SYNTHESIS AND BIOACTIVITY EVALUATION OF AMOXICILLIN DERIVATIVES

Ph.D Thesis

By

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UNIVERSITY OF PESHAWAR

PAKISTAN

(NOVEMBER 2012)
SYNTHESIS AND BIOACTIVITY EVALUATION OF AMOXICILLIN DERIVATIVES

By

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Dissertation

Submitted to the University of Peshawar in the partial fulfillment of the Requirements for the Degree of Doctor of Philosophy

In

Chemistry

INSTITUTE OF CHEMICAL SCIENCES
UNIVERSITY OF PESHAWAR
PAKISTAN
(NOVEMBER 2012)
It is recommended that this dissertation prepared by Mr. Abdul Hameed entitled “Synthesis and bioactivity evaluation of amoxicillin derivatives” be accepted as fulfilling this part of the requirements for the degree of “DOCTOR OF PHILOSOPHY IN CHEMISTRY”.

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### TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>S. No.</th>
<th>TITLE</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>ACKNOWLEDGEMENTS</td>
<td>xiv</td>
</tr>
<tr>
<td>II</td>
<td>ABSTRACT</td>
<td>xv</td>
</tr>
<tr>
<td>III</td>
<td>LIST OF ABBREVIATIONS</td>
<td>xviii</td>
</tr>
<tr>
<td>IV</td>
<td>LIST OF TABLES</td>
<td>xx</td>
</tr>
<tr>
<td>V</td>
<td>LIST OF FIGURES</td>
<td>xxi</td>
</tr>
<tr>
<td>VI</td>
<td>LIST OF SCHEMES</td>
<td>xxx</td>
</tr>
</tbody>
</table>

**CHAPTER-1  INTRODUCTION TO BETA-LACTAM ANTIBIOTICS  1-14**

1  Beta-lactam antibiotics  1

1.1  Cephalosporin  1

1.1.1  Cephalosporin nucleus  1

1.1.2  History  1

1.1.3  Basic Structure  2

1.1.4  Classification  2

1.1.4.1  First generation cephalosporin  2

1.1.4.2  Second generation cephalosporin  3

1.1.4.3  Third generation cephalosporin  4

1.1.4.4  Fourth generation cephalosporin  4

1.1.4.5  Fifth generation cephalosporin  4

1.1.5  Mechanism of action  5

1.1.6  Spectrum of activity  5
2.5 Properties

2.5.1 Optical properties

2.5.2 Magnetic properties

2.5.3 Thermal properties

2.5.4 Electronic properties

2.5.5 Energy properties

2.5.6 Biomedical properties

2.6 Review on Recent Advancements in Metal Nanoparticles

2.6.1 Review on Gold Nanoparticles

2.6.2 Review on Silver Nanoparticles

CHAPTER 3 METAL NANOPARTICLES STABILIZED WITH AMOXICILLIN

3.0 Materials and measurements

3.1 Experimental

3.1.1 Synthesis of gold nanoparticles stabilized with Sodium salt of amoxicillin

3.1.2 Gold nanoparticles formation using different protocols

3.1.2.1 Gold nanoparticles formation at room temperature

3.1.2.2 Gold nanoparticles formation at high temperature

3.1.2.3 Gold nanoparticles formation using microwave irradiation

3.1.2.4 Gold nanoparticles formation by sonication
| 3.1.2.5 | Gold nanoparticles formation at low temperature | 50 |
| 3.1.3  | Synthesis of gold nanoparticles stabilized with amoxicillin | 50 |
| 3.1.4  | Synthesis of silver nanoparticles stabilized with sodium salt of amoxicillin | 51 |
| 3.1.5  | Synthesis of silver nanoparticles stabilized with amoxicillin | 51 |
| 3.1.6  | Synthesis of nano-alloys stabilized with amoxicillin | 52 |
| 3.2    | Results and discussion | 53 |
| 3.2.1  | Characterization and optical properties of Au-Nanoparticles | 56 |
| 3.2.2  | Variation in HAuCl₄ and ligand ratios | 60 |
| 3.2.3  | Formation of Gold nanoparticles using different protocols | 60 |
| 3.2.4  | Stability check of gold nanoparticles | 62 |
| 3.2.5  | Synthesis of Au-NPs using Trimethylamine | 64 |
| 3.2.6  | Reactions involved in reduction of HAuCl₄ | 65 |
| 3.2.7  | Characterization through TEM images | 65 |
| 3.2.8  | Analytical protocol of amoxicillin stabilized Au-NPs | 67 |
| 3.2.9  | Ag nanoparticles stabilized with Amoxicillin | 68 |
| 3.2.10 | Characterization with UV-visible spectroscopy | 70 |
| 3.2.11 | Characterization with Transmission Electron Microscope | 73 |
| 3.2.12 | Stability check of silver nanoparticles | 75 |
3.2.13 Ag and Gold nano-alloys stabilized with Amoxicillin

3.2.14 Characterization of nano-alloys with AFM

CHAPTER 4 METAL NANOPARTICLES STABILIZED WITH CEFUROXIME

4.1 Experimental

4.1.1 Synthesis of gold nanoparticles stabilized with Cefuraxime using NaBH₄ as a reducing agent

4.1.2 Trimethylamine as a reducing agent

4.1.3 Synthesis of silver nanoparticles stabilized with Cefuroxime

4.1.4 Synthesis of silver and gold nano-alloys stabilized with cefuroxime

4.2 Results and discussion

4.2.1 Gold nanoparticles stabilized with cefuroxime

4.2.2 Characterization of AuNPs with TEM

4.2.3 Silver nanoparticles stabilized with cefuroxime

4.2.4 Characterization of AgNPs with TEM

4.2.5 Silver-gold nano-alloys stabilized with cefuroxime

4.2.6 Characterization of nano-alloys with TEM

CHAPTER 5 METAL NANOPARTICLES STABILIZED WITH CEPHRADINE

5.1 Experimental

5.1.1 Synthesis of gold nanoparticles Caped with Cephradine using NaBH₄ as a reducing agent
6.2.2 AFM characterization of AuNPs 124
6.2.3 Silver nanoparticles stabilized with ceftriaxone 125
6.2.4 Characterization of AgNPs with AFM 128
6.2.5 Synthesis of nano-alloys stabilized with ceftriaxone 129
6.2.6 Characterization of nano-alloys with AFM 131

CHAPTER 7 METAL NANOPARTICLES STABILIZED WITH CEFIXIME 132-147

7.1 Experimental 133
7.1.1 Synthesis of gold nanoparticles stabilized Cefixime using NaBH₄ as a reducing agent 133
7.1.2 Using trimethylamine as a reducing agent 133
7.1.3 Synthesis of silver nanoparticles stabilized Cefixime 134
7.2 Results and discussion 134
7.2.1 Gold nanoparticles stabilized with cefixime 136
7.2.2 Characterization of AuNPs with AFM 140
7.2.3 Silver nanoparticles stabilized with cefixime 141
7.2.4 Characterization of AgNPs with AFM 144
7.3 Applications of AuNPs caped with cefixime 145
7.5 Applications of AgNPs caped with cefixime (reduced with TMA) 148

CHAPTER 8 BIOASSAYS 150-165

8.1 Introduction 151
8.1.1 Xanthine oxidase 151
| 8.1.2 | Urease | 151 |
| 8.1.3 | Carbonic anhydrase-ii | 152 |
| 8.1.4 | α-Chymotrypsin | 153 |
| 8.1.5 | Anti-glycation | 153 |
| 8.2 | Experimental | 154 |
| 8.2.1 | Protocol for xanthine oxidase assay and inhibition | 154 |
| 8.2.2 | Urease assay and inhibition | 155 |
| 8.2.3 | Carbonic anhydrase-ii inhibition assay | 155 |
| 8.2.4 | *in Vitro* α-chymotrypsin assay | 156 |
| 8.2.5 | Antiglycation | 156 |
| 8.3 | Results and discussions | 157 |
| 8.3.1 | Amoxicillin stabilized metal nanoparticles | 157 |
| 8.3.2 | Cefuroxime stabilized metal nanoparticles | 160 |
| 8.3.3 | Cephradine stabilized metal nanoparticles | 162 |
| 8.3.4 | Ceftriaxone stabilized metal nanoparticles | 164 |
| 8.3.5 | Cefixime stabilized metal nanoparticles | 166 |

**Conclusion**

**References**

170
Acknowledgements

First of all I thank to Almighty Allah the most Beneficent, and the most Merciful, Who enabled me to complete this work.

I am thankful to Higher Education Commission Pakistan for their scholarship throughout my research project of 5000 indigenous fellowships.

I wish to express my sincere gratitude, heartiest obligation and appreciation to my worthy, learned and experienced supervisor, Prof. Dr. Nazar Ul Islam, Institute of Chemical Sciences, University of Peshawar, for his guidance, helping attitude and valuable suggestions throughout my research work.

My special thanks are due to Prof. Dr. Imdadullah, Director of Institute of Chemical Sciences, University of Peshawar, Prof. Dr. Mohammad Nisar, and Prof. Dr. Ikhtiar Khan, Dr. Rasool Khan and Dr. Faiz Ur Rehman for their motivation and inspiration. I am also thankful to my other worthy professors and teachers at the Institute of Chemical Sciences for their help and cooperation.

I wish to pay my sincere thanks to my lab fellows Muhammad Arshad, Nasir Ullah, Waqas Ahmed, Faridoon, Usman Ali, Muhammad Ikram, Sohela Naz and Andaleeb Azam for their valuable assistance, guidance and fruitful suggestions. I am thankful to Mr. Zulfiqar Ali (lab. Assist) and Osama for their help.

I ardently extend my thanks to Dr. Muhammad Raza shah, Dr. Shamsa Kanwal HEJRIC University of Karachi, Karachi and Dr. Muhammad Irshad (LUMS) whose guidance and encouragement helped me to achieve my goals during my visit to HEJ and LUMS. I am also thankful to Burhan khan, Jariq khan, Saifullah Afridi and Dr. Nadeem for their nice company in Karachi.

Finally, I express my heartiest thanks to my great parents, sisters, brothers for their cooperation and encouragement to achieve this target.

Abdul Hameed
ABSTRACT

The work presented in this thesis consists of “synthesis and bioactivity evaluation of amoxicillin derivatives” which includes the synthesis, optimization, applications and bioactivity evaluation of new metallic derivatives (silver and gold nanoparticles) of amoxicillin and some other beta lactam antibiotics.

For a wide spread resistive strains of pathogenic bacteria, there is a need for some new antibacterial agents to treat the patients, infected with such resistive pathogenic bacteria. Here we synthesized new gold and silver derivatives of amoxicillin and some other beta lactam antibiotics like cefuraxime, cephradine, ceftriaxone etc belonging to beta lactam antibiotics family; some of them were penicillins while others are cephalosporins. We synthesized metallic derivatives of amoxicillin in the form of gold and silver nanoparticles and their nano-alloys using NaBH₄ and TMA as reducing agents. Reducing agents of different nature (organic and inorganic), NaBH₄ and trimethylamine were used for the synthesis of gold and silver nanoparticles to study their effect on sizes and shapes of nanoparticles as well as to avoid the sodium salt formation of amoxicillin and other beta-lactam antibiotics using TMA. It was found that nanoparticles synthesized with NaBH₄ were of small size compared to nanoparticles synthesized with TMA.

Gold nanoparticles stabilized with amoxicillin were prepared using NaBH₄ and TMA as reducing agents. The applications for metallic sensing like Cu, Fe, Co, Ni, Pb, Cd, and Hg etc were studied. Gold nanoparticles stabilized with amoxicillin were fluorescent and were used for the detection of Cu²⁺ ions in water solution. Amoxicillin and gold nanoparticles (AuNPs) stabilized with amoxicillin were screened for enzymes inhibition studies as well antiglycation and results were compared. These AuNPs showed very nice inhibition for urease, carbonic anhydrase and xanthine oxidase enzymes and were found very potent inhibitor for xanthine oxidase enzyme with inhibition of 90% and IC₅₀ value of 2.0±0.01. Amoxicillin showed antiglycation activity while AuNPs were inactive.
Silver nanoparticles stabilized with amoxicillin were also prepared using NaBH$_4$ and TMA as reducing agents. AgNPs were screened for enzymes inhibition studies and antiglycation activity and results were compared with amoxicillin and it was found that AgNPs were active against urease and xanthine oxidase enzymes while inactive against carbonic anhydrase and no antiglycation activity. Nano-alloys of AgNPs and AuNPs stabilized with amoxicillin were also prepared. These were also screened for enzymes inhibition and antiglycation activity but were inactive.

Gold and silver nanoparticles using other stabilizing agents like cefuroxime, cephradine, ceftriaxone and cefixime were also synthesized. Their nano-alloys were also prepared stabilized with the above mentioned antibiotics except cefixime. Their applications for metals sensing like Cu, Fe, Co, Ni, Pb, Cd, and Hg etc. were studied. The metallic sensing application of cefixime was investigated and was found that AgNPs stabilized with cefixime detected Ni ion in water solution while its AuNPs detected Cu ion in water solutions. Cephradine stabilized gold nanoparticles detected Hg and Cd ions in water solution upto micro molar concentration. The comparative bioassays of gold and silver nanoparticles stabilized with cefuroxime, cephradine, ceftriaxone and cefixime as well as their parent compounds were also studied and compared. Cefuroxime and their gold and silver nanoparticles showed best results in case of urease inhibitions. Cefuroxime was also found to be active by inhibiting urease enzymes along with its antibiotic activities. AuNPs stabilized with cefuroxime were also active against carbonic anhydrase enzymes. While its silver and nan-alloys were active by inhibiting in Vitroα-Chymotrypsin enzymes. Cephradine stabilized silver nanoparticles and nano-alloys were active for xanthine oxidase enzyme with an inhibition of 87.9% and 85.9% with IC$_{50}$ value 6.9±1.5 µg/mL and IC$_{50}$ value 6.76±0.07 µg/mL respectively. AuNPs and parent compound were inactive against XO enzymes inhibition. AuNPs, AgNPs, nano-alloys and parent compound cephradine were active for urease enzymes inhibition and inactive for antiglycation activity except the parent compound. AuNPs and AgNPs were active for carbonic anhydrase inhibition while Nano-alloys and parent compound were inactive. All were inactive for In Vitroα-Chymotrypsin enzyme inhibition. Ceftriaxone caped
gold and silver nanoparticles were active for urease inhibition except its nano-alloy while only AgNPs were active for xanthine oxidase enzyme inhibition and rest of members were inactive. All were inactive for the remaining enzymes as well as antiglycation activity. Cefixime is active against xanthine oxidase enzyme inhibition and antiglycation activity while gold and silver nanoparticles were active for urease enzyme inhibition only.
## LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-APA</td>
<td>6-aminopenicillinic acid</td>
</tr>
<tr>
<td>TEA</td>
<td>Triethylamine</td>
</tr>
<tr>
<td>TMA</td>
<td>Trimethylamine</td>
</tr>
<tr>
<td>DCC</td>
<td>N,N'-Dicyclohexylcarbodiimide</td>
</tr>
<tr>
<td>CDI</td>
<td>Carbonydiimidazole</td>
</tr>
<tr>
<td>HAuCl₄</td>
<td>Tetrachloroaauric acid</td>
</tr>
<tr>
<td>Lys</td>
<td>Lysine</td>
</tr>
<tr>
<td>Asp</td>
<td>Aspartic acid</td>
</tr>
<tr>
<td>Cyst</td>
<td>Cysteine</td>
</tr>
<tr>
<td>PhMe</td>
<td>Toluene</td>
</tr>
<tr>
<td>AcOH</td>
<td>Acetic acid</td>
</tr>
<tr>
<td>EtOH</td>
<td>Ethanol</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>(Ac)₂O</td>
<td>Acetic anhydride</td>
</tr>
<tr>
<td>MeCN</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>NaOEt</td>
<td>Sodium ethoxide</td>
</tr>
<tr>
<td>ESI – MS</td>
<td>Electrospray ionization mass spectrum</td>
</tr>
<tr>
<td>Ceph</td>
<td>Cephradine or Cefradine</td>
</tr>
<tr>
<td>Cefix</td>
<td>Cefixime</td>
</tr>
<tr>
<td>Ceft</td>
<td>Ceftriaxome</td>
</tr>
<tr>
<td>Amox</td>
<td>Amoxicillin</td>
</tr>
</tbody>
</table>
AuNPs  Gold nanoparticles
AgNPs  Silver nanoparticles
GNPs  Gold nanoparticles
mM  Milli molar
TEM  Transmission Electron Microscope
HR-TEM  High resolution Transmission Electron Microscope
SEM  Scanning Electron Microscope
XRD  X-ray diffraction
Rpm  Rounds per minute
SPR  Surface Plasmon Resonance
LSRP  Local Surface Plasmon Resonance
BSA  Bovine serum albumin
MTT  3-(4,5-Dimethylthiazol-2-YI)-2,5-Diphenyltetrazolium Bromide
DLS  Dynamic light scattering
EDX  Energy Dispersive X-ray Microanalysis
TGA  Thermo Gravimetric Analysis
KSV  Stern–Volmer quenching constant
ICP  Inductively Coupled Plasma
SWAXS  Small and wide angle X-ray scattering
ZO  Xanthine oxidase
SAED  Selected Area Electron Diffraction
CI  Crystalline index
PL  Photoluminescence
FDA  Food and Drug administration
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table No.</th>
<th>Description</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 3.1</td>
<td>Variations in the amount of NaBH&lt;sub&gt;4&lt;/sub&gt;</td>
<td>57</td>
</tr>
<tr>
<td>Table 3.2</td>
<td>Optimization of Silver nanoparticles reduced with trimethylamine</td>
<td>72</td>
</tr>
<tr>
<td>Table 3.3</td>
<td>Shows the optimization reactions of silver nanoparticles reduced with NaBH&lt;sub&gt;4&lt;/sub&gt;</td>
<td>73</td>
</tr>
<tr>
<td>Table 8.1</td>
<td>Enzymes inhibition and anti-glycation activities of amoxicillin and amoxicillin stabilized gold and silver nanoparticles</td>
<td>159</td>
</tr>
<tr>
<td>Table 8.2</td>
<td>Enzymes inhibition and anti-glycation activities of cefuroxime and cefuroxime stabilized gold and silver nanoparticles</td>
<td>161</td>
</tr>
<tr>
<td>Table 8.3</td>
<td>Enzymes inhibition and anti-glycation activities of cephradine and cephradine stabilized gold and silver nanoparticles</td>
<td>163</td>
</tr>
<tr>
<td>Table 8.4</td>
<td>Enzymes inhibition and anti-glycation activities of ceftriaxone and ceftriaxone stabilized gold and silver nanoparticles</td>
<td>165</td>
</tr>
<tr>
<td>Table 8.5</td>
<td>Enzymes inhibition and anti-glycation activities of cefixime and cefixime stabilized gold and silver nanoparticles</td>
<td>167</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure No.</th>
<th>Description</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chapter 1</strong></td>
<td><strong>Introduction to beta lactam antibiotics</strong></td>
<td></td>
</tr>
<tr>
<td>Figure 1.1</td>
<td>Cephalosporin nucleus</td>
<td>1</td>
</tr>
<tr>
<td>Figure 1.2</td>
<td>Cephalosporins</td>
<td>2</td>
</tr>
<tr>
<td>Figure 1.3</td>
<td>Basic structure of cephalosporin for 1(^{\text{st}}) generation</td>
<td>3</td>
</tr>
<tr>
<td>Figure 1.4</td>
<td>Basic structure of cephalosporin for 2(^{\text{nd}}) generation</td>
<td>3</td>
</tr>
<tr>
<td>Figure 1.5</td>
<td>Basic structure of cephalosporin for 3(^{\text{rd}}) generation</td>
<td>4</td>
</tr>
<tr>
<td>Figure 1.6</td>
<td>Penicillin nucleus</td>
<td>6</td>
</tr>
<tr>
<td>Figure 1.7</td>
<td>Structural formula of ampicillin</td>
<td>8</td>
</tr>
<tr>
<td>Figure 1.8</td>
<td>Structural formula of amoxicillin</td>
<td>8</td>
</tr>
<tr>
<td>Figure 1.9</td>
<td>Structural of penicillin</td>
<td>11</td>
</tr>
<tr>
<td>Figure 1.10</td>
<td>Monobactam nucleus</td>
<td>11</td>
</tr>
<tr>
<td>Figure 1.11</td>
<td>Carbapenem nucleus</td>
<td>12</td>
</tr>
<tr>
<td>Figure 1.12</td>
<td>Basic structure for Cephemycins</td>
<td>12</td>
</tr>
<tr>
<td>Figure 1.13</td>
<td>Basic structure for Oxacephem</td>
<td>13</td>
</tr>
<tr>
<td><strong>Chapter 2</strong></td>
<td><strong>Introduction to nanotechnology</strong></td>
<td></td>
</tr>
<tr>
<td>Figure 2.1</td>
<td>Diagrams for Silver and Gold nanoparticles</td>
<td>16</td>
</tr>
</tbody>
</table>
Figure 2.2  Dark field microscopy image of 60 nm silver nanoparticles

Figure 2.3  color of nanoparticles depends on the Size of particles

Chapter 3  Metal nanoparticles stabilized with amoxicillin

Figure 3.1  PL spectra for AuNPs, Au(III), Amoxicillin

Figure 3.2  Images of AuNP and Amoxicillin showing fluorescence

Figure 3.3  Absorption spectra for the confirmation of salt of amoxicillin

Figure 3.4  Effect of Sodium Borohydride on the size and shape of AuNPs

Figure 3.5  FTIR spectra of amoxicillin (upper) and Au-3NPs (lower)

Figure 3.6  UV-vis spectra of Au-NPs with different ligand ratios in 5mL deionized water

Figure 3.7  Synthesis of AuNPs in different protocols

Figure 3.8a  Effect of pH on the stability of amoxicillin stabilized AuNPs

Figure 3.8b  Effect of NaCl on the stability of amoxicillin stabilized AuNPs

Figure 3.9  UV-vis spectra showing different gold to ligand ratios
using trimethylamine as reducing agent

Figure 3.10  Inset shows AuNPs-3 and trimethylamine reduced Au-NPs in day light (a) under UV illumination (b) 65

Figure 3.11  TEM images for AuNPs reduced with trimethylamine 66

Figure 3.12  TEM images for AuNPs reduced with NaBH₄ 67

Figure 3.13  PL spectra for the detection of Cu²⁺ 68

Figure 3.14  AgNPs having different ratios of Ag and sodium salt of Amoxicillin 69

Figure 3.15  Amoxicillin stabilized AgNPs Reduced with TMA 69

Figure 3.16  FTIR spectra for amoxicillin (upper) and AgNPs (lower) 70

Figure 3.17  UV-Visible spectra for the amoxicillin stabilized AgNPs Reduced with NaBH₄ 71

Figure 3.18  UV-Visible spectra for the amoxicillin stabilized AgNPs Reduced with trimethylamine 72

Figure 3.19  AFM images of Amox caped AgNPs reduced with trimethylamine 74

Figure 3.20  AFM images of amoxicillin caped AgNPs reduced with NaBH₄ 74

Figure 3.21  UV results for Ag and Au nano-alloys 76

Figure 3.22  AuNPs-AgNPs alloy (ratio shows AuNPs: AgNPs) 76

Figure 3.23  Amoxicillin stabilized Ag-Au nanoalloys 77
Chapter 4  **Metal nanoparticles stabilized with cefuroxime**

Figure 4.1  Structure of Cefuroxime  81

Figure 4.2  UV-visible spectra for AuNPs stabilized with Cefuroxime  82

Figure 4.3  UV-visible spectra for AuNPs stabilized with Cefuroxime reduced with TMA  83

Figure 4.4  Gold nanoparticles stabilized with cefuroxime (reduced with NaBH₄)  83

Figure 4.5  AuNPs stabilized with Cefuroxime Reduced with TMA  84

Figure 4.6  FTIR data for cefuroxime (upper), AgNPs (middle) and AuNPs (lower)  85

Figure 4.7  pH effect on the stability of AuNPs capped with cefuroxime  86

Figure 4.8  TEM images for Cefuroxime capped AuNPs (reduced with NaBH₄)  87

Figure 4.9  TEM images for Cefuroxime capped AuNPs (reduced with TMA)  88

Figure 4.10  UV-VIS data for AgNPs stabilized with cefuroxime (NaBH₄)  89

Figure 4.11  UV-VIS data for AgNPs stabilized with cefuroxime (TMA)  90

Figure 4.12  Silver nanoparticles stabilized with cefuroxime (reduced with NaBH₄)  90
Figure 4.13  pH effect on the stability of AgNPs caped with cefuroxime

Figure 4.14  TEM images for Cefuroxime caped AgNPs (reduced with NaBH₄)

Figure 4.15  TEM images for Cefuroxime caped AgNPs (reduced with TMA)

Figure 4.16  UV data for nano-alloys stabilized with Cefuraxime

Figure 4.17  Silver and gold nano-alloys stabilized with cefuroxime

Figure 4.18  TEM images of gold and silver nano-alloys (reduced with TMA)

Figure 4.19  TEM images of gold and silver nano-alloys (reduced with NaBH₄)

Chapter 5  Metal nanoparticles stabilized with cephradine

Figure 5.1  Structure of Cephradine

Figure 5.2  UV-visible spectra for AuNPs stabilized with Cephradine

Figure 5.3  Gold nanoparticles stabilized with cephradine (NaBh₄)

Figure 5.4  Cephradine caped gold nanoparticles reduced with TMA

Figure 5.5  UV-visible spectra for AuNPs stabilized with Cephradine reduced with TMA

Figure 5.6  FTIR data for cephradine (upper), AuNPs (middle) and AgNPs (lower)
Figure 5.7 pH effect on Gold nanoparticle Stabilized with Cephradine

Figure 5.8 Spectral data for pH effect on Gold nanoparticle Stabilized with Cephradine

Figure 5.9 AFM images of AuNPs stabilized with cephradine

Figure 5.10 Silver nanoparticles stabilized with cephradine

Figure 5.11 Spectral data for Silver nanoparticles stabilized with cephradine

Figure 5.12 pH effect on Silver nanoparticle Stabilized with Cephradine

Figure 5.13 Spectral data for pH effect on Silver nanoparticle Stabilized with Cephradine

Figure 5.14 AFM images of AgNPs stabilized with cephradine

Figure 5.15 3D form of AFM image of AgNPs stabilized with cephradine

Figure 5.16 Spectral data gold-silver nano-alloys stabilized with cephradine

Figure 5.17 Gold-silver nano-alloys stabilized with cephradine

Figure 5.18 AFM images of Au-Ag nano-alloys stabilized with cephradine

Figure 5.19 3D format of AFM images of cephradine stabilized nano-alloys

Figure 5.20 Detection of Cd ions in aqueous solution using AuNPs caped with cephradine
Figure 5.21 Detection of Cd ions in aqueous solution using AuNPs caped with cephradine with a coefficient of detection of $R^2=0.9915$

Figure 5.22 Detection of Hg ions in aqueous solution using AuNPs caped with cephradine

Figure 5.23 Detection of Cd ions in aqueous solution using AuNPs caped with cephradine with a coefficient of detection of $R^2=0.9992$

Figure 5.24 Comparative detection of metallic ions in aqueous solution using AuNPs caped with cephradine

Chapter 6 **Metal nanoparticles stabilized with ceftriaxone**

Figure 6.1 Spectral data for Gold nanoparticles stabilized with ceftriaxone

Figure 6.2 Gold nanoparticles stabilized with ceftriaxone

Figure 6.3 FTIR data of Ceftriaxone (upper), AuNPs (Middle) and AgNPs (lower)

Figure 6.4 pH stability of AuNPs stabilized with ceftriaxone

Figure 6.5 Spectral data for pH stability of AuNPs stabilized with ceftriaxone

Figure 6.6 Salt stability of AuNPs stabilized with ceftriaxone

Figure 6.7 AFM images of ceftriaxone stabilized AuNPs

Figure 6.8 Silver nanoparticles stabilized with ceftriaxone (Ratio: Silver: ceftriaxone)

Figure 6.9 Absorption peak for Silver nanoparticles stabilized
with ceftriaxone (Ratio: Silver: ceftriaxone)

Figure 6.10  pH stability of AgNPs stabilized with ceftriaxone  128
Figure 6.11  AFM images of ceftriaxone stabilized AgNPs  129
Figure 6.12  Spectral data for Gold-silver nano-alloys stabilized with ceftriaxone  130
Figure 6.13  Gold-silver nano-alloys stabilized with ceftriaxone (Ratio: AuNPs: AgNPs)  130
Figure 6.14  AFM images of ceftriaxone stabilized nano-alloys  131
Figure 6.15  3D format of ceftriaxone stabilized nano-alloys  131

Chapter 7  Metal nanoparticles stabilized with cefixime

Figure 7.1  Structure of Cefixime  134
Figure 7.2  Spectral data for AuNPs stabilized with Cefixime  137
Figure 7.3  Spectral data for AuNPs stabilized with Cefixime using TMA  138
Figure 7.4  Cefixime stabilized AuNPs, reduced with TMA  138
Figure 7.5  FTIR data for cefixime (upper) AuNPs (middle) and AgNPs (lower)  139
Figure 7.6  pH stability of Cefixime capped AuNPs  140
Figure 7.7  AFM images of cefixime stabilized AuNPs  141
Figure 7.8  Spectral data for Cefixime stabilized AgNPs, reduced with NaBH₄  142
Figure 7.9  Spectral data for Cefixime stabilized AgNPs, reduced with TMA

Figure 7.10  Cefixime stabilized AgNPs, reduced with TMA

Figure 7.11  pH stability of Cefixime capped AgNPs

Figure 7.12  AFM images of cefixime stabilized AgNPs

Figure 7.13  Detection Cd\(^{2+}\) in aqueous solution using cefixime capped AuNPs, with a detection range of 0.000001M

Figure 7.14  Detection Cu\(^{2+}\) in aqueous solution using cefixime capped AuNPs with detection range of 0.000001M

Figure 7.15  Detection of metals in aqueous solution using cefixime capped AuNPs (0.0001M metallic Salts)

Figure 7.16  Detection of 0.0001M Ni\(^{2+}\) in aqueous solution using AgNPs of Cefixime

Figure 7.28  Comparative detection of metal ions in aqueous solution using AgNPs of Cefixime
# LIST OF SCHEMES

<table>
<thead>
<tr>
<th>Scheme No.</th>
<th>Description</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scheme 3.1</td>
<td>Gold nanoparticles stabilized with amoxicillin</td>
<td>53</td>
</tr>
<tr>
<td>Scheme 4.1</td>
<td>Formation of Gold and Silver nanoparticles with Cefuroxime</td>
<td>81</td>
</tr>
<tr>
<td>Scheme 5.1</td>
<td>Gold and Silver nanoparticles caped with Cephradine</td>
<td>100</td>
</tr>
<tr>
<td>Scheme 6.1</td>
<td>Synthesis of gold and silver nanoparticles caped with ceftriaxone</td>
<td>120</td>
</tr>
<tr>
<td>Scheme 7.1</td>
<td>Synthesis of gold and silver nanoparticles caped with cefixime</td>
<td>135</td>
</tr>
</tbody>
</table>
CHAPTER-1

INTRODUCTION TO

BETA-LACATAM ANTIBIOTICS
Chapter-1

Introduction to beta-lactam antibiotics

1. Beta-lactam Antibiotics
Beta-lactam (1, 4-cyclic amide) antibiotics consists of six main classes which are given in the following

1.1 Cephalosporins
The cephalosporins belong to a class of β-lactam antibiotics which was derived originally from the fungus Acremonium, which was previously known as "Cephalosporium".

1.1.1 Cephalosporin nucleus
Its consists of 1, 4-cyclic amide nucleus fused with 1, 3-thiazolidine ring as shown in the figure 1.1

\[
\begin{align*}
\text{R} & \hspace{1cm} \text{H} \\
\text{O} & \hspace{1cm} \text{N} \\
\text{O} & \hspace{1cm} \text{S} \\
\text{O} & \hspace{1cm} \text{N} \\
\text{O} & \hspace{1cm} \text{CO}_{2}\text{OH}
\end{align*}
\]

\text{Figure 1.1: Cephalosporin nucleus}

1.1.2 History
Cephalosporin antibiotics were isolated for the first time from Cephalosporium acremonium cultures in Sardinia in 1948 by Giuseppe Brotzu (Italian scientist)[1]. He found that the substances produced by this culture were active against Salmonella typhi, which causes typhoid fever and had beta-lactam. At the Pathology, University, Guy Newton and Edward Abraham isolated cephalosporin C. The cephalosporin nucleus, 7-aminocephalosporanic acid (7-ACA), was originated from cephalosporin C and showed to be similar to the penicillin nucleus of 6-aminopenicillanic acid (6-APA), but it was not satisfactorily powerful for clinical use. Derivatizing the side chain of 7-ACA lead to the development of very nice antibiotic agents, and for the first time the first agent cefalotin (cephalothrin) was introduced by Eli Lilly and Company in 1964.
1.1.3 Basic Structure

The cephalosporin molecule contains two ring systems which comprises a β-lactam ring attached with dihydrothiazine ring. The main body itself can also be called as 7-ACA (7-aminopenicillanic acid); can be synthesized by hydrolysis of its natural compound cephalosporin C. Analogous of 7-ACA are comparatively stable to acid hydrolysis and tolerant to β-lactamases (enzymes). Cephalosporin C consists of a side-chain which is derived from D-aminoadipic acid.

![Cephalosporins](image)

**Figure 1.2: Cephalosporins**

1.1.4 Classification

The cephalosporins can be classified on the basis of chemical and activity features for instance chemical structure, side chain properties, pharmacokinetic, spectrum of activity or clinical properties. But the most common classification for cephalosporins is to classify them into different generations which are based on different antimicrobial activities of different cephalosporins.

1.1.4.1 First generation cephalosporins

The first generation of cephalosporins is the one, introduced in the market for the first time. The members of this generation have worthy antimicrobial activity against gram-positive bacteria but against gram-negative species they have limited activities. These have very simple chemical structures. Cephalexin,
Cephradine and Cefadroxil are members of this class and all have a single methyl group at position C-3. Small and uncharged (non-polar) specie is attached at position 3 in this class. Cefaclor is often classified as first generation due to its C-7 side chain, although at position C-3 a Cl atom (not an agreement as a first generation cephalosporin). Cefaclor also show best activity.

\[ \text{Figure 1.3: The basic structure of cephalosporins of first generation} \]

### 1.1.4.2 Second generation cephalosporins

Early class of second generation cephalosporins is very similar in basic structure to the first generation of cephalosporins. The introduction of α-iminomethoxy group to the C-7 side chain is an important change in the structure of second generation. This side chain increases the resistance to β-lactamase enzymes, which is mainly due to the stereo chemical hindering of the beta-lactam ring. This side chain was firstly incorporated in Cefuroxime which is a second generation antibiotics. The introduction of aminothiazole ring to the C-3 side chain is another important change which increases its antimicrobial activity dramatically. The aminothiazole ring is present in the structure of Cefotiam.

\[ \text{Figure 1.4: Basic structure of 2nd generation cephalosporins} \]
1.1.4.3 Third generation cephalosporins

Cephalosporins of third generation have the aminothiazole functional group at C-7 position. Various groups are also placed at 7-α-position like 7-α-iminohydroxy and 7-α-iminomethoxy. Ceftibuten have 7-α-ethylidene group which has given Ceftibuten a maximum resistive activity against β-lactamase enzymes. Most of the orally absorbed drugs are ester but some of the drugs can be absorbed orally without the need of esterification. For example Cefixime and Cefdinir when modified by placing a vinyl group at C-3 position.

![Figure 1.5: The basic structure of 3rd generation cephalosporins](image)

1.1.4.4 Fourth generation cephalosporins

The cephalosporins of this class have high resistance against gram-negative bacteria than the 2nd and 3rd generation cephalosporins. This difference is due to the zwitterion effect of these compounds. The side chain at position C-7 is similar to third generation cephalosporins generally containing iminomethoxy-aminothiazole group while in case of Cefclidine aminothiadiazole is present. Members of this group diffuse easily through the cell membrane of gram-negative bacteria because of the positively charged quaternary nitrogen present in the side chain at position C-3.

1.1.4.5 Fifth generation cephalosporins

Ceftobiprole and Ceftaroline are the two drugs which are currently present in this class, which are the only effective β-lactam antibiotics against MRSA.
(methicillin-resistant-*Staphylococcus-aureus*). Ceftaroline was derived from Cefozopran, a fourth-generation cephalosporin. It preserves the alkoxyimino group at position C-7 from earlier generations and thus has high resistance to β-lactamases.

### 1.1.5 Mechanism of action

1. The enzymes which are necessary for the development of the cell wall of bacteria are inhibited by these drugs and thus preventing cell wall synthesis.
2. Bactericidal activities are concentration independent and kill bacteria at maximum level.
3. Post-antibiotic effect is not noticed which is clinically significant.

### 1.1.6 Spectrum of activity

Generally first generation has very good activity against gram positive bacteria and less activity against gram negative bacteria. Third generation cephalosporins have better activity against gram positive bacteria with few deficiencies and less activity against gram positive activity. Fourth generation cephalosporins have both gram positive and gram negative activities.

### 1.2 Penicillin

Penicillin nucleus consist of beta lactam ring fused with thiozolidine ring with nitrogen being common to both rings as shown in the **figure 1.6**. Penicillin is a group of beta-lactam antibiotics derived from a fungus specie *Penicillium*[2]. Penicillin V, Ampicillin and amoxicillin are few examples of this group.
1.2.1 History of penicillin

Penicillin was discovered for the first time by a French medical student Ernest Duchesne (in 1896). This was re-discovered by bacteriologist Alexander Fleming in 1928. Dr. Fleming published the results of his research in 1929, giving the name penicillin to the substance[2].

Sir Alexander Fleming (in 1928) observed that groups of the bacterium *Staphylococcus aureus* might be ruined by the mold *Penicillium-notatum*, showing that there is an antibacterial agent in it. This was later confirmed as a medicine, suitable for killing some types of bacteria which cause diseases inside bodies. Because of the unawareness of the importance of Alexander Fleming’s discovery, penicillin was not used until 1940s, but when Howard Florey and Ernst Chain collected it in the powder form and used it as a medicine.[3-5]

The structural arrangement of atoms and the molecular shape of penicillin were confirmed by Hodgkin by using x-rays.

Dr. Howard Florey and three colleagues began a research at Oxford University to find out the ability of penicillin to kill bacteria. Because of the war with Germany and lack of resources, the British scientists failed to produce such an amount of penicillin to be used for clinical trials and they asked United States for help. British scientists were quickly provided Peoria Lab; where scientists were already busy in working on the fermentation techniques for increasing the developmental rate of fungal cultures. Howard Florey and Norman Heatley went
to the U.S on July 9, 1941, with a little amount of penicillin to begin work[6]. The yield of penicillin was increased 10 times by Andrew J. Moyer in November, 26, 1941. The clinical trials were performed in 1943 and were proved that penicillin is the most active antibacterial agent. As a result of this research work, two members of the British group were awarded Nobel Prize.

### 1.2.2 Some widely used Penicillin

Some of the penicillin antibiotics are

1. Amoxicillin
2. Ampicillin
3. Carbenicillin
4. Dicloxacillin
5. Oxacillin
6. Penicillin V, G, N and O

#### 1.2.3 Amoxicillin in Brief

A semisynthetic amino-penicillin beta lactam antibiotic related to ampicillin (Figure 1.7) in structure is amoxicillin (Figure 1.8). Amoxicillin is a moderate-spectrum beta lactam antibiotic which is very effective for a large variety of Gram-positive, and some Gram-negative pathogens.

##### 1.2.3.1 History

Scientists at Beecham Research Laboratories had discovered amoxicillin in 1972. It is sold by GlaxoSmithKline under the trade name of Amoxil[7].
Ampicillin was developed which have a broader spectrum of activity than either of the original penicillins which have a narrow spectrum of antimicrobial activity. Now ampicillin could be used to treat a broad range of infections caused by Gram-positive and Gram-negative microbes. By the discovery of amoxicillin, duration of action was improved. However it has only an additional –OH group at para position of benzene ring on ampicillin.

**Figure 1.7:** Structural formula of Ampicillin

**Figure 1.8:** Structural formula of Amoxicillin

### 1.2.3.2 FDA (Food and Drug administration) approved uses of amoxicillin

Amoxicillin is designated for the treatment of the succeeding infections:

1. **Infections of Ear, Throat and Nose**: for the middle ear infection (otitis media), for sore throat (pharyngitis), for the infection of sinus (sinusitis), for tonsillitis, and for the treatment of tonsil-pharyngitis.
2. **Infections genito-urinary territory**: for the infections of vagina, uterus, fallopian tubes or ovaries (genital organs) and for the kidneys, ureters, bladder and urethra (urinary tract).

3. **Infections of Skin**: for the infection of dermis and subcutaneous tissue (cellulitis), infections of superficial form of cellulitis (erysipelas), for the inflammation of the hair follicles (folliculitis),

4. **Infections in Lower respiratory tract**: For inflammation of the lower respiratory tract (tracheobronchitis), inflammation of the mucous membranes of the bronchi (bronchitis), inflammation of the lungs (pneumonia).

5. **For the diseases transmitting Sexually**: Gonorrhea, ano-genital and urethral infections (acute uncomplicated)[8]

1.2.3.3 Other uses

Amoxicillin is also used for Chlamydial infections (*Chlamydia trachomatis*) [9, 10], Post exposure prophylaxis for anthrax and inflammation of the inner lining of the heart (Endocarditis)[11]. It is also used for Typhoid Fever (enteric fever), which is fatal if left untreated[12].

1.2.3.4 Advantages

1. Amoxicillin is better absorbed from the gastro-intestinal area compared to other beta lactam antibiotics (ampicillin etc.).

2. It is excellent for the infections of middle ear (Otitis media)[13].

3. It easily diffuses into the tissues and fluids of body, while does not penetrate to brain and spinal fluid.

4. It could be used and is safe to be used in pregnancy[14] for this reasons it is widely used for the treatment of pregnant women.

5. Children could use it safely.
1.2.3.5 Disadvantages

1. The penicillin allergy which causes a hypersensitivity (anaphylactic) reaction, is its main disadvantage
2. It cause diarrhea compared to other beta lactam antibiotics
3. It may interact in urinary glucose test using cupric sulfate (Benedict’s solution)
4. It is ineffective against beta-lactamase creating bacteria
5. Amoxicillin may cause tooth enamel defects in permanent teeth if used during in babyhood[15].

1.2.3.6 Mode of action

Amoxicillin kills bacteria. It inhibits the synthesis of cell walls of the bacteria. This results a defect in the formation of cell wall and thus causing death to the cell.

These are easily penetrated to gram-negative bacteria because of free amino group in their structure compared to the natural penicillins[8].

1.2.3.7 Clearing out of the system

The amoxicillin has a half-life of about 61.3 minutes but in the case of lacking renal function it approaches to 7-10 hours. Generally it takes 5-6 hours to leave the system.

1.2.3.8 Chemistry of Amoxicillin

The amoxicillin structure consists of a nucleus of 6-aminopenicillinic acid and side chain of 2-amino-2-(4-hydroxyphenyl) acetyl attached with the amino group of 6-APA at position 6 (Figure 1.8). The nucleus of 6-aminopenicillinic acid
contains a 4 membered amide ring called β-lactam ring, and a thiazolidine ring attached to beta-lactam ring. The side chain is different from ampicillin only by a hydroxyl group on the para position of phenyl ring (figure 1.7).

![Figure 1.9: Structure of Penicillin](image)

1.3 Monobactam antibiotics

These are also the β-lactam compounds used as antibiotics similar to other β-lactam antibiotics but here in this case only beta-lactam ring is present which is not fused to other rings as in other β-lactams antibiotics. Monobactams are active only against Gram negative bacteria and are rarely used. Examples are aztreonam, tigemonam, nocardicin A and tabtoxin.

![Figure 1.10: monobactam nucleus](image)
1.4 Carbapenem antibiotics

Carbapenem antibiotics were basically derived from thienamycin (a product, derived naturally from *Streptomyces cattleya*). These are broad spectrum β-lactam antibiotics. Their structures make them highly resistant to most β-lactamase enzymes. Carbapenems are the antibiotics for multiple bacterial infections, such as *E. coli* and *Klebsiella pneumoniae*.

![Carbapenem nucleus](image)

**Figure 1.11:** Carbapenem nucleus

1.5 Cephamicins antibiotics

These are β-lactam antibiotics very similar to cephalosporins. Some times cephamycins are classified as cephalosporins. Cephamicins are generally based upon the cephem nucleus and are very similar to cephalosporins. The difference is that cephamycins are a very effective antibiotic against anaerobic pathogens than cephalosporins.

![Basic structure for cephamcins](image)

**Figure 1.12:** Basic structure for cephamcins
1.6 Oxacephem antibiotics

In case of oxacephem it has similar molecule to a cephem, but the difference is oxygen substituted for the sulfur in the ring. These are synthetic compounds and not found in nature. Examples are moxalactam and flomoxef.

![Basic structure for oxacephem](image)

**Figure 1.13**: Basic structure for oxacephem
CHAPTER-2

INTRODUCTION

TO

NANOTECHNOLOGY
2. What is nanotechnology?

Nanotechnology refers to the technology which uses materials with nano scale sizes, usually ranging from one to hundred (100nm) nanometers at least in one dimension. There are a number of definitions for ‘nanotechnology’, in all definitions, dimensional measurement in the nanometer scale has been used rather than other physical properties. The best of all definitions and most accepted one is that nanotechnology is the exploitation of materials of 100 nanometers or less at least on one axis. This definition of nanotechnology does not refer to unintentionally formed nano sized materials that occur naturally in the universe, i.e. viruses, volcanic ash, diesel exhaust etc. Nanomaterial shows novel and different chemical and physical properties because of their size and shape. There are some nanomaterial categorized on the basis of shape and size like nano-layers, nanowires and nanotubes etc. Thus the particles shape and size has been used as origin for classification of nanoparticles.

2.1 Metal Nanoparticles

In the recent few decades, the field of metal nanoparticles has a wonderful growth. Fe nanoparticles were synthesized for the first time, from Fe (CO)$_5$ in organic solvents; similar approach was used to make Co nanoparticles. Amorphous Fe nanoparticles may be prepared at low temperature and the carbonyl is decomposed sonochemically[16]. Silver and gold nanoparticles as shown in figure-2.1, can be prepared with different methods and have a huge contribution to the field of nanotechnology.
2.2 Synthesis

Metal-nanoparticles can be synthesized using different physical and chemical methods [17-23] i.e. citrate reduction method[24], two-phase synthesis[25], and a one phase synthesis in organic solvents[26]. A recent development in the field of methodology of nanoparticles is the use of amino acids as reducing as well as capping agents [27, 28]. The chemical procedures involve reduction of metal ions to metal atoms, followed by stabilization with a capping or stabilizing agent. The chemical method is more appropriate for obtaining small and uniform nanoparticles than the others; furthermore, the size and uniformness of the nanoparticles depend upon the kind and amount of the reducing agents. Metals from the transition series, especially luxurious metals show very nice catalytic activities for many organic reactions. These substances show properties of both heterogeneous and homogeneous catalysis. The active sites of the surface of metal nuclei are important for catalysis, which is similar to the mechanism of conventional heterogeneous catalysis. While nanoparticles melt in reaction medium similar to conventional homogeneous catalysis. The best advantage of the nanoparticles is; the catalyst can be easily recovered from the reaction medium. The problem of nanoparticles is the flocculation in the presence of electrolytes. To overcome this problem, the chemical reduction of the corresponding metal salts is carried out in a solution in the presence of suitable capping agents or stabilizers, which neutralize the electrostatic repulsive force.
Also these stabilizers help in obtaining functionalized metal-nanoparticles. For instance, thiol derivatives (-SH) have been used as strong stabilizers or capping agents for gold nanoparticles; thiol derivatives have strong coordination affinity to functionalize the gold surface by formation of a monolayer on the surface. Amines have been used in the synthesis of nanoparticles as reducing agents as well as stabilizing agents [27-32]. Various amines have been used, including simple primary amines[31, 32], amino acids[27-29] and multifunctional amino polymers[30]. Gold and silver nanoparticles have been studied in great detail [33-36]. Especially, the size effect on the Plasmon band in connection with the Mie theory and its modifications has been of major interest[36]. Alloy nanoparticles, on the other hand, have been studied because of their catalytic effects[37, 38]. As gold has low catalytic activity compared to platinum or palladium, the structural and catalytic changes have been examined for the admixture of platinum or palladium to gold[39, 40]. Liz-Marzan et al [41] used inorganic fibers in aqueous solution for the stabilization of gold-silver particles with diameters of 2 to 3 nm after the simultaneous reduction of gold and silver salts by NaBH₄. Teo et al. [42] synthesized a 38 atom gold-silver cluster (Au18Ag20). The solution of this cluster showed a single absorption peak at 495 nm. Mulvaney et al. [35, 43] and also Sinzig et al. [44] prepared silver nanoparticles coated with an over layer of gold (core-shell nanoparticles). These particles have two distinct Plasmon absorption bands and their relative intensities depend on the thickness of shell but also alloy formation within the shell was suggested on the basis of optical absorption spectra. Several reports are there in which natural materials like plants; bacteria, fungi, yeast and honey etc. have been used for synthesizing gold and silver nanoparticles. For synthesizing gold and silver nanoparticles stabilized with plant extracts, Daizy Philip et al. have reported facile, rapid and single-pot aqueous biosynthesis of silver and gold nanoparticles using the leaf extract of Krishna tulsi (Ocimum sanctum). It is used for the treatment of bronchitis, bronchial asthma, malaria, diarrhea, dysentery, skin diseases, arthritis, painful eye diseases, chronic fever and insect bites. It has also been suggested to possess
Chapter 2  Introduction to nanotechnology

antifertility, anticancer, antidiabetic, antifungal, antimicrobial, hepatoprotective, cardioprotective, analgesic, adaptogenic and diaphoretic actions. The important ingredients present in the leaf extract are triterpenes, flavonoids and eugenol [45].

2.3 Characterization Techniques
Nanoparticles after preparation are characterized with some techniques to confirm their shape and size. The characterization techniques which may be used are:
1. UV-Visible spectroscopy
2. Scanning Electron Microscopy (SEM)
3. Transmission Electron Microscopy (TEM)
4. X-rays Diffraction (XRD)
5. Atomic Force Microscopy (AFM)
6. Photon Correlation Spectroscopy (PCS)
7. Nanoparticle Surface Area Monitor (NSAM)
8. Condensation Particle Counter (CPC)
9. Differential Mobility Analyzer (DMA)
10. Aerosol Time of Flight Mass Spectroscopy (ATFMS)
11. Scanning Mobility Particle Sizer (SMPS)
12. Nanoparticle Tracking Analysis (NTA)
13. Aerosol Particle Mass Analyzer (APM)

2.4 Applications
Nanoparticles have applications in the fields of analytical chemistry for example optoelectronics or chemical and bio sensing. Some of the metal-nanoparticles (e.g. platinum) are very suitable for catalyzing the redox reaction of some molecules with analytical interest, which can be monitored through electro analytical techniques. The large surface area of the metal nanoparticles is responsible for their different chemical, optical, mechanical, magnetic and
catalytic properties as compared to bulk materials [46, 47]. AgNPs and AuNPs have a vital role in the fields of antibiotics where complete mechanisms of action have already been explained by experts [48-51]. Similarly AuNPs have a vital role in the areas ranging from chemical separations [52] and sensing [53-62] to applications in the medical community [63-66], such as the diagnosis and treatment of certain cancers [67-70].

Nanoparticles are so small that they can easily interact with microbes such as bacteria or viruses. Since nanoparticles can exactly be the particles of any substance, so they are sufficiently adaptable and can be used in many scientific applications i.e. from mild electronics to innovative biomedical procedures [71]. The nanoparticles have more medical applications as their small size is very feasible to fight against various unwanted attackers inside the human body. They can work against viruses and bacteria in much the same way as immune system’s cells do in the body [71].

Nanoparticles can be armed with sensors and cameras as well as cancer-killing medicines. These equipped nanoparticles then flow in the blood stream; their sensors locate the affected site of the cancer. The cameras could beam back images to viewers. The nanoparticles could also be conjugated with fluorescent materials so that they can be seen both on optical imaging devices and MRI [71].

2.5 Properties
Metal nanoparticles exhibit diverse important properties which may be utilized in different fields of medicines, optical activity catalysis and analytical chemistry etc. some of the properties are given below
2.5.1 Optical properties

Nanoparticles could be used for coating anti-reflection products, thus produce a refractive index for various surfaces, and also provide light based sensors for use in diagnosing cancer. The optical properties of silver nanoparticles can be used as a useful constituent in different sensors. Silver nanoparticles have efficient properties of absorbing and scattering light and in contrast of many pigments, dyes and colors, have a size and shape dependent color of the particles. The strong interaction of the silver nanoparticles with light occurs because the transmission of electrons on the metal surface undergo a combined oscillation when excited at a definite wavelengths of light known as a surface plasmon resonance (SPR), which results in extraordinarily strong scattering and absorption properties. 60 nm silver nanoparticles when strike with white light, illuminate as bright blue points under a dark field microscope (Figure 2.2). This blue color illumination is due to a peak at 450 nm wavelength which is a characteristics wavelength for silver nanoparticles. Spherical silver nanoparticles may also show illuminations at 400 nm (violet light) to 530 nm (green light) according to their particle size. Illumination is even observed in the infrared region with rod or plate shaped silver nanoparticles[72]. Thus the color of the nanoparticles depend on size and shape of the particles (Figure 2.3)

Figure 2.2: Dark field microscopy image of 60 nm silver nanoparticles
Figure 2.3: Color of nanoparticles depends on the Size of particles

2.5.2. Magnetic properties
Magnetic nanoparticles can be synthesized, their magnetic properties can be utilize in different applications for example if the nanoparticles are magnetized then they can improve the detail and contrast of MRI images.

2.5.3. Thermal properties
Particularly modified particles may increase the conduction of heat from gatherers of solar energy to the storing containers. They can also be used to increase the cooling effect of coolant system presently used by transformers in these types of processes[71].

2.5.4. Electronic properties
Small sizes of nanoparticles are very suitable for the production of high performance delicate electronics as nanoparticles not only deliver constituents with a high conductivity, but also smoother parts for small size electronics like cell phones and laptops etc. Nanoparticle electronics can be used to make digital displays which are less expensive and brighter in color[71].
2.5.5. Energy properties
Nanoparticle may be utilized in energy storage batteries which would be longer-lasting and have more energy density. Metal nanoparticle could have applications for storage of hydrogen gas; and extremely efficient fuel cells could be produced by using the property of electro-catalysts for these devices. In combustion engines, when nanoparticles are used as catalysts, make the engine more efficient and more economic[71].

2.5.6. Biomedical properties
Antibacterial coated nanoparticles (both silver and gold nanoparticles) may be used for wound directly. Also the nanoparticles stabilized with antibiotics may increase their activity against microbes providing new types of drugs. “Quantum dots,” which can identify or diagnose diseases and could be used to load drugs on it for carrying drugs to a particular affected area like cancer cells and tumor etc. But most of quantum dots are toxic therefore other non-toxic fluorescent nanoparticles could be used for the same purposes[71].
2.6 Review on Recent Advancements in Metal Nanoparticles

2.6.1 Review on Gold Nanoparticles

Liu et al. reported the synthesis of Au–Ag alloy nanoparticles using axial amine 5 poly amido-amine cascade molecules (dendrimer) as stabilizing agent and sodium borohydride as reducing agent. Different molar ratios of gold, silver and dendrimer were used. The axial amines of the dendrimer were acylated. Different methods were used to characterize newly synthesized nanoparticles. Newly synthesized nanoparticles were stable at pH 5-8 and at a temperature range of 4-50 °C. It was observed that the size and optical activity of nanoparticles were dependent upon metal composition, for example an increase in gold content decrease the size of nanoparticles. X-ray absorption coefficient measurements disclosed that X-ray attenuation intensity of nanoparticles was enhanced by increasing the gold ratio in alloy nanoparticles and by acetylation. Cytotoxicity assays revealed that cyto-compatibility of nanoparticles was also improved by acetylation. With these properties the Au–Ag alloy nanoparticles could be successfully used in many medical applications containing CT imaging [73].

Cubillana-Aguilera et al. synthesized gold nanoparticles in aqueous solution at room temperature and high power ultrasounds, sodium citrate was used as capping and reducing agent. Whole synthetic process was completed in 5.5 minutes. The synthesized nanoparticles were spherical with a homogenous size distribution. The size ranged from 5 to 17 nm having average diameter of 10 ± 1 nm. The half-life of nanoparticles was more than 30 days. Due to use of ultrasounds in their synthesis, nanoparticles were called sono-nanoparticles. UV–Vis spectroscopy was used to study the stability of nanoparticles, which were characterized by XRD and Transmission Electron Microscopy (TEM) [74].

Granmayeh Rad et al. synthesized gold nanoparticles at room temperature using laser ablation with first harmonic output. TEM was used for the determination of
size and optical properties of gold nanoparticles. The Laser used in this study was Nd: YAG operated between 5 Jcm-2 and 15 Jcm-2. The application of nanoparticles obtained was studied in many different fields [75].

Hien et al., reported the synthesis of gold nanoparticles by γ-irradiation using hyaluronan as stabilizing agent. UV-Vis spectroscopy was used to measure the maximum absorption wavelength at 517–525 nm which is a region for gold nanoparticles. TEM was used to determine the size and size distribution of nanoparticles. The diameter of nanoparticles was in the range of 4-10 nm. The size of nanoparticles was depending upon concentration of hyaluronan, dose amount and concentration of Au$^{3+}$. The nanoparticles in colloidal solution containing hyaluronan were found to be stable at ambient conditions for more than six months. These nanoparticles found many applications in cosmetics and biomedicines[76].

Mishra et al. synthesized gold nanoparticles using biological systems including live cell filtrate, supernatant broth of Penicillium rugulosum and potato dextrose broth. Nanoparticles were characterized using different techniques including TEM, FTIR, XRD, UV-Vis spectroscopy and X-ray photoelectron spectroscopy (XPS). Shapes and sizes of nanoparticles were determined by pH and gold content. Increasing gold salt concentration increased the size and aggregation of nanoparticles. Synthesis was favored at pH 4 to 6. Genomic DNA of bacteria Escherichia coli and Staphylococcus aureus were used to conjugate with gold nanoparticles. DNA was observed to stabilize the nanoparticles against aggregation [77].

Nalawade et al. synthesized multi spiked gold nanoparticles by reduction of gold ions in ethylene glycol using NaOH to accelerate the reaction. Average diameter of nanoparticles obtained was 75 ± 10 nm. Nanoparticles were characterized using different techniques including UV-Vis spectroscopy, TEM and XRD. Nanoparticles when obtained in the presence of PVP stabilizing agents were used
as a catalyst in the reduction of o-nitro aniline to benzenediamine with NaBH$_4$ following a pseudo first order kinetics [78].

Nguyen et al. reviewed the application of functionalized gold nanoparticles in cell imaging, metal ions, protein, small organic compounds, DNA and RNA on the bases of controllable sizes and shapes. Gold nanoparticles were also observed to be used as biosensors due to their optical properties and the ability of these nanoparticles to generate immobilization of biomolecules without affecting their bioactivities. It was noticed that the optical properties of gold nanoparticles were dependent upon their shapes, sizes, functional groups and aggregation [79].

Misra et al. synthesized gold nanoparticles by gamma radiolysis of HAuCl$_4$·3H$_2$O precursor in aqueous solution of PVP in the presence of acetone, 2-propanol and small amount of Ag$^+$. Formation of nanoparticles was affected by concentration of reactant and molecular weight of PVP. Nanoparticles were characterized using TEM and it was observed that at 360,000 Da molecular weight of PVP, gold nanoparticles were spherical. Gold nanoparticles were used in the determination of H$_2$O$_2$, as they react with the reaction product of H$_2$O$_2$ and o-PDA and enhance the absorption peak of the reaction. The absorbance of the peak was dependent upon H$_2$O$_2$ concentration [80].

Noruzi et al. synthesized gold nanoparticles within 5 min using aqueous extract of rose petals. Different characterization techniques, including UV-VIS spectroscopy, XRD, FTIR, EDX, dynamic light scattering and TEM were used to observe the properties of nanoparticles. TEM determined the various shapes of nanoparticles, XRD determined the face centered cubic structure and purity and FTIR determined the functionalization with biomolecules having –OH, –NH$_2$ and other stabilizing functional groups. Particle size was determined by dynamic light scattering and was observed to be 10 nm [81].

Peng et al. synthesized dendrimer entrapped gold nanoparticles and increased their biocompatibility of CT imaging by modification with Polyethylene glycol.
The size of the nanoparticles was controlled at the range of 2–4nm by adjusting the salt and dendrimer molar ratio. The resultant nanoparticles were stable at pH range of 5–8 in aqueous solution and at temperature range of 0–50°C. High attenuation intensity as shown by X-ray absorption coefficient measurements and long half decay time shown by pharmacokinetics studies makes nanoparticles very effective for CT blood pool imaging particularly in cancer diagnosis [82].

Akhavan et al. synthesized the gold nanoparticles using γ-irradiation. Stabilizer used was bovine serum albumin protein. Gold nanoparticles were characterized using different techniques. The size and shape of nanoparticles was demonstrated from UV-Vis spectroscopy, TEM and XRD and was found to be in the range of 2 to 7 nm with spherical shape. The size depended upon the irradiation doses. Dynamic light scattering and FTIR indicated the conjugation of BSA to nanoparticles. SDS-PAGE analysis demonstrated the effect of irradiation on BSA structure. MTT assay indicated the non-cytotoxic nature of gold nanoparticles to mammalian cells. It was observed that aggregation and degradation of BSA was protected partially by nanoparticles synthesized in the experiment [83].

Bach et al. described the synthesis of gold nanoparticles grafted with biocompatible poly (2-hydroxyethyl methacrylate) and chemically anchored adenosine. Atom transfer radical polymerization was used to synthesize the disulfide containing poly (2-hydroxyethyl methacrylate). $^1$H-NMR and FTIR confirmed the formation. Gold nanoparticles were synthesized by grafting of disulfide. Grafting was confirmed by different techniques including TGA, EDX, FTIR and XPS. HR-TEM demonstrated the size of resultant nanoparticles to be 5.0 nm. Nanoparticles were functionalized by boronic acid. The strong interaction of free –OH of adenosine with boronic acid resulted in covalent immobilization and led to the formation of AuNPs. UV-Vis spectroscopy, EDX, XPS and FTIR indicate
significant changes in the surface properties of gold nanoparticles after immobilization with PHEMA-adenosine [84].

Zeng et al. reported the solution phase synthesis of symmetrical hexagonal PbO nano-sheets by anisotropic growth using gold nanoparticles at room temperature. In the absence of gold nanoparticles, nano-sheets were not formed. Nanoparticles provided nucleation sites to seed the growth of nano-crystals which aggregated in the form of nano-sheet. The method yielded high quality and highly symmetrical PbO nano-sheets with controlled edge length[85].

Larguinho et al. reviewed the use of nanotechnology in clinical diagnostics and therapy. Many nano scale devices have been developed for single molecule characterization of proteins, DNA and RNA. With the use of nanotechnology much advancement has been made in suitable biomedical diagnostics with high resolution in much less time[86].

Seol et al. reported the microwave synthesis of gold nanoparticles with a uniform size of 12.04 ± 1.35nm. By adjusting different reaction parameters like temperature and pH, size of the nanoparticles were produced with improved quality and uniformity. Temperature ramping rate was changed using high power microwave. The reaction was completed within a few minutes of time [87].

Tarnawski and Ulbricht synthesized gold nanoparticles containing two different thiols, dodecane-thiol and 1-mercaptoundec-11-yl-hexa. Different characterization techniques were used. Composition of thiol in the nanoparticle shell was determined by FT-IR and contact angle measurement (CA) and ratio was found to be (EG6:C12 = 72:28). The size of nanoparticles was determined by UV-Vis spectroscopy, TEM and AFM and was found to be ≤10 nm. Mixtures of C12 and EG6 on planar gold films were used for the preparation of self-assembled monolayers nanoparticles. Surface functionalization was carried out by the adsorption of ligand from aqueous solution and the substrate used was
spin coated films of polystyrene on gold coated surface and silicon wafers surface plasmon resonance sensor disk. AFM and CA results indicated that topography and hydrophobicity/ hydrophilicity properties are dependent on the concentration of ligand used. SPR analysis revealed the adsorption of nanoparticles and myoglobin to the PSt-b-PEGylated surfaces. Amount adsorbed could be controlled for both kinds of colloids by the degree of PEGylation. SPR analysis revealed that amount of nanoparticles adsorbed to the SAM surface was strongly dependent upon composition of surface. Because of their interaction with polymer surfaces, the synthesized gold nanoparticles were reported to be used as colloids for investigation of different kinds of polymers and could be used in many other biological and analytical applications when modified slightly [88].

Pałławski et al. reported controlled synthesis of AuNPs using flow micro-reactor system. Glucose was used for the reduction of AuCl₃ ions in the presence of varying amount of PVP as a stabilizing agent. Batch reactor was used to optimize the flow rate and concentration of different components in the micro-reactor. UV-Vis spectroscopy and Dynamic Light Scattering (DLS) techniques were used to determine the effect of temperature and reducing agent concentrations upon the rate constants of nanoparticles growth and nucleation. Rate law of nanoparticles was formulated using kinetic data from the reaction and synthesis conditions were established from the law. T-type geometry was applied in connection of micro-channels for application of PVP and different reactant at the proper time. It was reported that AuNPs with narrow size distribution and well-defined spherical shapes could be prepared and stabilized by using this method[89].

Streszewski et al. studied the kinetics and mechanisms of AuNPs formation from hydrazine sulfate and [AuCl₄]⁻. The effect of reducing agent concentration on the nanoparticle formation was determined by using UV-Vis spectroscopy and DLS techniques. Several steps were observed in the reaction mechanism. Step wise
mechanism includes the bimolecular reduction of Au (III) complex ions followed by autocatalytic reduction of Au (I) ions. Formation of metallic gold activated the AuNPs formation by nucleation and growth. Values of rate constants were determined by using the kinetic data and modified Finke–Watzky model. The autocatalytic reduction was confirmed by TEM and DLS studies. Rate law was also determined for the formation of gold nanoparticles [90].

Arshi et al. synthesized AuNPs by one step microwave irradiation method. Citric acid and cetyl-trimethyl ammonium bromide were used as reducing and binding agents respectively. Two types of AuNPs were produced by using two irradiation times in the reaction i.e. 40 and 70 seconds. UV-Vis spectroscopy and TEM were used for the characterization of synthesized AuNPs. Absorption maxima from UV-Vis studies were found to be at 590 nm for 40s and 560 nm for 70s gold nanoparticles. Shape was observed to be spherical with particle size distribution in the range of 1–10 nm for 40s and 1–2 nm for 70s time. The effect of concentration on the antibacterial activity of AuNPs was observed against *E. coli*. Antibacterial activity of the two nanoparticles was quite high about 22mm zone of inhibition. Antibacterial activity was almost similar for two kinds of nanoparticles but was slightly better for gold nanoparticle synthesized at 70s irradiation time [91].

Rudolf et al. synthesized organometallic Fe complex. The disulfide group of metallo carbonylsuccinimide complex was responsible for covalent binding with noble metals. This complex was adsorbed on the surface of AuNPs of different sizes. Different characterization techniques including IR, UV-Vis spectroscopy, TEM, DLS and ICP-MS were used to determine the properties of AuNP-metallo carbonyl conjugates. IR analysis revealed a surface enhancement IR absorption effect of AuNP-metallo carbonyl conjugates[92].

Mandal et al. developed multi-functionalized gold nanoparticles and coated them with multilayers of boron phenylalanine, fluorescent dye and folic acid. The functionalized AuNPs were used to deliver boron to cancer cells. Uptake of NPs
in cancer cell and its tracking upto cellular level was demonstrated by in vitro confocal fluorescence microscopy [93].

Fayaz et al. described biosynthesis of gold and silver NPs by exposing the cell free extract of *Geobacillus stearothermophilus* to the metal salts in solution. Different characterization techniques including FTIR, UV-Vis spectroscopy, XRD and TEM were used to study the properties of nanoparticles. The absorption maximum of gold nanoparticles was 522 nm and that of silver was 423 nm. The gold nanoparticles were observed to be mono dispered and that of silver were poly dispersed as revealed by TEM. The bacterium present in the reaction mixture secreted certain proteins. SDS PAGE and FTIR confirmed the presence of these proteins in the reaction mixture. These proteins were responsible for the high stability of NPs [94].

Berzina et al. synthesized and analyzed the structure and electrical properties of AuNP polyaniline composite materials. Nanoparticles obtained were of different sizes in the range of 5–10 nm depending on different stabilizing agents used. With mixture of AuNPs stabilized by 2-mercaptoethanesulfonic acid and dodecyl benzene sulfonic acid were used for polymerization of anilinium salt. The obtained composite material revealed the best results. Schottky barriers were observed between gold particles and polyaniline and were demonstrated by electrical properties. Because of this property these composite materials could be applied in bio-inspired complex information handling materials [95].

Chu et al. modified the surface of gold nanoparticles with luminescent polymers and studied the morphological behavior of the particles by TEM and photoluminescence quenching. Polymer and AuNPs were hydrogen bonded with polymer acting as H-acceptor and AuNPs were H-donor. The degree of dipole-dipole interaction and hydrogen bonding was different due to different electron donating abilities of Me and MeO substituents present on the side chains of polymers (PBOT1–PBOT3) and (PBT1–PBT3). This was demonstrated by the addition of AuNPs contained in acid (AuSCOOH) and acid-free (AuSC10)
surfactants. The nano-composite obtained from PBOT1 and AuSCOOH exhibited highest $K_{SV}$ (Stern–Volmer quenching constant). $K_{SV}$ values were predicted from the fittable exponential equations developed by titration of NP quenchers with other fluorescent polymers having pyridyl-conjugated units in different ratios. These observations were also confirmed by the TEM images [96].

Gupta et al. reported the synthesis of gold nanoparticles capped with 16-Mercaptohexadecanoic acid by single phase synthesis and their control on silicon surfaces. Absence of unreacted thiol in AuNPs was confirmed by FTIR and XPS. XPS confirmed the presence of AuNPs on silicon surface after immobilized and HR-TEM confirmed the attachment of AuNPs to Si surface. These studies could be used for further nano-structuring [97].

Ara et al. synthesized stable AuNPs with soluble starch as stabilizing agent as well as reducing agent for 5h at 40°C. UV-Vis, TEM and z-scan techniques were used to characterize AuNPs. The size of nanoparticles was determined by TEM and was ranged between 12–22 nm. The surface plasmon resonance was confirmed by optical properties. Continuous wave He–Ne single laser beam technique was used to study the non-linear optical properties. Laser beam was used at three different incident intensities at a wavelength of 632.8 nm. Close holes-scan studies exposed the non-linear refractive indices of AuNPs to be in order of $10^{-7}$ cm$^2$/W. Comparison was made with diffraction patterns detected in far-field. Open hole z-scan studies exposed the non-linear absorption of AuNPs in order of $10^{-1}$ cm/W[98].

Kumar et al. reported biosynthesis of AuNPs using green technique. Reducing and stabilizing agent used was the aqueous extract of *Terminalia chebula*. UV-VIS spectroscopy revealed the surface plasmon resonance at 535 nm. The size of anisotropic AuNPs was found to be in range of 6 to 60 nm as observed from TEM studies. The main component responsible for reduction and stabilizing of AuNPs in the aqueous extract of *T. chebula* were hydrolysable tannins. Standard well diffusion method used to check the antimicrobial activities of AuNPs...
demonstrated that activity toward gram positive \textit{S. aureus} was much better than the gram negative \textit{E. coli} [99].

Ali et al. biosynthesized gold and silver nanoparticles by reduction of \( \text{HAuCl}_4 \) and \( \text{AgNO}_3 \) using the extract of \textit{Mentha piperita}. \( \text{HAuCl}_4 \) and \( \text{AgNO}_3 \) were mixed with extract and incubated. The resultant nanoparticles were studied using UV-Vis spectroscopy, SEM equipped with EDS and FTIR. The size of gold and silver nanoparticles was 150 nm and 90 nm respectively. Spherical shape was observed in case of silver nanoparticles. Activities of synthesized nanoparticles were very high for \textit{Escherichia coli} and \textit{Staphylococcus aureus}[100].

Kumar et al. reported biosynthesis of AuNPs using aqueous extract of \textit{Cassia auriculata} leaves. Gold nanoparticles were formed by reduction of \( \text{AuCl}_3 \) at room temperature within 10 min and the reaction rate was much higher compared to other chemical methods. UV-Vis spectroscopy, XRD, SEM-EDAX, TEM and FTIR were used for the characterization of AuNPs. Nanoparticles synthesized with \textit{C. auriculata} were stable, ranged from 15-25 nm with triangular and spherical crystalline geometry. The effect of pH on the stability of gold nanoparticles was also checked. The plant used for reducing and stabilizing of AuNPs was an anti-diabetic plant and the resulting nanoparticle might help to promote anti-hyperglycemic activity upon testing [101].

Philip et al. synthesized silver and gold nanoparticles using leaf extracts of \textit{Murraya Koenigii} as reducing and stabilizing agent. Reaction was carried out at ambient conditions for silver nanoparticles and at 373 K for gold nanoparticles. UV-Vis spectroscopy, FTIR, XRD and TEM were used to characterize the synthesized nanoparticles. The synthesis of silver nanoparticles was completed within 5 min at room temperature and the size of resultant NPs was about 10 nm with SPR band at 411 nm. Gold nanoparticles were synthesized by mixing extract to the boiling solutions of \( \text{HAuCl}_4 \). AuNPs synthesized were spherical in shape with a size of about 20 nm and SPR at 532 nm. TEM, XRD and SAED confirmed
the crystalline morphology of nanoparticles. FTIR exposed that capping molecules in silver nanoparticles are different from gold nanoparticles [102].

Chili et al. used ultraviolet irradiation technique to synthesize multi twinned gold nanoparticles. Polyvinyl pyrrolidone was used for the reduction of gold salt [103].

Gupta et al. synthesized gold nanoparticles capped with short chain thiol (4-aminothiophenol) and functionalized with amine-termination. The effect of reaction conditions and dispersion medium was studied on the morphology of the particles. Anhydride capping agent was used to stabilize the nanoparticles subjected to aggregation due to amine-amine hydrogen bonding. SiO₂ substrate was used for immobilization of stabilized nanoparticles through covalent bonding. The absence of unreacted thiol and binding of nanoparticles on silicon surfaces was confirmed by XPS studies. Particle morphology was studied by using UV-Vis spectroscopy and TEM. Binding of particles to Si surfaces was also confirmed by cross-sectional HR-TEM images [104].

Bahadur et al., synthesized silica coated gold nanoparticles and functionalized the surface of the synthesized particles with different groups. Citrate reduction method was applied to prepare AuNPs having mean size of 16 nm. Microwave irradiation of the prepared gold solution with ammonia and tetra-ethoxyxilane resulted in silica coating within 5 min. The advantages of the reported method over other conventional methods were size uniformity, mono-dispersity and short time reaction without any pre-coating step. By changing the concentration of tetra-ethoxyxilane, particles were prepared with different coating thickness ranging from 5-105 nm. DLS and TEM confirmed the size uniformity and mono-dispersity of the particles. The synthesized silica coated gold nanoparticles were functionalized with different functional groups including alkyl, carboxylate and amino groups which enhanced their use in many applications. Zeta potential measurements and XPS identified the functional groups on the surface of nanoparticles [105].
Aromal et al. reported the synthesis of spherical AuNPs in the size range of 14–17 nm using aqueous extract of *Macrotyloma uniflorum*. Synthesis of AuNPs was studied under varied experimental conditions by changing pH, temperature and quantity of extract. Different characterization techniques including UV-Vis spectroscopy, FTIR, XRD and TEM were used to study the prepared AuNPs. Nanoparticles were highly crystalline having face-centered cubic structure was confirmed by XRD, SAED patterns and HRTEM images. Different functional groups were present in the bio-molecule capping the AuNPs was confirmed by FTIR spectrum [106].

### 2.6.2 Review on Silver Nanoparticles

Guidelli et al. synthesized silver nanoparticles by thermally treating aqueous solution of Ag with dl-alanine used as reducing and capping agent. The resultant nanoparticles were spherical in shape having an average size of 7.5 nm with a narrow size distribution and arranged in face-centered cubic crystalline structure. Silver ions on the surface of NPs were reduced by the amine group of dl-alanine and resulted in the stability of colloid. The resultant nano-composites were used as ESR dosimeter. Tissue equivalency was not lost considerably with the concentration of silver NPs in composites. The bio-hybrid composite containing nanoparticles had lower energetic dependence and higher sensitivity compared to pure dl-alanine lead to the manufacture of small-sized dosimeters [107].

Ghaseminezhadet al. synthesized silver nanoparticles by adding fungal supernatant to the mixture of AgNO₃ and starch solution. Characteristics of nanoparticles prepared by the reported novel green method were compared to the nanoparticles prepared by modified polysaccharide method and microbial method. Average sizes of the AgNPs prepared by the reported method were found to be 15 nm compared to 20 and 84 nm prepared by modified
polysaccharide method and microbial method. The size was confirmed by DLS technique. Nanoparticles synthesized by the reported method were the most stable. High crystallinity of the prepared AgNPs was confirmed by XRD spectrum. Presence of the functional groups was confirmed by FTIR and it was revealed that there were a combination of the functional groups present in nanoparticles synthesized by the other two methods[108].

Abdel-Halim and Al-Deyab synthesized silver nanoparticles by a green method using hydroxy-propyl cellulose samples. Alkalization of cellulose followed by etherification with propylene oxide led to the formation of hydroxy-propyl cellulose samples with different molar substituents. Effect of propylene oxide concentration, alkali concentration and duration and temperature of etherification reaction on the formation of hydroxy-propyl cellulose were studied extensively. Water solubility studies showed that samples with 0.4 or higher molar substitution were completely soluble. The prepared samples were used to reduce AgNO₃ solution in synthesis of AgNPs. UV-Vis spectra confirmed that at pH 12.5, full reduction of Ag⁺ to AgNPs was accomplished. Reaction conditions were optimized and maximum yield was obtained by utilizing 0.3% hydroxy-propyl cellulose solution having 0.42 molar solution for reduction at 90 °C for 90 min [109].

Kouvaris et al. reported a green method for synthesis of AgNPs using leaf broth of Arbutus unedo. Leaf broth was used as reducing and stabilizing agent simultaneously. Surface functionalized AgNPs were prepared by exposing AgNO₃ solution to leaf broth. Single crystalline nature having narrow size distribution was revealed by different characterization techniques. The resultant discrete AgNPs were stable over long periods of time and the stability was due to coating of particles with the organic leaf extract leading to the formation of small aggregates. The synthesized AgNPs were found to be very appropriate for many applications in biotechnology and coating procedures [110].
Wei et al. synthesized AgNPs by solar irradiation method using *Bacillus amyloliquefaciens* leaf extract which was mixed with silver nitrate solution and irradiated. Synthesis of nanoparticles was affected by different conditions including extract concentration, light intensity and addition of sodium chloride. Reaction conditions were optimized and reduction of 98.23 ± 0.06% of 1 mM silver ions to nanoparticles was accomplished in 80 min with 3 mg/mL extracts concentration, 70,000 lx solar intensity and 2 mM sodium chloride content. The ζ-potential of synthesized nanoparticles reached −70.84 ± 0.66 mV under optimum conditions. Prepared nanoparticles were found to be circular and triangular crystalline having an average diameter of 14.6 nm as confirmed by TEM and XRD. Enzymatic reactions were considered not to be involved due to involvement of heat-inactivated extracts in the formation of nanoparticles. High absolute ζ-potential value was responsible for the stability and was considered to be due to interaction with proteins present in extracts. Synthesized nanoparticles were found to be active against *Escherichia coli* and *Bacillus subtilis* in liquid and solid medium[111].

Vasileva et al. reported a green synthetic method for the synthesis of AgNPs having mean diameter of 14.4 ± 3.3 nm. Ultrasound technique was used for the reduction of AgNO₃ in the presence of d-glucose. The starch was used for the stabilization of AgNPs. AgNPs were characterized by different techniques including UV-Vis spectroscopy, XRD, TEM, differential scanning calorimetry and thermo gravimetric/differential thermal analysis. The synthesized AgNPs had catalytic activity in the reduction of H₂O₂. Due to degradation of AgNPs in the said reduction, absorbance strength of localized surface plasmon resonance band observed was changed and the change was dependent upon the concentration of H₂O₂. An improvised plasmon resonance-based optical sensor was characterized and calibrated with good sensitivity and linear response over a wide range of concentrations, i.e. 10⁻¹–10⁻⁶ mol/L H₂O₂. The quantification limit of sensor was lower than many enzyme based biosensors and was found to be 0.9 μM H₂O₂.
Due to lower quantification limit, the optical sensor for H$_2$O$_2$ could also be used in determination of many other reactive oxygen species[112].

Abdel-Mohsen et al. reported the synthesis of hyaluronan fibers containing AgNPs by an eco-friendly chemical method. Hyaluronan fibers were prepared by wet spinning method from a transparent solution of aqueous solution of NaOH containing dissolved hyaluronic acid. Fibers having AgNPs were also prepared in the same method. Effect of different parameters was studied upon the fibers containing AgNPs i.e. concentration of AgNO$_3$ and hyaluronan fiber, temperature, duration of reaction and pH of the mixture. The presence of AgNPs in fibers was confirmed by different techniques including TEM, UV-Vis spectroscopy, XRD, SEM, ICP OES and 2D SWAXS. Mechanical properties of the fibers containing AgNPs were measured[113].

Shukla et al. reported the use of agar, extracted from the red alga Gracilaria dura for the synthesis of silver nanoparticles and nanocomposite material. UV-VIS spectroscopy, TEM, SAED, EDX and XRD analysis were used to characterize AgNPs. Agar/silver nanocomposites were found to be thermally stable as compared to extracted agar and silver nitrate as determined by DSC and TGA studies. The synthesized AgNPs were found to be spherical, with a size of 6 nm and uniformly dispersed as revealed by TEM analysis. Prepared AgNPs destroy 99.9% bacteria when compared to control value, thus exhibiting a greater bactericidal activity. Increased temperature reduced the time required for the synthesis of AgNPs. Crystalline index (Cl$_{DSC}$ 0.73) was confirmed by XRD and DSC studies [114].

Quang et al. reported the use of silica beads for the synthesis of AgNPs having size in the range of 0.5mm -1 mm. Sodium borohydride was used for the reduction of silver ions contained within the pores of silica beads. The effect of pore size and porous structure on AgNPs synthesis was determined by using silica beads with different pore diameters in the range of 3.8-20 nm. Increase of pore volume was observed to increase the amount of silver contained inside the
pores. The particle size was dependent upon the pore size and porous structure and was mainly in the range of 8-15 nm [115].

Rani and Rajasekharreddy synthesized silver-protein (core–shell) nanoparticles by an eco-friendly method using leaf extract of the betle, *Piper betle* L. (Piperaceae) as reducing agent. The size of AgNPs and shell thickness on the AgNPs surface was controlled mainly by the exposure time. Synthesized AgNPs were revealed to be spherical in shape from TEM studies and the shell size was confirmed by increased intensity of fluorescence emission peak. Crystalline structure was confirmed by XRD and SAED results. Stability of the particles was increased by the protein anchored to silver surfaces. The presence of protein on the surface of nanoparticles was confirmed by XPS and FTIR analysis. Acute toxicity of biosynthesized AgNPs against aquatic organism *Daphnia magna* were evaluated and compared to that of chemically synthesized silver nanoparticles. The toxicity of biosynthesized nanoparticles were less than that of chemically synthesized nanoparticle. Protein shell on the silver core was thought to decrease the toxicity of biosynthesized nanoparticles. Biogenic AgNPs were more superior and environment friendly due to smaller toxicity to non-target organisms[116].

Shivaji et al. synthesized silver nanoparticles using cell-free culture supernatants of psychrophilic bacteria i.e. *Pseudomonas meridiana*, *Pseudomonas proteolytica*, *Pseudomonas Antarctica*, *Arthrobacter gangotriensis* *Arthrobacter kerguelensis* and two mesophilic bacteria i.e. *Bacillus cecembensis* and *Bacillus indicus*. UV-Visible spectroscopy, AFM and TEM techniques were used to characterize synthesized AgNPs. The synthesized AgNPs were stable in dark for eight months and had sizes in the range of 6-13 nm. Effect of pH, temperature and bacterial species used for synthesis was evaluated on the formation and stability of silver nanoparticles. The effect of cell-free culture supernatants was thus found to be varying from one bacterial specie to other. The synthesized silver nanoparticles were found to be bactericidal. Reported work demonstrated
the use of culture supernatants of psychrophilic bacteria and genus Arthrobacter for the first time in the synthesis of silver nanoparticles [117].

Bhatte et al. reported an environmental friendly method for the synthesis of AgNPs in powder form. Hydrogen was used for the reduction of aqueous solution of AgNO₃ in the presence of PVA as stabilizer. PVA is fully biodegradable with very low cytotoxicity which makes the method eco-friendly. UV-Vis spectroscopy, XRD, TEM, EDAX and DLS techniques were used to characterize the nanoparticles. Activities of synthesized AgNPs as recyclable catalyst were studied in the formation of different enaminones [118].

Abou-Okeil et al. synthesizes silver nanoparticles using amine containing β-cyclodextrin for reduction of silver nitrate and stabilization of the resultant AgNPs. 2-chloroethylamine was reacted with cyclodextrin under different conditions (temperature, time, concentration of 2-chloroethylamine and sodium hydroxide) to synthesize Aminated β-cyclodextrin. Synthesis of AgNPs was studied under different factors including time, temperature, pH, concentration of aminated β-cyclodextrin and extent of amination. TEM and UV-Vis spectroscopy was used for characterization of AgNPs. Reaction conditions were optimized and it was observed that AgNPs having size range of 1-9 nm could be synthesized by reacting 0.1 M AgNO₃ with 0.6 g β-cyclodextrin derivative at pH 12 and carrying out the reaction for 20 min at 70 °C [119].

Tuan et al. reported sono-electrodeposition method for synthesis silver nanoparticles colloid using silver plate as a source of silver ions. The size of synthesized nanoparticles ranged from 4 to 30 nm and were dispersed in non-toxic solution. Coconut hust was used to produce activated carbon on which silver nanoparticles were loaded. The best activated carbon had a surface area of 890 m²/g. Morphology and methylene blue adsorption ability of the activated carbon was not affected by the presence of AgNPs. Activated carbon loaded with AgNP was very active against bacteria Escherichia coli with a strong As (V) adsorption and minimal inhibitory concentration of 16 μg/ml. The synthesized
materials were found to have many applications in treatment and prevention of microbial infections and also in treatment of environmental contaminations [120].

Gunawidjaja et al. combined AgNPs with Eu: Lu$_2$O$_3$ by a core-shell approach. Eu: Lu$_2$O$_3$ acted as the phosphor shell component while metal core component was AgNPs. Metallic NP core was separated from the phosphor shell by an optically transparent SiO$_2$ layer. The interaction between core and shell was varied by varying the thickness of the SiO$_2$ layer, normally in the range of nanometers. Besides spherical AgNPs, wavelength-tunable plasmonic Ag nanoplates were also used as core component. Core-shell NPs were embedded into a transparent polymeric matrix to fabricate nano-composite phosphor. Reported design was used as a framework for the construction of low cost scintillator composed of nano-composites [121].

Rastogi and Arunachalam synthesized highly stable AgNPs by a green synthetic route using aqueous extract of garlic. $0.1 \text{ M } [\text{Ag(NH}_3\text{)}_2]^+$ was mixed with aqueous extract and exposed to bright sunlight for 15 min. Sunlight served as catalyst while extract was used as reducing and capping agent in the preparation of AgNPs. UV-Visible spectroscopy, FTIR, GA-XRD and TEM were used for the characterization of AgNPs. The size of synthesized nanoparticles were found to be $7.3 \pm 4.4$ nm, having spherical shape and poly-dispersed. Presence of protein as capping agents was confirmed by FTIR studies. AgNPs were synthesized in a good yield of around 80% by dry weight and 85% ICP-AES method. Antibacterial activities of AgNPs were determined by well diffusion assay and were found to be active against both gram positive and gram negative bacteria. Colloidal solutions containing AgNPs were stable and retained their bactericidal property for very long period of time usually more than a year [122].

Lah and Johan reported a simple chemical reduction method for the synthesis of anisotropic mono-dispersed AgNPs using Daxad 19 surfactant. The shape of AgNPs was affected by temperature and weight ratios of the reactants. Under
controlled temperature, increasing weight ratios of both AgNO₃ and Daxad 19 enhanced the reduction of silver ions to silver nanoparticles. Resultant AgNPs were uniform in shapes, sizes and were well-dispersed; however the dimensions could be controlled easily. The results of UV-Vis spectra were compared to that obtained from Mie’s Scattering Theory. The optical behavior of the experimental UV-Vis spectra was confirmed by the simulated spectrum [123].

Li et al. reported a process analogous to silver mirror reaction to synthesize colloidal AgNPs without the addition of Tollens reagent. C₁₉H₄₂BrN was used as alternative to NH₃ to react with silver ions. UV-Vis spectroscopy, TEM, FTIR and XRD techniques were used to characterize the synthesized AgNPs and the reaction conditions. Concentration of Ag in colloidal AgNPs was as high as 0.05 mol/L. The colloidal solution was found to be stable for more than 45 days. The bromine present in C₁₉H₄₂BrN acted as surfactant counter ion and resulted in the formation of C₁₉H₄₂NBr⁻Ag⁺ complex. The complex was reduced to AgNPs using glucose as reducing agent. The effect of molar ratios of C₁₉H₄₂NBr to Ag on the shape and dispersion of AgNPs were also studied[124].

Zahir and Rahuman reported the synthesis of AgNPs using aqueous leaf extracts of *Euphorbia prostrata* Ait. (Euphorbiaceae) against the haematophagous fly *Hippobosca maculata* Leach (Diptera: Hippoboscidae) and the adult cattle *Haemaphysalis bispinosa* Neumann (Acarina: Ixodidae). Anitparasitic activities were also determined for chloroform, hexane, acetone, methanol, ethyl acetate and leaf extract. Silver nanoparticles were characterized using UV-Visible spectroscopy, FTIR, XRD and SEM techniques. Parasites were exposed to plant extracts of different concentrations and AgNPs for one day. Toxic effects were observed on the parasites for all extracts with maximum mortality in chloroform, hexane, acetone, methanol, ethyl acetate and leaf extracts of *E. prostrata* and nanoparticles synthesized against *H. maculata* and *H. bispinosa*. Silver nanoparticles showed 100% mortality at a concentration of 10 mg l⁻¹. UV-Visible spectrograph was recorded for the colloidal nanoparticle solution against time.
Leaf extracts of *E. prostrata* revealed maximum absorption at 420 nm after 6 h. FTIR confirmed the formation of AgNPs. SEM revealed the average size of AgNPs to be 52.4 nm having rod shaped structures. GC–MS analysis of the aqueous leaf extract confirmed the presence of 2-phenylethanol. It was suggested that the synthesized AgNPs, leaf extracts of *E. prostrata* and leaf methanol could be used for the control of *H. maculata* and *H. bispinosa*. Toxicological effects of nanoparticles were determined with no toxicity observed on *Ceriodaphnia dubia*, *Daphnia magna* and animals against *Bos indicus* after 24-h exposure[125].

Kawai et al. described the synthesis of a new water soluble stabilizer, 4-diazoniumcarboxylbenzene fluoroborate, for the formation of AgNPs. Sodium borohydride was used for the reduction of AgNO₃ and diazonium salt to produce stable dispersion of nanoparticles. Synthesized AgNPs were characterized using UV-visible spectroscopy, XRD, TEM and XPS techniques. It was observed that nanoparticles were stabilized by Ag–C σ-bonds. Antimicrobial activities of the synthesized AgNPs were determined against *Staphylococcus aureus* and were found to be very active against the said bacteria [126].

Hebeish et al. reported the use of hydroxypropyl starch as reducing and stabilizing agent in the synthesis of AgNPs having size of 6 to 8 nm with 500 ppm concentration. The resultant nanoparticle solution was diluted to 50 and 100 ppm with distilled water and applied to cotton fabrics in the absence and presence of binder. SEM was used to study the morphological behavior and surface properties of the resultant fabrics and the presence of AgNPs on the fabric surface was confirmed. Antibacterial activity of resultant fabric was studied against gram positive *Staphylococcus aureus* and gram negative *Escherichia coli*. It was revealed that bacterial concentration was reduced by more than 95% without washing in the presence or absence of binder and irrespective of the nanoparticle concentration. However binder fixed the nanoparticles onto the fabric surface and it was confirmed by the preservation of
antibacterial properties of the fabrics in presence of binder even after twenty washing cycles [127].

Krishnaraj et al. studied the effect of biologically synthesized AgNPs on growth metabolism of hydroponically grown *Bacopa monnieri* (Linn.) Wettst. Plants grown in hydroponic solution were analyzed for total phenols, carbohydrates, proteins, antioxidant enzymes, peroxidise and catalase. Aqueous extracts of *Acalypha indica* Linn. Leaf was used for the reduction of silver nitrate solution for the synthesis of AgNPs. AgNO₃ induced the stress conditions due to increased activity of catalase and peroxidase. SEM analysis was used to study the morphological behavior of the plants and it was revealed that AgNPs treated plants did not show any severe toxic effects. Light microscopic evaluations revealed structural aberrations in stem and root anatomy. AAS technique confirmed the presence of silver in stem and root tissue of the plants [128].

De Matos et al. synthesized AgNPs by irradiating a solution of AgNO₃ and *Euphorbia mili*. Xenon lamp light was used for irradiation followed by ultra-short laser pulses. No additive like reducing agents, surfactants or solvents were used in this method. TEM and UV-visible spectroscopy confirmed the synthesis of AgNPs. Irradiation with xenon lamp was used to synthesize AgNPs and by laser pulses were used to reduce the size of AgNPs to 10–50 nm. The effect of concentration of AgNO₃, concentration of leaf extract, time and energy of laser irradiation upon the formation and size of AgNPs were studied. The reduction in size of nanoparticles due to laser irradiation was explained on the basis of particles breaking due to multi-photon process which promoted the photo-excitation of the surface plasmon band leading to charge accumulation [129].

Manivel and Anandan reported the synthesis of AgNPs coated with silica by an eco-friendly method using N-[3-(trimethoxysilyl)propyl]-ethylenediamine as reducing and stabilizing agent and also described the interaction of the synthesized AgNPs with human serum albumin (HSA) and bovine serum albumin (BSA). UV-visible spectroscopy, STEM, EDX and TEM were used to characterize
Chapter 2  Introduction to nanotechnology

the synthesized silica coated AgNPs. The binding between serum albumins and silica coated AgNPs were studied using UV-visible and fluorescence studies. Benesi–Hildebrand equation was used to calculate the association constant of HSA \((1.63 \times 10^4 \text{ M}^{-1})\) and BSA \((3.23 \times 10^4 \text{ M}^{-1})\) from the changes in absorption bands of serum albumin in the presence of silica coated AgNPs. Fluorescence of serum albumins was quenched by the addition of silica coated AgNPs. The fluorescence quenching data was used to measure the quenching rate constant \((k_q)\), Volmer constant \((K_{SV})\), the apparent binding constant and the number of binding sites. Quenching process was suggested to be dynamic in nature from the reduction in life time as studied from fluorescence decay analysis of albumins in the presence of AgNPs. Circular dichroism spectroscopy was used to study the conformational changes in protein due to AgNPs [130].

Vidhu et al. described the green synthesis of AgNPs using aqueous extract of \textit{Macrotyloma uniflorum} seed. The synthesis of AgNPs was studied under different conditions like pH, temperature and amount of extract. Different characterization techniques including UV-Visible, TEM, FTIR and XRD were used to study properties of AgNPs. UV-Visible spectra and appearance of brown color confirmed the synthesis of AgNPs. Face centered cubic structure was revealed from XRD studies. TEM analysis revealed the size of particle to be about 12 nm with anisotropic morphology and well-dispersed in nature. Different functional groups in the capping AgNPs was confirmed by FTIR [131].

Chinnapongse et al., described the synthesis of silver nanoparticles capped with citrate and 20 nm in size and investigated their persistence in synthetic aquatic media and natural fresh water by determining their colloidal stability. Colloidal stability was determined by using UV-Visible spectroscopy, AFM and DLS analysis. Stability was determined for different sources of water including pond water, sea water, hard water, water with natural organic matter and synthetic sea water. In seawater and other waters containing higher than 20 mmol L\(^{-1}\) NaCl, singly dispersed nanoparticles were unstable with almost zero optical
absorbance in the first 10 h of mixing. The disappearance rate of singly dispersed nanoparticles was tested on the basis of rate of aggregation as a function of natural organic matter and water chemistry. Stability was maintained in other waters having lower quantity of natural organic matter and salinity upto 48 h. It was revealed that fresh water could sustain nanoparticles to enter successfully in marine and estuarine systems. The study predicted the potential risk of soil and aquatic section to exposure of citrate capped silver nanoparticles and could be able to design nano-EHS risk assessment experiments [132].

Guzman et al. demonstrated the synthesis of silver nanoparticle by reducing aqueous AgNO$_3$ solution using a mixture of sodium citrate and hydrazine hydrate as reducing agent and sodium dodecyl sulfate as a stabilizing agent. The size of individual particles was observed to be in the range of 10-20 nm, however they were found in the form of agglomerates with a size in the range of 40-60 nm. Kirby-Bauer method was used to determine the antibacterial activity of the synthesized AgNPs and was found active against Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli. Preliminary results of the minimal inhibitory concentration using standard dilution micro-method were also reported [133].

Darroudi et al. reported the synthesis of colloidal AgNPs by a sono-chemical method using aqueous gelatin solution as an eco-friendly stabilizing agent. The effect of different reaction parameters including concentration of silver ions, reducing agent, ultrasonic amplitude and ultrasonic time was studied on the size of nanoparticles. Particle size increased with ultrasonic time and decreased with amplitude. Synthesized nanoparticles were 3.5 nm in size, spherical shaped and well-dispersed. The method was suggested to be applied to other noble metals including Pd, Au and Pt and in many industrial, medicinal and technological processes [134].

Dipankar and Murugan described the synthesis and characterization of AgNPs using Iresine herbstii and determined their cytotoxic, antioxidant and
antibacterial activity. The reaction mixture was incubated for 7 days and showed maximum absorption at 460 nm. Poly dispersed pure AgNPs having size of 44 to 64 nm was confirmed by SEM and EDX studies. Cubic and face centered cubic crystals were indicated by XRD analysis. Capping of AgNPs with plant materials was demonstrated by FTIR. Biosynthesized AgNPs were found active against human pathogenic bacteria and phyto-synthesized AgNPs were showed antioxidant as well as cytotoxic activity against Hela cervical cell lines. Due to cytotoxic, antioxidant and antibacterial activities of synthesized AgNPs, they could be used in many clinical applications[135].

Chmielewska and Sartowska described the synthesis of cotton nanocomposites containing AgNPs in its matrix. AgNPs were synthesized in cotton structures by electron beam irradiation. The effect of concentration of Ag salt on the synthesized Ag nanostructures was studied. EDS and SEM-BSE techniques were used for the characterization of nano-composites. The effect of AgNPs size and irradiation upon the thermal properties was carried out using DSC and TGA analysis. Antimicrobial activities of the silver-cotton nanostructures were studied[136].

El-Shishtawy et al. synthesized stable aqueous dispersion of AgNPs in water/micelles system containing glucose and cetyltrimethylammonium bromide as reducing and stabilizing agents respectively. The effect of different reaction conditions including concentration of glucose, NaOH, AgNO₃ and cetyltrimethylammonium bromide, and the reaction time on the evolution of AgNPs plasmon band was determined. Synthesized AgNPs were characterized using different techniques including FTIR, TEM and UV-Visible spectroscopy. It was revealed that the obtained aqueous AgNPs dispersion was quite stable with less than 30 nm diameter and well controlled shaped by the described simple procedure[137].
CHAPTER-3

METAL NANOPARTICLES

STABILIZED

WITH AMOXICILLIN
3.0 MATERIALS AND MEASUREMENTS

Tetrachloroauric acid trihydrated, yellow (HAuCl$_4$.3H$_2$O) purchased from Merck, sodium borohydride (NaBH$_4$) from Fisher chemicals UK and amoxicillin from local pharmaceutical company. Doubly distilled water was used throughout experiments for the preparation of gold nanoparticles and further analysis. UV-vis spectra were measured in the same solvent (water). Photoluminescence spectra were taken on Perkin-Elmer spectrophotometer in the same solvent. FT-IR spectra were recorded on 8900 shimadzu HYPER, using KBr pellets of gold nanoparticles. AFM and TEM images were recorded.

3.1 EXPERIMENTAL

3.1.1 Synthesis of gold nanoparticles stabilized with Sodium salt of amoxicillin

Gold nanoparticles (AuNPs) were prepared using a classical method known as Turkevich Method[24]. Amoxicillin was used as a stabilizer to synthesize gold nanoparticles. For the preparation of gold nanoparticles (Au-NPs) the following conditions were used; an amount of 160 µL (varied for various reactions) of tetrachloroauric (III) acid (from 50 mM stock solution of HAuCl$_4$.3H$_2$O) and the desired amount of amoxicillin (from 50 mM stock suspension of amoxicillin) sonicated well, before use. Reaction mixture was kept on stirring for 1h. 80 µL were added (varied for various reactions) of sodium borohydride (freshly prepared 880 mM solution of NaBH$_4$) at once with vigorous stirring.

Following the addition of reducing agent the color of the reaction mixture changed to brown or dark brown and with the passage of time turned to grayish purple depending upon the ratios of Au (III), amoxicillin and NaBH$_4$. By the addition of NaBH$_4$, sodium salt of amoxicillin formed during the reaction and...
thus the resulting nanoparticles were gold nanoparticles stabilized with sodium salt of amoxicillin. Reaction of different ratios were checked out for complete optimization, i.e. 1:1, 2:1, 3:1.......20:1 etc. The resulting mixtures were stirred for 3h at room temperature and UV-vis spectra were recorded for the mixture. The gold nanoparticles (Au-NPs) were isolated by freeze drying the reaction mixtures. The solid Au-NPs were collected for FT-IR, and TEM studies.

3.1.2 Gold nanoparticles formation using different protocols

Reaction with optimized ratio for the synthesis of gold nanoparticles were then applied to check different protocols/conditions for synthesizing nanoparticles with best results i.e. synthesis of AuNPs at room temperature, high temperature, cold state and sonication etc.

3.1.2.1 Gold nanoparticles formation at room temperature

At room temperature (30 °C) best results were obtained with clear solutions. Gold nanoparticles obtained in such conditions were used for further analysis i.e. TEM, IR, NMR etc.

3.1.2.2 Gold nanoparticles formation at moderate temperature

The results of gold nanoparticles formation at higher temperature (60 °C) were not so attractive because precipitates were noticed in all cases.
3.1.2.3 Gold nanoparticles formation using Microwave irradiation

We also tried to prepare gold nanoparticles with microwave radiations but the results were not good and black precipitates were obtained in this case.

3.1.2.4 Gold nanoparticles formation by sonication

Gold nanoparticles were also tried to be prepared by sonication but the results were poor in this case.

3.1.2.5 Gold nanoparticles formation at low temperature

We also tried to prepare gold nanoparticles in the ice cold conditions. It gave good result but was not comparable with the results obtained at room temperature.

Thus it was assumed that the formation of gold nanoparticles at room temperature using Turkevich method gave best results and thus it might be the most suitable method for the formation of gold nanoparticles especially in case of beta-lactams stabilizing gold nanoparticles.

3.1.3 Synthesis of gold nanoparticles stabilized with amoxicillin

Gold nanoparticles (AuNPs) stabilized with amoxicillin were also prepared by classical method known as Turkevich Method with a little modification. Amoxicillin was used as a stabilizer to synthesize the gold nanoparticles. For the preparation of gold nanoparticles (Au-NPs) the following conditions were used; an amount of 300 µL (varied for various reactions) of tetrachloroauric (III) acid (from 50 mM stock solution of HAuCl₄·3H₂O) and the desired amount of
amoxicillin (from 50 mM stock suspension of amoxicillin) were taken in a vial. Reaction mixture was kept on stirring for 1h then added 0.5 to 1mL trimethylamine at once along with vigorous stirring. Stirring was kept continue for 3 hours and then UV spectra were recorded. Reactions with different ratios (Gold: amox) were carried out to check the optimized ratio for AuNPs. The nanoparticles prepared were then separated from solution with the help of centrifuge (12000rpm) which were then used for further analysis.

3.1.4 Synthesis of Silver nanoparticles stabilized with sodium salt of amoxicillin

For the preparation of Ag nanoparticles (Ag-NPs); an amount (varied for various reactions, table 3) of AgNO₃ (from 1mM stock solution of AgNO₃) and the desired amount of amoxicillin (from 1mM stock solution of amoxicillin were prepared by the addition of 1mM solution of NaOH or Na₂CO₃ for complete dissolution), as amoxicillin has low solubility in water. The whole reaction mixture was managed not to be more than 10ml. Reaction mixture was kept on stirring for 30 minutes then 1ml of sodium borohydride (freshly prepared 40 mM solution of NaBH₄) was added at once. After the addition of reducing agent the color of the reaction mixture changed to brown, dark brown, yellowish brown and purple colors, depending upon the ratios of silver and amoxicillin. The resulting mixture was stirred for 4h at room temperature and UV-vis spectra were recorded. The silver nanoparticles (Ag-NPs) were isolated by centrifuge for further studies like characterization and bioassays.

3.1.5 Synthesis of Silver nanoparticles stabilized with amoxicillin

Amoxicillin stabilized silver nanoparticles (AgNPs) were also prepared, for the preparation of Ag nanoparticles (Ag-NPs); an amount (varied for various reactions table 2) of AgNO₃ (from 1mM stock solution of AgNO₃) was taken in
Chapter 3  

Metal nanoparticles stabilized with amoxicillin

vial, and a desired amount of amoxicillin (from 1mM stock solution of amoxicillin, prepared in double distilled water with vigorous stirring), as amoxicillin has low solubility in water. Reaction mixture was kept on stirring for 30 minutes then 1ml of 0.5 mL of trimethylamine was added at once. Following the addition of reducing agent the color of the reaction mixture changed according to the ratios of Silver to amoxicillin. The resulting mixture was stirred for 4h at room temperature and UV-vis spectra were recorded. For fully optimized AgNPs a number of reactions were carried out with different ratios. The silver nanoparticles (Ag-NPs) were isolated by centrifuge. The solid Ag-NPs were collected for FT-IR, TEM and other studies.

3.1.6 Synthesis of nano-alloys stabilized with amoxicillin (AuNPs +AgNPs)

Gold and silver nanoparticles stabilized with amoxicillin were prepared. Reactions of different ratios of AuNPs and AgNPs were carried out by taking a constant amount of AuNPs in different vials, kept on stirring. Then different amounts of AgNPs solution were added. The reaction mixtures were stirred for 30 minutes and then a reducing agent was added i.e. trimethylamine or NaBH₄ reactions were stirred for 3 to 4 hours. Different colors of the solutions were noticed which were due to the different sizes of the nanoparticles because of ratios of AuNPs and AgNPs in the reaction mixtures. After completion of the reaction time, UV spectra were recorded. Same process was repeated again by taking AgNPs constant and varying the amounts of AuNPs. The nano-alloys particles were then separated from the solution through centrifuge at 12000 rpm for TEM and other analysis.
3.2 Results and discussion

The synthesized gold nanoparticles stabilized with amoxicillin (scheme 3.1) were fluorescent and their fluorescence was first checked out and then was utilized for sensing transition metals. Photoluminescence (PL) spectra of the synthesized nanoparticles reduced by NaBH₄, have an excitation peak at 360 nm, exhibit maximum emission around 435-440 nm. After careful observation of reaction conditions it was found that prior to reduction, the mixture of gold and amoxicillin was non-fluorescent (Figure 3.1, line e). But once the gold nanoparticles were formed by the reduction of gold/amoxicillin solution with NaBH₄, these showed improved photoluminescence (PL) (Figure 3.1, line a) with slight blue shift as compared to PL of amoxicillin (Figure 3.1, line c). This blue shifted photoluminescence value was probably due to the PL intensity of sodium salt of amoxicillin (Figure 3.1, line d) which has been decreased after sequestering with Au-NPs. Figure 3.2 shows Fluorescence of Gold nanoparticles.
Figure 3.1: PL spectra of (a) Au-3 NPs (diluted Au-3), (b) Gold (Au (III)), (c) Amoxicillin (amox), (d) Amoxicillin+Sodium Borohydride (Amox-NaBH4) and (e) Gold+amoxicillin (Au-amox).

Figure 3.2: (a) Amoxicillin solution (left), Gold nanoparticles (right) under visible light (b) Amoxicillin solution (left), Gold nanoparticles (right) under UV light
Chapter 3  
*Metal nanoparticles stabilized with amoxicillin*

The gold-amoxicillin stock solution, once incubated at 37 °C with continuous stirring, produces nanoparticles of various sizes without addition of reducing agent which gives precipitates if kept overnight. Peptide linkage, phenolic and amino groups in this case were probably involved in the reduction of Au (III).

As for as fluorescence of Au-NPs was concerned, it was confirmed that the fluorescence at 440 nm was because of the salt of amoxicillin which was formed during reaction. Na\(^+\) of the NaBH\(_4\) or of Na\(_2\)CO\(_3\) combines with amoxicillin and gives its salt which was indicated by its absorbance peak at 350 nm in the UV spectra as shown in **figure 3.3**.

![Absorption spectra confirming the formation of salt of amoxicillin](image)

**Figure 3.3**: Absorption spectra confirming the formation of salt of amoxicillin
3.2.1 Characterization and Optical properties of Au-nanoparticles

Absorbance spectra recorded during synthesis and an absorbance maximum in the range of 520-570 nm which is typically ascribed to the plasmon resonance band of gold nanoparticles was observed. The peak position was an indicative of the particles size and shape (Figure 3.4).

Gold nanoparticles (Au-NPs) stabilized with amoxicillin antibiotic were prepared in aqueous medium using different ratios of gold, ligand and reducing agents as well as at different pH of reaction mixture and various amounts of reaction solvent (water). The resulting gold nanoparticles were stable at room temperature. The effect of various parameters on the size of gold nanoparticles and their stability were studied using uv-visible spectroscopy in initial observations.

Gold nanoparticles obtained from the reaction samples in which the HAuCl₄ was reduced with different amounts of NaBH₄; Table 3.1 shows variations in the absorbance as well as in the wavelength directly affecting the size and shape of the nanoparticles. Under optimum conditions, by increasing NaBH₄ ratio there was a gradual decrease in size uniformity of Au-NPs. Thus it means that for the reduction of HAuCl₄, a particular amount of NaBH₄ may be required. Decrease in absorption of Au-NPs was probably due to incomplete reduction of Au (III), in which less amount of NaBH₄ was added. I also noted that excess amount of NaBH₄ leads to decomposition of gold nanoparticles which was confirmed by the decline in absorption band in UV-vis spectra (Figure 3.4), and also excess of NaBH₄ precipitates out the gold from the solution.
### Table 3.1: Variations in the amount of NaBH₄

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Sample code</th>
<th>NaBH₄(µL)</th>
<th>Au:ligand:NaBH₄ (Molar ratio)</th>
<th>λ_{abs}/ abs_{max}(nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Au-3-40</td>
<td>40</td>
<td>8 : 5 : 35</td>
<td>532/1.640</td>
</tr>
<tr>
<td>2</td>
<td>Au-3-60</td>
<td>60</td>
<td>8 : 5 : 53</td>
<td>536/1.817</td>
</tr>
<tr>
<td>3</td>
<td>Au-3-80</td>
<td>80</td>
<td>8 : 5 : 70</td>
<td>544/1.820</td>
</tr>
<tr>
<td>4</td>
<td>Au-3-100</td>
<td>100</td>
<td>8 : 5 : 88</td>
<td>537/1.734</td>
</tr>
<tr>
<td>5</td>
<td>Au-3-120</td>
<td>120</td>
<td>8 : 5 : 105</td>
<td>537/1.780</td>
</tr>
<tr>
<td>6</td>
<td>Au-3-160</td>
<td>160</td>
<td>8 : 5 : 140</td>
<td>546/1.730</td>
</tr>
</tbody>
</table>

Conditions: HAuCl₄; 50mM, Ligand; 50mM, NaBH₄; 880mM
FTIR spectra of amoxicillin and Au-NPs showed that broadening of peaks in the spectrum of Au-NPs was an indication of gold nanoparticles formation. Another indication from the figure was the clue of the formation of amoxicillin salt, as the peak of carboxylic ketonic group shifted from 1776 cm\(^{-1}\) to 1600 cm\(^{-1}\) and 1400 cm\(^{-1}\) and the intensity was decreased and also broad peak for –OH group of carboxylic acid disappears. Thus the carboxylate group was also involved in the stabilization of gold nanoparticles.
**Figure 3.5:** FTIR spectra of amoxicillin (upper) and AuNPs (lower)
3.2.2 Effect of variation in HAuCl₄ and ligand ratios

Gold nanoparticles obtained from various ratios of ligand and gold show various results; 1.6:1 of gold to ligand ratio gives very good result as shown in Figure 3.6.

![Figure 3.6: UV-vis spectra of Au-NPs with different ligand ratios in 5mL deionized water](image)

3.2.3 Formation of Gold nanoparticles using different protocols

The modified classical Turkevich method was used for the synthesis of gold nanoparticles. However for best results I tried five various synthetic protocols, which include room temperature synthesis, at low temperature (i.e. Synthesis at 5°C, sonication at 35°C, moderate temperature synthesis at 60°C) and microwave
irradiation. Best results were obtained with clear solutions at room temperature (30 °C), which were stable for couple of weeks. Gold nanoparticles obtained in such conditions were used for further analysis i.e. stability check of gold nanoparticles at different parameters, and characterization via SEM, FTIR, UV-vis and photoluminescence spectroscopy.

As shown in figure 3.7, results of gold nanoparticles formation at higher temperature (60 °C) were not noteworthy. Same was the case under microwave irradiation followed by appearance of black precipitates. Sonication gave slight better results but it’s rather time compromising technique. Ice cold conditions, was the second best route for synthesis of Au-NPS using amoxicillin which improved the efficiency as more nanoparticles were formed under cold conditions while synthesis at room temperature gave best results. Therefore it was assumed that the formation of gold nanoparticles at room temperature using Turkevich method was the most suitable, facile and rapid synthetic route for the formation of gold nanoparticles using amoxicillin in the presence of NaBH₄.

![Figure 3.7: Synthesis of AuNPs in different protocols](image-url)
3.2.4 Stability check of gold nanoparticles

Stability of prepared gold nanoparticles was checked by varying the pH of the solution containing gold nanoparticles. In the basic medium (pH=12-13) they were primarily stable for two days (Figure 3.8a) and retained their absorbance for couple of weeks. While changing the pH to acidic medium, their absorbance gradually decreased and microscopic coagulation started appearing at pH 3. This may be due to the protonation of amoxicillin involved in stabilizing Au-NPs, whose carboxylate groups were probably involved in the stabilization of Au-NPs.

![Figure 3.8a: Effect of pH on the stability of amoxicillin stabilized GNPs](image)

Stability of gold nanoparticles was checked with different concentrations of NaCl salt solution. 2M, 3M and 4M salt solution were added to gold nanoparticles solution but no obvious changes were observed even for a week in 2M NaCl solution.
solution (Figure 3.8b). After few weeks, microscopic precipitates were noticed at the bottom of vials which showed that they were stable to salts solution for quite a long time.

Stability of gold nanoparticles was also checked at different temperatures i.e. 50 °C, 60 °C, 70°C and 80 °C. Gold nanoparticles were stable upto 80°C. The gold nanoparticles were heated for 30 minutes at each temperature and the stability was checked by observing absorbance as well as by the formation of precipitates as seen from naked eye. Above 80 °C the gold nanoparticles started coagulation and formed precipitates which showed instability of gold nanoparticles at higher temperature.

Figure 3.8b: Effect of NaCl on stability of GNPs (M =Molar)
3.2.5 Synthesis of Au-NPs using Trimethylamine

To support the idea of utilization of amoxicillin as stabilizing agent for Au-NPs synthesis, I tried trimethylamine as reducing agent. Trimethylamine clearly avoids salt formation because in case of NaBH₄, hydride ion released which acted as a strong base and abstracted hydrogen from the carboxylic group of amoxicillin and sodium salt formation became easy while in case of trimethylamine no sodium salt formation occurred. Absorbance maximum in the range of 520-570 nm was also observed in this case (Figure 3.9) which is typically ascribed to the plasmon resonance region of gold nanoparticles. Varying the Au (III) to amoxicillin ratios lead to different shapes of Au-NPs synthesis.

![UV-vis spectra showing different gold to ligand ratios using triethylamine as reducing agent](image_url)

**Figure 3.9:** UV-vis spectra showing different gold to ligand ratios using triethylamine as reducing agent
Figure 3.10: Inset shows AuNPs-3 and trimethylamine reduced Au-NPs in (a) day light (b) under UV illumination

3.2.6 Reactions involved in reduction of HAuCl₄

Reduction with Sodium borohydride

\[
8\text{HAuCl}_4 \cdot 3\text{H}_2\text{O} + 3\text{NaBH}_4 \rightarrow 8\text{Au}^{0} + 3\text{NaB(OH)}_4^- + 12\text{H}_2\text{O} + 32\text{HCl}
\]

Reduction with Trialkylamines

\[
\text{HAuCl}_4 + 3\text{NR}_3 \rightarrow \text{Au}^{0} + 3\text{NR}_3^- + \text{H}^+ + \text{Cl}^-
\]

3.2.7 Characterization through TEM images

The gold nanoparticles stabilized with amoxicillin and sodium salt of amoxicillin reduced with NaBH₄ and Trimethylamine were then characterized using TEM
(transmission electron microscope) to find their sizes. The TEM images showed that the nanoparticles have different sizes and shapes depending upon the reducing agent. As NaBH$_4$ is a strong reducing agent and due to its high speed reaction the size of nanoparticles did not grow so much and there was particles of only 4 to 5 nm as shown in the (Figure 3.12) while trimethylamine is an organic reducing agent just giving an electron from the lone pair of nitrogen and the reaction was not so fast and thus allowed the particles to grow more in size as compared to NaBH$_4$. Thus the nanoparticles obtained through the reduction of trimethylamine had a size of about 20 nm (Figure 3.11)

Figure 3.11: TEM images for AuNPs reduced with trimethylamine
3.2.8 Analytical protocol of amoxicillin stabilized Au-NPs

The analytical potential of prepared amoxicillin stabilized gold nanoparticles was explored by utilizing the fluorescence properties of Au-NPs. The effect of different heavy metals were checked i.e. Fe, Mn, Co, Hg, Cd, Ni etc. Cu$^{2+}$ had a prominent quenching effect on the fluorescence of Au-NPs as shown in figure 3.13. Under the optimum conditions, Cu$^{2+}$ was detected in the concentration
range of 0.5µM–0.1mM with linear regression equation of 
$I=412.56315+0.5307\times10^{-6}$ with $R^2= 0.996$. Limit of detection (3σ) was 9 nM, with 
relative standard deviation (n = 11) of 1.7%. (Figure 3.13)

![Figure 3.13: PL Spectra for detection of Cu$^{2+}$ Conditions: 1x10-6M Cu2+](image)

3.2.9 Ag nanoparticles stabilized with Amoxicillin

Silver nanoparticles stabilized with amoxicillin were also prepared with very 
good results as known from their color appearance (figure 3.14 reduce with 
NaBH$_4$ and Figure 3.15 reduced with TMA) as well as from their UV-VIS spectra 
(Figure3.17 Reduced with TMA) and (figure 3.18 Reduced with NaBH$_4$). UV-
Visible spectra suggested that the particles formed with trimethylamine 
reduction were uniform in size which was probably due to its mild reducing 
action while those formed with reduction of NaBH$_4$ were not so uniform due to
vigorous reducing action. Both types of nanoparticles have same plasmon resonance band at about 400nm which is a characteristic peak for silver nanoparticles. For NaBH₄ the λₘₐₓ value was about 400nm similar to that reduced with trimethylamine but with a little broadness which showed an indication of nanoparticles of different sizes and shapes. Different ratios of amoxicillin and silver gave nanoparticles of different sizes and shapes as indicated by different colors of solutions.

**Figure 3.14:** AgNPs having different ratios of AgNO₃ and sodium salt of Amoxicillin

**Figure 3.15:** AgNPs having different ratios of AgNO₃ and Amoxicillin (TMA)

FTIR spectra of amoxicillin and AgNPs showed that broadening of peaks in the spectrum of AgNPs was an indication of silver nanoparticles formation. Another indication from the figure was the shifting of peak of carboxylic ketonic group from 1776 cm⁻¹ to 1600cm⁻¹ and 1400cm⁻¹. Thus the carboxylate group was also involved in the stabilization of gold nanoparticles (**Figure 3.16**).
3.2.10 Characterization with UV-visible spectroscopy

UV/Visible spectra of AgNPs stabilized with amoxicillin showed a strong absorbance band near 400 nm which was sharp for AgNPs reduced with trimethylamine (Figure 3.17) and a little broadness for AgNPs reduced with NaBH₄ (Figure 3.18). The broad spectrum resulting from NaBH₄ reduction showed variations in particles shapes, while the AgNPs with TMA were uniform.
in shape as UV spectra suggested and results were very interesting. Resonance plasmon band in the region of 400nm to 500nm was an indication of the presence of silver nanoparticles. Optimization was carried out by doing a number of reactions by varying the amounts of ligands to metal ratio (table 3.2 and table 3.3). The best optimized ratio selected for further studies was 5:1 in case of reduction with NaBH$_4$ while 6:1 in case of reduction with trimethylamine (Figure 3.17 and Figure 3.18).

![Uv visible spectra of amoxicillin stabilized AgNPs reduced with NaBH4](image-url)
Chapter 3  
Metal nanoparticles stabilized with amoxicillin

Figure 3.18: UV visible spectra of amoxicillin stabilized AgNPs reduced with Triethylamine

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Ligand (1mM) mL</th>
<th>AgNO₃ (1mM) mL</th>
<th>Trimethylamine mL</th>
<th>λ_{abs}/λ_{abs max} (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>400/2.5</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>410/3.5</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>8</td>
<td>1</td>
<td>410/3.4</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>10</td>
<td>1</td>
<td>410/3.1</td>
</tr>
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</table>

Table 3.2: Optimization of Silver nanoparticles reduced with trimethylamine
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<th>S.No.</th>
<th>Ligand (1mM) mL</th>
<th>AgNO$_3$ (1mM)</th>
<th>NaBH$_4$ (40mM) (mL)</th>
<th>$\lambda_{\text{abs}}$ / $\lambda_{\text{abs max}}$ (nm)</th>
</tr>
</thead>
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<td>1</td>
<td>5</td>
<td>1</td>
<td>410 / 3.8</td>
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<td>1</td>
<td>6</td>
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<td>1</td>
<td>7</td>
<td>1</td>
<td>380 / 2.3</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>8</td>
<td>1</td>
<td>380 / 1.8</td>
</tr>
</tbody>
</table>

**Table 3.3:** Shows the optimization reactions of Silver nanoparticles reduced with NaBH$_4$.

### 3.2.11 Characterization with Transmission Electron Microscope (TEM)

The size obtained from TEM images for silver nanoparticles caped with amoxicillin by the reduction of NaBH$_4$ was about 15 to 20nm (Figure 3.20). But the particles were not uniform in sizes. The nanoparticles reduced with trimethylamine were more spherical in shape with a size range of 15 to 25nm as compared to those formed with the reduction of NaBH$_4$ (Figure 3.19).
Figure 3.19 TEM images of Amox caped AgNPs reduced with trimethylamine

Figure 3.20 TEM images of Amox caped AgNPs reduced with NaBH₄
3.2.12 Stability check of Silver nanoparticles

Stability of prepared silver nanoparticles stabilized with amoxicillin were checked by varying the pH of the solution. They were stable in basic medium (pH=12-13) for two days and retained their absorbance for a couple of weeks. While at lower pH (acidic medium) their absorbance gradually decreased and microscopic ppt started appearing at pH 3. This may be due to protonation of carboxylate group of amoxicillin, which was probably involved in the stabilization of Ag-NPs.

Stability of silver nanoparticles was checked with different concentrations of NaCl salt solutions i.e. 1M and 2M. Various amounts of NaCl solution were added to the silver nanoparticles solution but no obvious changes were observed even for a week. Procedure followed was; five vials were taken and 3ml of 1mM solution of AgNPs were taken in each vial. Salt solution (1M and 2M) was added in each vial starting from 0.1ml salt solution and shake well. Recorded their UV- VIS spectra at the same time and then after 24hrs, 48hrs and even after a week. Absorption spectra showed that AgNPs were stable against 2M NaCl solution.

Stability of AgNPs were also checked at different temperatures i.e. 50 °C, 60 °C, 70 °C and 80 °C. These particles were kept heated at mentioned temperature for 30 minutes and found that silver nanoparticles were stable up to 80°C.

3.2.13 Silver and Gold nano-alloys stabilized with Amoxicillin

UV results of the nano-alloy synthesized by the combination of AuNPs and AgNPs showed a confirmation towards the nano-alloy formation. The plasmon absorption bands of different ratio showed that best results may be obtained with the ratio of 1:1 as this absorption plasmon band was in between the two regions specific for silver nanoparticles (400nm-500nm) and gold nanoparticles.
Chapter 3  
*Metal nanoparticles stabilized with amoxicillin*

500nm-600nm) (Figure 3.21). There was also color changes which indicated the formation of Au-Ag nano-alloys (Figure 3.22).

![Ultraviolet-Visible absorption spectrum of AuNPs:AgNPs alloyed](image1)

**Figure 3.21:** UV-Visible data for nano-alloys stabilized with amoxicillin

![Image of solution samples](image2)

**Figure 3.22:** Au-Ag nano-alloys stabilized with amoxicillin
3.2.14 Characterization of nano-alloys with AFM

The AFM image of nano-alloys showed that the particles size was about 60-70nm while the size of their corresponding nanoparticles was smaller; as the particles size of AuNPs was 10-15nm and of AgNPs it was about 20nm (Figure 3.23 and 3.24)

Figure 3.23: AFM images of Amoxicillin stabilized Ag-Au nanoalloys

Figure 3.24: 3D format of AFM images of nano-alloys
CHAPTER-4

METAL NANOPARTICLES STABILIZED WITH CEFUROXIME
4.1 EXPERIMENTAL

4.1.1 Synthesis of gold nanoparticles stabilized with Cefuroxime using NaBH₄ as a reducing agent

A volumetric amount of HAuCl₄ (1mM solution) was taken in a vial (various amounts for various reactions) and also a volumetric amount of cefuroxime solution (1mM aqueous solution) was added to the vial containing gold solution while stirring. The reaction mixture was stirred for 30 minutes and NaBH₄ (0.1 mL from 40mM solution) was added as a reducing agent. The color changes for each reaction were observed and noticed which gave a primary indication of nanoparticles formation. After the addition of reducing agent UV-Visible spectra were recorded on spectrophotometer after stirring the reaction for 3-4 hours. A particular absorption plasmon band in the region of 500 to 600nm showed the presence of gold nanoparticles. After completion of reaction, the nanoparticles were separated by centrifuging the solution at 10000-12000 rpm. Which were then used for further analysis like TEM, FT-IR, bioassays etc.

4.1.2 Trimethylamine as a reducing agent

Same procedure was repeated here. HAuCl₄ (1mM), and Cefuroxime (1mM) was used throughout the reactions. Gold solution and ligand were mixed while stirring and after half an hour, 0.1 to 1mL of reducing agent (trimethylamine in this case) was added and stirred for 4 hours. Reactions were managed by observing colors first and then by recording their UV-visible spectra. Nanoparticles were isolated by centrifuge for further analysis.
4.1.3 Synthesis of Silver nanoparticles stabilized with Cefuroxime

AgNO₃ (1mM) solution and cefuroxime (1mM) solution were prepared. Particular amounts of both solutions (different ratios for various reactions) were taken in a vial. Kept on stirring for thirty minutes and a reducing agent (1mL NaBH₄ (40mM) or 0.5 to 1mL TMA) were added. The reaction was kept stirring for 3 to 4 hours. Observing the colors of the solution the ratio of new reaction has been increased to get better results. After completion of reaction time, absorption spectra were recorded on UV-Visible spectrophotometer. The nanoparticles were then separated with the help of centrifuge for characterization and other analysis.

4.1.4 Synthesis of silver and gold nano-alloys stabilized with Cefuroxime

Already prepared gold and silver nanoparticles stabilized with cefuroxime were mixed together with different ratios in different reactions. Stirred for half an hour and then a reducing agent was added (0.5ml NaBH₄ (40mM) or 0.5mL TMA). After 3 to 4 hours stirring, reaction mixtures with different colors were obtained their UV-Visible absorption spectra were recorded.

4.2 Result and discussions

Cefuroxime (Figure 4.1), a beta-lactam antibiotic belongs to the cephalosporin group of antibiotics, was used as a stabilizing agent for the synthesis of gold and silver nanoparticles and their alloys. This was selected for the purpose to have sulphur in the structure formula, as sulphur has high affinity for stabilizing gold and silver to form nanoparticles (scheme 4.1). Along with sulphur cefuroxime also have amino group and carboxylate group which may stabilized gold and silver metals easily during nanoparticles formation[138].
4.2.1 Gold nanoparticles stabilized with cefuroxime

Reactions of synthesizing gold nanoparticles using cefuroxime were carried out with positive results. To find out the optimized ratio using NaBH₄ or TMA as reducing agents, reactions with different ratios of metal to ligand ratios were carried out; keeping metal (gold) constant and varying the amount of ligand and vice versa. The ratio which gave best result in respect of having the sharpest absorption peak was selected (figure 4.2 and 4.3) for further studies which was 6:1 (metal: ligand) for AuNPs. UV-Visible results showed the presence of gold because of particular peak in the region of 500-600nm as shown in the figures 4.2 and 4.3. The color of gold nanoparticles stabilized with cefuroxime was
different for different reactions having different ratios (metal to ligand) as shown in the **figure 4.4** (NaBH₄) and **figure 4.5** (TMA) which dependent on their particle sizes. Gold nanoparticles stabilized with cefuroxime showed an absorption plasmon band in the region of about 530nm which is a particular region (500-600nm) for gold nanoparticles absorption. Nanoparticles of spherical shape give an absorption band at 520nm while in this case it was 530nm which means that shape was not fully spherical. Gold nanoparticles stabilized with cefuroxime were also tried to be synthesized by the reduction with trimethylamine (TMA). Better results were obtained with this reducing agent (**figure 4.3**).
Chapter 4  

Metal nanoparticles stabilized with Cefuroxime

Figure 4.3: Cefuroxime stabilized AuNPs reduced with TMA

Figure 4.4: Gold nanoparticles stabilized with cefuroxime (reduced with NaBH₄)
FTIR spectra of Cefuraxime, AuNPs and AgNPs showed that the shifting of peak of carboxylic ketonic group from 1784 cm\(^{-1}\) to 1606 cm\(^{-1}\) and 1384 cm\(^{-1}\) in case of AuNPs while from 1784 cm\(^{-1}\) to 1600 cm\(^{-1}\) and 1383 cm\(^{-1}\) in case of AgNPs. Thus the carboxylate group was involved in the stabilization of gold and silver nanoparticles (Figure 4.6).
Figure 4.6: FTIR data for cefuroxime (upper), AgNPs (middle) and AuNPs (lower)
Stability of gold nanoparticles stabilized with cefuroxime were checked with temperature, NaCl salt (1M) and pH. It was found that these nanoparticles were stable with temperature up to 80 °C and also stable with NaCl up to 1ml of 1M.

Gold nanoparticles were stable in the pH range of 6-9 while unstable completely in acidic medium (pH of 2-4) and stable in basic region (figure 4.7).

4.2.2 Characterization of AuNPs with TEM

Gold nanoparticles were characterized with TEM to confirm their sizes. Gold nanoparticles reduced with NaBH₄, have an average size of 5-8nm (figure 4.8) while those reduced with TMA have an average size of 12-15nm (Figure 4.9).
**Figure 4.8**: TEM images for Cefuroxime capped AuNPs (reduced with NaBH₄)
Figure 4.9: TEM images for Cefuroxime capped AuNPs (reduced with TMA)
4.2.3 Silver nanoparticles stabilized with cefuroxime

Reactions of synthesizing silver nanoparticles were carried out with positive results. To find out the optimized ratio using NaBH$_4$ or TMA as reducing agents, reactions with different ratios of metal to ligand were carried out; keeping metal (silver) constant and varying the amount of ligand and vice versa. The ratio which gave best result in respect of having the sharpest absorption peak was selected (figure 4.10) for further studies which was 8:1 (metal: ligand) for AgNPs. UV-Visible results showed the presence of silver nanoparticles, as they have a particular peak in the region of 400-500 nm as shown in the figures 4.11 and Figure 4.12.

![UV-Visible data for AgNPs stabilized with Cefuraxime](image-url)

**Figure 4.10:** UV-Visible data for AgNPs stabilized with Cefuroxime
Chapter 4  

Metal nanoparticles stabilized with Cefuroxime

Figure 4.11: Cefuroxime stabilized AgNPs reduced with TEA

Figure 4.12: Silver nanoparticles stabilized with cefuroxime (reduced with NaBH₄)
Stability of silver nanoparticles stabilized with cefuroxime were checked with temperature, NaCl salt (1M) and pH. It was found that these nanoparticles were stable with temperature up to 80 °C and also stable with NaCl up to 1ml of 1M.

Silver nanoparticles were stable in basic region (pH=9-12) while highly unstable in highly acidic medium (pH=2-3). While in neutral region these showed relatively good stability (figure 4.13).

![Figure 4.13: Effect of pH on the stability of cefuroxime capped AgNPs](image)

4.2.4 Characterization of AgNPs with TEM

Silver nanoparticles reduced with sodium borohydride have an average size of 8nm according to the TEM images (figure 4.14), while those reduced with TMA have an average size of 15-20nm (figure 4.15).
Figure 4.14: TEM images for Cefuroxime capped AgNPs (reduced with NaBH₄)
Figure 4.15: TEM images for Cefuroxime capped AgNPs (reduced with TMA)
Chapter 4  

**Metal nanoparticles stabilized with Cefuroxime**

### 4.2.5 Gold and silver nano-Alloys stabilized with cefuroxime

Gold and silver nano-alloys capped with cefuroxime were prepared by carrying different reactions of AuNPs and AgNPs capped with cefuroxime with various ratios to get the best results. Result obtained with a ratio of 3:3 observed in their UV-Visible spectra was the best (figure 4.16) and also the colors of solutions (figure 4.17). Absorption plasmon band of the ratio 3:3 has two peaks; one peak was in the region of 400 nm and other was in the region of 535 nm was evidence towards the formation of nano-alloys of gold and silver nanoparticles.

![UV-VIS data for nano-Alloys stabilized with cefuraxime](image)

**Figure 4.16**: UV-VIS data for nano-Alloys stabilized with cefuroxime
Figure 4.17: Silver and gold nano-alloys stabilized with cefuroxime

4.2.6 Characterization of nano-alloys with TEM

The sizes of nano-alloys were 8nm (in case of NaBH₄) and 10 to 12 nm (in case of TMA) according to the TEM images and their shapes were almost spherical (figure 4.18 and 4.19).

Figure 4.18: TEM images of gold and silver nano-alloys (reduced with TMA)
Figure 4.19: TEM images of gold and silver nano-alloys (reduced with NaBH₄)
CHAPTER-5

METAL NANOPARTICLES STABILIZED WITH CEPHRADINE
5.1 EXPERIMENTAL

5.1.1 Synthesis of gold nanoparticles stabilized with Cephradine using NaBH₄ as a reducing agent

Aqueous solution of HAuCl₄ (1mM solution) was taken in a vial and a particular amount of cephradine solution (1mM aqueous solution) was added to the vial containing gold solution while stirring. The reaction mixture was stirred for 30 minutes and NaBH₄ (0.1 mL from 40mM solution) was added as a reducing agent. Change in color for each reaction was noticed which gave a primary indication of nanoparticles formation. After the completion reaction time 3 to 4 hours, UV-Visible spectra were recorded on spectrophotometer. A particular absorption plasmon band in the region of 500 to 600nm showed the presence of gold nanoparticles. After completion of reaction, the nanoparticles were separated by centrifuge at 10000-12000 rpm which were then used for further analysis like TEM, bioassays etc.

5.1.2 Trimethylamine as a reducing agent

HAuCl₄ (1mM), and Cephradine solutions (1mM) were used to synthesized gold nanoparticles. Gold solution and ligand were mixed while stirring and after half an hour, 0.1 to 1mL of reducing agent (trimethylamine in this case) was added and stirred for 4 hours. Reactions were managed by observing colors and by recording UV-visible data for them. Nanoparticles were then isolated by centrifuge for further analysis.
Chapter-5  

**Metal nanoparticles stabilized with cephradine**

5.1.3 **Synthesis of Silver nanoparticles stabilized with Cephradine**

1mM solution of Cephradine was prepared in basic medium (Na$_2$CO$_3$) for complete solubility. AgNO$_3$ (1mM) solution was also prepared for the reaction. Both solutions were taken in vials with different molar ratios for different reactions. For example 1:1, 1:2, 1:10...10:1 etc. Stirred for half an hour and a reducing agent (1mL NaBH$_4$ (40mM)/0.5 to1mL TMA) was added. Different colors of the reactions were obtained. After completion of reaction time, their absorption spectra were recorded. Nanoparticles for characterization and other analysis were collected using centrifuge.

5.1.4 **Synthesis of silver and gold nano-alloys stabilized with Cephradine**

Nano-alloys stabilized with cephradine were also synthesized. AuNPs and AgNPs stabilized with cephradine were reacted together in different ratios for full optimization. Reaction mixtures of different colors were obtained. UV-Visible spectra were recorded for them. Method of synthesis applied was the same as used for other nano-alloys synthesis. Nanoparticles were collected for TEM, AFM and other analysis through centrifuge at 10000 to 12000 rpm.

5.2 **Results and discussion**

Cefradine or cephradine is a beta-lactam antibiotic belonging to cephalosporin family. It also has a sulphur atom in the six membered ring (figure 5.1) which was basically assumed to take part in the synthesis of gold and silver nanoparticles.
The schematic representation of gold and silver nanoparticles caped with cephradine may be shown as (scheme 5.1). It was assumed that sulphur is responsible for the stabilization of gold and silver metals but carboxylate acid and amine groups also have affinity for metals and thus may involve in the stabilization to form gold and silver nanoparticles.

Scheme 5.1: synthesis of gold and silver nanoparticles caped with cephradine
5.2.1 Gold nanoparticles stabilized with Cephradine

A number of reactions having different metal to ligand ratios were carried out. For example metal to ligand ratios of 1:1, 1:2......1:20, and also 2:1, 3:1......20:1 were examined to get best results. The sharpest peak was selected among the obtained peaks for further studies. According to the UV-Visible absorption results, it was 7:1 in case of gold nanoparticles (figure 5.2) which was the optimized ratio with sharpest peak in case of NaBH₄ reduction.

Gold nanoparticles prepared in different ratios had different colors of the solutions (figure 5.3). Their absorption bands were also different in sharpness of their peaks. Generally the gold nanoparticles caped with cephradine gave an absorption band in the region of 540nm which is a characteristic region for gold nanoparticles (500-600nm). This is a strong indication for gold nanoparticles sequestered with cephradine, a beta-lactam antibiotic with a strong reducing agent ‘NaBH₄’.

Gold nanoparticles were also prepared by reduction with a mild reducing agent trimethylamine. Very good results were obtained as compared to NaBH₄ reduced gold nanoparticles. In comparison with NaBH₄ reduction, lots of variations in their colors of solutions (figure 5.4) and absorption plasmon bands were present (figure 5.5). There was also a blue shift in case of trimethylamine which appeared at 520-525nm. More sharpness of a peak shows more uniformity in the particles shapes while broadness of peak means particle with different shapes. Thus broad peak is actually a combination of a number of sharp peaks which means that there were particles of different shapes.
Figure 5.2: UV data for AuNPs stabilized with Cephradine

Figure 5.3: Gold nanoparticles stabilized with cephradine (Ratio = gold: cephradine) (NaBH₄)
**Figure 5.4:** Cephradine capped gold nanoparticles reduced with TMA

**Figure 5.5:** UV-VIS data for Cephradine capped gold nanoparticle reduced with TEA

FTIR spectra of Cephradine, AuNPs and AgNPs showed that broadening of peaks in the spectrum of nanoparticles was an indication of gold and silver
nanoparticles formation. Another indication from the figure was the shifting of peak of carboxylic ketonic group from 1750 cm⁻¹ to 1645 cm⁻¹ and 1473 cm⁻¹ in case of AuNPs while from 1745 cm⁻¹ to 1626 cm⁻¹ and 1400 cm⁻¹ in case of AgNPs. Thus the carboxylate group was involved in the stabilization of gold and silver nanoparticles (Figure 5.6).

Figure 5.6: FTIR data for cephradine (upper) AuNPs (middle) and AgNPs (lower)
Stability of gold and silver nanoparticles was checked with NaCl solution (1M), heat and pH.

It was found that 3 ml of gold and silver nanoparticles each were stable with the addition of up to 1mL of NaCl solution (1M). While on heating they were stable up to near boiling point of water.

Gold nanoparticles were highly stable in neutral, slightly basic and basic medium (pH 7 to 11) (figure 5.7 and 5.8). In acidic medium nanoparticles were precipitated out.

**Figure 5.7**: pH effect on Gold nanoparticle Stabilized with Cephradine
5.2.2 Characterization of AuNPs with AFM

AFM images showed that the particles size was 100nm to 120nm with various shapes (Figure 5.9).

Figure 5.8: pH effect on the stability of Cephradine tabilized AuNPs

Figure 5.9: AFM images of AuNPs stabilized with cephradine
5.2.3 Silver nanoparticles stabilized with Cephradine

Same procedure was followed for the optimization purposes. A number of reactions having different metal to ligand ratios were carried out. For example metal to ligand ratios of 1:1, 1:2......1:20, and also 2:1, 3:1......20:1 were examined to get better results. After recording their absorption spectra, the sharpest peak was selected for further studies which was 10:1 according to the UV-Visible absorption results (figure 5.10 and Figure 5.11)

![Figure 5.10: Silver nanoparticles stabilized with cephradine](image)

![Figure 5.11: Spectral data for Silver nanoparticles stabilized with cephradine](image)
Stability of silver nanoparticles stabilized with cephradine was checked. It was found that silver nanoparticles were stable in slightly basic pH of 8-11, while slightly stable in slight acid medium (pH 5-6) and very unstable in highly acidic medium (pH 2-4) (figure 5.12 and 5.13).

**Figure 5.12:** pH effect on Silver nanoparticle Stabilized with Cephradine

**Figure 5.13:** pH effect on Silver nanoparticle stabilized with Cephradine
5.2.4 Characterization of AgNPs with AFM

AFM images suggested that the particles size is about 50 to 100nm with an oval shape (Figure 5.14 and Figure 5.15)

![AFM images of AgNPs stabilized with cephradine](image1)

**Figure 5.14:** AFM images of AgNPs stabilized with cephradine

![3D form of AFM image](image2)

**Figure 5.15:** 3D form of AFM image of AgNPs stabilized with cephradine
Chapter-5  

Metal nanoparticles stabilized with cephradine

5.2.5 Gold and silver nano-alloys stabilized Cephradine

Gold and silver nano-alloys sequestered with cephradine were also tried giving positive results. It was confirmed by the absorption plasmon band that there was the presence of nano-alloys (Figure 5.16 and Figure 5.17). An optimized reaction of AuNPs and AgNPs caped with cephradine led to the formation of nano-alloys. Absorption bands also showed the presence of nano-alloys, i.e. two peaks in the same spectrum; one was in the silver nanoparticle region at about 410nm and the other peak was in the gold nanoparticle region at about 540nm (figure 5.16). For complete optimization various reaction with various ratios were carried out, like 1:1, 1:2, .1:5 etc. The best ratio obtained was 1:2 (AgNPs: AuNPs).

![Figure 5.16: Gold and Silver nano-alloys stabilized with Cephradine](image_url)
Figure 5.17: Gold-Silver nano-Alloys stabilized with cephradine

5.2.6 AFM characterization of nano-alloys

AFM images showed that various sized particles were present with oval shapes. The particles size was about 70-80 nm (Figure 5.18 and Figure 5.19).

Figure 5.18: AFM images of Au-Ag nano-alloys stabilized with cephradine
Figure 5.19: 3D format of AFM images of cephradine stabilized nano-alloys

5.2.7 Applications

Nanoparticles stabilized with cephradine were checked for their sensing application. They were checked against pharmaceutical drugs as well as some heavy and toxic metals. The AuNPs capped with cephradine were active for sensing some of metals in aqueous solutions. The best results were obtained for Cd ions and Hg ions in their aqueous solutions of 0.0001M. For detection purposes, added 20 µL in 2mL of AuNPs and thus by dilution formula it can be calculated the concentration of Metals ion which will be 0.000001M or $1\times10^{-6}$M. Thus AuNPs caped with cephradine can be used to detect Cd (Figure 5.20) and
Hg ions (Figure 5.22) in an aqueous solution up to 0.000001M or 1×10^{-6}M. For Cd the coefficient of detection was 0.9915 (Figure 5.21) while for Hg it was 0.9992 (Figure 5.23).

Figure 5.20: Detection of Cd ions in aqueous solution using AuNPs capped with cephradine
Figure 5.21: Detection of Cd ions in aqueous solution using AuNPs capped with cephradine with a coefficient of detection of \( R^2 = 0.9915 \)

Figure 5.22: Detection of Hg ions in aqueous solution using AuNPs capped with cephradine
Similarly AuNPs were also used for sensing of other metals like Ni, Co, Cu etc. they were also sensed but had very less effect on the absorption peak of AuNPs. A comparative bar graph shows sensing of different heavy metals (Figure 5.24).

Figure 5.23: Detection of Hg ions in aqueous solution using AuNPs caped with cephradine with a coefficient of detection of $R^2 = 0.9992$

Figure 5.24: Detection of metallic ions in aqueous solution using AuNPs caped with cephradine
CHAPTER-6

METAL NANOPARTICLES STABILIZED WITH CEFTRIAXONE
6.1 EXPERIMENTAL

6.1.1 Synthesis of gold nanoparticles stabilized with Ceftriaxone using NaBH₄ as a reducing agent

A particular amount of HAuCl₄ (1 mM solution) was taken in a vial (various amounts for various reactions) and a particular amount of Ceftriaxone solution (1 mM aqueous solution, prepared in basic medium of Na₂CO₃ for complete dissolution) was added to the vial containing gold solution while stirring. The reaction mixture was stirred for half an hour and NaBH₄ (0.1 mL from 40 mM solution) was added as a reducing agent. The color changed for each reaction were noticed which gave a primary indication of nanoparticles formation. A reducing agent was added and UV-Visible spectra were recorded on spectrophotometer after stirring the reaction mixture for 3-4 hours. A particular absorption plasmon band in the region of 500 to 600 nm showed the presence of gold nanoparticles. After completion of reaction time, the nanoparticles were separated by centrifuge at 10000-12000 rpm which were then used for further analysis like TEM, FT-IR, bioassays etc.

6.1.2 Using trimethylamine as a reducing agent

Same procedure was repeated again. HAuCl₄ (1 mM) and Ceftriaxone (1 mM) were used, reduced with TMA. Gold solution and ligand were mixed while stirring and after half an hour, 0.1 to 1 mL of reducing agent (trimethylamine in this case) was added and stirred for 4 hours. Reactions were managed by recording UV-visible spectra. Nanoparticles were isolated with the help of centrifuge for further analysis.
6.1.3 Synthesis of Silver nanoparticles stabilized with Ceftriaxone

Ceftriaxone stabilized silver nanoparticles were also prepared using the same method. Different reactions were carried out for full optimization of the nanoparticles synthesis. Different colors were obtained with different ratios of the reaction mixtures showing different particle sizes. Absorption spectra were recorded with UV-Visible spectrophotometer. Nanoparticles were collected with the help of centrifuge at 10000-12000 rpm for TEM, AFM and other analysis.

6.1.4 Synthesis of silver and gold nano-alloys stabilized with ceftriaxone

Gold nanoparticles stabilized with ceftriaxone and silver nanoparticles stabilized with Ceftriaxone were mixed together in vials with different ratios in different reactions. Stirred for half an hour and a reducing agent were added (0.5ml NaBH₄ (40mM)/0.5mL TMA). After 3 to 4 hours stirring, reaction mixtures with different colors were obtained. Their UV-Visible absorption spectra were recorded. Nano-alloys were isolated with the help of centrifuge at 10000 to 12000 rpm for TEM, AFM and other analysis.
Chapter-6  

Metal nanoparticles stabilized with Ceftriaxone

6.2 Results and discussion

Ceftriaxone is a member of cephalosporin family of antibiotics having molecular structure shown in the scheme 6.1 below

6.2.1 Gold nanoparticles stabilized with Ceftriaxone

1mM solution of Gold (HAuCl₄) and 1mM solution of ceftriaxone was prepared. Reactions between ceftriaxone and gold were carried out with the use of NaBH₄ as strong reducing agent. 1Mm solution of gold and 1mM solution of Ceftriaxone was taken in the ratio of 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1 and 8:1 (Gold: Ceftriaxone) and their UV spectra were recorded after the reaction completion. It was found in UV-Visible results that there was a peak in the region, characteristics of gold nanoparticles (figure 6.1) showing an absorption band at 540-545nm. It was observed during the optimization process that the formation of gold nanoparticles were very poor at low ratios of gold contents but as the ratio increased to 4:1(Gold: Ceftriaxone) and 5:1(Gold: ceftriaxone) it improved the results. UV-VIS spectra were taken which confirmed the formation of Gold nanoparticles (figure 6.1 and 6.2) Observing absorption spectra, the formation of gold Nanoparticles was best in 10:1 (gold: Ceftriaxone).
Scheme 6.1: Synthesis of gold and silver nanoparticles capped with ceftriaxone
Figure 6.1: Absorption band for gold nanoparticles stabilized with Ceftriaxone

Figure 6.2: Gold nanoparticles stabilized with ceftriaxone (Ratio: Gold: ceftriaxone)

FTIR spectra of Ceftriaxone, AuNPs and AgNPs showed that broadening of peaks in the spectrum of nanoparticles was an indication of gold and silver nanoparticles formation. Another indication from the figure was the shifting of
peak of carboxylic ketonic group from $1776 \text{ cm}^{-1}$ to $1664 \text{ cm}^{-1}$ and $1473 \text{ cm}^{-1}$ in case of AuNPs while from $1776 \text{ cm}^{-1}$ to $1588 \text{ cm}^{-1}$ and $1383 \text{ cm}^{-1}$ in case of AgNPs. Thus the carboxylate group was involved in the stabilization of gold and silver nanoparticles (Figure 6.3)

Figure 6.3: FTIR data of Ceftriaxone (upper), AuNPs (Middle) and AgNPs (lower)
Chapter 6  Metal nanoparticles stabilized with Ceftriaxone

*Figure 6.4:* pH stability of AuNPs stabilized with ceftriaxone

*Figure 6.5:* pH stability of AuNPs stabilized with ceftriaxone
The stability of AuNPs capped with ceftriaxone was checked with heat, pH and Salt. These were stable on heating up to 80 °C.

For pH stability, it was observed that the gold nanoparticles were almost stable in basic range while more stable in acidic range (figure 6.4 and 6.5).

The stability of gold nanoparticles was also checked with NaCl solution (1M). Uv-Visible results showed that on higher concentration of NaCl, AuNPs showed low stability and their particle sizes were affected (figure 6.6)

![Figure 6.6: Effect of NaCl salt on Ceftriaxone capped Gold nanoparticles](image)

6.2.2 AFM characterization of AuNPs

AFM images showed that the particles were spherical in shape with an average size of 50nm (figure 6.7)
Figure 6.7: AFM images of ceftriaxone stabilized AuNPs

6.2.3 Silver nanoparticles stabilized with Ceftriaxone

1mM solution of Silver (AgNO₃) and 1mM solution of ceftriaxone as stabilizing agent were prepared in distilled water. Reactions between ceftriaxone and Ag solution were carried out with different ratios using NaBH₄ as strong reducing agent (figure 6.8). This was confirmed by the absorption peak in the region
specified for silver nanoparticles. Thus for silver nanoparticles there was a peak at about 400nm (figure 6.9).

**Figure 6.8**: Silver nanoparticles stabilized with ceftriaxone (Ratio: Silver: ceftriaxone)
Stability of silver nanoparticles was checked with heat, pH and Salt. These nanoparticles were stable on heating up to boiling. The pH stability was checked from pH 2-3 to 12-13. The Silver nanoparticles were mostly unstable in acidic medium (pH 2-3 and 4-5) and ppt. was formed (figure 6.10). The salt stability was also checked with NaCl solution (1M). It was observed that silver nanoparticles were stable with 1M NaCl solution.
6.2.4 AFM characterization of AgNPs

AFM images showed that the sizes of particles were 60-70 nm and shape is nearly spherical (Figure 6.11).
6.2.5 Gold and silver nano-alloy stabilized with Ceftriaxone

Gold and Silver nano-alloys were also synthesized. Different reactions having different ratios of AuNPs and AgNPs stabilized with ceftriaxone were carried out. The best result for alloy formation was at a ratio of 1:1 (AgNPs: AuNPs). The UV spectra were taken to confirm their formation (figure 6.12). The absorption spectrum for the ratio of 1:1 had two peaks. One peak was in the silver nanoparticles region at about 380nm while the other peak was in the region of gold nanoparticles at about 530nm.
Figure 6.12: Gold and silver nano-alloys caped with ceftriaxone

Figure 6.13: Gold-silver nano-alloys stabilized with ceftriaxone (Ratio: AuNPs: AgNPs)
6.2.6 AFM characterization of AuNPs

AFM images showed that the particles size in this case was 250nm in one dimension and about 500nm in the other direction showing that the particles are oval in shape (Figure 6.14 and Figure 6.15).

Figure 6.14: AFM images of ceftriaxone stabilized Au-Ag nano-alloys

Figure 6.15: 3D format of ceftriaxone stabilized nano-alloys
CHAPTER-7

METAL NANOPARTICLES STABILIZED WITH CEFIXIME
Chapter 7  
Metal nanoparticles stabilized with cefixime

7.1 EXPERIMENTAL

7.1.1 Synthesis of gold nanoparticles stabilized with Cefixime using NaBH₄ as a reducing agent

A particular amount of HAuCl₄ (1mM solution) was taken in a vial (various amounts for various reactions depending upon the ratios of Gold to ligand). A particular amount of Cefixime solution (1mM aqueous solution, prepared in Na₂CO₃ solution for complete dissolution) was added to the vial containing gold solution while stirring. The reaction mixture was stirred for 30 minutes and NaBH₄ (0.1 mL from 40mM solution) was added as a reducing agent. The color change for each reaction was noticed which gave a primary indication of nanoparticles formation. After the addition of reducing agent and stirring for 3-4 hours the UV-Visible spectra were recorded. A particular absorption plasmon band in the region of 500 to 600nm showed the presence of gold nanoparticles. After completion of reaction, the nanoparticles were separated by centrifuge at 10000-12000 rpm which were used for further analysis like TEM, bioassays etc.

7.1.2 Cefixime stabilized AuNPs Using Trimethylamine as a reducing agent

Same procedure was repeated here. HAuCl₄ (1mM) and Cefixime (1mM) was used throughout the reactions. Gold solution and ligand were mixed while stirring and after half an hour, 0.1 to 1mL of reducing agent (trimethylamine) was added and stirred for 4 hours. Reactions were managed by recording UV-visible data. Nanoparticles were then isolated by centrifuge for further analysis.
7.1.3 Synthesis of Silver nanoparticles stabilized with Cefixime

Silver nanoparticles stabilized with Cefixime were also prepared using the same method mentioned above. Different reactions with different ratios were carried out for complete optimization with NaBH₄ and TMA. UV-Visible spectra were recorded in which the absorption bands were in the region, particular for silver nanoparticles. Nanoparticles were collected through centrifuge for TEM, AFM and other analysis.

7.2 Results and discussion

Cefixime a beta-lactam antibiotic belong to the family of cephalosporins having molecular structure given below (Figure 7.1)

![Cefixime Structure](image-url)
Scheme 7.1: synthesis of gold and silver nanoparticles capped with cefixime
7.2.1 Gold nanoparticles stabilized with Cefixime

Using Cefixime as a stabilizing agent, gold nanoparticles were synthesized. In initial observations like color changes it was assumed that gold nanoparticles were possible to be synthesized using Cefixime as a stabilizing agent.

Gold nanoparticles were synthesized using the same Turkevich method. All reactions were carried out in aqueous medium using a reducing agent like NaBH₄ and TMA. For the optimization purposes a number of reactions were carried out in different ratios of metal to ligand. For example 1:1, 2:1, 3:1, 4:1…………20:1 (gold: Cefixime) and also 1:2, 1:3……..20:1 etc. Optimized conditions were selected by the sharpest peak in UV-VIS spectra for further process. According to the absorbance peaks in the region of 540-550nm showed the successful formation of gold nanoparticles (figure 7.2) as this region is specific for the gold nanoparticles.
The optimized ratio of AuNPs stabilized with cefixime was 8:1 (gold: ligand) with an absorption band at 540nm in case of NaBH₄ reduction (Figure 7.2) while in case of trimethylamine it was 8:1 (gold: ligand) again with an absorption band at 530nm (Figure 7.3).
Figure 7.3: AuNPs capped with cefixime using TMA as reducing agents

Figure 7.4: Cefixime stabilized AuNPs, reduced with TMA

FTIR spectra of Cefixime, AuNPs and AgNPs showed that shifting of peak of carboxylic ketonic group from 1771 cm\(^{-1}\) to 1604 cm\(^{-1}\) and 1383 cm\(^{-1}\) in case of AuNPs while from 1771 cm\(^{-1}\) to 1593 cm\(^{-1}\) and 1383 cm\(^{-1}\) in case of AgNPs and
disappearance of broad peak of -OH group of carboxylic acid was indications that carboxylate group was also involved in the stabilization of gold and silver nanoparticles (Figure 7.5)

**Figure 7.5:** FTIR data for cefixime (upper) AuNPs (middle) and AgNPs (lower)
Stability of cefixime capped gold nanoparticles was checked with salts, heat and pH. It was found that cefixime capped gold nanoparticles were stable against 1M NaCl solution and also upon heating upto 80 \(^{\circ}\)C. While in case of stability against pH, it was noticed that in acidic medium they were unstable while highly stable in the neutral medium and less stable in basic medium. (Figure 7.6)

![Figure 7.6: pH stability of cefixime-Gold NPs](image)

**7.2.2 AFM characterization of AuNPs**

AFM images showed that the particles size was 10nm to 12nm with irregular shapes (Figure 7.7)
Figure 7.7 AFM images of cefixime stabilized AuNPs

7.2.3 Silver nanoparticles stabilized with Cefixime

Silver nanoparticles stabilized with cefixime were also synthesized. For complete optimization, a number of reactions were carried out in different ratios of Ag to ligand. Successful results were obtained which were confirmed through absorption peaks in the region of silver nanoparticles. Thus I got absorbance plasmon bands for silver nanoparticles at about 400-410nm (Figure 7.8) in case of NaBH₄.

Silver nanoparticles were also prepared using trimethylamine. Best results were obtained with mild reducing agent like trimethylamine (figure 7.9 and Figure 7.10).
Figure 7.8: Silver Nanoparticles stabilized with Cefixime (NaBH₄)
Stability of silver nanoparticles was also checked with NaCl salt solution (1M), heat and temperature. It was found that silver nanoparticles stabilized with cefixime were stable with 1M NaCl solution and heat up to boiling temperature of water. While in stability with pH it was noticed that in highly acidic medium
(pH 2-3) they were unstable, while highly stable at slight basic (pH 8-9) and less stable in basic medium (figure 7.11).

Figure 7.11: pH stability of AgNPs stabilized with Cefixime

7.2.4 AFM characterization of AgNPs

AFM images showed that the size of AgNPs was 25nm with oval shape as shown in the figure 7.12.
Chapter 7  Metal nanoparticles stabilized with cefixime

Figure 7.12: AFM images of cefixime stabilized AgNPs

7.3 Applications of AuNPs caped with cefixime

Cefixime caped AuNPs were checked for chemo sensing and metallic sensing properties. These nanoparticles were used to sense heavy metals in water solutions i.e. Cd, Co, Ni, Fe, Cu etc. These were found good sensors for Cd ions and Cu ions in aqueous solutions. The coefficient of detection for Cd was $R^2 = 0.9628$ and detect Cd ions in an aqueous solution upto $1\times10^{-6}$M of concentration (Figure 7.13).
Figure 7.13: Detection Cd\(^{2+}\) in aqueous solution using cefixime caped AuNPs, with a detection range of 0.000001M

Similarly AuNPs were also used for the detection of Cu ions and can sense Cu ions in aqueous solution of about 1\(\times\)10\(^{-6}\)M of concentration (Figure 7.14) with a coefficient of detection of 0.9859. The comparative sensing power with other metals of AuNPs is shown in Figure 7.15.
Figure 7.14: Detection Cu$^{2+}$ in aqueous solution using cefixime caped AuNPs with detection range of 0.000001M

Figure 7.15: Detection of metals in aqueous solution using cefixime caped AuNPs (0.0001M metallic Salts)
Chapter 7  

Metal nanoparticles stabilized with cefixime

7.5 Applications of AgNPs capped with cefixime (reduced with TMA)

AgNPs stabilized with cefixime were used for sensing heavy metals ions. These had the ability to sense Ni ions in the aqueous solution up to micro molar concentration with a coefficient of 0.9858 (Figure 7.16). They had very less sensing ability for other metals except Ni ions (Figure 7.17). The sensing applications were performed on UV-Visible spectrophotometer.

Figure 7.16: Detection of 0.0001M Ni2+ in aqueous solution using AgNPs of Cefixime
Figure 7.17: Detection of metal ions in aqueous solution using AgNPs of Cefixime
CHAPTER-8

ENZYMES INHIBITION STUDIES
8.1 INTRODUCTION

8.1.1 Xanthine Oxidase

The Xanthine Oxidase (XO) (EC 1.2.3.2) is a cytosolic molybdenum containing enzyme which is responsible for the formation of uric acid from the purines hypoxanthine and xanthine, and production of reactive oxygen species. Excessive accumulation of uric acid in the joints can cause gout and free radicals are involved in the pathologic process of inflammation, mutagenesis, atherosclerosis (Hardening of arteries), cancer and aging [139, 140]. One of the therapeutic approaches to treat gout is the use of XO inhibitors that block the production of uric acid. Allopurinol is the sole XO inhibitor under the clinical application in the past three decades[141]. Unfortunately, this drug has many severe adverse effects such as hepatitis, nephropathy, allergic reactions and 6-mercaptopurine toxicity [142-144]. Therefore, there is an urgent need to explore new XO inhibitors.

8.1.2 Urease

The coordination compounds are of considerable interest because metal ions are found in the active sites of a large number of metallo-proteins such as hemocyanin, and also in metallo-enzymes like in ureases, tyrosinase, laccase and ascorbate oxidase[145-159].

Urease (urea amidohydrolase EC 3.5.15) is a nickle containing metallo-enzyme which catalyzes the hydrolysis of urea to ammonia and carbon dioxide. Urease is an enzyme responsible for an organism to use urea as nitrogen source[160]. In plants urease also acts as defense protein in systemic nitrogen transport pathways[161]. Urease is known to be one of the major causes of pathogenesis induced by H pylori, thus allow them to survive at low pH of the stomach and,
therefore, play an important role in the pathogenesis of gastric and peptic ulcer, apart from cancer [162]. Urease is directly involved in the formation of infection stones and contributes to the pathogenesis of urolithiasis, pyelonephritis, ammonia and hepatic encephalopathy, hepatic coma and urinary catheter encrustation [157, 158, 162].

Due to the diverse functions of this enzyme, its inhibition by potent and specific compounds could provide an invaluable addition to new way of treatment to infections.

8.1.3 Carbonic anhydrase-II

Carbonic anhydrase (EC 4.2.1.1 CA-II) catalyzes a very simple physiological reaction that is the interconversion between carbon dioxide and the bicarbonate ion. It is also involved in certain physiological process connected with respiration and transport of CO$_2$/bicarbonate between metabolizing tissues and lungs. CA-II also play an important in pH and CO$_2$ homeostasis, electrolyte secretion, biosynthetic reactions such as gluconeogenesis, lipogenesis and ureogenesis, bone resorption, calcification, tumorigenicity, and many other physiological or pathological processes[163, 164]. Cancer cells have a higher replication rate than the normal cells. This requires a high flux of bicarbonate in the metabolic pathway[165], hence the expression of CA’s is increased in many tumors, where they act to acidify the extra cellular milieu and provide tumors a growth advantage over normal tissues. It was reported that expression of CA-I and CA-II correlate well with the aggressiveness of colorectal cancer and synchronous distant metastasis[166].

Most inhibitors of carbonic anhydrase belong to the sulfonamide or sulphamate classes of compounds. These inhibitors are clinically used for the treatment or
prevention of a variety of disorders such as glaucoma, acid-base disequilibria, epilepsy, anticancer, and other minor neuromuscular disorders.

8.1.4 α-Chymotrypsin

α-Chymotrypsin (EC 3.4.21.1), a serine protease mainly secreted from pancreas, catalyzes the breakdown of polypeptide, and proteins. This enzyme not only digests proteins from foods, but also catalyzes the degradation of body’s own tissues in uncontrolled manner in disease such as pancreatitis, and cirrhosis[167]. α-Chymotrypsin activates epithelial sodium channel (EnaC) by proteolytic cleavage which results in cystic fibrosis[168]. Chymotrypsin, along with cathepsin, is responsible for the cleavage of interleukin 1-β (IL-1β) precursor into functional active IL-1β which causes inflammatory arthritis[169]. Inhibition of α-chymotrypsin is therefore an important strategy to control the on-set, and for treatment of certain disease e.g., cystic fibrosis, and pancreatitis.

8.1.5 Anti-glycation

Diabetes mellitus is the most prevalent metabolic syndrome world-wide with an incidence varying between 1 to 8%. Diabetes is characterized by hyperglycemia resulting in various short-term metabolic changes in lipid and protein metabolism, and long-term irreversible vascular changes[170].

Glycation is the reaction that takes place when simple sugar molecules (such as fructose, glucose, ribose, etc.) become attached to proteins or lipids without the moderation of an enzyme. Glycation process, also known as the Maillard reaction, is divided into three key stages:

1. The early reactions resulting in the formation of a Schiff base and Amadori products,
The rearrangements of these chemical groups, and

The final reactions forming the classical Maillard browning products or now known as AGEs

Since glycation disrupts normal metabolic pathways, and if the state of hyperglycemia is still persists, the circulation of AGEs increases resulting in the promotion of certain health risks. These include diabetes-specific complications of the micro-vasculature system (retinopathy, nephropathy, and neuropathy) and complications of the macro-vasculature (atherosclerosis leading to heart disease, stroke and peripheral vascular disease), which are present in the non-diabetic population, but have a two to five-fold increase in diabetic subjects [171].

8.2 EXPERIMENTAL

8.2.1 Protocol for Xanthine oxidase assay and inhibition

"The XO inhibitory activity of test compounds was determined by measuring the rate of hydroxylation of the substrate (xanthine) and subsequent formation of uric acid, which is a colorless end product of the reaction and shows absorption at 295 nm[172]. Briefly, the reaction mixture containing 10 μL of 1 mmol.L-1 pure sample was dissolved in DMSO, 150 μL of phosphate buffer (0.05 mol • L-1, pH 7.4), 0.003 units of Xanthine Oxidase dissolved in buffer (20 μL), and 20 μL of 0.1 mmol.L-1 xanthine as substrate for enzyme. After addition of xanthine oxidase, the mixture was incubated for 10 min at room temperature and pre-read in the UV region (λ max 295 nm). The substrate was added to reaction mixture, and continuous reading for 15 min at an interval of 1 min was observed (Spectra MAX-340). The percentage inhibitory activity induced by the samples were determined against a DMSO blank and calculated using the following formula. Inhibition (%) = 100 - [(OD test compound /OD control) × 100]. The IC50 of the compounds as calculated using EZ-Fit windows-based software
(Perrella Scientific Inc. Amherst, U.S.A.). To compare the inhibitory activities of the compounds, allopurinol was used as standard. The reaction for each compound was performed in triplicate”.

8.2.2 Urease assay and inhibition

“Reaction mixture s comprising 25μL of enzyme (jack bean urease) solution and 55 μL of buffers containing 100mM urea were incubated with 5 μL of test compounds (0.5 mM concentration) at 30 °C for 15 min in 96-well plates. Urease activity was determined by measuring ammonia production using the indophenol method as described by weather burn. Briefly, 45 μl each phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70 μL of alkali reagent(0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to each well. The increasing absorbance at 630 nm was measured after 50min, m using a micro plate reader (Molecular Device, USA). All reactions were performed in triplicate in a final volume of 200 μL. The results (change in absorbance per min) were processed by using softMax Pro software (molecular Device, USA). The entire assays were performed at pH 6.8. Percentage inhibitions were calculated from the formula 100-(ODtestwell/ODcontrol) ×100. Thiourea was used as the standard inhibitor of urease”[173].

8.2.3 Carbonic Anhydrase-II inhibition assay

“4-NPA, which is colorless, in this assay, on hydrolysis converted to 4-nitrophenol and carbon dioxide and which is followed by measuring the formation of 4-nitrophenol, a yellow colored compound. The reaction was carried out at 25-28 °C. The experiment was carried out in buffer solution having HEPES an acidic and tris alkaline at a total concentration of 20 mM and pH
ranges from 7.2-7.9. For every test compound the reaction tube consisted of 140 mL of the HEPES-Tris solution, 20 mL of newly prepared aqueous solution of purified bovine erythrocyte CA-II (0.1-0.2mg/2000 mL of deionized water for 96-well plates), sigma Aldrich. 20 mL of test compound which was dissolved in DMSO and 20 mL of substrate 4-NPA at concentration of 0.7 mM diluted in ethanol[174]. The experiment was carried out in triplicates”.

8.2.4 In Vitro α-Chymotrypsin Assay

“The inhibitory activity of α-chymotrypsin was performed in 50 mM Tris-HCl buffer pH 7.6 with 10 mM CaCl₂, as mentioned by Cannell et al.[175] with the slight modification. The enzyme α-chymotrypsin (12 units/mL prepared in buffer mentioned above) with the 0.5 mM test compound prepared in DMSO, was incubated at 30°C for 25 min. The reaction was initiated by the addition of the chromogenic substrate, N-succinyl-L-phenylalanine-p-nitroaniline (SPPNA; 0.4 mM final concentration prepared in the buffer as above). The change in absorbance by release of p-nitroanilide was continuously monitored at 410 nm. The positive control without test compound was replaced by DMSO (final concentration 7%). The percentage of inhibition based upon initial velocity and calculated as:

% inhibition = (1 - (OD of test/OD of control))*100”

8.2.5 ANTIGLYCATION

“Bovine Serum Albumin (BSA) was purchased from Merck Marker Pvt. Ltd. Rutin, Methyl glyoxal (MGO) (40% aqueous solution), sodium dihydrogen phosphate (NaH₂PO₄), disodium hydrogen phosphate (Na₂HPO₄) and Dimethyl sulphoxide (DMSO) were purchased from Sigma Aldrich.

Assay was performed by using the method described by Rahbar et al., (2005) with slight modifications[176]. Triplicate samples of BSA 10 mg/ml, 14 mM MGO,
Enzyme Inhibition Studies

0.1M phosphate buffer (pH 7.4) containing NaN\textsubscript{3} (30 mM) was incubated under aseptic conditions, (in such a way that each well of 96-well plate contain 50\(\mu\)L BSA solution, 50\(\mu\)L MGO, and 20\(\mu\)L test sample) at 37°C for 9 days. After 9 days of incubation, each sample was examined for the development of specific fluorescence (excitation, 330 nm; emission, 440 nm), against sample blank, on a microtitre plate spectrophotometer (Spectra Max, Molecular Devices). Rutin was used as a positive control (IC\textsubscript{50} = 294 \(\mu\)M ± 1.50 SEM).

The percent inhibition of AGE formation in the test sample versus control was calculated for each inhibitor compound by using the following formula:

\[
\% \text{ inhibition} = \left(1 - \frac{\text{fluorescence of test sample}}{\text{Fluorescence of the control group}} \right) \times 100
\]

8.3 Results and discussions

8.3.1 Amoxicillin stabilized metal nanoparticles

Amoxicillin stabilized gold and silver nanoparticles and their alloys were screened for enzymes (urease, Xanthine oxidase, carbonic anhydrase and chymotrypsin) inhibition studies to explore their inhibition potentials of these nanoparticles as well as their antiglycation studies were carried out. The urease enzymes are Nickel containing enzymes while Xanthine oxidase is a molybdenum containing enzyme. After screening of these nanoparticles against the xanthine oxidase enzymes the AuNPs and AgNPs were found significantly active with inhibition of 90.4% and 82.2% respectively having IC\textsubscript{50} value 2.0±0.01 \(\mu\)g/mL and IC\textsubscript{50} value 2.73±0.05 \(\mu\)g/mL respectively.

In case of urease enzymes the AuNPs were active having inhibition of 97% having IC\textsubscript{50} value 28.83±0.38 \(\mu\)g/mL and AgNPs were also active having IC\textsubscript{50} value 82.66±1.06 \(\mu\)g/mL with inhibition of 93%.
It is known that -NH$_2$ group is mainly responsible for antiglycation activity as it attacks the electron deficient carbonyl carbon of methyl glyoxal compound. It was found that AuNPs, AgNPs and nano-alloys were inactive in the antiglycation studies while the parent compound amoxicillin showed an inhibition of 52.69% with an IC$_{50}$ value of 466.20±7.8. This might be the involvement of -NH$_2$ group in the stabilization of nanoparticles.

All nano products stabilized with amoxicillin and parent compound were screened for In Vitro α-Chymotrypsin enzyme inhibition but were inactive.

In case of carbonic anhydrase AuNPs showed 68.4% inhibition and IC$_{50}$ value of 143.03±4.40 while AgNPs and Alloys stabilized with amoxicillin and the parent compound were inactive. (See table 8.1 for all above results)
Table 8.1: Shows the enzymes inhibition and anti-glycation activities of amoxicillin and amoxicillin stabilized gold and silver nanoparticles

<table>
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<tr>
<th>S.No</th>
<th>Enzymes /other activities</th>
<th>%Inhibition</th>
<th>Amoxicillin</th>
<th>AuNPs</th>
<th>AgNPs</th>
<th>Alloys</th>
<th>Standard(µM)</th>
<th>Standard (µg/mL)</th>
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<td>Xanthine oxidase</td>
<td>-9.5</td>
<td>90.4</td>
<td>82.2</td>
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<td>1.598±0.011</td>
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8.3.2 Cefuroxime stabilized metal nanoparticles

Cefuroxime stabilized gold and silver nanoparticles and their alloys were screened for enzymes (urease, Xanthine oxidase, carbonic anhydrase and chymotrypsin) inhibition studies to explore their inhibition potentials of these nanoparticles. The ureases are Nickel containing enzymes while, the Xanthine oxidase is a molybdenum containing enzyme. After screening of these nanoparticles against these enzymes the nanoparticles were found selective against Jack bean urease enzyme. Among the nanoparticles the AgNPs were found more active having IC$_{50}$ value 23.96±0.27 µg/mL than AuNPs having IC$_{50}$ value 82.66±1.06 µg/mL. The alloy of these nanoparticles was also found significantly active having IC$_{50}$ value 15.63±0.59 µg/mL. None of these nanoparticles were active against Xanthine oxidase enzyme, which shows selectivity of these nanoparticles against urease enzymes.

Gold, silver, nano-alloys and parent compound were inactive in the antiglycation studies. The parent compound cefuroxime showed inhibition more than AuNPs and AgNPs.

All nano products stabilized with cefuroxime and parent compound were screened for In Vitro α-Chymotrypsin enzyme inhibition parent compound and AuNPs were inactive while AgNPs and nano-alloys were active with inhibition of 63.8% and 73.8% respectively with IC$_{50}$ values of 480.8± 8.4 and 410.7± 7.3 respectively.

In case of carbonic anhydrase AuNPs showed 57.5% inhibition with IC$_{50}$ value of 464.53 ± 11.68 while other AgNPs and Alloys stabilized with cefuroxime were inactive. (See table 8.2 for all above results)
<table>
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<th>S.No</th>
<th>Enzymes /other activities</th>
<th>Enzymes /other activities</th>
<th>Cefuroxime</th>
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<th>AgNPs</th>
<th>Alloys</th>
<th>Standard(µM)</th>
<th>Standard (µg/mL)</th>
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<td>Xanthine oxidase</td>
<td>%Inhibition</td>
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<td>NA</td>
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<td>Urease</td>
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<td>23.96±0.27</td>
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<td>Carbonic Anhydrase-II</td>
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<td>IC&lt;sub&gt;50&lt;/sub&gt;±S.E.M(µg/mL)</td>
<td>NA</td>
<td>464.53±11.68</td>
<td>NA</td>
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<td>0.12 ± 0.03</td>
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<tr>
<td>4</td>
<td>In Vitro α-Chymotrypsin</td>
<td>%Inhibition</td>
<td>15</td>
<td>45.4</td>
<td>63.8</td>
<td>73.8</td>
<td>98.6%</td>
<td>98.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IC&lt;sub&gt;50&lt;/sub&gt;±S.E.M(µg/mL)</td>
<td>NA</td>
<td>NA</td>
<td>480.8±8.4</td>
<td>410.7±7.3</td>
<td>5.7 ± 0.1</td>
<td>3.45± 0.1</td>
</tr>
<tr>
<td>5</td>
<td>Antiglycation</td>
<td>%Inhibition</td>
<td>22.21</td>
<td>4.5</td>
<td>6.2</td>
<td>2.3</td>
<td>86</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IC&lt;sub&gt;50&lt;/sub&gt;±S.E.M(µg/mL)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>294.5±1.5</td>
<td>17.093±1.5</td>
</tr>
</tbody>
</table>
8.3.3 Cephradine stabilized metal nanoparticles

Cephradine stabilized gold and silver nanoparticles and their alloys were screened against the xanthine oxidase (XO) and were found that AgNPs and nano-alloys were active with inhibition activity of 87.9% and 85.9% respectively having IC$_{50}$ value 6.9±1.5 µg/mL and IC$_{50}$ value 6.76±0.07 µg/mL respectively. AuNPs and parent compound were inactive against XO enzymes.

In case of urease enzymes the parent compound and its all nano products were active having inhibition of 89% (cephradine), 54% (AuNPs), 98% (AgNPs) and 97% (Alloys) with good IC$_{50}$ values.

All nano products stabilized with cephradine and parent compound were screened for antiglycation activity but were inactive.

These were also screened for carbonic anhydrase enzymes and found that AuNPs showed 64.2% inhibition and IC$_{50}$ value of 170.66±1.39 while AgNPs and Alloys stabilized with cephradine and the parent compound were inactive.

All nano products stabilized with cephradine and parent compound were screened for In Vitro α-Chymotrypsin enzyme inhibition but were inactive. (Please see the table 8.3)
Table 8.3: Activities of cephradine and cephradine stabilized gold and silver nanoparticles and their alloys

<table>
<thead>
<tr>
<th>S.No</th>
<th>Enzymes /other activities</th>
<th>Cephradine</th>
<th>AuNPs</th>
<th>AgNPs</th>
<th>Alloys</th>
<th>Standard(µM)</th>
<th>Standard in µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Xanthine oxidase</td>
<td>%Inhibition</td>
<td>-4.6</td>
<td>-0.7</td>
<td>87.9</td>
<td>85.9</td>
<td>98.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IC50±S.E.M(µg/mL)</td>
<td>NA</td>
<td>NA</td>
<td>6.9±1.5</td>
<td>6.76 ± 0.07</td>
<td>2.0±0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.27±0.01</td>
</tr>
<tr>
<td>2</td>
<td>Urease</td>
<td>%Inhibition</td>
<td>89</td>
<td>54</td>
<td>98</td>
<td>97</td>
<td>98.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IC50±S.E.M(µg/mL)</td>
<td>38.16±0.92</td>
<td>177.73±5.20</td>
<td>8.5±0.047</td>
<td>4.43±0.13</td>
<td>21±0.011</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.598±0.011</td>
</tr>
<tr>
<td>3</td>
<td>Carbonic Anhydrase-II</td>
<td>%Inhibition</td>
<td>38.7</td>
<td>64.2</td>
<td>-19.7</td>
<td>22.9</td>
<td>89.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IC50±S.E.M(µg/mL)</td>
<td>NA</td>
<td>170.66 ± 1.39</td>
<td>NA</td>
<td>NA</td>
<td>0.12 ± 0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.027±0.03</td>
</tr>
<tr>
<td>4</td>
<td>In Vitro α-Chymotrypsin</td>
<td>%Inhibition</td>
<td>22.3</td>
<td>26.2</td>
<td>33.5</td>
<td>5.0</td>
<td>98.6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IC50±S.E.M(µg/mL)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>5.7 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.45± 0.1</td>
</tr>
<tr>
<td>5</td>
<td>Antiglycation</td>
<td>%Inhibition</td>
<td>8.21</td>
<td>9.98</td>
<td>22.2</td>
<td>16.5</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IC50±S.E.M(µg/mL)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>294.5±1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>17.093±1.5</td>
</tr>
</tbody>
</table>
8.3.4 Ceftriaxone stabilized metal nanoparticles

Ceftriaxone stabilized gold and silver nanoparticles and their alloys were screened against the xanthine oxidase and were found that AgNPs were active with inhibition of 90.8% having IC$_{50}$ value 1.8±0.12 µg/mL. AuNPs, alloys and parent compound were inactive against XO enzymes.

In case of urease enzymes the parent compound, AuNPs and AgNPs were active having inhibition of 93 % (ceftriaxone), 97% (AuNPs), 97% (AgNPs) with good IC$_{50}$ values (please see the table 8.4) while alloys were inactive which may be because of higher concentration of metals in the alloys.

All nano products stabilized with ceftriaxone and parent compound were screened for antiglycation activity but were inactive.

These were also screened for carbonic anhydrase enzymes and found that AuNPs, AgNPs and Alloys stabilized with ceftriaxone and the parent compound were inactive. (See table 8.4 for all above results)

All nano products stabilized with ceftriaxone and parent compound were screened for In Vitro α-Chymotrypsin enzyme inhibition but were inactive
Table 8.4: Activities of ceftriaxone and ceftriaxone stabilized gold and silver nanoparticles and their alloys

<table>
<thead>
<tr>
<th>S.No</th>
<th>Enzymes /other activities</th>
<th>Enzymes /other activities</th>
<th>Ceftriaxone</th>
<th>AuNPs</th>
<th>AgNPs</th>
<th>Alloys</th>
<th>Standard( µM)</th>
<th>Standard in µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Xanthine oxidase</td>
<td>%Inhibition</td>
<td>32.7</td>
<td>9.3</td>
<td>90.8</td>
<td>4.2</td>
<td>98.6</td>
<td>98.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IC_{50}±S.E.M(µg/mL)</td>
<td>NA</td>
<td>NA</td>
<td>1.8±0.12</td>
<td>NA</td>
<td>2.0±0.01</td>
<td>0.27±0.01</td>
</tr>
<tr>
<td>2</td>
<td>Urease</td>
<td>%Inhibition</td>
<td>93</td>
<td>97</td>
<td>97</td>
<td>34.2</td>
<td>98.2</td>
<td>98.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IC_{50}±S.E.M(µg/mL)</td>
<td>80.5±0.66</td>
<td>37.53±1.46</td>
<td>4.53±0.027</td>
<td>NA</td>
<td>21±0.011</td>
<td>1.598±0.011</td>
</tr>
<tr>
<td>3</td>
<td>Carbonic Anhydrase-II</td>
<td>%Inhibition</td>
<td>3.6</td>
<td>30.3</td>
<td>-8.2</td>
<td>4.6</td>
<td>89.0</td>
<td>89.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IC_{50}±S.E.M(µg/mL)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0.12±0.03</td>
<td>0.027±0.03</td>
</tr>
<tr>
<td>4</td>
<td>In Vitro α-Chymotrypsin</td>
<td>%Inhibition</td>
<td>1.4</td>
<td>2.9</td>
<td>-2.9</td>
<td>-</td>
<td>98.6%</td>
<td>98.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IC_{50}±S.E.M(µg/mL)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>-</td>
<td>5.7±0.1</td>
<td>3.45±0.1</td>
</tr>
<tr>
<td>5</td>
<td>Antiglycation</td>
<td>%Inhibition</td>
<td>44.04</td>
<td>3.03</td>
<td>3.03</td>
<td>12.2</td>
<td>86</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IC_{50}±S.E.M(µg/mL)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>294.5±1.5</td>
<td>17.093±1.5</td>
</tr>
</tbody>
</table>
8.3.5 Cefixime stabilized metal nanoparticles

Cefixime stabilized gold and silver nanoparticles and the parent compound were screened for the inhibition of xanthine oxidase enzymes and were found that cefixime was active with an inhibition of 95.0% having $\text{IC}_{50}$ value $133.2 \pm 3.7 \mu\text{g/mL}$. AuNPs and AgNPs were inactive against XO enzymes.

In case of urease enzymes the parent compound, AuNPs and AgNPs were active having inhibition of 93 % (ceftriaxone), 97% (AuNPs), 97% (AgNPs) with good $\text{IC}_{50}$ values (please see the table 8.4) while alloys were inactive which may be because of higher concentration of metals in the alloys.

All nano products stabilized with cefixime and parent compound were screened for antiglycation activity. All Nanoparticles were inactive while the parent compound was active with an inhibition of 52.37% having $473.26\pm4.0 \text{ IC}_{50}$ value.

These were also screened for carbonic anhydrase enzymes and found that AuNPs and AgNPs stabilized with cefixime and the parent compound were inactive. (See table 8.5 for all above results)

All nano products stabilized with cefixime and parent compound were screened for \textit{In Vitro} $\alpha$-Chymotrypsin enzyme inhibition but were inactive
Table 8.5: Activities of cefixime and cefixime stabilized gold and silver nanoparticles

<table>
<thead>
<tr>
<th>S.No</th>
<th>Enzymes/other activities</th>
<th>Cefixime</th>
<th>AuNPs</th>
<th>AgNPs</th>
<th>Standard (µM)</th>
<th>Standard in µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Xanthine oxidase</td>
<td>%Inhibition</td>
<td>95.0</td>
<td>3.4</td>
<td>0.5</td>
<td>98.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IC$_{50}$±S.E.M (µg/mL)</td>
<td>133.2 ± 3.7</td>
<td>NA</td>
<td>NA</td>
<td>2.0±0.01</td>
</tr>
<tr>
<td>2</td>
<td>Urease</td>
<td>%Inhibition</td>
<td>48</td>
<td>74</td>
<td>88</td>
<td>98.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IC$_{50}$±S.E.M (µg/mL)</td>
<td>NA</td>
<td>43.23±1.63</td>
<td>41.76±0.77</td>
<td>21±0.011</td>
</tr>
<tr>
<td>3</td>
<td>Carbonic Anhydrase-II</td>
<td>%Inhibition</td>
<td>40.6</td>
<td>45.0</td>
<td>13.5</td>
<td>89.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IC$_{50}$±S.E.M (µg/mL)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0.12±0.03</td>
</tr>
<tr>
<td>4</td>
<td>In Vitro α-Chymotrypsin</td>
<td>%Inhibition</td>
<td>2.0</td>
<td>16.5</td>
<td>20.1</td>
<td>98.6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IC$_{50}$±S.E.M (µg/mL)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>5.7±0.1</td>
</tr>
<tr>
<td>5</td>
<td>Antiglycation</td>
<td>%Inhibition</td>
<td>52.37</td>
<td>14.52</td>
<td>17.47</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IC$_{50}$±S.E.M (µg/mL)</td>
<td>473.26±4.0</td>
<td>NA</td>
<td>NA</td>
<td>294.5±1.5</td>
</tr>
</tbody>
</table>

165
Conclusion

Thus I concluded that I have synthesized gold and silver nanoparticles using beta-lactam antibiotics (amoxicillin, cefuroxime, cephradine, ceftriaxone and cefixime) as caping agents. Their nano-alloys were also prepared except cefixime. NaBH₄ and Trimethylamine (TMA) were used as reducing agents and studied their effect on size and shapes of nanoparticles

- Different sized gold and silver nanoparticles were obtained for different caping agents.
- FTIR spectra showed that carboxylate group of all these drugs were involved in stabilizing gold and silver nanoparticles.
- The nanoparticles formed with the reduction of TMA were normally bigger in size as compared to those formed with the reduction of NaBH₄.
- AuNPs stabilized with amoxicillin were fluorescent and its fluorescent property was used to detect Cu²⁺ ions in water solution up to 20 x 10⁻⁹M. AgNPs and nano-alloys stabilized with amoxicillin were not fluorescent.
- Gold nanoparticles (AuNPs) stabilized with amoxicillin showed very nice inhibition for urease, carbonic anhydrase and xanthine oxidase enzymes. Silver nanoparticles stabilized with amoxicillin were active against urease and xanthine oxidase enzymes while inactive against carbonic anhydrase.
- Cefuroxime and their gold and silver nanoparticles showed best results in case of urease inhibitions. AuNPs stabilized with cefuroxime were also active against carbonic anhydrase enzymes.
- Cephradine stabilized gold nanoparticles detected Hg and Cd ions in water solution up to micro molar concentration.
- Cephradine stabilized silver nanoparticles and nano-alloys were active for xanthine oxidase enzyme, while AuNPs and parent compound were inactive against XO enzymes inhibition. AuNPs, AgNPs, nano-alloys and parent compound “cephradine” were active for urease enzymes inhibition and inactive for antiglycation activity except parent compound. AuNPs and AgNPs stabilized with cephradine
were active for carbonic anhydrase inhibition while nano-alloys and parent compound were inactive. All were inactive for *In Vitro* α-Chymotrypsin enzyme inhibition.

- Ceftriaxone capped gold and silver nanoparticles were active for urease inhibition except its nano-alloy. Only AgNPs stabilized with ceftriaxone were active for xanthine oxidase enzyme inhibition and rest of members were inactive.
- AgNPs stabilized with cefixime detected Ni\(^{1+}\) ion in water solution. AuNPs stabilized with cefixime detected Cu\(^{2+}\) ion in water solutions. Cefixime was active for xanthine oxidase enzyme inhibition and antiglycation activity while gold and silver nanoparticles were active for urease enzyme inhibition only.
References


References


References


References


References


References


References


References


References


References


References


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References


References


References


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