DEPARTMENT OF CHEMISTRY AND CENTRE FOR RESEARCH

ST. TERESA'S COLLEGE (AUTONOMOUS)

ERNAKULAM

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B.Sc. CHEMISTRY PROJECT REPORT

Name : **IRIN JOGI**
Register Number : **AB21CHE005**
Year of Work : **2023-2024**

This is to certify that the project **"BACOPA MONNIERI EXTRACT MEDIATED GREEN SYNTHESIS, CHARACTERISATION AND APPLICATIONS OF ZINC OXIDE NANOPARTICLES"** is the work done by **IRIN JOGI.**

Dr. Saritha Chandran A.
Head of the Department

Mr. Linto Anto
Staff-member in charge

ST. TERESA'S COLLEGE (AUTONOMOUS) DEPARTMENT OF CHEMISTRY

Submitted to the Examination of Bachelor's Degree in Chemistry

Date: 4/5/24

Examiners: Dr. Ajith James Jose, SB College Chgy
: Dr. Nisha P.P.

PROJECT REPORT

On

BACOPA MONNIERI EXTRACT MEDIATED GREEN SYNTHESIS, CHARACTERISATION AND APPLICATIONS OF ZINC OXIDE NANOPARTICLES

Submitted by

IRIN JOGI (AB21CHE005)

In partial fulfilment for the award of the

Bachelor's Degree in Chemistry

DEPARTMENT OF CHEMISTRY AND CENTRE FOR RESEARCH

ST. TERESA'S COLLEGE (AUTONOMOUS)
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CERTIFICATE

This is to certify that the project work entitled **"BACOPA MONNIERI EXTRACT MEDIATED GREEN SYNTHESIS, CHARACTERISATION AND APPLICATION OF ZINC OXIDE NANOPARTICLES"** is the work done by **IRIN JOGI** under my guidance in the partial fulfilment of the award of the Degree of Bachelor of Science in Chemistry at St. Teresa's College (Autonomous), Ernakulam affiliated to Mahatma Gandhi University, Kottayam.

MR. LINTO ANTO
Project Guide

DECLARATION

I hereby declare that the project work entitled **"BACOPA MONNIERI EXTRACT MEDIATED GREEN SYNTHESIS, CHARACTERISATION AND APPLICATION OF ZINC OXIDE NANOPARTICLES"** submitted to Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous) affiliated to Mahatma Gandhi University, Kottayam, is a record of an original work done by me under the guidance of **MR. LINTO ANTO, ASSISTANT PROFESSOR,** Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous), Ernakulam and this project work is submitted in the partial fulfilment of the requirements for the award of the Degree of Bachelor of Science in Chemistry.

IRIN JOGI

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IRIN JOGI

Contents

Chapter 1

Introduction

1.1 NANOCHEMISTRY

Nanochemistry is the combination of chemistry and nanoscience that deals with designing and synthesis of materials of nanoscale of different shapes and size, structure and composition. It is being used in chemical, materials and physical science as well as engineering, biological and medical applications. It is relatively a new branch of chemistry concerned with the unique properties associated with assemblies of atoms or molecules of nanoscale (1-100 nm), so the size of nanoparticles lies somewhere between individual atoms or molecules and larger assemblies of bulk materials which we are more familiar with. Nano chemistry has also been used to study the health and safety effects of airborne and waterborne nanosized particulates, and nanoparticles have been used to clear up or neutralize pollutants. Nano chemistry can be characterized by concept of size, shape, self-assembly, defects etc. So, the synthesis of any new nano-construct synthesis is associated with all these concepts. Nano –construct synthesis is dependent on how the surface, size and shape will lead to self-assembly of the building blocks into the functional structures. Nanotechnologies involve the creation and manipulation of materials at the nanometer scale, either by scaling up from single group of atoms or by refining or reducing bulk materials.

In 1959 Richard Feynman gave a lecture at the American Physical Society titled “There’s plenty of room at the bottom: an invitation to enter a new field of physics,” proposing to focus on manipulating matter on atomic- or nano- scale to build devices from bottom up. It went largely unnoticed then, but since the 1980s the lecture has been regarded as an inception point of nanotechnology and nanoscience. To systematize and categorize the areas of studies “at the bottom,” plenty of nano terminology has been introduced since, with just a few examples named below. The relatively new field of nano chemistry has been developing from the convergence of the parts of many mature chemistry subfields relevant to nanomaterials, including, but not limited to, colloid and interface chemistry, surface chemistry, inorganic, organic, polymer, physical chemistry, and material chemistry. As a result of this amalgamation of many branches of chemistry to attempt to fully grasp all the chemical phenomena occurring on the nanoscale, nano chemistry has become a complex and multifaceted field of study with blurred boundaries with other chemical subdisciplines.

Since nanomaterials can significantly improve the properties/functions of an object, we hope to see this technology used more going forward. But in order for more nanomaterials to be moved out of laboratories and into the market, many parties have a role to play:

1. First and foremost, it is the role of a nano chemist to achieve facile and tailored synthesis of nanomaterials with desired functions at a low cost.

2. Because nanoscience is an interdisciplinary field, scientists from various fields, such as physics and biology, need to work together to achieve the goals.

3. And finally, the general public and policymakers should give their input regarding the commercialization and utilization of nanotechnology.

1.1.1 Nanoparticles

Nanoparticles are particles between 1-100 nanometres. Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structures. Nanoparticles are usually distinguished from microparticles (1-1000 μm), "fine particles" (sized between 100 and 2500 nm), and "coarse particles" (ranging from 2500 to 10,000 nm), because their smaller size drives very different physical or chemical properties, like colloidal properties and ultrafast optical effects or electric properties.

Nanoparticles occur widely in nature and are objects of study in many sciences such as chemistry, physics, geology and biology.[1] Being at the transition between bulk materials and atomic or molecular structures, they often exhibit phenomena that are not observed at either scale. They are an important component of atmospheric pollution, and key ingredients in many industrialized products such as paints, plastics, ceramics and magnetic products. The production of nanoparticles with specific properties is a branch of nanotechnology.

1.1.2 Synthesis Approaches

In the field of nanochemistry, the synthesis of nanomaterials plays a crucial role in bridging dimensions from the micro to the nano scale. Two primary approaches, namely the bottom-up and top-down approaches, are employed for the fabrication of nanomaterials. Each approach offers distinct advantages and limitations, making them suitable for different applications. This section provides an in-depth overview of these approaches and presents a comprehensive comparison between them.

1. Bottom-Up Approach

Atomic or molecular components are assembled from the “bottom” to create complex nanostructures. This method precisely controls nanomaterial composition, structure, and properties. Bottom-up chemical synthesis creates nanoscale structures. Chemical synthesis involves many nanomaterial fabrication methods. Sol-gel synthesis produces nanoparticles, thin films, and porous materials. This method hydrolyses and condenses a precursor solution to form a gel, which can be processed into nanomaterials. Self-assembly uses molecular interactions to form hierarchical structures. This method spontaneously organizes nanoscale building blocks into complex architectures. Electrostatic, van der Waals, and hydrogen bonding can cause self-assembly. Researchers can precisely control nanostructures by designing molecular components and interactions. Bottom-up approaches use chemical synthesis, self-assembly, vapour deposition, atomic layer deposition, and molecular beam epitaxy to make nanomaterials with precise properties. Layer-by-layer material deposition or growth allows for atomically controlled structures.

2. Top-Down Approach

The top-down approach fabricates nanomaterials by trimming bulk materials from the “top” down. This method breaks down larger structures to create nanoscale structures. Top-down lithography, which etch or print nanoscale features on a substrate, is widely used in the semiconductor industry. Photolithography and electron beam lithography use masks or focused particles or light to selectively modify or remove nanoscale material. These methods allow precise material patterning and nanoscale device and integrated circuit creation. Another top-down method for nanoparticle and nanocomposites production is mechanical milling. Grinding, crushing, or milling bulk materials to nanoscale size is this method. Mechanical milling works well for materials that are difficult to synthesize chemically or require specific mechanical properties. Top-down methods also use laser ablation and nanoscale etching to shape and modify materials. Laser ablation creates nanoparticles by vaporizing and removing material. Reactive ion etching use chemical reactions to selectively remove material, creating intricate nanostructures.

1.1.3 Uses of nanoparticles

Nanomaterials are at the leading edge of the rapidly developing field of nanotechnology. Their unique size-dependent properties make these materials superior and indispensable in many areas of human activity.

1. Nanoparticles are being used in the manufacture of scratchproof eyeglasses, crack resistant paints, anti-graffiti coatings for walls, transparent sunscreens, stain-repellent fabrics, self-cleaning windows and ceramic coatings for solar cells

2. Nanoparticles as fillers in tyres can improve adhesion to the road, reducing the stopping distance in wet conditions.

3. Nanoparticles are used increasingly in catalysis to boost chemical reactions. This reduces the quantity of catalytic materials necessary to produce desired results, saving money and reducing pollutants. Two big applications are in petroleum refining and in automotive catalytic converters.

4. Nanostructured ceramic coatings exhibit much greater toughness than conventional wear-resistant coating for machine parts. Nanotechnology enabled lubricants and engine oils also significantly reduce wear and tear, which can significantly extend the lifetimes of moving parts in everything from powerful tools to industrial machinery.

5. Nanoscale materials are beginning to enable washable, durable “smart fabrics” equipped with flexible nanoscale sensors and electronics with capabilities for health monitoring, solar energy capture, and energy harvesting through movement.

6. Nanoscale materials are also being incorporated into a variety of personal care products to improve performance. Nanoscale titanium dioxide and zinc oxide have been used for years in sunscreen to provide protection from sun while appearing invisible on the skin.

7. Nanoscale additives to or surface treatment of fabrics can provide lightweight ballistic energy deflection in personal body armour, or can help them resist wrinkling, staining and bacterial growth.

1.2 ZINC

Zinc is an essential trace element for humans, plants and for microorganisms and is necessary for the prenatal and postnatal development. It is the second most abundant trace metal in humans after iron and it is the only metal which appears in all enzyme classes. Zinc is also known for its strong anti-bacterial and anti-fungal activities.

1.2.1 Zinc oxide

Zinc oxide is a largely inert, white compound which is used very widely as a bulking agent or filler, and as a white pigment. It is found in some rubber, glass and ceramic products, and finds use in the chemical industry as a catalyst. It is also used in paints as a corrosion inhibitor and for mildew control. Zinc is an essential trace element, and zinc oxide is added to fertilizers, animal feed, and vitamin supplements. It is also used in a many cosmetic and medical products and in toiletries, as it has antibacterial and deodorant properties. It is found in, for example, baby powder and anti-dandruff shampoos, in calamine lotion and in sticking plasters and dental cement. Its strong absorption of ultra-violet (UV) light has led to its use in sunscreen lotions.[2] It blocks both UVA (longer wavelength) and UVB (shorter wavelength) radiation, protecting against sunburn, skin damage and cancer.

1.3 ZINC OXIDE NANOPARTICLES

Zinc oxide nanoparticles are nanoparticles of zinc oxide that have diameters less than 100 nanometres. They have a large surface area relative to their size and high catalytic activity. The physical and chemical properties of zinc oxide nanoparticles depend on the different ways they are synthesised. Some of the techniques available for the production of zinc oxide nanoparticles are laser ablation, hydrothermal methods, electrochemical decompositions, etc. ZnO is a wide-bandgap semiconductor with an energy gap of 3.37 eV at room temperature.[2]

1.3.1 Uses of zinc oxide nanoparticles

Zinc oxide nanoparticles is known for its low toxicity and high UV absorption making it a good material to be used in the biomedical field.it also has a hard and rigid structure making it useful in the ceramic industry. Zinc oxide nanoparticles is naturally known as a strong resistance to microbes. Due to these reasons zinc oxide nanoparticles is extensively used for biological labelling, biological sensing, drug delivery, and nanoscience.

1.3.2 Synthesis of zinc oxide nanoparticles

Nanoparticles are conventionally prepared by physical and chemical methods (ultrasonication, sol-gel method, laser vaporisation routes etc). Conventional methods of synthesising nanoparticles are costly, use toxic reagents, require complicated procedures, and expensive equipment, consume a large amount of time and energy, require organic solvents and non- biodegradable stabilization agents and are thus environmentally harmful. Out of these methods there has been a study progress in developing reliable and cost-effective strategies for the clean production of zinc oxide nanoparticles (ZnO-NPs) owing to their structural and functional characteristics. A more promising and different form of synthesis from chemical and physical synthesis is the biological or green production of nanoparticles. The use of safe reagents, such as water and natural extracts, eliminated the need to use hazardous ingredients and was an amazing way to create metal nanoparticles. The use of plant and plant extracts is an appealing, innovative, and safe method choice. Plant extracts facilitate the control and precise synthesis of nanoparticles with proper shape and size.[3] Hence, we opted the leaves of Bacopa monnieri for the synthesis of zinc oxide nanoparticles. The zinc nitrate hexahydrate is the metal precursor. In this work, we present a green method for the synthesis of zinc oxide nanoparticles using the leaves of Bacopa monnieri.

1.4 BACOPA MONNIERI

Fig 1.1 Bacopa monnieri

Bacopa monnieri is a plant species in the genus Bacopa and family Plantaginaceae. It is a perennial, creeping herb native to the wetlands of southern and eastern India, Australia, Africa, Asia, and north and south America. It is commonly known by the name water hyssop, Brahmi, thyme-leafed gratiola, herb of grace and Indian pennywort. It is a plant that has been used for centuries in traditional and ayurvedic medicine. Bacopa might increase certain brain chemicals that are involved in thinking, learning and memory. The leaves of this plant are succulent, oblong and 4-6 mm thick.it can even grow in brackish conditions. Very few studies were reported on the chemical and medicinal applications of Bacopa monnieri.

1.4.1 BENEFITS OF BACOPA MONNIERI

1. Bacopa monnieri contains powerful compounds that may have antioxidant effects. These are substances that protect against cell damage caused by potentially harmful molecules called free radicals.

2. Bacopa monnieri have potent anti-inflammatory properties and suppress pro-inflammatory enzymes and cytokines.

3. Bacopa monnieri can improve memory, attention, and the ability to process visual information.

4. Bacopa monnieri can be used to reduce stress and anxiety by elevating mood and reducing cortisol levels.

5. Bacopa monnieri helps to keep the blood pressure within a healthy range.

6. High levels of antioxidants and compounds like bacosides in Bacopa monnieri is responsible for its anti-cancer properties.

1.5 CHARACTERISATION

UV-Visible Spectroscopy

UV-visible spectroscopy (UV-vis) is a technique widely employed to detect the formation of complexes between the molecules of the inhibiting compounds and the metal in the electrolyte. Information on the various adsorption bands and transitions based on the existence of various functional groups are provided with the use of UV-visible spectroscopy. This technique is commonly used to characterize nanoparticles. It allows to confirm the nanoparticle formation by measuring the Surface Plasmon Resonance (SPR). This procedure can provide information about the size, stability, and aggregation of the NPs. UV-Visible spectroscopy is an analytical technique commonly used for the characterization of materials, including zinc oxide (ZnO). In this introduction, we'll focus specifically on how UV-Visible spectroscopy is employed to study the properties of ZnO. It is a non-destructive method that investigates the interaction of ultraviolet and visible light with a substance. It measures the absorption of light in the UV (200-400 nm) and visible (400-750 nm) regions of the electromagnetic spectrum. This technique is widely utilized in various scientific disciplines to study the electronic transitions and optical properties of materials.

Zinc oxide (ZnO) is a semiconductor material with a wide bandgap energy (approximately 3.3 eV) and exhibits unique properties. It has a diverse range of applications, including in optoelectronic devices, sensors, catalysts, and sunscreen formulations. ZnO's optical and electronic properties make it an ideal candidate for study using UV-Visible spectroscopy. When ZnO nanoparticles or thin films are exposed to UV or visible light, electronic transitions occur. Electrons in the material absorb energy from incident photons, moving from the ground state to excited states. The energy of the absorbed light corresponds to the energy gap between electronic energy levels in the material. The spectrophotometer comprises a light source, a monochromator to select specific wavelengths, a sample holder or cuvette, and a detector. The instrument measures the intensity of light before and after it passes through the ZnO sample. The absorbance spectrum is then recorded. By measuring the absorbance of the ZnO sample at various wavelengths across the UV and visible spectrum.

The resulting UV-Visible absorption spectrum will exhibit characteristic peaks, which provide information about the electronic transitions and optical properties of ZnO. The position and intensity of these peaks can be analysed to understand the bandgap energy, particle size, and other relevant optical features.

X-Ray Diffraction spectroscopy

X-ray diffraction (XRD) is a non-destructive analytical technique that provides detailed information about the atomic and molecular structure of crystalline materials. It is widely used in material science, chemistry, and physics to determine the arrangement of atoms within a substance. Zinc oxide (ZnO) is a semiconductor material with a hexagonal wurtzite crystal structure. Its unique structural properties, including the lattice constants and crystal symmetry, make it a compelling material for various applications.

XRD spectroscopy works on the principle of Bragg's Law, which describes the scattering of X-rays by a crystalline lattice. When a monochromatic X-ray beam is directed onto a crystalline sample, the X-rays are scattered by the crystal lattice, and they interfere constructively at specific angles, resulting in diffraction peaks on a detector. The positions and intensities of these diffraction peaks are related to the crystal structure and spacing between the atomic planes within the material. An XRD spectrometer consists of an X-ray source (usually a copper Kα radiation source), a sample holder, and a detector. The X-ray beam is directed at the sample, and the resulting diffraction pattern is recorded on the detector. The XRD patterns collected from ZnO nanoparticles show a series of diffraction peaks at various angles (2θ). The positions and intensities of these peaks are used to identify the crystal structure of ZnO.

Scanning electron microscopy

Scanning Electron Microscopy (SEM) is an analytical technique for characterizing the morphology, size, and surface features of materials, including zinc oxide (ZnO) nanoparticles. Scanning Electron Microscopy (SEM) is an advanced imaging technique that utilizes a focused electron beam to probe the surface of a specimen. It offers high-resolution, three-dimensional imaging of the sample and provides valuable insights into its topographical and morphological features. SEM is widely used in materials science, nanotechnology, and various scientific fields. Zinc oxide (ZnO) nanoparticles are of great interest due to their unique properties, such as a wide bandgap energy, high surface area, and excellent optical and electrical characteristics. They find applications in diverse areas, including sensors, photocatalysis, nanocomposites, and electronics.

In SEM, a focused electron beam is scanned across the sample's surface and interactions between the electrons and the sample generate various signals. The primary signal used for imaging is the secondary electrons emitted from the sample's surface. These electrons provide information about the sample's topography and can create detailed, high-resolution images.

SEM instrument consists of an electron gun, a series of electromagnetic lenses to focus the electron beam, a specimen chamber, and detectors for various signals. The electron beam scans across the sample, and the emitted signals are collected and used to create images of the sample's surface. During SEM analysis of ZnO nanoparticles, high-resolution images are obtained, revealing the particle's size, shape, distribution, and surface morphology. The SEM images provide detailed information about the size distribution of ZnO nanoparticles, their shape (spherical, rod-shaped, etc.), and any agglomeration or aggregation. The surface features, such as cracks, pores, and surface roughness, can also be observed.

Scanning Electron Microscopy is a valuable tool for characterizing ZnO nanoparticles, providing detailed insights into their morphology, size distribution, and surface features. This information is critical for designing and tailoring ZnO nanoparticles for a wide range of technological applications.

1.6 APPLICATIONS OF ZINC OXIDE NANOPARTICLES

Zinc oxide nanoparticles are being used in numerous technologies and is incorporated into wide array of consumer products that make use of its optical, conductive and antibacterial properties.

1. Zinc oxide nanoparticles have various applications in food industry. They can be used as food preservatives and additives due to their antibacterial properties and nutritional functions.

2. Zinc oxide nanoparticles have therapeutic activity against cancer, diabetes, microbial infection and inflammation. It can be also used as drugs, imaging tools and biosensors.

3. Zinc oxide nanoparticles have potential application in the field of nanoscience. They have the ability to remove heavy metals from wastewater, making them useful as nano adsorbents.

4. Zinc oxide nanoparticles has a unique optical, chemical sensing, semiconducting, electrical conductivity and piezoelectric properties. Due to these properties, it is applied in solar cells, photocatalysis and chemical sensors.

1.7 OBJECTIVES

- To synthesis zinc oxide nanoparticles using Bacopa monnieri plant extract.
- Characterization of zinc oxide nanoparticles by spectroscopic methods.
- To study the antibacterial activity of the synthesised zinc oxide nanoparticles.

Chapter 2

Materials and Methods

This chapter gives a brief description of the materials and experimental procedures adopted for the present investigation.

2.1 MATERIALS

2.1.1 Zinc nitrate hexahydrate

Zinc nitrate hexahydrate manufactured by Nice Chemicals (P) Ltd, Kochi, Kerala was used in this study.

2.1.2 Sodium Hydroxide

Sodium hydroxide manufactured by Nice Chemicals (P) Ltd, Kochi, Kerala was used in this study

2.2 EXPERIMENTAL METHODS

2.2.1 Preparation of leaf extract

Fresh leaves of Brahmi (Bacopa monnieri) are collected from the Cochin estuary area. Leaves weighing 15g were thoroughly washed thrice using sterile distilled water and cut into small pieces. Then it was mixed with 100 mL of sterile distilled water and then transferred into a 250 mL beaker and the content was heated to 60°C for 20 min. After boiling, it was cooled and filtered by means of filter paper and the clear filtrate was stored in a sterile beaker at 4°C for further use.

2.2.2 Synthesis of zinc oxide nanoparticles

For the synthesis of zinc oxide nanoparticles, 20 ml of prepared leaf extract was taken and heated for 10-15 min at 60-70 degree Celsius using a magnetic stirrer. Then 50 ml of 91mM Zinc nitrate hexahydrate solution was added drop wise to it under constant stirring. The colour of the solution turned yellow. 50 ml of 1 M of NaOH was added drop wise till the cream-coloured precipitate of Zinc Hydroxide was formed. The reaction mixture was left for 5-10 min under stirring for the complete reduction to Zinc Hydroxide. Now the reaction mixture was left undisturbed for 1 hour. The precipitate was collected by centrifugation at 1100 rpm for 10 min. The precipitate was washed three times with double distilled water and collected in a ceramic crucible. It was heated in an air heated furnace at 200 degrees Celsius for 1 hour. A light-yellow colored powder was obtained which was collected and mashed in a mortar-pestle so as to get a finer nature of the material for characterisation

Fig 2.1 The powdered form of the prepared ZnO nanoparticles

2.2.3 Characterization

2.2.3.1 Ultraviolet-Visible spectroscopy

Ultraviolet-visible spectroscopy (UV-Visible) refers to absorption spectroscopy or reflectance spectroscopy in part of the ultraviolet and the full adjacent visible spectral regions. 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. Absorption measures transition from the ground state to the excited state. UV-Visible spectrum was recorded using Evolution 201/220 Thermofischer Scientific UV VIS Spectrophotometer in the range of 200-500 nm.

2.2.3.2 Scanning Electron Microscopy (SEM)

SEM was taken using Jeol 6390LA/ OXFORD XMX N spectrometer with Accelerating voltage: 0.5 to 30 Kv, Filament: Tungsten, Magnification x 300000.

2.2.3.3 X-ray diffraction (XRD)

X-ray diffraction is a powerful technique used to analyze the crystal structure of materials. It involves shining X-rays onto a crystalline sample and measuring the angles and intensities of the X-rays that are scattered by the sample. This information is then used to determine the arrangement of atoms within the crystal lattice.

2.3 Applications

2.3.1 Antibacterial activity

Preparation of nutrient media

Nutrient broth was prepared by dissolving 1.3 gram of it in 100 ml distilled water. Test tubes were filled with 5ml of nutrient broth and were sterilized using an autoclave. Nutrient agar media was prepared by mixing 1.3gm of nutrient broth and 2gm of agar-agar in 100 ml distilled water. The media was autoclaved and 20ml each poured into sterile petri plates under aseptic conditions.

Preparation of microbial cultures

Escherichia coli (E. coli) was inoculated into 5ml of sterilized nutrient broth and kept for overnight incubation at 37°C.

Well diffusion method

A lawn culture of each bacterium was prepared using sterilized cotton swabs. A sterilized swab was dipped into the bacterial suspension and moved side to side from top to bottom leaving no space uncovered. The plate is rotated to 90 degrees and the same procedure was repeated so that the entire plate was coated with bacteria. Once the lawn had been prepared, wells of 6 mm diameter was cut into agar plates using sterile well cutter. The wells were labelled and 20µL of zinc oxide nanoparticles were loaded into corresponding wells. The antibacterial activity of zinc oxide nanoparticles were compared with standard antibiotics available. This plate was incubated at 37°C for 24 hrs. The radius of each zone was measured using a standard ruler in centimeters. If the compound is effective against bacteria at certain concentration, no colonies will grow. This is the zone of inhibition, which is a measure of the compound effectiveness, the larger the clear area around the well, the more effective the compound.

Killing and disposing

After the experiment, the bacteria are destroyed by autoclaving the plates for 20 min. All the glassware used for the experiment were also autoclaved to remove any bacteria if present.

Chapter 3

Results and discussion

3.1 CHARACTERISATION OF ZINC OXIDE NANOPARTICLES

3.1.1 UV visible absorption spectroscopy

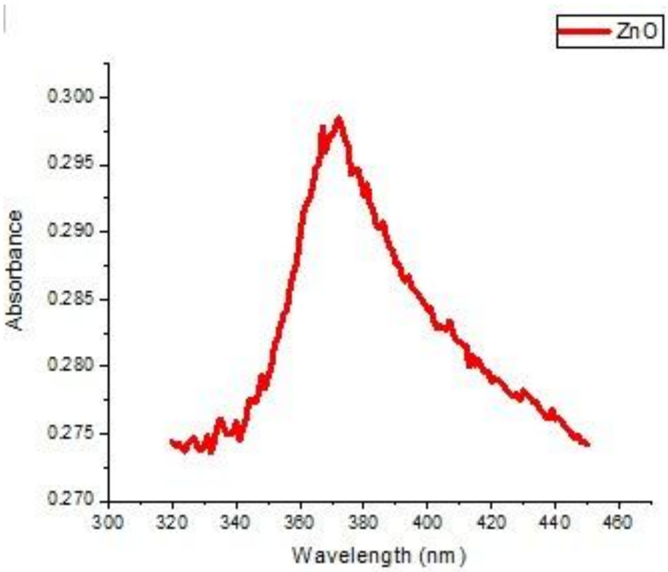

Fig 3.1 UV graph of the synthesised ZnO nanoparticles

UV visible spectroscopy was done for the preliminary confirmation of the synthesised nanoparticles. The given UV spectrum (Fig 3.1) shows absorbance at a wavelength of 372 nm.[4] The characteristic absorbance of ZnO nanoparticles shows absorbance in the range 350-400 nm. Thus, we confirm the synthesis of zinc oxide nanoparticles.

3.1.2 Scanning Electron Microscopy (SEM)

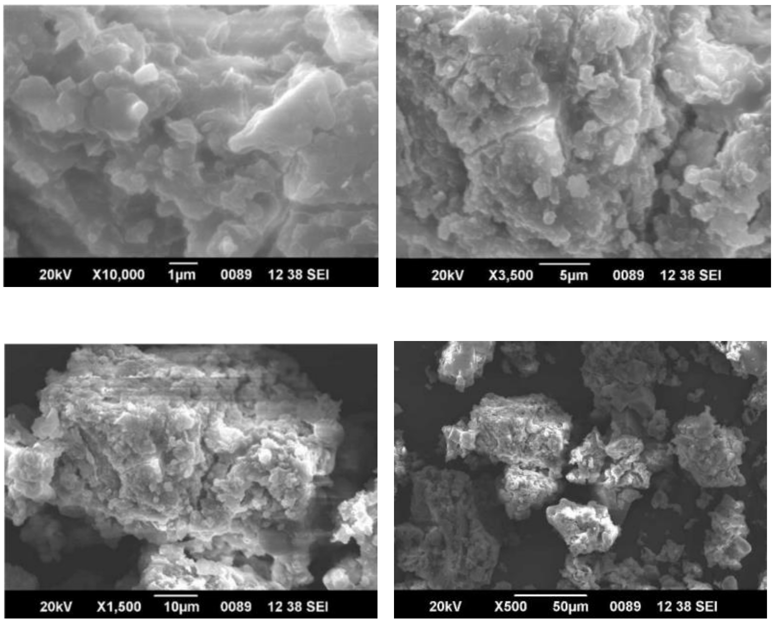

Fig 3.2 SEM images of the synthesised ZnO nanoparticles

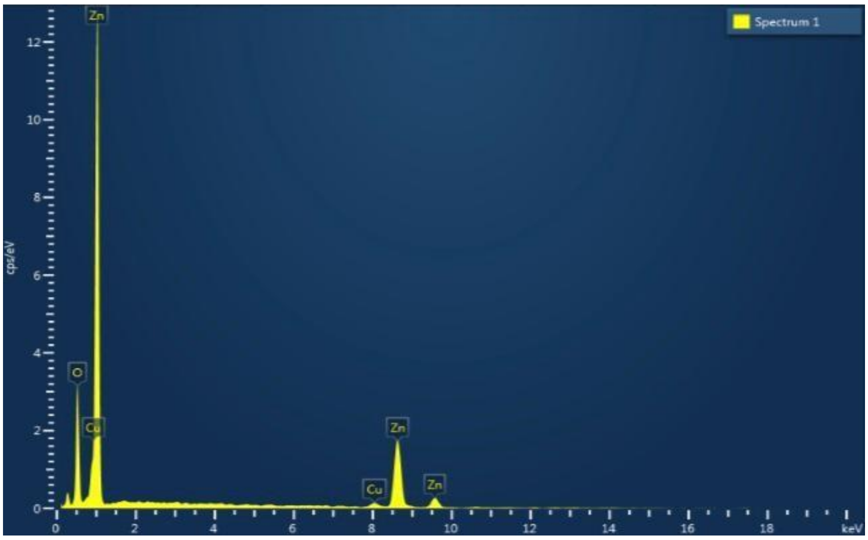

Fig 3.3 SEM graph of synthesised ZnO nanoparticles

Element	Line Type	Wt%	Atomic %
O	K series	25.93	58.82
Cu	K series	2.8	1.6
Zn	K series	71.27	39.58
Total:		100	100

Fig 3.4 EDX spectrum of the synthesised ZnO nanoparticles

The scanning electron microscope analysis was performed to determine the shape and morphology of the prepared zinc oxide nanoparticles under various magnifications and the results are shown in the figure. The SEM images shows the irregular shapes of the zinc oxide nanoparticles.[5] The observation of large nanoparticles in SEM image is attributed to agglomeration. The elemental composition of the green synthesised nanoparticles was determined in EDX. It was found that ZnO nanoparticles synthesised with brahmi leaf extract has 71.27% zinc and 25.93% of oxygen. The EDX graph also shows the presence of copper impurities in small amounts.

3.1.3 X-ray diffraction (XRD)

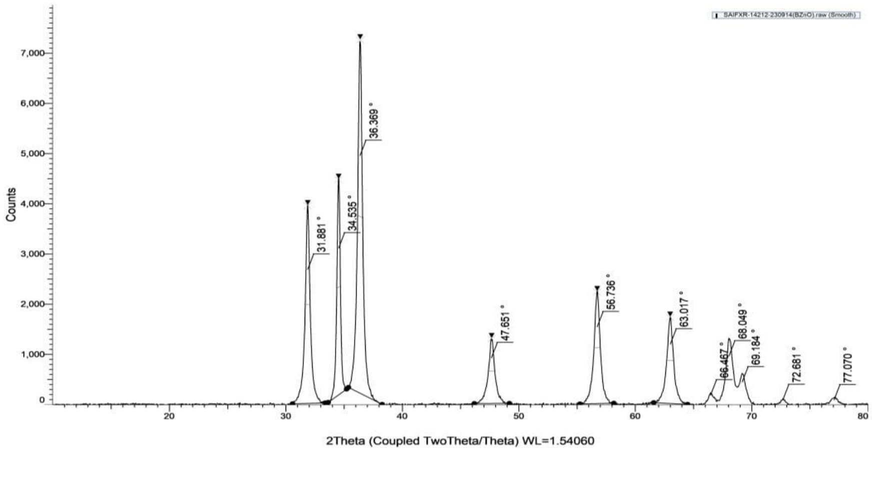

Fig 3.5 XRD graph of the synthesised ZnO nanoparticles

XRD pattern of synthesised sample is shown in figure. The sharp and narrow peaks suggests that synthesised product has good crystallinity with relatively large crystallites. The XRD pattern shows the noticeable peaks corresponding to 2θ values of 31.881°, 34.535°, 36.369 °, 47.651°, 56.736°, 63.017°, 66.467°, 69.184°.[6] The average crystalline size of nanoparticles was estimated using the Debye-Scherer formula $D = (0.89 \times \lambda) / (\beta \times \cos\theta)$, where λ (1.54 A°) is the wavelength of x-ray, θ being x-ray diffraction angle. The size of the prepared zinc oxide nanoparticles was 33 nm.

3.2 APPLICATION

3.2.1 Antibacterial activity

The bacterial activities of the prepared zinc oxide nanoparticles were tested against the clinical pathogen E. coli (gram negative pathogen). The figure illustrates the zone of inhibition of E. coli against standard drug nalidixic acid (NA 30) and biosynthesised zinc oxide nanoparticles. After 24 hours of incubation, the inhibition zone was measured around 1cm. The existence of inhibition zone clearly indicates the involvement of membrane disruption and leads to the death of pathogens. The large inhibition zone of biosynthesised nanoparticles when compared to the inhibition zone of antibiotic (2cm) proves the antibacterial property of zinc oxide nanoparticles. [7] [8]

Fig 3.6 Antibacterial activity of synthesised ZnO nanoparticles

Chapter 4

Conclusions

This study successfully reports the green synthesis of zinc oxide nanoparticles using Bacopa monnieri(brahmi) leaf extract. It proves to be a rapid green approach for the synthesis providing a cost effective and an efficient way for the synthesis of zinc oxide nanoparticles. Therefore, this reaction pathway satisfies all the conditions of a 100% green chemical process. Zinc oxide nanoparticles were completely synthesized through simple and eco-friendly pant mediated green synthesizing technique from the leaves of Bacopa monnieri. XRD, SEM-EDX, and UV-visible spectroscopy were accustomed to characterize the produced nano powders. Crystal structures of synthesized zinc oxide nanoparticles were with particle sizes were observed from XRD results. According to scherrer's equation, the produced ZnO nanoparticles have a particle size of 33nm. Small agglomeration of the extracted nanoparticles was observed from SEM results. It was also proven that the produced ZnO possessed an antibacterial property against E.Coli bacteria. Zinc oxide nanoparticles can be used as intelligent weapons toward a wide range of drug-resistant microbes, as well as a capable antibiotic replacement.

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