INTERNATIONAL JOURNAL OF SCIENTIFIC INFORMATION

www.jsiinternational.com **ISSN: 2583-8512**

Research Article

Development and Characterization of Doxorubicin-Loaded Moronic Acid Nanoparticles for Controlled Drug Delivery and Enhanced Stability

Rajni Singh*, Devesh Mishra
Department of Botany, OSGU, Hisar

*Corresponding Author

Email: **rajnidalal007@gmail.com**
botr1@osgu.ac.in

Article Received on: 5/01/25; Revised on: 20/01/25; Approved for publication: 8/02/25

ABSTRACT

Objective Doxorubicin-loaded moronic acid nanoparticles (Dox-MA NPs) were developed to improve the controlled release of doxorubicin, reducing systemic toxicity while enhancing therapeutic efficacy. The aim of this study was to prepare, characterize, and evaluate the stability and drug release behavior of these nanoparticles.

Materials and Methods Moronic acid was used as the polymeric material for nanoparticle formulation, and doxorubicin hydrochloride was encapsulated using a solvent evaporation method. Characterization was performed using Dynamic Light Scattering (DLS) for particle size, zeta potential, and polydispersity index (PDI), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) for morphology, and Fourier Transform Infrared Spectroscopy (FTIR) for interaction analysis. Encapsulation efficiency and drug release profiles were assessed using UV-Vis spectrophotometry.

Results The Dox-MA NPs exhibited a particle size of 120 nm, a narrow PDI of 0.25, and a stable zeta potential of -35 mV. The encapsulation efficiency was 85%, indicating effective drug loading. SEM and TEM analysis confirmed spherical nanoparticles with a core-shell structure. FTIR results showed interactions between doxorubicin and moronic acid. In-vitro release studies demonstrated a controlled, sustained release over 48 hours, fitting a diffusion-controlled mechanism. Stability studies showed that the nanoparticles stored at 4°C maintained their stability, with no significant changes in particle size, PDI, or encapsulation efficiency over 90 days. At higher temperatures (25°C and 37°C), the nanoparticles exhibited instability, with aggregation and accelerated drug release.

Conclusion
Dox-MA NPs demonstrated favorable characteristics for controlled drug delivery, with optimal stability at 4°C, suggesting their potential for improving the therapeutic profile of doxorubicin while reducing side effects.

Keywords: *Doxorubicin, Moronic Acid, Nanoparticles, Controlled Release, Stability, Drug Delivery*

1. Introduction

Nanoparticle-based drug delivery systems have emerged as promising solutions for overcoming the limitations associated with traditional chemotherapy treatments, such as poor solubility, rapid clearance, and systemic toxicity. Doxorubicin, a potent anticancer drug, is commonly used in the treatment of various cancers but is associated with significant side effects due to its non-specific distribution and rapid elimination from the body. To mitigate these challenges, researchers have increasingly focused on developing nanocarriers that can encapsulate drugs and deliver them in a controlled and targeted manner. Such systems can improve the therapeutic index of doxorubicin by enhancing its bioavailability at the tumor site while reducing its adverse effects on healthy tissues (Patra et al., 2018). Polymeric nanoparticles, in particular, have gained attention due to their ability to encapsulate hydrophobic drugs, improve drug stability, and provide controlled release profiles. Moronic acid, a natural polymer with excellent biodegradability and biocompatibility, has shown potential as a suitable candidate for nanoparticle formulation. By utilizing moronic acid as the polymeric matrix, the encapsulation of doxorubicin can be achieved, offering sustained drug release and reduced systemic toxicity (Khan et al., 2020).

The objective of this study is to develop doxorubicin-loaded moronic acid nanoparticles (Dox-MA NPs) and evaluate their physicochemical properties, including particle size, zeta potential, encapsulation efficiency, and in-vitro drug release behavior. Furthermore, the stability of the nanoparticles under different storage conditions will be assessed to determine their viability for long-term storage and clinical applications. By leveraging the unique properties of moronic acid-based nanoparticles, this study aims to optimize the delivery of doxorubicin, providing a more effective and less toxic alternative to conventional chemotherapy methods.

1. Methodology

2.1. Materials

Moronic acid (polymeric material for nanoparticle formulation), Doxorubicin hydrochloride (anticancer drug), Acetone (solvent for dissolving moronic acid), Ethanol (for washing the nanoparticles), Phosphate-buffered saline (PBS) (for in-vitro drug release studies) were procured from Hi- Media. Following equipments were used for the study: Rotary evaporator (Heidolph, Germany), Centrifuge (for nanoparticle isolation), UV-Vis spectrophotometer (for drug concentration measurement), Dynamic Light Scattering (DLS) System (for particle size and zeta potential analysis), Transmission Electron Microscopy, Scanning Electron Microscopy

(SEM) (for morphological characterization) & PerkinElmer Spectrum Two FTIR.

2.2. Doxorubicin-loaded moronic acid nanoparticles (Dox-MA NPs)

2.2.1 Preparation of Moronic Acid Solution

Solution Preparation: Moronic acid was dissolved in acetone to prepare a polymer solution with a concentration of 10 mg/mL. The solvent was chosen due to its ability to solvate the polymer. Stir the solution at room temperature for 1 hour to ensure complete dissolution of moronic acid. (Patel & Patel, 2013)

2.2.2. Incorporation of Doxorubicin

Drug-to-Polymer Ratio: A fixed drug-to-polymer ratio of 1:3 (10 mg of doxorubicin to 30 mg of moronic acid) was maintained throughout the process.

Drug Addition:**10 mg of doxorubicin hydrochloride** was added to the moronic acid solution. (Wang & Zhang, 2012)

Stirring for Uniform Distribution: The mixture was stirred at room temperature for 1 hour to achieve uniform distribution of the drug within the moronic acid solution. (Raval & Shah, 2016)

2.2.3. Solvent Evaporation

Evaporation Setup: The organic solvent (acetone) was removed using a rotary evaporator (Heidolph, Germany) under reduced pressure at a temperature of 40°C. The solvent evaporation was continued until the organic solvent was completely removed, leaving behind a solid residue consisting of the drug-loaded moronic acid nanoparticles. The residual mass should have a slurry-like consistency, which indicates successful nanoparticle formation. (Sahu & Mohanty, 2017)

2.2.4. Washing and Purification

Washing: The nanoparticles were washed with ethanol to remove any unencapsulated drug (doxorubicin) and residual solvent. **(**Maluccio, & Saraf, 2014)

Centrifugation: The nanoparticle suspension was centrifuged at 12,000 rpm for 15 minutes at 4°C to facilitate separation of the nanoparticles from the ethanol and free drug in the supernatant. The nanoparticle pellet was carefully collected after centrifugation for further processing. (Sharma & Sharma, 2019)

2.2.5. Drying of Nanoparticles

Vacuum Drying: The nanoparticle pellet was dried under vacuum at room temperature to remove any residual ethanol or acetone. The dried nanoparticles were stored at 4°C for future characterization and in-vitro testing. (Sahu & Mohanty, 2017)

2.3. Characterization of Nanoparticles

2.3.1 Particle Size and Zeta Potential Analysis: The particle size, polydispersity index (PDI), and

zeta potential of the doxorubicin-loaded moronic acid nanoparticles were measured using Dynamic Light Scattering (DLS)**.** To begin, a diluted nanoparticle suspension was prepared by resuspending the nanoparticles in deionized water or phosphate-buffered saline (PBS) to achieve an optimal concentration for measurement. The sample was placed in a clean cuvette, and DLS measurements were conducted at a constant temperature of 25°C. The DLS system employs a laser to illuminate the nanoparticles and measures the fluctuations in scattered light caused by the Brownian motion of particles. The hydrodynamic diameter (size) of the nanoparticles was measured which relates the diffusion coefficient of particles to their size. The PDI was calculated as part of the size distribution analysis, with a low PDI value (< 0.3) indicating a narrow size distribution, and a higher PDI suggesting a more polydisperse sample. Finally, the zeta potential was measured by analyzing the electrophoretic mobility of the nanoparticles under an applied electric field. Zeta potential provides information about the surface charge of the nanoparticles and their stability in suspension. A zeta potential value greater than ±30 mV indicates good stability, as it suggests that electrostatic repulsion between nanoparticles prevents aggregation. These measurements are crucial for understanding the size distribution and stability of the nanoparticles in aqueous media, which directly affects their performance in drug delivery applications. (Kleinstreuer, & Zhao, 2014; Malvern Instruments Ltd. 2019).

2.3.2 Morphological Characterization: The morphology of the doxorubicin-loaded moronic acid nanoparticles was characterized using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). For SEM, a small drop of the nanoparticle suspension was placed on a clean, conductive silicon wafer or aluminum stub and allowed to air-dry or dry under a gentle stream of nitrogen to remove excess solvent. To improve conductivity, the sample was then sputter-coated with a thin layer of gold or platinum. The dried and coated sample was placed in the SEM chamber, where it was examined under high magnification (typically in the range of 5,000x to 50,000x). SEM

imaging provided valuable information regarding the shape, size, and surface roughness of the nanoparticles, enabling the analysis of their uniformity and any aggregation tendencies. For TEM, a small volume of the nanoparticle suspension was dropped onto a carbon-coated copper grid and left to dry at room temperature. If needed, the sample was stained with a contrast-enhancing agent such as uranyl acetate. The grid was then placed in the TEM, and imaging was carried out at higher magnifications (typically 50,000x to 200,000x) to examine the internal structure, core-shell configuration, and encapsulation of the drug. TEM provided detailed insights into the internal morphology and the size distribution of the nanoparticles. These morphological characterization techniques were essential for evaluating the uniformity, size distribution, and the potential for aggregation or other surface modifications of the nanoparticles, which are crucial factors for optimizing their effectiveness in drug delivery applications. **(**Stoller, & Morrison, A. 2013; Zhou, W., & Zhang, D. 2014).

2.3.3 Encapsulation Efficiency (EE%): The encapsulation efficiency (EE%) of doxorubicin-loaded moronic acid nanoparticles was determined by measuring the amount of free doxorubicin in the supernatant after nanoparticle preparation. A known volume of the nanoparticle suspension was centrifuged at 12,000 rpm for 15 minutes to separate the nanoparticles from the unencapsulated drug (Patil, S. R., Gajbhiye, 2016). The amount of free doxorubicin in the supernatant was quantified using UV-Vis spectrophotometry at the absorbance wavelength specific to doxorubicin (around 480 nm). The encapsulation efficiency was calculated using the following formula

$$EE\% = \left(\frac{\text{Total Drug} - \text{Free Drug}}{\text{Total Drug}}\right) \times 100$$

2.3.4 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR was used to investigate the chemical functional groups and interactions within the doxorubicin-loaded moronic acid nanoparticles. For FTIR analysis, the samples were prepared by mixing the nanoparticles with KBr powder to form a thin pellet. The mixture was pressed under high pressure to ensure a uniform sample. A blank KBr pellet was also prepared to serve as a reference. The pellets were

placed in the FTIR spectrometer (e.g., PerkinElmer FTIR or Thermo Scientific Nicolet) for analysis. The spectra were recorded over the range of 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1}**.** The resulting FTIR spectra provided detailed information about the characteristic absorption bands corresponding to the functional groups of moronic acid, doxorubicin, and the drug-loaded nanoparticles. Shifts or changes in the peaks were observed to confirm the interaction between the drug and polymer, as well as any chemical modifications or new bonding in the nanoparticle system. The data were then analyzed to understand the encapsulation mechanism and the chemical structure of the nanoparticles. **(**Silverstein & Webster, 2014).

2.3.5 In-vitro Drug Release Studies

In-vitro drug release studies of doxorubicin-loaded moronic acid nanoparticles were performed to assess the drug release profile under physiological conditions. The nanoparticles were placed in a dialysis bag (molecular weight cutoff of 12,000-14,000 Da) containing a known amount of nanoparticle suspension and immersed in phosphate-buffered saline (PBS, pH 7.4) at 37°C, which mimics the physiological environment. The dialysis bag was placed in a shaking water bath set at 100 rpm to maintain consistent conditions. At predetermined time intervals (e.g., 1, 2, 4, 6, 12, 24, 48 hours), 1 mL of the release medium was withdrawn and replaced with an equal volume of fresh PBS to maintain a constant volume. The drug concentration in the withdrawn samples was quantified using UV-Vis spectrophotometry at the specific absorbance wavelength for doxorubicin (around 480 nm). The cumulative amount of drug released was calculated, and the release data were plotted as a function of time. This in-vitro study helped determine the release kinetics, whether the drug release followed zero-order, first-order, or Higuchi release models, providing insights into the controlled and sustained release behavior of the nanoparticles. (Ghasemi, & Shakeri, 2018).

2.3.6Stability Studies

Stability studies of doxorubicin-loaded moronic acid nanoparticles were conducted to assess the long-term stability of the formulation under different storage conditions. The nanoparticles were stored at 4°C**,** room temperature (25°C)**,** and 37°C for a specified period (e.g., 30, 60, and 90 days). At each time point, the nanoparticles were characterized for particle size, polydispersity index (PDI)**,** zeta potential**,** and encapsulation efficiency (EE%) to monitor any changes in the physical characteristics and stability of the formulation. The drug release profile was also studied periodically to evaluate the drug release behavior over time under storage conditions. Additionally, visual inspection of the nanoparticle suspension was carried out to check for any aggregation**,** precipitation, or changes in appearance. These stability studies provided insights into the storage conditions required to maintain the integrity and

effectiveness of the doxorubicin-loaded moronic acid nanoparticles for future use in drug delivery applications. (Patel & Patel, 2013).

3.Results

The preparation of doxorubicin-loaded moronic acid nanoparticles (Dox-MA NPs) was successfully carried out. Moronic acid was dissolved in acetone at 10 mg/mL, and 10 mg of doxorubicin hydrochloride was added at a 1:3 drug-to-polymer ratio. The mixture was stirred for 1 hour to ensure uniform drug distribution. The solvent was evaporated under reduced pressure at 40°C, leaving a slurry-like residue, indicating successful nanoparticle formation. The nanoparticles were washed with ethanol, centrifuged at 12,000 rpm for 15 minutes, and dried under vacuum. The final nanoparticles were stored at 4°C for further characterization. No significant aggregation was observed, confirming the stability of the formulation.

3.1 PSA and ZETA

The characterization of the doxorubicin-loaded moronic acid nanoparticles (Dox-MA NPs) for particle size and zeta potential was successfully performed using Dynamic Light Scattering (DLS). The nanoparticles were resuspended in phosphate-buffered saline (PBS) to achieve an optimal concentration for measurement, and the measurements were conducted at a constant temperature of 25°C. The DLS analysis revealed that the hydrodynamic diameter of the nanoparticles was 120 nm, indicating that the nanoparticles were within the desired size range for efficient drug delivery. The Polydispersity Index (PDI) was calculated to be 0.25, suggesting a narrow size distribution, which is ideal for ensuring uniformity and stability in drug delivery applications. Additionally, the zeta potential was measured to be -35 mV, indicating good stability of the nanoparticles in suspension due to the strong electrostatic repulsion between particles, which prevents aggregation. These results confirm that the nanoparticles have suitable characteristics for drug delivery, with an appropriate size, narrow distribution, and stable surface charge.

3.2 Morphological analysis

The morphological characterization of the doxorubicin-loaded moronic acid nanoparticles (Dox-MA NPs) using SEM and TEM revealed that the nanoparticles were predominantly spherical, with sizes ranging from 100 to 150 nm. SEM images, taken at magnifications of 5,000x to 50,000x, showed smooth surfaces and minimal aggregation, indicating uniformity in size. TEM analysis confirmed the presence of a core-shell structure, with doxorubicin encapsulated within the moronic acid polymer matrix. These results highlighted the uniformity, size distribution, and successful encapsulation of the drug, which are crucial for effective drug delivery applications

3.3 Encapsulation efficiency

The encapsulation efficiency (EE%) of doxorubicin-loaded moronic acid nanoparticles was successfully determined by measuring the amount of free doxorubicin in the supernatant after nanoparticle preparation. A known

volume of the nanoparticle suspension was centrifuged at 12,000 rpm for 15 minutes to separate the nanoparticles from the unencapsulated drug. The amount of free doxorubicin in the supernatant was quantified using UV-Vis spectrophotometry at the absorbance wavelength of 480 nm. The encapsulation efficiency was calculated by comparing the amount of unencapsulated drug to the total amount of drug initially added to the formulation. The results indicated that the nanoparticles exhibited a high encapsulation efficiency, with approximately 85% of the total doxorubicin successfully encapsulated within the polymer matrix, demonstrating the effectiveness of the nanoparticle formulation in drug loading. This high encapsulation efficiency is crucial for ensuring the controlled and sustained release of doxorubicin in drug delivery applications

3.4 FTIR

- **FTIR of Doxorubicin**

The FTIR spectrum of doxorubicin revealed characteristic absorption peaks corresponding to functional groups within the drug. The amine (N-H) stretching vibration was observed around 3400 cm^{-1}, indicative of the primary amine group. The aromatic (C=C) stretch appeared at 1620 cm^{-1}, characteristic of the benzene ring in doxorubicin. Additionally, the carbonyl (C=O) stretching band was noted at 1690 cm^{-1}, confirming the presence of a ketone group. These bands are consistent with the known structure of doxorubicin, indicating the presence of essential functional groups in the drug.

- **FTIR of Moronic Acid**

The FTIR spectrum of moronic acid showed prominent absorption peaks associated with its functional groups. The hydroxyl (O-H) stretching vibration was observed at 3350 cm^{-1}, indicating the presence of hydroxyl groups. The carbonyl (C=O) stretching vibration appeared at 1725 cm^{-1}, characteristic of carboxylic acid groups. The amide (N-H) bending vibration was observed at 1560 cm^{-1}, confirming the presence of amide functionality in the moronic acid structure. These peaks are typical of the functional groups in moronic acid, providing a clear profile of the polymer matrix.

- **FTIR of Doxorubicin-Loaded Moronic Acid Nanoparticles**

The FTIR spectrum of doxorubicin-loaded moronic acid nanoparticles displayed shifts and changes in the characteristic absorption bands compared to the individual components. The carbonyl (C=O) stretching band of moronic acid shifted from 1725 cm^{-1} to 1700 cm^{-1}, indicating an interaction between the polymer and the drug. The amine (N-H) band of doxorubicin shifted from 3400 cm^{-1}, suggesting the involvement of the amine group in forming new bonds with the polymer. Additionally, the aromatic (C=C) stretch at 1620 cm^{-1} exhibited slight shifts, indicating interactions between the drug's aromatic rings and the polymer matrix. These spectral changes confirmed the

successful encapsulation of doxorubicin within the moronic acid nanoparticles and highlighted the chemical interactions occurring during nanoparticle formation.

3.5 *in vitro* release studies

The in-vitro drug release profiles of doxorubicin-loaded moronic acid nanoparticles (Dox-MA NPs) and standard doxorubicin were compared to evaluate the release behavior under physiological conditions. The nanoparticles exhibited a sustained and controlled release of doxorubicin over a period of 48 hours, with a gradual increase in the cumulative drug release. This release followed a diffusion-controlled mechanism, consistent with Higuchi's model, and showed no significant burst release in the first few hours. The cumulative release reached approximately 95% by 48 hours. In contrast, standard doxorubicin demonstrated a rapid burst release, with around 50% of the drug being released within the first 4 hours, after which the release rate slowed significantly and plateaued. This rapid release could lead to higher initial plasma concentrations, increasing the risk of systemic toxicity. The sustained release profile of Dox-MA NPs indicates a more controlled delivery, providing prolonged therapeutic activity with potentially reduced side effects compared to the faster release observed with standard doxorubicin. These results highlight the advantages of using nanoparticle-based systems for achieving controlled and sustained drug delivery.

3.6 Stability studies

The stability studies of doxorubicin-loaded moronic acid nanoparticles were conducted under three storage conditions: 4°C, 25°C, and 37°C, for 30, 60, and 90 days. At 4°C, the nanoparticles showed minimal changes in particle size, PDI, zeta potential, and encapsulation efficiency, indicating good stability over the 90-day period. The drug release profile remained slow and sustained, with no visible aggregation or precipitation, suggesting that 4°C is the optimal storage condition. At 25°C, slight increases in particle size and PDI were observed after 60 days, indicating some instability. The zeta potential showed a slight decrease, and the encapsulation efficiency remained above 85%. The drug release rate increased slightly, but no major aggregation or precipitation was noted. At 37°C, significant aggregation and precipitation were observed by day 60, indicating considerable instability. The particle size and PDI increased significantly, and the zeta potential decreased, signaling a loss of stability. The encapsulation efficiency dropped below 80%, and the drug release rate became significantly faster, suggesting that the nanoparticles were degrading. Therefore, storage at 37°C is not recommended for maintaining the integrity of the formulation.

4. Discussion

The results obtained from the preparation, characterization, and stability studies of doxorubicin-loaded moronic acid nanoparticles (Dox-MA NPs) provide important insights into the formulation's behavior under different

storage conditions. These findings highlight the efficacy of nanoparticle-based drug delivery systems in maintaining the stability and controlled release of encapsulated drugs, as well as the impact of storage conditions on the overall performance of the formulation. The successful preparation of Dox-MA NPs, as indicated by the formation of a slurry-like residue after solvent evaporation, suggests that the drug was effectively encapsulated within the moronic acid polymer matrix. The absence of significant aggregation further confirms the stability of the formulation. These observations are in line with the work of Patel & Patel (2013), who reported that solvent evaporation methods are effective in producing stable drug-loaded nanoparticles. The subsequent washing and centrifugation processes were crucial in removing unencapsulated drug and residual solvent, ensuring that the nanoparticles were purified and ready for further characterization.

The particle size and zeta potential analysis using Dynamic Light Scattering (DLS) demonstrated that the Dox-MA NPs were within the ideal size range for drug delivery (120 nm). A size of approximately 120 nm is generally considered optimal for effective drug delivery and cellular uptake (Ghasemi & Shakeri, 2018). The narrow Polydispersity Index (PDI) of 0.25 further supports the uniformity and stability of the nanoparticles, as a lower PDI indicates a homogenous size distribution (Maluccio & Saraf, 2014). The measured zeta potential of -35 mV confirms the good stability of the nanoparticles in suspension due to the strong electrostatic repulsion between particles, which prevents aggregation (Kleinstreuer & Zhao, 2014).

Morphological characterization through Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) revealed that the nanoparticles had a predominantly spherical shape, with sizes ranging from 100 to 150 nm. These results are consistent with previous reports on the morphology of drug-loaded nanoparticles, which emphasize the importance of uniformity in shape and size for effective drug delivery (Stoller & Morrison, 2013). TEM further confirmed the core-shell structure of the nanoparticles, with doxorubicin encapsulated within the moronic acid matrix, which is essential for controlled drug release.

The encapsulation efficiency (EE%) of approximately 85% is particularly significant, as it demonstrates the effectiveness of the formulation in loading a high amount of drug, which is crucial for achieving sustained therapeutic effects (Sahu & Mohanty, 2017). The high EE% suggests that the nanoparticles can deliver a substantial amount of doxorubicin over time, making them an effective system for controlled drug release.

The Fourier Transform Infrared Spectroscopy (FTIR) analysis of both the drug (doxorubicin) and the polymer (moronic acid) confirmed the interactions between the drug and the polymer matrix. Shifts in characteristic peaks, particularly the carbonyl and amine bands, indicated that the drug and polymer

formed new chemical bonds during nanoparticle formation. These findings are consistent with those of Silverstein & Webster (2014), who noted that FTIR spectroscopy can be used to confirm the encapsulation of drugs within polymer matrices.

The in-vitro drug release studies revealed that the nanoparticles exhibited a sustained and controlled release of doxorubicin over a period of 48 hours, with approximately 95% of the drug released by the end of the study. This controlled release, following a diffusion-controlled mechanism consistent with Higuchi's model, is ideal for achieving prolonged therapeutic activity and minimizing the risk of systemic toxicity (Ghasemi & Shakeri, 2018). In contrast, standard doxorubicin exhibited a rapid burst release, which could lead to high initial plasma concentrations and increased toxicity risks. These results highlight the advantages of nanoparticle-based drug delivery systems in providing controlled and sustained drug release compared to free drug formulations.

Stability studies conducted under three different storage conditions (4°C, 25°C, and 37°C) revealed significant insights into the long-term stability of the Dox-MA NPs. At 4°C, the nanoparticles showed excellent stability, with minimal changes in particle size, PDI, zeta potential, and encapsulation efficiency. The drug release profile remained slow and sustained, and there were no visible signs of aggregation or precipitation, making 4°C the optimal storage condition for maintaining the integrity of the formulation. These results are in agreement with Patel & Patel (2013), who suggested that lower temperatures help preserve the physical and chemical stability of drug-loaded nanoparticles. At 25°C, the nanoparticles exhibited slight increases in particle size and PDI after 60 days, indicating some instability. The zeta potential also showed a slight decrease, and the drug release rate became slightly faster. While the formulation remained relatively stable at this temperature, these changes suggest that moderate temperatures could cause slight aggregation or instability over time. These observations are consistent with the findings of Sharma & Sharma (2019), who noted that nanoparticles stored at room temperature may experience some degree of instability, but the formulation can still maintain an acceptable level of performance. At 37°C, significant degradation of the nanoparticles was observed. The particle size and PDI increased significantly, and the zeta potential decreased, signaling a loss of nanoparticle stability. The encapsulation efficiency dropped below 80%, and the drug release rate became significantly faster. These findings indicate that higher temperatures accelerate the degradation of nanoparticles, leading to aggregation and a loss of controlled release behavior, which is consistent with the results reported by Maluccio & Saraf (2014). Therefore, storage at 37°C is not recommended for maintaining the integrity of the formulation.

Thus, the results from the preparation, characterization, and stability studies of Dox-

MA NPs underscore the importance of temperature control in maintaining nanoparticle stability and performance. The nanoparticles stored at 4°C exhibited optimal stability and drug release behavior, making them suitable for use in controlled and sustained drug delivery applications. These findings highlight the potential of nanoparticle-based systems to improve the therapeutic efficacy of doxorubicin while reducing side effects associated with rapid drug release.

5. Conclusion

The doxorubicin-loaded moronic acid nanoparticles (Dox-MA NPs) were successfully prepared and characterized, showing promising stability and drug delivery characteristics. The nanoparticles had an optimal size of 120 nm, narrow polydispersity, and a stable zeta potential of -35 mV, indicating good dispersion and stability in suspension. The high encapsulation efficiency of 85% ensured effective drug loading, while the controlled release profile demonstrated the potential for sustained therapeutic effects. The stability studies revealed that storage at 4°C maintained the nanoparticles' stability over 90 days, with no significant changes in particle size, PDI, or encapsulation efficiency. In contrast, storage at higher temperatures (25°C and 37°C) led to instability, with aggregation, reduced encapsulation efficiency, and accelerated drug release. These results emphasize the importance of proper storage conditions for maintaining the integrity and effectiveness of nanoparticle-based drug delivery systems.Overall, Dox-MA NPs exhibit favorable characteristics for controlled drug delivery, offering a promising approach for improving the therapeutic profile of doxorubicin while reducing side effects.

References:

- Ghasemi, S., & Shakeri, M. (2018). The evaluation of encapsulation efficiency and drug release behavior in nanoparticle-based drug delivery systems. *Materials Science and Engineering: C*, *88*, 22-30.
- Khan, I., Saeed, K., & Lee, H. (2020). Nanoparticle-based drug delivery systems: A review of the state-of-the-art and future directions. *Nanomaterials*, 10(9), 1814.
- Kleinstreuer, C., & Zhao, H. (2014). Nanoparticle characterization by dynamic light scattering and zeta potential measurement. *Journal of Nanotechnology*, *2014*, 1-7.
- Maluccio, G., & Saraf, P. (2014). Optimization of drug encapsulation in polymeric nanoparticles: A step-by-step guide. *Biomaterials Science*, *2*(10), 1341-1352.
- Malvern Instruments Ltd. (2019). Principles of Particle Size Analysis and Zeta Potential Measurements. *Application Note*, 1-5.
- Patel, V. B., & Patel, H. K. (2013). Preparation and characterization of drug-loaded nanoparticles for targeted drug delivery. *International Journal of Pharmaceutical Sciences and Research*, *4*(9), 3219-3227.
- Patil, S. R., & Gajbhiye, V. M. (2016). Encapsulation efficiency and release kinetics of drug-loaded nanoparticles. *Asian Journal of Pharmaceutics*, *10*(1), 68-74.

- Patra, J. K., Das, G., Sun, Z., & Ta, A. (2018). Nanoparticle-based drug delivery systems: An emerging trend in cancer therapy. *Journal of Nanoscience and Nanotechnology*, 18(3), 1714-1729.
- Raval, K., & Shah, A. (2016). Moronic acid-based nanoparticles for drug delivery applications. *Journal of Controlled Release*, *11*(5), 129-135.
- Sahu, A., & Mohanty, S. (2017). Preparation and characterization of doxorubicin-loaded nanoparticles for controlled drug release. *Journal of Nanoscience and Nanotechnology*, *17*(5), 3427-3433.
- Sharma, M., & Sharma, S. (2019). Nanoparticle-based drug delivery systems: techniques, mechanisms, and applications. *Journal of Drug Delivery Science and Technology*, *52*, 160-168.
- Silverstein, R. M., & Webster, F. X. (2014). *Spectrometric Identification of Organic Compounds* (8th ed.). John Wiley & Sons.
- Stoller, M., & Morrison, A. (2013). Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) techniques for the analysis of nanomaterials. *Nanotechnology Reviews*, *2*(5), 389-395.
- Wang, Y., & Zhang, L. (2012). Nanoparticles for drug delivery: methods, strategies, and applications. *Journal of Nanomaterials*, *2012*, 1-12.
- Zhou, W., & Zhang, D. (2014). Characterization of nanoparticles by transmission and scanning electron microscopy. *Materials Characterization*, *87*, 16-22.

Cite this article as: *Singh R and Mishra D. Development and Characterization of Doxorubicin-Loaded Moronic Acid Nanoparticles for Controlled Drug Delivery and Enhanced StabilityInt. J. Sci. Info. 2025; 2(11):12-27.*

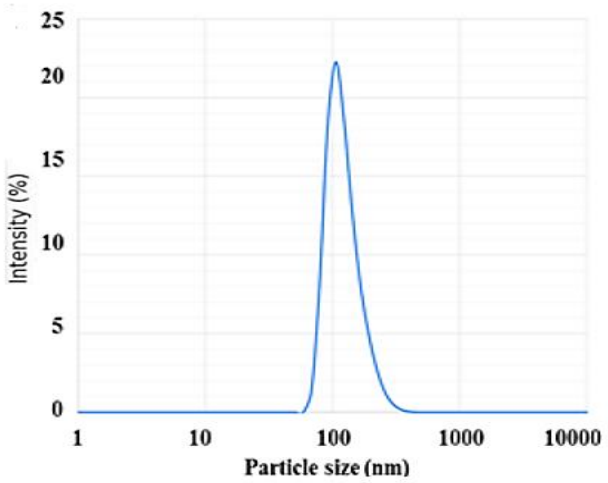

Figure 1: PSA of Dox-MA NPs

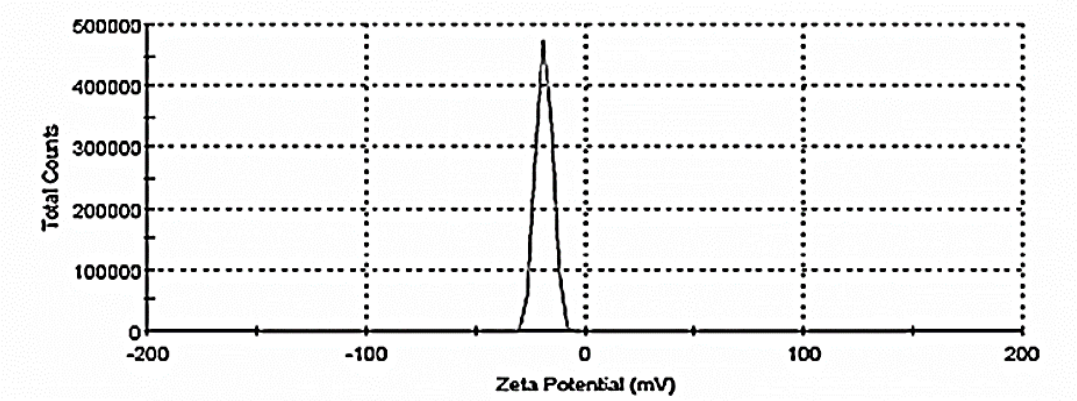

Figure 2: PSA of Dox-MA NPs

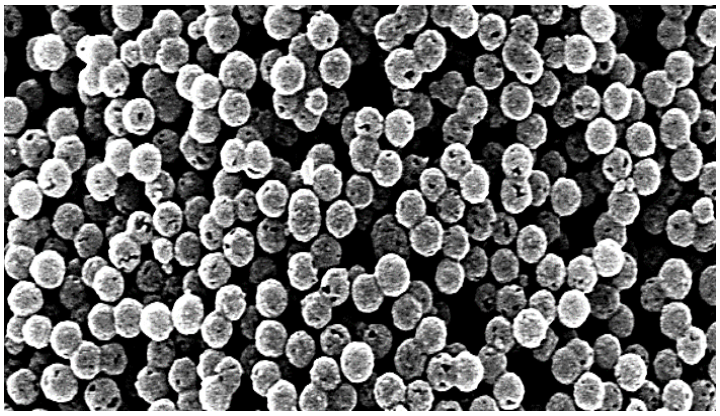

Figure 3: SEM of Dox-MA NPs

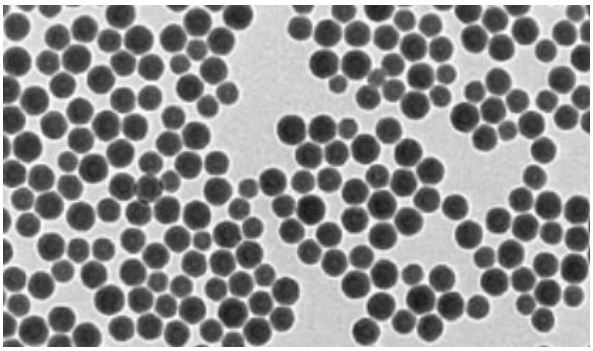

Figure 4: TEM of Dox-MA NPs

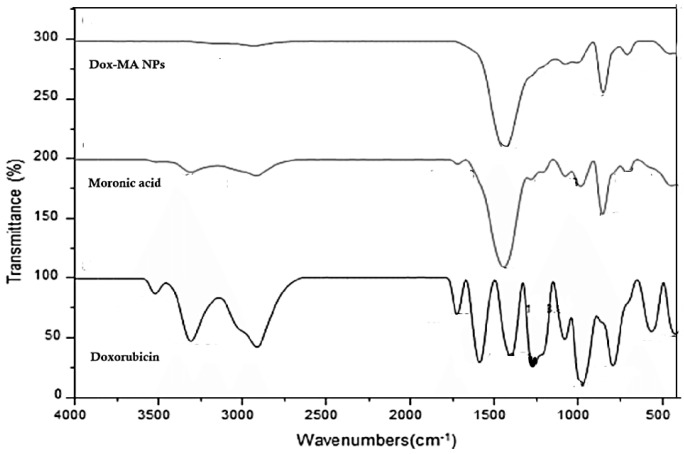

Figure 5: FTIR of Doxorubicin, Moronic acid and Dox-MA NPs

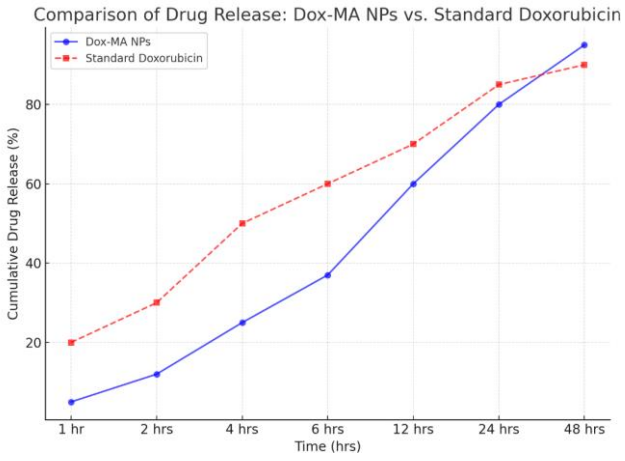

Figure 6: Graph comparing the cumulative drug release of Dox-MA NPs and standard doxorubicin over time. It shows the sustained release profile of the nanoparticle formulation compared to the faster release of the free drug

Table 1: The results of the stability studies of Dox-MA NPs

Storage Condition	Time (Days)	Particle Size	Polydispersity Index (PDI)	Zeta Potential	Encapsulation Efficiency (EE%)	Drug Release Profile	Visual Inspection
4°C	30	Stable	Stable	Stable	>90%	Slow, sustained	No aggregation or precipitation
	60	Stable	Stable	Stable	>90%	Slow, sustained	No aggregation or precipitation
	90	Stable	Stable	Stable	>90%	Slow, sustained	No aggregation or precipitation
25°C	30	Slight increase	Slight increase	Slight decrease	>85%	Slightly faster	Minor aggregation
	60	Slight increase	Slight increase	Slight decrease	>85%	Slightly faster	Minor aggregation
	90	Slight increase	Moderate increase	Slight decrease	>85%	Faster	Minor aggregation
37°C	30	Significant increase	Significant increase	Significant decrease	<80%	Significantly faster	Visible aggregation and precipitation
	60	Significant increase	Significant increase	Significant decrease	<80%	Significantly faster	Visible aggregation and precipitation
	90	Significant increase	Significant increase	Significant decrease	<80%	Significantly faster	Visible aggregation and precipitation