



## **Synthesis, Characterization and Cytotoxic Estimation of Cobalt Nanoparticles against Pathogenic Bacteria**

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**Abstract :** In this investigation cobalt nanoparticles have used as antibiotic to inhibit the growth of bacteria. The size depend property is the key issue that the antimicrobial nanoparticles relaying on, as well as the size of these submicron nanoparticles compared with the volume ratio. Many researchers in the time being focusing on the use of the innovated property of nanoparticles to inhibit the growth of various types of bacteria due to their stability, easiness to synthesis and its high efficiency compare with the others antimicrobials. Cobalt nanoparticles were synthesis by reduction method .the size and morphology of the nanoparticles were characterized by Uv- visible, AFM and SEM and which showed size ranging between 10-30 nm in diameter. Staphylococcus aureus and E-coli were used to examine the cytotoxicity of cobalt nanoparticles, both bacteria have shown significant zone inhibition 14 and 15 mm consecutively after 24 hours incubation. That indicate the high efficient of cobalt nanoparticles to inhibit the growth of such pathogenic microorganisms.

### **Introduction**

Myriad of researches now a days are focusing on the use of conventional and innovated nanoparticles on several biological systems because of their new properties and consequently their applications. When the size of the materials will be changed their physical and chemical properties will be changed too and that will lead to new materials with new properties and applications. Estimating the potential effect of nanoparticles on the environment has the priority in nanoscience<sup>1,2,3</sup>. One of the most efficient ways to estimate the cytotoxicity of nanoparticles is to study their ability to inhibit the microorganisms<sup>4</sup>. The ability of microorganisms to genetically resist the antibiotics over last few decades have forced the scientist and pharmaceutical companies to develop new strategies to overcome this issue<sup>5</sup>. Recently many successful attempts have been formulated new antimicrobial agents with high efficiency, safe and less cost. Which encourage the researchers to alter some notorious antimicrobials which are well knowing with their exceptionally irritants and lethal.<sup>6</sup> have found that cobalt nanoparticles with low molecular weight have the potential to inhibit the growth of several types of bacteria as well as antifungal activities. <sup>7</sup> have revealed that metal and metal oxide nanoparticles have high efficiency to inhibit the growth of both gram negative and gram positive bacteria. Yet many methods have been adopted to prepare the metal nanoparticles such us co-precipitation <sup>8</sup>reduction<sup>9</sup>, sol-gel <sup>10</sup>, photochemical <sup>11</sup>, laser ablation<sup>12</sup> electrochemical reduction<sup>13</sup> and biological method<sup>14</sup> ect. The ability of design, control and manipulate metal nanoparticles have revealed new generation of high efficient antimicrobial materials with functionalized, targeted and surface modified properties<sup>15</sup>. This study however was aimed to synthesis Co NPs, to characterize the NPs and to study its efficiency on gram positive and gram negative pathogenic bacteria .

**Materials and methods**

**Synthesis of cobalt nanoparticles.**

Cobalt nanoparticles were prepared by dissolving 0.1 M of cobalt chloride  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  in 50 ml of 50% ethanol along with 0.1 M PEG, solution of 25 mL of 0.2 M NaOH and 0.1 ml of 0.1 M  $\text{NaBH}_4$  was added to the mixture under vigorous stirring at room temperature. After about 30 s, gray solid particles occur. The precipitate was then washed several times with distilled water and ethanol. After centrifugation at 5000 rpm for 10 minutes. Finally the precipitated nanoparticles was dried in a vacuum oven at 70 °C. the obtained powder was grinded and further used in the experiments.

**Charecterization of cubalt nanopaticles.**

Cobult nanoparticles have been characterized firstly by uv-vis spectra where the to measure the peak, scanning Electronic Microscopy SEM have been used to measure the morphology , size and shape of cobalt nanoparticles and Atomic Focce Microscope AFM have been used to measue the topograpgy of the nanoparticles

**Examination of antibacterial activity of cobalt nanoparticles**

The antibacterial activity of cobalt nanoparticles have been examine *in-vitro* against gram negative *E-coli* and gram positive *staphylococcus aurous* by well diffusion method <sup>16</sup> pure culture of both strains have been cultured in Muller Hinton Agar agar and incubated at 37°C for 24 hours. The wells have been made using gel puncture and 1 ml of Co NPs have been added to the wells. After the incubation the zone of inhibition have been measured.

**Result and disscussion.**

Synthesis and characterization of cobalt nanoparticles Gray suspention have been observed after adding sodium hydroxide followed by sodium borohydrate to cobalt chloride, which indicate the formation of cobalt nanopaticlesas shown in figur 1 a and b.



**Figure 1:A is cobalt chloride suspension B Gray color of Co NPs**

**Table 1: Characterization of cobalt nanoparticle**

No.	Wavelength(nm)	Absorbance
1	410	0.55
2	420	0.72
3	430	0.867
4	440	1.286
5	450	1.065
6	460	0.8
7	470	0.68
8	480	0.42

Characterization of cobalt nanoparticles was done using UV-Spectroscopy and showed a peak at 440 nm as shown in Table 1 and Figure 2 which indicated total conversions of cobalt ions to cobalt nanoparticles.

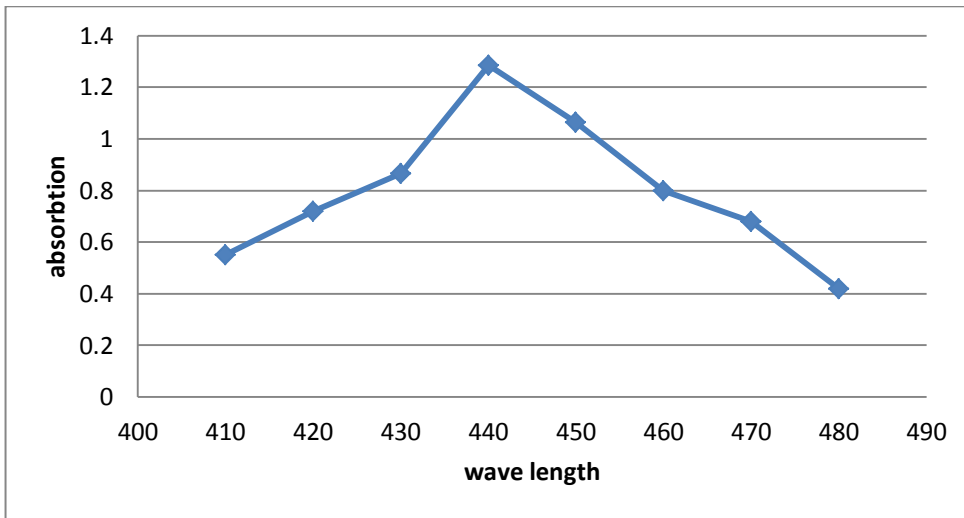
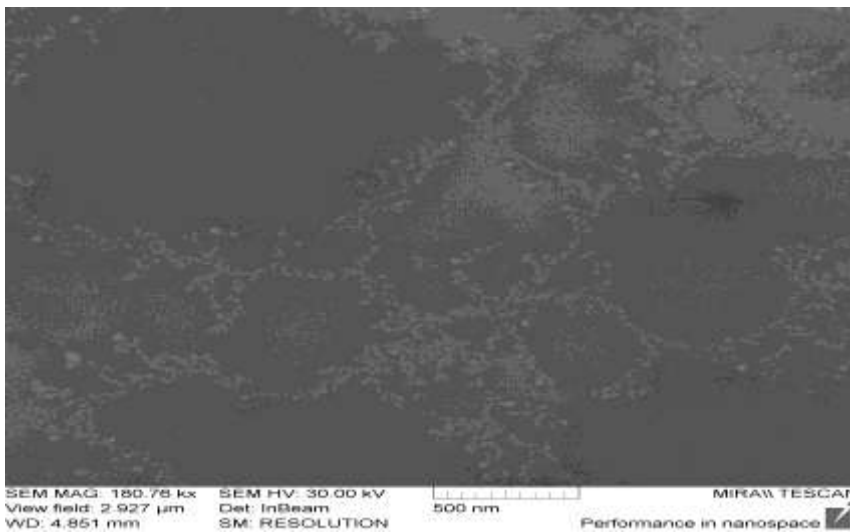


Figure 2:Characterization of cobalt nanoparticles using UV-Spectra showing the peak at 440 nm

**Scanining Electrtonic Microscope.**

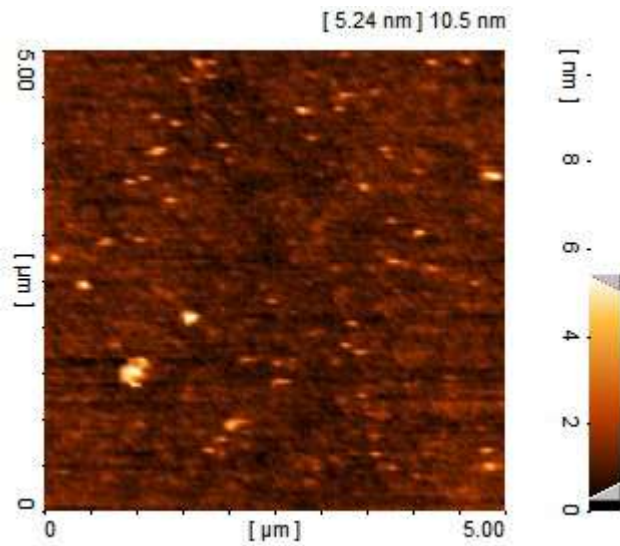
Scanining Electrtonic Microscope have been used to measure the size, shape and morphology of Co NPs. The result showed Co NPs have been fomed in spehical shape with size anging between 10 to 30 nm in diameter as it shown in figur 3.



Figur 3. Scanning electron microscopy (SEM) image of Conanoparticles shown that the spherical shape of Co NPs with size ranging between 10 – 30 nm.

**Atomic Foce Microscope AFM of Co NPs**

The surface topography analysis of AFM revealed a thin and flat morphology of Co NPs with thickness ranging between 5.24 to 10.5 nm as it revealed in figure 4



**Figure 4. AFM image of Co NPs**

**The antibacterial activity of cobalt nanoparticles**

The antibacterial activity of cobalt nanoparticles have been estimated *in-vitro* on gram negative bacteria *E-coli* and gram positive bacteria *staphylococcus aureus* by standard well diffusion method in Muller Hinton Agar<sup>16,2,3</sup> where both bacteria have been cultured 24 hours along with one ml of the synthesized cobalt nanoparticles. Both bacteria have resulted significant zone of inhibition where *E-coli* have shown 15 mm while *staphylococcus aureus* have shown 14 mm as its clarify in figure 5



**Figure 5: the antibacterial activity of Co NPs against *E-coli* and *staphylococcus aureus***

**Discussion**

The present study was aimed to synthesis and characterize Co NPS and to estimate the cytotoxic efficiency of Co NPs on gram positive and gram negative bacteria. Co NPs have been prepared successfully by chemical reduction method<sup>17</sup> using cobalt chloride which is violet in color but after adding sodium hydroxide followed by sodium borohydride the color turned gray which indicate the total conversion of Co ions to Co NPs<sup>18,19</sup> Co NPs have been characterized by UV-Visible spectra , the peak obtained at 440 nm which conform the formation of<sup>20</sup> SEM have been being used to measure the size, shape and morphology of Co NPs which shows spherical shape with size ranging between 10- 30 nm in diameter<sup>21,22</sup> AFM has being resulted a thin and

plane morphology of the Co NPs. The antimicrobial activity of Co NPs have been estimated on *E-coli* and *Staphylococcus aureus* both bacteria have showed significant zone of inhibition due to the formation of concentrated Co ions which have been formed on the surface of Co NPs due to the increase surface ratio comparing with the volume<sup>2,3</sup>

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