REDOX STATE DEPENDENT TUNING OF ANTIBACTERIAL ACTIVITY OF CERIUM OXIDE NANOPARTICLE

To be submitted as Major Project in partial fulfillment of the requirement for the degree of

M. Tech. BIOMEDICAL ENGINEERING

Submitted by

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ACKNOWLEDGEMENT

I **Bhakti Sargia**, student of M.TECH BIOMEDICAL ENGINEERING, 2K14/BME/12 is presenting a project report on "**REDOX STATE DEPENDENT TUNING OF ANTIBACTERIAL ACTIVITY OF CERIUM OXIDE NANOPARTICLE**" under the supervision of **Dr. Sanjay Singh** (Assistant Professor), AU-ILS. He encouraged me to undertake this very interesting topic and even gave me valuable suggestion and information which were mandatory for the completion of the project.
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CERTIFICATE

This is to certify that the dissertation entitled **"REDOX STATE DEPENDENT TUNING OF ANTIBACTERIAL ACTIVITY OF CERIUM OXIDE NANOPARTICLE"** in the partial fulfillment of the requirements for the reward of the degree of **Master of Technology in Biomedical Engineering,** Delhi Technological University (Formerly Delhi College of Engineering, University of Delhi), is an authentic record of the candidate's own work carried out by her under our guidance. The information and data enclosed in this thesis is original and has not been submitted elsewhere for honoring of any other degree.

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Declaration

I declare that my major project dissertation entitled "**REDOX STATE DEPENDENT TUNING OF ANTIBACTERIAL ACTIVITY OF CERIUM OXIDE NANOPARTICLE**" submitted to Department of Biotechnology, Delhi Technological University as a result of the work carried out by me at Institute of Life Science as Major project. The information and data enclosed in this thesis is original and has not been submitted elsewhere for honoring of any other degree.

Date:

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ABBREVIATION

CeNPs	Cerium oxide nanoparticles
mM	Mili Molar
μM	Micro Molar
PO_4	Phosphate species
MM	Minimal Media
Aq.	Aqueous environment
PL	Photoluminescence
CFU	Colony formation unit
AlOH	Aluminum hydroxide
$CaCO_3$	Calcium carbonate
$SrCO_3$	Strontium carbonate
NiO_2	Nickel oxide
MoO_3	Molybdenum trioxide
H_2O_2	Hydrogen peroxide
PBS	Phosphate buffered saline

Title - Redox state dependent tuning of antibacterial activity of cerium oxide nanoparticle

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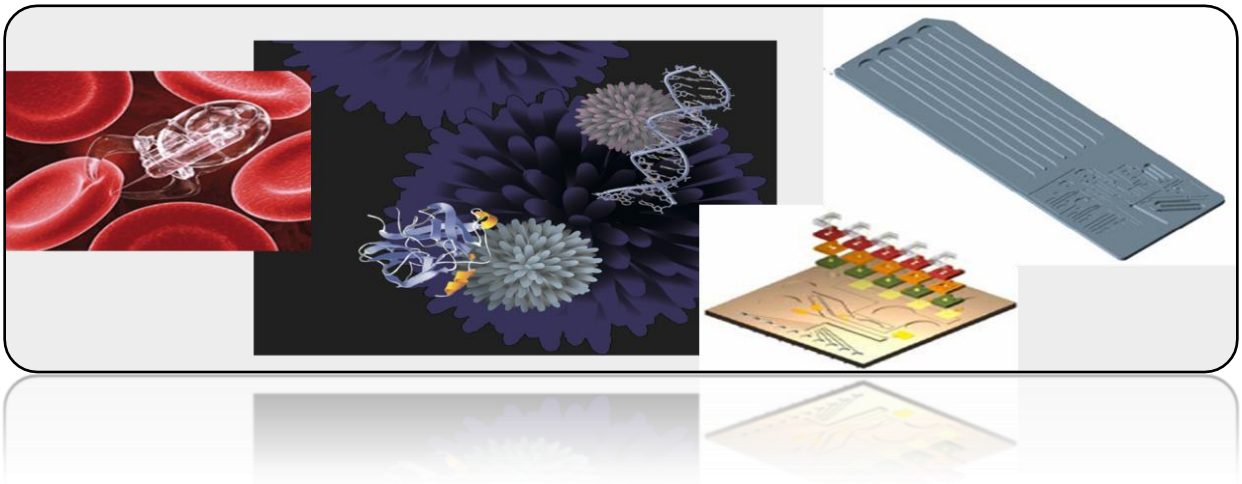
Abstract

Cerium oxide nanoparticles have been used to scavenge reactive oxygen and nitrogen species. Cerium oxide poses the biological catalytic antioxidants property both as superoxide dismutase and catalase mimetic due to rapid change in their oxidative state between +3 and +4. CeNPs (3+) and CeNPs (4+) both exhibit superoxide dismutase and catalase mimetic activity, respectively. CeNPs will interact with components of biologically relevant buffers and medium, which could affect their SOD activity and catalytic properties. The + 3 state of CeNPs highly interaction with phosphate ions and thus loses its SOD activity. The exposure of phosphate anions to CeNPs (4+) did not persuade any significant change in its surface chemistry and therefore there is no much deviation in catalase mimetic activity. Exposure of phosphate anions to CeNPs (4+) did not block its redox cycling between 3+ and 4+ which was seen in +3 oxidative states. Binding of phosphate anions to cerium oxide nanoparticle (+3) can significantly distort the characteristics feature of CeNPs nanomaterials and thus shift their catalytic behavior because of it. Due to availability of phosphate ions in abundant quantity in biological systems in an inorganic form, it is possible that the action of CeNPs as a catalyst can strongly influenced by the local concentration of the phosphate ion in the cells in which it has been introduced. Due to the abundance of phosphate anions in the biological environment; it is likely that internalized CeNPs will be greatly influenced by cytoplasmic and nucleoplasmic concentration of phosphate. Here in this study we will target the antibacterial activity of Cerium oxide nanoparticle by reducing the phosphate environment in growth media. The toxic effect need to be demonstrated of interaction of cerium nanoparticle with phosphate at different concentration which will result in decline of the growth rate of bacteria in media.

Key words: CeNPs, Antibacterial property, SOD, Phosphate, Escherichia coli.

CHAPTER 1

Introduction



INTRODUCTION TO NANOSCIENCE AND NANOTECHNOLOGY

The term "nano" refers to the metric prefix 10^{-9}. It means one billionth of something. "Nano" can be ascribed to any unit of measure. For example, a small mass in nanograms, the amount of liquid present in cell in terms of nanoliters. Nano is a branch of science which includes study of structures and materials on the scale of nanometers [1, 2, 3]. It can be made more exciting and can be converting into useful properties when its structures are converted into nanometer size range. Nano scale structures existed in nature long before studying them in laboratories by scientist. For example single strand of DNA, the building block of all living things, is about three nanometers wide. The scales on a butterfly's wings contain nanostructures. Peacock feathers and soap bubbles get their sparkling coloration from light interacting having structures just tens of nanometers thick. Scientists created nanostructures in the lab that mimic nature's amazing nanostructures.

Because nanostructures are so small, specific methods needed to make objects in nano size range [4]. Scientists have use beams of electrons or ions to carve and fabricate structures as small as 25 nanometers into metal, silicon and carbon-based materials. To form these solid material surfaces, nanostructures can be formed in liquids. The reaction is carried out with chemicals in liquids and gases form to generate nanofibers, nanocrystals and quantum dots, as small as one nanometer wide nanostructures. Scientists are learning how to build three-dimensional structures at the nanoscale level. Nano-electro-mechanical systems, or NEMS, these device might use for microscopic robots to carry out tasks too small for humans to do. For example, NEMS can be used to carry out surgery on a single cell or can act as automatic actuators to move individual molecules. To magnify and visualized nanoscale structures, high-powered microscopes are required.

Nanoscience has impacted our lives with creative innovations such as stain-resistant fabrics motivated by nanoscale features of lotus plants and computer hard drives, which store information having structure nanometer thick. Several disciplines including physics, chemistry, biology and materials science use nanoscience principles for wide range of applications in energy, medicine, information storage, computing.

The word 'nano' is derived from the Greek word for dwarf. The conceptual underpinnings of nanotechnologies were first laid out in 1959 by the physicist Richard Feynman, in his lecture 'There's plenty of room at the bottom' (Feynman 1959). Feynman explored the opportunity of manipulating material at the scale of individual atoms and molecules, imagining the whole of the Encyclopedia Britannica written on the head of a pin and foreseeing the rising ability to examine and control matter at the nanoscale. The primary motivating force for miniaturization came from the electronics industry, which aimed to develop tools for creating smaller electronic devices on silicon chips. The size range that holds so much curiosity and perfection is typically from 100nm down to the atomic level because it is in range that materials can have different properties compared with the same materials having larger size [5, 6].

These effects were observed due to an increased in relative surface area and quantum effects. Chemical reactivity follows inverse relationship with respect to surface area, making nanomaterials useful. As the size of the matter gets reduced to ten times or less, here quantum

effects can begin to play an important role, and change material's optical, magnetic or electrical properties. Nanotechnologies aim to create structures, devices and new systems with new properties and functions.

Nanotechnologies can be regarded as an authentically interdisciplinary branch which encouraged the collaboration between researchers to share knowledge, idea, tools and techniques. An understanding of the physics, biology, medicine, engineering and chemistry along with its processes at the nanoscale is required Indeed, it could be argued that evolutionary developments in all of these fields towards investigating matter at small size scales has now come to be known as 'nanotechnology'.

APPROACHES OF NANOMATERIAL SYNTHESIS

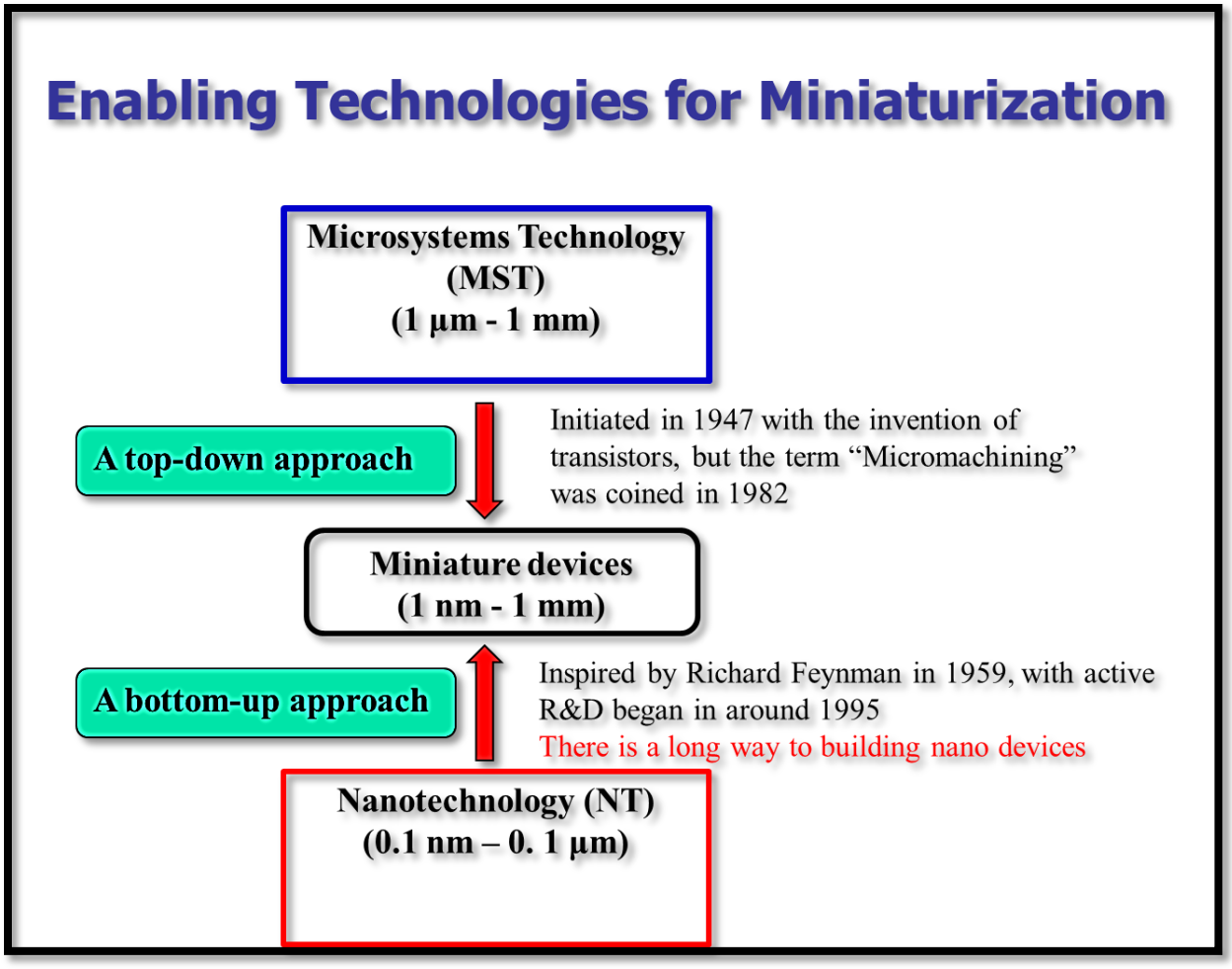


Figure 1.1. Techniques of nanomaterial synthesis

Basically two types of approach is used

i) Top-down approach

Here the breaking down of a system to gain insight into its compositional sub-systems in a reverse engineering order. Here an overview of the system is formulated, but not detailing first-level subsystems. Each subsystem is then refined and characterized in yet greater detail, until the entire specification is known upto to its base elements. A top-down model is often specified with the help and support of "black boxes" and be detailed enough for practical validation of the model. The top down approach starts with big picture [7]. It breaks down from larger to smaller segments.

ii) Bottom-up approach

It is the piecing together of systems to give rise to more and more complex systems, thus making the original sub-systems of the emergent system. Bottom-up is a type of information processing based on incoming data from the environment source to form a perfect perception. In this approach the individual base elements of the system are first studied and specified in great detail. The specified elements are then linked together to form a larger subsystems. This strategy resembles as a "seed" model, in which the early stages are small size but eventually after sometime it grows into complexity.

THE SIGNIFICANCE OF THE NANOSCALE

A nanometer (nm) is defined as one thousand millionth of a meter. Like a red blood cell is approx. 7,000 nm wide and water molecule is almost 0.3nm. People are paying attention to nanoscale because properties of materials start differing from larger scale. Nanoscience and nanotechnologies are actually not new. Chemists makes polymers, which are large molecules made up of small nanoscale subunits. Advancement in the tools now allow atoms and molecules to be examined with great precision have enabled us for the growth and development of nanoscience and nanotechnologies.

The bulk properties of materials can change dramatically with nano ingredients. Composites made from particles which are of nano-size ceramics or metals can unexpectedly become much stronger than predicted by existing materials-science ideas. For example, metals with a grain size of around 10 nanometers are seven times harder and tougher than their ordinary structure. The bulk properties of any material are simply define as an average of all the quantum forces affecting all the atoms present in it. When we make things smaller and smaller, we eventually reach a point where the averaging works no longer. The properties of materials are different at the nanoscale for two main reasons.

First, nanomaterials have fairly larger surface area present with respect to the same mass of material produced in its larger form. This makes nanomaterials more chemically reactive and can affect their strength and electrical properties [69].Second, quantum effects begin to

dominate the behavior of the matter at nanoscale particularly at a lower end affecting its optical, electrical and magnetic behavior of the materials.

TABLE 1.1PROPERTY OF NANOPARTICLE [70]

Properties	Examples
Electrical	Increased electrical conductivity in ceramics and magnetic nanocomposite , increased electric resistance in metals
Magnetic	Increased magnetic core activity up to a critical grain size, super paramagnetic behavior
Optical	Spectral shift of optical absorption and florescence properties ,increased quantum efficiency of semiconductor crystals.
Sterical	Increased selectivity, hollow spheres for specific drug transportation and controlled release
Biological	Increased permeability through biological barriers(membrane, blood –brain barrier etc.) improved biocompatibility
Catalytic	Better catalytic efficiency through higher surface-to-volume ratio

CLASSIFICATION OF NANOMATERIALS

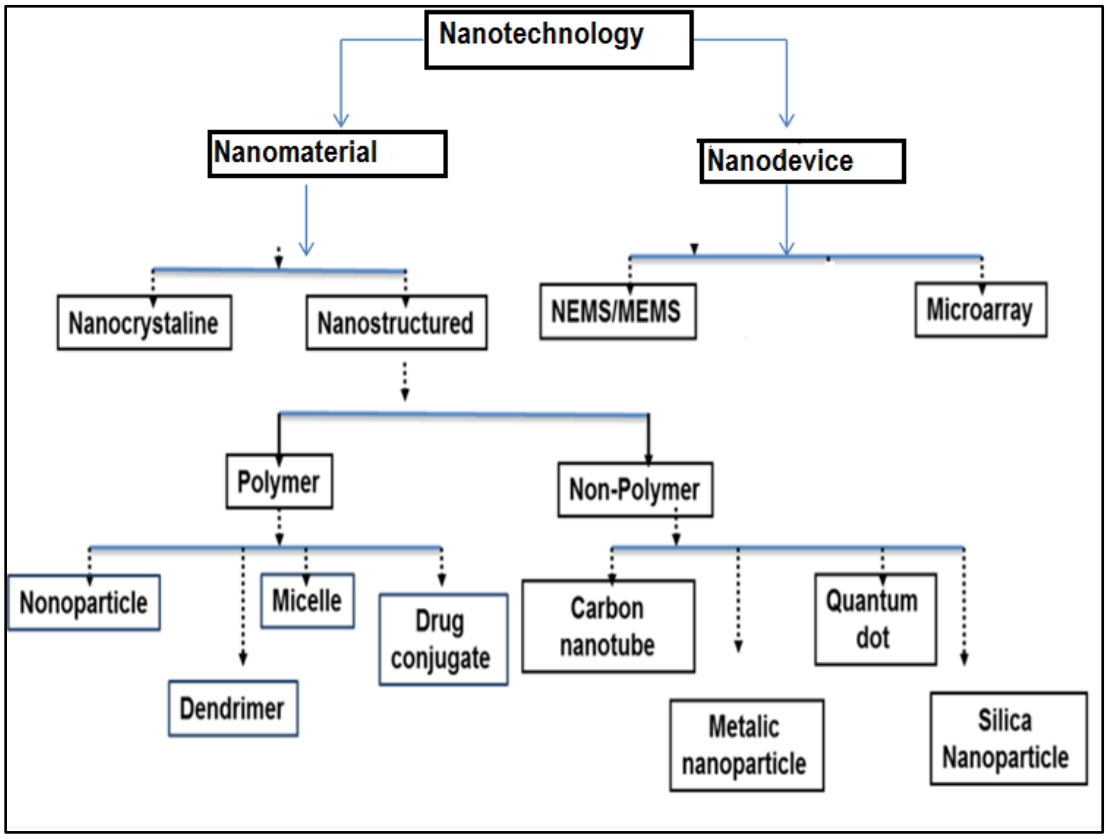


Figure 1.2. Broad classification of nanotechnology

CLASSIFICATION OF NANOMATERIALS

Nanomaterials are categorized as different methods of classification which include origin, dimensions and structural configuration [71].

A. BASED ON ORIGIN:

1. NATURAL NANOMATERIALS:

Nanomaterials which belong to resource of nature are defined as a natural nanometer. For examples virus, protein molecules which include antibody originated from nature are some of the natural nano structured materials. In addition some other examples are mineral such as clays, natural colloids, such as milk and blood, fog, gelatin ,mineralized

natural materials, such as shell, coral and bones, Insect wings and opals, Spider silk, Lotus leaf , Gecko feet, volcanic ash, ocean spray etc.

2. **ARTIFICIAL NANOMATERIAL:**

This type of nanoparticles is prepared deliberately through a well-defined mechanical process and fabrication process. The examples of artificial materials are carbon nanotubes and semiconductor nanoparticles like quantum dots etc.

B. BASED ON DIMENSION:

1. ZERO DIMENSIONAL (0-D):

This type of nanomaterials has Nano dimensions in all three directions. Metallic nanoparticles like gold, silver nanoparticles and semiconductor such as quantum dots are the perfect example of it. These types of nanoparticles are spherical in size and the diameter of nano particles is in the range of 1-45 nm. Cubes and polygons shapes can also come under such kind of nanomaterials.

2. ONE DIMENSIONAL (1-D):

In this type of nanostructures, one dimension of nanostructure will be outside the nanometer range. The example of it includes nanowires, nanorods, metal oxide and nanotubes. These materials are long, but with diameter of only few nanometers.

3. TWO DIMENSIONAL (2-D):

In this type of nanomaterials, two dimensions are outside the nanometer range. Example includes different kind of Nano films such as coatings, thin-film-multilayers, nano sheets and nano-walls. The area of the nano films can be large like in micrometer, but the thickness always exits in nano range.

4. THREE DIMENSIONAL (3 D):

All dimensions of 3 D are outside the nano meter range. Example of this includes bulk materials composed of individual blocks which are in nanometer scale.

C. BASED ON MATERIAL

1. CARBON BASED NANO MATERIALS:

Example of this kind of nanomaterials is hollow spheres, ellipsoids, and tubes. Spherical and ellipsoidal configured as carbon nanomaterials defined as fullerenes, while cylindrical ones are described as nanotubes.

2. SEMICONDUCTOR BASED NANO MATERIALS:

Quantum dots (QDs) are a semiconducting materials consisting of semiconductor core (CdSe), coated by shell (e.g., ZnS) to improve its optical properties, and a cap enabling it to improved solubility in aqueous buffers. They are neither defining as atomic nor bulk semiconductors. Their properties generally originate from their physical size, which ranges from 9–100 Å in radius. Due to their bright fluorescence, narrow emission, broad UV excitation and high photo stability QDs have been implemented for in vitro bioimaging for real time monitoring and tracking of intracellular process for longer period of time. Quantum-dots have a large impact on important development in different medical field like diagnostic tools (magnetic resonance imaging and MRI), in vitro and in vivo detection, analysis of biomolecules, antibody immunoassays, DNA hybridization, development of non-viral vectors for gene therapy, transport vehicles for DNA, protein, drugs or cells, Fluorescence imaging of tissue, labeling of cells and as therapeutic tools for cancer treatment.

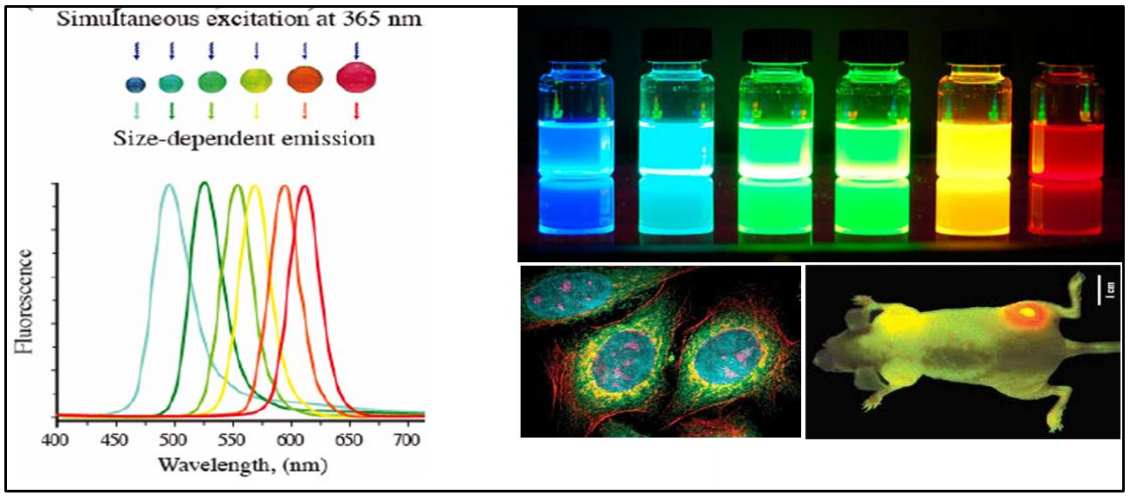


Figure 1.3 Semiconductor based on quantum dots [78]

3. METALLIC BASED NANOMATERIALS:

The main component of these particles is metal. It basically includes nanogold, nanosilver and metal oxides, such as titanium dioxide Metallic nanoparticles are developing as good delivery carrier for drug and biosensor. Although nanoparticles of various metals have been made however silver and gold nanoparticles are of prime importance for biomedical use. They have been used for active delivery of bioactive, drug discovery, immuno bioassays, detection, imaging and many other applications due to the surface functionalization ability, as an alternative to quantum-dots.

4. DENDRIMERS:

Dendrimers are highly branched macromolecules with the dimensions size in nanometer-scale. The surface of dendrimer possesses much chain which can be modified to perform specific chemical functions by it. PAMAM dendrimer is the best illustration of such kind of materials**.** Dendrimers are generally hyperbranched, tree-like structures and have specific compartmentalized chemical polymer. Dendrimer contain three different regions: core, branches, and surface. Dendrimers have a high degree of molecular uniformity, narrow molecular weight distribution; have specific size shape characteristics, and a very highly-functionalized terminal surface. They can be tailored and modified into biocompatible compounds with very low cytotoxicity and a high biopermeability. They bear promising properties aimed at delivery of bioactives ranging from different drugs, vaccines, metal, and genes to desired target. Their hollow interior provides space to incorporate drugs and other bioactive physically. Most important applications of dendrimers are solubilization, gene therapy, drug delivery, immunoassay and MRI contrast agent.

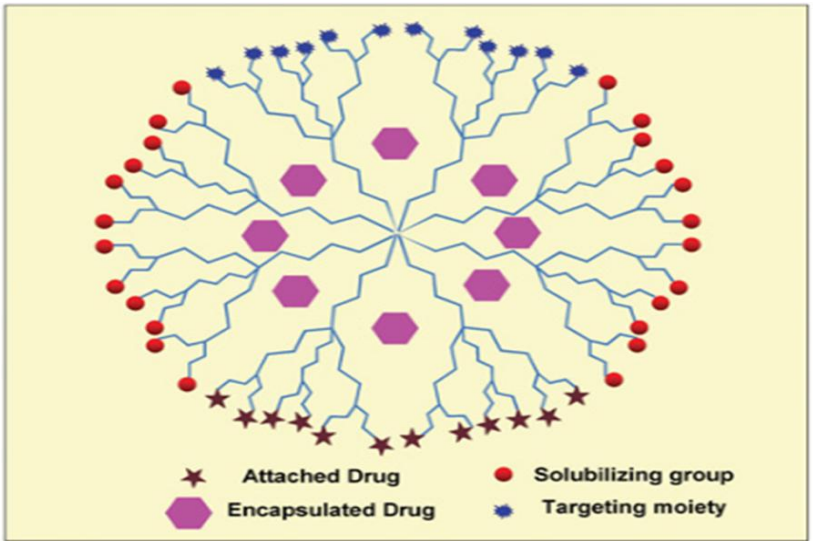


Figure 1.4 Dendrimer*

[**Shishu and majul mehesgwari, university of pharmaeceutical science Chandigarh (2009)*]

1. POLYMERIC BASED NANOMATERRIAL :

This type of nanoparticles consists of inherent properties such as biocompatibility, no immunogenicity, nontoxicity and biodegradability. Applications of polymer is in drug targeting to particular organs/tissues, as carriers of DNA in gene therapy, their ability to deliver proteins, peptides and genes through oral route of administration. They are colloidal carrier, 10 nm -1μm in size, consisting of synthetic or natural polymers. Nanocapsules, Nanospheres and Nanoshells are composed of insoluble organic polymers (methacrylic acid and PEG) and can bypass digestive tract and can act as pharmacologic vectors directly in the intestine. Polyplexes are assemblies, which form spontaneously between nucleic acids and polycations or cationic liposomes, and can be used in transfection activity. The shape, size distribution, and the transfection capability of these nano complexes depend on their composition and the charge ratio of nucleic acid to that of the cationic polymer. Examples of polycations that can be used in gene transfer or therapy are poly-L-lysine, linear/branched poly (ethyleneimine), poly- (amidoamine), poly-amino esters, and last cationic cyclodextrin.

2. LIPIDS BASED NAOMATERIAL :

Exterior lipid bilayer is very highly chemically reactive, providing a means to conveniently couple tags on covalent basis. Tags can be antibody, antigen, cell receptors, nucleic acid probes, etc. This they provides significant versatility in assay formats that is receptor-based, nucleic acid probe, etc. With diameters ranging in size approximately from 50 nm to 800 nm, their aqueous containing core encapsulates up to millions of molecules of signal generating specific “markers” that can be used to detect in a variety of different way. A variety of different encapsulates, including visually detectable dyes, optically and fluorescence emitting detectable dyes, enzymes, and electroactive compounds.

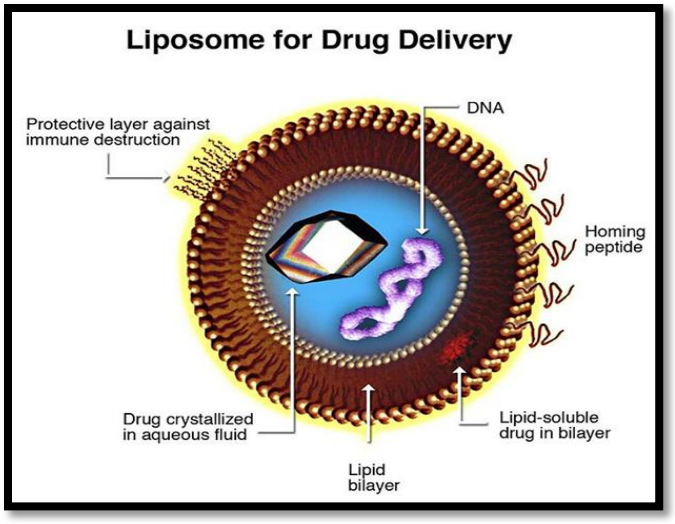


Figure 1.5. Lipid based drug delivery*

[**Softpedia nanobiotechnology*]

1. NANOCOMPOSITES:

Nanocomposite is defined as multiphase solid type material where at least one of its phases has either one, two or three dimensions in nanoscale. The most common examples of such materials are colloids, gels and copolymers.

2. FULLERENES :

This are interconnected carbon atoms similar to graphite, but not have planar 28 to >100 C atoms .Carbon can be subjected to extreme heat and regain original shape. It is as hard as or even harder than diamond. Carbon nanotubes entering the nuclei of cells and can be used to deliver drugs and vaccines. There are two types of nanotubes generally known as first are single-walled nanotubes (SWNTs) and second are multi-walled nanotubes (MWNTs), which differ in the arrangement of their graphene cylinders. Nanotubes offer more distinct advantages over other types of drug delivery and diagnostic systems due to very interesting physicochemical properties like ordered structure with has high aspect ratio, ultra-light weight, high mechanical strength, high electrical conductivity along with high thermal conductivity also, metallic or semi-metallic behavior and high surface area.

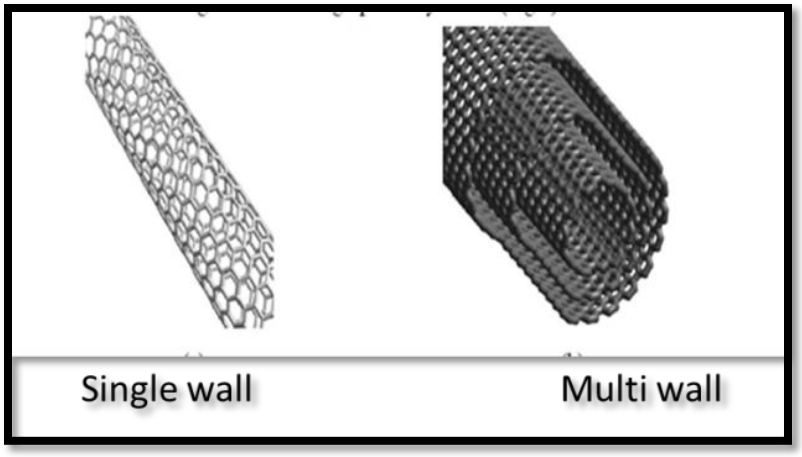


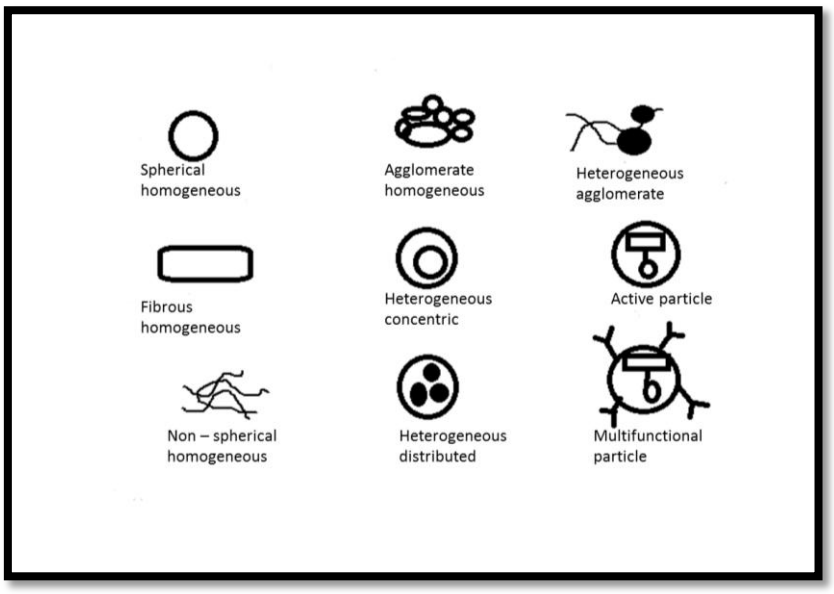
Figure 1.6. Carbon nanotube*

[**Polymer/carbon nanotube nanocomposite veena choudhary and anju IIT delhi(2011)*]

5. METAL OXIDE NANOPARTICLES:

It mainly consist of pure metals or various inorganic, oxide or alloys, they display unique mechanical, catalytic and electrical properties. Example is $CaCO_3$, SiO_2, AlOH, $SrCO_3$, MgO, ZnO, CeNPs and TiO_2. It helps drugs to pass blood brain barrier. It has extensive range of application in biomedicine.

Figure 1.7. Different structure of nanoparticle



APPLICATION OF NANOTECHNOLOGY IN BIOMEDICINE

As nanometer-sized particles have different optical, magnetic, chemical and structural properties from bulk solids they have wide potential applications in medicine [75].

Potential applications

a. DRUG DELIVERY

Nanoparticles use for drug delivery can be either metal or polymer, or lipid based. In Drug Delivery, because of their small sizes, nanoparticles are taken up by cells where large particles can be excluded and is cleared out from the body. A nanoparticle carries the pharmaceutical agent inside its main core, while its shell is functionalized with a binding agent. Through binding agent, the targeted nanoparticle recognizes the target cell. The functionalized nanoparticle shell interacts with the membrane. The nanoparticle is ingested inside the cell membrane, and will interact with biomolecules inside the cell. The nanoparticle particles get break, and the pharmaceutical agent present inside it is released.

Table 1.2 Drug delivery methods

Types of Nanosystems	Size	Characteristics	Applications
Polymeric nanoparticles	10-1000 nm	Biocompatible, biodegradable, offer complete drug protection	Excellent carrier for controlled and sustained delivery of drugs. Stealth and surface modified nanoparticles can be used for active and passive delivery of bioactives.
Nanocrystals Quantum dots	2–9.5 nm	Semi conducting material synthesized with II-VI and III-V column element; Size between 10-100 Å; Bright fluorescence, narrow emission, Broad UV excitation and high photo stability	Long term multiple color imaging of liver cell; DNA **hybridization**, **immunoassay**; receptor mediated **endocytosis**; labeling of breast cancer marker HeR_2 surface of cancer cells
Carbon nanotubes	0.5–3 nm diameter and 20–1000 nm length	Third allotropic crystalline form of carbon sheets either single layer (single walled nanotube, SWNT) or multiple layer (multi-walled nanotube, MWNT). These crystals have remarkable strength and unique electrical properties (conducting, semi conducting, or insulating)	Functionalization enhanced solubility, penetration to cell cytoplasm and to nucleus, as carrier for gene delivery, peptide delivery
Dendrimer	<10 nm	Highly branched, nearly monodisperse polymer system produced by controlled polymerization; three main parts core, branch and surface	Long circulatory, controlled delivery of bioactives, targeted delivery of bioactives to **macrophages**, liver targeting

b. MEDICAL IMAGING

Optical properties of nanoparticles depend greatly on its structural appearance. Particularly, the color emitted by a quantum dot depends on its diameter. The quantum dots (QD) can be injected to a targeted subject, and then it will be detected by exciting them to emit light.

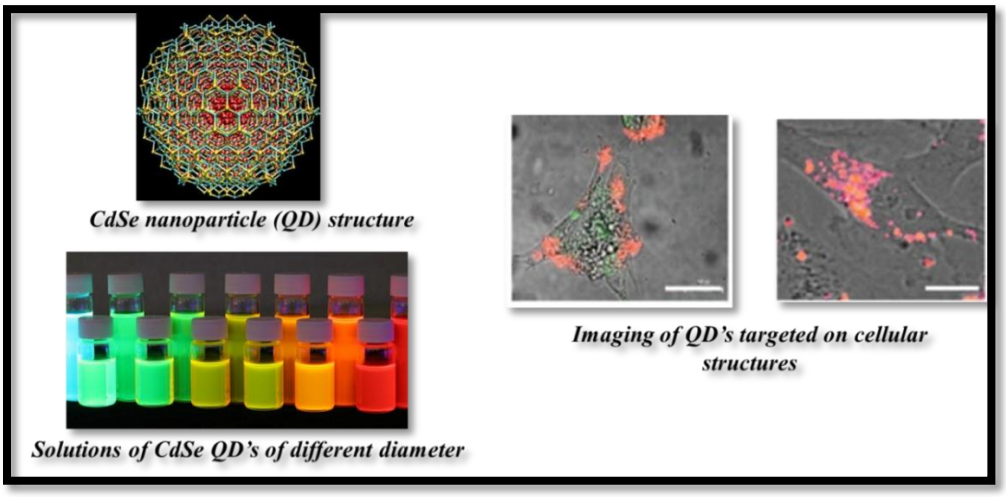


Figure 1.8. Imaging technique [79]

c. THERAPY

Nanoshells coated with a metallic outer layer and a silica core. They are selectively attracted to cancer shells either through process of enhanced permeation retention or due to molecules coated on the shells. The Nanoshells are then heated with an external energy source like NIR and thus result in killing the cancer cells.

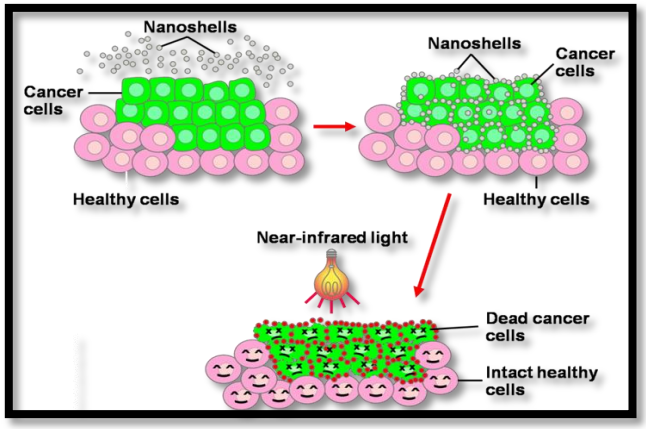


Figure 1.9. Thermal ablation of cancer cells assisted by nanoshells coated with metallic layer and an external energy source*.[**Indian institute of biochemical jadavpur,2011]*

Table 1.3. Cancer therapeutics by nanotechnology

Nanosystem	Applications in cancer therapeutics
Carbon nanotubes	DNA mutation detection, disease protein **biomarker** detection
Dendrimers	Controlled release drug delivery, image contrast agents
Nanocrystals	Improved formulation for poorly-soluble drugs
Nanoparticles	MRI and ultrasound image contrast agents, targeted drug delivery, permeation enhancers, reporters of **apoptosis**, **angiogenesis**, etc.
Nanoshells	Tumor-specific imaging, deep tissue thermal ablation
Nanowires	Disease protein biomarker detection, DNA mutation detection, gene expression detection
Quantum dots	Optical detection of genes and proteins in animal models and cell assays, tumor and lymph node visualization.

d. DETECTION AND DIAGNOSIS

Lab on chips helps in detection and diagnosis of diseases. Nanowire and cantilever are used in lab on chips for early detection of cancer biomarkers.

Table 1.4 Different modality of detection

Modality	Compound	Use
Imaging Agents	superparamagnetic iron oxide nanoparticle	MRI agent
	Dendrimer-based MRI agents	MRI agent-cardiovascular

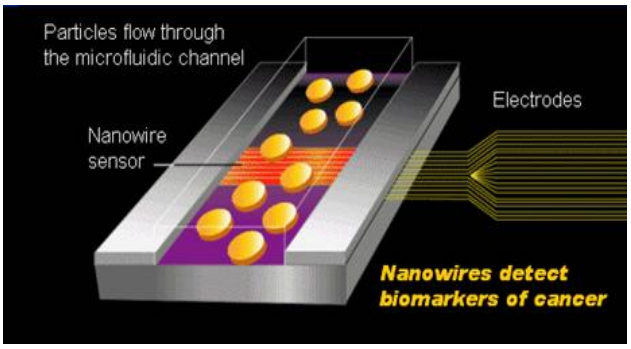


Figure 1.10. The microfluidic channel with nanowire sensor can detect the presence of altered genes associated with cancer.*[**NCI alliance for nanotechnology in cancer]*

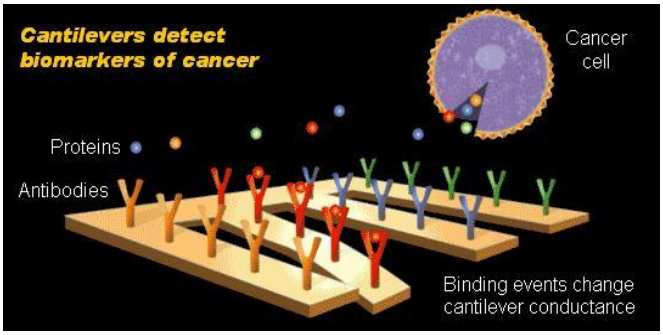


Figure 1.11. The nanoscale cantilever detects the presence and concentration of various molecular expressions of a cancer cell.*[**Cancer nanotechnology IEEE]*

METAL OXIDE NANO PARTICLES

Metal oxides plays a very interesting and noteworthy role in many areas specifically in field of chemistry, physics and materials science [28, 29, 30, 31, 32, 33]. The metal elements are able to form a large variety of oxide compounds. They have adopted a huge number of structural geometry with an electronic structure that can exhibit metallic, semiconductor or insulator character. In scientific applications, oxides can be used as fabrication of microelectronic circuits, sensors, piezoelectric, fuel cells, coatings for the passivation of surfaces against corrosion, and as a catalysts. In the field of nanotechnology the main goal is to make nanostructures, nanoarrays with special properties with respect to bulk or single particle species. Oxide nanoparticles can exhibit distinctive physical and chemical properties because of its limited size and a very high density of corner and edge surface sites. Particle size is expected to persuade three important groups of basic properties in any of material. The first one comprises structural characteristics, namely the lattice symmetry and cell parameters. Bulk oxides are usually robust and have stable systems with well-defined crystallographic structures. However, the rising significance of surface free energy oh nanoparticle and stress with decreasing particle size can be considered. Changes in thermodynamic stability associate with the size can induce in various modifications of cell parameters or structural transformations and in extreme cases the nanoparticle can disappear due to their interactions with its surrounding environment and interacting with a high surface free energy. In order to display mechanical and a structural stability, a nanoparticle must have a low surface free energy. As a result of this requirement, phases that are low in stability at bulk materials can become very stable in the Nano level. This structural phenomenon has been detected in TiO_2, VO_x, Al_2O_3 and MoO_x oxides. Size-induced structural distortions linked with changes in cell parameters have been observed in this. As the nanoparticle size decreases, the increasing number of surface atom and boundary atoms generates a stress and its association with structural perturbations. Beyond intrinsic strain, there can be extrinsic strain associated with a particular synthesis process which can be partially comforted by annealing or calcination. Non-stoichiometry is a common phenomenon. While, interactions with the substrate on which the nanoparticles are supported make situation difficult and can induce structural perturbations or phases not seen for the bulk state of the oxide. The second important effect of size is relationship with the electronic properties of the oxide. In any of the nano material, the nanostruture produces

the quantum size effects which basically arise from the presence of discrete, atoms like electronic states. From a solid-state point, these states can be considered and evaluated as being one of the superposition of bulk states with a simultaneous increase in oscillator strength. On addition, generally electronic effects of quantum confinement experimentally probed on the oxides are related to the energy shift of exciton levels and optical band gap. An important factor has to be considering and evaluate when dealing with the electronic properties of a bulk oxide surface which have long-range effects of the Madelung field, which are not generally present in a nanostructured oxide. According to the theoretical studies for oxides it show a redistribution of charge when going from large periodic structures to small clusters which must be approximately considered to be relatively small for ionic solids while significantly larger for covalent ones. The degree of ionicity or covalency in a metal oxygen bond can strongly depend on size in systems with a partial ionic or a covalent character; an increase in the ionic component to the metal-oxygen bond in parallel to the size decreasing. Structural and electronic properties clearly drive the physical and chemical properties of the solid, the third group of properties influenced by size in a simpler classification. In their bulk state, many oxides have wide band gaps and have low reactivity. A decrease in the average size of an oxide particle does change the magnitude of the band gap, with strongly influence in the conductivity and chemical reactivity. Surface properties are subjected due to their importance significant in chemistry. Solid-gas or solid-liquid chemical reactions can mostly be confined to the surface or sub-surface regions of the solid. The two dimension nature of the surfaces has notable structural, typically a rearrangement or reconstruction of bulk geometries, and electronic, for example presence of mid-gap states, consequences. In the case of nano structured, surface properties are strongly modified with respect to its 2D defined infinite surfaces, producing solids with UN standard sorption or acid/base characteristics. The presence of under coordinated atoms or O vacancies in an oxide nanoparticle produce specific geometrical arrangements as well as a well occupied electronic states located above the valence band of the corresponding bulk material, enhancing the chemical activity of the system.

PROPERTIES OF NANOPARTICULATED OXIDES

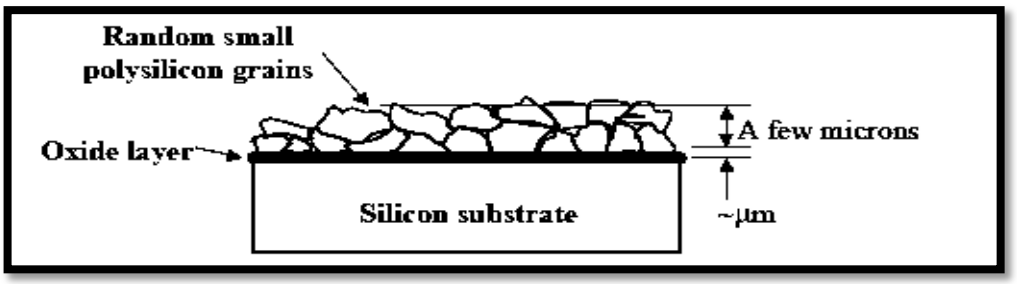
The current knowledge about oxide materials allows affirming that most of the physico-chemical properties display acute size dependence. Physical along with its chemical properties there is special significance in Chemistry are mostly related to industrial use of oxides as sensors, ceramics, absorbents or catalysts. The new application in this field will depend on the size dependence of the optical, mechanical and, chemical properties of oxide nanomaterials. The size effects in oxide have often two interrelated faces, structural, electronic quantum-size and size defect effects.

1. **SILICON OXIDES**

   There are 3 principal silicon compounds used in nanotechnology and microsystems: Silicon dioxide (SiO_2), Silicon carbide (SiC) and silicon nitride (Si_3N_4) each has distinct characteristic and unique applications. Silicon dioxide is least expensive material which offers a good thermal and electrical insulation. It is also used as a low-cost material for

masks in micro fabrication technique such as etching, deposition and diffusion. Used as a sacrificial material in surface micromachining.

Above all, it is very easy to produce:
$Si + O_2 \rightarrow SiO_2$ (by dry heating of silicon)
$Si + 2H_2O \rightarrow SiO_2 + 2H_2$ (by oxide silicon in wet steam)
Silicon carbide (SiC) is having very high melting point and resistance to chemical reactions make it ideal applicant material for being masks in micro fabrication processes. It has superior dimensional stability. Silicon nitride (Si_3N_4) is used as excellent barrier to diffusion to water and ions. Its ultra-strong resistance to oxidation and many etchants form make it a superior material for masks in deep etching. It is also use as high strength electric insulators.
Polysiliconis are aggregation of pure silicon crystals with randomly orientations deposited on the top of silicon substrates. These are highly doped silicon. They are deposited to the substrate surfaces to produce localized resistors and gates for transistors. Being randomly oriented, polysilicon is even stronger than single molecule silicon crystals.



2. MgO AND OTHER ALKALINE-EARTH OXIDES

Magnesium oxide (MgO) is widely used in chemical industry as a scrubber for air pollutant gases (CO_2, NOx, SOx) and as a catalyst support [35]. It exhibits as a rock salt structure like oxides of other alkaline earth metals. The non-polar face is the most stable surface, and particles of magnesium oxide usually display a cubic shape. For example, when Mg metal is burned in air or oxygen, the Magnesium oxide smoke particles are formed are almost perfect cubes having faces. Special measures to prepare MgO nanoparticles exhibiting and faces have been somewhat successful, but in general they tend to facet to surfaces containing planes. The rock salt surface is non-polar, but its surface energy is twice that of surface. The situation is more complex because it will either content a layer of Mg cations or a layer of O anions. Neither of these planes is having neutral charge. MgO nano particles exhibiting faces are intrinsically unstable and should undergo a structural transformation.

3. **ZIRCONIUM OXIDES:**

Zirconium dioxide (ZrO_2) is very fascinating material from a technological point of view; it can be used as structural ceramic, a solid electrolyte, a gas sensor, and as a catalyst. Decreasing the size of zirconia-based particles to nanometric levels provides significant changes in their physical and chemical properties due to modifications produced at structural or electronic levels. Pure bulk ZrO_2 exhibits three structures in different ranges of temperature at an atmospheric pressure. The most stable form of Zr is monoclinic [36, 37].

4. **CERIUM OXIDES:**

Ceria (CeO_2) is an oxide with applications in areas of catalysis, electrochemistry, photochemistry and materials science. The most stable state of CeO_2 adopts a fluorite-type Fm3m crystal structure in which each metal cation is surrounded by an eight oxygen atoms. The band gap of pure ceria is found to be ~ 5 eV, but due to crystal defects or impurities it can be transform into a good n-type semiconductor. Experimental and theoretical studies show that a bulk CeO_2 is not a fully ionic oxide. CeO_2 is an ion covalent compound or a covalent insulator. One of the most important properties of ceria is its ability to undergo conversion between "+4" and "+3" formal oxidation states. Ceria is a key component in catalysts commonly used to reduce the emissions of Carbon monoxide, NOx, and hydrocarbons from automobile exhaust. It is used as base material of electrolytes and electrodes in solid oxide fuel cells.

CE-CONTAINING MIXED-METAL OXIDES:

The performance of ceria in automotive catalysts and fuel cells can be enhanced by doping Ce oxide with a second metal (M= Zr, Ca, Cu, Au, Pt, Tb, La, Mn, etc.). Mixed-oxides maintain fluorite-type structures, particularly the cation sub-lattice, up to a high level of doping [38, 39, and 40]. The doping element in many cases will enhance the thermal stability of the support system and favors the transport of oxygen. The doped-ceria nanoparticles become very active catalysts for reactions such as the water-gas shift or the devastation of SO_2. This effect can be achieved by doping with noble metals like Cu, Au or Pt. These properties of ceria-based systems are mainly driven by two physico-chemical phenomena; the local M-O ordering and distance and the way the systems achieve charge neutrality, which in case of ceria is mainly due to presence of the oxygen vacancies.

5. **TITANIUM OXIDES**:

The Ti-O bond appears to have an rising covalent character with oxygen content of oxide, so the departure of Ti^{n+} from formal oxidation state grows from +2 to +4.[41] Titanium dioxide (TiO_2) is one of the most prominent oxide materials for performing variety of industrial applications specially related to catalysis (among which the selective reduction of NO_x in stationary sources, and photocatalysis for pollutant elimination or organic synthesis,), its use as a white pigment in paintings, as part of photovoltaic

devices, or electrochromic devices, sensors, as a food additive, in cosmetics and a potential tool in cancer treatment.

6. **OTHER OXIDES**:

Nanostructured have been prepared from many oxides. Here we will briefly describe other single oxide systems containing Zn, Fe, and Sn. Zinc oxide presents the quartzite structure and displays a high covalent Zn-O[42] bond.300Fe and O form a number of phases, for example FeO ; Fe3O4 (magnetite), α- Fe_2O_3 (hematite), γ-Fe_2O_3 (maghemite), and ε-Fe_2O_3. The latter two phases are synthetic while remaining oxides occur in nature form. The prevalence of the Fe_2O_3 has stoichiometry effect for different temperature and pressure preparation conditions. The magnetic properties of the Fe oxides have been broadly studied and analyzed; the enhancing magnetic recording properties of magnetite and maghemiteTin (IV)[44,45,46,47] oxide adopts the tetragonal rutile structure with the surface being the most stable one.

CERIUM OXIDE NANO-PARTICLE

Rare earth, which is also known as an 'industrial vitamin' and a 'reserves' of new materials, has played an important role in technical progress, development of conventional industries, and they are also used in high-technology industries such as information and biotechnology. Main reason behind difference in chemistry of rare earth elements from other main group elements and transition metals is due to the nature of the 4f orbital's, which are 'covered' inside the atom and are protected from the atom's environment by the 4d and 5p electrons [63,64]. These orbital's give them unique catalytic, magnetic and electronic properties. These unusual behaviors of rare earth are used to achieve new types of applications that are not achievable with transition and main group metals. Its first element, Cerium in the lanthanide group with 4f electrons, has attracted great attention from researchers from various field of science. When it is allowed to react with oxygen in a nano particle formulation, its oxide adopts a fluorite crystalline structure that emerges as a mesmerizing material. When cerium oxide is used in Nano particle form it has various applications in engineering and biological field, such as solid-oxide fuel cells, elevated-temperature oxidation protection materials, catalytic materials, solar cells and potential pharmacological agents [65]. Although it has various applications, its main application is in the field of catalysis, and stems from their special structure and atomic properties compared with other materials. Nowadays, CeNPs and CeNPs-containing materials main application are catalyst, structural and electronic promoter of heterogeneous catalytic reactions. In industry, it is used as an active component with various applications such as three-way catalysts for automobile exhaust-gas treatments, oxidative coupling of methane and water-gas shift reaction. Nowadays, due to CeNPs multi enzyme properties, including superoxide oxidase, catalase and oxidase, mimetic properties, it has emerged as a charming and beneficial material in biological fields such as in bio analysis, biomedicine, drug delivery and bio scaffolding [66, 67].This review gives a complete introduction to CeNPs's catalytic mechanisms, multi enzyme like activities and potential application in biological fields.

CHEMISTRY OF CERIUM OXIDE

Bulk CeO can adopt a fluorite-type crystal structure in which each metal cation is bounded by eight oxygen atoms, in its most steady phase [26]. One of the most unique feature of ceria is its capacity to undergo a facile conversion between "+4" and "+3" formal oxidation states.

Due to their exceptional catalytic activities, which are resultant from rapid and expedient mutation of the oxidation state between Ce4þ and Ce3þ, cerium oxide nano particles are getting huge notice, nowadays. The cerium atom can easily and significantly adjust its electronic configuration which is best fits its direct environment. It can also exhibit oxygen vacancies, or defects in the lattice structure through loss of oxygen or its electrons, alternating between CeO_2 andCeO_2^{-x} during redox reactions. Being a established engineered nanoparticle with different industrial applications, CeNPs was nowadays found to have multi-enzyme, including superoxide oxidase, catalase and oxidase, mimetic properties that can be used to produce a variety of biological effects, such as being potentially antioxidant towards almost all noxious intracellular reactive oxygen species.

The basis for both the enzyme-mimetic and ROS/RNS scavenging ability of CeNPs , to a certain degree, depends upon various factor such as the inbuilt physicochemical properties of nano scale materials, the explicit ability of CeNPs to absorb/release oxygen and the relative thermodynamic efficiency of redox cycling between Ce^{3+} and Ce^{4+} ions on the surface of CeNPs . It can exist as pure CeO_2 or Ce_2O_3 at the bulk scale. However, at the nano scale, it contains a mixture of both Ce^{4+} and Ce^{3+} on the CeNPs surface. With decrease in the complete CeNPs diameter, there is a measureable loss of oxygen atoms and an increase in the number of Ce^{3+} sites on the NP surface. Due to loss of oxygen atoms from the CENPS surface, there is a reduction in the oxidation state of Ce ($Ce^{4+}Ce^{3+}$) and an increase in the number of oxygen vacancies on the CeNPs surface. The ratio of Ce^{3+}/Ce^{4+} sites on the surface is directly connected with the antioxidant activity of the CeNPs. Under aqueous solution conditions, it can switch between the two valences states (Ce^{4+} and Ce^{3+}) on the surface of CeNPs. The formula CeO_2^{-x} can be used to describe the overall structural stability of the CeNPs which is used to demonstrate the occurrence of oxygen vacancies under these conditions. In order to maintain electronic charge stability each oxygen vacancy must be accompanied by reduction of two surface Ce^{4+} ions. Earlier, it was postulate that both oxygen vacancy sites and the Ce redox couple (Ce^{3+}/Ce^{4+}) were equally involved in the CeNPs biological antioxidant activity. An experiment was performed by doping of CeNPs to discriminate between biological antioxidant activities driven by oxygen vacancies versus the Ce^{3+}/Ce^{4+} redox couple. The experiment uses the fact that Sm^{3+} is non-catalytic and when it is substituted for Ce^{3+} in the crystal lattice, the Ce^{3+}/Ce^{4+} redox couple is disrupted, but the number of oxygen vacancies is unchanged. The result from experiment proved that the Sm^{3+} doped CeNPs no longer displayed biological antioxidant activity, providing considerable evidence that the Ce^{3+}/Ce^{4+} redox couple is the main location of the intrinsic antioxidant properties of CeNPs.

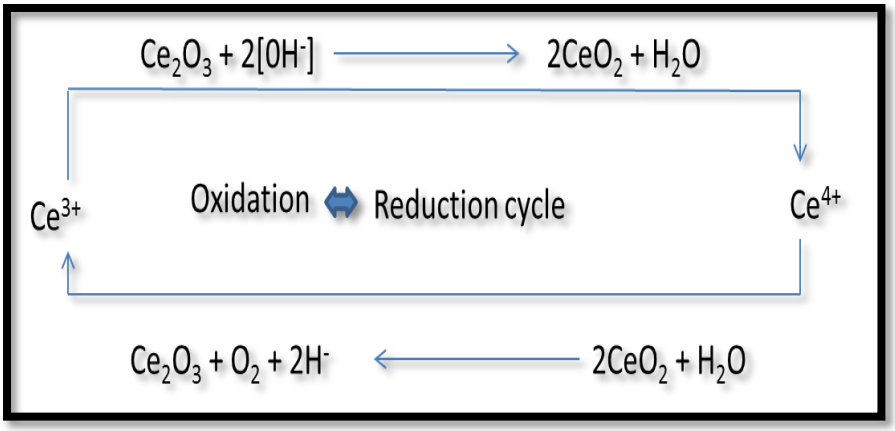


Figure 1.13 Oxidation and reduction cycle of cerium oxide

BIOMEDICAL APPLICATION

Cerium oxides (CeO_2) are an important oxide with huge applications in areas of catalysis, electrochemistry, photochemistry and materials science. Nano particles gives us opportunity for many new developments in the field of biosensors, biomedicine and bio nanotechnology. Nowadays, ceria nano particles have been known as a possible agent to control cellular aging, among various science research communities. It has emerged as exciting and valuable material in biological fields such as bio analysis, biomedicine, drug delivery, and bio scaffolding.

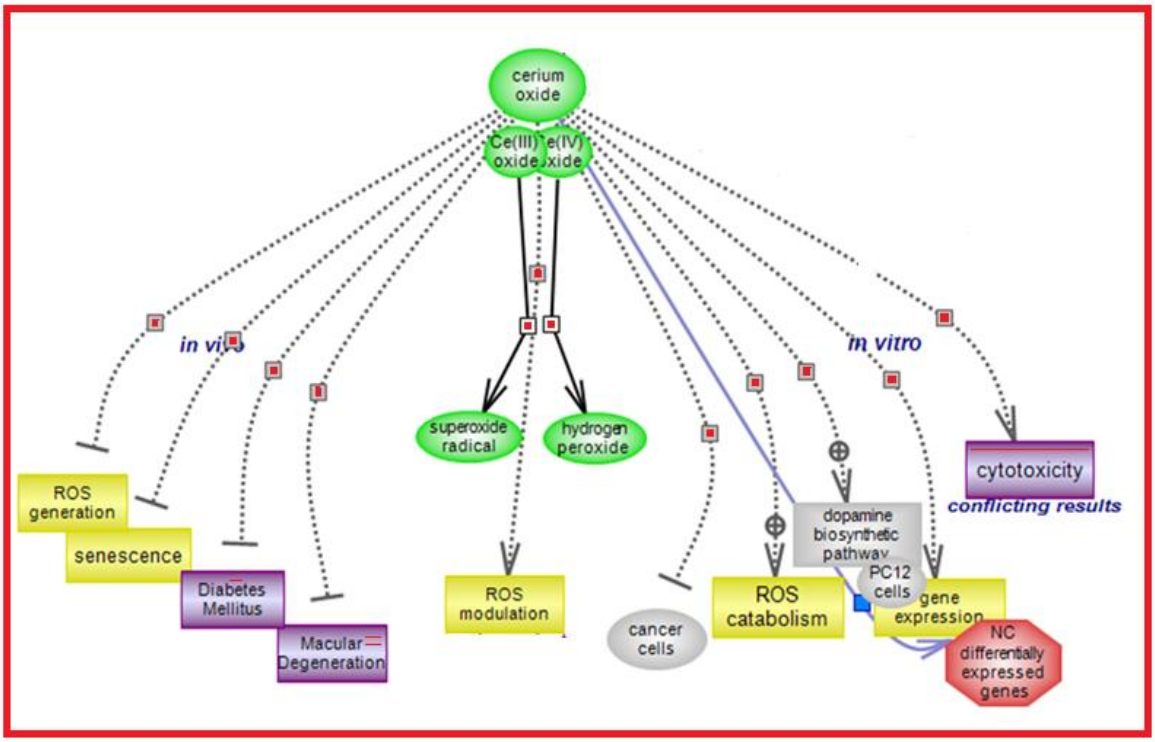


Figure 1.14 Application of CeNPs at different oxidative state.*

[**CeNP response pathway PW: 0001439, gene editing rat resource center]*

A.BASED ON MULTIENZYME ACTIVITY BIO ANALYSIS:

Nanotechnology has already to a great extent impacted bio analysis. Due to its stable physical and chemical properties make inorganic nano particles suitable for use in biological assay to eradicate the shortcomings of organic fluorophores, radioactive labeling or natural enzymes, which are photo bleachable, lethal, costly and easily degradable, [13] respectively. These properties have been successfully used for biological detection and analysis.

PH-DEPENDENT BIO ENZYME MIMETIC ACTIVITIES:

By using optical and magnetic resonance imaging, CeNPs based device is used for the detection of chronic inflammation. Chronic inflammatory conditions are usually symptoms linked with high local concentrations of ROS and decreases in pH, which could be used as pro-inflammatory markers. By taking benefit of the pH-dependent and antioxidant activities of CeNPs it can be used as a sensor for ROS and pH conditions.

COLORIMETRIC PROPERTY:

Catalyzing the colorimetric reactions of organic dyes, CeNPs can also be used as a colorimetric agent itself due to its good feature of color-changing ability corresponding with a shift in redox state [14].

SUPEROXIDE DISMUTASE MIMETIC ACTIVITY

By using an enzyme called Superoxide dismutase [68] we can repair cells and reduces the damage done to them by superoxide, the most common free radical in the body. It catalyzes the disproportionation of superoxide to H_2O_2 and O_2.

Due to $Ce^{3þ}$ /$Ce^{4þ}$ valence switch ability of CeNPs makes it possible for CeNPs to be used as a SOD mimic.

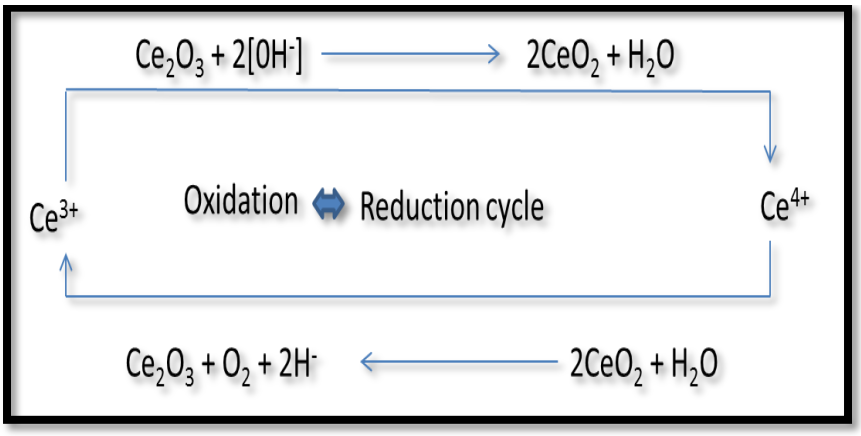


Figure 1.15 SOD activity.

CATALASE MIMETIC ACTIVITY:

Catalase [69] is a protective enzyme found in nearly all living organisms that are exposing to oxygen. It degrades, powerful and potentially dangerous oxidizing agent known as H_2O_2. Although, the complete mechanism of catalase is not currently known, it is believed CeNPs can act as catalase mimic in a redox-state-dependent manner, and higher levels of cerium in the þ4 state exhibited more important activity. The molecular mechanism involves two half reactions namely: oxidation and reduction. In oxidative half reaction one molecule of H_2O_2 reacts with $Ce^{4þ}$, thereby reducing it to $Ce^{3þ}$ and releasing protons and O_2 and in the reductive half reaction second H_2O_2 molecule attach to the oxygen vacancy site , thereby oxidizes the $Ce^{3þ}$ back to the initial $Ce^{4þ}$ state and release H_2O. These two processes are similar to the one found in catalases. It is important to note that H_2O_2 was produced when CeNPs acted as SOD. In vivo, it is believed that surplus H_2O_2 is more toxic potential than superoxide because it is the substrate for the Fenton reaction and generates hydroxyl radicals which are one of the most destructive reactive

oxygen species. Luckily, CeNPs has both catalase- and SOD-like activities. The generated, H_2O_2 in the SOD-mimetic process can go through the catalase-mimetic dismutation cycle and produce harmless H_2O and O_2. Due to this, CeNPs is an excellent antioxidant. However, its anti-oxidative quality is optimum only when the two enzyme-like activities of CeNPs are coordinated. One of the most important elements which must be taken into account when CeNPs is used in cell culture or animal studies is phosphate, as it is a basic substance in biological systems. The other factor which is important is pH value, as it can manipulate the catalytic properties of CeNPs [81, 82]. Under acidic pH the catalase-mimetic activity of CeNPs is appreciably diminish, whereas the SOD activity of CeNPs is not greatly affected over a variety of pH changes. This suggests that at a more acidic pH, CeNPs cannot detoxify the hydrogen peroxide at the same rate as it can at neutral pH, whereas the rate for superoxide converting to peroxide is not affected, this gives us an very important result that CeNPs could be harmful in an environment with low pH.

HYDROXYL RADICAL SCAVENGING:

The hydroxyl radical [8] is one of the strongest oxidants and most biologically active free radicals. There are two methods by which living systems remove hydroxyl radicals: blocking hydroxyl radical initiation by antioxidant enzymes and by breaking the hydroxyl radical chain reaction using non enzymatic antioxidants [8]. It is revealed that ultrafine CeNPs (2–5 nm) having mixed valence states has a significant neuro protective effect on H_2O_2 treated adult spinal cord model systems consider to prevent oxidative injury. As it provides a resource of hydroxyl radicals, which has a key role in oxidative injury, it was proposed that the protective effect of CeNPs on the spinal cord involved its free-radical scavenging effect. When we use H_2O_2 to treat CeNPs directly, it was observed a noteworthy color change from light yellow to deep orange, which indicates that $Ce^{3þ}$ can used as an antioxidant to react with the free radicals generated from H_2O_2 and oxidize to $Ce^{4þ}$.

NITRIC OXIDE RADICAL SCAVENGING:

The nitric oxide [9] radical is a gaseous free radical that shows multifaceted biological effects, both beneficial and damaging. It can react quickly with the superoxide radical, thus forming the highly reactive peroxy nitrite anion, which is more powerful and toxic oxidant. Due to unusually high NO production, which can result in huge number of diseases, so by removing excess NO can have beneficial effects. Due to CeNPs's NO exhaust gas treatment ability and NO adsorption and decomposing ability in industry, it was explore and demonstrated the NO scavenging ability of CeNPs under physiologically relevant conditions . The NO scavenging ability is present in CeNPs with a lower $Ce^{3þ}/Ce^{4þ}$ ratio; in contrast the superoxide scavenging properties which prefer a higher level of cerium in the $Ce^{3þ}$ state.

PEROXIDASE MIMETIC ACTIVITY:

Peroxidases [10] are a class of oxide reductive that are commonly distributed among living organisms. They can catalyze the reduction of peroxide and oxidize .Due to this biochemical function present on them, provide them an important role in many different and vital biological processes, such as defense mechanisms, immune responses and pathogeny. Peroxidase occupies a prominent position in biotechnology [10]. Nowadays, many nano materials have peroxidase-like activity and its potential to replace nature peroxidase by virtue of their stability, low cost and obtain ability. For peroxidase mimics based on transition metal oxide material, their behavior was mostly derived from the metal ions that have a catalytic activity using H_2O_2 as substrate through a mechanism analogous to that of the Fenton reaction. It was found that cerium ions could also perform a Fenton-like reaction. Due to analogous of cerium with Fenton –like reaction cerium containing materials can to be used as peroxidase mimics. Due to its peroxidase-like

activity, CeNPs has huge potential applications in environmental science especially in chemistry, biotechnology and medicine.
With the use of PNC, we can faster up the rate of the oxidation of luminol by H_2O_2 and greatly improve the chemiluminescence intensity, based on this they designed a sandwich method for detection of human thrombin.

OXIDASE MIMETIC ACTIVITY:

An oxidase [11] is an enzyme that catalyzes an oxidation–reduction reaction involving molecular oxygen as the electron acceptor. Due to its multiple physico-logical roles, oxidase is greatly important to us. Studies revealed that CeNPs obtain from an in situ aqueous-phase method showed oxidase-like activities, because they could quickly oxidize several organic substrates without oxidizing agents.
Due to oxidase-like property of CeNPs, which facilitate the oxidation of organic molecules to yield chromogenic products [83] developed strong and dependable colorimetric immunoassays. They attached folic acid to poly acrylic acid-coated CeNPs (PNC). As folic acid can exclusively recognize certain tumor cells with elevated expression of folate receptors on the surface, it can be used as an alternative of the antifolate receptor antibody normally used in enzyme-linked immune sorbent assay. Target cancer cells will capture the folic-acid-modified PNC. A usual ELISA assay was also carried out in which horseradish peroxidase-labeled secondary antibodies was used to evaluate the binding of a fixed primary antibody to a particular target or surface receptor and evaluated by catalyzing the oxidation of tetramethyl benzidine by H_2O_2. CeNPs based on ELISA assays have several advantages:

(1) Both the antibody and natural enzyme used in conventional ELISA assay were unstable and easy to denature and inactivate, whereas those used in CeNPs-based ELISA assay were more stable.
(2) The H_2O_2 used in conventional ELISA assay for horseradish peroxidase can simply decompose and lose its oxidative ability, whereas CeNPs could oxidize the substrate without H_2O_2, thus eliminating the difficulty.
(3) The cost of this method is much lower and the operation is much easier, thus it is more robust than the conventional ELISA assay.

PHOSPHATASE-MIMETIC ACTIVITY:

A phosphatase [12] is an enzyme that removes phosphate groups from their substrates by hydrolyzing the phosphoric acid monoesters into the phosphate ions. Phosphates have various important roles in many biological processes, like signal transduction and cell proliferation, differentiation, metabolism and communication. Nowadays, it is reported that some metal ions and their complexes can be used as artificial phosphoesterases [84] that can speed up the rate of phosphate ester hydrolysis in different ways, including Lewis acid activation, nucleophile activation and leaving group activation. Because of Lewis acid, some of lanthanide ions and their complexes have been shown to be particularly reactive for hydrolyzing phosphate esters. Cerium, the only lanthanide which can easily oxidized to the tetravalent state, has received great notice. Nowadays, studies revealed that CeNPs can be used to hydrolyze phosphate ester bonds of many biologically relevant molecules. CeNPs can also be used to efficiently mediate the dephosphorylation of phosphopeptides.

B. DISEASES:

CERIUM COMPOUND APPLICATIONS IN BIOMEDICINE:

The pharmacological properties of cerium had been exposed more than a century ago and have been used to treat many types of diseases[15].Cerium(III) oxalate has been commonly used to treat vomiting associated diseases such as sea-sickness, chronic diarrhea ,pregnancy phase and neurological disorders, including epilepsy and chorea. Cerium nitrate can be used in place of silver sulfadiazine cream for the topical treatment of extensive burns. According to clinical reports from the revolutionary era of cancer chemotherapy, cerium (III) iodide can be used for tumor reduction and better quality of life. Due to these different biomedical effects give to the bio related properties of cerium, such as a similarity to calcium, bacterio static and bactericidal activities, and immune modulatory properties.

Although the cerium compounds can be used in biomedicine, there are several drawbacks: (1) Due to its small molecule it cannot be easily taken up directly by cells; (2) Due to its short blood circulation time and nonspecific bio distribution of cerium compounds it can cause many useless side effects; and (3) Insolubility in water of cerium oxalate makes their absorption by organisms difficult. These drawbacks can be overcome by use of nano materials, because they can be taken up by mammalian cells easily through various pathways system. As nano material are easy to modify and has long blood circulation times which makes them potentially targetable with a convenient distribution in vivo and the insolubility problem can be overcome by wrapping the material in a layer of water-soluble polymers.

OXIDATIVE STRESS-RELATED DISEASES:

Oxidative stress in biological systems is due to imbalance between ROS and nitrogen species production and antioxidant levels [16]. ROS and reactive nitrogen species are strong oxidizing and nitrating agents that include O_2^{-2}, HO, H_2O_2, NO and $ONOO^{-1}$. These reactive species are generally by products and play dual roles in the organism, depending on concentration, location and intracellular conditions such as causing toxicity or else acting as signaling molecules. In normal biological systems, when extra reactive species are produced to a limited degree, cells are able to neutralize the damage through natural mechanisms, including enzyme antioxidants and endogenous reductants. However, problem can arise when the production of reactive species exceeds the capability of cellular defense systems. Such extra and unusual active species can cause huge damage to molecular structures in biological organisms. Oxidative stress plays a vital role in aging and in a variety of human disease states, such as inflammatory and autoimmune diseases, arthritis, cardiovascular and neurodegenerative diseases.

Due to its multiple antioxidant-enzyme-like activities, catalase, peroxidase-like activities, hydroxyl radical and nitric oxide radical scavenging properties, CeNPs was probable to scavenge almost all types of reactive species. Due to this CeNPs is better than any antioxidant enzyme because they have capacity to scavenge only a single type of free radical before being inactivated. Nowadays, CeNPs has been getting a great deal notice as a potential antioxidant agent in vivo and as a potential therapeutic tool in the treatment of stress related diseases.

NEURODEGENERATIVE DISORDER

The brain and central nervous system are main parts of body which are vulnerable to oxidative stress because of high oxygen utilization, low levels of endogenous antioxidant systems and high levels of poly unsaturated form of fatty acids, which are focus to lipid peroxidation [17]. With the increase in oxidative stress and free radical production can cause several neurodegenerative diseases, such as trauma, Alzheimer's disease and Parkinson's diseases, ischemic stroke and aging. Therapeutic efforts can be used for removal of ROS and prevention of their formation which can be helpful in neurodegenerative diseases. Yet, use of molecular antioxidants to reduce these pathological conditions has met only partial victory, which was attributed to the limits of molecular antioxidants:

(1) They cannot be easily taken up directly by cells, which can results in a lack of ability to reach practical levels of antioxidants at the site of injury.
(2) Many can scavenge only one type of reactive species, whereas diseases often produce mixed reactive species of more than one type.
(3) They cannot cross the blood–brain barrier (BBB).

Therefore, there is a serious need to design new classes of antioxidants that are better-quality than molecular antioxidants. Due to above mention drawbacks CeNPs come into picture as an antioxidant, because it can scavenge almost all types of reactive species and has brilliant regeneration ability and can also cross the BBB because of its nano size. It can protect neurons from free-radical-mediated damage initiated by UV light, H_2O_2, irradiation and excitotoxicity.

DIABETES

Diabetes is a metabolic disorder caused due to hyperglycemia and inadequate secretion or action of endogenous insulin [18]. Increase in oxidative stress is a generally accepted as a cause of development and progression of diabetes .The combination of CeNPs and sodium selenium can be used to overcome diabetic.

RETINAL DAMAGE

Photoreceptor cells has the maximum rate of oxygen metabolism of any cells in the body and are constantly exposed to the detrimental effects of oxidative stress and constant attack by photons [19]. It was believed that ROS that damage the sensitive cells in retina thought to play a central role in retinal diseases, including inherited retinal degeneration, diabetic retinopathy, macular degeneration and retinal detachment, which can lead to partial or complete loss of vision. To overcome ROS dangerous effect, CeNPs can be used to stop retinal degeneration induced by intracellular peroxides and thus preserve retinal morphology and prevent loss of retinal function.

CHRONIC INFLAMMATION

Chronic inflammation is a multifaceted immunological disorder that can result in irreversible organ damage, and leads to the development of many major diseases [20]. Overproduction of the free radical nitric oxide has been occupied as a significant mediator of inflammation. The ability

of CeNPs to scavenge $NO^{\cdot}$ makes it a feasible anti-inflammatory agent.

CANCER

CeNPs is cytotoxic to cancer cells and can induce oxidative stress which can cause lipid peroxidation and cell membrane leakage. It can protect normal cells but not cancer cells from ROS damage. This may be recognized to cancer cells having a more acidic cytosolic pH than normal cells because of higher glycolysis and considerably higher production of lactate. The antioxidant ability of CeNPs is gone in acidic condition and it behaves as a strong oxidant, which can help the oxidation of intracellular and extracellular components to induce cell apoptosis. It is revealed that CeNPs can influence tumor–stromal interactions to detract tumor development and invasion while benefiting stromal cells, which was attributed to the bi functional character of CeNPs. In addition it is believed CeNPs can grant radioprotection to normal cells while growing toxicity to cancer cells, which is essential in radiation therapy. Even though RT remains a helpful modality for the control and cure of malignant cancer, many dangerous side effects are linked with it, including fatigue, nausea and dermatitis. Recently, many recent research studies have been focusing on the improvement of radiation damage on surrounding healthy cells. Amifostine is the only clinically available radio protectant, even though there are side effect such as nausea and hypotension. The dual character of CeNPs gives it the likely to be used as an adjuvant for RT. Several researches have revealed that treatment with CeNPs before RT exposure decreases the RT-induced cell damage and death in normal tissues of the gastrointestinal tract, lung, breast, and head and neck. On the contrary, CeNPs could improve the efficiency of RT to induce tumor cell death because of its acidic cytosolic environment.

C.DRUG DELIVERY DEVICES AND BIOSCAFFOLD:

Recently, nanoparticles have shown incredible potential use in biotechnology as drug delivery systems and bioscaffolds. CeNPs, a nanomaterial with pharmacological potential, can be used as a nanocarrier or scaffold and also act as a therapeutic agent [21]. Combining the two different properties will increases CeNPs's potential application in biotechnology. Spired by its unique and versatile properties of CeNPs, a multifunctional CeNPs capped mesoporous silica nanoparticle anticancer drug delivery agent.

D .ANTIBACTERIAL AGENT

The Microbes such as bacteria, yeast and fungi plays an important role in remediation of toxic metals through reduction of the metal ions and is consider interesting as nanofactories[27]. Bactericidal behavior of nano particles is known due to the presence of electronic effects that are come into account as a result of change in their local electronic structure of surface because of its smaller sizes. These effects are considered to be contributing towards the improvement of the reactivity of nano particles.

The introduction of the antimicrobial agents had a significant role in diminishing the total deaths from infectious diseases. The emergence of bacterial resistance to antibacterial drugs has become a serious problem nowadays for public health. Many traditionally originated used antibiotics are not fruitful anymore in managing drug resistant bacteria. This can lead to the re-appearance of the controlled microbial diseases. Moreover, most of the bacteria escape antibiotic treatments

and host defense systems by establishing a protective matrix of exopolymeric substances called as biofilm. Additionally, the horizontal gene transfer between bacteria within the biofilms can also increase the spread of antibiotic resistance. The antibiotic resistance problem is the decline in the development of new antibacterial agents, with only some of newly approved agents introduced to the pharmaceutical. Therefore, there is an excessive need to develop new antibacterial agents. Many researchers are evaluating potential antibacterial effect of metals in their nanoparticle form. Metals like zinc, silver, and copper have been used as antibacterial agents for long period of time. The advantage of using these metals in their nanoparticle form is that these nanoparticles prepared will have very small diameter, and hence we can have high surface area to volume ratio. It is believed that high surface area to volume ratios and the resultant distinctive chemico-physical properties of the nanoparticles could add on to their antimicrobial activities. Antibacterial nanoparticles can influence several structures and biological pathways found in a wide range of pathogenic bacteria. This will makes it harder for bacteria to develop any resistance against nanoparticle.

Cerium oxide nanoparticles are metal oxide nanoparticles that have been exploited in a wide number of biomedical applications. For example, they are used as a UV light absorber in sunscreens lotion. CeO_2 nanoparticles act as antioxidant activity at physiological pH level, and thus protect cells against oxidative stress, inflammation, or damage caused due to radiation. Studies on antibacterial activity of CeO_2 nanoparticles shows mixed results also. While some suggested antibacterial activity for CeO_2 nanoparticles.

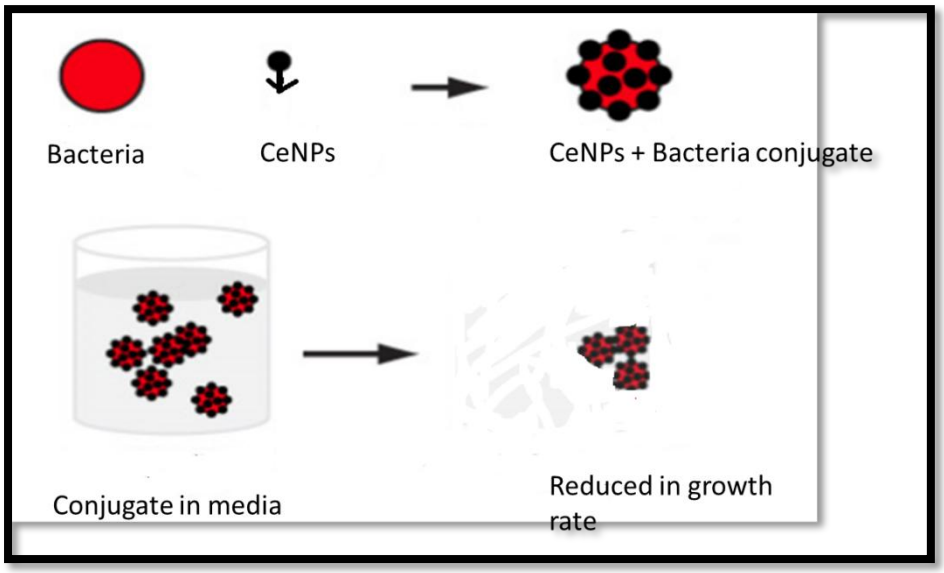


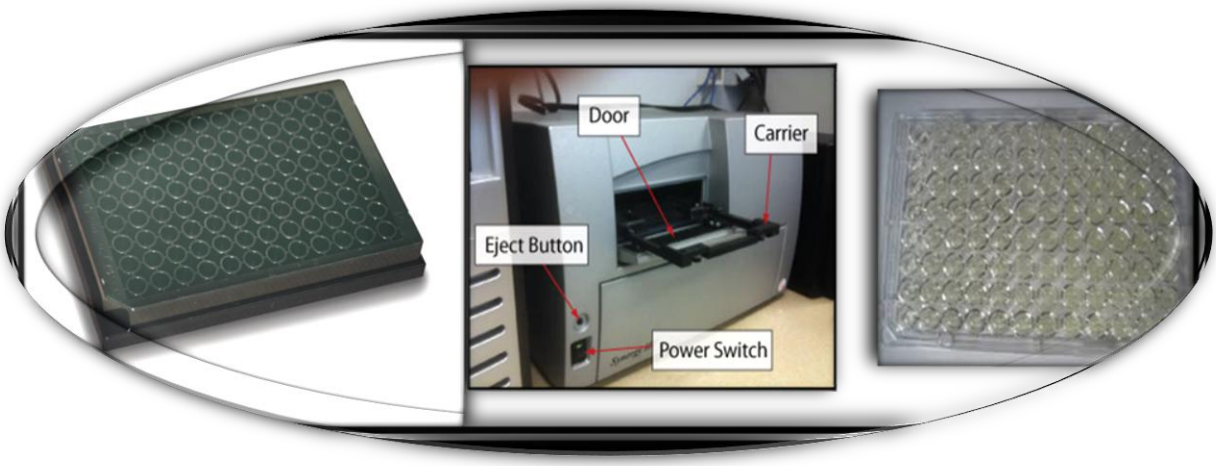
Figure 1.16. Mechanism behind bacteria nanoparticle interaction.

The use of the antimicrobials has reduced the morbidity and mortality associated with many of the infectious diseases [56]. The rise of antimicrobial resistance in bacteria poses a major threat to public health and has even threatened the antibiotic efficacy in treating the infectious diseases [56, 58]. Many bacteria have become resistant to most of commonly used antimicrobial treatments, which not only increases the risk of serious infections in human society but also spread antimicrobial resistant bacteria all over the human population [58]. In addition to the development of novel and new antimicrobials, faster and much more sensitive antimicrobial resistance screening tests are required. These improved tests will not only help in expedite the diagnosis of antimicrobial susceptibility in infected person but also allow the proper antimicrobial treatment which need to be administered rather than using broad-spectrum antimicrobial based on different disease symptoms or diagnosis without having knowledge of antimicrobial susceptibility of infectious agent. A novel method is needed that is much faster and does not compromise sensitivity or specificity. In recent times, new antimicrobial resistance detection methods have been developed that show results in as little as three hours [60, 61]. These methods of using nanoparticles as diagnostic agents to detect antimicrobial resistance are based on shifts in surface resonance or magnetic relaxometry. These methods require specialized, expensive materials and equipment.

Cerium oxide nanoparticles offer a useful role in molecular diagnostics. Cerium oxide nanoparticles exist in a mixed valence state making cerium oxide nanoparticles useful both as reducing and oxidizing agents, depending on its ionic state [62, 63]. Cerium oxide nanoparticles show a shift in absorbance in the ultraviolet region upon oxidation and reduction, which allows us to check the change in valence state, detected using a spectrophotometer [64]. Hence, a spectrophotometer-based diagnostic assay can be developed that detects changes in the redox state of the CeNPs and thus identify the presence of antimicrobial resistance. These cerium oxide nanoparticles have the capability to be used in range of molecular diagnostic assays, like antimicrobial resistance detection.
To detect the presence of antimicrobial resistance, assays that can either oxidize or reduce the cerium oxide nanoparticles is required, and then measure the change spectrophotometrically.

Chapter 2

Instrumentation



Introduction

Increasing contagious diseases and the increase in incidence of drug resistance among pathogenic bacteria have made to search for new antimicrobials strategy. One of the most promising therapeutic agents is nanoparticles. The distinguishing physical and chemical properties of nanoparticles combined with the growth inhibitory capacity against microorganisms has led us for the research on nanoparticles and their potential application to work as antimicrobials. Metals like silver use for treatment and cure of burns and chronic wounds, and copper has been used to make water potable. Some of the metallic compounds possess antimicrobial property. The convergence of nanotechnology and biology has brought metals in the form of nanoparticles as antimicrobial agents. Nanoparticles have distinct and a well definite physical and chemical properties which can be easily change according to our desired applications. Additionally, their potent antimicrobial efficacy due to their large surface area to volume ratio has provided them as an edge over their chemical counterparts which are facing trouble of drug resistance. The application of nanoparticles as antimicrobials is gaining relevance and importance especially in prophylaxis and therapeutics, in medical devices, food industry and textile industry.

Nanomaterials hold huge promising element for improving existing diagnosis, imaging, therapy and designing. It act as a novel approaches to treat a variety of human ailments especially in the field of biomedical engineering. While some applications of nanotechnology has been translated into clinical settings. Since a variety of materials can be made nanosized, the scope of nanomedicine is a huge and larger. At the same time, the impact of nanomaterials on cellular and animal models has to be carefully evaluated under both acute and chronic condition at toxicological level and pharmacological doses. It is extremely important to evaluate the basic issues like the fate of nanomaterials in biological systems, and how the cells and tissues react with the exposure of nanomaterials in developing new nanomedicines. It is a challenge to correlate reports on one type of nano particles to reports on other types of nanoparticle due to their intrinsic differences in their physical properties and chemical properties, methods of preparation, and their biological targets. The biological processes can also occur at the nanoscale and due to their flexibility to biological functionalization; the nanoparticles are discovering importance in the field of medicine. The latter have gained extensive importance due to their ability to tolerate adverse processing environments. Currently, the metallic nanoparticles are systematically being explored and broadly examined as potential antimicrobials agent. Antimicrobial activity of the nanoparticles has been known to be a function of the surface area in contact with the microbes. The small size and the high surface to volume ratio that is large surface area of the nanoparticles will enhance their interaction with microorganisms to carry out a broad range of feasible antimicrobial activities. Metal oxide nanoparticles with antimicrobial activity when embedded and coated on to the surfaces can find huge applications in water treatment, synthetic textiles, biomedical, surgical devices, food processing and packaging. Furthermore, the composites which were prepared using metal nanoparticles and polymers can be better utilized due to enhanced antimicrobial activity.

We focus here on the role of various metallic nanoparticles as possible antimicrobials and the promising mechanism of their inhibitory actions. The growing application of nanoparticles as antimicrobials in industries, medicine, cosmetics, textiles and food packaging which requires the

evaluation of toxicity and risks associated with these nanoparticles. The metal based NP constitute very effective antimicrobial agent against pathogenic microorganisms. Some of the nanoparticles such as silver, titanium dioxide, cerium oxide and zinc oxide are getting significant attention as antimicrobials and additives in consumer, health-related and industrial products.

Instrumentation

UV-Visible spectroscopy

UV-Visible spectroscopy is established on the Beer-Lambert law, which states that when a beam of monochromatic light is passed through a solution of an absorbing substance, rate of decrease of intensity of radiation with thickness of the absorbing solution which is proportional to the incident radiation as well as the concentration of the solution. UV-Vis spectroscopy does not show the structural information about molecules but can be used to determine the concentration of a solution. [77]

The expression of Beer-Lambert law is…
$A = \log (I_0/I) = ECL$
Where, A = stands for Absorbance
I_0 = intensity of light incident upon sample cell
I = intensity of light leaving sample cell
C = molar concentration of solute
L = length of sample cell (cm.)
E = molar absorptive

The value of $\log10 (I_0/I)$ is known as absorbance of the solution. Molar extinction coefficient is independent of concentration and path length, whereas absorbance depends upon both. The wavelength at which maximum absorbance occurs is called λmax. In conventional spectrophotometer electromagnetic radiation is passed through the sample which held in a small square section cell. Radiations across the whole of the ultraviolet/visible range is scanned over a period of approximately 30 s, and radiation of the same frequency and intensity is simultaneously passed through reference cell containing only solvent. Photocells then detect radiation transmitted and the spectrometer records the absorption by comparing it with the difference between the intensity of the radiation passing through the sample and the reference cells. Two lamps are used. A hydrogen/deuterium discharge lamp covers the ultraviolet range, and a tungsten filament covers the whole visible range. The radiation is separated according to its frequency/wavelength by a diffraction grating followed by a narrow slit. The slit ensures the radiation is of a very narrow waveband. Detection of the radiation passing through the sample or the reference cell can be then achieved by either a photomultiplier or a semiconducting cell followed by an electron multiplier. The spectrum is produced by comparing the currents generated by the sample and the reference beams.

Figure 2.1. UV vis spectrophotometer[*AU-ILS]

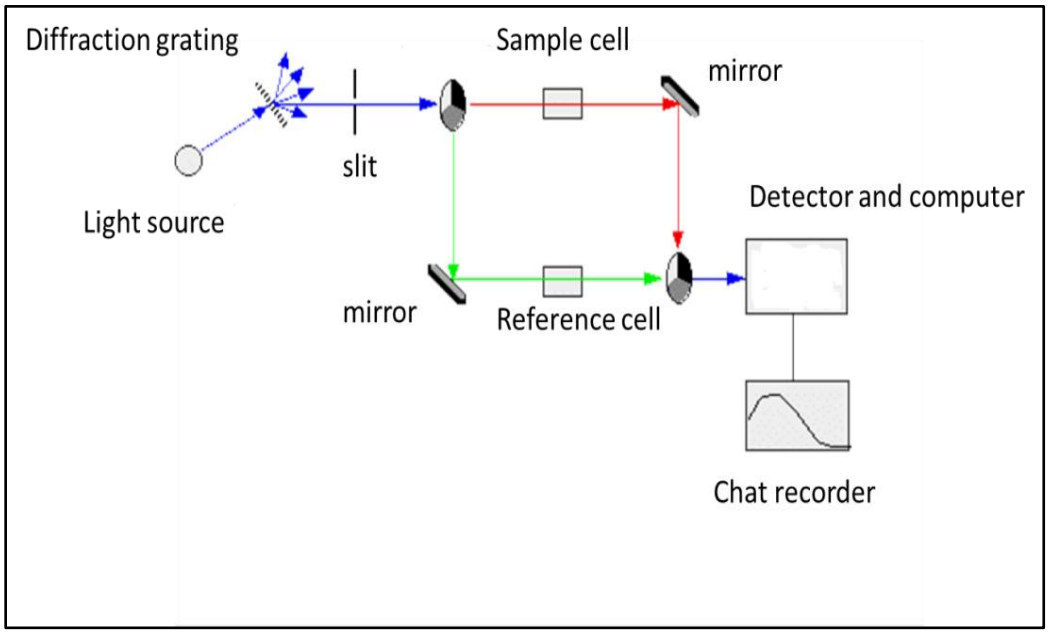


Figure 2.2. Working of spectrophotometer

Photoluminescence (PL)

Photoluminescence spectroscopy is defined as a contactless, non-destructive method of probing electronic structure of materials. Initially light is directed on top of the sample, where it get absorbed and imparts excess amount of energy into the material in a process known as photo-excitation. The excess energy can be dissipated by the sample through the emission of light called as luminescence. With respect to photo-excitation, this luminescence is photoluminescence. Photo-excitation causes electrons within a material to move into permissible excited states. The minute these electrons return to their equilibrium states, the excess energy is released and may contain the emission of light or may not. The energy of the emitted light relates to the variance in the energy levels between two electron states involved in the transition between excited state and equilibrium state. The quantity of emitted light is associated to the relative contribution of the radiative process. When chemical substrate undergoes internal energy transitions formerly relaxing to its ground state by emitting photons, some of the absorbed energy is dissipated so that the light photons which are emitted are of lower energy than those absorbed. One of such most familiar phenomenon is known as fluorescence, having short lifetime (10^{-8} - 10^{-4} s).

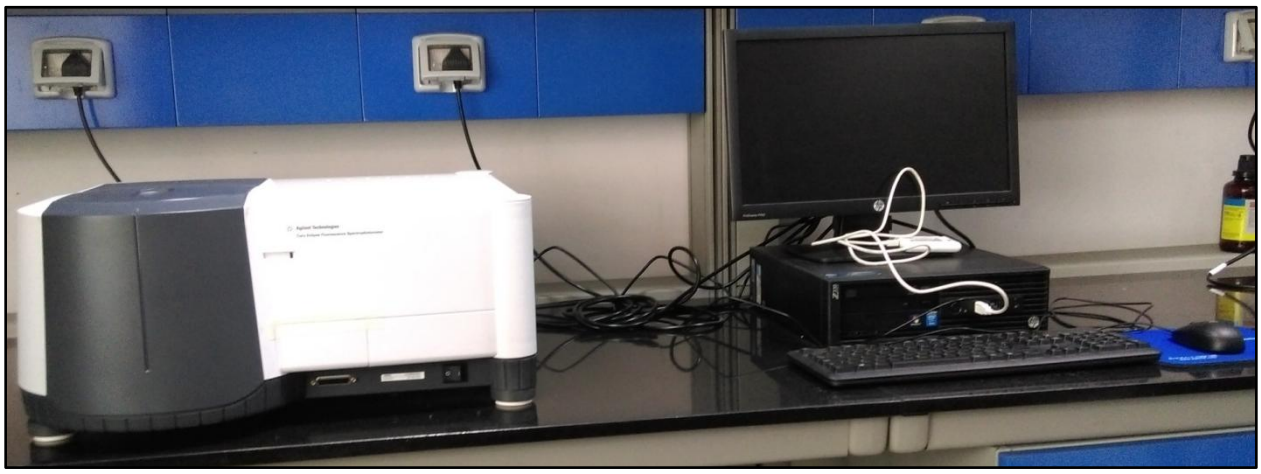


Figure 2.3 PL instrument[*AU-ILS]

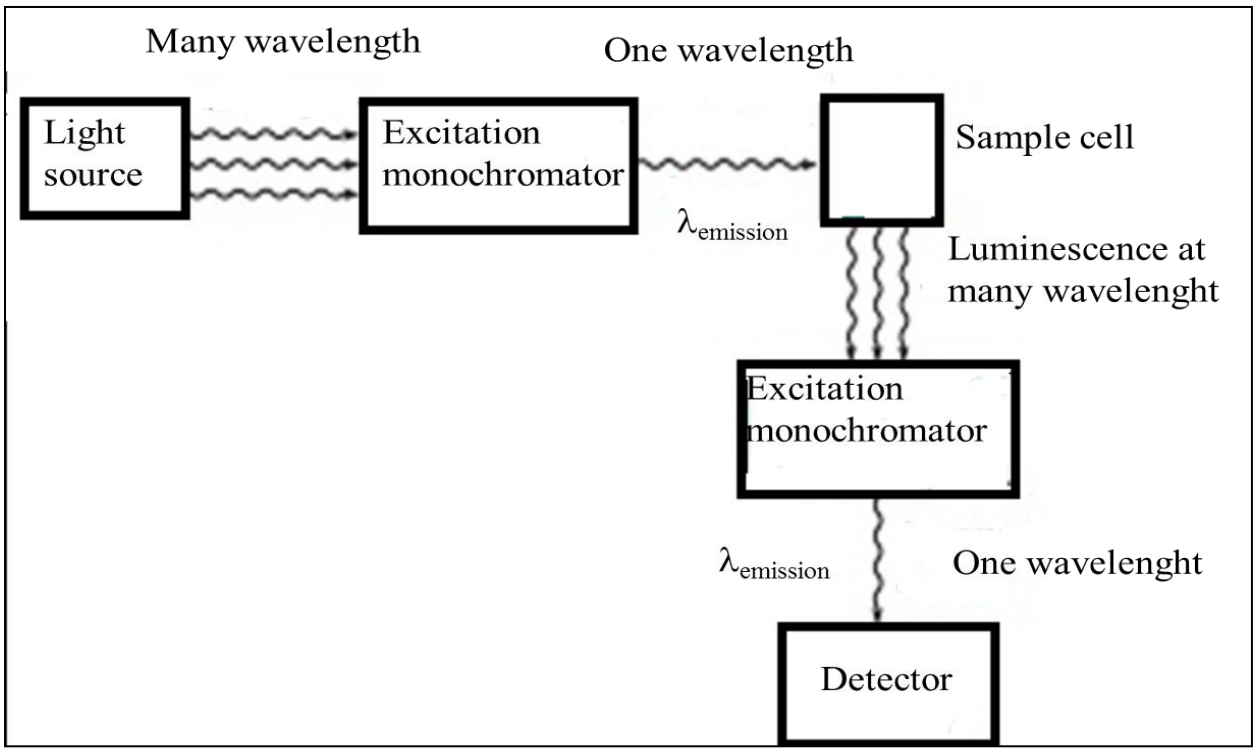


Figure 2.4. Working of PL

Material

Cerium oxide nanoparticles, E.coli (strain name dh5-α cell lines), di-sodium hydrogen orthophosphate, PBS buffer, cuvette and 96 well plate.

Figure 2.5 Sample of CeNPs (+4 and +3 state) [*AU-ILS]

Preparation of LB media
10g of tryptone was dissolved along with 5g yeast extract and 10 g NaCl in 950 ml of milliQ water. It was sterilized by autoclaving at 121^0C for 15 mins.

Preparation of Minimal media
It was produced from 5g glucose, 1g NH_4CL, 5.4g NaCl, 0.1 g $MgSO_4$ and 0.02 g $Cacl_2$ hexahydrated filled upto 1 liter with milliQ water. It was sterilized by autoclaving at 121^0C for 15 mins.

Methods

Characterization of CeNPs

UV visible spectra of CeNP (+3 and +4 oxidative state) were obtained using quartz cuvette. The peak of both was different. The phosphate peak was also analysis. The interaction of CeNP (100μM) with phosphate at different concentration level (5μM, 10μM, 100μM, 1000μM) and also with equal concentration (1:1) was taken .The peak shift was characterized.

Analysis of SOD activity

The SOD activity of CeNPs (both oxidative state +3 and +4) was done. The SOD activity of phosphate (alone) and with CeNPs at different concentration (1000μM, 500μM, 100μM) was done using UV Vis spectrophotometer.

Procedure of SOD

SOD

- Remove all enzymes (Cytochrome c, Xanthine oxidase, Catalase) needed from the SOD Kit in the fridge and place them in a beaker containing ice
- Collect Hypoxanthine, H_2O_2, CeNPs and Tris Buffer
- In a clean 96 well plate, make three sets of each control and test as given in the table below

	TEST (microliter)	**CONTROL (microliter)**
Cytochrome c	5	5
Xanthine oxidase	3	3
Tris Buffer (pH 7.5)	38.5	41.5
Catalase (mix and change tip)	0.5	0.5
CeNPs	3	-

Hypoxanthine (always last)	50	50
	100	**100**

- Place the lid on the plate and set the multiplate reader (Take3, Shake: 3 seconds, Enzyme kinetics: 20 minutes run; 30 seconds interval, Read: absorbance; 550 nm; rapid; select cells)
- Place the plate in the reader and run the samples
- Remove the plate, export the data in an excel file and switch off the instrument

Growth pattern

The growth pattern of E.coli was observed in different environment condition that is in minimal media, CeNPs, PO_4, and in CeNPs plus PO_4 complex at different concentration (1000μM, 500μM, 100μM).

Procedure

- E.coli was grown overnight in LB media at 37^0c for incubation.
- Next day the samples where set in 96 well plate. The final OD of inoculated sample was set at 0.1 ($\approx 10^8$ CFU/ml).
- Per well concentration of plate was set to 200μl.
- The absorbance was taken at various time intervals 1hr, 3hrs upto 6 hrs.

DCFDA

The cell-permeant name as 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA) (also known as dichlorofluores *cin* diacetate) is a chemically reduced form of the fluorescein used as an indicator for reactive oxygen species (ROS) in cells, for example, to detect the generation of the reactive oxygen intermediates in neutrophils and macrophages. Upon cleavage of acetate groups by the intracellular esterases and oxidation, the nonfluorescent H_2DCFDA is converted to the highly fluorescent 2', 7'-dichlorofluorescein (DCF).

Procedure

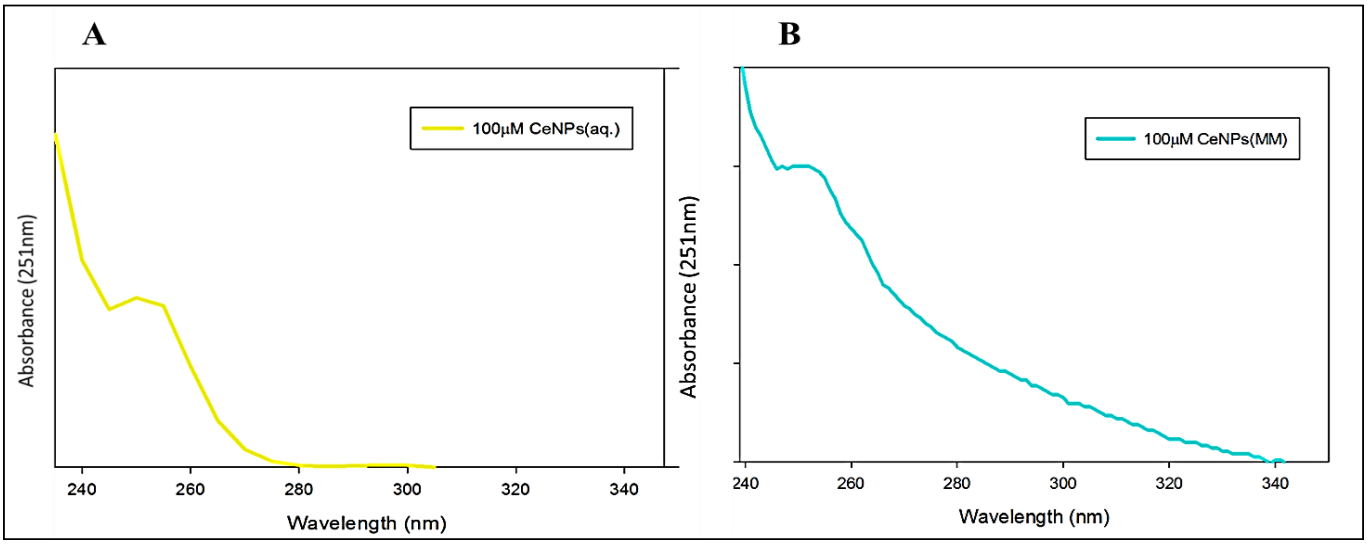
- After 6 hours incubation of bacterial sample, 100μl was taken and transfer to 96 well plate.
  Note: Each step proper vortexing has to be done.
- DCFDA (10μl) was added in 5 ml filtered PBS buffer.
- 100μl of diluted DCFDA was added to each well and the plate was kept at 37^0c in incubator.
- The fluorescence intensity was measured.

Chapter 3

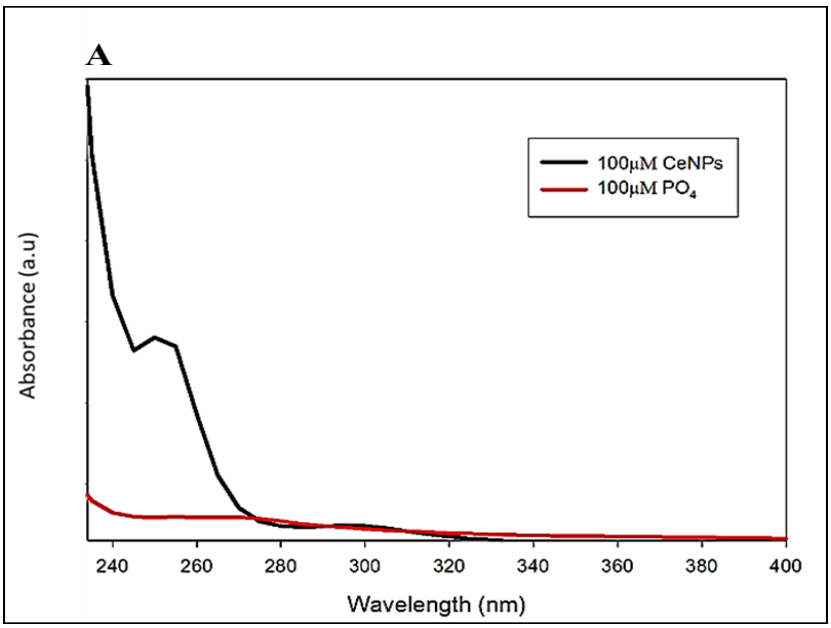
A redox state dependent antibacterial activity of CeNPs

(Results and Discussions)

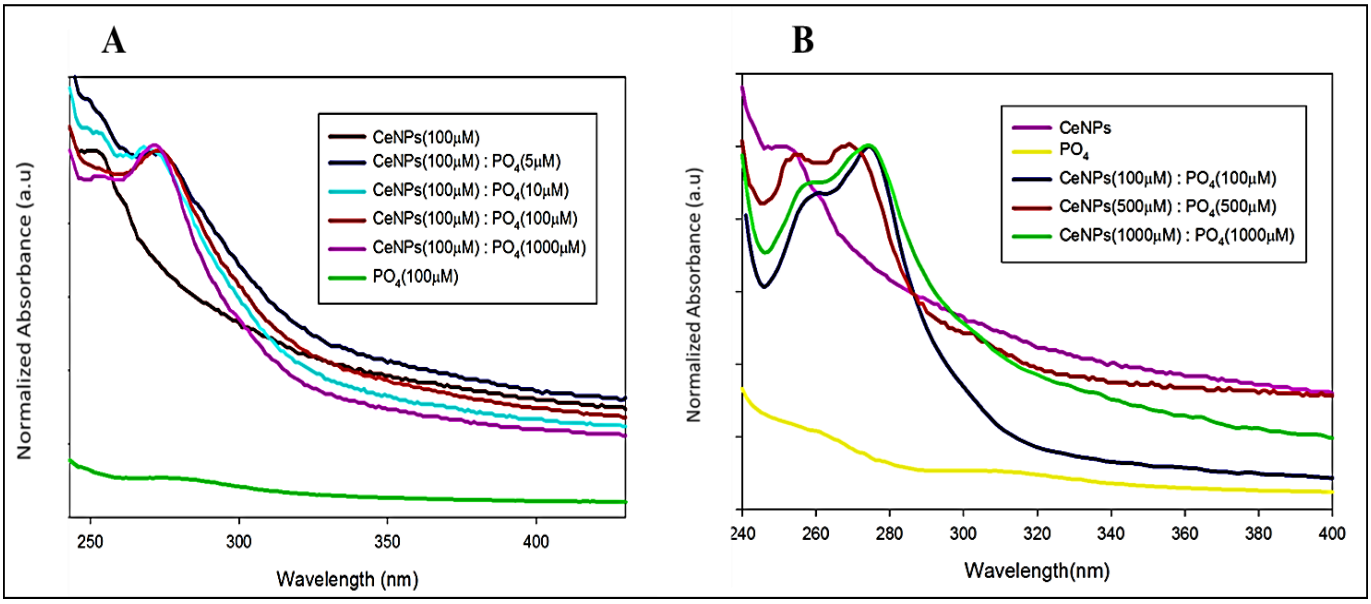
Results



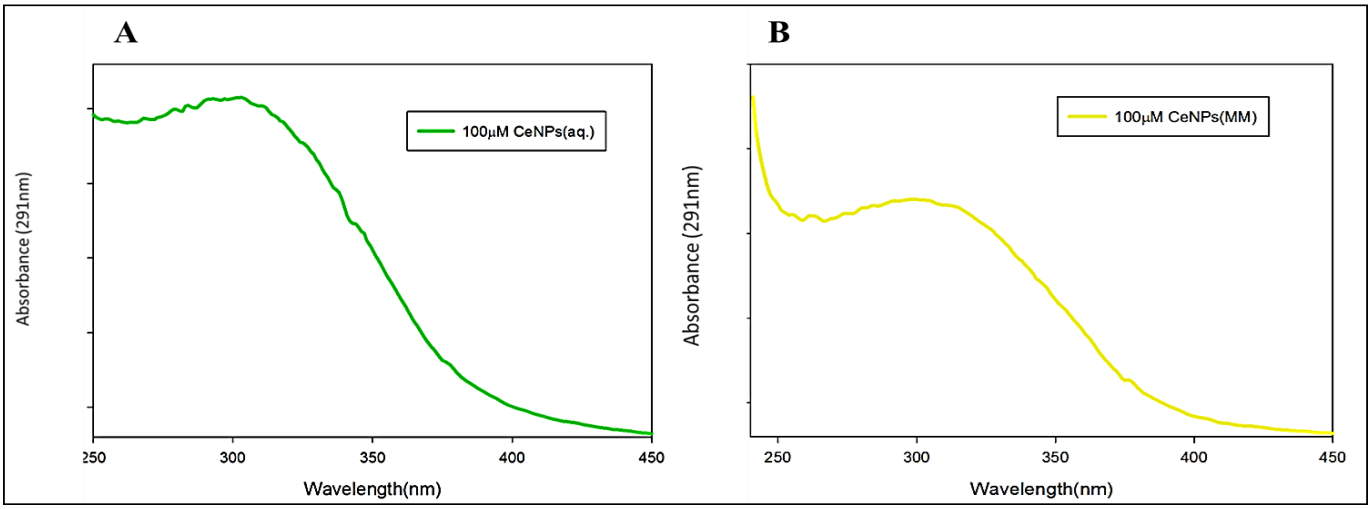
Graph 3.1 The peak of CeNPs (+3 oxidative states) in water and minimal media at 251 nm. 150 µl of CeNPs was taken from stock (1mM) and dissolved in 1335 µl of milliQ water.



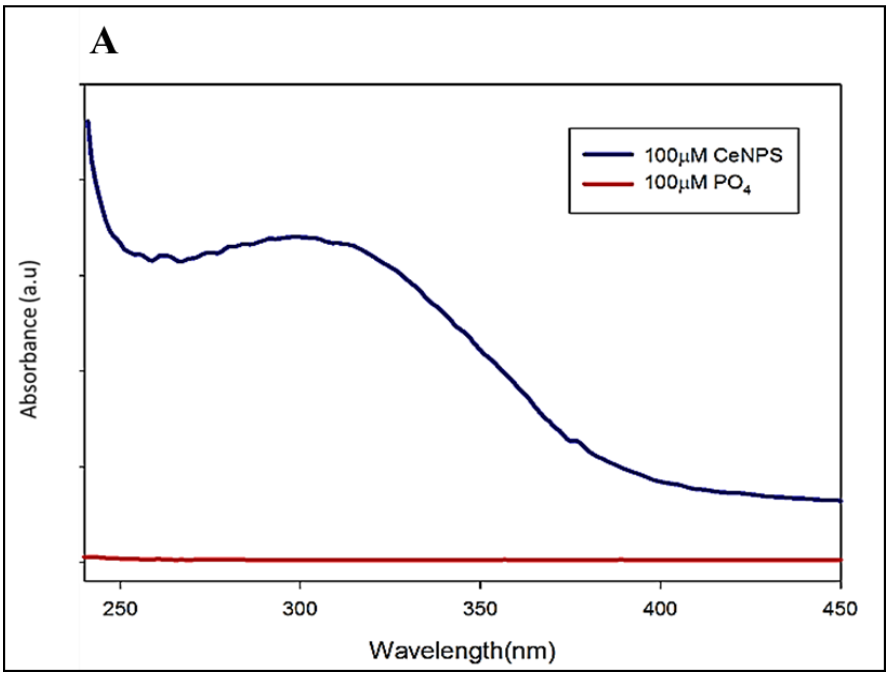
Graph 3.2 Peak of cerium oxide and phosphate. No peak of PO_4 was seen. 15µl of PO was taken from stock of 10mM (PO_4) and final volume was made upto 1.5 ml.



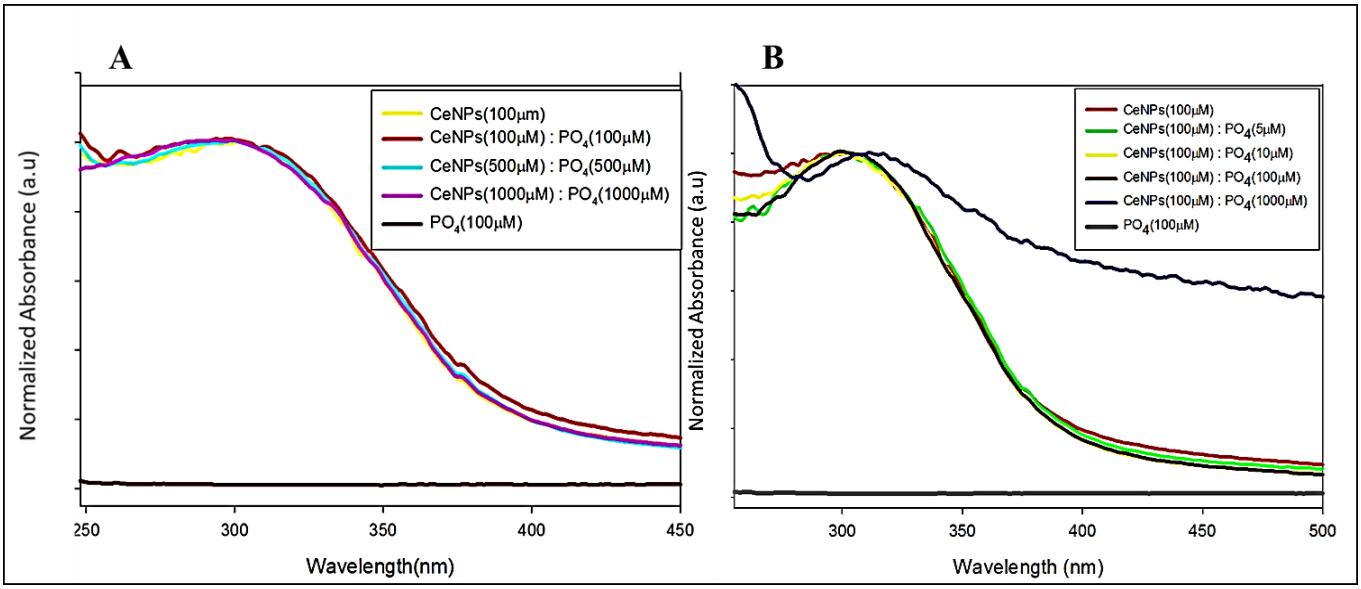
Graph 3.3 A. Interaction of constant cerium (+3) particle (100µM) with phosphate at different concentration.150µl of cerium nanoparticle is taken from 1mM stock and is mix with PO_4 (.75µl,1.5µl,15µl,30µl respectively) from 10mM stock. B. Interaction of cerium(+3) particle with phosphate at equimolar concentration. Their was a peak shift from 251 to 275 nm.



Graph 3.4 The peak of CeNPs (+4 oxidative states) in water and minimal media at 299 nm. 150 µl of CeNPs was taken from stock (1mM) and dissolved in 1335 µl of milliQ water.

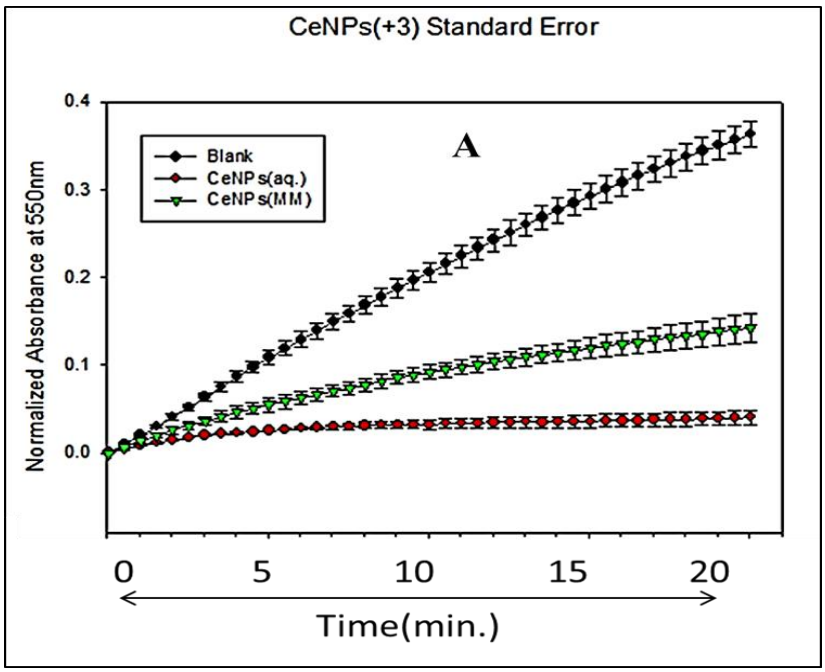


Graph 3.4 Peak of cerium oxide and phosphate. No peak of PO_4 was seen. 15μl of PO was taken from stock of 10mM (PO_4) and final volume was made upto 1.5 ml.

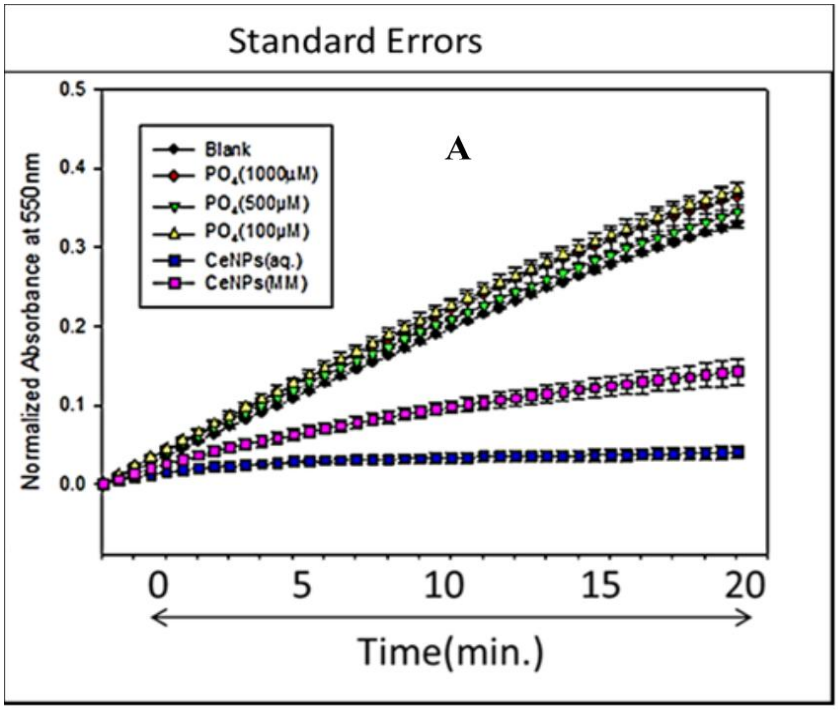


Graph 3.5 A. Interaction of constant cerium (+4) particle (100μM) with phosphate at different concentration.150μl of cerium nanoparticle is taken from 1mM stock and is mix with PO_4 (.75μl,1.5μl,15μl,30μl respectively) from 10mM stock. B. Interaction of cerium(+4) particle with phosphate at equimolar concentration.Their was no peak shift at 299 only.

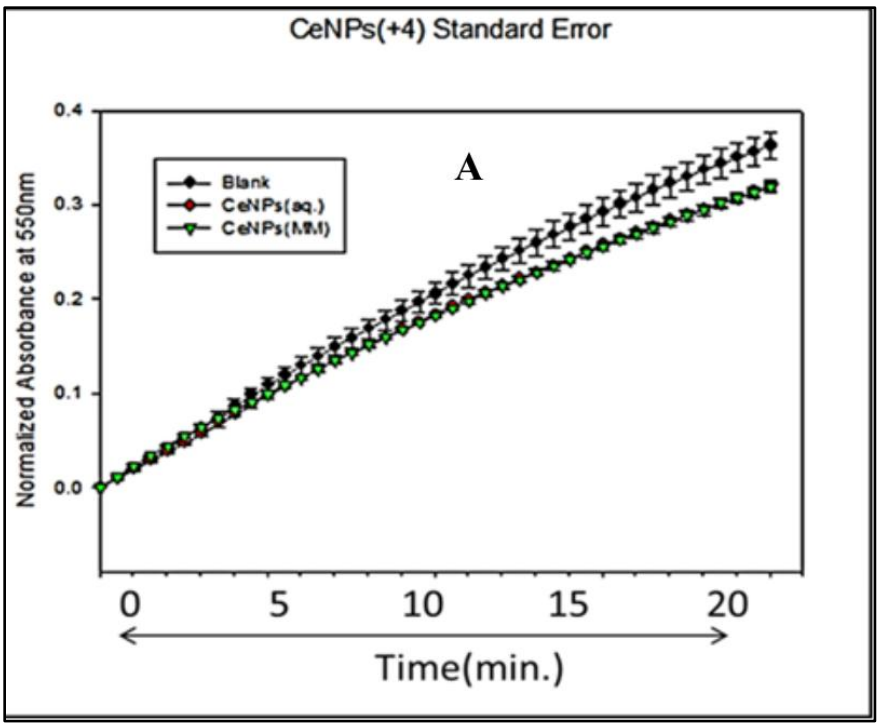
SOD



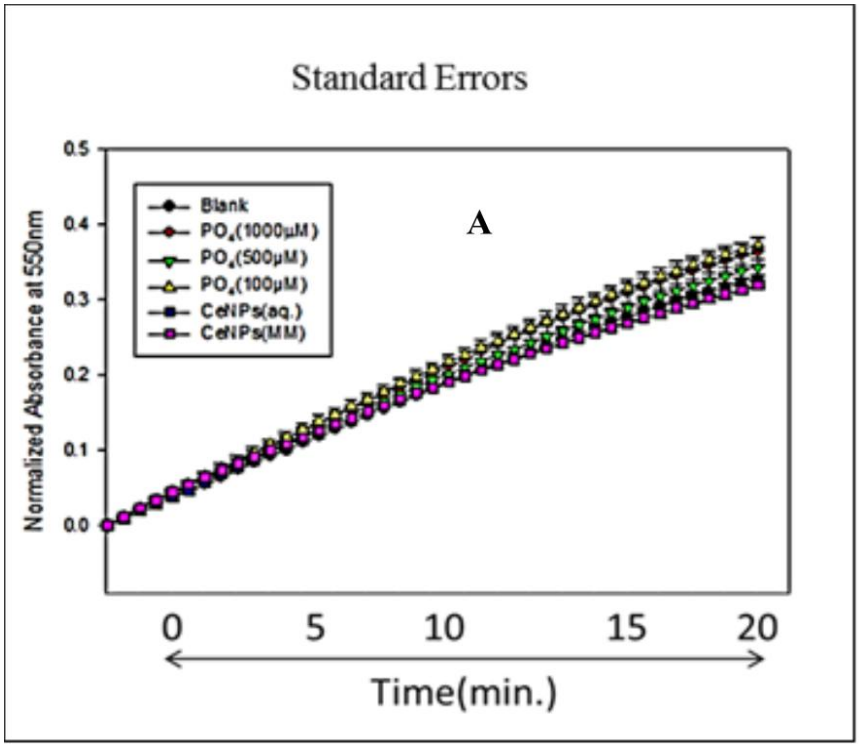
Graph 3.5 SOD activity of cerium oxide nanoparticle (+3 oxidative states) in aqueous and MM



Graph 3.6 SOD activities of PO_4 at different concentration (100µM, 500µM, and 1000µM), CeNPs (+3) as control.

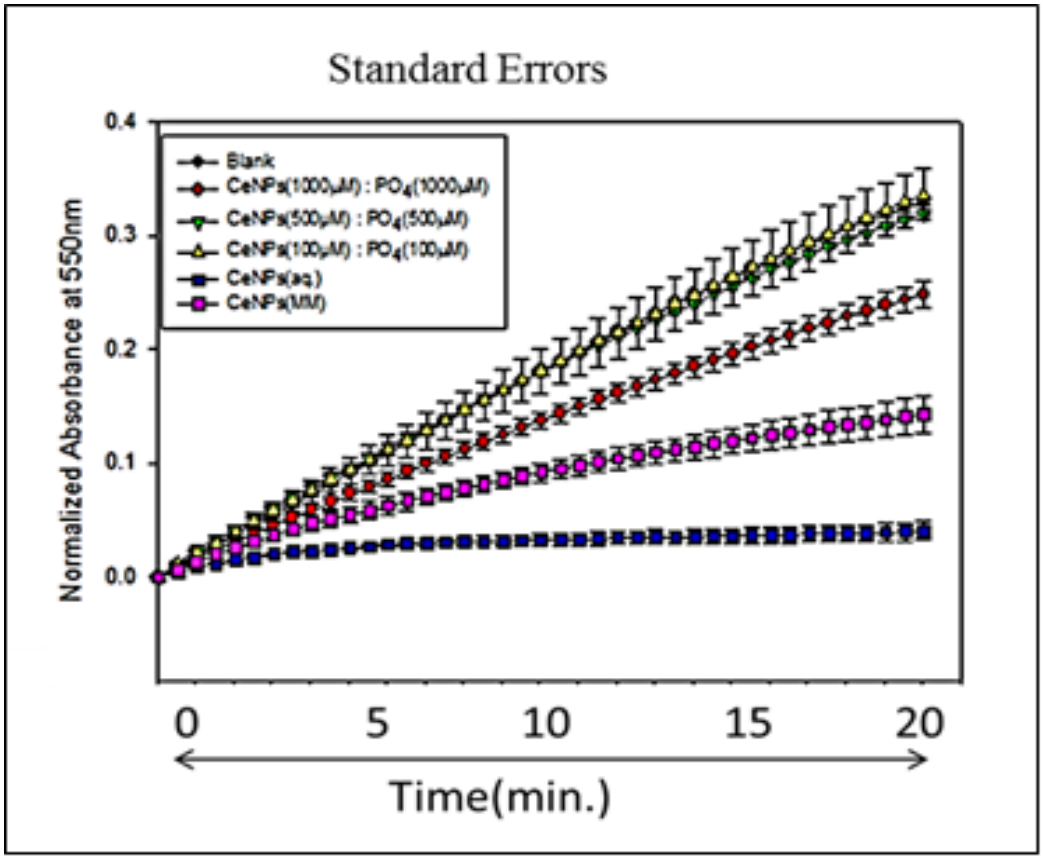


Graph 3.7 SOD activity of cerium oxide nanoparticle (+4 oxidative states) in aqueous and MM



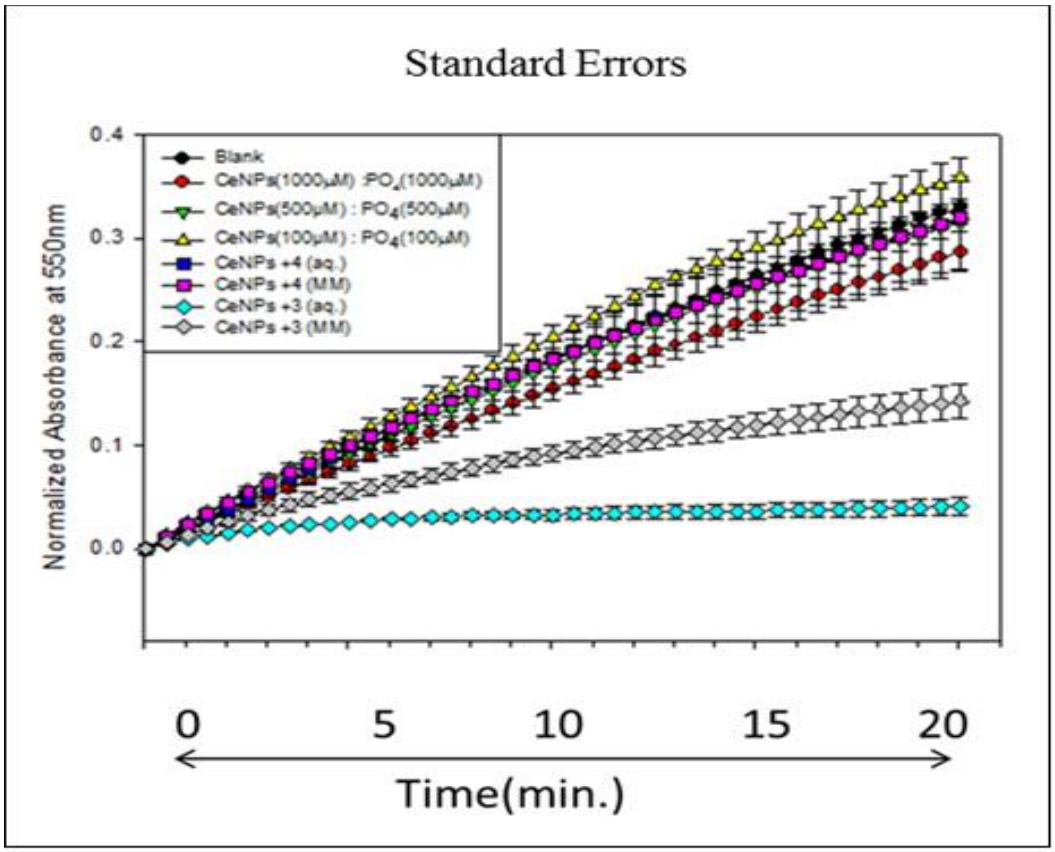
Graph 3.8 SOD activities of PO_4 at different concentration (100μM, 500μM, and 1000μM), CeNPs (+4) as control.

A



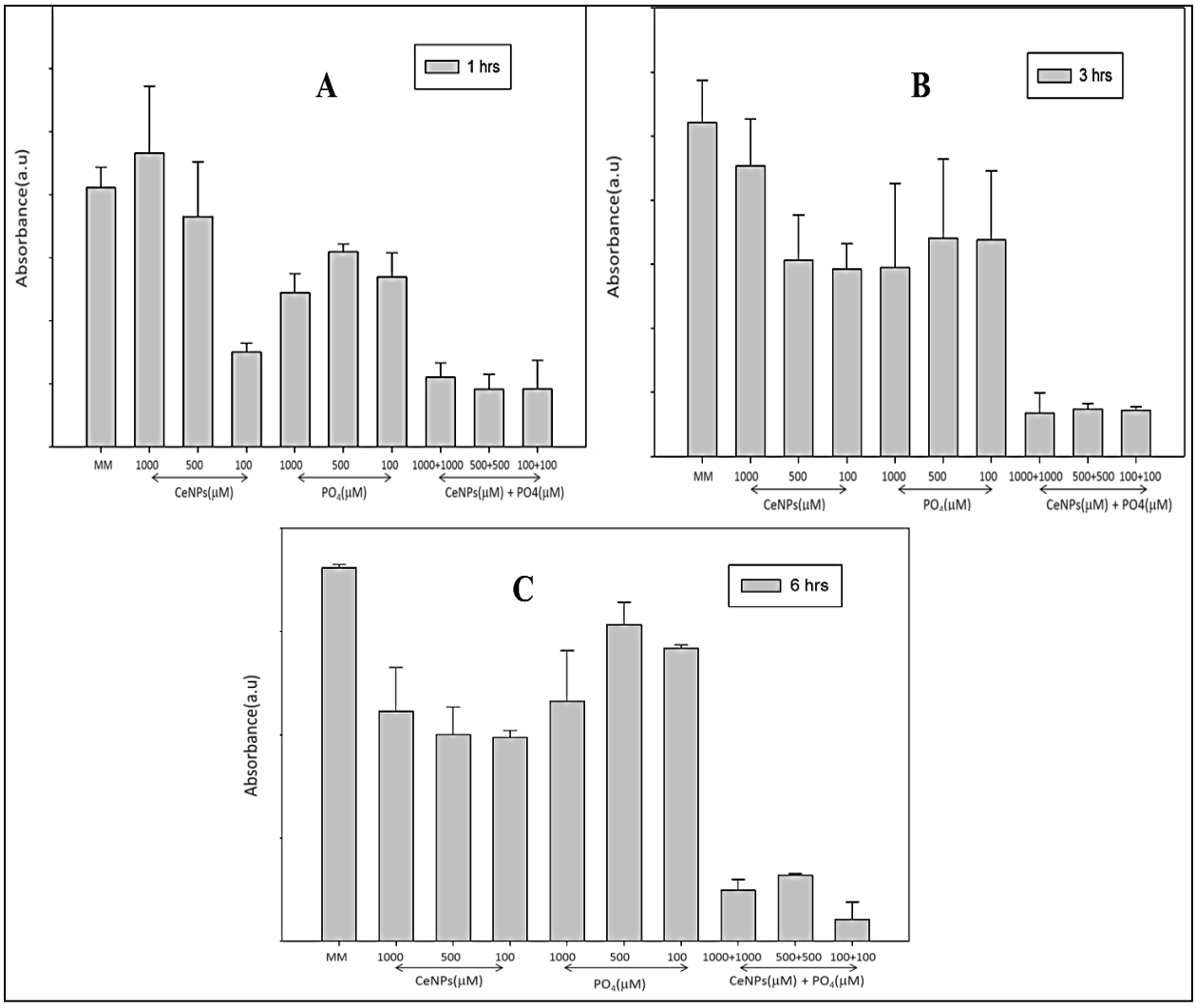
Graph 3.9SOD activity of cerium nanoparticle (+3 oxidative state) with phosphate at equimolar concentration (at 1000μM, 500μM, 100μM), CeNPs (+3) peak was kept as control.

B

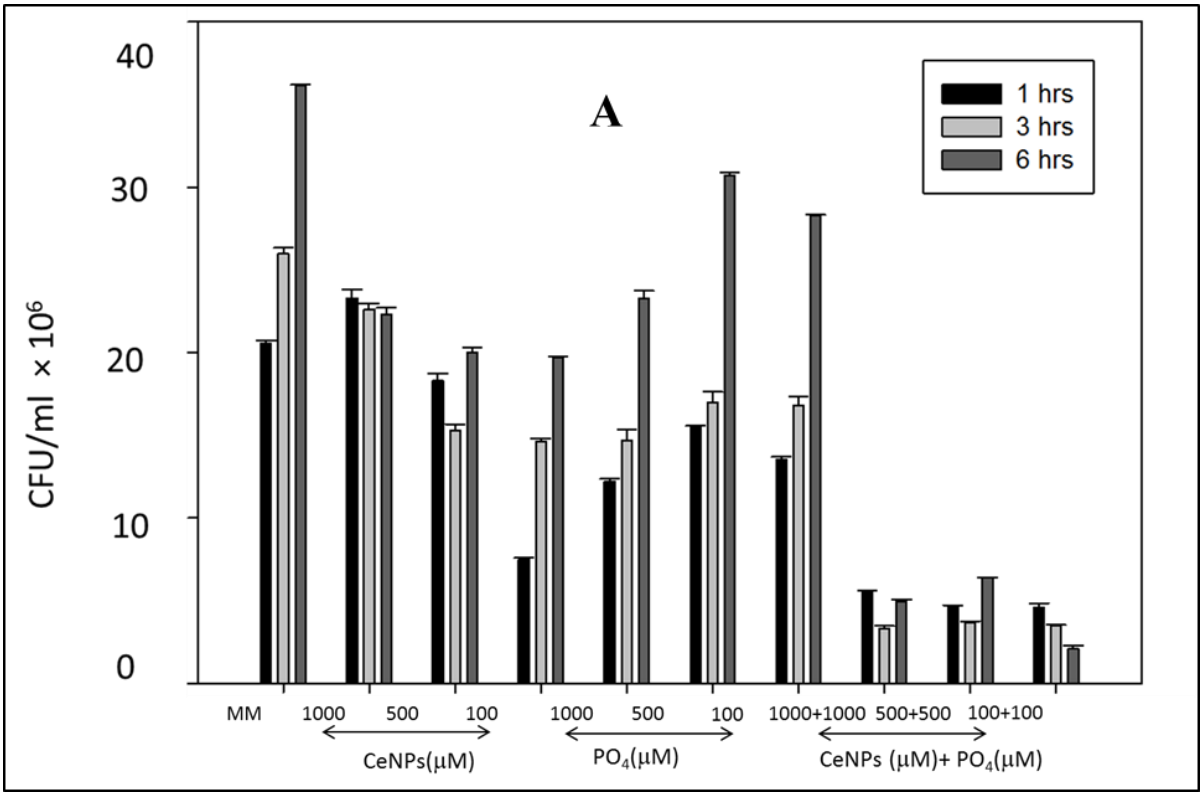


Graph 3.10 SOD activity of cerium nanoparticle (+4 oxidative state) with phosphate at equimolar concentration (at 1000μM, 500μM, 100μM), CeNPs (+3) and CeNPs (+4) peak were kept as control.

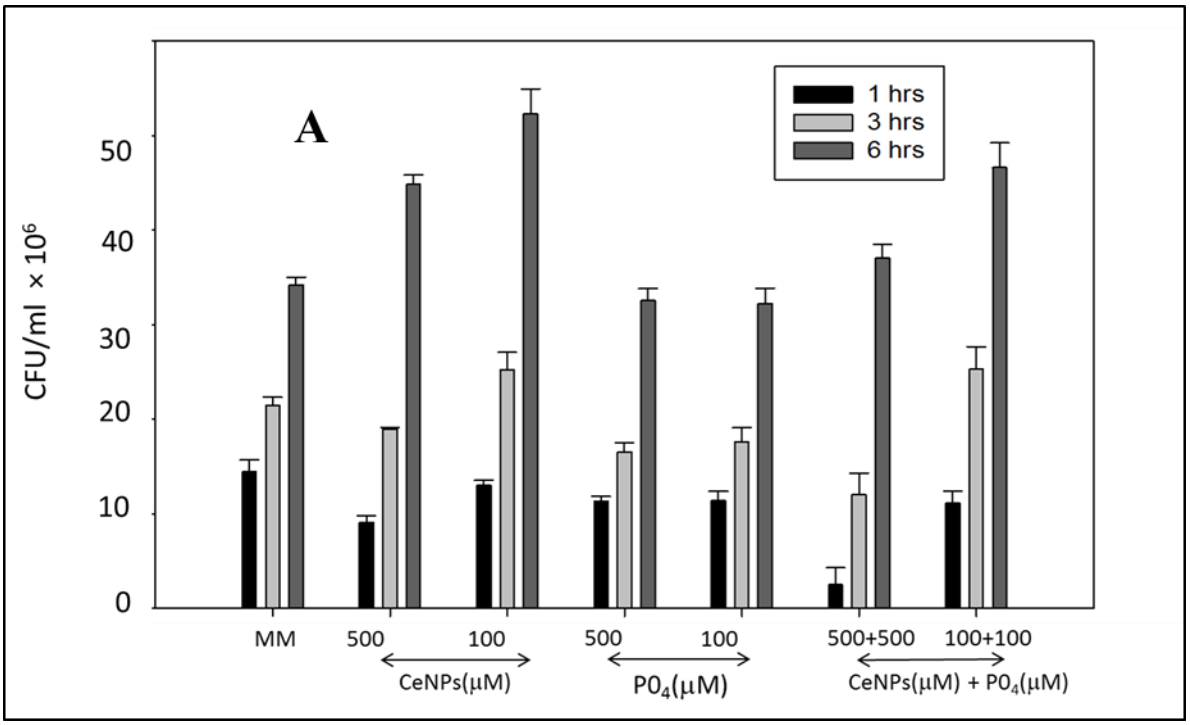
Growth pattern



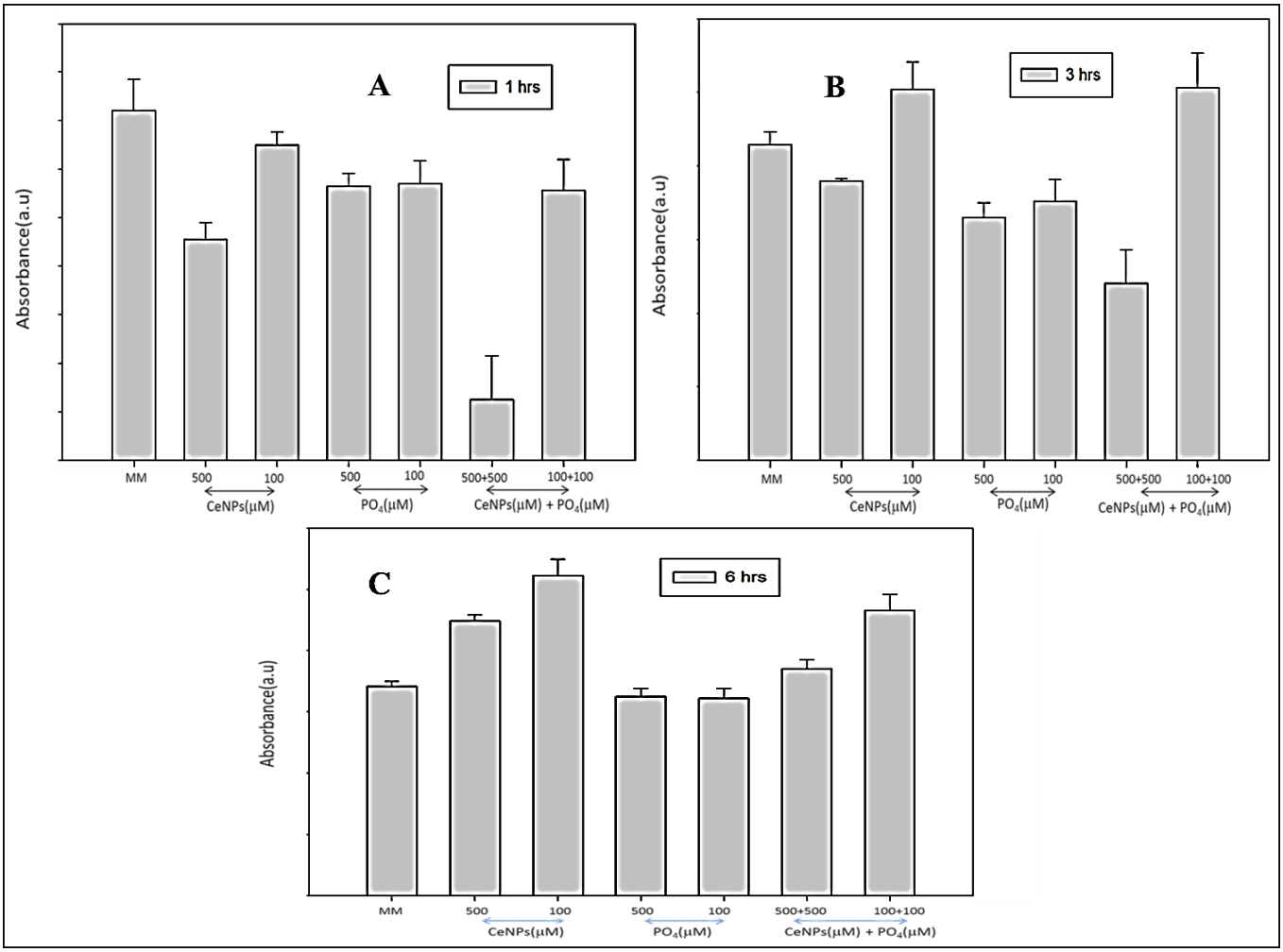
Graph 3.11Study of Growth rate of E.coli at different time interval. E.coli was incubated for 1 hr, 3 hrs and 6 hrs in different growth media. MM media was used as a control. CeNPs (+3) was used in 3 different concentration (1000μM,500μM,100μM) and PO_4 was also used at 3 different concentration (1000μM,500μM,100μM) . The interaction of CeNPs and PO_4 was also used as growth media at equimolar concentration.



Graph 3.12 Colony formation unit was check of E.coli at different time interval (CeNPs used here is + 3 oxidation state)

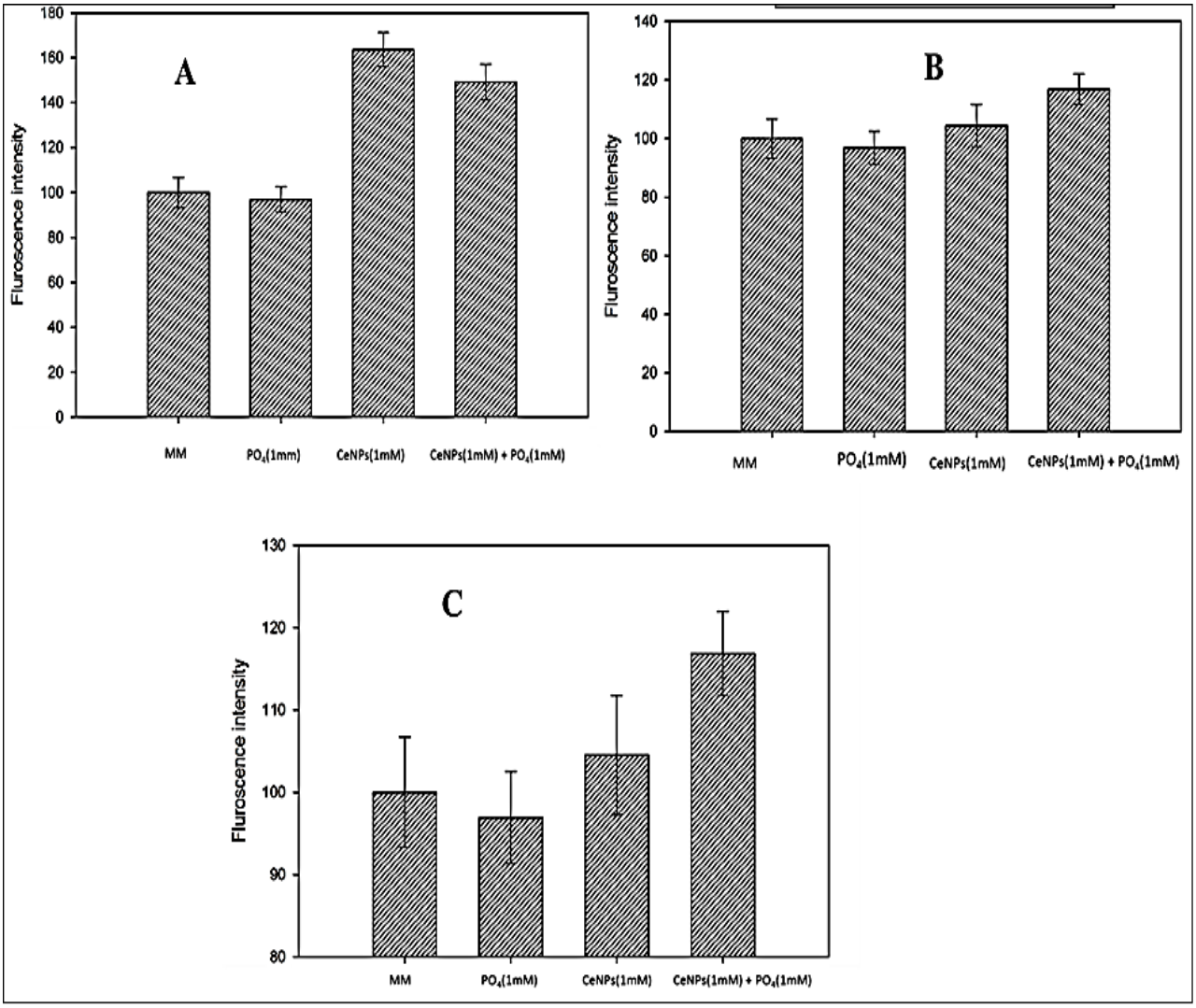


Graph 3.14. Colony formation unit was check of E.coli at different time interval (CeNPs used here is + 4 oxidation state)

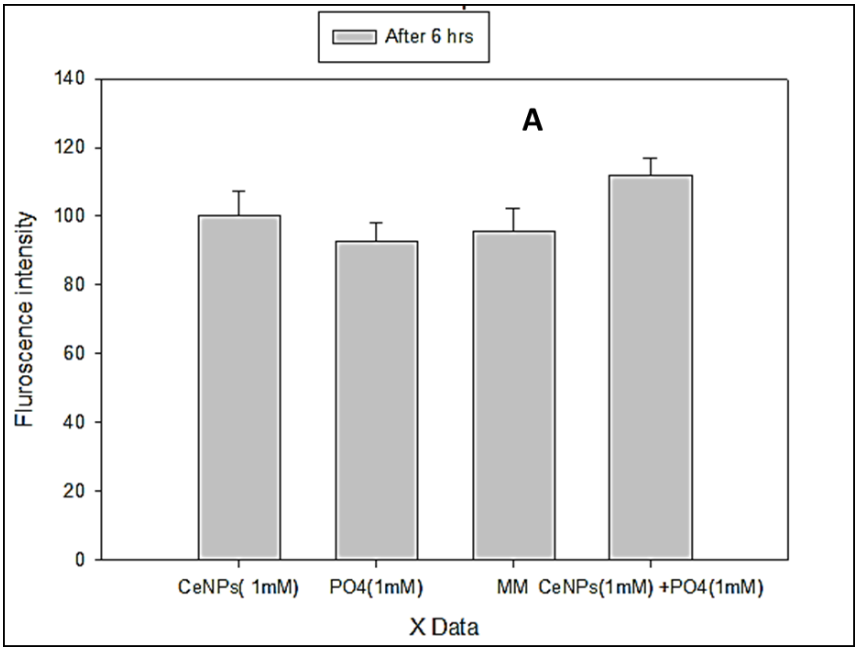


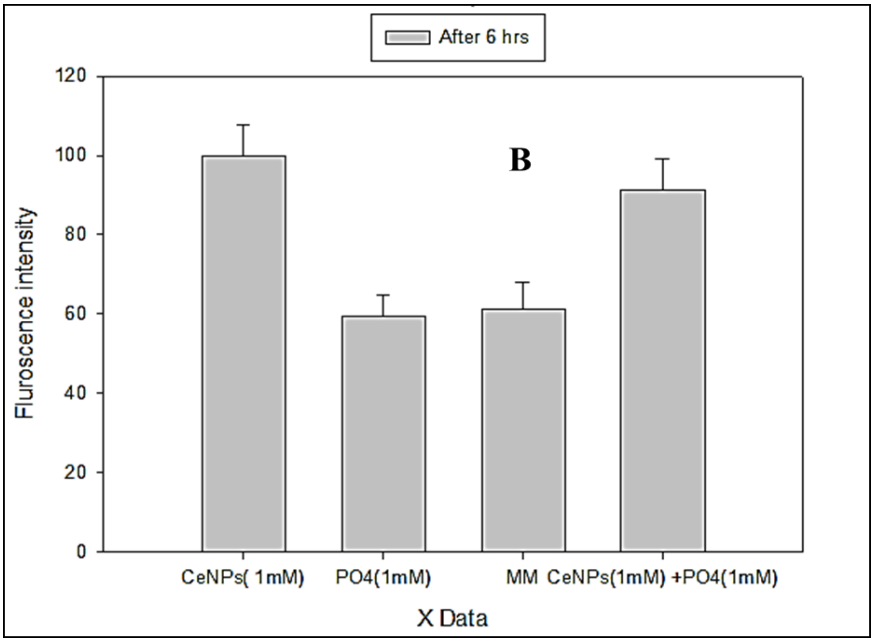
Graph 3.15 Study of Growth rate of E.coli at different time interval. E.coli was incubated for 1 hr, 3 hrs and 6 hrs in different growth media. MM media was used as a control. CeNPs (+4) was used in 3 different concentration (1000μM,500μM,100μM) and PO_4 was also used at 3 different concentration (1000μM,500μM,100μM) . The interaction of CeNPs and PO_4 was also used as growth media at equimolar concentration.

DCFDA



Graph 3.16 The cell where treated with fluorescence dye. The fluorescence intensity was me seared. Here MM was kept as control in all 3 cases. **A.** CeNPs (+4) **B.** CeNPs (+3) **C**.CeNPs (+3) [All sample where taken after 6 hrs of incubation of cells in media at 37^0c]





Graph 3.17 A CeNPs (+3) was kept as control **B.** CeNPs (+4) was kept as control

Discussion

Cerium oxide nanoparticle can be promising antimicrobial strategy by limiting the phosphate availability in system. We demonstrated the affinity of cerium nanoparticle with phosphate at different concentration. The toxic effect of cerium oxide nanoparticle depends on presence of phosphate concentration. Graph 3.1 and 3.2 describe the normal Cerium (at 251nm) and phosphate (straight line) peak respectively. Graph 3.3 describe there is a peak shift in CeNPs (+3) while interaction with phosphate which suggest that there is chemical interaction of CeNP and PO_4 forming a milky appearance complex and thus block redox cycle. Whereas in Graph 3.4, 3.5 and 3.6 we have observed +4 peaks at 299nm and with phosphate the peak remains same at 299nm there was no significance change. In Graph 3.5, 3.6, 3.7, 3.8, 3.9 and 3.10 we have study the chemical stability of cerium oxide nanoparticle. SOD mimetic activity of CeNPs was found out. The SOD kinetic activity was study upto 20 min we found out CeNPs (+3) in aqueous environment show best SOD activity whereas in MM SOD was less. The PO_4 does not show any SOD activity but as it get interact the SOD activity get reduce of Cerium oxide (+3) state .Whereas in +4 states there was no SOD activity neither with PO_4. However the presence of phosphate alters the chemistry and SOD activity of CeNPs even at micro molar concentrations. Taken in account, the results in this observation give idea about mechanism behind the SOD mimetic activity of nanoceria and make a strong case for surface associated CeNPs(+3) sites as the active site for SOD mimetic activity. The exposure of phosphate anions did not induce any change in the surface chemistry of CeNPs (4+). Cerium phosphate formation blocks the redox cycling between Ce3þ/Ceþ4, which is essential for antioxidant activity of CeNPs. Graph 3.11,3.12,3.14,3.15 show the growth rate of E.coli in different nutrient containing media ,it was found that in MM the growth was moderate but as we add PO_4 additionally to MM the growth rate increase. The growth was found maximum in highest concentration of PO_4 that is in 1000μM .As soon as the CeNPs (+3) was added to PO_4 the growth start retarding due to deficiency of phosphate. The same experiment was performing with CeNPs (+4) but we found opposite result as there is no interaction of +4 with PO_4 the growth was normal as in MM and PO_4 environment. In Graph 3.16 and 3.17 the stress level condition of cell was check .If the cell get proper nutrient for its growth then there is no stress and no fluorescence was seen .The fluorescence dye use is DCFDA dye. The maximum fluorescence is seen in CeNPs (+3) mix with PO_4 because there is no nutrient source whereas in all other media the fluorescence was less due to presence of PO_4. Since our results tells that these interactions with phosphate can affect the cell growth taken this concept into account we can design experiments for cell studies and CeNPs will be great useful for biomedical applications.

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