

BIOLOGICAL PROPERTIES OF Cr₂O₃ NANOPARTICLES

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ABSTRACT

Among the many inorganic NPs that have stood out for their exceptional properties in several areas of contemporary science and technology, chromic oxide nanoparticles (Cr₂O₃ NPs) stand out. In order to fulfil its vital potential, Cr₂O₃ NPs have been manufactured using a variety of manufacturing techniques. The majority of the methods for synthesizing Cr₂O₃ NPs have economic and ecological drawbacks, which has led to the pursuit of more sustainable alternatives. Given the many physiological, environmental, economic, and medical benefits of Cr₂O₃ NPs, it is surprising that biological production methods have been shown to be appropriate for their synthesis. Crucial features of Cr₂O₃ NPs synthesized from biomass and broths enhanced their antioxidant, antifungal, and antibacterial applications. There has been a lot of interest in phytosynthesized Cr₂O₃ NPs from the electronics industry, chemistry, and medicine for their potential use as microelectronic circuit components, sensors, fuel cells, solar energy collectors, antioxidants, and photocatalytic agents. The difficulties in understanding the formation reaction persist despite the mentioned benefits of biogenically generated Cr₂O₃ NPs. Recent advances in the synthesis, characterisation, and medical and pharmacological uses of phytosynthesized Cr₂O₃ NPs were summarized in this study.

KEYWORDS: Cr₂O₃ NPs; Anti-microbial activity; Anti-inflammatory activity; Antioxidant

INTRODUCTION

Chromic oxide nanoparticles, also known as Cr₂O₃ NPs, are a kind of inorganic nanoparticle that stands out due to its exceptional properties that are applicable to a variety of fields of current science and technology. In order to make the most of the critical potential of Cr₂O₃ NPs, a number of different manufacturing techniques have been used in their synthesis. Chromic oxide nanoparticles, also known as Cr₂O₃ NPs, are a kind of inorganic nanoparticle that stands out due to its exceptional properties

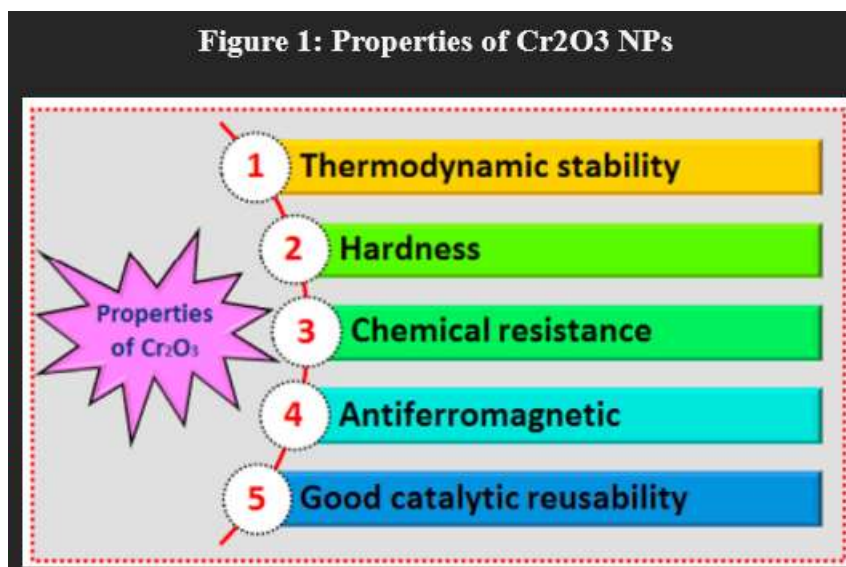
that are applicable to a variety of fields of current science and technology (Khan et al., 2019). In order to make the most of the critical potential of Cr₂O₃ NPs, a number of different manufacturing techniques have been used in their synthesis. Because of the negative effects on the environment and the financial challenges associated with the majority of the methods for producing Cr₂O₃ NPs, more and more people are looking for ways to improve the economy and the environment without sacrificing either. Interestingly, it has been shown that biological resource-based manufacturing processes are perfect for synthesizing Cr₂O₃ NPs. This is owing to the many benefits that these nanoparticles provide in terms of health, the environment, the economy, and medicine. Metal and metal oxide nanomaterials have recently attracted a lot of interest, and several synthetic techniques have been established. Metal or metal oxide NPs may be easily shaped and sized to meet specific requirements thanks to the wide range of applications for these nanomaterials, which has boosted their production. Metal ions and oxides at the nanoscale, such as "cadmium, cobalt, zirconium, calcium, aluminum, silver, sulfur, bismuth vanadate, stannic oxide, selenium, zinc" (Yaqoob et al., 2020), etc., are developing a lot of interest due to their low toxicity, excellent catalytic activity, outstanding antimicrobial activity, and ease of synthesis using economical and up-scalable methods. Cr₂O₃ NPs is one of the most remarkable metal oxides because of its antiferromagnetic properties, high hardness, high chemical resistance, and exceptional thermodynamic stability (Fig.1).

Additionally, Cr₂O₃ NPs is behaving like a semiconductor in both the n-type and p-type modes. Solar cells, fuel cells, piezoelectric devices, green pigments and protective coatings, sensors, photocatalysis, and catalysis are just a few of the many uses that have been explored in the past using Cr₂O₃ NPs. Because of its dependable curative biomedical uses, Cr₂O₃ NPs also play a significant role in the pharmaceutical and medical fields as agents that inhibit enzymes, fight cancer, antileishmanial, and antibacterial infections. Many people are interested in finding a more sustainable way to synthesize Cr₂O₃ NPs because of their many useful uses and attractive characteristics. Cr₂O₃ NPs have been suggested to be manufactured using a wide variety of physical and chemical processes, including mechanical grinding, electrochemical methods, thermal breakdown, microwave, sol-gel, solvothermal, the combustion synthesis (Jamkhande et al., 2019), microemulsion, solution plasma departure, chemical precipitation, mechanical grinding again, arc discharge method, co-precipitation, as well as template method. Up until now, the physical processes have been successful, but they have been hindered by the high energy costs and equipment requirements. In contrast, procedures based on wet chemistry have the potential to produce harmful byproducts that endanger the environment. For this reason, bio-reductants made extracts are quickly becoming the preferred technique of chemical reduction in environmentally conscious practices. Nanotechnology is a relatively young field within biomedical sciences, but it is finding more and more uses in the health care industry (Ying et al., 2022). Nanotechnology is a rapidly developing area that makes use of nanoparticles for the aim of conducting diagnostic and therapeutic procedures. An

interesting new development in the treatment of many different illnesses is the fabrication of nanomaterials, including infections caused by fungi and bacteria, as well as a number of different forms of cancer. Due to their tiny size, high surface-to-volume ratio, along with size-dependent optical properties, nanoparticles (NPs) have captivated many with their remarkable physicochemical features, especially in light of their possible expanded biological uses. Nanomedicine and nanotechnology rely on nanoparticles (NPs) as its fundamental building component. Diagnostics, detection, medicine delivery, and many more fields have made use of them, and therapy of a number of illnesses, as well as the treatment of several forms of cancer. For instance, nanoparticles of metal oxide (NPs) find extensive usage in several products for functions such as enzyme inhibition, photocatalysis adsorption, and more (Gupta et al., 2019).

Chromium oxide nanoparticles (Cr_2O_3) have a remarkable particularity among metal oxide nanoparticles (NPs) because of their exceptional durability, hardness, high resistivity, high melting temperature, as well as broad bandgap of 3.4 eV: these characteristics make them stand out. As a result of their one-of-a-kind capabilities, Nanoparticles of Cr_2O_3 NPs may find use in the development of catalytic materials, photocatalysis, super capacitors for lithium-ion batteries, sensing, as well as other biological activities. A crucial factor in determining whether or not Cr_2O_3 NPs are suitable for usage in a variety of biological systems is their biocompatibility (Ribeiro et al., 2022). A wide range of medicinal applications have made great use of Cr_2O_3 NPs, such as drug delivery, antioxidant defense, antibacterial, anti-cancer, anti-viral, along with anti-diabetic properties, among others. There are a number of drawbacks associated with the preparation of Cr_2O_3 NPs using standard chemical or physical processes. These drawbacks include the significant use of toxic substances, both as solvents as well as starting materials, as well as significant changes in temperature and pH during the process. The use of such parameters results in the imparting of additional harmful features, including as carcinogenicity as well as environmental toxicity, this limits the use of nanoparticles in several biological and therapeutic contexts (Renuka et al., 2020). Thus, nanoparticles produced by environmentally friendly synthesis have emerged as a reasonable substitute for traditional physical and chemical processes, with the potential to lessen the negative aspects of the former. The production of nanoparticles by green synthesis is an intriguing technology due to the fact that it is simple, economical, and environmentally benign. Metallic nanoparticles that have been biologically produced are cytotoxic agents that combat cancer. Producing nanoparticles using extracts is a simple and easy way to make nanoparticles on a large scale, in contrast to processes using microbes and fungi. The environmentally friendly production of chromium oxide nanoparticles (Cr_2O_3 NPs) by the use of extracts is now in the focus of a large number of researchers. *Callistemon viminalis* (Bottle Brush), flower extracts, cactus (*Opuntia ficus-indica*), and other substances have been used in the manufacturing of Cr_2O_3 NPs (Wang et al., 2021).

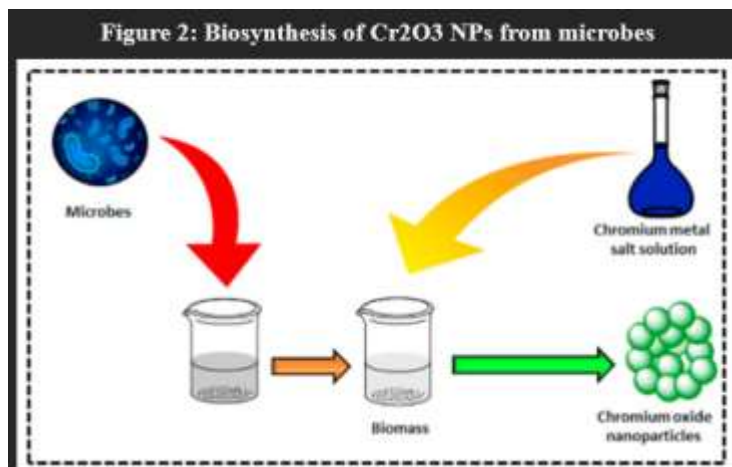
Because of its analgesic as well as anti-inflammatory properties, which is found all over the worldwide. It has been used in conventional medicine for the treatment of cardiovascular problems. The green production of Cr₂O₃ NPs has previously made use of a variety of extracts (Ananda et al., 2022). In the current research, Cr₂O₃ NPs that were used to prove the above maintain properties by were generated and characterized using methods such as UV/Vi's spectroscopy, EDX, SEM, and XRD. Additionally, the in vitro activity of these nanoparticles was evaluated. With that in mind, the purpose of this perspective review is to provide studies on the biosynthesis, characterization methodologies, and modern uses of Cr₂O₃ NPs utilizing microorganisms.



EXPERIMENTAL DETAILS

Nanoparticles (NPs) made of precious metals such as palladium, silver, platinum, as well as gold have been created by using bacterially produced nanostructures, either within or outside of cells. The setup used for fabricating Cr₂O₃ NPs from bacterial biomass is shown in Fig. 2. Researchers using *Aspargillus niger* biomass, Cr₂O₃ NPs were recently produced. Making 4-50 nm-sized Cr₂O₃ NPs by treating *Bacillus subtilis* biomass with an aqueous solution of potassium dichromate. Cr₂O₃ NPs were synthesized using *Bacillus subtilis* culture supernatants. Used *Bacillus cereus* to fabricate Cr₂O₃ NPs, and then researchers used TEM, EDS, SEM, and XPS to characterize them. Plus, environmentally friendly production of Cr₂O₃ NPs using *Erwinia amylovora*. It was proven that Cr₂O₃ NPs were formed by their orange to green hue. The MR-1 strain of *Shewanella oneidensis* bacterial biomass was used to produce Cr₂O₃ NPs. However, using microbes for Cr₂O₃ NPs biosynthesis results in a sluggish creation rate and a restricted variety of NP sizes

and forms when compared to recognized conventional methods. Therefore, in order to biosynthesize Cr_2O_3 NPs, decrease and stabilize agents based on a variety of material extracts are being investigated.

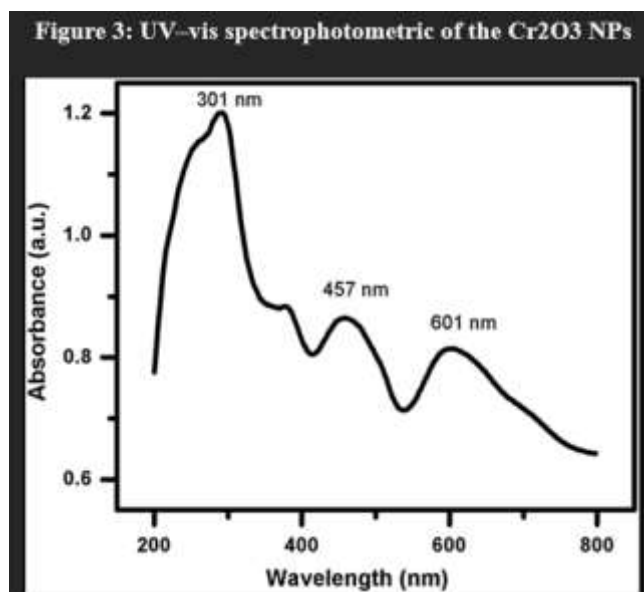


CHARACTERIZATION TECHNIQUES FOR Cr_2O_3 NPs

It is essential to characterize Cr_2O_3 NPs since their features are vital to their multifunctional uses. Some of these factors include surface area, fractal dimensions, magnetism, size, purity, and pore size. What follows is a discussion of the primary analytical techniques used to determine the characteristics of the synthesized Cr_2O_3 NPs.

UV-vis SPECTROPHOTOMETRIC ANALYSIS of Cr_2O_3 NPs

Spectrophotometers that are classified as ultraviolet-visible (UV-Vis) make use of a light source that illuminates a sample with light that spans the visible to ultraviolet (UV) wavelength range, which is generally between 190 and 900 nanometers. The near-infrared (NIR) spectrum is accessible via UV-Vis-NIR spectrophotometers, which is between 800 and 3,200 nanometers (Tao et al., 2022). The SPR of the manufactured NPs may be determined using this instrument. Absorption measurements of liquids and a wide variety of other substances often need this. The SPR for Cr_2O_3 NPs falls anywhere between 250 and 450 nanometers. Discoveries have shown that the SPR of Cr_2O_3 NPs, which in turn impacts its morphological characteristics, varies depending on the extraction method, temperature, pH, and sort of extract used. The investigational approach provides vital details on the size of the Cr_2O_3 NPs, stability, structure, or aggregation.



XRD ANALYSIS of Cr₂O₃ NPs

Table 1: Biosynthesis of Cr₂O₃ NPs using microbes with size and shape

Name of bacterial strains	Characterization techniques	Shape	Size (nm)
<i>Aspargillus niger</i>	XRD, UV-Vis, SEM, EDS	Hexagonal	36
<i>Bacillus subtilis</i>	TEM, EDS, FTIR, ICP-OES, TGA, DSC	Circular	4-50
<i>Bacillus subtilis</i>	UV-Vis, FTIR, XRD, SEM	Spherical	50-78
<i>Bacillus cereus</i>	SEM, EDS, TEM, XPS	Discrete-spherical	8
<i>Erwinia amylovora</i>	UV-Vis, TEM	—	—
<i>Shewanell oneidensis</i> MR-1	SEM, EDX, Raman	—	50

A non-destructive technique for studying a material's physical properties, chemical make-up, and crystallographic structure is X-ray diffraction analysis, or XRD analysis.

Biosynthesized Cr₂O₃ NPs are subjected to XRD-based crystallographic and structural characterization. The JCPDS file Number. 00-038-1479 was used to perform the X-ray diffraction research. It is possible to determine the average size of Cr₂O₃ NPs crystallites by using Scherrer's equation.

$$d = \frac{K\lambda}{\beta \cos \theta}$$

"D is the average size of Cr₂O₃ NPs in nanometers.

λ - Wavelength of the X-ray radiation,

K- Scherrer's constant,

β - Full width at half-maximum (FWHM) in radians,

θ - Bragg's angle."

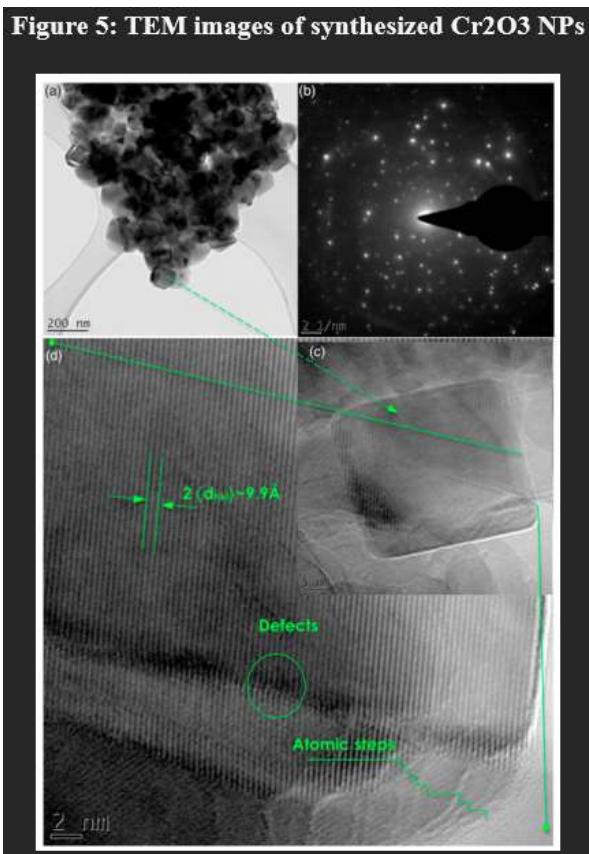
SEM ANALYSIS of Cr₂O₃ NPs

Scanning electron microscopy analysis, often known as SEM analysis, is a method that creates a high-resolution picture of the surface of a material by using a focussed electron beam at a certain wavelength. It is a method that is quick, accurate, and does not cause any disruptions when it comes to studying the surface composition along with topography of a material. Topographical study of Cr₂O₃ NPs is carried out using the SEM method. Due to the lack of information, it gives on the precise form and average size distribution, this is limited to morphological studies. It has been reported that different nanopolymers may be disrupted using the SEM method (Hassan et al., 2019). Looking back, it's true that this instrument is expensive and time-consuming. According to scanning electron microscopy (SEM) research, big clusters included most of the nanoparticles. The agglomerates were discovered to be in the shape of flakes with uneven shapes and were not evenly distributed. The diameters of several of these aggregates were even within the micrometre range, and they were much larger than the average.

TEM (TRANSMISSION ELECTRON MICROSCOPY) ANALYSIS OF THE Cr₂O₃ NPs

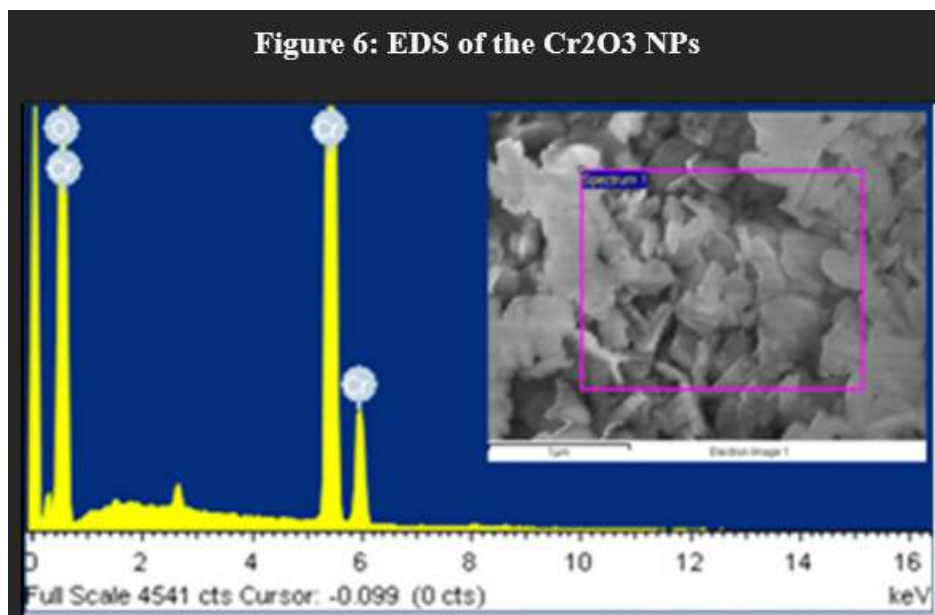
Use of an electron beam in TEM allows for the acquisition of high-resolution pictures of structures at the atomic level. TEM is a vital analytical instrument used in the fields of biology, nanotechnology, microbiology, and materials science domains, chemical, along with physical sciences. The most reliable method for examining the structure of nanoparticles (NPs), such as Cr₂O₃ NPs, is TEM. The most cutting-edge kind of TEM, known as Higher Definition Transmission Electron Microscopy (HRTEM), enables the

study of nuclear crystallographic structures. Fig. 5 displays the results obtained from the morphological evaluation of Cr₂O₃ NPs generated via photosynthesis using the HRTEM method.



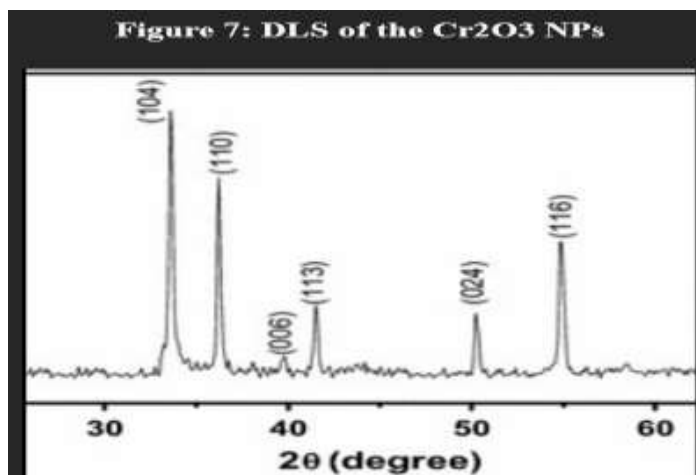
EDS STUDIES OF THE Cr₂O₃ NPs

Energy-dispersive X-ray spectroscopy, or EDS for short, is a non-destructive analytical technique that may determine the chemical composition of a material without harming it. Both EDX and XEDS are other names for it. Cr₂O₃ NPs as well as other metal NPs may have their elemental composition described quantitatively and qualitatively using EDS. Quantitative analysis involves measuring the chromium and oxygen concentrations in the Cr₂O₃ NPs by analysing the signal intensities, qualitative analysis, on the other hand, requires guessing where each EDS X-ray peak will be located. In order to determine and quantify the elemental composition, the integration of EDS with TEM along with SEM has been enhanced by contemporary technological developments.



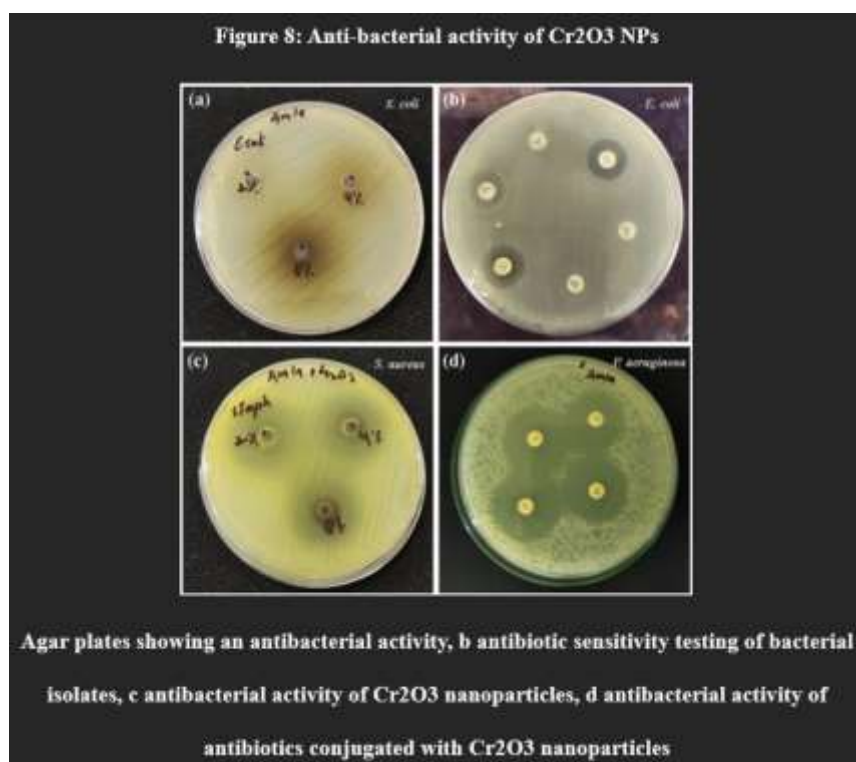
DLS ANALYSIS OF Cr₂O₃ NPs

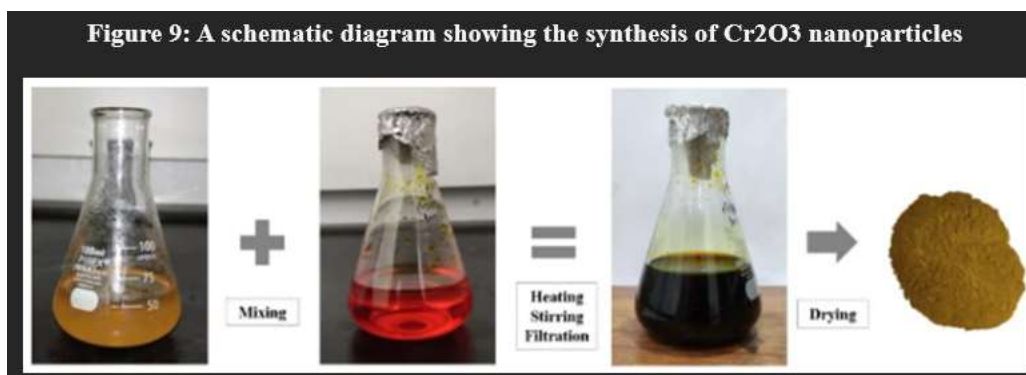
One non-invasive method for studying molecular and particle sizes and shapes in liquids is dynamic light scattering (DLS). Quantum elastic light scattering is another name for this technique, along with photon correlation spectroscopy (PCS). A common way of looking of DLS is as a dispersion of quasi-elastic light. Its function is to aggregate selected NPs and distribute their sizes. Despite its minor drawbacks, this method is sensitive, quick, has the ability to determine the average size of particles at the macro as well as nano scopes. The size of the particles determines how fast the DLS method works. The characterisation of Cr₂O₃ NPs has also made use of several cutting-edge methods developed by numerous research specialists.



ANTI-MICROBIAL ACTIVITY

Figure 8a shows that solution exhibit any antibacterial activity. Cr2O3 NPs mediated, in contrast, demonstrated potent antibacterial action against the fungal isolate, in addition to strains of bacteria that are Gram-positive and those that are Gram-negative (Fig. 8c). The engineered nanoparticles exhibited remarkable efficacy against every single bacterial isolate examined; for example, they exhibited a zone of inhibition as low as 30 mm against *E. coli* along with high as 53 mm against *A. baumannii* (Fig. 9a). Researchers provide the average zone sizes for *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*, all of which were studied with two isolates each. There was only one isolation for every other kind of microorganism. For *P. aeruginosa* and *E. aerogenes*, the concentrations of Cr2O3 NPs were found to be 0.2 µg/ml. For *K. pneumoniae* and *S. aureus*, it was 0.6 µg/ml. For "*S. enterica*, *A. baumannii*, *S. aureus*, *E. coli*, *P. vulgaris*, along with *C. albicans*", it was 0.8 µg/ml.





ANTIOXIDANT ACTIVITY OF Cr₂O₃ NPs

The researchers used the phosphomolybdenum technique to determine the total antioxidant capacity (TAC) of Cr₂O₃ NPs. In a balanced ratio of 4 mM ammonia molybdate, 28 mM sodium phosphate, which as well as 0.6 M sulfuric acid, the working reagent was prepared. Nanoparticles were created in distilled water at concentrations ranging from 50 to 200 µg/ml. Various dilutions of nanoparticles were combined with reagent solution in test tubes. A control was established using ascorbic acid. For 90 minutes, the test tubes were placed in a water bath that was heated to 95 °C. As a result of the decrease of the phosphomolybdate ion along with the production of the phosphomolybdenum (V) complex, a greenish-blue hue occurs when antioxidants are present. Every sample had a wavelength at 695 nm measured after the incubation time. The approach that was used to calculate the proportion of TAC is shown below.

$$\text{TAC \%} = (\text{OD}_{\text{control}} - \text{OD}_{\text{sample}}) / \text{OD}_{\text{control}} \times 100$$

The average value was obtained by repeating the experiment three times.

ANTI-INFLAMMATORY ACTIVITY OF Cr₂O₃ NPs

To assess the anti-inflammatory properties of Cr₂O₃ NPs, researchers created a functional solution containing 0.2% bovine serum albumin (BSA). Before adding BSA, nanoparticles were created in several test tubes with concentrations varying from 200 to 800 µg/ml. The tubes were cooled after incubating at 75 °C for 5 minutes in the water bath. The control group was given ascorbic acid. The anti-inflammatory activity % was determined by recording the absorbing capacity of each sample at 660 nm and using the following formula:

$$\text{Anti - inflammatory activity \%} = (\text{OD}_{\text{control}} - \text{OD}_{\text{sample}}) / \text{OD}_{\text{control}} \times 100$$

The experiment was repeated three times to get the average value.

CONCLUSION AND FUTURE PERSPECTIVES

Environmental identities, morphological and physiochemical features/properties, and potential catalytic and biological applications of Cr₂O₃ NPs are largely impacted by the production procedures utilized. Cr₂O₃ NPs provide great opportunities to research centers as well as pharmaceutical firms owing to their multifunctional biological performance, thus researchers are critically examining biological techniques to producing them from plants and microorganisms. The analysis of Cr₂O₃ NPs production has been thoroughly shown using contemporary characterisation methods. The antiviral, antioxidant, antibacterial and protein kinase inhibitory properties of biogenically produced Cr₂O₃ NPs have been adequately emphasized. Research into the potentially harmful effects of Cr₂O₃ NPs should focus on practical ways to preserve and improve their biological characteristics in order to increase their multifunctional biological features. Further development of these biological methods is possible with more focus on elucidating the exact processes at work, in the meantime concentrating on optimizing the shape and size of the Cr₂O₃ NPs by manipulating experimental conditions.

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