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Research Article

SYNTHESIS, CHARACTERIZATION, AND ANTIBACTERIAL ACTIVITIES OF CHROMIUM OXIDE NANOPARTICLES AGAINST *KLEBSIELLA PNEUMONIAE*

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ABSTRACT

Objective: This paper consists of synthesis of chromium oxide (Cr_2O_3) nanoparticles by sol-gel technique, their characterization and investigation of antibacterial activity of these nanoparticles against pathogenic bacteria by measuring zone of inhibition, colony forming units, and optical density (OD) on solid agar media as well as in liquid medium.

Methods: The Cr_2O_3 nanoparticles were synthesized by sol-gel technique using tetraethylorthosilicate as precursor. The synthesized Cr_2O_3 nanoparticles were characterized by X-ray diffraction (XRD), Fourier-transform infrared spectroscopy, ultraviolet-visible spectroscopy, and transmission electron microscopy (TEM) techniques. The antibacterial effect of these Cr_2O_3 nanoparticles against *Klebsiella pneumoniae* was investigated both on the solid agar plates and in liquid medium supplemented with different concentrations of Cr_2O_3 nanoparticles. The antibacterial activity of Cr_2O_3 nanoparticles was also compared with the antibacterial activities of the standard antibiotics such as ampicillin, chloramphenicol, penicillin G, streptomycin, sulphatriad, and tetracycline which were taken in the form of hexa discs.

Results: Average particle size of the Cr_2O_3 nanoparticles was found to be 24.0 nm. It was observed that *K. pneumoniae* is resistant to the penicillin G and ampicillin, but Cr_2O_3 nanoparticles show good antibacterial property. The minimum inhibitory concentration of Cr_2O_3 for *K. pneumoniae* is 2.5 mg/ml. The bacterial growth was monitored by measuring zone of Inhibition, colony formation unit, and OD method.

Conclusion: Sol-gel technique is a convenient and easy technique for the synthesis of metal nanoparticles. Nanosized Cr_2O_3 particles showed an effective antibacterial activity against *K. pneumoniae*. Therefore, Cr_2O_3 nanoparticles due to its low manufacturing cost and high effectiveness in antimicrobial properties may find wide applications in various industries to address safety issues.

Keywords: *Klebsiella pneumoniae*, Chromium oxide nanoparticles, X-ray diffraction, Transmission electron microscopy.

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INTRODUCTION

Transition metal oxide nanoparticles represent a broad class of materials that have been researched extensively due to their interesting catalytic, electronic, magnetic, and medicinal properties. The nanobiotechnology studies have focused on the medical applications of nanoparticles for treatments and antibacterial effect. Recently, chromium oxides (Cr_2O_3) have attracted much attention due to their importance in science as well as in technology. Chromia (Cr_2O_3), possess specific applied applications such as in high-temperature resistant materials [1], corrosion resistant materials [2], liquid crystal displays [3], green pigment [4], heterogeneous catalysts [5,6], coating materials [7,8], and so on. The intrinsic properties of inorganic materials are mainly determined by their composition, structure, crystallinity, size and morphology; great efforts have been devoted to the investigation of different Cr_2O_3 materials synthesis [9-11].

Cr_2O_3 nanoparticles have been synthesized by different methods such as hydrothermal [12,13] solid thermal decomposition [14], sol-gel [15], combustion [16], precipitation-gelation [17], microwave irradiation [3,18], inverse-microemulsion [19], oxidation of chromium in oxygen [20], and precipitation [21] methods. Negahdary *et al*. [22] synthesized Cr_2O_3 nanoparticles with chemical methods. Characterization of nanoparticles studied with ultraviolet (UV)-visible spectrophotometer, X-ray diffractometer, and transmission electron microscopy (TEM) microscope. Ramesh *et al*. [23] synthesized Cr_2O_3 nanoparticles by reduction of potassium dichromate solution with *Arachis hypogaea* leaf extract. The antibacterial effect of Cr_2O_3 nanoparticles against *Escherichia coli* was investigated as a model for gram-negative bacteria. Khatoon *et al*. [24] reported the internalization of Cr_2O_3 nanoparticles in *Escherichia coli* cells by flow cytometry using light scattering method. El-Ajaily *et al*. [25] reported the antibacterial activity of Cr (VI) and Cr (III) complexes against *P. aeruginosa* bacteria. Singh *et al*. [26] reported viability of an environmentally relevant bacterium, *E. coli* exposed to varying concentrations of Cr_2O_3 nanoparticles was evaluated propidium mono-azide assisted quantitative-polymerase chain reaction. Rakesh *et al*. [27] synthesized the Cr_2O_3 nanoparticles by reduction of potassium dichromate solution with *Mukia maderaspatana* plant extract. The resulting Cr_2O_3 nanoparticles were characterized by X-ray diffraction (XRD), SEM, UV-visible absorption and Fourier-transform infrared (FTIR) spectroscopy. The antibacterial effect of Cr_2O_3 nanoparticles against *E. coli* was investigated. Khalil [28] investigated the antibacterial activity of chromium nanoparticles on two phytopathogenic bacteria, namely, *Erwinia carotovora* and *Pseudomonas fluorescens*. Therefore, the transition metal nanoparticles have been researched widely because of their good antimicrobial activity [29,30].

METHODS

Materials

Klebsiella pneumoniae (MTCC 3384) was obtained from microbial type culture collection (MTCC), Institute of Microbial Technology, Chandigarh. All other chemicals used in the experiment were of AR grade and obtained from standard chemical sources.

Synthesis of Cr_2O_3 nanoparticles

Cr_2O_3 nanoparticles were synthesized using sol-gel method. The procedure uses chromium trioxide solution of pH 1-2, ethanol and tetraethylorthosilicate (TEOS) as the precursor material. The Cr_2O_3 nanoparticles were prepared by mixing chromium trioxide solution drop by drop into the flask containing 1:4 TEOS and ethanol solution with continuous stirring. The resulting solution was heated at 70.0°C with continuous stirring in a closed container for 6.0 hrs. The resulting solution was then kept in the oven at 100.0°C for 10-15 days, and after that, the particles were kept in muffle furnace at 400.0°C for 4.0 hrs. Blackish green Cr_2O_3 nanoparticles were obtained.

Characterization techniques

The size, structure, morphology and magnetic properties of as-prepared metal nanoparticles were characterized by FTIR (shimadzu corp-02014) in the wavelength range 400-4000/cm, UV-visible spectroscopy (Shimadzu 1800) in the wavelength range 200-1000/cm, XRD (Rikagu mini-2 using Cuα1, λ=0.15406 nm radiations), and TEM (FEI-Philips, Morgagni 286D with magnification up to 2,80,000x, Acc. Voltage: 100 Kv).

Antibacterial study

The antibacterial activity of Cr_2O_3 nanoparticles against *K. pneumoniae* was tested by measuring zone of inhibition (ZOI), evaluating colony forming unit (CFU) on solid medium, and by measuring the optical density (OD) of culture solution. The zone of inhibition (ZOI) was measured by agar well-diffusion method. Nutrient broth/agar (0.1 g beef, 0.2 g yeast extract, 0.5 g peptone, 0.5 g NaCl dissolved in 100 ml of double distilled water) was used to cultivate bacteria. The media was autoclaved and cooled. The media was poured in the previously sterilized petri plates and kept for 30 minutes for solidification. After 30 minutes, the plates were left overnight at room temperature to check for any contamination to appear. The bacterial test organism *K. pneumoniae* were grown in nutrient broth at 37°C for 24.0 hrs. A 100 µl of the fresh overnight nutrient broth culture was spread onto solidified nutrient agar plates. Wells of 8.0 mm diameter were prepared with the help of a sterilized stainless steel cork borer. Using a micropipette, different concentrations of the Cr_2O_3 nanoparticles solution (2.5, 3.0, 3.25, 3.5, 3.75, 4.0, 6.0, 8.0, 10.0, 12.0 mg/ml) was poured into each well on the plates. Various antibiotics in the form of hexa discs were used as a positive control for bacteria to compare the inhibition of bacterial growth with Cr_2O_3 nanoparticles. The plates containing bacteria solutions of nanoparticles and antibiotic discs were incubated at 37.0°C for 24.0 hrs. After 24.0 hrs of incubation, the different level of zone of Inhibition produced by Cr_2O_3 nanoparticles against *K. pneumoniae* was measured in mm.

CFU and OD measurement

K. pneumoniae was used for colony forming units (CFU) measurement on the solid medium plate. Serial dilutions of the broth culture were prepared. 0.1 ml of 10^{-6} dilution of the bacterial culture was tested with different concentration (1.0, 2.0, 3.0 mg/ml) of Cr_2O_3 nanoparticles. After incubation at 37.0°C, the number of CFU was counted. The growth behavior of the *K. pneumoniae* was also investigated by measuring OD through the administration of the Cr_2O_3 nanoparticles at different concentrations into the dilute solution of the broth culture.

RESULTS AND DISCUSSION

The average particle size was calculated from XRD data using Scherrer's equation. Particle morphology of the sample was investigated by a TEM. FTIR spectroscopy was performed to know the synthesis condition, and UV-visible spectroscopy was carried out for the optical study of metal nanoparticles. Fig. 1 shows XRD pattern of the Cr_2O_3 nanoparticles. The inspection of XRD pattern revealed that Cr_2O_3 thus formed is of rhombohedral phase (JCPDS no. 38-1479 with a=4.9587 A°c=13.594 A°). The major peaks at 2θ values of 24.62, 33.8, 36.4, 41.8, 50.32, 55.16, 63.6, and 65.32 are indexed as (012), (104), (110), (113), (024), (116), (214), (300), respectively. Average particle size of the Cr_2O_3 nanoparticles was found to be 24.0 nm using Scherrer's formula d = Kλ/βCos θ where the constant K is taken to be 0.94, λ is the wavelength of X-ray and β, and θ are the full width at half maximum and Braggs angle, respectively.

Fig. 2 shows the TEM image of the Cr_2O_3 nanoparticles. The microstructural characterization studies were conducted to determine the size of nanoparticles and examine the homogeneity and size distribution. The particles were observed to be almost spherical. It can be seen from the Fig. 2 that there is a uniform distribution of the particle with mean particle size 21.36 nm which is in close agreement with the XRD result.

Fig. 3 shows FTIR spectra of Cr_2O_3 nanoparticles synthesized by sol-gel technique. FTIR spectroscopy was carried out to ascertain the purity and nature of metal or metal oxide nanoparticles. The band between 3200 and 3400/cm and 1622/cm are due to the -OH stretching and bending vibrations of adsorbed water molecule on the sample, band at 2929/cm may be due to $-CH_3$ stretching vibrations 1072/cm is due to Cr-O-Cr vibrations 952/cm and 902/cm are assigned to Cr=O vibrations. The two peaks at 550 and 617/cm are assigned to Cr-O str. modes are evidence for the presence of crystalline Cr_2O_3 nanoparticles [31].

The optical characterization of the sample was recorded on UV-visible absorption spectrophotometer. Fig. 4 shows UV-visible spectra of Cr_2O_3 nanoparticles as a function of wavelength. The UV-visible absorption spectroscopy of Cr_2O_3 nanoparticles shows an absorption peak at about 351.2 and 255.1 nm. The band energy gap was found to be 4.19 eV.

Fig. 5 shows the zone of inhibition of bacterial growth produced by different concentration of Cr_2O_3 nanoparticles on agar plates. The minimum inhibitory concentration (MIC) of Cr_2O_3 nanoparticles for *K. pneumoniae* was observed at 2.5 mg/ml. Table 1 shows the average zone of inhibition produced at different concentrations of the synthesized Cr_2O_3 nanoparticles, and these results reveal the strong efficiency of these Cr_2O_3 nanoparticles to inhibit the bacterial growth.

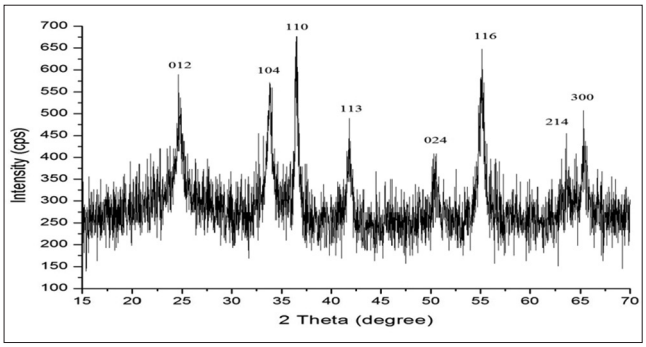

Fig. 1: X-ray diffraction pattern of chromium oxide nanoparticles

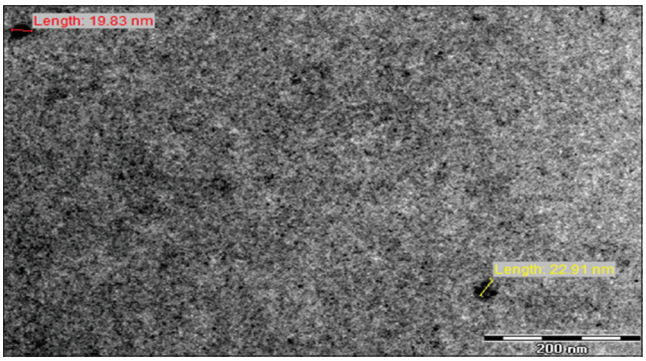

Fig. 2: Transmission electron microscopy image of chromium oxide nanoparticles

The zone of inhibition increases gradually as the concentration of Cr_2O_3 nanoparticles increases. Results clearly demonstrate that synthesized Cr_2O_3 nanoparticles act as promising antimicrobial agent.

Fig. 6 shows the zone of inhibition produced by different antibiotics such as ampicillin (10 mcg), chloramphenicol (25 mcg), penicillin G (1 unit), streptomycin (10 mcg), sulphatriad (300 mcg), and tetracycline (25 mcg) which are taken in the form of hexa discs. It was found that *K. pneumoniae* is resistant to the penicillin G and ampicillin.

Fig. 7 shows the colony forming unit measurement on the solid medium plate. The serial dilutions of the broth culture were prepared. 0.1 ml of 10^{-6} dilution of the bacterial culture were spread on the plate (a), plates (b), (c) and (d) consists of 0.1 ml of diluted broth with different concentrations (1.0 mg/ml, 2.0 mg/ml, 3.0 mg/ml) of Cr_2O_3 nanoparticles. These plates were incubated at 37.0°C for 24.0 hrs. After 24.0 hrs, the numbers of CFU were counted. There were 185 colonies were counted on plate (a) and the number of CFU decreases as the concentration of metal nanoparticles increases. This shows that the Cr_2O_3 exhibited good antibacterial property.

Aqueous dispersion of these nanoparticles at desired concentrations was made. The 50 ml of diluted bacterial cells were taken in different flasks. The solutions were taken in real life situations. Shaking provided bacteria aeration and homogeneity. Control flask containing all the initial reaction components except the Cr_2O_3 nanoparticles showed no antibacterial activity. Cr_2O_3 nanoparticles were added in the solution at the beginning of bacterial cell growth. Optical densities as a function of time measured periodically up to 24.0 h of control and solutions containing different concentrations of Cr_2O_3 nanoparticles as shown in Fig. 8 and it is observed that as the concentration of Cr_2O_3 nanoparticles increases, the growth decreases.

CONCLUSION

Cr_2O_3 nanoparticles of average particle size 24.0 nm were synthesized using Sol-gel method. Antibacterial study of Cr_2O_3 nanoparticles was investigated against *K. pneumoniae* by using zone of inhibition, CFU measurement, and OD methods. The zone of inhibition shown by the Cr_2O_3 nanoparticles against *K. pneumoniae* was compared with well-known antibiotics. It is observed that *K. pneumoniae* is resistant to the penicillin G and ampicillin, but Cr_2O_3 nanoparticles show good antibacterial property. The MIC of Cr_2O_3 for *K. pneumoniae* is 2.5 mg/ml. The zone of inhibition, CFU estimation and OD curves shows that the bacterial growth reduces significantly with the increase in the concentration of Cr_2O_3 nanoparticles. The results obtained from zone of inhibition, CFU and OD curves were in close agreement with each other. Therefore, it is concluded that the Cr_2O_3 nanoparticles are easy to synthesize and possess good antibacterial activities.

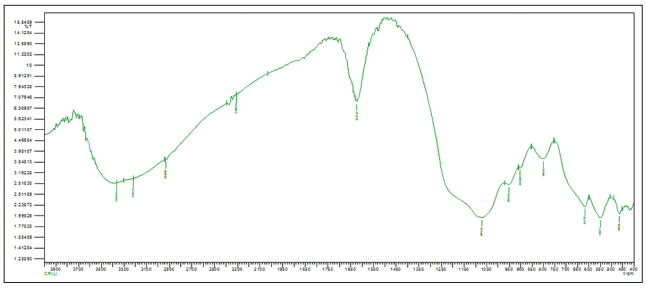

Fig. 3: Fourier-transform infrared spectra of chromium oxide nanoparticles

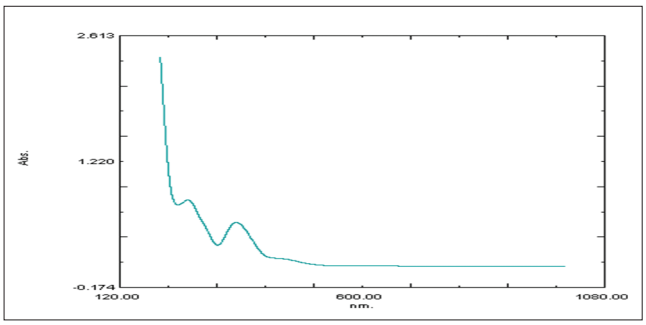

Fig. 4: Ultraviolet-visible absorption spectra of chromium oxide nanoparticles

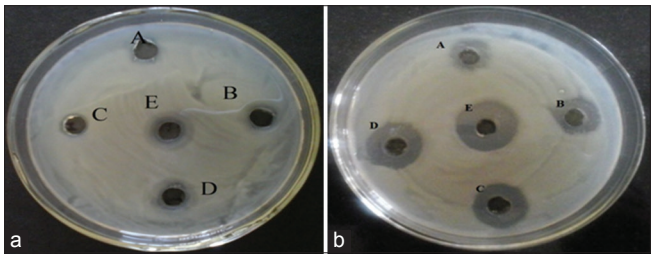

Fig. 5: Zone of inhibition produced by different concentrations of chromium oxide nanoparticles. (a) (2.5 mg/ml, 3.0 mg/ml, 3.25 mg/ml, 3.5 mg/ml, 3.75 mg/ml), (b) (4.0 mg/ml, 6.0 mg/ml, 8.0 mg/ml, 10.0 mg/ml, 12.0 mg/ml) against *Klebsiella pneumoniae*

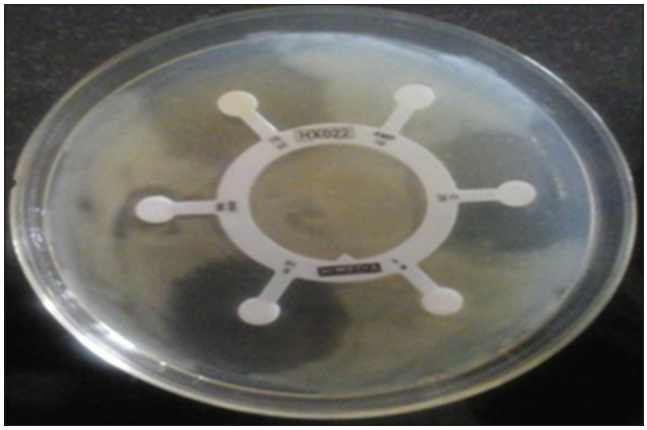

Fig. 6: Zone of inhibition produced by different antibiotics (ampicillin [10 mcg], chloramphenicol [25 mcg], penicillin G [1 unit], streptomycin [10 mcg], sulphatriad [300 mcg], tetracycline [25 mcg]) against *Klebsiella pneumoniae*

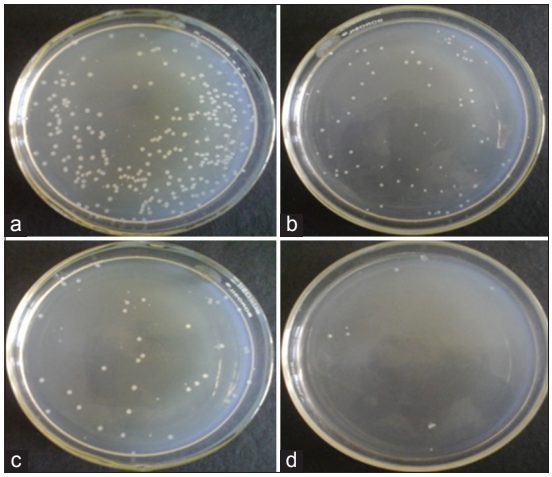

Fig. 7: Agar plates showing CFU count at different concentrations of chromium oxide nanoparticles against *Klebsiella pneumonia.* (a) 0.1 ml of diluted broth, (b) Diluted broth+1.0 mg Cr NP, (c) diluted broth+2.0 mg Cr NP, (d) diluted broth + 3.0 mg Cr NP

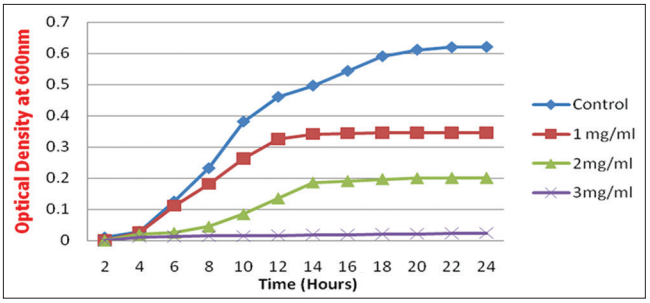

Fig. 8: Effect of chromium oxide nanoparticles on the growth of *Klebsiella pneumoniae*

Table 1: Result of agar well-diffusion method

Concentration of Cr_2O_3 nanoparticles mg/ml	Average zone of inhibition (mm)
2.5	8.7±0.58
3.0	9.3±0.58
3.25	9.7±1.15
3.50	10.0±0.0
3.75	10.2±0.76
4.0	11.8±0.6
6.0	14.5±0.5
8.0	16.6±0.0
10.0	17.3±0.6
12.0	19.6±0.6

Cr_2O_3: Chromium oxide

ACKNOWLEDGMENTS

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