



*Chapter 1*  
*Introduction &*  
*Review of Literature*



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## GRAPHICAL OVERVIEW OF THE CHAPTER

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### HIGHLIGHTS OF THE CHAPTER

- **Introduction of nanotechnology**
- **History of nanotechnology**
- **Classification and types of nanoparticles**
- **Synthesis methods of M-NPs**
- **Characterization techniques of M-NPs**
- **Application fields of M-NPs**

# CHAPTER 1

## INTRODUCTION AND REVIEW OF LITERATURE

### 1.1 GENERAL INTRODUCTION

Change is a spontaneous and unavoidable process. The spontaneous processes lead to rise in the overall entropy of the system and its surroundings and are irreversible in both natural and thermodynamic sense. The entropy rise explains both the asymmetry between the past and the future and the irreversibility of natural processes [1]. Nanotechnology is a fast-evolving sector of science and technology that has changed our way of life in a variety of ways. In our daily lives, we drink water coming from water filters that uses 15-20 nm particles to filter viruses and other microbial pathogens and at work we often use computers that uses 45 nm CMOS integrated circuits. Nanotechnology makes use of nanoscience knowledge to modify and utilise nanoscale objects for the production of valuable commodities. Nanotechnology and nanoscience are two words that frequently confound people and are inextricably linked. The function of physical systems with sizes near to the atomic or nanoscale is the subject of nanoscience. Nanoscale objects have radically different magnetic, electrical, and chemical characteristics than bulk matter [2], [3]. Nanoscience is the study of the properties and behaviour of nanoparticles, whereas nanotechnology is the use of nanoscience knowledge to produce useful products [4].

Prior to actually going into the realm of nanotechnology, it's vital to understand the some of the most commonly used terms in the domain. The word nano comes from the Greek word nanos, which means "dwarf". According to International Organisation for Standardization (ISO) term nanomaterial is used for the materials having any of its dimension (external or internal) in nanoscale [5] and covers size from 1-100 nm. The term nanoparticle is used for the nano-objects having all the dimensions in the nanoscale [6], [7]. Another most commonly used term in the field of nanotechnology is nanostructures which stands for the structure consist of discrete functional parts either externally or internally with more than one dimensions in the nanoscale range [8]. The word "nanocomposite" refers to multiphasic constructions with at least one dimension restricted to the nanoscale range [9]. The process of developing a consistent language to eliminate ambiguities and assist legislation and regulations, risk analysis, and communication is referred to as term standardisation. In the track of standard terminology

development, an international committee ASTM published standards to define terminologies in the field of nanotechnology [10].

## **1.2 BIRTH AND DEVELOPMENT OF NANOTECHNOLOGY**

Nanotechnology's imprints are similar to that of science. During the ninth century in Mesopotamia, craftsmen provided evidence of the earliest usage of nanoparticles [11]. Nanoparticles during that period was used as decorative purpose to glitter the pot surfaces. Nanoparticles of silver and copper were used to generate shiny effects on ceramics [12]. The ceramics of Middle Ages and Renaissance were decorated with copper or gold particles [13]. Alloy of gold and silver (Au-Ag) was used in AD 400 by Roman people for making Lycurgus cup to exhibit green colour on reflection and red colour on transmission of light [14]. Nanoparticles during AD 300-1700 were used by Damascans and Romans for making steel swords with features like exceptional strength, crack proof, and sharp edges [15]. During AD 800 in the Mayan city of Chichen Itza a corrosion resistant azure pigment commonly called Maya Blue was discovered [16]. The production of nanocomposites and the characteristics of the finished products were not fully understood in these times. However, as nanotechnology advanced, a number of scientists started to link these ancient products to the nanotechnology [17], [18].

However, scientifically Michael Faraday published the first scientific paper on the subject in 1857. This report explained the synthesis of colloidal solution of gold nanoparticles (AuNPs) by the name “activated gold” at Royal Society of London. He proposed his statement on the “activated gold” that “gold reduced in exceedingly fine particles, which becoming diffused, produce a ruby red fluid, the various preparations of gold, whether, ruby, green, violet or blue consist of that substance in a metallic divided state” [19]. He also demonstrated the Tyndall effect possessed by colloidal gold. In 1940s, silica nanoparticles were produced and sold in the United States and Germany [20]. “There is plenty of room at the bottom” Feynman said in a lecture in 1959, referring to the latent potential of dimensionally tiny matter. Furthermore, he also claims that “The principles of physics, as far as I can see, do not speak against the possibility of manoeuvring things atom by atom” [21]. His words, however, were not well received at the time and were dismissed as speculative and science fiction. The first report of the production of a citrate stabilised silver colloid was published in 1889 [22], which began the scientific history of silver nanoparticles (Ag-NPs). Commercial production of colloidal Ag-NPs for medicinal applications began in 1897 under the name "Collargol" [23].

However, the stabilization of Ag-NPs using proteins was reported in the year 1902 [24]. Furthermore, the synthesis of gelatine stabilized Ag-NPs was reported in 1953 [25]. A detailed developments in the field of nanotechnology have been represented in Figure 1.1 [26], [27].

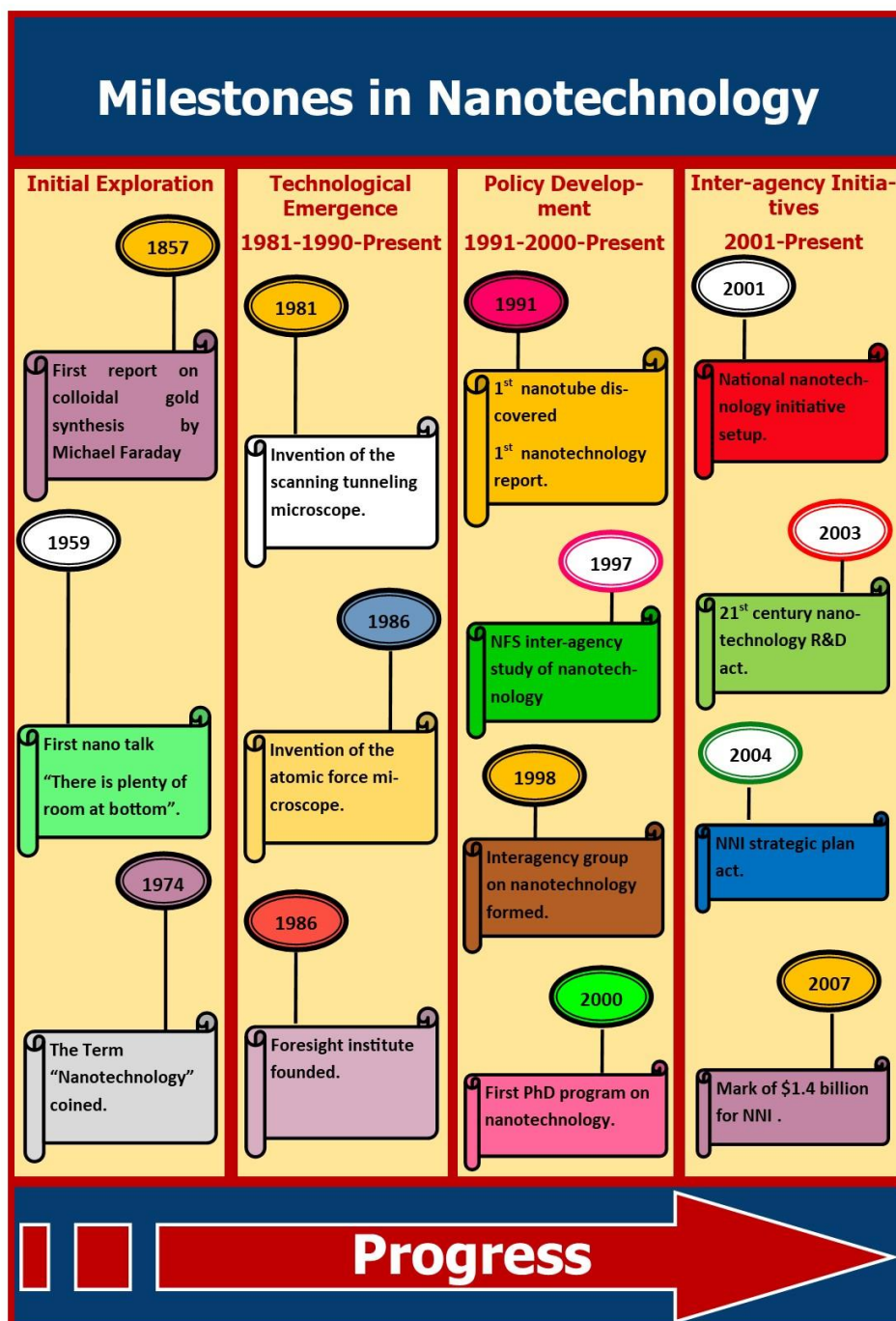
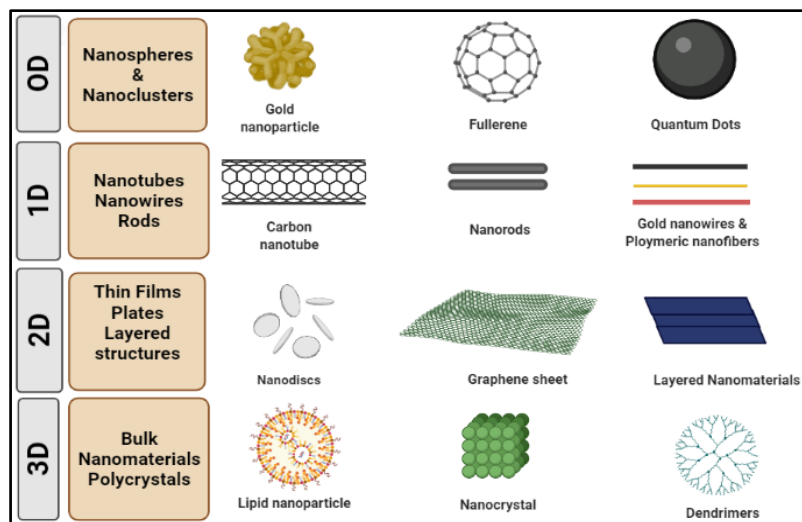


Figure 1.1: Milestone in the development of nanotechnology [27].

### 1.3 CLASSIFICATION OF NANOMATERIALS

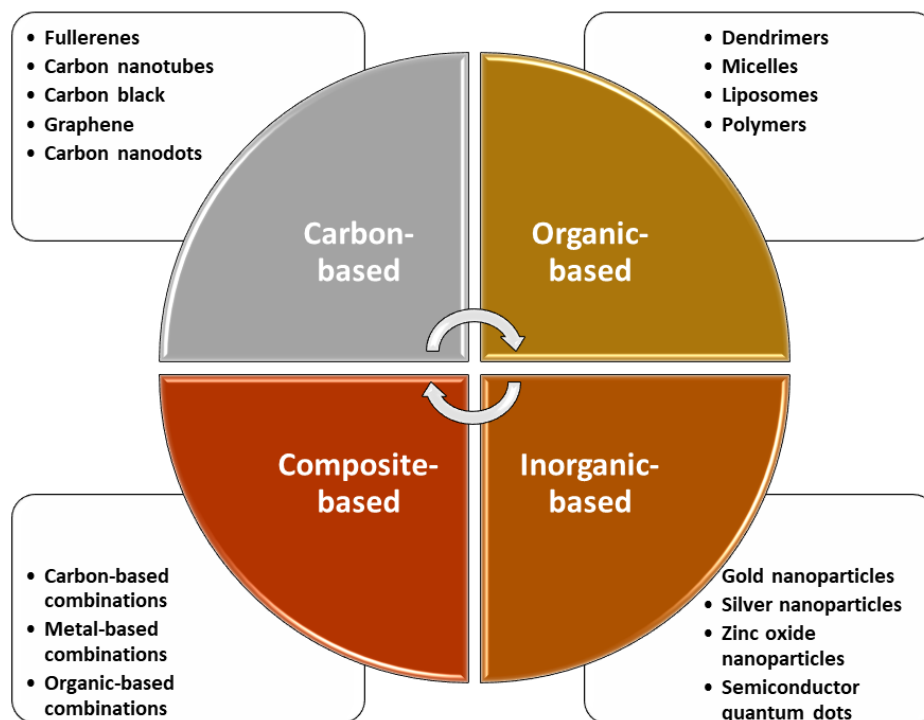
In order to classify the nanomaterial, it becomes important to understand its physical as well as chemical nature. Nanomaterials may be categorised into four groups based on their dimensions, as illustrated in Figure 1.2, namely zero-dimensional (0D), one-dimensional (1D), two-dimensional (2D), and three-dimensional (3D) [28]. It was Gleiter in 1995 who first time proposed the idea to classify the nanomaterials [29]. He classified them based on their crystalline nature and chemical compositions but the idea was incomplete as he did not consider the dimensions of the materials for the classification. After 20 years, Pokropivny and Skorokhod proposed a new classification method based on dimensions i.e., 0D, 1D, 2D, and 3D nanomaterials [30]. Zero-dimensional nanomaterial: 0D nanomaterials are those in which all of their dimensions limit themselves in nanoscale range (0-100 nm). Nanoparticles generally comes under this category. One dimensional nanomaterial: 1D nanomaterials have one of their dimensions outside nanoscale range. Nanotubes, nanowires and nanorods are examples of this category. Two-dimensional nanomaterials: 2D nanomaterials exhibits two of its dimensions outside the nanoscale range. Nanofilms, nanolayers, nanocoating's and graphene are examples of this category nanomaterials. Three dimensional nanomaterials: (3D) structures are materials having three arbitrary dimensions beyond the nanoscale (>100 nm). However, these materials possess a nanocrystalline structure or involve the presence of peculiarities at the nanoscale. They can be composed of multiple arrangements of nanosize crystals, most typically in different orientations. Bulk powders, bundles of nanowires, multiple layers of nanotubes are some examples of this category [30].



**Figure 1.2:** Nanomaterial's classification based on dimension [28].

## 1.4 TYPES OF NANOPARTICLES BASED ON COMPOSITION

Nanoparticles may be divided into several categories depending on their geometries, sizes, dimensions, homogeneity, and aggregation. The shapes of nanoparticles are diverse due to multiple methods of their synthesis. Some of the important characteristics that are accounted during shape-based classification are: sphericity, aspect ratio and flatness. Nanoparticles having a large aspect ratio generally achieve helical, zigzag and belt form morphology while nanoparticles having a small aspect ratio generally exhibit spherical, oval, cubical, triangular, spiral and pillar morphologies [30], [31]. Further based on the composition the nanomaterials can be categorized into four major groups viz. carbon-based nanomaterials [32], organic-based nanomaterials [33], [34], inorganic-based nanomaterials [35], [36], and composite-based nanomaterials [32], [37]–[40] (Figure 1.3).



**Figure 1.3:** Nanomaterial's classification based on composition.

### 1.4.1 Carbon-based nanomaterials

Carbon-based materials are one of the most well-known types of nanomaterials. Carbon nanotubes, graphene, fullerenes, carbon nanofibers, carbon dots, and carbon black are among them. Graphene is made up of carbon atom sheets that are one atom thick [41]. Although its industrial production process is in development, so many possible uses have been already

recognised. Some of them are electrodes for solar cells and organic light-emitting diodes (LEDs), electronic components with a faster electron speed than silicon, hydrogen storage, for supercapacitors electron storage, in membranes for gas separation, and sensors. They're also employed as composite reinforcement, atomic force microscope tips, and in bone growth scaffolding [42].

#### **1.4.2 Organic-based nanomaterials**

Natural or synthesized organic compounds serve as templates for organic nanoparticles. Liposomes, dendrimers, ferritin and micelles are examples of organic nanoparticles. These nanoparticles are biodegradable, non-toxic, and may be consists of hollow core (nanocapsules). These capsules are sensitive to thermal and electromagnetic radiation. Both "top-down" and "bottom-up" methodologies can be used to make organic nanoparticles [43]–[46].

#### **1.4.3 Inorganic-based nanomaterials**

Metal or non-metal element could be used to make an inorganic nanomaterial, which can also be in the type of a hydroxide, oxide, semiconductor quantum dots, and chalcogenide. Photonics, chemical sensors, electronics, biomedical devices, and biosensors are only a few of the applications for these materials in modern society. The applications take use of the nanomaterials unique electrical, optical, and catalytic capabilities, which can be tweaked by changing its shape, size, and composition [47]–[49]. Inorganic nanoparticles can be synthesised using a variety of ways. Inorganic nanomaterials have been synthesised using physical methods such as microwave irradiation, mechanical milling, and sonication, chemical methods such as sol–gel synthesis, co-precipitation, and hydrothermal methods [50], and biological methods.

#### **1.4.4 Composite-based nanomaterials**

Composite nanomaterials are novel materials that have recently earned a lot of appreciation because of their scientific and technical significance. Catalysts with high activity and selectivity, metal semiconductor junctions, optical sensors, and modifiers of polymeric films for packaging are only a few of their uses. By integrating the appropriate, desirable traits of several materials to build a new material with a broad spectrum of desirable characteristics, composite nanomaterials can attain versatility [51]. Presently, polymer-based nanocomposites are front runners in among many nanocomposites [52]–[56].



## 1.5 NANOPARTICLES CHARACTERISTIC PROPERTIES

Nanoparticles/nanomaterials exhibits unique physico-chemical characteristics due to their shape and size as compared to their bulk counterpart [57]. The physico-chemical properties of nanoparticles like surface area, mechanically strength, optical activity and chemically reactivity contributes for extraordinary properties of nanoparticles [58]. Characteristics properties of various nanoparticles have been listed in Table 1.1.

**Table 1.1:** Characteristics of different kind of nanoparticles.

| Type                     | Properties  | Examples  | References |
|--------------------------|---|---|------------|
| Carbon-based             | High surface area to volume ratio, inert, semi-conductor, conductor, superconductor, light absorption and transmission, thermal conductivity, tensile strength, elasticity and flexibility, UV-resistant, high mechanical properties, strength, and biocompatibility. | Fullerenes, graphene, carbon nanotubes, carbon nanofibers and black carbon.   | [59]–[62]  |
| Inorganic-based (metals) | High surface area to volume ratio, reactivity, moisture sensitive, heat, light absorption and scattering, semi-conductor, stable, ductile, thermal, and electrical conductivity, and toxicity.  | Gold, silver, iron, aluminium, cobalt, cadmium, lead, copper, and zinc.   | [63]–[70]  |
| Metal oxide-based        | High surface area to volume ratio, magnetic, reactive, UV-filtering, moisture sensitive, and heat.  | Titanium, iron, zinc, cerium, aluminium, and magnetite oxide, and silicon dioxide.  | [71]–[76]  |
| Organic based (polymer)  | High surface area to volume ratio, easy synthesis, cost-effective, bio-compatible, easily bio-degradable, and non-toxic.  | Chitosan, poly D, L-lactic-co-glycolic acid, poly methyl methacrylate, poly phenyl acetylene, and poly $\gamma$ -glutamic acid nanoparticles. | [77]–[85]  |

Although all types of nanoparticles have their own value and have been utilized in the various sectors but in the current piece of work, we are focused on metal nanoparticle due to their

distinctive properties. In the subsequent sections detailed description of metal nanoparticles, their characteristics, synthesis and applications have been described.

## **1.6 METAL NANOPARTICLES (M-NPs)**

In the field of modern nanotechnology, M-NPs have attracted the scientific community towards itself with more enthusiasm since their discovery by Faraday in 1857 [19]. The reason for this huge focus of interest is due to its unique properties. Nanoparticles of metals like zinc (Zn), copper (Cu), iron (Fe), gold (Au), silver (Ag) etc has been reported from various methods [86]–[89]. Any of the synthesis process (physical, chemical, and biological processes) can be used to make these nanoparticles, which can then be modified with various functional groups of interest [90], [91]. The surface qualities such as greater surface area to volume ratio, surface charge, shape, size, crystallinity, and reactivity are some of the functional aspects of M-NPs. These properties enable them for applications in the field such as biosensors [92], imaging [93], drug delivery systems [89], [94]–[98], conformational studies of biomolecules [99], cosmetics [100], foods [101], feeds [102], mechanics [103], electronics [104], energy devices [105], and medicinal applications [106], [107]. The physiochemical properties, stability, reactivity, photothermal, and plasmonic properties of M-NPs advocates them as suitable carriers for therapeutic drugs. The plasmonic properties of M-NPs such as gold nanoparticles have been utilized in photothermal therapy for killing brain tumor cells [108], [109]. Similarly, the metallic quantum dots and magnetically active iron nanoparticles have been used in bioimaging and drug delivery [89], [110]–[112].

### **1.6.1 Characteristic properties of M-NPs**

M-NPs have earned great scientific interest in past couple of decades due to their particular properties [113], [114]. The properties of materials at the nanoscale usually get changed due to the quantum size effect [114]. It is well known that when the size of a substance shrinks to nanoscale, the number of atoms on its surface rises due to increase in the surface-to-volume ratio. Substantial surface area to volume ratio, greater surface energy, quantum confinement, and surface plasmon resonance (SPR) are all characteristics of M-NPs.

In comparison to bulk material, nanoparticles with a larger surface area to volume ratio have more active sites on their surface per unit area which influences the surface-related properties of the materials [114]. M-NPs have a higher surface energy, which effects particle nucleation and development, and hence has a significant impact on their application and

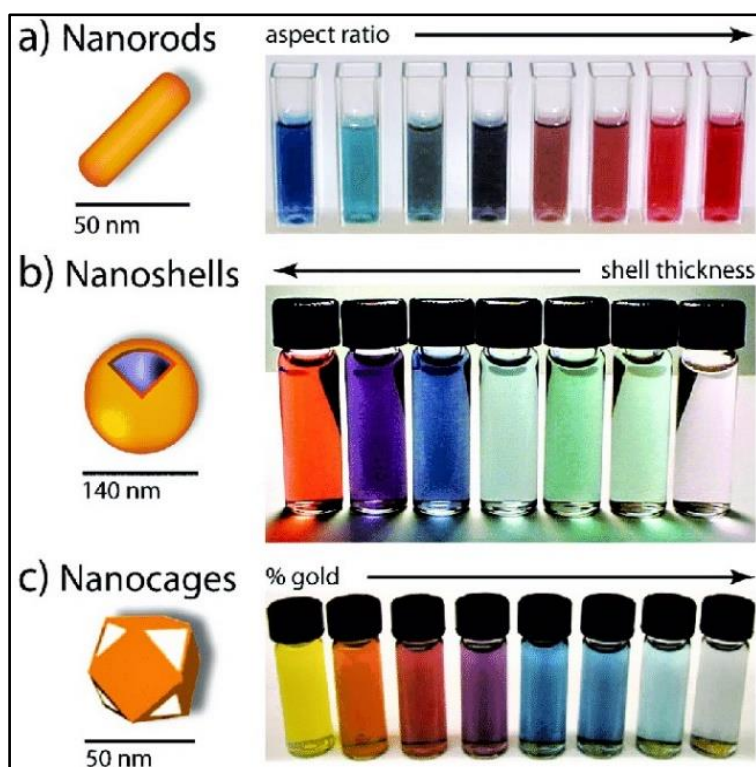
environmental impact. Surface energies of M-NPs can also be size dependent [115]. The surface energies of M-NPs also play a great role in understanding its thermodynamics [116]. Quantum confinement is a key feature of M-NPs, particularly in semiconductors. Quantum confinement is the energy of confined electrons (electrons or electron-hole). The quantum confinement effect is observed when the size of the particle is too small to be comparable to the wavelength of the particles. So as the size of a particle decrease till we reach a nano scale the decrease in confining dimension makes the energy levels discrete and this increases or widens up the band gap and ultimately the band gap energy also increases [117], [118]. These discrete energy band gaps are very suitable for the transport of photogenerated charge carriers, hence have great importance in field of photocatalysis. Quantum confinement is exhibited by semiconductors in all the three directions [119]. SPR is described as the cumulative vibration of M-NPs conduction band (CB) electrons in resonance with incoming photons. However, the size and structure of the M-NPs have a significant impact on the interaction. Furthermore, the type and chemistry of the M-NPs dispersion medium have an impact on the interaction [120]. A thorough understanding of mechanism of the interaction of nanoparticle with various morphologies and sizes can be used for biosensing applications and imaging purposes. The SPR property of M-NPs has been greatly explored in developing colour sensors and sensor devices [121]. High zeta potential of M-NPs helps them in preventing aggregation in solution, hence provides greater stability to nanoparticles [122]. In the subsequent sections M-NPs from some of the most widely used metals have been discussed.

### **1.6.2 AuNPs**

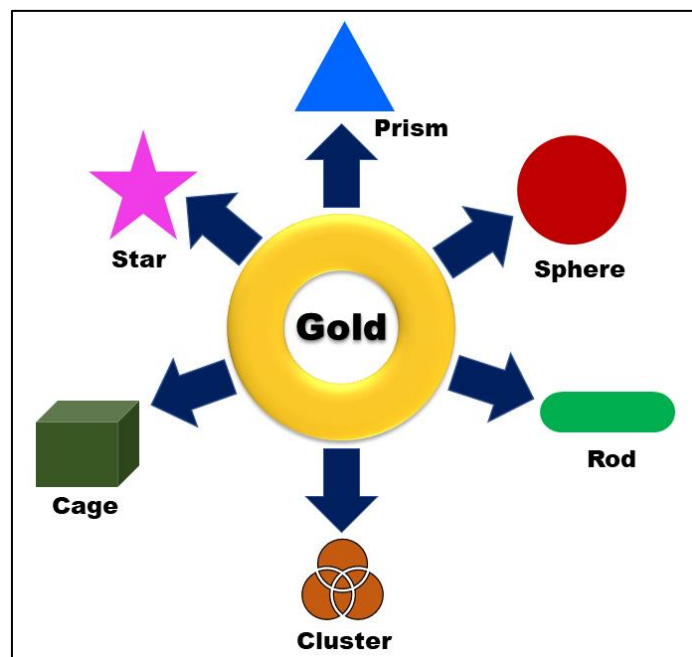
Gold (atomic number 79, atomic mass 196.966) is a rare metal with relatively stable chemical properties. It is a rich and opulent metal with a lengthy history [123]. Due to its aqueous solubility, high stability, and size and morphology controllability, gold nanoparticles (AuNPs) are an interesting type of nanoparticles among all nanoparticles [124]. AuNPs because of unique SPR and physical characteristics, widely employed for labelling, imaging, and sensing purposes. However, due to progressive advancements they are also being used for disease diagnosis and therapeutics [123]–[126]. Manipulation of AuNPs' size, shape, surface chemistry, and aggregation state can readily change their optical and electrical characteristics [127], [128]. The surface chemistry of AuNPs, as well as their underlying molecular principles, are crucial for applicability. Proteins, polymers, nucleic acids, medicinally important molecules, and polysaccharides are commonly used to functionalize AuNPs for a variety of purposes [128]–

[132]. AuNPs may be produced in two ways: top-down or bottom-up.

The size of AuNPs varies in nanoscale, while it can have shapes like spheres, rods, nanotriangles nanocages, nanoshells, and nanostars [133], [134]. The shape and size of AuNPs highly depend on the method used for synthesis [124], [133]. The bulk gold has bright yellow colour. However, the colour of AuNPs varies on the basis of their size and shapes (Figure 1.4,1.5) [134]. The variations in the properties of AuNPs are because of band gap. The band gap increases as particle size decreases. The energy levels of AuNPs become distinct at the size of a fermi wavelength (0.5 nm), which has a significant impact on their electrical, magnetic, and optical characteristics [135], [136]. AuNPs possess excellent bio-compatibility and colloidal stability, which advocates them for various kind of biomedical applications [137]–[139]. The conjugation of AuNPs with biomolecules also known as bioconjugation enables its uses in biosensors for diagnostic purposes [139], [140].



**Figure 1.4:** Variations in the colour of gold nanospheres, nanorods, and nanocages with respect to change in size [141].



**Figure 1.5:** Different shapes of AuNPs [134].

### 1.6.3 Ag-NPs

Silver (Ag) is a durable metallic element with atomic mass of 107.86. It ranks 67<sup>th</sup> among all elements in terms of abundance. Silver also has a white metallic shine, which has led to its widespread usage in jewellery, money coins, and cutlery [142]. Silver vessels were used to keep the water and wines pristine in ancient times. The use of silver for medicinal reasons may be traced all the way back to antiquity. In 17<sup>th</sup> and 18<sup>th</sup> centuries ulcers were treated with silver nitrate [143]. In 1884, silver nitrate (1%) solution was used by German obstetrician Crede in new-born baby to inhibit gonococcal conjunctivitis [144]. Later in 1967, silver sulfadiazine was introduced by Fox for the treatment of burn patients. However, silver sulfadiazine cream is also used in current time to treat serious burn wounds [145]. Lea published the first scientific paper on the manufacture of Ag-NPs (using citrate as a reductant and stabilizing agent) in 1889. However, at that time it was known as silver colloid [146]. Since their initial synthesis in 1897, silver colloids have been used in medicine for over a century, according to a literature review. These were referred to as "Collargol" rather than "nanoparticles" at that time [147]. However, the first bactericidal silver substance, known as "Algaedyn," was approved to be used as a disinfectant in the United States in 1954 [24].

Ag-NPs possess several novel properties as compared to the bulk metal, hence has drawn great scientific interest. Elemental silver possesses high thermal and electrical

conductivity, hence it is greatly used in electronics. Ag-NPs due to their small size possess large surface area, surface energies and more reactive sites. Because of the significant surface plasmon resonance (SPR) that Ag-NPs display in response to incoming light, they may be used for a variety of biosensing and diagnostic applications [148]. Antibacterial activity of Ag-NPs against Gram-positive (*Staphylococcus aureus*), Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*) [149], [150], multidrug-resistant bacteria (*Pseudomonas aeruginosa*), and erythromycin-resistant bacteria (*Streptococcus pyogenes*) [151] and many other types of bacteria has been recognised. Due to the prolonged release of silver ions, Ag-NPs have been shown to have strong antifungal potency (*Aspergillus fumigatus*, *Mucor*, and *Candida tropicalis*) and antiviral activities [152], [153]. Ag-NPs have a number of advantages over other M-NPs, including higher electrical conductivity, higher temperature stability, good chemical stability, outstanding catalytic activity, and lower cost than gold and platinum [154]–[159]. Ag-NPs can be produced by photochemical synthesis [160], electrochemical synthesis [161], chemical synthesis [162], physical synthesis [163], and green synthesis [164]. The cost of the synthesis process, the instrumentation facilities, the possible dangers involved, and the applications all play a role in the method selection. To address the bulk of market demands for Ag-NPs and other M-NPs, physical and chemical manufacturing methods are being utilised [165].

#### **1.6.4 Copper nanoparticles (CuNPs)**

Copper (Cu) is a metal having atomic number of 29 and atomic mass of 63.55. Electrical conductivity, thermal conductivity, good ductility, malleability, corrosion resistance and great tensile strength are some of its characteristics to list. These properties of copper make it an essential element in the society from thousands of years [166], [167]. They find their applications in the optics, electronics, and medical fields. However, it also has uses in preparation of lubrications, nanofluids, and conductive films [168], [169]. The preference of CuNPs over Ag-NPs is because of its low cost and availability [169]. Although CuNPs of smaller size possess high activity but some time it may form clusters which decreases its essential properties [170].

The size of CuNPs has a significant impact on features such as electron affinity, electronic transitions, magnetic properties, melting temperature, and affinity towards compounds. Quantum size of CuNPs along with coulomb charging are responsible factors for quantum effects [169]. The quantum effects in CuNPs are generally prominent in spherical

nanoparticles and sharp edge nanoparticles [166]. CuNPs are more difficult to synthesise than gold or Ag-NPs because of their higher redox potential [166].

### **1.6.5 Platinum nanoparticles (Pt-NPs)**

Platinum (Pt) is a chemical element having atomic number 78 and atomic mass of 195.084. It is a dense, malleable, ductile, unreactive, and precious transition metal [171]. The abundance of Pt in earth is very low i.e., 0.01 ppm. Along with Pt, Pt-NPs are outstanding tools in various biotechnological, nano-medicinal, and pharmacological fields. Pt-NPs individually and in combination with alloys exhibit excellent catalytic properties due to significance of large surface area. Pt-NPs are widely used as catalyst to reduce pollutants, for organic acid production [172], dehydrogenation of compounds [173], [174], and hydrogenation processes for the production of biofuels [175], vitamins, and fats [176]. They're also utilised in environmentally friendly technologies like remediation of pollutant aromatic compounds [177], in harvesting solar energy [178], and for waste water processing and purification [179]. Pt-NPs also exhibit great antimicrobial, antioxidant, and anticancer properties, which makes them valuable in medicine and diagnostics [176].

Pt-NPs preparation has progressed significantly in recent years. Pt-NPs with cubical, cubooctahedron, octahedron, nanorods and other shapes have been synthesized. The nanotubes and hollow nanostructure of Pt-NPs were obtained using templates or through galvanic replacements [180]. In contrast to pure Pt-NPs, the alloys of Pt-NPs are also very useful [181]. The bulk of Pt-NPs were created via physical and chemical approaches throughout the early phases of research. On the other hand, green synthesis of Pt-NPs has emerged as a promising alternative to conventional techniques for manufacturing Pt-NPs that are both cost-effective and ecologically benign [182]. The properties of Pt-NPs in combination with materials such as metals, polymers, targeting agents, etc could be utilized for as photothermal-therapy, radiotherapies, and to control pathogenic microorganisms [172].

### **1.6.6 Zinc oxide nanoparticles (ZnO)**

Zinc takes its name from the German word "zink," which means "tip" or "point," since when zinc ores are heated, they form sharp pointed crystals. Zinc has five stable isotopes while sphalerite is most common ore of it. It was used to make brass from the first century BC (Roman civilisation) (copper and zinc alloy). Coinage and other beautiful metal works were made from brass. [183]. The Hindu ruler Madanapala (1374) termed zinc "Yasada or Jasada" in his medical

dictionary [184], [185]. However, its biomedical usage as a therapeutic skin cream dates back to ancient Egypt, when it was used to cure skin ailments and heal wounds [186]. Zinc was also used in lotions and cosmetics during Roman civilization [187]. For the very first occasion, Paracelsus (the father of pharmacology, 1493-1549) recommended zinc sulphate as a medicinal agent, revolutionising biomedical science [185]. However, it was Gaubius in 1771, highlighted zinc therapy named “luna fixa” in his scientific paper for the first time for the treatment of convulsions and spasms [188]. This “luna fixa” was nothing but ZnO. It is a semiconductor-class inorganic molecule. At typical room temperature, it has 3.37 eV energy gap. The catalytic, optoelectronic, electrical, and photochemical characteristics of zinc oxide are well recognised [189]. Because of its enormous surface area and high catalytic activity, ZnO nanoparticles/clusters are a popular catalyst for a variety of catalytic processes. In addition to the qualities listed above, ZnO is a distinct antibacterial agent. The scientific community is very interested in improving the antimicrobial potential of ZnO through their combination with other semiconductor materials and doping them. Moreover, a variety of capping agents (polymers and plant extracts) may be utilised to modify the shape and size of ZnO, hence improving its antibacterial action[185], [190]–[192].

## **1.7 Quantum dots**

The credit for the discovery of quantum dots goes to Brus, Efros, and Ekimov (1980) according to Optical Society of America [193]. Quantum dots covers size range from 2 to 10 nm. Quantum dots stand out for their distinctive size and shape dependent optoelectronic capabilities, which have recently found applications in bioimaging as well as many other fields including photodetectors, light-emitting diode (LED) manufacturing, solar cell technology, and computing [194], [195]. Quantum dots can be of various types depending upon their modifications with several polymeric, inorganic, organic, or biological materials [196].

### **1.7.1 Properties of quantum dots**

A quantum dot or semiconductor is differentiated by its bandgap energy, which is the power needed to drive an electron from one electronic band into another at a higher energy level. An exciton, a pair of electrons and holes, is the result of this excitation process. The exciton releases energy as a fluorescence photon after regaining relaxation and returning to its ground state [193], [195]. Quantum size effect is an exciting phenomenon shown by quantum dots or semiconductor materials. The quantum size effect occurs when the excitation and emission



wavelengths drop as the size of the nanocrystal decreases, the energy band gap widens, and vice versa. By changing the particle size, it is possible to adjust the fluorescent colour that the quantum dots emit when exposed to UV light [197].

## 1.8 APPROACHES FOR M-NPs SYNTHESIS

Literature studies display that there are numerous methods of nanoparticles synthesis. The synthesis of M-NPs may be divided into two types: top-down and bottom-up (Figure 1.6). Top-down approach can be defined as approach in which nanoparticles are produced from the breakdown of bulk material by various physical, chemical, and mechanical approaches. This technique encompasses all physical methods of nanoparticle production. A bottom-up strategy is one in which atoms or molecules are used as precursors for the production of M-NPs. This methodology encompasses both chemical and biological nanoparticle production approaches (Figure 1.6) [90]. All though all types of nanoparticles can be synthesized from top-down and bottom-up approaches, however subsequent sections emphasize M-NPs synthesis using both the approaches.

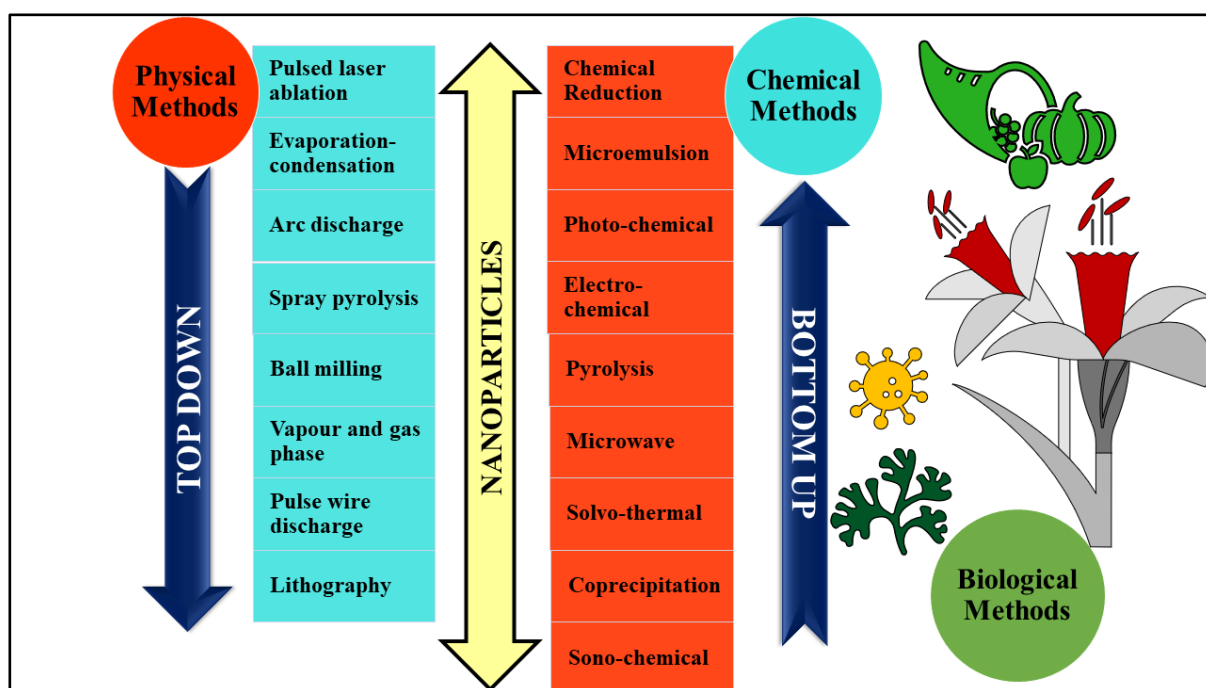


Figure 1.6: Approaches of M-NPs synthesis (adapted from [198]).

### **1.8.1 Top-down approach of M-NPs synthesis**

Top-down approach utilizes various physical and chemical treatments to reduce the size of bulk material. Generally top-down methods are operationally easy but production of small size and desired shape is very difficult to achieve from this approach [90], [199]. Mechanical milling also called physical milling, nano-lithography, sputtering, laser ablation technique, and temperature dependent breakdown (thermal breakdown) techniques of M-NPs manufacturing are some of the examples of this approach.

#### **1.8.1.1 Mechanical milling**

Mechanical milling involves basic principle of reduction in the size of particle due to high energy in ball milling. The ball milling technique was developed in 1970 by John Benjamin [200], [201]. Milling may be classified into two types based on the amount of mechanical energy used throughout the operation. However, one of the two processes, high intensity ball milling, is known to yield nano-scale particles [90]. Mechanical milling is most commonly used top-down method to produce M-NPs. The milling process involves addition of bulk powder and heavy rotating balls into a container followed by implementation of high mechanical energy. A number of high-energy mills (attrition ball mills, planetary ball mills, vibrating ball mills, high-energy ball mills, etc.) can be used to make nanoparticles [200]. Mechanical milling has the major benefit of being able to generate high-purity nanoparticles on a wide scale. However, this approach has numerous drawbacks, including a high energy need, a long processing time, and contamination from milling balls. Aluminium, magnesium, and carbon are often blended using this method. Additionally, it is used to generate elemental aluminium powder and beta-silicon carbide [90].

#### **1.8.1.2 Physical vapour deposition (PVD)**

PVD is a vaporisation coating process that includes atomic-level material transfer. The following sequence of stages can be used to explain the process. Firstly, the material that need to be deposited is transformed to a vapour either by gaseous plasma or high-temperature vacuum. In the next step, the vapour is transferred to a low-pressure zone followed by the vapours condensation on suitable substrate to produce a thin layer. PVD methods are often used to deposit thin films with variable thicknesses [90].

### **1.8.1.2.1 Laser ablation**

To decrease particles to nanoscale, laser ablation methods use laser irradiation. The target substance (typically a solid material) is put beneath a thin layer in this process. The substance is then irradiated with a pulsed laser. Laser irradiation causes solid material to fragment, eventually resulting in the production of nanoparticles [202]. Some of the most popular lasers used to make nanoparticles include the neodymium-doped yttrium aluminium garnet laser, titanium-doped sapphire laser, and copper vapour-based laser [202]–[204]. The size, yield, and stability of nanoparticles are influenced by factors such as laser pulse time, wavelength used, the time period of ablation, laser fluency, and surrounding liquid media. Surfactants in the surrounding medium have a big influence on the ablation efficiency and attributes of the M-NPs that are being produced [204]. Laser ablation is a good way to get a lot of nanoparticles in a small amount of time. This method is commonly used to produce  $\text{Al}_2\text{O}_3$  nanoparticles coating and silicon nanoparticles [205], [206]. For the industrial scale production of nano-size particles in suspension, this process is very easy and efficient. The characteristics of nanoparticles may be altered by adjusting the laser setting and liquid type. Nanoparticles can be formed in liquid media without the need of a surfactant.

### **1.8.1.2.2 Nanolithography**

The term “Lithography” can be defined as transfer of an image from a template to a substrate. In 1700s lithography was primarily used in printing. Nanolithography is a Greek word and has meaning “tiny writing on a stone”. Thus, nanolithography can be defined as a process of producing/imprinting nano size patterns on the substrate. Additionally, it is involved in the research and use of nanoparticles in nanofabrication and nanopatterning [207]. Lithography can be categorised into two categories masked and mask less lithography techniques. Masked lithography technique required a mask with projected / grooved pattern. This grooved or projected patterns on mask eventually transferred to a substrate. Masked lithography can be executed by three types photolithography, soft lithography, and nano-imprint lithography. In contrast, non-masked or direct write lithography has cost and convenience advantages [208]. Scanning probe lithography and scanning electron beam lithography and are examples of mask less lithography technologies. The primary benefit of lithography is that it is an inexpensive, simple to implement, inherently parallel, and high throughput method to produce large variety of nanoparticles. Furthermore, soft lithography can make nanostructures from a variety of

materials, including the complex organic molecules that are required for biological research [209].

### **1.8.1.2.3 Ion sputtering**

The ejection of atoms from the surface (known as target) due to the shelling of high energy particles is known as sputtering. Hence, sputtering can also be called a momentum transfer process. The sputtered atoms travel to a substrate and form the desired layer. The discharge of glow because of applied electric field between the two electrodes (at low gas pressure) is the common and simplest source of ions for the sputtering process. Gas breaks down to carry electricity (called plasma) at a specific point of lowest voltage. Plasma ions are propelled towards the target and deposit on the substrate. This kind of sputtering process is known as “DC sputtering”. In most of sputtering cases, an inert gas (argon) is used. Reactive sputtering, on the other hand, occurs when a reactive gas is introduced along with inert argon to form the plasma with the goal of depositing a specific chemical compound (for example, oxide and nitride) [90], [210]. There are some of the advantages of ion sputtering to produce nanoparticles. The sputtered material's composition is unchanged and identical to the target substance for non-reactive sputtering while for reactive sputtering the sputtered materials composition changes. For refractory metals and intermetallic complexes, this approach is preferred over evaporation and laser ablation. Sputtering equipment is less costly than electron-beam lithography systems, making this a cost-effective approach. Process produces fewer contaminants. The sputtering methods are very good for making alloy nanoparticles due to better control on composition as compared to other chemical reduction methods [90], [211].

## **1.8.2 M-NPs synthesis through a bottom-up technique**

The bottom-up method (constructive method) of nanoparticle synthesis is the polar opposite of the top-down method. Nanoparticles are created using this process using relatively basic materials. Pyrolysis, CVD, spinning, sol-gel, and biological synthesis are all examples of bottom-up methods.

### **1.8.2.1 Chemical vapour deposition method of M-NPs synthesis**

Chemical vapor deposition (CVD) technique of M-NPs production is a vacuum deposition method to produces high-quality, high-performance, and solid materials. The synthesis in CVD method includes chemical reaction between an organometallic or halide compounds (deposited

on substrate) and gases to produce non-volatile solid thin films on substrates (glass, and quartz). The major difference between CVD and physical vapor deposition (PVD) is that in case of CVD the deposition of material onto the substrate is multidirectional, whereas in the PVD methods the deposition is line-of-site impingement [212], [213]. Metal and carbon-based nanoparticles may both be made via CVD. The most significant benefit of CVD procedures is that the nanoparticles generated are hard, robust, uniform, and very pure, while downsides include the need for specialised equipment and the generation of highly hazardous gaseous by-products [213].

### **1.8.2.2 Sol-gel procedure of M-NPs synthesis**

M-NPs production using the sol-gel technique is a flexible soft chemical process. The operational simplicity of the sol-gel approach makes it more exciting to produce nanoparticle. Briefly, the sol-gel process involves the transition of a solution system from a liquid "sol" (mostly colloidal) into a solid "gel" phase. The starting materials used in the preparation of the "sol" are usually inorganic metal salts or metal organic compounds such as metal alkoxides. In a typical sol-gel process, the precursor is subjected to a series of hydrolysis and polymerization reactions to form a colloidal suspension, or a "sol". Further processing of the "sol" makes it possible to make materials in different forms. In a nutshell, the synthesis process involves a sequence of hydrolysis and polycondensation processes that result in a colloidal suspension called "sol". To obtain the metal oxide nanoparticle, the created "gel" is dried by calcination at various temperatures. Metal oxides, ceramics, and glass nanoparticles are commonly synthesised using this method [214], [215]. Sol-gel method has several advantages such as metastable nanoparticle, purity, homogeneity, and low temperature synthesis. The low temperature synthesis of TiO<sub>2</sub> nanoparticles has been reported using precursor such as TiCl<sub>3</sub>, TiCl<sub>4</sub>, Ti(OBu)<sub>4</sub>, and Ti[OCH(CH<sub>3</sub>)<sub>2</sub>]<sub>4</sub> (TTIP) [216], [217].

### **1.8.2.3 Spinning method of M-NPs synthesis**

To make desired nanoparticles, the spinning process employs a spinning disc reactor (SDR). Physical factors of SDR may be changed to produce nanoparticles of the appropriate shape and size. To prevent oxygen and other chemical reactions from occurring within the SDR chamber, it is usually filled with inert gases like nitrogen [218], [219]. At the point when the precursor (liquid) and water are introduced to reactor, the rotation speed of disc can be changed. The spinning of the disc brings atoms and molecules closer together. The fused molecules are

thereafter precipitated and collected followed by drying. The operating parameters such as flow rate of liquid, speed of disc rotation, the ratio of liquid/precursor, feed location, and disc surface governs the characteristics of synthesized nanoparticles [220], [221]. For example, Vilardi et al [218] produced iron nanoparticles (0.24-24 kg/day) using SDR at a rotational velocity of 1400rpm of an average size of  $28 \pm 2.1$  nm. Similarly, ZnO (57 kg/day) of approximately 47-56 nm has been reported using SDR [219].

#### **1.8.2.4 Pyrolysis method of M-NPs synthesis**

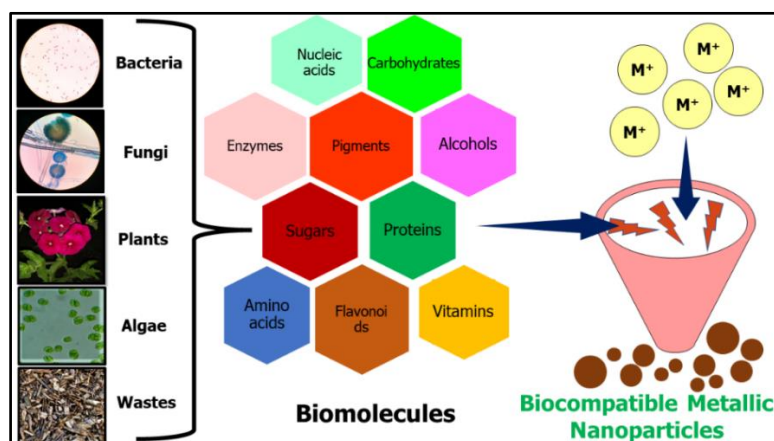
Pyrolysis can be defined as an endothermic process used to thermally convert organic material into valuable fuels and chemicals in an oxygen-deficient environment due to accelerated heating [222]. Pyrolysis is among the most prevalent processes for producing nanoparticles on a large scale. In the pyrolysis method, the precursor is burned (flame/resistive heating) to produce nanoparticles. The nature of the precursor can be liquid or gas in this method. Thus, precursors in the furnace are exposed to high pressure and temperature. However, the flame can be replaced with laser or plasma in this method. The biggest advantage of using high temperature is fast evaporation [223], [224]. The major advantages of the pyrolysis method for nanoparticles synthesis are efficiency, cost effectiveness, simplicity, and continuous process with high yield [225]. For example, the magnetic iron-oxide nanoparticles ( $\text{Fe}_2\text{O}_3$ ,  $\text{Fe}_3\text{O}_4$ , and  $\text{FeO}$ ) have been reported to be prepared by flame spray pyrolysis (FSP) method under controlled atmospheric conditions [226]. In another finding, adsorption low-temperature pyrolysis for the Ru nanoparticles on carbon has been reported for the production of hydrogen [227].

#### **1.8.2.5 Chemical reduction method**

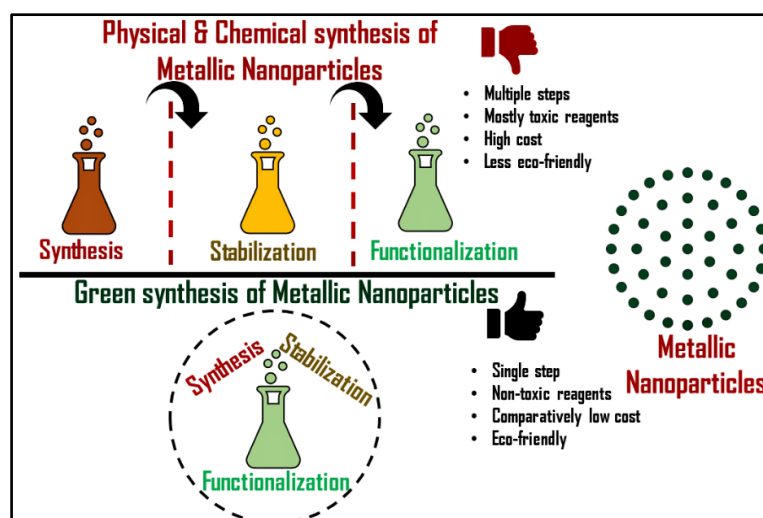
The production of M-NPs through chemical reduction methodology is extremely prevalent. The chemical reduction approach entails reducing ionic salt in a suitable media with a variety of reducing agents [198], [228]. The reduction of metal salts into M-NPs has been achieved through variety of agents viz. trisodium citrate, sodium borohydride, and N,N dimethylformamide [88], [229]–[233]. In addition, chemical approaches use a stabilizer or capping ingredient to keep M-NPs stable viz. trisodium citrate, riboflavin, citrate/l-cysteine, polyvinyl alcohol, and thioglycolic acid [234]–[245]. Particle size and shape, on the other hand, may be modified by adjusting variables such as metallic salt concentration, reducing agents, pH of system, and temperature.

### 1.8.2.6 Biological methods

The biological method to M-NPs production is also known as "green synthesis," and it often employs biological sources as reducing and capping agents, such as bacteria, algae, fungus, lichens, and plant extracts or compounds from these sources (Figure 1.7) [198], [246]. In terms of reaction steps, hazardous chemicals, and biocompatibility, present physical and chemical procedures have limitations. These techniques require different reagents in each of the phases, such as a reducing agent, a stabilising agent, and a functional reagent, due to the multistep synthesis. Biological synthesis, in contrast to conventional approaches, has several benefits, such as reducing the amount of chemicals required in the synthesis process and shortening the synthesis stages (Figure 1.8) [247]–[250]. Furthermore, green synthesized nanomaterials are eco-friendly and more biocompatible [251]–[253].



**Figure 1.7:** Various biological sources and biomolecules explored for M-NPs synthesis (adapted from [198]).



**Figure 1.8:** Comparison among various synthesis approaches of M-NPs (adapted from [198]).

#### **1.8.2.6.1 Bacteria**

Bacteria have capacity to convert metallic ions into nanoparticles, making them a promising source for M-NPs synthesis. Bacteria are a fascinating source for M-NP synthesis since they are easy to handle and grow quickly. Furthermore, microorganisms for the biomineralization (metal ions) process can also be genetically modified [254]. The unique capabilities of bacteria for synthesis of M-NPs may be attributed by adaptations to continuous exposure to harsh and toxic environment. In addition, bacteria have a variety of ways to deal with stress conditions. They can sequester metallic ions from the surroundings, presence of efflux pumps helps to ooze out toxic ions, and precipitation of ions in the extracellular environment (Figure 1.9a).

#### **1.8.2.6.2 Fungi**

Production of M-NPs through fungi as biological source is widely known as myco-synthesis, however the technology of nanoparticle synthesis can be referred as myco-nanotechnology [255]. Fungi have several benefits over other biological sources for M-NPs synthesis, including high tolerance to heavy metals, simplicity of culturing, and extracellular synthesis (reduces down-stream cost). Fungi have many proteins, enzymes, cofactors, and other metabolites in their cells and surfaces that help to reduce metal ions into nanoparticles [256]. Nanoparticles of metals such as silver, cadmium, copper, zinc, palladium, barium, platinum, gold, iron, titanium, selenium, zirconium, and cobalt has been reported from various types of fungi [257].

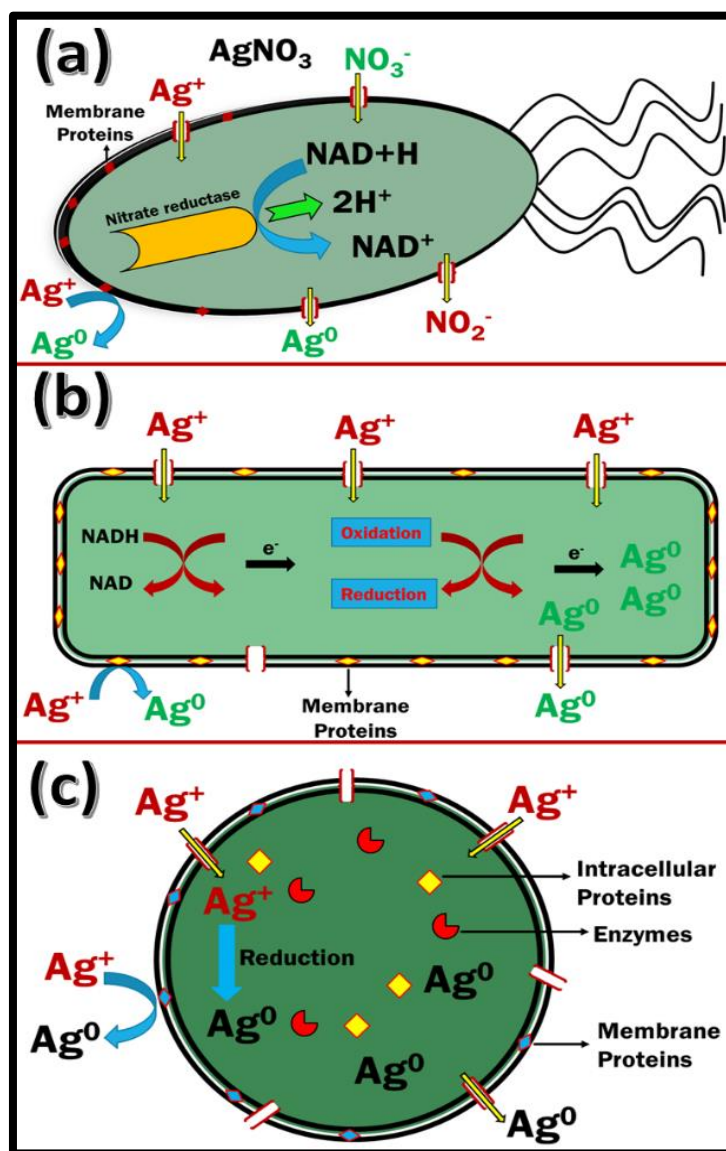
Mechanisms of M-NPs from fungi are not very clearly understood. There are various reported mechanisms for synthesis of AgNPs. Mukherjee et al. [258] have reported that AgNPs synthesis involves two main steps. The first step is the absorption of  $\text{Ag}^+$  ions on topmost layer of fungi, while the second step is the reduction of  $\text{Ag}^+$  ions to  $\text{Ag}^0$  to produce nanoparticles (Figure 1.9b) [259].

#### **1.8.2.6.3 Algae**

Algae have great tendencies to synthesize M-NPs because of their extraordinary capabilities to withstand and remediate toxic metals [260]. Algal cells contain a lot of carbohydrates, enzymes and proteins, minerals, fatty acids, and other molecules, hence can be involved in M-NPs synthesis as potential reducing and stabilising moieties [261], [262]. The rate, size, and stability are greatly controlled by temperature, pH, and type of light and its intensity of synthesis [263], [264]. M-NPs can be produced by algal cells in their periplasm, nucleus, cytoplasm, and



pyrenoid. The precise method by which algae produce M-NPs is yet unknown. The most likely processes of nanoparticle formation involve the reduction of metal ions by algal biomolecules and their bioaccumulation inside the cell (Figure 1.9c) [265]. The algal bio-reduction of metal salts into nanoparticles is three step phenomena viz. activation, growth, and termination (Figure 1.9c) [266], [267].



**Figure 1.9:** Mechanisms of metal (silver) nanoparticle synthesis by (a) bacteria, (b) fungi, and (c) algae [198].

#### 1.8.2.6.4 Lichens

Lichens are quite rich in bioactive compounds and have been reported for their bioactivity against many pathogens. Considering the bioactivity of lichens, researchers have focused on searching possibility of synthesizing metal and other nanoparticles as efficient antimicrobial

agents [268]. M-NPs from *Usnea longissima* [269], *Parmotrema tinctorum* [270], *Parmelia sulcata* [271], *Protoparmeliopsis muralis* [272], *Ramalina sinensis* [273], *Pseudevernia furfuracea*, *Lobaria pulmonaria* [274], *Xanthoria elegans*, *Usnea antarctica* [275], and *Lecanora muralis* [276] have been reported.

#### **1.8.2.6.5 Plants**

Plants are most exciting biological source to produce M-NPs. Employing plants of medicinal aspects for M-NPs synthesis provides some extra advantages. Any part of plant can be utilised to make nanoparticles since they are high in biological chemicals that function as reducing and stabilising agents. Silver, copper, titanium dioxide, zinc oxide, gold and platinum nanoparticles have all been successfully synthesised using metal solutions of silver nitrate, titanium tetrabutoxide, zinc nitrate and zinc acetate, hydrogen tetrachloroaurate, hexachloroplastic acid, and copper (II) sulphate [266–268]. Priyadarshini et al. [280] used *Cissus quadrangularis* extract to make titanium dioxide nanoparticles to inhibit growth of *Escherichia coli* and *Staphylococcus*. Similarly, cinnamon was also employed to produce AgNPs [281] and *Justicia wynaadensis* to produce ZnO [282] with potent antibacterial properties.

#### **1.8.2.6.6 Wastes**

Superfluous developments in cities have led to rapid rise in urban population that eventually has increased consumer demands along with increase in the load of municipal solid wastes (MSW) [283]. According to the reports of Environmental Protection Agency [284], alone United States has generated ~267.8 million tons of MSW in 2017. However, the global amount of MSW >2 billion tonnes annually. Recently, the biogenic synthesis of nanoparticles using waste substances, or its components is a thrust area of research. Synthesis of M-NPs using wastes from agriculture, fruits wastes, plant residues, and wastes from wood is such a sustainable technology [285], [286]. These wastes include a lot of carbohydrates, proteins, sugars, alcohols, phenols, lignans, flavonoids, and unsaturated fats, all of which may be utilised to make M-NPs. AgNPs made from citrus fruit peel (*Citrus limon*, *Citrus sinensis*, and *Citrus limetta*) were found to exhibit good antibacterial efficacy [287]. However, before proceeding to synthesis, the waste material needed to be washed or treated with some reagents to remove external impurities. Furthermore, complex substrates are subjected to pre-treatments for making availability of biomolecules for reduction and stabilization.

Literature study shows that generally nanoparticles synthesis involves multiple steps. Initially, precursor metal salt (in case of metallic nanoparticles) is reduced with suitable reducing agent, followed by stabilization using a stabilizing agent. In the final step stabilized nanoparticle is functionalized using suitable ligand or molecule (application dependent) either by direct conjugation or with the help of linker molecules [130], [288]. Additionally, existing approaches are also costly, and time consuming. Thus, in order to overcome these constraints in our current work we have used one-pot synthesis approach for the synthesis of M-NPs. The details and significance of one-pot synthesis has been discussed in subsequent section.

## 1.9 ONE-POT SYNTHESIS AND ITS ADVANTAGES

Synthesis of nanomaterials by one-pot approach is gaining much attention in recent years due to operational simplicity and good efficiency. Organic, inorganic and hybrid all kind of nanomaterials can be synthesized using one-pot approach. Hence, it can be said that one-pot is a direct approach for synthesis of nanomaterials. The procedure is simple and appealing to researchers since it eliminates the need for prolonged separation and purification of intermediate chemical components. As a result, it saves a lot of time, enhances reaction efficiency, and also improves yield. One-pot approach is very popular in the field of chemistry, however it is also receiving huge attention in the field of nanotechnology. Green synthesis is most common example of one-pot synthesis for producing nanoparticles. There are many reports on the green synthesis of nanoparticles through one-pot methods. The advantages of green one-pot method over other methods have been represented in Figure 1.8. The synthesis of CsPbBr<sub>3</sub>@SiO<sub>2</sub> nanoparticle (core-shell) through one-pot method have been documented with high stability [289]. Similarly, branched core-shell Pt@Ag through wet chemical approach have been reported. The particles were reported to have good catalytic activity for the degradation of 4-nitrophenol [290]. One-pot synthesis of AuNPs using *Sansevieria roxburghiana* have been reported for the degradation of pollutants like congo-red, 4-nitrophenol, phenol red, acridine orange, methylene blue, and bromothymol [291]. Recently, *Flacourtia jangomas* fruit extract mediated one-pot synthesis of Ag-NPs have been reported with strong antimicrobial activity [292].

## 1.10 CHARACTERIZATION TECHNIQUES OF M-NPs

Nanoparticles shows variations in their properties depending on shape, and size. Hence it becomes very important to characterize nanoparticles. Some of the important characterization techniques are discussed in subsequent sections.

### 1.10.1 UV-visible (UV-vis) spectrophotometry

Light and matter interactions is the basis for the all forms of spectroscopy. UV-vis absorption spectroscopy is very useful to measure the change in the light passes through sample. UV and visible lights are energetic and excite electrons of samples to higher energy levels resulting in the formation of distinct spectra. Generally, M-NPs possess a characteristic  $\lambda$  maximum. UV-vis spectroscopy can be used to determine the concentration of a compound in solution based on absorption employing the Beer-Lambert law. According to this law the absorbance in a sample is directly proportional to the compound present in it. The law can be written as  $A = \epsilon cl$ , where  $A$  is absorbance,  $l$  is path length,  $c$  is the concentration of compound in solution and  $\epsilon$  is the molar extinction at particular wavelength for the compound. For example, Sharma and Chaudhary [130] synthesized aminoglycoside drug conjugated gold nanoparticle through one-pot synthesis approach. The synthesized gold nanoparticle exhibited characteristic peak of AuNPs at 520 nm. Similarly, Ag-NPs produced through the aqueous extract solution of *Citrus lemon* leaf exhibited absorption maxima at 429 nm [293]. In another example, ZnO synthesized using microalgae *Arthrospira platensis* exhibited UV-vis maxima at 370 nm [294].

### 1.10.2 Fourier transform infrared (FTIR) spectroscopy

FTIR spectroscopy makes use of infra-red (IR) light that alters the dipole moments of molecules which correspond to a certain vibrational energy. The bond spring constant and reduced mass are the two variables responsible for resulting vibrational energy. Vibrations are distinctive to classes and groups of functional groups because they are made up of distinct atoms with variable binding strengths. Infrared spectroscopy like FTIR is an exciting tool for determining the functional groups on a nanoparticle's surface. The technique is useful for analysing the chemical composition of a wide range of molecules and substances, including organic chemicals, semiconductor materials, polymers, gases, biological samples, and minerals [295]. Functional groups present on the nanoparticle surface determined based on their molecular rotational degree and movements (stretching, bending, scissoring, and twisting [296]. A typical

dispersive IR spectrophotometer usually takes 3 min to scan from 4000 to 400  $\text{cm}^{-1}$ , while most of the new FTIR instruments can do same analysis in 1 second or in less than one second.

### 1.10.3 X-ray diffraction (XRD) pattern analysis

XRD analysis is a wonderful technique to determine the crystal structure of nanomaterials and other materials. Constructive interference among monochromatic X-rays and sample (crystalline) is the basis of XRD. XRD working involves irradiating nanomaterial with incident X-rays followed by measurement of the intensities and scattering angles of the X-rays leaving the nanomaterial. The diffraction of X-rays is explained by "Bragg's Law of Diffraction". The equation  $n\lambda = 2d \sin\theta$  is commonly used to explain Bragg's diffractions, where  $n$  is diffraction order,  $\lambda$  is wavelength (constant),  $\theta$  is the angle of incidence,  $d$  is the distance among atomic layers. The crystallite size from XRD data can be calculated using Scherrer's equation:

$$D = \frac{k\lambda}{\beta \cos\theta}$$

where  $D$  is crystalline size,  $k$  is dimension-less shape factor (0.9),  $\lambda$  is wavelength,  $\beta$  is full width half maximum of the peak (FWHM), and  $\theta$  is Bragg's angle. Primarily in the XRD analysis material is identified based on their diffraction pattern. XRD is also a very good technique for phase identification. Moreover, XRD analysis is also helpful in revealing differences between the actual structure and deviated structure due to internal stresses and defects [297], [298].

### 1.10.4 Dynamic light scattering (DLS) analysis

DLS analysis is an important technique to characterize nanoparticles, proteins, latex, and colloids. The detection of light dispersed by a material's contact with light is thought to offer important information about the material's or sample's physical qualities. Working of DLS includes direction of a monochromatic beam towards sample followed by analysis by detector using scattered light at a specific angle [299], [300]. DLS actually determines the hydrodynamic radius of the nanoparticle ( $R_H$ ).  $R_H$  is calculated using diffusion coefficient by implementing Stokes-Einstein equation i.e.,

$$D_f = k_B T / 6\pi\eta R_H.$$

In the equation where  $k_B$  is Boltzmann constant,  $T$  correspond to the temperature of the suspension, and  $\eta$  is the viscosity of the surrounding media of the nanoparticles [299].

### **1.10.5 Zeta potential analysis**

Zeta potential determination of nanomaterials is very important characterization technique. The technique is generally used to estimate the surface charge of nanomaterials that eventually governs the physical stability of nanomaterials [301]. If a sample of nanomaterials exhibits large positive or negative value of zeta potential (-30 mV to +30 mV) then it is assumed that nanoparticles have good physical stability attributed by the electrostatic repulsion of individual nanoparticles. In contrast if nanomaterials possess small value of zeta potential it means that the particles may lose their stability due to aggregation and flocculation [302], [303].

### **1.10.6 Scanning electron microscopy (SEM) analysis**

SEM is a technique that can be used to find out size, shape, and texture of nanomaterials and other kind of materials. The basic working of field emission scanning electron microscopy (FESEM) includes projection of a fine beam of electrons on the sample or material in a series of parallel tracks. The projected electrons interact with the sample, after interactions material produces several different signals that are detected by specific detectors and displayed on the screen. In SEM analysis most of the electrically insulating samples are generally coated with a thin layer of conducting material (carbon, gold, other metals). Mostly carbon is the material of choice as conductive coating for the elemental analysis. Metal coatings in SEM are generally used for high resolution electron imaging application purposes [304]. In biology and nanotechnology most of researchers now days use (FESEM) for better resolution. In the FESEM electrons are liberated by a field emission source. Nindawat and Agrawal [305] has revealed spherical, rod, triangle, hexagon, and polygon shapes of Ag-NPs synthesized using *Arnebia hispidissima* through FESEM analysis. In another report, Roy et al. [306] has revealed spherical, rod, triangular, and flower shapes of AuNPs through FESEM analysis.

### **1.10.7 Transmission electron microscopy (TEM) analysis**

TEM works on the same principles as light microscope. The main distinction between the two is that light microscopes work with light rays, whereas the TEM works with electrons in a beam. The beam electrons of TEM possess shorter wavelength and high energy as compared to light rays of light microscope that have long wavelength and lesser energy. The basic mechanism of

the light microscope is that its resolution power increases with decrease in the wavelength of light, but in case of TEM the resolution power increases with the increase in wavelength of the electron transmission. An electron gun, an image forming system, and an image recording system are all part of a conventional TEM. The wavelength of the electron beam in TEM is about 0.005 nm which is 100,000X shorter than that wavelength of light, hence TEM provides much better resolution (1000 times) as compared to the light microscope [307], [308]. TEM widely used in the field such as cancer research, virology, materials science, palaeontology, and palynology. TEM is generally used to analyse the shape, size, and texture of nanomaterials similar to SEM analysis. Let us consider an example, Marychuk et al. [308] determined the spherical, triangular, and hexagonal shapes of AuNPs (8-200 nm) using TEM. Recently, Das et al. [309] revealed spherical, triangular and star shapes of AuNPs using TEM.

#### **1.10.8 Inductively coupled plasma mass spectrometry (ICP-MS) analysis**

ICP-MS is an exciting characterization tool, helpful in providing of nanoparticle size, density of particles and their mass concentration, and elemental composition analysis. ICP-MS is very helpful in the analysis of multielement with greater sensitivity. Single particle ICP-MS (spICP-MS) is useful to detect metal or metal oxide nanoparticles up to the very low concentrations [310]. ICP-MS studies are very useful in cellular uptake studies of nanoparticles. In a study, citric acid conjugated and DNA conjugated AuNPs of 15, 30, and 60 nm were studied for cellular uptake study on HeLa cells using on-line droplet chip ICP-MS hyphenated technique [311]. ICP-MS technique has higher speed, precision and sensitivity as compared to atomic absorption spectroscopy.

#### **1.10.9 Atomic force microscopy (AFM) analysis**

AFM is a characterization instrument of scanning probe microscopes family [312]. A typical AFM consists of four major components viz. AFM probe (cantilever) to directly sense the force between the probe and a sample, a piezo scanner (for controlling the probe-sample position), software (for controlling operational parameters and result monitoring), and a feedback control system. AFM is very effective in measuring the surface morphology and mechanical properties of nanomaterials [313]. Furthermore, AFM is very effective to fabricate nanomaterials with absolute precautions, control, and reproducible fashion [314]. Therefore, AFM can be considered as an appropriate and pivotal nanotechnology tool.

## 1.11 APPLICATIONS OF M-NPs

Literature study shows that M-NPs originated from various types of metal precursors can be utilized for large number of applications. M-NPs have been evaluated for antimicrobial, antifungal, antiviral, anti-cancer, antioxidant, catalytic, drug delivery, energy devices, and biosensors. Nanotechnology's introduction into biomedicine, on the other hand, has opened up new diagnostic and therapeutic possibilities. Drug transport, anti-cancer action, delivery of gene to target, fluorescent labelling, protein determination, enhancement in the MRI contrast, DNA probing, engineering of tissue, phagokinetic studies, and hyperthermia all uses nanomaterials [315]–[320].

### 1.11.1 Antimicrobial activity

Resistance in microbes to routinely used antibiotics has increased exponentially, posing a serious danger to people's health and well-being all over the world [321]. To combat antimicrobial resistance researchers are focusing to develop new effective antimicrobials, combination of antibiotics, and conjugation of antibiotics with M-NPs with better antimicrobial potential. In this context Ag-NPs have gained significant attraction. Burns and wounds have traditionally been treated with silver to prevent microbial development. As a result, silver and its nanoparticles in ointments are quite popular in burn cases and open wounds to block infection [230], [322], [323]. Silver and other M-NPs exhibit antimicrobial activity by mechanisms such as creating damage to cellular structures of microbe, inactivating the enzymes and proteins of microbial cells, by condensing the genetic material, by creating error in DNA replication process, adversely affecting ATP synthesis, by creating obstacles in ribosomal interactions, and by accumulating themselves in the cells. However, M-NPs antimicrobial action has been linked to reactive oxygen species i.e. reactive oxygen species (ROS) generation, cell membrane damage, and disturbance in microbial cell signalling [149], [324]–[327]. The biggest advantage of nanoparticles as antimicrobial agent is that most of the antibiotics acts only by one or two mechanisms whereas M-NPs shows their antimicrobial activity by multiple ways.

Pt, Ag, Cu, Au, TiO<sub>2</sub>, and ZnO are most commonly used M-NPs as antimicrobial agents against gram-negative and gram-positive bacteria [328], [329]. CuNPs (size <2 nm) synthesized by using l-ascorbic acid were found to have good antimicrobial potential [330]. CuNPs produced from endophytic actinomycete *Streptomyces capillispiralis* Ca-1 exhibited



antimicrobial activity gram-positive *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, and *Bacillus dimenuta* ATCC 19146, and gram-negative *Pseudomonas aeruginosa* ATCC 9022, and *Escherichia coli* ATCC 8739 [331]. CuNPs generated by mycobacteria were also shown to be efficient against *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 605 [332]. Similarly, AgNPs produced through extract of *Lysiloma acapulcensis* exhibited excellent antimicrobial potential [333]. In another work, Vazquez-Muñoz et al. [334] displayed that AgNPs with antibiotics have synergistic antimicrobial efficacy. AgNPs produced through tea leaf extract were observed for remarkable activity against food-borne pathogens viz. *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, and *Salmonella enteritidis* [335]. AuNPs also have been documented for strong antimicrobial activity. AuNPs produced through *Annona muricata* extract have been reported with antimicrobial activity [336]. Additionally, gentamicin attached AuNPs have been observed effective against *Escherichia coli* DH5 $\alpha$ , *Escherichia coli* ATCC 25922, and *Staphylococcus aureus* MTCC 3160). Surprisingly, AuNPs were shown to have antibacterial action against *Escherichia fergusonii* ATCC 35469 [130]. There are many more examples of M-NPs with antimicrobial activity.

### 1.11.2 Antifungal activity

M-NPs has also been reported to possess antifungal activity. Nanoparticles of metal such copper, silver, iron, and gold has been studies for their antifungal potential. Iron oxide nanoparticles were observed to be effective against a number of fungi by Parveen et al. [337]. Nanoparticles exhibited remarkable antifungal potential against *Trichothecium roseum*, *Cladosporium herbarum*, *Penicillium chrysogenum*, *Alternaria alternata*, and *Aspergillus niger*. In another report biosynthesized CuNPs from *Streptomyces griseus* has been reoprted for antifungal activity against red root-rot disease causing *Poria hypolateritia* [338]. A similar kind of activity by AgNps produced from *Phyllanthus urinaria*, *Pouzolzia zeylanica*, and *Scoparia dulcis* leaf extracts towards *Aspergillus niger*, *Aspergillus flavus*, and *Fusarium oxysporum* has been recorded [339]. In another study, zinc oxide nanoparticles synthesized using *Brassica oleracea* var. *Italica*, *Beta vulgaris*, *Cinnamomum verum* and *Cinnamomum tamala* extract with strong antifungal activity against *Candida albicans* and *Aspergillus niger* [340]. More recently, AgNPs synthesized using *Penicillium verrucosum* and *Nigrospora oryzae* has been reported for antifungal activity against *Fusarium chlamydosporum* and *Aspergillus flavus*, and *Fusarium moniliforme* and *Fusarium oxysporum* respectively [341], [342].

### **1.11.3 Antiviral activity**

M-NPs has also been reported for the antiviral activity. Galdiero et al. [343] has documented strong antiviral activities of Ag-NPs against several families such as retroviridae, hepadnaviridae, paramyxoviridae, herpesviridae, poxviridae, orthomyxoviridae and arenaviridae. In another work, iron oxide nanoparticles have been reported for antiviral activity towards pandemic influenza strain (A/H1N1/Eastern India/66/PR8-H1N1) [344]. Similarly, algal synthesized AuNPs and AgNPs has been found effective against *Herps simplex* (HSV-1) virus. In addition to this, a surface coating consisting of copper nanoparticle-graphene composite has been found for the antiviral activity against influenza virus [345]. In another study, CuNPs made from *Syzygium alternifolium* were found to have antiviral action against Newcastle disease virus [346].

### **1.11.4 Anticancer activity**

M-NPs due to their unique physicochemical properties has been reported for anticancer/antitumor effects. M-NPs exhibit anticancer activity either due to the antioxidant action or due to an external stimulus (hyperthermia). Due external stimulus cells generate reactive oxygen species (ROS) that have potential to kill oncogenic cells. In addition to this nanoparticle can also interact with tumour environments, hence can interfere in the development of tumour mass [347]. Let us consider some examples, Aswathanarayan et al. [348] has reported anticancer potential of zinc oxide nanoparticles on colorectal cancer cell HT 29. In another work, AgNPs synthesized from *Cleome viscosa* L. has been reported for anticancer activity (lung (A549) and ovarian (PA1) cell lines) [349]. Similarly, AuNPs from *Lonicera japonica* have been reported for anticancer activity against HeLa cells [350]. Likewise, AuNPs synthesized from leaf extract of *Panax notoginseng* has been observed for similar kind of activity on PANC-1 (pancreatic cancer) cell lines [351]. Copper oxide nanoparticles decorated by graphene oxide were found to exhibit anticancer activity against HCT-116 cell lines [352]. Furthermore, AGNPs from *Eucalyptus Camaldulensis* leaf extract have been reported for anticancer activity against Caco-2 cell line [353].

### **1.11.5 Catalytic activity**

M-NPs have received huge attention in the field of catalysis. The size, shape, and surface content of produced M-NPs plays a crucial role on their activity and selectivity. The crucial interactions during catalysis occur at the interface between the substance and the reaction

media, thus role of capping ligands during the reaction is also a key factor [354]. The reacting molecules adsorb on the catalytically active solid surface in heterogeneous catalysis. Chemical bonds are destroyed and new ones are created on the surface, and the products are finally discharged into the liquid or gas phase [355]. Let us consider some reports on catalytic activity of M-NPs. Cobalt-oxide nanoparticles (40-80 nm) synthesized from *Punica granatum* peel extract were observed for photo-catalytic activity for the degradation (78.45%) of RBO 3R in 50 min irradiation time [356]. Likewise, nickel nanoparticles synthesized from *Camellia sinensis* have been reported for catalytic activity for the crystal violet dye with 99.5% of degradation [357]. In addition to this core shell nanoparticle have also been reported with catalytic attributes. Catalytic activity for the degradation of 4-nitrophenol has been demonstrated in hollow nanospheres of metal oxides such as NiO, CuO, and NiO/CuO covered with a porous carbon shell [358]. In another finding Au-Ag alloy stabilized by polymer has been reported for catalytic degradation of 4-nitrophenol [359]. CuNPs on cotton cellulosic nanocomposite were able to produce hydrogen more efficiently ( $H_2$  generation rate  $1253 \text{ mLg}^{-1} \text{ min}^{-1}$ ) as compared to other catalysts. Furthermore, nanoparticles were also observed for fast degradation (4 minutes) of 4-nitrophenol [360]. In another study, palladium coated anisotropic AuNPs were shown to have 1.5-fold greater catalytic activity to reduce 4-nitrophenol [361]. Similarly, light driven AuNPs and AgNPs synthesized through extract of *Derris trifoliata* were observed for catalytic degradation of 4-nitrophenol ( $1744 \text{ s}^{-1} \text{ g}^{-1}$ ) [362]. More recently, carbon nanotubes (multi-walled) decorated with tungsten trioxides nanoparticles has been reported for 4-nitrophenol reduction [363]. Mt-NPs can be used to immobilize enzyme. In a study, magnesium nanoparticles have been used to immobilize lipase for n-propyl gallate synthesis [364].

### 1.12.6 Biosensor

Nanomaterials had a significant influence in biosensing in recent decades. Noble M-NPs, in particular, have enabled the creation of novel biosensing systems with improved capabilities in the selective detection of bio-analytes [140], [365]. Noble M-NPs offer unique physicochemical features that have encouraged the creation of a slew of biosensing platforms. Furthermore, they give an extra or improved layer of application to regularly utilised techniques (fluorescence, infrared, and Raman spectroscopy) [366]. Let's us consider couple of reports where M-NPs have been effectively used as biosensor. AuNPs have reported for detection of immunoglobulins in real urine samples with detection limit of  $8 \mu\text{g/L}$  [367]. In another work,

heterogeneous Au@C-Co<sub>3</sub>O<sub>4</sub> dodecahedrons synthesized through sacrificial template method have been observed for electrochemical biosensing of H<sub>2</sub>O<sub>2</sub> with detection limit of 19 nM [368]. In another study, nucleic acid-functionalized metal-organic framework have been used as homogeneous electrochemical biosensor for multiple tumor biomarkers [369]. Likewise, coupled well-exfoliated graphene oxide with Ag or Ag-Fe<sub>3</sub>O<sub>4</sub> used for electrochemical detection of ascorbic acid in blood (detection limit 74 nM) [370]. Anisotropic AuNPs in combination with lysine and dithiothreitol as aggregation promoters have been used in colorimetric assay for detection of mercury (Hg<sup>2+</sup>) with ultra-low detection limit (27 pM in deionized water and 58 pM in tap water) [371]. Work by Halkare et al. [372] developed a biosensor by using *Escherichia coli* B40 bacteria as receptor for sensing heavy metal ions (Hg<sup>2+</sup> and Cd<sup>2+</sup>) in water system with detection limit 0.5 ppb. Recently, electrochemical biosensor using AuNPs on nanotubes of TiO<sub>2</sub> decorated with CdS quantum dots has been developed with higher (6-fold) sensing potential for cholesterol (10790  $\mu\text{A mM}^{-1} \text{cm}^{-2}$ ) and hydrogen peroxide (78833  $\mu\text{A mM}^{-1} \text{cm}^{-2}$ ) [373]. More recently, DNA nanostructure biosensor has been developed for the electrochemical detection of cyanazine herbicide with detection limit of 0.8 nM [374].

The work of dissertation is presented in following sections:

- **Chapter 1:** Introduction and review of literature.
- **Chapter 2:** Aim and objectives of the study.
- **Chapter 3:** Development of gentamicin conjugated gold nanoparticles and evaluation of their antimicrobial potential.
- **Chapter 4:** Development of silver nanoparticles using quercetin for the colorimetric sensing of l-cysteine.
- **Conclusion, future prospects and new findings.**
- **Bibliography.**