576–82
- Sharma V, Park K, Srinivasarao M. Colloidal dispersion of gold nanorods: Historical background, optical properties, seed-mediated synthesis, shape separation and self-assembly. Mater Sci Eng R Rep. 2009;65:1–38.

- I.L. Medintz, *et al.*Quantum dot bioconjugates for imaging, labelling and sensing
- Nikoobakht, B., & El-Sayed, M. A. (2003). Preparation and growth mechanism of gold nanorods (NRs) using seed-mediated growth method. *Chemistry of Materials*, *15*(10), 1957-1962.
- Xu, W., Wang, B., Zhang, Y., An, Q., Zhou, R., Peng, C., ... & Xing, Y. (2021). Recent advances on application of gold nanorods in detection field. *Materials Research Express*.
- Ma, J., Wang, X., Feng, J., Huang, C., & Fan, Z. (2021). Individual Plasmonic Nanoprobes for Biosensing and Bioimaging: Recent Advances and Perspectives. *Small*, *17*(8), 2004287.
- Yin, Y., Zhu, J., Wang, Z., & Yuan, H. (2020, December). Plasmon tuning and electromagnetic field enhancement of Au@ Ag nanorod-gold film nanostructure. In *2020 IEEE MTT-S International Conference on Numerical Electromagnetic and Multiphysics Modeling and Optimization (NEMO)* (pp. 1-4). IEEE.
- Yang, N., You, T. T., Gao, Y. K., Zhang, C. M., & Yin, P. G. (2018). Fabrication of a flexible gold nanorod polymer metafilm via a phase transfer method as a SERS substrate for detecting food contaminants. *Journal of agricultural and food chemistry*, *66*(26), 6889-6896.
- Chang, H. H., & Murphy, C. J. (2018). Mini gold nanorods with tunable plasmonic peaks beyond 1000 nm. *Chemistry of Materials*, *30*(4), 1427-1435.
- Mallick, K., Wang, Z. L., & Pal, T. (2001). Seed-mediated successive growth of gold particles accomplished by UV irradiation: a photochemical approach for size-controlled synthesis. *Journal of Photochemistry and Photobiology A: Chemistry*, *140*(1), 75-80.
- Khan, I., Saeed, K., & Khan, I. (2017). Nanoparticles: Properties, applications and toxicities. *Arabian Journal of Chemistry*.
- Daniel, M. C., & Astruc, D. (2004). AuNPs: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. *Chemical reviews*, *104*(1), 293-346.
- Veith, M. (2002). Molecular precursors for (nano) materials—a one step strategy. *Journal of the Chemical Society, Dalton Transactions*, (12), 2405-2412.

- Chen, X., Liu, L., Peter, Y. Y., & Mao, S. S. (2011). Increasing solar absorption for photocatalysis with black hydrogenated titanium dioxide nanocrystals. *Science*, 1200448.
- Bhardwaj, V., Ankola, D. D., Gupta, S. C., Schneider, M., Lehr, C. M., & Kumar, M. R. (2009). PLGA nanoparticles stabilized with cationic surfactant: safety studies and application in oral delivery of paclitaxel to treat chemical-induced breast cancer in rat. *Pharmaceutical research*, *26*(11), 2495-2503.
- Williams, D. B., & Carter, C. B. (1996). The transmission electron microscope. In *Transmission electron microscopy* (pp. 3-17). Springer, Boston, MA.
- Takahashi H, Niidome Y, Yamada S. Controlled release of plasmid DNA from gold nanorods induced by pulsed near-infrared light. Chem Commun 2005, 17:2247–2249
- Stone, J., Jackson, S., & Wright, D. (2011). Biological applications of gold nanorods. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*, *3*(1), 100-109.
- Majdalawieh, A., Kanan, M. C., El-Kadri, O., & Kanan, S. M. (2014). Recent advances in gold and silver nanoparticles: synthesis and applications. *Journal of nanoscience and nanotechnology*, *14*(7), 4757-4780.
- Bindhu MR, Umadevi M (2014) Antibacterial activities of green synthesized AuNPs. Mater Lett 120: 122-125.
- Elswaifi, S. F., Palmieri, J. R., Hockey, K. S., & Rzigalinski, B. A. (2009). Antioxidant nanoparticles for control of infectious disease. *Infectious Disorders-Drug Targets (Formerly Current Drug Targets-Infectious Disorders)*, *9*(4), 445-452.
- Gordon, M. H. (1990). The mechanism of antioxidant action in vitro. In *Food antioxidants* (pp. 1-18). Springer, Dordrecht.
- Raghupathi, K. R., Koodali, R. T., & Manna, A. C. (2011). Size-dependent bacterial growth inhibition and mechanism of antibacterial activity of zinc oxide nanoparticles. *Langmuir*, *27*(7), 4020-4028.
- Kumar, S., Lather, V., & Pandita, D. (2015). Green synthesis of therapeutic nanoparticles: an expanding horizon. *Nanomedicine*, *10*(15), 2451-2471.

- Elswaifi, S. F., Palmieri, J. R., Hockey, K. S., & Rzigalinski, B. A. (2009). Antioxidant nanoparticles for control of infectious disease. *Infectious Disorders-Drug*

- Abdal Dayem, A., Hossain, M. K., Lee, S. B., Kim, K., Saha, S. K., Yang, G.-M., . . . Cho, S.-G. (2017). The role of reactive oxygen species (ROS) in the biological activities of metallic nanoparticles. *International journal of molecular sciences, 18*(1), 120.
- Alekseeva, A. V., Bogatyrev, V. A., Khlebtsov, B. N., Mel'nikov, A. G., Dykman, L. A., & Khlebtsov, N. G. (2006). Gold nanorods: Synthesis and optical properties. *Colloid Journal, 68*(6), 661-678. doi: 10.1134/s1061933x06060019
- Ali, M. R. K., Wu, Y., Tang, Y., Xiao, H., Chen, K., Han, T., . . . El-Sayed, M. A. (2017). Targeting cancer cell integrins using gold nanorods in photothermal therapy inhibits migration through affecting cytoskeletal proteins. *Proceedings of the National Academy of Sciences, 114*(28), E5655-E5663. doi: 10.1073/pnas.1703151114
- Altintas, Z. (2017). *Biosensors and nanotechnology: applications in health care diagnostics*: John Wiley & Sons.
- Amri, C., Shukla, A. K., & Lee, J.-H. (2021). Recent Advancements in Nanoparticle-Based Optical Biosensors for Circulating Cancer Biomarkers. *Materials, 14*(6), 1339.
- An, L., Wang, Y., Tian, Q., & Yang, S. (2017). Small Gold Nanorods: Recent Advances in Synthesis, Biological Imaging, and Cancer Therapy. *Materials (Basel, Switzerland), 10*(12), 1372. doi: 10.3390/ma10121372
- Bala, S., Kumar, D., Chand, S., Wadhwa, H., Mahendia, S., & Kumar, S. (2019). Synthesis and characterization of gold nanorods encapsulated in reduced graphene oxide. *AIP Conference Proceedings, 2093*(1), 020011. doi: 10.1063/1.5097080
- Buluş, E., Bulus, G. S., & Yakuphanoglu, F. (2020). Production and Characterization of Novel Nature-Friendly Organic Fertilizer Covers Based on Nanotechnology for the Agricultural Sector. *JOURNAL OF MATERIALS AND ELECTRONIC DEVICES, 5*(1), 12-16.

- Buzea, C., Pacheco, I. I., & Robbie, K. (2007). Nanomaterials and nanoparticles: sources and toxicity. *Biointerphases, 2*(4), MR17-MR71.
- Centi, S., Tatini, F., Ratto, F., Gnerucci, A., Mercatelli, R., Romano, G., . . . Pini, R. (2014). In vitro assessment of antibody-conjugated gold nanorods for systemic injections. *Journal of Nanobiotechnology, 12*(1), 55. doi: 10.1186/s12951-014-0055-3
- Cheetham, M. R., Griffiths, J., Nijs, B. d., Heath, G. R., Evans, S. D., Baumberg, J. J., & Chikkaraddy, R. (2020). Out-of-Plane Nanoscale Reorganization of Lipid Molecules and Nanoparticles Revealed by Plasmonic Spectroscopy. *The journal of physical chemistry letters, 11*(8), 2875-2882.
- Chen, G., Qian, Y., Zhang, H., Ullah, A., He, X., Zhou, Z., . . . Shen, J. (2021). Advances in cancer theranostics using organic-inorganic hybrid nanotechnology. *Applied Materials Today, 23*, 101003.
- Choudhary, H. K., Kumar, R., Pawar, S. P., Sundararaj, U., & Sahoo, B. (2020). Effect of morphology and role of conductivity of embedded metallic nanoparticles on electromagnetic interference shielding of PVDF-carbonaceous-nanofiller composites. *Carbon, 164*, 357-368.
- Choudhury, S. R., & Goswami, A. (2017). Non-metallic nanoparticles & their biological implications. *Basic Appl. Aspects, Nanobiotechnology*, 61.
- Corsi, I., Winther-Nielsen, M., Sethi, R., Punta, C., Della Torre, C., Libralato, G., . . . Fiordi, L. (2018). Ecofriendly nanotechnologies and nanomaterials for environmental applications: Key issue and consensus recommendations for sustainable and ecosafe nanoremediation. *Ecotoxicology and environmental safety, 154*, 237-244.
- Crespo, J., Guari, Y., Ibarra, A., Larionova, J., Lasanta, T., Laurencin, D., . . . Richeter, S. (2014). Ultrasmall NHC-coated gold nanoparticles obtained through solvent free thermolysis of organometallic Au (I) complexes. *Dalton Transactions, 43*(42), 15713-15718.
- Deb, P. K., Odetallah, H. a. M. A., Al-Jaidi, B., Akkinepalli, R. R., Al-Aboudi, A., & Tekade, R. K. (2019). Chapter 11 - Biomaterials and Nanoparticles for Hyperthermia Therapy. In R. K. Tekade (Ed.), *Biomaterials and Bionanotechnology* (pp. 375-413): Academic Press.

- Devlin, E. J., Denson, L. A., & Whitford, H. S. (2017). Cancer Treatment Side Effects: A Meta-analysis of the Relationship Between Response Expectancies and Experience. *Journal of Pain and Symptom Management, 54*(2), 245-258.e242. doi: 10.1016/j.jpainsymman.2017.03.017
- Dikshit, M. K., Pathak, V. K., & Srivastava, A. K. (2019). *Preface: Proceeding of the 1st International Conference on Advances in Mechanical Engineering and Nanotechnology (ICAMEN).* Paper presented at the AIP Conference Proceedings.
- Evans, E. R., Bugga, P., Asthana, V., & Drezek, R. (2018). Metallic nanoparticles for cancer immunotherapy. *Materials Today, 21*(6), 673-685.
- Fang, N., Cheng, X., Chen, K., Dong, B., Yang, M., Filbrun, S., . . . Wang, G. (2021). Dynamin Action at the Final Stage of Clathrin-Mediated Endocytosis.
- Gao, C., Zhang, Q., Lu, Z., & Yin, Y. (2011). Templated Synthesis of Metal Nanorods in Silica Nanotubes. *Journal of the American Chemical Society, 133*(49), 19706-19709. doi: 10.1021/ja209647d
- Gericke, M., & Pinches, A. (2006). Biological synthesis of metal nanoparticles. *Hydrometallurgy, 83*(1-4), 132-140.
- Ghaffari, M., & Dolatabadi, J. E. N. (2019). Chapter 17 - Nanotechnology for pharmaceuticals. In S. Thomas, Y. Grohens & Y. B. Pottathara (Eds.), *Industrial Applications of Nanomaterials* (pp. 475-502): Elsevier.
- Giljohann, D. A., Seferos, D. S., Daniel, W. L., Massich, M. D., Patel, P. C., & Mirkin, C. A. (2010). Gold nanoparticles for biology and medicine. *Angewandte Chemie International Edition, 49*(19), 3280-3294.
- Gole, A., & Murphy, C. J. (2004). Seed-Mediated Synthesis of Gold Nanorods: Role of the Size and Nature of the Seed. *Chemistry of Materials, 16*(19), 3633-3640. doi: 10.1021/cm0492336
- Guo, H., Yi, S., Feng, K., Xia, Y., Qu, X., Wan, F., . . . Zhang, C. (2021). In situ formation of metal organic framework onto gold nanorods/mesoporous silica with functional integration for targeted theranostics. *Chemical Engineering Journal, 403*, 126432.
- Guo, R., Liu, Q., Wang, W., Tayebee, R., & Mollania, F. (2021). Boron nitride nanostructures as effective adsorbents for melphalan anti-ovarian cancer drug. Preliminary MTT assay and in vitro cellular toxicity. *Journal of Molecular Liquids, 325*, 114798.

- Hameed, A., Fatima, G. R., Malik, K., Muqadas, A., & Fazal-ur-Rehman, M. (2019). Scope of nanotechnology in cosmetics: dermatology and skin care products. *Journal of Medicinal and Chemical Sciences, 2*(1), 9-16.
- Han, H.-W., Joe, A., & Jang, E.-S. (2021). Reduced cytotoxicity of CTAB-templated silica layer on gold nanorod using fluorescence dyes and its application in cancer theranostics. *Journal of Industrial and Engineering Chemistry*, *96*, 202-212.
- Hlapisi, N., Motaung, T. E., Linganiso, L. Z., Oluwafemi, O. S., & Songca, S. P. (2019). Encapsulation of Gold Nanorods with Porphyrins for the Potential Treatment of Cancer and Bacterial Diseases: A Critical Review. *Bioinorganic Chemistry and Applications, 2019*, 7147128. doi: 10.1155/2019/7147128
- Hu, Q., Li, H., Wang, L., Gu, H., & Fan, C. (2018). DNA nanotechnology-enabled drug delivery systems. *Chemical reviews, 119*(10), 6459-6506.
- Huang, T., Holden, J. A., Reynolds, E. C., Heath, D. E., O'Brien-Simpson, N. M., & O'Connor, A. J. (2020). Multifunctional Antimicrobial Polypeptide-Selenium Nanoparticles Combat Drug-Resistant Bacteria. *ACS Applied Materials & Interfaces, 12*(50), 55696-55709.
- Jakhmola, A., Vecchione, R., Onesto, V., Gentile, F., Profeta, M., Battista, E., . . . Netti, P. A. (2021). A theoretical and experimental study on L-tyrosine and citrate mediated sustainable production of near infrared absorbing twisted gold nanorods. *Materials Science and Engineering: C, 118*, 111515.
- Jana, N. R., Gearheart, L., & Murphy, C. J. (2001). Evidence for Seed-Mediated Nucleation in the Chemical Reduction of Gold Salts to Gold Nanoparticles. *Chemistry of Materials, 13*(7), 2313-2322. doi: 10.1021/cm000662n
- Jiang, K., & Pinchuk, A. O. (2015). Chapter Two - Noble Metal Nanomaterials: Synthetic Routes, Fundamental Properties, and Promising Applications. In R. E. Camley & R. L. Stamps (Eds.), *Solid State Physics* (Vol. 66, pp. 131-211): Academic Press.
- Jin, L., Shen, S., Huang, Y., Li, D., & Yang, X. (2021). Corn-like Au/Ag nanorod-mediated NIR-II photothermal/photodynamic therapy potentiates immune checkpoint antibody efficacy by reprogramming the cold tumor microenvironment. *Biomaterials, 268*, 120582.

- Jin, R., Cao, Y., Mirkin, C. A., Kelly, K. L., Schatz, G. C., & Zheng, J. (2001). Photoinduced conversion of silver nanospheres to nanoprisms. *science, 294*(5548), 1901-1903.
- Jin, X., Yao, S., Qiu, F., Mao, Z., & Wang, B. (2021). A multifunctional hydrogel containing gold nanorods and methylene blue for synergistic cancer phototherapy. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 614*, 126154.
- John, C. L., Strating, S. L., Shephard, K. A., & Zhao, J. X. (2013). Reproducibly synthesize gold nanorods and maintain their stability. [10.1039/C3RA41521J]. *RSC Advances, 3*(27), 10909-10918. doi: 10.1039/c3ra41521j
- Kah, J. C. Y., Yeo, E. L. L., He, S., & Engudar, G. (2015). 12 - Gold nanorods in photomedicine. In M. R. Hamblin & P. Avci (Eds.), *Applications of Nanoscience in Photomedicine* (pp. 221-248). Oxford: Chandos Publishing.
- Khan, F. A. (2020). Nanomaterials: types, classifications, and sources *Applications of Nanomaterials in Human Health* (pp. 1-13): Springer.
- Khan, I., Saeed, K., & Khan, I. (2019). Nanoparticles: Properties, applications and toxicities. *Arabian journal of chemistry, 12*(7), 908-931.
- Khan, Q. A., & Luo, Z. (2021). Advances in Optical Aptasensors for Early Detection and Diagnosis of Various Cancer Types. *Frontiers in Oncology, 11*, 19.
- Hulla, J. E., Sahu, S. C., & Hayes, A. W. (2015). Nanotechnology: History and future. *Human & experimental toxicology, 34*(12), 1318-1321.
- Kim, M. R., Lee, D. K., & Jang, D.-J. (2010). Template-Based Electrochemically Controlled Growth of Segmented Multimetal Nanorods. *Journal of Nanomaterials, 2010*, 203756. doi: 10.1155/2010/203756
- Kim, T.-H., Lee, K.-B., & Choi, J.-W. (2013). 3D graphene oxide-encapsulated gold nanoparticles to detect neural stem cell differentiation. *Biomaterials, 34*(34), 8660-8670.
- Kiss, A., Baksa, V., Bege, M., TÁlas, L., BorbÁs, A., Bereczki, I., . . . SzemÁn-Nagy, G. (2021). MTT Test and Time-lapse Microscopy to Evaluate the Antitumor Potential of Nucleoside Analogues. *Anticancer Research, 41*(1), 137-149.
- Krifa, M., & Prichard, C. (2020). Nanotechnology in textile and apparel research–an overview of technologies and processes. *The Journal of The Textile Institute, 111*(12), 1778-1793.

- Kuo, C.-H., & Huang, M. H. (2005). Synthesis of branched gold nanocrystals by a seeding growth approach. *Langmuir, 21*(5), 2012-2016.
- Li, P., Wu, Y., Li, D., Su, X., Luo, C., Wang, Y., . . . Zhang, W. (2018). Seed-Mediated Synthesis of Tunable-Aspect-Ratio Gold Nanorods for Near-Infrared Photoacoustic Imaging. *Nanoscale research letters, 13*(1), 313-313. doi: 10.1186/s11671-018-2734-8
- Liang, H., Wang, B., Wang, H., Yu, M., Zheng, L., Wang, M., . . . Feng, W. (2018). Inhibition of Lysozyme Fibrillation by Gold Nanorods and Nanoparticles. *J Nanosci Nanotechnol, 18*(5), 3087-3094.
- Liopo, A., Wang, S., Derry, P. J., Oraevsky, A. A., & Zubarev, E. R. (2015). Seedless synthesis of gold nanorods using dopamine as a reducing agent. [10.1039/C5RA19817H]. *RSC Advances, 5*(111), 91587-91593. doi: 10.1039/c5ra19817h
- Lu, F., & Astruc, D. (2020). Nanocatalysts and other nanomaterials for water remediation from organic pollutants. *Coordination Chemistry Reviews, 408*, 213180.
- Lu, M., Zhu, H., Lin, M., Wang, F., Hong, L., Masson, J.-F., & Peng, W. (2021). Comparative study of block copolymer-templated localized surface plasmon resonance optical fiber biosensors: CTAB or citrate-stabilized gold nanorods. *Sensors and Actuators B: Chemical, 329*, 129094.
- Mahmoud, N. N., Aqabani, H., Hikmat, S., & Abu-Dahab, R. (2021). Colloidal Stability and Cytotoxicity of Polydopamine-Conjugated Gold Nanorods against Prostate Cancer Cell Lines. *Molecules, 26*(5), 1299.
- Mao, W., Son, Y. J., & Yoo, H. S. (2020). Gold nanospheres and nanorods for anti-cancer therapy: comparative studies of fabrication, surface-decoration, and anti-cancer treatments. [10.1039/D0NR01690J]. *Nanoscale, 12*(28), 14996-15020. doi: 10.1039/d0nr01690j
- McNamara, K., & Tofail, S. A. (2017). Nanoparticles in biomedical applications. *Advances in Physics: X, 2*(1), 54-88.
- McNeil, S. E. (2005). Nanotechnology for the biologist. *Journal of leukocyte biology, 78*(3), 585-594.

- Meng, L., Zhang, J., Li, H., Zhao, W., & Zhao, T. (2019). Preparation and Progress in Application of Gold Nanorods. *Journal of Nanomaterials*, *2019*, 4925702. doi: 10.1155/2019/4925702
- Mousavi, S. M., Zarei, M., Hashemi, S. A., Ramakrishna, S., Chiang, W.-H., Lai, C. W., & Gholami, A. (2020). Gold nanostars-diagnosis, bioimaging and biomedical applications. *Drug metabolism reviews*, *52*(2), 299-318.
- N'Konou, K., & Torchio, P. (2019). Chapter 13 - Optical Absorption Modeling of Plasmonic Organic Solar Cells Embedding Ag–SiO2 Core–Shell Nanoparticles. In S. Mohapatra, T. A. Nguyen & P. Nguyen-Tri (Eds.), *Noble Metal-Metal Oxide Hybrid Nanoparticles* (pp. 265-282): Woodhead Publishing.
- Nakamoto, M., Kashiwagi, Y., & Yamamoto, M. (2005). Synthesis and size regulation of gold nanoparticles by controlled thermolysis of ammonium gold (I) thiolate in the absence or presence of amines. *Inorganica chimica acta*, *358*(14), 4229-4236.
- Barros, H. R., Piovan, L., Sassaki, G. L., de Araujo Sabry, D., Mattoso, N., Nunes, Á. M., ... & Riegel-Vidotti, I. C. (2016). Surface interactions of gold nanorods and polysaccharides: From clusters to individual nanoparticles. *Carbohydrate polymers*, *152*, 479-486.
- Sandhiya, S., Dkhar, S. A., & Surendiran, A. (2009). Emerging trends of nanomedicine–an overview. *Fundamental & clinical pharmacology*, *23*(3), 263-269.
- Meng, L., Zhang, J., Li, H., Zhao, W., & Zhao, T. (2019). Preparation and Progress in Application of Gold Nanorods. *Journal of Nanomaterials*, *2019*
- Nima, Z. A., Alwbari, A. M., Dantuluri, V., Hamzah, R. N., Sra, N., Motwani, P., ... & Biris, A. S. (2017). Targeting nano drug delivery to cancer cells using tunable, multi-layer, silver-decorated gold nanorods. *Journal of applied toxicology*, *37*(12), 1370-1378.
- Sharma, V., Park, K., & Srinivasarao, M. (2009). Colloidal dispersion of gold nanorods: Historical background, optical properties, seed-mediated synthesis, shape separation and self-assembly. *Materials Science and Engineering: R: Reports*, *65*(1-3), 1-38.
- Gole, A., & Murphy, C. J. (2004). Seed-mediated synthesis of gold nanorods: role of the size and nature of the seed. *Chemistry of Materials*, *16*(19), 3633-3640.

- Scarabelli, L., Sánchez-Iglesias, A., Pérez-Juste, J., & Liz-Marzán, L. M. (2015). A "tips and tricks" practical guide to the synthesis of gold nanorods.
- Long, N. N., Kiem, C. D., Doanh, S. C., Nguyet, C. T., Hang, P. T., Thien, N. D., & Quynh, L. M. (2009, September). Synthesis and optical properties of colloidal gold nanoparticles. In *Journal of Physics: Conference Series* (Vol. 187, No. 1, p. 012026). IOP Publishing.
- Medhe, S., Bansal, P., & Srivastava, M. M. (2014). Enhanced antioxidant activity of gold nanoparticle embedded 3, 6-dihydroxyflavone: a combinational study. *Applied Nanoscience*, *4*(2), 153-161.
-
- Niidome, Y., & Niidome, T. (2007). Chapter 20 - Gold nanorods: application to bioscience and medicine. In H. Masuhara, S. Kawata & F. Tokunaga (Eds.), *Handai Nanophotonics* (Vol. 3, pp. 297-307): Elsevier.
- Nikoobakht, B., & El-Sayed, M. A. (2003). Preparation and Growth Mechanism of Gold Nanorods (NRs) Using Seed-Mediated Growth Method. *Chemistry of Materials, 15*(10), 1957-1962. doi: 10.1021/cm020732l
- Nikzamir, M., Akbarzadeh, A., & Panahi, Y. (2021). An overview on nanoparticles used in biomedicine and their cytotoxicity. *Journal of Drug Delivery Science and Technology, 61*, 102316. doi: https://doi.org/10.1016/j.jddst.2020.102316
- Oliveira, J. P., Prado, A. R., Keijok, W. J., Antunes, P. W. P., Yapuchura, E. R., & Guimarães, M. C. C. (2019). Impact of conjugation strategies for targeting of antibodies in gold nanoparticles for ultrasensitive detection of 17β-estradiol. *Scientific reports, 9*(1), 1-8.
- Ovais, M., Khalil, A. T., Islam, N. U., Ahmad, I., Ayaz, M., Saravanan, M., . . . Mukherjee, S. (2018). Role of plant phytochemicals and microbial enzymes in biosynthesis of metallic nanoparticles. *Applied microbiology and biotechnology, 102*(16), 6799-6814.
- Patil, M. P., & Kim, G.-D. (2018). Marine microorganisms for synthesis of metallic nanoparticles and their biomedical applications. *Colloids and Surfaces B: Biointerfaces, 172*, 487-495.
- Pérez-Juste, J., Pastoriza-Santos, I., Liz-Marzán, L. M., & Mulvaney, P. (2005). Gold nanorods: Synthesis, characterization and applications. *Coordination*

Chemistry Reviews, 249(17), 1870-1901. doi: https://doi.org/10.1016/j.ccr.2005.01.030

- Pokropivny, V., Lohmus, R., Hussainova, I., Pokropivny, A., & Vlassov, S. (2007). *Introduction to nanomaterials and nanotechnology*: Tartu University Press Ukraine.
- Poole Jr, C. P., & Owens, F. J. (2003). *Introduction to nanotechnology*: John Wiley & Sons.
- Pugazhendhi, A., Edison, T. N. J. I., Karuppusamy, I., & Kathirvel, B. (2018). Inorganic nanoparticles: a potential cancer therapy for human welfare. *International journal of pharmaceutics, 539*(1-2), 104-111.
- Requejo, K. I., Liopo, A. V., & Zubarev, E. R. (2018). Synthesis of Gold Nanorods Using Poly(vinylpyrrolidone) of Different Molecular Weights as an Additive. *ChemistrySelect, 3*(43), 12192-12197. doi: https://doi.org/10.1002/slct.201803337
- Roach, L., Ye, S., Moorcroft, S. C. T., Critchley, K., Coletta, P. L., & Evans, S. D. (2018). Morphological control of seedlessly-synthesized gold nanorods using binary surfactants. *Nanotechnology, 29*(13), 135601. doi: 10.1088/1361-6528/aaa99d
- Sachdeva, S., Singh, D., & Tripathi, S. K. (2020). Structural characterizations of gold nanorods synthesized using seed mediated method. *AIP Conference Proceedings, 2220*(1), 020053. doi: 10.1063/5.0001844
- Saha, K., Agasti, S. S., Kim, C., Li, X., & Rotello, V. M. (2012). Gold nanoparticles in chemical and biological sensing. *Chemical reviews, 112*(5), 2739-2779.
- Saldan, I., Dobrovetska, O., Sus, L., Makota, O., Pereviznyk, O., Kuntyi, O., & Reshetnyak, O. (2018). Electrochemical synthesis and properties of gold nanomaterials. *Journal of Solid State Electrochemistry, 22*(3), 637-656. doi: 10.1007/s10008-017-3835-5
- Samal, A. K., Sreeprasad, T. S., & Pradeep, T. (2010). Investigation of the role of NaBH4 in the chemical synthesis of gold nanorods. *Journal of Nanoparticle Research, 12*(5), 1777-1786. doi: 10.1007/s11051-009-9733-8
- Sardar, R., Funston, A. M., Mulvaney, P., & Murray, R. W. (2009). Gold nanoparticles: past, present, and future. *Langmuir, 25*(24), 13840-13851.
- Sardar, R., & Shumaker-Parry, J. S. (2008). Asymmetrically functionalized gold nanoparticles organized in one-dimensional chains. *Nano letters, 8*(2), 731-736.

- Sardoiwala, M. N., Kaundal, B., & Roy Choudhury, S. (2018). Chapter 37 - Development of Engineered Nanoparticles Expediting Diagnostic and Therapeutic Applications Across Blood–Brain Barrier. In C. Mustansar Hussain (Ed.), *Handbook of Nanomaterials for Industrial Applications* (pp. 696-709): Elsevier.
- Scarabelli, L., Sánchez-Iglesias, A., Pérez-Juste, J., & Liz-Marzán, L. M. (2015). A "Tips and Tricks" Practical Guide to the Synthesis of Gold Nanorods. *The Journal of Physical Chemistry Letters, 6*(21), 4270-4279. doi: 10.1021/acs.jpclett.5b02123
- Shah, N. J. (2021). Drug Used in Rheumatoid Arthritis 20. *Introduction to Basics of Pharmacology and Toxicology: Volume 2: Essentials of Systemic Pharmacology: From Principles to Practice*, 353.
- Shams, S., Bakhshi, B., Tohidi Moghadam, T., & Behmanesh, M. (2019). A sensitive gold-nanorods-based nanobiosensor for specific detection of Campylobacter jejuni and Campylobacter coli. *Journal of Nanobiotechnology, 17*(1), 43. doi: 10.1186/s12951-019-0476-0
- Shan, C., Huang, Y., Wei, J., Chen, M., & Wu, L. (2021). Ultra-high thermally stable gold nanorods/radial mesoporous silica and their application in enhanced chemo-photothermal therapy. *RSC Advances*, *11*(18), 10416-10424.
- Shi, X., Perry, H. L., & Wilton-Ely, J. D. (2021). Strategies for the functionalisation of gold nanorods to reduce toxicity and aid clinical translation. *Nanotheranostics, 5*(2), 155.
- Shi, Y., Jiang, R., Liu, M., Fu, L., Zeng, G., Wan, Q., . . . Wei, Y. (2017). Facile synthesis of polymeric fluorescent organic nanoparticles based on the self-polymerization of dopamine for biological imaging. *Materials Science and Engineering: C, 77*, 972-977.
- Smith, D. K., & Korgel, B. A. (2008). The Importance of the CTAB Surfactant on the Colloidal Seed-Mediated Synthesis of Gold Nanorods. *Langmuir*, *24*(3), 644-649. doi: 10.1021/la703625a
- Soulé, J.-F., Miyamura, H., & Kobayashi, S. (2011). Powerful Amide Synthesis from Alcohols and Amines under Aerobic Conditions Catalyzed by Gold or Gold/Iron, -Nickel or -Cobalt Nanoparticles. *Journal of the American Chemical Society, 133*(46), 18550-18553. doi: 10.1021/ja2080086
- Sperling, R. A., Gil, P. R., Zhang, F., Zanella, M., & Parak, W. J. (2008). Biological applications of gold nanoparticles. *Chemical Society Reviews*, *37*(9), 1896-1908.

- Sudri, S., Duadi, H., Altman, F., Allon, I., Ashkenazy, A., Chakraborty, R., . . . Hirshberg, A. (2021). Diffusion Reflection Method for Early Detection of Oral Squamous Cell Carcinoma Specifically Targeted by Circulating Gold-Nanorods Bio-Conjugated to Anti-Epidermal Growth Factor Receptor. *International Journal of Nanomedicine, 16*, 2237-2246.
- Wang, S., Chen, R., Yu, Q., Huang, W., Lai, P., Tang, J., & Nie, L. (2020). Near-Infrared Plasmon-Boosted Heat/Oxygen Enrichment for Reversing Rheumatoid Arthritis with Metal/Semiconductor Composites. *ACS Applied Materials & Interfaces, 12*(41), 45796-45806. doi: 10.1021/acsami.0c13261
- Wei, X. Z., & Mulvaney, P. (2014). Chapter 3 - Optical Properties of Strongly Coupled Plasmonic Nanoparticle Clusters. In N. V. Richardson & S. Holloway (Eds.), *Handbook of Surface Science* (Vol. 4, pp. 75-108): North-Holland.
- Xu, D., Du, Y., Ge, F., He, Y., & Yeung, E. S. (2021). Unveiling cellular internalization dynamics of single gold nanorods by tracking their orientational and translational motions. *CCS Chemistry*, 1-23.
- Xu, W., Wang, B., Zhang, Y., An, Q., Zhou, R., Peng, C., . . . Xing, Y. (2021). Recent advances on application of gold nanorods in detection field. *Materials Research Express*.
- Yan, H. H., Zhang, Q., Cheng, R., Zhu, F., Liu, J. J., Gao, P. F., . . . Wang, J. (2021). Size-Dependent Plasmonic Resonance Scattering Characteristics of Gold Nanorods for Highly Sensitive Detection of microRNA-27a. *ACS Applied Bio Materials*.
- Yang, G., Phua, S. Z. F., Bindra, A. K., & Zhao, Y. (2019). Degradability and clearance of inorganic nanoparticles for biomedical applications. *Advanced Materials*, *31*(10), 1805730.
- Yang, W., Liang, H., Ma, S., Wang, D., & Huang, J. (2019). Gold nanoparticle based photothermal therapy: Development and application for effective cancer treatment. *Sustainable Materials and Technologies, 22*, e00109. doi: https://doi.org/10.1016/j.susmat.2019.e00109
- Yao, J., Li, P., Li, L., & Yang, M. (2018). Biochemistry and biomedicine of quantum dots: from biodetection to bioimaging, drug discovery, diagnostics, and therapy. *Acta biomaterialia, 74*, 36-55.

- Ye, D., Zuo, X., & Fan, C. (2018). DNA nanotechnology-enabled interfacial engineering for biosensor development. *Annual Review of Analytical Chemistry, 11*, 171-195.
- Zaorsky, N. G., Churilla, T. M., Egleston, B. L., Fisher, S. G., Ridge, J. A., Horwitz, E. M., & Meyer, J. E. (2017). Causes of death among cancer patients. *Annals of Oncology*, *28*(2), 400-407. doi: 10.1093/annonc/mdw604
- Zhang, H., Liu, Y., Yao, S., Shang, M., Zhao, C., Li, J., & Wang, J. (2021). A multicolor sensing system for simultaneous detection of four foodborne pathogenic bacteria based on Fe3O4/MnO2 nanocomposites and the etching of gold nanorods. *Food and Chemical Toxicology*, *149*, 112035.
- Zhou, H., Liu, J., Dai, T., Mundo, J. L. M., Tan, Y., Bai, L., & McClements, D. J. (2021). The gastrointestinal fate of inorganic and organic nanoparticles in vitamin D-fortified plant-based milks. *Food Hydrocolloids*, *112*, 106310.
- Ziegler, C., & Eychmuller, A. (2011). Seeded growth synthesis of uniform gold nanoparticles with diameters of 15− 300 nm. *The Journal of Physical Chemistry C, 115*(11), 4502-4506.

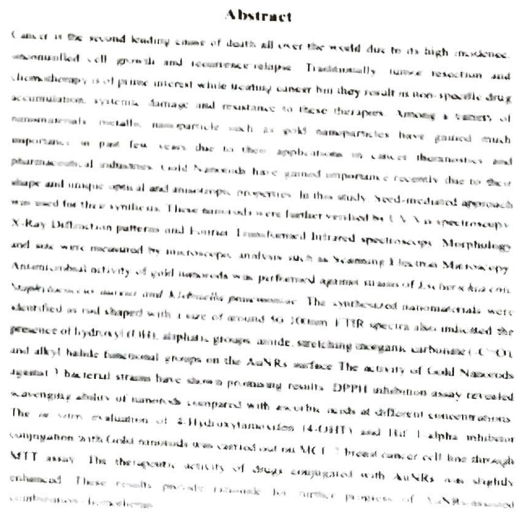
turnitin

Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author:	**Huzaifa Tahir**
Assignment title:	**Plagiarism detection (June 2021) Part 1 (Moodle TT)**
Submission title:	**thesis huzaifa**
File name:	**102318_Huzaifa_Tahir_thesis_huzaifa_715833_954140428.docx**
File size:	**1.55M**
Page count:	**68**
Word count:	**19439**
Character count:	**110446**
Submission date:	**24-Jun-2021 04:10PM (UTC+0500)**
Submission ID:	**1597857445**



Abstract

Cancer is the second leading cause of death all over the world due to its high incidence uncontrolled cell growth and recurrence relapse. Traditionally tumor resection and chemotherapy is of prime interest while treating cancer but they result in non-specific drug accumulation, systemic damage and resistance to these therapies. Among a variety of nanomaterials metallic nanoparticle such as gold nanoparticles have gained much importance in past few years due to their applications in cancer theranostics and pharmaceutical industries. Gold Nanorods have gained importance recently due to their shape and unique optical and nanoscopic properties. In this study Seed-mediated approach was used for their synthesis. These nanorods were further verified by U.V Vis spectroscopy X-Ray Diffraction patterns and Fourier Transformed Infrared spectroscopy. Morphology and size were measured by microscopic analysis such as Scanning Electron Microscopy Antimicrobial activity of gold nanorods was performed against strains of *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. The synthesized nanomaterials were identified as rod shaped with a size of around 50-200nm. FTIR spectra also indicated the presence of hydroxyl (OH), aliphatic groups, amide, stretching inorganic carbonate C=O and alkyl halide functional groups on the AuNRs surface. The activity of Gold Nanorods against 3 bacterial strains have shown promising results. DPPH inhibition assay revealed scavenging ability of nanorods compared with ascorbic acids at different concentrations. The in vitro evaluation of 4-Hydroxytamoxifen (4-OHT) and Tat-1 alpha inhibitor conjugation with Gold nanorods was carried out on MCF-7 breast cancer cell line through MTT assay. The therapeutic activity of drugs conjugated with AuNRs was slightly enhanced. These results provide rationale for further progress of AuNRs-assisted combinatorial hyperthermia

DR. RUMEZA HANIF
Associate Professor
Deptt of Healthcare Biotechnology
Abdur-Rahman School of Applied
(ASAB), NUST Islamabad

ORIGINALITY REPORT

15%	9%	12%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	John Stone. "Biological applications of gold nanorods", Wiley Interdisciplinary Reviews Nanomedicine and Nanobiotechnology, 01/2011 Publication	1%
2	Alkilany, A.M.. "Gold nanorods: Their potential for photothermal therapeutics and drug delivery, tempered by the complexity of their biological interactions", Advanced Drug Delivery Reviews, 201202 Publication	<1%
3	worldwidescience.org Internet Source	<1%
4	Submitted to University of Bridgeport Student Paper	<1%
5	openaccess.city.ac.uk Internet Source	<1%
6	link.springer.com Internet Source	<1%
7	www.tandfonline.com	

DR. RUMEZA HANIF
Associate Professor
Deptt of Healthcare Biotechnology
Atta-ur-Rahman School of Applied Biosciences (ASAB), NUST Islamabad

	Internet Source	<1%
8	www.mdpi.com Internet Source	<1%
9	iopscience.iop.org Internet Source	<1%
10	www.hindawi.com Internet Source	<1%
11	"Bio-manufactured Nanomaterials", Springer Science and Business Media LLC, 2021 Publication	<1%
12	Manoranjan Arakha, Suman Jha. "Interfacial Phenomena on Biological Membranes", Springer Science and Business Media LLC, 2018 Publication	<1%
13	tel.archives-ouvertes.fr Internet Source	<1%
14	"Handbook of Ecomaterials", Springer Science and Business Media LLC, 2019 Publication	<1%
15	dokumen.pub Internet Source	<1%
16	etd.auburn.edu Internet Source	<1%

17	ar.scribd.com Internet Source	<1%
18	pubs.acs.org Internet Source	<1%
19	repository.nwu.ac.za Internet Source	<1%
20	"Nanomaterials and Plant Potential", Springer Science and Business Media LLC, 2019 Publication	<1%
21	Jing, Hao, Li Zhang, and Hui Wang. "Geometrically Tunable Optical Properties of Metal Nanoparticles", UV-VIS and Photoluminescence Spectroscopy for Nanomaterials Characterization, 2013. Publication	<1%
22	www.ncbi.nlm.nih.gov Internet Source	<1%
23	"Nanomaterial Biointeractions at the Cellular, Organismal and System Levels", Springer Science and Business Media LLC, 2021 Publication	<1%
24	"Handbook of Environmental Materials Management", Springer Science and Business Media LLC, 2019 Publication	<1%

25	Sanvicens, N.. "Multifunctional nanoparticles - properties and prospects for their use in human medicine", Trends in Biotechnology, 200808 Publication	<1 %
26	epdf.tips Internet Source	<1 %
27	Hongwei Liao, Jason H. Hafner. "Gold Nanorod Bioconjugates", Chemistry of Materials, 2005 Publication	<1 %
28	users.itk.ppke.hu Internet Source	<1 %
29	Submitted to New Art College Student Paper	<1 %
30	archiv.ub.uni-marburg.de Internet Source	<1 %
31	www.coursehero.com Internet Source	<1 %
32	moam.info Internet Source	<1 %
33	ro.uow.edu.au Internet Source	<1 %
34	www.jrheum.org Internet Source	<1 %

35	"Microbial Nanobiotechnology", Springer Science and Business Media LLC, 2021 Publication	<1 %
36	Bartosz Sikora, Aleksandra Skubis-Sikora, Agnieszka Prusek, Joanna Gola. "Paracrine activity of adipose derived stem cells on limbal epithelial stem cells", Research Square Platform LLC, 2021 Publication	<1 %
37	baadalsg.inflibnet.ac.in Internet Source	<1 %
38	www.dtic.mil Internet Source	<1 %
39	www.nicnas.gov.au Internet Source	<1 %
40	Erica Sharpe, Daniel Andreescu, Silvana Andreescu. "Artificial Nanoparticle Antioxidants", American Chemical Society (ACS), 2011 Publication	<1 %
41	Submitted to University College London Student Paper	<1 %
42	jcovm.uobaghdad.edu.iq Internet Source	<1 %
43	"Nanotheranostics", Springer Science and Business Media LLC, 2019	<1 %

Publication

44	updatepublishing.com Internet Source	<1 %
45	docplayer.net Internet Source	<1 %
46	docshare.tips Internet Source	<1 %
47	repository.ntu.edu.sg Internet Source	<1 %
48	Anindita Behera, Bharti Mittu, Santwana Padhi, Nabanita Patra, Jagvir Singh. "Bimetallic nanoparticles: Green synthesis, applications, and future perspectives", Elsevier BV, 2020 Publication	<1 %
49	Ayse Busra Sengul, Eylem Asmatulu. "Toxicity of metal and metal oxide nanoparticles: a review", Environmental Chemistry Letters, 2020 Publication	<1 %
50	Submitted to Higher Education Commission Pakistan Student Paper	<1 %
51	Submitted to Indian Institute of Technology, Kharagpure Student Paper	<1 %
52	Submitted to KYUNG HEE UNIVERSITY	

	Student Paper	<1%
53	Submitted to University of Queensland Student Paper	<1%
54	www.scientific.net Internet Source	<1%
55	"Approaches in Bioremediation", Springer Science and Business Media LLC, 2018 Publication	<1%
56	Submitted to Hong Kong Baptist University Student Paper	<1%
57	Sardar, Rajesh, and Jennifer S. Shumaker-Parry. "Spectroscopic and Microscopic Investigation of Gold Nanoparticle Formation: Ligand and Temperature Effects on Rate and Particle Size", Journal of the American Chemical Society, 2011. Publication	<1%
58	Submitted to University of Birmingham Student Paper	<1%
59	Submitted to University of Liverpool Student Paper	<1%
60	etheses.bham.ac.uk Internet Source	<1%
61	research.brighton.ac.uk Internet Source	

<1 %

62 Dandan Huang, Jingchen Zhao, Minglin Wang, Shuhua Zhu. "Snowflake-like gold nanoparticles as SERS substrates for the sensitive detection of organophosphorus pesticide residues", Food Control, 2020 <1 %
Publication

63 Del Villar, Ignacio, Javier Goicoechea, Carlos Zamarreño, and Jesus Corres. "Nano-Materials and Nano-Structures for Chemical and Biological Optical Sensors", Series in Sensors, 2012. <1 %
Publication

64 Handbook of Nanoelectrochemistry, 2016. <1 %
Publication

65 Saroj Bala, Devender Kumar, Subhash Chand, Heena Wadhwa, Suman Mahendia, Shyam Kumar. "Synthesis and characterization of gold nanorods encapsulated in reduced graphene oxide", AIP Publishing, 2019 <1 %
Publication

66 Valeria V. Kleandrova, Feng Luan, Alejandro Speck-Planche, M. Natália D. S. Cordeiro. "chapter 61 QSAR-Based Studies of Nanomaterials in the Environment", IGI Global, 2017 <1 %
Publication

67	przyrbwn.icm.edu.pl Internet Source	<1 %
68	www.pulsus.com Internet Source	<1 %
69	"Antibacterial Drug Discovery to Combat MDR", Springer Science and Business Media LLC, 2019 Publication	<1 %
70	"Computational Approaches in Biomedical Nano ‐ Engineering", Wiley, 2018 Publication	<1 %
71	"Nanoscience in Food and Agriculture 2", Springer Science and Business Media LLC, 2016 Publication	<1 %
72	"New Frontiers of Nanomaterials in Environmental Science", Springer Science and Business Media LLC, 2021 Publication	<1 %
73	Amina Rahat, Shumaila Bashir, Khanzadi Fatima Khattak, Bashir Ahmad, Ibrar Khan, Sadiq Azam, Javid Ali, Jamshaid Ahmad. "Antifungal, Antibacterial, Phytotoxic and Insecticidal Activities of Nigella sativa Seeds", Asian Journal of Chemistry, 2015 Publication	<1 %

74	Avinash Singh Patel, C.K. Yeung, Charles Brennan, Haotian Zheng. "Ingredients & Formulation", Elsevier BV, 2020 Publication	<1 %
75	BNRC.springeropen.com Internet Source	<1 %
76	J. Shadmehr, F. Sedaghati, S. Zeinali. "Efficient elimination of propiconazole fungicide from aqueous environments by nanoporous MIL-101(Cr): process optimization and assessment", International Journal of Environmental Science and Technology, 2021 Publication	<1 %
77	Mahendra Rai, Alka Yadav, Aniket Gade. "CRC 675—Current Trends in Phytosynthesis of Metal Nanoparticles", Critical Reviews in Biotechnology, 2008 Publication	<1 %
78	Mena Aioub, Sajanlal R. Panikkanvalappil, Mostafa A. El-Sayed. "Platinum-Coated Gold Nanorods: Efficient Reactive Oxygen Scavengers That Prevent Oxidative Damage toward Healthy, Untreated Cells during Plasmonic Photothermal Therapy", ACS Nano, 2017 Publication	<1 %

79 Molly M Miller. "Sensitivity of metal nanoparticle plasmon resonance band position to the dielectric environment as observed in scattering", Journal of Optics A Pure and Applied Optics, 04/01/2006 <1%
Publication

80 Phat Trong Huynh, Giang Dang Nguyen, Khanh Thi Le Tran, Thu Minh Ho, Bich Thi Duong, Vinh Quang Lam, Thanh Vo Ke Ngo. "One-Pot, Surfactant-Free Synthesis of Gold Nanostars and Evaluation of Their Antibacterial Effects against Propionibacterium acnes", Journal of Nanomaterials, 2021 <1%
Publication

81 Shen Li, Joseph P. Garay, Colby A. Tubbs, Hector L. Franco. "CRISPR-based knockin mutagenesis of the pioneer transcription factor FOXA1; optimization of strategies for multi-allelic proteins in cancer cells", Cold Spring Harbor Laboratory, 2020 <1%
Publication

82 Siraj Uddin, Luqman Bin Safdar, Javed Iqbal, Tabassum Yaseen et al. " Green synthesis of nickel oxide nanoparticles using leaf extract of : Characterization, and diverse biological applications ", Microscopy Research and Technique, 2021 <1%

Publication

83	Sofia Bisso, Jean-Christophe Leroux. "Nanopharmaceuticals: A focus on their clinical translatability", International Journal of Pharmaceutics, 2020 Publication	<1 %
84	Springer Handbook of Nanomaterials, 2013. Publication	<1 %
85	Susanne Koeppl, Felix P. V. Koch, Walter Caseri, Ralph Spolenak. "Usage of the isotope effect for the synthesis of ultrahigh aspect ratio gold nanorods", Journal of Materials Chemistry, 2012 Publication	<1 %
86	Travis Jennings, Geoffrey Strouse. "Chapter 3 Past, Present, and Future of Gold Nanoparticles", Springer Science and Business Media LLC, 2007 Publication	<1 %
87	Submitted to University of Strathclyde Student Paper	<1 %
88	Vikas Pandey, Aditya Ganeshpurkar, Abhilasha Thakur, Megha Sharma et al. "Gold nanoparticles: An advanced drug delivery and diagnostic tool", Elsevier BV, 2020 Publication	<1 %

89	Zhi-Chuan Xu. "Wet chemical synthesis of gold nanoparticles using silver seeds: a shape control from nanorods to hollow spherical nanoparticles", Nanotechnology, 03/21/2007 Publication	<1 %
90	dspace.lib.cranfield.ac.uk Internet Source	<1 %
91	eprints.uthm.edu.my Internet Source	<1 %
92	followscience.com Internet Source	<1 %
93	kar.kent.ac.uk Internet Source	<1 %
94	kclpure.kcl.ac.uk Internet Source	<1 %
95	mafiadoc.com Internet Source	<1 %
96	mkon.nu Internet Source	<1 %
97	researchbank.rmit.edu.au Internet Source	<1 %
98	s-space.snu.ac.kr Internet Source	<1 %
99	tesisenxarxa.net Internet Source	<1 %

100	www.frontiersin.org Internet Source	<1 %
101	www.issp.ac.ru Internet Source	<1 %
102	www.victorcastano.net Internet Source	<1 %
103	"Bio‐inorganic Hybrid Nanomaterials", Wiley, 2007 Publication	<1 %
104	"Plant Nanobionics", Springer Science and Business Media LLC, 2019 Publication	<1 %
105	Adrián Fernández-Lodeiro, Jamila Djafari, Javier Fernández-Lodeiro, Maria Paula Duarte et al. "Synthesis of Mesoporous Silica Coated Gold Nanorods Loaded with Methylene Blue and Its Potentials in Antibacterial Applications", Nanomaterials, 2021 Publication	<1 %
106	Alaaldin M. Alkilany, Pratik K. Nagaria, Cole R. Hexel, Timothy J. Shaw, Catherine J. Murphy, Michael D. Wyatt. "Cellular Uptake and Cytotoxicity of Gold Nanorods: Molecular Origin of Cytotoxicity and Surface Effects", Small, 2009 Publication	<1 %

107	Arooma Jannat, Peter John, Attya Bhatti, Muhammad Qasim Hayat. "Tomorou attenuates progression of rheumatoid arthritis through alteration in ULK-1 independent autophagy pathway in collagen induced arthritis mice model", Cell Death Discovery, 2019 Publication	<1 %
108	Eric Singh, M. Meyyappan, Hari Singh Nalwa. "Flexible Graphene-Based Wearable Gas and Chemical Sensors", ACS Applied Materials & Interfaces, 2017 Publication	<1 %
109	Ghaemi, Ferial, Amirhassan Amiri, and Robiah Yunus. "Methods for coating solid-phase microextraction fibers with carbon nanotubes", TrAC Trends in Analytical Chemistry, 2014. Publication	<1 %
110	Gopalakrishnan, Sandhya, Kannan Vaidyanathan, and Nandakumar Kalarikkal. "RECENT ADVANCES IN NANOMEDICINE : APPLICATIONS IN DIAGNOSIS AND THERAPEUTICS", Advanced Nanomaterials, 2014. Publication	<1 %
111	Hemali Padalia, Sumitra Chanda. "Antioxidant and Anticancer Activities of Gold	<1 %

Nanoparticles Synthesized Using Aqueous Leaf Extract of Ziziphus nummularia", BioNanoScience, 2021
Publication

---

112 Huang, H.. "An approach for selectively oxidizing spherical nanoparticles in polydispersion gold nanorods", Colloids and Surfaces A: Physicochemical and Engineering Aspects, 20080320
Publication

<1 %

---

113 Katherinne I. Requejo, Anton V. Liopo, Paul J. Derry, Eugene R. Zubarev. "Accelerating Gold Nanorod Synthesis with Nanomolar Concentrations of Poly(vinylpyrrolidone)", Langmuir, 2017
Publication

<1 %

---

114 Leonardo Scarabelli, Ana Sánchez-Iglesias, Jorge Pérez-Juste, Luis M. Liz-Marzán. "A “Tips and Tricks” Practical Guide to the Synthesis of Gold Nanorods", The Journal of Physical Chemistry Letters, 2015
Publication

<1 %

---

115 Mani Alagiri, Perumal Rameshkumar, Alagarsamy Pandikumar. "Gold nanorod-based electrochemical sensing of small biomolecules: A review", Microchimica Acta, 2017
Publication

<1 %

116	Marya Ahmed, Ravin Narain. "Rapid Synthesis of Gold Nanorods Using a One-Step Photochemical Strategy", Langmuir, 2010 Publication	<1 %
117	Pei Liu, Rongying Zhou, Tingting Yin, Qiang Wang et al. "Novel bio-fabrication of silver nanoparticles using the cell-free extract of Lysinibacillus fusiformis sp. and their potent activity against pathogenic fungi", Materials Research Express, 2020 Publication	<1 %
118	Saito, Naoto, Hisao Haniu, Yuki Usui et al. "Safe Clinical Use of Carbon Nanotubes as Innovative Biomaterials", Chemical Reviews Publication	<1 %
119	Shengnan Liao, Wang Yue, Shuning Cai, Quan Tang, Weitong Lu, Lingxiao Huang, Tingting Qi, Jinfeng Liao. "Improvement of Gold Nanorods in Photothermal Therapy: Recent Progress and Perspective", Frontiers in Pharmacology, 2021 Publication	<1 %
120	Shu Ting Zhang, Zhi Ang Liu, Ming Yue Hu, Sulan Ma, Min Li, Xiao Li Liu. "Synthesis of Gold Nanorods by Using H_2O_2 as the Weak Reducer in a Binary Surfactant Mixture", Journal of Nano Research, 2020 Publication	<1 %

121	Siddik Sarkar, Suraj Konar, Puvvada Naga Prasad, Shashi Rajput et al. "Micellear Gold Nanoparticles as Delivery Vehicles for Dual Tyrosine Kinase Inhibitor ZD6474 for Metastatic Breast Cancer Treatment", Langmuir, 2017 Publication	<1 %
122	Weerakanya Maneeprakorn, Suwussa Bamrungsap, Natpapas Wiriyachaiporn, Kajornsak Faungnawakij. "Inorganic nanoparticle-based biosensors for point-of-care diagnostics", Elsevier BV, 2021 Publication	<1 %
123	Xin Hu, Noriharu Takada, Siti Machmudah, Wahyudiono, Hideki Kanda, Motonobu Goto. "Ultrasonic-Enhanced Fabrication of Metal Nanoparticles by Laser Ablation in Liquid", Industrial & Engineering Chemistry Research, 2020 Publication	<1 %
124	Yucai Wang, Kvar C. L. Black, Hannah Luehmann, Weiyang Li et al. "Comparison Study of Gold Nanohexapods, Nanorods, and Nanocages for Photothermal Cancer Treatment", ACS Nano, 2013 Publication	<1 %
125	Zhou, Rui, Tianyuan Zhou, Min Li, Hongmei Gong, and Qian Tao. "Synthesis of gold	<1 %

nanorods with longitudinal surface plasmon resonance wavelength up to 1245 nm using gallic acid as a reductant in the presence of a binary surfactant system", Micro & Nano Letters, 2015.
Publication

126	book.medazma.ir Internet Source	<1 %
127	cdn.intechopen.com Internet Source	<1 %
128	d-nb.info Internet Source	<1 %
129	dx.doi.org Internet Source	<1 %
130	eprints.soton.ac.uk Internet Source	<1 %
131	eprints.utar.edu.my Internet Source	<1 %
132	etd.lib.metu.edu.tr Internet Source	<1 %
133	hdl.handle.net Internet Source	<1 %
134	idoc.pub Internet Source	<1 %
135	iwaponline.com	

	Internet Source	<1%
136	pt.scribd.com Internet Source	<1%
137	publikationen.bibliothek.kit.edu Internet Source	<1%
138	repositories.lib.utexas.edu Internet Source	<1%
139	repository.bilkent.edu.tr Internet Source	<1%
140	shodhganga.inflibnet.ac.in Internet Source	<1%
141	shura.shu.ac.uk Internet Source	<1%
142	"Bioelectrochemical Interface Engineering", Wiley, 2019 Publication	<1%
143	"Functional Chitosan", Springer Science and Business Media LLC, 2019 Publication	<1%
144	"Fungal Nanotechnology", Springer Science and Business Media LLC, 2017 Publication	<1%
145	Guler, Bahar, Bilal Demir, Emine Guler, Kadri Gulec, Ozan Yesiltepe, Dilek Odaci Demirkol,	<1%

and Suna Timur. "Targeting and imaging of cancer cells using nanomaterials", Nanobiomaterials in Medical Imaging, 2016.
Publication

146	Handbook of Nanomaterials Properties, 2014. Publication	<1 %
147	Ken-Tye Yong. "Templated Synthesis of Gold Nanorods (NRs): The Effects of Cosurfactants and Electrolytes on the Shape and Optical Properties", Topics in Catalysis, 03/2008 Publication	<1 %
148	Liu, Q.. "Antioxidant activities of barley seeds extracts", Food Chemistry, 2007 Publication	<1 %
149	Maria Gorbunova, Vladimir Apyari, Stanislava Dmitrienko, Yury Zolotov. "Gold nanorods and their nanocomposites: synthesis and recent applications in analytical chemistry", TrAC Trends in Analytical Chemistry, 2020 Publication	<1 %
150	Mengxiao Yu, Jie Zheng. "Clearance Pathways and Tumor Targeting of Imaging Nanoparticles", ACS Nano, 2015 Publication	<1 %
151	Nanotechnology in Drug Delivery, 2009. Publication	<1 %

152	Sania Arif, Aamina Batool, Nauman Khalid, Iftikhar Ahmed, Hussnain Ahmed Janjua. "Comparative analysis of stability and biological activities of violacein and starch capped silver nanoparticles", RSC Advances, 2017 Publication	<1 %
153	"Biomaterials in Clinical Practice", Springer Science and Business Media LLC, 2018 Publication	<1 %
154	"Nanostructures for Antimicrobial and Antibiofilm Applications", Springer Science and Business Media LLC, 2020 Publication	<1 %
155	Advanced Sciences and Technologies for Security Applications, 2016. Publication	<1 %
156	Katherinne I. Requejo, Anton V. Liopo, Eugene R. Zubarev. "Gold Nanorod Synthesis with Small Thiolated Molecules", Langmuir, 2020 Publication	<1 %
157	Linfeng Gou, Catherine J. Murphy. "Fine-Tuning the Shape of Gold Nanorods", Chemistry of Materials, 2005 Publication	<1 %
158	Mariam Abubaker, Che Wan Zanariah Che Wan Ngah, Musa Ahmad, Bambang Kuswandi.	<1 %

"Functionalized gold nanoparticles as optical nanosensors for determination of aluminum (III) ions in water samples", Elsevier BV, 2019
Publication

159 Nikhil R. Jana. "Gram-Scale Synthesis of Soluble, Near-Monodisperse Gold Nanorods and Other Anisotropic Nanoparticles", Small, 2005 <1%
Publication

160 S. Dhara. "Formation, Dynamics, and Characterization of Nanostructures by Ion Beam Irradiation", Critical Reviews in Solid State and Materials Sciences, 1/2007 <1%
Publication

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	On		