CHAPTER I

INTRODUCTION

1.1 NANOTECHNOLOGY

Nanotechnology broadly refers to a field of applied science and technology whose special and unique properties could be attributed to the small size and large surface area (**Nanda et al., (2009)).** It is the application of science and technology to manipulate the matter at atomic and molecular scale. It has the ability to build micro and macro materials and products with atomic precision. An important aspect of nanotechnology is the development of experimental processes for the synthesis of nanoparticles with different size, shape and controlled dispersity. Currently it is employed as a tool to explore the murkier avenues of medical sciences like imaging, sensing, targeted drug delivery, gene delivery, and artificial implants. Nanoparticles are being sighted as fundamental building blocks of nanotechnology. In the recent years, research is focused on metal nanoparticles due to their unique optical, electronic, mechanical, magnetic, and chemical properties that are significantly different from those of bulk materials (**Mazur (2004)).**

Nanoparticles because of their small size have distinct properties compared to the bulk form of the same material, thus offering many new developments in the field of biosensors, biomedicine, and bio nanotechnology. Nanotechnology is a powerful technology, which up holds immense scope for the design and development of many types of novel products with potential medical applications on early disease detection, treatment, and prevention (**Alan and Jan (2009)).**

1.2 ONE-DIMENSIONAL NANOSTRUCTURES

This is the era of global competition for possessing state-of-the-art technologies and hence there is a surging interest in the preparation and characterisation of novel 1D nanoparticle such as nanotubes and nanofibres because of their unique optical, electrical and mechanical properties. Over the past few years, considerable research work is focused on metal nanoparticles due to their potential

applications in diverse fields including catalysis, magnetic recording media and microelectronics. Nanoparticulate transition metal oxides can exhibit improved optical, magnetic and electrical properties compared with their bulk counterparts, rendering such nanoparticles suitable for a variety of applications, e.g. as electrodes in energy storage devices, as catalysts and as magnetic storage devices (**Hyeon et al., (2001))**. Generally, nanoparticles display a distribution in their size; however, synthesis routes leading to the formation of monodispersed particle sizes are becoming available more.

The most challenging part of research in the field of nanotechnology involves the cost effective and environmentally safe procedures for nanomaterial synthesis. The approach towards nanomaterial synthesis could be broadly divided into two groups following the principle of either "top-down approach" or "bottom-up approach". The top-down approach seeks to fabricate nanodevices on silicon (or other semiconductors) chips directly using electron beam or X-ray lithography. In the bottom-up approach, nanostructures are synthesized from atoms or molecules. In the latter, the synthesis protocols can be further divided into physical methods, chemical methods and biological/bio-inspired methods. Various physical methods have been successfully employed for the synthesis of nanomaterial such as vapor deposition (**Oha et al., (2004))**, thermal decomposition (**Teng. X., (2003))**, spray pyrolysis (**Suh and Suslick (2005))**, photoirradiation (**Li et al., (2000))**, laser ablation (**Amendola and Meneghetti (2006))**, ultrasonication (**Chen et al., (2001))**, radiolysis (**Kurihara et al., (1983))** and solvated metal atom dispersion (**Ponce and Klabunde (2005))**.

However, chemical methods have several advantages over physical methods; therefore chemical methods are widely used for nanomaterial synthesis. Nanomaterials such as metals, metal oxides and semiconductor nanoparticles can be synthesized by chemical methods like reduction or oxidation of metal ions or precipitation of the desired composites (by carrying out the appropriate chemical reaction).

In general, nanoparticles are unstable in solution; so special precautions should be taken to avoid their aggregation or precipitation. Nanoparticle synthesis also involves the use of "stabilizing agents" which associate themselves with surface of the particle providing charge or solubility properties to keep the nanoparticle suspended and thereby preventing aggregation (**Thi et al., (2011)**).

1.3 SYNTHESIS OF NANOPARTICLES

Metallic nanoparticles have been examined for their use as tools for a new generation technological devices and many techniques for producing nanoparticles are now available. In the last decade many successful synthesis methods to produce metal nanostructures in a variety of shapes such as sphere, spheroid, cube, octahedron, tetrahedron, bipyramid, plate, hollow, and rod have been developed. The methods for making nanoparticles can generally involve either a top-down approach or a bottom-up approach (**Ventra et al., (2006)).**

In top-down synthesis nanoparticles are produced by size reduction from a suitable starting material. Top-down production methods introduce imperfections in the surface structure of the product and this is a major limitation because the surface chemistry and the other physical properties of nanoparticles are highly dependent on the surface structure.

In bottom-up synthesis the nanoparticles are built from smaller entities, for example by joining atoms, molecules and smaller particles. In bottom-up synthesis, the nanostructured building blocks of the nanoparticles are formed first and then assembled to produce the final particle. The bottom-up synthesis mostly relies on chemical and biological methods of production.

1.4 OVERVIEW OF IRON NANOPARTICLES

Iron and its compounds are widespread in nature and can be readily synthezised in the laboratory. Iron and its compounds are present in the hydrosphere, the lithosphere and (as pollutants) in the atmosphere. Iron is a biogenic element, present in all biota, but some iron compounds can cause harmful effects to human

beings, animals, and environment (**Gurzau et al., (2003))**. In occupational exposure of human beings, iron and iron oxides are known to produce benign siderosis – but iron oxides have been also implicated as a vehicle for transporting high concentration of carcinogens and sulfur dioxide deep into the lungs, thereby enhancing the activity of these pollutants. Iron oxides also cause damage by staining materials. Analysis of urban air samples showed that the probable sources of iron compounds are the iron and steel industry and urban transport such as underground railways. Tunnel dust – generated by the interaction of brakes, wheels and rails – contains about 90% iron, 1–2% quartz and the remnants of other metals in the underground rail system.

1.4.1 Iron in the human body

The content of iron in the human body is regulated by a complex mechanism for maintaining homeostatsis. During childhood, pregnancy or blood loss, the need for iron is more and so is the absorption. Absorption occurs in two steps: absorption of ferrous ions from the intestinal lumen into the mucosal cells, and transfer from the mucosal cell to the plasma, where it is bound to transferrin for transfer to storage sites.Transferrin is a β1-globulin (and is) produced in the liver. As the Fe^{2+} ion is released into plasma, it becomes oxidized by oxygen in the presence of ferroxidase I. There are 3–5 g of iron in the body, about two-thirds of which is bound to haemoglobin, 10% to myoglobin and iron-containing enzymes, and the remainder is bound to the iron storage proteins ferritin and hemosiderin. Exposure to iron induces synthesis of apoferritin, which then binds ferrous ions. Iron may be released slowly from ferritin by reducing agents such as ascorbic acid, cysteine, and reduced glutathione. Normally, excess ingested iron is excreted, but some remains within shed intestinal cells, in bile, in urine, and in even smaller amounts in sweat, nails, and hair. Total iron excretion is usually ~ 0.5 mg/day. With excess exposure to iron or iron overload, there may be a further increase in ferritin synthesis in hepatic parenchymal cells. In fact, the ability of the liver to synthesize ferritin exceeds the rate at which lysosomes can process iron for excretion. Lysosomes convert the protein from ferritin to hemosiderin, which then remains in situ. The formation of hemosiderin from ferritin is not physciologically well understood, but it seems to

involve denaturation of the apoferritin molecule. With increasing iron loading, ferritin concentration appears to reach the maximum and a greater portion of iron is found in hemosiderin. Both ferritin and hemosiderin are in fact storage sites for intracellular metal and are protective in that they maintain intracellular iron in bound form.

1.4.2 Iron – the most essential micronutrient

Iron is involved in many functions within the human body and is a vital constituent of hemoglobin and myoglobin, which are involved in oxygen transport and supply within the tissues. It is also involved in electron transfer, hydroxylation, and acts as catalyst for oxygenation, cell proliferation and disposal of oxygen radicals. Iron's most important property is the reversible one electron oxidation reduction reaction between the two common oxidation states, Fe^{2+} and Fe^{3+}, allowing it to coordinate electron donors and to participate in redox processes. Reactions with oxygen can lead to the formation of intermediates with unpaired electrons. The human body has developed complicated metabolic processes to absorb, transport and store iron ensuring a ready supply for cellular growth and function, but limits its participation in reactions that produce free radicals and its availability to invading pathogens (**Bashiri et al., (2003)).** Human beings normally have 40–50 mg Fe/kg body weight. Approximately 75% is present in metabolically active compounds. The remaining 25% constitutes a dynamic store that is turned over constantly. It ensures an adequate supply for normal physiological functions despite short term variations in absorption or loss from the body. The store also supplies the immediate needs when requirements are increased (e.g., by rapid growth or pregnancy). Iron reserves that have been utilized are then gradually replaced by increased absorption (**Kraemer and Zimmermann (2007)).**

1.5 IRON DEFICIENCY ANAEMIA

Anaemia is one of the most common and intractable nutritional problems in the world today. The World Health Organization (WHO) estimates that some two billion people are anaemic defined as haemoglobin concentrations are below recommended thresholds. The main causes of anaemia are dietary iron deficiency,

infectious diseases such as malaria, hookworm infections and schistosomiasis, deficiencies of other key micronutrients including folate, vitamin B12 and vitamin A or inherited conditions that affect red blood cells (RBCs). Iron deficiency affects energy metabolism, causing symptoms like fatigue, lack of concentration with decreased mental, physical and cognitive performance.

Although anaemia has been recognized as a public health problem for many years, there has been little progress towards improvement and the global prevalence of anaemia remains unacceptably high. It has been estimated that around two billion people in the world are anaemic, mostly in the countries of Africa and Asia (**Jamil et al., (2008)).**

One of the reasons for the apparent failure to reduce the prevalence of anaemia is that many programmes and their interventions have been designed with the assumption that the only cause of anaemia is iron deficiency. This means that, when trying to control anaemia, other causes have been underestimated and that iron deficiency without anaemia is addressed as a major and common health problem (**Worwood (2007)).** To plan effective interventions to combat both iron deficiency and anaemia there is an urgent need to have better information on the iron status of various populations.

1.5.1 Deleterious effects of iron deficiency

Many infants and women of childbearing age, particularly in the economically downtrodden countries of the developing world, are iron deficient. About half of these iron deficient individuals develop Iron Deficiency Anaemia (IDA), the most advanced form of the disease, which has several major negative impacts on health and contributes substantially to the risk of early death or disability.

Iron deficiency is therefore a major health problem in the developing world. Recently WHO ranked it as 7th out of the 10 major global preventable risks for disease, disability and death, those together accounts for 40% of the 56 million

deaths that occur worldwide each year and for one third of the global loss of healthy life years. It has been estimated that if iron deficiency is eliminated worldwide, more than 35 million people would have one additional year of healthy life (**Hurrell et al., (2009)).**

1.5.2 Stages of iron deficiency

The first stage of iron deficiency is characterised by the absence of a measurable iron store, the second (iron deficient eythropoiesis) by evidence of a restricted iron supply in the absence of anaemia and the third (iron deficiency anaemia) by a haemoglobin concentration that falls below the normal threshold for age and sex. The iron indicators that can be used to identify the three stages of iron deficiency are discussed.

In the first stage, a depletion of iron stores occurs with no apparent symptoms and hemoglobin level is in the normal range. Serum ferritin and bone marrow iron are become less and there is a consequent increase in iron absorption. The second stage is when a slower erythropoiesis takes place due to the lack of iron availability; the hemoglobin level starts to decrease with the adjuvant decrease of serum ferritin, bone marrow iron, low serum iron and an increase in total iron binding capacity. At this stage the hematocrit remains unchanged. The third and last stage is when iron deficiency of anemia develops. Ferritin levels and transferrin saturation levels become very low, iron stores are depleted, serum iron and hemoglobin levels become low and the total iron binding capacity is elevated (**Kraemer and Zimmermann (2007)).**

1.5.3 Hematological parameter associated with iron deficiency

The effect of iron depletion without anemia on adaptation to training has not been fully characterised but laboratory evaluation of iron status is necessary and helpful in defining anemic states. Iron containing compounds in the body are one of these three types: a) functional compounds that serve in metabolic or enzymatic functions and b) compounds that serve as transport and c) storage forms for iron. There are a number of markers that describe these functional, transport and storage

compartments for iron: serum iron, total iron binding capacity, red blood cell count, hemoglobin, hematocrit, red blood cell indices (MCV, MCH, MCHC, RDW), transferrin, transferrin saturation, and serum ferritin. These laboratory tests are essential to an accurate diagnosis of ID and the evaluation of therapy (Fallon. 2004). Three RBC measurements are routinely carried out: packed cell volume (PCV), the proportion of whole blood volume occupied by the RBC, haemoglobin (Hb) concentration of whole lysed blood and RBC count, which is the number of RBC per unit volume of whole blood.

1.5.3.1 Packed cell volume (PCV)

PCV is the variable normally used to assess the basic status of the erythron increased in polycythemia, decreased in anemia although if a sample is too hemolyzed to allow measurement of PCV, a meaningful Hb measurement may still be obtained. RBC count as such should not be interpreted clinically. An abnormally high PCV (polycythemia) may be relative, due to a change in the proportion of circulating RBC to blood plasma without any alteration in the size of the erythron, or absolute, due to a real increase in erythron size. Absolute polycythemia may be primary (e.g. polycythemia vera or rarely, erythropoietin producing tumors) or secondary (a consequence of disease in another organ system).

1.5.3.2 Haemoglobin

Hemoglobin constitutes the major fraction of body iron (functional iron) with a concentration of about 0.5 mg iron/mL blood. Iron is distributed within the body via transferrin in the plasma, a transport protein that mediates iron exchange between tissues. Ferritin is the primary storage compound for the body's iron and serum ferritin concentration is a reliable index of iron stores (1 ng/ml of serum ferritin indicates about 8 mg of storage iron). Serum ferritin does not exhibit diurnal variations as seen with serum iron levels (**Fallon (2004)).** The serum ferritin level is reduced in all stages of ID and may be the first indication of a developing ID. Serum ferritin is generally considered the single best test to detect iron deficiency. Although highly trained athletes usually have normal and absolute levels of hemoglobin, they often have ID, generally latent, that implies no decrease in

hemoglobin. Swimmers with low ferritin levels may not be anemic, but their performance at maximal intensities may be compromised. Anemia in swimmers, like in other athletes, has negative effects on physical exercise capacity and their ability to train from day to day.

1.6 CHOICE OF THE IRON COMPOUNDS

The choice of the iron compound is often a compromise between reasonable cost, bioavailability and the acceptance of any sensory change. When selecting the most appropriate chemical form of a given micronutrient, the main considerations and concerns are enumerated below: (**Kraemer and Zimmermann (2007)).**

Sensory problems: The iron compound fortified to the dietary substance must not cause unacceptable sensory problems (e.g. colour, flavour, odour or texture) at the level of intended fortification or segregate out from the food matrix and they must be stable within given limits.

Interactions: The likelihood or potential for interactions between the added micronutrient and the food vehicle along with other nutrients (either added or naturally present), in particular, any interactions that might interfere with the metabolic utilization of the iron compound supplement, needs to be assessed and checked prior to the implementation of a fortification programme.

Cost: The cost of fortification must not affect the affordability of the food nor its competitivity with the unfortified alternative.

Bioavailability: Most importantly, the iron compound fortified must be sufficiently well absorbed from the food vehicle and be able to improve the micronutrient status of the target population.

Safety is also an important consideration. The level of consumption that is required for fortification to be effective must be compatible with a healthy diet. According to the conclusion of the Sharing United States Technology to Aid Improvement of Nutrition (SUSTAIN) Task Force reports (1997), only electrolytic iron powders (diameter <45 microns or 325 meshes) have been proven to be sufficiently bioavailable to humans. The data indicates that carbonyl iron and some

hydrogen reduced (H-reduced) iron powders have comparable bioavailability to electrolytic iron.

Atomized iron and carbon monoxide reduced (CO-reduced) iron are not recommended at the present time because of their low bioavailability. Elemental iron with a large particle size (diameter >149 microns or 100 mesh) is probably too insoluble in the intestine and is therefore not generally recommended for use as a food fortificant (**Hurrell et al., (2009)).**

1.7 OVERVIEW OF SILVER (Ag)

Silver nanoparticles are one of the promising products in the nanotechnology industry. Silver refers to any specified form of the element silver or to the mixture of forms which occur in that particular environmental setting. The Silver ion is the most fundamental entity of silver. It is an atom in which the number of electrons is one less than the number of protons, creating a positively charged cation (thus written Ag+). The ionic radius of a silver ion is ~0.1 nm. A silver ion is not usually considered a particle, and its surface area is irrelevant. But ions are highly reactive because they are charged. An ion can associate with other ions, but the ion itself is inherently persistent and cannot be destroyed. Complex interactions blur precise boundaries among macromolecules, nanoparticles, colloids and particles (**Lead and Wilkinson (2006))**. Silver nanomaterial or nanoparticles are made up of many atoms of silver in the form of silver ion-clusters of metallic silver atoms and or silver compounds (**Balogh et al., (2001))** engineered into a particle of nanoscale size. High surface area is a particularly important property for nano silver, because it increases the rate at which silver ions are released.

Nano silver or silver nanoparticles refer to a nanoparticle or a nanocoating comprised of many atoms of silver engineered for a specified use. Silver nanoparticles are usually engineered to release silver ions, which are the source of antibacterial activity.

Silver has been widely known for its many properties useful to human beings. It is however, an element of many faces. It has the highest electrical conductivity, a property useful in electrical contacts and conductors. Its chemical traits allow uses ranging from dental alloys to explosives. The way it reacts to light (photochemistry) was manipulated to develop traditional photography. Claims of medicinal properties have followed silver since the time of Hippocrates, the father of medicine. Most importantly, silver has long been used as a disinfectant; for example, in treating wounds and burns, because of its broad-spectrum toxicity to bacteria and, perhaps, to fungus and viruses, as well as its reputation of limited toxicity to human beings.

Most of the emerging products exploit silver's effectiveness in killing a wide range of bacteria (thus the term broad-spectrum biocide), including some of the strains that have proven resistant to modern antibiotics. Perhaps most importantly, nano silver particles deliver toxic/ silver ions in large doses directly to sites where they most effectively attack microbes. The technology appears to be cost-effective. To date, silver is used in consumer products than any other nanomaterial.

Nonliving surfaces that penetrate the body and are implanted within the body are prone to support the growth of microbial biofilms. Nanosilver coatings on the surfaces of artificial joints, pacemakers, artificial heart valves and teflon sleeves for the repair of blood vessels and catheters, among other devices, have great potential to prevent these deadly microbial growths.

Silver-impregnated bandages and dressings are the treatment of choice for serious burns and are now available over the-counter for local treatment of wounds and elimination of pathogenic bacteria (**Vermeulen et al., (2007))**. Ceramic filters that incorporate a coating of nanosilver for water purification are proposed as a solution to the drinking water purification problem of billions of people (**Lubick (2008)).**

Nanocrystalline Silver's extremely small size and large surface area makes it to have different properties than the bulk material. Nano-silver possesses a high extinction coefficient, high surface plasmon resonance and anti-microbial properties and is also less toxic then the bulk form (**Kumar et al., (2007) and Lesniak et al., (2005)).**

Currently silver nanomaterials have been widely used everyday in consumer's products such as: nanosilver infused storage containers, nanosilver coated surfaces of medical devices to reduce hospital related infections (**Alt et al., (2004)),** bandages, footwear (**Huang et al., (2008))** and countless household items which claim to be anti-microbial. Nanosilver is a popular additive in many health products as listed previously due to its unique ability to fight infectious diseases, slow the growth of bacterium, mold and germs. While all of these properties appear to make nanosilver the new "wonder-drug" of the nanotechnology world, problems too arise.

The development of consistent processes for the synthesis nanocrystalline of silver is an important aspect of the current nanotechnology research. Silver nanoparticles can be synthesized by several physical, chemical and biological methods. However in the past few years, various rapid chemical methods have been replaced by green synthesis because of avoiding toxicity of the process and increased quality.

1.8 APPLICATIONS OF SILVER NANOPARTICLES

- Silver nanoparticles are used for purification and quality management of air, biosensing, imaging, drug delivery system.
- Biologically synthesized silver nanoparticles have many applications like coatings for solar energy absorption and intercalation material for electrical batteries, as optical receptors, as catalysts in chemical reactions, for biolabelling and as antimicrobials.

- ❖ Though silver nanoparticles are cytotoxic they have tremendous applications in the field of high sensitivity bimolecular detection and diagnostics, antimicrobials and therapeutics, catalysis and micro-electronics.
- ❖ It has some potential applications like diagnostic biomedical optical imaging, biological implants (like heart valves) and medical applications like wound dressings, contraceptive devices, surgical instruments and bone prostheses.
- ❖ Many major consumer goods manufacturers are already producing household items that utilize the antibacterial properties of silver nanoparticles. These products include nano silver lined refrigerators, air conditioners and washing machines.

1.8.1 Silver nanoparticles as an antimicrobial agent

Ag nanoparticles are highly antimicrobial to several species of bacteria, including the common kitchen microbe, E. coli. According to the mechanism reported, silver nanoparticles interact with the outer membrane of bacteria, and arrest the respiration and metabolic pathway that leads to the death of the bacteria. New technology advances in reducing silver compound chemically to nanoscale sized particles have enabled the integration of this valuable antimicrobial into a large number of materials including plastics, coatings, and foams as well as natural and synthetic fibers. Current research in inorganic nanomaterials having good antimicrobial properties has opened a new era in pharmaceutical and medical industries. Silver is the metal of choice as it holds the promise to kill microbes effectively. Silver nanoparticles have been recently known to be a promising antimicrobial agent that acts on a broad range of target sites both extracellularly as well as intracellarly. Silver nanoparticles shows very strong bactericidal activity against gram positive as well as gram negative bacteria including multi-resistant strains.

1.9 INTRODUCTION FOR *ab initio* STUDIES

The understanding of the behavior of electrons in solids is one of the keys in understanding materials. The electron theory of solids is capable of explaining optical, magnetic, thermal and electronic properties of materials. The electron theory

provides important fundamental ideas for a technology which is often considered to be the basis for modern electronic devices. The main tools used in *ab initio* solid state computer experiments are density functional theory (DFT) and periodic boundary conditions. The DFT methods are used to calculate structural, optical, electronic and vibration properties of low dimensional system. Density functional theory is an alternative for predicting the properties with good agreement. Thus *ab initio* theoretical calculations have gained recognition, because they have shown to be important when predicting the properties of new materials. Currently this technique is too demanding computationally to apply to large structures. It is clear, however that with the continuing increase in the available computing power and rapid development of new theoretical approaches, the range of investigations is going to grow dramatically. So in the present study, attempts have been made to know about the structural properties of the chosen metal nanoparticles iron (Fe) and silver (Ag) using *ab initio* studies. Thus in the present work, "*ab initio*" simulation studies on elemental nano sized iron and silver particles were carried out and the results were compared with the experimental results. An attempt has been made to attribute the electronic structure of the material to its effect on biological cells. An attempt to study elemental iron and silver nanoparticles using *ab initio* technique to link the electronic structure of the material with its biological effect is also made. The results obtained from the theoretical study were compared and are in agreement with the corresponding experimental results.

1.10 INTRODUCTION FOR BIOLOGICAL STUDIES

In the present work iron and silver nanoparticles have been studied and an attempt has been made to use them for biological applications such as antibiotic, antibacterial study and animal study.

The antibacterial activity of the prepared iron and silver nanoparticles against two Gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa) and two Gram-positive bacteria (Bacillus subtilis and Staphylococcus aureus) were investigated. The bactericidal property of nanoparticles depends on their size, stability, and concentration added to the growth medium, since this provides greater

retention time for bacterium nanoparticles interaction. In general, bacterial cells are in the micron-size range. Since bacteria are ubiquitous, present in the soil, air and water, some of the bacteria may be beneficial; while some of them may bacteria cause illness and even death. Pathogenic bacteria, that cause disease, relentlessly bombard the human body daily. An overview of some of the bacteria is given in the following sections.

1.10.1 *E. coli* Bacteria

E. coli is short for *Escherichia coli*. *E. coli* is gram-negative, facultative anaerobic and non-sporulating. Cells are typically rod-shaped and are about 2.0 microns (μm) long and 0.5 μm in diameter, with a cell volume of 0.6 - 0.7 $(\mu m)^3$. *E. coli* bacteria usually live in human or animal intestinal tracts, and their presence in drinking water is a strong indication of recent sewage or animal waste contamination. *E. coli* comes from human and animal wastes. *E. coli* and related bacteria possess the ability to transfer DNA via bacterial conjugation, transduction or transformation, which allows genetic material to spread horizontally through an existing population. It is also responsible for food poisoning when they enter the intestinal tract.

1.10.2 *Bacillus* Bacteria

Bacillus is a genus of rod-shaped bacteria and a member of the division Firmicutes. *Bacillus* species are obligate aerobes, and test positive for the enzyme catalase. Ubiquitous in nature, *Bacillus* includes both free-living and pathogenic species. Under stressful environmental conditions, the cells produce oval endospores that can stay dormant for extended periods. These characteristics originally defined the genus, but not all such species are closely related, and many have been moved to other genera. Members of the genus *Bacillus* are gram-positive, rod-shaped, spore-formers that require oxygen. However, this is a very diverse group of organisms and some species are actually gram-negative or facultative. The cell wall of *Bacillus* is a structure on the outside of the cell that forms the second barrier between the bacterium and the environment and at the same time maintains triangle shape and withstanding the pressure generated by the cell's turgor.

1.10.3 *Pseudomonas* Bacteria

Pseudomonas is a genus of gamma proteobacteria, belonging to the larger family of *pseudomonas*. *Pseudomonas* literally means 'false unit'. Because of their widespread occurrence in water and in plant seeds such as Dicots, the pseudomonades were observed early in the history of microbiology. The generic name *Pseudomonas* created for these organisms was defined in rather vague terms in 1894 as a genus of gram-negative, rod-shaped, aerobic, non-spore forming, positive catalase test and polar-flagella bacteria. Soon afterwards, Pseudomonades was isolated from many natural niches and a large number of species names were originally assigned to the genus. Being Gram-negative bacteria, most Pseudomonas species are naturally resistant to penicillin. *Pseudomonas aeruginosa* is a highly relevant opportunistic pathogen. As a result of their metabolic diversity, ability to grow at low temperatures and ubiquitous nature, many Pseudomonades can cause food spoilage. Notable examples include dairy spoilage by P. fragi, mustiness in eggs caused by P. taetrolens and P. mudicolens, and P. lundensis, which causes spoilage of milk, cheese, meat, and fish.

1.10.4 *Staphylococcus* Bacteria

Staphylococcus is a genus of Gram-positive bacterias. Under the microscope they appear round (cocci) and form grape-like clusters. Two major divisions of the genus *Staphylococcus* are separated by the bacteria's ability to produce coagulase, an enzyme that can clot blood. All species grow in the presence of bile salts and are catalyzed positive. *Staphylococcus* can cause a wide variety of diseases in humans and other animals through either toxin production or penetration. These bacteria can reach the bloodstream (bacteremia) and end up in many different body sites, causing infections (wounds, abscesses, osteomyelitis, endocarditis, and pneumonia) that may severely harm or kill the infected person. Staphylococcal bacteria, also called staph, multiply rapidly and can lead to severe infections. When staph bacteria enter the bloodstream, they can cause inflammation, swelling, pus formation and elevated blood pressure. Coma and death can occur if the infected person is left untreated.

1.11 INTRODUCTION FOR ANIMAL STUDIES

Iron deficiency anaemia is a global public health issue affecting human beings. Among domestic animals, the effect of iron deficiency is more pronounced in piglets. Anaemia in baby pigs occurs, almost without exception, if no supplemental iron is provided during the first few days after farrowing. Hence an attempt was made using nanoparticles of elemental iron to give an alternate supplement for the treatment for anaemia.

Currently silver is applied in the treatment of wounds either in the form of impregnated bandages or as a cream containing silver sulfadiazine. A bactericidal agent with greater potency and less likelihood of developing bacterial resistance is an urgent need in therapeutic management of wounds. Recently, silver nanoparticles have been suggested as having high bactericidal property with less development of resistance to silver nanoparticles in pathogenic microbes. This would be especially beneficial in veterinary medicine to keep the wound area clean and hygienic. Keeping the above facts into consideration, the present study was formulated to test utilization of silver nanoparticles in wound dressings in rabbits as model.

1.12 LITERATURE SURVEY

1.12.1 Literature Survey for Iron (Fe)

A thorough and systematic study has been carried out on the past works done on Iron and Silver nanoparticles and is presented in this part of the chapter in a detailed manner.

Jafari et al., (2010) have investigated the Fe core-shell nanoparticles through a reverse micelle method. They have observed that the particles are almost free of oxides. The average particle size increased with increasing water to surfactant molar ratio (w). The particle size distribution also became broader with increasing w-value. Moreover, the agglomeration type changed from magnetic beads to magnetic nanocrystal clusters with increasing w. They have also found that the nanoparticles have superparamagnetic properties. They have further reported that the core/shell nanoparticles were functionalized by creating Au-S bonds followed by

linking to polyglycerol. The cell toxicity of the nanoparticles was investigated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. At low concentration (3 mM/l), the uncoated nanoparticles exhibited a reasonable biocompatibility, offering suitable carriers for biomedical applications.

Pham et al., (2010) have synthesized boronic acid functionalized magnetic nanoparticles (B-f-magnetic nanoparticles) using a surface of gold layer coated on the shell of iron nanoparticle. Due to the boronic acid attachment onto the gold layer, the covalent immobilization of adenosine onto the surface of Fe@Au nanoparticle was obtained through the formation of boroester bonds. They have reported that the covalent immobilization of B-f-magnetic nanoparticles with adenosine makes novel hybrid magnetic nanoparticles for potential applications in the separation of adenosine and hydroxyl groups containing biomolecules.

Yang et al., (2006) have prepared Co-coated Fe nanoparticles by a direct chemical reduction method. They have studied the magnetic behavior of the prepared sample. The as-prepared Co coated Fe particles displayed a 50% enhanced Hc value and a 12% reduced Ms as compared with that of uncoated particles.

Suslick et al., (1996) have synthesized Fe particles in the presence of polyvinylpyrrolidone (PVP) and oleic acid by sonochemical decomposition technique. They have observed that the iron particles have a relatively narrow range in size from 3 to 8 nm for polyvinylpyrrolidone, while oleic acid gives an even more uniform distribution of 8 nm. The magnetic properties of nanocrystalline iron particles are superparamagnetic in character with a saturation magnetization of 101 emu/g at 290 K.

Zhang et al., (2005) have synthesized rod-like Fe particles by reduction of Fe salts using hydrazine hydrate in the presence of cetyl trimethylammonium bromide (CTAB). They have found that the CTAB is a key factor for the formation of the nanorods. They have also synthesized Fe particles without using CTAB, the

reaction produced very irregular particles with diameters of several hundreds of nanometres. The coercive force value of Fe nanorods is 183 Oe.

Fe nanoparticles were synthesized via a simple one-pot thermal decomposition of $Fe(CO)_5$ in the presence of oleylamine by **Peng et al., (2006),** They have found that the controlled oxidation of the Fe surface leads to a crystalline Fe_3O_4 shell and results in a dramatic increase in the chemical and dispersion stability of the nanoparticles. The surface ligand exchange transfers the core/shell nanoparticles from hydrophobic to hydrophilic, forming stable aqueous dispersion of the nanoparticles in phosphate-buffered saline. They have further demonstrated that the prepared nanoparticles can serve as promising magnetic labels for highly efficient bioseperation/drug delivery and highly sensitive biodetection.

Silica coated iron nanostructures were prepared through a simple surfactant controlled chemical reduction route by **Ni et al., (2010).** They have reported the prepared iron particles act as cores and insulating silica as shells. The silica shell played the double roles as thermal barrier coating that protected the metallic iron from oxidation and as dielectric coating that separated the magnetic metal particles for suppressing the eddy current loss. The prepared Fe composite particles exhibited slightly changed magnetic properties, improved oxidation resistance and much better electromagnetic wave absorption performance than that of the iron counterparts without silica coating.

Prema and Selvarani (2012) have synthesized zerovalent iron nanoparticles by chemical reduction method using aqueous solution of ferrous sulfate heptahydrate ($FeSO_4.7H_2O$) with sodium borohydride ($NaBH_4$) as a reducer. The average size of the particles was determined from X-ray diffraction line broadening and the diffractogram was compared with the standard JCPDS data to identify the crystallographic phase as well as the structure of the particles. The size of the particles was in the range of 18-30 nm. The morphology of the nanoparticles was studied by scanning electron microscopy (SEM). The antibacterial activity of the zero-valent iron nanoparticles was carried out by agar well diffusion method.

Bacterial sensitivity of nanoparticles was found to vary depending on the microbial species. Vibrio parahaemolyticus showed the highest antibacterial efficacy to iron nanoparticles than the other bacterial pathogens used in the experiment. Two-way ANOVA test revealed that the functions of iron nanoparticles on bacterial pathogens are statistically significant.

Changhalee et al., (2008) have worked on zero-valent iron nanoparticles (nano-Fe^0) in aqueous solution rapidly inactivated *Escherichia coli.* A strong bactericidal effect of nano-Fe^0 was found under deaerated conditions, with a linear correlation between log inactivation and nano-Fe^0 dose (0.82 log inactivation/mg/L nano-Fe^0·h). The inactivation of *E.coli* under air saturation required much higher nano-Fe^0 doses due to the corrosion and surface oxidation of nano-Fe^0 by dissolved oxygen. Significant physical disruption of the cell membranes was observed in E.coli exposed to nano-Fe^0, which may have caused the inactivation or enhanced the biocidal effects of dissolved iron. The reaction of Fe (II) with intracellular oxygen or hydrogen peroxide also may have induced oxidative stress by producing reactive oxygen species.The bactericidal effect of nano-Fe^0 was a unique property of nano-Fe^0,which was not observed in other types of iron-based compounds.

Saba A. Mahdy et al., (2012) have reported on the toxicity of ZVIN nanoparticles on gram-negative and gram-positive bacterial systems, *Escherichia coli and Staphylococcus aureus.* Detailed characterisation of the nanoparticles using x-ray diffraction (XRD), scan electron microscopy (SEM) confirmed the presence of 31.1nm sized ZVIN particles. St.aureus, *E.coli* were grown in the presence of different ZVIN nanoparticles concentrations for 24 hours. FeO nanoparticles MIC of *E. coli and St.aureus* at concentrations 30 μg/ml, where as growth is completely inhibited at concentrations 60 μg/ml.

Melanie Auffa et al., (2008) have proposed iron-based nanoparticles for increasing number of biomedical and environmental applications although in vitro toxicity has been observed. The aim of the study was to understand the relationship between the redox state of iron based nanoparticles and their cytotoxicity toward a

Gram-negative bacterium, *Escherichia coli.* While chemically stable nanoparticles (γ-Fe_2O_3) have no apparent cytotoxicity, nanoparticles containing ferrous and, particularly, zerovalent iron are cytotoxic. The cytotoxic effects appear to be associated principally with an oxidative stress as demonstrated using a mutant strain of E.coli completely devoid of super oxide dismutase activity. This stress can result from the generation of reactive oxygen species with the interplay of oxygen with reduced iron species (FeII and/or Fe^0) or from the disturbance of the electronic and/or ionic transport chains due to the strong affinity of the nanoparticles with the cell membrane.

Sukdeb Pal et al., (2007) have investigated the antibacterial properties of differently shaped silver nanoparticles against the gram-negative bacterium *Escherichia coli*, both in liquid systems and on agar plates. Energy-filtering transmission electron microscopy images revealed considerable changes in the cell membranes upon treatment, resulting in cell death. Truncated triangular silver nanoplates with a {111} lattice plane as the basal plane displayed the strongest biocidal action, compared to spherical and rod-shaped nanoparticles and with Ag^+ (in the form of $AgNO_3$). It is proposed that nanoscale size and the presence of a {111} plane combine to promote this biocidal property. This is the first comparative study on the bactericidal properties of silver nanoparticles of different shapes, and the results demonstrate that silver nanoparticles undergo a shape-dependent interaction with the gram-negative organism *E. coli.*

1.12.2 Literature Survey for Silver (Ag)

Zhao et al., (2010) have reported that Ag ions and Ag-based compounds are highly toxic to microorganisms, showing strong biocidal effects on as many as 12 species of bacteria including *Escherichia coli.*

Sondi and Salopeck (2004) have reported the antimicrobial activities of Ag-NPs against the growth of *E. coli* on Luria–Bertani agar plates. They have stated that the E. coli bacterial strain served as a model of Gram-negative bacteria. Results showed that the growth inhibition of *E. coli* was dependent on the concentration of

Ag-NPs and the initial concentration of cultivated bacteria. The growth inhibitory concentrations were found to be about 50–60 and 20μgCm^{-3} for 105 CFU and 104 CFU of *E. coli*, respectively. They have also reported that the bacterial cells were damaged and destroyed along with the accumulation of Ag-NPs in the bacterial membrane.

Morones et al., (2005) have used different types of Gram-negative bacteria to test the antibacterial activities of Ag nanoparticles having a crystallite size in the range of 1–100nm. It has been reported that the antibacterial activity of Ag nanoparticles against Gram-negative bacteria can be divided into three process: (i) nanoparticles mainly having crystallite size in the range of 1–10nm attach to the surface of the cell membrane and drastically disturb its proper functions, such as permeability and respiration; (ii) they are able to penetrate inside the bacteria and cause further damage by possibly interacting with sulfur- and phosphorus-containing compounds such as DNA; (iii) nanoparticles release silver ions, which will have an additional contribution to the bactericidal effect of Ag nanoparticles.

Kim et al., (2007) have used a model of both Gram-negative (*E. coli*) and Gram-positive (*S.aureus*) bacteria to investigate the antibacterial activities of Ag nanoparticles. Their studies revealed that *E. coli* is inhibited at a low concentration of Ag nanoparticles (3.3 nM), and ten times lesser than the minimum inhibitory concentration on *S. aureus* (33nM).

Shrivastava et al., (2007) have described the strong antibacterial potency of novel Ag nanoparticles having size in the range of 10–15nm with improved stability against some strains of non-resistant and drug-resistant bacteria. They have also observed and concluded that the antibacterial effect is dose-dependent and is more pronounced against Gram-negative than Gram-positive bacteria and it was also independent of acquisition of resistance by the bacteria against antibiotics. They have also reported that the major mechanism in which Ag nanoparticles manifested antibacterial properties was by anchoring to and penetrating the bacterial cell wall, and modulating cellular signaling by dephosphorylating putative key peptide

substrates on tyrosine residues. They have also made a comparative study of the effect of Ag nanoparticles with different shapes on the Gram-negative bacterium.

Pal et al., (2007) have demonstrated that Ag nanoparticles undergo shape-dependent interaction with *E.coli*.Truncated triangular silver nanoplates with a {111} lattice plane as the basal plane displayed the strongest biocidal action, compared to spherical and rod-shaped nanoparticles.

Guzman et al., (2009) have reported the results of antibacterial activities of synthesized Ag nanoparticles against *E. coli, P. aeruginosa and S. aureus* around 14.38, 6.74, and 14.38ppm, respectively.

Sathishkumar et al., (2009) have discussed by the green synthesis of nanocrystalline Ag particles and their bactericidal activity. They have reported the biosynthesis of Ag nanoparticles from Ag precursors using the bark extract and powder of novel Cinnamon zeylanicum. They used aqueous 1 mM $AgNO_3$ solutions and different dosages of C. zeylanicum bark powder (CBP) for the bioreduction of nano-scale Ag particles.

Lok et al., (2007) have investigated the mode of antibacterial action of nano-Ag against *E. coli*. They have synthesized Ag nanoparticles by the borohydride reduction of $AgNO_3$ in the presence of citrate as a stabilizing agent. $NaBH_4$ (50 mg) was added to a vigorously stirred $AgNO_3$ solution (16 mg in 1 litre) and sodium citrate (0.7 mM) at room temperature. Then the solution was concentrated further to 100 ml under vacuum. TEM analysis revealed that the prepared Ag nanoparticles are spherical in shape with a diameter of 9.3 ± 2.8 nm. The proteomic signatures of nano-Ag-treated E. coli cells have been studied using an accumulation of envelope protein precursors. This indicated that the Ag nanoparticles may target the bacterial membrane, leading to a dissipation of the proton motive force.

Hoang Vinh Tran et al., (2010) have synthesized monodisperse chitosanbased Ag nanoparticles and then investigated their antibacterial activities.

They used a ‘green’ synthesis method to prepare Ag nanoparticles using non-toxic chitosan agent. They added 25 ml of fresh solution of 0.1 M $AgNO_3$ to 100 ml of chitosan and then dissolved it in a solution of 1 wt% acetic acid to reach an appropriate concentration. TEM analysis revealed that the nanoparticle size was in the range of 5–7 nm. They have also studied the bactericidal effect against Gram-negative (*E. coli and Pseudomonas aeruginosa*) and Gram-positive (*Lactobacillus fermentum, Staphylococcus aureus and Bacillus subtilis*) bacterial strains, and yeast (*Candida albicans*). They have shown that the bactericidal activity of Ag nanoparticles was affected by the cell membrane structure; Gram-negative bacteria were inhibited more strongly than Gram-positive bacteria.

Sadeghi et al., (2010) have reported the antibacterial activity of Ag nanoparticles against *S. aureus and E. coli*. They have synthesized Ag nanoparticles by a chemical method using reduction of $AgNO_3$ in the presence of $NaBH_4$. To investigate the antimicrobial activity of Ag nanoparticles on the bacterial growth, the authors have determined the MIC of Ag nanoparticles for *S. aureus and E. coli* by optical density of the bacterial culture solution containing different concentrations of Ag nanoparticles after 24hrs. In addition, the effect of Ag nanoparticles on the morphological changes of S. aureus and E. coli was observed by SEM. They have reported that the Ag nanoparticles had high antibacterial efficiencies, and this activity was quite strong and durable. It was also shown by them that Ag nanoparticles require a lower concentration to inhibit development of the *Streptococcus* mutans and *E. coli* strains.

Chamakura et al., (2011) have shown that efficiencies is dependent upon the ratio of E. coli to nanoparticles rather than the concentration of Ag nanoparticles. They have synthesized Ag nanoparticles by a chemical reduction method. They used Ag nitrate and various reducing agents (ascorbic acid ($C_6H_8O_6$), sodium citrate ($C_6H_5ONa_3$), sodium borohydride ($NaBH_4$), and dimethylamine borane (DMAB) in order to complete the reduction of Ag cations. The researchers have treated the E. coli with different Ag nanoparticle concentrations and proposed a summary of known modes of inactivation of the bacteria by Ag nanoparticles. They have also

compared the effect of Ag nanoparticles with other chemical disinfectants. It was found that higher ratio of Ag nanoparticles to bacteria resulted in a faster bactericidal effect, and that Ag nanoparticles exhibit persistent and efficient bactericidal activity even for the lowest concentration compared with the chemical disinfectant.

Kumar et al., (2004) have studied the generation of Ag nanoparticles on active carbon by an electrochemical deposition method and they have also studied the utilization of Ag nanoparticles in controlling the microorganisms in water. The Ag nanoparticles were generated electrochemically by passing a DC current through Ag electrodes dipped in distilled water. A 2 wt% of Ag deposited on activated carbon (AgC-EC) was prepared by suspending around 5 g of carbon in distilled water with vigorous stirring and maintaining 40 V DC through the Ag electrodes for a sufficient amount of time. SEM results showed the formation of Ag nanoparticles with size (50–200 nm) after a run time of 5 min. They have also qualitatively studied the antibacterial activity of the Ag nanoparticles by testing the presence of coliforms in water after contact with the catalyst, using a Readycult reagent. They have showed that the Ag nanoparticles increased the intrinsic activity (activity per site) and hence had superior activity. The high intrinsic activity of the catalyst is known from its effectiveness in controlling the microorganism in water with lower weight.

Akhavan and Ghaderi (2010) have reported the antibacterial activity of a sol–gel synthesized Ag–TiO_2 nanocomposite layer against *E. coli* bacteria. Their TEM images showed that the size of Ag nanoparticles is in the range of 5–10 nm. They have reported that Ag– TiO_2 / (a) TiO_2 nanocomposite thin films have excellent antibacterial activity against *E. coli* bacteria. By comparing the antibacterial activity of Ag–TiO_2 / TiO_2 nanocomposite and TiO_2 thin films, they have shown that the relative rate of reduction of the viable bacteria by Ag-TiO_2 nanocomposite thin film is greater than the corresponding value for the parent TiO_2 thin film.

1.13 SCOPE OF THE PRESENT WORK

The present work deals with three main topics and they are preparation and characterisation of iron and silver nanoparticles, theoretical analysis and the biological applications of Fe and Ag nanoparticles. It is possible to synthesize metallic nanoparticles with many different shapes and sizes. It is also possible to control the size of these particles to some extent. Using analytic techniques it is possible to determine the characteristics of the nanoparticles.

A thorough review of literature has showed that various preparation techniques have been used for the synthesis of Fe and Ag nanoparticles. It is of great interest to synthesize and develop metallic and non-metallic nanocrystals using the chemical precipitation methods, given that direct chemical approaches are generally compatible for large-scale production. Hence in the present study an attempt has been made to prepare Fe and Ag nanoparticles using chemical precipitation method. When the particulate size decreased to nano level, iron and silver nanoparticles gain the ability to terminate bacteria because they can easily penetrate into the cell wall of bacteria.

The structure of the prepared nanoparticles is characterised using X-ray diffraction analysis and Raman Study. The surface morphology of the prepared nanoparticles is investigated using Scanning Electron Microscope and Transmission Electron Microscope. The chemical composition of the prepared samples is identified using Energy Dispersive X-ray Analysis.

Simulation studies for iron and silver nanoparticles have been carried out by using *ab initio* software. The band structure calculations based on *ab initio* density functional methods have been used to calculate the full dispersion of the band. As a consequence of the effective masses using the first-principle calculations the electronic structures and band parameters for Fe, and Ag nano particle were obtained within the LDA approximations.

The bactericidal applications of iron and silver nanoparticles on different types of bacteria such as *Escherichia Coli, Pseudomonas aeruginosa, Bacillus Subtilis and Staphylococcus aureas* and evaluation of the antibacterial activity has been carried out using standard Zone of Inhibition (ZOI) microbiology assay.

A detailed literature survey shows that only very limited studies have been carried out on Fe and Ag nanoparticles in animal model. Iron deficiency anemia is a global public health issue affecting human beings. Among domestic animals, the effect of iron deficiency is more pronounced in piglets. Anemia in baby pigs occurs, almost without exception, if no supplemental iron is provided during the first few days after farrowing **(Lipinski et al., (2010)).** Hence piglets are more prone to develop iron deficiency anemia than any other animal species. An attempt has been made to overcome the deficiency of anemia in piglets and in the present work. Iron was given to piglets by different methods like iron injection, oral supplementation of ferric ammonium citrate and oral supplementation of nano iron particles with different concentration levels and the clinical results were studied.

A bactericidal agent with greater potency and less likelihood of developing bacterial resistance is an urgent need in therapeutic management of wound. Recently, silver nanoparticles have been found to have high bactericidal property with lesser development of resistance. This would be especially beneficial in veterinary medicine to keep the wound area clean and hygienic. Keeping the above facts into consideration, the present study is formulated to test the utilization of silver nanoparticles in wound dressings in rabbits as model.

1.13.1 ORGANIZATION OF THESIS

The dissertation consists of **seven** chapters.

Chapter 1 starts with an overview of the background of iron and silver nanostructures and their physicochemical properties, and synthesis methodology and tuning of various parameters to get agglomeration free iron and silver nanostructures.

A detailed review of the recent developments of metallic nanoparticles is carried out to study the potential biological applications. The final part of this chapter explains the scope of the present research and organization of the thesis.

Chapter 2 narrates briefly about the different methods used to prepare nanoparticles and gives a detailed description about the experimental techniques used for the preparation of iron (Fe) and silver (Ag) nanoparticles.

Chapter 3 describes about the different characterisation techniques used to study the structural, morphological, compositional and optical properties of the prepared iron (Fe) and silver (Ag) nanoparticles. The X-ray diffraction and Raman spectroscopy studies were carried out on prepared iron and silver nanoparticle samples. The morphology and their compositional analysis of the as-prepared samples have been studied using Scanning Electron Microscopy (SEM) and EDAX respectively. Optical studies of prepared iron and silver nanoparticle samples were carried out using UV-Vis spectrophotometer.

Chapter 4 describes the theoretical analysis carried out for the as prepared Iron (Fe) and Silver (Ag) nanoparticles using *ab initio* technique. The results obtained by the theoretical methods were similar to the corresponding experimental results. The electronic properties such as band structure, conductivity, dielectric loss and resistivity and the optical properties such as reflectivity, refractive index and absorption of the Iron (Fe) and Silver (Ag) nanoparticles were also studied theoretically. The self-consistent method of convergence of the structure has been used to optimize the lattice parameter. An attempt was made to attribute the electronic structure and energy band of the material for its effect on bacteria and on the cells of piglets and rabbits.

The antibacterial activity applications of iron (Fe) and silver (Ag) nanoparticles on different types of bacteria such as gram positive and gram negative bacteria are described in **Chapter 5**. The model gram negative bacteria used in the present study are *Escherichia coli*, *Pseudomonas aeruginosa* and gram positive

bacteria used are *Bacillus subtilis and Staphylococcus aureus*. It is based on its cell wall structure and dye absorption character.

In-vivo biological applications of iron and silver nanoparticles on piglet and rabbit models have been described in **Chapter 6.** This work has been carried out at the Department of Veterinary Physiology, Veterinary College and Research Institute, Namakkal-Dt, Tamil Nadu.

In the present work, iron was given to piglets by different methods like iron injection, oral supplementation of ferric ammonium citrate and oral supplementation of nano iron particles with different concentration level. The weight of piglets increased appreciably when iron was given by oral supplementation of iron nanoparticles when compared to the other methods used for supplementing iron. The present study was formulated to test utilization of silver nanoparticles in wound dressings in rabbits as model.

The salient features of the current research work are summarized and presented in **Chapter 7.**