3.0 INTRODUCTION:

Nanoparticles of noble metals are of great interest due to their possible applications in different fields like medical [1,2], biological [3,4], catalysis [5,6], optical [7], microelectronics [8] etc. In recent years, with higher integrated density of the electronic components, there are growing demands for the decrease in the thickness of the conductive films, narrowing of the width of printed circuits and the space between these circuits. It is thus required that the powders composed in the paste should have as small diameter as possible. Therefore synthesis of such particles is a challenging task.

A number of chemical and physical methods are being used to synthesize transition metal nanoclusters. These chemical methods [9-11] are generally based on the reduction of metal salts by a variety of reducing agents such as citrate, hydrazine, ferric ions, formaldehyde, ethanol, boron hydrides and ascorbic acid. Depending upon the reducing power of these reagents, the synthesis reactions are carried out at various temperature and other conditions to achieve reasonable rates. To prevent undesired agglomeration with formation of large particles in the range of micrometer or metal powders, stabilizers such as special ligands, polymers [12,13] or tetra alkyl ammonium salts generally need to be used. In the physical method, metal nanoparticles are formed through free radical mechanism. These methods have been discussed in details in Chapter I.

Of all the methodologies developed for the production of metal nanoparticles, the electrochemical method offers an alternative simple means within reverse micelles in organic solvent systems. Now we have successfully applied it to the preparation of transition metal mono and bimetallic nanocluster within suitable mixture of organic solvents and have developed a unique synthetic route in preparing sufficient yields of these nanoparticles. Our synthetic approach is to control the growth and particle size by introducing ‘size control’
reagent in to the electrochemical system in which appropriate surfactants or ligands are employed as both supporting electrolyte and the stabilizer for the resulted nanoparticles. Other parameter for controlling the size of nanoparticles is current density in electrochemical reduction method.

Pure and size selective nanoparticles can be prepared by electrochemical reduction method. This process makes use of an inexpensive two electrode set up for 25-30ml electrolyte solution. A sacrificial anode consisting of the bulk metal transformed into metal nanocluster. Inert platinum sheet is used as a cathode. The supporting electrolyte used is tetra alkyl ammonium salt which also serves as stabilizer for metal clusters. Organic solvents are used to prevent possible metal hydride formation.

During the synthesis the bulk metal anode is oxidized and converted into metal cations. These cations migrate to the cathode and reduction takes place with the formation of metal in zero oxidation state.

\[
\begin{align*}
\text{Anode} & \quad - \quad \text{M bulk} \rightarrow \text{M}^{n+} + \text{ne}^- \\
\text{Cathode} & \quad - \quad \text{M}^{n+} + \text{ne}^- + \text{stabilizer} \rightarrow \text{M} \text{ coll/Stabilizer} \\
\text{M bulk+stabilizer} & \quad - \quad \text{M} \text{ coll/stabilizer}
\end{align*}
\]

Where, M bulk = metal bulk sheet,
M coll/stabilizer = ammonium salt stabilized colloidal metal cluster.

The transition metal cluster in the range 1-20 nm range has been prepared by this method for fundamental and practical reason. The particle size of nanostructured metal clusters can be controlled by varying parameters such as types of ligands, their concentration, solvent and current density in electrochemical synthesis.

Quaternary ammonium compounds are a group of ammonium salts in which organic radicals are substituted for all four hydrogen of the
original ammonium cation. They have a central nitrogen atom, which is joined to four organic radicals and one acid radical. The organic radicals may be alkyl or aryl and the nitrogen can be part of a ring system. They are prepared by treatment of an amine with an alkylating agent. They show a variety of physical, chemical and biological properties and most compounds are soluble in water and act as a strong electrolyte. In electrochemical synthesis, the choice of tetra alkyl ammonium surfactant is governed by following constraints:

i) It should be absorbed on the metal oxide surface to stabilize the synthesized nanoparticles.

ii) It should be highly soluble and dissociated in the solvent to play the role of electrolyte.

iii) The hydrophilic–lipophilic balance of the chosen surfactant should be sufficiently high to prevent any precipitation, due to the presence of water even in small quantities in the medium.

The surfactants that satisfied the above constraints such as tetra propyl ammonium bromide (TPAB), tetra ethyl ammonium bromide (TEAB), and tetra butyl ammonium bromide (TBAB) are used in the present work and their structures are shown in Fig.3.0.1.

![Figure 3.0.1](image)

**Fig. 3.0.1.** (a) Tetra ethyl ammonium bromide (TEAB), (b) Tetra propyl ammonium bromide (TPAB), (c) Tetra butyl ammonium bromide (TBAB)
A controlled current electrolysis is used throughout the synthesis process. The basic working principle is simple viz. the electrochemical reduction of metal salts in presence of surfactants which serves as the electrolyte and as the stabilizer.

The salient features of these types of metal colloids are

i) High yield,

ii) Absence of undesired side products such as metal hydrides or boron impurities,

iii) Easy isolation,

iv) Variation of solubility of proper choice of ammonium salt,

v) Control of particle size by adjustment of the current density.

Metal alloy nanoparticles are of great interest in surface science since they can exhibit electronic, optical and catalytic properties that are quite different from those of their constituents [14-18]. An important phenomenon that occurs on alloy surface is the segregation by which the surface chemical composition is completely modified. Some of the factors [18] affecting the surface segregation of an alloy AB are

i) Bond strengths of A-A, A-B and B-B,

ii) Atomic size of the individual components A and B,

iii) Enthalpies of sublimation and

iv) Surface energies.

Since bimetallic oxide clusters are of great interest in catalysis and electrocatalysis [19]. The copper based nanomaterials have been attracted a lot of attention in the past due to their non-toxicity, interesting electronic structures and promising applications in photovoltaic and solar energy cells [20-43]. Also they have full miscibility and high chemical stability so that they can be used in almost any environment with no risk of rapid chemical reactions.

Bimetallic nanoparticles have been obtained using different synthetic methods including replacement reaction [44], biosynthesis [45], green
synthesis [46], alcohol reduction [47], borohydride reduction [48], ultrasound irradiation [49] and metal evaporation-condensation method [47]. However, relatively few methods [50-52] produce true alloy nanoparticles due to phase separation at the atomic level leading to the formation of core shell particles.

### 3.1 Synthesis of Monometallic Copper, Silver and Molybdenum Nanoparticles:

The electrochemical reduction method for the size selective preparation of tetra-alkyl ammonium salt stabilized transition metal clusters in the size range of 1-50 nm has been reported [53,54]. The tetra-alkyl ammonium salts are very effective phase transfer catalysts which are used as stabilizers in the synthesis of nanoclusters of transition and noble metals. They have weak interaction with the nanoparticles and can be exchanged. They also help to prevent oxidation of the metal atoms.

In the present work we have prepared tetra-alkyl ammonium salt stabilized copper, silver and molybdenum (oxide) monometallic nanoparticles separately using the electrochemical reduction method of Reetz [54]. The mechanism proposed for this method is shown in Fig. 3.1.1. In this work we have also verified the effect of concentration and nature of tetra-alkyl ammonium salt and the effect of the current density on the particle size.

The electrolysis cell designed for the solution volumes of about 25-30 ml was used in electrolysis process. The sacrificial anode of pure bulk metal sheet (Cu/Ag/Mo) 1cm x 1cm and an inert cathode of platinum sheet 1cm x 1cm were arranged parallel to one another 1cm apart from each other in the electrolysis cell. Dry oxygen free solvent consisting of a mixture of acetonitrile (ACN) and tetra hydro furan (THF) in the ratio of 4:1 respectively was used for preparing the solution of supporting electrolyte cum stabilizer which is either TBAB or TPAB or TEAB. Trials were conducted to determine the optimum
concentration to be used during electrolysis. It was observed that a concentration of 0.01M was most effective for better synthesis. This 30 ml solution of supporting electrolyte was taken in a vessel and electrolysis was carried out by passing an electric current of constant current densities for two hours. Stirring of the solution was done by bubbling U.H.P. nitrogen through the solution. All the while electrolysis process was carried out at room temperature. In the electrolysis process whatever physical changes that were produced in the solutions were carefully recorded and the formation of metal nanoparticles was observed by monitoring the change of color. The electrolysis duration was kept constant for 2 hours, when quantitative dissolution of the anode metal occurred. After allowing the suspension to settle for another two hours the tetra-alkyl ammonium bromide stabilized metal nanoparticles formed agglomerates and settled at the bottom of the electrolysis vessel. The solid particles settled at the bottom of the bottle were separated from the solution by simple decantation and the decanted solid product was washed with dry THF for three to four times to remove excess tetra-alkyl ammonium bromide. The supernatant was also collected and preserved for further analysis. The products were then dried under vacuum in desiccators. The samples were preserved in air tight capsule for further analysis. The supernatant solution after first decantation was used for UV-visible spectroscopic analysis and the optical properties were observed as a function of current density. The solid obtained as metal nanoparticles were preserved for further characterization.
Reaction Mechanism for Electrochemical Synthesis of Metal Clusters:

\[
\text{Anode} \quad \text{Met}_{\text{bulk}} \quad \rightarrow \quad \text{Met}^{n+} + \text{ne}^- \\
\text{Cathode} \quad \text{Met}^{n+} + \text{ne}^- + \text{stabilizer} \quad \rightarrow \quad \text{Met}_{\text{col}/\text{stabilizer}}
\]

**Fig. 3.1.1** Mechanism for the electrochemical reduction synthesis of metal colloids and nanoparticles
3.2 Variation of Parameters in Synthesis of Cu, Ag and Mo Monometallic Nanoparticles:

a) Effect of nature of stabilizer:-
In order to study the effect of nature of stabilizer, the electrolysis process was carried out using different quaternary ammonium salts like TBAB, TPAB and TEAB. Nanoclusters prepared by using these quaternary ammonium salts were characterized by different analytical techniques.

b) Effect of concentration of stabilizer:-
The concentration of each stabilizer was varied to study the concentration effect on the particle size and effective formation of copper, silver and molybdenum (oxide) nanoclusters. Optimization of concentration of the stabilizer was made and it was found that 0.01 M solution of each stabilizer is proper for efficient formation of metal oxide nanoclusters.

c) Effect of current density:-
In order to study the effect of current density on particle size, the current in the electrolysis process was varied for each stabilizer from at 6 to 10 mA/cm². At optimum concentration (0.01M) of each stabilizer, the effect of current density was studied by characterizing the samples, using different analytical techniques such as UV visible spectroscopy, FTIR, XRD, SEM-EDS and TEM. It was found that variation in current density influences the potential at cathode and ultimately it affects the particle size of nanoclusters in the electrolysis process.

d) Effect of pH:-
pH measurements have been made before and after electrolysis for each solutions using different stabilizer and the results were compared. As can be seen from table 3.2.1 that basicity of ammonium ion goes on increasing with increase in number of alkyl groups. This is
due to electron donating nature of alkyl group which reduces the strength of positive charge present on ammonium ion and hence it creates more tendencies to donate unshared electron pair which is responsible for increase in pH for higher alkyl quaternary ammonium salts in organic solvent. The same trend of further increase in basicity with increase in number of alkyl groups was observed after electrolysis confirming the formation of coordinate covalent bond of metal with ligand.

Table 3.2.1: Comparison of pH effect on electrolysis as a function of capping agent

<table>
<thead>
<tr>
<th>Capping agents</th>
<th>pH before electrolysis</th>
<th>pH after electrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEAB</td>
<td>6.80</td>
<td>8.77</td>
</tr>
<tr>
<td>TPAB</td>
<td>7.00</td>
<td>9.25</td>
</tr>
<tr>
<td>TBAB</td>
<td>7.56</td>
<td>11.5</td>
</tr>
</tbody>
</table>

3.3 Synthesis Of Cu-Ag And Cu-Mo Bimetallic Nanoparticles

Electrochemical reduction method has been adopted for synthesis of bimetallic nanoparticles in the present study. The method employed was similar to the one used for the monometallic nanoparticles as discussed in fig 3.1.1.

Electrolysis cell (for solution volume of 25-30 ml) was designed to carry out electrolysis process. In this cell, two anodes (Cu-Ag or Cu-Mo) were positioned in such a way that they lie parallel to each other and apart from each other by a distance of 1x1 cm. The inert cathode, platinum foil was placed between the two anodes and it was perpendicular to both of them.

In typical procedure, the above electrolysis vessel having anode (Cu-Ag or Cu-Mo) 1 x 1cm and a platinum cathode (1 x 1cm) were employed in the preparation of Cu-Ag or Cu-Mo bimetallic nanoclusters. A
mixture of acetonitrile and tetrahydrofuran (4:1) was used as solvent. The supporting electrolyte consisted of solution of ligand, tetra-alkyl ammonium bromide (TEAB/TPAB/TBAB) at 0.01M. The supporting electrolyte used also act as stabilizer in the formation of bimetallic nanoclusters.

Electrolysis was carried out at room temperature by passing the current of various current densities (6 or 10 mA/cm²) for two hours in each experimental set. The colour changes were observed and noted during the electrolysis process. After two hours of electrolysis process the concentration of the nanoparticles increased sufficiently leading to their agglomeration process. After the electrolysis time, the solid particles of the nanoparticles settled at the bottom of the vessel. The reaction mixture was transferred into the clean and dry bottle along with solid particles and then after 15 to 20 minutes decantation process was followed for separation. The solid as well as the supernatant were collected separately. The solid sample was washed 2 to 3 times with THF to remove the excess ligand. The samples were then dried in vacuum desicator and further stored in airtight capsules for characterization. The supernatant obtained after first step of decantation was preserved for UV visible analysis.

### 3.4 Variation Of Process Parameters In Synthesis Of Cu-Ag And Cu-Mo Bimetallic Nanoparticles

In the preparation of the bimetallic nanoparticles, a number of parameters have been varied in order to observe the effect on the synthesis of nanoparticles. These parameters are same as those studied in the synthesis of monometallic nanoparticles and discussed at Fig. 3.2. They include,

a) Effect of nature of stabilizer

b) Effect of concentration of stabilizer

c) Effect of current density

d) Effect of pH
3.5 Sample Preparation for Characterization Techniques:

a) **UV visible spectroscopic analysis:**
Supernatant samples were used to record colour and study surface plasmon absorption in the UV-visible region using Jasco UV-Visible Spectroscopy (UV 540).

b) **Fourier Transform Infrared Spectroscopy:**
The synthesized solid nanoclusters were mixed with KBr and FTIR spectra were recorded in the range of 400 to 4000 cm\(^{-1}\) on Jasco FTIR 8400 Spectrophotometer.

c) **X-ray Diffractometry:**
The powdered X-ray diffraction patterns were obtained out on X-ray powder diffractometer PW-1840, using CuK\(_\alpha\) radiation (\(\lambda = 1.54 \, \text{Å}\)). The samples were scanned from 20-82° at the scan rate of 5 x 10\(^4\) CPS. The study was carried out at the Department of Physics, Pune University, Pune.

d) **Scanning Electron Microscopy:**
The scanning electron microscopy study was carried out on JEOL make JSM 63608A microscope at the Department of Physics, Pune University, Pune.

e) **Transmission Electron Microscopy:**
The synthesized nanoclusters were ultrasonicated in ethanol and then a drop of the dispersed nanoparticles was placed onto a carbon coated 400 mesh copper grid with format coating over it, followed by natural evaporation. HR-Transmission electron microscopic study and electron diffraction were carried out on Philips CM 200kv at the Indian Institute of Technology, Mumbai and UGC sponsored DAE LAB Indore.
f) **X-ray Photoelectron Microscopy:**

X-ray Photoemission Spectroscopy studies were performed on VG Micro Tech ESCA-3000 Electron Spectrometer at a pressure of 1.33 x 10\(^{-8}\) Pa, with MgK\(\alpha\) exciting radiation. This study was carried out at the UGC sponsored DAE LAB Indore.

3.6 **Antimicrobial Study Of Monometallic And Bimetallic Nanoparticles:**

**A) Bacteria and Health:**

Bacteria are involved in many aspects of ecology and health, for example we use bacteria for making yoghurt, curd, cheese and other fermented foods and also large numbers of bacteria live on the skin and in the digestive tract. The human gut contains more than 1000 bacterial species which are beneficial [55]. Gut bacteria synthesize vitamins such as Folic acid, Vitamin K and biotin and they ferment the complex, indigestible carbohydrates. Other useful bacteria in the gut flora include Lactobacillus species which convert milk sugar into Lactic acid. Bacteria also play important role in the medicine such as vaccine component and in the production of antibiotics, drugs, hormones, antibodies. On the other hand a pathogenic bacterium causes an enormous level of spoilage, suffering and death through the infection [56].

![Cell Structure](image)

**Fig. A.1** Cell Structure
B) **Bacterial Infection:**

Many microorganisms are pathogenic to human or animals and cause various diseases resulting in extensive morbidity and mortality. The most common bacterial disease is the Tuberculosis caused by Mycobacterium tuberculosis, a leading cause of the death worldwide [57]. Each pathogenic species participates in a characteristic spectrum of interactions with its host. Some microorganisms such as *Staphylococcus* or *Streptococcus* species cause skin infection and other forms of surface infection [58]. On the other hand, many organisms are also part of normal flora exist on the skin and in the nose without causing any disease at all. Obligate intracellular parasites such as, Rickettesia and Chlamydia are able to grow and reproduce only within host cells [59]. Finally some species such as *Pseudomonas aeruginosa* are opportunistic pathogens and cause disease in the individual suffering from immune suppression or cystic fibrosis cells [60]. Other common diseases include upper respiratory tract infections (URTI) such as acute otitis media and sinusitis, lower respiratory tract infections (LRTI) such as pneumonia and bronchitis, urinary tract infections (UTI), gastrointestinal tract infections, sexually transmitted diseases, endocarditis, septicemia, sepsis and Lyme disease [61].

C) **Antibacterial activity:**

The microorganisms have minute body size and simple structure. They reproduce at rapid rate and produce physical and chemical changes in their environment and causes beneficial as well as harmful effect on human being. The microorganisms are the main causes for any metabolic disturbance in humans. The disease causing microorganisms are called pathogens. The growth of pathogens and other harmful bacteria controlled by antibacterial agents are natural as well as synthetic molecules. The antibacterial agents which are obtained from natural sources are termed as antibiotics. The antibacterial agents may be biotic just inhibiting the growth of bacteria or biocidal and kill the bacteria.
Antibacterial activity of monometallic and bimetallic nanoparticles has been tested against two gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* and two gram negative bacteria *i.e.* *Escherichia coli* and *Salmonella typhi*.

Highly purified samples of the synthesized nanoparticles were tested for their *in vitro* antibacterial activity by Agar-agar plate diffusion technique using acetonitrile and THF as a solvent.

**i) Bacillus subtilis:**

It is **Gram-positive** bacteria. A species of bacillus found in soil and decomposing organic matter, some strains of its produce antibiotics. A member of the genus *Bacillus*, *B. subtilis* is rod-shaped and has the ability to form a tough, protective **endospore** allowing the organism to tolerate extreme environmental conditions.

![Bacillus subtilis](image)

**Fig. C. 1:** *Bacillus subtilis*

It produces the proteolytic enzyme **subtilisin**. Its spores can survive the extreme heating that is often used to cook food and it is responsible for causing *ropiness*, a sticky, stringy consistency caused by bacterial production of long-chain **polysaccharides** in spoiled bread dough. Its other uses include the following:

1. *B. subtilis* strain QST 713 has a natural fungicidal activity and is employed as a biological control agent.
2. It is popular worldwide before the introduction of consumer antibiotics as an immunostimulatory agent to aid treatment of gastrointestinal and urinary tract diseases. It is still widely used in Western Europe and the Middle East as an alternative medicine.

3. It can convert explosives into harmless compounds of nitrogen, carbon dioxide and water plays a role in safe radionuclide waste [e.g. Thorium (IV) and Plutonium (IV)] disposal with the proton binding properties at its surfaces.

4. Recombinants *B. subtilis* str. pBE2C1 and *B. subtilis* str. pBE2C1AB were used in production of polyhydroxyalkanoates (PHA) and that they could use malt waste as carbon source for lower cost of PHA production.

**ii) Staphylococcus aureus:**

*Staphylococcus aureus* the and most common cause of staph infections is a spherical bacterium frequently living on the skin or in the nose of a healthy person that can cause a range of illness from minor skin infections (such as pimples, boils and cellulitis) and abscesses to life-threatening diseases such as pneumonia, meningitis, Endocarditi Toxic Shock Syndrome (TSS) and septicemia.

*S. aureus* was discovered in Aberdeen Scotland in 1880 by the surgeon Sir Alexander Ogston in pus from surgical abscesses [62].

*S. aureus* is a gram-positive coccus which appears as grape like clusters when viewed through a microscope and has largened round golden-yellow colonies often with β-hemolysis when grown on blood agar plates [63]. The golden appearance is the etymological root of the bacteria’s name: aureus means “golden” in Latin.
iii) *Escherichia coli:*

*Escherichia coli* (*E.coli*) is one of the main species of bacteria living in the lower intestines of mammals known as gut flora. When located in the large intestine it actually assists with waste processing vitamin K production and food absorption. It was discovered in 1885 by Theodor Escherich a German pediatrician and bacteriologist [64]. The *E.coli* strain O157:H7 is one of hundreds of strains of the bacterium that causes illness in humans [65]. As with all gram negative organisms *E.coli* are unable to sporulate. Thus treatments which kill all active bacterial such as pasteurization or simple boiling are effective for their eradication without requiring the more rigorous sterilization which also deactivates spores. As a result of their adaptation to mammalian intestine *E.coli* grow best *in vivo* or at the higher temperatures characteristic of such an environment rather than the cooler temperatures found in soil and other environments.

*E.coli* can generally cause several intestinal and extra-intestinal infections such as urinary tract infections meningitis, mastitis septicemia and gram negative pneumonia. The enteric *E. coli* are divided on the basis of virulence properties into enterotoxigenic (ETEC causative agent of diarrhea in humans, pigs, sheep, goats, cattle, dogs and horses) enteropathogenic (EPEC causative agent of diarrhea in
humans, rabbits, dogs, cats and horses); enteroinvasive (EIEC found only in humans) verotoxigenic (VTEC found in pigs, cattle, dogs and cats); enterohaemorrhagic (EHEC found in humans, cattle and goats attacking porcine strains that colonize the gut in a manner similar to human EPEC strains) and enteroaggregative E. coli (EAggEC found only in humans).

![Image](image_url)

**Fig. C. 3:** *Escherichia coli*

### iv) *Salmonella typhi:*

It is **Gram-negative bacterium** posses rod-shaped, **flagellated** and aerobic structure. It is a member of the genus *Salmonella*, which are pathogenic, causing food poisoning, typhoid and paratyphoid fever in humans and other infectious diseases in domestic animals [66] *Salmonella enterica typhi* or *S. typhi* is an obligate parasite which has no known natural reservoir, outside of humans.
Salmonella enterica Serovar Typhimurium (also known as S.Typhimurium) can lead to a form of human gastroenteritis sometimes referred to as salmonellosis.

The genome sequences of serovar Typhimurium LT$_2$ [67] have been established. Also an analysis of the proteome of Typhimurium LT$_2$ under differing environmental conditions has been performed [68].

Salmonella enterica Serovar Paratyphi A has been identified [69]. It is associated with paratyphoid fever. It is sometimes known as Salmonella Paratyphi.

### 3.6.1 Experimental Procedure For Antibacterial Testing:

The antibacterial activity of monometallic and bimetallic nanoparticles was screened in vitro against gram positive and gram negative human pathogens using Ampicillin as standard.

#### a) Types of Bacteria:

I. Gram (+ve) bacteria–*Bacillus Subtilis* (ATCC No. 6633), *Staphylococcus aureus* (ATCC No. 25923).

II. Gram (-ve) bacteria–*Escherichia coli* (ATCC No. 25922), *Salmonella typhi* (ATCC No. 23564).
b) **Materials:**

i) Nutrient agar (12-15 mL)

ii) Sterile petri dishes

iii) Old grown culture (24 hours) in test tube.

iv) Sterile pipettes

v) Test tube containing solution of the compound to be tested with known concentration in ACN:THF.

c) **Preparation of Sub–Culture:**

Antibacterial activities were studied by the paper disc plate method [70] A uniform suspension of test organism of 24 hours old culture was prepared in test tube containing sterile saline solution. A sterile nutrient agar was then added in each of the petri dishes. The dishes were related to ensure the uniform mixing of the micro organism in the agar medium which was then allowed to solidify. The agar cups were prepared with sterile cork borer bearing suitable dimension. The solution of each compound to be tested for aseptically into each cup. The ACN: THF was used a control of the solvent incubated at 37°C for 24 hours. The concentration of the test compound in ACN:THF was 25 and 50µg ml⁻¹. Amphicillin was used as a standard compound for comparison. After incubation the inhibitory zones around the agar cups were observed. The diameter of inhibition zones were measured in terms of mm.

d) **Zones of Inhibition:**

i) Strong growth inhibitor (zone size 15- 20mm)

ii) Moderate growth inhibitor (Zone size 9-14mm)

iii) Less growth inhibitor (Zones size 6-8 mm)

iv) No growth inhibitor

Keeping above mentioned points in view, the screening of the metal nanoparticles for antimicrobial activity has been performed.
3.7 REFERENCES:


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