"Synthesis, Characterization, and Wound-Healing Applications of SFNPs for medicated Fabrics"

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ABSTRACT:

Silk fibroin (SF), derived from the *Bombyx mori* is a promising biopolymer for biomedical and pharmaceutical applications, such as the clothing industry, textile industry, trade, and commerce. This is because silk is well-known for its biocompatibility, softness in texture, anticancer, anticoagulant, antioxidant, wound healing, medicinal properties, etc. Nanoparticles are characterized for their drug delivery and cancer treatment properties. Hence, silk can be used to derive the fibroin nanoparticles. This project includes the procedure of extraction of SNFPs and their characterization using various methods like UV-VIS spectrophotometer, FTIR, EDS, FE-SEM, SEM, and XRD. This study also focuses on the manufacturing of the medicated fabric coated with SNFPs and proving its blood clotting property which throws light on its medical uses in making bandages, sanitary napkins, wipes, gauges, etc. due to its excellent wound healing properties proved in this paper.

Keywords: SFNPs, Characterization, Nanotechnology, Wound healing, Medicated fabrics

INTRODUCTION:

Silk plays a vital role in numerous commercial applications. Apart from it silk also has its applications in fields like cosmetics and medicines. Silk mainly consists of two proteins Fibroin and Sericin, in a ratio of 75:35% respectively. Silk Fibroin is well known bio material because of its mechanical properties, bio-degradability, bio-compatibility and amino acid composition. Silk fibroin (SF), derived from *the Bombyx mori* silkworm, is a natural polymer known for its unique amphiphilic nature, compatibility with biological systems, ability to degrade naturally, strong mechanical strength across different forms, and versatile processing capabilities

The mechanical properties of the silk fibroin nano-particles can be enhanced by the modifications in the crystalline structures of its associated amino acids by changing various parameters. Fibroin protein

consists of 18 various types of amino acids, mainly Alanine, Glycine, Serine, etc.[1] Silk fibroin is considered to be the linear, water-insoluble protein with high tensile strength that makes up about 75% of the silk cocoon. Silk Fibroin protein contains 3 major components which comprise a heavy chain, a light chain, and a glycoprotein of molecular weight 391kDa, 26kDa, 25-30kDa, respectively. Fibroin also contains two domains one is hydrophilic and another one is hydrophobic. The entire poly-peptide chain approx. consists of 5,263 amino acids, organized into two distinct domains This fibroin protein was used for obtaining fibroin-based nanoparticles.[3]

Paul Ehrlich for the first time proposed a tiny submicron named ‘magic bullet’ which were known for its targeted delivery to a cell avoiding the unwanted tissues or cells. And one of these magic bullets is the nanoparticles. These nanoparticles provide large clinical benefits when they are encapsulated, entrapped, absorbed or chemically bonded to some other drug molecules. Functions that nanoparticles provide include: i) protection to the drug molecules from degradation, ii) increasing the permeability of the drug molecule, iii) targeting the site of interest only, iv) increasing the cellular uptake.[5]

Nanoparticles are typically classified into three main types: a) polymer-based nanoparticles b) inorganic nanoparticles c) lipid- based nanoparticles. The inorganic nanoparticles have wide applications but a major drawback, its non- biodegradability. While the lipid-based nanoparticles on the other hand are biodegradable but structurally complex making it a difficult task to synthesis it. But the polymer-based nanoparticles are biodegradable as well as have a stable profile, which makes them an ideal choice for their use. The Fibroin, being a protein, also falls under the polymer-based nanoparticles.[5]

The fibroin-based nanoparticles are synthesized taking into consideration various properties which have already been studied by various researchers, like biodegradability, bio-compatibility, targeted drug delivery, anti-cancerous property etc.[4][5]

Fibroin based nanoparticles show their applications in various fields like Tissue engineering, suture material, artificial vascular and ligament treatment, cornea, wound healing, bone graft, agriculture, medicine, cosmetics, etc.[1]

There are a variety of methods used when it comes to the synthesis of nanoparticles, like spray-drying, lyophilizing, electro-spraying, capillary microdot technique, salting out, supercritical fluid technologies, nano-precipitation, etc.[6][5]

After nanoparticle synthesis, characterization techniques are employed to analyze the properties of the produced nanoparticles. Various processes like FE-SEM, SEM, XRD, UV-VIS, FTIR, EDS, etc., are used for the characterization of nanoparticles. These characterization techniques are based on the factors such as surface morphology (FE-SEM, SEM), functional group (FTIR), absorption of UV light by the particles (UV-VIS, Zeta potential, DLS), presence of the different elements in the molecules (EDS), crystalline structure (XRD) etc. [7]

This characterization methods allow us to study the surface properties, morphological properties, also some chemical and physical properties which further allows its applications in the required fields depending upon its properties.

This paper follows the nano-precipitation method for the synthesis of nanoparticles as it is flexible, reliable, and cost-effective. This project lime lights the Synthesis and characterization of fibroin nanoparticles for its further applications in various fields in various fields including research but mainly manufacturing of fabric coated with SNFPs.

MATERIALS AND METHODOLOGY:

A) Methodology for Synthesis of Fibroin Nanoparticles:

Silk fibroin nanoparticles were synthesized using a standard nanoprecipitation method adapted from Wongpinyochit *et.al. (2016).* Silk fibers were obtained from the cocoons of the *Bombyx mori* silkworm and dissolved in a $CaCl_2$ solution (9.3M) at 60 °C under constant stirring for 4 h to obtain a homogeneous silk fibroin solution. The resulting solution was transferred into a dialysis membrane (molecular weight cut-off: 12–14 kDa) and dialysed against distilled water for 72 h at room temperature, with frequent water changes at 6 h intervals to ensure complete removal of salts. Following dialysis, the silk fibroin solution was centrifuged at 10,000 rpm for 10 min at 4 °C to remove insoluble impurities, yielding a clear supernatant. Briefly, the purified silk fibroin solution (1% w/v) was added dropwise into cold acetone at a solvent-to-non-solvent ratio of 1:5 (v/v). The resulting nanoparticle suspension was collected by centrifugation at 12,000 rpm for 30 min at 4 °C. The pellet was redispersed in distilled water and subjected to ultrasonication (40 kHz, 10 min) followed by vortex mixing for 5 min to reduce particle aggregation and ensure uniform dispersion. A final centrifugation step was carried out under the same conditions to obtain purified and well-dispersed silk fibroin nanoparticles, which were stored at 4 °C for further characterization and application studies as shown in fig.9

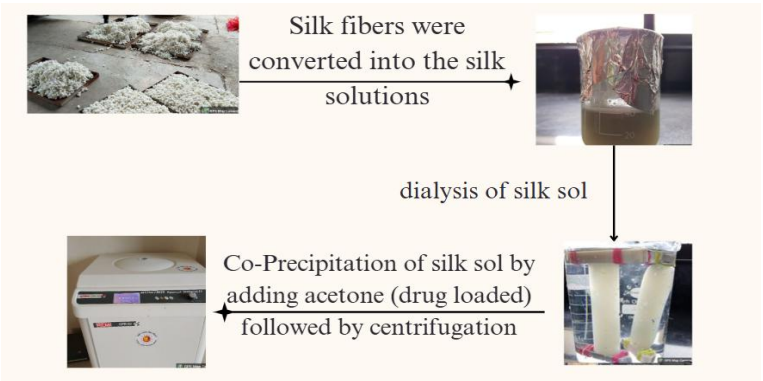


Fig 9: Synthesis of the SFNPs

B) Manufacturing of medicated fabric with wound healing properties:

The synthesized nanoparticles were used in the making of fabric coated with SFNPs. This was done by dipping and soaking the fabric into the SNFPs solution for 3 to 4 times and then drying it in the laminar air flow for 3 to 4 hrs. This fabric treated with SNFPs and an untreated fabric as a control were sent for the SEM characterization for further analysis.

C) Methodology for Characterization techniques of SFNPs:

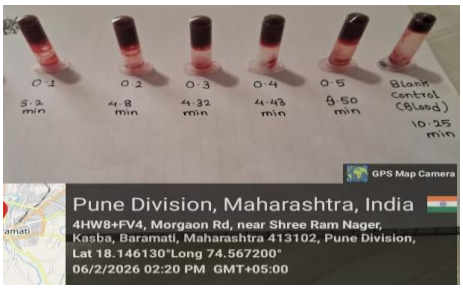
1) **FE-SEM:** Sample preparation for field-emission scanning electron microscopy (FE-SEM) involved both thin-film and solid-state approaches. For thin-film analysis, a small aliquot of the silk fibroin nanoparticle suspension was uniformly smeared onto a clean glass slide and allowed to air-dry at low temperature to prevent structural deformation. The dried films were subsequently used for FE-SEM imaging. For solid-state analysis, the nanoparticle suspension was dehydrated to obtain a dry powder, which was then mounted onto conductive sample holders for microscopy.[10]
2) **XRD:** This technique supports two sample configurations, namely powdered specimens and large, flat samples. The analysis is based on the identification of crystalline structures. In this method, the powdered sample is placed in the XRD instrument to obtain characteristic intensity peaks arising from atomic lattice arrangements. These diffraction patterns provide information regarding the crystallinity, size, and structural dimensions of the analyzed particles.[11]
3) **UV- VIS**: A clear, transparent, and homogeneously dispersed SFNP solution was placed in the spectrophotometer for analysis. Ultraviolet radiation was passed through the sample, and the absorbed or transmitted light was recorded. The resulting absorption spectrum was obtained based on the measured absorbance and transmittance and was further analyzed accordingly.
4) **Biuret reagent**: The method used for the detection of the presence of protein in any compound. The principle of this method mainly revolves around the reaction of the biuret reagent with the peptide bonds which results in the formation of purple color.
5) **FTIR**: The prepared powdered and liquid samples were analyzed sequentially using FTIR spectroscopy. Infrared radiation interacting with the sample enabled the identification of characteristic functional groups, which were represented by distinct absorption peaks in the resulting spectra.[12]
6) **EDS**: This technique is fully integrated with FE-SEM analysis, wherein elemental composition is determined through software-assisted detection. The powdered nanoparticle samples were analyzed in the FE-SEM, enabling simultaneous surface imaging and energy-dispersive X-ray spectroscopy (EDS) analysis.[13]
7) **Nanodrop spectrophotometry:** Quantitative estimation of nanoparticle concentration was performed using a Nanodrop UV–Visible spectrophotometer. The sample was analyzed by placing a micro-volume aliquot onto the instrument pedestal, allowing rapid absorbance-based measurement without further dilution.

D) Methodology for detection of clotting time of blood:

The stopwatch was started immediately after transferring 1 mL of blood into each Eppendorf tube. The instant at which the blood sample was withdrawn was designated as time zero, and all subsequent measurements were calculated from this point. The filled Eppendorf tubes were held in the palm of the hand to maintain the sample at physiological temperature (~37 °C). At one-minute intervals, the tubes were gently tilted to assess the onset of blood coagulation. This observation was continued until visible clot formation was detected. The time at which clotting occurred was recorded, and the stopwatch was stopped accordingly.

Fig. No. 8.a Control blood sample

Fig.No 8.b. SFNP's infused blood sample



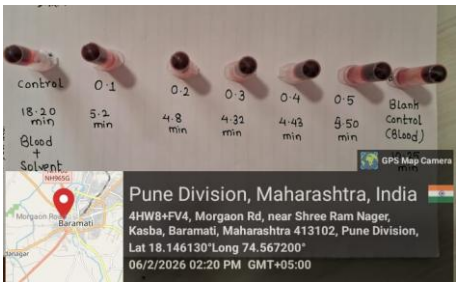


Fig No. 11.a,b Conc. Dependent analysis of Blood clotting property of fibroin nanoparticles

RESULTS AND DISCUSSION:

Variety of methods are available for the synthesis of silk fibroin nano-particles which include polyvinyl alcohol bending, spray drying, salting out, capillary microdot printing, supercritical CO_2 precipitation, nano-precipitation. This study focuses on nano-precipitation method used for the synthesis of fibroin nanoparticles. This method resulted in the formation of spherical nanoparticles that ranged from 30nm to 110nm in size. Characterization of the nanoparticles was done using FE-SEM, FTIR, UV-VIS spectrophotometer, EDS, XRD. The results are as follows:

a) Biuret Reagent Test: Protein Detection:

Biuret reagent reacts with the peptide bonds present in the protein and results in the purple color formation. Here the fig. 10.b is distilled water and acetone (control solvent) mixture while the fig 10.a represents SFNPs sol with leaves purple color. This specifies the presence of the peptide bonds and the protein in the fig. 10.a while the colorless solution of solvent has no protein in fig.10.b

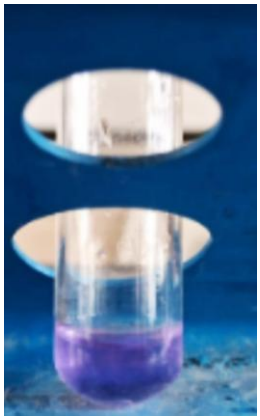


Fig. No. 10.a SFNPs

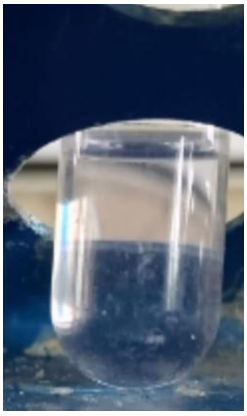


Fig.No. 10.b Control

b) FE-SEM (Field Emission Scanning Electron Microscopy):

Field Emission Scanning Electron Microscopy (FE-SEM) was utilized to examine the morphology and size of SFNPs at high resolution. The images obtained using FE-SEM for dried smear sample projects the spherical nanoparticles with a size of 30nm to 110nm, as shown in Fig.1a,b,c. While for the solid powdered sample of SNFPs, the image shows abrupt crystalline particles which range in µm size as shown in Fig.2a,b.

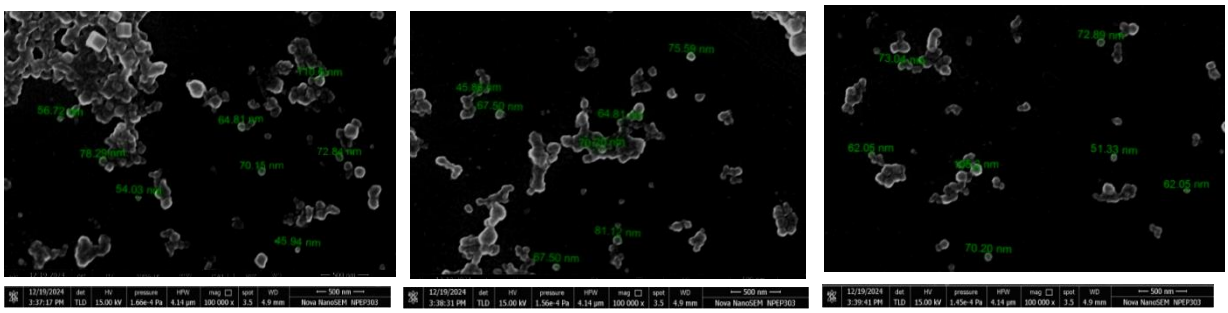


Fig: 1.a,b,c) (SNFPs): FE-SEM images of obtained using liquid sample

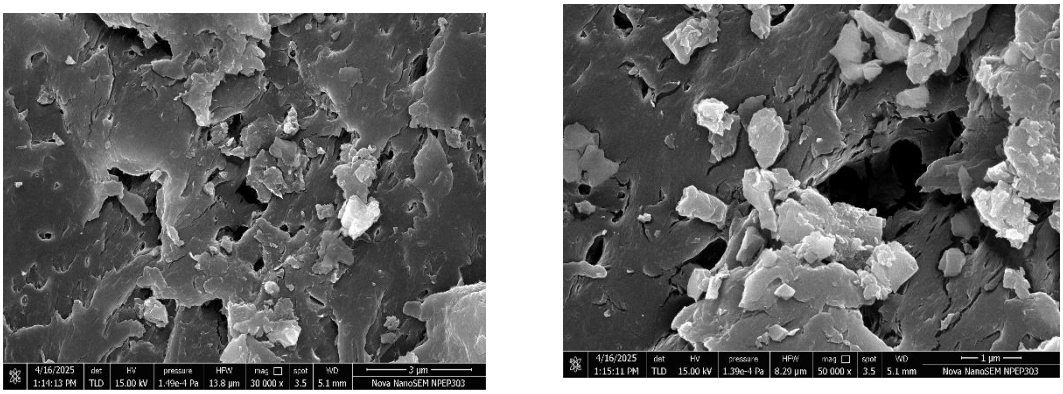
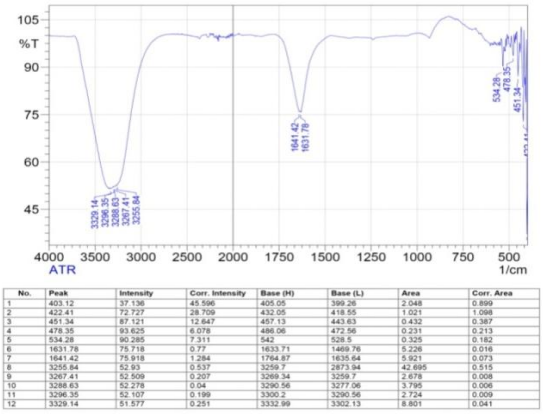


Fig: 2.a, b) (SNFPs): FE- SEM images obtained using powdered sample.

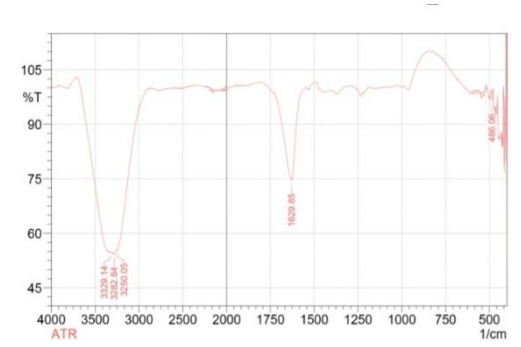
c) FTIR (Fourier-transformed infrared spectroscopy):

To detect the functional groups present in the fibroin nanoparticles, the FTIR was performed as a characterization method based on the functional groups present in the given sample. The following test was performed for 2 samples, solid as well as the liquid solution of SFNPs. a) The FTIR spectrum for liquid state showed the peaks at 403.12, 422.41, 451.34, 478.35, 534.28, 1631.78, 1641.42, 3255.84, 3267.41, 3288.63, 3296.35, 3329.14 cm^{-1} as shown in the Fig.3.a. b. The FTIR spectrum for solid state showed the peaks at 486.06, 1629.85, 3250.05, 3282.84, 3329.14 as shown in the figure. 3.b. These peaks represent the specific functional groups depending upon the wavelength of the functional group, as shown in Table 1. Comparing Table 1 and the peaks in Fig. 3 a and Fig. 3 b of the sample (SNFPs) presence of various functional groups such as -OH, -COOH, -NH, S=S, etc. was detected. Hence, the sample shows the presence of OH group, Amide I, and disulfide linkages. This confirms the presence of fibroin protein.[8]



No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	403.12	37.136	45.596	405.05	399.26	2.048	0.899
2	422.41	72.727	28.709	432.05	418.55	1.021	1.098
3	451.34	87.121	12.847	457.13	443.63	0.432	0.387
4	478.35	93.625	6.078	486.06	472.56	0.231	0.213
5	534.28	90.285	7.311	542	528.5	0.325	0.182
6	1631.78	75.718	0.77	1633.71	1469.76	5.226	0.016
7	1641.42	75.918	1.284	1764.87	1635.64	5.921	0.073
8	3255.84	52.93	0.537	3259.7	2873.94	42.695	0.515
9	3267.41	52.509	0.207	3269.34	3259.7	2.678	0.008
10	3288.63	52.276	0.04	3290.56	3277.06	3.795	0.006
11	3296.35	52.107	0.199	3300.2	3290.56	2.724	0.009
12	3329.14	51.577	0.251	3332.99	3302.13	8.801	0.041

Fig 3.a FTIR of SNFPs (liquid sample)



No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	486.06	96.38	3.094	489.92	480.28	0.086	0.073
2	1629.85	74.473	24.949	1726.29	1548.84	9.101	8.61
3	3250.05	55.258	0.61	3253.91	2920.23	34.915	0.298
4	3282.84	54.233	0.096	3284.77	3255.84	7.585	0.029
5	3329.14	54.6	0.238	3332.99	3318.49	3.532	0.012

Fig 3.b FTIR of SNFPs (solid sample)

Table No. 1: Functional groups and their wavelengths

Wavelength (nm)	Functional group
3650- 3250	H_2O, OH (for OH, it should be followed by a 1600-1300)
1630-1650	Amide I
430-620	Disulfide linkage (S=S)

d) UV- VIS (Ultraviolet Visible spectrophotometer):

The UV absorption spectra of silk fibroin nanoparticles were carried out by the UV-VIS spectrophotometer by recording absorption spectra in a wavelength range of 300nm to 400nm. The λ max in this spectrum was recorded to be 339.62nm, as shown in Fig. 4.1, through which we can confirm the extraction of the fibroin protein. Silk Fibroin solution absorption peak may range from 250 to 400nm. [9]

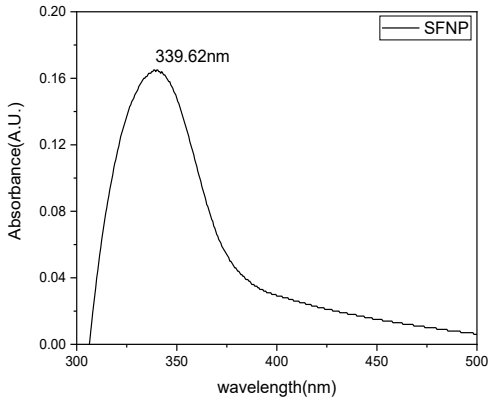


Fig 4. a: UV- VIS Spectroscopy of SNFPs

e) XRD (X-Ray Diffraction):

The XRD was carried out to analyze the particle size and shape of the synthesized SFNPs (Fig. 5. a, b, c., and Table 2). These results were further used for interpreting the particle size and shape with the help of the Debye-Scherrer formula (i.e. D= 0.9ƛ/ ß Cos θ. where, ƛ= the wavelength of the X-rays, ß= the width at half maximum of a peak) and the fig. 5.a. The analyses of the table lead and its further calculation from the formula give the particle size of ≈231nm, and the graph shows the amorphous structure of the particle.[15]

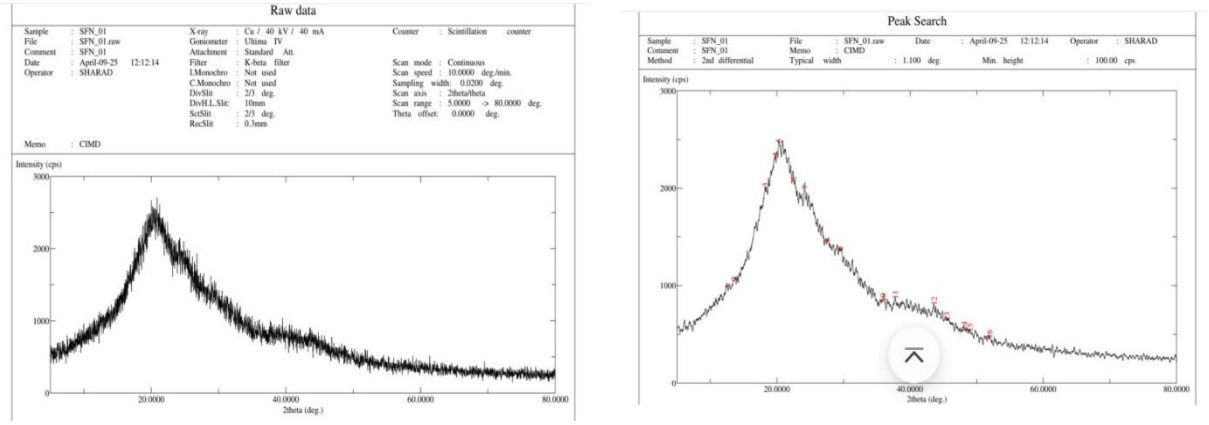


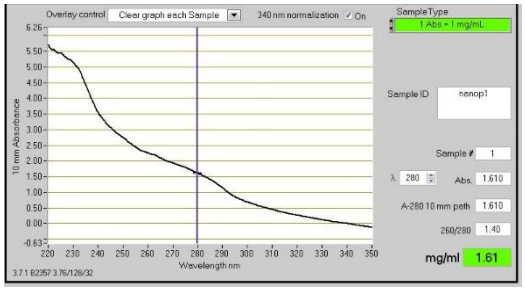
Fig 5.a, b, c: XRD images of SNFPs

Table No. 2: XRD results of SNFPs

Parameters	***Particle size (D)***	**2θ**	**Cos θ**	**Sinθ**	***FWHM***	***Beta (radian)***
Sample (SFNPs)	231nm	20.9^0	0.983^0	0.181^0	21.42^0	0.37

f) Nano-Drop Spectrophotometry:

The concentration of the synthesised silk fibroin nanoparticles was determined using a spectrophotometric method(A-280nm). A small aliquot of the nanoparticle suspension was subjected to analysis, and the instrument, calibrated with standard references, provided a direct estimation of concentration. Upon analysis, the concentration of nanoparticles in the prepared sample was found to be **1.61 mg/mL**, reflecting an adequate yield and uniform dispersion of the synthesized nanoparticles.



g) EDS (Energy Dispersive Spectroscopy):

The EDS test was carried out to do the elemental analysis of the SFNPs. Fig. 6 and Table 3 show the presence of different elements and their conc. The elements having a high amount of concentration in SFNPs are C>O>N with their conc. 51.83, 28.53, 14.37 respectively. While the elements which show negligible conc. are Ca> Al> Fe> Si> Mg> Cl> Au with their conc.as 1.59, 1.55, 1.06, 0.69, 0.22, 0.16, 0.00 respectively. These conc. are considered in %. Fig. 6.a,b,c. show the accumulation of the O, C and N atoms, respectively.

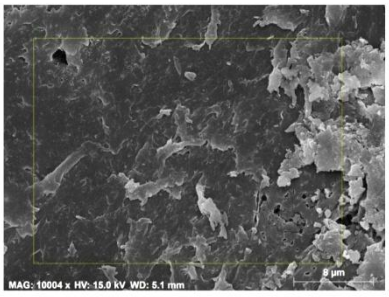
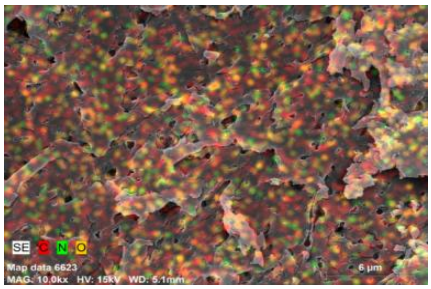


Fig No.6.1 Selected area for EDS



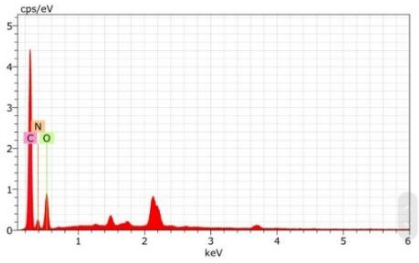


Fig No. 6.a,b: Elemental analysis by EDS of SNFPs

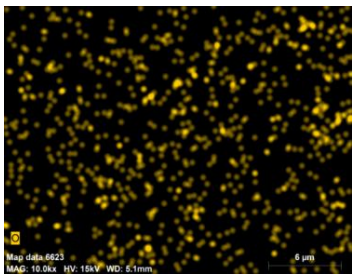


Fig. No. 6.a EDS of SNFPs- O atoms

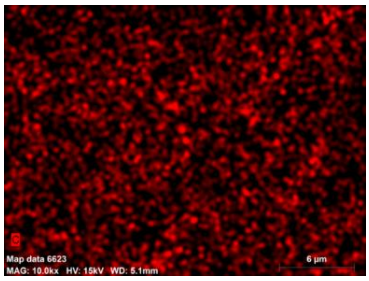


Fig. No. 6.b EDS of SNFPs- C atoms

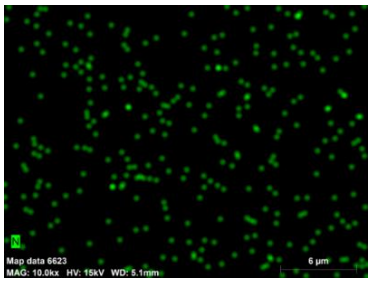


Fig. No. 6.c EDS of SNFPs- N atoms

Table No. 3: EDS results interpreting the element conc. of SNFPs

Elements	*Atomic number*	*Series*	*Unnormalized weight [%]*	*Normalised weight [%]*	*Atomic Conc.[%]*	*(One Sigma)*
C	6	K	53.78	53.78	60.96	6.39
O	8	K	26.32	26.32	22.40	3.67
N	7	K	15.16	15.16	14.74	2.70
Ca	20	K	1.43	1.43	0.49	0.08
Al	13	K	1.38	1.38	0.70	0.10
Fe	26	K	0.91	0.91	0.22	0.07
Si	14	K	0.69	0.69	0.33	0.06
Mg	12	K	0.18	0.18	0.10	0.04
Cl	17	K	0.14	0.14	0.05	0.03

**One sigma in EDS refers to the standard deviation or measurement error, representing the statistical precision of the elemental composition analysis.*

h) SEM (Scanning Electron Microscopy):

For the manufacturing of the fabric coated with SFNPs, the fabric was treated with the SNFPs solution and dried in a sterilized environment in a laminar air flow. SEM was used to capture a high-resolution image of the fabric coated with SFNPs. It was observed that the SFNPs were loaded on the fabric as shown in fig: resulting in the successful manufacturing of SNFPs SFNPs-coated fabric. This fabric can be further used for various medical applications e.g. Manufacturing of bandages, sanitary napkins, gauges etc. due to its excellent wound healing property.[1]

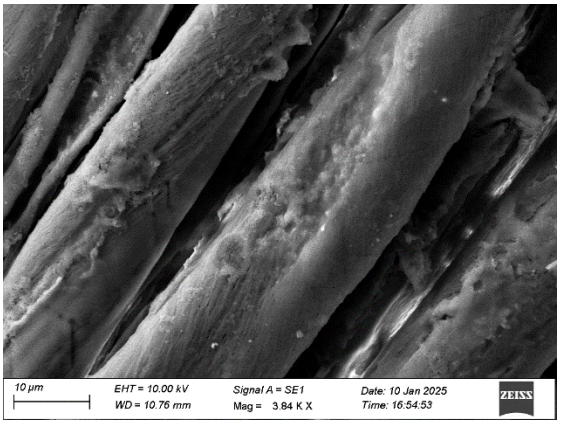


Fig.No 7.a SEM of untreated fabric

Fig.6.b SEM of treated fabric with SNFPs

i) Wound Healing:

The blood clotting assay was performed on initial bases to examine the presence of blood clotting ability in the SFNPs. And the results obtained are as follows: Control group required a mean time of 8.596 mins while the Nanoparticles infused group required 5.266 mins for the clotting of blood. This experiment proved the presence of blood clotting ability of the Fibroin nanoparticles.

Table No. 4: Wound healing efficiency

Sample type	No. of samples	Time required for clotting	Mean
Control blood sample	Sample C1	7 mins 34sec	8.596 (approx. 9mins)
	Sample C2	8 mins 45sec	
	Sample C3	10 mins	
Nanoparticles infused blood sample	Sample N1	4mins 23sec	5.266 (approx. 5mins)
	Sample N2	5mins 15 sec	
	Sample N3	6 mins 33 sec	

Further, the detailed experimental set up was used to find the exact concentration at which the SFNPs showed better performance followed by its confirmation. The results of the wound-healing assay demonstrated a pronounced improvement in clotting efficiency upon treatment with nanoparticle-

infused blood samples. For the second set up the control group required a mean clotting time of 9.79 minutes (table no. 5), while the nanoparticle-treated samples exhibited a significantly reduced mean clotting time of 5.27 minutes.

Table No.5. Conc. dependent analysis of blood clotting property in fibroin nanoparticles.

Silk sol conc. (ml)	Distilled water (ml)	Blood (ml)	Time				Mean Time
			Set A	Set B	Set C	Set D	
0.1	0.4	0.5	6.2 min	9.7 min	14.10 min	6.15 min	9.03 min
0.2	0.3	0.5	5.8 min	8.3 min	10.6 min	6.20 min	7.73 min
0.3	0.2	0.5	5.3 min	7.16 min	8.10 min	6.19 min	6.69 min
0.4	0.1	0.5	5.4 min	5.28 min	6.46 min	6.15 min	5.82 min
0.5	0.0	0.5	6.5 min	6.23 min	5.38 min	4.49 min	5.65 min
Control (0.0)	0.0	0.5	11.3 min	9.8 min	9.52 min	8.55 min	9.79 min

Control value = **9.79**

Mean 0.5 conc. values = **5.65**

Decrease in the blood clotting time in control to 0.5 highest conc.**= 9.79 - 5.65**

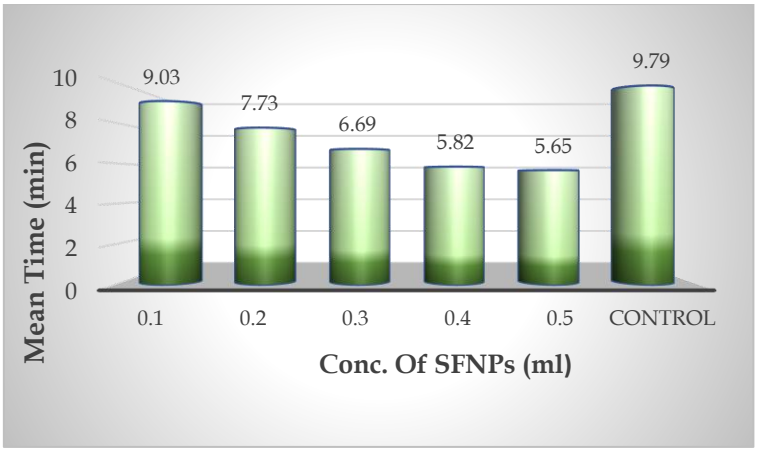
= 4.14

$$Reduction\ \% = \frac{4.14}{9.79}\ x\ 100$$

$$= 0.4229 \times 100$$

$$= 42.29\%$$

The Blood clotting time required decreased by approximately ~ 42.29%



Graph no. 1 Conc. dependent analysis of the blood clotting property of fibroin nanoparticles.

This reduction of approximately 42.29% highlights the ability of the nanoparticles to promote rapid haemostasis. Blood clot formation is a critical early event in the wound-healing cascade, as it prevents excessive blood loss and provides a provisional matrix for subsequent cellular migration and tissue regeneration. Prolonged clotting time is often associated with delayed wound repair, whereas faster clot formation enhances healing efficiency. The observed decrease in clotting duration in the nanoparticle-treated samples suggests improved interaction between the nanoparticles and blood components, potentially facilitating platelet activation or fibrin network formation. Consequently, the nanoparticle-infused system demonstrates superior wound-healing efficiency compared to the natural healing process observed in the control group.

CONCLUSION:

The silk fibroin nanoparticles were successfully synthesized in a flexible, reliable, and cost-effective way. These synthesized nanoparticles were characterized with the help of various techniques and images obtained through various instruments. This study resulted in a soft-driven protocol for the synthesis of fibroin nanoparticles and their characterization using multiple techniques. The required blood clotting time was reduced by ~42.29%. Also, it was successfully applied in the manufacturing of the fabric coated with these SFNPs which shows significant wound healing properties hence can further be used for multiple purposes like manufacturing of bandages, sanitary napkins, wipes, etc.

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