CHAPTER- 2

REVIEW OF LITERATURE

2.1 Background

Despite the fact that we exist in a period of advanced and modern technologies for illuminating underlying mechanisms of diseases and molecularly designing new drugs, infectious diseases continue to be one of the greatest health challenges worldwide. In the beginning of the 20th century, infectious diseases were the leading root of death worldwide (Cohen, 2000). The decreases in morbidity and mortality from infectious diseases over the last century were attributed primarily to an initiation of antimicrobial factors. Today, nevertheless, the expansion of antibiotic resistance is a growing menace due to the indiscriminate usage of antibiotics, invalidating major antimicrobial drugs that are presently employed in the clinic. It has resulted in the proliferation of selective pathogenic bacteria which are resistant to multiple drugs (Tenover, 2006). Changes in societal activities, the advancement of technology, and evolving microorganisms themselves are cooperatively contributing to the escalation of emerging and re-emerging infectious diseases, and to the evolution of antimicrobial resistance. A combination of increased pressure of antibiotic selections and a reduction in the development of new antibiotics has produced the misery that once treatable infections become untreatable (Rice, 2009). Resistance to antimicrobial drugs becomes a threatening problem not just in hospitals, but also in communities, resulting in less effective drugs available to curb infections by “old” established bacteria such as Streptococcus pneumonia (Lode, 2009). Drug-resistant Neisseria gonorrhoeae and Haemophilus influenzae were already recognized worldwide in the 1970s (Berkowitz, 1995). Of late, more than 1500 people in Germany have been infected with a new virulent form of Escherichia coli, which was not previously known to be affected in any outbreaks, but turned out to be extremely infectious and toxic, leading to a number of fatal cases. Moreover, bioinformatics analysis revealed that this deadly bacterial strain carries several antibiotic resistance genes, including resistance to aminoglycosides, macrolides, and β-lactam antibiotics. This implicates that treating this virulent bacteria with antibiotics is extremely difficult (Hyde, 2011; Tuffs, 2011). The resistance to antimicrobial drugs has been tried to be solved by discovering new antibiotics and chemically modifying existing antimicrobial drugs. Also, in that respect there is no confidence that the maturation of novel antimicrobial drugs can catch up to the microbial pathogen's fast and frequent development of resistance in a timely way. Moreover, drug resistance enforces high dose administration of antibiotics, often generating intolerable toxicity, growth of new antibiotics, and requests for significant
economic, labour, and time investments. In the ongoing race of the development of antimicrobial agents, however, microbes appear to be the victor, and the pipeline for new drugs is verging on empty. Very few new antibiotics have been found in the past 40 years due to the cost and complexities connected with drug discovery and maturation (Projan, 2003). Despite extensive efforts in research and enormous investment of resources, the pace of drug development has not kept up with the growth of immunity. The spread of opposition to many currently used antimicrobial agents among fungi, viruses, and parasites is a high level alert to find paradigm-shifting approach for treating microbial infections as a top priority in medicine. This challenging and dynamic form of infectious diseases and the emergence of strains resistant to many currently used antibiotics demand for longer-term solutions to this ever-growing and probable problem (Taylor et al., 2002). Therefore, design, discovery, and delivery of antimicrobial drugs with improved efficacy and avoidance of resistance are highly demanded.

To address these issues, in the present scenario nanoscale materials has emerged up as novel antimicrobial agents. Nanoparticles exist in the natural world and are also created as a result of human activities. Nanotechnology provides a means to modify key features of different materials. “Nanotechnology” is the application of science to control matter at the molecular level. It is the most promising field for generating new applications in medicine (Shirley et al., 2010). Discoveries in the past decade have shown that once materials are prepared in the form of very small particles, they change significantly their physical and chemical properties, sometimes to the extent that completely new phenomenon are established. Nanotechnology can be termed as the synthesis, characterization, exploration and application of nanosized (1-100nm) materials for the development of science. It deals with the materials whose structures exhibit significantly novel and improved physical, chemical, and biological properties, phenomena, and functionality due to their nano scaled size. The history of nanomaterials is quite long; nevertheless, major developments within nanoscience have taken place during the last two decades. The idea of nanotechnology was first highlighted by Noble laureate Richard Feynman, in his famous lecture at the California Institute of Technology, (Feynman, 1959). Richard Feynman in one of his articles published in 1960 titled, “There is plenty of room at the bottom” discussed the idea of nanomaterials. He pointed out that if a bit of information required only 100 atoms, then all the books ever written could be stored in a cube with sides 0.02 inch long. Norio Taniguchi (1974) first defined the term Nanotechnology. According to him, “Nanotechnology mainly consists of the
processing of, separation, deformation, and consolidation of material by one atom or by one molecule”.

The role of nanoscale metals allows achieving hundred-time decreased concentration and at the same time increase in antimicrobial properties: reduction of a particle size from 10 μ to 10 nm increases the contact surface area by $10^9$ times (Pal et al., 2007). Small sizes of nanoparticles contribute their easy penetration into the microorganism cell. In gain, unitary of the positive characteristics of nanoparticles is also their thermal stability. This property is useful in the evolution of different composites with nanoparticles, e.g. dressing materials and antimicrobial coatings for medical devices, etc. (Krutyakov et al., 2008). The intrinsic attributes of metal nanoparticles are mainly defined by size, anatomy, composition, crystallinity and morphology. Nanoparticles, because of their minuscule size, have distinct properties compared to the bulk phase, thus providing many new growths in the subject areas of biosensors, biomedicine, and bio nanotechnology. It is an enormously powerful technology, which takes a vast promise for the invention and evolution of many types of novel products with its potential medical applications in early disease detection, prevention and treatment.

2.2 Properties of nanoparticles

They act as link between bulk materials and atomic or molecular structures. They have small size and large surface to volume ration which offers several advantages like easy diffusion, greater absorption and greater interaction. Due to their unexpected optical properties they produce quantum effects. Nanoparticles can enhance the strength of polymers e.g. clay nanoparticles increase the rigidity of plastics. In the free state, they are highly mobile (e.g., silica nanoparticles (10 nm) has sedimentation rate under gravity of 0.01 mm/day in water, in the absence of some other additional influence). Nanoparticles have a wide range of compositions, depending on the use or the product. They can be categorized as hard (e.g., titanium dioxide nanoparticles, fullerenes etc) or as soft (e.g., liposomes, vesicles and nanodroplets). Hydrophilic and hydrophobic parts of nanoparticles are helpful in stabilizing emulsions. They can self-assemble at water/oil interfaces and act as solid surfactants. High doses of nanoparticles can have adverse effect on human health and environment (Vollath, 2008; Sun et al., 2010).
Chapter 2

Review of Literature

2.3 Application of Nanoparticles

Once materials are made in the shape of very small atoms, they change significantly their physical and chemical attributes. In fact, in nanosize as surface area as compared to bulk molecule is high, this raises the activity of the nanoparticles and therefore, the normal properties of the particle like heat treatment, bulk transfer, catalytic activity all increases. Some nanoscale materials have been used for decades (e.g. in window glass, sunglasses, car bumpers, paints), whereas others are newly identified (e.g. Those used in sunscreens and cosmetics, textiles, explosives, propellants) or their applications are presently under development (e.g. in batteries, electronic storage media, drug delivery systems, medical implants and new organs) (Deutscher et al., 2008). Applications of nanoparticles in different areas are described below and also summed up in Figure 1.

![Diagram of Nanoparticles used in different areas]

**Figure 2.1:** Nanoparticles used in different areas.
2.3.1 Electronics

**Nanoprocesors:** By using nano processors in electronic circuit elements computations can be obtained at faster speed e.g. carbon nanotubes (CNTs) (Jacoby, 2002).

**Displays:** Nanocrystalline materials can be used to enhance the resolution of television or a monitor due to reduction in pixel size at much lower cost. CNTs are being investigated for low voltage field-emission displays (Carey, 2003).

**Data storage:** Discs and tapes containing engineered nanomaterials can store large amounts of information. Future possibilities for data storage include spintronics and nanowires (Buzea et al., 2007).

**High energy density batteries:** New nanomaterials have higher capacity and better cycle life to be used in batteries than their larger-particle counterparts e.g. nanocrystalline alloys, nanosized composite materials, carbon nanotubes, and nanosized transition metal oxides (Liu et al., 2006).

2.3.2 Transportation and telecommunication

**Car tyres:** Nanoparticles of carbon black ranging between 10 nm - 500 nm act as filler in the polymer matrix of tires, and are used for mechanical reinforcement.

**Car bumpers:** Clay particle based composites containing plastics and nano-sized clay are used to make car exteriors that are lighter and twice more resistant to scratches than usual materials (Buzea et al., 2007).

2.3.3 Biomedical Applications

Nanoparticles are important scientific tools that have been and are being explored in various biotechnological, pharmacological and pure technological uses.

**Bioseparation:** Nanotube membranes can act as channels for highly selective transport of molecules and ions between solutions that are present on both side of the membrane (Jirage et al., 1997). For example, membranes containing nanotubes with inside diameters (less than 1 nm) separate small molecules on the basis of molecular size, while nanotubes with larger inside diameters (20–60 nm) can be used to separate proteins (Martin and Kohli, 2003).
**Nasal vaccination:** Antigen-coated polystyrene nano spheres, used as vaccine carriers targeting human dendritic cells, have been researched for nasal vaccination (Matsusaki *et al.*, 2005). Nanospheres had a direct effect on human dendritic cells, inducing transcription of genes important for, e.g., phagocytosis as well as an immune response.

**Imaging and diagnosis:** Nanoparticles and nanostructures are becoming a part in human medical application, including imaging or the delivery of therapeutic drugs to cell, tissues and organs. Molecular imaging is an important study in biology and medicine with ability to detect, measure, and display molecular and cellular alterations that fall out *in vitro* and *in vivo*. Using nanoparticle contrast agents, images such as ultrasound and MRI have a favorable distribution and improved contrast. Furthermore, the potential for coating the NPs with antibodies, collagen, and other micro molecules makes them biocompatible for detection and diagnosis. Nanotechnology based diagnosis techniques provide two major advantages. Firstly they allow rapid testing and secondly early detection of the disease, thus preventing disease to spread and cause harm to the patient (Niemeyer, 2001):

**Cancer:** Presently, both nanoparticles diagnostics and therapeutics are actively being applied to cancer therapy, cancer imaging and immunology (Banu *et al.*, 2010; Perrault and Chan, 2010; Shi *et al.*, 2010). Properties of nanoparticle like small size and high surface area to volume ratio, allows many functional groups to be bonded to a nanoparticle, which can seek out and preferentially accumulate at tumor sites (Shuming *et al.*, 2007). Conventional anticancer treatments are nonspecific to target killing of tumor cells, may cause severe systemic toxicity, and develop drug resistant. Nanoparticles have ability to overcome these impediments. An exciting potential use of nanotechnology in cancer treatments is the exploration of tumor-specific thermal scalpels to heat and burn tumors. Palcitaxel is widely known anti cancerous drug but it has side effects which include hypersensitivity reactions, asking the administration of steroids and antihistamines. In early 2005, Abraxane was approved for clinical use. Abraxane is nothing but paclitaxel loaded in nanoparticles of a natural polymer, albumin, using a high-pressure emulsification process. Altered palcitaxel i.e. Abraxane not only removed the side effects connected with the previous form but also provided some extra benefits like higher drug dosing (Micha *et al.*, 2006). Another study led by the National University of Singapore (NUS) found that attaching chemotherapy drug Epirubicin to nano diamonds (EPND- nano diamond-Epirubicin drug delivery complex) effectively eliminates chemo resistant cancer stem cells. The researchers found that with both standards Epirubicin as well as EPND normal cancer cells were killed, but only EPND
was capable of killing chemoresistant cancer stem cells and preventing secondary tumour formation in xenograft models of liver cancer (Wang et al., 2014).

**Drug delivery**: Targeted encapsulated drug delivery using NPs is more effective for improved bioavailability, minimal side effects, decreased toxicity to other organs, and is not costly. Drug delivery systems, lipid- or polymer-based nanoparticles, can be designed to improve the pharmacokinetics and biodistribution of the drug (Chu et al., 2013). Combining nanoparticles with antibiotics is another way of transporting drugs. It helps in transportation as well as enhances the activity of nanoparticles and drug in combination. More than one antimicrobial agent can be packed within the same nanoparticle. Further development of resistance to multiple agents within the same nanoparticle is unlikely because it will require multiple simultaneous gene mutations within the same microbial cell (Friedman et al., 2013). Many types of lipophilic and water-soluble antibiotics can be conjugated inside or on the surface of nanoparticles, or carried via encapsulation. Drug loaded nanoparticles interact with organs and tissues and are rented up by cells through physical encapsulation, adsorption or chemical conjugation (Abeylath et al., 2008).

**Tissue Engineering**: It is really fast growing scientific area in this era and used to create, repair, and/or replaces cells, tissues and organs. Lack of functional cells, low mechanical strength of engineered cells, not immunologically compatible with the host and nutrient limitation are the hindrances in the field of tissue and tissue engineering (Rivron et al., 2008). Nanotechnology is being used in stem cell tissue engineering, neural cell tissue engineering, cartilage cells tissue engineering, bone cells tissue engineering, vascular cells tissue engineering and hepatic cells tissue engineering (Kingsley et al., 2013). Scaffolds with biochemical, mechanical, and electrical properties similar to those of native tissues have been nanoengineered to enhance cell adhesion, proliferation, differentiation, and even maturation, thereby fostering cell function and tissue growth (Yang et al., 2014).

**Gene therapy**: Gene therapy is a technique for correcting defective genes responsible for disease development. The major challenge of gene delivery is to improve the transfection efficiencies of the nonviral carriers. Among various nonviral gene vectors, nanoparticles (NPs) offer an ideal platform for the incorporation of all the desirable characteristics into a single gene delivery system. Conventional uses of viral vectors are associated with adverse immunologic, inflammatory responses, and diseases. Nanoparticles are more secure, more flexible in their range of cellular targets, and have larger capacity than viruses (Sharma et al.,
Researchers in US have shown that nanoparticles can be utilized to deliver genetic material to the cells effectively and safely, potentially defeating the main obstruction to the growth of gene therapy (Singh et al., 2015).

**Medical Devices:** Nanotechnology in medical devices is a speculative subfield of nanotechnology regarding the possibility of engineering, molecular assemblers, machines which could re-order matter at a molecular or atomic scale. Nanomedicine would make use of nanorobots, introduced into the body, to repair or detect damages and infections (Freitas and Havukkala, 2005). Various surgical tools using nanotechnology are pacemakers, hearing aids, wound dressings, carbon nanotube catheters, nanoneedles (undergoing in vitro studies), contrast agents for molecular imaging, bone replacement material, DNA/protein microarrays and lab-on-a-chip for in vitro molecular diagnostics. Use of nanotechnology in medical science besides resulting in minimal invasive surgery also introduces nanosurgery and femtosecond laser systems in neurosurgery, ophthalmology and dermatology (Roszek et al., 2005).

**Antimicrobial agents:** It is unadorned that the metal based nanoparticles constitute an effective antimicrobial agent against common pathogenic microorganisms. Consequently, more or less of the nanoparticles such as silver, titanium dioxide and zinc oxide are receiving considerable attention as antimicrobials and additives in consumer, health-related and industrial products (Dibrov et al., 2002). Silver nanoparticles (Ag NPs) are being used as additives in health related merchandise such as bandages, catheters, and other fabrics to prevent infection, especially during the healing of lesions and burns (Sarkar et al., 2007). An antibacterial Ag/Na carboxymethyl cotton burns dressing by the partial cation exchange of sodium with silver has been evolved and these can find applications in surgical dressings (Baker et al., 2005). Nanoparticles of titanium dioxide are used in cosmetics, disinfection, filters that exhibit strong germicidal properties and remove smells, and in conjugation with silver as an antimicrobial agent. Moreover, due to the photocatalytic activity, it has been applied in effluent treatment. It is considered non-toxic and has been sanctioned by the American Food and Drug Administration (FDA) for utilization in human food, drugs, cosmetics and food contact materials (Wist et al., 2004). Copper oxide nanomaterials due to their antimicrobial property are being integrated into a mixture of medical and skin coatings. Nanoparticles of gold (Au) are currently being added to many common household and daily
use products such as bedding, washers, water purification systems, tooth paste, shampoo, fabrics, deodorants, filters, paints, kitchen utensils, toys, creams, lotions and ointments on account of its antibacterial properties (Baker et al., 2005). Chitosan nanoparticles are used as bacteria immobilizer; microbicide in biomedical products (Rabea et al., 2003; Qi et al., 2004). Nitric oxide (NO) releasing nanoparticles are used in treatment of infected wound and diabetic foot (Weller, 2009).

**Nano biosensors:** Nanoparticle-based biosensors are quite popular these days as they can be easily synthesized in bulk by standard chemical techniques, and do not involve advanced fabrication approaches. The nanomaterials based sensing tools can be applied to determine the toxins and other unwanted materials prevailing in the surroundings. Gold, silver, platinum, palladium, copper, cobalt and other nanoparticles are being extensively explored in the development of biosensors. The various applications of nano biosensors include the detection of glucose in diabetic patients (Pickup et al., 2005), detection of urinary tract bacterial infections (Drummond et al., 2003), detection of nitrates, inorganic phosphates (Larsen et al., 1997), detection of HIV-AIDS (Alterman et al., 2001), and the diagnosis of cancer (Gao et al., 2004). Gold nanoparticle modified DNA has been used to develop a microcantilever-based DNA biosensor to detect DNA even at very low concentration through a hybridization reaction (Su et al., 2003). Real-time electricity based sensors for biological and chemical species have been made using boron-doped silicon nanowires (SiNWs) (Cui et al., 2001). Nano biosensors can be used for feeding of nutrient media and substrate mixtures into the bioreactors, preparations and separation of various compounds in different industrial operations. For example, in the metallurgical operations requiring separation of impurities existing in a complex form, nano biosensors can be used to separate the impurities (Malik et al., 2013).

### 2.3.4 Pollution remediation

**Elimination of pollutants:** Due to their enhanced chemical activity, nanomaterials can be used as catalysts to react with toxic gases (such as carbon monoxide and nitrogen oxide) in automobile catalytic converters and power generation equipment (Joshi, 2009). Paints that absorb noxious gases from vehicle exhaust have already been developed. They contain 30 nm spherical nanoparticles of titanium oxide and calcium carbonate mixed in a silicon-based polymer, polysiloxane, and absorbs nitrogen oxide gases from vehicle exhaust. The porous polysiloxane lets the nitrogen oxide gases diffuse and adhere to the titanium dioxide particles.
UV radiation from sunlight converts nitrogen oxide to nitric acid, which is then neutralized by the calcium carbonate. The lifetime of the paint is said to be up to 5 years (Hogan, 2004).

**Water Remediation:** Iron nanoparticles with a small content of palladium are tested to transform harmful products in groundwater into less harmful end products (He and Zhao, 2005). The nanoparticles are able to remove organic chlorine (a carcinogen) from water and soil contaminated with the chlorine-based organic solvents (used in dry cleaners) and convert the solvents to harmless hydrocarbons. Chitosan nanoparticles are used in drinking water as disinfectants (Li et al., 2008).

### 2.3.5 Cosmetics

Due to the recent development of nanotechnology, engineered nanomaterials have been embraced by the cosmetics industry for several reasons. Because of their ability to penetrate deeper into the protective layers of skin than any cosmetic before, they are used as delivery agents for skin nutrients, such as synthetic peptides that instruct cells to regenerate. Some nanoparticles have antioxidant properties, feature that helps maintain a youthful appearance of the skin (Xiao et al., 2005). Due to their small size and specific optical properties, they are thought to conceal wrinkles and small creases. Titanium dioxide and zinc oxide become transparent to visible light when formed at the nanoscale, however are able to absorb and reflect UV light. Therefore, currently they are being used in sunscreens and in the cosmetic industry. Functionalized fullerenes are now incorporated into cosmetic products, such as creams, claiming radical scavenging properties. Alumina nanopowder is used for optical reduction of fine lines (Lohani et al., 2014).

### 2.3.6 Coatings

Nanomaterials have been used for very thin coatings for decades. Self-cleaning windows are coated with highly hydrophobic titanium dioxide. The titanium dioxide nanoparticles speed up, in the presence of water and sunlight, the breakdown of dirt and bacteria that can then be washed off the glass more easily (Buzea et al., 2007). Nanoparticles are already being used in coating textiles such as nylon, to provide antimicrobial characteristics. Also the control of porosity at the nanoscale and surface roughness in a variety of polymers and inorganic materials led to ultra hydrophobic - waterproof and stain resistant fabrics (Joshi and Bhattacharyya, 2011).

### 2.3.7 Industrial Usage

**Insulation materials:** Nanocrystalline materials synthesized by the sol-gel technique exhibit a foam-like structure called an "aerogel". Aerogels are porous, extremely lightweight, and have low thermal conductivity (Hrubesh and Poco, 1995).
**Nanocomposites:** Composites are materials that combine two or more components and are designed to exhibit overall the best properties of each component. Nanocomposites containing CNT (carbon nanotube) and polymers are being used in wide range of applications, such as super capacitors, sensors, solar cells, etc. (Baibarac and Gomez-Romero, 2006).

**Paints:** The wear resistance of the paints based on encapsulated nanoparticles is claimed to be ten times greater than that for conventional acrylic paints. TiO₂ because of its white colour is used in paints (Borup and Leuchtenberger, 2002).

**2.3.8 Food industry:** Nanotechnology holds many interesting applications in the food industry, for instance in terms of food safety and quality control. Nanosieves are used to filter out bacteria and nanosensors for the detection of contaminants or micro-organisms. These sensors can also be incorporated in food packaging materials to detect food deterioration. Nanoscale iron powder can be employed in the operation of water purification and soil cleanup. In food storage and food packaging, silver, silica, magnesium and zinc oxide nanoparticles are used. Nano-silver has also been employed to coat packaging materials and interior surfaces of fridges and dishwashers, as well as being integrated into plastic food containers. Nanoclays are used to increase the shelf life of drinks in plastic bottles by preventing oxygen from migrating through the plastic bottle walls and destabilising the drink (Wang et al., 2008). The use of nanotechnologies can lead to the development of products that are lower in fat, sugar and salt, and can help overcome technical and sensory problems that food developers come across when using formal methods. Like mayonnaise that is much lower in fat but tastes equally good as the high fat product can be prepared. Canola Active Oil contains nanocapsules or nanomicelles of phytosterols, which are believed to cut back the consumption of cholesterol from the digestive tract. The chocolate slim shake is a dietary product, where silica nano-molecules are included that are coated with cocoa particles to make a creamy coffee taste with reduced fat content. Toffee is made up of fat droplets surrounded by a thin nanoscale protein membrane in a matrix of sugar containing milk protein. Titanium dioxide is a white solid used in certain candy-coated chocolates (Pray and Yaktine, 2009). Higher intake of salt is considered dangerous and unhealthy. Surveys have indicated that the size of salt particles dominates the salt intensity and how quickly the salt is tested. By using smaller, and potentially nano-sized, salt particles, the level of salt in products such as crisps and snacks could be brought down, yielding a healthier product (Rama et al., 2013).
2.3.9 Mechanical engineering

Cutting tools: Made of nanocrystalline materials (such as tungsten carbide, WC) are much harder than their conventional due to the fact that the micro hardness of nanosized composites is increased compared to that of micro sized composites (Stiglich et al., 1996).

Lubricants: Nanospheres of inorganic materials could be used as lubricants, acting as nanosized ball bearings (Fleischer et al., 2003).

2.4 Zinc Oxide Nanoparticle

Of the inorganic materials, metal oxides such as titanium dioxide (TiO$_2$), zinc oxide (ZnO), magnesium oxide (MgO) and calcium oxide (CaO) are of particular interest as they are not only stable under harsh process conditions but also generally regarded as safe materials to human beings and animals (Fu et al., 2005). Some of the metal oxides e.g. MgO and CaO are essential minerals for human health. Other metal oxides such as TiO$_2$ and ZnO have been used extensively in the formulation of personal care products (Roselli et al., 2003). Among the nanoparticles exploited in nanomedicine, zinc oxide nanoparticles (ZnO NPs) are very promising because of their unique properties. It usually appears as a white powder and is nearly insoluble in water. The powder is widely used as an additive for numerous materials and products including plastics, ceramics, glass, cement, rubber (e.g. car tyres), lubricants, paints, ointments, adhesives, sealants, pigments, foods (source of Zn nutrient), batteries, ferrites, fire retardants, etc. ZnO is present in the earth crust as a mineral zincite; however, most ZnO used commercially is produced synthetically. ZnO is nontoxic and is compatible with human skin making it a suitable additive for textiles and surfaces that come in contact with human body. The increase in surface area of nanoscale ZnO compared to bulk has the potential to improve the efficiency of the material function (Li et al., 2006).

2.4.1 Synthesis

There are several new routes developed to synthesize ZnO nanoparticles, such as a wet polymerization method (Ying et al., 2009), sol-gel combustion (Zak et al., 2011), precipitation or co-precipitation method (Wang et al., 2010), hydrothermal (Lu et al., 2000), solvo thermal method (Sangkhaprom et al., 2010), chemical vapor deposition (CVD) (Yousefi et al., 2010), microwave assisted (Cao et al., 2011), sonochemical method (Mishra et al., 2010) and thermal oxidation method (Xu et al., 2011). Co-precipitation is one of the most successful techniques for synthesizing ZnO NPs. It is fractional precipitation of a
specified ion in a solution. Precipitation is not only of the target ion but also of other ions existing side by side in the solution. The additional precipitation of unwanted ions is of course an impediment to the analytical process. Some of the most commonly used substances in co-precipitation operations are hydroxides, carbonates, sulfates and oxalates (Rajendran et al., 2010). The nanocrystalline particles of ZnO are synthesized using ultrasonic irradiation and the particle sizes are controlled using different solvents during the sonication process.

2.4.2 Physical Properties

ZnO is a semiconductor with a wide band gap (3.3 eV), large exciton binding energy (60 meV) and n-type conductivity. ZnO can absorb UV light with the wavelength equal or less than 385 nm. ZnO semiconductor has several favourable properties, including good transparency, high electron mobility, wide band gap and strong room-temperature luminescence (Sivakumar et al., 2012). It has density and molar mass of 5600 kg/m$^3$ and 81.40 g/mol respectively. Melting point of ZnO nanoparticles is 1975°C and boiling point is 2360°C.

2.4.3 Chemical Properties

Zinc oxide nanoparticles are available as white powder as well as dispersions (Moezzi et al., 2012). ZnO exhibits three crystallize structures namely, wurtzite, zinc-blende and an occasionally noticed rock-salt. The wurtzite structure is most stable at ambient conditions and thus most common. It is nearly insoluble in water and alcohol, but it is soluble in (degraded by) most acids, such as hydrochloric acid (Norman and Alan, 1997). Though ZnO shows light covalent character, it has very strong ionic bonding in the Zn–O. Its longer durability, higher selectivity, and heat resistance are preceded than organic and inorganic materials (Ni et al., 2005).

2.4.4 Optical Properties

Zinc oxide nanoparticles possesses high optical absorption in the UVA (315–400 nm) and UVB (280–315 nm) regions which is beneficial in antibacterial response and used as a UV protector in cosmetics (Rodrigues-Paez et al., 2001).

2.4.5 Applications of Zinc Oxide nanoparticles (ZnO NPs)

It is used in paints, cosmetics, sunscreens, plastic and electronics and pharmaceuticals products etc. It is used in the walls of hospitals as antimicrobials. Zinc oxide is used in the
manufacture of rubber and cigarettes (used as a filter). It is also potentially used to treat leukemia and carcinoma cancer cell. It is an active ingredient for dermatological application in cream, lotions and ointments on account of its strong antibacterial property e.g calamine solution. In ceramic industry it is used as an additive in the manufacture of concrete. It is also used as drug carrier. ZnO nanoparticles are also used in industrial sectors including environmental, synthetic textiles, food, packaging, medical care, healthcare, as well as construction and decoration. They are also used as catalysts and as a component of paints, wave filters, UV detectors, transparent conductive films, gas sensing monitors, solar cells, sunscreens, and other cosmetic products. It has been used in waste water treatment due to their photocatalytic activity (Wist et al., 2004). It is also used as an additive in food products such as breakfast cereals and also in animal feed. Producing and packing meat products (e.g., meat and fish) and vegetable products (e.g., Sweet corn and peas) also involve use of zinc oxide nanoparticles (Espitia et al., 2012). ZnO nanostructures have become very attractive as UV-protective textile coatings. ZnO nano rod arrays provided excellent UV protection (Wang et al., 2004). ZnO nanoparticles are also used for the production of typographical and offset inks. It is also used as an artificial fertilizer (Moezzi et al., 2012). It also has uses in criminology, in mechanical fingerprint analysis (Pitkethly, 2009).

2.5 Nanoparticles as Antimicrobials

Metals have been used as antimicrobials for thousands of years. For example vessels made up of copper and silver has been used for water disinfection and food preservation since the time of the Persian Kings (Alexander, 2009). Japanese soldiers during the Second World War dropped silver coins into transport containers to conserve water to prevent the spread of dysentery (Borkow and Gabbay, 2009). Silver was used as storage devices during historical periods and silver nitrate solution was immediately used for wound healing during the Second World War (Law et al., 2008). The healing function of gold can be drawn backward to the Chinese medical history in 2500 BC. Red colloidal gold is even practiced in the Indian Ayurvedic medicine for rejuvenation and revitalization during old age under the name of Swarna Bhasma (“Swarna” meaning amber, “Bhasma” meaning every bit) (Mahdihassan, 1985). Over the past two centuries, physicians have used the, magnesium (Mg), arsenic(As) oxides as well as copper(Cu) and mercury (Hg) salts to treat diseases such as leprosy, tuberculosis, gonorrhea and syphilis (Frazer and Edin, 1930). The medicinal use of metals was prevalent until the discovery of antibiotics by Nobel laureate Sir Alexander Fleming in the 1920s, at which point these applications rapidly diminished. At the start of the 21st century with the rapidly increasing threat of multidrug resistance and the shortage of new antibiotics in the pipeline, the function of antimicrobial metals is undergoing resurgence.
Nanoparticles are called “a marvel of modern medical specialty”. Nanoparticles have antimicrobial properties that may be employed to control microbial populations, including those that have developed immunity to antibiotics (Rai et al., 2009). Various properties of nanoparticles which make them an ideal antimicrobial agent is summed up in figure 2. Besides these they have properties such as biodegradability, biocompatibility, conjugation, complexation or encapsulation properties and their ability to be functionalized.

Figure 2.2: Desirable properties of nanoparticle to be an antimicrobial

Among metal nanoparticles silver (Ag) nanoparticles have been extensively studied and applied as effective antimicrobial agents (Prabhu and Poulase, 2012). Silver nanoparticles are used as antimicrobial agents in most of the public places such as elevators and railroad stations in China. Besides, they are used as antimicrobial agents in surgically implanted catheters in order to reduce the infections caused during surgery and are proposed to possess anti-fungal, anti-inflammatory, anti-angiogenic and antipermeability activities (Kalishwaralal et al., 2009; Gurunathan et al., 2009; Sheikpranbabu et al., 2009). Recently, effective antimicrobial activity of silver nanoparticles has been observed against multidrug resistant and highly pathogenic bacteria, including *Staphylococcus aureus*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Escherichia coli*. 
Methicillin resistant *Staphylococcus aureus* (MRSA), Methicillin resistant *Staphylococcus epidermidis* (MRSE) and *Streptococcus pyogenes* (Ingle et al., 2008; Nanda and Saravanan, 2009; Lara et al., 2010). Ag nanoparticles also have fungicidal and fungistatic effects on the dermatophytes *Trichophyton mentagrophytes* (*T. mentagrophytes*) and *Candida* species (Kim et al., 2009; Gajbhiye et al., 2009). There are also several studies which reported the antiviral activity of silver nanoparticles against the hepatitis B virus, respiratory syncytial virus, HIV 1 and monkeypox virus (Sun et al., 2008; Rogers et al., 2008; Lara et al., 2010).

Besides silver nanoparticles, nitric-oxide-releasing nanoparticles (NO NPs) also possess broad spectrum antibacterial activity which can inhibit the growth of many antibiotic resistant and sensitive bacteria (Fang, 1997). Recently in a study NO-NPs significantly reduced all Gram-negative bacteria (*E. coli, K. pneumoniae* and *P. aeruginosa*) growth within 24 h, while in case of Gram positive (*S. pyogenes* and *E. faecalis*) bacteria lower NO-NP concentrations were able to achieve 90–100% growth inhibition within 8–16 h of exposure (Friedman et al., 2011). NO- nanoparticles have also shown activity against *C. albicans* and *T. mentagrophytes* infections (Schairer et al., 2012).

Chitosan nanoparticles have antimicrobial activity against bacteria, fungi, and viruses but have greater efficacy against fungi and viruses than against bacteria (Friedmann et al., 2013). Activity of chitosan nanoparticles against *S. aureus* and *E. coli* was found to be better than chitosan by itself, acetic acid, and certain antibiotics including doxycycline (Blecher et al., 2011).

Nanoparticles of gold (Au) have reported to be effective against killing of MRSA, Vancomycin resistant *Enterococci* (VRE), *E. coli* and *S. aureus* (Rai et al., 2010; Pissuwan et al., 2009). Recent works have focused on functionalizing the gold nanoparticles as geothermal agents for hyperthermically killing pathogens (Norman, 2008). The gold nanoparticles can be employed to coat a wide range of surfaces for instance, implants, fabrics for treatment of wounds and glass surfaces to maintain hygienic conditions in the home, in hospitals and other places (Das, 2009). Gold nanoparticles have also been researched for their anti-HIV activity as well as against influenza virus (Giancivincenzo et al., 2010; Sametband et al., 2011).

Cu (Cu) nanoparticles have shown activity against a wide range of bacterial pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli, B.subtilis, L. monocytogenes* and also fungi especially *S. cerevisae* (Ruparelia et al., 2010).
This microbiocidal effect increases with higher doses of copper-containing nanoparticles (Blecher et al., 2011).

X-ray treatment with Bismuth (Bi) nanoparticles is effective against multidrug resistant (MDR) *P. aeruginosa*, which is the most common gram negative bacteria causing nosocomial infections (Luo et al., 2013).

Inorganic metal oxide nanoparticles can be utilized as effective antimicrobial agents in view of their non-toxic profile, stability and antibacterial properties (Gordon et al., 2011). MgO nanoparticles have exhibited biocidal activity against certain gram positive, gram negative bacteria and even spores (Richards et al., 2000; Kooper et al., 2002). The germicidal effect of iron oxide nanoparticles on *Staphylococcus aureus* was evaluated by Tran and coworkers in 2010. Though less effective but copper oxide (CuO) nanoparticles have found to be effective against various organisms responsible for causing hospital acquired infections like *P. aeruginosa*, *S. aureus*, *B. subtilis* when used in high concentration (Ruparelia et al., 2008; Ren et al., 2009). Studies have shown that TiO$_2$ nanoparticles also inactivates various microorganisms that are highly resistant to desiccation and UV radiation, which makes TiO$_2$ a promising agent for improving process hygiene and product safety in food industry and cosmetics (Muranyi et al., 2010). Photocatalytic activity of TiO$_2$ nanoparticles against fungi have also been reported (Maneerat and Hayata, 2006). The antimicrobial effect of TiO$_2$ nanoparticles on *E. coli*, *S. aureus*, *L. monocytogenes* and *L. acidophilus* have also been demonstrated (Hu et al., 2006; Choi et al., 2007; Chorianopoulos et al., 2011). In order from higher to lower antimicrobial activity, TiO$_2$ nanoparticles damage viruses, then bacterial cell walls, and then bacterial spores (Huh and Kwon, 2011). Recent surveys have shown alumina (Al) nanoparticles to be efficient not only to bacteria (*E. coli*, *S. aureus*) but other microbial forms like micro algae also (Bala et al., 2011; Sadiq et al., 2011). Researchers from Cornell University and Rensselaer Polytechnic Institute have developed a new type of nanoscale surface to which bacteria can’t stick. This holds promise for applications in the food processing, medical and even shipping industries. Technology uses an electrochemical process called anodization to create nanoscale pores (15 nm) that change the electrical charge and surface energy of a metal surface, which in turn exerts a repulsive force on bacterial cells and prevents attachment and biofilm formation. It has been tested for alumina against well-known pathogens, *Escherichia coli* O157:H7 and *Listeria monocytogenes*, from attaching (Feng et al., 2014).
Zinc Oxide has proved to be a powerful antimicrobial agent in the formulation of the microscale and nanoscale systems for therapeutic applications. It has also been stated that ZnO nanoparticles showed greater antimicrobial activity apparently than micro particles. For example, a study that used particle supplements to liquid cell suspensions to investigate the antibacterial effect of both micron-scale and nanoscale ZnO particles concluded that nanoparticles had a greater antibacterial effect (Jiang et al., 2009). The stability of ZnO nanoparticles under harsh processing conditions and relatively low toxicity combined with the potent antimicrobial properties favours their application as antimicrobials (Stoimenov et al., 2002). Further ZnO nanoparticles, are nontoxic and biocompatible, therefore they have been utilized as drug carriers, cosmetics ingredients, and medical filling materials (Zhou et al., 2006). ZnO nanoparticles have bactericidal effects on both Gram-positive and Gram-negative bacteria and even against spores which are resistant to high temperature and high pressure (Kumar et al., 2011; Raghupathi et al., 2011). Studies have shown that ZnO nanoparticles exhibit minimal effect on human cells (Brayner et al., 2006; Zhang et al., 2007). The ZnO nanoparticles have exhibited good antimicrobial activity against B. subtilis, E. coli O157:H7, P. fluorescens, L. monocytogenes, enterotoxigenic E. coli, S. enteritidis, S. typhimurium, S. aureus, P. aeruginosa and C. jejuni (Sinha et al., 2011; Arabi et al., 2012; Xie et al., 2012). The nano-ZnO multilayer deposited on cotton fabrics showed excellent antibacterial activity against S. aureus (Ugur et al., 2010). 90% of the growth of B. subtilis was inhibited by the ZnO nanoparticles at the concentration of 10ppm whereas growth of E. coli was partially inhibited at the same concentration of zinc oxide nanoparticles in a study conducted by Adams et al., 2006. Previous reports using ZnO nanoparticles have also suggested stronger antibacterial effect on Gram-positive bacteria (S. aureus) as compared with Gram-negative bacteria (E. coli, P. aeruginosa) (Jin et al., 2009; Premanathan et al., 2011) where as contradicting results has been proposed by Sinha et al. (2011). The nanotoxicity was more pronounced on gram negative bacteria. ZnO nanoparticles reduce the growth of Enterobacter sp. by 50% while 80% reduction was observed in Marinobacter sp. Nanotoxicity towards gram positive cells was significantly less, due to the presence of thicker peptidoglycan layer. This fact was further supported by Nair et al. (2009) using PEGylated ZnO nanoparticles. Ansari et al. (2012a) have shown that the ZnO- nanoparticles inhibited bacterial growth of methicillin-sensitive S. aureus (MSSA), MRSA and methicillin –resistant
S. epidermidis (MRSE) strains independent of drug resistant mechanisms of MRSA and MRSE. Similar results were supported by the research of Hajipour et al. (2012) which also depicted antibacterial activity against methicillin-resistant Streptococcus agalactiae and methicillin-resistant S. aureus. These nanoparticles were also found to be effective against extended spectrum β-lactamases producing E. coli and K. pneumoniae (Ansari et al., 2012b). ZnO NPs have also been observed to possess significant antifungal activity against Fusarium sp. in a concentration dependent manner (Sharma et al., 2010). This is further backed by the study in which antifungal activity of zinc oxide (ZnO) nanoparticles was evaluated for Trichophyton mentagrophyte, Microsporum canis, Candida albicans and Aspergillus fumigatus. The maximum inhibition in all the tested fungi was observed at largest ZnO nanoparticles concentration i.e. 40 mg/mL (El-Diasty et al., 2013). Sivakumar and Senthilkumar, (2014) also supported good antifungal activity against Aspergillus fumigatus, Aspergillus flavus, Penicillium sp. and Aspergillus niger. ZnO nanoparticles affect the viability of the pathogenic yeast, Candida albicans also in concentration-dependent manner (Lipovsky et al., 2011). Shi et al. (2010) mentioned that ZnO nanoparticles had fungicidal effect against yeasts and also a fungistatic action against moulds. Lili et al. (2011) also investigated antifungal activity of zinc oxide nanoparticles against two postharvest pathogenic fungi (Botrytis cinerea and Penicillium expansum). ZnO nanoparticles, causing deformation in fungal hyphae, significantly inhibited the growth of B. cinerea and in case of P. expansum, ZnO nanoparticles prevented the development of conidoophores and conidia eventually leading to the death of fungal hyphae.

2.6 Mechanism of action

Drug resistance principally the multidrug is serious concern as it can result in treatment failure which can have severe consequences, especially in case of critical patients. Before going into mechanism(s) by which nanoparticles inhibit/inactivate microorganisms it is essential to study their mechanisms of resistance of microorganisms also. The mechanisms of antibiotic resistance of microorganisms are summed up in figure 3 and described below.

1) Decreased uptake and increased efflux of drug from the microbial cell: It prevents the concentration of antimicrobial agent from increasing to toxic levels within the microbial cell (Knetschand Koole, 2011).
2) Expression of resistance genes that code for an altered version of the substrate to which the antimicrobial agent binds: These types of resistance genes confer resistance to antibiotics such as beta-lactams, glycopeptides (including vancomycin), sulfonamides, quinolones, macrolides, aminoglycosides, tetracyclines, linezolid, and rifampin (Knetsch and Koole, 2011).

3) Covalent modification of the antimicrobial drug molecule which inactivates its antimicrobial activity: Microbes can also express drug resistance genes that code for enzymes that covalently modify the antimicrobial drug, thereby reducing its antimicrobial activity. Covalent modification of drug is used as a resistance mechanism against beta-lactams, aminoglycosides, chloramphenicol, tetracyclines, macrolides, quinolones, and streptogramins (Poole, 2002).

4) Increased production of a competitive inhibitor of antibiotic: For example, in case of resistance of sulfonamide, bacteria synthesis para-aminobenzoic acid (PABA), which compete with the sulfonamide drug for the binding site of bacterial dihydropteroatesynthetase (Deck and Winston, 2012).

5) Drug tolerance of metabolically inactive persisters: In a population of bacterial cells, a tiny fraction (~1 in every $10^6$ cells) randomly switches on expression of toxin–antitoxin (TA) genes, which cause their metabolic activity to slow or stop. These cells are called persisters, and their slower metabolic activity makes them more tolerant to antibiotics. Therefore, when an infecting population of bacterial cells is exposed to antibiotics, most of the cells that are drug-sensitive are eradicated, while the few persisters remain unaffected. This gives the appearance that the infection is cured. However, at some point, the persisters randomly switch back on their metabolic activity and resume growth, causing the infection to recur, despite the previous antibiotic treatment (Hajipour et al., 2012).

6) Biofilms: They result in tolerance of bacteria to very high concentrations of multiple antibiotics, resulting in chronic infections despite antibiotic treatment. Biofilm formation occurs in the pathogenesis of many infectious diseases, including gingivitis, otitis media, and lung infections (Huh and Kwon, 2011).

7) Swarming: Planktonic bacterial cells differentiate into elongated cells with multiple flagella, called swarm cells. These swarm cells stay in close proximity to each other
and migrate on surfaces as a single unit, analogous to a raft. These swarm cells are also tolerant to antibiotics (Friedman and Pelgrift, 2013).

![Image of mechanisms of antibiotic resistance]

**Figure 2.3: Mechanisms of antibiotic resistance (Singh et al., 2014)**

Famous microbiologist Alexander Fleming once said that “There is probably no chemotherapeutic drug to which in suitable circumstances the bacteria can not react by in some way acquiring fastness.” Therefore, there is a high probability of microorganism becoming resistant to newly developed drugs at later stages; further these drugs are highly expensive also. Therefore nanoparticles are considered to be good antimicrobial agents and may overcome the barrier of MDR owing to their multifunctional mechanisms to intervene normal cell functionality as it is unlikely for microorganisms to undergo series of mutation for developing resistance (Huh and Kwon, 2011).

Different nanoparticles exert different mechanism for antimicrobial activity. Some show multiple mechanisms whereas others follow one or two antimicrobial mechanisms. Nitric Oxide (NO) releasing nanoparticles exerts its antimicrobial action largely through reactive nitrogen oxide intermediates (RNOS), which are formed after NO reacts with superoxide ($O_2^-$). RNOS cause nitrosative damage to DNA, including breaking of strand,
formation of abasic sites, and deamination of cytosine, adenine and guanine. RNOS also cause increased generation of hydrogen peroxide ($\text{H}_2\text{O}_2$) and alkylating agents, which themselves damage DNA. They inhibit DNA repair enzymes, including DNA alkyl transferases and also react with prosthetic groups of proteins, like Fe–S clusters and heme, leading to removal of heme from the protein and Fe from the bacterial cell (Schairer et al., 2012). They also inactivate zinc metalloproteins, thereby inhibiting microbial cellular respiration as well as causing lipid peroxidation. NO can also react with thiols, producing S-nitrosothiols (RSNO), which are powerful nitrosylating agents. Finally, they can also stimulate the innate immune response in the human host (Blecher et al., 2011).

At pH lower than 6.5, amino group on chitosan nanoparticles becomes positively charged. These positive charges have an antimicrobial effect by associating with the negatively charged cell walls and plasma membranes of microbial cells. This leads to increased permeability of the microbial cell envelope, osmotic damage, and flow of cytoplasmic contents (including ions and proteins) out of the microbial cell (Huang et al., 2011). Chitosan also inhibits transcription of mRNA by binding to DNA in bacterial and fungal cells, and thereby hindering protein translation. Chitosan nanoparticles might also act by chelating metals, thereby decreasing the activities of metalloproteins (Huh and Kwon, 2011). They also inhibits release of inflammatory cytokines and increases production of fibroblasts and deposition of collagen III, thereby causing faster wound healing. Faster wound healing drops the probability of infection of the wound (Friedmann et al., 2013).

The antimicrobial activities of silver (Ag) nanoparticles are due to Ag$^+$ ions, which are formed when Ag is dissolved in aqueous solution (Knetsch and Koole, 2011). Ag$^+$ binds to negatively charged parts of the membrane creating holes in the membrane, allowing cytoplasmic contents to flow out of the cell, dissipating the $\text{H}^+$ gradient across the membrane, and sometimes causing cell death (Huh and Kwon, 2011). Otherwise, these interactions allow Ag$^+$ to pass through the cell wall and plasma membrane into the cytoplasm of the bacterial cell, where Ag$^+$ applies additional antimicrobial effects like inhibiting cytochromes of the electron transport chain of microbes (Huang et al., 2011). Ag$^+$ also binds to and damages DNA and RNA of microbes along with inhibiting DNA replication, thereby inhibiting cell division (Hindi et al., 2009). They also denature the 30S ribosomal subunit, thereby preventing protein translation. Further they also inhibit cell wall synthesis in Gram positive bacteria (Lara et al., 2010). Ag$^+$ ions can also exert antimicrobial effect by generation of
reactive oxygen species (ROS), which are toxic to both bacterial cells and eukaryotic host cells (Brown et al., 2012).

Copper (Cu) nanoparticles interact with amine and carboxyl groups on the surfaces of microbial cells (Blecher et al., 2011). At sufficiently high concentrations, free Cu$^{2+}$ ions induce formation of reactive oxygen species (ROS), which inhibit both DNA replication and amino acid synthesis in microbes (Huh and Kwon, 2011).

Upon exposure to near-UV and UVA radiation, TiO$_2$ generates reactive oxygen species (ROS), including hydrogen peroxide (H$_2$O$_2$) and hydroxyl radicals (•OH) resulting in damage of bacterial cell membranes, thereby compromising membrane semi permeability, interfering with oxidative phosphorylation, and sometimes causing cell death (Blecher et al., 2011). Some antimicrobial activity has also been shown by TiO$_2$ nanoparticles in the absence of irradiation, suggesting that they use other antimicrobial mechanisms unrelated to photocatalysis that have not yet been discovered (Huh and Kwon, 2011).

Magnesium-containing nanoparticles, including magnesium halogen-containing nanoparticles (MgX$_2$ NP) and magnesium oxide-containing nanoparticles (MgO NP), also use multiple mechanisms to combat microbes, thereby making resistance to them unlikely. In general, metal–halogen complexes inhibit certain enzymes of microbial cells. They may induce formation of reactive oxygen species (ROS), which cause lipid peroxidation of the microbial cell envelope, causing flow of cytoplasmic contents out of the cell (Blecher et al., 2011). MgF$_2$ Nanoparticles cause a drop in cytoplasmic pH which raises membrane potential by lipid peroxidation (Lellouche et al., 2012). They also inhibit growth and biofilm formation of E. coli and S. aureus.

Gold (Au) nanoparticles generally are considered to be biologically inert but can be engineered to possess chemical or photothermal functionality. It is also possible that gold nanoparticles bind to the DNA of microorganism and inhibit the uncoiling and transcription of DNA (Rai et al., 2010).

Aluminum oxide-containing nanoparticles (Al$_2$O$_3$ Nanoparticles) are a type of metal nanoparticle that might actually increase the likelihood of development of drug resistance. However, even at very high concentrations, Al$_2$O$_3$ NP damages the cell wall of microbes but causes only low levels of inhibition of microbial growth (Huh and Kwon, 2011). Al$_2$O$_3$ NP causes oxidative damage to the membrane of the microbial cell, and triggers an increase in
expression of genes that promote conjugation and a decrease in expression of genes that inhibit conjugation (Qiu et al., 2012).

Upon irradiation of Bismuth (Bi) with X-rays, Bi releases electrons and free radical forms. Both these electrons and free radicals heavily damage microbial DNA leading to cell death. Packaging Bi into nanoparticles shortens the average distance that the free radicals travel from Bi to microorganism, thereby significantly increasing the antimicrobial effect of bismuth nanoparticles (Luo et al., 2013).

![Mechanisms for antimicrobial activity of nanoparticles](image)


The mechanism of the antibacterial activity of ZnO particles is still not well understood. From the literature it is evident that the antimicrobial activity of ZnO nanoparticles depends on the surface area and concentration, while the crystalline structure and particle shape have little effect (Wang and Song, 2006; Hajipour et al., 2012). ZnO
nanoparticles use multiple mechanisms to combat microbes, thereby making resistance to them unlikely. These mechanisms are as follows:

1) ZnO nanoparticles bind strongly to bacterial cell membranes and destroy both the lipids and proteins of the membrane. This causes increased membrane permeability and flow of cytoplasmic contents out of the cell, which can cause cell death (Blecher et al., 2011). Huang et al. (2008) demonstrated disorganization of cell walls of both Gram-positive and Gram-negative bacteria when zinc oxide nanoparticles were used. In another study, ZnO nanoparticles (12 nm) inhibited the growth of *E. coli* by disintegrating the cell membrane and increasing the membrane permeability (Jin et al., 2009). SEM analyses of the bacteria before and after treatment with ZnO nanofluids show that the presence of ZnO nanoparticles damages the membrane wall of the bacteria. Electrochemical measurements using a model 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) monolayer suggest some direct interaction between ZnO nanoparticles and the bacteria membrane (*E.coli*) at high ZnO concentrations (Zhang et al., 2007). Study by Brayner et al. (2006) suggested that synthesized ZnO nanoparticles (average particles diameter 12 nm) are able to slow down the bacterial growth (100% with 3mM) as a result of disorganization of the *E. coli* membranes, which increases the membrane permeability leading to the accumulation of nanoparticles in the bacterial membrane and cytoplasm regions of the cells. It has been observed that nanoparticles get internalized in the cell resulting in cell wall disorganization and cell membrane damage. The cellular internalization of ZnO nanoparticles are also known to increase the oxidative stress inside the bacterial cell and cause damage to all components of the cell including proteins, lipid and DNA (Huang et al., 2008).

2) ZnO nanoparticles also cause formation of Zn$^{2+}$ ions and reactive oxygen species (ROS), including hydrogen peroxide (H$_2$O$_2$), which damage bacterial cell by binding of the particles on the bacterial surface due to the electrostatic forces (Huh and Kwon, 2011). Zinc ions are known to inhibit multiple activities in the bacterial cell, such as glycolysis, transmembrane proton translocation and acid tolerance (Phan et al., 2004). In contrast to the presence of ZnO nanoparticles, the presence of zinc ions is likely to inhibit bacterial proliferation (bacteriostatic), rather than killing bacteria (bactericidal). The production of reactive oxygen species and the disruption of cell membranes caused by ZnO nanoparticles may actually be bactericidal.
ZnO nanoparticles coated glass surfaces generate reactive oxygen species (ROS), which inhibit biofilm formation by *S. aureus* and *E. coli* (Applerot *et al.*, 2012). There are some reports on the considerable antibacterial activity of ZnO, which is attributed to the generation of reactive oxygen species (ROS) on the surface of these oxides (Sawai and Yoshikawa, 2004; Yamamoto, 2001). ZnO nanoparticles have weak mutagenic potential that induces frame shift mutation in *Salmonella typhimurium* (−) (TA98 and TA1537) (Pan *et al.*, 2010). The ability of ZnO nanoparticles to induce frame shift mutation is dependent on the presence of S9 fraction. It is possible that the S9 fraction increases the internalization of nanoparticles and then increases the generation of ROS that induce frame shift mutation in the bacteria. The toxicity of oxide nanoparticles (e.g., ZnO and CuO) does not always depend on the bacteria internalization. The nanoparticles can locally change microenvironments near the bacteria and produce ROS or increase the nanoparticles solubility, which can induce bacterial damage (Heinlaan *et al.*, 2008). As a direct proof the formation of hydroxyl radicals and singlet oxygen species of a suspension of ZnO was determined by electron spin resonance while H$_2$O$_2$ formation was evident by direct quantification (Sondi and Salopek-Sondi, 2004; Simoes *et al.*, 2006). Sawai *et al.*, 1996 studied the antibacterial behavior of ZnO particles by using a chemiluminescence and oxygen electrode analysis. They reported that H$_2$O$_2$ was produced in ZnO slurry and the concentration of H$_2$O$_2$ produced was linearly proportional to the ZnO particle concentration of the slurry.

3) When coated with polyvinyl alcohol (PVA), ZnO nanoparticles increase membrane permeability and then enter the cytoplasm of the bacterial cell, where they impose oxidative stress (Huh and Kwon, 2011; Huang *et al.*, 2008; Hajipour *et al.*, 2012). For bacteria grown in suspension in vitro, literature suggests that smaller diameter particles are more effective at reducing bacteria activity than larger particles with identical chemistry (Nair *et al.*, 2009).

4) They are also believed to interact with phosphorus, sulphur groups resulting in inactivation of DNA replication as well as inhibited enzyme function (Rai and Bai, 2011).

5) Antifungal activity of ZnO NPs is due to electrostatic interaction between the NPs and fungal cell surface leading to destruction of the cell membrane integrity (Shi *et al.*, 2010).
Incongruous and overuse of antibiotics have resulted in drug resistance. Microorganisms have successfully developed numerous strategies for resisting the action of practically all antibiotics (Kuroda et al., 2001). Currently used antifungals also have toxic side-effects, interact with other drugs, and become ineffective as a consequence of the rapid growth of fungal resistance (Shahi et al., 1999). Moreover, the therapeutic responses are slow, and thus inappropriate for treatment of patients with severe or rapidly progressive mycoses. This resistance to antimicrobial agents has resulted in distress and death from treatment failures, and increased health care costs. Drug resistance enforces high dose administration of antibiotics, often generating intolerable toxicity, development of new antibiotics, and requests for significant economic, labor, and time investments. Therefore, there has been increasing interest in the role of inhibitors of antibiotic resistance for combination therapy (Gibbons, 2005). Likewise, the development of vaccines and new antimicrobial agents has not kept pace with resistance; therefore, the search for other methods of therapy, such as synergistic combinations, is necessary. The increased clinical response to
combination therapy is explained to be due to synergism between the antibiotics used. Synergism has been defined as a phenomenon in which two different compounds are combined to enhance their individual activity. If the combination results in worsening effect, it is called antagonism. Effect which is less than synergistic but not antagonistic is termed as additive or indifference (Rani et al., 2009). Combination therapy is used to expand the antimicrobial spectrum, minimizing toxicity, preventing the emergence of resistant mutants during therapy and obtaining synergistic antimicrobial activity (Eliopoulos and Moellering, 1991). The combination of nanoparticles with existing antibiotics seems to be a very fascinating option by combining the two treatment modalities. Availability of different types of nanoparticles has opened up the avenue for probing the impact of this association on various pathogens. Combining antibiotics with nanoparticles restores their ability to destroy microorganisms that have acquired resistance to them. Furthermore, nanoparticles tagged with antibiotics have been shown to increase the concentration of antibiotics at the site of microbe-antibiotic interaction and facilitate the binding of antibiotics to bacteria (Allahverdiyev et al., 2011). Also with the nanoparticles targeting intracellular bacteria, the high local dose at the site of infection kills the infecting bacteria before they can develop resistance, while the total lower dose decreases the probability that bacteria outside of the site of action of these nanoparticles will develop drug resistance (Pelgrift and Friedman, 2013). Moreover, more than one antimicrobial compound can be packed within the same nanoparticle. Packing more antimicrobial within the same nanoparticle increases potency, antimicrobial efficacy and can overcome resistance mechanism in the microbes related to using each drug alone (Zhang et al., 2010). Many types of lipophilic and water-soluble antibiotics can be conjugated inside or on the surface of nanoparticles, or carried via encapsulation (Abeylath and Turos, 2008). Antibiotics loaded nanoparticles enter host cells through endocytosis, releases the content to eliminate intracellular microbes (Alphandary et al., 2000).

When mixed with antibiotics, silver nanoparticles (Ag Nanoparticles) increases the antimicrobial effects of the antibiotics, including penicillin G, amoxicillin, vancomycin, clindamycin, chloramphenicol, kanamycin, ampicillin and erythromycin against gram positive and gram negative bacteria (Blecher et al., 2011). In a study by Fayaz et al. (2009) enhanced effect was observed for ampicillin against Gram negative bacteria when combined with Ag NPs. The cross linking and rigidity of peptidoglycan of gram positive cell wall make it difficult for silver nanoparticles to penetrate. However, in case of Gram negative bacteria there is a thin layer of peptidoglycan that can be easily attacked by Ag NP-Ampicillin
conjugate leading to the internalization of these particles and in turn the cell lysis. Birla et al. (2008) reported the combined effect of silver nanoparticles with five antibiotics (ampicillin, gentamicin, kanamycin, streptomycin and vancomycin) on the three most common human pathogens— *S. aureus*, *E. coli* and *P. aeruginosa*. The antibacterial activities of ampicillin, gentamycin, streptomycin and vancomycin were found to be increased in combination with Ag-Nanoparticles against the Gram-negative micro-organisms, i.e., *E. coli* and *P. aeruginosa* as compared with *S. aureus* which is a gram positive bacterium (Pal et al., 2007). It has also been reported that the antibacterial activity of cefoperazone against methicillin resistant *Staphylococcus aureus* (MRSA) was boosted when it was used with colloidal silver (De Souza et al., 2006). In an experiment by Huang et al. (2011) chitosan was incorporated into Ag nanoparticles which decreased the growth and killed MRSA, *P. aeruginosa*, *P. mirabilis* and *A. bauminii*. The effect of Ag-chitosan complex was more pronounced than chitosan and Ag Np alone. This increased efficacy may be due to the fact that chitosan increases permeability of microbial cell and allow Ag NPs to enter more easily and have microbiocidal effect. Nanoparticles containing both Titanium dioxide (TiO$_2$) and Ag were more effective against *Candida albicans* and *Aspergillus* than Ag NPs and fluconazole alone (Blecher et al., 2011).

Packaging magnesium oxide (MgO) in MgO nanoparticles increases the number of halogen molecules that can be adsorbed onto the MgO by upto five fold, which increases microbiocidal activity of halogens. MgO Np with Chlorine (Cl$_2$) and bromine (Br$_2$) were highly bactericidal to *E. coli* and *Bacillus megaterium*, though less so to endospores of *B. subtillis* (Blecher et al., 2011).

Many studies have reported strong antimicrobial effects against Gram-positive and Gram-negative bacteria, including antibiotic-resistant strains, by Au/drug nanocomposites (e.g., Au Nanoparticles coated with antibiotics such as streptomycin, gentamicin, and neomycin) (Huang et al., 2009).In a recent study by Brown et al. (2012), gold (Au) nanoparticles combined with ampicillin (AMP) killed multi drug resistant bacteria including MRSA, *Enterobacter aerogenes*, *P. aeruginosa*, *E. coli* K-12 substrain DHS-alpha (pPCR-Script AMP SK+). Activity of gold capped vancomycin was 64 times higher than using vancomycin alone against vancomycin resistant *Enterococci* (VRE) and *E. coli*. Au Np has also revealed increased activity against *E. coli* when coated with ciprofloxacin (Huh and Kwon, 2011). In medical applications, gold nanoparticles have been used in treating B-
chronic lymphocytic leukemia (CLL). CLL is an incurable disease predominantly characterized by apoptosis resistance. Earlier CLL treatment was with anti-VEGF antibody; however, treatment was found to be more effective when VEGF antibody was attached to the gold nanoparticles (Mukherjee et al., 2007). Selective killing of *S. aureus* by Au Nanoparticles conjugated with anti-protein A antibodies, which target the bacterial surface, has been demonstrated (Mühling et al., 2009). Chamundeeswari et al. (2010) demonstrated that, chitosan-capped gold nanoparticles when coupled with ampicillin shows 2-fold increase in antimicrobial activity compared with that of free ampicillin show antibacterial activities against multidrug-resistant clinical isolates of *E. coli* and *P. aeruginosa*. The coating of aminoglycosidic antibiotics with gold nanoparticles has an antibacterial effect on a range of Gram-positive and Gram-negative bacteria (Saha, 2007). Cefaclor (a second-generation β-lactam antibiotic) reduced gold nanoparticles have potent antimicrobial activity on both Gram positive (*S. aureus*) and Gram-negative bacteria (*E. coli*) compared to cefaclor and gold nanoparticles alone (Rai et al., 2010). Fayaz et al. (2011) coated gold nanoparticles with vancomycin forming vancomycin bound gold nanoparticles (VBGNP) and tested it against *E. coli*, *S. aureus* and vancomycin resistant *S. aureus*. The VBGNP exhibited notable antibacterial activity against *E. coli* which is normally resistant to vancomycin. This implies that gold nanoparticles facilitated the binding of vancomycin to the bacterial cell surface independent of its structure in both Gram-positive and Gram-negative bacteria. Perni et al. (2009) demonstrated the ability of gold nanoparticles to increase photo dependent oxidative action of methylene blue embedded in polysiloxane polymers on MRSA and *E. coli*. According to findings of Zhao et al. (2010) amino-substituted pyrimidines, which themselves do not possess any antibiotic action, in the presence of gold nanoparticles exhibit antibacterial activity against multidrug-resistant clinical isolates (e.g., *E. coli*, *P. aeruginosa*) without any additional source of energy such as light irradiation.

Conjugating bismuth (Bi) nanoparticles to antibodies against the target microbe shortens the average distance between the Bi Nanoparticles and bacterial cells, thereby increasing the bactericidal effect even more (Luo et al., 2013). Bi nanoparticles combined with polyclonal antibodies were effective against MDR *P. aeruginosa* when irradiated with X-rays of low dose.

Vancomycin encapsulated in chitosan nanoparticles is effective against VRSA (Huh and Kwon, 2011). Chitosan alginate nanoparticles containing benzyl peroxide has increased activity against *Propioniobacterium acne* as compared to their activity alone (Friedman et al.,
2013). A study also showed chitosan's synergistic antimicrobial activity against drug resistant *P. aeruginosa* when used with sulfamethoxazole (Tin *et al.*, 2009). Benzyl penicillin-encapsulating cationic liposomes and NO-releasing silica Nanoparticles showed antimicrobial and antibiofilm activities (Martinez *et al.*, 2009).

One of the commonly used non-silver nanoparticles is titanium dioxide (TiO$_2$). The efficacy of twenty two different antibiotics with TiO$_2$ nanoparticles has been studied against MRSA by Roy *et al.* (2010). The authors studied activities of antibiotics such as penicillins, cephalosporins, glycopeptides, aminoglycosides, fluoroquinolones, azalides, macrolides, lincosamides and sulfonamides. They found that the antibacterial activities of all antibiotics have been increased in the presence of nanosize titanium dioxide against test strains and optimum results were observed with penicillin and amikacin. The lowest increase in activity was detected for chloramphenicol followed by norfloxacin and clarithromycin. With nalidixic acid TiO$_2$ nanoparticles showed no beneficial antibacterial effect. TiO$_2$ Ag NPs when irradiated with visible light has more antibacterial and antiviral activity when TiO$_2$ irradiated by visible light alone (Huh and Kwon, 2011).

Therapeutic roles for zinc in different diseases have been established in recent years. Zinc oxide has a very good potential to get into the clinic. Studies have showed that the antimicrobial activity of ZnO nanoparticles combined with other antimicrobial agents have better activity compared with that of uncombined ZnO nanoparticles. They have antifungal activity and synergistic activities when combined with antifungal agents’ fluconazole (FLU) and caspofungin (CASPO). It has potential as a combination therapeutic agent for the treatment of infections caused by resistant *Candida* isolates (Albarrag *et al.*, 2014). Studies have revealed improved activity of nano ZnO when used in combination with cephalosporins, beta lactums and amino glycosides against different pathogenic microorganisms (Tillotson and Theriault, 2013). Luo *et al.* (2013) investigated the synergistic role of ZnO nanoparticles against gentamicin, clarithromycin, ceftriaxone and ofloxacin. Compared to other three antibiotics, ZnO nanoparticles achieved synergistic antibacterial effects with ceftriaxone against *E. coli*. The results of colony-forming capability test, infrared ray (IR) spectrum, and detection of reactive oxygen species (ROS) indicated that ceftriaxone facilitate the entry of ZnO nanoparticles into bacterial cell. The combined effect of ZnO nanoparticles and antibiotics was promising against, *Enterococcus sp.*, *Staphylococcus aureus*, *Proteus mirabilis* for vancomycin, erythromycin, ofloxacin and tigecycline (Chauhan *et al.*, 2014).
Banoee et al. (2010) demonstrated beneficial interactions of ZnO nanoparticles with ciprofloxacin, in the presence of ZnO nanoparticles a total of 27 and 22% increase in inhibition zone areas was observed against S. aureus and E. coli, respectively. Gaddad et al. (2010) found that the antimicrobial activity of beta lactums, cephalosporins, aminoglycosides, glycopeptides, erythromycin, clindamycin and tetracycline against S. aureus increased by ZnO nanoparticles in the sub-inhibitory concentration of 100 μg/disc. The antibacterial activities of all antibiotics have increased from minimum 2 mm to a maximum of 10 mm.

Increased activity of antibiotic in presence of ZnO nanoparticles can be attributed to inhibition of antibiotic efflux from the cell due to interference of ZnO nanoparticles with pumping NorA protein, activation of antibiotic uptake by influencing activity of membrane Omf protein, and by binding reaction between antibiotic and ZnO nanoparticles stabilizing the antibiotic–ZnO nanoparticle complex. They induce faster electron transfer kinetics in its active site which hinders with the activity of NorA protein and helps restoring antibiotic action (Banoee et al., 2010).

2.8 Doping

Doping is the process of adding controlled impurities to a semiconductor material. There have been significant changes in physical, chemical, and biological properties of host material on doping depending on types of dopant and its concentration. It has also been found from some previous studies that doping may increase antimicrobial effect (Rekha et al., 2010). Dopant impurities like Cu$^{2+}$, Mn$^{2+}$, Co$^{2+}$, Ni$^{2+}$, rare earth and transition elements, play an important role in changing the electronic structure and transition possibilities of the host material (Zhang et al., 2006). Ag doped with polymer chitosan and iron oxide results in high antimicrobial efficacy against E. coli, B. subtilis and S. aureus (Banerjee et al., 2010). Silver and nitrogen doped TiO$_2$ was found to be more effective against E. coli biofilms and B. subtilis as compared to pure TiO$_2$ nanoparticles (Matsunga et al., 1988). Yuan and coworkers in 2010 studied the antibacterial properties of silver (Ag)- and nitrogen (N)-doped TiO$_2$ nanoparticles by agar diffusion method toward Escherichia coli and Bacillus subtilis. The results indicated that both Ag and N doped TiO$_2$ increased the antibacterial properties of TiO$_2$ nanoparticles under fluorescent light irradiation. A 1% Ag-N-TiO$_2$ had the highest antibacterial activity with a clear inhibition zone of 33.0 mm toward Escherichia coli and 22.8 mm toward Bacillus subtilis. Very interestingly, Ag doped TiO$_2$ nanoparticles
showed strong light independent antimicrobial activities against both *E. coli* and *B. subtilis* spores, by exploiting the combined bactericidal activity of Ag and TiO$_2$ together (Hamal *et al.*, 2010). Increase in antibacterial property of Ag doped TiO$_2$ is due to the effect of doped metal (Ashkarran *et al.*, 2010). In another study also, Ag-doped TiO$_2$ nanoparticles exhibits an excellent toxicity against the *E. coli* bacteria (Thiel *et al.*, 2007). The photocatalytic activity by UV-A and the potential activation by visible light, when doped with novel metals, make TiO$_2$-mediated disinfection especially useful in developing countries where electricity is not available for sterilization (Li *et al.*, 2008). The antibacterial effect of those TiO$_2$ nanoparticles, gold capped TiO$_2$ naonocomposites, and vanadium (V)-doped TiO$_2$ nanoparticles were evaluated on two types of bacteria, *E. coli* and *B. megaterium*, and it was found that they have strong oxidizing ability and photocatalytic activity. The good antibacterial effect may be due to small particles size, large surface area, large band gap energy, and more active sites for carrying out catalytic reactions (Fu *et al.*, 2005). Gold (Au) nanoparticles doped with toluene blue O and certain antibodies are effective against Methicillin Resistant *S. aureus* (Zharov, 2006). MgO nanoparticles doped with halogens (Cl$_2$, Br$_2$) have shown antimicrobial activity against Gram positive, Gram negative bacteria and even spores (Stoimenov *et al.*, 2002). Chen *et al.* (2008) studied antibacterial activity of nanoparticles composed of iron and titanium oxides with immobilized succinic anhydride by dopamine linker on the surface of nanoparticles and also subsequent immobilization of IgG against *Staphylococcus saprophyticus*, *Streptococcus pyogenes*, and antibiotic-resistant bacteria–multidrug-resistant *S. pyogenes*and MRSA. The authors demonstrated not only antibacterial action but also effective inhibition of the bacterial growth by nanoparticles under irradiation of a low-power ultraviolet (UV) lamp during short period. The antibacterial activity of the Zn-doped CuO in a colloidal suspension or deposited on the fabric was tested against *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive) bacteria. A substantial enhancement of 10,000 times in the antimicrobial activity of the Zn-CuO nanocomposite compared to the pure CuO and ZnO nanoparticles was observed after 10 min exposure to the bacteria. Similar activities were observed against multidrug resistant *E. coli* and Methicillin-resistant *S. aureus* (Malka *et al.*, 2013). The prepared bimetal (Cu and Ag) nanoparticles doped beads exhibited significantly larger antibacterial activities than single (Cu or Ag) metal doped beads for both Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* bacteria. The prepared bimetal beads remained effective for 120 h, completely inhibiting the bacterial growth, and therefore, they can be considered potential antibacterial agents for water purification (Khare *et al.*, 2014). Doping of palladium
(Pd) nanoparticles with gold (Au) can be a promising approach for the reductive treatment of wastewaters containing halogenated contaminants (De Corte et al., 2012). Furthermore, the ability of CuO nanoparticles to reduce bacterial population to zero was enhanced in the presence of silver (Ren et al., 2009; Maniprasad and Santra, 2012). Kunkalekar et al. (2013) found the chemically synthesized silver doped MnO$_2$ nanoparticles have antibacterial activity against MRSA. Significant increase in antibacterial activity ($E. coli$) and antifungal activity ($A. niger$) was observed for silver doped bismuth oxide (Bi$_2$O$_3$) (Raj et al., 2014).

ZnO is one of the most promising materials since it has a high mechanical and thermal stability. Recently, it has been reported that the doping of ZnO nanostructures with other elements can enhance its various properties (Jung et al., 2011). Therefore, doping with selective elements offers an effective method to enhance and control the electrical and optical properties of ZnO nanostructures, which is crucial for its practical applications (Wang et al., 2008). Commonly, different elements as a dopant in ZnO can be categorized into two groups: one group can substitute for zinc (Zn) and the second group for oxygen (O). These different dopants can tune various properties of ZnO nanostructures.

Antimicrobial study of selenium (Se) doped ZnO nanoparticles and undoped ZnO was done against $E. fecalis$, $K. pneumonia$, $E. aerugenosa$, $P. aerugenosa$, $A. Fecalis$, Fusarium and Aspergillus. Se doped ZnO demonstrated better activity and largest inhibition was observed with Se-doped ZnO, against $E. fecalis$. Further, around 80-90 % of inhibition was observed for $K. pneumonia$ and $P. aerugenosa$ by Se-doped ZnO. Again, very good inhibition was recorded for Se-doped ZnO against fungi Fusarium and Aspergillus, while ZnO nanoparticles resulted in moderate zone of inhibition against tested fungi (Sowbhagya and Ananda, 2014). Regarding Ag-doped ZnO thin films, it was verified that increasing the silver content decreases the growth rate of Escherichia coli and decreases the amount of bacterial cells present at the end of the experiment (Carvalhoa et al., 2014). Nano-ZnO was twice as potent in killing Aspergillus, as compared to its non-nano-counterpart and loading of nano-ZnO with 5% nano-Pd (palladium) further increased its activity, four times that of micro-ZnO. Nano-ZnO doped with 5 % nano-Pd, pure nano-ZnO and micro-ZnO, showed antifungal activity against Aspergillus niger with an MIC of 1.25, 2.5 and 5mg/mL, respectively. However, Candida albicans yeasts were relatively resistant to these compounds, with an MIC of 2.5, 5 and 10 mg/mL for Pd doped nano-ZnO, nano-ZnO and micro-ZnO, respectively (Gondal et al., 2012). Pristine doped ZnO nanoparticles showed complete inhibition of growth of $E. coli$ as compared to 51% inhibition by Se doped ZnO nanoparticles.
The antibacterial activity of undoped ZnO and tin (Sn) doped ZnO nanostructures synthesized by a simple, versatile, and wet chemical technique have been investigated against *Escherichia coli*, methicillin-resistant *Staphylococcus aureus*, and *Pseudomonas aeruginosa* bacterial strains. It has been interestingly observed that Sn doping enhanced the inhibitory activity of ZnO against *S. aureus* more efficiently than the other two bacterial strains (Jan et al., 2013). It has also been reported that tyrosine assisted addition of Ag to ZnO nanoparticles enhanced photodegradation of organic dye pollutants and destruction of bacteria (Lu et al., 2008). Research by Karunakaran et al. (2010, 2011a, 2011b) synthesized Ag ZnO nanoparticles using microwave, sono-chemical and sol-gel methods. The results showed that Ag-doped ZnO nanoparticles had better antibacterial activity compared with ZnO nanoparticles. The Ag (7.5%) doped ZnO nanoparticles inhibit the growth of *E. coli* more than 88 percent (Venkatasubramanian and Sundaraj, 2014). Femi et al. (2011) reported great antimicrobial potential of gold (Au) doped ZnO nanoparticles against *S. aureus, K. pneumoniae, Enterococci sp., S. typhi*. Increased membrane permeability, cellular internalization, and intracellular structural change of polyvinyl alcohol (PVA)-coated ZnO nanoparticles were also reported (Huang et al., 2008). Bacteriological study showed the enhanced antibacterial activity of transition metals (Co and Mn) doped ZnO nanoparticles than undoped ZnO indicating the great potential of ZnO nanoparticles in relevant clinical and biomedical applications (Nirmala and Anukaliani, 2011). Dutta et al. (2010) also reported that Fe- or Co-doped ZnO nanoparticles had enhanced antibacterial activity against *E. coli*. Nair et al. (2011) and Manjula et al. (2011) also showed increased antibacterial activity of Co doped ZnO nanoparticles against Gram negative *E. coli, K. pneumoniae, S. dysenteriae, S. typhi, P. aeruginosa* and Gram positive *B. subtilis, S. aureus* and *B. atrophaeus*. Gordon et al. (2011) combined ZnO with iron oxide to produce magnetic nanoparticles with antibacterial activity. The results showed that the antibacterial activity of the combined nanoparticles was dependent on the weight ratio [Zn]/[Fe], i.e., the higher the ratio, the higher the antibacterial activity. Desselberger (2000) synthesized Mn doped ZnO nanoparticles and found that doped ZnO nanoparticles had an increased antibacterial activity against both Gram-negative and Gram-positive bacteria than undoped ZnO nanoparticles. Similarly enhanced antibacterial activity of Mn doped ZnO against *Escherichia coli, Salmonellae typhi, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Serratiamar ceseus* was reported by Rekha et al. (2010) as well as Sonia et al. (2011).

One possible explanation of the antimicrobial effect of doped ZnO is based on the abrasive surface texture of ZnO. ZnO nanoparticles have been found to be abrasive due to
surface defects (Stoimenov, 2002). The reason is that doping of metal oxide and/or transition metals (like Mn) increases the surface defects.

2.9 Toxicity of nanoparticles

Nanoparticles due to their unique physico-chemical and biological properties have far reaching industrial and medical applications. But there is a dearth of knowledge about the effect of the prolonged exposures to nanoparticles on human health and environment. Humans have evolved to cope with most naturally occurring nanoparticles. However, some nanoparticles, account for many premature deaths. The potential human benefits of nanotechnology are innumerable and include many aspects of human life with wide a diversity of merchandise. Apart from positive effect, uncontrollable use of nanotechnology/nanoparticles will have deep impact on health, environment and society. Therefore, profound knowledge about the potential effect of nanoparticles on health, environment and society needs to be assessed completely before their large-scale production and application in various fields (Canesi et al., 2008).

2.9.1 Effect of nanoparticles on Human Health

Knowledge of the toxicity effects of these small substances is limited, but is rapidly growing. Several reports have addressed the harmful impact of nanomaterials on living cells (Nel et al., 2006; Singh et al., 2009). Studies conducted on the nanoparticles-induced toxicity have revealed that the metal-based nanoparticles can affect the biological behavior at the organ, tissue, cellular, subcellular and protein levels. It has also been shown that intravenously injected nanoparticles can be accumulated in colon, lung, bone marrow, liver, spleen, and lymphatics (Hagens et al., 2007). Inhaled nanoparticles can also enter the systemic circulation and reach lung, liver, heart, spleen, and brain (Poma et al., 2008), which is particularly facilitated for small size nanoparticles because of efficient cellular uptake and transcytosis across epithelial and endothelial cells into blood and lymph circulation (Rabea et al., 2003). Inflammation and fibrosis are effects observed on an organism level, whereas oxidative stress, antioxidant activity and cytotoxicity are observed effects on cellular level (Oberdorster et al., 2005). Studies also suggest the possibility of multi-organ nanotoxicity that therapeutically administered antimicrobial nanoparticles may generate. For example, free radical-mediated oxidative stress generated by the interaction of antimicrobial nanoparticles with cells may result in hepatotoxicity and pulmonary toxicity (De Jong and Borm, 2008). Various metabolic changes suggest mitochondrial failure, and enhanced ketogenesis, fatty
acid β-oxidation, and glycolysis, contributing to hepatotoxicity and nephrotoxicity (Lei et al., 2008). Besides, some classes of nanoparticles can affect the circulatory system by altering heart rate (Chalupa et al., 2004) as well as reproductive system by increased detachment of seminiferous epithelium and possible spermatoxicity (Yoshida et al., 2010).

Factors affecting toxicity of nanoparticles are: small particle size, a large surface area and the ability to generate reactive oxygen species (Nel et al., 2006). The size of the nanoparticles are small and these can easily access the skin, lungs and brain and cause adverse effects (Kennedy et al., 2009). For example, exposure of metal based nanoparticles to human lung epithelial cells leads to the generation of reactive oxygen species and result in oxidative stress and cellular damage. Although the role of nanoparticles in toxicity is uncertain, experimental evidence has shown that very small particles inhibit phagocytosis function more than larger particles. Donaldson et al. (2001) have shown that very small metal particles cause more inflammation than larger inhaled particles. Some researchers have shown that most of the nanoparticles can release active oxygen and cause oxidative stress and inflammation by the RES (reticuloendothelial system). Acute toxicity resulting from nanoparticles has been investigated in the mouths of rats. The results indicated that toxicity depends on the size, coating, and chemical component of the nanoparticles (Ai et al., 2011). Also, the systemic effects of nanoparticles have been shown in different organs and tissues. The effects on inflammatory and immunological systems may include oxidative stress or pre-inflammatory cytotoxin activity in the lungs, liver, heart, and brain. The effects on the circulatory system can include prethrombosis effects and absurd effects on heart function. Genotoxicity, carcinogenicity, and teratogenicity may occur as a result of the effects of nanoparticles. Some nanoparticles could pass the blood–brain barrier and cause brain toxicity (Muhlfeld et al., 2008).

Researchers’ have investigated the effects of nanoparticles on microorganisms, invertebrates and vertebrates. There is motivation to do more studies on microorganisms due to their importance in industry and medicine. Li et al. (2005) discovered the killing features of TiO₂ nanoparticles and carbon nanotubes on endospore bacteria, which were coated with multi-wall nanotubes. They found that combining UV radiation with TiO₂ is more effective, although the combined TiO₂ and nanotubes caused endospore concentration. Therefore, some spores survived because of this protection. Nano silver crystals are used in bandages as antimicrobial agents, but the use of silver nanoparticles depends on counteracting their positive (antimicrobial effect) and negative (cellular toxicity) effects. Results have shown that
carbon nanotubes at high dose are toxic for organisms, and accordingly, health scientists have defined them as dangerous and suggested manipulation of the nanoparticles (Willems et al., 2000). Also, an increase in the functional degree of single-wall carbon nanotubes displays lower toxicity than multi-wall nanotubes in in vitro tests on fibroblasts. Diamond is accepted in the scientific community as a biomaterial in the 21st century and is used in coatings for synthetic heart valves, orthopathy designs, joint substitutes, catheters, stent orthopedic pins, and tooth roots. Diamond nanoparticle coating on a hip implant increases strength, but the release of nanoparticles as a result of scratching or abrasion can cause problems for the body. The monocyte morphology changed after silicon carbide and HA (Hydroxyapatite) digestion, but no differences were seen after diamond absorption (Bauer et al., 2003). Magnetic nanoparticles have been used in photogene, targeting drug delivery, cell separation, cancer therapy, imaging, and magnetic hyperthermia for cancer therapy, and also for tissue engineering. Super paramagnetic nanoparticles, 2 to 30 nm, with citric acid or methyl carboxyl dextrin were tested on rats and showed that these nanoparticles cause diarrhea and may lead to animal death while citrate itself does not cause toxicity (Mahmoudi et al., 2009). The effects of bio adjusted coating layers have been studied on magnetic nanoparticles. Uncoated iron oxide particles of 20 nm cause toxicity in human skin fibroblasts. Iron oxide nanoparticles of 9 nm were coated with polyvinyl alcohols (PVA), which have thiol, amine, and carboxylic acid functional groups. The results show that PVA nanoparticles, when combined with thiol, carboxyl, and PVA, poison melanoma cells (Rishikesh, 2009). Using 2 nm gold cationic particles in microbiological assays and in vitro hemolysis show slight toxicity (Robe, 2008). Diseases associated with inhaled nanoparticles are asthma, bronchitis, emphysema, lung cancer, and neurodegenerative diseases, such as Parkinson’s and Alzheimer’s diseases. Nanoparticles in the gastro-intestinal tract have been linked to Crohn’s disease and colon cancer. Nanoparticles that enter the circulatory system are related to occurrence of arteriosclerosis, and blood clots, arrhythmia, heart diseases, and ultimately cardiac death. Translocation to other organs, such as liver, spleen, etc. may lead to diseases of these organs as well. Exposure to some nanoparticles is associated to the occurrence of autoimmune diseases, such as: systemic lupus erythematosus, scleroderma, and rheumatoid arthritis.

The toxicological evaluation of zinc oxide reported by National Institute for Occupational Safety and Health (NIOSH) have been showed that the LD₅₀ of normal ZnO for rats is more than 8 g/kg body weight and belongs to non-toxic chemicals demonstrated by a
single oral ingestion (NIOSH, 2010). 30-min half maximal effective concentration (EC50) of ZnO nanoparticles indicates that the toxic effects of ZnO Nanoparticles, bulk ZnO particles and Zn$^{2+}$ are similar (Mortimer et al., 2010). Several studies on the potential toxicity of ZnO nanoparticles or powders in various animal systems, such as rats, guinea pigs, mice, human skin, zebrafish, Daphnia etc., have been reported (Zhu et al., 2009; Ma et al., 2009; Bai et al., 2010). Ma-Hock and Burkhardt (2008) found that ZnO nanoparticles could induce inflammatory reactions or oxidative stress responses in the respiratory tracts and lungs after inhalation. Zvyagin et al. (2008) found that ZnO nanoparticles stayed in the stratum corneum and accumulated into skin folds and/or hair follicle roots after exposure to human skin with 20–30 nm ZnO nanoparticles. As ZnO nanoparticles are widely used in sunscreen, human skin exposure to ZnO nanoparticles is one of the most important routes. Cross et al. (2007) reported the dermal adsorption of ZnO nanoparticles. When Franz-type diffusion cells were exposed to a novel, transparent nano-ZnO sunscreen formulation for 24 h, there was no sign of penetration of ZnO nanoparticles penetration. Moreover, electron microscopy indicated that no nanoparticles could be detected in the lower stratum corneum or viable epidermis. Oral, inhalation, and intra tracheal instillation routes have also been used to evaluate the acute toxicity of ZnO nanoparticles. Zheng et al. (2009) assayed the toxicity of ZnO nanoparticles in mice exposed via the digestive tract. Compared with the blank group, the spleen and brain cells were normal, whereas other primary organs (including heart, lung, liver, and kidney) were damaged. These results were supported by findings of Wang et al. (2008), who showed that the pathological changes induced by ZnO nanoparticles were both size- and dose-dependent. When mice were treated via the intra tracheal tract, histopathological observation revealed serious pulmonary inflammation, proliferation, and alveolar wall thickening in the lungs of all the treated mice groups. Moreover, all of these changes were more serious in animals that received higher dosages.

The cytotoxicity of both bulk and nanoparticles of ZnO in several cell cultures including mouse neural stem cells, mouse embryo fibroblast cells, epithelial, NIH3T3 fibroblast, endothelial cells, human lung epithelium cells, human liver cells, human bronchial epithelium cells, human cardiac microvascular endothelial cells and human kidney cells (Huang et al., 2010; Hsiao and Huang, 2011; Sharma et al., 2012) has also been studied. There is no doubt that ZnO nanoparticles have cytotoxicity against different culture cells mostly due to the induction of oxidative and inflammatory responses. Toxicity studies in Caenorhabditis elegans showed that ZnO nanoparticles can inhibit growth and reproductive
capability (Wang et al., 2009). Deng et al. (2009) found that toxic effect of ZnO nanoparticles (10, 30, 60 and 200 nm) in mouse neural cells were dose dependent rather than size dependent. Zn ions from ZnO nanoparticles at 12 ppm or higher concentration in the culture after 24 h of treatment could induce cell damage. Heng et al. (2010) evaluated the cellular association, cytotoxic and inflammatory potential of spherical and sheet-shaped ZnO nanoparticles on mouse and human cell lines as well as with primary cultures of mouse bone marrow-derived dendritic cells. The results also demonstrated dose-dependent effects on the cytotoxicity of spherical and sheet-shaped ZnO nanoparticles on human cell lines. Brunner et al. (2006) found that almost all human or rodent cells died following exposure to ZnO nanoparticle concentrations above 15 ppm. This was further supported by Sharma et al. (2009) who reported that ZnO nanoparticle-induced cytotoxicity was concentration-and time-dependent. ZnO nanoparticles can induce cytotoxicity by increasing oxidative stress in the human colon cancer cell line LoVo (De Berardis et al., 2010); results indicated that ZnO nanoparticles caused a time- and dose-dependent decrease of cell number compared with untreated cells. ZnO nanoparticles induced increased levels of hydrogen peroxide and hydroxyl radicals, decreased levels of molecular oxygen and glutathione, and reduced interleukin-8 (IL-8) release (a signal for proinflammatory mediator release). In study by Premnathan et al., (2011) ZnO nanoparticles exhibited a preferential ability to kill cancerous human myeloblastic leukemia cells (HL60) as compared with normal peripheral blood mononuclear cells (PBMCs). These findings indicate that ZnO nanoparticles might be used for carcinoma therapy in the future.

ZnO nanoparticles are soluble to some extent in aqueous media. It is the key issue in the toxicity of nanoparticles. Toxicity is due to dissolution of ZnO (Bondarenko et al., 2012). Mechanisms of cellular toxicity such as elevated ROS production that exceeds the capacity of the cellular antioxidant defense system cause cells to enter a state of oxidative stress, which results in damage of cellular components such as lipids, proteins, and DNA (Xia et al., 2006). The oxidation of fatty acids leads to the generation of lipid peroxides that initiate a chain reaction resulting in disruption of plasma and organelle membranes and subsequent cell death. Interestingly, bacteria and immortalized T cells (Hanley et al., 2008) produce greater levels of ROS than primary T cells, a phenomenon that may mechanistically underlie the greater susceptibility of cancerous T cells to nanoparticle-mediated toxicity. There is increasing evidence that elevated ROS act as critical signaling molecules in the induction of apoptosis, induced by different stimuli (Carmody and Cotter, 2001; Ryter et al., 2007) and hence studies were performed to determine if ZnO nanoparticles induced cytotoxicity through
an apoptotic pathway. ZnO started the apoptosis process with fragmentation of DNA. Cleavage of DNA at the inter nucleosomal linker sites yielding DNA fragments is regarded as a biochemical hallmark of apoptosis (Compton, 1992). ZnO nanoparticles induce apoptosis in myeloblastic leukemia cells. Collectively, these studies indicated that a primary mechanism of ZnO nanoparticles cytotoxicity might proceed by inducing the generation of ROS, which then were responsible for the induction of apoptosis. These observations may provide the basis for the development of new rational strategies to enhance the destruction of disease-causing cell types such as cancer cells.

2.9.2 Effect of nanoparticles on Environment

Nanoparticles can enter the environment in several ways:

- During manufacturing
- During product use
- Accidental spill during transportation and storage
- End of life disposal
- Land applications of biosolids, waste water and surface run off
- Leaching from the site of disposal

Nanopollution is a generic name for all waste generated by nanodevices or during the nanomaterials manufacturing process. This sort of waste may be very serious because of its size. Nanoparticles tend to agglomerate and change into larger microparticles, which are more active and highly mobile in the environment. These microparticles can contaminate soil and ground water when released into the water or air. It can drift in the gentle wind and might easily penetrate animal and plant cells causing unknown effects. Screens raises concerns about nano-pollution, and indicates that it is not presently possible to “precisely predict or control the ecological impacts of the departure of these nano-products into the environment.” Once the nanoparticles enter one ecosystem they could proceed to another, for instance, the movement of nanoparticles between water and sediments, or the absorption of atmospheric particles in urine. The primary business will be if any of the nanoparticles entering the environment are toxic or could become toxic to living species in the surroundings. In other words problems related to ecotoxicity arises when nanoparticles attacks non target species also along with target species. For instance, on that point is the possibility of nanoparticles being toxic to microorganisms in the land and groundwater. Nanoparticles are classified with respect to their environmental toxicity according to the response of the most sensitive of the three test organisms: algae, crustaceans and fish (Ta et al., 2011). Nanoparticles entering the environment may not initially be toxic to living species in the surroundings, but they could in
their lifecycle become toxic. For instance, waste nanoparticles from a manufacturing plant entering a stream could alter the pH of the watercourse. Altering the pH of a stream can lead to metals that are not normally soluble dissolving, such as aluminum. Aluminum in the water supply would in turn be toxic to living things in the watercourse. Dr. Anne Kahru from the National Institute of Chemical Physics and Biophysics in Estonia and Henri-Charles Dubourguier from Institut Supérieure d’Agriculture in France identified in 2009 the most harmful NPs and most sensitive organism groups through evaluation of existing information on NPs toxicity in different species. The organisms included were bacteria, algae, crustaceans, nematodes, yeasts, fish, and ciliates. They stand for primary food-chain levels. The evaluated NPs were TiO$_2$, CuO, MWCNs, SWCNTs, C60-fullerenes, ZnO and Ag (Oestand, 2010). It is significant to remember that nanotechnology can be utilized in a positive way in the environment, for instance, the use of nanoparticles of groundwater and contaminated land remediation. Nevertheless the risks of the nanoparticles in their life cycle in the environment must be built (Karn et al., 2009).

![Figure 2.6: The fate of nanoparticles in the environment (Williams et al., 2006 (SCENIHR)).](image-url)
ZnO nanoparticles are most toxic to algae (<0.1 mg/l) followed by crustaceans, fish, bacteria, and protozoa. The (lethal concentration) LC 50 values of ZnO nanoparticles are between 10 and 100 mg/L for nematodes, yeast and mammalian cells (Bondarenko et al., 2013). ZnO nanoparticles are widely used in consumer products such as sunscreen and can make its way into aquatic ecosystem from domestic and commercial waste water. A study suggested negative impact on the cardiorespiratory function in adult fish Catostomus commersonii (Bessemer et al., 2014). Priyanka Gajjar and colleagues at Utah State University also studied CuO and ZnO NPs, but they desired to ascertain out if these metals-containing NPs and Ag NPs were dangerous to beneficial soil microorganisms. These microorganisms are important in plant growth and pollutant degradation. Both CuO and Ag NPs killed the microorganisms while the ZnO NPs inhibited microorganism growth and reproduction (Oestrand, 2010). Watching along from this would be possible hazards from the nanoparticles or from consuming the microorganisms affected by the nanoparticles for fish, insects or mammals. Another toxicity study showed that ZnO nanoparticles exposed terrestrial isopods Porcellioscaber died following bioaccumulation (Pipan-Tkalec et al., 2010). In that respect it is also a danger to plants from nanoparticles, which again could have a follow-on effect on the food chain. Toxicity studies of ZnO nanoparticles are developing rapidly; however, it is still not sure whether ZnO nanoparticles are safe for health and the environment due to the lack of environmentally relevant conditions used in the experiments (Franklin et al., 2007). Lin and Xing (2007) showed that Zn and ZnO nanoparticles obviously inhibited plant root growth and seed germination. Nanoparticles of Zn are less toxic than their ionic forms evaluated. ZnO NPs caused tunneling effect in the primary roots of maize. Metal NPs including their ions, impair the growth and development in plants by altering their cellular structures (Pophrel and Dubey, 2012). Blinova et al., (2010) performed experiments in artificial freshwater and natural water to test and compare the acute toxicity of CuO and ZnO nanoparticles to crustaceans species Daphnia magna and Thamnocephalus platyurus, and protozoan Tetrahymena thermophile. The results showed that the half maximal lethal concentration (LC50) values of nano and bulk ZnO were lower than that of CuO nanoparticles.

2.9.3 Effect of nanoparticles on Society

Beyond the toxicity risks to human health and the environment which are associated with first-generation nanomaterials, nanotechnology has broader societal impact and poses broader social challenges. Nanotechnologies may provide fresh results for the millions of
people in producing nations who lack access to basic services, such as safe water, reliable energy, wellness maintenance, and education but all this will be done using less labor. Unskilled labor workers will be first to be dismissed by the incessant usage of nanotechnology in the workplace. There is fear that the world's educational systems have lagged behind in educating students for the "Nanotech Age" resulting in future of unemployment. Claimed benefits of nanotechnology will not be evenly distributed, and that whatever benefits (including technological and/or economic) associated with nanotechnology will only reach affluent nations (Invernizzi et al., 2008). The legal age of nanotechnology research and development - and patents for nanomaterials and products - is concentrated in developing nations (including the United States, Japan, Germany, Canada and France). In summation, most patents related to nanotechnology are concentrated amongst a few multinational corporations, including IBM, Micron Technologies, Advanced Micro Devices and Intel. This has contributed to concerns that developing nations will not receive access to the infrastructure, funding and human resources needed to support nanotechnology research and evolution, and that this is likely to exacerbate such inequalities. Producers in developing nations could also be disadvantaged by the substitution of natural products (including rubber, cotton, deep brown and tea) by developments in nanotechnology. These natural products are important export crops for developing nations, and many farmers' livelihoods depend on them. Their substitution with industrial nano-products could negatively affect the economic systems of developing lands that have traditionally relied on these export crops (Invernizzi et al., 2008). Outlaws and terrorists with stronger, more sinewy, and a good deal more compact devices could cause grave harm to society. Nano-built products may be vastly overpriced relative to their cost, perpetuating unnecessary poverty. New products and lifestyles may make significant social disruption. Products which are banished in some societies like guns in Britain, seedless watermelon in Iran, hashish in the United States and alcohol in Muslim societies can now be easily made up using personal factories could be required to be at least somewhat disruptive to society, and could provide an impetus for knee-jerk and overly broad restrictions on the technology.

Considering all factors, testing the effects of nanomaterials on mammals and the environment is necessary. The toxicity of nanoparticles can be assessed by a number of in vitro and in vivo studies. For example, the toxic effects of nanoparticles can be carried out using zebrafish as a model due to its fast development and transparent body structure. Cell culture based assays are used as a pre-screening tool to understand the biological effects of
nanoparticles. However, along with the \textit{in vitro} assays it is necessary to confirm the \textit{in vivo} biological activities of nanoparticles in animal models to study the suitability of their application (Shaw \textit{et al.}, 2008). There is an increasing use of microarray and real-time reverse transcription polymerase chain reaction for gene expression analyses as these are very sensitive and reliable methods to assess the changes in the expression levels of thousands of genes simultaneously under a wide variety of experimental conditions (Schrand \textit{et al.}, 2010). Only with more research, and using scientific evidence, microscopy tools, and modern analysis methods, we can discover the advantages or disadvantages of their applications. There are methods for improving the performance and reducing toxicity of nanoparticles in medical design, such as biocompatible coating materials or biodegradable/biocompatible nanoparticles. These are readily absorbed and assimilated by the cells and reduce toxicity. Cytotoxicity is related to cell line and can be reduced by changing nanomaterial compositions and sizes.

Generally, toxicity studies, helps to find out maximum tolerated dose (MTD) in preclinical evaluation, which can be used to evaluate the clinical potential of compound or new drug molecule. Other equally appropriate limiting doses include those that achieve the use of maximum feasible dose (MFD). These limit doses helps to calculate the clinical safety value of the drug. These recommendations are reliable for reproduction and carcinogenicity in rodents and non rodents for acute, subchronic, and chronic toxicity studies of 1000 milligrams (mg)/kilogram (kg)/day (Maneewattanapinyo \textit{et al.}, 2011; Whitehead and Stallard, 2004). In the few situations where a dose of 1000 mg/kg/day does not result in a mean exposure margin of 10-fold to the clinical exposure and the clinical dose exceeds 1 gram (g) per day, then the doses in the toxicity studies should be limited by a 10-fold exposure margin or a dose of 2000 mg/kg/day or the MFD, whichever is lower. In some cases, the dose of 2000 mg/kg/day results in an exposure that is less than the clinical exposure, a higher dose up to the MFD can be considered (Robinson \textit{et al.}, 2008). Doses providing a 50-fold margin of exposure to the clinical systemic exposure generally are also considered acceptable as the maximum dose for acute and repeated-dose toxicity studies in rodents and non rodents (Robinson \textit{et al.}, 2008).

It is evident that nanoparticles due to their biological and physiochemical properties are promising as antimicrobials and therapeutic agents. They can be used to address a number of challenges in the field of nanomedicine. Like fullerene derivatives and nanoparticles made of compounds holding oxygen vacancies (CeO$_2$, and Y$_2$O$_3$) have demonstrated
neuroprotective properties and antiapoptotic activity (Bosi et al., 2003). They have been shown to prevent apoptosis in hepatic, kidney, and neuronal cells, a fact attributed to their antioxidant properties (Schubert et al., 2006). But it must be remembered that they can also possibly cause adverse biological effects at the cellular and subcellular levels. Therefore, after the cytotoxicity and clinical studies the nanoparticles can find immense application as antimicrobials in the consumer and industrial products. By gaining control over dangerous particles, we can increase the use of nanoparticles by reducing their harmful effects, and thus allowing them to be used in the curing of diseases (Linkov et al., 2008; Suh et al., 2009).