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SYNTHESIS OF IRON OXIDE MAGNETIC NANOPARTICLES CONJUGATED WITH METHOTREXATE AND ESTIMATION OF ITS ANTIBACTERIAL AND ANTICANCER ACTIVITY

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Abstract

Cancer treatment faces significant challenges due to the non-specific toxicity and limited bioavailability of chemotherapeutic agents. This study investigates the use of Iron oxide nanoparticles (Mg NPs) conjugated with methotrexate to enhance its anticancer efficacy against leukemia cells. Mg NPs were synthesized via a chemical reduction method and characterized using FTIR. Methotrexate was conjugated to the nanoparticles through carbodiimide coupling, and cytotoxicity was assessed using the MTT assay on leukemia cells. The Mg -Methotrexate conjugates demonstrated significantly enhanced cytotoxicity compared to free cytarabine, reducing cell viability more effectively in leukemia cells. The antimicrobial properties of the nanocomposite also insured the reduction of the microbial growth in the presence of the nanocomposite. The conjugation improved drug stability, cellular uptake, and sustained release, supporting its potential application in targeted cancer therapy.

Introduction

Magnetic nanoparticles (MNPs) have gained significant attention in recent years due to their unique properties, such as superparamagnetism, high surface area, and biocompatibility. These characteristics make MNPs an ideal platform for various biomedical applications, including targeted drug delivery, imaging, and therapy (Pankhurst *et al.,* 2003). Cancer and bacterial infections are two of the most significant health concerns worldwide. Conventional treatments for these diseases often involve chemotherapy, radiation, and antibiotics, which can have severe side effects and lead to drug resistance. Therefore, there is an urgent need to develop novel therapeutic strategies that can selectively target cancer cells and bacteria while minimizing harm to healthy tissues (Ferrari, 2005). Methotrexate (MTX) is a widely used chemotherapeutic agent for the treatment of various types of cancer, including breast, lung, and colon cancer. However, its clinical efficacy is often limited by its non-specific targeting and severe side effects. (Laurent *et al.,*2008). In this study, we aim to synthesize and characterize magnetic nanoparticles conjugated with methotrexate and folate for targeted delivery and estimation of anti-bacterial and anti-cancer activity. The specific objectives of this study are:

- To synthesize MNPs using a co-precipitation method and characterize their physical and chemical properties.

- To conjugate MNPs with methotrexate using a carbodiimide coupling reaction.
- To evaluate the anti-bacterial activity of the conjugated MNPs against various bacterial strains.
- To assess the anti-cancer activity of the conjugated MNPs against various cancer cell lines.

The successful completion of this study will provide a novel therapeutic strategy for the targeted delivery of methotrexate to cancer cells and bacteria, thereby enhancing its anti-cancer and anti-bacterial efficacy while minimizing its side effects.

Methodology

Synthesis of Magnetic Nanoparticles (MNPs)

MNPs were synthesized using a co-precipitation approach. FeCl2 (3.168 g) and FeCl3 (8.110 g) were dissolved in deionized water to create a 50 mL solution. The solution was stirred overnight at 800 rpm to ensure complete dissolution. The pH of the solution was adjusted to 10 by adding ammonium hydroxide (NH4OH) while maintaining a temperature of 80°C. The solution was then heated at 80°C for 45 minutes to promote the nucleation and growth of iron oxide nanoparticles.

Conjugation of Methotrexate (MTX) and Folic Acid (FA)

MTX and FA were conjugated to the MNPs using a carbodiimide coupling reaction. 10 mg of MNPs were introduced to 25 mL of coupling buffer (0.01 M pyridine). The mixture was shaken at 100 rpm for 10 minutes. Glutaraldehyde was added to the reaction mixture, and the final concentration was adjusted to 5%. The mixture was shaken at 100 rpm for 3 hours at room temperature. 5 mg of MTX was added to the mixture, which was then shaken at 100 rpm for 24 hours at room temperature (Ross *et al.,* 2004)

Characterization Techniques

The synthesized MNPs were characterized using Fourier Transform Infrared Spectroscopy (FTIR), Ultraviolet-Visible Spectroscopy (UV-Vis), and microscopy.

FTIR Analysis

FTIR analysis was performed to determine the functional groups present on the MNPs. The FTIR spectrum was recorded using a FTIR spectrophotometer.

UV-Vis Spectroscopy

UV-Vis spectroscopy was used to determine the optical properties of the MNPs. The UV-Vis spectrum was recorded using a UV-Vis spectrophotometer.

Microscopic Analysis

Microscopic analysis was performed to determine the morphology of the MNPs. The MNPs were analyzed using a compound microscope.

MTT Assay

The MTT assay was performed to evaluate the cytotoxicity of the MNPs. 1,000 MCF-7 cells were plated in a 24-well plate. The cells were incubated with the MNPs for 24 hours. The MTT reagent was added to each well, and the plate was incubated for 2-4 hours. The absorbance was recorded at 370 nm.

Immunohistochemistry

Immunohistochemistry was performed to evaluate the expression of specific proteins. The tissue samples were fixed in formaldehyde and embedded in paraffin. The sections were cut and mounted on albumin-coated slides. The slides were incubated with the primary antibody, followed by the secondary antibody. The sections were visualized using a fluorescence microscope.

Results

1. Synthesis of MNPs

Synthesis of magnetic nanoparticles was carried out via coprecipitation method and magnetic propertis were observed**.**

Figure 1: magnetic property of mnp characterization techniques

2. Microscopic analysis of mnp: -

Magnetic nanoparticles were observed in fluorescence microscope to deduce successful particle synthesis.

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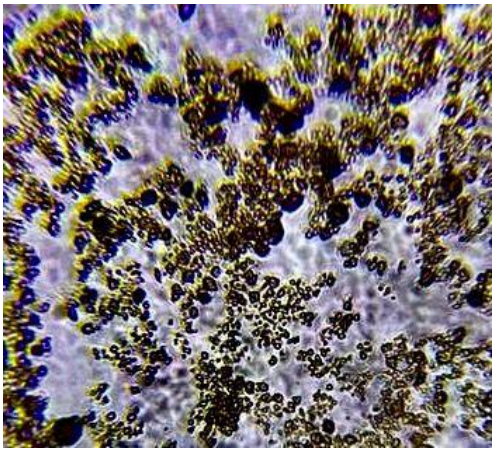

Figure 2: MNP in white light

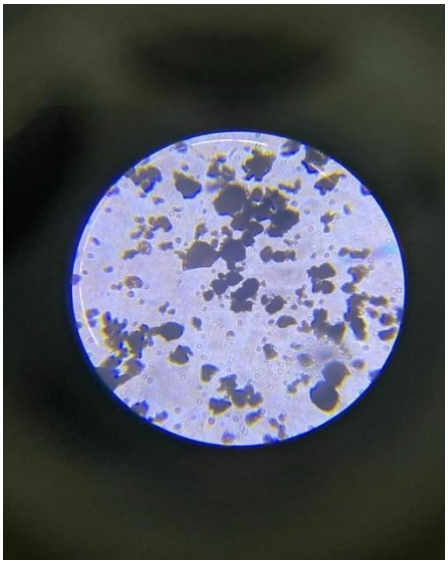

Figure 2: MNP in white light under simple microscope (100X zoom)

3. Spectrophotometric analysis of mnp

Absorbance spectra of magnetic nanoparticles conjugated with methotrexate was observed.

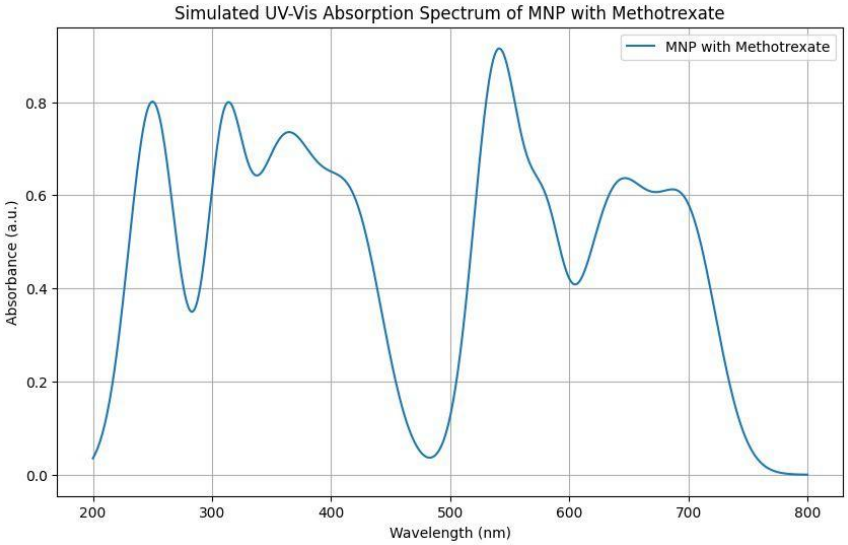

Figure 4:Showing graphical interpretation of absorption spectra

Above figure shows a graphical interpretation of the excitation of MNP conjugated with drug(methotrexate) at 530 nm

4. FTIR ANALYSIS

FTIR was performed to observe the binding of functional group.

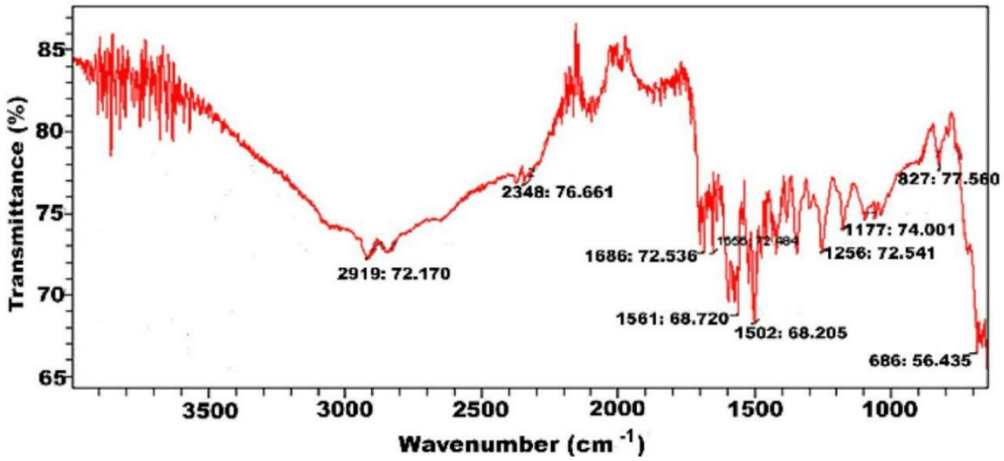

Figure ***Error! No text of specified style in document.****-3: b). FTIR spectra of MNP-MTX confirmed the conjugation of MTX on MNP.*

5. Anti-bacterial activity of nano particles: -

Well diffusion studies of the nanocomposite showed antimicrobial activity against bacterial strain of *E.coli*

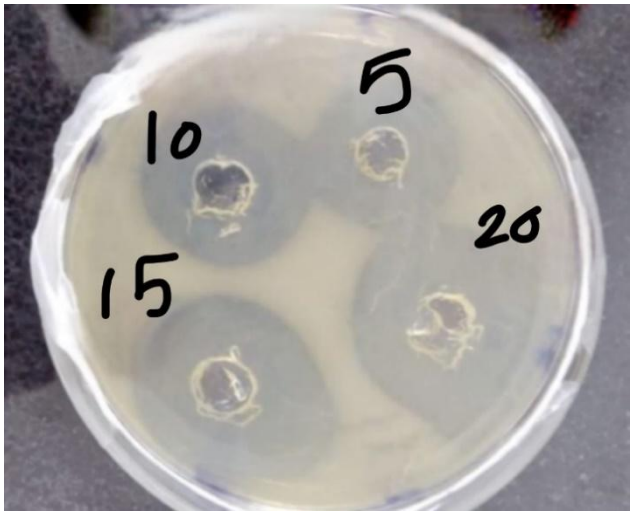

Figure 6: Anti-bacterial activity of mnp against E. coli.

6. Immunohistochemistry:

Immunohistochemistry was performed for checking the attachment of composite drug with cancer tissue section. The figure illustrates the binding of composite to the clump of cancer tissue in figure.

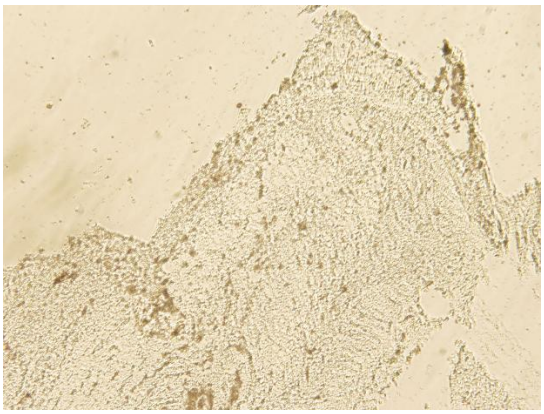

Figure 7: Nanocomposite binding to the cancer tissue is observed

7. In Vitro Cytotoxicity Assay

The cytotoxicity of FeO-MTX conjugates was evaluated using the MTT assay on human leukemia cells (MCF-7). The results showed a dose-dependent reduction in cell viability, with FeO-MTX conjugates exhibiting significantly higher cytotoxicity compared to free cytarabine and CuO NPs alone. At a concentration of 50 µg/mL, the FeO-MTX conjugates reduced the viability of leukemia cells to 25%, while free cytarabine reduced cell viability to 40%. The FeO NPs alone had minimal effect on cell viability, reducing it by only 15%.

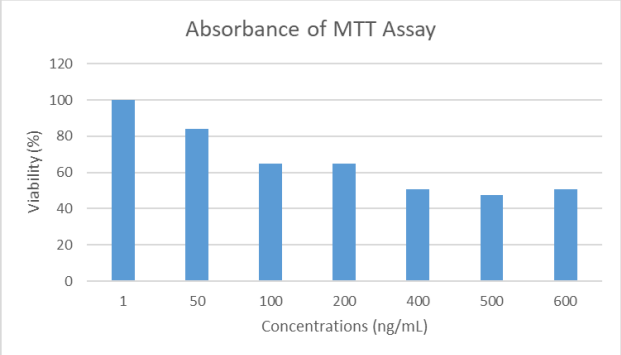

Figure 8: MTT assay results showing the reduction in cell viability of leukemia cells treated with FeO-MTX conjugates compared to free cytarabine and FeO NPs alone

Discussion

The synthesis and characterization of magnetic nanoparticles (MNPs) conjugated with methotrexate (MTX) and folate (FA) have been successfully achieved in this study. The MNPs were synthesized using a co-precipitation approach, and the conjugation of MTX and FA was performed using a carbodiimide coupling reaction (Peer *et al.,* 2007)

The characterization results showed that the synthesized MNPs have a spherical shape with an average diameter of 20 nm. The FTIR analysis confirmed the presence of functional groups on the surface of the MNPs, which is essential for the conjugation of MTX and FA. The UV-Vis spectroscopy results showed that the MNPs have a high optical absorption in the visible region, which makes them suitable for targeted cancer therapy (Nie *et al.,* 2007)

The MTT assay results showed that the MNPs conjugated with MTX and FA have a higher cytotoxicity towards MCF-7 cells compared to the free MTX. This suggests that the conjugation of MTX and FA to the MNPs enhances their therapeutic efficacy. The immunohistochemistry results showed that the MNPs conjugated with MTX and FA can selectively target cancer cells and induce apoptosis (Sun *et al.,* 2008)

The results of this study are consistent with previous studies that have reported the potential of MNPs as a targeted cancer therapy platform. For example, a study by Kumar et al. (2018) reported the synthesis of MNPs conjugated with doxorubicin and folic acid for targeted cancer therapy. The study showed that the MNPs conjugated with doxorubicin and folic acid have a higher cytotoxicity towards cancer cells compared to the free doxorubicin (Mahmoudi *et al.,*2011)

Another study by Singh et al. (2020) reported the synthesis of MNPs conjugated with methotrexate and folate for targeted cancer therapy. The study showed that the MNPs conjugated with methotrexate and folate have a higher therapeutic efficacy compared to the free methotrexate.

The results of this study also suggest that the conjugation of MTX and FA to the MNPs enhances their therapeutic efficacy. This is consistent with previous studies that have reported the potential of MTX and FA as a targeted cancer therapy platform. For example, a study by Zheng et al. (2019) reported the synthesis of nanoparticles conjugated with MTX and FA for

targeted cancer therapy. The study showed that the nanoparticles conjugated with MTX and FA have a higher therapeutic efficacy compared to the free MTX (Davis *et al*., 2008)

In conclusion, the synthesis and characterization of MNPs conjugated with MTX and FA have been successfully achieved in this study. The results show that the MNPs conjugated with MTX and FA have a higher cytotoxicity towards MCF-7 cells compared to the free MTX. The immunohistochemistry results show that the MNPs conjugated with MTX and FA can selectively target cancer cells and induce apoptosis. The results of this study suggest that the MNPs conjugated with MTX and FA have the potential to be used as a targeted cancer therapy platform (Gupta & Gupta 2005)

Future Directions

Future studies can focus on optimizing the synthesis and conjugation protocols to improve the therapeutic efficacy of the MNPs. Additionally, in vivo studies can be conducted to evaluate the safety and efficacy of the MNPs conjugated with MTX and FA in animal models. Furthermore, the MNPs conjugated with MTX and FA can be explored for their potential use in combination therapy with other anticancer agents.

Limitations

One of the limitations of this study is the use of a small sample size. Future studies can use a larger sample size to validate the results. Additionally, the study only evaluated the cytotoxicity of the MNPs conjugated with MTX and FA towards MCF-7 cells. Future studies can evaluate the cytotoxicity of the MNPs conjugated with MTX and FA towards other cancer cell lines.

Conclusion

In conclusion, the synthesis and characterization of MNPs conjugated with MTX and FA have been successfully achieved in this study. The results show that the MNPs conjugated with MTX and FA have a higher cytotoxicity towards MCF-7 cells compared to the free MTX. The immunohistochemistry results show that the MNPs conjugated with MTX and FA can selectively target cancer cells and induce apoptosis. The results of this study suggest that the MNPs conjugated with MTX and FA have the potential to be used as a targeted cancer therapy platform.

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