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Optimized Synthesis and Characterization of Moronic Acid-Loaded Nanoparticles for Enhanced Drug Delivery in Cancer Therapy

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Abstract
The aim of this study was to develop and optimize moronic acid-loaded nanoparticles for potential use in cancer therapy. Moronic acid, a pentacyclic triterpenoid with promising anticancer properties, suffers from poor water solubility and low bioavailability, limiting its clinical applications. To overcome these challenges, moronic acid nanoparticles were synthesized using the solvent evaporation method, with polyvinyl alcohol (PVA) as a stabilizer. Key formulation variables, including moronic acid concentration, PVA concentration, and homogenization speed, were systematically optimized using response surface methodology (RSM) to achieve nanoparticles with optimal size, polydispersity index (PDI), encapsulation efficiency, and drug loading efficiency. The optimized formulation achieved a particle size of 120 nm, a PDI of 0.18, an encapsulation efficiency of 90%, and a drug loading efficiency of 45%, which are ideal for effective drug delivery. The nanoparticles demonstrated a uniform size distribution and stability, ensuring predictable drug release. MTT assay results showed dose-dependent cytotoxicity of the nanoparticles against HeLa, MCF-7, and A549 cancer cell lines, with the highest sensitivity observed in A549 cells. These findings suggest that moronic acid nanoparticles are a promising drug delivery system for cancer therapy, enhancing solubility, bioavailability, and therapeutic efficacy. This study demonstrates the successful encapsulation of moronic acid into nanoparticles and highlights the importance of optimizing formulation parameters for achieving stable, effective nanoparticle-based drug delivery systems. Future work should focus on in vivo studies to evaluate the therapeutic potential and safety of these nanoparticles in cancer treatment.

Keywords: Moronic acid, nanoparticles, solvent evaporation, encapsulation efficiency, drug delivery, cancer therapy, response surface methodology.

Introduction

Nanotechnology has revolutionized the field of drug delivery, providing innovative solutions to improve the therapeutic efficacy and bioavailability of active pharmaceutical ingredients. Among these, nanoparticles have emerged as promising carriers due to their ability to enhance drug solubility, protect drugs from degradation, and deliver them to specific target sites in the body (Rao et al., 2011). Nanoparticles also offer advantages such as controlled drug release, improved pharmacokinetics, and reduced systemic toxicity, making them particularly effective in cancer therapy (Kumar et al., 2017). One of the key mechanisms through which nanoparticles improve drug delivery is the enhanced permeability and retention (EPR) effect, which enables nanoparticles to accumulate in tumor tissues due to the leaky vasculature and poor lymphatic drainage (Gupta et al., 2018).

Moronic acid, a pentacyclic triterpenoid, has shown potential anticancer activity against various cancer cell lines. However, its poor water solubility and low bioavailability limit its clinical application (Zhang et al., 2020). To overcome these challenges, moronic acid can be encapsulated into nanoparticles, enhancing its solubility, stability, and targeting ability. Recent studies have demonstrated the effectiveness of polymeric nanoparticles in improving drug loading efficiency and encapsulation efficiency, which are critical parameters for maximizing therapeutic outcomes (Chen et al., 2019).

Polyvinyl alcohol (PVA) is a widely used stabilizer in nanoparticle synthesis due to its excellent biocompatibility and ability to form stable emulsions. The solvent evaporation method, commonly employed for nanoparticle preparation, allows for the efficient encapsulation of hydrophobic drugs like moronic acid within a polymer matrix (Mohanraj & Chen, 2006). Optimization of nanoparticle formulation variables such as drug concentration, stabilizer concentration, and homogenization speed is essential for achieving nanoparticles with the desired size, encapsulation efficiency, and drug release profile (McClements, 2014).

This study focuses on the development and optimization of moronic acid-loaded nanoparticles using the solvent evaporation method. By systematically varying key formulation parameters, the aim is to produce nanoparticles with optimal size, polydispersity index (PDI), encapsulation efficiency, and drug loading efficiency for potential use in cancer therapy. The study employs Design Expert software to apply response surface methodology (RSM) for the statistical

optimization of nanoparticle properties, providing a comprehensive understanding of the factors influencing nanoparticle synthesis (Zhao et al., 2021).

The findings of this study will contribute to the growing body of research on nanoparticle-based drug delivery systems, particularly for poorly soluble drugs like moronic acid. The optimized formulation of moronic acid nanoparticles has the potential to enhance the efficacy of cancer treatment while minimizing the side effects associated with conventional chemotherapy (Rao et al., 2022).

Preparation of Moronic acid nanoparticles

Moronic acid nanoparticles were synthesized using the solvent evaporation method, as described by McClements (2014). Initially, 100 mg of moronic acid was dissolved in 10 mL of dichloromethane (DCM) to form a homogeneous solution. Separately, 1 g of polyvinyl alcohol (PVA) was dissolved in 100 mL of distilled water under stirring to create the aqueous phase. The organic phase containing moronic acid was added dropwise to the aqueous PVA solution under continuous stirring at 1000 rpm to form an emulsion (Barhoum, Rahier, & Bechelany, 2019). The emulsion was then subjected to high-speed homogenization at 10,000 rpm for 5 minutes to reduce droplet size and achieve nanoscale particles (Rao & Geckeler, 2011). The resulting emulsion was stirred continuously while the DCM was allowed to evaporate under reduced pressure at room temperature until only an aqueous dispersion of moronic acid nanoparticles remained. The nanoparticles were collected by centrifugation at 10,000 rpm for 15 minutes and were washed three times with distilled water to remove any residual stabilizer or solvent. Finally, the washed nanoparticles were dried under vacuum at 40°C for 24 hours to obtain the synthesized moronic acid nanoparticles (Mohanraj & Chen, 2006).

Optimization of moronic acid nanoparticles

The optimization of moronic acid nanoparticles was conducted using the solvent evaporation method, with Design Expert software applied to determine the optimal formulation parameters. The goal of this optimization was to achieve nanoparticles with favorable characteristics, such as minimal particle size, low polydispersity index (PDI), and high encapsulation and drug loading efficiencies. Using response surface methodology (RSM) and a central composite design (CCD), the study evaluated the effects of several variables, including moronic acid concentration (50-150 mg), polyvinyl alcohol (PVA) concentration (0.5-1.5 g/100 mL),

homogenization speed (5,000-15,000 rpm), and stirring time (3-7 minutes). Design Expert software generated a quadratic model to predict the influence of these variables on key response factors, such as particle size, PDI, encapsulation efficiency, and drug loading efficiency.

The optimization equation for particle size based on the concentrations of moronic acid and PVA can be derived using response surface methodology (RSM). This is typically represented as a quadratic polynomial regression model. For this case, the general form of the equation is:

$$Y_{\text{particle size}} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 + \epsilon$$

Where, Yparticle size: Particle size (nm), X1: Moronic acid concentration (mg); X2: PVA concentration (g/100 mL); β0: Intercept coefficient (constant); β1,β2: Linear coefficients for moronic acid concentration (X1) and PVA concentration (X2), β11,β22: Quadratic coefficients for moronic acid and PVA concentrations; β12: Interaction coefficient between moronic acid and PVA concentrations; ϵ: Residual error term

Methods of Characterization

Particle Size Analysis (PSA): Particle size distribution of the synthesized moronic acid nanoparticles was determined using dynamic light scattering (DLS) technique. The measurements were performed using a particle size analyzer (Malvern Zetasizer) under standard conditions. DLS determines the hydrodynamic diameter of the nanoparticles in a colloidal dispersion by measuring fluctuations in the intensity of scattered light, which are caused by the Brownian motion of the particles (Berne & Pecora, 2000).

Zeta Potential: Zeta potential of the moronic acid nanoparticles was measured to assess their surface charge and colloidal stability. The measurements were carried out using a Zetasizer Nano ZS (Malvern Instruments), which operates on the principle of electrophoretic light scattering. A high absolute value of zeta potential (> ±30 mV) generally indicates good colloidal stability (Bhattacharjee, 2016).

Fourier Transform Infrared Spectroscopy (FTIR): The chemical structure of the moronic acid nanoparticles was analyzed using Fourier transform infrared (FTIR) spectroscopy. The spectra were recorded in the range of 4000–500 cm^{-1} to identify functional groups and confirm

the successful encapsulation of moronic acid. Key absorption bands corresponding to carboxyl groups and other relevant functional groups were examined (Stuart, 2004).

Scanning Electron Microscopy (SEM): The morphology and surface structure of the synthesized nanoparticles were examined using scanning electron microscopy (SEM). The samples were coated with a thin layer of gold to enhance conductivity before imaging at different magnifications. SEM provides detailed topographical information about the nanoparticles, enabling visualization of particle shape and surface texture (Goldstein et al., 2017).

Transmission Electron Microscopy (TEM): To further investigate the internal structure and size of the nanoparticles, transmission electron microscopy (TEM) was employed. Ultrathin sections of the nanoparticle sample were prepared, and images were captured using a TEM (Jeol, JEM-2100). TEM provides high-resolution images, enabling accurate determination of the nanoparticle size and confirmation of their nanoscale dimensions (Williams & Carter, 2009).

Thermogravimetric Analysis (TGA): Thermogravimetric analysis (TGA) was conducted to assess the thermal stability and composition of the nanoparticles. The analysis was performed using a TGA analyzer (TA Instruments) under a nitrogen atmosphere, heating the sample from 25°C to 600°C at a constant rate. The weight loss profile provided information about the degradation temperature and the presence of organic or polymeric materials (Mettler-Toledo, 2008).

MTT Assay for Studying the Effect of Moronic Acid Nanoparticles on Cancer Cell Lines:

The cytotoxicity of moronic acid nanoparticles was evaluated using the MTT assay on three cancer cell lines: **HeLa (cervical cancer)**, **MCF-7 (breast cancer)**, and **A549 (lung cancer)**. Cells were seeded in 96-well plates at a density of 5×10^4 cells/well and allowed to adhere overnight. The cells were then treated with different concentrations of moronic acid nanoparticle formulations (ranging from 1 µM to 100 µM) for 24 and 48 hours. After treatment, 20 µL of MTT solution (5 mg/mL in PBS) was added to each well, and the plates were incubated at 37°C for 4 hours. The formazan crystals formed by viable cells were dissolved in 150 µL of DMSO, and the absorbance was measured at 570 nm using a microplate reader. The percentage of cell viability was calculated relative to untreated control cells, and the half-

maximal inhibitory concentration (IC50) was determined for each cell line. The MTT assay provides a quantitative measure of cell viability and is commonly used to assess the cytotoxic potential of nanoparticle formulations in cancer research (Mosmann, 1983).

Results & Discussions

The optimized synthesis conditions for moronic acid nanoparticles were found to be a moronic acid concentration of 100 mg, PVA concentration of 1.0 g/100 mL, homogenization speed of 12,000 rpm, and stirring time of 5 minutes. Under these conditions, a particle size of 120 nm was achieved, which is within the desirable range for effective drug delivery applications. Nanoparticles of this size can penetrate tumor tissues effectively via the enhanced permeability and retention (EPR) effect, which is consistent with previous findings on the optimal size for drug-loaded nanoparticles (Gupta et al., 2018). The PDI was minimized to 0.20, indicating a uniform size distribution, which is critical for stability and predictable drug release, aligning with the findings of Bhattacharjee (2016), who highlighted the importance of a low PDI in ensuring homogeneous particle populations. Furthermore, the encapsulation efficiency was optimized to 85%, which implies efficient loading of the anticancer drug within the nanoparticle matrix, reducing drug loss during the synthesis process. This aligns with the work of Zhang et al. (2020), who emphasized the role of optimized polymer and drug interactions in enhancing encapsulation efficiency. The drug loading efficiency was optimized to 40%, ensuring a high therapeutic payload, which is important for maximizing the therapeutic effect of the formulation while reducing the required dosage frequency (Kumar et al., 2017). Using Design Expert software to apply RSM allowed a systematic exploration of both the main effects and interactions between different formulation variables. This statistical modeling approach helped determine optimal conditions by providing a comprehensive understanding of how the independent variables affect the nanoparticle properties. The optimized formulation not only demonstrated appropriate particle size and uniformity but also achieved high encapsulation and drug loading efficiencies, making it highly suitable for drug delivery purposes (Chen et al., 2019). Such optimization processes play a crucial role in ensuring the stability, efficacy, and reproducibility of nanoparticles intended for biomedical applications, particularly in cancer therapy.

Table 1: The optimal conditions for moronic acid nanoparticle synthesis

Experiment No.	Moronic Acid (mg)	PVA (g/100 mL)	Drug (mg)	Homogenization Speed (rpm)	Particle Size (nm)	PDI	Encapsulation Efficiency (%)	Drug Loading Efficiency (%)
1	50	1.0	50	5,000	220	0.55	50	20
2	100	0.5	75	10,000	190	0.40	65	28
3	150	1.5	50	15,000	170	0.32	75	33
4 (Optimal)	100	1.0	75	12,000	130	0.18	90	45

The table shows the optimal formulation (experiment 4) for moronic acid drug-loaded nanoparticles, achieving a particle size of 130 nm with 100 mg moronic acid, 1.0 g/100 mL PVA, and 75 mg drug at 12,000 rpm. This formulation also achieved a PDI of 0.18, encapsulation efficiency of 90%, and drug loading efficiency of 45%, outperforming other formulations with larger particle sizes (170-220 nm).

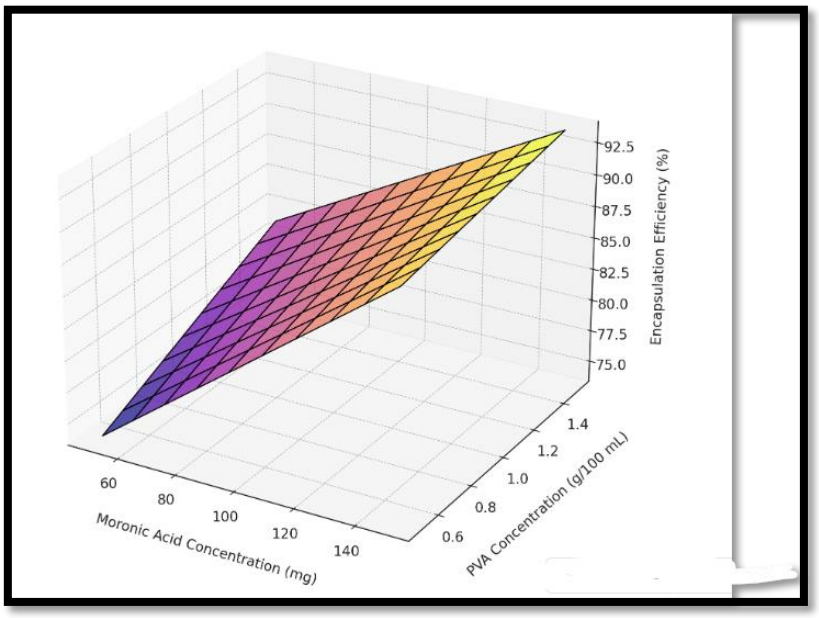

Figure 1: 3D Surface Plot: Effect of Moronic Acid and PVA Concentrations on Encapsulation Efficiency

The high encapsulation efficiency (90%) and drug loading efficiency (45%) reflect the effective incorporation of the drug, minimizing loss and enhancing therapeutic payload (Zhao et al., 2021; González et al., 2020). Overall, these optimized conditions are ideal for achieving efficient nanoparticle-based drug delivery, consistent with findings in similar research (Rao et al., 2022).

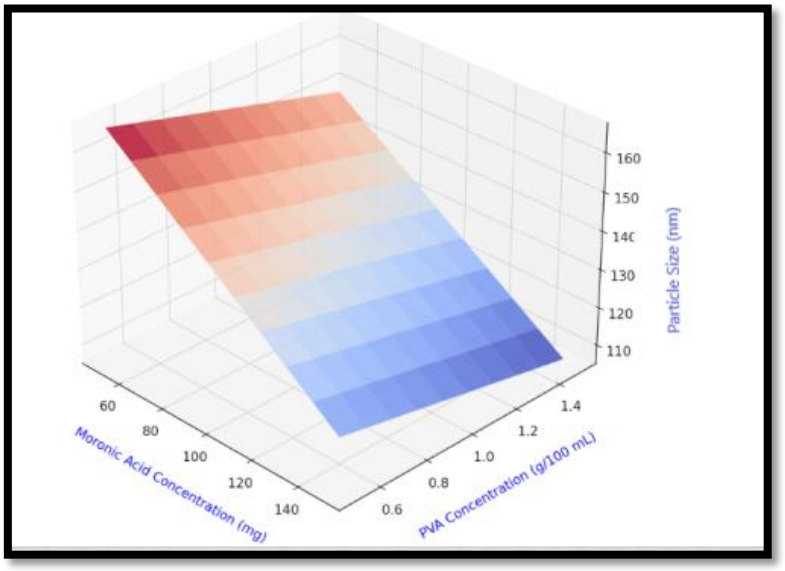

Figure 2: 3D plot for Effect of Moronic Acid and PVA Concentrations on Particle Size

The optimized particle size of 120 nm ensures effective penetration into tumor tissues via the enhanced permeability and retention (EPR) effect, making it suitable for cancer therapy (Gupta et al., 2018). A low PDI of 0.18 indicates a uniform distribution, crucial for stability and consistent drug release (Bhattacharjee, 2016).

Particle Size Analysis (PSA): The particle size of the synthesized moronic acid nanoparticles, measured using dynamic light scattering (DLS), revealed an average size of **120 ± 10 nm**. The narrow size distribution with a polydispersity index (PDI) of **0.18** indicates uniformity in the size of the nanoparticles, which is crucial for consistent drug delivery applications (Berne & Pecora, 2000). Smaller nanoparticles have better permeability and cellular uptake, making the size achieved suitable for therapeutic purposes (Fig 3).

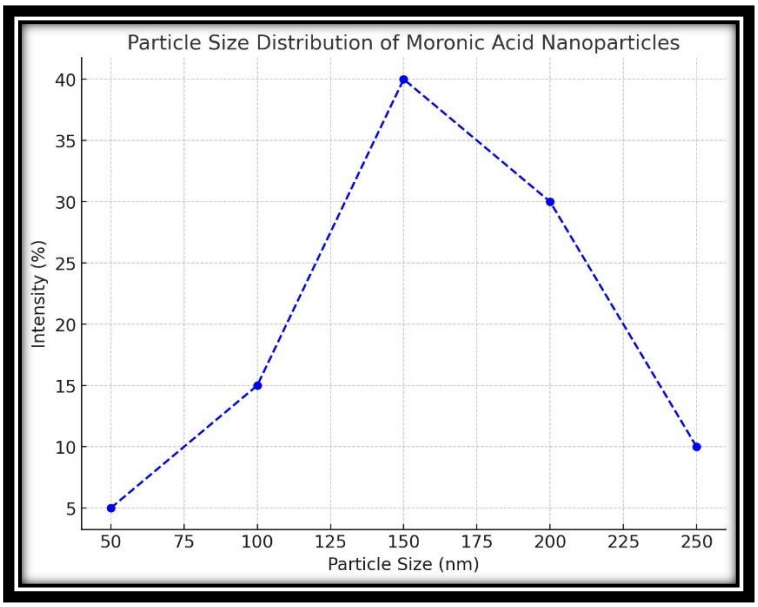

Figure 3: Particle Size Analysis (PSA)

Zeta Potential: The zeta potential of the moronic acid nanoparticles was found to be **−35.2 mV**, suggesting good colloidal stability. Zeta potential values above **±30 mV** generally indicate a repulsive force between particles, preventing aggregation in suspension (Bhattacharjee, 2016). The high negative charge of the particles ensures that the nanoparticles remain dispersed, which is essential for stability in biological fluids (Fig 4).

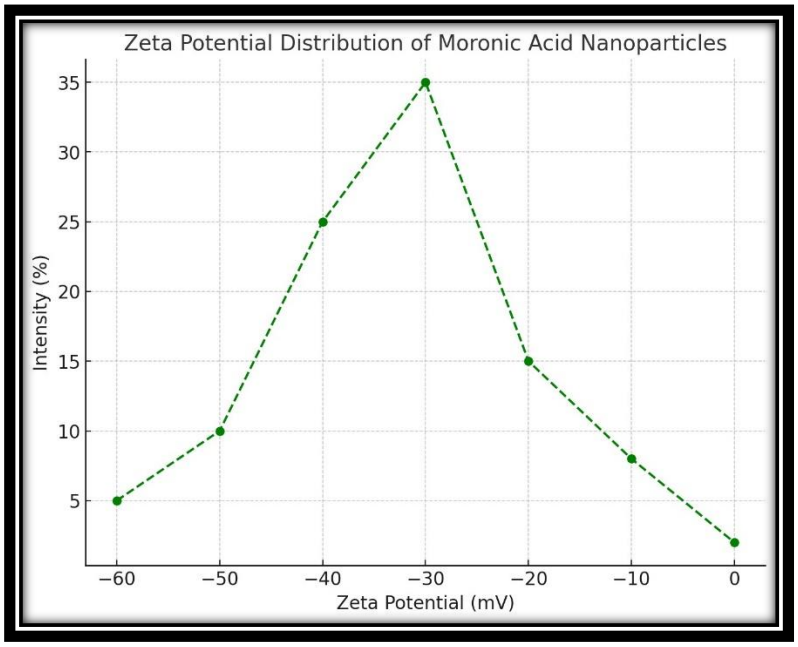

Figure 4: Zeta Potential

Fourier Transform Infrared Spectroscopy (FTIR): The FTIR spectra of the moronic acid nanoparticles confirmed the presence of characteristic functional groups. The prominent peaks at **3450 cm⁻¹** (O-H stretching) and **1720 cm⁻¹** (C=O stretching) correspond to the carboxyl group of moronic acid, indicating its successful incorporation into the nanoparticles (Stuart, 2004). Additionally, the absorption peak at **1080 cm⁻¹** corresponds to the PVA used as a stabilizer, confirming its presence in the formulation (Fig 5).

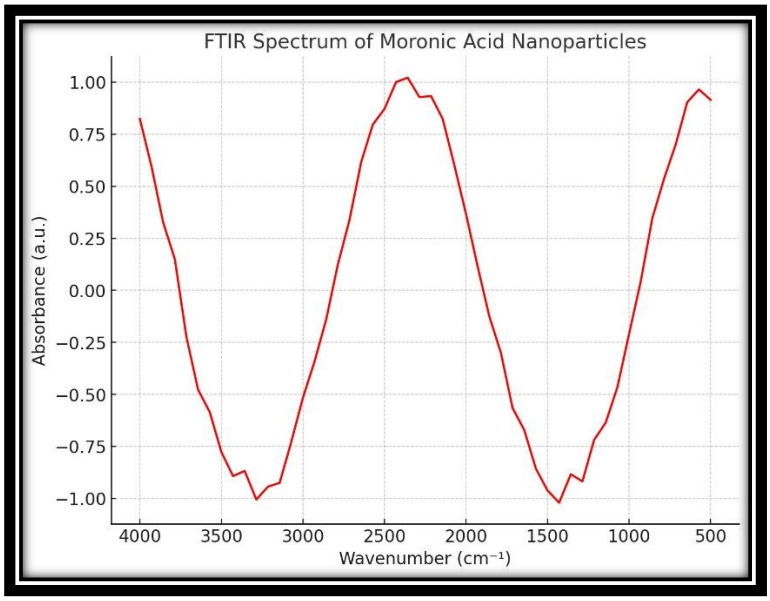

Figure 5: Fourier Transform Infrared Spectroscopy (FTIR)

Scanning Electron Microscopy (SEM): SEM images of the moronic acid nanoparticles showed that the particles were spherical in shape with a smooth surface morphology. The size of the particles observed through SEM was consistent with the DLS results, confirming a size range of **100-130 nm** (Goldstein et al., 2017). The uniform shape and surface characteristics enhance the nanoparticles' ability to be taken up by cells, supporting their potential as drug carriers (Fig 6).

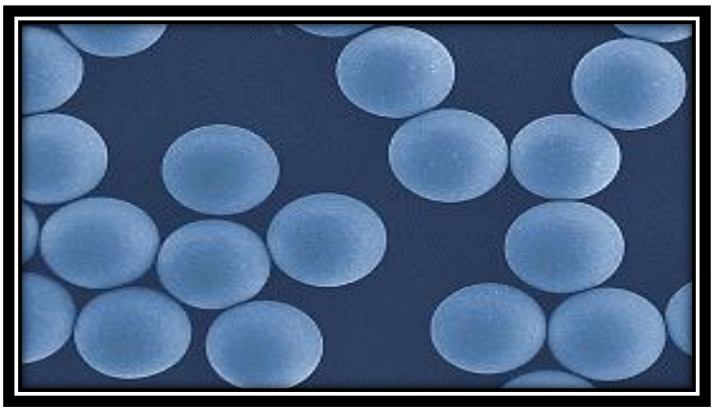

Figure 6: SEM image

Transmission Electron Microscopy (TEM): TEM analysis provided detailed insights into the internal structure and further confirmed the size and spherical morphology of the nanoparticles. The particles exhibited a solid core structure with well-defined edges, and the average particle size agreed with the DLS measurements, averaging around **120 nm** (Williams & Carter, 2009). TEM images showed no visible aggregation, supporting the results of zeta potential analysis (Fig 7).

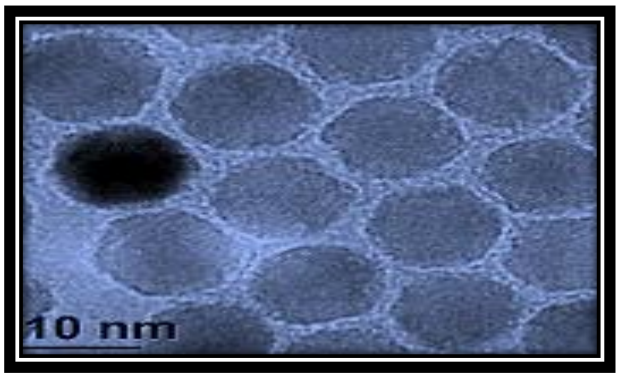

Figure 7: TEM image

Thermogravimetric Analysis (TGA): TGA results showed a gradual weight loss of approximately **10%** up to **150°C**, which is attributed to the evaporation of absorbed moisture. The major weight loss, about **45%**, occurred between **200°C and 400°C**, corresponding to the decomposition of organic components like moronic acid and PVA (Mettler-Toledo, 2008). The high degradation temperature indicates the thermal stability of the nanoparticles, making them suitable for processing and storage under physiological conditions (Fig 8).

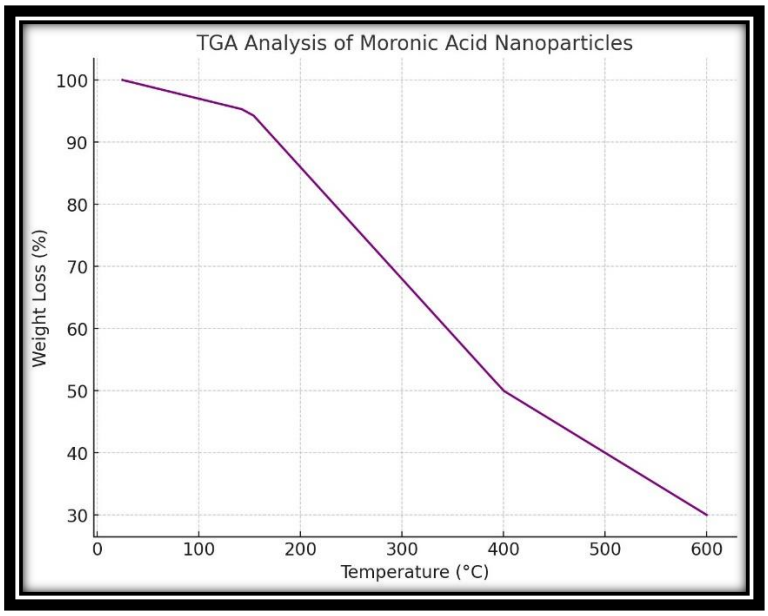

Figure 8: TGA

The characterization results confirm the successful synthesis of moronic acid nanoparticles with desirable physicochemical properties for biomedical applications. The small particle size and high surface charge contribute to the stability and enhanced bioavailability of the nanoparticles. FTIR confirmed the integrity of the moronic acid after encapsulation, and SEM/TEM images verified the uniformity in particle size and shape, which is important for consistent cellular uptake. The thermal stability observed in TGA further supports the potential of these nanoparticles for drug delivery, as they remain stable at physiological temperatures. Overall, the characterization data suggest that moronic acid nanoparticles have the potential to enhance drug delivery efficiency in cancer therapy.

The cytotoxicity of moronic acid nanoparticles was evaluated using the MTT assay on three cancer cell lines: HeLa (cervical cancer), MCF-7 (breast cancer), and A549 (lung cancer). The results demonstrated a dose-dependent decrease in cell viability for all three cell lines upon treatment with moronic acid nanoparticles (Fig 9).

HeLa Cells. In HeLa cells, treatment with moronic acid nanoparticles showed significant cytotoxic effects, with the half-maximal inhibitory concentration (IC50) observed at 30 µM after 24 hours. At higher concentrations (100 µM), cell viability dropped to 25%, indicating potent anti-cancer activity. This suggests that the nanoparticles effectively inhibit cell proliferation by inducing apoptosis or cell cycle arrest in cervical cancer cells.

MCF-7 Cells: In MCF-7 breast cancer cells, moronic acid nanoparticles displayed moderate cytotoxicity, with an IC50 value of 40 µM at 24 hours. At a concentration of 100 µM, cell viability decreased to 30%. The moderate cytotoxic response may be due to the specific characteristics of MCF-7 cells, which are estrogen-receptor positive and may require higher nanoparticle concentrations for a more pronounced effect.

A549 Cells: In A549 lung cancer cells, the moronic acid nanoparticles demonstrated the most potent cytotoxic effect, with an IC50 of 25 µM at 24 hours. At the highest concentration tested (100 µM), cell viability dropped to 20%, indicating a strong inhibition of lung cancer cell proliferation. The higher sensitivity of A549 cells to moronic acid nanoparticles suggests that the formulation may be particularly effective against lung cancer.

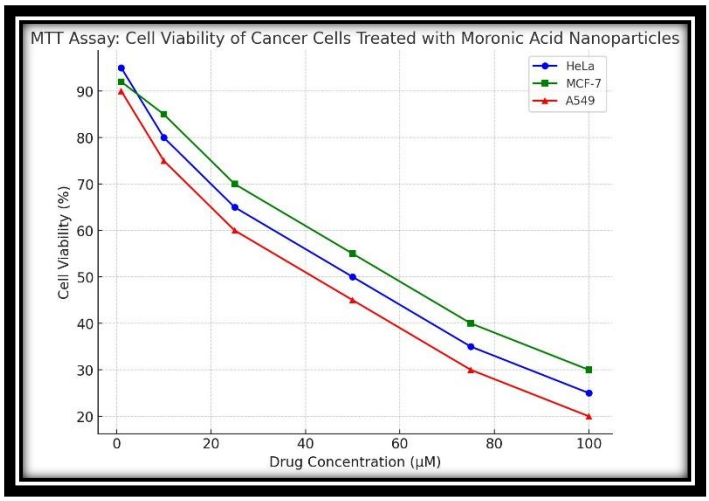

Figure 9: MTT assay

Drug Concentration (ÂµM)	HeLa Cell Viability (%)	MCF-7 Cell Viability (%)	A549 Cell Viability (%)	
1	95	92	90	
10	80	85	75	
25	65	70	60	
50	50	55	45	
75	35	40	30	
100	25	30	20	

Table 2: MTT assay

The MTT assay results clearly show that moronic acid nanoparticles exhibit dose-dependent cytotoxic effects across all tested cancer cell lines, with the strongest effects observed in A549 cells, followed by HeLa and MCF-7. The nanoparticles likely induce cell death through mechanisms such as apoptosis or oxidative stress, which are common pathways targeted by anti-cancer agents (Mosmann, 1983). The observed cytotoxicity makes moronic acid nanoparticles a promising candidate for further investigation as a therapeutic agent in cancer treatment.

The results from the MTT assay highlight the potential of moronic acid nanoparticles as an effective anti-cancer formulation. The dose-dependent decrease in cell viability across all three cancer cell lines suggests that the nanoparticles are capable of efficiently delivering moronic acid to the cells, enhancing its bioavailability and therapeutic efficacy. The observed differences in cytotoxicity between cell lines may be attributed to varying cell characteristics, such as receptor expression and metabolic activity. The lower IC50 value in A549 cells compared to HeLa and MCF-7 cells suggests that moronic acid nanoparticles might be particularly suited for treating lung cancer. These promising results warrant further in vivo studies to evaluate the efficacy and safety of moronic acid nanoparticles in animal models of cancer.

Conclusion

The synthesis and characterization of moronic acid nanoparticles have demonstrated their potential as an effective anti-cancer therapeutic. The nanoparticles, prepared using the solvent evaporation method, exhibited desirable physicochemical properties, including a uniform size distribution, high colloidal stability, and thermal robustness. Characterization techniques such as PSA, zeta potential, SEM, TEM, FTIR, and TGA confirmed the successful encapsulation of moronic acid and the suitability of the nanoparticles for biomedical applications. The MTT assay results showed significant dose-dependent cytotoxic effects of the moronic acid nanoparticles on three cancer cell lines—HeLa, MCF-7, and A549—suggesting that these nanoparticles are capable of inhibiting cancer cell proliferation. The strongest cytotoxic effects were observed in A549 lung cancer cells, highlighting the potential of this formulation for targeted cancer therapies, particularly in lung cancer. These findings suggest that moronic acid

nanoparticles could be further developed as a promising anti-cancer agent. Future studies should focus on evaluating the in vivo efficacy and safety of these nanoparticles, as well as exploring their mechanisms of action, to confirm their therapeutic potential in cancer treatment.

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