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Utilisation of egg shells for green synthesis of ZnO nanoparticles and analysis of their antibacterial efficacy

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Abstract

Nanotechnology has emerged as a rapidly growing field because of the unique properties of materials at nanoscale. In the current research we have discussed on green synthesis of zinc oxide (ZnO) nanoparticles using egg-shell and have assessed their anti-bacterial efficacy. The egg-shell which is otherwise a waste, is used in this research, as a capping agent, templating agent, stabilizer and nano particle growth controller. The green ZnO nanoparticles have high antibacterial properties and in this study its efficacy has been tested against Bacillus cereus and Escherichia coli bacteria. This study aims to put forward an efficient green synthesis route for ZnO nanoparticle synthesis with wide range of potential application especially in biomedical field as it is non-toxic, eco-friendly.

Keywords: *Green synthesis, Zinc oxide, non-toxic, eco-friendly, antibacterial.*

INTRODUCTION

Currently, harmful microbes possess an ability to grow even in presence of drugs that were earlier used to inhibit them. This puts forth a critical global health concern and necessitates to evolve alternate pathways to deal with these microbes. This alternate pathway is paved by nanotechnology. Nanotechnology is an advance field of science that studies about the materials whose sizes are in nanometres i.e. from 1-100nm, these materials also show excellent physical and chemical properties. From the list of various nanomaterials observed till date, zinc oxide (ZnO) nanoparticles have gained valuable attention because of its high surface area, chemical resistance and also because of its anti-bacterial properties [1]. Above mentioned features of zinc oxide nanoparticles make it a perfect and a first choice to be used in fields like medicine, food preservatives, cosmetics and also for environmental remediation [2]. The priorly used conventional methods for preparation of ZnO nanoparticles involves the use of toxic reagents and also has a high energy demand which makes the overall procedure very complex [3].

To overcome these limitations, we need to design an eco-friendly non-toxic and sustainable method called as "Green synthesis".

In this method we will use biological waste to act as a stabilizing and templating agent.

Egg shell contains calcium carbonate ($CaCO_3$) as well as organic proteins and are biowaste material in large scale. Hence use of egg-shell has double advantage of waste utilization and eco-friendly production [5]. This helps to synthesize the Zinc-oxide nano-particle which will have no side-effects, will be least harmful and can be easily used in the field of medical science with the help of which, toxicity free and more effective drug materials can by synthesized which can be used to treat harmful bacteria.

This study is motivated by:

1) The need of green and sustainable way to synthesize nanoparticle.
2) The use of egg-shell biological waste in best possible way.
3) Making down an alternative-antimicrobial agents.

METHODOLOGY

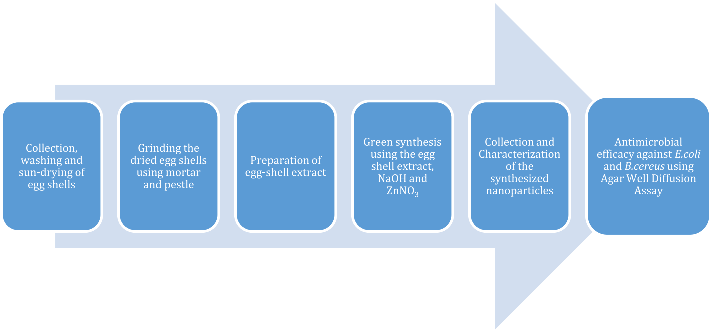

Figure 1: Flow Chart showing the methodology used in this study

Collection, washing and sun-drying of egg shells

Egg-shells were collected and washed with distilled water to remove impurities. Further, the shells were dried in sunlight for 2 days.

Grinding the dried egg shells using mortar and pestle

The sun-dried shells were ground into fine powder using mortar and pestle and stored in an air tight container at room temperature [7].

Preparation of egg-shell extract

4.5 g of finely powdered egg-shell was added in 5%(v/v) solution of dilute acetic acid. The mixture was stirred for 2 hours at 60°C using a magnetic stirrer. While stirring, bubbles of CO_2 evolved. This signifies that the egg-shell contains calcium carbonate $CaCO_3$ which reacts with dilute acetic acid (CH_3COOH) in the following manner:

$CaCO_3 + 2CH_3COOH\ Ca^{2+} + CO_2 + H_2O + CH_3COO^-$

After the bubbles stop evolving and settle down, the solution was filtered using Whatmann filter paper and the clear filtrate was stored at 4°C.

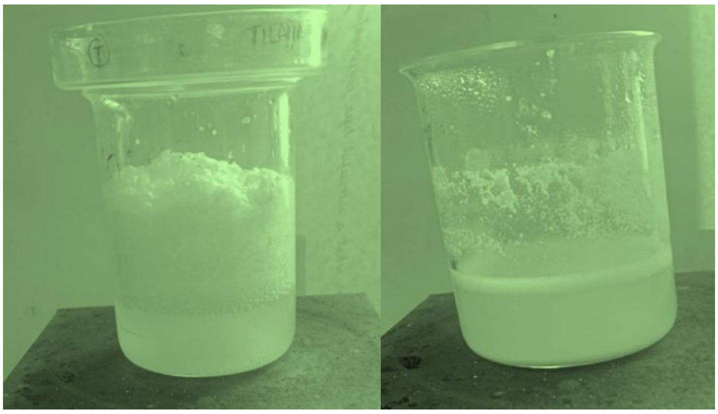

Figure 2: Formation of CO2 bubbles followed by Settling of CO2 bubbles

Green synthesis of ZnO nano-particles:

29.8g of $Zn(NO_3)_2.6H_2O$ (Zinc Nitrate Hexahydrate) was dissolved in 200 ml of distilled water. This solution was stirred for around 30-45min which ensures the proper mixing of the two components [16]. Once a homogeneous solution of Zinc Nitrate was obtained, 15ml of egg-shell extract was added to it and stirred for 2 hours at room temperature. This was done to ensure proper interactions between zinc ions and the biomolecules present in the egg-shell extract. The resultant mixture was then left undisturbed for 30 mins. Sodium Hydroxide (NaOH) solution was then added to the mixture as precipitating agent and to increase the pH of the reaction mixture. The solution of NaOH was added dropwise in the mixture of Zinc-nitrate and egg-shell extract [15]. The addition of NaOH continued till pH reached 10-11. With the addition of NaOH, the nano-particle formation starts appearing as white colour precipitate. After about an hour, when proper precipitation has occurred, the mixture was left in an incubator at 60°C overnight. This results in overall growth of nano-particle and proper nucleation. After incubation, the mixture is centrifuged through which the precipitate was collected. The collected precipitate was then washed with ethanol once and water thrice [5]. After obtaining the precipitate, it was spread on a petri dish and was heated at 150°C for 2 hours. This resulted in drying of precipitate and the formation of ZnO nanoparticles. After drying the powder was collected in mortar and pestle and then it was ground finely and then the fine powder was collected and stored in airtight condition at 4°C.

Figure 3: Drying of the ZnO precipitate to obtain ZnO nanoparticle powder

The fine powder was then characterized using FTIR.
Once characterized, the ZnO nanoparticles were characterized, they were used for an antibacterial efficacy analysis by the Agar Well Diffusion method against Gram-positive *Bacillus cereus* and Gram-negative *Escherichia coli*.

RESULTS

The Formation of ZnO nanoparticle was confirmed by FTIR test performed at BIT Mesra, Ranchi, Jharkhand, India.

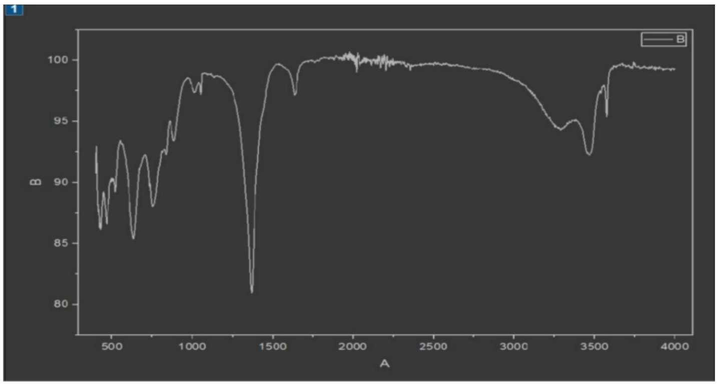

Figure 4: FTIR graph confirming the formation of ZnO nanoparticles

Table 1: Zone of Inhibition for Bacillus cereus

Sample	Zone of inhibition(cm)
Zinc Oxide nanoparticles (Test-sample)	1.0± 0.1 cm
Ampicillin (Positive-control)	1.5± 0.1 cm
Water (Negative-control)	0 cm

Table 2: Zone of Inhibition for Escherichia coli

Sample	Zone of inhibition(cm)
Zinc Oxide nanoparticles (Test-sample)	0.6± 0.1 cm
Ampicillin (Positive control)	1.0± 0.1 cm
Water (Negative control)	0 cm

DISCUSSION

FTIR Analysis

The Spectrum obtained by FTIR is shown in the figure 4. From the FTIR graph we can infer that the fundamental vibrational mode obtained at 3400-3500 cm^{-1} corresponds to O-H stretching vibration, which indicates the presence of hydroxyl group on the surface of ZnO nano-particle[9][4]. Another peak at 1600-1650 cm^{-1} is for H-O-H bending vibration of water molecules or weak C=O stretching which suggest that it may contain some residue of organic compounds also [4]. 1000-1200 cm^{-1} range signifies the presence of C-O and C-N stretching vibrations which again tells us about the presence of organic residues which may be acting as stabilizing agents [5].

The 500-700 cm^{-1} is the most important peak obtained which confirms the ZnO nanoparticles formation. This is the region of strong Zn-O stretching vibration [3].

Antimicrobial Efficacy

The antibacterial activity of the synthesized ZnO nanoparticles was evaluated against *Bacillus cereus* and *Escherichia coli* using the agar-well diffusion method. The ZnO nanoparticle sample produced a zone of inhibition of approximately 1.0± 0.1 cm, indicating good antibacterial activity against *Bacillus cereus*. The positive control (Ampicillin) exhibited a comparatively larger zone of inhibition, demonstrating its superior antibacterial efficacy. However, the inhibition zone produced by the ZnO nanoparticles confirms that the synthesized nanoparticles possess significant antibacterial potential against this Gram-positive bacterium.

The antibacterial activity of ZnO nanoparticles can be attributed to several mechanisms, including the generation of reactive oxygen species (ROS) such as hydroxyl radicals, superoxide radicals, and hydrogen peroxide, which induce oxidative stress and damage bacterial cell components. In addition, the release of Zn^{2+} ions, direct interaction of nanoparticles with the bacterial cell membrane, increased membrane permeability, and leakage of intracellular constituents collectively contribute to bacterial cell death. The obtained zone of inhibition demonstrates that the eggshell-mediated green synthesized ZnO nanoparticles exhibit effective antibacterial activity against *Bacillus cereus*, suggesting their potential application in biomedical, pharmaceutical, and antimicrobial coating applications. The antibacterial activity of the synthesized ZnO sample was tested against *Escherichia coli* also using the same method. For the ZnO nanoparticles, the zone of inhibition was found to be 0.6± 0.1 cm, which indicates a moderate antibacterial activity against *Escherichia coli* [17]. For the current study, the obtained ZnO nanoparticles exhibit better antibacterial efficacy against Gram-positive *Bacillus cereus* as compared to Gram-negative *Escherichia coli*.

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