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Synthesis, characterization and antibacterial properties of nano-sized cobalt particles

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ABSTRACT

Nano-sized particles exhibit unique and fascinating physical, chemical and biological properties owing to their large surface to volume ratio. The study of nanoparticles interaction with microorganisms is of great importance to consider them for various biomedical applications. The aim of the present study was to study the antibacterial behavior of cobalt oxide (Co_2O_4) nanoparticles against gram positive and gram negative bacterial strains. Solution based chemical reduction method was used to synthesize the Co_2O_4 nanoparticles taking cobalt chloride as precursor. The prepared Co_2O_4 nanoparticles were characterized by X-ray diffraction (XRD) and Variable Sample Magnetometer (VSM) techniques to study the structural and magnetic properties. Kirby-Bauer disk diffusion susceptibility method was used to investigate the in-vitro antimicrobial activity of cobalt nanoparticles. *Escherichia coli (E.coli), Pseudomonas aeruginosa (P. aeruginosa)* and *Bacillus subtilis (B. subtilis)* were used to determine the antibacterial performance of our samples at different concentration of nanoparticles. It was noticed that prepared Co_2O_4 nanoparticles exhibited the concentration dependent antibacterial activity against all tested bacterial strains.

1. INTRODUCTION

Nano-sized particles owing to high surface area to volume ratio and quantum confinement effects are considered promising candidates for nanodevices of modern technology (Raza 2016). Metal and metal oxide nanoparticles are of great attraction for researcher due to their fascinating biological, optical, and chemical properties and possible applications in many fields including nano-biotechnological (Khan 2015). By

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virtue of ingesting magnetic properties, cobalt and cobalt oxide nanoparticles are extensively used technological applications such as electrochemistry, sensors, magnetic fluids, energy storage devices, catalysts and biomedicine (Allaedini 2013, Jun 2008, Zhang 2015).

One of the global health and environmental concerns is the increasing antimicrobial resistance of many microorganisms against drugs because some pathogens which were curable in past now are becoming untreatable such as methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococcus (VRE) (Henderson 2006). Humans have always been in a state of war and struggle against the microorganisms that cause infection and disease to Human health.

To win this battle and to overcome this frightening situation of microbial resistance to antibiotics, the search and discovery of alternative means against these microorganisms is only way forward to survive. Metals based nanoparticles and investigation of their antimicrobial activity could be one of such alternatives. Many studies are being done to check the structural and chemical effects of various metals nano-complexes to discover an alternative of the drugs which used against microorganisms (Saha 2009). Cobalt is also among those materials which can be used for antimicrobial activity so the synthesis, characterization and antimicrobial performance of Cobalt based nanoparticles of great importance.

The present study aims at the preparation of cobalt oxide nanoparticles in facile chemical route, their characterization and concentration dependent antibacterial tests aganist gram negative and gram positive bacterial strains such as *Escherichia coli (E.coli), Pseudomonas aeruginosa (P. aeruginosa)* and *Bacillus subtilis (B. subtilis).* We are confident that our results will contribute to figure out alternative ways for antibiotics.

2. EXPERIMENTAL DETAILS

2.1 Preparation and Characterization of Cobalt oxide Nanoparticles

Cobalt oxide nanoparticles were synthesized at room temperature following facile chemical method reported by Liang and Zhao (2012) with some modifications. At first stage, 0.235 g of tri-sodium citrate. trihydrate ($C_6H_5Na_3O_7.3H_2O$) was mixed in 10 ml of deionized water under constant stirring (150 rpm). Then 0.2 g of cobalt chloride hexahydrate ($CoCl_2.6H_2O$) and 0.1 g of sodium borohydride ($NaBH_4$) were added at the same time under constant stirring (150 rpm). A boil in the solution can be noticed when large amount of hydrogen released during the reduction reaction. When hydrogen release stopped the grayish-black powder of nanoparticles was collected by a magnet and washed serval times with deionized water and ethanol before drying at room temperature in air for 24 hours.

To study the magnetic behavior of prepared as prepared cobalt oxide nanoparticles, Lakeshore 7407 Vibrating Sample Magnetometer (VSM) was used at room temperature. For structural analysis and to determined crystallite size X-ray powder diffractometer (D-maxIIA, Rigaku, Japan) was used. Before conducting XRD of sample, the as-prepared cobalt oxide nanoparticles powder was annealed at 500 °C in inert atmosphere and finely ground using mortar and pestle at room temperature. The diffractometer was operated at 30 kV in the range of 2θ from 20° to 80° with CuKα radiations having wavelength (WL) λ= 1.54060 Å.

2.2 Antibacterial activity Test

Antibacterial activity of the Ni nanoparticles was determined using agar disk diffusion method (Asghari 2012). Antibacterial activity of the as prepared cobalt oxide (Co_2O_4) nanoparticles at three different concentrations C-1 (1mg/ml), C-2(50mg/ml) and C-3 (100mg/ml) was carried out against three different bacterial strains (*B. subtilis, E.coli* and *P. aeruginosa*) using disk diffusion method. Bacteria were firstly grown in nutrient broth overnight. 50µl of the bacterial suspension was thoroughly spread on the nutrient agar plates until plates appeared dry. 5mm sterile paper disks were then put on the plates. Antibiotic Ciprofloxacin was used as the positive control (Ab) and water was used as the negative control (con). About 20µl of each the Co_2O_4 nanoparticles suspension, water, and antibiotic solution was then poured on the disks. Plates were incubated at 37°C for 24 hours. Antibacterial activity was measured by calculating the diameter of zone of inhibition (ZOI) around the disks.

3. RESULTS AND DISCUSSION

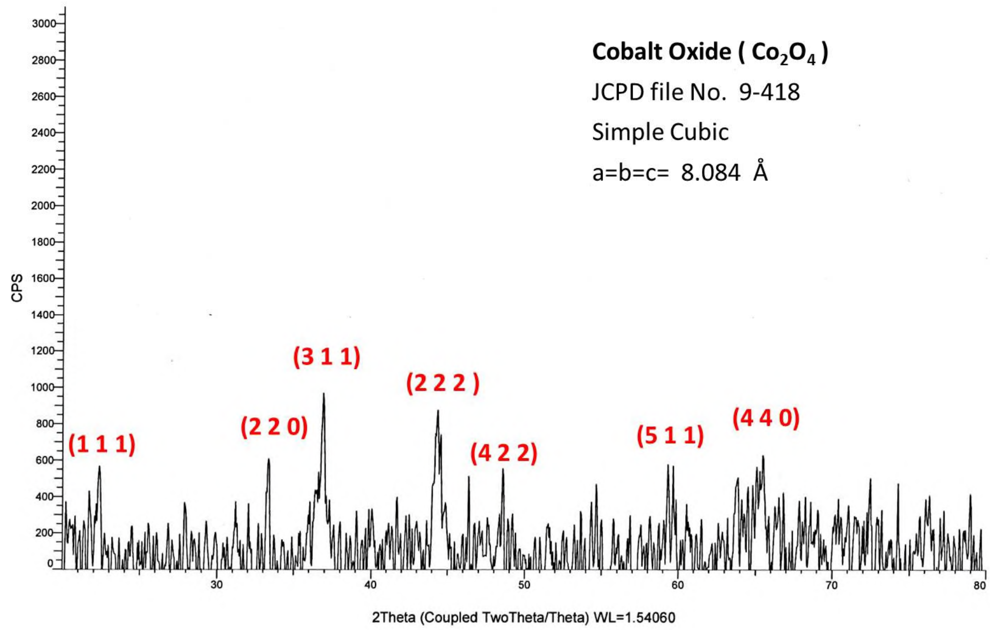

Fig. 1 X-ray diffraction (XRD) pattern of cobalt powder sample, revealing the simple cubic crystalline metallic cobalt oxide nanoparticles. The intensity in vertical axis is measured counts per second (CPS) and diffraction angle (2θ) measured is taken along horizontal axis.

In Fig. 1, the XRD pattern of our cobalt sample is illustrated which confirmed the crystalline nature of prepared cobalt oxide nanoparticles.
The diffraction peaks appearing at 2θ = 22.7°, 33.4°, 36.9°, 44.5°, 48.7°, 59.3°, and 65.7° can be assigned to the (111), (220), (311), (222), (422), (511) and (440) planes of simple cubic structure of cobalt oxide (Co_2O_4), respectively according to the JCPDS File No. 9-418. The lattice parameter was found 8.084 Ǻ. The crystalline size of the prepared nanoparticles was calculated using the Debye–Scherrer formula (Adeela 2015) as shown in Eq. (1)

$$D = \frac{\delta\lambda}{\beta cos\theta} \text{ ------------------------ (1)}$$

where D is the crystallite size, β is full-width half-maximum (FWHM) of the X-ray diffraction peaks in radians, λ is the wavelength of x-rays, δ is the shape factor and its value depends on the shape of the crystallite size and for circular shape ~0.9, and θ Bragg's diffraction angle. Using the broadening of maximum intensity XRD peak (311) appearing at 2θ =36.9°, the crystalline size was found 21.8 nm.

Fig. 2, shows the magnetic hysteresis loop of the as-prepared Co_2O_4 nanoparticles measured at room temperature. The saturation magnetization (M_s) of the Co_2O_4 particles was found 0.133 emu/g with retentively (M_r) of 0.0307 emu/g. The value of coercivity (H_c) was found 157.68 Oe. The results of VSM indicated the ferromagnetic nature of prepared Co_2O_4 nanoparticles.

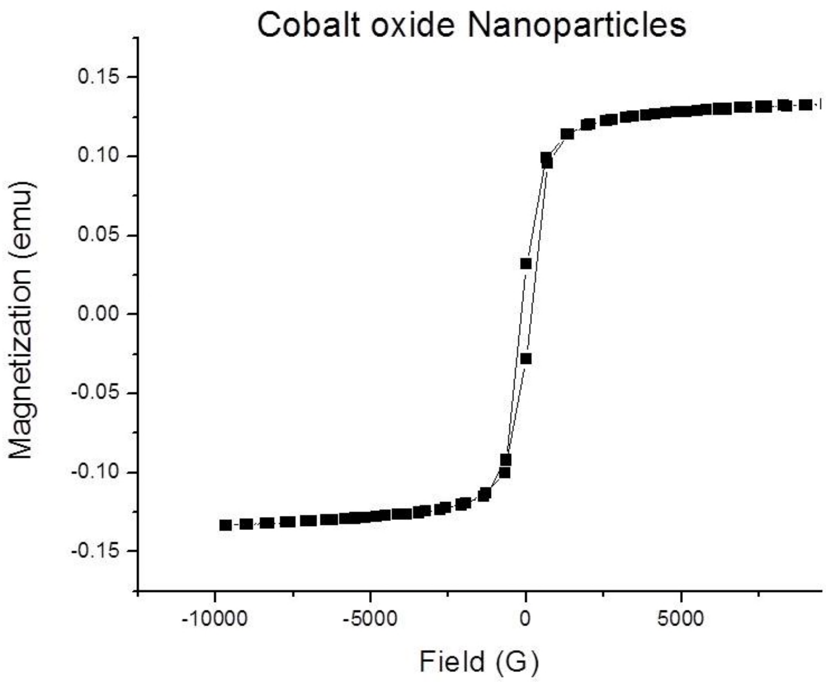

Fig. 2 Magnetization versus magnetic field plot of as prepared cobalt oxide nanoparticles at room temperature displaying the ferromagnetic nature of particles.

The antibacterial efficacy of cobalt oxide nanoparticles (Co_2O_4 NPs) at three different concentrations (C-1, C-2, and C-3) along with positive (Ab) and negative conrtol (con) against *B. subtilis* is shown in figure 3 (left). It can be clearly seen that as the Co_2O_4 NPs has exhibited better antibacterial efficiency at higher concentration. The same trend was also noticed for other two bacterial strains (*E.coli and P. aeruginosa*). The antibacterial activity of Co_2O_4 NPs with highest concentration C-3 (100mg/ml) for each bacterial strain in graphical form is shown in right panel of Fig. 3.

The highest antibacterial activity of Co_2O_4 NPs was observed against B. subtilis (ZOI, 8mm). Particles also showed good antibacterial activity against *E. coli* (ZOI, 6mm) and *P. aeruginosa* (ZOI, 5mm). It was interesting to note that *E. cloi* were susceptible to cobalt oxide particles while they were resistant to antibiotic. Therefore, based on these results it can be suggested that Co_2O_4 NPs can be a good bactericidal agent for antibiotic resistant bacterial strains. It will be of great interest for the future studies to find the antibacterial potential of Co_2O_4 NPs against other antibiotic resistant bacteria as well.

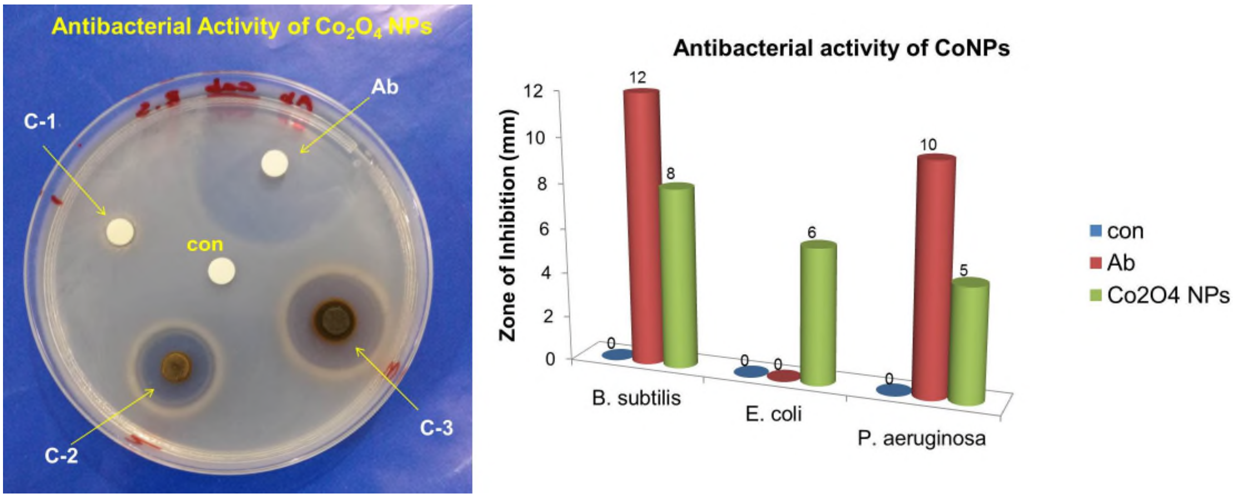

Fig. 3 (left) *B. subtilis* zones of inhibition (ZOI) around cobalt oxide nanoparticles impregnated disks. The distance of the bacterial lawn from disk can clearly be noticed, (right), graphs showing the results of ZOI formed around the highest particles concentration (C-3) soaked disks against B. subtilis, E. coli and P. aeruginosa.

3. CONCLUSIONS

We synthesized the cobalt oxide nanoparticles by a facile chemical reduction route. The prepared nanoparticles were found cobalt oxide (Co_2O_4) having simple cubic crystalline structure as determined by XRD. The VSM confirmed the ferromagnetic nature of these nanoparticles. The antibacterial activity tests showed that obtained Co_2O_4 NPs were toxic to all three bacterial strains which were examined in this work. At higher concentrations particles exhibited even better antibacterial performance than antibiotic used as positive control.

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