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

POONAM SANGWAN*, HARISH KUMAR

1Department of Chemistry, GDC Memorial College, Bahal, Haryana, India. 2Department of Chemistry, Chaudhary Devial University, Sirsa, Haryana, India. Email: poonam.sangwan35@gmail.com

Received: 14 September 2016, Revised and Accepted: 27 October 2016

ABSTRACT

Objective: This paper consists of synthesis of chromium oxide (Cr$_2$O$_3$) 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$_2$O$_3$ nanoparticles were synthesized by sol-gel technique using tetraethylorthosilicate as precursor. The synthesized Cr$_2$O$_3$ 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$_2$O$_3$ nanoparticles against Klebsiella pneumoniae was investigated both on the solid agar plates and in liquid medium supplemented with different concentrations of Cr$_2$O$_3$ nanoparticles. The antibacterial activity of Cr$_2$O$_3$ nanoparticles was also compared with the antibacterial activities of the standard antibiotics such as ampicillin, chloramphenicol, penicillin G, streptomycin, sulphathiazide, and tetracycline which were taken in the form of hexa discs.

Results: Average particle size of the Cr$_2$O$_3$ nanoparticles was found to be 24.0 nm. It was observed that $K$. pneumoniae resistant to the penicillin G and ampicillin, but Cr$_2$O$_3$ nanoparticles show good antibacterial property. The minimum inhibitory concentration of Cr$_2$O$_3$ for $K$. pneumoniae is 2.5 mg/ml. The bacterial growth was monitored by measuring zone of inhibition, colony formation units, and OD method.

Conclusion: Sol-gel technique is a convenient and easy technique for the synthesis of metal nanoparticles. Nano-sized Cr$_2$O$_3$ nanoparticles showed an effective antibacterial activity against $K$. pneumoniae. Therefore, Cr$_2$O$_3$ 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.

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$_2$O$_3$) have attracted much attention due to their importance in science as well as in technology. Chromia (Cr$_2$O$_3$), 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$_2$O$_3$ materials synthesis [9-11].

Cr$_2$O$_3$ 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$_2$O$_3$ 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$_2$O$_3$ nanoparticles by reduction of potassium dichromate solution with Arachis hypogaea leaf extract. The antibacterial effect of Cr$_2$O$_3$ nanoparticles against Escherichia coli was investigated as a model for gram-negative bacteria. Khatoot et al. [24] reported the internalization of Cr$_2$O$_3$ 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 $E$. aeruginosa bacteria. Singh et al. [26] reported viability of an environmentally relevant bacterium, $E$. coli exposed to varying concentrations of Cr$_2$O$_3$ nanoparticles was evaluated propidium mono-oxide assisted quantitative-polymerase chain reaction. Rakesh et al. [27] synthesized the Cr$_2$O$_3$ nanoparticles by reduction of potassium dichromate solution with Mucia madraspatana plant extract. The resulting Cr$_2$O$_3$ nanoparticles were characterized by X-ray diffraction (XRD), SEM, UV-visible absorption and Fourier-transform infrared (FTIR) spectroscopy. The antibacterial effect of Cr$_2$O$_3$ 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 Cr₂O₃ nanoparticles

Cr₂O₃ nanoparticles were synthesized using sol-gel method. The procedure uses chromium trioxide solution of pH 2-3, ethanol and tetraethylorthosilicate (TEOS) as the precursor material. The Cr₂O₃ 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 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 hrs. Blackish green Cr₂O₃ 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 Cr₂O₃ nanoparticles against K. pneumoniae was tested by measuring zone of inhibition (ZOI), evaluating colony forming units (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 Cr₂O₃ 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 Cr₂O₃ 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 Cr₂O₃ 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 Cr₂O₃ 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 Cr₂O₃ 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 Cr₂O₃ nanoparticles. The inspection of XRD pattern revealed that Cr₂O₃ thus formed is of rhombohedral phase (JCPDS no. 38-1479 with a=4.9587 Å, c=13.594 Å). The major peaks at 2θ values of 20 varying from 26.22, 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 Cr₂O₃ 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, θ are the full width at half maximum and Bragg’s angle, respectively.

Fig. 2 shows the TEM image of the Cr₂O₃ 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 Cr₂O₃ 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 1200 and 1400/cm and 1622/cm are due to –OH stretching and bending vibrations of adsorbed water molecule on the sample, band at 2929/cm may be due to –CH₃ stretching vibrations 2902/cm is due 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 Cr₂O₃ nanoparticles [31].

The optical characterization of the sample was recorded on UV-visible absorption spectrophotometer. Fig. 4 shows UV-visible spectra of Cr₂O₃ nanoparticles as a function of wavelength. The UV-visible absorption spectroscopy of Cr₂O₃ 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 Cr₂O₃ nanoparticles on agar plates. The minimum inhibitory concentration (MIC) of Cr₂O₃ 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 Cr₂O₃ nanoparticles, and these results reveal the strong efficiency of these Cr₂O₃ nanoparticles to inhibit the bacterial growth.

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

![Fig. 2: Transmission electron microscopy image of chromium oxide nanoparticles](image2.png)
The zone of inhibition increases gradually as the concentration of Cr₂O₃ nanoparticles increases. Results clearly demonstrate that synthesized Cr₂O₃ 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 Cr₂O₃ 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 Cr₂O₃ exhibited good antibacterial property.

CONCLUSION

Cr₂O₃ nanoparticles of average particle size 24.0 nm were synthesized using Sol-gel method. Antibacterial study of Cr₂O₃ nanoparticles was investigated against K. pneumoniae by using zone of inhibition, CFU measurement, and OD methods. The zone of inhibition shown by the Cr₂O₃ 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 Cr₂O₃ nanoparticles show good antibacterial property. The MIC of Cr₂O₃ 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 Cr₂O₃ 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 Cr₂O₃ nanoparticles are easy to synthesize and possess good antibacterial activities.

Hou X, Choy KL. Synthesis of Cr nanoparticles mg/ml

Table 1: Result of agar well-diffusion method

<table>
<thead>
<tr>
<th>Concentration of CrO&lt;sub&gt;2&lt;/sub&gt; nanoparticles mg/ml</th>
<th>Average zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>8.7±0.58</td>
</tr>
<tr>
<td>3.0</td>
<td>9.3±0.58</td>
</tr>
<tr>
<td>3.25</td>
<td>9.7±1.15</td>
</tr>
<tr>
<td>3.50</td>
<td>10.0±0.0</td>
</tr>
<tr>
<td>3.75</td>
<td>10.2±0.76</td>
</tr>
<tr>
<td>4.0</td>
<td>11.8±0.6</td>
</tr>
<tr>
<td>6.0</td>
<td>14.5±0.5</td>
</tr>
<tr>
<td>8.0</td>
<td>16.6±0.0</td>
</tr>
<tr>
<td>10.0</td>
<td>17.3±0.6</td>
</tr>
<tr>
<td>12.0</td>
<td>19.6±0.6</td>
</tr>
</tbody>
</table>

CrO<sub>2</sub>: Chromium oxide

ACKNOWLEDGMENTS

This work was supported by the Department of Biotechnology, Chaudhary Devi Lal University, Sirsa and financial support was provided by University Grant Commission (UGC), New Delhi.

REFERENCES