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DEVELOPMENT AND EVALUATION OF SILVER SULPHADIAZINE LOADED NANOFIBROUS SCAFFOLD FOR WOUND DRESSING APPLICATIONS

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Keywords:

Medicated nanofiber, Biodegradability, Wound healing, Electrospinning, Drug delivery

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ABSTRACT: Electrospinning is the fiber-forming process using an electric field where electrostatic forces are employed. By this process, continuous nanofibers range from 0.01 to 10 micrometers can be obtained. Nanofibers produced by the electrospinning technique has application in various fields such as tissue engineering, wound dressing, filtration, protective clothing, drug delivery, and sensor applications. This study aimed to develop a novel bio medicated nanofibrous scaffold for wound dressing applications. Nanofiber of polyvinyl alcohol and sodium alginate loaded with silver sulphadiazine were successfully prepared by the electrospinning method. The formulations were subjected to various physicochemical evaluations such as weight variation, surface pH, folding endurance, moisture evaluation studies, biodegradability, and in vitro drug release studies. All the prepared nanofibers were found to be transparent, flexible with a smooth surface. The physicochemical characteristics of Medicated nanofibers were satisfactory concerning weight variation, surface pH, folding endurance, moisture loss, and moisture uptake studies. The drug entrapment was in the acceptable range for all the formulations, indicating a uniform distribution of the drug. Nanofibers prepared using sodium alginate, and polyvinyl alcohol showed satisfactory biodegradability. Thus, a polyvinyl alcohol-SA wound dressing system containing silver sulphadiazine could be a good candidate in wound care.

INTRODUCTION: Chronic non-healing wounds show delayed and incomplete healing processes and in turn expose patients to a high risk of infection. Treatment currently focuses on dressings that prevent microbial infiltration and keep a balanced moisture and gas exchange environment.

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Electrospinning is a simple, cost-effective, and reproducible process that can utilize both synthetic and natural polymers to address these specific wound challenges [1]. Electrospinning is a fiber-forming process by applying electric potential where electrostatic forces are employed to control the production of fibers.

It is closely related to the more established technology of electro spraying, where the droplets are formed. Electrospinning readily leads to the formation of continuous fibers ranging from 0.01 to 10 μm. The development of nanofibers by electrospinning process has led to potential

applications in filtration, protective clothing, and biological applications such as tissue engineering scaffolds and drug delivery devices [2]. Electrospun nanofiber mat is a good wound dressing candidate because of its unique properties: the highly porous mat structure and well-interconnected pores are particularly important for exuding fluid from the wound; the small pores and very high specific surface area not only inhibit the exogenous microorganism invasions but also assist the control of fluid drainage; in addition, the electrospinning process provides a simple way to add drugs into the nanofibers for any possible medical treatment and antibacterial purposes [3]. Many natural and synthetic polymers have been tried to obtain fiber-based scaffolds. This formulated scaffold could mimic the extracellular matrix (ECM) environment of a specific tissue by surface modifications.

These fibers allow easy adherence to seeded cells, potent proliferation, and differentiation with the growth factors. These nanofibers are highly porous with proper interconnection among their pores, exhibit an increased ratio of surface area to volume, are highly flexible, and show blending with other copolymers during the process of electrospinning. Alginate is a natural heteroglycan formed by the combination (1, 4)-b-D-mannuronic acid and (1, 3)-a-L-guluronic acid and obtained from brown seaweed. Sodium alginate is water-soluble, biocompatible, biodegradable polyelectrolyte used as a natural polymer in various applications related to tissue engineering, drug delivery, wound dressing, etc.

Attempts have been made to electrospun alginate solutions blended with several other polymers to fabricate nanofibers as pure alginate cannot be electrospun. Different polymers blended with alginate can be glycerol, polyvinyl alcohol (PVA), polyethylene oxide (PEO), *etc*., to enhance its mechanical behavior and electro spinnability 4, 5. In this work, we have blended SA with PVA for making them into nanofibers. Polyvinyl alcohol (PVA) is a polyhydroxy synthetic polymer that provides easy access to sodium alginate, making it elecrospinnable by forming an intermolecular hydrogen bonding. PVA is soluble in water and has chemical stability, high chemical resistance, and biodegradability. PVA has been replicated in many studies as an additive synthetic copolymer with natural polymers to undergo easy electrospun nanofiber fabrication processes. In SA/PVA electro-spinning, PVA interacts with SA through hydrogen bonds to form SA/PVA composite fibers. In this work, we developed SA/PVA/AgSD nanofibrous mats for wound dressing applications.

MATERIALS AND METHODS:

Materials: Silver Sulphadiazine was obtained as a gift sample from Galentic India Pvt. Ltd Mumbai. Sodium alginate (SA) (medium molecular weight) and Poly (vinyl alcohol) (PVA) (medium molecular weight) were purchased from Nice chemicals, Kochi. Lysozyme was obtained from Yarrow Chem Pvt. Ltd, Mumbai. Gluteraldehyde was purchased from Chemdyes Corporation, Rajkot. All other agents were analytically pure. The polymer and the solvent were used without further purification.

Fabrication of Medicated Nanofibrous Scaffold [6, 7]: 2% sodium alginate solution was prepared by adding 2 grams of sodium alginate powder into 100 ml of purified water with constant agitation for 4 h at room temperature. 10% PVA solution was prepared by dissolving 10 grams of PVA into 100 ml of purified water at 60 ºC baths with gentle magnetic stirring for 4 h. To prepare the blend solutions, mix the above solutions of Sodium alginate and PVA in the volume ratios of 65/35, 75/25 and 70/30 separately. Calculated quantity of Silver Sulfadiazine dissolved in a minimal quantity of ammonia solution was added to the blend to contain 1% drug to the dry polymer weight.

The blend was stirred continuously for 2 h at room temperature for proper mixing and internal binding in the solution. The as-prepared solutions were loaded into the plastic syringe (10ml), and this was placed inside the electrospinning machine Espin Nano. A blunt-ended 22-gauge stainless steel needle was connected to the syringe, which acted as the nozzle. The emitting electrode from a Gamma High Voltage Research ES30P power supply capable of generating DC voltages up to 30 kV was attached to the needle. The grounding electrode from the same power supply was attached to a rectangular piece of aluminium foil which acted as a collector plate. Upon application of a high voltage ranging between 15-30 kV across the needle and the collector plate, a fluid jet was initiated from the nozzle and accelerated towards

the collector. The solvent gradually evaporated, leaving only ultrathin fibers on the collector. AS this process continued for a considerable time period, the deposited fiber turns into a thin film. The obtained nanofibrous scaffold was left exposed to air for complete drying.

Crosslinking of Sodium Alginate/PVA/ Nanofibrous Scaffold [6]: As both Sodium alginate and PVA are readily soluble in water; their hydrophilic property should be reduced for its prolonged use as wound protective film and drug depot. The fibrous scaffold was first treated with 2% glutaraldehyde vapor in a desiccator for 48 h and then dipped in 1% $CaCl_2$ in ethanol for 1 h in order to crosslink the polymer, thereby decrease their hydrophilic property. Glutaraldehyde is a universal crosslinking agent for polymers, and calcium chloride is reacted with sodium alginate to form insoluble calcium alginate. The crosslinked nanofibers were then dipped in water for 48 h to confirm their water-insoluble nature.

Selection of Range of Critical Parameters For Optimization: A number of trials were run to identify the critical parameters that could affect fiber diameter, biodegradability, and drug release. The factors that varied during the trial were polymer ratio, tip to collector distance, and voltage. Based on the fiber diameter, the polymer solution ratio was fixed at 65/35, 70/30 and 75/25. Similarly, the voltage was fixed at 17, 20, and 23 kV. The tip to collector distance was kept at 20, 24, and 28 cm Formulation table for the Medicated nanofibrous scaffold as per the Factorial design given in **Table 1.**

TABLE 1: FORMULATION TABLE FOR THE MEDICATED NANOFIBROUS SCAFFOLD AS PER THE FACTORIAL DESIGN

S. no	Formulation code	Polymer solution ratio (PVA:SA) (coded)	Voltage (coded)	Distance (coded)	Polymer solution ratio (PVA:SA)	Voltage (kV)	Distance (cm)
1	F1	1	-1	-1	75:25	17	20
2	F2	1	1	1	75:25	23	28
3	F3	-1	1	-1	65:35	23	20
4	F4	-1	-1	1	65:35	17	28
5	F5	-1	-1	-1	65:35	17	20
6	F6	-1	1	1	65:35	23	28
7	F7	1	1	-1	75:25	23	20
8	F8	1	-1	1	75:25	17	28
9	F9	0	0	0	70:30	20	24
10	F10	0	0	0	70:30	20	24
11	F11	0	0	0	70:30	20	24
12	F12	0	0	0	70:30	20	24

Evaluations of the Medicated Nanofibrous Scaffold:

Physical Appearance: All the nanofibrous scaffolds were visually inspected for color, clarity, flexibility, and smoothness.

Morphological Characterization by SEM Analysis [8, 9]: Fiber morphology, texture, and dimensions of the fibers were studied using a scanning electron microscope with an accelerating voltage of 20 kV. Obtained images were analyzed using Image J software for the calculation of the average diameter of the nanofibers.

Determination of Porosity [10, 11]: The porosity was determined using image analysis algorithms by MATLAB (Math Works, Version 7) based on the global thresholding method. According to the global thresholding, the binary images were created, and a single constant threshold was used to segment the images. All pixels up to and equal to the threshold were considered the object, and the remaining ones belonged to the background. Percent of porosity was determined as below:

$$P = (1-n/N) \times 100$$

Where, 'n' is the number of white pixels, 'N' is a total number of pixels in the image and 'P' is the percent of porosity.

Weight Variation [12]: Individual batches of Nanofibrous scaffold of size (2 × 2 cm²) were cut exactly at three different places, and the weight of each scaffold was taken on an electronic balance

and the average weight and standard deviation were calculated.

Surface pH [13]: The surface pH of nanofibrous scaffold was determined in order to investigate the possibility of any adverse effects in-vivo. The nanofibrous scaffold (1 cm × 1 cm) was allowed to swell in a closed Petri dish at room temperature for 30 min in 5 ml of distilled water. A swollen scaffold was removed and placed under digital pH meter to determine the surface pH.

Folding Endurance [14]: Folding endurance was determined to find the flexibility of nanofibrous scaffold, which is essential for the handling, comfort, and secured application of nanofiber on the wound. Folding endurance was measured by folding the nanofibers at the same place till it breaks. The number of times it could be folded at the same place without breaking gave the exact value of folding endurance.

Percentage Moisture Content [15]: The prepared nanofibers were weighed individually and kept in a desiccator containing calcium chloride at room temperature for about 24 h. The nanofibers were weighed repeatedly until they showed a constant weight. Values for the percentage of moisture content were calculated using the formula

Percentage of moisturecontent = (Initial weight - Final weight) / (Final weight) × 100

Percentage Moisture Uptake: The weighed nanofibers were kept in a desiccator at room temperature for 24 h and then exposed to 84% RH using a saturated solution of potassium chloride.

The nanofibers were weighed repeatedly until they showed a constant weight. Values for the percentage of moisture uptake were calculated using the formula.

Percentage of moisture uptake = (Final weight-Initial weight) / (Initial weight) × 100

Drug Content Estimation: Nanofibers were cut into 2 × 2 cm and taken in a 100 ml standard flask. Dissolved the contents in ammonia solution, made up to the volume with distilled water, and subjected to continuous shaking in a shaker for 3 h. After proper dilution, the absorbance was measured at 254 nm using a UV Visible Spectrophotometer.

Percentage drug content = (Practical content) / (Theoretical content) × 100

Biodegradability Test [16, 17]: The In- vitro degradation of Medicated nanofibers (2 × 2 cm) was carried out in 1mlas phosphate buffer solution (PBS, PH 7.4) at 37 °C containing 1.5 µg/ml lysozyme. The concentration of enzyme was chosen to correspond to the concentration in the human serum. Briefly, nanofibers of known dry weights were sterilized by autoclaving (120 °C, 20 min) and incubated in the lysozyme solution with gentle mechanical agitation during the study period. The lysozyme solution was refreshed daily to ensure continuous enzyme activity. The samples were withdrawn at definite time intervals from the medium, rinsed with distilled water, dried under vacuum, and weighed. The in vitro degradation was expressed as a percentage of weight loss of the dried nanofibers on every alternate day during the lysozyme treatment. To separate between enzymatic degradation and dissolution, control samples were stored under the same conditions as described above, but without the addition of lysozyme.

Cumulative Drug Release: The cumulative drug release study was done simultaneously with the biodegradability test. The drug content in the replaced enzymatic media of the biodegradability test was estimated spectrophotometrical at 254 nm.

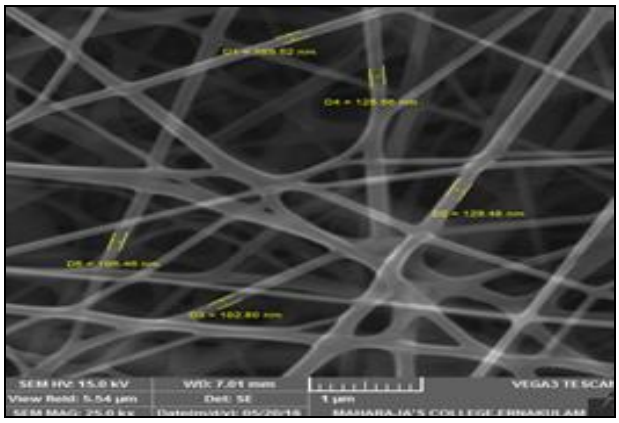
RESULTS AND DISCUSSION:

Physical Appearance: All the drug-loaded nanofibers were found to be transparent, flexible with a smooth surface.

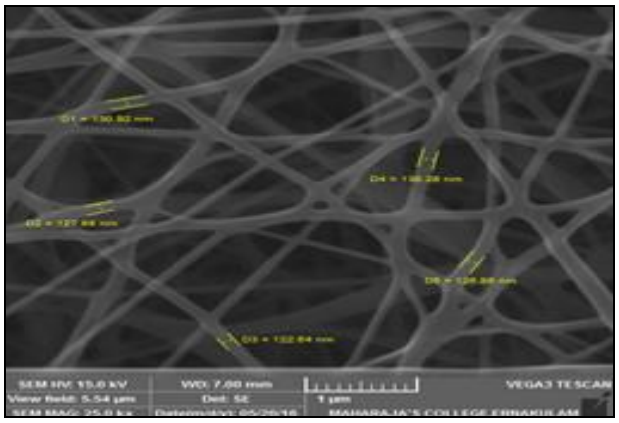
Morphological Characterization by SEM Analysis Fiber Diameter: Morphology of nanofibrous scaffold was examined by their SEM images. Uniform fibers were formed at different blended ratios.

A decrease in fiber diameters was observed when the volume ratio of sodium alginate was increased into the polymer blend.

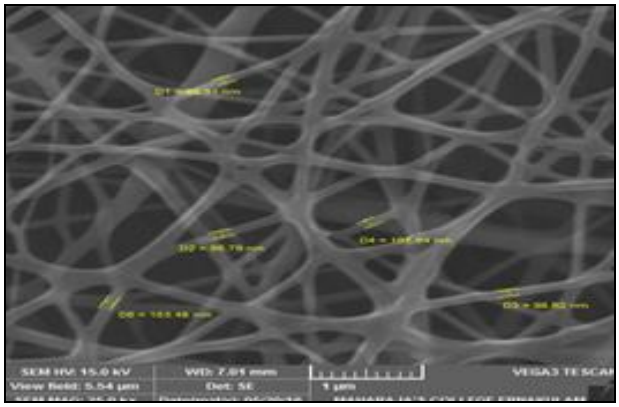
It was found that the fibers obtained from different formulations have a diameter range of 60 nm-200 nm. The formulation F4 has the minimum diameter without any imperfections (beads formation). SEM images of formulations was given in **Fig. 1.**



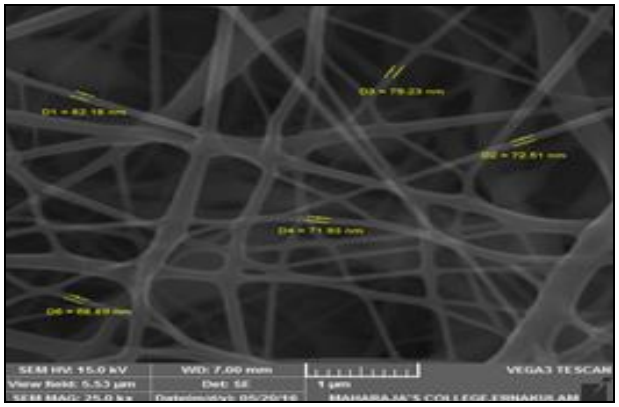
SEM IMAGE OF FORMULATION F1



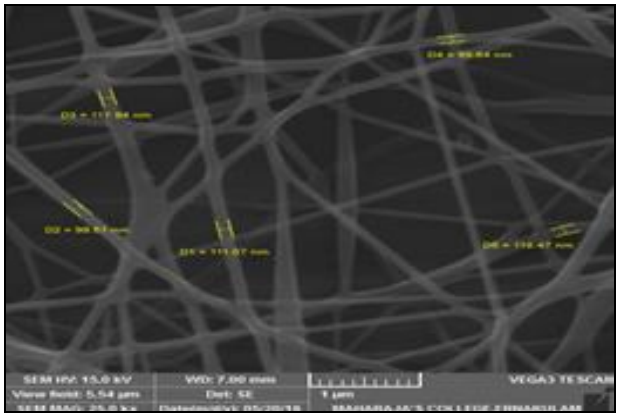
SEM IMAGE OF FORMULATION F2



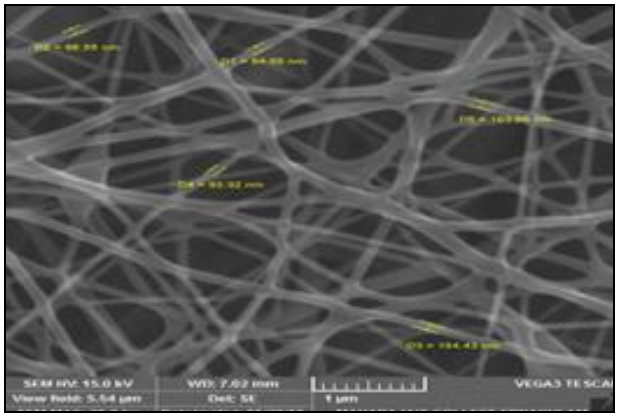
SEM IMAGE OF FORMULATION F3



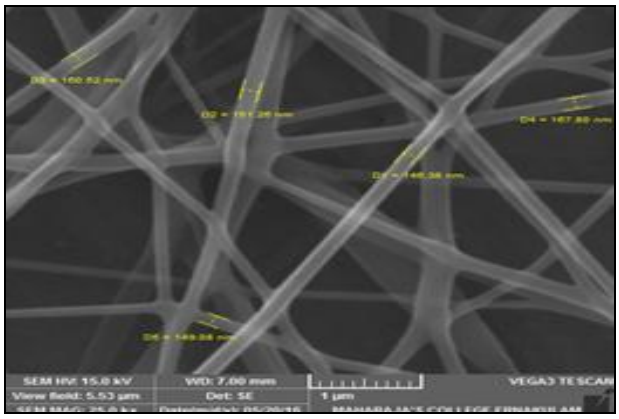
SEM IMAGE OF FORMULATION F4



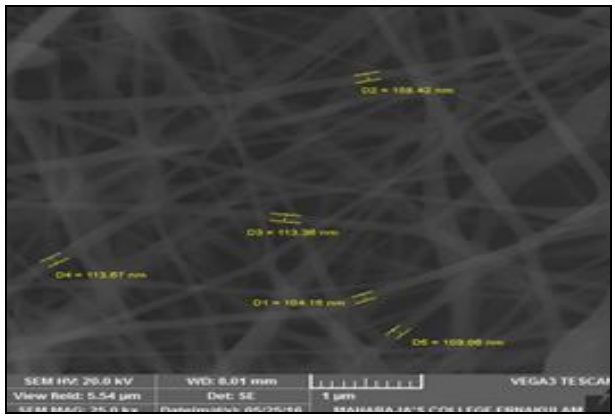
SEM IMAGE OF FORMULATION F5



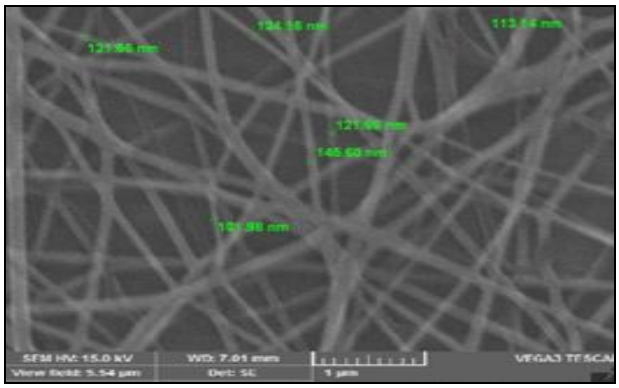
SEM IMAGE OF FORMULATION F6



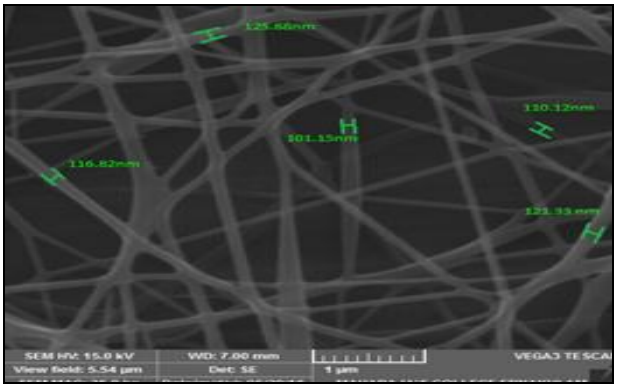
SEM IMAGE OF FORMULATION F7



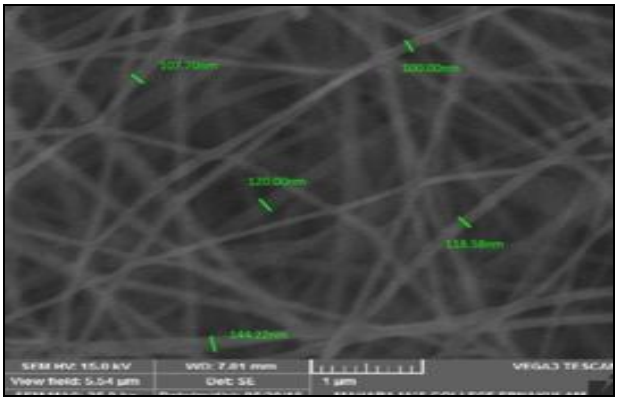
SEM IMAGE OF FORMULATION F8



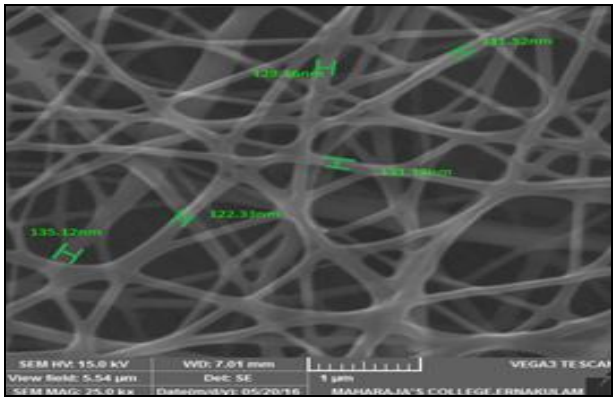
SEMIMAGE OF FORMULATION F9



SEM IMAGE OF FORMULATION F10



SEM IMAGE OF FORMULATION F11



SEM IMAGE OF FORMULATION F12

FIG. 1: SEM IMAGE OF PREPARED FORMULATIONS

Percentage Porosity: The porosity was determined using image analysis algorithms by MATLAB. Results revealed that the porosity of all formulations varied from 36.55 to 62.48%. It was observed that formulation F4 showed maximum percentage porosity, and formulation F7 showed minimum percentage porosity.

Weight Variation: Weight variation studies were conducted on all the formulations and reported in the table. Results showed that the mean weight of the nanofibrous scaffold ranged from 19.84 ± 1.54 mg to 24.08 ± 0.43 mg. The weight uniformity data for all the formulations showed no significant difference in the weight of individual formulations from the average value, and the variations were within low standard deviation. Low deviation values in weight variation measurements ensured uniformity of the nanofibrous scaffolds.

Surface pH: The surface pH of all the formulations ranged from 6.28 ± 0.27 to 7.31 ± 0.06. Since the surface pH of the nanofibrous scaffold was around the neutral range, there will not be any irritation or allergic reaction on the skin, wound and surrounding area.

Folding Endurance: In order to evaluate the brittleness/flexibility, the nanofibrous scaffolds were subjected to a folding endurance test. The values were in between 337 ± 0.96 to 459 ± 0.69 foldings. This revealed that the prepared nanofibrous scaffolds had the capacity to withstand the mechanical pressure along with good flexibility.

Moisture Content, Moisture Uptake: Moisture content and moisture uptake studies were conducted on all the formulations and reported in the table. Moisture evaluation studies indicated that the formulation F4 showed high percent moisture content and maximum moisture absorption than the other formulations.

Moisture content and moisture uptake studies indicated that the increasing conc. of sodium alginate may be attributed to the hygroscopic nature of the polymeric nanofibers.

Drug Entrapment: The percentage of drugs entrapped in various formulations ranged from 90.23% to 97.75%. All the formulations showed the presence of high drug entrapment. The drug

entrapment was found to be in the acceptable range for all the formulations, indicating the uniform distribution of the drug.

Results of physico chemical evaluations of medicated nanofibrous scaffold given in **Table 2.**

TABLE 2: PHYSICO CHEMICAL EVALUATIONS OF MEDICATED NANOFIBROUS SCAFFOLD

Formulation Code	Average Diameter (nm)	Percentage porosity (%)	Weight Variation (mg/4cm^2)	Surface pH	Folding endurance	Moisture Content (%)	Moisture Uptake (%)	Entrapment efficiency (%)
F1	114.76	38.96	22.96±0.63	6.53±0.03	337±0.96	2.55±0.98	6.66±0.64	93.38
F2	127.71	43.70	23.24±1.24	7.21±0.09	350±1.85	2.39±1.12	7.03±1.75	93.59
F3	100.68	45.39	21.64±0.75	6.73±0.17	397±4.51	3.96±0.85	8.11±0.75	94.41
F4	74.94	65.48	21.84±0.57	6.81±0.26	442±3.04	5.01±1.05	10.67±0.89	97.75
F5	107.72	51.28	24.08±0.43	6.92±0.11	459±0.69	4.90±1.31	9.22±1.54	96.82
F6	98.78	59.81	21.12±1.15	7.31±0.06	415±2.21	4.67±0.95	9.89±0.56	96.12
F7	155.00	35.55	21.53±1.52	6.72±0.19	338±3.25	2.11±1.16	6.28±0.91	90.23
F8	109.73	48.65	20.69±0.27	6.69±0.23	371±1.55	3.89±0.66	7.61±1.21	94.67
F9	123.03	51.59	22.27±1.74	6.28±0.27	380±0.88	3.79±0.35	8.52±0.88	95.99
F10	115.02	53.51	22.93±0.57	6.81±0.68	351±1.63	4.06±0.53	7.34±0.45	90.31
F11	118.1	44.92	19.84±1.54	7.11±0.55	402±4.22	3.51±1.26	7.57±0.96	92.77
F12	125.69	47.33	21.47±0.47	6.49±0.13	388±0.84	3.75±1.63	7.88±1.94	95.18

Biodegradability Test and Cumulative Drug Release: The in-vitro degradation study revealed that the polymers can be degraded by lysozyme, which indicated their controllable biodegradability. Formulation F4 shows maximum biodegradability since its weight loss was 86.12% on the10th day. And F4 also shows maximum drug release, which is 97.68% on the 7th day. Results of bio degradation study was given in **Table 3.**

Formulation F4 also shows maximum drug release, that is 97.68% at the 7th day. Results of Percentage cumulative drug Release of medicated nanofibers was given in **Table 4.**

TABLE 3: RESULTS OF BIODEGRADATION STUDY

Formulation Code	Percentage (%) weight loss at different days.				
	2nd day	4th day	6th day	8th day	10thday
F1	18.46	27.77	45.41	60.46	72.81
F2	14.79	27.71	44.17	61.19	73.25
F3	17.87	35.12	47.93	62.78	77.64
F4	18.35	34.18	50.53	69.12	86.12
F5	17.80	33.10	49.54	64.08	80.39
F6	19.27	33.72	50.12	65.33	82.73
F7	14.35	26.13	43.89	58.97	70.55
F8	16.61	28.25	43.21	63.26	75.04
F9	15.53	30.82	48.33	64.27	75.22
F10	14.33	27.92	46.87	63.74	78.49
F11	16.64	30.55	43.25	60.29	76.13
F12	15.74	28.79	47.53	64.98	75.88

TABLE 4: PERCENTAGE CUMULATIVE DRUG RELEASE OF MEDICATED NANOFIBERS

Formulation code	Time in days						
	1		1		1		1
F1	11.99	20.96	33.02	47.56	60.68	70.56	82.8
F2	9.88	22.45	32.65	45.67	58.82	70.77	78.03
F3	12.45	23.48	41.61	53.18	63.88	77.64	87.56
F4	13.73	28.21	44.97	57.4	71.55	85.03	97.68
F5	15.24	28.81	39.46	52.01	60.39	78.54	91.25
F6	14.39	25.87	40.63	55.67	67.8	81.07	92.83
F7	8.34	18.33	30.82	45.72	60.77	72.07	81.72
F8	11.79	25.38	40.23	50.84	62.54	74.64	86.45
F9	10.16	25.92	42.92	53.49	65.05	78.33	88.9
F10	12.21	20.62	33.79	48.96	62.07	74.63	87.68
F11	13.54	20.14	37.81	52.51	66.39	80.58	90.12
F12	10.55	22.72	30.76	50.38	63.73	73.13	85.18

CONCLUSION: Medicated nanofibrous scaffold of silver sulphadiazine was successfully developed and evaluated. All the prepared nanofibers were found to be transparent, flexible with a smooth surface. Their SEM images examined the morphology of electrospun nanofibers. Uniform fibers were formed at different blended ratios. The percentage porosity of the nanofibrous scaffold was found to range between 36.55 to 62.48%. The results of physicochemical characteristics of Medicated nanofibrous scaffold were satisfactory concerning thickness, weight variation, surface pH, folding endurance, and moisture loss and moisture uptake studies.

The drug entrapment was in the acceptable range for all the formulations, indicating the uniform distribution of the drug. Nanofibrous scaffold prepared using sodium alginate, and PVA showed satisfactory biodegradability. The *in-vitro* drug release studies showed formulation F4 has high drug release as compared to other formulations.

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CONFLICTS OF INTEREST: The authors declare that there is no conflict of interest.

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