Journal of Chemical Health Risks

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JCHR (2024) 14(2), 1694-1701 | ISSN:2251-6727

Fabrication and Evaluation of Mesoporous Silica Nanoparticles Loaded with *Adiantum Philippines* Fraction and Thuja Oil as a Wound Healing Agent.

Swati A. Pagar[1*], Nilima Abhijeet Thombre[2]

[1]Research Scholar, MET's Institute of Pharmacy (Affiliated to Savitribai Phule Pune University) Adgaon, Nashik. MS, India.

[2]HOD, Department of Pharmaceutics, MET's Institute of Pharmacy, (Affiliated to Savitribai Phule Pune University) Adgaon, Nashik. MS, India.

(Received: 07 January 2024 Revised: 12 February 2024 Accepted: 06 March 2024)

KEYWORDS
Mesoporous Silica Nanoparticles, Adiantum Philippines, Thuja Oil, Drug Delivery, Entrapment Efficiency, In-vitro Drug Release.

ABSTRACT:

Background: The research focuses on developing Mesoporous Silica Nanocarriers for delivering Adiantum Philippines and Thuja Oil, aiming to enhance their therapeutic effects.

Methods: The study meticulously formulates MSNs loaded with Adiantum Philippines and Thuja Oil. The MSNs were prepared using the method described by Fayez Hamam. Detailed characterization of the nanoparticles is performed for Particle size, morphology, entrapment efficiency, drug release properties, FTIR, and DSC studies.

Results: The results reveal a significant entrapment efficiency of 83.38 ± 0.26% for the APTO-MSNs, indicating a high level of Adiantum Philippines and Thuja Oil encapsulation within the nanocarriers. The in-vitro drug release profile over 12 hours is noteworthy, with a gradual and controlled release pattern, culminating in a peak release of 93.33%. These findings highlight the efficiency of MSNs in maintaining a sustained release of the loaded substances.

Conclusion: The study concludes that MSNs are an effective delivery system for Adiantum Philippines and Thuja Oil, enhancing their therapeutic efficacy. The research suggests potential applications in pharmaceuticals, particularly in improving the delivery and effectiveness of natural therapeutic agents.

Introduction

The burgeoning realm of nanotechnology has unfolded myriad advancements in various scientific domains, significantly in drug delivery systems. Among the nanostructured materials, Mesoporous Silica Nanoparticles (MSNs) have emerged as promising carriers for drug delivery owing to their high surface area, tenable pore size, and ease of functionalization. [1,2] The unique mesoporous structure of MSNs facilitates the effective loading of drugs and their subsequent controlled release at the target site, rendering them superior to traditional drug nanocarriers. [3,4]

Exploring natural therapeutics has led to scrutinizing diverse plant extracts and essential oils with potential medicinal benefits. *Adiantum Philippines*, a fern endemic to the Philippines, has been documented for its antimicrobial, wound healing, anti-diabetic, and anti-inflammatory properties, among others. [5,6] Similarly, Thuja oil, derived from Thuja occidentalis, has been traditionally hailed for its antibacterial, antifungal, anti-inflammatory, and other therapeutic properties. [7,8]

The innovative formulation of MSNs loaded with *Adiantum Philippines* fraction and Thuja oil unveils a novel avenue toward harnessing the synergistic therapeutic effects of these natural substances. The encapsulation of these bioactive compounds in MSNs ensures their stability and facilitates a sustained release at the target site, potentially augmenting their therapeutic

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index. Moreover, the nano-sized dimension of MSNs permits superior penetration and distribution in biological tissues, which is pivotal for achieving a desirable therapeutic outcome.

This research endeavors to formulate, develop, and evaluate mesoporous silica particles loaded with *Adiantum Philippines* fraction and Thuja oil. The objective is to scrutinize the potential of this innovative formulation as a wound-healing agent, thereby contributing to the burgeoning repertoire of nanotechnology-based therapeutic solutions. The meticulous characterization and evaluation of the formulated MSNs, in terms of their physicochemical properties, release kinetics, and therapeutic efficacy, are quintessential for elucidating their potential as an advanced drug delivery system. Furthermore, this study seeks to lay a scientific foundation for the potential commercialization of these formulated nanoparticles, bridging the gap between traditional natural medicine and modern nanotechnology.

Materials and Methods

Materials

The materials utilized in this study were sourced from various locations. *Adiantum Philippines* and *Thuja occidentalis* were locally collected from the local area and authenticated by the Botanical Survey of India, Pune. Silica Gel 60, Sodium Hydroxide, Sodium Chloride, Sulfuric Acid, Distilled Water, Ethanol, and Chloroform were procured from Research Lab Fine Chem Industries, Mumbai. Each material was meticulously chosen to ensure the highest quality and consistency throughout the research process.

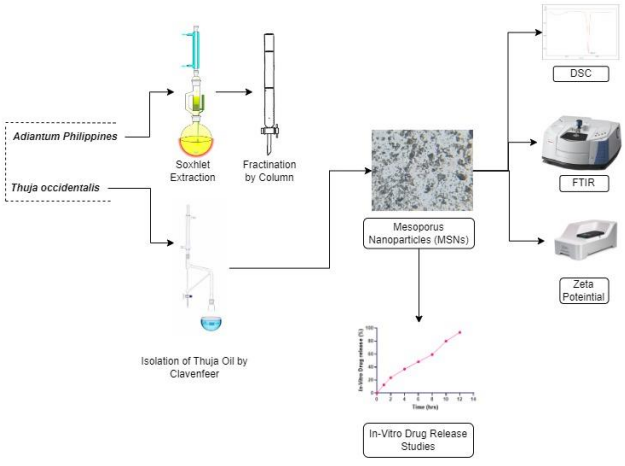
Methods

Preparation of *Adiantum Philippines* (AP) Fraction

Adiantum Philippines Fraction (Chloroform) was prepared with an ethanolic extraction using a Soxhlet apparatus, a commonly employed method for extracting bioactive molecules from plant materials. [9] Following this, the resultant ethanolic extract was subjected to fractionation via column chromatography to obtain the chloroform fraction, a technique analogous to the fractionation processes explored in studies involving other Adiantum species. [10–12] The fractionation was meticulously performed to ensure a high degree of purity and concentration of the *Adiantum Philippines* Fraction (Chloroform), which was critical for the subsequent phases of the research. This approach aligns with established practices in phytochemical studies, where solvent extraction and chromatographic fractionation are pivotal steps in isolating desired compounds from plant materials. [13]

Isolation of Essential oil from *Thuja occidentalis* (TO)

The isolation of essential oil from *Thuja occidentalis* (TO) was carried out using a Clevenger apparatus. Initially, fresh leaves of Thuja occidentalis were collected and subjected to hydrodistillation in the Clevenger apparatus for a stipulated period, typically an hour, ensuring the thorough extraction of the essential oil. The process involves the steam passage through the plant material, evaporating the steam volatile compounds. The vapor mixture then condenses in a cooling system within the apparatus, separating the essential oil from water due to their differences in density. The essential oil, being less dense, floats on the water surface and is collected in a graduated tube of the Clevenger apparatus, allowing for the measurement of the essential oil yield. [14,15]



Graphical Representation

Preparation of Mesoporous Silica Nanoparticles (MSNs)

The preparation of MSNs was carried out in alignment with the procedure delineated by Fayez

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JCHR (2024) 14(2), 1694-1701 | ISSN:2251-6727

Hamam *et al*.[16], albeit with certain modifications. In brief, silica gel 60 (48 g), sodium hydroxide (30 g), and sodium chloride (utilized to modulate the polymerization rate) were amalgamated in 500 ml of distilled water. Subsequently, sulphuric acid (H_2SO_4) was incrementally introduced at a 200 ml/min rate until the pH stabilized at approximately 7.0. The ensuing wet-gel slurry was meticulously rinsed with water to eliminate lingering salts. After that, the Buchner filtration method was employed to procure the wet cake. The MSNs were rendered into their dry, finely particulate form utilizing a hot air oven.

Loading of AP fraction and Thuja Oil in MPSPs

The AP fraction and thuja-loaded Mesoporous Silica Nanoparticles (MSNs) were prepared through 240 mg of the Chloroform fraction, and 0.3 ml of Thuja oil was dissolved in 10 ml of ethanol. This solution was then sonicated for 30 minutes in an ultrasonic water bath. Subsequently, 1 gram of MSNs powder was added to this sonicated mixture. The combination was thoroughly triturated using a pestle and mortar to ensure uniform mixing. After the trituration process, the resulting mixture was placed in an oven at room temperature. This step was critical for allowing the ethanol to evaporate, leaving behind the AP fraction and Thuja Oil-loaded MSNs.

Characterization of MSNs

Fourier transform infrared spectroscopy.

5 mg of each sample was triturated with 100 mg of potassium bromide powder. A Fourier transform infrared spectrophotometer (IRPrestige 21, Shimadzu, Kyoto, Japan) was used to scan each test sample at 400 to 4000/cm. The following samples were examined: the physical mixture, mesoporous silica nanoparticles, AP fraction, and Thuja oil. [17]

Differential scanning calorimetry

A calibrated STA449 F3 Jupiter was used to measure differential scanning calorimetry (DSC). A temperature range of 25°C to 350°C was scanned at 10°C/min. An empty pan served as a reference while 10–15 mg of the material was evaluated. The physical mixture, mesoporous silica nanoparticles, AP fraction, and Thuja Oil were examined for DSC analysis. [18]

Zeta potential measurements for particle size

The MSN and drug-loaded MSN particle sizes. A Horiba Scientific device measured the particle size three times at room temperature (30 seconds per run). This technique uses dynamic light scattering to measure size. [18]

% Entrapment efficiency

The samples were subjected to UV visible spectroscopy (UV-1800, Shimadzu) at 278 nm after being diluted with ethanol at 1:8 (v/v). As previously indicated, the reference experiment was conducted, but the mixture of MPS and chloroform fraction Thuja oil was dissolved in ethanol, and the absorbance was measured at 278 nm. [19]

$$Loading\ Efficiency = \frac{Wtotal - Wfree}{Wtotal} \times 100 \quad (1)$$

Where, W is the weight of fraction in mg.

***In-vitro* drug release study**

The dialysis bag diffusion approach was used to release AP and TO-loaded MSNs in vitro. In summary, 2 mg of samples were divided into 2 ml of PBS at a pH of 7.4, and they were securely sealed inside a dialysis bag made of cellulose acetate membrane with a molecular weight cut-off value of 10,000. [20] The dialysis bag was submerged in the compartment holding 50 ml of PBS pH 7.4 dissolving media, which was kept at 37°C±0.5°C and shaken in a water bath shaker at 100 rpm. Pooja Warule *et al*. (2017).[4] The required volume of samples (1 mL) was taken out at prearranged intervals and subjected to UV-visible spectrophotometer analysis at 278 nm. The exact amount of new released media was added to keep the specific volume. [21]

Results and Discussion

FTIR study

The Fourier-transform infrared spectroscopy (FTIR) studies for *Adiantum Philippines* (Chloroform fraction), *Thuja occidentalis* (Thuja Oil), and AP and TO loaded Mesoporous Silica Particles (MSNs) demonstrate distinct and consistent functional group characteristics, indicating no significant interactions between the constituents in the loaded MSNs. The hydrogen-bonded OH group shifts from 3436.53 cm^{-1} in the Chloroform

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JCHR (2024) 14(2), 1694-1701 | ISSN:2251-6727

fraction and 3790.4 cm^{-1} in Thuja Oil to 3530.08 cm^{-1} in the loaded MSNs. The free OH group also maintains consistency, with values moving from 3539.7 cm^{-1} and 3499.2 cm^{-1} to 3429.78 cm^{-1} in the loaded MSNs. The CH stretching is observed at 2972.73 cm^{-1} in the Chloroform fraction and aligns closely at 2974.66 cm^{-1} in the loaded MSNs. Similarly, the Carbonyl C=O group exhibits a minor variation, shifting from 1778.05 cm^{-1} and 1741.41 cm^{-1} to 1787.69 cm^{-1}. The C=C stretch and C-O stretch frequencies are stable in the loaded MSNs at 1656.55 cm^{-1} and 1024.02 cm^{-1}, respectively. The aromatic ring, noted in both the chloroform fraction and Thuja Oil, is not observed in the loaded MSNs, which might be due to its integration into the MSNs' structure. Lastly, out-of-plane C-H bending shifts minimally from 795.49 cm^{-1} and 785.85 cm^{-1} to 793.56 cm^{-1} in the loaded MSNs. These findings highlight the successful incorporation of the components into the MSNs without altering their fundamental molecular structures, affirming the stability and compatibility of the loaded MSNs.

Differential scanning calorimetry (DSC)

The DSC spectra of *Adiantum Philippines* exhibited a peak at 172.25°C, while *Thuja occidentalis* demonstrated a peak at 151.23°C, each indicating unique thermal events pertinent to their stability profiles. Notably, the physical mixture of loaded MSNs showed two peaks at 149.48°C and 171.52°C, closely mirroring the individual components. This suggests minimal interaction between the constituents at a molecular level, implying potential compatibility in the formulation. These findings provide critical insights into the thermal characteristics of these components, laying the groundwork for further exploration into their suitability and stability in pharmaceutical applications.

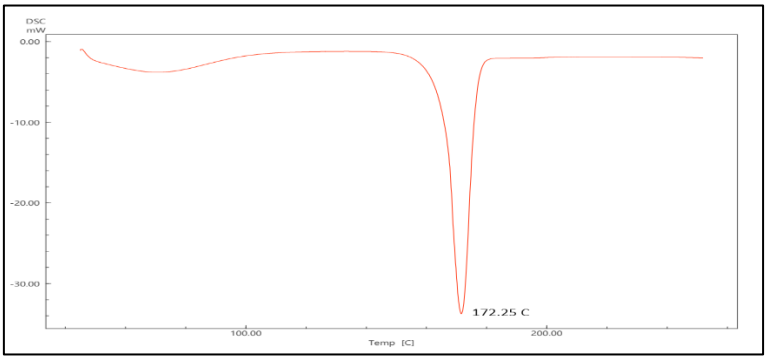

Figure 1: DSC spectra of *Adiantum Philippines*

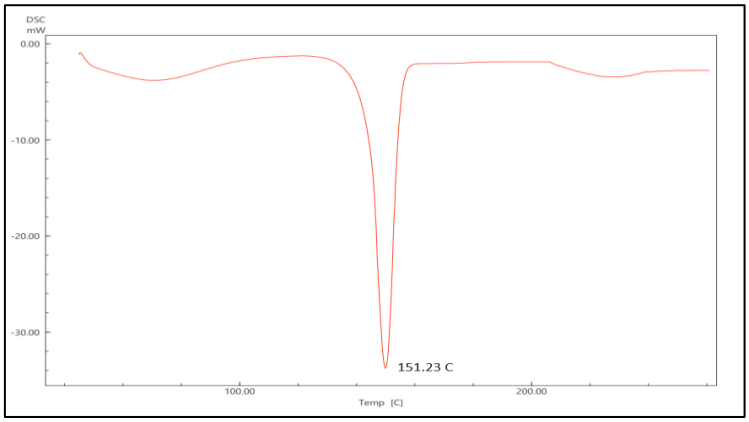

Figure 2: DSC spectra of *Thuja occidentalis* (Thuja Oil)

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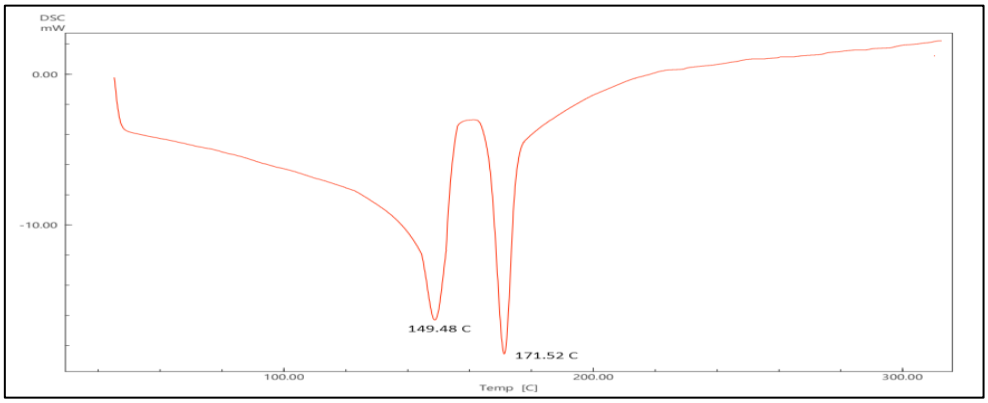

Figure 3: DSC spectra of the **physical mixture of MSNs**

Characterization of MSNs

% Entrapment efficiency

Table 1: % Entrapment efficiency of APTO -MSNs

Sr. No.	Formulation	% Entrapment efficiency
1	APTO -MSNs	83.38 ± 0.26%

Values are expressed in mean±SD (n = 3)

The entrapment efficiency of APTO-MSNs, at 83.38 ± 0.26%, reflects a highly effective encapsulation of the drug within the MSNs. This high efficiency ensures optimal drug retention for controlled release.

Particle Size Analysis

The particle sizes of the MSNs and AP and TO-loaded -MSNs were found to be 128.4 ± 67.2 nm and 171.2 ± 39.2 nm (Table 2)

Table 2: Particle Size Analysis of MSNs

Sr. No.	Formulation	Particle size (nm) (n = 3)
1	APTO -MSNs	171.2 ± 39.2
2	MSNs	128.4 ± 67.2

Values are expressed in mean±SD (n = 3)

Zeta Potential

Table 3: Results of Zeta Potential

Sr. No.	Formulation	Zeta potential (mV) (n = 3)
1	APTO -MSNs	15.2 ± 4.11
2	MSNs	28.6 ± 3.11

Values are expressed in mean±SD (n = 3)

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The Zeta potential of samples is shown in Table 3. The APTO -MSNs showed a zeta potential of 15.2 ± 4.11 mV (Figure 4), indicating moderate stability due to sufficient surface charge to prevent particle aggregation. In contrast, MSNs exhibited a higher zeta potential of 28.6 ± 3.11 mV, suggesting superior colloidal stability due to stronger electrostatic repulsion. This variation likely results from surface interactions in the loaded MSNs.

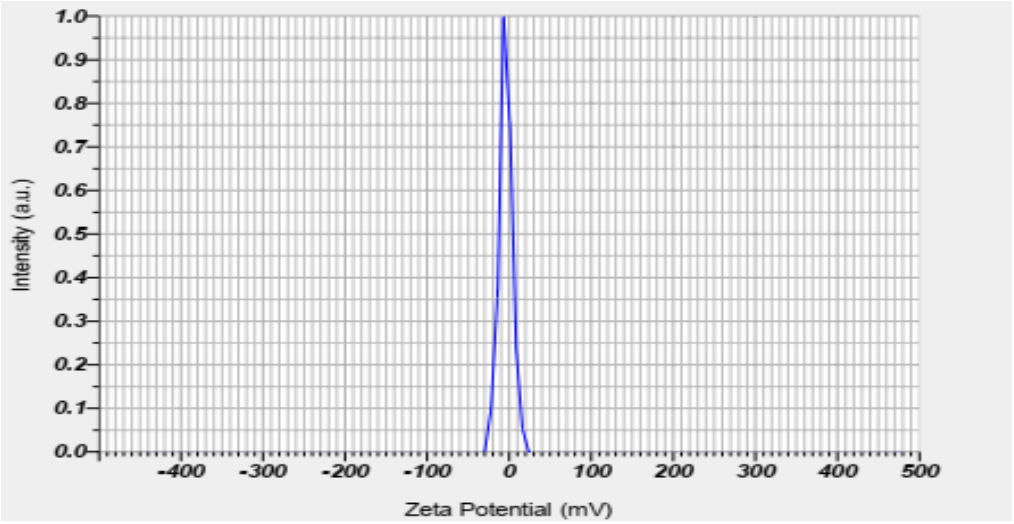

Figure 4: Zeta Potential of APTO-MSNs.

***In-vitro* drug release**

The *In-vitro* drug release profile of APTO-MSNs over 12 hours showed a controlled and incremental release of the drug (Figure 5). At 1 hour, it was progressing to 23.57% by the 2-hour mark. This trend of steady release continued, with the drug release reaching 36.91% at 4 hours and approximately half (48.1%) of the drug being released by 6 hours. A notable increase was observed at 8 hours with a release of 59.17%, followed by a significant to 79.91% at 10 hours. By the end of the 12 hours, the release reached its peak at 93.33%, indicating a near-complete release of the drug.

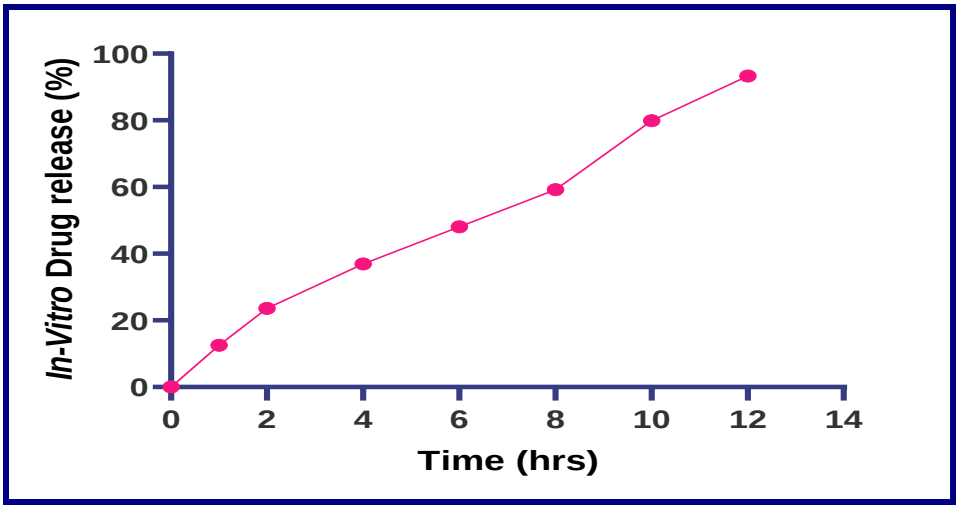

Figure 5: *In-Vitro* Drug release study of APTO-MSNs

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JCHR (2024) 14(2), 1694-1701 | ISSN:2251-6727

Conclusion

The research conclusion emphasizes the effective formulation and characterization of Mesoporous Silica Nanoparticles (MSNs) loaded with *Adiantum Philippines* and Thuja Oil. The study highlights the nanoparticles' high entrapment efficiency, better stability, and controlled drug release over 12 hours. These findings suggest the potential of this novel nanocarrier system in enhancing the therapeutic efficacy of loaded natural substances, providing a significant contribution to the field of pharmaceutical nanotechnology and paving the way for future applications in drug delivery systems.

Acknowledgment

The authors would like to express their sincere gratitude to their research guide, Dr. Nilima Avinash Thombre, for their invaluable guidance, support, and mentorship throughout the research process. Their expertise and insights have been instrumental in shaping this study. The authors also acknowledge the MET's Institute of Pharmacy, Adgaon, Nashik. MS, India, for providing the necessary facilities and resources that enabled the successful completion of this research. Their support and encouragement are greatly appreciated.

Author's Contribution

All authors contributed equally.

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