RESEARCH ARTICLE

Designing Mesoporous Silica Nanoparticles for Dual Delivery of Artemisinin and Amphotericin B: An Innovative Strategy for Therapeutic Intervention

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ABSTRACT

The present investigation assesses the potential of mesoporous silica nanoparticles (MSNPs) as dual drug carriers for artemisinin and amphotericin B. These two therapeutic agents were chosen due to their established pharmacological activities—artemisinin for its antimalarial efficacy and amphotericin B for its strong antifungal action. The study primarily concentrated on designing, developing, and optimizing MSNP-based formulations to achieve efficient co-delivery of these agents. Comprehensive synthesis and characterization procedures were undertaken to analyze the encapsulation performance of MSNPs. Key physicochemical parameters such as particle diameter, polydispersity index (PDI), surface charge, drug loading capacity, and entrapment efficiency were meticulously evaluated. Synthesized nanoparticles exhibited an average size of 199.0 ± 14.0 nm, a PDI value of 0.256 ± 0.21, and a zeta potential of –34.1 ± 0.21 mV. Additional structural and surface examinations were conducted. Entrapment efficiency studies revealed that artemisinin ranged between 69.30 ± 1.4% and 72.44 ± 1.0%, while amphotericin B demonstrated an efficiency of 60.23 ± 1.8% to 68.15 ± 1.4%, confirming successful incorporation of both agents into the MSNP framework. In-vitro release profiles evaluated in PBS (pH 7.4) exhibited a biphasic drug release, characterized by an initial rapid release followed by a controlled release over 48 hours. The observed release behaviour aligns with controlled drug delivery principles. Overall, the findings suggest that MSNPs co-loaded with artemisinin and amphotericin B offer promising attributes for targeted and sustained drug delivery, potentially enhancing therapeutic outcomes.

Keywords: Mesoporous silica nanoparticle, Artemisinin, Noval drug delivery system, Amphotericin B

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INTRODUCTION

Noval drug delivery platforms are designed to deliver therapeutic substances directly from the administration site to the specific location of infection or illness. Among the various delivery routes, oral administration It is still considered the most preferred method owing to its straightforward administration, better patient adherence, and economical nature. It is estimated that oral drug formulations account for approximately 90% of pharmaceutical production and nearly half of the globally[1]. However, despite its benefits, oral drug delivery encounters major obstacles in attaining optimal therapeutic effectiveness. These difficulties stem from the numerous physiological barriers that drugs must overcome while traversing the body. Major challenges involve the stomach's highly acidic conditions, enzymatic breakdown of drugs. Many drugs suffer from poor water solubility, which slows their dissolution in bodily fluids and negatively impacts their absorption[2]. Traditional delivery methods may also produce undesirable side effects, requiring higher doses to achieve the intended therapeutic effect. Furthermore, these systems often lack precise control over drug distribution and release profiles to address these limitations, Noval DDS technologies are being developed to protect drugs from harsh internal environments, improve their systemic absorption, and enable controlled and site-specific release[3,4]. Nanotechnology significantly enhances drug development through precise delivery to targeted sites, reduction of therapeutic agent toxicity, and decreased overall healthcare costs. A diverse range of nanocarriers has been developed for drug delivery applications, each exhibiting distinct properties. Inorganic nanoparticles are being extensively investigated in drug delivery research owing to their superior loading capacity, relative to organic alternatives. MSNPs stand out for their tunable physicochemical features, such as customizable surface area, pore diameter, making them highly suitable for targeted therapeutic applications. Due to their tunable characteristics, MSNPs can efficiently accommodate a wide range of molecules. Additionally, their surfaces can be easily functionalized with various chemical groups to improve both the loading efficiency and controlled release of therapeutic agents. Compared to other inorganic carriers, MSNPs stand out for their excellent biocompatibility, degradability. Emerging research supports the potential of MSNPs as promising platforms for biomedical purpose. Numerous studies have also explored MSNPs-based drug loading strategies to create stable amorphous formulations,

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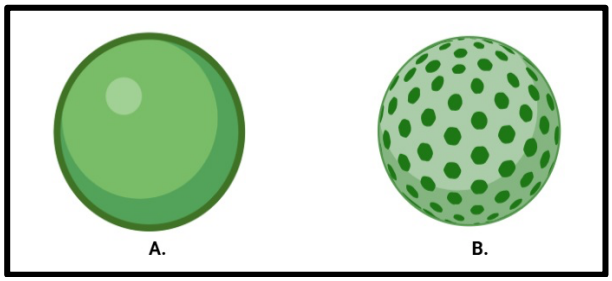

Figure 1: A Non porous silica nanoparticles B Mesoporous silica nanoparticles.

leading to improved drug solubility and enhanced bioavailability[5,6,7]. Mesoporous silica nanoparticles (MSNPs) are gaining significant attention as drug delivery vehicles, owing to their versatile physicochemical characteristics like wide surface area and adjustable pore dimensions and volume, chemical stability, and ease of surface functionalization. These features enable the encapsulation and controlled release of diverse therapeutic agents, while functionalization strategies can further enhance biocompatibility, targeting, and pharmacokinetic profile. MSNPs have demonstrated the ability to improve dissolution rates, and facilitate the co-delivery of multiple agents, thereby offering the potential for synergistic therapeutic effects[8].

Artemisinin is a sesquiterpene endoperoxide lactone, often derived from the Chinese plant Artemisia annua, commonly referred to as Qinghao or Sweet wormwood. Artemisinin derivatives are extensively utilized in the treatment of malaria. Artemisinin-based combination treatment is more successful and exhibits less resistance than a solitary artemisinin medication, as previously documented. Notwithstanding its remarkable characteristics, it is imperative to advance novel technology for prompt detection and secure treatment solutions for illnesses. In this context, a multi-faceted therapeutic agent utilizing advanced materials such as nanoparticles might enhance their therapeutic efficacy more robustly. MSNPs are recognized for demonstrating superior efficacy compared to individual pharmaceuticals[9].

Amphotericin B is an antifungal drug. Also, a cornerstone for severe fungal infections for more than five decades. It was originally derived from a species of soil-dwelling *Actinomycete*. This compound remains the preferred

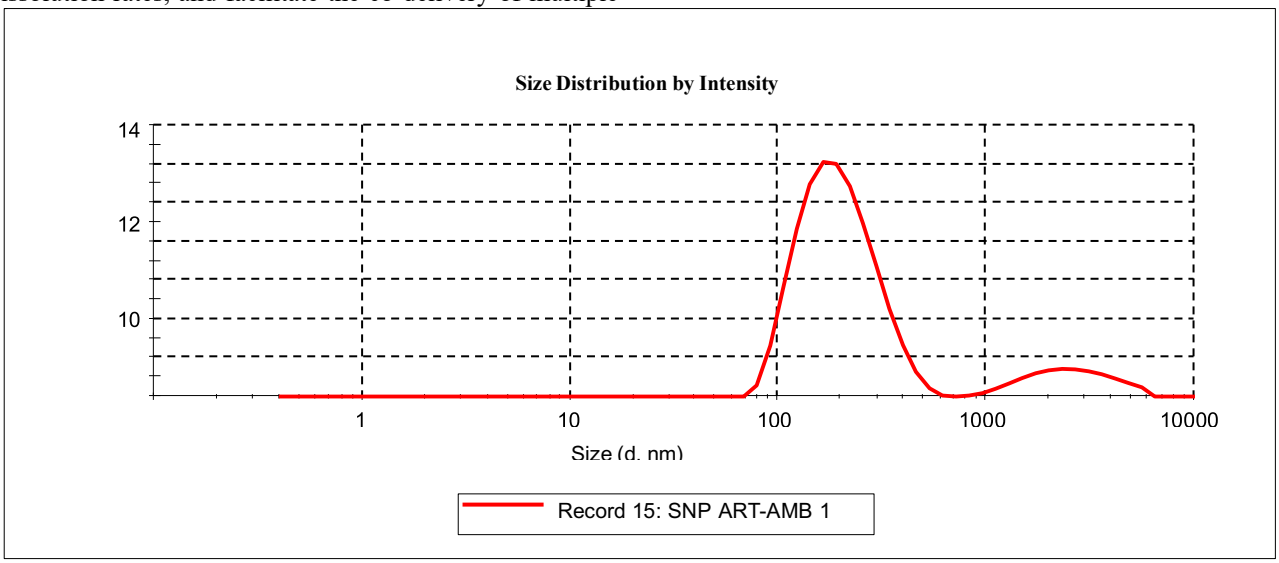

Figure 2: Particle size distribution and PDI of ART-AMB loaded MSNP

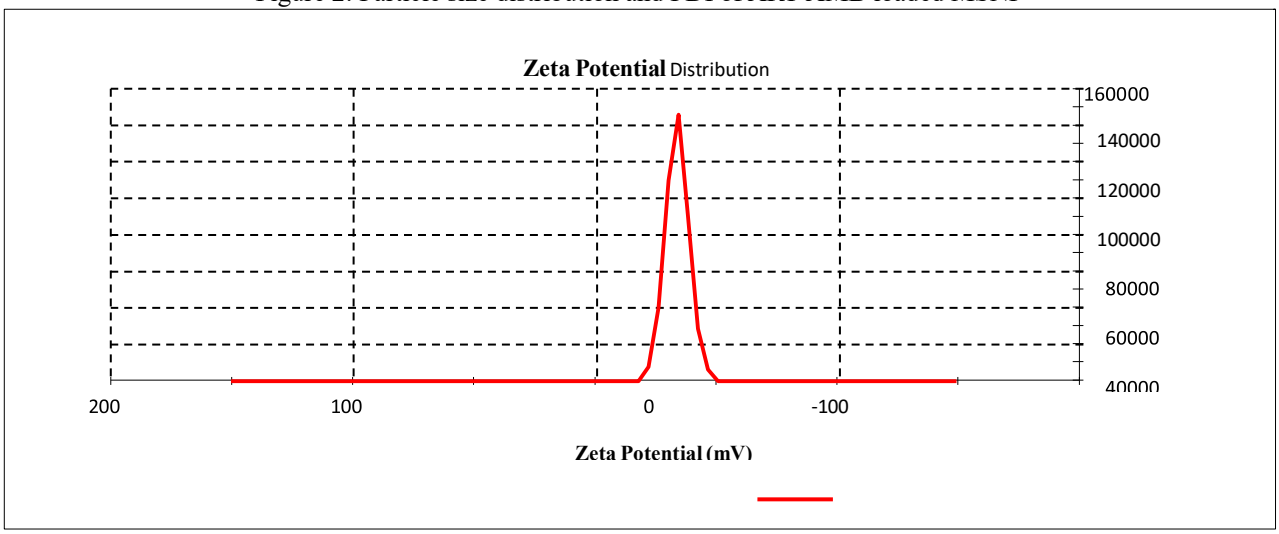

Figure 3: Zeta potential of ART-AMB loaded MSNP

treatment for both systemic fungal infections and leishmaniasis. Currently available formulations of amphotericin B are administered intravenously, further contributing to the expense of treatment. Developing an oral version of the drug could significantly cut costs and enhance patient adherence. However, achieving this is complex due to the unique and challenging physicochemical characteristics of the compound[10].

Mesoporous silica nanoparticle loaded with Drugs

Although artemisinin is a significant prospective medicine for malaria, it can also be employed in the treatment of many inflammatory illnesses. The isolated impact of this medicine has shown several drawbacks, including low bioavailability, inadequate site specificity, and delivery issues. Nonetheless, nano-formulated artemisinin and its derivatives can surmount these challenges by enhancing bioavailability, customization, and effectiveness relative to traditional medications. This research succinctly discusses the application of nanotechnology, specifically in medication delivery as therapeutic agents, using artemisinin and amphotericin B as a carrier drugs[11,12].

Material and method

Tetra ethyl orthosilicate (TEOS of 208.33 g/mol) and surfactant Cetyltrimethylammonium bromide (CTAB) C19H42Br obtained from the Merk. Ethyl alcohol absolute ((CH3)2CO), Poly (vinyl alcohol) (PVA) and Isopropanol from Sigma Aldrich. Amphotericin B and Artemisinin, from TCI Mumbai, India. Dimethyl Sulphoxide (DMSO), Poly ethylene glycol 200, monobasic potassium phosphate, sodium hydroxide (NaOH), Tween 80 was obtained from Sigma Aldrich, Humberg Germany and Deionized water.

Surface Functionalization of MSNP

MSNPs were used for the functionalization. Briefly, a of MSNP were added to polyethene glycol 200 and an amount of deionized water and dispersed using magnetic stirrer followed by keeping the suspension for 30 min in sonicator, resulting mixture was then subjected to high-speed centrifuge at 12000 rpm for 30 min. After centrifugation, the supernatant was discarded and the powder was collected in a petri plate and dried in an oven at 80°C for overnight. After drying, the sample was transferred to a container and stored and further use in the formulation development[13,14,15,16].

Formulation Development of Drug-Loaded MSNP

A known quantity of dried functionalized MSNP (FMSNP) were taken in a beaker and deionized water was added. Mixture was sonicated for 30 min under bath sonication. formulation optimization was carried out with varying the concentration of drugs, sonication time and centrifugation speed. Formulations were prepared using varying proportions of Amphotericin B (AMB) and Artemisinin (ART) by adding drugs to the MSNP and kept for 6 hours. on a magnetic stirrer at 600 rpm. After 6 hours the drug loaded onto the MSNP was analysed for under particle size analyser[17,18,19].

Characterizations

The developed formulations of MSNPs loaded with ART and AMB were thoroughly analysed using multiple

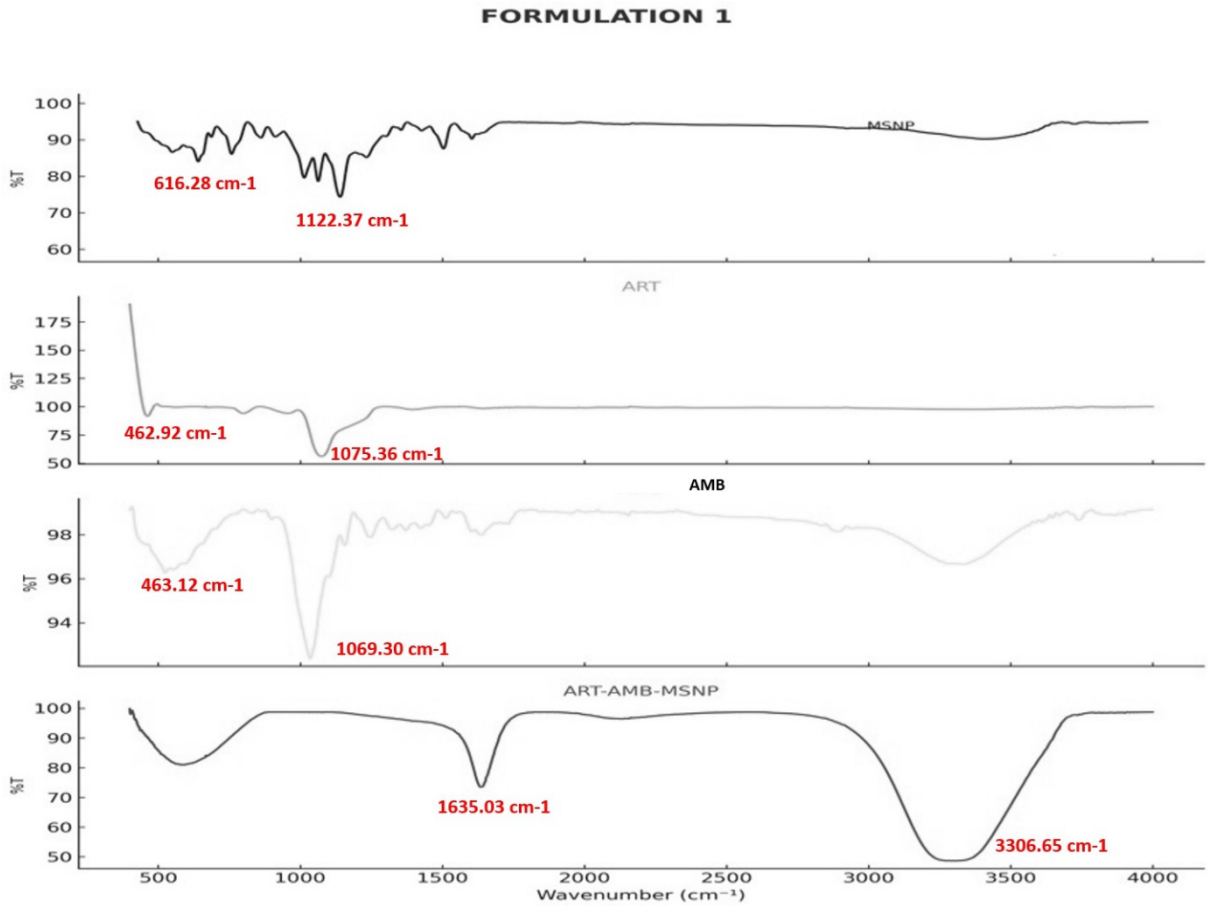

Figure 4: (1) *FTIR* Spectra of MSNP (2) *FTIR* Spectra of ART (3) *FTIR* Spectra of AMB (4) *FTIR* Spectra of physical mixture of ART-AMB-MSNP

analytical techniques to evaluate key parameters, including particle size, distribution, PDI, Zeta Potential, shape. Particle size analysis was carried out with a nano zetasizer from Instruments (Malvern, UK), which provided insights into how various factors affected the properties of the formulations. Drug-encapsulated mesoporous nanoparticles were diluted and assessed through DLS using the Zetasizer to determine particle size and zeta potential. Additionally, a UV-visible spectrophotometer was employed to measure the amount of drug incorporated into the silica nanoparticles. Entrapment efficiency and release behaviour were established using a standard calibration curve[20,21,22].

Size, polydispersity index and zeta potential

The prepared formulations were placed in cuvettes and analysed for particle size, PDI and zeta potential at ambient temperature utilizing a Malvern particle size analyser (Model EN1690, Malvern Instruments, UK). All evaluations were carried out in triplicate at a fixed scattering angle of 90°. (23)

FTIRAnalysis

Fourier-transform infrared spectroscopy (Nicolet 380) was employed to investigate possible interactions between the drugs and mesoporous silica nanoparticles. Spectral analysis was performed within the wavelength range of 4000 cm^{-1} to 400 cm^{-1} [24].

Scanning Electron Microscopy (SEM)

Silica nanoparticles were thoroughly analysed using high-resolution field-emission scanning electron microscopy (FESEM). This novel imaging technique provided detailed micrographs, allowing for a clear observation of the nanoparticles' morphology and structural characteristics. These particles demonstrated a uniform spherical shape, reflecting their well-organized and consistent structural properties. Conversely, only ensured precise size measurements but also offered valuable insights into the spherical architecture of the produced nanoparticles[25].

Entrapment Efficiency

To assess the encapsulation efficiency (EE) of different mesoporous silica nanoparticle (MSNP) formulations loaded with artemisinin (ART) and amphotericin B (AMB), the concentration of unencapsulated drug in the surrounding medium was determined. A 3 mL sample of the formulation was transferred into a centrifuge tube and subjected to centrifugation at 21,000 rpm for 30 minutes. Following this process, the supernatant was carefully filtered. The resulting filtrate was then rinsed, appropriately diluted, and analysed using a UV–visible spectrophotometer (Model

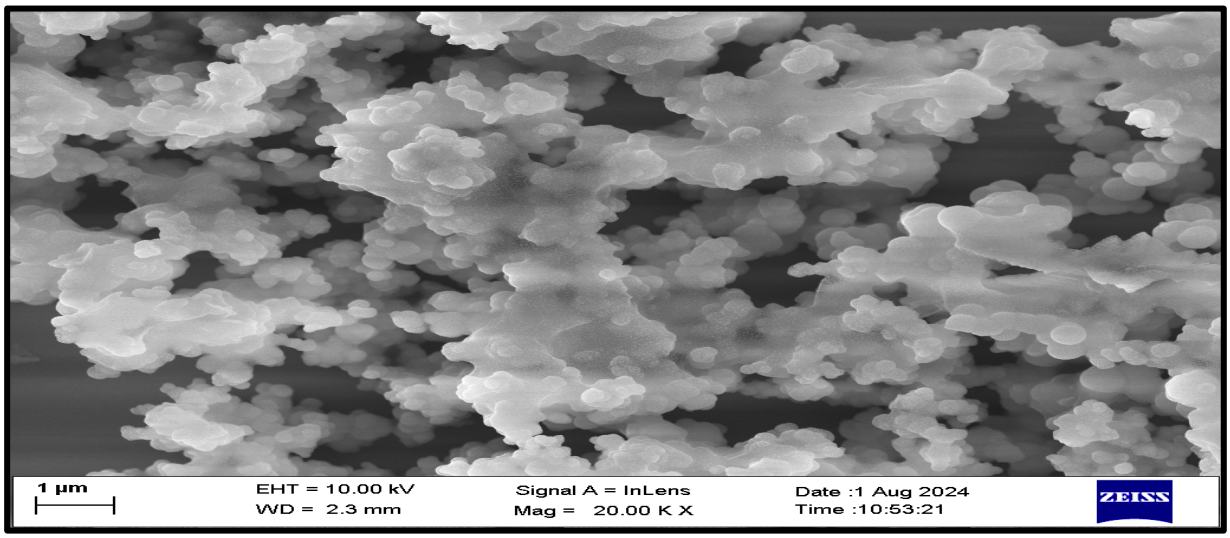

Figure 5: SEM image of ART-AMB loaded MSNP

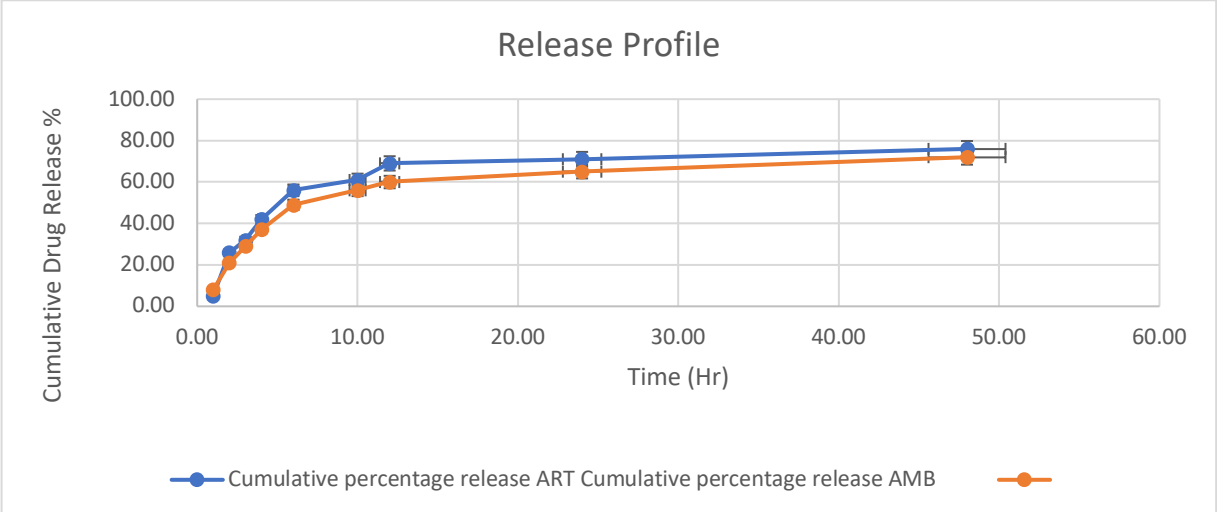

Figure 6: Measured cumulative percentage release vs. time profile.

1700, Shimadzu, Japan) at wavelengths of 258 nm for ART and 405 nm for AMB. The proportion of drug encapsulated within the nanoparticles was calculated using the following formula[26].

Encapsulation efficiency (%) =

$$\frac{\text{Amount of drug use} - \text{Amount of untrapped drug}}{\text{Amount of drug use in formulation}} \times 100$$

***In-Vitro* Drug Release Studies**

The drug release profile of the prepared formulations examined under physiological conditions at pH 7.4. In this procedure, 5 mL of the formulation was placed inside a dialysis membrane and immersed in 100 mL of PBS with 2ml of 1% Tween 80 at 37°C within a shaking water bath. Samples of 2 mL were withdrawn from the PBS medium at predetermined intervals between 15 minutes and 48 hours for analysis using a UV-visible spectrophotometer. To preserve the system's volume, an equal amount of fresh PBS was replenished after each sampling[27].

RESULTS AND DISCUSSION

Particles Size and PDI

Particle sizes of various formulations containing ART-AMB within MSNP were evaluated. Among these, the optimized formulation demonstrated size of 199± 1.5 nm and a PDI of 0.256. ± 0.012. Across all tested silica nanoparticles, the range of optimized formulation was size from 111 ± 2.5 nm to 199 ± 8.22 nm, with PDI values between 0.211 ± 0.026 and 0.256 ± 0.017. Specifically, formulation F5, identified as optimal, showed a particle size of 199± 1.5 nm and a PDI of 0.256. ± 0.012. This range gives better stability of silica nanoparticle formulation, thus preventing aggregation. Generally, PDI values above 0.7 indicate a wide particle size distribution, which is undesirable. Nanoparticles with PDI values below 0.4 are considered to have a more uniform size distribution and reduced aggregation tendencies[28].

Zeta potential Determination

The zeta potential of various ART-AMB-loaded silica nanoparticle formulations was assessed to evaluate colloidal stability. Among them, the optimized formulation exhibited a zeta potential value of -34mV ± 4.5 mV. It serves as a crucial parameter for determining the stability of nanoparticulate systems, as it reflects the surface charge and the extent of electrostatic repulsion between particles. Nanoparticles exhibiting a zeta potential exceeding ±20 are considered highly stable. Strong positive or negative surface charges enhance interparticle repulsion, effectively preventing aggregation and thereby improving the steric stability of the nanoparticles[29].

FTIR study

Artemisinin, displays distinct IR absorption bands corresponding to its functional groups. The presence of the characteristic peroxide bridge (–O–O–) is usually inferred from bands in the 880–900 cm^{-1} range, although this signal can be weak. C–H stretching vibrations from the aliphatic chains typically appear between 2850–2950 cm^{-1}, while C–O stretching is observed near 1050–1150 cm^{-1}. Amphotericin B, a polyene antifungal antibiotic, shows a more complex spectrum due to its amphipathic nature and multiple functional groups. The strong peaks between 1700–1720 cm^{-1} are associated with C=O stretching vibrations from carboxylic acid and ketone functional groups. Furthermore, bands appearing within 1500–1650 cm^{-1} are linked to C=C stretching in conjugated polyene structures. Vibrations due to C–H bending typically emerge around 1400–1450 cm^{-1}, while C–O stretching bands are generally present in the 1000–1300 cm^{-1} region. Artemisinin and amphotericin B are co-formulated or incorporated into delivery systems like mesoporous silica nanoparticles, shifts or changes in these characteristic FTIR bands can suggest interactions such as hydrogen bonding or encapsulation. These spectral changes are valuable for confirming successful drug loading and assessing potential alterations in molecular structure. This suggests that there were no chemical interactions between two drugs, indicating their compatibility[30].

SEM Analysis

The refined preparation method effectively generated MSNPs loaded with ART-AMB, utilizing Tween 80 as the surfactant. SEM imaging confirmed the morphological features of the resulting nanoparticles. These silica nanoparticles were smooth in morphology and with desired size range[31].

Entrapment Efficiency

The encapsulation efficiencies of artemisinin and amphotericin B were notably high approximately 72.44±1.3 to 69.30±1.4 % and 68.15±1.4 to 60.23±1.8 % respectively. The entrapment efficiency can also influence the characteristics of silica nanoparticles, as the entrapment efficiency increases, the number of drugs going into the system per particles absorbed get increases, and can also result in reduced dosing requirements[32].

***In-vitro* release study**

Release profile investigation was conducted in triplicate using the equilibrium dialysis membrane technique for both the optimized ART-AMB formulation and its equivalent drug solution. The study was performed at 37 °C in PBS with a pH of 7.4. Samples were collected at predetermined intervals and analysed through UV spectrophotometry. To maintain sink conditions, the withdrawn medium was replenished with fresh PBS. The concentration of the drug was plotted over time to assess its release behaviour. The release pattern of ART and AMB occurred in two distinct phases. Initially, a controlled release took place over 6 hours, characterized by a quick release accounting for approximately 25% of the encapsulated ART and AMB. This was subsequently followed by a control release phase lasting for 48 hours with a slower release rate, reaching cumulative release levels of around 65–75 % for ART and 60–70 % for AMB. These results indicated the prolonged retention of the drugs within the MSNPs at pH 7.4[33,34,35].

6. Conclusion:

In this study, MSNP and their functionalized counterparts are utilized as drug delivery systems because of their ability to co-load multiple agents, their biocompatibility, and their controlled release characteristics. This approach facilitates the regulated release of numerous medications at diverse rates, presenting significant potential for improving the efficacy and safety of drug delivery systems. It additionally presents a prospective approach for developing effective

and secure therapies for a range of diseases. Our findings indicate that ART-AMB encapsulated in mesoporous silica nanoparticles yields significant results, thereby facilitating to develop the safe and effective formulations for the treatment of various life-threatening diseases with minimal adverse effects. Artemisinin is generally regarded as a safe pharmaceutical agent, with experimental investigations reporting no signs of toxicity under the specified treatment protocols. The introduction of advanced drug delivery systems has successfully addressed these challenges, representing a promising approach to enhance the therapeutic effectiveness of artemisinin and amphotericin B based treatments. These multifunctional platforms have demonstrated remarkable effectiveness with growing research interest and ongoing efforts to overcome the key scientific challenges associated with MSNPs in biomedical applications.

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