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Design, Development and Characterization of Asiaticoside loaded nanofibers.

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ABSTRACT
Asiaticoside, a triterpenoid derived from Centella asiatica, has significant antibacterial, collagen-stimulating, and wound-healing effects, its low skin penetration and poor solubility restrict its clinical use. This study used centrifugal spinning to create and assess Asiaticoside-loaded nanofibers as a modified-release wound dressing. Using a cotton-candy-style spinneret (2000–2400 rpm), polyvinyl alcohol (8–12% w/w) was mixed with secondary polymers (sodium alginate or chitosan) and propylene glycol to create nanofibers. TLC, UV, FTIR, and DSC were used to screen nine formulations (F1–F9) for morphology, thickness and weight homogeneity, entrapment efficiency, and physicochemical compatibility. The improved formulation (NF5) showed high drug entrapment (≈89%), sustained release (>85% over 24 h), and smooth, bead-free fibers with diameters of 217–558 nm. Stability tests validated the preservation of physicochemical characteristics over 90 days, and antimicrobial testing against Staphylococcus aureus showed higher effectiveness compared to similar medication gel. These findings suggest that centrifugal-spun Asiaticoside nanofibers are a viable approach for enhanced wound healing applications and provide a feasible, solvent-effective platform for localized, controlled drug administration, improving therapeutic efficacy and antibacterial activity.. .

Keywords: *Asiaticoside, centrifugal spinning, nanofibers, PVA, wound healing, modified release, entrapment efficiency.*

1. INTRODUCTION

Transdermal drug delivery systems (TDDS), which restrict gastrointestinal degradation and avoid first-pass hepatic metabolism while delivering therapeutic agents directly to the systemic circulation in a controlled and sustained manner, have emerged as a promising substitute for traditional drug administration routes.[1] Patient compliance, fewer doses, and tailored therapy are all major benefits of this medication delivery method, especially for wound care and chronic illnesses.[2] Nanofiber-based systems have generated a lot of interest among the many TDDS platforms because of their particular structural and functional characteristics. Because to their nanometer-scale diameters, high surface-area-to-volume ratios, customizable porosity, and structural resemblance to the extracellular matrix (ECM), nanofibers promote improved tissue regeneration, cell adhesion, and proliferation.[3] Because of these qualities, nanofibers are a perfect scaffold for applications involving wound healing, allowing for immediate interaction with the wound bed, effective exudate control, and prolonged medication release. Furthermore, a variety of therapeutic compounds, including both hydrophilic and hydrophobic medications, can be encapsulated by nanofibers without losing their stability or bioactivity.[4] Due to its adaptability and capacity to create fibers with continuous morphology, electrospinning is currently the main method used to create nanofibers. Even though electrospinning is widely used, its low production rates, high voltage requirements, and solvent-related safety and environmental issues make it difficult for large-scale and commercial use.[5]

Melt blowing, bicomponent fiber spinning, phase separation, template synthesis, and self-assembly are examples of alternative nanofiber production techniques that have been studied. Even so, these techniques sometimes have complicated process parameters and limited compatibility with various polymer systems.[6] Centrifugal spinning has developed into a viable and scalable substitute in recent times. This method, which is sometimes compared to a cotton candy machine, uses centrifugal force to propel a polymer solution out of a revolving head. When the liquid jet is deposited on a collector, it stretches and solidifies. This high-throughput technique is appropriate for translational applications because it uses less energy, performs at low voltages, limits solvent exposure, and allows effective processing of thermosensitive pharmaceuticals and polymer blends.[7]

Asiaticoside is a pentacyclic triterpenoid originally extracted from Centella asiatica. Its wound-healing, anti-inflammatory, antioxidant, antibacterial, and collagen synthesis-stimulating characteristics have been extensively researched. By modulating the TGF-β1 and VEGF signaling pathways, which are essential in the wound healing cascade, it stimulates fibroblast proliferation, angiogenesis, and extracellular matrix remodeling. Asiaticoside's limited bioavailability, low permeability, and poor water solubility limit its clinical use despite its therapeutic effectiveness. By improving solubility, shielding the medication from deterioration, and facilitating long-term, targeted distribution to the wound site, incorporating Asiaticoside into nanofiber matrices provides a way to get around these restrictions.[8] Optimizing the performance of nanofibers depends on the choice of polymeric carriers. A naturally occurring, biocompatible, and biodegradable polymer, chitosan has intrinsic antimicrobial activity and expedites through tissue repair by influencing inflammatory mediators like prostaglandin E, interleukin-8, and interleukin-1β. It also promotes angiogenesis and wound contraction. Brown algae are the source of sodium alginate, a polyanionic polymer that maintains structural integrity, retains moisture, and encourages the development of granulation tissue. However, in conventional electrospinning, the electrostatic repulsion between alginate polyanions may prevent the emergence of nanofibers. By combining alginate with high molecular weight polymers like polyvinyl alcohol (PVA), which lowers repulsive forces, stabilizes the polymer jet, and permits homogeneous nanofiber creation with desired mechanical characteristics, this restriction can be addressed. PVA, chitosan, and sodium alginate work together to create a synergistic polymer matrix that promotes prolonged drug release, antimicrobial defense, ECM mimicking, and faster wound healing.[9] Incorporating a permeation enhancer, such as propylene glycol, into the nanofiber formulation further improves cutaneous absorption of Asiaticoside, ensuring therapeutic drug concentrations at the wound site. The resulting nanofibers not only provide a breathable and flexible dressing that protects the wound from environmental contaminants but also create an optimal microenvironment for tissue regeneration, exudate management, and scar prevention.[10] Advanced wound dressing systems that combine regulated bioactive administration with structural support are desperately needed since wound healing is complicated and multifactorial, especially in chronic situations like diabetic foot ulcers. To address these

needs, centrifugally spun nanofibers provide a scalable, secure, and effective platform. The formulation and optimization of Asiaticoside-loaded nanofibers utilizing PVA as a fundamental polymer and sodium alginate and chitosan as secondary polymers made by centrifugal spinning are the main objectives of this work.The work further characterizes the nanofibers in terms of morphology, drug loading, in vitro drug release, mechanical and thermal properties, biocompatibility, and stability according to ICH guidelines. The ultimate aim is to develop a clinically translatable, nanofiber-based wound dressing capable of enhancing the healing process while minimizing complications and scarring.[11]

2. MATERIALS AND METHODS

Material

Asiaticoside (Sunpure Pharma, Ghaziabad); Polyvinyl alcohol (Research-Lab Fine Chem Industries); Sodium alginate, chitosan, PVP, propylene glycol and other analytical reagents (Research-Lab Fine Chem). All solvents analytical grade.

Method

Shimadzu UV-Vis 1800; Shimadzu IRAffinity-1S FTIR; Remi centrifuge; Remi magnetic stirrer; Carl Zeiss Supra 5 SEM; DSC instrument; cotton candy centrifugal spinner (JK-M06, modified); stability chamber (LABLINE); digital vernier and analytical balance.

Polymer Selection and Optimization

By analyzing several combinations of polyvinyl alcohol (PVA, 10% w/w), chitosan, and sodium alginate, along with propylene glycol as a permeation enhancer, the polymeric composition for Asiaticoside-loaded nanofibers was customized. The selection criteria were designed to ensure compatibility with Asiaticoside (100 mg) and provide a homogenous, viscous spinning dope appropriate for centrifugal spinning. Fiber morphology using optical microscopy and scanning electron microscopy (SEM), the lack of beads, mechanical integrity, repeatability in mat handling, entrapment effectiveness, in vitro drug release profile, and consistency in weight and dimensions were all taken into consideration while evaluating formulations. High drug entrapment effectiveness, a smooth and bead-free fiber shape, and an ideal sustained release profile were found in the improved formulation (NF5).[12]

Preparation of Asiaticoside-Loaded Nanofibers

Using a cotton-candy type centrifugal spinning approach, asiaticoside-loaded nanofibers were created. To generate homogeneous, bead-free fibers with high drug entrapment efficiency and desired release properties, polymer selection and formulation optimization were carried out. To create a transparent, uniform solution (Solution A), polyvinyl alcohol (PVA, 10% w/w) needed to dissolve in distilled water while being constantly stirred and gently heated. Depending on the drug's solubility, an appropriate amount of water or methanol was used to dissolve the secondary polymer (either chitosan or sodium alginate), Asiaticoside (100 mg), and propylene glycol (Solution B). To create a viscous, homogenous polymer-drug combination appropriate for centrifugal spinning, Solution B was progressively added to Solution A despite being continuously stirred. Aliquots of 10 mL of the polymer-drug combination were added into the spinneret after the centrifugal spinner was warmed for 10 minutes. In order to facilitate fiber ejection and subsequent deposition on an aluminum foil-wrapped collector, spinning was carried out at 2000–2400 rpm with the spinner head reaching temperatures of 150–180 °C. Blank fibers have been utilized as controls; they were made in the same way but without medication. Fiber morphology (evaluated using optical and scanning electron microscopy), mechanical integrity, lack of beads, reproducibility in mat handling, entrapment efficiency, in vitro drug release profile, and weight uniformity were the criteria used to optimize the polymeric formulation and spinning parameters. The optimized batch (NF5) was chosen for additional characterization because of its prolonged release characteristics, high drug entrapment, and smooth shape. [13]

Characterization of Nanofibers

Thickness and Weight Variation:

The nanofiber mats had been cut into uniform samples measuring 2 by 2 cm. A Vernier caliper was used to measure thickness at six different locations to account for variations in fiber deposition, and an analytical balance was used to calculate the average weight. To guarantee repeatability, all measurements were carried out in triplicate, yielding information on the consistency and homogeneity of the produced nanofibers.[14]

Drug Entrapment Efficiency(EE%):

The entrapment efficiency was calculated by measuring the quantity of Asiaticoside integrated into the nanofibers. After dissolving a 10 mg sample of dry nanofiber in 10 mL of methanol while stirring for five minutes, the insoluble residues were filtered off. UV spectrophotometry was used to determine the drug concentration at 210 nm (or, in the case of release studies, at 237 nm) using a standard calibration curve. Using the formula, entrapment efficiency was determined.[15]

Microscopy and Scanning Electron Microscopy (SEM):

Initially, optical microscopy was used for evaluating surface roughness and fiber morphology. SEM examination allowed for high-resolution imaging of the nanofiber structure, which made it possible to estimate the diameter of the fiber and evaluate its uniformity. Depending on the formulation, fiber diameters ranged from 217 to 558 nm, as determined by digital image analysis using ImageJ software.[16]

Fourier Transform Infrared Spectroscopy (FTIR):

To determine functional groups along with potentially chemical interactions between Asiaticoside and the polymer matrix, FTIR spectra were obtained across 500–4000 cm^{-1}.

Differential Scanning Calorimetry (DSC):

DSC was used to determine the drug's physical state and assess its thermal characteristics, differentiating between its crystalline and amorphous states.[17]

In-Vitro Drug Release:

Using a Franz diffusion cell to simulate drug diffusion under physiological circumstances, the release of Asiaticoside from the optimized nanofiber formulation (NF5) was assessed. A synthetic diffusion membrane was used to properly mount nanofiber samples. This membrane acted as a barrier between the receptor compartment, which was filled with phosphate-buffered saline (PBS, pH 7.4) kept at 37 ± 0.5 °C, and the donor compartment, which contained the nanofiber mat. Aliquots of 1 mL were taken out of the receptor compartment at predefined intervals, such as 0.5, 1, 2, 4, 6, 8, 12, and 24 hours, and subsequently replaced with an equivalent amount of fresh, pre-warmed PBS to guarantee a constant concentration gradient and preserve sink conditions. A UV-visible spectrophotometer was used to measure the amount of Asiaticoside in the retrieved samples at 237 nm, which is the drug's maximum absorbance. To guarantee accuracy and repeatability, the process was carried out in triplicate. Careful handling was used to prevent disturbing the nanofiber mat when sampling. This technique gave extensive insight into the formulation's sustained release behavior under physiologically relevant circumstances and enabled continuous monitoring of drug release over time, reflecting the diffusion of Asiaticoside from the polymeric nanofiber matrix into the aqueous media.[18]

Antimicrobial Activity:

The agar diffusion technique was used to assess the antibacterial effectiveness of the Asiaticoside-loaded nanofibers against the common wound pathogen Staphylococcus aureus ATCC 6538. For creating a confluent lawn of growth, Mueller-Hinton agar plates were prepared and adequately loaded with bacterial suspensions. The infected agar surface was carefully covered

with 1 mg of the optimized drug-loaded nanofiber mat, blank nanofiber mat, and a standard Asiaticoside gel (equal to 52 µg of the drug). The medication was then allowed to diffuse from the nanofibers into the surrounding agar media by incubating the plates at 37°C for an extended period of time. After incubation, a calibrated ruler was used to measure the zones of inhibition, which are transparent regions that show suppression of bacterial growth, in millimeters. This configuration made it possible to directly compare the nanofiber formulation's antibacterial efficacy to that of blank fibers and traditional gel. The drug-loaded nanofibers' capacity to successfully impede bacterial growth was demonstrated by the observed inhibition zones, which offered both qualitative and quantitative proof of the antibacterial activity. This was probably made possible by the regulated and localized release of Asiaticoside from the nanofiber matrix.[19]

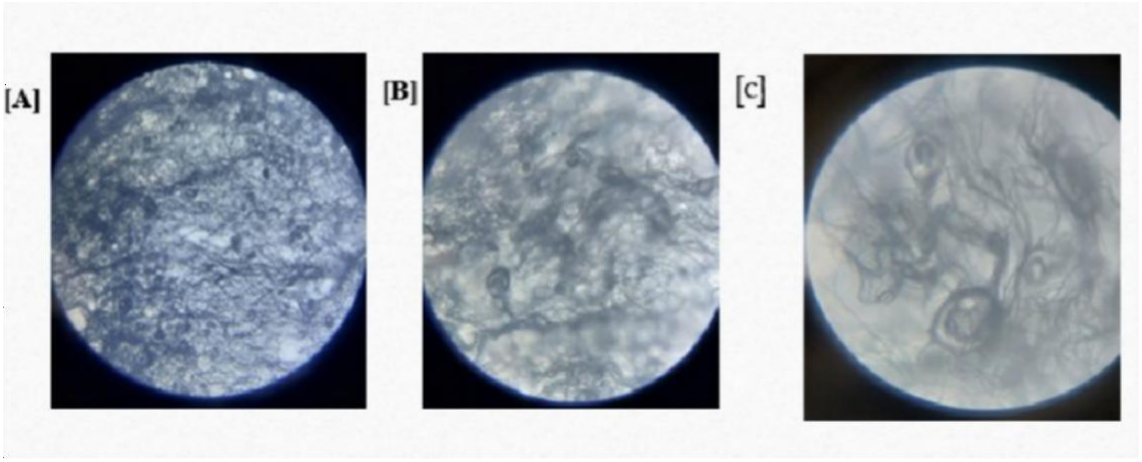
Stability Testing:

The ICH Q1A (R2) criteria were used to evaluate the nanofibers' long-term stability. For ninety days, samples were kept at 25 ± 2 °C and 75% ± 5% relative humidity. pH, entrapment efficiency, in-vitro drug release, and visual/microscopic examination were assessed at 0, 30, 60, and 90 days. The NF5 formulation data were emphasized to show how physicochemical characteristics remain the same throughout time.[20]

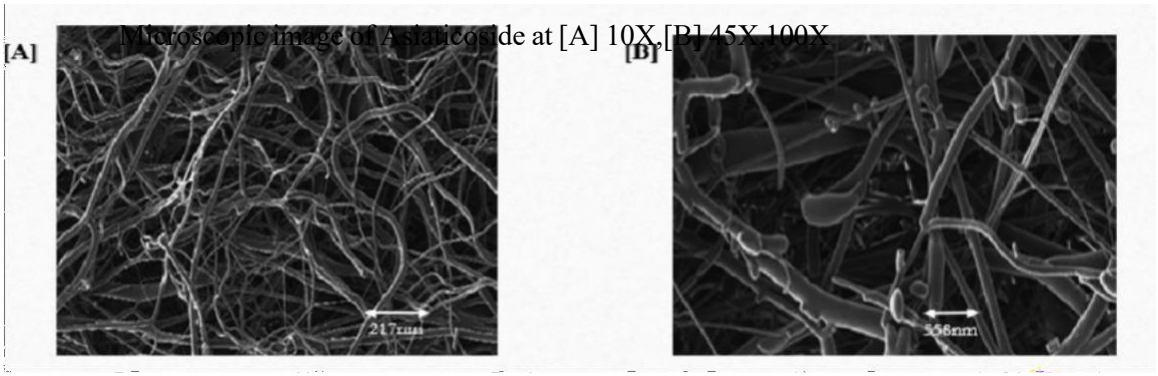
Preformulation and Identification

To verify the identification, purity, and compatibility of Asiaticoside with particular excipients, preformulation experiments were carried out. The presence of Asiaticoside in the sample was confirmed by thin-layer chromatography (TLC) analysis, which showed a typical Rf value of around 0.48 that was compatible with the reference standard. To calculate the maximum absorbance (λmax) and create calibration curves for quantitative quantification, UV-visible spectroscopic spectroscopy was used. The calibration curve for methanol followed the equation $y = 0.2053x - 0.0649$ with a correlation coefficient (R^2) of 0.9934, while the curve for phosphate-buffered saline (PBS, pH 7.4) was $y = 0.1797x + 0.0675$ ($R^2 = 0.9983$). These results showed excellent linearity and allowed for accurate drug quantitation in further research. The structural integrity of Asiaticoside was further confirmed by Fourier-transform infrared (FTIR) spectroscopy, which revealed distinctive absorption bands corresponding to broad O–H stretching vibrations around 3705 cm^{-1}, ester C=O stretching at about 1734 cm^{-1}, and glycosidic C–O stretching vibrations near 1242 and 1032 cm^{-1}. Although slight band shifts were seen, most likely as a result of physical interactions between the drug and polymers, the spectra of drug-excipient combinations showed no substantial peak shifts, suggesting the lack of major chemical interactions or incompatibilities. In combination, our preformulation and identification findings confirmed that Asiaticoside is suitable for use in nanofiber-based delivery systems.[21]

Morphology (Optical/SEM)



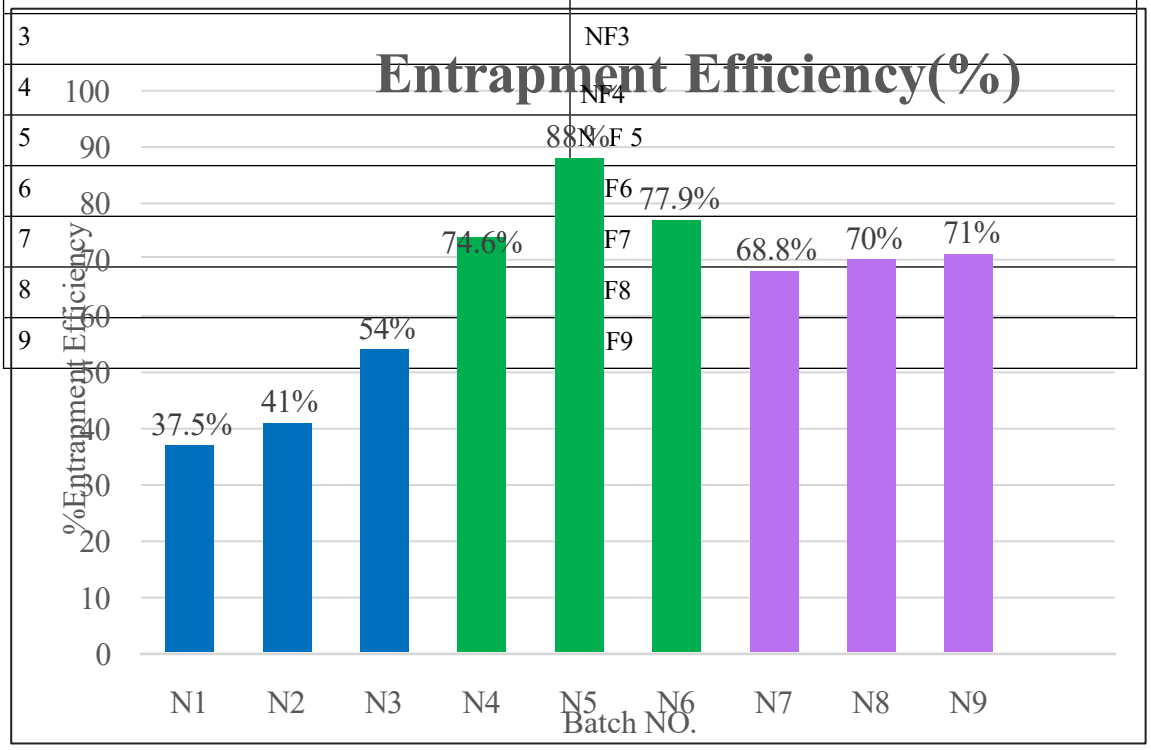
Microscopic image of Asiaticoside at [A] 10X,[B] 45X,100X



SEM images for Asiaticoside –loaded Nanofibers

Optical microscopy demonstrated continuous fibers lack of apparent errors. Optimized batches (NF5) showed smooth, bead-free fibers with

Sr no	Formulation Code
1	NF1
2	NF2
3	NF3
4	NF4
5	NF 5
6	F6
7	F7
8	F8
9	F9



diameters ranging from around 217 to 558 nm based on spinner settings and PVA content, according to SEM imaging. Optimal speed and polymer viscosity resulted in homogenous fibers, whereas higher polymer concentration tended to increase the average diameter.[22]

Entrapment Efficiency (EE%)

The optimized nanofiber formulation (NF5) showed a high entrapment efficiency of around 88%, suggesting that Asiaticoside was effectively incorporated and distributed uniformly throughout the polymeric matrix. Asiaticoside's compatibility with the chosen polymers—PVA, chitosan, and sodium alginate—as well as the uniform mixing produced by centrifugal spinning are responsible for this high EE. The prolonged release and effectiveness of the nanofiber-based wound

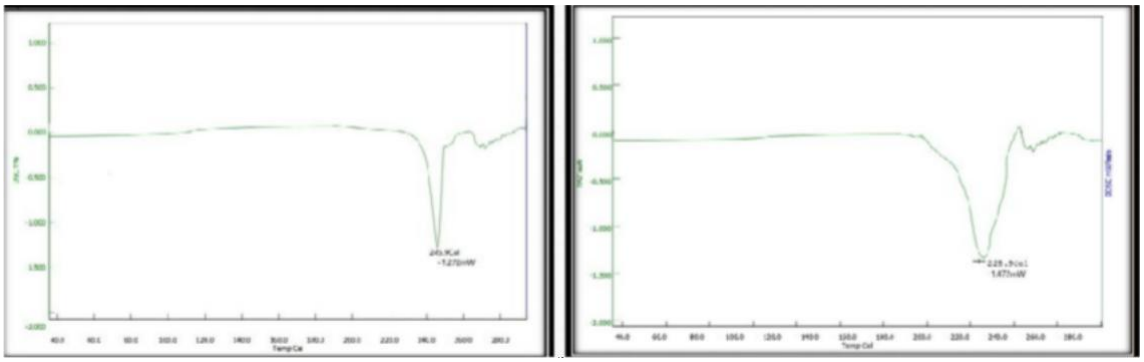
% Entrapment efficiency of prepared formulation

dressing depend on the constant delivery of a therapeutic dosage, which is ensured by effective drug encapsulation .[23]

Weight and Thickness Uniformity

The weight and thickness of 2 × 2 cm samples were measured in order to evaluate the physical homogeneity of the NF5 nanofiber mats. Consistent fiber deposition, repeatable mat production, and dependable structural integrity were confirmed by the minimal variance seen across samples. In order to ensure the practical usability and quality of the nanofiber formulation, such homogeneity is necessary for constant mechanical qualities, predictable medication dosage, and appropriate management during application as a wound dressing.[24]

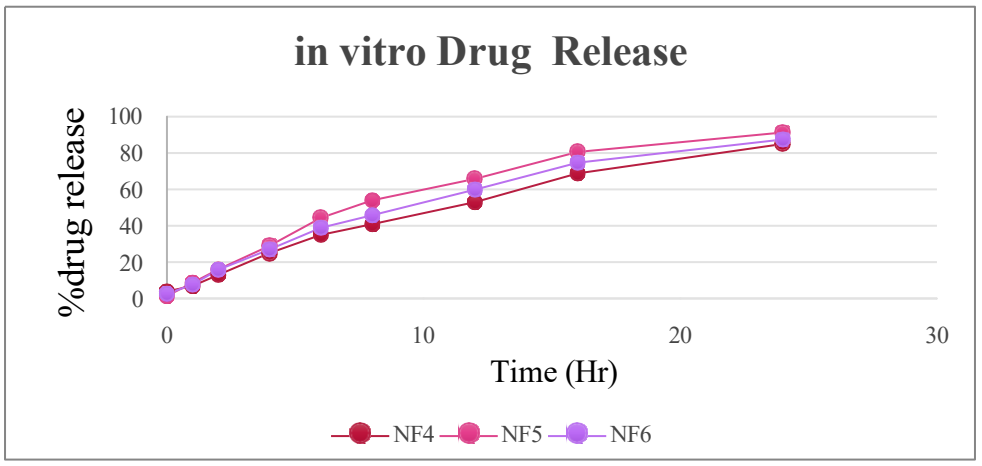
Thermal Analysis (DSC)



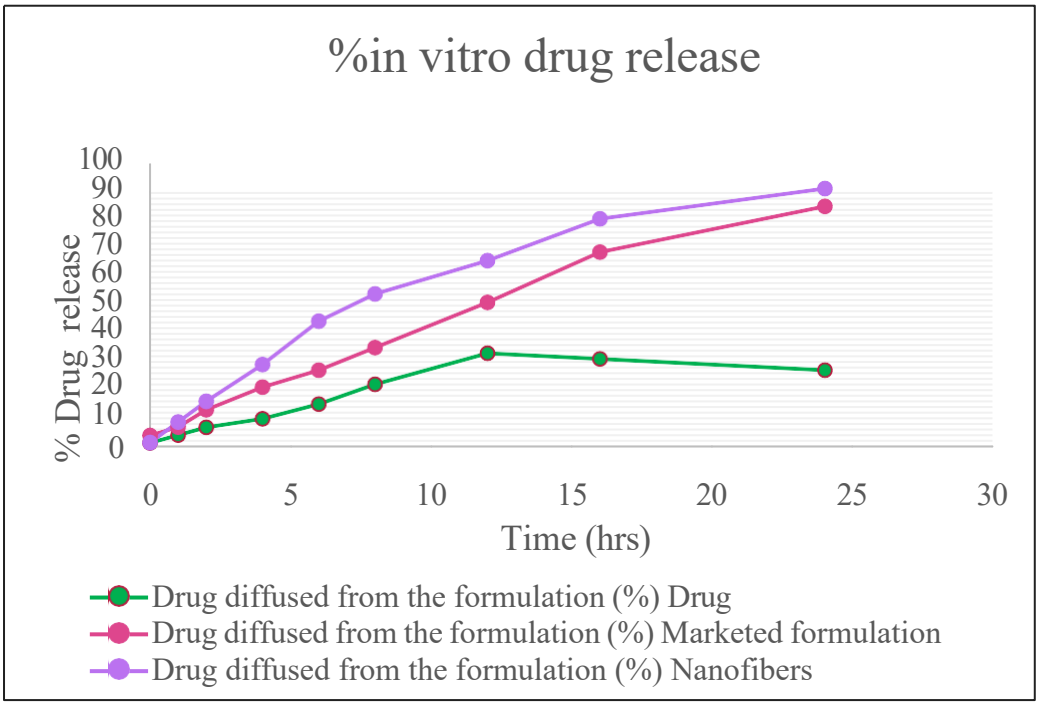
DSC of Asiaticoside nanofibers

The thermal characteristics of Asiaticoside and its nanofiber formulation (NF5) was examined using differential scanning calorimetry (DSC), which reveals light on the drug's physical condition inside the polymeric matrix. Native Asiaticoside's DSC thermogram showed a distinct endothermic melting peak, which is indicative of its crystalline structure. The thermogram of NF5, on the different alternative, showed that this melting peak was either entirely lacking or greatly diminished, indicating that Asiaticoside was either molecularly diffused throughout the polymeric network or transformed into an amorphous form during the centrifugal spinning process. Because it can increase the solubility and rate of dissolution of medications that are poorly soluble in water, such as Asiaticoside, this transition from crystalline to amorphous state is very desirable. Furthermore, small thermal changes that corresponded to the polymers (PVA, chitosan, and sodium alginate) were noted; these transitions were compatible with the polymers' known thermal characteristics and showed no appreciable degradation or unanticipated interactions during processing. Overall, the DSC findings demonstrate that the nanofiber formulation efficiently includes Asiaticoside in an amorphous or molecularly dispersed state, which is likely to increase its bioavailability and therapeutic effectiveness in wound healing applications.[25]

In Vitro Drug Release and Kinetics



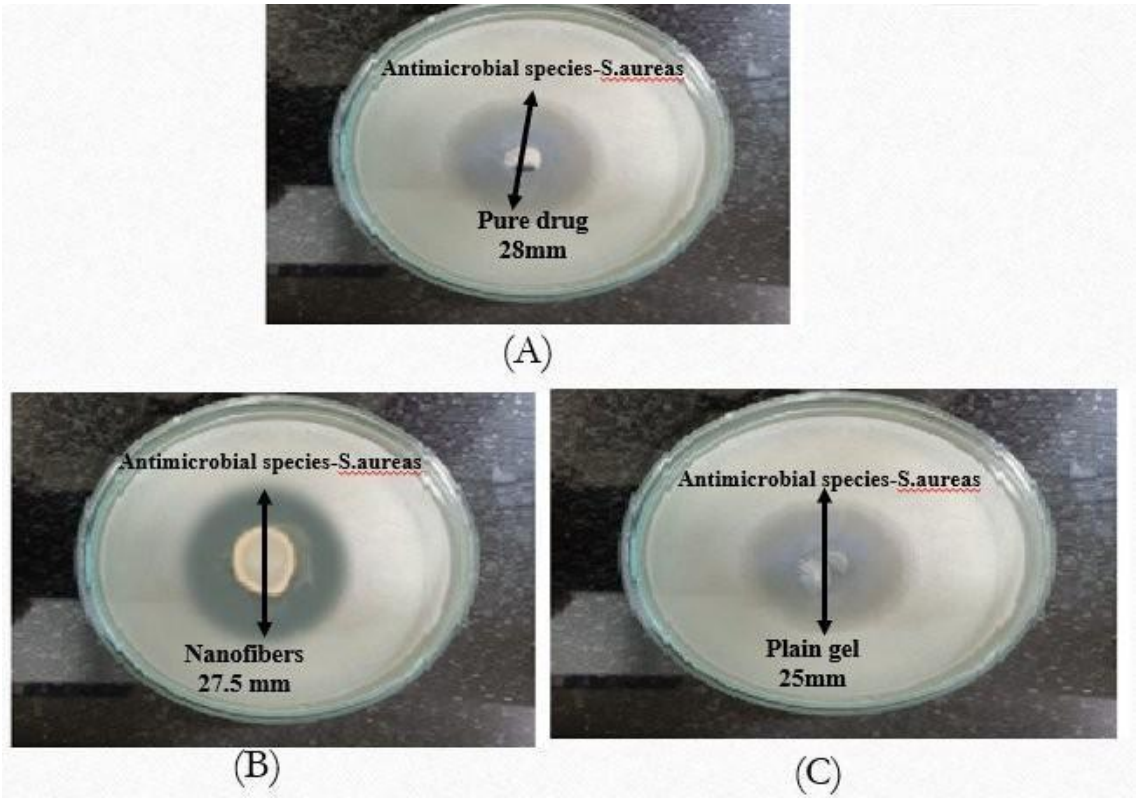
F *In vitro* drug release study of different batches of Nanofibers



Comparative study in vitro drug released a) Standard (pure) Asiaticoside b) Marketed formulation c) Nanofibers

Asiaticoside from the improved nanofiber formulation (NF5) showed a biphasic release pattern in the in vitro release tests, with an initial burst release followed by a sustained drug release over a 24-hour period. Surface-associated drug molecules that quickly diffuse into the receptor milieu and have an early therapeutic action are responsible for the initial burst. By this stage was completed, much of the Asiaticoside was released gradually; by 24 hours, the total drug release reached around 85–88%. The benefit of the nanofiber matrix in regulating drug release was demonstrated by the sustained release from NF5, which was much higher than that seen for pure drug or traditional Asiaticoside gel formulations. The release data corresponds to diffusion-controlled models, such as the Higuchi and Korsmeyer-Peppas equations, according to kinetic analysis. The release mechanism involved Fickian diffusion at early time points and non-Fickian/anomalous diffusion at later stages, representing a mix of drug diffusion and polymer relaxation processes, according to the computed release exponent (n) values. These results verify that NF5 nanofibers distribute Asiaticoside in a regulated and maintained manner, which is essential for long-term therapeutic action at the wound site.[26]

Antimicrobial Activity



Zone of inhibition of (A) Pure drug , (B) nanofiber and (C) Plain gel

Using the agar diffusion technique, the antibacterial activity of the Asiaticoside-loaded nanofiber mats was assessed against Staphylococcus aureus ATCC 6538. The findings showed that, in comparison to both blank nanofiber mats and equal dosages of Asiaticoside in gel form, the drug-loaded nanofibers generated significantly greater zones of inhibition. The large surfac e area of the nanofibers, which allows for precise interaction with bacterial cells, and the continuous release of Asiaticoside from the fiber matrix, which sustains effective local drug concentrations over time, are responsible for this increased antibacterial activity. It was confirmed that the observed activity was exclusively caused by the encapsulated medication because the blank nanofiber mats had a weak antibacterial action. The ability of NF5 nanofibers to deliver both localized and extended antibacterial activity is demonstrated by quantitative measures of inhibition zones, which substantiate the qualitative observation of increased antimicrobial efficacy. This is especially advantageous for infected wound conditions.[27]

Stability Studies

Through an interval of 90 days, the improved NF5 nanofiber formulation's physical and chemical durability was assessed under accelerated storage conditions (25 °C/75% relative humidity). From 89% on day 0 to 84.9% on day 90, the entrapment efficiency diminished minimal, suggesting negligible drug loss or degradation. The standard sustained release pattern was maintained in the in vitro release profiles following the storage period, with just a few modifications from the original data. This suggests that the polymeric matrix successfully kept the medication within the fibers. Throughout the course of of the investigation, no significant modifications in the nanofiber dispersion's visual appearance, fiber shape, or structural integrity were observed and its pH maintained approximately neutral (about 7.2–7.3).Together, these results show that the NF5 nanofiber formulation maintains its drug loading, release behavior, and compatibility for possible therapeutic uses while being physically and chemically stable under ambient storage conditions.[28]

3. CONCLUSIONS

Using a cotton-candy type centrifugal spinning approach, Asiaticoside-loaded nanofiber mats were effectively manufactured in this work. The optimized formulation (NF5) showed numerous important characteristics that are suitable for topical wound healing applications. A scanning electron microscope (SEM) analysis verified smooth, bead-free fiber shape with diameters ranging from 217 to 558 nm, showing homogeneity and structural integrity appropriate for prolonged drug release. The nanofibers also showed good entrapment efficiency (~89%), assuring adequate drug loading. More than 85% of the encapsulated Asiaticoside was released over the course of 24 hours, according to in vitro experiments that demonstrated a regulated release profile, suggesting the possibility of long-term therapeutic effect at the wound site. With respect to the high surface area, immediate contact with bacterial cells, and sustained local drug concentrations provided by the nanofiber matrix, the formulation also maintained strong antimicrobial activity against Staphylococcus aureus, compared with

equivalent drug doses in conventional gel form. The flexibility of the nanofiber system for short-term storage was demonstrated by stability experiments carried out under ICH-like circumstances (25°C/75% RH) for 90 days, which revealed only slight changes in entrapment efficiency, release profile, pH, and fiber integrity. Furthermore, centrifugal spinning eliminated the high voltage needs and solvent-related risks associated with conventional electrospinning, proving to be a scalable, energy-efficient, and safe manufacturing technique. All things considered these results point to Asiaticoside-loaded nanofiber mats as viable options for the targeted, regulated administration of bioactive compounds in wound healing applications. To confirm their clinical promise and facilitate their conversion into therapeutic wound care products, more preclinical research is necessary, including in vivo effectiveness and safety analyses.

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