

Synthesis of Mn_2O_3 Nanoparticles using Different Preparation Methods and their Cytotoxic Effects on MCF-7 Breast Cancer Cells

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ABSTRACT

Background: Manganese (III) oxide (Mn_2O_3) nanoparticles have attracted significant interest due to their potential applications in biomedical research, particularly in cancer therapy. The synthesis method plays a crucial role in determining their physicochemical properties, which can influence their cytotoxicity against cancer cells. **Objective:** This study aimed to synthesize and characterize α - Mn_2O_3 and β - Mn_2O_3 nanoparticles using two different preparation methods—chemical precipitation and sol-gel—and evaluate their cytotoxic effects on MCF-7 breast cancer cells. **Methods:** The synthesized Mn_2O_3 nanoparticles were structurally and morphologically characterized using X-ray diffraction (XRD) to determine crystallite size and lattice parameters. Field Emission Scanning Electron Microscopy (FE-SEM) and Transmission Electron Microscopy (TEM) were employed to examine the nanoparticles' morphology, while Energy Dispersive X-ray (EDX) analysis was used for chemical composition assessment. The impact of the preparation method on nanoparticle shape, size, and biological effectiveness was analyzed. The cytotoxic activity of both α - Mn_2O_3 and β - Mn_2O_3 nanoparticles was assessed against the MCF-7 breast cancer cell line using standard in vitro cell viability assays. **Results:** The study demonstrated that the synthesis method significantly influenced the structural and morphological characteristics of Mn_2O_3 nanoparticles. Variations in nanoparticle size and shape affected their biological activity, with differences in cytotoxicity observed between α - Mn_2O_3 and β - Mn_2O_3 against MCF-7 cells. The nanoparticles exhibited dose-dependent cytotoxic effects, suggesting their potential as therapeutic agents in cancer treatment. **Conclusion:** The findings highlight the influence of the preparation method on Mn_2O_3 nanoparticle properties and their cytotoxic potential against breast cancer cells. Further investigations into their mechanism of action and biocompatibility are recommended to explore their potential applications in nanomedicine and targeted cancer therapy.

Keywords: Mn_2O_3 Nanoparticles, Chemical Precipitation and Sol-Gel Method, MCF-7 Breast Cancer

INTRODUCTION

Nanotechnology manipulates the size and shape of structures, and systems on nanometer scale, specifically 1 nm to 100 nm (10 to 9 meters) [1, 2]. The term "nanometer" originates from the Greek word "nanos" meaning very small [3]. Nanoparticles have bigger surface areas than their bulk counterparts, as well as increased reactivity and adjustable characteristics [4-6]. The unique characteristics mentioned have driven the progress of nanoscience and the adoption of nanoparticles (NPs) in diverse fields including biomedicine, food analysis, environmental remediation, and paint [7-12]. Nanoscale science provide unprecedented levels of comprehension and manipulation of matter at the atomic and molecular scales [13]. Nanoscale particles have received much attention due to their remarkable electrical, and magnetic properties [14]. These dimensions make them excellent candidates for nanoengineering [15, 16]. The hunt of breakthrough technological uses bioscience, and Advancements in medicine delivery are propelling nanoparticle research. [17-20] Nanomedicine, a science that combines nanotechnology and medicine, has various benefits over traditional cancer therapies, including multifunctionality, efficient drug transport, and controlled release of chemotherapy drugs. These advantages are made feasible by the unique physical and chemical qualities of nanoparticles (NPs), such as small size, configurable chemical composition, vast surface area, and adjustable shape. [21, 22] Metal-based nanoparticles are especially appealing in nanomedicine due to their restricted size and shape distribution, long

circulation half-life, rich surface functions, and potential for photothermal or photodynamic therapeutic methods. Compared to non-metallic nanoparticles of similar size, the increased density of metal-based nanoparticles have for quicker cellular absorption, which proves useful for cancer therapy tactics [23]. Furthermore, metal nanoparticles have been shown to improve targeting and drug delivery, particularly when activated with targeted ligands that give regulated deposition within cancer cells [24]. Metal nanostructures can change the tumor microenvironment by transforming unfavorable state into a treatable state.

For example, external stimuli such as light, heat, ultrasound radiation, and magnetic fields can alter the redox potential of biological systems and generate reactive oxygen species (ROS), thus increasing the sensitivity of targeted tissues [25]. Raj, Asiri et al. (2015) and Rasheed, Al-Algawi et al. (2018) [26, 27] This study seeks to synthesis nano Mn_2O_3 particles using two methods: chemical precipitation and sol-gel, and to analyze the properties of the resulting product using a variety of physical techniques.

EXPERIMENTAL PART

Analytically pure materials chemicals (BDH) Manganese sulfate monohydrate ($\text{MnSO}_4 \cdot \text{H}_2\text{O}$) (99%), sodium hydroxide (NaOH), ethanol, Deionized water.

Instruments and Apparatus

All sample analyses were conducted at the College of Science, University of Tehran, Iran. Siemens model D500 was used to irradiate the sample to obtain XRD patterns. For surface studies, an scanning electron microscope ZEISS model: Sigma VP was used and elemental analysis of α - Mn_2O_3 NPs studies by EDX, TEM: Philips model: CM120.

MATERIALS AND METHODS

Preparation of β - Mn_2O_3 by Chemical Precipitation Method

The manganese salt (3.5 g) was dissolved in 200 mL of deionized water. A dropwise addition of NaOH (0.1M) solution was made while stirring continuously to obtain $\text{pH} = 10$. The brown-black precipitate of manganese hydroxide $\text{Mn}(\text{OH})_2$ was formed after a few minutes. This precipitate is collected and repeatedly cleaned with ethanol and distilled water until $\text{pH} = 7$ is reached. Following that, the precipitate dried for 16 hours at 80°C , then calcination of precipitate at 500°C for 5 hours to obtain β - Mn_2O_3 nanoparticles.

Preparation of α - Mn_2O_3 by Sol-Gel Method

The preparation of α - Mn_2O_3 NPs was carried out using the sol-gel method. Initially, 10 grams of manganese sulfate were dissolved in 100 mL of deionized water. This solution was stirred using a magnetic stirrer for 15 minutes at room temperature to ensure complete dissolution and clarity. Similarly, 7.5 g of sodium bicarbonate were dissolved separately in 60 mL of deionized water, also stirred for 15 minutes until completely transparent.

The next step involved placing the manganese sulfate solution on the magnetic stirrer and continuously stirring it. A burette was filled with sodium bicarbonate solution, and it was slowly added to the

manganese sulfate solution at a rate of 2-3 drops per minute, while maintaining continuous stirring. This resulted in the formation of a white precipitate from $\text{Mn}(\text{OH})_2$. The addition of sodium bicarbonate solution continued while monitoring the pH reached 8. Subsequently, the precipitate was washed with ethanol and deionized water to remove impurities. It was then dried in an oven at 100°C to obtain manganese sulfate. Afterward, the calcination process was carried out at 500°C for 5 hours to obtain α - Mn_2O_3 NPs.

RESULTS AND DISCUSSION

The XRD data depicted Figure 1 (a and b) for Mn_2O_3 NPs prepared using chemical precipitation and sol-gel method respectively. The 2 θ values at (23.20° , 33.007° , 36.13° , 38.25° , 40.74° , 45.63° and 47.39°) and (23.25° , 33.07° , 38.35° , 45.30° , 49.42° and 55.10°) indicates the formation of crystal system is cubic from β - Mn_2O_3 and crystal system as an orthorhombic from α - Mn_2O_3 nanoparticles with the average crystallite size at 29.08 nm and 30.97 nm for chemical precipitation and sol-gel method respectively. These peaks are in good agreement to those reported in standard (JCPDS card, file no. 00-151-4107 and JCPDS card, file no. 00-151-4103) to β - Mn_2O_3 and α - Mn_2O_3 nanoparticles respectively. The lattice constants obtained from the XRD data are ($a = 9.4100 \text{ \AA}$) with cell volume about ($833.24 \text{ \AA}^3$) and cell parameters ($a = 9.4118 \text{ \AA}$, $b = 9.4177 \text{ \AA}$ and $c = 9.4233 \text{ \AA}$) with Cell volume ($835.26 \text{ \AA}^3$), which are consistent β - Mn_2O_3 and α - Mn_2O_3 phases respectively. FE-SEM images of the β - Mn_2O_3 and α - Mn_2O_3 nanoparticles are shown in Figures (2 and 3). The spherical and tubular nanoparticles shapes belong to the β - Mn_2O_3 nanoparticles prepared by chemical precipitation method, with average diameters 80.50 nm. While, in the case of α - Mn_2O_3 nanoparticles synthesized using sol gel method, we noticed an almost oval and cubic shapes with average diameters around 50.99 nm

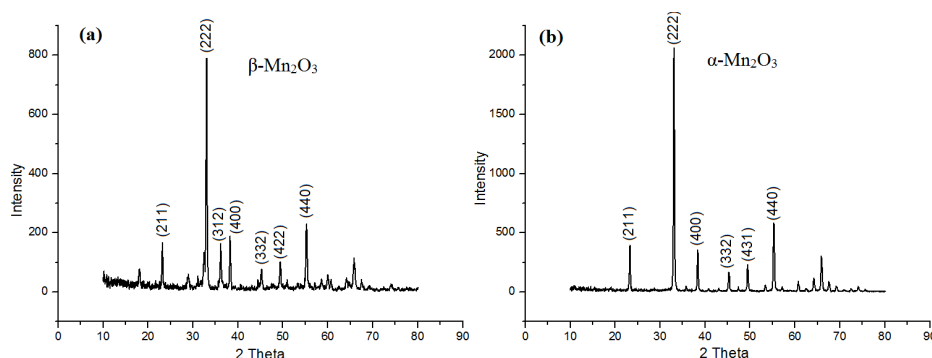


FIGURE 1: XRD Pattern of Mn_2O_3 Nps Using (A) Chemical Precipitation and (B) Sol-Gel Method.

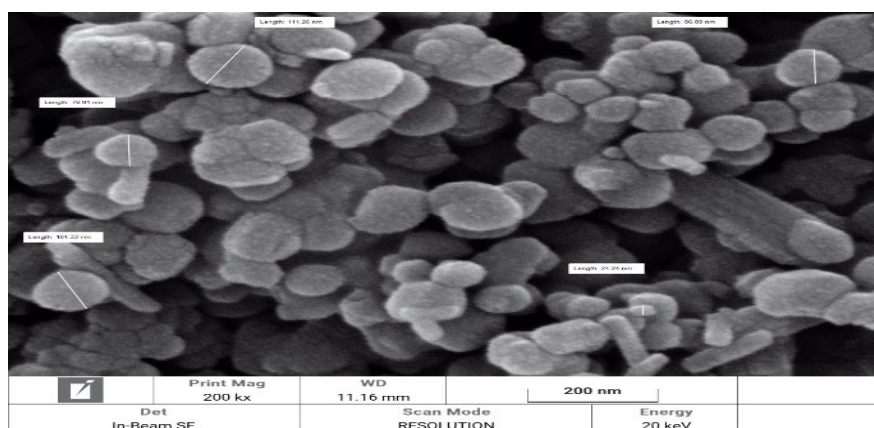


FIGURE 2: FE-SEM Image of β - Mn_2O_3 Nanoparticles Synthesis by Chemical Precipitation Method

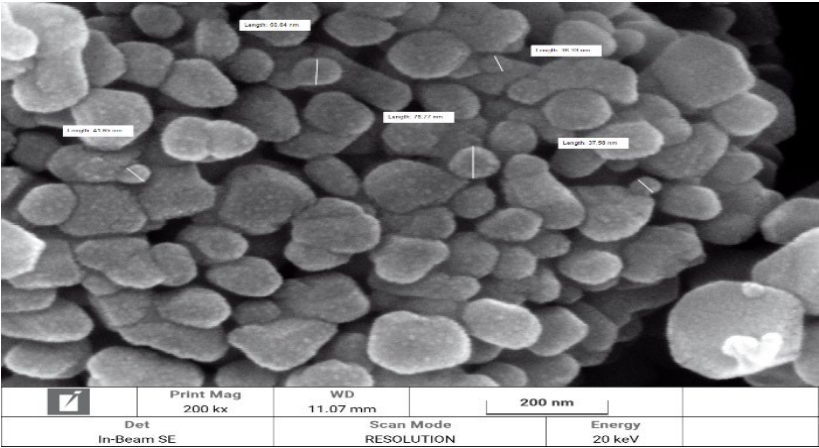


FIGURE 3: FE-SEM Image of α - Mn_2O_3 Nanoparticles Synthesis by Sol-Gel Method

Energy Dispersive X-ray Spectroscopy (EDX) results of β - Mn_2O_3 nanoparticles synthesis by chemical precipitation method and α - Mn_2O_3 nanoparticles synthesis using sol-gel method are illustrated in Figures 4 and 5 respectively.

For the chemical precipitation method have the elemental composition is 76.61% manganese (Mn) and 23.39% oxygen (O). In contrast, the sol-gel method yields a composition of 72.75%

manganese (Mn) and 27.25% oxygen (O). The analysis showed the formation of β - Mn_2O_3 and α - Mn_2O_3 with good purity. Figures (6 and 7) shows a Transmission Electron Microscopy (TEM) images at two magnifications (100 and 200 nm) of β - Mn_2O_3 and α - Mn_2O_3 nanoparticles. The nanoparticles appear highly agglomerated with heterogeneous shapes and their sizes range approximately from 13 to 23 nm and 11 to 24 nm respectively.

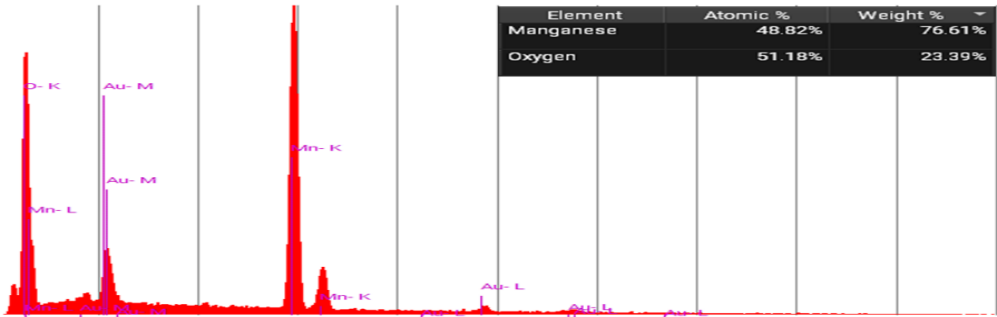


FIGURE 4: EDX Analysis of β - Mn_2O_3 Nanoparticles Prepared by Chemical Precipitation Method

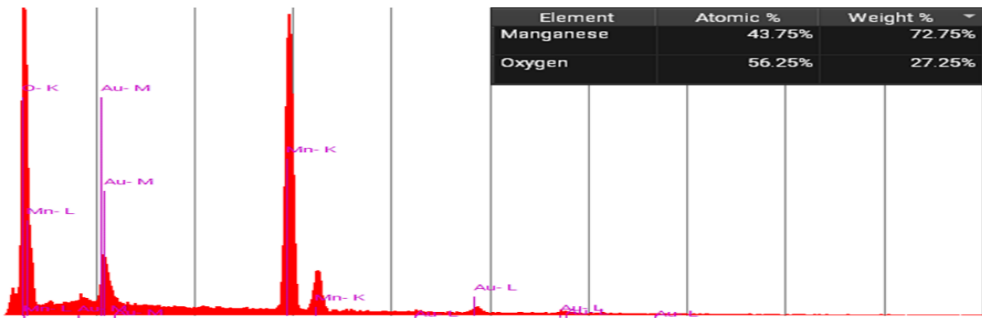


FIGURE 5: EDX Analysis of α - Mn_2O_3 Nanoparticles Prepared by Sol-Gel Method

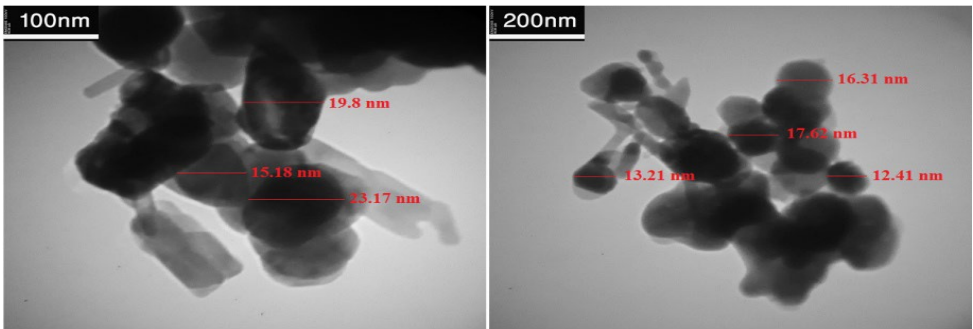


FIGURE 6: TEM Image of β - Mn_2O_3 Nanoparticles Prepared via Chemical Precipitation Method.

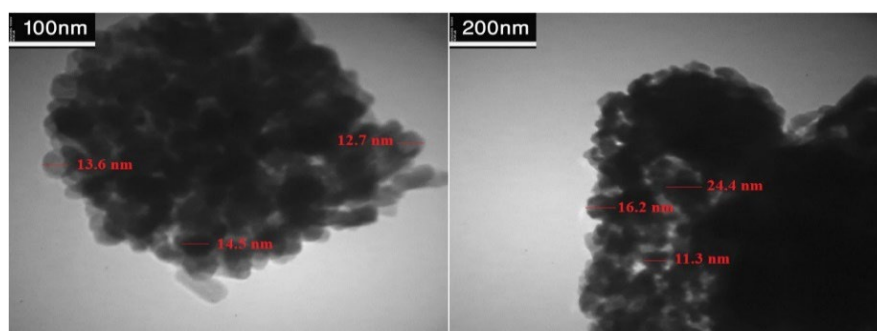


FIGURE 7: TEM Image of $\alpha\text{-Mn}_2\text{O}_3$ Nanoparticles Prepared via Sol-Gel Method.

Study cytotoxicity against MCF-7 Breast Cancer Cell Line

A study was conducted to assess the influence of Mn_2O_3 NPs on MCF-7 cancer cells, commonly used in cancer research. Several concentrations of Mn_2O_3 NPs (20, 40, 80, 160, and 320 $\mu\text{g/mL}$) were used to expose the cells for 24 hours. The IC_{50} value, representing the concentration causing inhibition or death of half of the cancer cells, was calculated to be 58.64 $\mu\text{g/mL}$ and 85.46 $\mu\text{g/mL}$ for $\beta\text{-Mn}_2\text{O}_3$ and $\alpha\text{-Mn}_2\text{O}_3$ nanoparticles respectively. The $\beta\text{-Mn}_2\text{O}_3$ and $\alpha\text{-Mn}_2\text{O}_3$

nanoparticles results demonstrated a concentration-dependent effect of the material, showing increased cancer cell death at higher concentrations. Figure 8 and table 1 shows $\beta\text{-Mn}_2\text{O}_3$ effect on the MCF-7 Breast Cancer Cell line, while figure 9 and table 2 illustrate the impact $\alpha\text{-Mn}_2\text{O}_3$ on the MCF-7 Breast Cancer Cell line. From results above, this indicates that both Mn_2O_3 nanoparticles exhibit potent cytotoxicity against MCF-7 cells that mean their potential use as a targeted therapeutic agent for cancer at appropriate concentrations.

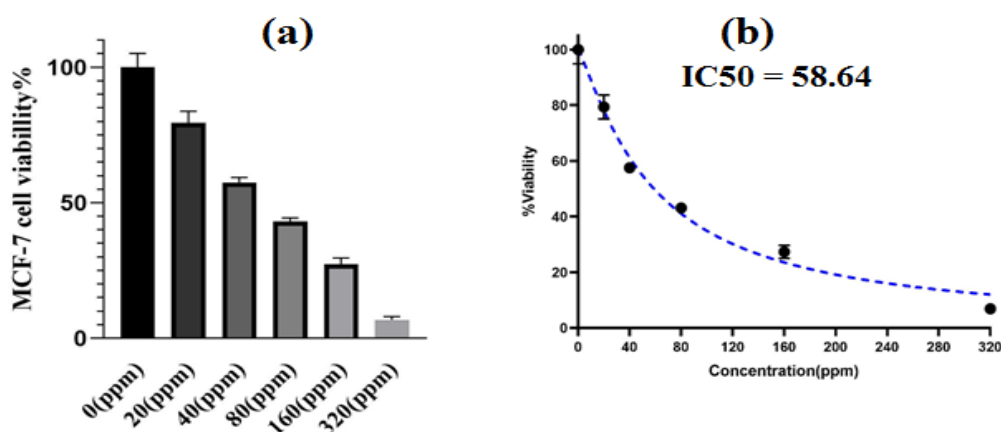


FIGURE 8: (a) Illustrates the Percentage of Cancer Cell Killing, and (b) Shows the IC_{50} for the $\beta\text{-Mn}_2\text{O}_3$ NPs Prepared Using Chemical Precipitation.

Table 1: Showing $\beta\text{-Mn}_2\text{O}_3$ NPs Concentrations, Killing Percentage of Cancer Cells, and Cell Viability.

Concentration ($\mu\text{g/mL}$)	Cell Death (%)	Cell Viability (%)
20	20.5865	79.4135
40	42.4654	57.5346
80	56.8939	43.1061
160	72.6807	27.3193
320	93.12861	6.87139

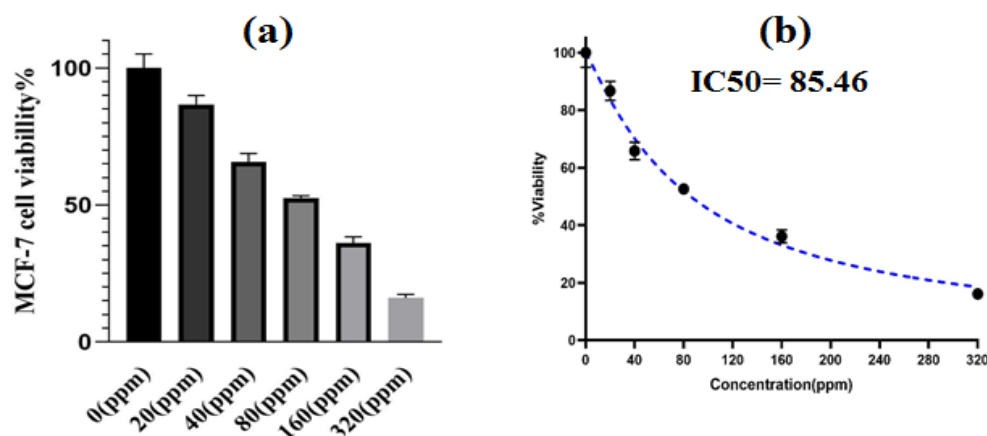


FIGURE 9. (a) Illustrates the Percentage of Cancer Cell Killing, and (b) shows the IC_{50} for the $\alpha\text{-Mn}_2\text{O}_3$ NPs Prepared Using Sol-Gel.

Table 2: Showing α -Mn₂O₃ NPs Concentrations, Killing Percentage of Cancer Cells, and Cell Viability.

Concentration ($\mu\text{g/mL}$)	Cell Death (%)	Cell Viability (%)
20	13.3009	86.6991
40	34.1913	65.8087
80	47.4116	52.5884
160	63.9306	36.0694
320	83.902	16.098

Conclusion

In this study, Two Mn₂O₃ NPs types were successfully prepared using two methods chemical precipitation and sol-gel. The Mn₂O₃ NPs were confirmed using several techniques (XRD, FE-SEM, EDX and TEM). The XRD spectra confirmed the effect of the method type used on Mn₂O₃ NPs phase formed, as it was a cubic from β -Mn₂O₃ and crystal system as an orthorhombic from α -Mn₂O₃ nanoparticles. FE-

SEM and TEM images of the β -Mn₂O₃ and α -Mn₂O₃ nanoparticles were confirmed effect of method type on the size and shape nanoparticles.

EDX spectra confirmed the found of magnesium and oxygen in the two samples prepared by both methods. Results indicate that α -Mn₂O₃ and β -Mn₂O₃ had good anticancer activities against human breast cancer cell lines in a nanoparticles concentration-dependent manner.

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