

Antibacterial performance of chromium nanoparticles against *Escherichia coli*, and *Pseudomonas aeruginosa*

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ABSTRACT

To make use of nano-sized particles for biomedical applications such nanomedicines for different diseases, it is important to study how nanoparticles behave when they interact with microorganisms. In the present study, we investigated the antibacterial performance of chromium oxide nanoparticles (Cr_3O_4 NPs) against two gram negative bacterial strains, *Escherichia coli*, and *Pseudomonas aeruginosa*. Chromium chloride was used as precursor to synthesize the chromium oxide nanoparticles by solution based chemical method. To study the structural analysis of prepared nanoparticles X -ray diffraction (XRD) was carried out and for magnetic behavior, Variable Sample Magnetometer (VSM) was used at room temperature. The antibacterial activity of chromium oxide nanoparticles at different concentrations was investigated against *Escherichia coli* (*E.coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) by Kirby-Bauer disc diffusion susceptibility method. The antibacterial investigation of as prepared nanoparticle showed that chromium oxide nanoparticles (Cr_3O_4 NPs) can be used as antibacterial agents depending upon the concentrations used.

1. INTRODUCTION

Dramatic change in physical, chemical and biological properties of all materials including metal and transition metal oxides is very attraction for researchers when the size of the particles is reduced into nanometer range. This drastic variation in characteristics of nano-sized materials is attributed to their large surface to volume ratio and various quantum mechanical effects (Babukutty 2015). Chromium oxide nanoparticles are considered very important owing to fundamental and technological point of view. They can be used for many applications such as liquid crystal displays, corrosive resistant materials, high temperature resistant materials, catalysts, and device fabrication in microelectronic, piezoelectric and sensors, (Ramesh 2012).

Different methods have been reported for the preparation of chromium oxide

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nanoparticles such as sol gel (El-sheikh 2009), hydrothermal (Pie 2008), precipitation method (Kim 2004), electrochemical method (Rakesh 2013), and microwave plasma assisted preparation (Vollath 1996) and chemical reduction method. Every method has its merits and demerits however chemical reduction method is considered a low cost, simple and suitable to be extended for further applications (Ramesh 2012).

Owing to the increasing antimicrobial resistance of many microorganisms against drugs, 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). Thus it is need of hour to search of alternatives against these microorganisms. Antibacterial activity investigations on nanoparticles of different materials aim at this goal. Various studies are being done to find out potential antimicrobial agents against different microorganisms, for example Khatoon (2011), El-ajaily (2007), Singh (2011) and Rakesh (2013), made efforts to study the antibacterial performance of chromium based nanomaterials against some bacterial strains including *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. But still need is there to study toughly the concentration dependent antibacterial activity of chromium oxide nanoparticles so that these particles may be used as an effective antibacterial agent in various products. The present work focused to investigate the concentration dependent antibacterial properties of chromium oxide nanoparticles.

2. EXPERIMENTAL DETAILS

2.1 Preparation and Characterization of Cobalt oxide Nanoparticles

Chromium oxide nanoparticles were prepared by the solution based chemical method. Chromium chloride ($\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$) was used as precursor and ethylene diamine tetra acidic acid (ETDA) was used as solvent for the synthesis of chromium oxide nanoparticles. $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ was dissolved in 0.2 M solution of Ethylene diamine tetra acidic acid (ETDA) at room temperature and stirred for 30 min. Sodium hydroxide (NaOH) was added to above solution to adjust pH to 9.0. Resultant green precipitates were centrifuged at 4000 rpm for 60 sec. Centrifuged particles were dried at 400°C for 60 min to obtain chromium oxide nanoparticles.

The crystalline nature of the as prepared chromium oxide nanoparticles was investigated by X-ray powder diffractometer (D-maxIIA, Rigaku, Japan). The diffractometer was operated at 30 kV in the range of 2θ from 20° to 80° with $\text{CuK}\alpha$ radiations having wavelength (WL) $\lambda = 1.54060 \text{ \AA}$. To study the magnetic behavior of prepared as prepared cobalt oxide nanoparticles, Lakeshore 7407 Vibrating Sample Magnetometer (VSM) was used at room temperature.

2.2 Antibacterial Activity Tests

The antibacterial activity of chromium oxide nanoparticles using three different concentrations, C1 (1 mg/ml), C2 (50 mg/ml) and C3 (100 mg/ml) was investigated against two gram negative bacterial strains: *E.coli* and *P. aeruginosa*. Disk diffusion method was used for antibacterial activity tests. Both bacterial strains were grown overnight in nutrient broth. A 50 μl bacterial suspension was taken and completely

spread over the nutrient agar plates. Small (5 mm diameter) sterile disks were placed on the dry plates and 20 μ of the chromium oxide nanoparticles solution was added on the disk. Same volume of antibiotic Ciprofloxacin and water was also put on the positive control (Ab) and negative control (con) disks. After that the plates were incubated at 37°C for 24 hours. Antibacterial activity was checked by measuring the diameter of zone of inhibition (ZOI) around each disk.

3. RESULTS AND DISCUSSION

The XRD pattern of as prepared chromium oxide nanoparticles powder sample is shown in Fig. 1 which revealed the crystalline nature of prepared chromium oxide nanoparticles. The XRD peaks appeared at $2\theta = 23^\circ, 32.7^\circ, 41.3^\circ, 46.9^\circ, 53^\circ, 58.2^\circ, 68.5^\circ,$ and 78° can be assigned to the (220), (202), (400), (004), (242), (333), (602) and (335) diffraction planes respectively according to the JCPDS File No. 12-559. The XRD results indicated that prepared nanoparticles were chromium oxide (Cr_3O_4) having tetragonal crystalline structure. The lattice parameters were found $a=b= 8.72\text{\AA}$ and $c= 7.5\text{\AA}$.

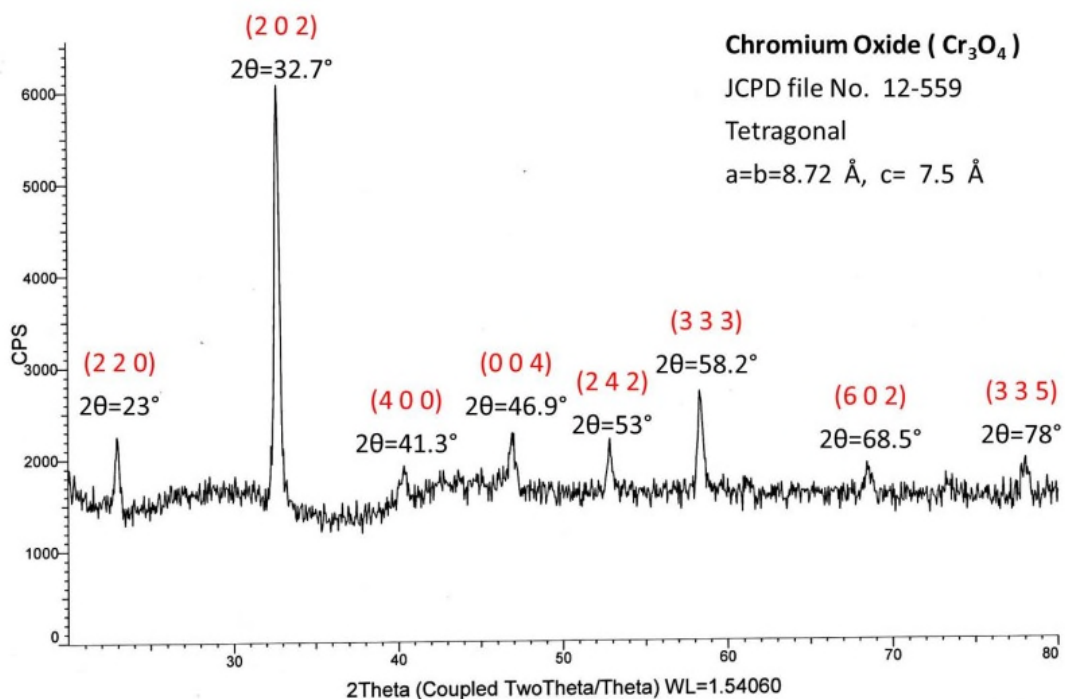


Fig. 1 X-ray diffraction (XRD) pattern of chromium oxide powder sample, indicating the tetragonal crystalline nature of prepared nanoparticles.

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 (202) appearing at $2\theta = 32.7^\circ$, the crystalline size was found 15.7 nm.

Magnetic hysteresis loop of the as-prepared chromium oxide nanoparticles measured at room temperature is shown in Fig. 2. This plot simply revealed the paramagnetic behavior of chromium oxide nanoparticle at room temperature.

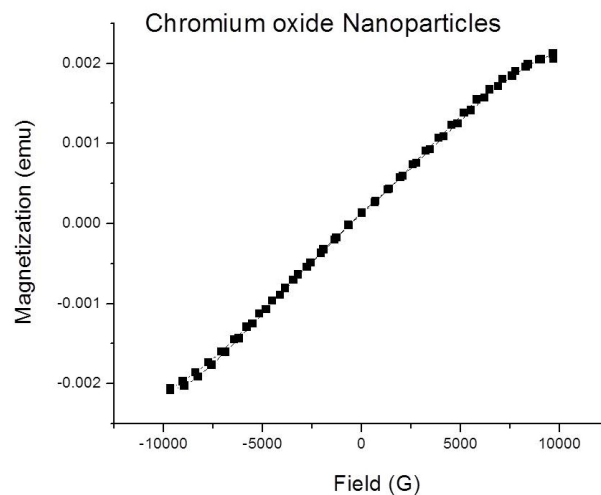
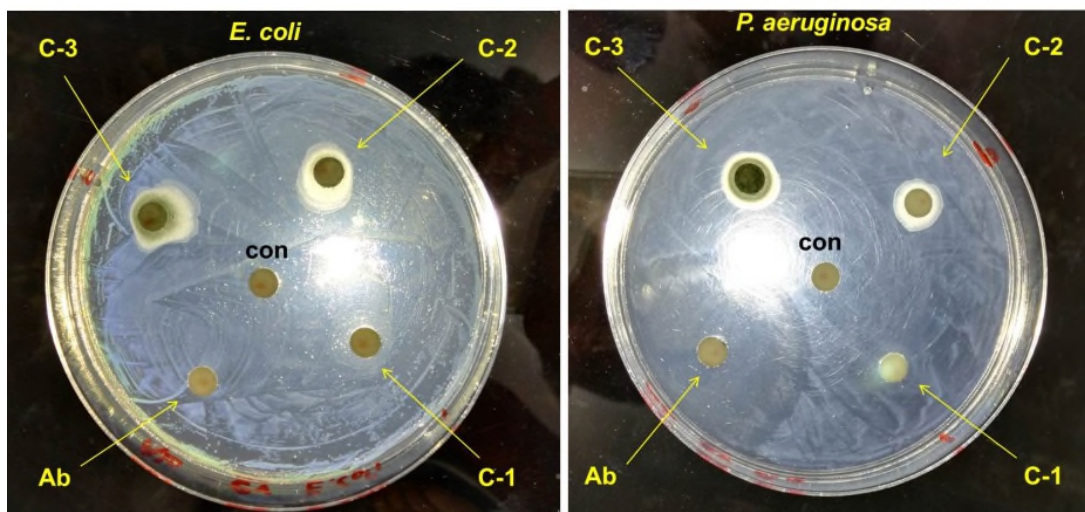


Fig. 2 Magnetization versus magnetic field plot of as prepared chromium oxide nanoparticles at room temperature displaying the paramagnetic nature of particles.

The antibacterial activity results of Cr_3O_4 NPs for its different concentrations (C1, C2, and C3) and positive and negative controls are shown in Fig 3. It can be seen from zone of inhibitions of bacterial growth on the agar plate (upper panel of Fig. 3) that Cr_3O_4 NPs at all concentrations showed at least some bactericidal activity against both *E.coli* and *P. aeruginosa*. However, at higher concentrations antiseptic efficiency of Cr_3O_4 NPs increased. Interestingly, *E. coli* were observed to be completely resistant to the antibiotic, ciprofloxacin (ZOI: 0mm) but they were susceptible to Cr_3O_4 NPs. Therefore, it can be suggested that Cr_3O_4 NPs can be a good bactericidal agent for antibiotic resistant bacterial strains. It will be very interesting for future studies to find the antibacterial action of Cr_3O_4 NPs against other antibiotic unsusceptible bacterial strains. In lower panel of Fig 3, the antibacterial activity results of Cr_3O_4 NPs at highest concentration (C3) in comparison with positive and negative controls are illustrated in graphical forms.



Antibacterial activity of Chromium oxide NPs

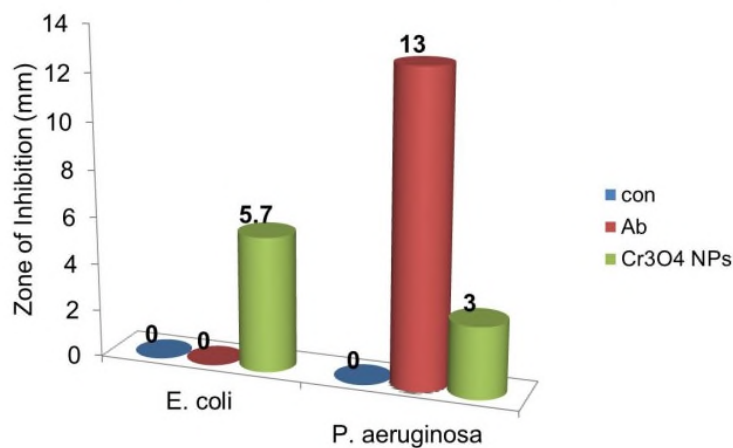


Fig. 3 Images of agar plates ((upper panel) containing antibiotic (ab), water (con) and different concentrations of Cr₃O₄ NPs impregnated disks and exhibition of inhibitory zones of *E. coli* and *P. aeruginosa*. The lower panel depicts the graphical representation of ZOI measurements for highest concentration of Cr₃O₄ NPs (C3) and both controls against *E. coli* and *P. aeruginosa*.

3. CONCLUSIONS

Chromium oxide nanoparticles synthesized by solution based chemical methods were characterized by XRD and VSM which revealed the tetragonal crystalline structure and paramagnetic nature of the Cr₃O₄ nanoparticles. The antibacterial activity investigations showed that prepared Cr₃O₄ NPs were good bactericidal agent even for antibiotic resistant bacterial strains.

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