SYNTHESIS, CHARACTERIZATION, AND ANTIBACTERIAL ACTIVITIES OF CHROMIUM OXIDE NANO Particles AGAINST KLEBSIELLA PNEUMONIAE

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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 (CrO3) have attracted much attention due to their importance in science as well as in technology. Chromia (Cr2O3), 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 CrO3 materials synthesis [9-11].

Cr3O4 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 Cr3O4 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 Cr3O4 nanoparticles by reduction of potassium dichromate solution with Arachis hypogaea leaf extract. The antibacterial effect of Cr3O4 nanoparticles against Escherichia coli was investigated as a model for gram-negative bacteria. Khatoo et al. [24] reported the internalization of Cr3O4 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 Klebsiella pneumoniae bacteria. Singh et al. [26] reported viability of an environmental relevant bacterium, E. coli exposed to varying concentrations of Cr3O4 nanoparticles was evaluated propidium mono-oxide assisted quantitative-polymerase chain reaction. Rakesh et al. [27] synthesized the Cr3O4 nanoparticles by reduction of potassium dichromate solution with Mukia maderaspatana plant extract. The resulting Cr3O4 nanoparticles were characterized by X-ray diffraction (XRD), SEM, UV-visible absorption and Fourier-transform infrared (FTIR) spectroscopy. The antibacterial effect of Cr3O4 nanoparticles against E. coli was investigated. Khalil [28] investigated the antibacterial activity of CrO3 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$_2$O$_3$ nanoparticles

Cr$_2$O$_3$ nanoparticles were synthesized using sol-gel method. The procedure uses chromium trioxide solution of pH 1-2, ethanol and tetraethyorthosilicate (TEOS) as the precursor material. The Cr$_2$O$_3$ 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$_2$O$_3$ 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 2,800,000x, Acc. Voltage: 100 kV).

Antibacterial study

The antibacterial activity of Cr$_2$O$_3$ 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 (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 into the previously sterilized petri plates and kept for 30 minutes for solidification. After 30 minutes, the plates were kept overnight at room temperature to check for any contamination to appear. The bacterial test organism K. pneumoniae was 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$_2$O$_3$ 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$_2$O$_3$ 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$_2$O$_3$ 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$_2$O$_3$ 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$_2$O$_3$ 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$_2$O$_3$ nanoparticles. The inspection of XRD pattern revealed that Cr$_2$O$_3$ thus formed is of rhombohedral phase (JCPDS no. 38-1479 with a=9.9587, c=13.594 A$^\text{a}$). The major peaks at 20 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$_2$O$_3$ 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 Bragg’s angle, respectively.

Fig. 2 shows the TEM image of the Cr$_2$O$_3$ 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$_2$O$_3$ 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 bending vibrations. The two peaks at 550 and 617/cm are assigned to Cr=O vibrations. The optical characterization of the sample was recorded on UV-visible absorption spectrophotometer. Fig. 4 shows UV-visible spectra of Cr$_2$O$_3$ nanoparticles as a function of wavelength. The UV-visible absorption spectroscopy of Cr$_2$O$_3$ 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$_2$O$_3$ nanoparticles on agar plates. The minimum inhibitory concentration (MIC) of Cr$_2$O$_3$ 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$_2$O$_3$ nanoparticles, and these results reveal the strong efficiency of these Cr$_2$O$_3$ nanoparticles to inhibit the bacterial growth.

![Fig. 1: X-ray diffraction pattern of chromium oxide nanoparticles](image1)

![Fig. 2: Transmission electron microscopy image of chromium oxide nanoparticles](image2)
The zone of inhibition increases gradually as the concentration of Cr$_2$O$_3$ nanoparticles increases. Results clearly demonstrate that synthesized Cr$_2$O$_3$ 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$_2$O$_3$ 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$_2$O$_3$ 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$_2$O$_3$ nanoparticles showed no antibacterial activity. Cr$_2$O$_3$ nanoparticles were added in the solution at the beginning of bacterial cell growth. Optical densities as a function of time were measured periodically up to 24.0 h of control and solutions containing different concentrations of Cr$_2$O$_3$ nanoparticles as shown in Fig. 8 and it is observed that as the concentration of Cr$_2$O$_3$ nanoparticles increases, the growth decreases.

CONCLUSION

Cr$_2$O$_3$ nanoparticles of average particle size 24.0 nm were synthesized using Sol-gel method. Antibacterial study of Cr$_2$O$_3$ nanoparticles was investigated against K. pneumoniae by using zone of inhibition, CFU measurement, and OD methods. The zone of inhibition shown by the Cr$_2$O$_3$ 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$_2$O$_3$ nanoparticles show good antibacterial property. The MIC of Cr$_2$O$_3$ 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$_2$O$_3$ 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$_2$O$_3$ nanoparticles are easy to synthesize and possess good antibacterial activities.
Alumina foam coated with nanostructured chromia aerogel: The antibacterial effect of cerium oxide nanoparticles on Klebsiella pneumoniae. Abecassis-Wolfovich M, Rotter H, Landau MV, Korin E, Erenburg AI, Hou X, Choy KL. Synthesis of Cr-based nanocomposite coatings with incorporation of inorganic fullerene-like nanoparticles. Thin Solid Films 2008;516(23):8620-4.

Table 1: Result of agar well-diffusion method

| Concentration of CrO$_3$ 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                        |

CrO$_3$: Chromium oxide

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