

Biosynthesis, Characterization, Adsorption and Antimicrobial studies of Manganese oxide Nanoparticles Using Punica Granatum Extract

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Abstract

Manganese sulfate and Punica granatum plant extract were used to create MnO_2 nanoparticles, which were then characterized using techniques like Fourier transform infrared spectroscopy, ultravioletvisible spectroscopy, atomic force microscopy, X-ray diffraction, transmission electron microscopy, scanning electron microscopy, and energy-dispersive X-ray spectroscopy. The crystal's size was calculated to be 30.94nm by employing the Debye Scherrer equation in X-ray diffraction. MnO_2 NPs were shown to be effective in adsorbing M(II) = Co, Ni, and Cu ions, proving that all three metal ions may be removed from water in one go. Ni(II) has a higher adsorption rate throughout the board. Co, Ni, and Cu ion removal efficiencies were 32.79%, 75.00%, and 30.20%, respectively. Two species of bacteria and one type of fungus were examined at three different use concentrations if possible of MnO_2 nanoparticles. Antibiotics like Amoxicillin and Metronidazole were used as a control group to see how the findings stacked up.

Keywords: Adsorption, Antimicrobial, Biosynthesis, Manganese oxide, Removal.

Introduction

Nanotechnology is a burgeoning area of study in medicine. It is the responsibility of nanoparticles, an intermediary between micro materials and atomic structures, to improve the physical qualities such as surface area and volume ratio¹. The development of novel medications has used the therapeutic characteristics that herbal plants and their derivatives contain². MnO₂ has attracted the attention of numerous researchers due to its impact and electromagnetic properties³. MnO₂ has been synthesized using various techniques, including self-reacting microemulsion, deposition, and solid

reaction ⁴. However, using natural compounds to decrease and stabilize Mn metal into nanoparticles is more environmentally friendly, less expensive, and more straightforward than the preceding 5 procedures Escherichia coli. Klebsiella and Pseudomonas aeruginosa are pneumonia, pathogenic microorganisms that can harm individuals with weakened natural defenses and result in severe systemic disease ⁶. Due to their nanoscale size, NPs can penetrate biofilms and bacterial cell walls and have a cytotoxic effect. They can also increase the effectiveness of current

antibiotics by preventing their detection and providing a method of targeted delivery to microorganisms to maximize their topical concentration and bactericidal effects ⁷. In this study, we sought to synthesize MnO₂ NPs using

Materials and Methods

Obtaining samples Punica granatum was gathered and marked from a nearby source, and we used hydrated manganese sulfate. MnSO₄.H₂O was purchased from England, NaOH from Alpha India's Alpha Chemical, ethanol from Samka Aldaraj, and copper, cobalt, and nickel sulfate. Several spectroscopic and microscopic methods were used to make and identify the compounds, including the following: a magnetic stirrer, a sensitive electronic balance model As 220C1, a centrifuge type PLC, and an electric oven type (FAITHFUL) model -WHL. And an XRD diffraction type PW1730 (Phillips/ Holland). Shaking Water Bath type (SCL FINETEDI), PH-type UV-visible tape measure (160/Uv) Shimadzu using the deionized water as a solvent, FT-IR (8500S) type spectroscopy in 400cm⁻¹, and (centre of examinations). 4000 Application of an X-ray energy dispersion device, SEM type **FESEM-EDS** Model MIRAIII, manufacturer TESCAN, and country of manufacture Czech (EDX). TEM with the EM10C-100Kv model number and Atomic Force Microscopes AFM.

Preparation of Punica Granatum extract and MnO₂ NPs

Deionized water has been used to wash the fresh pomegranate peel and eliminate dust. To create homogeneous powders, the dry pomegranate peel is carefully blended in a mixer. After that, 20 g were ground up and combined with 200 ml of deionized water. The mixture was heated for 30 minutes at 60 °C while being stirred. After filtering, the solution was placed in the refrigerator. From the preparation of MnO₂, NPs were created using the green synthesis method. As a result, 0.1M, 50 ml of MnSO₄.H₂O, and 100 ml of pomegranate peel extract were added slowly (one drop per second) and stirred for 30 minutes. The solution was then given 40 ml of 1 N NaOH. The pH level rose to 10-12. The result was a dark black crystal precipitate Punica Granatum extract as the reducing and capping agents, characterize the synthesized MnO_2 NPs, and assess their antibacterial effectiveness versus bacteria, both on their own and in combination with other antibiotics.

cleaned with deionized water (all steps were done with centrifuge and then decantation). They were dried for 4 hours at 120 °C before being dried again for 4 hours at 250 °C. Manganese oxide nanoparticles were produced as a black powder.

Adsorption study

To create a stock solution, 10g of CoCl₂.6H₂O were dissolved in one litre of distilled water to produce 10000 ppm. The stock concentration for NiCl₂.4H₂O and CuCl₂.2H₂O was 5000 ppm because the stock was made by dissolving 5g in 1 litre of distilled water.

Adsorption of metal ions on the surface of MnO₂ NPs was performed by adding 0.1 g of the adsorbent nanoparticle to 50 ml of a 1000 ppm metal ion solution in a shaker water bath at 26 °C and shaking at 150 revolutions per minute (rpm). The adsorbent was then separated from the solution at specific times by centrifuging. A visible spectrophotometer measured the remaining clear solution to determine the remaining concentration after adsorption using the calibration curve

Biological Activity Study

Using the disc diffusion method in a nutrient medium (jellos medium) type Muller Hinton agar, the antimicrobial activity of the synthetic MnO₂ NPs in concentrations of about (25, 50, and 75) mg/L, was tested against two reference bacterial strains (G+) Staphylococcus aurous, and (G-), Escherichia coli, and the fungus Candida albicans. Likewise, the antifungal activity of a nutrient medium based on potato dextrose was measured using the same method.





Results and Discussion

FT-IR analysis

The FTIR of MnO_2 shows in Fig. 1, bands at 590 and 532 cm⁻¹ in the FTIR spectra of MnO_2 are attributed to the Mn-O stretching mode, proving the presence of the Mn-O bond in the MnO₂ structure ⁸, ⁹. Absorption bands at 1643.35, 1411.89, and 1554.83 cm⁻¹ stretching correspond to O-H bending

vibrations linked with Mn atoms. In contrast, the absorption band at 3425.58 cm^{-1} arises from varying degrees of hydrogen bonding within the sample. O-H vibrations in the FTIR spectrum, were observed, and are indicative of water molecules being absorbed into the MnO₂ structure.



Figure 1. FT-IR spectrum of MnO₂ NPs

UV-Visible analysis

The UV-Vis absorption spectrum of the biosynthesis of MnO_2 NPs is shown in Fig. 2. The

absorption peak in this spectrum at 348.0 nm was due to the transition holes process between Mn and O.



Figure 2. UV-Visible spectrum of MnO₂NPs

XRD analysis

According to XRD analysis, orthorhombic MnO₂ consistent with 110, 200, 310, 201, 301, 500 and

451 crystal planes are allocated to a series of diffraction peaks at 2θ of 12.8657, 18.2382, 28.8139, 37.5886, 42.0775, 43.1011, 49.9468,



56.8433, 59.6661 and 72.8652, respectively, JCPDS card (no. 44–0141). XRD measurements, which showed no impurity peaks, are evidence that MnO₂ NPs samples are highly crystalline. The average crystal size was calculated using the Debye Scherrer

equation (D = 0.9 $\lambda/\beta \cos \theta$) where D= the average crystalline size, the Cu K X-ray radiation (λ = 1.5418 °A), was discovered to be 35.74nm in Fig. 3 and Table 1.



Figure 3.	XRD of	f MnO ₂	NPs
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Pos.	Height	FWHM	Particle size (nm)	Average c	rystal	size
[°2Th.]	[cts]	[°2Th.]		(nm)		
12.8657	519.51	0.246	33.97			
18.2382	525.75	0.1476	56.98			
28.8139	2023.78	0.3444	24.89			
37.5886	1472.47	0.2952	29.71	30.94		
42.0775	309.71	0.3936	22.60	50.74		
43.1011	181.99	0.246	36.29			
49.9468	318.60	0.3936	23.27			
56.8433	646.58	0.1968	47.98			
59.6661	421.39	0.492	19.46			
72.8652	220.68	0.72	14.33			

EDX analysis

The EDX spectrum of MnO_2 Nps shows the expected peaks for manganese and oxygen. Having a 1:1 ratio between them Fig. 4. The results

demonstrate how exceptionally pure the generated nanoparticles are—the outcomes of the EDX measurement's accurate estimations and fundamental theoretical calculations also agree⁹.





Figure 4. EDX of MnO₂ Nps.

SEM and TEM analysis

SEM and TEM were used to determine the Morphology and shapes of nanomaterials. Low amounts of rods in nanostructured, unconsolidated forms of MnO_2 NPs can be seen in SEM and TEM measurements of Figs. 5 and 6. In the TEM picture, the MnO_2 nanoparticles emerged as UN-consolidated structures at the nanoscale. It should also be highlighted that the samples exhibit high pore content, which distinguishes them in

adsorption applications. The shape of Mno₂ nanoparticles was found to be packed together in the TEM image. Due to the accuracy of the measurement, the sample's shape cannot be determined with absolute certainty, but it appears to contain measurements of the sample's spherical internal structure that are zero-dimensional (all of its dimensions are nanoscale), which is highly preferred in surface chemistry for nanomaterial's¹⁰.



Figure 5. SEM of MnO₂ NPs.



Figure 6. TEM of MnO₂ NPs

AFM analysis

AFM surface analysis must be thoroughly examined due to numerous factors, such as deformations or image artifacts resulting from a tip and contamination, which may produce misleading results. The decision to operate in contact or without contact is one of the critical factors. The contact mode, or degree of surface contact, between the sample and its tip, damages MnO_2 NPs severely. The tip is placed very close to the sample but not in contact with it; hence the only mode necessary for this task is the non-contact one. In terms of optical



behaviour, Figs. 7 and 8 shows the development of three-dimensional spherical clusters of MnO₂ Nps following metallization. Due to the environmentally friendly synthesis of the nanomaterials, the sample's surface has pores, is highly rough, and tends to have an amorphous shape^{11, 12}. The size of the prepared oxide nanoparticles ranges between 15.00 to 50.00 nm, as shown by the Height Accumulation Distribution Report of MnO₂ NPs. This confirms that the manganese oxide made using pomegranate oxide¹². peel extract is а nano



Figure 8. Height Cumulation Distribution Report.



Adsorption Study

For each ion, the adsorption time profile was shown in a comparison of the adsorption behaviour of the prepared MnO_2 nanoparticles. The fact that Co (II) exhibits continuous adsorption growth suggests that the process is far from equilibrium and that this is not a straightforward type of adsorption. Instead, it is a precipitation process in which metal oxide nanoparticles act as crystallization nuclei to

cause the crystallization of the cobalt chloride salt. The plateau of equilibrium is more distinct for Ni (II) and Cu (II), particularly for Ni (II) Fig. 9. The MnO₂ surface is the largest in an alternate form. This arrangement may be caused by convergences between the atomic radius of the element and that of adsorbate metal ions, which make them easily incorporate with the metal oxide's lattice active sites¹³⁻¹⁵.



Figure 9. Adsorption time evolution of the metal ions on the MnO₂ surfaces.

The adsorption rate of Ni(II) is the highest in the time scale and conditions of our experiment at all surfaces, whereas Co(II) and Cu(II) ions are close in magnitude, as shown by the above figures. The adsorption process' rate is influenced by (i) charge, (ii) size, and (iii) electronic interactions. Since all ions have the same charge, the first factor (charge) cannot be the leading cause of this difference. Size influences the diffusion process in both the bulk of the solution and the adsorbent mass¹⁶. According to this theory, Co (II) ought to have the highest adsorption rate, followed by Ni (II) and then Cu (II). However, the observed decrease in the Co (II) adsorption rate and the unrestricted linear growth of the adsorbed portion suggest that there is still another process occurring along with adsorption, which is the Co (II) oxidation by metal oxide¹⁷; the adsorption percentage of ions on the surface of

manganese oxide of mixed Co, Ni, and Cu were 32.79 %, 75.00%, and 30.20%.

Antimicrobial Study

The antibacterial activity of the synthesized MnO₂ nanoparticles was tested using the agar well diffusion method against the bacteria Escherichia coli, Staphylococcus aureus, and the Candida fungus in different concentrations of 25, 50, and 75 mg/L ^{18,19} and compared with Amoxicillin and Metronidazole as a drug, DMSO solvent medium served as the controls as antibiotics. The antimicrobial activities of the MnO₂ nanoparticles were evaluated by examining the inhibition zone of growth against the used pathogens and adjusting the concentration of the nanoparticles. Table 2, Fig. 10 show the inhibition zone of growth in (mm) of MnO₂ NPs against the bacterial pathogens, two 20-24 Bactria. and one fungus

 Table 2. The Inhibition Zone (mm) of MnO2 NPs against Different Microbial.

Conc.(mg/L)	Escherichia coli	Staphylococcus aureus	Candida albicans
25	5	6	5
50	4	5	4
75	16	12	30





Figure 10. The Inhibition zone of growth.

Conclusion

MnO₂ NPs with a crystal size of 30.94nm were synthesized by a biosynthetic method using Punica Granatum extract and MnSO₄.H₂O salt as starting materials. The product was diagnosed and confirmed to be a nanocomposite by several techniques, including EDX, AFM, SEM, TEM, EDX, UV-vis and IR. It was found that manganese oxide has a thin cluster morphology in its total form. They exhibit antimicrobial activity that

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Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for

Authors' Contribution Statement

A. T. A. and L. K. A. K. certify that we have participated title of MS (Biosynthesis, Characterization, Adsorption and Antimicrobial significantly slows down bacterial species *Escherichia coli* and *Staphylococcus aureus* growth. As well as antifungals. The adsorption of three metal ions, Co, Ni, and Cu, was also studied at the same time with the removal from water by MnO₂ NPs, i.e. they are percent effective at 32.79 %, 75.00%, and 30.20% in removing salts and heavy elements that are considered water pollutants.

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- re-publication, which is attached to the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

studies of Manganese oxide Nanoparticles Using Punica Granatum Extract) in different roles as follows: Conception, design, acquisition of data, analysis, interpretation, drafting the MS, revision

and proofreading.

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التحضير الحيوي والتشخيص ودراسات الامتزاز ومضاد الميكروبات لجسيمات اوكسيدالمنغنيز النانوية باستخدام مستخلص قشور الرمان

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الخلاصة

تضمن العمل في هذا البحث تحضير اوكسيد المنغنيز النانوي من مستخلص النباتي لقشور الرمان و كبريتات المنغنيز في وسط قاعدي وشخص بطرق مختلفة مثل ؛ FT-IR ، الأشعة فوق البنفسجية المرئية ، الفحص المجهري للقوة الذرية وحيود الأشعة السينية ، والمجهر المجهري الفاذ ، مجهر المسح الإلكتروني ، مطيافية الأشعة السينية المشتنة للطاقة. في حيود الأشعة السينية ، تحديد حجم المجهر الإلكتروني النافذ ، مجهر المسح الإلكتروني ، مطيافية الأشعة السينية المشتنة للطاقة. في حيود الأشعة السينية ، تحديد حجم المجهر الإلكتروني النافذ ، مجهر المسح الإلكتروني ، مطيافية الأشعة السينية المشتنة للطاقة. في حيود الأشعة السينية ، تم تحديد حجم المجهر المسح الإلكتروني ، مطيافية الأشعة السينية المشتنة للطاقة. في حيود الأشعة السينية ، تم تحديد حجم البلورة باستخدام معادلة ديباي شيرر ، والتي وُجد أنها 30.94 نانومتر. تم امتصاص أيونات المعادن التالية M(II) = O و N و البلورة باستخدام معادلة ديباي شيرر ، والتي وُجد أنها 30.94 نانومتر. تم امتصاص أيونات المعادن التالية M(II) البلورة باستخدام معادلة ديباي شيرر ، والتي وُجد أنها 30.94 نانومتر. تم امتصاص أيونات المعادن التالية مع وقت واحد, Ni و II) للني معدل امعادن الثلاثة من الماء في وقت واحد, Ni البلورة بلعلى معدل امتصاص خلال الفترة الزمنية. كانت كفاءة إز الة أيونات المعادن الثلاثة من الماء في وقت واحد, 30.27 %. 30.20 % معدل امتصاص خلال الفترة الزمنية كانت كفاءة إز الة أيونات المعادن الثلاثة من الماء في معان معادن التالية مع أعلى معدل امتصاص خلال الفترة الزمنية. كانت كفاءة إز الة أيونات O ، و N ، و D أيونات 75.00% ، 75.00% %. 30.20 % معلي معدل من المعادي المامي ين من البكتيريا ونوع واحد من الفطريات بثلاثة تراكيز مختلفة, تمت معارنة النتائج مع تم اختبار جسيمات 200 المادات الحيوية متل أموكسيسيلين وميترونيدازول للمقارية الناتية محمالية منائية معامي معان معادن المامية مع معادام المصاص خلال الفترة النتائي وميترونيدا وح واحد من الفطريات بثلاثة تراكيز مختلفة, تمت معارنة النتائج مع تم اختبار حسيما معادات الموية مال أموكسيسيلين وميترونيدازول المقارية النتائية النتائيم.

الكلمات المفتاحية: الامتزاز ، مضادات الميكروبات ، التخليق الحيوي ، أوكسيد المنغنيز, از الة.