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ANTIBACTERIAL ACTIVITY OF CHEMICALLY SYNTHESIZED CHROMIUM OXIDE NANOPARTICLES AGAINST ENTEROCOCCUS FAECALIS

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ABSTRACT

Metal nanoparticles have been intensively studied with in the past decades. In this paper chromium oxide nanoparticles were synthesized by Sol-gel method. The average particle size, crystalline structure, morphology, optical behaviour and formation of chemical bonds in resulting chromium oxide nanoparticles were characterized by X-Ray Diffraction (XRD), Transmission electron microscopy (TEM), UV-Visible spectroscopy and Fourier-transform infrared (FTIR) spectroscopy. The size of the synthesized Cr_2O_3 nanoparticles was found to be 24.0 nm by using Debye-Scherrer formula. The antibacterial effect of Chromium Oxide nanoparticles was carried out both in liquid and solid growth medium against Enterococcus faecalis. The bacterial growth was monitored by measuring zone of inhibition (ZOI), colony forming unit (CFU) and optical density (OD) method.

Keywords*: *Enterococcus Faecalis, Chromium Oxide Nanoparticles, XRD, TEM.

I. INTRODUCTION

Nanoparticles are regarded as highly reactive species because of large surface area. Nanoparticles, especially transition-metal oxides play a significant role in many areas of chemistry, physics and materials science [1].Transition metal oxide nanoparticles have been researched extensively due to their interesting catalytic, electronic, magnetic and medicinal properties. Among the transition metal oxide nanoparticles chromium (III) oxide (Cr_2O_3) nanoparticles have received great attention due to its various applications in many areas such as green pigments [2], heterogeneous catalysts [3], coating materials for thermal protection [4], hydrogen storage [5] and antibacterial activity [6-10] . The Cr_2O_3 nanoparticles are synthesized by various methods such as sol-gel technique [11], sonochemical method [12], precipitation-gelation [13], mechanochemical process [14], microwave plasma [15] and gas condensation [16] etc.

In the present study we synthesize the Cr_2O_3 nanoparticles by sol-gel technique using TEOS as precursor.

II. EXPERIMENTAL

2.1. Materials

All of the chemicals used in experiment were of analytical grade and obtained from standard chemical sources. The *Enterococcus faecalis* (MTCC NO. 6845) was obtained from microbial type culture collection (MTCC), Institute of microbial technology, Chandigarh..

2.2. Synthesis of Cr_2O_3 nanoparticles

In present study the chromium oxide nanoparticles were synthesised by 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˚c with continuous stirring in a closed container for 6 hrs. The resulting solution was then kept into the oven at 100˚c for 10-15 days and after that the particles were kept in muffle furnace at 400˚c for 4 hrs. Blackish green Cr_2O_3 nanoparticles were thus obtained.

2.3. Characterization techniques

The size, structure, morphology and magnetic properties of as prepared metal nanoparticles were characterised by FTIR (shimadzu corp-02014) in the wavelength range 400-4000 $cm^{-1,}$ UV-Visible spectroscopy (Shimadzu 1800) in the wavelength range 200-1000 cm^{-1}, XRD (Rikagu mini-2 using Cuα1, λ=0.15406 nm radiations) and Transmission Electron Microscopy (TEM) (FEI-Philips, Morgagni 286D with magnification up to 2,80,000x, Acc. Voltage : 100 Kv).

2.4. Antibacterial Study

The Cr_2O_3 nanoparticles were tested for antibacterial activity by agar well diffusion method against *Enterococcus faecalis*. Nutrient broth/agar was used to cultivate bacteria. The media was autoclaved and cooled. The bacterial test organism *Enterococcus faecalis* were grown in nutrient broth at 30°c for 24 hrs. The fresh overnight nutrient broth culture was spread on to solidified nutrient agar plates. Wells of 8 mm diameter were prepared on the agar media. The different concentrations of the samples of nanoparticles solution 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 for 24 hrs. After 24 h of incubation the different level of zone of inhibition of bacteria was investigated.

2.5. CFU Measurement

Colony forming units (CFU) of *Enterococcus faecalis* were counted by using solid agar media. 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, 2, 3 mg/ml) of Cr_2O_3 nanoparticles. After incubation at 30˚c the number of colony forming

unit were counted. The growth behaviour of the *Enterococcus faecalis* was also investigated by administration of the Cr_2O_3 nanoparticles at different concentrations into the dilute solution of the broth culture.

III. 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 in order to know the synthesis condition and UV-visible spectroscopy was carried out for the optical study of metal nanoparticles. Figure 1 shows XRD pattern of the Cr_2O_3 nanoparticles. X-ray diffraction pattern revealed that the synthesized Cr_2O_3 is of rhombohedral phase (JCPDS no. 38-1479 with a=4.9587 A˚ b= 13.594 A˚ and space group R3-c). The major peaks at 2θ values of 24.62, 33.8, 36.4, 41.8, 50.32, 55.16, and 65.32 are indexed as (012), (104), (110), (113), (024), (116), (214) respectively [17]. Average particle size of the Cr_2O_3 nanoparticles was found to be 24 nm using Scherrer's formula $d = K \lambda / \beta \cos\theta$.

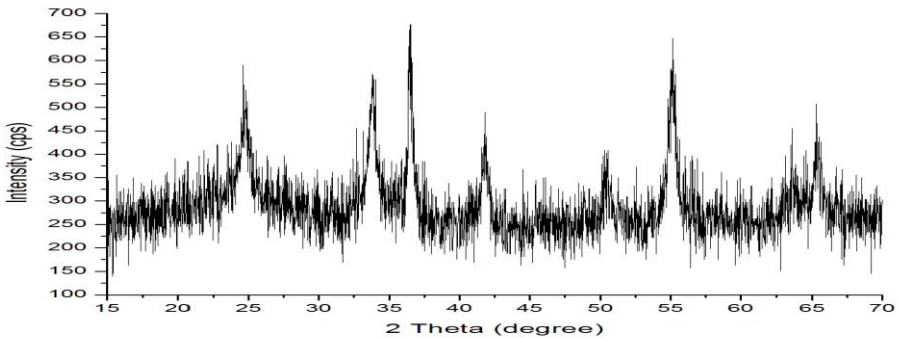

Fig.1 XRD pattern of Cr_2O_3 nanoparticles

Figure 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. It can be seen from the Figure 2 that there is a uniform distribution of particle size with mean particle size 21 nm which is in agreement with the XRD result.

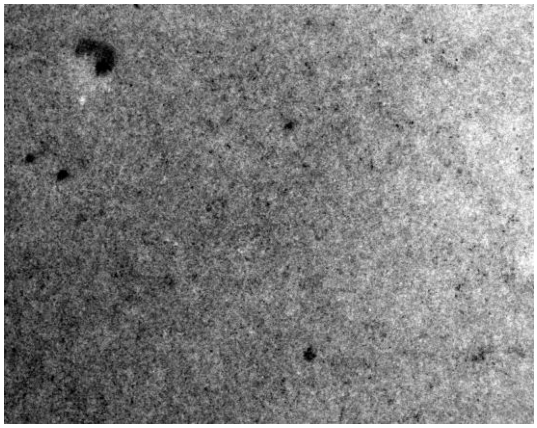

Fig.2 TEM image of Cr_2O_3 nanoparticles

Figure 3 shows FTIR spectra of Cr_2O_3 nanoparticles synthesized by sol-gel technique. FTIR spectroscopy was carried out in order to ascertain the purity and nature of metal or metal oxide nanoparticles. The band at 3215 cm^{-1}-3361 cm^{-1} is due to the -OH stretching vibrations, band at 2929 cm-1 may be due to $–CH_3$ stretching vibrations 1072 cm^{-1} is due to Cr-O-Cr vibrations 952 cm^{-1} and 902 cm^{-1} are assigned to Cr=O vibrations. The two peaks at550 cm^{-1} and 617 cm^{-1} are assigned to Cr-O str. modes are evidence for the presence of crystalline Cr_2O_3 nanoparticles [7].

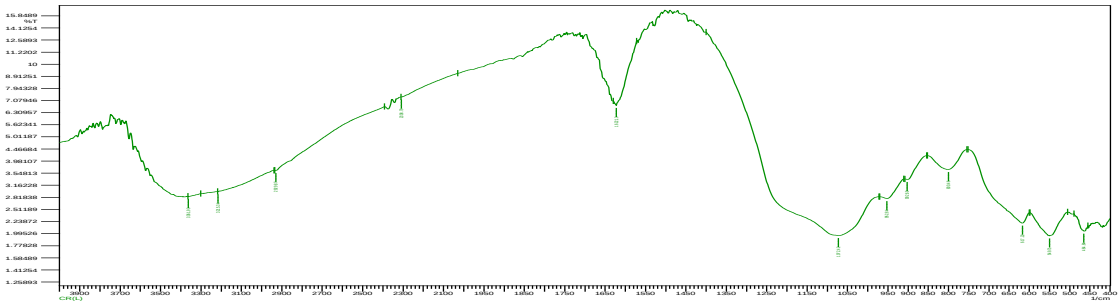

Fig.3 FTIR Spectra of Cr_2O_3 nanoparticles

The optical characterisation of the sample was recorded on UV-Visible absorption spectrophotometer. Figure 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 a absorption peak at about 351.2 nm.

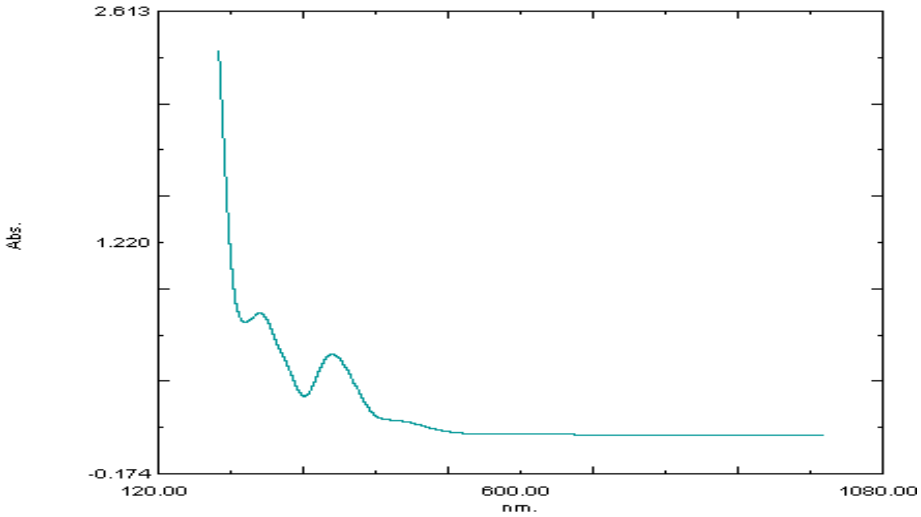

Fig.4 UV-Vis absorption spectrum of Cr_2O_3 nanoparticles in aq. Solution

The Figure 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 of Cr_2O_3 nanoparticles for *Enterococcus faecalis* was observed at 2.5 mg/ml. The zone of inhibition increases gradually as the concentration of Cr_2O_3

nanoparticles increases. Results clearly demonstrate that the synthesized Cr_2O_3 nanoparticles was promising antimicrobial agent.

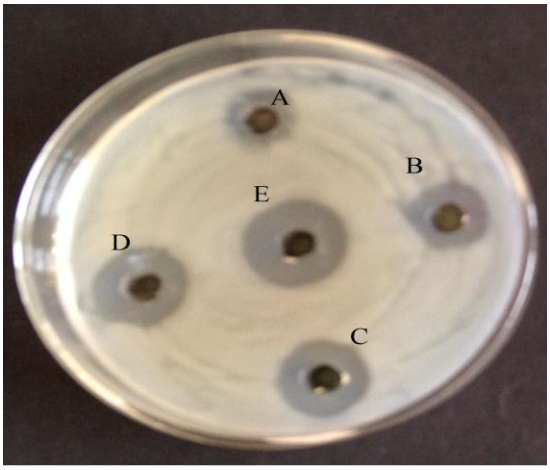

Fig.5 Images of Antibacterial Activity Of Different Concentration Of Cr_2O_3 Nanoparticles (4mg/Ml, 6mg/Ml, 8mg/Ml, 10mg/Ml, 12mg/Ml) On *Enterococcus Faecalis*

Figure 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), tetracycline (25 mcg) which are taken in the form of hexa discs. It was found that *Enterococcus faecalis* is resistant to the Penicillin G, sulphatriad and Ampicillin.

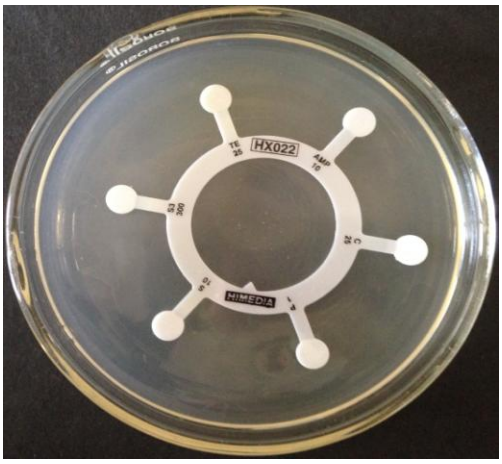

Fig.6 Image of Agar plate containing antibiotic discs and appearance of inhibitory zones of *Enterococcus faecalis* with different antibiotics.

Figure 7 shows the Colony Forming Unit of *Enterococcus faecalis*. It is clear from the graph that the number of bacterial colonies decreases with increase in the concentration of the metal nanoparticles.

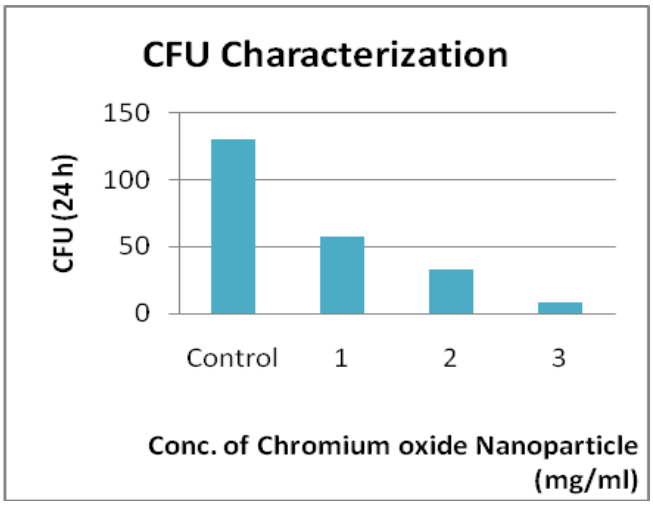

Fig.7 shows CFU with different concentrations of Cr_2O_3 nanoparticles.

Aqueous dispersion of these nanoparticles at desired concentrations was made. The 50 ml of Diluted bacterial cells 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. Chromium oxide 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 h of control and solutions containing different concentrations of chromium oxide nanoparticles as shown in Fig.8. From the Figure 8 it was noticed that when the concentration of Cr_2O_3 nanoparticles was increased the growth was reduced.

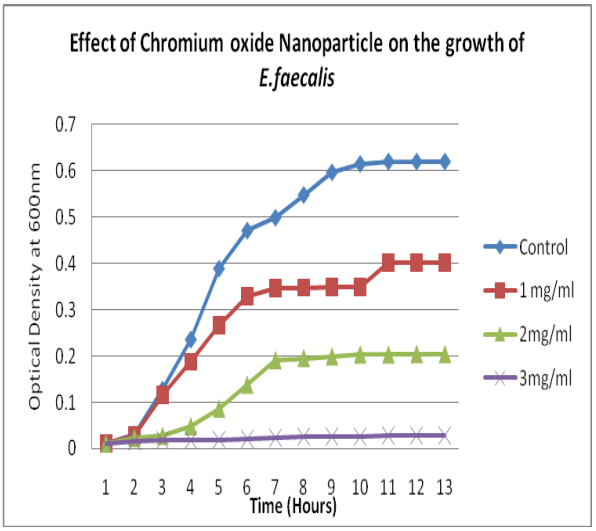

Fig.8 The effect of Cr_2O_3 nanoparticles on the growth of *Enterococcus faecalis*.

IV. CONCLUSION

In the present study Cr_2O_3 nanoparticles of mean size 24 nm were synthesized by using Sol-gel method. Antibacterial study of these chromium oxide nanoparticles were investigated against *Enterococcus faecalis* by using zone of inhibition, CFU measurement and optical density methods. The zone of inhibition produced by the Cr_2O_3 nanoparticles against *Enterococcus faecalis* was compared with well known antibiotics. It was found that *Enterococcus faecalis* is resistant to the penicillin G, sulphatriad and ampicillin but Cr_2O_3 nanoparticles shows good antibacterial property. The MIC of Cr_2O_3 for *Enterococcus faecalis* is 2.5 mg/ml. The zone of inhibition, CFU estimation and optical density curves shows that the bacterial growth reduces significantly with the increase in concentration of Cr_2O_3 nanoparticles.

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