A

PROJECT REPORT

on

"Antioxidant activity of biosynthesized of CeO_2nanoparticles mediated by *Aegle marmelos* leaves"

By

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Certificate

This is to certify that the project entitled

"Antioxidant activity of biosynthesized of CeO_2 nanoparticles mediated by *Aegle marmelos*leaves"

has been successfully completed by

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Seat no:-_____

During the academic year 2021-22 for fulfilment of

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ABSTRACT

In the recent year, we observed that nanotechnology developed all over the world. Nanoparticle demonstrate prospective for various uses such as biomedical and drugs delivery, Agriculture and food, defense, energy storage, antibacterial etc. there are many methods of synthesis of nanoparticles but biotechnology based synthesis approach has gained considered safe, eco-friendly and cheap. Green synthesis has been confirmed the formation of CeO_2 NPs using biological method aqueous leaf extract of Aeglemarmelos (bael leaf) which act as reducing and stabilizing agent.Synthesized nanoparticles are characterized by using X-ray diffraction (XRD), UV-visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), and Scanning electron microscopy (FESEM) , Raman Spectroscopy and Photoluminicence. Characterization reveals that the synthesized CeO_2 NPs having Cubic structure with 5.3 nm average nanoparticle size. UV-visible spectroscopy shows characterized peak at 349.95nm and bang gap is 3.4eV. Fourier transform infrared spectroscopy (FTIR) which shows presence of functional group such as hydrooxyle , Nitrile , Aromatic, Alkanes and Alkenes .Ramanspectroscopy shows active mode at 628 cm -1 .Photoluminence shows different emisions. Field emission sccaning electron microscopy (FESEM) shows the surface morphology with slight agglomeration.

Keywords:-Green synthesis , nanotechnology, functional groups , Morphology, AegelMarmelos.

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CHAPTER 1

INTRODUCTION

1.1 NENOTECHNOLOGY

Nanotechnology has been defined as being "concerned with materials and systems whose structures and components exhibit novel and significantly improved physical, chemical and biological Properties, phenomena and processes due to their Nano scale Size". Nanotechnology is a multidisciplinary field, as it combines the knowledge from different disciplines: chemistry, physics, and biology amongst others Nanotechnology is the art and science of manipulating matter at the atomic or molecular scale and holds the promise of providing significant improvements in technologies. The word "nanotechnology" was introduced for the first time by Norio. Taniguchi at the International Conference on Industrial Production in The word "nanotechnology" was introduced for the first time by Norio Taniguchi at the International Conference on Industrial Production in Tokyo in 1974 in order to describe the super thin processing of materials with nanometer accuracy and the creation of nano-sized mechanisms.Ideas of nanotechnological strategy, which were put forward by Richard Feynman (known as "Father of Nanotechnology") in his lecture delivered in 1959 at the session of the American Physical Society, were developed by Eric Drexler in 1986. Nanoscale materials are defined as a set of substances where at least one dimension is less than approximately 100 nanometers. A nanometer is one millionth of a millimeter - approximately 100,000 times smaller than the diameter of a human hair.Nanomaterials are of interest because at this scale unique optical, magnetic, electrical, and other properties emerge. These emergent properties have the potential for great impacts in electronics, medicine, and other fields. Nanoscience deals with the scientific study of objects with sizes in the 1 – 100 nm range in at least one dimension.

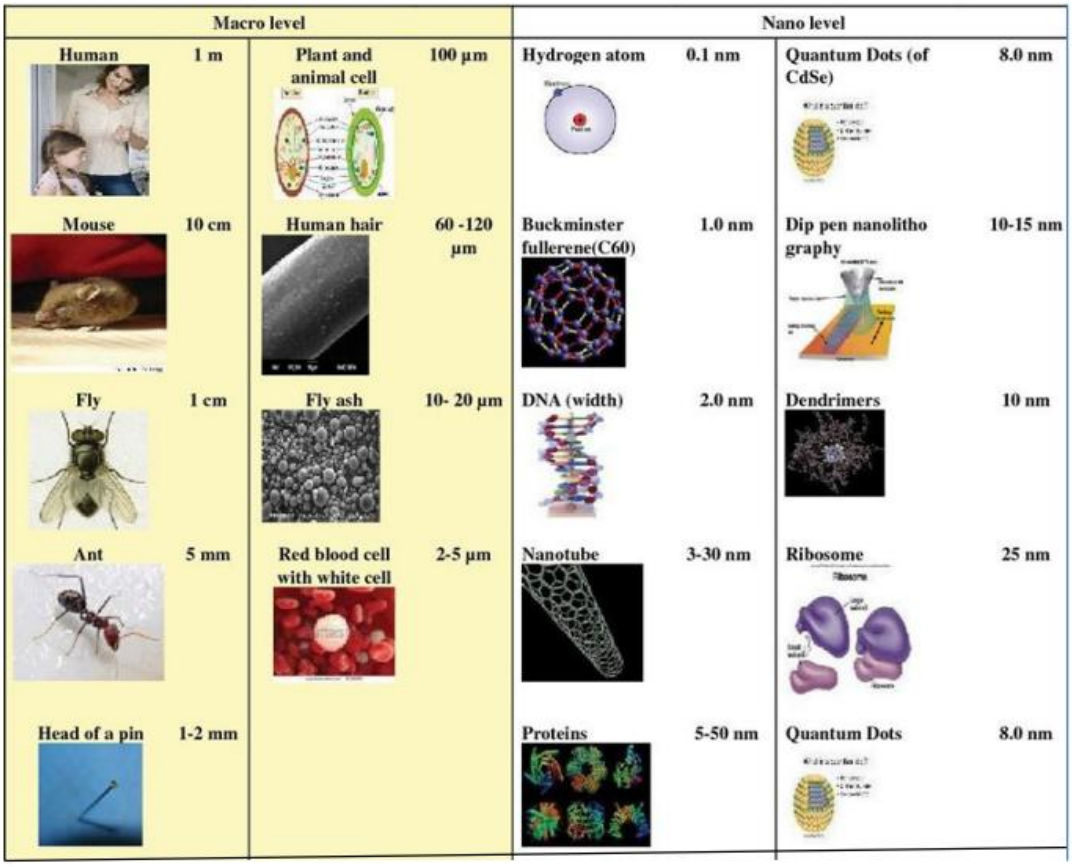


Fig 1.1 Nanoscale for micro level and nano level.

1.1.1 Challenges in Nanotechnology

1. To build such novel tools that will easily measure Nano dimension.

2. The technique may require new innovations in metrological technology.

3. Measurements of physical properties of nanomaterials require extremely sensitive instrumentation.

4. To maintain the noise level as low as possible

5. Random doping fluctuations are very important at nanometer scale.

6. To overcame the huge surface energy, due to the increase the surface to volume ratio.

7. To make all nanomaterials with desired size, uniform size distribution controlled, morphology crystallinity, chemical composition and micro structure etc.

8. To avoid the contamination.

1.1.2 Application of Nanotechnology:-

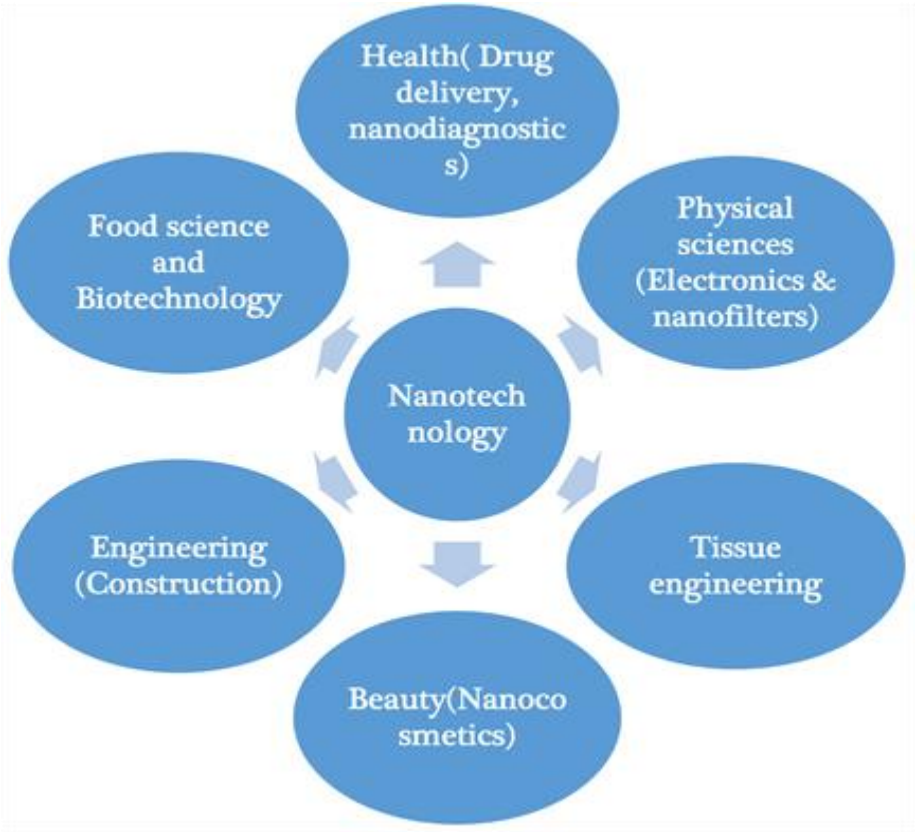


Fig:1.2 Application of Nanotechnology

1.2 NANOSCIENCE

"**Nano science is the study of phenomena on a nanometer scale. Nano science is the studyof phenomena and manipulation of materials at atomic, molecular and macromolecular scales, where properties differ significantly from those at a larger scale."**

Nano science is the study of structures and materials on an ultra-small scale, and the unique and interesting properties these materials demonstrate. Nano science is cross disciplinary,

meaning scientists from a range of fields including chemistry, physics, biology, medicine, computing, materials science and engineering are studying it and using it to better understand our world.

1.3 NANOMATERAILS

"In practice nanostructures are the smallest solid things, which are practically possible tomake or ever has made. Any engineered object with at least one of its dimensions less than 100 nm is called as a 'Nanomaterial''

By patterning matter on the Nano scale, it is possible to vary fundamental properties of materials without changing the chemical composition. There are two primary types of Nano scale building blocks that may be used for further device fabrication and applications.

They range from zero Dimensional atom clusters to 3D equated grain structure.

(i) 0D (e.g., nanoparticles, Nano clusters, Nano crystals)

(ii) (ii) 1D (e.g., nanotubes, Nano fibers, nanowires)

The direct incorporation of these Nano architectures in existing materials to improve their properties is often referred to as incremental nanotechnology. However, the self-assembly of these Nano sized building blocks into 2D (e.g.-films, plates, network) and3D (e.g.-nanomaterial‟ s) architectures may yield entirely new devices and functionalities–referred to as evolutionary .

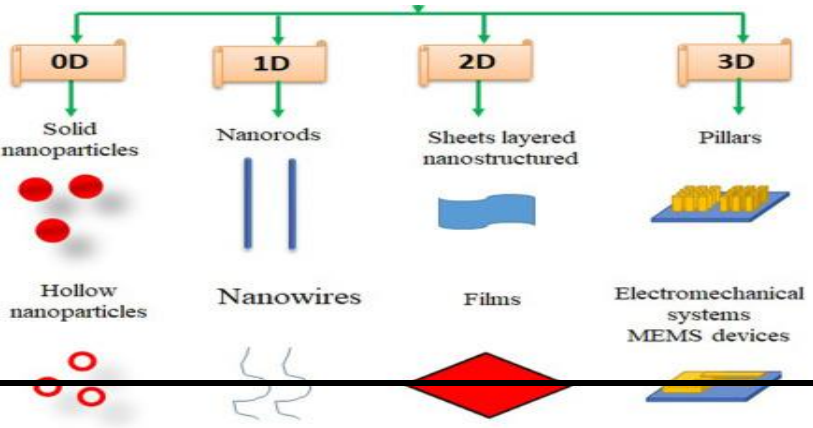


Fig 1.3:-Various kinds of nanomaterials. (A) 0D spheres and clusters. (B) 1D nanofibers, wires, and rods. (C) 2D films, plates, and networks. (D) 3D nonmaterial

1.4Properties of Material

As we know, the typical dimension range of nanomaterial's is lie in between 1-100 nm. Due to this, bulk properties and nanomaterial properties are different. Thedifferent properties at nan scale arise due to some origins. These are as given as followingLarge surface to volume ratio, Quantum confinement, large surface energy, reduced imperfection or defects. The following are the changes in the properties of the materials at nanometer size.

- **Mechanical Properties**

The magnetic properties of nanomaterial‟ s differ from those of bulk due to, the increase in surface to volume ratio and the increase in surface energy. Due to this reasons the ferromagnetism of bulk material disappears and transfers to super magnetism in the nano scale.

. **•Optical properties**

In nanomaterial's, the colour variation arises from changes in the compositions, sizeand other properties. These effects are due to the phenomena called surface Plasmon resonance. In this effects the frequency at which, the conduction electrons oscillate in response to the changing electric field of incident electromagnetic waves on scattering. The gold, silver, and copper nanoparticles possess Plasmon

resonances. The band gap of semiconductor increases, therefore there is shifting of absorption peak of nanoparticles shifts towards the shorter wavelength side.

- **Thermal Properties**

In nanomaterial there is huge fraction of surface atoms and also large surface energy. Also the lattice constants are reduced due to decrease in dimensions. So the nanomaterial‟s have lower melting point or phase transition temperature. The selfpurification is an intrinsic thermodynamic property of nanomaterial. Due to a reduction in size, there is an increase in perfection.

- **Electrical Properties**

As the dimension of nanomaterials is reduced,the surface scattering is increased inside thematerial. Due to this the electrical conductivity decreases. Also, electrical conductivity is enhanced appreciable due to the better ordering in microstructure.

- **Magnetic Properties**

The magnetic properties of nanomaterials differ from those of bulk due to, the increase in surface to volume ratio and the increase in surface energy. Due to this reasons the ferromagnetism of bulk material disappears and transfers to super magnetism in the Nanoscale.

- **1.4.1 Cerium oxide and use:**

Cerium oxide nanoparticles (CeO_2NPs, nanoceria) are widely used in chemical mechanical polishing/planarization corrosion protection, solar cells, fuel oxidation catalysis and automotive exhaust treatment**.** It is typically a white to off-white crystalline solid. It is composed of Cerium and oxygen where cerium has the oxidation state of +3 or +4 .CeO_2 reacts with acid as well as base. So $CeO_2$is an amphoteric.

Formula:CeO_2

Atomic number:58

Density: 7.22 g/cm³

Melting point: 2400 °C

Molecular weight:172.115 /mole

Boiling point :3500°C

Cerium oxide nanoparticles (CeO_2NPs) have received much attention because of their excellent catalytic activities, which are derived from quick and expedient mutation of the oxidation state between Ce^{4+} and $Ce^{3+.}$ The cerium atom has the ability to easily and drastically adjust its electronic configuration to best fit its immediate environment. It also exhibits oxygen vacancies, or defects, in the lattice structure; these arise through loss of oxygen and/or its electrons, alternating between CeO_2 andCeO_2during redox reactions. Being a mature engineered nanoparticle with various industrial applications, CeO_2 was recently found to have multi-enzyme, including superoxide oxidase, catalase and oxidase, mimetic properties that produce various biological effects, such as being potentially antioxidant towards almost all noxious intracellular reactive oxygen species. CeO_2 has emerged as a fascinating and lucrative material in biological fields such as bioanalysis, biomedicine, drug delivery, and bio scaffolding. This review provides a comprehensive introduction to CeO_2 catalytic mechanisms, multi-enzyme-like activities, and potential applications in biological fields.

♣**Chemical Properties**

1. Cerium(IV) oxide, also known as cericoxide, ceric dioxide, ceria, cerium oxide or cerium dioxide, is an oxide of the rare-earthmetal cerium.
2. It is a pale yellow-white powder with the chemical formula CeO_2.
3. It is an important commercial product and an intermediate in the purification of the element from the ores.
4. The distinctive property of this material is its reversible conversion to a non-stoichiometric oxide

Uses of Cerium Oxide

Cerium oxide nanoparticles (CeO_2NPs, nanoceria) are widely used in

1.chemical mechanical polishing/planarization ,

2.corrosion protection ,

3.solar cells ,

4.fuel oxidation catalysis ,

5. automotive exhaust treatment

1.4.1 Properties of cerium chloride: $CeCl_3$

Cerium(III) chloride ($CeCl_3$), also known as cerous chloride or cerium trichloride, is a compound of cerium and chlorine.

It is a white hygroscopic salt; it rapidly absorbs water on exposure to moist air to form a hydrate, which appears to be of variable composition,though the heptahydrate $CeCl_3 \cdot 7H_2O$ is known.

It is highly soluble in water, and (when anhydrous) it is soluble in ethanol and acetone.

$CeCl_3$ cerium chloride

Molecular weight of cerium chloride:172.115 g/mol (anhydrous)

Density of cerium chloride : 7.6 g/cm3 (anhydrous)

Boiling point of Cerium chloride : 1456°C

Melting point of Cerium chloride : 817°C

1.5 TYPES OF NANOMATERIAL'S

1. Carbon based nanomaterial
2. Metal nanoparticles
3. Semiconductor nanoparticles
4. Ceramic nanomaterial
5. Magnetic nanomaterial

1.5.1 Carbon based Nanoparticles:

Carbon-based nanoparticles include two main materials: carbon nanotubes (CNTs) and fullerenes. CNTs are nothing but graphene sheets rolled into a tube. These materials are mainly used for the structural reinforcement as they are 100 times stronger than steel. CNTs can be classified into single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). CNTs are unique in a way as they are thermally conductive along the length and non-conductive across the tube. Fullerenes are the allotropes of carbon having a structure of hollow cage of sixty or more carbon atoms. The structure of C-60 is called Buckminsterfullerene and looks like a hollow football. The carbon units in these structures have a pentagonal and hexagonal arrangement. These have commercial applications due to their electrical conductivity, structure, high strength, and electron affinity.

1.5.2 Metal Nanoparticle:

This group includes mainly noble metals such as silver and gold among others like Pt, Pod, and Cu etc. Especially, the Nobel metal nanostructure materials exhibit important unique physical phenomena called surface Plasmon resonance (SPR) and are ideal building blocks for engineering and tailoring nan scale structures for specific technological applications. By controlling their size, shape, architecture, composition, hybrid structures of these materials play an important role on revealing their new or enhanced functions and application potentials such as catalysts, absorbents, chemical, and biological sensors, as well as photonic, electronic devices and SERS detection. Metal nanoparticles are submicron scale entities made of pure metals (e.g., gold, platinum, silver, titanium, zinc, cerium, iron & thallium) or their compounds (e.g., oxide, hydroxide, sulphides, phosphates, fluorides & chlorides).

1.5.3 Ceramic Nanoparticles:

Ceramic nanoparticles are inorganic solids made up of oxides, carbides, carbonates and phosphates. These nanoparticles have high heat resistance and chemical inertness. They have applications in photocatalysis, photodegradation of dyes, drug delivery, and imaging. By controlling some of the characteristics of ceramic nanoparticles like size, surface area,

porosity, surface to volume ratio, etc., they perform as a good drug delivery agent. These nanoparticles have been used effectively as a drug delivery system for several diseases like bacterial infections, glaucoma, cancer, etc.

1.5.4 Semiconductor Nanoparticles:

Semiconductor nanoparticles have properties like those of metals and non-metals. They are found in the periodic table in groups II-VI, III-V or IV-VI. These particles have wide bandgaps, which on tuning shows different properties. They are used in photocatalysis, electronics devices, photo-optics and water splitting applications. Some examples of semiconductor nanoparticles are GaN, GaP, InP, InAs from group III-V, ZnO, ZnS, CdS, CdSe, CdTe are II-VI semiconductors and silicon and germanium are from group IV.

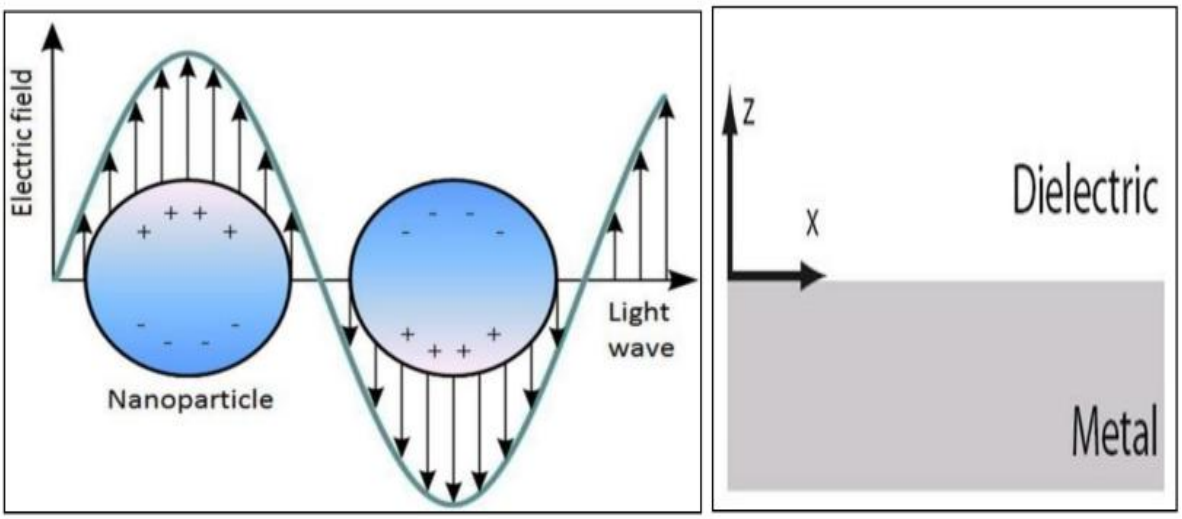


Figure1.4a)Metallic Interaction with the Electromagnetic Wavelength b): Propagation of SPR

The interaction of metals with electromagnetic radiation is largely dictated by the free conduction electrons in the metal. At optical frequencies the metals free electrongas can sustain surface and volume charge density oscillations, called Plasmon polarities or Plasmon's with distinct resonance frequencies. The surface charge density oscillations associated with surface Plasmons at the interface between a metal and a dielectric can give rise to strongly enhanced optical near-fields which are spatiallyconfined to the interface. Similarly, if the electron gas is confined in three dimensions, asin the case of a small sub wavelength particle, the overall displacement of the electrons with respect to the positively

charged lattice leads to a restoring force which in turn givesrise to specific particle Plasmon resonances depending on the geometry of the particle. Inparticles of suitable (usually pointed) shape, extreme local charge accumulations can occur that are accompanied by strongly enhanced optical fields. The study of optical phenomena related to the electromagnetic response of metals has been recently termed as plasmonic or nanoplasmonic. This rapidly growing field of Nano science is mostly concerned with the control of optical radiation on the sub wavelength scale. Surface Plasmon polarities are electromagnetic excitations propagating at the interface between a dielectric and a conductor, evanescently confined in the perpendicular direction. Theseelectromagnetic surface waves arise via the coupling of the electromagnetic fields to oscillations of the conductor's electron plasma When SPs couple with a photon, the resulting hybridized excitation is called a surface Plasmon polarity (SPP). The intensity enhancement near the interface due to the excitation of surface plasmon's can be obtained by evaluating the ratio of theincoming intensity and the intensity right above the metal interface. SPs are one of the best examples that things are different at the nan scale. When the size of a metallic particle is reduced to a few nanometres, the optical properties are dramatically modified by the appearance of SPs and its resulting behaviour is completelydifferent from the bulk metal one. SPs open the possibility to amplify, concentrate and manipulate light at the nan scale, overcoming the diffraction limit of traditional optics and increasing resolution and sensitivity of optical probes. Consequently, SPs can be used in a wide range of fields, including biomedical, energy, environment protection, and sensing and information technology applications. Nowadays, there are wellestablished applications of SPs that increase rapidly with the development of our capabilities to fabricate and manipulate nanomaterial. Why Surface Plasmon Resonance? The study of molecular binding processes is a key aspect to many fields ofresearch. From life science to environmental safety, determining which molecules interact, how they interact, and why they interact can ultimately lead to more effective drugs, higher performance materials, cleaner air/water quality, and much more. However, few encompass as many advantages as Surface Plasmon Resonance. SPR enables (1) High sensitivity, (2) Label-free detection, (3) Real-time monitoring, (4) Low volume sample consumption, (5) Quantitative evaluation Determination of kinetic rate constants. Furthermore, SPR is easy to perform and

can be a cost-effective solution. Surface to volume ratio Surface to volume ratio (SA/V) is important concept for understanding the change of properties at Nano-scale. SA/V is a pre requite for understanding of size dependent properties and behaviors and changes that are at the core of Nano science. Many of special properties that material exhibits at Nano scale results from the effect of size on the surface are to volume ratio (SA/V)/ when the reaction takes place at the surface of chemical or material, the grater the surface area to volume ratio the greater the reactivity. The equations for the surface area and volume of a sphere are: $S=4\pi r^2$ and $V = 4\pi r^3/ 3$ where r is the radius of the sphere.

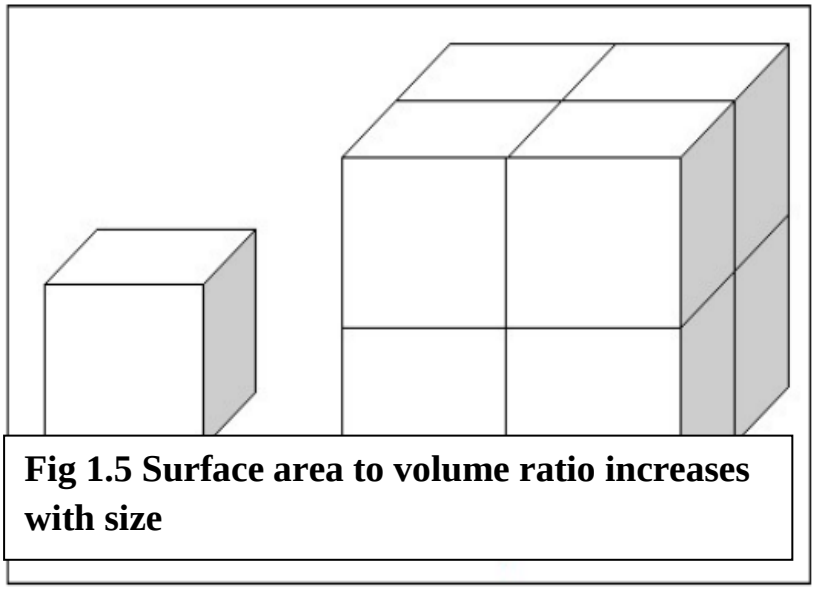


Fig 1.5 Surface area to volume ratio increases with size

Fig.1.7 surface area to volume ratio increases at nanoscale

NO. Of cubes(s)	Dimensions(cm)	Surface area $(l \times l \times 6cm^2)$	Volume $(l \times l \times lcm^3)$	Surface area/volum eratio
1	2 ×2	$(2 \times 2 \times 6) = 24$	$2 \times 2 \times 2) = 8$	3
8	1 × 1	$8(2 \times 2 \times 6) = 84$	$8(1 \times 1 \times 1) = 8$	6

1.6AegleMarmelosleaves

Scientific Classification:

Kingdom- Plantae.
Order - Sapindales.
Family - Rutaceae.
Subfamily -Aurantioideae.
Genus - Aegle.
Species - *AegleMarmelos*

Fig. 6 Aegle Marmelos leaf

*Aeglemarmelob*elongs to family Rutacceae, is commonly known as Bael in indigenous systems of medicine and has been regarded to possess various medicinal properties. The bael is one of the sacred trees of the Hindus. Leaves are offered in prayers to Shiva and Parvathi since ancient times. Bael is a deciduous sacred tree, associated with Gods having useful medicinal properties, especially as a cooling agent. This tree is popular in Shiva and Vishnu temples and it can be grown in every house.

Leaves are used as mild laxative, or the inflammation of the mucous membrane having a free discharge and for asthma. The decoction of the leaves is febrifuge, or helps in eliminating fever and is an expectorant, or promotes the removal of mucous secretion from the bronchial tubes. The leaf juice is given in dropsy or the abnormal accumulation of liquid in the cellular tissue accompanied with constipation and jaundice. A

hot poultice of the leaves is applied in ophthalmia or severe inflammation of conjunctiva with acute bronchitis and inflammation of the other body parts.

A.Marmelos has been reported to contain several phytoconstituents mainly marmenol, marmin, marmelosin, marmelide, psoralen, alloimperatorin, rutaretin, scopoletin, aegelin, marmelin, fagarine, anhydromarmelin, limonene, â-phellandrene, betulinic acid, marmesin, imperatorin, marmelosin, luvangentin and auroptene.

The essential oil isolated from the leaves of *A. marmelos* tree has proved to have antifungal activity against animal and human fungi like Trichophytonmentagrophytes, Trichophytonrubrum, Microsporumgypseum, Microsporumaudounii, Microsporum cookie, Epidermophytonfloccosum, Aspergillusniger, Aspergillusflavus and H

CHAPTER 2

SYNTHESIS OF NANOPARTICLES

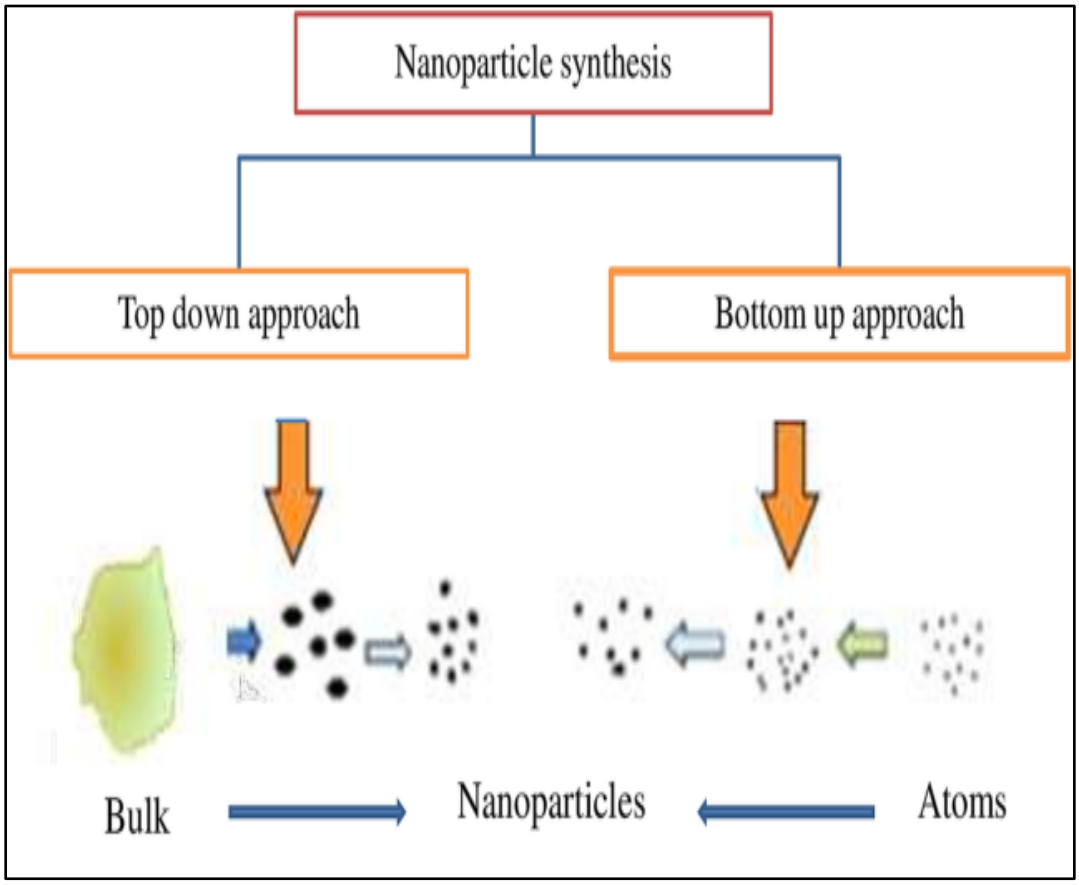


Fig 1.7 Nano particle sysnthesis

2.1 Preparation of Nanomaterial:

There are two approaches for synthesis of nanomaterial and the fabrication of nanostructure in "Top-down and Bottom-up approaches.

2.1.2 Top Down Approaches

Top-down approach involves the breaking down of the bulk material into nan sized structures or particles. Top-down synthesis techniques are extension of those that have been used for producing M.scProject(2021-2022) Suvarna Aher 22 micron sized particles. Top-down approaches are inherently simpler and depend either on removal or division of bulk material or on miniaturization of bulk fabrication processes to produce the desired structure

with appropriate properties. The biggest problem with the top-down approach is the imperfection of surface structure. For example, nanowires made by lithography are not smooth and may contain a lot of impurities and structural defects on its surface. Examples of such techniques are high-energy wet ball milling, electron beam lithography, atomic force manipulation, gas-phase condensation, aerosol sprayed.

2.1.2 Bottom – Up Approach

The alternative approach, which has the potential of creating less waste and hence the more economical, is the „bottom- up‟ . Bottom-up approach refers to the build up of a material from the bottom: atom-by-atom, molecule-by-molecule, or cluster-by cluster. Many of these techniques are still under development or are just beginning to be used for commercial production of nan powders. Organometallic chemical route, revere-micelle route, sol-gel synthesis, colloidal precipitation, hydrothermal synthesis, template assisted sol-gel, electro deposition etc. are some of the wellknown bottom–up techniques reported for the preparation of luminescent nanoparticle.

2.2 MOLAR MASS &WEIGHT PERCENT CALCUALTION

It is defined as the mass of give substance divide by the amount of substance.

Formula:-$weigh\ tingram$= concentration× molar mass× volume

1000

Materials	Molar Mass (g/mol)	Concentration (M)	volume (ml)	Weight of Materials in gram (gm)
1. $CeCl_3$	372.58	0.01	100	3.72

2.3**Synthesis of CeO_2:**

CeO_2 synthesized greens approach, typical synthesis 5 ml extract of *Aegle marmelos* leaves added 90 ml of distilled water in the proceeding step 3.72 gm of $CeCl_3$ was added and placed on hot plate with magnetic stirring at temperature 70 - 80^oC for 5 hrs. Centrifuge the solution for 10 min .Collect sample in petridish shake it through ethanol.Put it for calcination process at 400^oC. Then we will get White colour CeO_2 Powder.

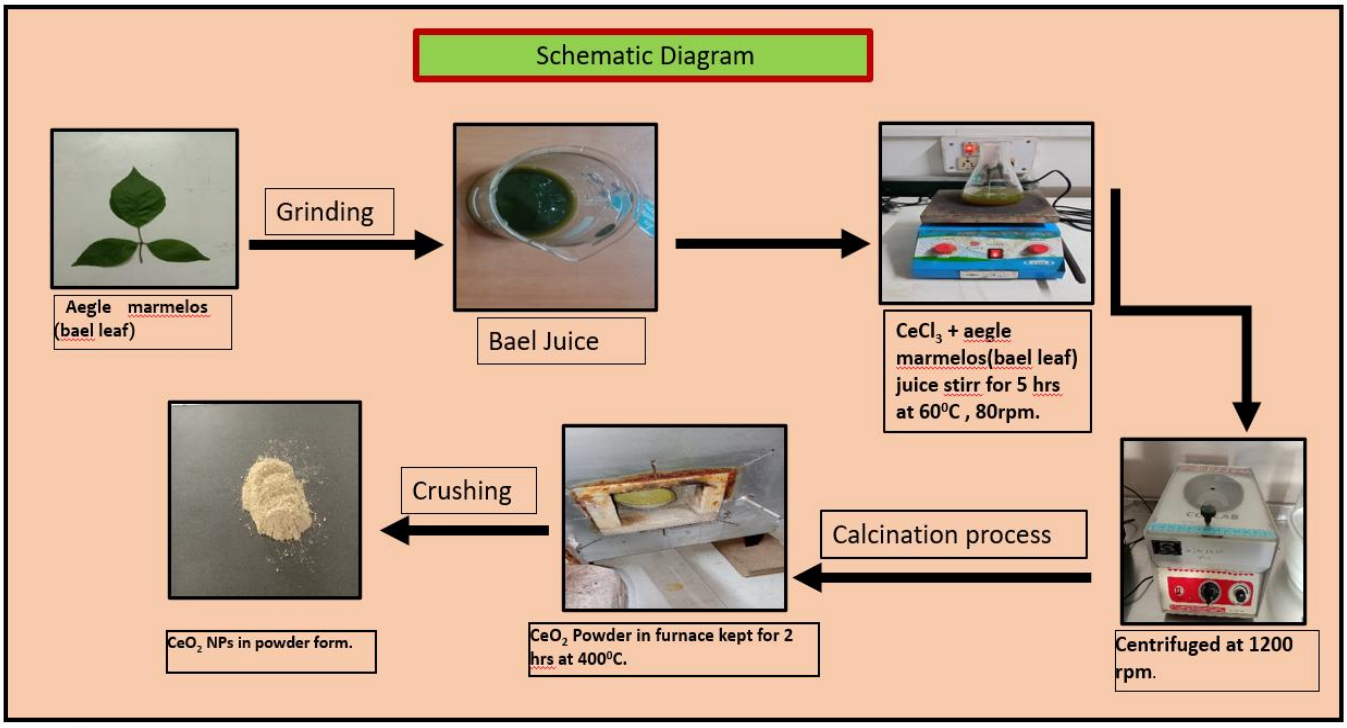


Fig : Schematic of green synthesis of CeO_2 NPs using Bael leaf extract

CHAPTER 3

Characterization Techniques

To understand the phase, morphology, crystal structure and composition of the synthesized material. it is very important to characterize the synthesized material better resolution, higher sensitivity and greater precision of characterization tools can give better insights of the materials, hence will be much helpful to exploit for suitable applications

3.1 Structural and Morphological Characterizations:

3.1.1 X-Ray Diffraction:

X-ray diffraction (XRD) is powerful non-destructive technique for characterizing crystalline material. It provides information on structures, phases, preferred crystal orientations (texture), & other structural parameters, such as average grain size, crystallanity, and stream & crystal defects This information can also help us to identify the unknown phase in the product. This technique is suitable for powders and thin films samples of various materials. High-energy accelerated electrons are made incident on target surface, which could eject core electrons in atoms through the ionization process. When an electron from higher energy orbital fills the shell, characteristic X-ray photons are emitted. This depends on atomic number usually targets used in X-ray tubes include Cu and Mo, which emits 8 keV and 14 keV. X-rays with corresponding wavelengths of 1.54 $A^o$and 0.8 A^o respectively. A collimated monochromatic beam of X-rays with a wavelength typically ranging from 0.7 to 2 A, is incident on a specimen and is diffracted by the crystalline planes in the specimen according to Bragg's equation which places the condition for the constructive interference for the diffracted X-ray from the successive atomic planes formed by the crystal lattice of the material The Bragg's condition is formulated by.

$$\mathbf{N\lambda = 2d \sin\theta}$$

Where,

λ is the wavelength of the incident X-ray, d is the interplanar distance, 0 is the scattering angle and is an integer the order of diffraction. The fundamental requirement is the coherence in diffracted X-rays that leads to intense signal.

POWDER METHOD:

This is one of the methods for characterization of crystal structure. This method was investigated by Debye and Scherrer Laue method and rotating crystal methods can be applied only if, single crystal of reasonable size is available. However most of the crystalline substances naturally available are in the form of polycrystals the method of preparation of single crystal is quite difficult. Debye and Scherrer method provides details about crystal structure even if the specimen taken for investigation is in the form of polycrystals. The crystalline substance be finally grounded and powdered so that its tiny crystals are randomly oriented. The powder specimen stuck on hair by means of gum and placed in the path of a narrow monochromatic X-rays.

To calculate average crystallite size of silver nanoparticles powder materials from XRD data based on Debye Scherer's formula,

$$\mathbf{D = K \lambda / \beta \cos \theta}$$

Where, D = Average crystallite size

β =FWHM (full width half maxima) 2-is wavelength of the x-ray radiation and (0.1542 nm).

θ =Is the Bragg's angle

K is constant (representing shape factor K is 0.89 for spherical, 0.94 for cubic and 0.9 for unknown size particles).

X-RAY DIFRACTOMETER:

Basic principle of this method is that millions of tiny crystals in the powder are randomly oriented All possible diffraction patterns will be available for Bragg's reflection. Such reflection will take place from many sets of parallel planes lying at different

angles to the incident X-ray beam. More ever, each set will give not only first order reflection but those of higher order as well. Since all orientations are equally likely. The reflected rays will form a cone whose axis lies along the direction of incident beam and whose semi vertical angle is twice the glancing angle For that particular set of lens is used.

For each set of planes and for each order, there will be such cone reflected X-rays. Their interactions with a photographic film form a series of concentric rings Radius of these rings are recorded and the film can be used to find the glancing angle and hence interplanar distance of crystalline substance. The advantage of this method is that it does not require large single crystals and almost any substance can be grinded into powder.

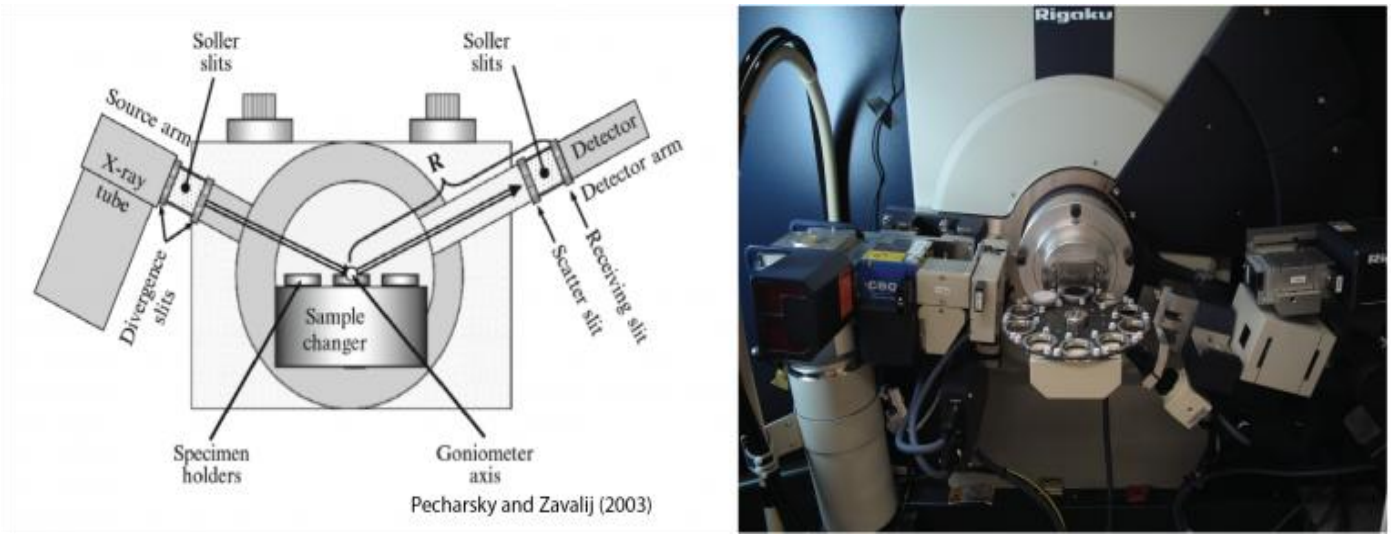


Fig 9: Schematic of the X-Ray Difractiometer and the images of X-ray difractiometer.

Advantages:

- Well established & accepted (the gold standard)
- Rapid & simple sample analysis
- Can readily differentiate polymorphs
- Can analyze mixed polymorphs, quantitative & qualitative
- X-rays are not absorbed very much by air, so the sample need not be in an evacuated chamber

Disadvantages:

- Sensitive to sample preparation technique
- Requires radiation license & fees to operate
- X-ray hazard, very expensive
- X-rays do not interact very strongly with lighter elements
- The intensity is 108 times less than that of electron diffraction

Applications:

- XRD is a non-destructive technique
- To identify crystalline phase and orientation
- To determine structural properties: strain, grain size, epitaxy, phase composition, preferred orientation, order-disorder transformation, thermal expansion
- To measure thickness of thin films & multilayer to determine atomic arrangement
- Detection limits: 3% in a two phase mixture, can be = 0.1% with synchrotron radiation
- Determination of linkage isomerism

3.1.2 Ultraviolet-visible spectroscopy (UV-Vis):

Ultraviolet-visible spectroscopy (UV-Vis) refers to absorption spectroscopy or reflectance spectroscopy in part of the ultraviolet and the full, adjacent visible regions of the electromagnetic spectrum. This means it uses light in the visible and adjacent ranges. The absorption or reflectance in the visible range directly affects the perceived colour of the chemicals involved. In this region of the spectrum, atoms and molecules undergo electronic transitions. Absorption spectroscopy is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions of electrons from the excited state to. the ground state, while absorption measures transitions from the ground state to the excited state.

Fig10: UV-Visible Spectroscopy

Principle:

The UV-Visible Spectroscopy is depend on the principal of beer Lambert law. The Beer-Lambert law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. Thus, for a fixed path length, UV/Vis spectroscopy can be used to determine the concentration of the absorber in a solution. It is necessary to know how quickly the absorbance charges with concentration. This can be taken from references (tables of molar extinction coefficients), or more accurately, determined from a calibration curve.

$$\mathbf{A = \varepsilon L c}$$

where,

A is the amount of light absorbed for a particular wavelength by the sample

ε is the molar extinction coefficient

L is the distance covered by the light through the solution

c is the concentration of the absorbing species

Molecules containing bonding and non-bonding electrons (n-electrons) can absorb energy in the form of ultraviolet or visible light to excite these electrons to higher anti-bonding molecular orbitals The more easily excited the electrons (i.e. lower energy gap between the HOMO and the LUMO), the longer the wavelength of light it can absorb. There are four possible types of transitions (π-π*, n-π*, σ – σ*, and n- σ *), and they can be ordered as follows σ- σ*>n- σ *>n-π *.

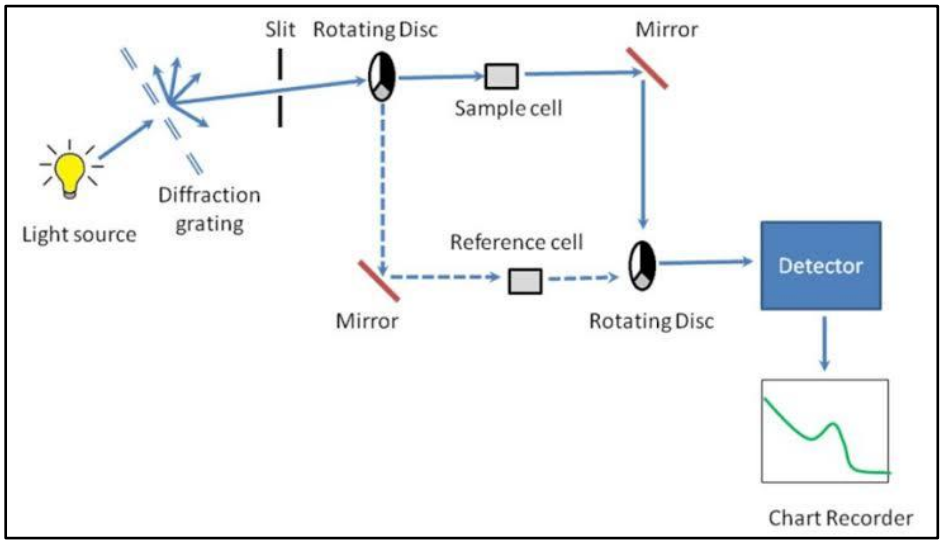


Fig 11: Schematics of UV-Visible Spectroscopy

Advantages:

- Analysis ability and easy to use.
- In astronomy research, an UV/Vis spectrophotometer helps the scientists to analyze the galaxies, neutron stars, and other celestial objects.
- A UV spectrum can provide rich information of the velocity and the elements of an astronomical object.
- In other industries, UV/Vis spectrophotometer also brought the high-tech spectral analysis possibilities

Disadvantages:

- The stray light of UV-Vis spectrophotometer that caused by the faulty equipment design and other factors could influence spectra measurement accuracy of the absorption in substance
- In addition, the electronic circuit design and the detectar circuit quality of spectrometer will affect the amount of noise that is coupled into the measurement signal, thereby affecting the measurement accuracy and reduce the sensitivity of the instrument

Applications:

- ❖ UV/Vis spectroscopy is routinely used in analytical chemistry for the quantitative determination of different analytes, such as transition metal ions, highly conjugated organic compounds, and biological macromolecules. Spectroscopic analysis is commonly carried out in solutions but solids and gases may also be studied.
- ❖ Solutions of transition metal ions can be colored (ie, absorb visible light) because d electrons within the metal atoms can be excited from one electronic state to another. The colour of metal ion solutions is strongly affected by the presence of other species, such as certain anions or ligands. For instance, the colour of a dilute solution of copper sulfate is a very light blue, adding ammonia intensifies the colour and changes the wavelength of maximum absorption (ms).

Organic compounds, especially those with a high degree of conjugation, also absorb light in the UV or visible regions of the electromagnetic spectrum The solvents for these determinations are often water for water-soluble compounds, or ethanol for organic-soluble compounds. (Organic solvents may have significant UV absorption, not all solvents are suitable for use in UV spectroscopy. Ethanol absorbs very weakly at most wavelengths) Solvent polanty and pll can affect the absorption spectrum of an organic compound Tyrosine, for example, increases in absorption maxima and molar extinction coefficient when pll increases from 6 to 13 or when solvent polarity decreases.While charge transfer complexes

also give rise to colors, the colors are often too intense to be used for quantitative measurement.

3.1.3 Fourier-transform infrared spectroscopy (FTIR):

FTIR is technique use to obtain an infrared spectrum of absorption or emission of a solid, liquid, gas. An FTIR spectrometer simultaneously collects high-spectral-resolution data over a wide spectral range. The term FTIR originates from the fact that a Fourier transform (a mathematical process) is required to convert the raw data into the actual spectrum.

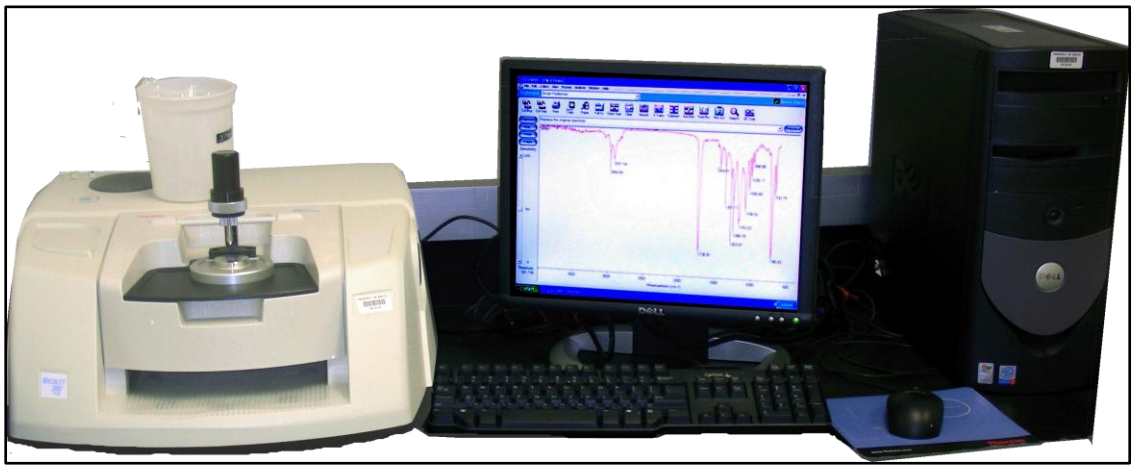


Fig 12: FT-IR Spectroscopy

Principle:

FTIR is provide information about the chemical bonding or molecular structure of material, whether organic or inorganic & identifier chemical bonds or functional group by absorption of IR radiation which excites vibrational mode in the bond. Frequency range range 400-600 am-1. measured as wave numbers typically over the background emission spectrum of IR source is first recorded, followed by the emission spectrum of the IR source with the simple place. The ratio of the sample spectrum to the background spectrum is directly related to sample's absorption spectrum The resultant absorption spectrum from the bond natural vibration frequencies indicates the presence of various chemical bond and functional groups present in the sample.

Construction:-

1. Source
2. An optical system which uses interferometer
3. Beam splitter
4. Stationary mirror
5. Moving mirror
6. Sample
7. Detector

1. **Source:**

   Global source, tungsten lamp, mercury arc.

2. **Beam splitter:**

   It is made up of material which is made up of reflective index

3. **Detector:**

   Polyelectric detector is used. It consists of two perpendicular mirrors, one of which is stationary mirror and the other is movable mirror. The position of movable mirror is controlled by He-Ne laser (632.8nm) and between these two mirrors, set a beam splitter a 450 from initial position of the movable mirror. A parallel beam of radiation from IR source is passed on the mirror though the beam splitter.

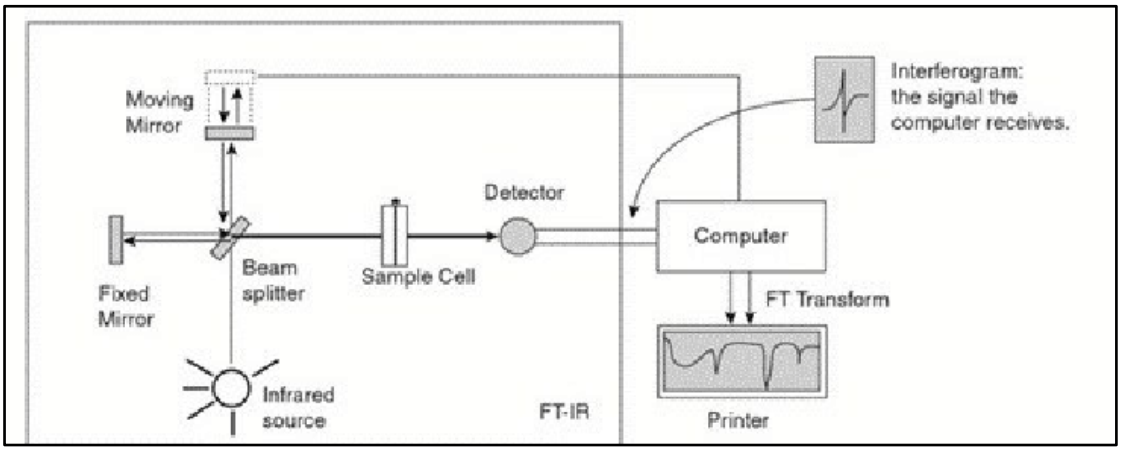


Fig13: FT-IR spectrometer schematic diagram

Working

The apparatus devices from classical attempt by Michelson to measure the ether wind by determining the velocity of light in two perpendicular directions. A parallel beam of radiation is directed from source to the interferometer, consisting of beam B splitter and two mirror m1 and m2, the beam splitter is a plate of suitably transparent material so as to reflect just 50% of radiation focusing on it. Thus half the radiation goes to m1 & half to m2 return from both these mirror along the same path & is then recombined to a single beam at the beam splitter. It well defined that if monochromatic radiation emitted by the source. The recombined beam leaving B shows constructive or destructive interference depending on the reflective path length 8 to m1 & B to m2.

Thus, if the path length is same or differs by integral multiple of 2. Constructive interference gives bright beam leaving B. whereas if the difference is a half integral number of 2. The beam cancel at B as mirror m2 moved smoothly away or toward from B.

A detector sees radiation alternating intensity. It's rather easy to visualize that if the source emits two separate monochromatic frequency vl& v2. Then interference caused by m1 & m2. The detector would see a more complicate intensity, fluctuation as m2 is moved but computing the Fourier transform of the resultant signal is very rapid way of obtaining original frequency & intensities emitted by the source. Taking the process further even white radiation emitted by source produce and interference pattern which can transformed back to original frequency distribution.

The production of a spectrum is a two stage process

1) Without the sample is a beam mirror m2 is move smoothly over period of time through a distance of about Icm, while to collect ito multichannel computer cames out the Fourier transformation of stored data to produce back ground spectrum.

2) A sample interferogram is record in exactly some way Fourier transformed spectrum. After natively the sample & background spectrum may each one be calculated in absorbance from the former to give an absorbance spectrum of alone sample.

Advantages:

- We can determine small quantity of analyte.
- Better sensitivity & brightness.
- Allows simultaneous measurement over the entire wave number range. 4. Required no shit device
- The resolution is better and constant across the entire region under study.
- High scanning by FT-IR is possible to measure the whole spectrum in a few seconds
- The detectors employed are much more sensitive
- Photometric accuracy advantage. These instruments employ a He-Ne laser as an internal wavelength calibration standard. These instruments are self-calibrating.

Disadvantages:

- Cannot detect molecules comprised of two identical atoms symmetric-such as N2 of 02
- Aqueous solution is very difficult to analyze-water is strong IR absorber.
- Complex mixture-samples give rise to complex spectra

Application:

- ❖ For opaque or cloudy samples
- ❖ Analysis of raw materials or finished products
- ❖ Kinetics reaction on the microsecond time-scale
- ❖ Analysis of chromatographic and the thermogravimetrie sample fractions

- ❖ Micro-samples Tiny samples, such as in forensic analysis:
- ❖ Identification of compounds

3.1.4 Field Emission Scanning Electron Microscopy (FESEM):

Filed Emission Scanning Electron Microscopy (FESEM) The field emission scanning electron microscope (FE-SEM) is a type of electron microscope that images the sample surface by scanning it with a high-energy beam of electrons in a raster scan pattern.

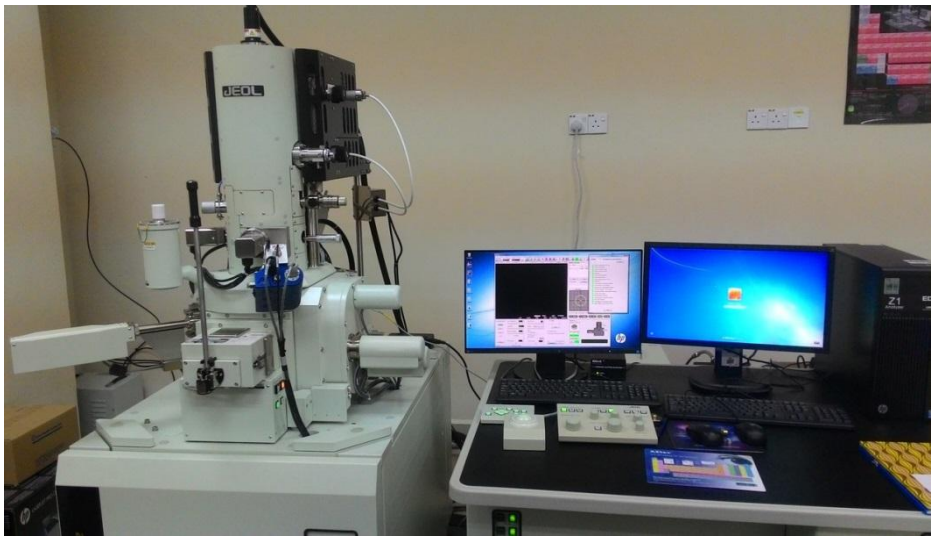


Fig 14: Instrumentation set-up of FESEM

Principle:

FESEM is the abbreviation of Field Emission Scanning Electron Microscope A FESEM is microscope that works with electrons (particles with a negative charge) instead of light. These electrons are liberated by a field emission source. The object is scanned by electrons according to a zig-zag pattern.

Working:

The field emission scanning electron microscope (FE-SEM) images a sample surface by raster scanning over it with a high-energy beam of electrons. The electrons interact with the atoms comprising the sample to produce signals that contain information about surface

topography, composition and other properties, such as electrical conductivity. There are two classes of emission source: thermionic emitter and field emitter. Emitter type is the main difference between the Scanning Electron Microscope(SEM) and the Field Emission Scanning Electron Microscope (FE-SEM).Thermionic Emitters use electrical current to heat up a filament; the two most common materials used for filaments are Tungsten (W) and Lanthanum hex boride (LaB6). When the heat is enough to overcome the work function of the filament material, the electrons can escape from the material. Thermionic sources have relative low brightness, evaporation of cathode material and thermal drift during operation. Field Emission is one-way of generating electrons that avoids these problems. A Field Emission Source (FES);also called a cold cathode field emitter, does not heat the filament. The emission is reached by placing the filament in a huge electrical potential gradient. The FESEM is usually wire of Tungsten (W) fashioned into a sharp point. The FE source reasonably combines with scanning electron microscopes (SEMs) whose development has been supported by advances in secondary electron detector technology. The acceleration voltage between cathode and anode is commonly in the order of magnitude of 0.5 to 30 kV, and the apparatus requires an extreme vacuum (~10–6 Pa) in the column of the microscope. Function of FESEM is Electrons are liberated from a field emission source and accelerated in a high electrical field gradient. Within the high vacuum column these so-called primary electrons are focused and deflected by electronic lenses to produce a narrow scan beam that bombards the object. As a result secondary electrons are emitted from each spot on the object. The angle and velocity of these secondary electrons relates to the surface structure of the object. A detector catches the secondary electrons and produces an electronic signal. This signal is amplified and transformed to a video scan-image that can be seen on a monitor or to a digital image that can be saved and processed further.

Advantages:

- FESEM provides topographical and elemental information at magnifications of 10x to 300,000x, with virtually unlimited depth of field.

- FESEM produces clearer, less electrostatically distorted images with spatial resolution down to 1 1/2 nanometers-three to six times better.
- The ability to examine smaller-area contamination spots at electron accelerating voltages compatible with energy dispersive spectroscopy (EDS).
- Reduced penetration of low-kinetic-energy electrons probes closer to the immediate material surface.
- High-quality, low-voltage images with negligible electrical charging of samples (accelerating voltages ranging from 0.5 to 30 kilovolts).
- Essentially no need for placing conducting coatings on insulating materials.
- For ultra-high-magnification imaging, we use in-lens FESEM

Disadvantages:

- The size and cost
- FESEMs are expensive, large and must be housed in an area free of any posible electric, magnetic or vibration interference.
- Maintenance involves keeping a steady voltage, currents to electromagnetic coils and circulation of cool water

Applications:

- ❖ Semiconductor device cross section analyses for gaste widths, gate oxides, film thicknesses, and construction details
- ❖ Advanced coating thickness and structure uniformity determination
- ❖ Small contamination feature geometry and elemental composition measurement.

3.1.5Photoluminescence

Photoluminescence (abbreviated as PL) is light emission from any form of matter after the absorption of photons (electromagnetic radiation).[1] It is one of many forms of luminescence (light emission) and is initiated by photo excitation (i.e. photons that excite electrons to a higher energy level in an atom), hence the prefix photo-.[2] Following excitation, various relaxation processes typically occur in which other photons are reradiated. Time periods between absorption and emission may vary: ranging from short femtosecond-regime for emission involving free-carrier plasma in inorganic semiconductors[3] up to milliseconds for phosphorescence processes in molecular systems; and under special circumstances delay of emission may even span to minutes or hours Observation of photoluminescence at a certain energy can be viewed as an indication that an electron populated an excited state associated with this transition energy. While this is generally true in atoms and similar systems, correlations and other more complex phenomena also act as sources for photoluminescence in many-body systems such as semiconductors. A theoretical approach to handle this is given by the semiconductor luminescence equations. Created with PDFBear.com M.Sc Project (2021-2022) Mansi Malpure Page 4 On this picture you can see fluorescence of different substances under UV light. Green is a fluorescent, red is Rhoda mine B, yellow is Rhoda mine 6G, blue is quinine, purple is a mixture of quinine and rhodamine 6g. Solutions are about 0.001% concentration in water.Photoluminescence spectroscopy is a contactless, nondestructive method of probing the electronic structure of materials. Light is directed onto a sample, where it is absorbed and imparts excess energy into the material in a process called *photo-excitation*. One way this excess energy can be dissipated by the sample is through the emission of light, or *luminescence*. In the case of photo-excitation, this luminescence is called *photoluminescence*.

Photo-excitation causes electrons within a material to move into permissible excited states. When these electrons return to their equilibrium states, the excess energy is released and may include the emission of light (a radiative process) or may not (a nonradiative process). The energy of the emitted light (photoluminescence) relates to the difference in energy levels

between the two electron states involved in the transition between the excited state and the equilibrium state. The quantity of the emitted light is related to the relative contribution of the radiative process.

The importance of photoluminescence

In most photoluminescent systems chromophore aggregation generally quenches light emission via aggregation-caused quenching (ACQ). This means that it is necessary to use and study fluorophores in dilute solutions or as isolated molecules. This in turn results in poor sensitivity of devices employing fluorescence, e.g., biosensors and bioassays. However, there have recently been examples reported in which luminogen aggregation played a constructive, instead of destructive role in the light-emitting process. This aggregated-induced emission (AIE) is of great potential significance in particular with regard to solid state devices. Photoluminescence spectroscopy provides a good method for the study of luminescent properties of a fluorophore.

Forms of photoluminescence

Resonant radiation

In resonant radiation, a photon of a particular wavelength is absorbed and an equivalent photon is immediately emitted, through which no significant internal energy transitions of the chemical substrate between absorption and emission are involved and the process is usually of an order of 10 nanoseconds.

Fluorescence

When the chemical substrate undergoes internal energy transitions before relaxing to its ground state by emitting photons, some of the absorbed energy is dissipated so that the emitted light photons are of lower energy than those absorbed. One of such most familiar phenomenon is fluorescence, which has a short lifetime (10^{-8} to 10^{-4} s).

Phosphorescence

Phosphorescence is a radiational transition, in which the absorbed energy undergoes intersystem crossing into a state with a different spin multiplicity. The lifetime of phosphorescence is usually from 10^{-4} - 10^{-2} s, much longer than that of Fluorescence. Therefore, phosphorescence is even rarer than fluorescence, since a molecule in the triplet state has a good chance of undergoing intersystem crossing to ground state before phosphorescence can occur.

Relation between absorption and emission spectra

Fluorescence and phosphorescence come at lower energy than absorption (the excitation energy). As shown in [link], in absorption, wavelength λ_0 corresponds to a transition from the ground vibrational level of S_0 to the lowest vibrational level of S_1. After absorption, the vibrationally excited S_1 molecule relaxes back to the lowest vibrational level of S_1 prior to emitting any radiation. The highest energy transition comes at wavelength λ_0, with a series of peaks following at longer wavelength. The absorption and emission spectra will have an approximate mirror image relation if the spacings between vibrational levels are roughly equal and if the transition probabilities are similar. The λ_0 transitions in Figure do not exactly overlap. As shown in Figure, a molecule absorbing radiation is initially in its electronic ground state, S_0. This molecule possesses a certain geometry and solvation. As the electronic transition is faster than the vibrational motion of atoms or the translational motion of solvent molecules, when radiation is first absorbed, the excited S_1 molecule still possesses its S_0 geometry and solvation. Shortly after excitation, the geometry and solvation change to their most favorable values for S_1 state. This rearrangement lowers the energy of excited molecule. When an S_1 molecule fluoresces, it returns to the S_0 state with S_1 geometry and solvation. This unstable configuration must have a higher energy than that of an S_0 molecule with S_0 geometry and solvation. The net effect in Figure is that the λ_0 emission energy is less than the λ_0 excitation energy.

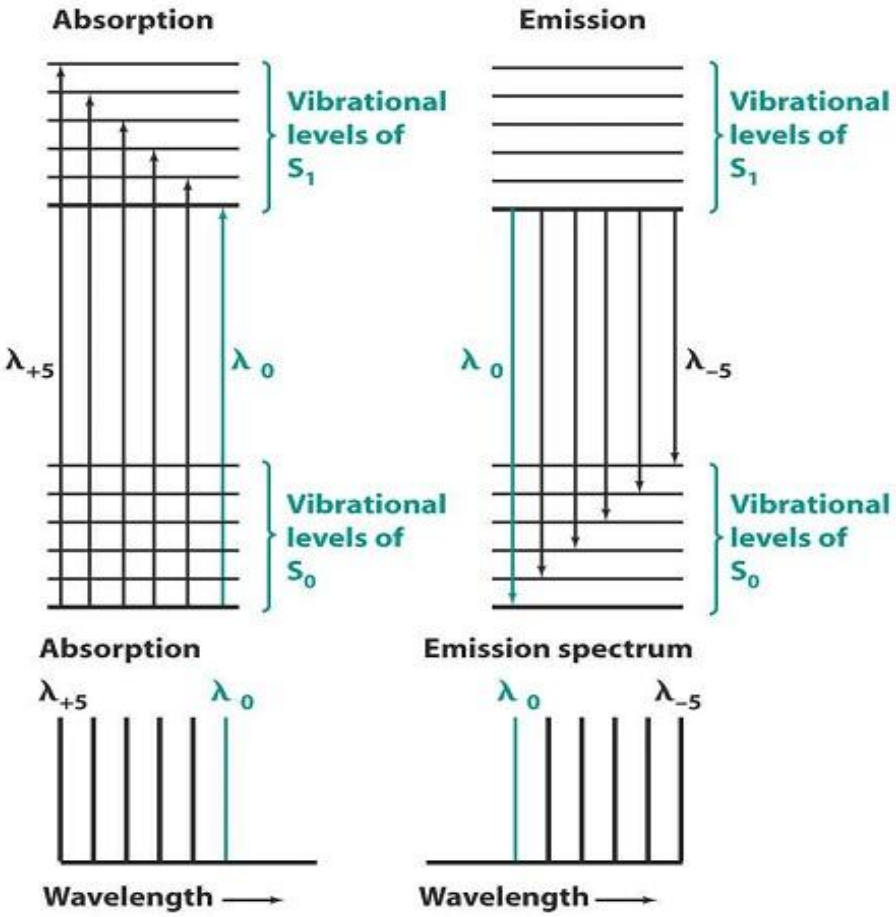


Fig 15 Energy-level diagram showing why structure is seen in the Absorption spectra.

Instrumentation

A schematic of an emission experiment is given in Figure. An excitation wavelength is selected by one monochromator, and luminescence is observed through a second monochromator, usually positioned at 90° to the incident light to minimize the intensity of scattered light reaching the dector. If the excitation wavelength is fixed and the emitted radiation is scanned, an emission spectrum is produced.Essentials of a luminescence experiment. The sample is irradiated at one wavelength and emission is observed over a range of wavelengths. The excitation monochromator selects the excitation wavelength and the emission monochromator selects one wavelength at a time .

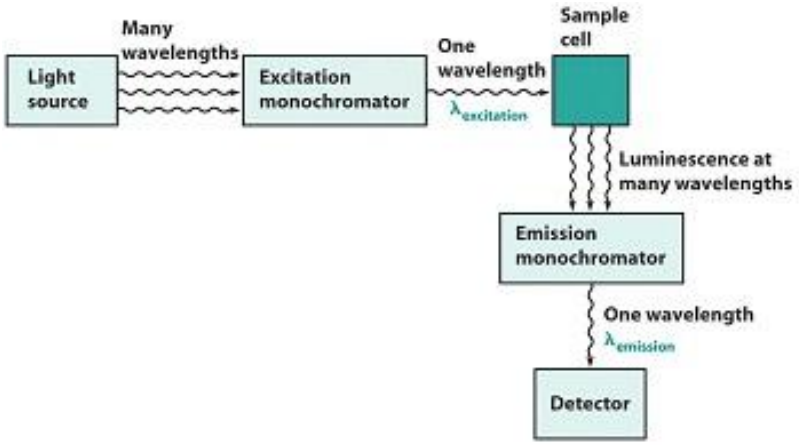


Fig 16 Schematic of Photoluminicence spectroscopy

Relationship to UV-visible spectroscopy

Ultraviolet-visible (UV-vis) spectroscopy or ultraviolet-visible spectrophotometry refers to absorption spectroscopy or reflectance spectroscopy in the untraviolet-visible spectral region. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In the UV-vis spectrum, an absorbance versus wavelength graph results and it measures transitions from the ground state to excited state, while photoluminescence deals with transitions from the excited state to the ground state.

An excitation spectrum is a graph of emission intensity versus excitation wavelength. An excitation spectrum looks very much like an absorption spectrum. The greater the absorbance is at the excitation wavelength, the more molecules are promoted to the excited state and the more emission will be observed.

By running an UV-vis absorption spectrum, the wavelength at which the molecule absorbs energy most and is excited to a large extent can be obtained. Using such value as the excitation wavelength can thus provide a more intense emission at a red-shifted wavelength, which is usually within twice of the excitation wavelength.

Principle

PL (Photoluminescence Spectroscopy) **uses a laser beam to capture light generated from a substance as it falls from the excited state to ground state when irradiated by a laser beam**. By measuring the luminescence spectrum, it is possible to observe material imperfections and impurities.

Advantages &Applications

Band gap determination

Impurity levels and defect detection

Recombination mechanisms

Surface structure and excited states

Disadvantages

Very low concentrations of optical centers can be detected using photoluminescence, but it is not generally a quantitative technique. The main scientific limitation of photoluminescence is that many optical centers may have multiple excited states, which are not populated at low temperature.

The disappearance of luminescence signal is another limitation of photoluminescence spectroscopy. For example, in the characterization of photoluminescence centers of silicon no sharp-line photolumine scence from 969 meVcenters was observed when they had captured self-interstitials.

3.1.6 Raman Spectroscopy

Raman Spectroscopy is a non-destructive chemical analysis technique which provides detailed information about chemical structure, phase and polymorphy, crystallinity and molecular interactions. It is based upon the interaction of light with the chemical bonds within a material.

Raman is a light scattering technique, whereby a molecule scatters incident light from a high intensity laser light source. Most of the scattered light is at the same wavelength (or color) as the laser source and does not provide useful information – this is called Rayleigh Scatter. However a small amount of light (typically 0.0000001%) is scattered at different wavelengths (or colors), which depend on the chemical structure of the analyte – this is called Raman Scatter.

A Raman spectrum features a number of peaks, showing the intensity and wavelength position of the Raman scattered light. Each peak corresponds to a specific molecular bond vibration, including individual bonds such as C-C, C=C, N-O, C-H etc., and groups of bonds such as benzene ring breathing mode, polymer chain vibrations, lattice modes, etc.

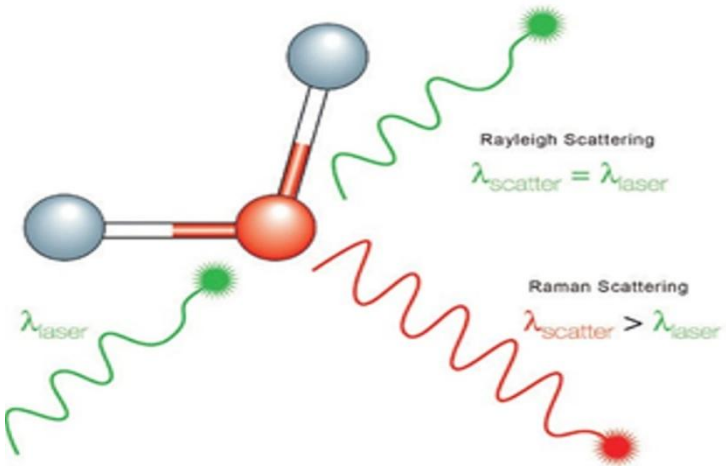


Fig. 17 Raman Scattering

CHAPTER 4

Result and Discussion

4.1.1Structural Analysis of X-ray Diffraction (XRD):

The XRD pattern of biosynthesized sample is depicted in Fig.1 (a). Notable peaks are observed at 28.50°, 33.25°, 47.36°, 56.50°, 59.05°, 69.35,76.75, and 79.18° corresponds to (111), (200), (220), (311), (222), (400), (331) and (420) planes, respectively which is in good agreement with the JCPDS file no: 89-8436 for CeO_2. The observed peaks are wellindexed to cubic fluorite structure of CeO_2.

The average particle size was found to be 5.3 nm which was determined by using the Debye Scherrer formula i.e. nanoparticle size (D) = (k λ)/ (d cosθ) Where, D is the particle in nm, K is crystallite shape factor a good approximation is 0.9 for spherical shape nanoparticles, λ is the X-ray wavelength used for X-ray diffraction, d is the full width at half the maximum (FWHM) in radians of the X-ray diffraction peak and θ is the Braggs' angle .

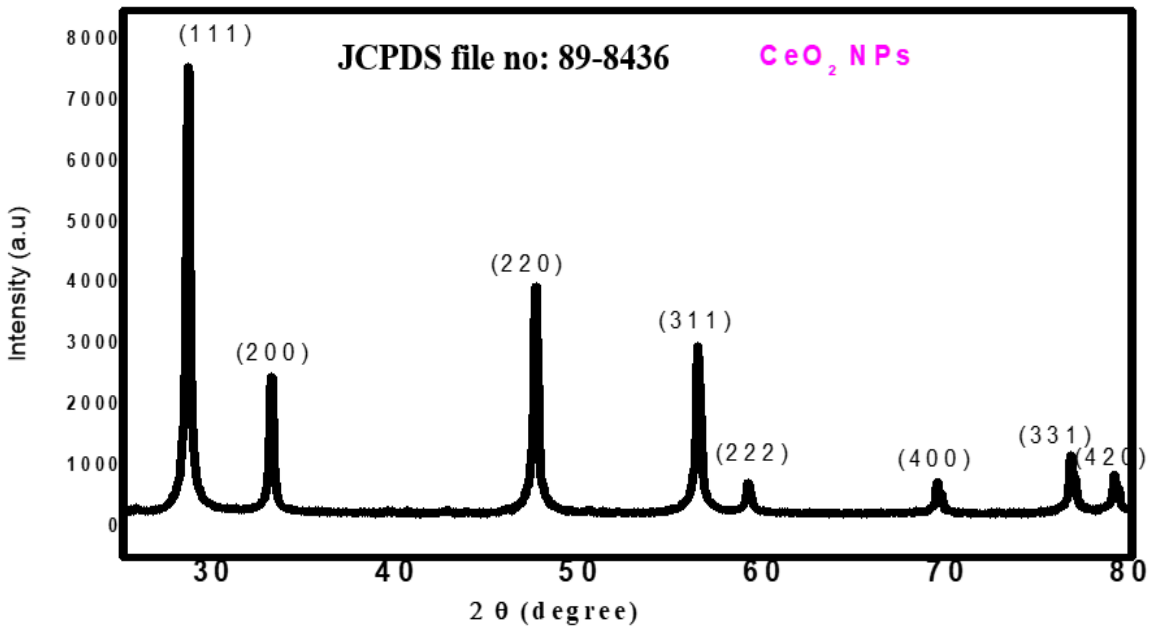


Fig 18 XRD Graph

4.1.2 UV-VISIBLE Spectroscopy Analysis:

UV-Vis. Spectrum is often used to study the effect of particle size on optical property of CeO_2. The absorption wavelength for synthesized CeO_2 was measured at 349.50nm. It is well known that UV-Visible absorption peak of CeO_2 material is about 350 nm. The Synthesized CeO_2 maximum wavelength occurred in 349.50 nm, we can conclude the band gap which is 3.52 eV by using Einstein's-energy equation.

$$E= hc/\lambda$$

Where, E is the band gap of NP's,

C is speed of light,

h is plank's constant

The absorption wavelength is in Ultra violet region.

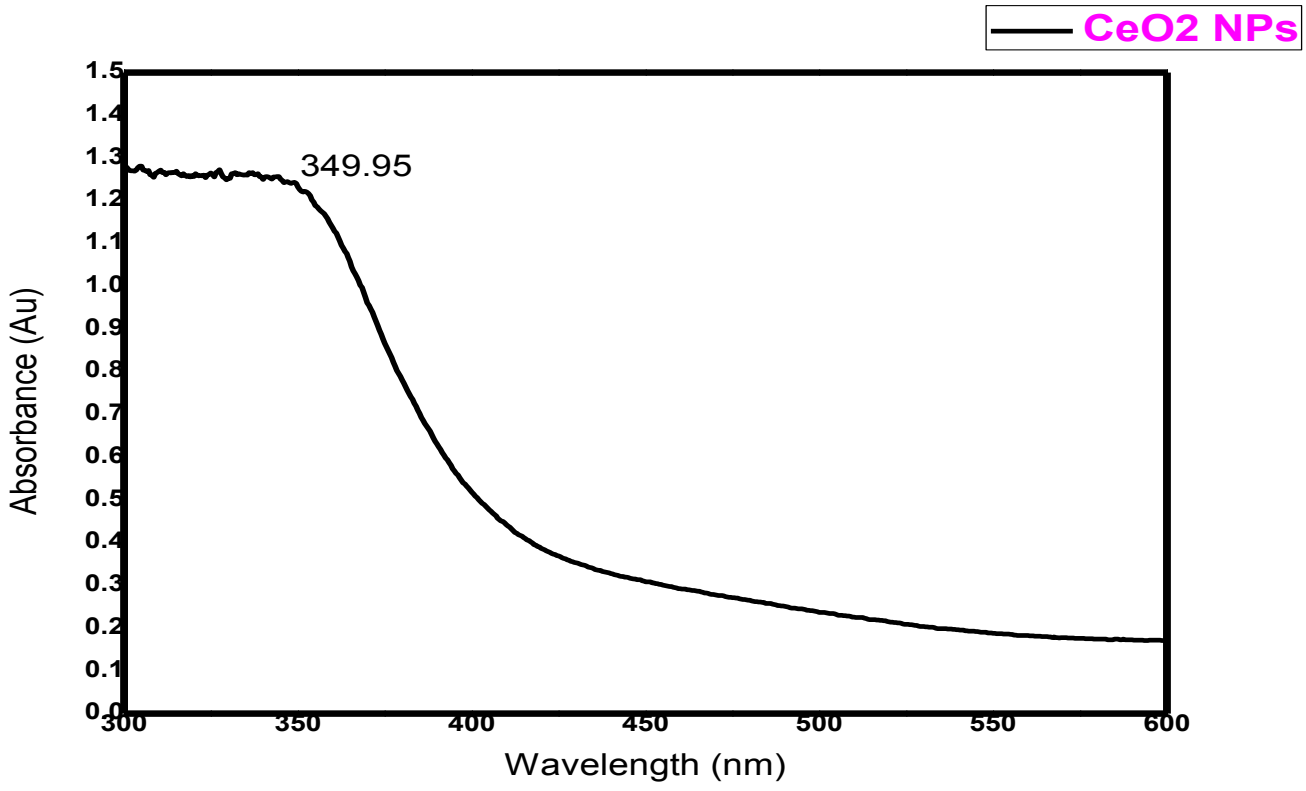


Fig 19 UV – Visible Graph

The band gap energy of CeO_2 Nps by Tauc plot method is 3.03 eV.

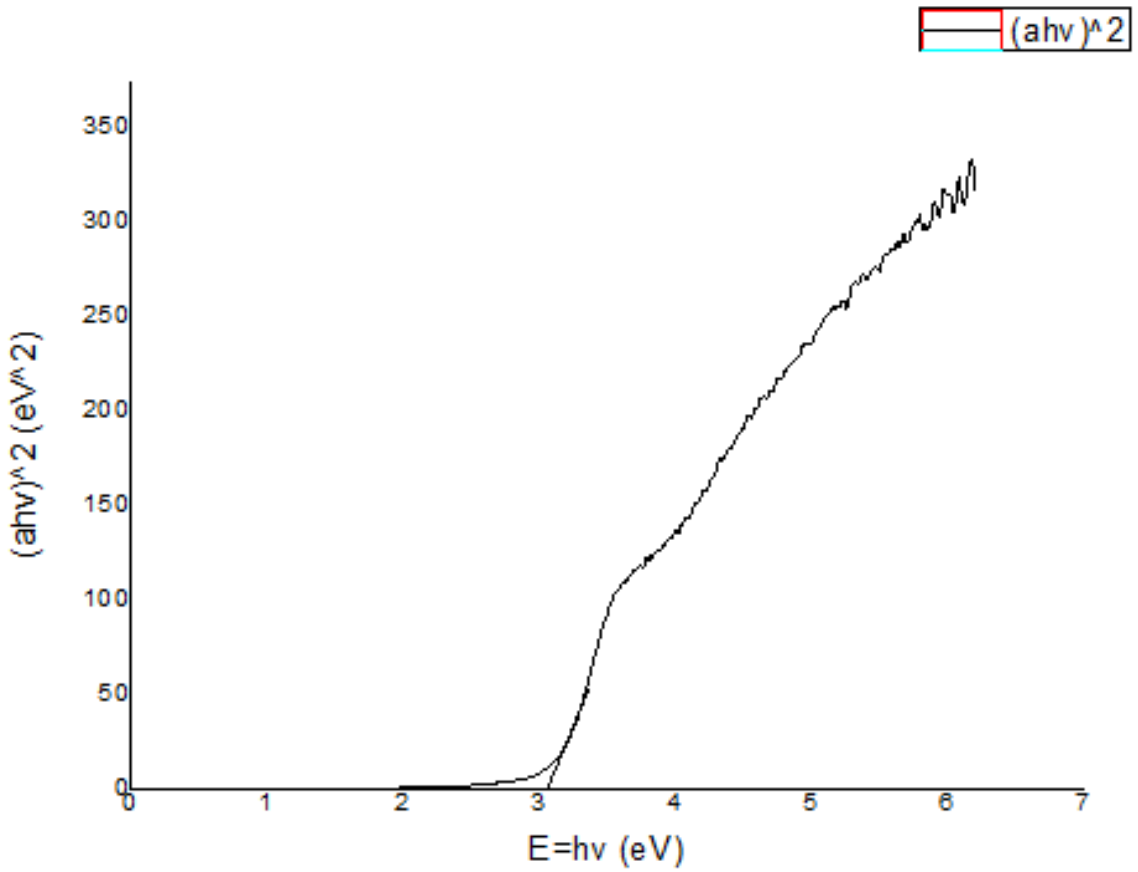
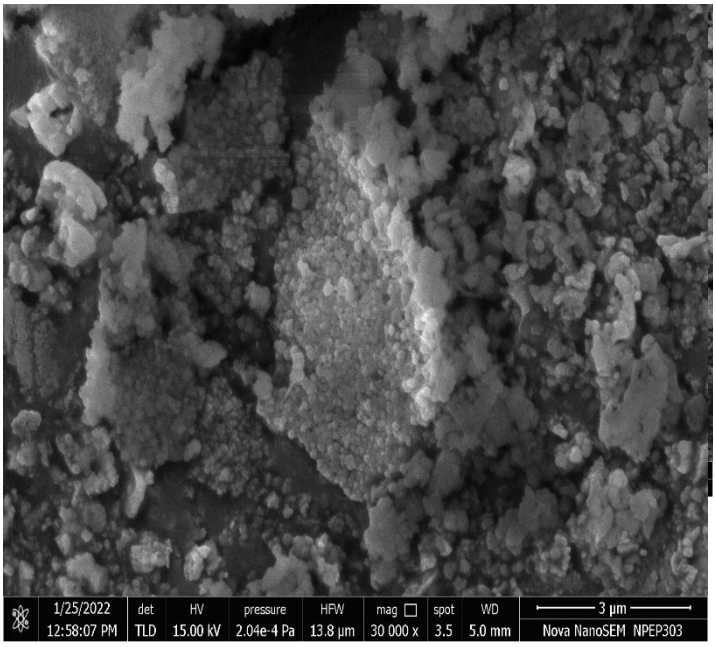
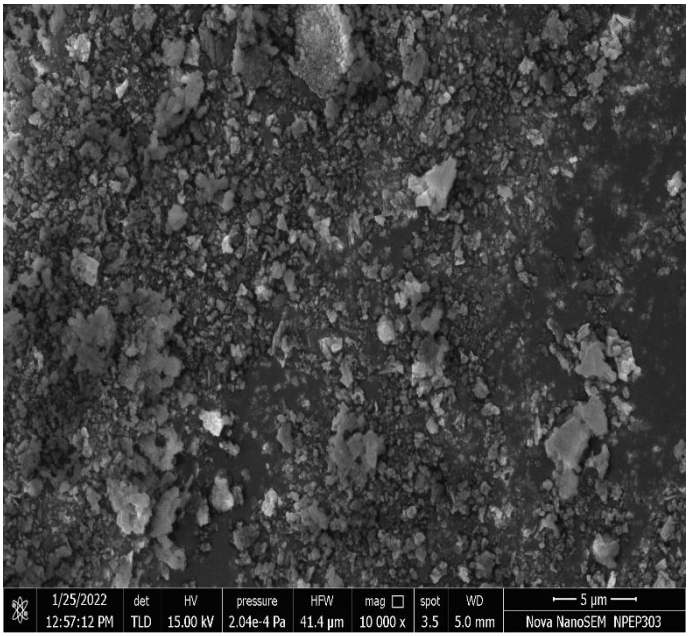


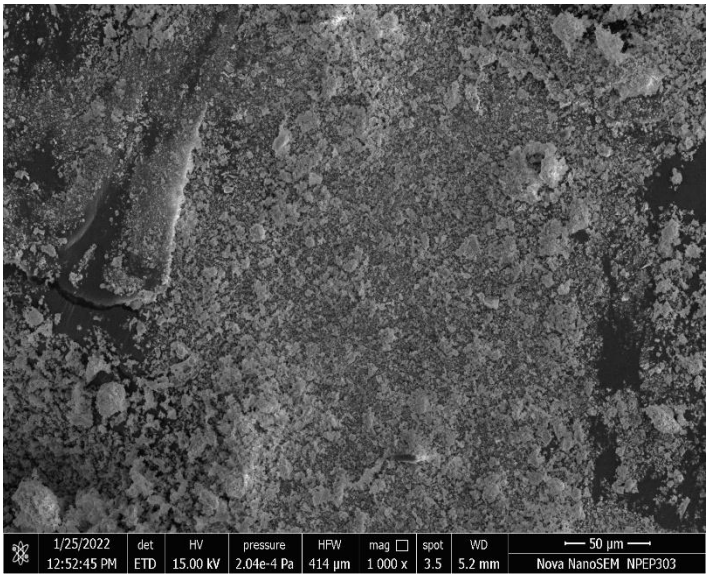
Fig 20 UV – Visible graph by tauc method

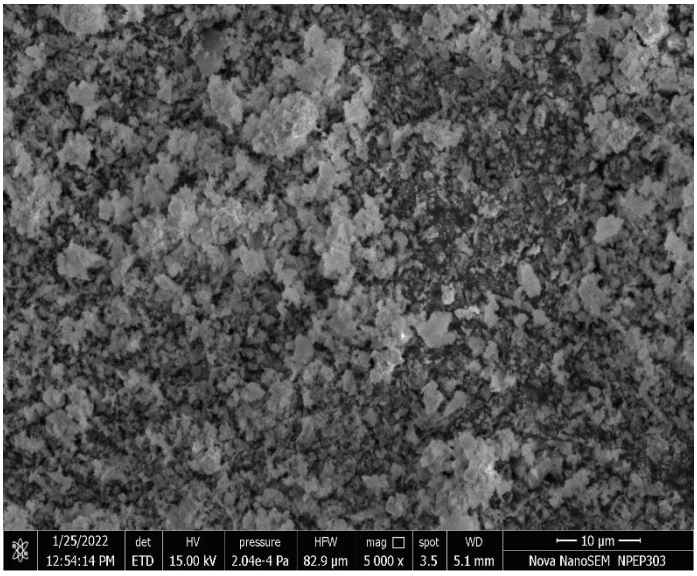
4.1.3 Field Emission Scanning Electron Microscope (FESEM) Study:

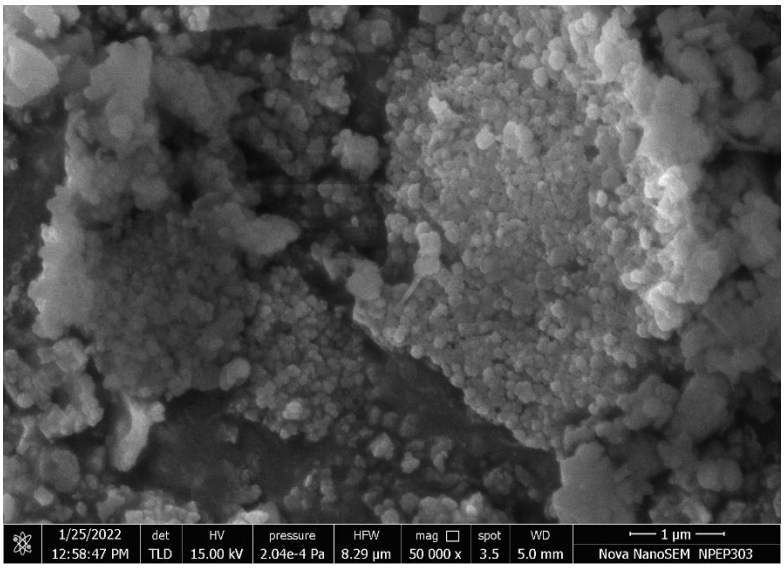
The synthesized product of CeO_2characterized by using FESEM technique which is used for the study of surface morphology. CeO_2shows particles are nearly spherical in shape morphology with slightly agglomeration as shown in the Figure.

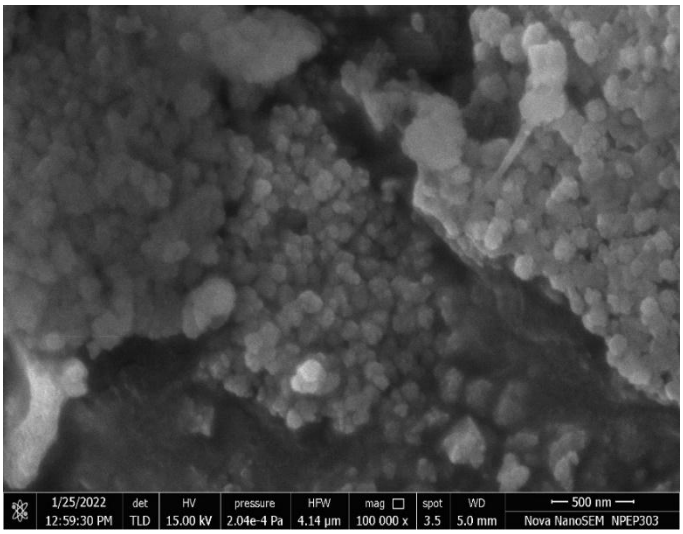












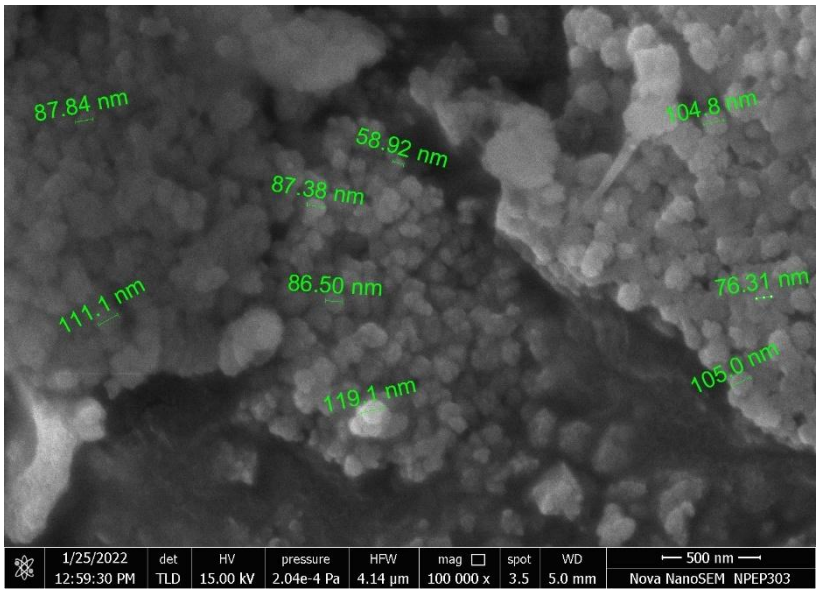
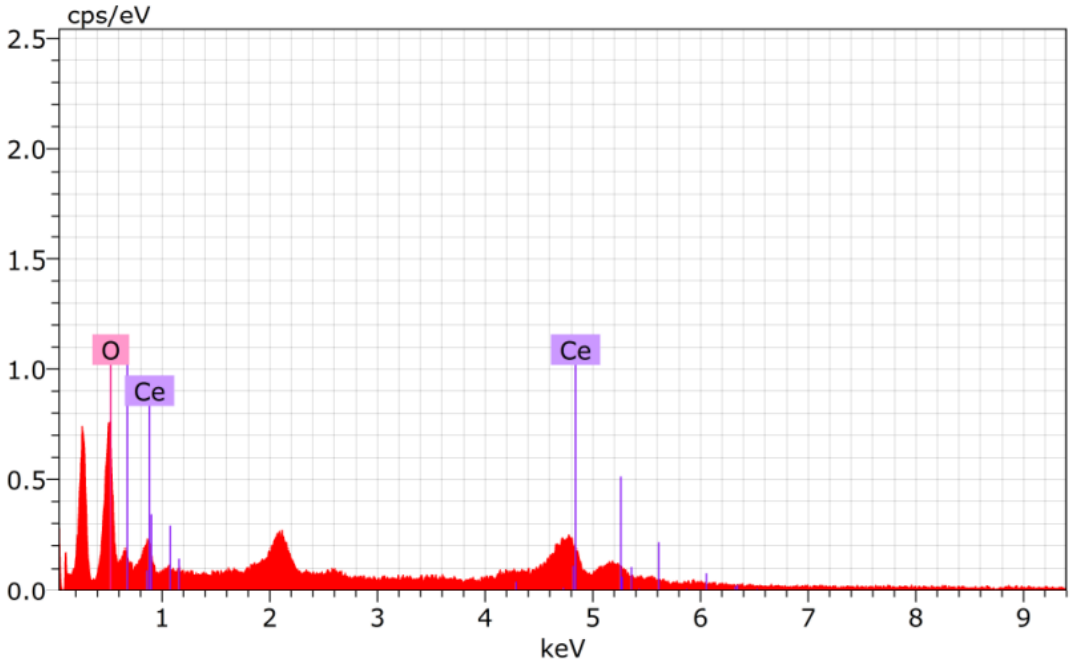


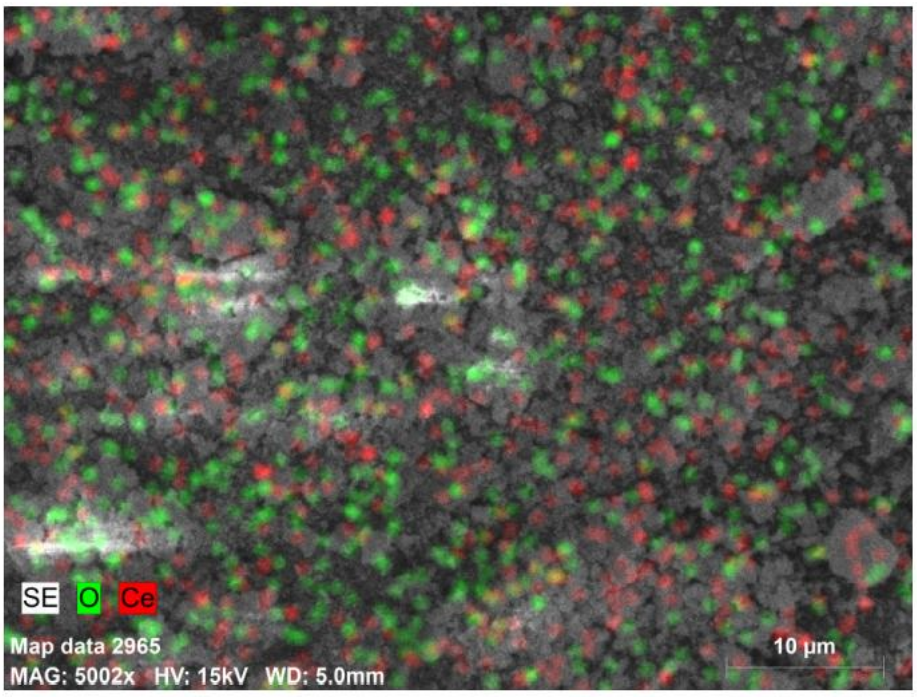
Fig 21 FESEM Images

EDS Analysis

From EDS Analysis we confirm the elements present in the sample . The elements which are present are Ce and O i.e. Cerium and Oxygen.



EDS Report



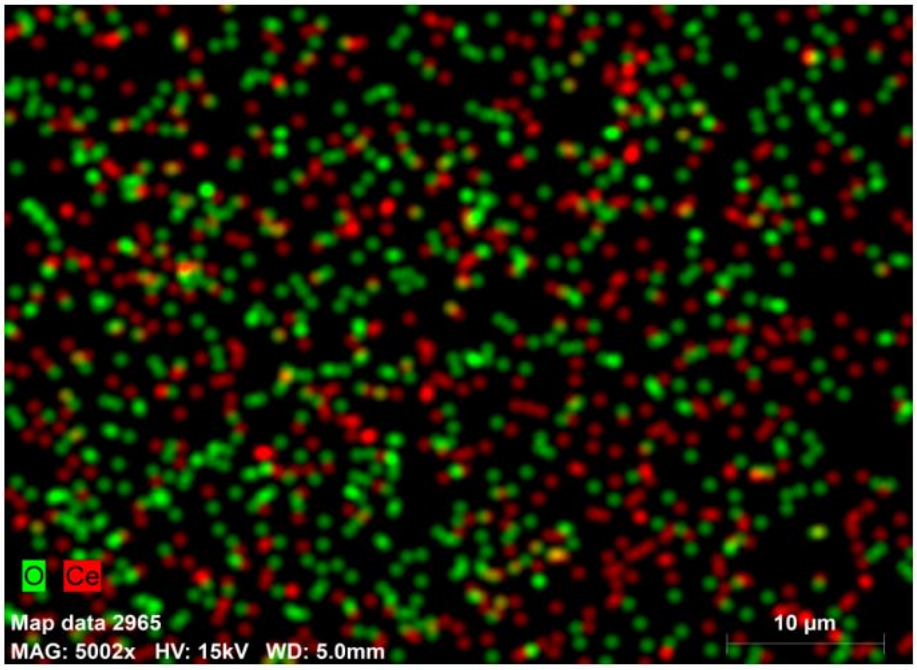


Fig 22 EDX Images

4.1.4 Fourier Transform Infrared (FT-IR) Spectroscopy:

FT-IR analysis was carried out to determine the possible biomolecules or Functional groups which responsible for stabilization of Synthesized CeO_2. From fig [4a] and [4b] we can compare the functional groups of both the leave extract and Synthesized CeO_2. The spectra shows that the there was a shift in the following peaks: 3340.71-3269cm^{-1},1398.39-1400.32 cm^{-1}, 1503.13-1026.13cm^{-1}, 599.86-555.50cm^{-1} and 374.19-376.12cm^{-1}. The highest peaks of both the FT-IR are 3340.71-3269$cm^{-1}$and they are attributed to -OH or N-H streching vibration. The peak shift implicates that it may involve in the formation of CeO_2. 1635.64 cm^{-1} gives the C=C stretching, peak 1398.34cm^{-1} can be assigned to the C-O, C-C, C=O groups. The NH and COOH groups which has been demonstrated to be excellent srabilizing effect [12]. Therefore, the stabilizing effect is seen due to the carboxylic, hydroxylic and amide groups of Aegle Marmelos leaves

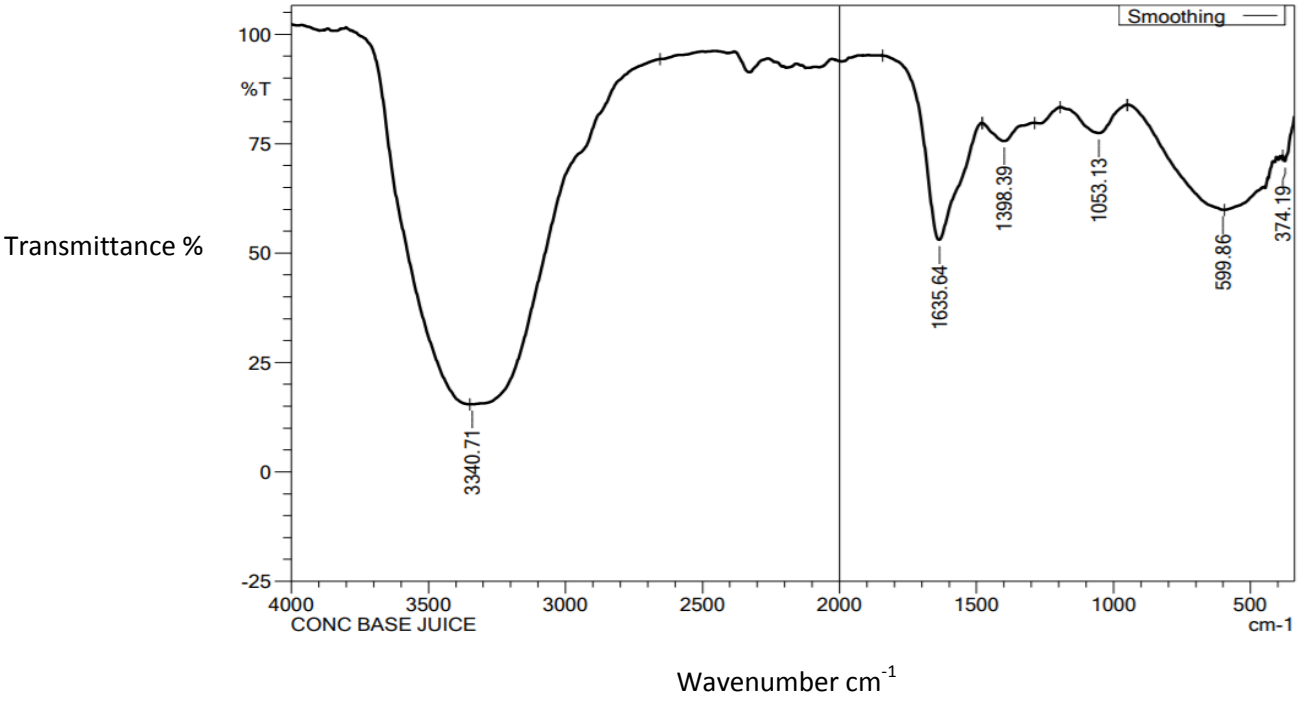


Fig 23 FTIRGraph of Bael Juice

Functional Group For Bael juice	Wavenumber cm^{-1}	Correspondance Frequency f = c*Wavenumber(GHz)
O-H (alcohol)	3340.71	1002.213
Primary amines	1635.64	490.69
Nitro Compound	1398.39	419.517
Aliphatic amines	1053.13	315.93
Alkyle halides	599.86	179.95

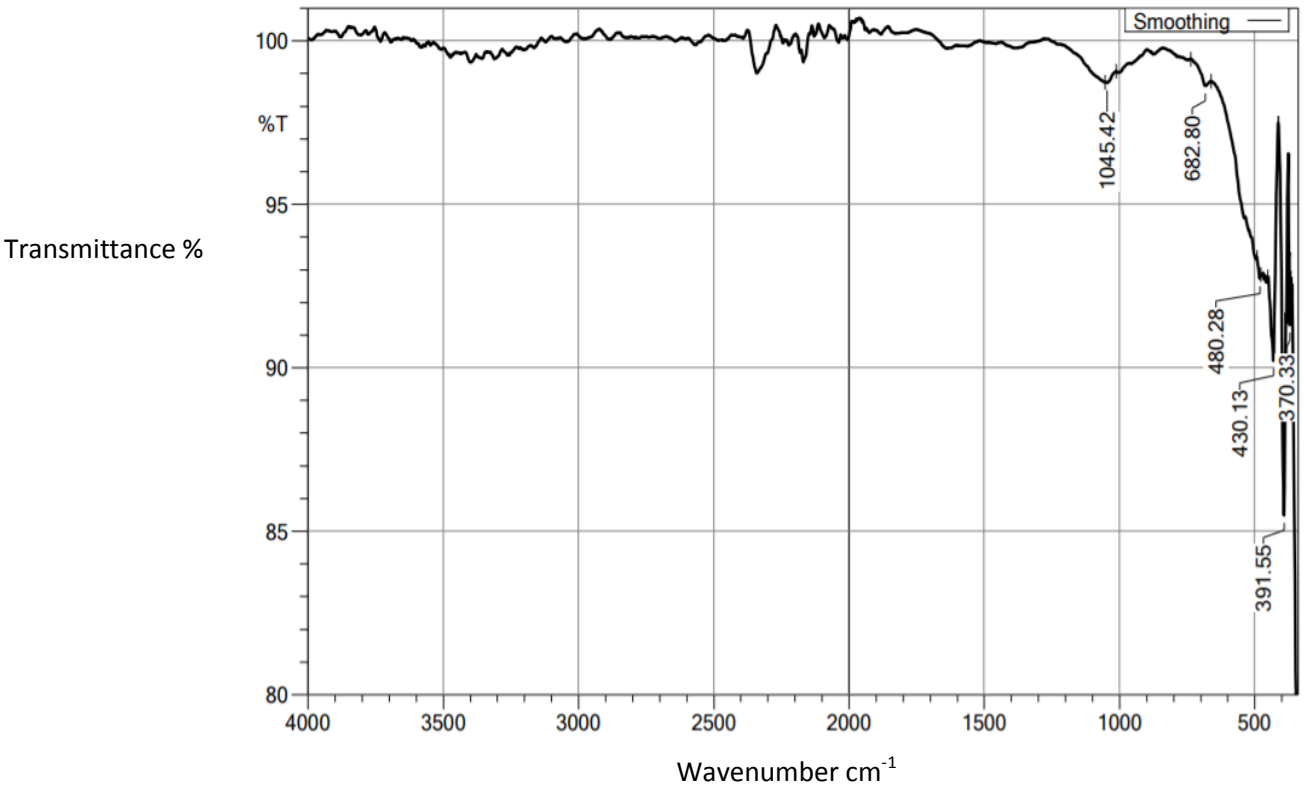


Fig 24 FTIR Grapg of CeO_2Nps

Functional Group For CeO_2	Wavenumber cm^{-1}	Correspondance Frequency f = c*Wavenumber(GHz)
C-O Stretching	1045.42	313.62
Aromatic alkenes	682.80	204.84
Ce-O stretching	480.28	144.08
Ce-O stretching	430.13	129.03
Ce-O stretching	391.55	117.46
Ce-O stretching	370.33	111.09

4.1.5 Raman spectroscopy

Raman Spectroscopy is a non-destructive chemical analysis technique which provides detailed information about chemical structure, phase and polymorphy, crystallinity and molecular interactions. It is based upon the interaction of light with the chemical bonds within a material. The raman shift is observed at 628.213 cm-1.

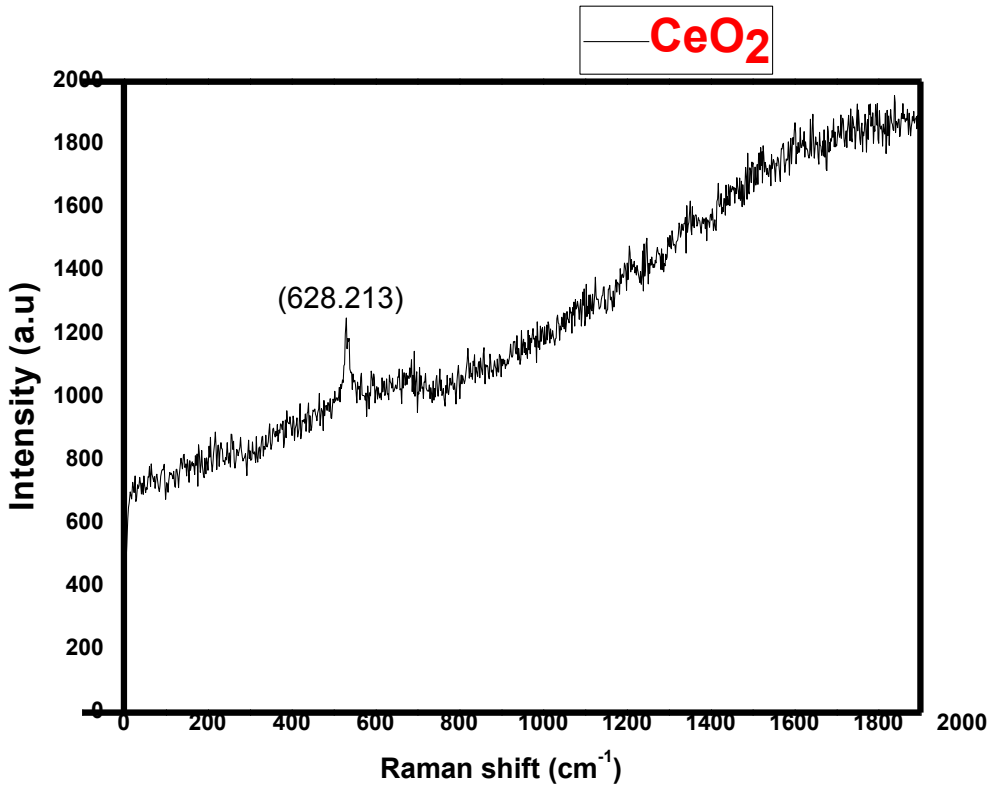


Fig 25 Graph of Raman

4.1.6 Photoluminicence

From Photoluminicence Analysis we confirm that the emmisions are taking place in Visible regionofElectromagneticradiations

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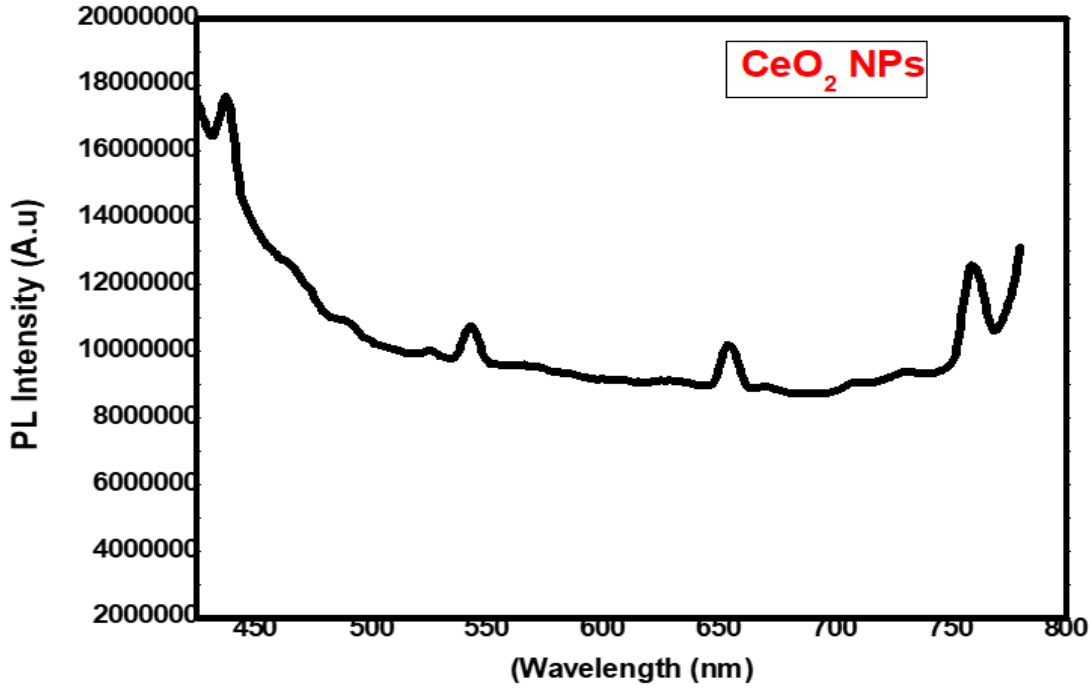
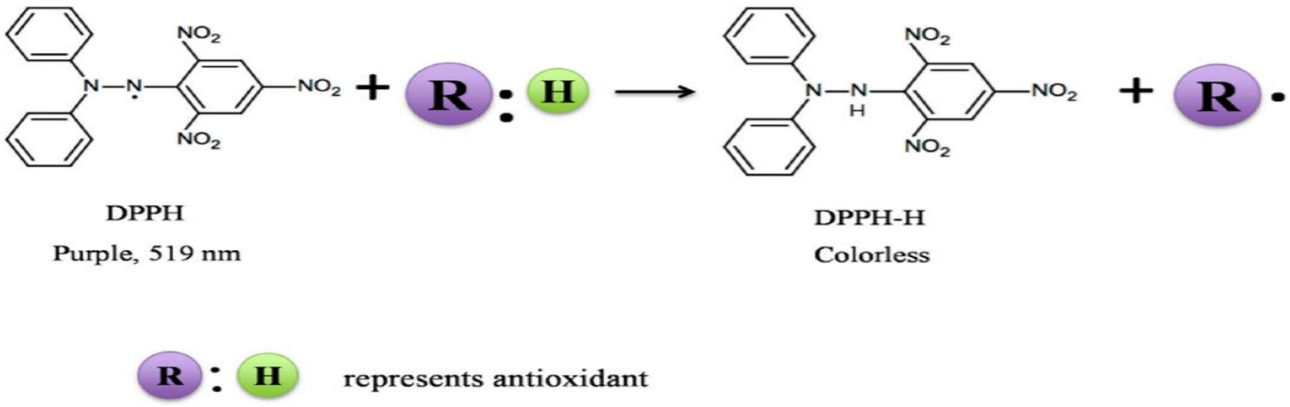


Fig 26 Graph of Photoluminicence

APPLICATION Of CeO_2

Antioxidant activities of CeO_2NPs

CeO_2 NPs were also claimed to contain antioxidant activity. Antioxidant activity is associated with the reduction of the 1,1-diphenyl-2-picrylhydrazile (DPPH) amounts as shown in the below figure To test the synthesized NPs, a method of scavenging DPPH free radicals was used. Generally, electrons/protons exchanged from CeO_2 NPs scavenge the DPPH free radicals. The transferred electrons/protons are derived from CeO_2 NPs -coated biomolecules.



Antioxidant activity of various test compounds by DPPH and NO radicals scavenging activity

Principle:

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical and is commonly used to evaluate the radical scavenging activity of antioxidant agents. The principle of this method is based on the fact that, decrement of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non radical form DPPH-H.

Various concentration dilutions

1 ml (1000 µg/ml) diluted to 2 ml to produce 500 µg/mL

1 ml (1000 µg/ml) diluted to 10 ml to produce 100 µg/mL

1 ml (1000 µg/ml) diluted to 100 ml to produce 10 µg/mL

1 ml (10 µg/mL)- diluted to 10 ml to produce 1 µg/mL

Procedure:

The free radical scavenging activity of test compound CeO_2 were determined by DPPH scavenging method as per procedure described by Shen et al., (2010).

Briefly,in this method, 0.1mM DPPH solution was prepared in methanol by adding 39.4 mg of DPPH in 1000 ml of methanol, and to 0.5 mL of this solution, 1.5 mL of test compounds

NISH01, SH402, SK03, AK04, and HP05 dissolved in DMSO were added at various concentrations (1, 10, 100, 500 & 1000 µg/mL).The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using non-coated 96 well plate on microplate reader (EPOCH, Agilent BioTek, US). Vitamin C was used as standard compound. Reduction in absorbance by test compounds indicates radical scavenging activity.

The scavenging activity by the DPPH radical was determined by

DPPH scavenging effect (% inhibition) = $\{(A_0 - A_1)/A_0) \times 100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance test compound or vitamin C.

Nitric oxide (NO) radical scavenging Activity

Principle:

Nitric oxide (NO) is a free radical that produced by interaction of NO with oxygen or reactive oxygen species.

It is free radical due to its unpaired electron and exhibits similar properties like superoxide free radicals.

Procedure:

Nitric oxide (NO) radical scavenging activity of CeO_2 were determined as

per the procedure described by Balakrishnan et al (2009).

Briefly, various concentrations of test CeO_2 (as 1, 10, 100, 500, and 1000 µg/ml) were dissolved in water. To which, 0.5 mL of 10 mM

sodium nitro prusside in phosphate buffered saline was added, then, 1 ml of various concentrations

1, 10, 100, 500, and 1000 µg/ml) of test compounds were mixed, and to this equal volume of freshly prepared

Griess reagent was added, solution was then incubated at 25°C for 3 hours.

From above solution, 100 µl of the reaction mixture was transferred to a non coated 96-well plate,

and the absorbance was read at 546 nm using a microplate reader (EPOCH, Agilent BioTek, US)

. Ascorbic acid was used as standard control.

The percentage of nitrite radical scavenging activity of test compounds was calculated by

Nitric oxide scavenging activity =

$$\frac{(\text{Absorbance of control} - \text{Absorbance of test compounds}) \times 100}{\text{Absorbance of control}}$$

Effects of various test compounds on DPPH radical scavenging Activity

Test compounds	Concentration (µg/mL)	Absorbance		Mean	Scavenging activity (%) DPPH
		1	II		
CeO_2					
	10	0.017	0.019	0.018	11.11
	100	0.023	0.036	0.0295	45.76
	500	0.067	0.066	0.0665	75.94
	1000	0.207	0.263	0.235	93.19

Chapter 5

Conclusion

- Plant mediated synthesis of nanoparticles have several advantages over physical and chemical methods.
- CeO_2 synthesized by green synthesis method with leaves extract of Aegle Marmelos. The leaves extract bioactive components play vital role for formation of CeO_2.
- Moreover, the prepared CeO_2 average nanoparticle size was found to be 5.3 nm determined by using XRD technique. UV- Visible shows band gap energy 3.4 eV . Ramanspectroscopy shows raman shift at 628.213 cm^{-1}.Photoluminicence shows emission in visible region and absortion in Ultraviolet region.
- Field emission scanning electron microscope (FE-SEM) reveals a spherical shape of CeO_2 with agglomerate morphology .
- Synthesized can be explored for antioxidant.

CHAPTER 6

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