Ciprofloxacin Loaded Gold Nanoparticles for the Eradication of *Enterococcus faecalis* in Liver and Kidney of Mice

By

SYED MOHSIN ALI

Reg. No. **00000205026**

Supervisor

Dr. Nosheen Fatima Rana

DEPARTMENT OF BIOMEDICAL ENGINEERING AND SCIENCES

SCHOOL OF MECHANICAL & MANUFACTURING ENGINEERING

NATIONAL UNIVERSITY OF SCIENCES AND TECHNOLOGY

ISLAMABAD

2020

Ciprofloxacin Loaded Gold Nanoparticles for the Eradication of *Enterococcus faecalis* in Liver and Kidney of Mice

By

Syed Mohsin Ali

Registration Number

00000205026

A thesis submitted in partial fulfillment of the requirements for the degree of

MS Biomedical Engineering and Sciences

Supervisor

Dr. Nosheen Fatima Rana

Thesis Supervisor's Signature: _____

DEPARTMENT OF BIOMEDICAL ENGINEERING AND SCIENCES

SCHOOL OF MECHANICAL & MANUFACTURING ENGINEERING

NATIONAL UNIVERSITY OF SCIENCES AND TECHNOLOGY, ISLAMABAD

2020

THESIS ACCEPTANCE CERTIFICATE

Certified that final copy of MS thesis written by Mr. Syed Mohsin Ali (Registration No. 00000205026), of SMME (School of Mechanical and Manufacturing Engineering) has been vetted by undersigned, found complete in all respects as per NUST Statutes / Regulations, is free of plagiarism, errors, and mistakes and is accepted as partial fulfillment for award of MS/MPhil degree. It is further certified that necessary amendments as pointed out by GEC members of the scholar have also been incorporated in the said thesis.

Signature: _____

Supervisor: Dr. Nosheen Fatima Rana

Date: _____

Signature (HOD): _____

Date: _____

Signature (Dean/Principal): _____

Date: _____

FORM TH-4

MASTER THESIS WORK

We hereby recommend that the dissertation prepared under our supervision by **Mr. Syed Mohsin Ali** 205026 Titled: **"Ciprofloxacin Loaded Gold Nanoparticles for the Eradication of *Enterococcus faecalis* in Liver and Kidney of Mice** "be accepted in partial fulfillment of the requirements for the award of **MS Biomedical Sciences** degree. (Grade_____)

Examination Committee Members

1. Name: Dr. Umar Ansari Signature: _____

2. Name: Dr. Saima Zafar Signature: _____

Supervisor: Dr. Nosheen Fatima Rana Signature: _____

Date: _____

_____ _____

Date Head of Department

COUNTER SIGNED

_____ _____

Date Principal

Declaration

It is hereby declared that this research study has been done for partial fulfillment of the requirement for the degree of Master of Sciences in Biomedical Sciences. This work has not been taken from any publication. I hereby also declare that no portion of work referred to in this thesis has been submitted in support of an application for another degree or qualification in this university or other institutes of learning.

Plagiarism Certificate (Turnitin Report)

This thesis has been checked for Plagiarism. Turnitin report endorsed by Supervisor is attached.

Signature of Student

Syed Mohsin Ali

Registration Number

00000205026

Signature of Supervisor

Copyright Statement

ACKNOWLEDGMENTS

Foremost, I want to offer this endeavor to **Allah Almighty** for the wisdom he bestowed upon me, the strength, peace of mind and good health in order to finish this research.

This thesis becomes a reality with the kind support and help of many individuals; I would like to extend my sincere thanks to all of them.

It is genuine pleasure to express my deep sense of thanks to my supervisor **Dr. Nosheen Fatima Rana** for her guidance, encouragement and constant supervision as well as providing necessary support in completing this endeavor. Her dedication and keen interest above all his overwhelming attitude to help me had been solely and mainly responsible for completing my work.

I would like to express my sincere gratitude toward my family for the encouragement which helped me in the completion of my research,

Finally, I would also like to thank all my colleagues, seniors and lab fellows for their wise counsel that hugely impacted various aspects of my research.

This study has indeed helped me to explore more knowledge avenues related to my topic and I am sure it will help me in my future

Dedicated to my exceptional parents whose tremendous support and cooperation led me to this wonderful accomplishment

ABSTRACT

The availability of diverse nanoparticles with controlled properties has generated widespread interest in their use in biological systems. Gold nanoparticles are widely used in the biomedical field because of their large surface area, and high conductivity. The modification of the nanometers is conducted to enhance the interaction of these nanoparticles with biological cells. *Enterococcus faecalis* a well know pathogen that causes nosocomial infection. It also shows multidrug resistance. This research includes the synthesis of gold nanoparticles then drug loading. After this different characterization techniques (UV-Vis, FTIR, SEM, Zeta potential, stability tests, etc.) were done to check these drugs loaded gold nanoparticles. Then evaluate the effect of an antibiotic Ciprofloxacin and its gold nanoparticles on *Enterococcus faecalis*. Study shows that metal nanoparticles could be useful carriers for ciprofloxacin, and ciprofloxacin loaded gold nanoparticles are stable. The bound ciprofloxacin is fluorescent, and this property could be used in biological investigations and they together are more effective in eradicating *Enterococcus faecalis* in the liver and kidney of mice.

List of Figures

List of Tables

Contents

CHAPTER 1

INTRODUCTION

1.1 NANOTECHNOLOGY

In nanotechnology, there are nanoparticles ranging in size between 1 and 100nm with a surrounding interfacial layer (Drexler and Eric 1986). Nanotechnology concept was first discussed by Richard Feynman in his lecture in 1959 in which he discussed the possibility of synthesis of nanoparticles via direct manipulation of atoms (Drexler and Eric 1992). In 1970, Japanese researcher defined nanotechnology for the very first time as "Nanotechnology majorly consist of the processing of, separation of, consolidation and deformation of materials by atom and molecules". (Corbett, McKeown, et al. 2000). After that many advancements in nanotechnology have been observed until now. Muslim scientists in the 9th century studied the synthesis of nanoparticles by carrying out the reduction of metal oxide upon heating at high temperatures which were already deposited on ceramic surfaces (Padeletti and Fermo 2003; Daniel and Astruc 2004).

According to the National Institute of Health, Nanomedicine is a formulation of a drug having a size less than a micron (Babu, Templeton, et al. 2013). Nanomedicine has become much important due to its ability to overcome many barriers such as blood-brain barrier etc. and it also enhances the bioavailability of drug (Lavan, McGuire, et al. 2003), effectively delivers hydrophobic therapies, and targets disease sites (Babu, Templeton, et al. 2013).

1.2 ANTIBIOTIC RESISTANCE

Antibiotics are used to treat bacterial infections. Change in bacteria response causes antibiotic resistance. Bacteria inside animals and humans become resistant. These bacteria may infect humans, due to this infection are difficult to treat than those caused by normal bacteria. This antibiotic resistance is very dangerous and leads to prolonged hospital stays, increased mortality, and higher medical cost. Behavior changes must include hand washing, proper vaccination method, good food hygiene, etc. These days new mechanism is emerging all over the world, threatening our ability to cure ordinary diseases. A growing list of infectious diseases includes tuberculosis, pneumonia, gonorrhea, and foodborne diseases. They are slowly becoming more and more difficult to treat as antibiotics are becoming less effective. The main cause of resistance is the misuse or overuse of antibiotics, as well as prevention.

1.3 CIPROFLOXACIN

Ciprofloxacin is a well-known antibiotic that is used against a huge number of infections of bacteria. The infections could be off joints or bones, abdomen or respiratory tract, skin infection or urinary tract infection. This antibiotic is approved by the World Health Organization to be the safest and most effective antibiotic for mankind against microbial diseases. These drugs are fluorescent and can be probed using different techniques even with the low concentration of the drug. Fluoroquinolones such as ciprofloxacin, norfloxacin, clinofloxacin, sparfloxacin, and levofloxacin are antibacterial which are being used against several microbial infections. The information available on the nature of the binding of gold nanoparticles and ciprofloxacin is very limited.

1.3.1 Mechanism of Action

As far as the mode of action is concerned, the fluoroquinolones are well known anti-bacterial agents that target two important enzymes of bacteria. One is the DNA topoisomerase IV and the other is the DNA Gyrase. The DNA gyrase introduces supercoiling in the DNA of bacteria. On the other hand, DNA topoisomerase IV causes the recognition of the crossovers in DNA and therefore is a decatenating enzyme. It restricts the repair, as well as the reproduction of the genetic material of bacteria, thus stopping the multiplication of the bacteria.

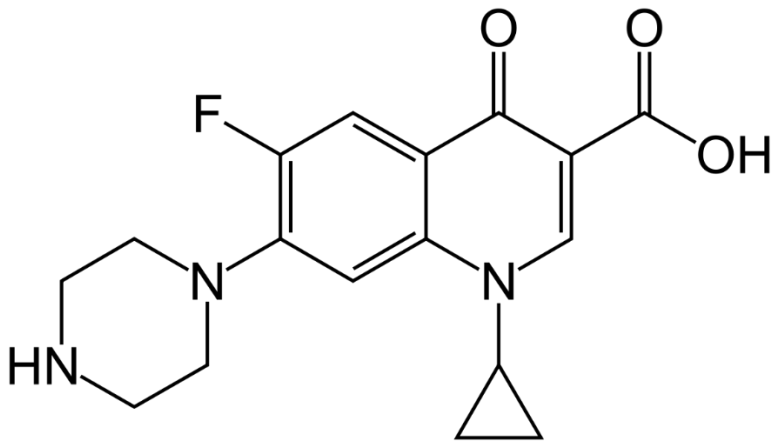


Figure 1.1 Chemical Structure of Ciprofloxacin

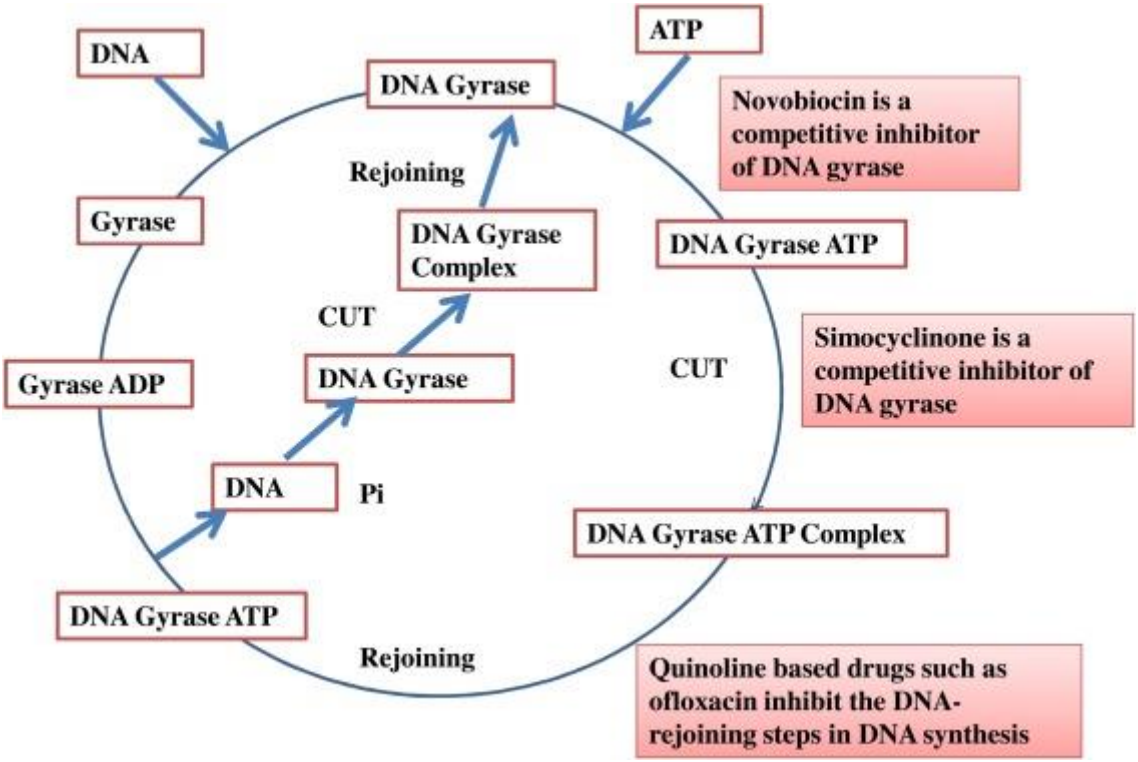


Figure 1.2 Pathway Showing DNA Gyrase in DNA molecule

1.4 *Enterococcus faecalis*

It is a facultative anaerobe bacterium that naturally inhabits the GI tract. *E. faecalis* can grow in a wide range of temperatures that have low G+ C content. These LAB group members have the ability to make bacteriocins. They are commonly used to enhance flavor and to ripen cheese. *E. faecalis* is also a commonly acquired infection from hospitals. E. faecalis is responsible for a variety of infections but mainly nosocomial infection other infections include urinary tract, bloodstream, surgical site, endocarditis, and visceral infection. It is resistant to drying, high temperatures, and strong chemicals. *Enterococcus faecalis* strains exhibit resistance to a wide range of antibiotics with a rising multidrug-resistant strain. It is

involved also in the transference of resistance genes to other bacteria. Now recently, the study of antibiotic-resistant microbes shows the connection between the bactericidal activity of antimicrobial and oxidative stress. Although *E. faecalis* can detoxify ROS, it gives ROS an advantage related to its surviving ability and to be more harmful.

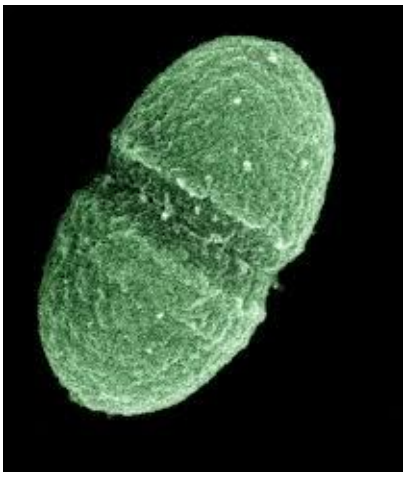


Figure 1.3 ***Enterococcus faecalis***

1.5 APPLICATIONS OF NANOPARTICLES IN MEDICINE

Nanoparticles are small particles having dimensions which resemble the building blocks of macromolecules such as DNA and proteins. Due to this feature nanoparticles have the benefit of being used for therapeutic purposes. Surface functionalization of particles can be done by various signaling molecules, functional groups, targeted molecules to make it target specific. Nanoparticles can also be made biocompatible by binding with different functional groups and it is loaded with drugs so that it can be used as a drug-delivering vehicle. Surfaces of nanoparticles are modified in such a way so that they can bind to different functional groups that determine the fate of the nanoparticles as to where they should be targeted.

Nanoparticles have a void or internal core where the material or the drug to be targeted is encapsulated. So not only the toxicity caused by the material or drug is minimized but also controlled release of the drug is achieved. Nanoparticles encapsulate other small molecules and radiolabeled molecules in its void or internal core to be used in imaging techniques. Such molecule encapsulated by nanoparticles do not cause harmful effects in the rest of the body due to its biocompatibility and target specificity

The important property of using nanoparticles in diagnostics and medicine is its biocompatibility. The outer surface of nanoparticles is modified by binding functional group molecule or encapsulating it with polyethylene glycol (PEG) to make it biocompatible and so it does not provoke immune reactions and so other inflammatory processes as well. It is considered as a self-molecule.

Surface functionalization of nanoparticle with small functional group molecules or with other ligands make nanoparticle highly targeted, also the controlled and sustained release of drug is because the surface group attached. Furthermore, functionalization has a lot to do with the biodistribution of drugs and plays an important part in its pharmacokinetic behavior. Nano-particle surface modified by small functional groups plays a huge role in the mode of excretion of nanoparticle from the body and its biodistribution gives an idea of the type of clearance the nanoparticle follows (Bharali, Khalil, et al. 2009).

1.6 AIMS AND OBJECTIVE

The purpose of this research is to synthesize ciprofloxacin loaded gold nanoparticles and then use this drug to remove *Enterococcus faecalis* more effectively from the kidney and liver of mice.

- Synthesis of Gold nanoparticles
- Drug Loading
- Characterization of ciprofloxacin loaded gold nanoparticles
- In-vitro and in-vivo studies.

This research is done in two different parts or categories. The first part is the formation of gold nanoparticles with drug loading and different characterization test. Drug ciprofloxacin is a fluoroquinolone an antibiotic that fights and kills different bacteria. This drug treats many types of bacterial infections including nosocomial infections, bone and joints infections, sinus or respiratory infections, skin infections and certain types of diarrhea.

The second part of our research is in-vitro and in-vivo studies in which we have used this drug to remove or eradicate *Enterococcus faecalis* from the liver and kidney of mice. We know that *E.faecalis* is becoming resistant to ciprofloxacin and many other antibiotics, so we have loaded this drug with gold nanoparticles and then check the results of this loading in the liver and kidney of mice.

CHAPTER 2

LITERATURE REVIEW

The most challenging and important thing is the controlling of the shape and size of nano-particles synthesis as this is an important consideration in determining the catalytic activity and shape of nanoparticles (Nikoobakht, B., et al. 2003). Different properties of nanoparticles of gold make them good drug-delivering vehicles. These nanoparticles can have a wide range of sizes from 1 to 150nm. They can be coated by several targeted agents. Moreover, essential properties include non-toxicity and biocompatibility. Gold nanoparticles also have great optical, chemical, and physical properties. These can be used to control pharmaceutical compound transport. Citrate reduction ability can be used for colloidal gold preparation. Gold nano-shells, nano-rods, and empty nanoparticles are some examples of several structures of gold nanoparticles in which they can be fabricated. Nobel nanoparticles (metal) are different from nano-platform like polymeric nanoparticles, magnetic nanoparticles, and semiconductor quantum dots. This feature strengthens each one of the irradiative and radiative properties of nanoparticles (Alaqad, K., & Saleh, T. A. 2016).

The increased number of atoms and the high surface to volume ratio give nanoparticles their uniqueness. They turned out to be important materials in the progression of many novel technological devices that are used in multiple pharmaceutical, physical and biomedical applications. Nanoparticles size is the reason for the phenomenal use of nanoparticles. Among different nanoparticles, gold nanoparticles are mainly used as a catalyst for diagnostic uses, medical treatment, and gene therapy. By chemical reduction method, gold nanoparticles are difficult to synthesize and have very low toxicity in variance with different nanomaterials.

To enhance the use of nanoparticles, different techniques have been used for the synthesis of different dimensions to functionalize their surface. The main problems in producing various techniques include their low polydispersity and high purity. Different solvents, stabilizers and reducing agents have been used to control the shape and size of nanoparticles. The effect of stabilizers on morphology and size has also been considered (Shamaila, S., et al. 2016).

Gold is being inquired for its antifungal and antibacterial properties. It has been used both as ionic states and as well as nanoparticles for checking its activities against fungal and bacterial strains. Gold complexes Au(I) and Au (III) both have been found to show antibacterial activity as well as antifungal properties. Prior research suggests that gold is not bactericidal. When weak and at higher concentrations, it shows some activity. One of the main reasons for antibacterial activity might be due to different chemicals that are used to synthesize nanoparticles that are not properly removed after washing or chemicals in gold ions or nanoparticles coating. Overall these gold nanoparticles are efficient carriers of drugs and antibiotics. They are important in enhancing the antibacterial activity of many antibiotics and drugs moreover reducing the side effects and effective doses that are usually caused by a high dosage of drugs drug (Zhang, Y., et al. 2015). Surface Plasmon peak property of gold nanoparticles is unique. This property is a feature of particles that have a small size close to lights wavelength. It is a physical concept in which electrons cause oscillations. Cytotoxicity can be minimized, and biocompatibility can be enhanced by capping and coating of gold nanoparticles. The toxic effect of nanoparticles can be harmful without coating, so capping is done to reduce this toxic effect (Matulionyte, M., et al. 2017).

The coating or capping of these nanoparticles can be of great benefit as it can increase the holding time of the desired nanoformulation in the tissues and cells thus increasing the effect of the formulation of the drug. Different Viral and non-viral vectors can also be used for synthesis. Many cationic vectors such as polypropyeneimine, polyethyleneimine (PEI), etc. have also been used. **(**Teimouri, M., et al. 2016).

Different techniques were used for the production of ciprofloxacin loaded solid nanoparticles. A conversion of ciprofloxacin HCL to a free base was done in situ for the purpose to efficaciously entrap the salt in the system. Positively charged solid lipid nanoparticles were synthesized by the addition of dodecyl dimethyl ammonium bromide (DDAB), a cationic lipid. This was done to ameliorate the interaction with the cell membrane of bacteria and to ensure physical stability to the carriers. Encapsulation efficiency was found out to be around 85% for many samples and particle-sizes of about 250-350 nm were made using both the methods. The physical stability of nanoparticles was found to be around nine months at 4 and 25°C. Ciprofloxacin encapsulation efficiency reduces slightly after 9 months. Cationic lipid which increases the antibacterial activity of ciprofloxacin in Nano-carriers were also formulated. (Rosario Pignatello et al.).

The main objective of this research was to develop a controlled drug delivery system by encapsulating the Ciprofloxacin. For the preparation of CIP-loaded SLNs, the ‘Emulsification method” was done. Results showed the successful synthesis of SLNs with average particle sizes ranging from 165 – 320 nm with 0.8 – 0.33 range of polydispersity index. All formulations showed high entrapment efficiency values. Spherical shape image having size range equivalent to the size observed by was shown by particle size analyzer. The CIP

release with various lipids shown the controlled-release behavior. The high and rapid release rate and strongest burst effect exhibited by Ciprofloxacin SLNP formula containing stearic acid. (Gamal A. Shazly)

Poly lactic-co-glycolic acid nanoparticles including ciprofloxacin were prepared by the emulsion solvent diffusion method. Probe sonication application effects in preparation of secondary emulsion beyond the high-pressure homogenization and its application were assessed during solidification. Their effect on the Zeta potential and size of the particle was analyzed. Furthermore, the effect of the introduction of 0.1 M NaCl and/or changing the extracting and external phases to pH 7.4 was analyzed. Xray, IR, SEM and DSC and in vitro drug release were examined by using the selected formula. Results: These formulations exhibited -0.839 to -6.81 mV (Zeta Potential), 135.7-187.85 nm particle size and encapsulation efficiency percentage (EE%) going from 35% to 69%. Conclusion: The presented data shows that using probe sonication is superior. The results showed that the elements that were investigated had a notable result in encapsulation efficiency percentage. (Doaa H Mubarak).

Nanoparticles commonly show antibacterial effects through feasible interaction with deoxyribonucleic acid (DNA) or with proteins inside the bacteria. Colony-forming unit (CFU) assay on bacteria cultured in silver nanoparticles or silver ions were conducted as a quantitative analysis on antimicrobial activities of nanoparticles remains uncertain. The comparison was done on different cultured media. One contained the nanoparticles while the other lacked them. It was found that the culture that lacked silver nanoparticles did not affect the growth rate of bacteria but instead, the lag time was changed. The growth rate was

extended in the culture which had the silver nanoparticles. Moreover, CFU assay confirmed that bacteria were killed exponentially in the presence of silver nanoparticles or ions. On experiment data, a quantitative analysis was established to describe the antimicrobial activity of silver nanoparticles. The prediction agrees well with the results from this model and dependence on different concentration of silver nanoparticles were also showed (Mohammad A. Haque et al.)

CHAPTER 3

MATERIAL AND METHODS

3.1 MATERIALS

All used chemicals were purchased. These chemicals were HAuCl4, trisodium citrate, 2-propanol, sodium bicarbonate, distilled water, and ciprofloxacin. A digital pH meter (model 510 Oakton, Eutech) was used.

3.2 SYNTHESIS OF GOLD NANOPARTICLES

Gold Nanoparticles of size 20-40nm were prepared for this purpose an 18 mL volume of distilled water was taken and 0.5mL of 10−2M tri-sodium citrate was mixed. 0.5mL chloroauric acid was added. The solution was stored overnight at 40°C. In this method, trisodium citrate behaved as the reducing agent itself. The color changed to wine red which indicates the formation of 20-40nm gold nanoparticles. The UV Vis spectrum of the gold nanoparticles was at 520nm. The solution was diluted for the UV-vis spectrum. These particles of 20-40nm size were further confirmed by Scanning Electron microscopy and Zetasizer. The concentration of the solution was 0.5mM.

3.3 SYNTHESIS OF CIPROFLOXACIN LOADED GOLD NANOPARTICLES

For Drug conjugation gold nanoparticle solution divided into 5 separate beaker containing 20mL each. Then each sample was mixed with 5mL of different concentration of ciprofloxacin (0.5mM, 1.0mM, 1.5mM, 2mM and 2.5mM) in 2-propanol and stirred well.

The pH was around 6.5 in all solutions. Then 8-hour stirring was done until the red color turned to blue color and the pH was acidic when observed. Time-dependent UV-VISIBLE spectra at different time intervals were recorded after mixing ciprofloxacin and gold nanoparticles. These solutions were kept overnight. Centrifugation was done and these solutions were washed with propanol 3 times to remove unabsorbed sodium citrate and ciprofloxacin. The Precipitate can be dispersed in organic solvents such as DMSO, DMF, butanol, and 2-propanol by sonication.

3.4 CHARACTERIZATION TEST

3.4.1 Stability test

Different stability tests were performed on gold nanoparticles like salt effect, temperature effect and pH effect. These tests were performed to check the stability of gold nanoparticles. The gold nanoparticles and drug-loaded nanoparticles were characterized by UV-2800 BMS Scientific Technical Corporation (PVT) Ltd.

3.4.2 Encapsulation Efficiency

Encapsulation Efficiency is the amount of drug that is successfully loaded into nanoparticle or micelle. Encapsulation efficiency was calculated with different drug concentrations to find the best suitable concentration of drugs in nanoparticles with the best encapsulation efficiency. For this different dilutions of the drug were taken to obtain the standard curve and this standard curve was further used in calculations.

Encapsulation Efficiency = (Total drug added – non entrapped drug)/total drug X 100

3.4.3 Drug Loading Capacity

It is the total drug loaded per unit weight of nanoparticles. It shows the mass of nanoparticles in percentage that is due to the encapsulated drug. For drug loading different concentration of drug loaded nanoparticles were taken to check the best suitable concentration. A standard curve was also used to calculate it. The formula of drug loading capacity is as follows,

Drug loading Capacity = Total entrapped drug / total nanoparticle weight X 100

3.4.4 Drug Release Efficiency

Drug release efficiency of different concentration of ciprofloxacin loaded gold nanoparticles were done. For this purpose 20mL of 20mM sodium bicarbonate solution was mixed well with 20mL ciprofloxacin loaded gold nanoparticles solution. This solution was then further divided into 5mL 8 fractions. Each fraction was centrifuged at different time intervals. One sample of the solution was used only once. The graph was plotted which gave the commutative drug release of ciprofloxacin.

3.4.5 UV-VIS SPECTROSCOPY

It is reflectance or absorbance spectroscopy in part of ultraviolet and visible spectral regions. It uses light in regions adjacent and visible. It works on the principle of a reflected beam of

light. The extent of absorption is measured when a beam of light falls on the sample and then the reflected light will give the absorbance which is measured by the light sensor. A glass cuvette containing the sample is placed in front of the lens and the beam of light will pass through it. The beam is split into two parts, one half passes through the sample and the other half through the solvent only. In this device, the absorption can be measured at the desired wavelength and the range can also be changed accordingly and can be adjusted as required. The plotted graph gives the wavelength on the x-axis and absorbance on the y-axis. The maximum absorption which is observed at a certain specific wavelength is called the lambda max. Lambda max obeys the Beer-Lambert Law. This law measures the electronic transition of molecules. The molar concentration of the sample is proportional to the absorbance of the sample. We call the absorption as the molar absorptivity. Beer-Lambert Law states that

$$A=EcL$$

So,

$$E=A/cL$$

Due to this law, the UV-vis is very helpful for the quantitative analysis

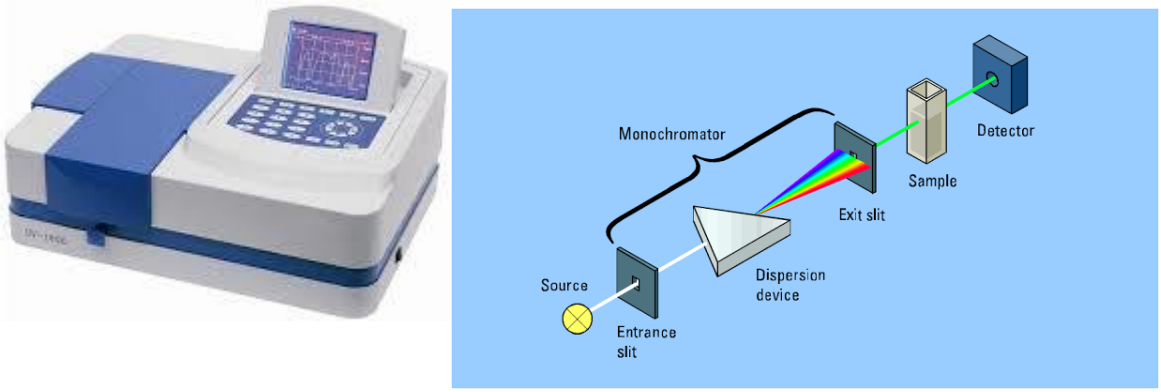


Figure 3.1 UV vis Spectrophotometer

3.4.6 Zeta potential

It is the charge in Millivolts (mV) that develops at the interface between a solid and liquid medium. It is a characterization technique to estimate surface charge so that physical stability can be employed of Nano suspension (Jiang et al., 2009). A high value of zeta potential indicates the physical stability of Nano suspensions which is because of the electrostatic repulsion of the particles. Zeta potential other than -30 millivolts to +30 millivolts is overall considered to have enough repulsive forces to have good physical colloidal stability. Whereas, low zeta potential value shows particle flocculation and aggregation because Van Der Waals forces act on them and that can result in physical instability (Hunter, 2013 et al.). Factors such as the presence of solution chemistry and material properties are also related to the physical stability of Nano suspension.

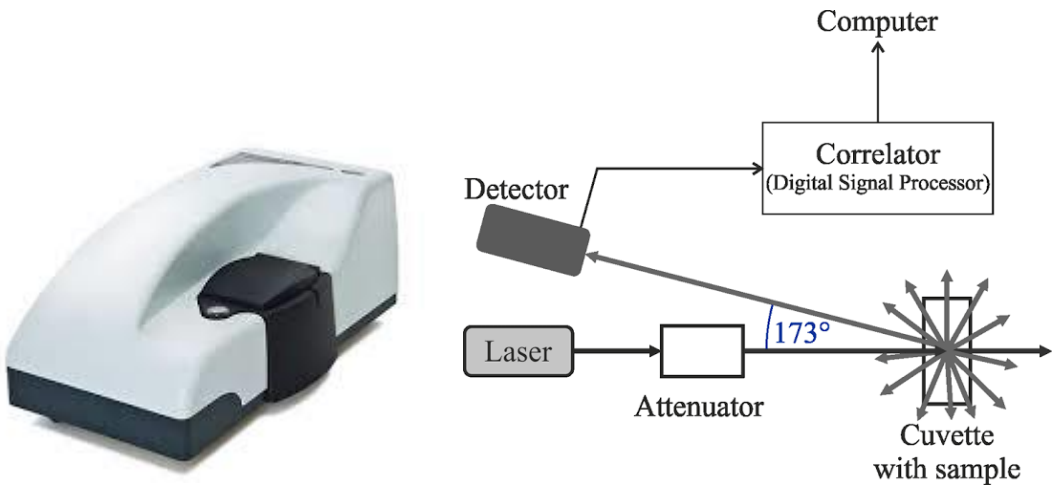


Figure 3.2 Zetasizer and Schematic diagram of Zetasizer

3.4.7 Fourier-transform infrared spectroscopy

It is a characterization technique used to obtain an Infrared (IR) spectrum of absorption or emission of solid, gas or liquid. It simultaneously gathers a spectral-resolution date over a

wide spectral range. The main goal of any absorption spectroscopy likes UV-Vis, spectroscopy, FTIR, etc. is to measure light absorbance at each wavelength. FTIR also used to record or obtain information on a material place in the IR beam. The FTIR tells us the spectra that can be used by analysts to quantify or identify materials.

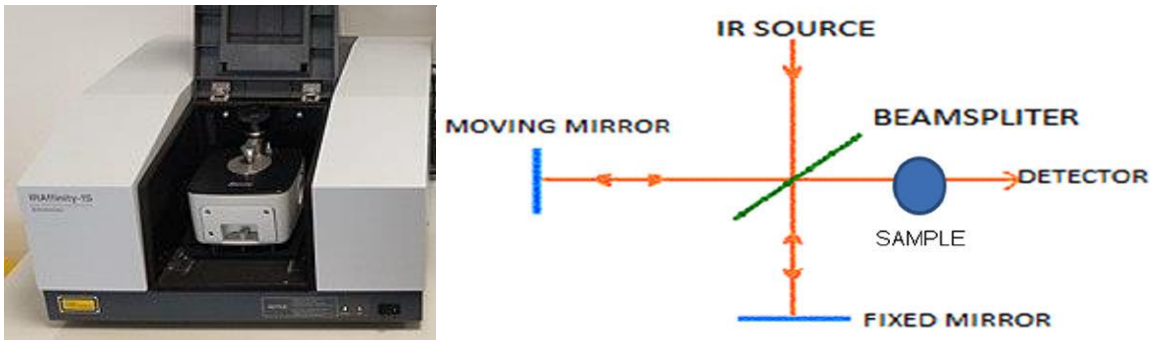


Figure 3.3 FTIR and Schematic Diagram of FTIR

3.4.8 SCANNING ELECTRON MICROSCOPE

SEM is a type of Electron microscope (EM) that gives images by scanning surfaces of a sample with a beam of electrons. It contains information about the composition of the sample and most importantly surface topography. In a raster scan pattern, a beam of an electron is scanned and the position of this beam along with the intensity of the signal produces images. SEM is used to measure area ranging from 1cm to 5 microns in width. Magnification ranges between 20X to 30 thousand X spatial resolution of 50nm to 100nm particles.

Figure 3.4 Scanning Electron Microscope

3.5 IN-VIVO STUDY

3.5.1 In Vivo Antibacterial Activity

For in vivo study 8 weeks old Balb/c mice were taken from ASAB-NUST animal house of almost equal weight. For control 2 mice were taken and the remaining mice were infected with *Enterococcus faecalis*. *E. faecalis* bacteria inhabit the gastrointestinal tracts of humans and other mammals, this causes urinary tract infection in mice. This infection was confirmed by the symptoms, behavior (feces) of mice. Now, these mice were further divided into three groups. Group one was not treated, group 2 mice were given 10mg/kg ciprofloxacin only and group 3 was given 500ug/kg drugs loaded Gold nanoparticles.

After the treatment, these mice were sacrificed and to check the results in the liver and kidney of the mice, we harvested these organs. Pestle and mortar along with PBS were used to crush the organs and then it was centrifuged, and a syringe filter was used later. The colony-forming unit was used to calculate the bacteria present in the respective organs. 10^{-9} Dilutions were done and then the sample was poured on a culture plate and incubated for 24 hours for *E. faecalis* to grow. Bacteria were counted after 24 hour. Significant growth is required for visual appearance, and during counting, it is uncertain whether the colony arose from a cell or group of cells. So, this uncertainty is present in CFU.

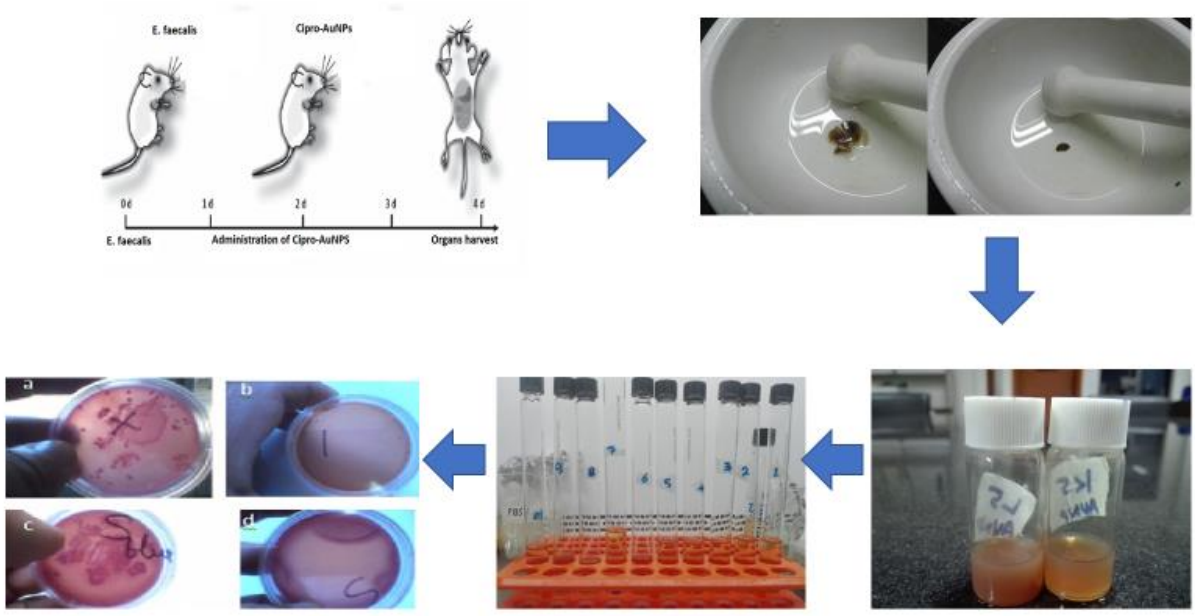


Figure 3.5 Schematic Diagram of In-Vivo Antibacterial study

Chapter 4

RESULTS AND DISCUSSION

4.1 VISUAL CONFIRMATION OF AuNPs

When 18 mL volume of distilled water was taken and 0.5mL of 10^{-2}M tri-sodium citrate was mixed. 0.5mL chloroauric acid was added. This solution was kept overnight at 40°C. Color changed to red which indicates the formation of 20-30nm gold nanoparticles as shown in the figure below.

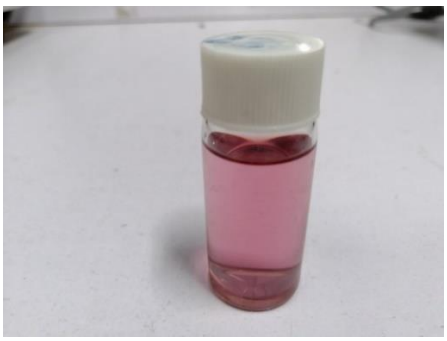


Figure 4.1 Wine red color Gold nanoparticles

4.2 VISUAL CONFIRMATION OF CIPROFLOXACIN LOADED GOLD NANOPARTICLES

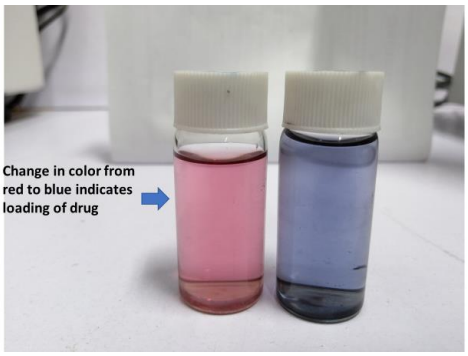


Figure 4.2 Ciprofloxacin Loaded Gold Nanoparticles

4.3 Characterization of Drug Loaded AuNPs

The characterization of ciprofloxacin loaded AuNP was done to evaluate and analyze their particle size and surface charge, drug release efficiency. Many other characterization tests were carried out, which include:

4.3.1 Encapsulation Efficiency

We loaded different concentrations of drugs with nanoparticles to check the best suitable and most effective concentration of a drug with nanoparticles. According to the table below it is clear that encapsulation efficiency increases with the increase in drug concentration but the problem is that as we keep on increasing the drug concentration the nanoparticles become unstable due to higher concentration of the drug. This can be check by performing different stability tests. The graph of nanoparticles loaded with ciprofloxacin deviates a lot and moves upward when UV-Vis spectroscopy was done of different drug concentrations. A more bluish color was observed when more ciprofloxacin was added, this confirms the increase in size. So gold nanoparticles are not effective in this condition. The best suitable value which we selected for the in vitro and in vivo study is 2mM ciprofloxacin conjugated with gold nanoparticles.

DRUG CONCENTRATION	PERCENTAGE
0.5mM	24.43%
1.0mM	29.30%
1.5mM	30.65%
2.0mM	48.92%
2.5mM	60.83%

Table 4.1 Encapsulation Efficiency of Drug Loaded Gold Nanoparticles

4.3.2 Loading Capacity

Similarly, different concentrations were also used to check the loading capacity. Different concentrations of drugs have different loading capacity and for calculation of loading capacity, we have to calculate the total drug and weight of nanoparticles. In the figure below, we can see that the loading capacity also increases with an increase in concentration. We know that higher concentration of drug will result in instability of nanoparticles as the agglomeration occurs and size also increases, so we have checked both encapsulation efficiency(EE) and the loading capacity to find the best suitable concentration of drug to be conjugated with our gold nanoparticles which are best suitable for in vitro and in vivo study with better efficacy. According to our research, we have selected a 2mM concentration of drugs for our research for this purpose.

DRUG CONCENTRATION	PERCENTAGE
0.5mM	8.85%
1.0mM	15.60%
1.5mM	28.85%
2.0mM	33.81%
2.5mM	34.54%

Table 4.2 Loading Capacity of Gold Nanoparticles

4.3.3 Optimized UV-Vis absorption spectroscopy

This was used to check the absorbance of gold nanoparticles. UV-2800 BMS Scientific Technical Corporation (PVT) Ltd is the device used. UV-vis spectroscopy is used widely in chemical, biochemical and clinical studies. Samples of gold nanoparticles, ciprofloxacin and ciprofloxacin loaded gold nanoparticles were observed and the results are as follow

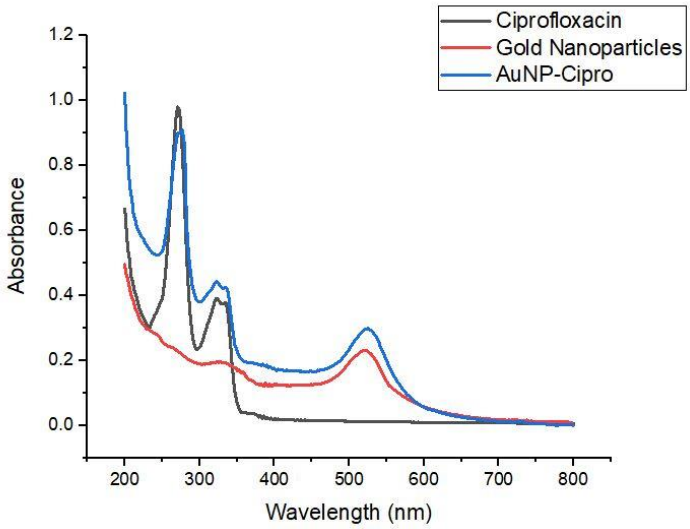


Figure 4.3 Comparative UV-Vis spectra of AuNP, Ciprofloxacin, and Drug Loaded AuNP

In the above figure red color line is of gold nanoparticles and the maximum absorbance is at 521nm. The black color line is of ciprofloxacin only and there are multiple peaks as shown above in the picture. The blue color line is of ciprofloxacin loaded gold nanoparticles and the peak shift confirms the conjugation of nanoparticles with ciprofloxacin. (271nm shifted to 277nm).

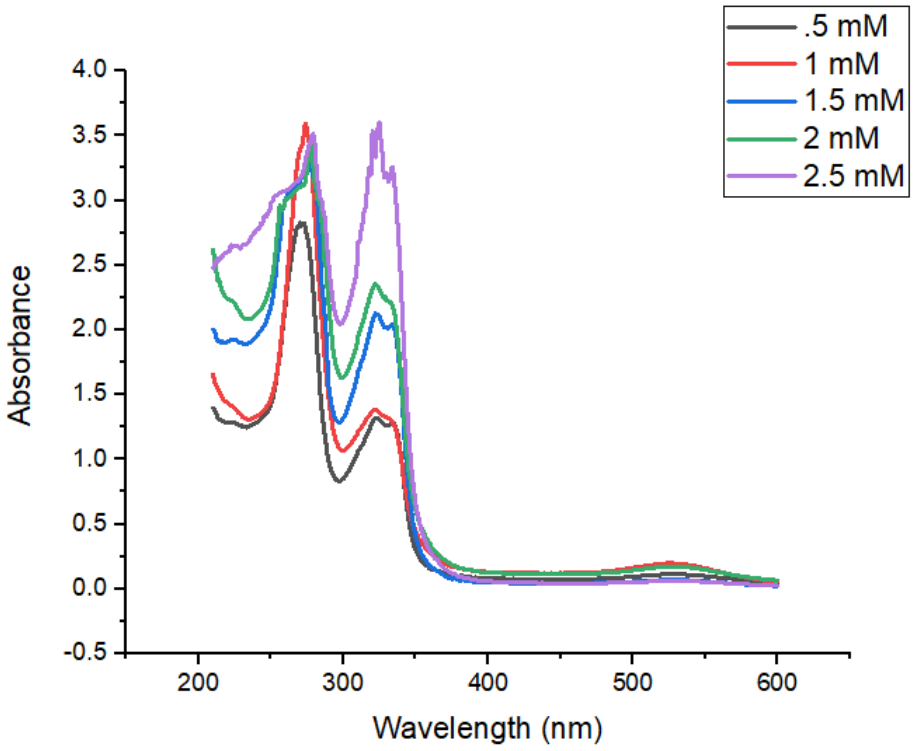


Figure 4.4 Comparative UV-Vis spectra of Optimized Drug Loaded Nanoparticles

UV-Visible spectroscopy of ciprofloxacin loaded AuNP. By increasing concentration Stability of nanoparticles is disturbed and the nanoparticles are not that effective as the size of nanoparticles increases by increasing concentration. The peak also shifted by increasing the concentration which indicates more loading of drugs with nanoparticles.

4.3.4 STANDARD CURVE

To obtain standard curve different dilutions were made and the UV-vis spectrum was done. The results are shown in the figure below and from this figure maximum absorbance peak point is selected to obtain the standard curve so that drug loading and drug release can be calculated from this standard curve.

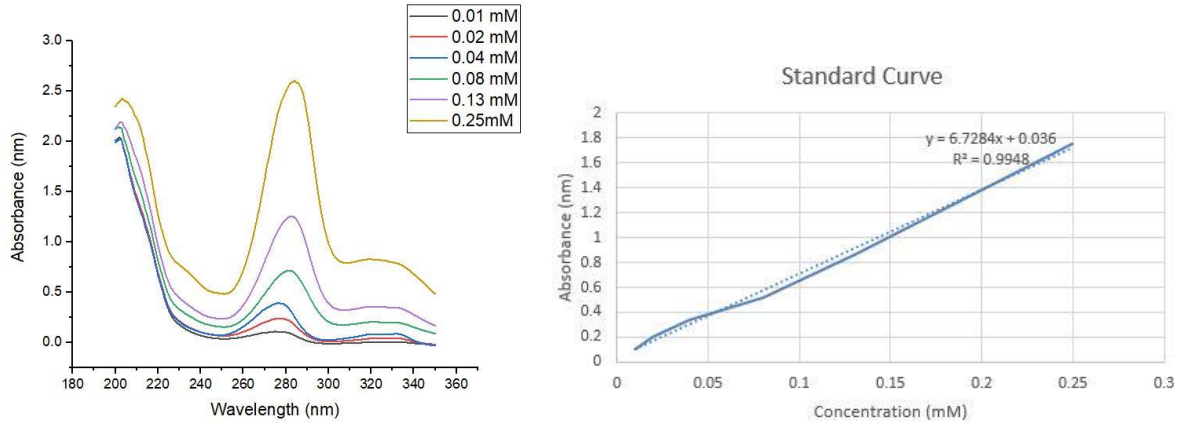


Figure 4.5 Dilutions and Standard Curve

4.3.5 Stability Test

This test was done to check the optimized drug concentration in nanoparticles. When we loaded the nanoparticles with a high concentration of drug the solution turned blue which depicts greater size and lower stability of gold nanoparticles. So, the higher concentration of drugs in nanoparticles, lower will be the stability as shown in the figure below. The higher concentration of the drug leads to the aggregation of the particles. Low concentration enhances the stability of the nanoparticles. Dark blue color shows a higher concentration of ciprofloxacin in the solution.

Figure 4.6 Ciprofloxacin loaded AuNP

4.3.5.1 Temperature Test

Temperature test was carried out to check the stability higher the temperature i.e. around 100°C, aggregation of nanoparticles will occur. UV-vis spectra of different ciprofloxacin loaded AuNP at different temperatures were carried out as shown below. The figure below indicates that color changes by increasing temperature. Moreover, the size of nanoparticles increases by increasing temperature. Best spectra are of 2mM ciprofloxacin loaded gold nanoparticles as shown in the figure below.

Figure 4.7 Colour change by increasing temperature

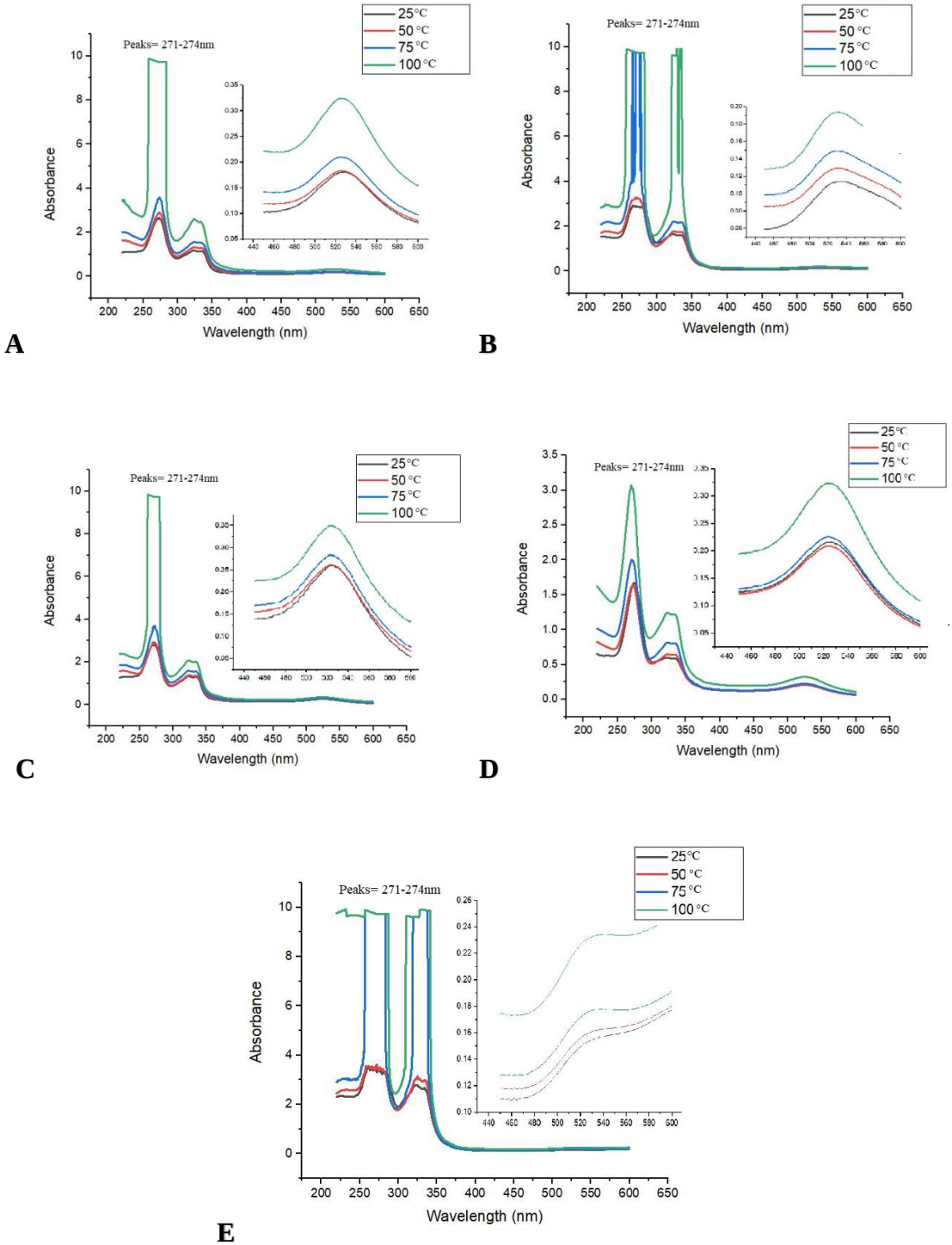
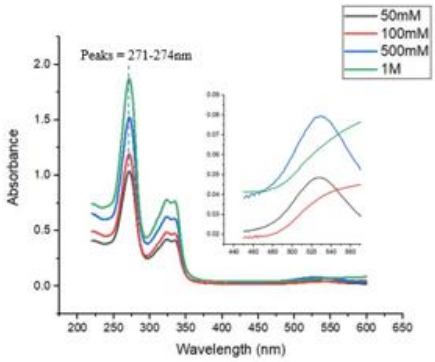
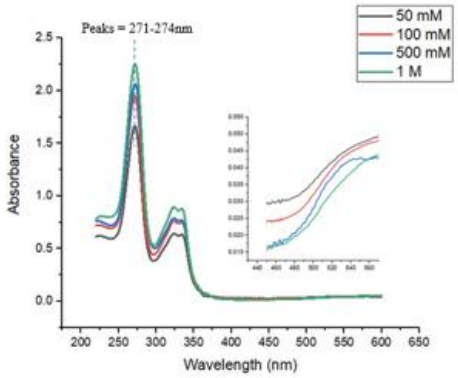


Figure 4.8 Temperature Effect on Gold Nanoparticles

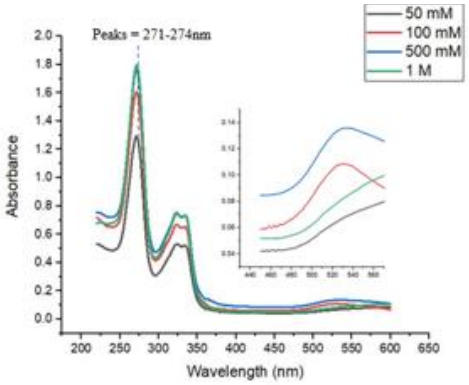
4.3.5.2 Salt test



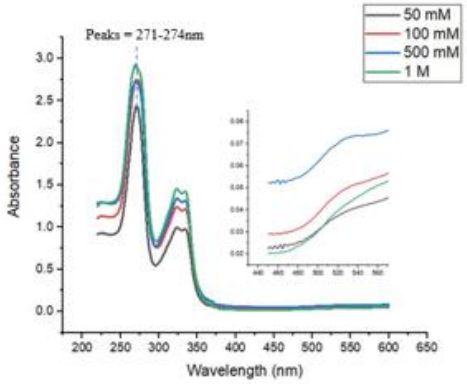
A



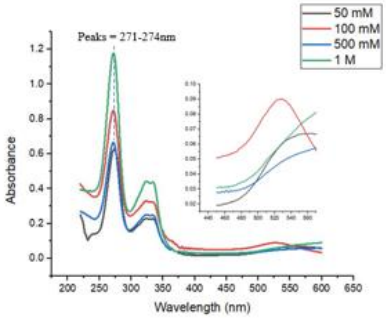
B



C



D



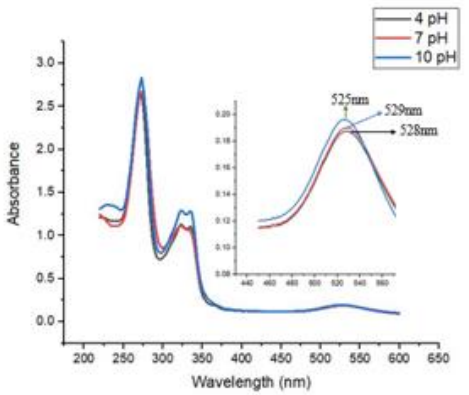
E

Figure 4.9 Salt effect on Gold nanoparticles

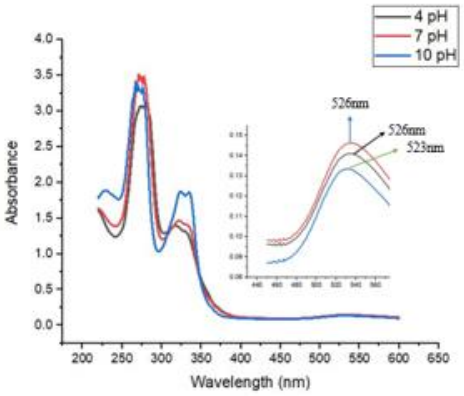
Different concentration of NaCl (50mM, 100mM, 500mM, and 1M) was mixed with different concentration of ciprofloxacin loaded gold nanoparticles to check the effect of salt on these nanoparticles. Colour of gold nanoparticles changes to blue upon mixing salt with gold nanoparticles. It was found that the size of nanoparticles increases with the addition of salt. UV-vis spectra of the salt test are shown in the above figures.

4.3.5.3 pH Test

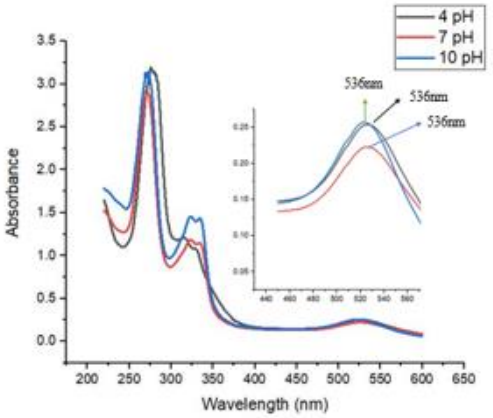
The pH test is done to check the effect of pH on different concentrations of ciprofloxacin loaded Gold nanoparticles. The UV-vis spectra of different pH i.e. 4, 7 and 10 pH were done and from this pH spectra, we concluded that the pH of ciprofloxacin is best in a basic medium (9.5 pH) rather than an acidic medium in which particles are unstable. At acidic pH ciprofloxacin is not activated so the reaction does not occur. Nanoparticles works under basic pH. The best drug concentration is also 2 mM as you can observe in the graphs.



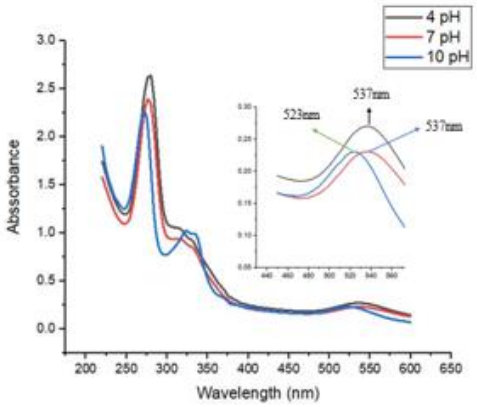
A



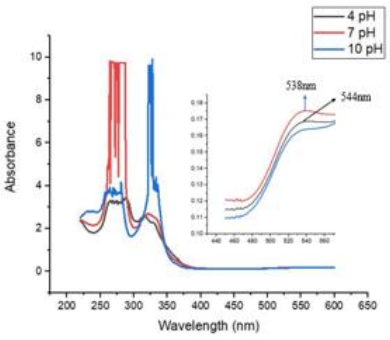
B



C



D



E

Figure 4.10 pH effect on Gold Nanoparticles

4.3.6 Drug Release

After the formation and washing of drug-loaded gold nanoparticles, sodium bicarbonate was used in the base-catalyzed desorption of the drug. A solution of ciprofloxacin capped gold nanoparticles were mixed with a 20mL aqueous bicarbonate solution (20mM) and this mixture was then split into 5mL 8 fractions. After each 1-hour period first 5 fractions were centrifuged and then centrifugate UV-vis spectroscopy was done and the remaining 3 fraction centrifugation and UV-vis spectroscopy were done after 12 and 24 and 48 hours respectively. The concentration and percentage were calculated, and this was plotted as shown in the figure. As we already know gold nanoparticles of smaller size releases drug faster than the large size nanoparticles.

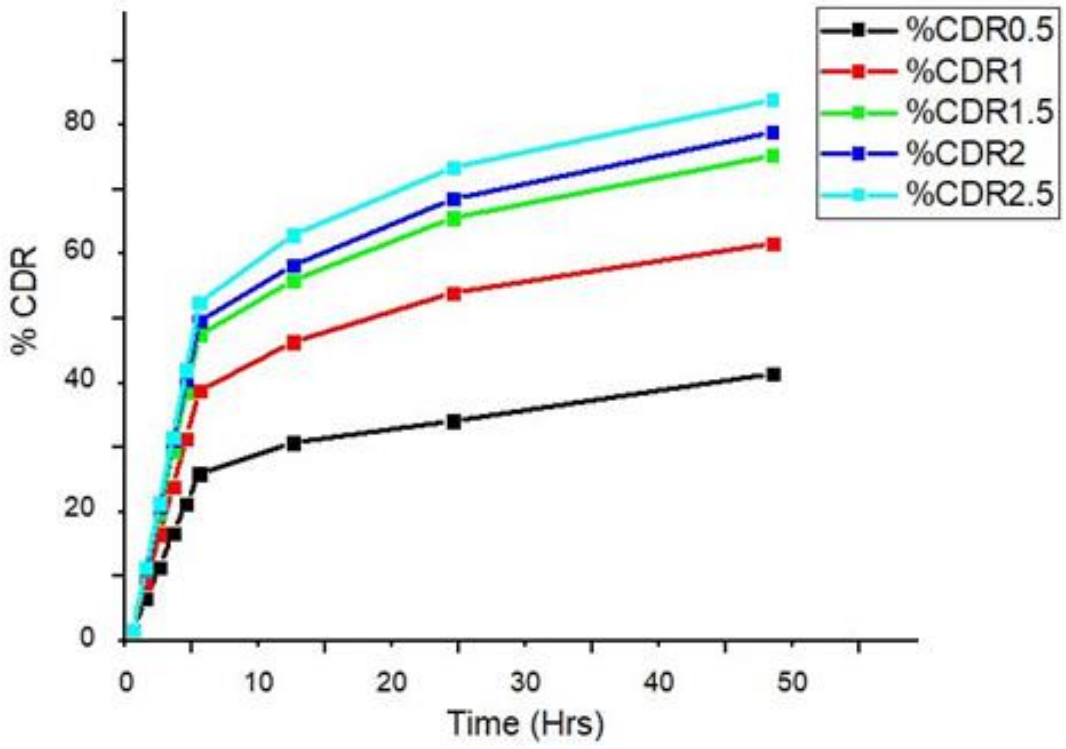


Figure 4.11 Drug Release Kinetics

Different drug-loaded nanoparticles were used as shown in the above figure and cumulative drug release increases with the rising concentration of the drug. But a higher concentration of the drug with nanoparticles is not that stable and nanoparticles size also increases due to higher concentration of the drug.

4.3.7 Fluorescent

As we know that ciprofloxacin is fluorescent, so we confirm that after conjugation of ciprofloxacin with gold nanoparticles by putting our particles on Benchtop 2UV transilluminator as shown in fig below and it is visible that gold nanoparticles are not fluorescent but when loaded with ciprofloxacin our formulation becomes fluorescent and this can be useful in biological investigation. Overall fluorescence is the best probe for observing the electronic properties of nanoparticles.

Figure 4.12 Fluorescent Drug Loaded Gold Nanoparticles

4.3.8 FTIR

The FTIR of nanoparticles, ciprofloxacin, and ciprofloxacin loaded AuNP are in figure 4.12 below. Absorptions at 1701 cm^{-1} and 1655 cm^{-1} are due to a carbonyl group (O-C-O) stretching of carboxylic acid and pyridine respectively. The absorption at 1288 cm^{-1} is because of C-N stretching. The above bands are almost present in both free and adsorbed ciprofloxacin. The bands at 3410 cm^{-1} are moved to 3443 cm^{-1} and slightly widened in absorbed ciprofloxacin case. 3410 cm^{-1} is due to O-H stretching. 3305 cm^{-1} is due to N-H stretching. The broad stretching band of O-H is due to water traces in the sample of nanoparticles. The bands around 2900 cm^{-1} are weak bands in the adsorbed species are because of traces of citrate. Absorptions around 1587 cm^{-1} and 1372 cm^{-1} in free ciprofloxacin are because of Symmetric and Asymmetric (O-C-O) stretching of the carboxyl. The bands around 1390 cm^{-1} are in both ciprofloxacin. The bands at 1587 cm^{-1} are partially hidden by the 1655 cm^{-1} band in the case of adsorbed ciprofloxacin. From the data, it can be concluded neither the carboxyl nor the keto is directly involved in binding to the gold as also it is clear from the spectra.

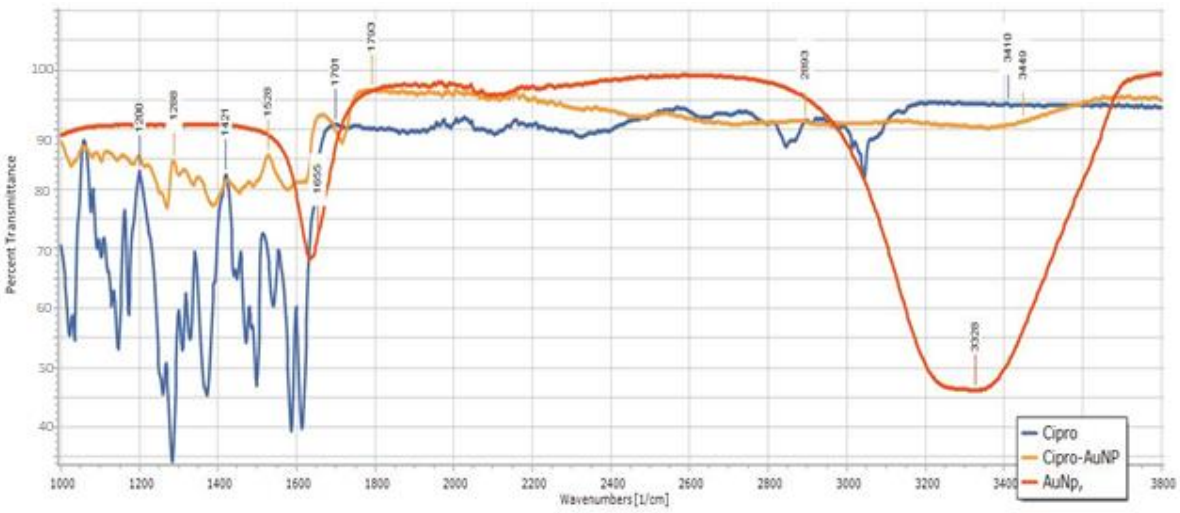


Figure 4.13 FTIR Spectra

4.3.9 Zeta Potential

Liquid samples of simple gold nanoparticles and drug-loaded gold nanoparticles were used for zeta potential in order to check the charge and size of our particles. The results show that particle size is around 20-30nm and the charge is around -32mV of AuNP.

Z-Average (d.nm)	**PDl**	**Intercept**	**Size (d.nm)**	**%Intensity**	**St Dev (d.nm)**
23.06	0.258	0.937	23.86	94.5	6.319
Zeta Potential (mV)	**Zeta Deviation (mV)**	**Conductivity (mS/cm)**	**Mean (mV)**	**Area %**	**St Dev (mV)**
-32.1	10.5	0.146	-32.2	98.4	9.76

Table 4.3 Zeta size and Zeta Potential

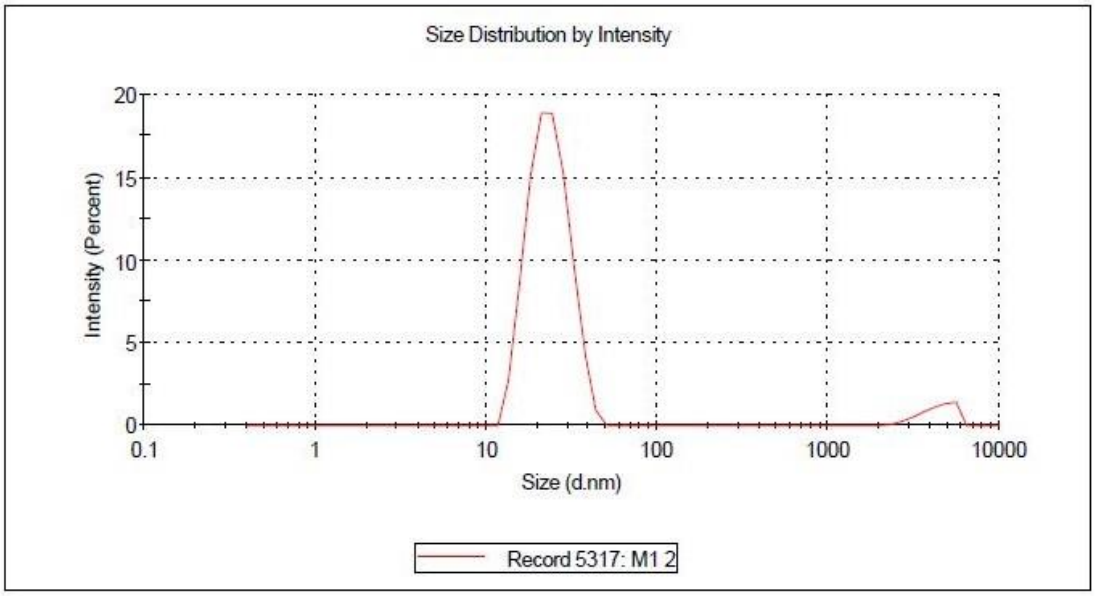


Figure 4.14 Zeta Charge on AuNP

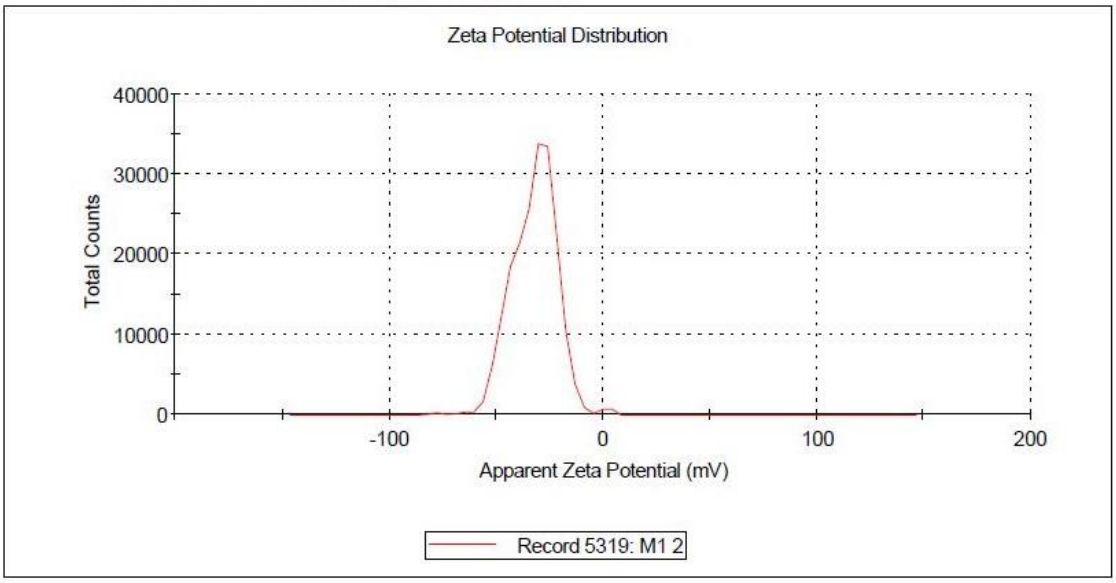
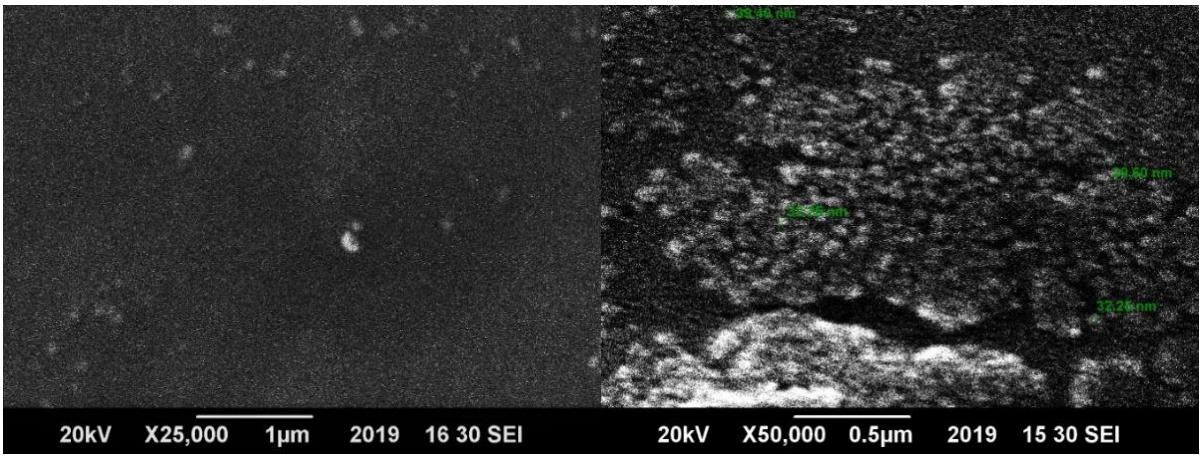


Figure 4.15 Zeta potential Size of AuNP

4.3.10 Scanning Electron Microscope

SEM was carried out to check the size and shape of gold nanoparticles and round shape was found. The liquid sample was dried on a glass slide to examine gold nanoparticles and drug-loaded gold nanoparticles. The size was around 30-40nm of Gold nanoparticles and 40-60nm of drug-loaded Gold nanoparticles as shown below in figure A. If we increase concentration of drug size of the nanoparticles increases and nanoparticles are not that much effective as shown below in figure B below. So formation of optimized ciprofloxacin loaded gold nanoparticles is necessary for improving efficacy of drug.

A.



B.

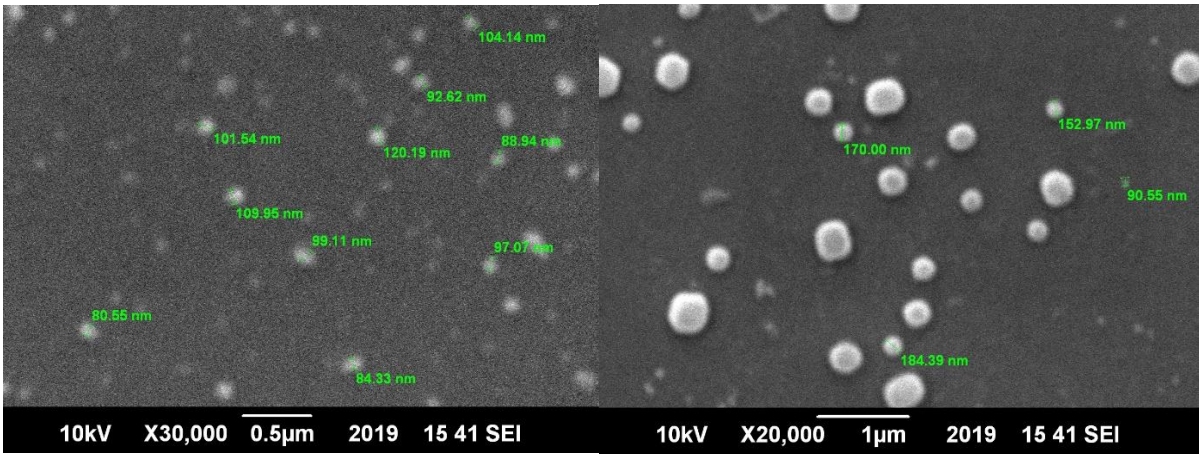


Figure 4.16 Scanning Electron Microscope (AuNP and Ciprofloxacin Loaded AuNP)

4.4 IN-VIVO RESULTS

Bacterial cells were counted after 24 hour incubation. Average cell counts in simple ciprofloxacin is 7.92 log10cfu/ug which is reduced to 2.722 log10cfu/ug in kidney. In liver ciprofloxacin count is 29.04906 log10cfu/ug which reduced to 28.43136 log10cfu/ug. So, it is clear from the average results that ciprofloxacin loaded gold nanoparticles are far better than

simple ciprofloxacin drug and CFU counts indicates clearly that *Enterococcus faecalis* are more eradicated by the use of optimized gold nanoparticles in both liver and kidney of infected mice.

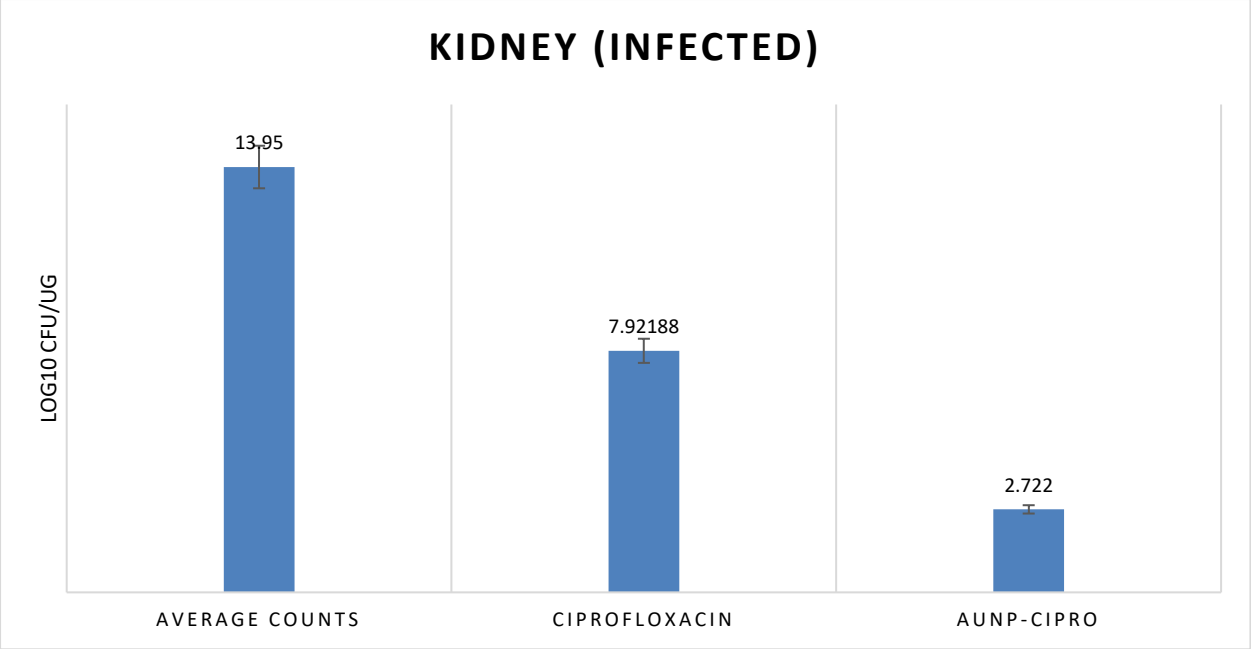


Figure 4.17 CFU of Infected Mice

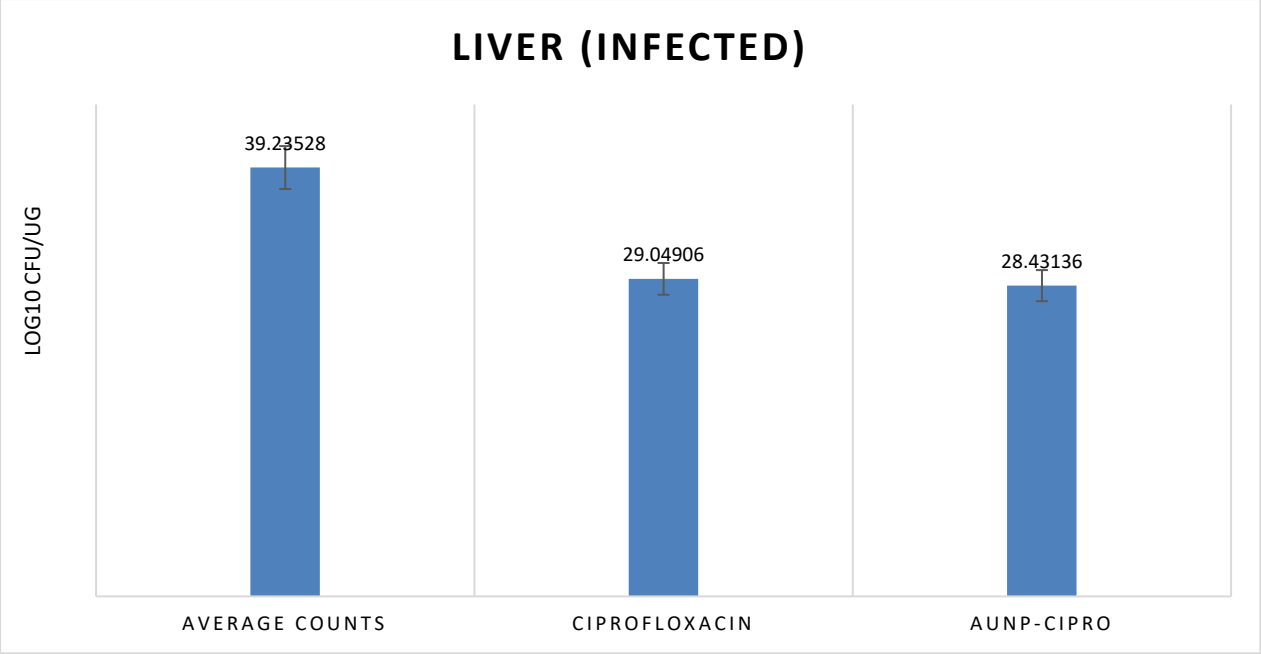


Figure 4.18 CFU of the liver of Infected Mice

CONCLUSION

Synthesis of drug-loaded gold nanoparticles that work more effectively against gram-positive bacterium *Enterococcus faecalis* was the main purpose of this research. As *Enterococcus faecalis* bacterium has shown resistance against many antibiotics. It was difficult to remove or inhibit its growth, so we used gold nanoparticles and loaded them with ciprofloxacin antibiotics. After that different characterization tests were performed to check the status of this formulation in comparison to ciprofloxacin only. Overall, we conclude that Ciprofloxacin loaded gold nanoparticles are less toxic and more compatible with cells and tissue. The biocompatibility of this formulation makes it preferable for the targeted drug delivery to the targeted tissues and cells. Furthermore, the side effect of this drug is less as compared to ciprofloxacin as the amount of effective dose has been reduced in this formulation. Our results confirm that ciprofloxacin loaded gold nanoparticles remove *Enterococcus faecalis* from organs of mice more effectively as compared to ciprofloxacin only.

REFERENCE

Renjis T. Tom, et al. (2003) Ciprofloxacin-Protected Gold Nanoparticles, Department of Chemistry and Regional Sophisticated Instrumentation Centre, Indian Institute of Technology Madras, Chennai 600 036, India: Langmuir 2004, 20, 1909-1914.

Gilho Lee (2013) Ciprofloxacin Resistance in *Enterococcus faecalis* Strains Isolated

From Male Patients with Complicated Urinary Tract Infection. Korean Journal of Urology .54.6.388

Sovan Lal Pal, Utpal Jana, et al. Nanoparticle: An overview of preparation and

Characterization. Journal of Applied Pharmaceutical Science 01 (06); 2011: 228-234

Erhirhie Earnest Oghenesuvwe, et al. Guidelines on dosage calculation and stock solution preparation in experimental animals' studies. Journal of Natural Sciences Research www.iiste.org ISSN 2224-3186 (Paper) ISSN 2225-0921 Vol.4, No.18, 2014.

Muhammad Nisar, Shujaat Ali Khan, et al. Robust Synthesis of Ciprofloxacin-Capped Metallic Nanoparticles and Their Urease Inhibitory Assay. Molecules 2016, 21, 411.

Claudia Gentry-Weeks, Monica Estay et al. Intravenous Mouse Infection Model for Studying the Pathology of *Enterococcus faecalis* Infections. INFECTION AND IMMUNITY (2003) p. 1434–1441

Weiwei Gao, Yijie Chen, Yue Zhang, Qiangzhe Zhang, Langfang Zhang, Nanoparticle-Based Local Antimicrobial Drug Delivery, Advanced Drug DeliveryReviews (2017), doi: 10.1016/j.addr.2017.09.015.

Shamaila, S., Zafar, N., Riaz, S., Sharif, R., Nazir, J., & Naseem, S. (2016). Gold nanoparticles: an efficient antimicrobial agent against an enteric bacterial human pathogen. Nanomaterials, 6(4),71.

Alaqad, K., & Saleh, T. A. (2016). Gold and silver nanoparticles: synthesis methods, characterization routes, and applications towards drugs. J. Environ. Anal. Toxicol, 6(384), 2161-0525.

Tyagi PK, Shruti, Sarsar V, Ahuja A. Synthesis of Metal Nanoparticles: A Biological Perspective for Analysis. International Journal of Pharmaceutical Innovations 2012; 2(4): 48-60

Zhang, Y., Shareena Dasari, T. P., Deng, H., & Yu, H. (2015). Antimicrobial activity of gold nanoparticles and ionic gold. Journal of Environmental Science and Health, Part C, 33(3), 286-327.

Matulionyte, M., Dapkute, D., Budenaite, L., Jarockyte, G., & Rotomskis, R. (2017). Photoluminescent Gold Nanoclusters in cancer cells: cellular uptake, toxicity, and generation of reactive oxygen species. International journal of molecular sciences, 18(2), 378.

Teimouri, M., Nia, A. H., Abnous, K., Eshghi, H., & Ramezani, M. (2016). Graphene oxide–

cationic polymer conjugates: Synthesis and application as gene delivery vectors. Plasmid, 84, 51-60.

Madu, A., Njoku, P., Iwuoha, G., & Agbasi, U. M. (2011). Synthesis and characterization of gold nanoparticles using 1-alkyl, 3-methyl imidazolium-based ionic liquids. International Journal of Physical Sciences, 6(4), 635-640.

Saleh, T. A. (2014). Spectroscopy: Between Modeling, Simulation and Practical Investigation. Spectral Analysis Review, 2(01),1.

Sershen, S. R., Westcott, S. L., Halas, N. J., & West, J. L. (2000). Temperature-sensitive polymer–nanoshell composites for photothermally modulated drug delivery. Journal of Biomedical Materials Research: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials, 51(3), 293-298.

Vasir, J. K., Reddy, M. K., & Labhasetwar, V. D. (2005). Nanosystems in drug targeting: opportunities and challenges. Current Nanoscience, 1(1), 47-64.

Chen, P. C., Mwakwari, S. C., & Oyelere, A. K. (2008). Gold nanoparticles: from nanomedicine to nanosensing. Nanotechnology, science and applications, 1, 45.

Yoon, K. Y., Byeon, J. H., Park, C. W., & Hwang, J. (2008). Antimicrobial effect of silver particles on bacterial contamination of activated carbon fibers. Environmental science &

technology, 42(4), 1251-1255.

São Pedro, A., Santo, I., Silva, C., Detoni, C., & Albuquerque, E. (2013). The use of nanotechnology as an approach for essential oil-based formulations with antimicrobial activity. Microb Path Strategy Combat, 1, 293-294.

Hameed, A., Khan, I., Azam, A., Naz, S. S., Khan, A., Saleem, M., ... & Islam, N. U. (2014). Stability and enzyme inhibition activities of Au nanoparticles using an aqueous extract of clove as a reducing and stabilizing agent. Journal of the Chemical Society of Pakistan, 36(3), 542-547.

Tedesco, S., Doyle, H., Blasco, J., Redmond, G., & Sheehan, D. (2010). Oxidative stress and toxicity of gold nanoparticles in Mytilus edulis. Aquatic Toxicology, 100(2), 178-186.

Mendoza, K. C., McLane, V. D., Kim, S., & Griffin, J. D. (2010). Invitro application of gold nanoprobes in live neurons for phenotypical classification, connectivity assessment, and electrophysiological recording. Brain Research, 1325, 19-27.

Leonhardt, U. (2007). Optical metamaterials: Invisibility cup. Nature Photonics, 1(4), 207.

Hartono, D., Yang, K. L., & Yung, L. Y. L. (2010). The effect of cholesterol on protein-coated gold nanoparticle binding to liquid crystal-supported models of cell membranes. Biomaterials, 31(11), 3008-3015.

Etame, A. B., Smith, C. A., Chan, W. C., & Rutka, J. T. (2011). Design and potential application of PEGylated gold nanoparticles with size-dependent permeation through brain microvasculature. Nanomedicine: Nanotechnology, Biology and Medicine, 7(6), 992-1000.

Lan, M. Y., Hsu, Y. B., Hsu, C. H., Ho, C. Y., Lin, J. C., & Lee, S. W. (2013). Induction of apoptosis by high-dose gold nanoparticles in nasopharyngeal carcinoma cells. Auris Nasus Larynx, 40(6), 563-568.

Mendoza, K. C., McLane, V. D., Kim, S., & Griffin, J. D. (2010). Invitro application of gold nanoprobes in live neurons for phenotypical classification, connectivity assessment, and electrophysiological recording. Brain Research, 1325, 19-27.

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.

Alaqad, K., & Saleh, T. A. (2016). Gold and silver nanoparticles: synthesis methods, characterization routes, and applications towards drugs. J. Environ. Anal. Toxicol, 6(384), 2161-0525.

Dykman, L., Khlebtsov, N. Gold nanoparticles in biomedical applications: recent advances and perspectives. Chemical Society Reviews (2012), 41(6), 2256-2282.

A. Hebeish1, M.A. Ramadan, et al, In vitro and in vivo antibacterial potential of chitosan - g – acrylonitrile silver nanocomposite against a pathogenic bacterium Int.J.Curr.Microbiol.App.Sci (2015) 4(3): 5-19. ISSN: 2319-7706.

T. Piercy, J. Steward et al. In vivo efficacy of fluoroquinolones against systemic tularaemia infection in mice. Journal of Antimicrobial Chemotherapy (2005), 56, 1069–1073.

Sagar R. Mudshinge, Amol B. Deore, Sachin Patil, Chetan M. Bhalgat. Nanoparticles: Emerging carriers for drug delivery. Saudi Pharmaceutical Journal (2011) 19, 129–141.

Khalid Alaqad and Tawfik A Saleh. Gold and Silver Nanoparticles: Synthesis Methods, Characterization Routes, and Applications towards Drugs. Journal of Environmental & Analytical Toxicology 2016, 6:4DOI: 10.4172/2161-0525.1000384

G. denotti, R. Piga et al. In Vitro Evaluation of *Enterococcus faecalis* Adhesion on Various Endodontic Medicaments. Open Dent J. 2009; 3: 120–124

R. Pignatello, A. Leonardi et al. A Method for Efficient Loading of Ciprofloxacin Hydrochloride in Cationic Solid Lipid Nanoparticles: Formulation and Microbiological Evaluation. Nanomaterials (Basel). 2018 May; 8(5): 304.

H. Pan, Y. Zhang et al. A comparison of conventional methods for the quantification of bacterial cells after exposure to metal oxide nanoparticles. BMC Microbiol. 2014; 14: 222.

M. A. Haque, Riku Imamura et al. An experiment-based model quantifying antimicrobial activity of silver nanoparticles on Escherichia coli. DOI: 10.1039/C7RA10495B (Paper) RSC Adv., 2017, 7, 56173-56182.

A. A. Navabi, A. A. Khademi et al. Comparative evaluation of *Enterococcus faecalis* counts in different tapers of the rotary system and irrigation fluids: An ex vivo study. Dent Res J (Isfahan). 2018 May-Jun; 15(3): 173–179.

Singh, Kushagri & Mishra, Abha & Singh, Amit. (2017). Synthesis Characterization and In Vitro Release Study of Ciprofloxacin-Loaded Chitosan Nanoparticle. BioNanoScience. 8. 10.1007/s12668-017-0470-7.

Page-Clisson, M & Pinto-Alphandary, H & Ourevitch, M & Andremont, Antoine & Couvreur, Patrick. (1999). Development of ciprofloxacin-loaded nanoparticles: Physicochemical study of the drug carrier. Journal of controlled release: official journal of the Controlled Release Society. 56. 23-32. 10.1016/S0168-3659(98)00065-0.

Thesis AuNP

ORIGINALITY REPORT

5%	0%	2%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Doaa H. Mobarak, Salwa Salah, Seham A. Elkheshen. "Formulation of ciprofloxacin hydrochloride loaded biodegradable nanoparticles: optimization of technique and process variables", Pharmaceutical Development and Technology, 2013 Publication	1%
2	"Rubber Nano Blends", Springer Science and Business Media LLC, 2017 Publication	<1%
3	Submitted to Georgia Perimeter College Student Paper	<1%
4	Submitted to Woodbridge High School Student Paper	<1%
5	Submitted to University of Edinburgh Student Paper	<1%
6	Submitted to Higher Education Commission Pakistan Student Paper	<1%

7	Submitted to University of Ulster Student Paper	<1%
8	Submitted to University of Liverpool Student Paper	<1%
9	Rosario Pignatello, Antonio Leonardi, Virginia Fuochi, Giulio Petronio Petronio, Antonio Greco, Pio Furneri. "A Method for Efficient Loading of Ciprofloxacin Hydrochloride in Cationic Solid Lipid Nanoparticles: Formulation and Microbiological Evaluation", Nanomaterials, 2018 Publication	<1%
10	Submitted to University of KwaZulu-Natal Student Paper	<1%
11	Submitted to Queen's University of Belfast Student Paper	<1%
12	Michael J. Richardson, James H. Johnston. "Sorption and binding of nanocrystalline gold by Merino wool fibres—An XPS study", Journal of Colloid and Interface Science, 2007 Publication	<1%
13	Submitted to Heriot-Watt University Student Paper	<1%
14	Submitted to Amity University Student Paper	<1%

15	Submitted to Chulalongkorn University Student Paper	<1%
16	Submitted to Charotar University of Science And Technology Student Paper	<1%
17	Submitted to Imperial College of Science, Technology and Medicine Student Paper	<1%
18	Submitted to Middle East Technical University Student Paper	<1%
19	Submitted to Queensland Academy for Health Sciences Student Paper	<1%
20	Submitted to Sheffield Hallam University Student Paper	<1%
21	Submitted to Sunway Education Group Student Paper	<1%

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		