International Journal of Engineering and Advanced Technology (IJEAT)
ISSN: 2249-8958 (Online), Volume-8 Issue-6, August 2019

Cobalt Nanoparticle As The Antibacterial Tool: In Vitro

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ABSTRACT--- In the present study carried out to investigate prophylactic activity of Cobalt nanoparticles towards isolated Escherichia coli (strain MTCC 723); the antimicrobial properties of Cobalt nanoparticles also determine by optimizing the concentration of Cobalt nanoparticles. A homogenized suspension was prepared by sonication Cobalt nanoparticles in water, and the infusion was prepared at concentrations of 5–35µg/ml. The pathogenic strain of microbial species was cultured under anaerobic conditions. Equalized standard dilutions of the cultured bacteria were used for testing. Spectroscopic and diffusion tests were conducted to quantify the antimicrobial properties of Cobalt nanoparticles against the bacteria. Cobalt nanoparticles of 200 nm diameter with concentration of 35µg/ml showed completely lysis of Escherichia coli cell. The results showed Cobalt nanoparticles with concentration of 35 µg/ml significant higher activity against Escherichia coli. Optimized concentration of Cobalt nanoparticles kills the pathogenic Escherichia coli cells.

Key words: Cobalt nanoparticle, Antimicrobial activity, Escherichia coli

I. INTRODUCTION

Metal nanoparticles are the significant materials nowadays established for usage in health-related applications [1-3]. Nanoparticles (NPs) are used as an alternative to antibiotics to target bacteria antibiotics. On the same time, Nanotechnology plays an important role for treating bacterial infections [4-5].nanoparticles are the nano-sized particles [6]. Metal nanoparticles are exceptionally appealing impetuses contrasted with mass materials because of their high surface-to-volume proportion [7-8]. There are many nanomaterials such as; silver, gold, and zinc nanoparticles exhibited their own structural properties important biological activities [9-10]. Bacterial cells have the resistance power towards antibiotics and metals and spreads their importance and creates different health related problems.

Revised Manuscript Received on August 30, 2019.

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Bacterial cells also have drug resistance which leads to the incompetence of medicine meant for treatment of number of diseases [11].For the development of human healthcare there has been care in the advance of firm antimicrobial metal nanoparticles [12]. Nowadays, Cobalt nanoparticles are more focused for human healthcare due to its antiseptic action and constancy [13-15]. Cobalt nanoparticles playacting as a possible antiseptic go-between from the sequence of other metal oxide like Al2O3, SiO2, and TiO2, to control many bacterial infections *Escherichia coli, B. Subtilis* and *Pseudomonas* Sp. cultivated product and foodstuff borne entity also controls the use of Cobalt nanoparticles [16-18].There is the synthesis and characterization of Nanomaterials of Co (11) showing antimicrobial action [19]. The mechanism behind antibacterial activity of Cobalt nanoparticles shows increasing lipophilicity, which later allows the dispersion of microbes over bilayer phospholipid of the cell membrane, through obstructive the metal-obligatory sites on the enzymes of microbes [20]. Considering the increase of bacterial infected diseases and human healthcare, the synthesis of compounds that have antibacterial effect, and, and have a low cytotoxicity is of great interest. It would be useful to use these nano materials, which require in a modest synthesis process and profitable way, are suitable for the formation of new types of bactericidal materials. Objective of this experimental study is to clarify bacterial inhibitory action of Cobalt nanomaterial through different experimental spectroscopic and diffusion methods. In this article we optimized the concentration of Cobalt nanoparticles as 35µg/ml for use as antibacterial agent. It acts as antibacterial tool for cell label.

II. MATERIAL AND METHODS

Materials and Test microorganisms

Cobalt nanoparticles with a 99% purity purchased from Sigma Aldrich. The particle size was 50 nm. Cobalt nanoparticles were suspended in sterile normal saline and constantly stirring until homogenized suspension was prepared. A uniform colloidal suspension was formed with powder concentration of 500 mg/ml. The morphology of Cobalt nanoparticles was determined by SEM. The surface morphology of the uncoated and coated beads was studied using Scanning Electron microscope (SEM, FEI ESEM Quanta 200). Fresh colonies of *Escherichia coli* were taken from MTCC (National Centre for Cell Science).

Escherichia coli were cultivated into nutrient broth containing flasks. For experimental study Nutrient broth medium was ready by dissolving 28 g of nutrient agar in 1000 ml of mili-Q-water. The prepared solution was autoclaved afterward at 121 ^{o}C, 15 lbs for 30 min.

Preparation and incubation of test inoculums

For preparing bacterial suspension from the stock bacterial culture single colony was taken and injecting 30 ml of sterile nutrient broth in a 100 ml flask. The flask was then raised at 37 °C at 110 rpm for 24 h. After growth, from overnight inoculum 0.4ml was transferred to a 100 ml Erlenmeyer flask having 25ml nutrient broth and raised in a shaking incubator for 3-4 h at 37 $^{\circ}$C and 100 rpm. The number of bacteria in the 4 h culture was estimated by measuring the optical density of the culture at 660 nm. An optical density of concerning 0.1 and 0.3 was roughly equal to a concentration of between 10^{8} CFU.ml-1.

Determination of zone of Inhibition

The bacterial inhibitory activities of the Cobalt nanoparticles were weighed by agar well diffusion method. Mueller Hinton agar medium was ready by autoclaving at 121 °C (15 lbs) and poured 30 ml for each Petri plates. Broth culture was aseptically spreaded by sterilized cotton swab over solidified Mueller Hinton agar plates. By sterilized gel borer Wells of equal distance and equal diameter (10mm) were made. 50µl of Cobalt nanoparticles of different concentrations from (5µg/ml, 15µg/ml, 25 µg/ml, and 35µg/ml) was filled in each well. The plates were incubated for growth of bacterial cell at 37 °C for 24 h. The sensitivities of the test organisms to the different concentrations of Cobalt nanoparticles were indicated by clear zone around wells (fig 3).

III. RESULTS AND DISCUSSION

Spectral analysis of Cobalt nanoparticles

The optical absorption spectra of Cobalt nanoparticles showed a sharp absorption band at 355 nm (fig.1).The SEM analyses also shows agglomerates of the nanoparticles. Scanning Electron Microscope (SEM) image of Cobalt nanoparticles (fig. 2) shows that, Cobalt nanoparticles are connected to each other with uneven pore dimensions and forms. Formation of Pore is as a result of large number of fugitive trips results in high surface to area of the nanoparticles. The pore size and shape formation related to various intrinsic defects formed during Cobalt nanoparticles provision and post treatment. Normally these imperfections are situated at the apparent of the cobalt nanostructures [18, 19]. It was observed that the synthesized nanoparticle size and shape depends upon the synthesis process including the type of ligand, attentiveness of reagents used, the solvent used, the overall concentration of reagents, time for reaction to complete, the evaporation time, and the temperature used for reaction/evaporation.

Antibacterial assay

Diffusion method and UV-Vis study

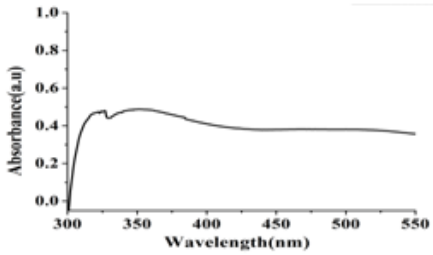
Rendering to standard reduction of bacteria criterion, less than 0–20% antibacterial effect indicates no bactericidal effect; between 20–50% reduction of bacterial cell indicates a low bactericidal effect; between 50–70% reduction indicates an expressive bactericide; greater than 70% reductions is considered strong antibacterial effect [22]. Giving to this, (fig.3) 5µg/ml Cobalt nanoparticles has no bactericidal effect. But, the plates containing concentration of 15 µg/ml, 25µg/ml, 35µg/ml shows 30% and 50% denote expressive bactericidal effect and plates with 35µg/ml concentrations has a dominant bactericidal result. Established on these outcomes, Cobalt nanoparticles have a sensitive antiseptic effect for the gram negative *Escherichia coli* strains. The antibacterial effect of prepared Cobalt nanoparticle at different concentrations was studied on *Escherichia coli* isolates in (fig. 3) and (table 1) showed the inhibition zone of different concentrations of metals nanoparticles. Results showed that, Cobalt exhibited inhibition zone (mm) of about 18, 20, 25, 27 mm in diameter for 5µg/ml 15µg/ml, 25µg/ml, and 35µg/ml of Cobalt nanoparticles concentrations, respectively. From the (fig.4) it was clearly understand the toxic effect of Cobalt nanoparticle from the absorbance study. The *Escherichia coli* cell with Cobalt nanoparticle showing less absorbance than that of only bacterial cell. The binding properties of Cobalt nanoparticles with bacterial cell due to tuning of Cobalt nanoparticles with the bacterial cells still now not clear but main cause is linked to the activity in the chelated complex, there is increases the lipophilic application of the metal and errands its infusion over the lipoid layers of the bacterial membranes. The dipole dipole interaction of positive charge of the metal is partly joint with the supporter atoms existing in the ligands and there is π-electron delocalization over the whole chelate ring. Apart from this, other factors for example solubility, conductivity and dipole moment predisposed by the presence of metal ions are also the likely motives for growing this inhibitory action of Cobalt nanoparticles [22-23].

IV. CONCLUSION

Cobalt nanoparticles possesses strong antibacterial, activity based on the above in vitro analysis. In higher concentrations, it has significant antimicrobial activity against pathogenic Gram negative *Escherichia coli* bacteria. From the experimental point of view it can be strongly concluded that cobalt nanoparticles the antibacterial tool for pathogenic microorganisms. Due to the beneficial effects of metal nanoparticles on microorganisms and control of many infectious diseases and prevent them from becoming chronic, many scientists believe that nanoparticles could create a great revolution in the field of health.

V. FIGURE

Fig.1: Uv-Visible Spectrum Of Cobalt Nanoparticles



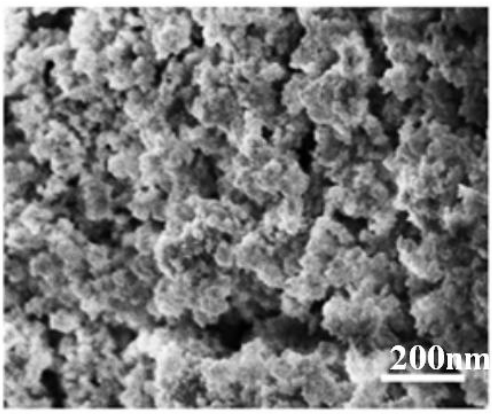


Fig.2: SEM of synthesized Cobalt nanoparticles

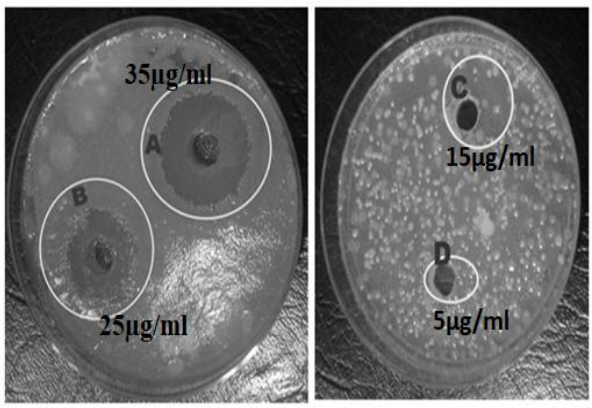


Fig. 3: Antibacterial activity of different

Table 1: Zone Of Inhibition Of Cobalt Nanoparticle Concentration Of Cobalt Nanoparticles. Against escherichia coli cells.

Cobalt Nanoparticle µg/L	Zone of Inhibition (mm)
5	18
15	20
25	25
35	27

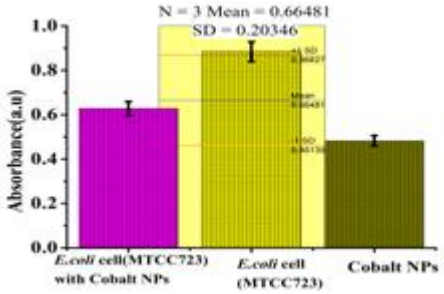


Fig 4: Uv-Visible Study Of Treated Escherichia Coli Cells

VI. ACKNOWLEDGMENT

Authors are thankful to BIHER; Chennai management provided the permission and facility to do the above work.

VII. AUTHORS CONTRIBUTIONS

All authors contributed equally to this manuscript

VIII. CLASHES OF INTEREST

There is no clash of concern.

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