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EFFECT OF ZINC OXIDE AND SILVER NANOPARTICLES IN BIOLOGICAL SYSTEM

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ABSTRACT

The purpose of this study was to examine the manufacture and characterisation of zinc oxide (ZnO) and silver nanoparticles (NP), as well as their use on harmful bacteria. ZnO NP were produced using a chemical reduction procedure with starch serving as the capping agent, while silver NP were made through an environmentally friendly technique beginning with AgNO3 solution and progressing through an extract of Citrus sinensis (sweet lime). The nanoparticles were subjected to UV-Vis spectroscopy, Dynamic Light Scattering (DLS) particle size analysis, Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD) analysis, and Thermogravimatric (TGA) analysis in order to complete the in-depth characterisation. Dynamic Light Scattering (DLS) particle size and scanning electron microscopy (SEM) image analysis led to the discovery that the average particle size of ZnO and silver nanoparticles, respectively, are 90 nm and 50 nm, respectively. The creation of nanoparticles was verified using techniques such as thermogravimetric analysis (TGA), X-ray powder diffraction (XRD), and UV-VIS spectroscopy. The antibacterial activity of artificially produced ZnO and silver nanoparticles was evaluated against four different pathogens using both liquid and solid growth media (Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, and streptococcus pneumonia). The development of the bacteria was tracked by determining the optical density (OD) of the culture solution and the number of colony forming units (CFU) that could be found on the solid medium. In addition to this, the impact that NP has on the amount of gene expression in E. coli was investigated. There was a significant increase in the amount of enzymatic activity expressed by beta-glucosidase when NP was added to the liquid E. coli culture.

keywords: *Zinc Oxide, Silver Nanoparticles*

introduction

Within the realm of contemporary materials science, the discipline of nanotechnology is recognised as one of the most active study fields. The size, distribution, and shape of nanoparticles all play a role in determining whether or not they display new or better features. There have been remarkable advancements in the field of nanotechnology in the recent past years, with numerous methodologies developed to synthesise nanoparticles of particular shape and size depending on specific requirements. These developments have been accompanied by an increase in the number of nanoparticles that can be manufactured. Nanoparticles and nanomaterials are finding an expanding number of uses in the modern world. Synthesis, characterisation, investigation, and use of nanosized (one-hundred nm) materials for the advancement of scientific knowledge are all examples of what might be referred to as nanotechnology. It focuses on the materials whose structures, as a result of their nanoscaled size, display considerably innovative and better physical, chemical, and biological characteristics, phenomena, and functioning.

Specifically, this is the case in biological systems. Nanoparticles, as a result of their size, have a surface area that is significantly greater than that of macro-sized materials. The size, shape, composition, crystallinity, and morphology of metal nanoparticles are the primary factors that influence the nanoparticles' inherent qualities. Because of their small size, nanoparticles exhibit various features in comparison to the bulk form of the same substance. As a result, nanoparticles provide numerous new opportunities for development in the disciplines of biosensors, biomedicine, and bio nanotechnology. Nanotechnology is also being exploited in the medical field for the purposes of illness diagnostics, the administration of therapeutic drugs, and the creation of remedies for a wide variety of ailments and conditions. Nanotechnology is an extremely strong technology that has a great deal of promise for the design and development of a wide variety of unique goods along with its possible uses in medicine for the early diagnosis, treatment, and prevention of disease.

Zinc oxide nanoparticles

It is almost completely insoluble in water and has a typical appearance of a white powder. The powder finds widespread application as an additive in a wide variety of materials and goods, such as plastics, ceramics, glass, cement, rubber (such as car tyres), lubricants, paints, ointments, adhesives, sealants, pigments, foods (as a source of the zinc nutrient), batteries, ferrites, fire retardants, and other products. Zinc oxide, or ZnO, can be found in the crust of the Earth in the form of the mineral zincite; however, the vast majority of ZnO that is used in industry is created synthetically. Because ZnO is not poisonous and does not react negatively with human skin, it is an excellent addition for materials like textiles and surfaces that are in direct touch with people. Because nanoscale ZnO has a greater surface area than bulk ZnO, there is a possibility that the material's function will be performed more effectively as a result.

Importance of ZnO nanoparticles

1) It is used in paints, cosmetics, sunscreens, plastic and rubber manufacturing, electronics and pharmaceuticals products etc.
2) It is also potentially used to treat leukemia and carcinoma cancer cell
3) It is also a strong antibacterial agent
4) It is also used as drug carrier
5) ZnO nanoparticles is also used in industrial sectors including environmental, synthetic textiles, food, packaging, medical care, healthcare, as well as construction and decoration.

Antibacterial activity of ZnO nanoparticles

Organic and inorganic antibacterial agents are the two primary categories of this class. When subjected to high temperatures and pressures, it has been discovered that organic antibacterial substances are less stable than inorganic antibacterial agents. As a result, zinc oxide has demonstrated its effectiveness as a potent antibacterial agent in the process of formulating microscale and nanoscale systems for use in therapeutic applications. The antibacterial activity of ZnO nanoparticles was shown to be much higher than that of micro particles. The precise methods by which the antibacterial agent inhibits bacterial growth are not yet entirely understood. Both Gram-positive and Gram-negative bacteria are rendered ineffective by the bactericidal actions of ZnO particles. They even exhibit antibacterial effect against spores, which are microorganisms that are resistant to high pressure and high temperature. According to the research that has been conducted, the antibacterial activity of ZnO nanoparticles is mostly determined by the surface area and concentration of the particles, while the crystalline structure and particle shape have a very minor impact. Additionally, it is indicated in the research that the antibacterial activity of ZnO particles is improved when their size is reduced. Therefore, the antibacterial activity of the nanoparticles is improved proportionally with both the concentration and the surface area of the nanoparticles. ZnO particles have been shown to be effective against bacterial growth, however the mechanism behind this effect is not yet completely understood. Some researchers have suggested in their study that the generation of hydrogen peroxide is the primary factor of the antibacterial activity, while the study also indicated that the binding of the particles on the bacteria surface due to electrostatic forces could be another factor in the antibacterial activity. Within the scope of this research project is an examination of the antibacterial activity of ZnO particles against pathogenic bacterial species such as Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, and Streptococcus pneumonia. Because of the following considerations, we have decided to employ these four distinct categories of harmful bacteria.

E. coli: Food poisoning, urinary tract infections, and newborn meningitis can all be caused by a virulent strain of gram-negative bacteria.

Pseudomonas aeruginosa: In addition to infecting the pulmonary tract and urinary tract, as well as burns and wounds, gram-negative rod-shaped bacteria are responsible for various blood illnesses.

Bacillus subtilis: Gram-positive coccoid bacterium causes pneumonia, meningitis, sepsis.

Streptococcus pneumonia: Gram-positive rod shaped bacteria causes food poisoning

Silver Nanoparticles

One of the most exciting developments in the field of nanotechnology is the discovery of silver nanoparticles. An essential component of the work that is being done in the field of nanotechnology right now is the investigation and development of reliable procedures for the production of silver nanoparticles. One of these potentially fruitful processes is known as green synthesis. Nanoparticles of silver may be manufactured using a variety of different physical, chemical, and biological processes. However, during the past several years, numerous quick chemical processes have been substituted by green synthesis due to the elimination of toxicity in the process as well as an increase in product quality.

Importance of Silver nanoparticles

1) It is utilised in the biosensing, imaging, and medication delivery systems, as well as the purification and quality control of air.
2) Silver nanoparticles that have been biologically produced can be used as coatings for solar energy absorption, as intercalation material for electrical batteries, as optical receptors, as catalysts in chemical processes, for biolabelling, and as antimicrobials, among other uses.
3) Silver nanoparticles are cytotoxic, yet they have significant uses in the fields of high-sensitivity bimolecular detection and diagnostics, antimicrobials and therapies, catalysis and microelectronics. This is despite the fact that silver nanoparticles are themselves harmful.
4) It may find use in diagnostic biomedical optical imaging, biological implants (such as heart valves), and medicinal applications (wound dressings, contraceptive devices, surgical equipment, and bone prosthesis, for example), among other possible applications.
5) A significant number of the world's largest producers of consumer products are already manufacturing things for the home that make advantage of the antibacterial capabilities of silver

nanoparticles. Nanosilver is used in a variety of consumer items, including air conditioners, washing machines, and refrigerators.

Silver nanoparticles as an antimicrobial agent

Ag NP is particularly effective against a wide variety of bacterial species, one of which being E. coli, a bacterium that is commonly found in kitchens. According to the proposed mechanism, silver nanoparticles engage with the outer membrane of bacteria, which halts respiration as well as some other metabolic process that ultimately results in the death of the bacterium. This beneficial antimicrobial agent can now be incorporated into a wider variety of materials, such as plastics, coatings, and foams, in addition to both natural and synthetic fibres, as a result of recent technological advancements that involve the chemical reduction of silver compounds to nanoscale-sized particles. Nanoparticles of silver already give an antibacterial protection that is more long-lasting and, in many cases, lasts for the lifetime of the product. The pharmaceutical and medical sectors have entered a new age as a result of the current research being conducted on inorganic nanoparticles that have strong antibacterial characteristics. Silver is the metal of choice since it possesses the potential to eradicate microorganisms in an efficient manner. Recently, it has come to light that silver nanoparticles have the potential to operate as an effective antibacterial agent. These particles act on a diverse variety of target locations, both extracellularly and intracellularly. (Shrivastava et al., 2007) and also it was discovered to be in few studies that silver nanoparticles exhibit extremely significant bactericidal activity against gramme positive as well as gramme negative bacteria including multiresistant strains (Zeng et al.,2007; Roe et al., 2008). Because of this, there has been a significant amount of progress made in the investigation of the biological applications of ZnO, Ag, and other metal NP.

β-glucosidase

It is a hydrolytic enzyme which produces the monosaccharide/glucose in bacterial/yeast cells which helps in the metabolism process. Its activity assay protocol is very simple and it is industrially important enzyme also. In E.coli this enzyme is produced by activation of the lac operon. This enzyme plays an important role in the study of genetics and molecular biology. Since it is highly expressed and accumulates in lysozyme in aging cells, it is also used as an aging biomarker both in vivo and in vitro in qualitative and quantitative assays.

Study on the interaction of protein and Nanoparticles

For a good number of years, one of the topics that has garnered a great deal of attention is the influence that the surface chemistry of biomaterials has on the process of protein adsorption. There has been a lot of research done on the topic of protein adsorption to different materials, and the results of that research have shown that certain factors, including electrostatic interactions, hydrophobic interactions, and specific chemical interactions between the protein and the adsorbent, play important roles. These interactions with the surface have the potential to readily wreak havoc on the protein's natural structure and, as a result, its function. The conjugation of protein with nanoparticles, on the other hand, not only makes it possible to stabilise the system, but, more importantly, it also enables the introduction of biocompatible functionalities onto the nanoparticles themselves, which can then be used for additional biological interactions or coupling. In the context of the current inquiry, -lactalbumin is serving as an important enzyme. In spite of the fact that the nanoparticles and protein interaction study was carried out with a select number of proteins, such as lysozyme, bovine serum albumin, and -lactalbumin, no interaction study was carried out with bovine -lactalbumin. With a molecular mass of 14 kDa, the protein is not very large and only exists as a monomer. The investigation of beta-ability lactalbumin's to unfold and refold in vitro was carried out extensively. Nevertheless, it is worthwhile to learn whether or not tiny metal nanoparticles might bring about changes in the structure as well as the function of the material. Another reason why this protein is so important is that, in its transitional state, beta-lactalbumin has the potential to operate as an anti-turmeric agent. It has been proven that the same protein can serve as an anti-tumor agent when it is formed into a conjugate with a fatty acid such as oleic acid. Rapid interactions can occur between nanoparticles and the proteins that are found in a variety of biological systems. However, to this day, there have only been a limited number of studies that have been carried out that have focused on the nanoparticles that the general population is frequently exposed to, such as the metals and metal oxides. For this reason, it is crucial to understand how and why proteins are adsorbed to these particles in order to have a proper knowledge of the biological effect they have. During the course of this examination, we carried out an in-depth study on the production and characterization of ZnO and Ag nanoparticles, as well as their use on biological systems. The effectiveness of ZnO and Ag nanoparticles as antibacterial agents against four distinct strains of bacteria, including Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, and Streptococcus pneumonia, has been investigated. The in vivo biological activity of an expressed beta-glucosidase enzyme in E. coli was measured by me in the presence of different amounts of zinc oxide nanoparticles and silver nanoparticles. To verify the production of protein-NP conjugate, a DLS particle size analyzer was used to monitor the interaction between the -lactalbumin protein and both ZnO and Ag nanoparticles. Tryptophan fluorescence and circular dichroism spectroscopy were used to observe the structural transformation that occurred in the protein as a result of its interaction with NP.

OBJECTIVES:

1. The production and detailed characterisation of silver nanoparticles and zinc oxide produced by a chemical reduction process (by green synthesis method).
2. Examine the antimicrobial activity/potential of ZnO and Ag nanoparticles against the four different human pathogenic bacteria like ; E. coli, Pseudomonas aeruginosa, Bacillus subtilis, Streptococcus pneumonia.

MATERIALS AND METHOD

Preparation and utilization of ZnO and Ag nanoparticles have been explored widely observe to the anti microbial effect in the fourth era. Synthesis of ZnO without using a protective agent was a problem. Due to the high polarity of the water, ZnO nanoparticles cause an immediate agglomeration during synthesis with water due to the Vander wall forces of attraction. To prevent aggloromation, soluble starch is now added before the reaction. The quick helical forms of the soluble starch protect and prevent the ZnO nanoparticles from agglomeration by the action of steric or electrostatic hindrance and stabilizing the ZnO nanoparticles. ZnO was used in paints, cosmetics, sunscreens, plastic and rubber manufacturing, electronics and pharmaceuticals products etc. It is also a strong antibacterial agent In recent years silver nanoparticles have been investigated by many research groups nationally and internationally. It is mainly because of the potential application of nanomaterials in biology, medicine, optics and in modern electronic devices Silver nanoparticles have advantage over other noble nanoparticles (e.g., gold and copper) because the surface Plasmon resonance energy of silver is situated far from the interband transition energy. Silver nanoparticles exhibit very strong bactericidal activity against gram-positive as well as gram-negative bacteria including multiresistant strains, as well as potential antifungal agent.

Chemicals:

In all of the studies, including the production of ZnO and silver nanoparticles and the fabrication of medium for the growth of bacterial cells, the chemicals that were utilised were of a pure and analytical quality. The following chemicals were obtained from Himedia labs Pvt. Ltd. in Mumbai, India: zinc nitrate hexahydrate, sodium hydroxide (NaOH), starch, and silver nitrate (AgNO3). The E. coli, Bacillus subtilis, Pseudomonas aeruginosa, and Streptococcus pneumonia bacterial cultures were acquired from the National Center For Cell Science (NCCS), which is located in Pune. Antibiotics, including Ampicilin, Tetracycline, and Ciprofloxacin, were obtained from Himedia in Mumbai, India. Tobramycin was also acquired. We made our purchases of beef extract, peptone, and sodium chloride (NaCl) from Merck in India.

Glassware and Apparatus

The entirety of the glassware, including but not limited to conical flasks, measuring cylinders, beakers, petri plates, and test tubes, was acquired from borosil in India.

Synthesis of ZnO nanoparticles

Wet chemical technique was used to manufacture ZnO nanoparticles utilising zinc nitrate and sodium hydroxides as precursors and soluble starch as a stabilising agent. After dissolving soluble starch at a concentration of 0.5 percent in 500 millilitres of distilled water, the mixture was put through a solubilization process in a microwave oven manufactured by Samsung and bearing the model number NoCE103VD. In the previous solution, there was an addition of 14.874 grammes (0.1 mol) of zinc nitrate. After that, the solution was stirred continuously using a magnetic stirrer (Tarson spinnot digital) for a whole hour while it was held at room temperature. After the zinc nitrate was completely dissolved, 300 millilitres (0.2 moles) of sodium hydroxide solution was added while the mixture was being continuously stirred. Each drop made contact with the sides of the jar. After the sodium hydroxide had been added in its entirety, the reaction was allowed to continue for a period of two hours. After the reaction was finished, the solution was left to settle for a whole day, and the next day, the supernatant solution was carefully discarded. The remainder of the solution was centrifuged at a speed of 10,000 g for ten minutes using a Remi cooling centrifuge apparatus with model number No-C30BL, and the supernatant was thrown away. The nanoparticles that were created in this manner were then subjected to three separate washes with distilled water.

RESULTS AND DISCUSSION

Synthesis and Characterization of ZnO and Ag nanoparticles

Synthesis of ZnO by chemical reduction method

The synthesis of ZnO nanoparticles was carried out in accordance with the method detailed in the section under "Material and Methods" (Section 3.4). As can be seen in Figure 3, the powder form of the nanoparticles have a white appearance.

Figure 3. Synthesized ZnO powder by chemical reduction method

Synthesis of Ag nanoparticles by green synthesis process (from Citrus sinensis)

Because of the activation of surface Plasmon resonance, Ag nanoparticles in aqueous solution have a hue that is somewhere between yellow and brown. After combining the extract with an aqueous solution of the Ag ion complex, an observation was made that the hue changed from being colourless to being a yellowish brown. 43 It was because of the decrease in levels of Ag+, which points to the creation of silver nanoparticles as seen in Figure 4.

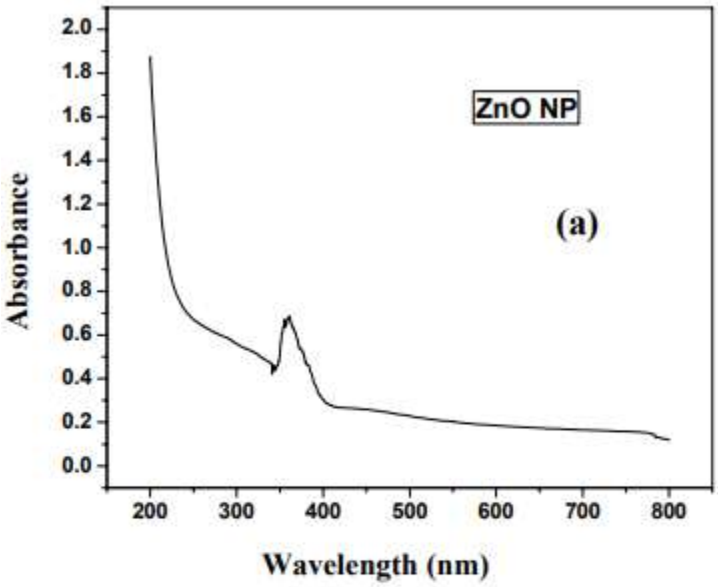
(a) (b) (c)

Figure 4. Digital photographs of (a) Citrus sinensis extract (b) 1 mM AgNO3 without citrus sinensis extract (c) 1 mM AgNO3 with Citrus sinensis extract after 10 hrs of incubation.

Characterization of ZnO and Ag nanoparticles

UV–Vis spectral analysis

Figure 5 displays the UV-Vis spectra of ZnO NP that was produced with a concentration of soluble starch of 0.5%. (a). It was determined that the nano ZnO that had been synthesised had an absorption peak at about 360 nm. Figure 5 displays the UV-Vis absorption spectra of the silver nanoparticles (Ag NP) (b). The absorbance spectra of the silver nanoparticles that were generated in the reaction medium showed that the maximum absorbance occurred at 421 nm. The particles are likely polydispersed if there is a significant widening of the peak between 350 and 480 nanometers in wavelength. With an increase in response time, it was discovered that the peak of the absorption spectra moved from 350 nm to 480 nm in a blue-shifted direction. The ZnO spectrum that was obtained was consistent with the result that was reported and published by (Vigneswaran et al., 2006). In addition, the spectra of the Ag nanoparticles matched the results that were reported and published in 2009 by Jain et al.



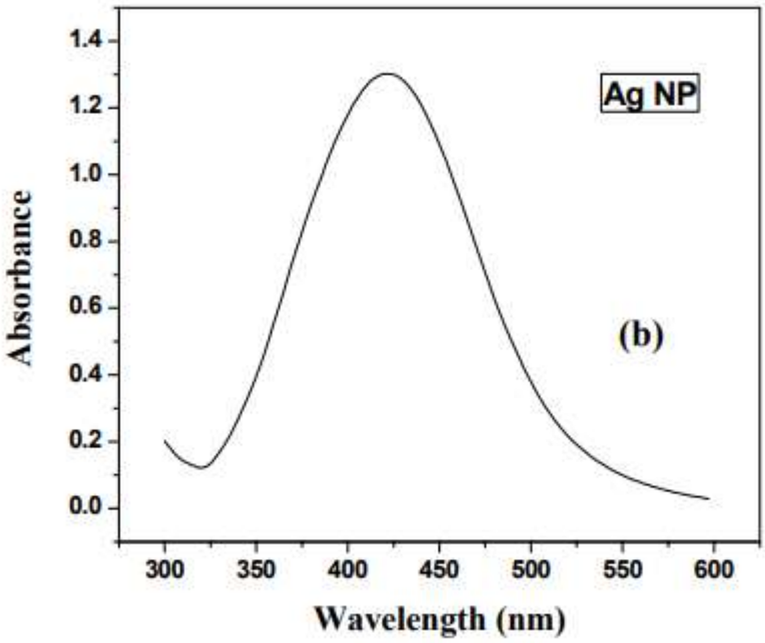
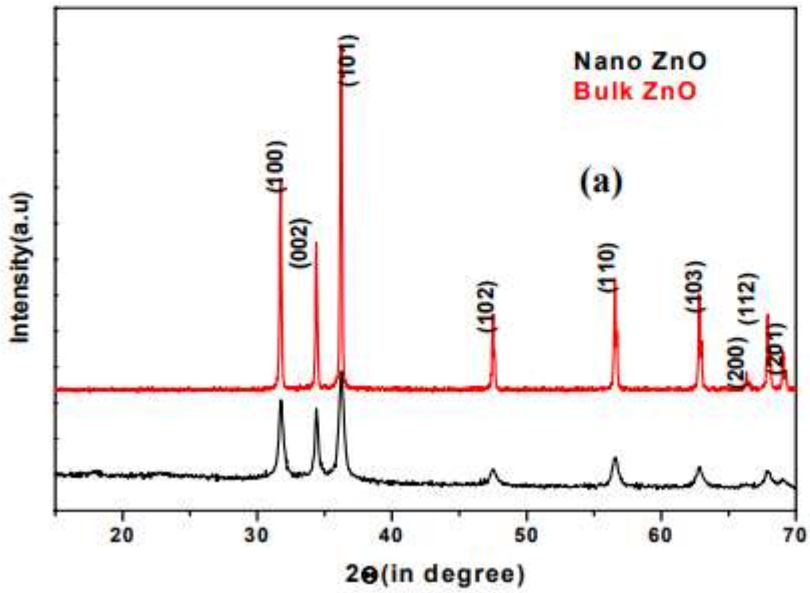


Figure 5. (a) UV-Vis spectra of the ZnO and (b) Ag nanoparticles prepared with 0.5% of soluble starch and 1mM aqueous AgNO3 solution with 10% Citrus sinensis extract.

X-ray diffraction pattern for ZnO and Ag nanoparticles

Figure 6 illustrates the XRD patterns of both bulk ZnO and nano ZnO. (a). Every peak was in the shape of a hexagon, and it was quite accurate to the information that was supplied (jcpds-79-0206). The most prevalent crystal habit for zinc oxide is hexagonal, and this is because of the symmetry of the crystal and the associated face velocities. Additionally, the ZnO NP crystallographic phase is the one that is thermodynamically stable. As a result of the quantum size effect, the diameter of the peaks that were produced by ZnO NP was significantly enlarged. Using the Scherer equation (which is covered in the section under "Material and Methods"), we were able to determine that the typical particle size was 42 nm. The distinctive peaks that were seen in the XRD picture that was displayed in Figure 6 provided as evidence that the Citrus sinensis extract-mediated synthesis of the silver nanostructure was successful (b). All of the peaks in the diffraction pattern correlate to the silver lines that are typical of face-centered cubic crystals (FCC). The diffraction lines that were seen at an angle of 2 of 32.80, 38.20, 55.10°, and 65.70, respectively, have been given the index numbers (111), (200), (220), and (311), respectively. In order to establish peak intensity, location, and width, XRD patterns were studied, and the full-width at half-maximum (FWHM) data was utilised in conjunction with the Scherer formula, which is described in the section under "materials and technique." The sample features structures of silver nanoparticles that are both cubic and hexagonal, as seen by the standard XRD pattern, which indicates that the sample comprises a mixed phase. Based on the whole width at half maximum of the peak corresponding to the 111 plane, the estimated average particle size of this sample was 50 nm. The form of the particles was cubic and hexagonal.



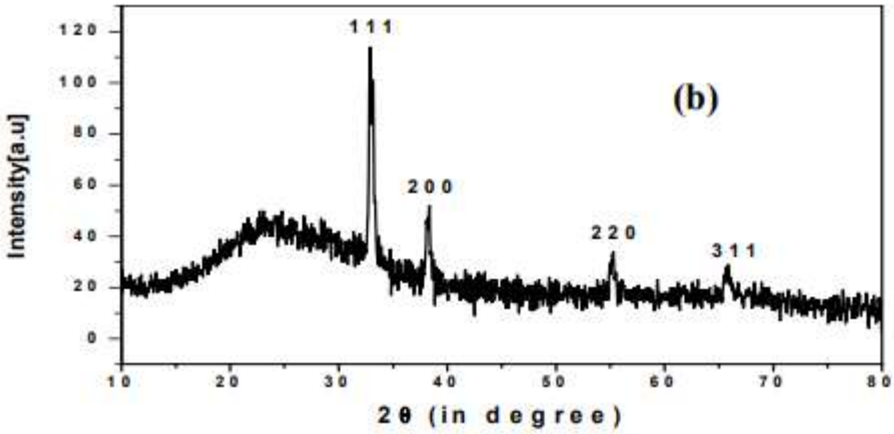


Figure 6. (a) XRD patterns of ZnO nanoparticles (red) bulk ZnO (black) ZnO synthesized using 0.5% starch. The peaks assigned to diffractions from various planes are of hcp ZnO. (b)The Ag nanoparticles synthesized by treating 10% Citrus sinensis extract with 1 mM aqueous AgNO3 solution.

CONCLUSION

ZnO and silver nanoparticles were effectively manufactured using chemical reduction and green synthesis techniques, respectively (using Citrus sinensis as the starting material). The nanoparticles were characterised in great detail by the use of UV-Vis spectroscopy, Dynamic Light Scattering (DLS) particle size analysis, Scanning Electron Microscopy (SEM), and X-Ray Diffraction (XRD) analysis. All of these techniques were utilised. Dynamic Light Scattering (DLS) particle size and scanning electron microscopy (SEM) image analysis revealed that the average particle size of ZnO was 90 nm, whereas the average particle size of Ag was 50 nm. In Escherichia coli, the in vivo biological activity of an expressed beta-glucosidase enzyme was evaluated in the presence of varied quantities of zinc oxide nanoparticles and silver nanoparticles. It was discovered that Escherichia coli grown in the presence of nanoparticles led to a significant increase in the biological activity of beta-glucosidase when the bacteria were allowed to incubate after being exposed to the particles. In addition, the tryptophan fluorescence and circular dichroism spectroscopy methods were utilised in order to investigate the interaction between the -lactalbumin protein and the nanoparticles. The measurement of the protein's tryptophan fluorescence uncovered the fact that, in the course of its interaction with NP, the protein goes through a structural alteration that may be described as chaotic. The tryptophan fluorescence quenching investigation showed that there are probably tryptophan residues in the binding site.

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