Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 11s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Formulation and Characterization of Nanofibers Incorporating Pravastatin for Potential Wound Healing Applications

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Cite this paper as: Swati R. Devkar, Manoj S. Tare, Rajesh B. Patil, (2025) Formulation and Characterization of Nanofibers Incorporating Pravastatin for Potential Wound Healing Applications. *Journal of Neonatal Surgery*, 14 (11s), 975-996.

ABSTRACT

This study explores the formulation and characterization of nanofibers incorporating Pravastatin, Aloe vera juice, and Onion oil for potential wound healing applications. The organoleptic properties, solubility, UV, FTIR, and DSC analyses confirmed the compatibility of the drug and excipients, with no significant interactions observed. Phytochemical screening revealed active bioactive compounds in Aloe vera and Onion oil, highlighting their therapeutic potential. Optimized nanofiber formulations exhibited excellent drug content, entrapment efficiency, and sustained drug release. Stability studies over three months showed minimal variation in drug properties. The in vivo burn wound healing study demonstrated a significant enhancement in healing (84.17%) with nanofiber treatment compared to the control group (44.32%). Histopathological evaluation showed normal skin architecture with mild epithelial changes in treated samples, further supporting the promising therapeutic potential of these nanofiber formulations for effective wound healing.

Keywords: *Nanofibers, Pravastatin, Aloe Vera Juice, Onion Oil, Histopathology, Burn Wound Healing*

1. INTRODUCTION

Nanoscience is the study of materials at the nanometer scale (1-100 nm), where atoms and molecules exhibit unique behaviors. The term "nano" comes from the Greek word for "dwarf," and nanoscience intersects various fields, including physics, biology, chemistry, and engineering. It focuses on phenomena at the atomic and molecular levels, with a nanometer being one billionth of a meter. While nanoscience explores structures at this scale, nanotechnology applies this knowledge to create practical devices. Nanotechnology utilizes nanoscience to manipulate, measure, and control matter at the nanoscale, enabling new applications in fields like medicine, electronics, and materials science. [(1-3)]

Nanofibers, first produced via electrospinning over a century ago, are fibers with diameters less than 100 nm, often created from polymers through a process involving high electrostatic forces. These nanomaterials have unique properties such as high surface area, tensile strength, and low thermal expansion, making them valuable for applications in healthcare (e.g., tissue engineering, wound care, and drug delivery), energy storage, and environmental remediation. Nanofibers can be made from natural or synthetic polymers, each offering distinct advantages. The use of nanofiber composites, combining different materials, enhances properties like mechanical strength and cellular compatibility, making them particularly useful in biomedical fields such as nerve tissue engineering. These materials hold significant promise for various industrial, medical, and high-tech applications. [(4-7)]

Nanofiber composites offer significantly larger surface areas compared to conventional composites, enhancing their strength without compromising volume fraction. Their high surface area compensates for imperfect bonding at the fiber-matrix interface. These composites can be surface-treated to impart new functional properties, such as improved drug release kinetics. For example, coaxial electrospinning of PCL nanofibers with bioactive molecules like FITC-BSA and PEG improves protein loading and sustainability. Nanofiber composites also exhibit tunable properties like biodegradability, electrical, magnetic, and thermal conductivity, which can be tailored for specific applications. Interface interactions between nanofibers and matrix materials critically influence composite properties, such as bonding strength and dislocation densities.[(8-10)]

2. MATERIALS AND METHODS [11-15]

i. MATERIALS:

The chemicals used in the formulation include Pravastatin, HPMC K 4M, HPMC K 15M, PVP, Aloe vera juice, Onion oil, and Tamarind gum, all supplied by Cosmo Chem Pvt. Ltd. Ethanol was supplied by Merck.

ii. METHODS: [11-15]

A. Continuous hot Soxhlet extraction

The extraction of Aloe vera, onion, and other plant materials was performed using a Soxhlet extractor with solvents like methanol, ethanol, and chloroform. The materials were first dried in the shade, ground into coarse powder, and sieved to remove fine particles. The extraction was carried out until all desired components were extracted, with completion verified through TLC analysis, where the absence of colored spots indicated full extraction. After each extraction, solvents were distilled off, and the concentrated extracts were air-dried and stored in airtight containers. The process was repeated with different solvents (ethanol and chloroform) to ensure complete extraction.

B. Preliminary phytochemical investigations

I. Physicochemical evaluation

The physico-chemical characterization of selected plant materials would be carried out.

a. Determination of foreign organic matter

Principal:

The determination of foreign organic matter in herbal drugs involves examining 5 grams of dried material under magnification to isolate contaminants like insects, mold, and sand. The proportion of foreign matter is calculated based on the weight of the drug.

b. Determination of moisture content

The glass stopper and measuring bottle were weighed, and 2 grams of the sample were dried in an oven. After cooling in a desiccator, the weight loss was calculated as a percentage of the initial weight.

c. Ash value

Ash content in crude drugs determines their consistency and purity, including both physiological and non-physiological components. Total ash, acid-insoluble ash, and water-soluble ash are measured to assess the quality and purity of the plant material.

d. Determination of Total ash

2gm of aerated crude drug was correctly measured in a tared silica dish and incinerated at not more than 450 livres, before carbon-free, cooled and weight free was taken. The ash percentage was measured using the air-dried medication.

e. Determination of Water- soluble ash

Ash was collected and boiled with 25 ml of water for 5 minutes, then washed in hot water and ignited at 350°C for 15 minutes on an ash-free filter paper. The insoluble content was purified and collected, and the difference in weight between the insoluble substance and the ash determined the water-soluble ash percentage. This value was measured for the air-dried medication.

f. Determination of Acid -insoluble ash

According to the mentioned process, the ash was collected, boiled 5 minutes in 25 mL hydrochloric acid, washed in warm water and ignited cooled in a desiccator, and weighted in solutions on ash less filtering paper. The acid-insoluble ash proportion with the air-dried medication was measured.

g. Extractive values

Different extractive values like alcohol soluble extractive, water soluble extractive values were performed by standard method

h. Determination of water-soluble extractive value

Five grams of air-dried powdered drug were macerated with 100 ml of chloroform for 24 hours, with shaking during the first 6 hours. The water-soluble extractive value percentage was determined by evaporating and drying 25 ml of the filtered extract.

i. Determination of Alcohol-soluble extractive value

Five grams of coarsely powdered air-dried medicinal substance were macerated with 100 ml of ethanol for 24 hours, with shaking during the first 6 hours. The ethanol-soluble extractive value, based on air-dried drugs, was determined by evaporating 25 ml of the filtrate, drying it at 105°C, and weighing it.

II. Preliminary phytochemical investigations

Phytochemical tests for various compounds include Molisch's test for carbohydrates, Benedict's and Fehling's tests for reducing sugars, and Biuret's test for proteins. Additional tests for steroids, terpenoids, glycosides, saponins, alkaloids, tannins, phenolic compounds, and flavonoids are conducted using specific reagents and methods to identify the presence of each compound in plant extracts.

3. FORMULATION DEVELOPMENT

i. Preparation of solutions:

Prepare a PVP solution by dissolving 10 mg of PVP in 10 ml of ethanol. Then, create four solutions by adding specific amounts of Pravastatin, Onion oil, Aloe vera juice, and a combination of all three drugs with HPMC K4M, HPMC K15M, and Tamarind gum to the ethanolic PVP solution.

ii. Formulation Table

A. Pravastatin

Table 01: Formulation table of Pravastatin Nanofiber

Ingredients	PF1	PF2	PF3	PF4
Pravastatin	100	100	100	100
HPMC K4M(mg)	60	65	70	75
HPMC K15M(mg)	75	70	65	60
Tamarind gum(mg)	100	100	100	100
Ethanolic PVP	10 ml	10 ml	10 ml	10 ml

B. Onion oil

Table 02: Formulation table of Onion Nanofiber

Ingredients	OF1	OF2	OF3	OF4
Onion oil	3.6 ml	3.6 ml	3.6 ml	3.6 ml
HPMC K4M(mg)	60	65	70	75
HPMC K15M(mg)	75	70	65	60
Tamarind gum(mg)	100	100	100	100
Ethanolic PVP	10 ml	10 ml	10 ml	10 ml

C. Aloe vera Juice

Table 03: Formulation table of Aloevera Nanofiber

Ingredients	AF1	AF2	AF3	AF4
Drug	1 ml	1 ml	1 ml	1 ml
HPMC K4M(mg)	60	65	70	75
HPMC K15M(mg)	75	70	65	60

Tamarind gum(mg)	100	100	100	100
Ethanolic PVP	10 ml	10 ml	10 ml	10 ml

D. Pravastatin+ Onion oil+ Aloevera Juice

Table 04 : Formulation table of Pravastatin+ Onion oil+ Aloevera Juice Nanofibre

Ingredients	**POAF1**	**POAPF2**	**POAPF3**	**POAPF4**
Pravastatin+ Onion oil+ Aloevera Juice	104.6	104.6	104.6	104.6
HPMC K4M(mg)	60	65	70	75
HPMC K15M(mg)	75	70	65	60
Tamarind gum(mg)	100	100	100	100
Ethanolic PVP	10 ml	10 ml	10 ml	10 ml

iii. Preparation of nanofibers

The electrospinning process was carried out using a Fluidnatek LE-50 benchtopline with a variable high-voltage 0–35 kV power supply. The system was equipped with a motorized injector able to scan towards a metallic collector (20 × 20 cm2) that allows to obtain an homogeneous electrospun deposition. The corresponding solution (drug sample) was first placed into a 3 mL syringe, connected by polytetrafluoroethylene (PTFE) tubes to a stainless-steel needle of 0.7 mm of diameter. The needle tip was connected to the positive terminal of the power supply, while the metal collector was connected to the negative one. A piece of aluminum foil was placed on the collector and the solution was electrospun for about 5–10 min under a steady flow rate in the range 0.06–0.2 mL/h, depending on the sample, using the motorized injector. The distance between the needle tip and the collector was 15 cm (based on preliminary tests), and the voltage was varied for each sample depending on its properties. The process was conducted at 25 ◦C and at 40% relative humidity (RH). All the solutions prepared for electrospinning were fixed to present a 10 wt% composition of polymer, either natural, synthetic or in combination of both of them according to the study carried out.

4. EVALUATION PARAMETERS: [16-17]

I. PREFORMULATION STUDY:

A preformulation research was carried out to ensure that the medication and polymer were in a stable and pure state before being formulated into a dosage form also a for determinations of characteristics which may play important role in dosage form development.

Characterization of drug sample:

a) Organoleptic properties:

The appearance and pH of the drug were visually observed, and a digital pH meter was used to determine its pH level. For the color evaluation, a small amount of the drug was placed in butter paper and examined under a well-illuminated area. To assess the odor, only a very small quantity of the drug was used in order to avoid overwhelming the senses.

b) Melting point determination:

The drug's melting point was determined using the capillary method with a Thiele tube, where a capillary tube filled with drug powder was heated in liquid paraffin. The melting temperature was recorded by taking triplicate readings with a thermometer.

The *λ*max determination:

A. Pravastatin

1. Preparation of stock solution

A quantity of drug (10 mg) was dissolved in 20 ml of ethanol contained in 100 ml volumetric flask and was made up to mark with the same solvent to produce a 100 µg/ml solution.

2. Determination of wavelength of maximum absorption

1 ml was withdrawn from the stock solution into a 10 ml volumetric flask and made up to mark with ethanol to produce 10 µg/ml solution. This was then scanned in the spectrophotometer through range wavelengths (200 – 400 nm) so as to obtain the wavelength of maximum absorption.

The wavelength of maximum absorption is found to be 360nm.

3. Preparation of calibration curve

From the stock solution of various concentrations (10-50 µg/mL) prepared by pipetting appropriate volumes (0.1, 0.2, 0.3, 0.4, 0.5 mL) of standard solution into 10 ml volumetric flasks and diluting to volume with ethanol.

4.Fourier-transform infrared spectroscopy analysis

Fourier Transform Infrared (FT-IR) spectroscopy records the IR spectrum of a drug sample, which is compared to reference spectra to identify chemical functional groups. The technique uses an interferometer to capture the interferogram, which is then transformed into a conventional spectrum to aid in qualitative and quantitative analysis.

5.Compatibility study

Compatibility studies of APIs and excipients are conducted during preformulation to assess potential interactions that may affect the stability and efficacy of the final product. The API and excipients are mixed, stored at 37°C for 14 days, and analyzed using FTIR spectroscopy to detect any changes or interactions.

6. Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) measures heat differences between a sample and reference to study physico-chemical interactions in formulation components. Using a DSC instrument, samples were analyzed under a dry nitrogen purge, heated and cooled at a constant rate to observe endothermic or exothermic effects.

7. Nuclear Magnetic Resonance Spectroscopy (NMR)

Nuclear Magnetic Resonance (NMR) spectroscopy is used to determine the structure of organic compounds by analyzing the magnetic properties of nuclei. It provides detailed information on chemical bonds, molecular dynamics, and conformation-activity relationships, with one-dimensional and two-dimensional techniques for studying simple and complex molecules.

B. Preformulation study of Aloevera

The organoleptic properties of Aloe vera, including appearance, pH, color, and odor, were assessed visually and with a pH meter. The melting point was determined using the capillary method with a Thiele tube, and the process was repeated thrice for accuracy. For λmax determination, a 100 µg/ml stock solution of Aloe vera was prepared, and its maximum absorption wavelength was found to be 478 nm. A calibration curve was prepared using various concentrations of the stock solution. Additionally, Fourier Transform Infrared (FT-IR) spectroscopy was used to record the IR spectrum of Aloe vera, identifying functional groups and providing a molecular fingerprint for qualitative and quantitative analysis

C. Preformulation study of Onion

A preformulation study was conducted to ensure the stability and purity of Onion before dosage form development. The organoleptic properties, including appearance, pH, color, and odor, were assessed visually and with a pH meter. The melting point was determined using the capillary method with a Thiele's tube, repeated for accuracy. For λmax determination, a 100 µg/ml stock solution of Onion was prepared, and its maximum absorption wavelength was found to be 410 nm. A calibration curve was also generated using various concentrations. Additionally, Fourier Transform Infrared (FT-IR) spectroscopy was used to record the IR spectrum of Onion, identifying functional groups and providing qualitative and quantitative information on the sample's composition.

5. POST FORMULATION [18-20]

Characterizations of Nanofibers

The preformulation and characterization of nanofibers involve several important analyses to assess drug content, loading, release, and stability. Drug content is determined by extracting the drug from nanofibers using a suitable solvent and quantifying it with UV-Vis spectroscopy. Drug loading is calculated based on the ratio of drug weight to nanofiber weight after electrospinning. Scanning electron microscopy (SEM) examines the nanofiber's surface morphology, while drug entrapment efficiency is measured using ultracentrifugation and UV-Vis spectroscopy. Production yield is determined by comparing the nanofiber mass to the total polymer and drug weight. In-vitro drug release studies use phosphate buffer saline (PBS) at 37°C, with samples analyzed by UV-Vis spectroscopy. Thermal stability is analyzed using TGA, and high-resolution images are obtained using Transmission Electron Microscopy (TEM). Nanofiber stability is tested over three

months at 4°C by assessing particle size, zeta potential, entrapment efficiency, and physical appearance.

6. INVIVO ACTIVITY: [21]

I. Wound healing activity

Experimental Animals:

The study was approved by the Institutional Animal Ethics Committee and conducted using healthy Wistar albino rats (200-220g), housed individually with free access to a standard pellet diet and water. Animals were anesthetized with ketamine (120 mg/kg) before experimental wounds were inflicted, and surgical procedures were carried out under sterile conditions. The rats were monitored for signs of infection, and any affected animals were excluded and replaced. Reagents and chemicals used included ethanol, ketamine (10 mg/kg), and diazepam (10 mg/kg), while instruments such as a Vernier caliper, shaving machine, and electrical heater were employed. The animal model focused on evaluating in vivo burn wound healing activity with test formulations: F1 (0.9% saline) and F2 (nanofibers formulation).

Experimental design

Sr. No	Name of group	Treatment	No. of animals
1	**Normal Control (**Group I**)**	0.9% saline	6
2	**Optimised Film (**Group II**)**	Nanofibers Formulate	8*

*****Minimum 08 rats are required to carry out the study instead of 06 rats, as there are chances of death due to burn.

Experimental condition: -

In this study, Wistar albino rats (250-300g) of either sex were housed in standard metabolic cages with free access to food and water. They were maintained under controlled room temperature and environmental conditions, with the animal protocol approved by the Institutional Animal Ethics Committee. On the day of wounding, rats were anesthetized with ketamine (1 ml/kg) and diazepam (1 ml/kg). A deep circular burn wound (15 mm diameter) was created on the dorsal thoracic region using an electrical heater (110°C for 15 seconds), and the wound was cleaned with normal saline. The animals were divided into groups and treated with an optimized film formulation (0.1g) applied once daily for 21 days. Wound healing was monitored by measuring the wound diameter, and the percentage of healing was calculated using the formula: $Px = ((d1^2 - dx^2) / d1^2) \times 100$, where d1 is the initial wound diameter and dx is the diameter at day-x. Healing was complete when new skin had formed and the wound was fully covered.

Histopathology of the skin tissue:

On the 21st day, the animals were sacrificed after being anesthetized, burned skin tissue samples were collected after sacrificing the rats for histopathological examination purposes. These tissue samples were fixed at 10% neutral buffered formalin solution, embedded in paraffin wax, cut into 5 µm-thick sections and stained with hematoxylin-eosin and Masson's trichrome stain for examination by light microscopy.

7. RESULT & DISCUSSION

1. PREFORMULATION STUDY:

a) Organoleptic properties:

The organoleptic properties of the substances were as follows: Pravastatin is a white to yellowish crystalline powder with no noticeable odor. Aloe vera juice, derived from succulent leaves arranged in a rosette, has a grey to green color and a mild, pungent odor. Onion oil appears as a brownish yellow oily globule with an obnoxious sulfide odor.

b) Melting point determination:

The melting points of the samples were as follows: Pravastatin had a melting point of 172.12°C, Aloe vera juice melted at 36.10°C, and Onion oil had a melting point of 40.13°C**.**

c) Solubility:

The solubility of Pravastatin in various mediums was as follows: Methanol (0.368 µg/ml), Ethanol (0.2415 µg/ml), DMSO (0.458 µg/ml), and Phosphate buffer pH 6.8 (0.125 µg/ml). For Onion oil, the solubility in Methanol was 0.547 µg/ml, Ethanol was 0.2915 µg/ml, DMSO was 0.258 µg/ml, and in Phosphate buffer pH 6.8, it was 0.09 µg/ml. The solubility of Aloe vera juice in various mediums was as follows: Methanol (0.347 µg/ml), Ethanol (0.2215 µg/ml), DMSO (0.358 µg/ml), and Phosphate buffer pH 6.8 (0.115 µg/ml).

d) UV Spectroscopy

Pravastatin:

UV Spectroscopy:

The *λ*max determination:

It is done by using UV-Spectrophotometer. The wavelength of maximum absorption is found to be 230 nm.

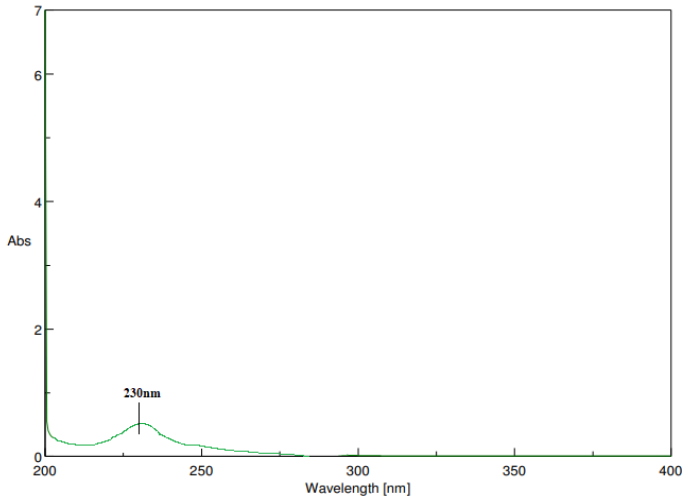


Fig no 1 : UV spectra of Pravastatin

Table 5: Absorbance of Pravastatin

Concentration(µgmL-1)	Absorbance(nm)
0.1	0.2587
0.2	0.5001
0.3	0.7241
0.4	0.9524
0.5	1.2017
0.6	1.4215

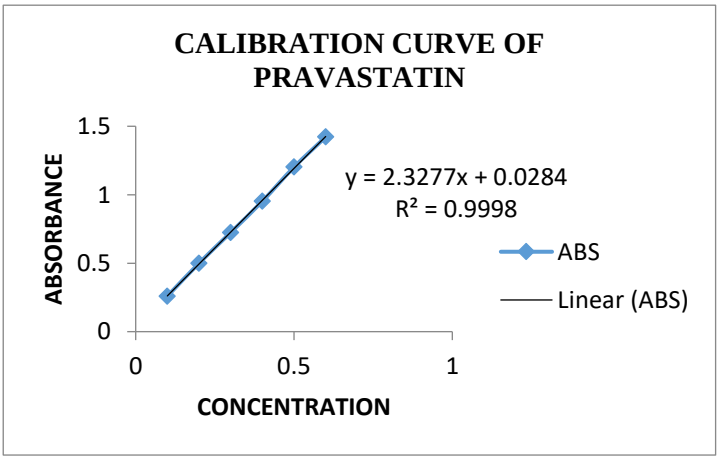


Fig no 3. Linearity Curve for Pravastatin

a) Fourier-transform infrared spectroscopy analysis

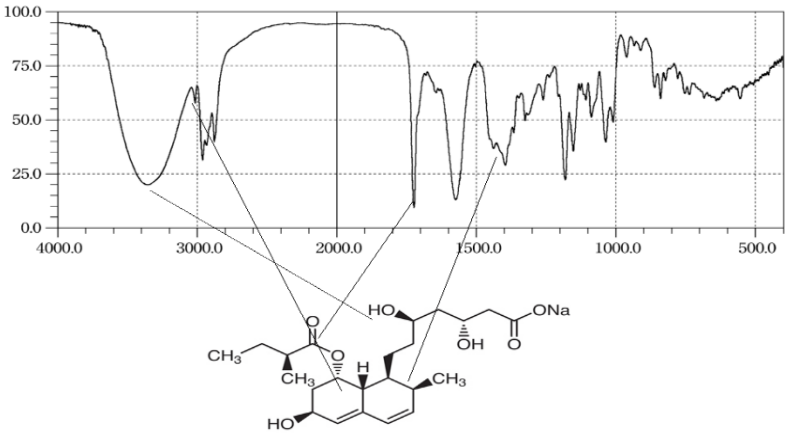


Fig no 4: FTIR Spectra of Pure Pravastatin

b) Compatibility study

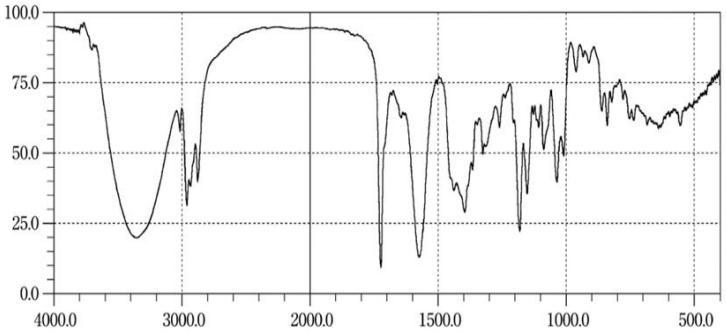


Fig no 5: Compatibility study by FTIR spectra

c) Differential Scanning Calorimetry

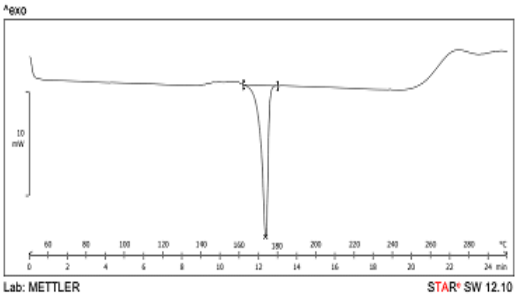


Fig no 6: DSC Graph of Pure Pravastatin

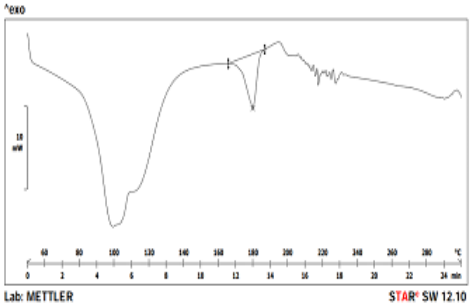


Fig no 7: Compatibility study by DSC Graph for mixture

B- Aloevera Juice

a) UV Spectroscopy:

The *λ*max determination:

It is done by using UV-Spectrophotometer. The wavelength of maximum absorption is found to be 478 nm.

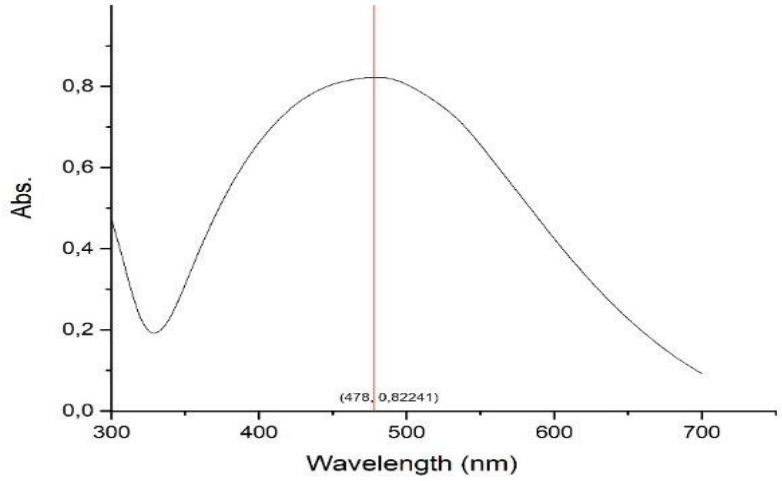


Fig no 8: UV spectra of Alovera

Table 6: Absorbance of Alovera

Concentration(µgmL-1)	Absorbance(nm)
0.1	0.2571
0.2	0.4501
0.3	0.6205
0.4	0.9504
0.5	1.1087
0.6	1.2279

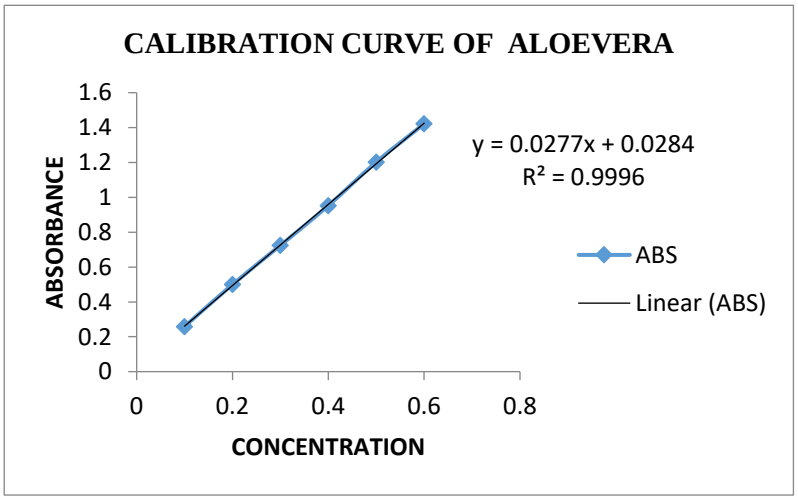


Fig no 9. Linearity Curve for Aloevera

b) Fourier-transform infrared spectroscopy analysis

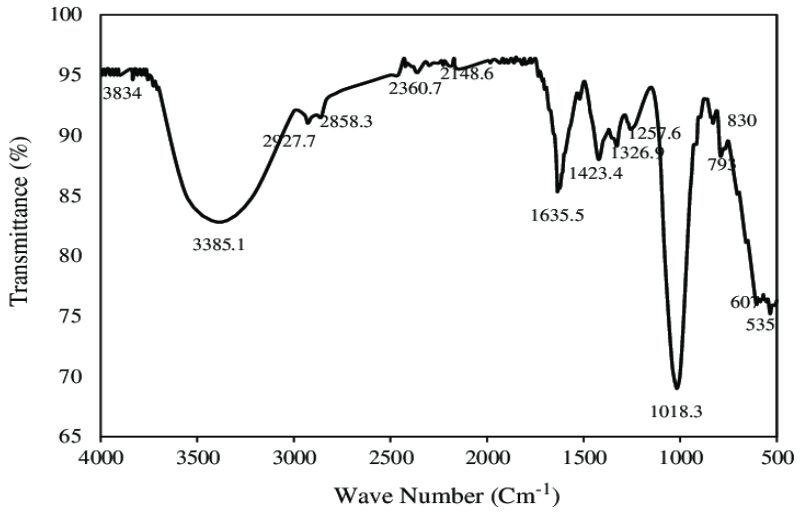


Fig no 10: FTIR Spectra of alovera

C. Onion oil

a) UV Spectroscopy:

The *λ*max determination:

It is done by using UV-Spectrophotometer. The wavelength of maximum absorption is found to be 410 nm.

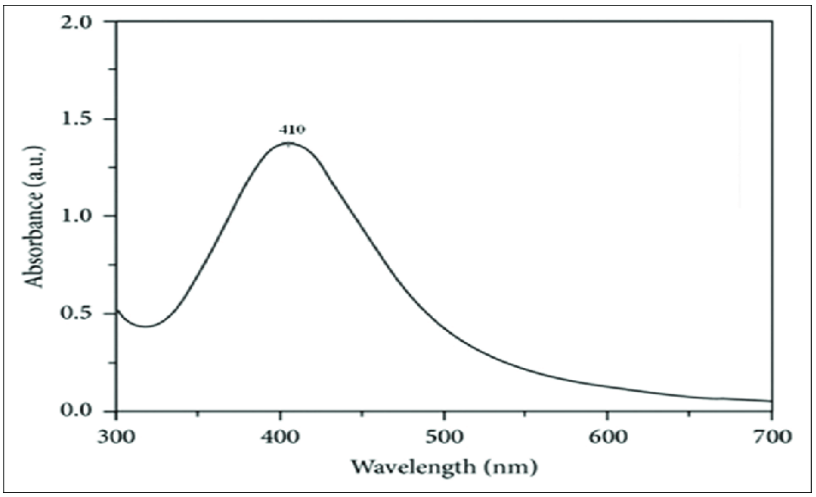


Fig no 11: UV of Onion Oil

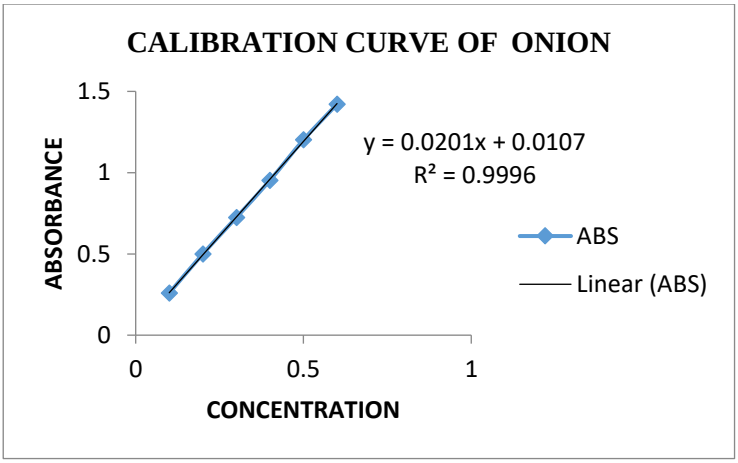


Fig no 12. Linearity Curve for Aloevera

Table 7: Absorbance of Onion

Concentration(µgmL-1)	Absorbance(nm)
0.1	0.2571
0.2	0.4581
0.3	0.7205
0.4	0.9504
0.5	1.2087
0.6	1.3279

b) Fourier-transform infrared spectroscopy analysis

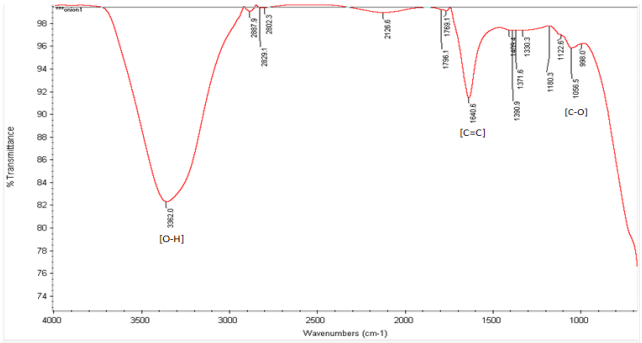


Fig no 13: FTIR Spectra of onion

Table No. 8: Spectrum interpretation of Aloevera

Functional group	**Wave number (cm^1)**
Hydroxyl group OH	3062.0
C–H asymmetric stretching in CH_2	2929.7
C=O Stretching	2858.3

C=C	1604.7
CO group	1056.5

D. Aloevera Juice and onion oil

1. Determination of foreign organic matter:

Foreign organic matter in Aloevera Juice and onion oil was found to be 0.42% w/w and 0.48 w/w when observed under 6X lens.

2. Determination of moisture content:

The observation of the loss on drying of Aloe vera juice showed the following moisture loss percentages: at 0 minutes, 0.00%; at 30 minutes, 0.320%; at 60 minutes, 0.210%; at 90 minutes, 0.170%; and at 120 minutes, 0.183%. Another trial recorded the following values: 0.00% at 0 minutes, 0.315% at 30 minutes, 0.209% at 60 minutes, 0.165% at 90 minutes, and 0.180% at 120 minutes. The values are expressed as mean ± SEM, with n=3.

3. Determination of Ash value:

Ash value is useful in determining authenticity and purity of sample and also these values are important qualitative standards.

The ash value of Aloe vera juice was evaluated in two trials. In the first trial, the total ash value was 2.7%, acid-insoluble ash was 0.32%, and water-soluble ash was 0.9%. In the second trial, the total ash value was 2.4%, acid-insoluble ash was 0.31%, and water-soluble ash was 0.15%. The values are presented as mean ± SEM, with n=3.

4. Determination of Extractive values: -

Ethanol-soluble extractive value was found to be greater than other extractive value; it indicates that compounds present in the leaves are soluble in alcohol in high amount. This might guide us for the isolation of maximum active components form plant.

The extractive values of Aloe vera juice were determined in two trials. In the first trial, the ethanol-soluble extractive value was 15%, and the water-soluble extractive value was 3.7%. In the second trial, the ethanol-soluble extractive value was 17%, and the water-soluble extractive value remained at 3.7%. The values are expressed as mean ± SEM, with n=3.

5.Preliminary Phytochemical Screening of Aloevera Juice

Aloevera were tested for the presence of active principles such as Terpenoid, Steroids, Glycosides, Saponins, Alkaloids, Flavonoids, Tannins, Proteins, Free Amino Acids, Carbohydrate and Vitamin C. Qualitative chemical test used to identify drug quality and purity. The identification, isolation and purification of active chemical constituents are depending chemical methods of evaluation. The preliminary phytochemical screening of Aloe vera juice extracts using methanol, ethanol, and chloroform revealed the presence of various phytoconstituents. Carbohydrates were detected by the Fehling test in all solvents, while other tests like Molish and Benedict were positive in ethanol. Proteins were present in methanol, ethanol, and chloroform extracts, and steroids were found in methanol and ethanol. Terpenoids were observed in methanol and ethanol, while glycosides were negative in all extracts except for the Killer-Killani test in ethanol. Saponins were detected in methanol, and flavonoids were present in ethanol. Alkaloids were found in both ethanol and chloroform, and tannins and phenolic compounds were present in all extracts

6. Preliminary Phytochemical Screening of Onion

Onion Extracts were tested for the presence of active principles such as Terpenoid, Steroids, Glycosides, Saponins, Alkaloids, Flavonoids, Tannins, Proteins, Free Amino Acids, Carbohydrate and Vitamin C. Qualitative chemical test used to identify drug quality and purity. The identification, isolation and purification of active chemical constituents are depending chemical methods of evaluation. The preliminary phytochemical screening of Onion extracts (methanol, ethanol, and chloroform) revealed the presence of various phytoconstituents. Carbohydrates were detected by the Fehling test in all solvents, while the Molish and Benedict tests showed positive results in ethanol. Proteins were present in methanol, ethanol, and chloroform, with steroids detected in methanol and ethanol. Terpenoids were observed in both methanol and ethanol, while glycosides were absent, except in ethanol for the Killer-Killani test. Saponins were found in methanol, and flavonoids were present in ethanol. Alkaloids were detected in ethanol and chloroform, and tannins and phenolic compounds were present in all extracts.

The all extracts were screened for the presence of various constituents. The result of this preliminary phytochemical examination is shown in Table.

The ethanol extract showed presence of significant metabolites like alkaloids, glycoside and flavonoids. So the ethanol extract will utilize for further plan of research work.

7. Characterizations of Nanofibers

After formulating the nanofiber with specified parameters, the batch is optimised i.e. NF2 is considered as optimised batch.

I. Drug Content:

A. Pravastatin Nanofibres

The drug content percentage in the formulation batches was as follows: PF1 had 91.42%, PF2 showed 97.37%, PF3 contained 92.34%, and PF4 had 90.14%.

B. Onion oil

The drug content percentages for the formulation batches were as follows: OF1 had a drug content of 92.04%, OF2 contained 96.28%, OF3 had 93.09%, and OF4 showed a drug content of 91.24%.

C. Aloevera Juice

The drug content percentages for the formulation batches were as follows: AF1 had a drug content of 90.19%, AF2 contained 98.91%, AF3 had 90.02%, and AF4 showed a drug content of 94.19%.

D. Pravastatin+ Onion oil+ Aloevera Juice

The drug content percentages for the formulation batches were as follows: POAF1 had a drug content of 90.37%, POAF2 contained 96.09%, POAF3 had 88.19%, and POAF4 showed a drug content of 94.29%.

II. Entrapment Efficiency (%)

A. Pravastatin Nanofibres

The entrapment efficiency percentages for the formulation batches were as follows: PF1 had an entrapment efficiency of 86.46%, PF2 showed 91.09%, PF3 had 85.12%, and PF4 exhibited an entrapment efficiency of 85.04%.

B. Onion oil

The entrapment efficiency percentages for the formulation batches were as follows: OF1 had an entrapment efficiency of 89.04%, OF2 showed 91.17%, OF3 had 87.01%, and OF4 exhibited an entrapment efficiency of 86.27%.

C. Aloevera Juice

The entrapment efficiency percentages for the formulation batches were as follows: AF1 had an entrapment efficiency of 89.12%, AF2 showed 91.81%, AF3 had 87.82%, and AF4 exhibited an entrapment efficiency of 86.72%.

D. Pravastatin+ Onion oil+ Aloevera Juice

The entrapment efficiency of the Pravastatin, Onion Oil, and Aloe Vera Juice nanofiber formulations (POAF1-POAF4) ranged from 86.61% (POAF3) to 94.84% (POAF2), with POAF1 and POAF4 showing efficiencies of 88.94% and 89.72%, respectively.

III. Drug Loading (%)

A. Pravastatin Nanofibres

The drug loading percentages for the formulation batches were as follows: PF1 had a drug loading of 6.06%, PF2 contained 8.21%, PF3 had 5.09%, and PF4 exhibited a drug loading of 5.19%.

B. Onion oil

The drug loading percentages for the formulation batches were as follows: OF1 had a drug loading of 4.28%, OF2 contained 8.14%, OF3 had 7.51%, and OF4 exhibited a drug loading of 6.19%.

C. Aloevera Juice

The drug loading percentages for the formulation batches were as follows: AF1 had a drug loading of 4.12%, AF2 contained 6.11%, AF3 had 5.08%, and AF4 exhibited a drug loading of 4.29%.

D. Pravastatin+ Onion oil+ Aloevera Juice

The drug loading percentages for the formulation batches were as follows: POAF1 had a drug loading of 4.90%, POAF2 contained 7.27%, POAF3 had 6.67%, and POAF4 exhibited a drug loading of 5.07%.

IV. Diffusion Study :

A. Pravastatin Nanofibres

Table 9: Drug Release of Provastine Fibre (PF1-PF4)

Time (hrs)	PF1	PF2	PF3	PF4
0	0	0	0	0
1	10.24	9.47	12.14	9.87
2	25.37	18.46	21.85	19.48
3	34.31	28.45	30.19	29.16
4	49.78	39.45	41.85	38.67
5	54.32	50.04	51.75	51.48
6	69.47	65.48	66.94	69.82
7	80.45	85.45	81.25	72.48
8	89.28	**94.37**	90.14	88.29

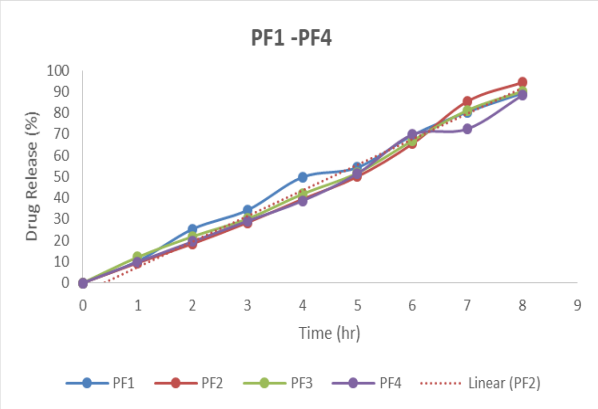


Fig no 14: Drug Release of Provastine Fibre (PF1-PF4)

.B. Onion oil

Table 10: Drug Release of Onion oil Fibre (OF1-OF4)

Time (hrs)	OF1	OF2	OF3	OF4
0	0	0	0	0
1	9.04	9.47	10.29	9.71
2	15.17	18.46	19.13	18.59
3	33.29	28.45	29.07	21.29
4	44.10	39.45	41.42	39.46
5	54.09	50.04	53.43	51.48
6	71.73	65.48	67.37	69.02
7	80.64	85.45	80.76	77.41
8	88.17	**95.37**	89.37	89.09

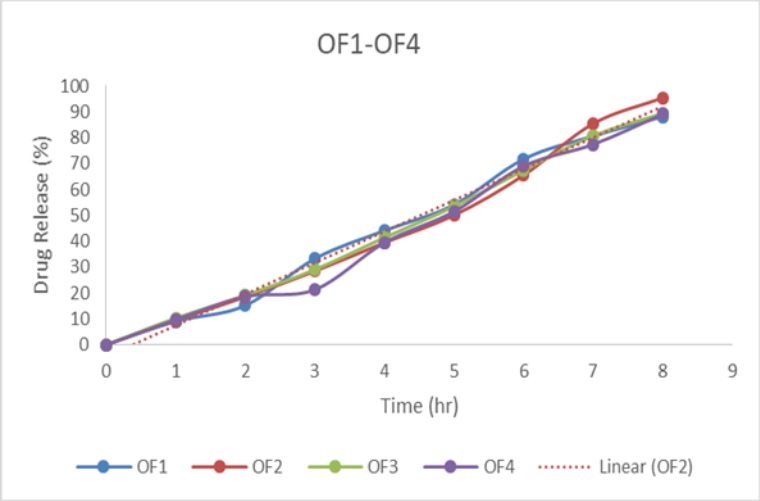


Fig no 15: Drug Release of Onion oil Fibre (OF1-OF4)

C. Aloevera Juice

Table 11 : Drug Release of Onion oil Fibre (AF1-AF4)

Time (hrs)	AF1	AF2	AF3	AF4
0	0	0	0	0
1	10.21	7.28	11.04	10.09
2	15.64	17.09	19.19	19.59
3	30.91	25.94	21.09	27.29
4	41.29	37.51	32.09	38.07
5	55.73	59.73	49.17	49.39
6	68.82	65.17	69.29	61.82
7	80.64	87.04	75.58	81.27
8	89.17	96.37	85.91	91.09

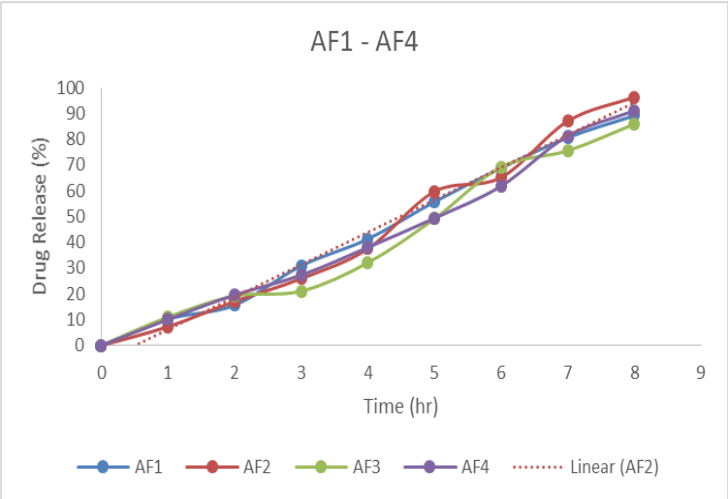


Fig no 16: Drug Release of Onion oil Fibre (AF1-AF4)

D. Pravastatin+ Onion oil+ Aloevera Juice

Table 12 : Drug Release of Onion oil Fibre (POAF1-POAF4)

Time (hrs)	POAF1	POAF2	POAF3	POAF4
0	0	0	0	0
1	8.26	7.23	13.04	9.09
2	19.21	15.73	21.46	17.47
3	35.64	29.10	31.76	21.64
4	45.09	39.43	38.34	35.41
5	59.54	59.73	43.86	47.27
6	65.16	67.37	61.07	69.31
7	79.34	82.79	71.07	79.91
8	87.39	94.04	81.27	89.07

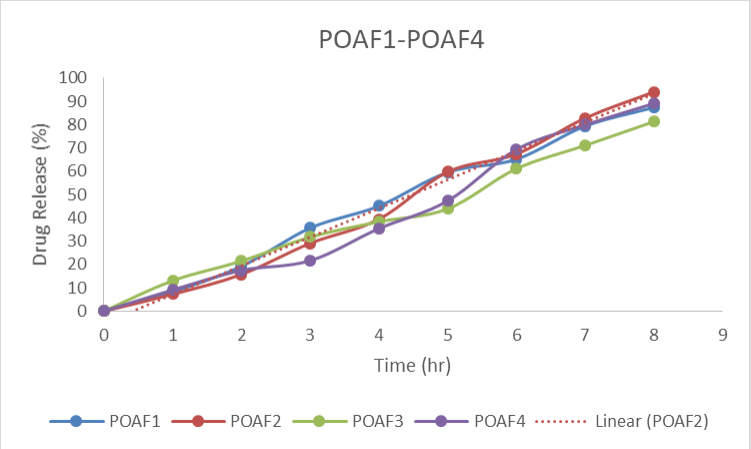


Fig no 17: Drug Release of Onion oil Fibre (POAF1-POAF4)

V. Scanning electron microscopy

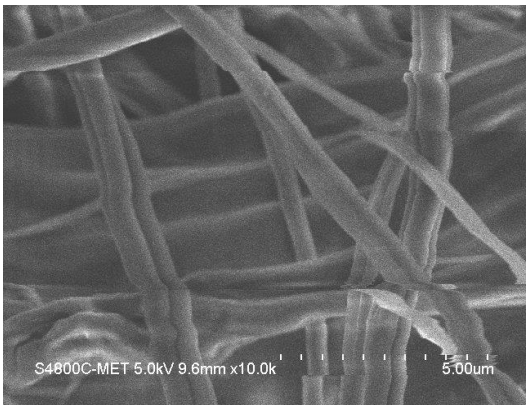


Fig no18: The Nanofibers view under X 5.00 K magnification

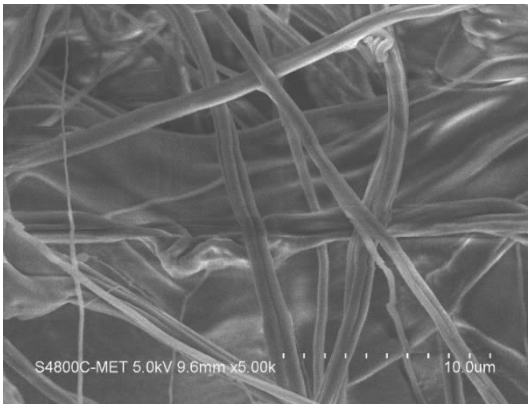


Fig 19: The Nanofibers under X 10.00 K magnification

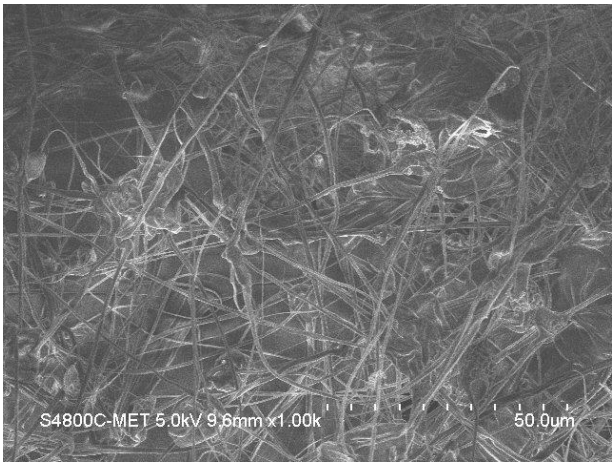


Fig no 20: The Nanofibers view under X 50.00 K magnification

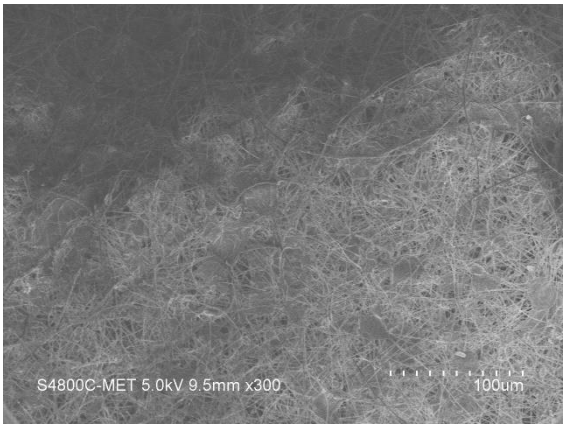


Fig no 21: The Nanofibers view under X 100.00 K magnification

VI. Stability Study :

1. Optimized Batch of Pravastatin nanofibre (PF2)

Table 13 : Optimized Batch of Pravastatin nanofibre (PF2)

Stability parameter	Initial	1 month	2 month	3 month
Drug Content (%)	97.37	97.37	97.36	97.36

Entrapment efficiency (%)	91.02	91.02	91.02	91.00
Drug release (%)	94.37	94.37	94.37	94.36

2. Optimized Batch of Onion oil nanofibre (OF2)

Table 14: Optimized Batch of Onion oil nanofibre (OF2)

Stability parameter	**Initial**	**1 month**	**2 month**	**3 month**
Drug Content (%)	96.28	96.28	96.27	96.27
Entrapment efficiency (%)	91.17	91.17	91.17	91.16
Drug release (%)	95.37	95.37	95.36	95.36

3. Optimized Batch of Aloevera nanofibre (AF2)

Table 15: Optimized Batch of Aloevera nanofibre (AF2)

Stability parameter	**Initial**	**1 month**	**2 month**	**3 month**
Drug Content (%)	98.91	98.91	98.90	98.90
Entrapment efficiency (%)	91.81	91.81	91.81	91.80
Drug release (%)	96.37	96.37	96.36	96.36

4. Optimized Batch Pravastatin+ Onion oil+ Aloevera nanofibre (POAF2)

Table 16: Optimized Batch Pravastatin+ Onion oil+ Aloevera nanofibre (POAF2)

Stability parameter	**Initial**	**1 month**	**2 month**	**3 month**
Drug Content (%)	96.09	96.09	96.08	96.08
Entrapment efficiency (%)	94.84	94.84	94.84	94.83
Drug release (%)	94.04	94.04	94.03	94.03

VII. Invivo activity:

Wound healing activity

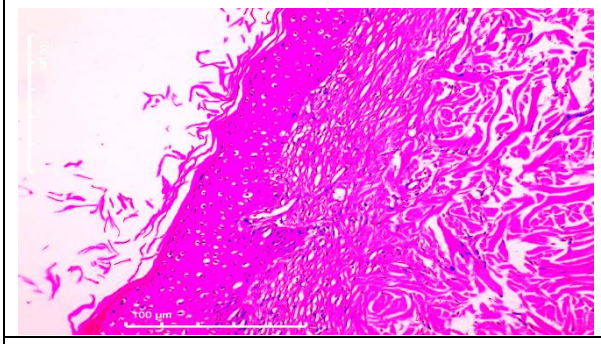
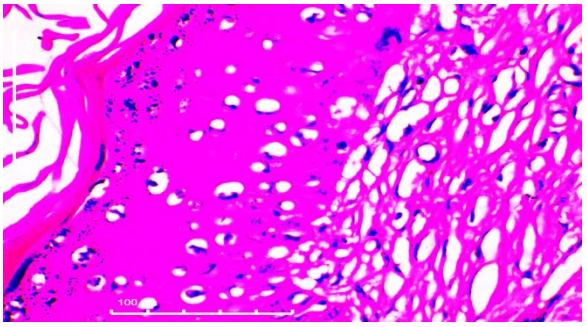
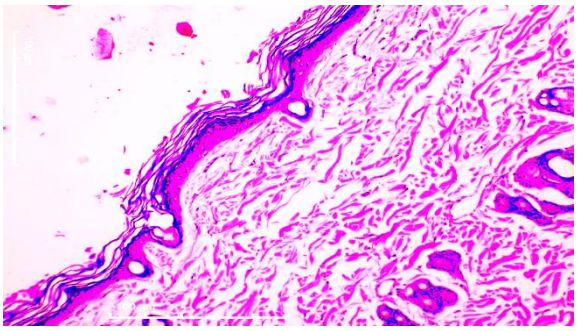
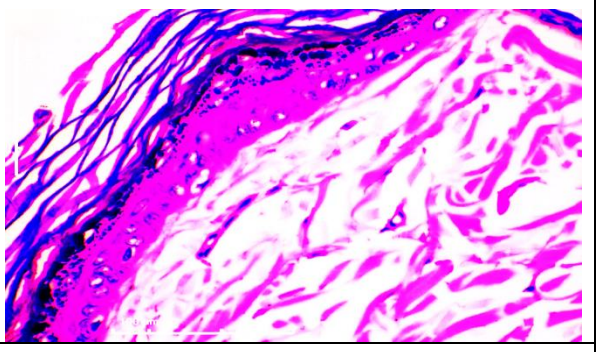
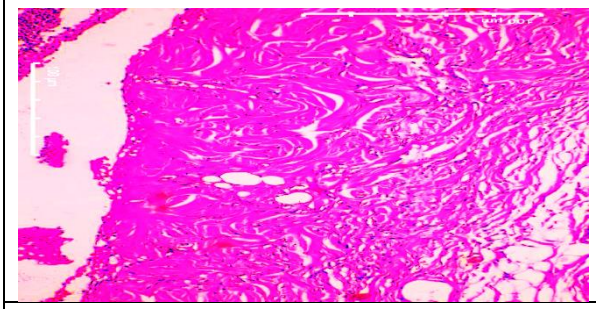
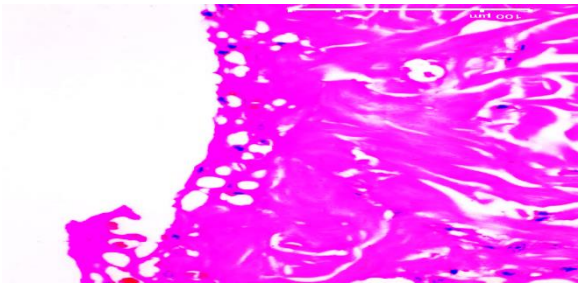
The burn wound healing study compares the efficacy of treatment with **0.9% saline (Group-I)** and **Optimized Film (Nanofiber Formulation) (Group-II)** over a 21-day period. In **Group-I** (Normal Control), the healing process shows a gradual reduction in wound size, with the percentage of healing reaching 44.32% by day 21. The wound size remains relatively larger throughout the study, with measurements consistently higher, ranging from 168.06 ± 12.09 mm on day 1 to 93.57 ± 19.17 mm on day 21. In contrast, **Group-II** (Optimized Film treatment) exhibits more significant healing, with a 21-day healing percentage of 84.17%. The wound size in this group decreases more rapidly, starting at 172.36 ± 10.02 mm on day 1 and reaching 27.28 ± 18.81 mm by day 21. The treatment with the nanofiber formulation shows a substantial improvement in wound healing compared to the saline group, demonstrating faster and more effective healing of burn wounds.

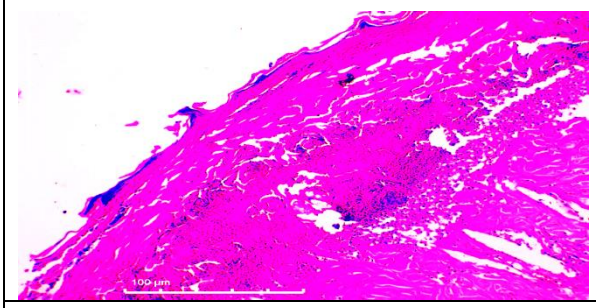
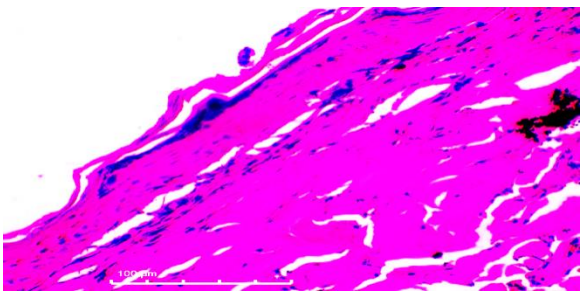
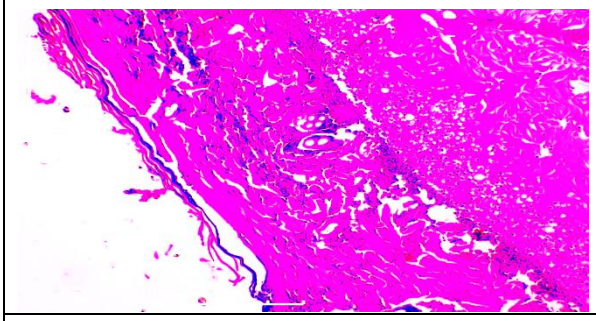
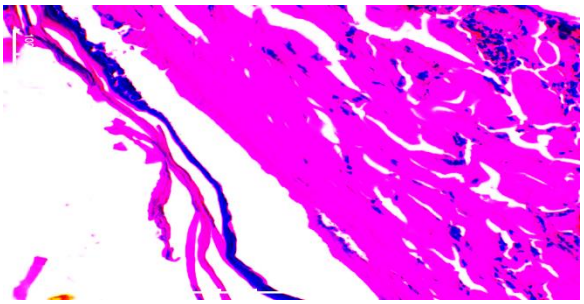
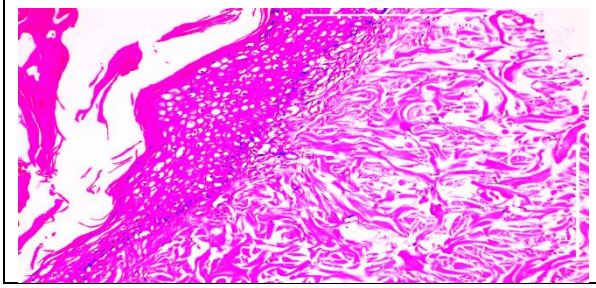
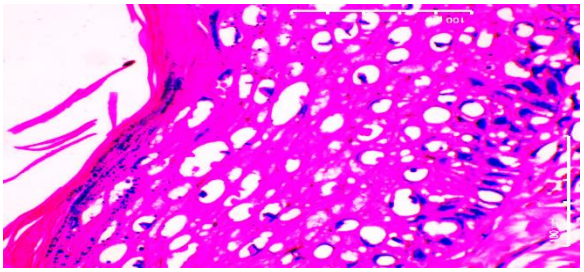
Sr. No	Name of group Treatment	The reading healing of burn wound mean + SD during the day.									percentage of reading healing of burn wound
		1	2	3	4	5	6	7	14	21	21 Days
1	**Normal Control** (Group-I) 0.9% saline	168.06± 12.09	167.34± 17.09	165.96± 19.31	160.72± 09.27	154.86± 11.19	142.78± 21.28	138.41± 21.34	102.48± 17.34	93.57± 19.17	44.32%
2	**Optimised Film** (Group-II) Nanofibers Formulate	172.36± 10.02	170.29± 16.12	161.28± 19.72	145.45± 27.32	122.67± 17.65	108.51± 13.42	94.34±21.01	56.67±12.04	27.28±18.81	84.17%

HISTOPATHOLOGY:

The histological examination of the skin samples from both the **Normal Control (NC)** and **Film-treated groups** reveals distinct findings. In the **Normal Control group (NC-1, NC-2, and NC-3)** at both 10X and 40X magnifications, the skin samples show normal structure and architecture, with no epithelial changes observed. All layers of the skin appear intact, and no abnormalities are detected, indicating healthy and undamaged tissue. In contrast, the **Film-treated groups (Film-1, Film-2, and Film-3)** exhibit similar overall structure, but **Film-2** shows mild changes in the epithelial layer, suggesting slight abnormalities. These changes are not severe, but the presence of mild epithelial alterations in **Film-2** indicates some degree of response or irritation, though no major structural damage is noted. Overall, while the **Film-treated groups** maintain a generally normal skin structure, mild changes are observed in the epithelial layer, particularly in **Film-2**, which may warrant further investigation.

Normal structure and architecture. No epithelial	**NC-1 (40X)**

changes were observed all the layers of skin were intact. No abnormality detected. **NC-1 (10X)**	
	
Normal structure and architecture. No epithelial changes were observed all the layers of skin were intact. No abnormality detected. **NC-2 (10X)**	**NC-2 (40X)**
	
Normal structure and architecture. No epithelial changes were observed all the layers of skin were intact. No abnormality detected. **NC-3 (40X)**	**NC-3 (40X)**
	
Normal structure and architecture. No epithelial changes were observed all the layers of skin were intact. No abnormality detected. **Film-1 (10X)**	**Film-1 (40X)**

	
Normal structure and architecture. No epithelial changes were observed all the layers of skin were intact. No abnormality detected. **Film-2 (10X)**	**Film-2 (40X)**
	
Normal structure and architecture but mild changes were observed in the epithelial layer. Mild abnormality detected. **Film-3 (10X)**	**Film-3 (40X)**
	

8. SUMMARY AND CONCLUSION:

The study assessed the organoleptic properties, solubility, UV, FTIR, DSC, and phytochemical components of Pravastatin, Aloe vera juice, and Onion oil. Pravastatin was white to yellowish, Aloe vera juice had a greenish hue with a mild odor, and Onion oil was brownish-yellow with a sulfide scent. Solubility testing showed Pravastatin was least soluble in phosphate buffer, while Onion oil was better soluble in methanol. UV and FTIR analyses confirmed no significant interactions between Pravastatin and excipients, ensuring compatibility for nanofiber formulation, which was further supported by DSC results. Phytochemical screening revealed bioactive compounds in Aloe vera and Onion oil, suggesting their therapeutic potential. The nanofiber formulations exhibited good drug content, entrapment efficiency, and sustained drug release. Stability studies showed minimal changes over three months, and the in vivo wound healing study demonstrated a significant improvement in burn healing (84.17%) compared to the control group (44.32%). Histopathology indicated mild epithelial changes in some treated samples, confirming the nanofibers' potential for effective wound healing.

9. CONFLICT OF INTEREST

Nil

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