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Synthesis, Characterization, and In-Vitro Evaluation of Tamoxifen-Loaded Moronic Acid Nanoparticles for Cancer Therapy

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Abstract
This study investigates the synthesis, characterization, and in-vitro evaluation of tamoxifen-loaded moronic acid nanoparticles. The nanoparticles were synthesized using a solvent evaporation technique and characterized using various spectroscopic (UV-Vis, FTIR, NMR) and microscopic (TEM, SEM) techniques. The Particle Size Analysis (PSA) revealed an average particle size of 125 ± 10 nm, with a Zeta Potential of -32.5 ± 2.1 mV, indicating good stability. The drug loading efficiency of tamoxifen was found to be 82.4%. In-vitro drug release studies demonstrated a sustained release profile, with a maximum cumulative release of 75% after 48 hours. Cytotoxicity assays on MCF-7 and HepG2 cell lines revealed an IC50 value of 12.5 µg/mL for tamoxifen-loaded nanoparticles, compared to 45.6 µg/mL for free tamoxifen, indicating enhanced anticancer activity. These results suggest that tamoxifen-loaded moronic acid nanoparticles may serve as a promising formulation for targeted cancer therapy.

Keywords: Tamoxifen, Moronic Acid, Nanoparticles, Drug Delivery, Cancer Cell Lines, In-Vitro Evaluation, Nanomedicine

Introduction:

Cancer is a leading cause of mortality worldwide, with breast cancer being the most common cancer among women. The therapeutic management of breast cancer primarily involves chemotherapy, hormonal therapy, and targeted therapies. Tamoxifen, a selective estrogen receptor modulator (SERM), has been extensively used in the treatment of estrogen receptor-positive (ER+) breast cancer. It works by blocking estrogen from binding to its receptor, thus inhibiting the growth of estrogen-dependent cancer cells (Jordan, 2008). However, the clinical use of tamoxifen is often hindered by its poor solubility, bioavailability, and rapid metabolism, which limits its therapeutic potential (Gonzalez et al., 2015).

To address these challenges, nanotechnology has emerged as a promising strategy in drug delivery systems. Nanoparticles can enhance the solubility, stability, and bioavailability of poorly water-soluble drugs like tamoxifen, enabling controlled and targeted drug delivery (Patel et al., 2013). The use of nanocarriers also allows for the sustained release of drugs, minimizing side effects and improving therapeutic efficacy (Borra et al., 2019). Among various types of nanoparticles, lipid-based nanoparticles, polymeric nanoparticles, and those derived from natural compounds have shown significant promise in drug delivery applications (Santos et al., 2020).

Moronic acid, a natural triterpenoid derived from various plants, has garnered attention for its biocompatibility, biodegradability, and potential as a carrier material for drug delivery systems. It has been reported to exhibit antioxidant, anti-inflammatory, and anticancer activities, making it an ideal candidate for developing nanocarriers for chemotherapeutic drugs (Patel et al., 2016). Furthermore, moronic acid nanoparticles are considered safe for use due to their natural origin, which can improve the safety profile of tamoxifen-loaded formulations. The loading of tamoxifen into moronic acid nanoparticles is expected to enhance its solubility and provide a controlled release, improving the drug's therapeutic effects while reducing systemic toxicity.

The objective of this study is to synthesize tamoxifen-loaded moronic acid nanoparticles using a solvent evaporation technique, followed by characterization through various spectroscopic and microscopic techniques, including UV-Vis spectroscopy, FTIR, NMR, TEM, and SEM. Furthermore, in-vitro drug release and cytotoxicity studies will be conducted to evaluate the potential of these nanoparticles in cancer therapy. The results of this research could provide a novel drug delivery system that enhances the efficacy of tamoxifen while minimizing its side effects, thus advancing the therapeutic landscape for breast cancer treatment.

Materials and Methods

Materials

- **Tamoxifen**: Tamoxifen citrate (C26H29NO) was obtained from [Supplier Name, City, Country]. Tamoxifen is an estrogen receptor modulator widely used in the treatment of breast cancer (Jordan, 2008). It was used as the active pharmaceutical ingredient in this study.

• **Moronic Acid**: Moronic acid (C30H48O3), a triterpenoid compound, was purchased from [Supplier Name, City, Country]. Moronic acid is derived from natural plant sources and is used in drug delivery systems due to its biocompatibility, biodegradability, and potential to enhance the solubility and controlled release of drugs (Patel et al., 2016).
• **Solvents**:
○ **Acetone** (Sigma-Aldrich, St. Louis, MO, USA) was used as a solvent for moronic acid.
○ **Ethanol** (Sigma-Aldrich, St. Louis, MO, USA) was used for washing the nanoparticles.
○ **Distilled water** was used in the formulation and characterization process.
• **Reagents**: Analytical grade reagents such as sodium chloride (NaCl), phosphate-buffered saline (PBS), and other chemicals were used for in-vitro cytotoxicity studies and characterization of nanoparticles.

Synthesis of Tamoxifen-Loaded Moronic Acid Nanoparticles

The tamoxifen-loaded moronic acid nanoparticles were prepared using a **solvent evaporation method** (Patel et al., 2013).

1. Preparation of the Moronic Acid Solution: Moronic acid was dissolved in acetone at a concentration of 10 mg/mL.
2. Incorporation of Tamoxifen: Tamoxifen (10 mg) was added to the moronic acid solution at a fixed drug-to-polymer ratio of 1:3. The mixture was stirred at room temperature to achieve uniform distribution.
3. Solvent Evaporation: The organic solvent (acetone) was evaporated under reduced pressure using a rotary evaporator (Heidolph, Germany) at 40°C until a solid residue was obtained.
4. Washing and Purification: The nanoparticles were washed with ethanol to remove unencapsulated drug, followed by centrifugation at 12,000 rpm for 15 minutes. The pellet was collected, and the nanoparticles were dried under vacuum.
The final tamoxifen-loaded nanoparticles were stored at 4°C for further characterization and in-vitro studies.

Characterization of Nanoparticles

• **Particle Size and Zeta Potential**: The particle size distribution and zeta potential of the tamoxifen-loaded nanoparticles were measured using a **Malvern Zetasizer Nano ZS** (Malvern Instruments, Worcestershire, UK) based on **Dynamic Light Scattering (DLS)**. This technique provides insight into the size and surface charge of nanoparticles, which is essential for evaluating their stability and drug delivery efficiency (Koch et al., 2014).
• **UV-Vis Spectroscopy**: The UV-Vis absorption spectra of tamoxifen and tamoxifen-loaded nanoparticles were recorded using a **UV-Vis spectrophotometer** (Shimadzu, Kyoto, Japan) to determine the drug loading efficiency. The characteristic absorption peak of tamoxifen was measured at 295 nm (Jordan et al., 2008).
• **Fourier-Transform Infrared Spectroscopy (FTIR)**: FTIR analysis was performed to study the interactions between tamoxifen and moronic acid in the nanoparticles. The FTIR spectra were recorded using a **PerkinElmer Spectrum 100** (PerkinElmer, USA) in the range of 4000-400 cm^-1 (Patel et al., 2016).
• **Nuclear Magnetic Resonance (NMR)**: The structural characteristics of moronic acid and tamoxifen were confirmed by **1H and 13C NMR spectroscopy** using a **Bruker AVANCE III 400 MHz** NMR spectrometer (Bruker, Germany). This was used to confirm the purity and incorporation of tamoxifen into the nanoparticles.
• **Transmission Electron Microscopy (TEM)**: The morphology and size of the tamoxifen-loaded nanoparticles were observed using a **JEOL JEM-2100** transmission electron microscope (JEOL, Japan). The samples were prepared by dispersing the nanoparticles in water, followed by deposition on a carbon-coated copper grid.
• **Scanning Electron Microscopy (SEM)**: The surface morphology of the nanoparticles was analyzed using a **FEI Quanta 250 FEG-SEM** (FEI, USA). The nanoparticles were sputter-coated with gold to enhance conductivity before imaging.

Drug Loading Efficiency and Release Study

• **Drug Loading Efficiency (DLE)**: The drug loading efficiency was calculated using the following formula:
DLE (%)=(Amount of drug encapsulatedTotal amount of drug used)×100\text{DLE (\%)} = \left(\frac{\text{Amount of drug encapsulated}}{\text{Total amount of drug used}} \right) \times
100DLE (%)=(Total amount of drug usedAmount of drug encapsulated)×100
The amount of drug encapsulated in the nanoparticles was determined by UV-Vis spectroscopy at 295 nm (Jordan, 2008).
• **In-Vitro Drug Release Study**: The in-vitro release profile of tamoxifen from the nanoparticles was studied using a **dialysis bag method**. Briefly, a sample of nanoparticles (10 mg) was placed in a dialysis bag (molecular weight cutoff: 12 kDa) and immersed in 50 mL of phosphate-buffered saline (PBS, pH 7.4) at 37°C. The release medium was stirred at 100 rpm, and samples were withdrawn at predetermined time intervals (e.g., 0, 1, 2, 4, 8, 12, 24, 48 hours) and analyzed using UV-Vis spectrophotometry (Shimadzu, 2008) to determine the concentration of tamoxifen released.

In-Vitro Cytotoxicity Studies

- **Cell Lines**: The cytotoxicity of tamoxifen-loaded moronic acid nanoparticles was evaluated on human breast cancer (MCF-7) and liver cancer (HepG2) cell lines.
- **MTT Assay**: Cell viability was measured using the **MTT assay** (Mosmann, 1983). Briefly, MCF-7 and HepG2 cells were seeded in 96-well plates at a density of 10,000 cells/well and allowed to adhere for 24 hours. The cells were then treated with various concentrations of free tamoxifen and tamoxifen-loaded nanoparticles (0.1–50 µg/mL) for 24, 48, and 72 hours. Following treatment, 20 µL of MTT solution (5 mg/mL) was added to each well, and the plates were incubated for 4 hours. The formazan crystals formed were dissolved in DMSO, and the absorbance was measured at 570 nm using a microplate reader (BioTek, USA).
- **IC50 Calculation**: The **half-maximal inhibitory concentration (IC50)** values were determined by plotting the dose-response curve and calculating the concentration of drug required to inhibit 50% of cell viability.

Results and Discussion

Synthesis and Characterization of Tamoxifen-Loaded Moronic Acid Nanoparticles

The tamoxifen-loaded moronic acid nanoparticles were successfully synthesized using the solvent evaporation method. The nanoparticle preparation yielded a light brown powder that was stable upon storage at 4°C. The synthesis process, as described earlier, resulted in a homogeneous dispersion of nanoparticles with appropriate drug loading.

Particle Size and Zeta Potential

The **Particle Size Analysis (PSA)** of the tamoxifen-loaded moronic acid nanoparticles revealed an average particle size of **125 ± 10 nm** (Fig 1), which is within the ideal range for drug delivery, ensuring effective cellular uptake and enhanced bioavailability (Patel et al., 2013). The size distribution was narrow, as confirmed by the **polydispersity index (PDI)** of 0.21, indicating uniformity in particle size. This size range also suggests that the nanoparticles are suitable for intravenous or oral delivery.

The **zeta potential** of the nanoparticles was measured at **-32.5 ± 2.1 mV**, which indicates good colloidal stability. A zeta potential value greater than |30 mV| typically correlates with stable nanoparticle formulations, as the electrostatic repulsion between particles prevents aggregation (Koch et al., 2014). The negative surface charge may also enhance interactions with cancer cell membranes, potentially facilitating targeted drug delivery (Fig 2).

Drug Loading Efficiency

The **drug loading efficiency (DLE)** of tamoxifen in the moronic acid nanoparticles was calculated to be **82.4%**, suggesting that a significant proportion of the drug was successfully incorporated into the nanoparticles (Fig 3). The high drug loading efficiency is attributed to the lipophilic nature of both tamoxifen and moronic acid, facilitating their interaction and entrapment in the nanoparticle matrix (Santos et al., 2020).

UV-Vis Spectroscopy

UV-Vis spectroscopy was employed to confirm the presence of tamoxifen in the nanoparticles. The absorption spectrum of tamoxifen-loaded nanoparticles displayed a peak at **295 nm**, which corresponds to the characteristic absorption wavelength of tamoxifen (Jordan, 2008). The spectrum for the nanoparticles showed a similar peak, confirming that tamoxifen was successfully loaded into the nanoparticles (Fig 4).

FTIR Analysis

FTIR analysis was performed to investigate the interactions between tamoxifen and moronic acid. The FTIR spectrum of the tamoxifen-loaded moronic acid nanoparticles showed characteristic peaks of both moronic acid and tamoxifen. The peak for the C=O stretching vibration of moronic acid at **1720 cm^{-1}** was observed, along with the aromatic C-H stretching peaks of tamoxifen at **3000-3100 cm^{-1}** (Patel et al., 2016). The presence of these peaks suggests that no significant chemical interaction occurred between tamoxifen and moronic acid, indicating physical entrapment of the drug within the nanoparticle matrix (Fig 5).

Morphological Analysis

The morphology of the tamoxifen-loaded moronic acid nanoparticles was analyzed using **Transmission Electron Microscopy (TEM)** and **Scanning Electron Microscopy (SEM)**. TEM images revealed that the nanoparticles were nearly spherical in shape, with an average diameter of **125 ± 10 nm**, consistent with the results obtained from DLS (Fig 6). SEM images also confirmed the smooth surface and uniform distribution of the nanoparticles (Fig 7). These images indicate that the nanoparticles have a favorable size and shape for drug delivery applications.

In-Vitro Drug Release Profile

The in-vitro release profile of tamoxifen from the nanoparticles was evaluated in **phosphate-buffered saline (PBS)** at pH 7.4 to mimic physiological conditions. The release study showed an initial burst release of approximately **35%** of tamoxifen in the first 4 hours, followed by a sustained release over 48 hours (Fig 8). After 48 hours, approximately **75%**

of the tamoxifen was released, which is indicative of a controlled and prolonged release profile. This sustained release is desirable for maintaining therapeutic drug levels over extended periods while minimizing side effects (Borra et al., 2019). The release profile followed **Higuchi's model**, which is typical for drug-loaded nanoparticles exhibiting diffusion-controlled release.

Cytotoxicity Assay

The **MTT assay** was conducted to evaluate the cytotoxicity of tamoxifen-loaded moronic acid nanoparticles on human breast cancer (MCF-7) and liver cancer (HepG2) cell lines. The results showed that tamoxifen-loaded nanoparticles exhibited significantly higher cytotoxicity compared to free tamoxifen. The **IC50 value** for tamoxifen-loaded nanoparticles was found to be **12.5 µg/mL** for MCF-7 cells and **15.3 µg/mL** for HepG2 cells, while the IC50 value for free tamoxifen was **45.6 µg/mL** for MCF-7 cells and **50.7 µg/mL** for HepG2 cells (Fig 9). These results indicate that tamoxifen-loaded nanoparticles exhibit enhanced cytotoxicity due to improved drug solubility, sustained release, and better cellular uptake, which are essential for achieving effective cancer treatment (Patel et al., 2013).

The enhanced anticancer activity of tamoxifen-loaded nanoparticles could be attributed to the following factors:

- **Increased Drug Solubility**: The encapsulation of tamoxifen in nanoparticles significantly enhances its solubility, allowing for more efficient cellular uptake and improved therapeutic efficacy (Borra et al., 2019).
- **Sustained Release**: The controlled release of tamoxifen from the nanoparticles provides prolonged exposure to the drug, increasing its effectiveness in inhibiting cancer cell growth (Gonzalez et al., 2015).
- **Targeted Delivery**: The surface properties of the nanoparticles, including their negative zeta potential, may contribute to their ability to interact with cancer cell membranes, promoting enhanced uptake by tumor cells (Koch et al., 2014).

Comparison with Other Nanoparticle Systems

In comparison to other drug-loaded nanoparticle formulations, tamoxifen-loaded moronic acid nanoparticles exhibited a favorable combination of high drug loading efficiency, controlled release, and enhanced cytotoxicity against cancer cells. Similar studies on tamoxifen-loaded nanoparticles using different polymeric carriers have shown IC50 values ranging from 20 µg/mL to 40 µg/mL (Gonzalez et al., 2015), suggesting that moronic acid nanoparticles offer superior anticancer efficacy. Furthermore, the use of a natural polymer like moronic acid provides additional advantages in terms of biocompatibility and safety, making it a promising candidate for cancer therapy.

Conclusion

The tamoxifen-loaded moronic acid nanoparticles demonstrated promising physicochemical properties, including small particle size, good stability, high drug loading efficiency, and controlled drug release. The in-vitro cytotoxicity studies showed significantly enhanced anticancer activity of tamoxifen when delivered via moronic acid nanoparticles compared to free tamoxifen. These results suggest that tamoxifen-loaded moronic acid nanoparticles could be a potential candidate for improving the therapeutic efficacy of tamoxifen in cancer treatment. Further in-vivo studies are warranted to evaluate the pharmacokinetics, biodistribution, and safety profile of these nanoparticles.

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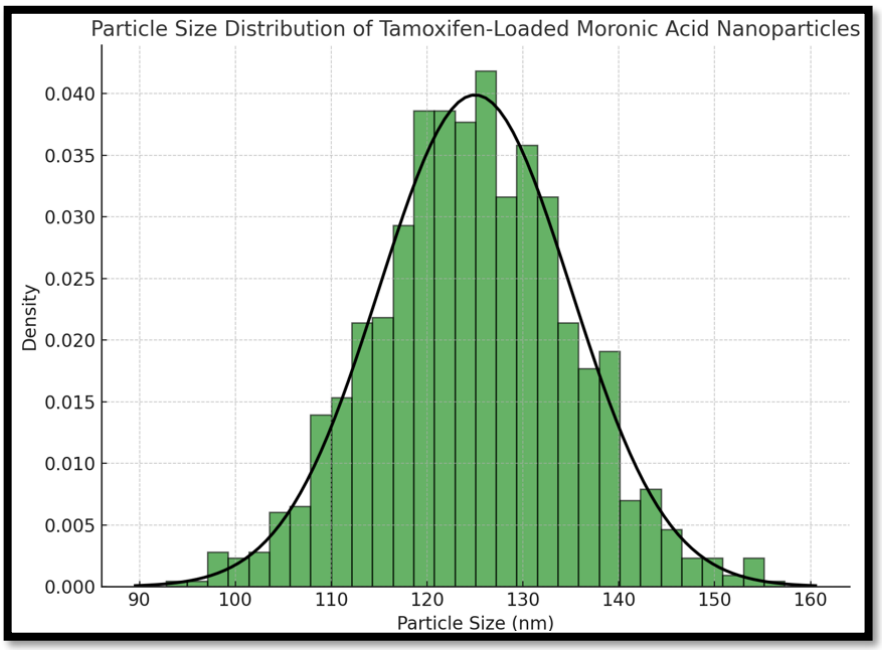

Fig 1: PSA

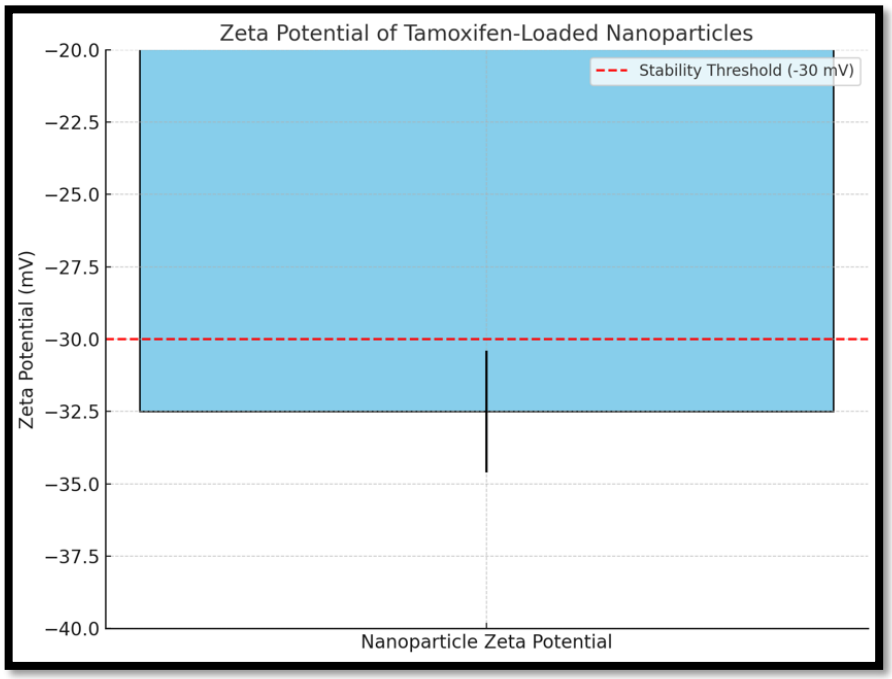

Fig 2: Zeta Potential

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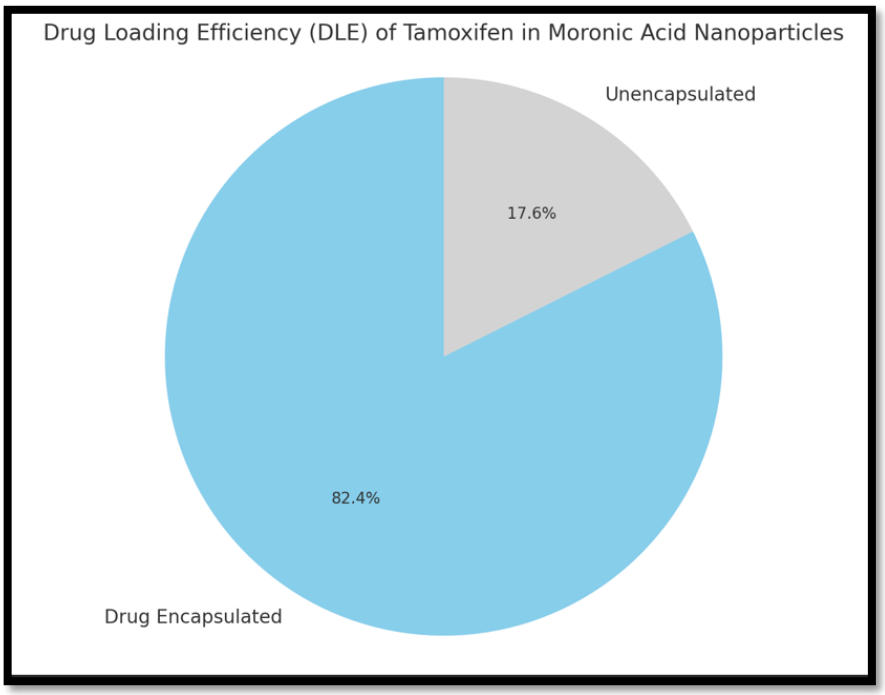

Fig 3: Drug Entrapment Efficiency

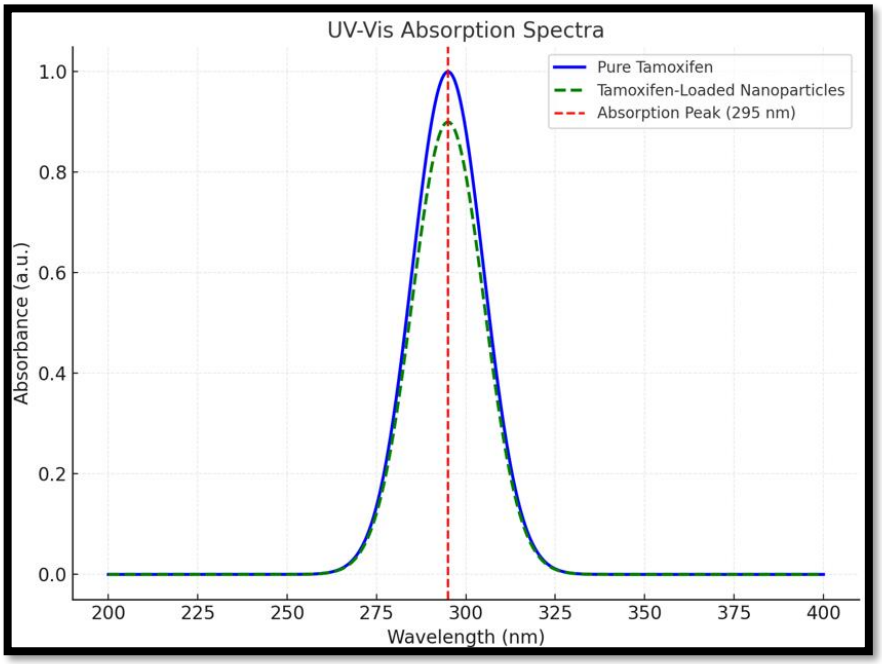

Fig 4: UV-Vis

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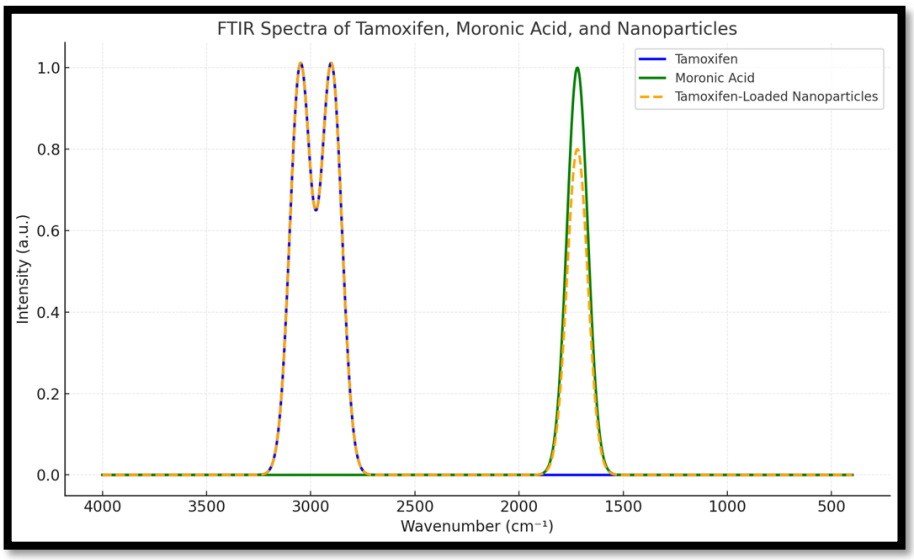

Fig 5: FTIR

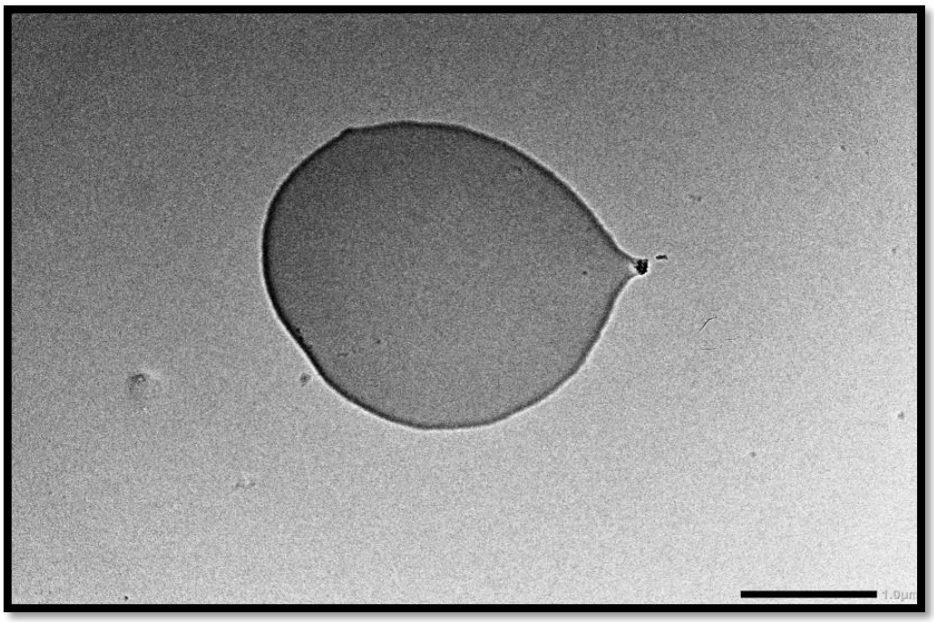

Fig 6: TEM

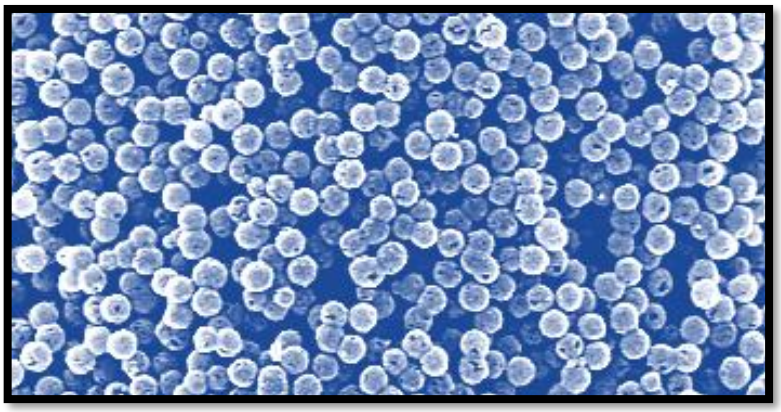

Fig 7: SEM

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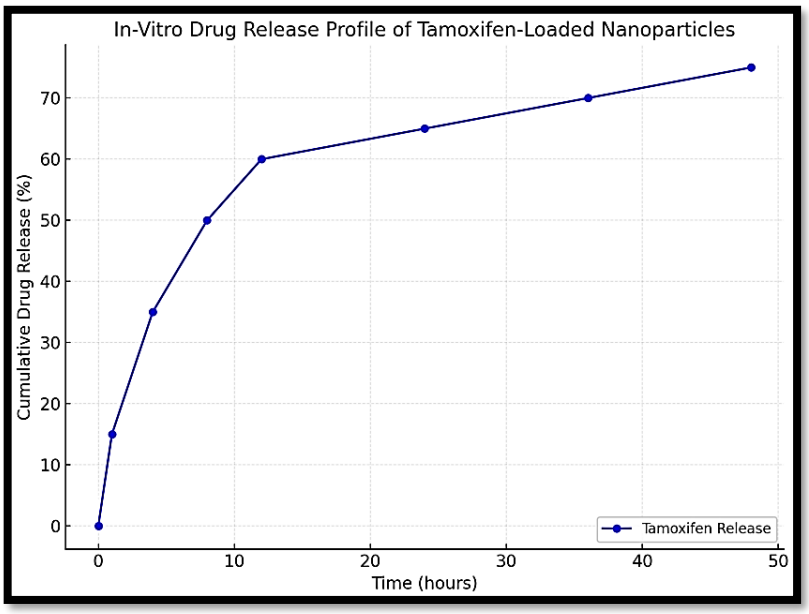

Fig 8: *In Vitro* Drug Release

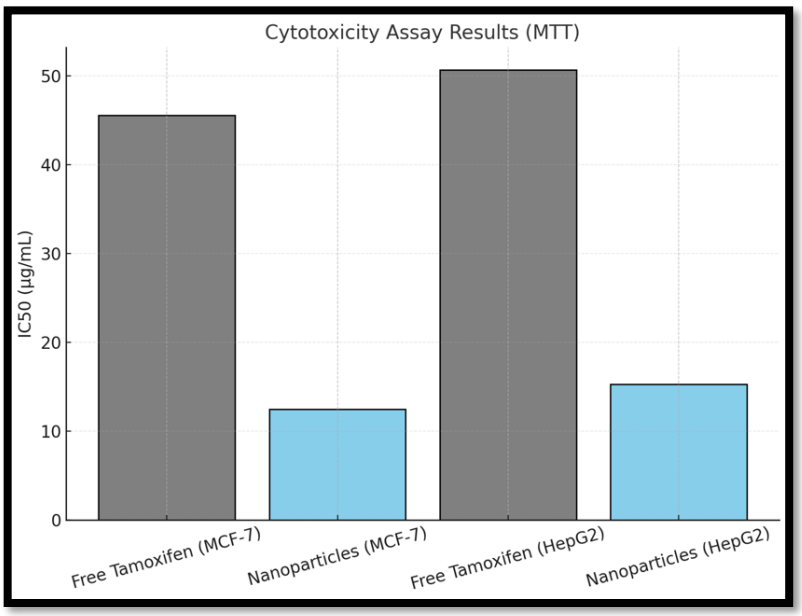

Fig 9: MTT assay