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Studies On Degumming and Microwave Assisted Formulations of Silk Fibroin Nanocomposites

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Abstract

It is well known that traditionally, silk fibroin has been used for wearable products for a pretty long time. Many more farmers are involved in sericulture industries, and it is the backbone of the silk industry in the country. In recent years, there is considerable amount of interest in innovative research on value-added products of silk fibroin bio nanocomposites. However, formulations of silk fibroin bio nanocomposites are complicated and time-consuming process. It takes more than 52 hours and may not be suitable for production of value-added products for the sericulture farmers. There is a need for research in the field of low degradation degumming, dissolving, and reproducible desalting methodology, which would allow the isolation of native-like silk fibroin and might be employed for the ultimate industrial manufacture of regenerated silk fibroin. The purpose of this paper is to report the Studies On Microwave Assisted Formulations of Silk Fibroin Nanocomposites. The first goal is to create a softer degumming approach that would allow us to begin the dissolution process with low to non-degraded silk fibroin material. This method is based on Chung et al observation's that the anionic detergent sodium dodecyl sulfate (SDS) improves degumming and the notion that shortening cooking time by employing microwave radiation minimizes degradation of the silk during the degumming process. Another goal of this effort is to reduce the time and temperature required for the complete dissolution of degummed silk fibroin, with the goal to destroy the silk fibroin as little as possible. The method developed is based on the results in the field of silk fibre quality control. So, this work looks into a new way to separate non-degraded regenerated silk fibroin, which requires the processing time of 52 hours with standard methods brings down to only 4 hours with the new method. When repeated short-term microwave treatments are used instead of the standard degumming protocol, degummed silk fibroin that didn't break down was made. Afterwards a ZnCl2 solution was used to completely dissolve the degummed fibroin in just 1 hour at a temperature of 45 °C. Gel filtration was used to get rid of the salt. Based on these changes, a cytocompatibility aqueous silk fibroin solution could be made from degummed silk in only 4 hours, cutting the total process time by 48 hours without affecting the quality of the isolated silk fibroin solution.

Keywords: Silkworm, *Bombyx mori*

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Introduction

Silk fibroin is the structural protein of the filament of the silkworm Bombyx mori and combines properties such as high mechanical strength, biocompatibility, and biodegrade- ability in vivo [1]. These properties also make silk fibroin attractive for the development of new advanced silk-based materials for soft bioelectronics. These soft systems include bioresorbable electronics for wearable sensors, electronic skins, and flexible energy devices and so many things [2]. To prepare a pure silk fibroin solution, the two protein components that make up the silk thread first are silk fibroin and second one is sericin, this both are must first be separated from each other. This process is called degumming. One are the traditional method for degumming is boiling cocoon s in distilled water 50 to 80 min. Then wash the silk fiber with distilled water. The most common method for degumming is boiling raw silk cocoons in 0.02 M sodium carbonate (Na2CO3) buffer for 30 to 60 min and after washing with distilled water. The modern and less time consuming method are microwave method [3]. In this method silk cocoons are boiled with Sodium dodecyl sulfate and sodium carbonate with 1:1 proportion of few minutes then wash the distilled water and separate the silk fiber and sericin. That the anionic detergent sodium dodecyl sulfate (SDS) improves the degree of degumming. and the hypothesis that reducing the cooking time by using microwave radiation reduces degradation of the silk during the degumming process. There is a need for research in the field of low degradation degumming dissolution, and reproducible desalting methodology [4]. native like silk fibroin and could eventually be used for the future production of regenerated silk fibroin on an industrial scale. Therefore, the aim of the present study was to first develop a gentler degumming. Degumming of silk involves the cleavage of peptide bonds of sericin, either by hydrolytic or enzymatic methods, and the subsequent removal of sericin from the silk fibroin. Hydrolysis of sericin can be carried out under neutral, alkaline, or acidic conditions to give four fractions, each having different properties.

Another objective of this work was the reduction of the required time and temperature for the complete dissolution of the degummed silk fibroin, with the aim of degrading the silk fibroin as little as possible. The method developed is originated from findings in the field of quality control of silk fibers. In 1935, Trotman and Bell described a method of detecting damage in silk by measuring the viscosity of a solution of silk in zinc chloride (ZnCl2) (58% w/w) after incubation for 6 h for 37 °C. On the basis of these findings, it was shown later that the incubation time of silk in Zncl2, could be reduced to 3 h by increasing the temperature to 45 °C .

Methodology

Materials - Cocoons of the silkworm Bombyx mori were purchased from the market yard Baramati as shown in figure: 1 & all chemicals utilized in this study were provided by t.c college Baramati,

Figure: 1- Cocoons of the silkworm Bombyx mori

Experimental Methods:

1. **Degumming of cocoons for silk fibroin:** In order to get silk fibroin, the cocoons are degummed by using three different methods namely A) Traditional method, B) Standard method and C) Microware method. These are briefly described as follows.

A) Traditional method: - For degumming. Bombyx mori cocoons were cut into small pieces and 2 gm cocoon pieces were heated in thermostat for 1 hour,

after that 2g cocoon pieces were boiled in 100 ml water for 70 to 80 min, the sericin will remove upto 25/30%, dried overnight or directly use for dissolution.

B) Standard method: In the standard degumming method, 2 g cocoon pieces were boiled under constant stirring in 1 L of 0.02 M Na2CO3 for 30 min and rinsed 3 times for 20 min.

C) Microwave method: - For degumming. Bombyx mori cocoons were cut into small pieces and 2 g cocoon pieces were heated for 1 min in 125 mL of an aqueous solution of 0.02 M Na2CO3 and 0.25% sodium dodecyl sulfate (SDS) in a 500 mL Duran bottle in a microwave at 187^{0}C. After microwave treatment the solution containing the silk was left for 10 min at room temperature. The temperature of the solution during this time was near to 80^{0}C. afterwards, the silk fibers were rinsed for min with distilled water. Excess water was removed by squeezing the silk fibers. Then, after 3 additional cycles (microwave heating in fresh degumming solution, 10 min rest, washing and squeezing) the degummed silk fibers were dried overnight or directly used for dissolution.

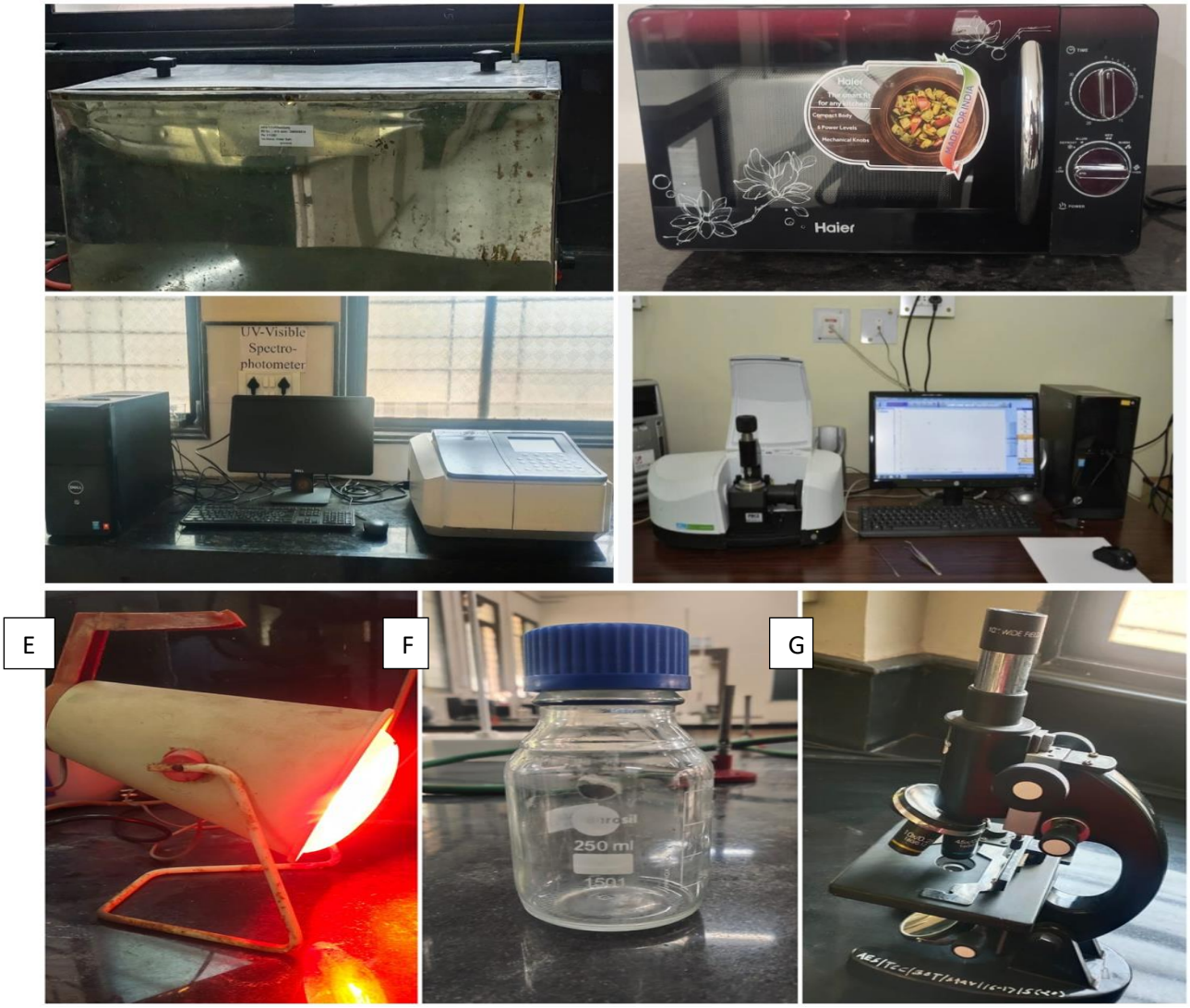


Figure: 2- A) Water Bath (for traditional method) B) Microwave oven (for microwave method) C) UV- Visible Spectrophotometer (for chemical parameter analysis) D) FTIR –Instrument (for chemical parameter analysis) E) IR Lamp (for drying samples) F) Duran bottle (for microwave method) F) Compound microscope (for physical parameter analysis).

Degumming efficiency was measured by determining the percentage weight loss of samples after degumming. It was expressed as a percentage of the initial weight. All the degummed samples were evaluated with an optical microscope to assess the extent of degumming and fiber damage Dissolution of Silk Fibroin Fiber > All dissolution experiments were carried out in a preheated oven with constant stirring. For this purpose, the solvent was always poured onto the silk previously placed in the vessel so that the silk was completely submerged in the solvent.

2. **Dissolution of silk fibroin by using Zinc Chloride (ZnCl2):** 0.1 g of degummed silk fibers were incubated in 10 mL of 56% (w/v) aqueous ZnCl2 solution at 45^{0}C for 1 h, yielding a 1% (w/v) silk fibroin solution, or directly boil the solution for few minutes to dissolve silk fibroin [5].

Result and Discussion

3.1 Studies on degumming of silk cocoons- For the sericulture business improvement require high quality silk fibroin cocoons which is used in silk sarees i.e., in fashion textile as well as biomedical sutures. The cost of fashion textile (like a silk sarees) depends on luster (shine), color, hardness i.e., strength and texture of silk which we get from the "degumming". Degumming is a process of removing the sericin, a sticky substance produced by the silk worm that holds the strands of the silk together.

*Degumming efficiency percentage-
Degumming efficiency percentage is measure of how much amount of sericin is removed and how much amount of silk fibroin get or degumming efficiency percentage stands for separation rate of a silk worm cocoons into sericin and fibroin

*Degumming efficiency % formula-
Degumming efficiency % =output (wt. of cocoons after degumming) ÷ input (wt. of cocoons before degumming)

3.1.1 Studies on degumming with traditional method
By the above Table: 1 and graphical representation (Figure: 4) we can conclude that according to the chemical kinetics temperature is inversely proportional to the degumming efficiency percentage. By the graphical observation (Figure: 4) if we increase the temperature at various time intervals (50, 60, 70, 80 min) the degumming efficiency percentage decreases. After studying graphical representation of traditional method as compare to 80^0,90^0,100^0 to the 70^0 temperature range we can get a good results on above discussion we can say that degumming efficiency percentage is depends on what temperature we can apply i.e. high temperature 80^0,90^0,100^0 favors the excellent result of degumming. We can conclude that at high temperature we can get good quality degumming efficiency percentage [6].

3.1.2 Studies on degumming with standard method
By the table: 2 and graphical representation (figure: 6) of standard method we can say that at 70^{0}C degumming efficiency percentage decreases near about 81 % at time interval 10 to 25 min. at 80^{0}C the degumming efficiency percentage decreases near about 65% at the same time interval.at 90^{0}C degumming efficiency percentage decreases near about 61% at same time interval.at 100^{0}C degumming efficiency percentage decreases near about 78% at same time interval. By these observations we can conclude that the standard method gives excellent result as compare to traditional method by comparing the parameters time and temperature [6].

3.1.3 Studies on degumming with Microwave method
After comparing above observations in table:3 and graphical representation of microwave method (figure:8) with traditional method as well as standard method, we can get on conclusion i.e. by the microwave method we can get 80 to 72% degumming efficiency percentage at the temperature 187^{0}C and the time interval 1 to 3 min. The microwave method strategy not only reduces time range but also generate economic value, which will accelerate its application in the silk and the pharmaceutical industries [7].

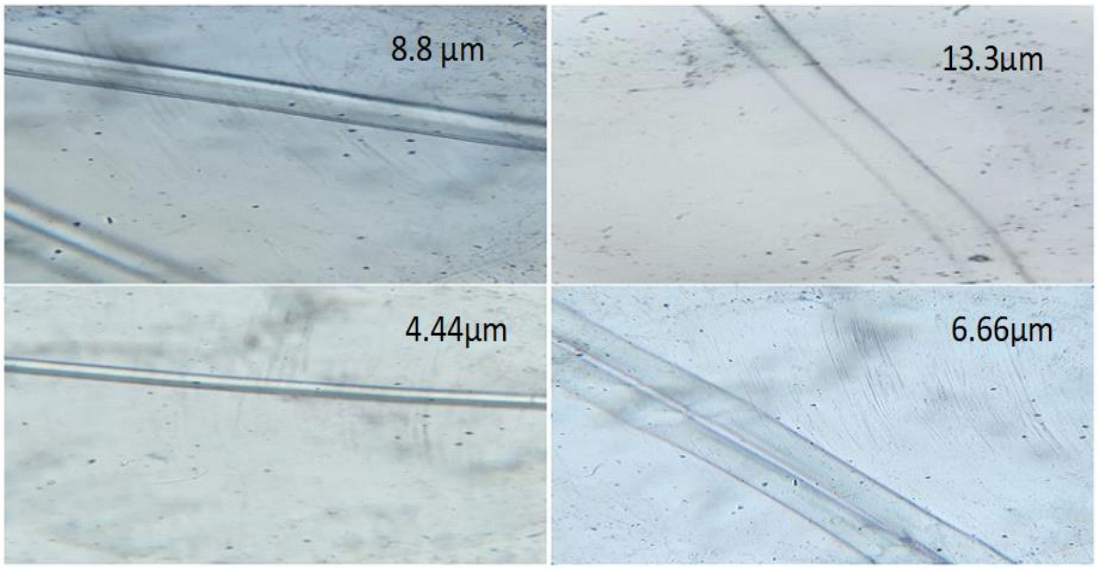


Figure :3(a)- At temperature 70^0C for four different times.

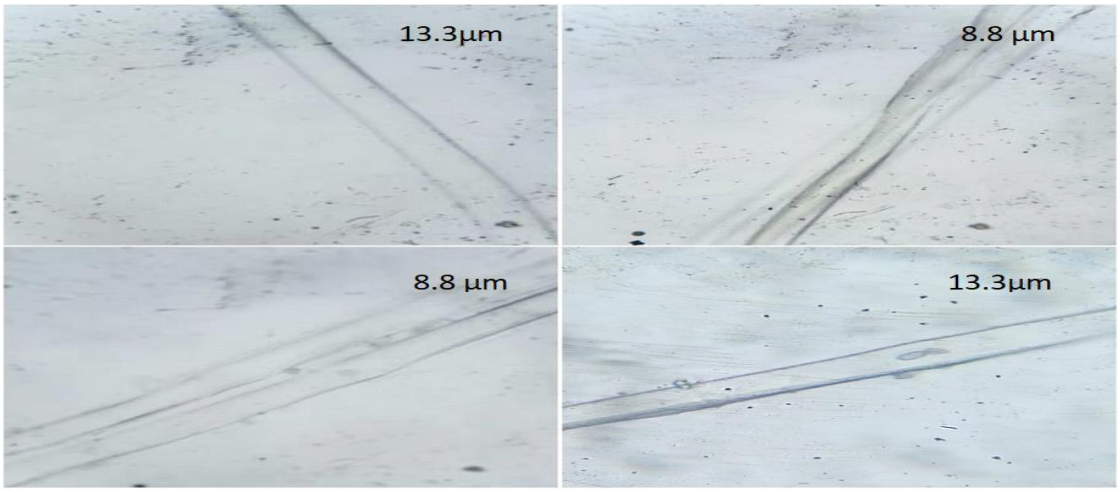


Figure: 3 (b) - At temperature 80^0C for four different times.

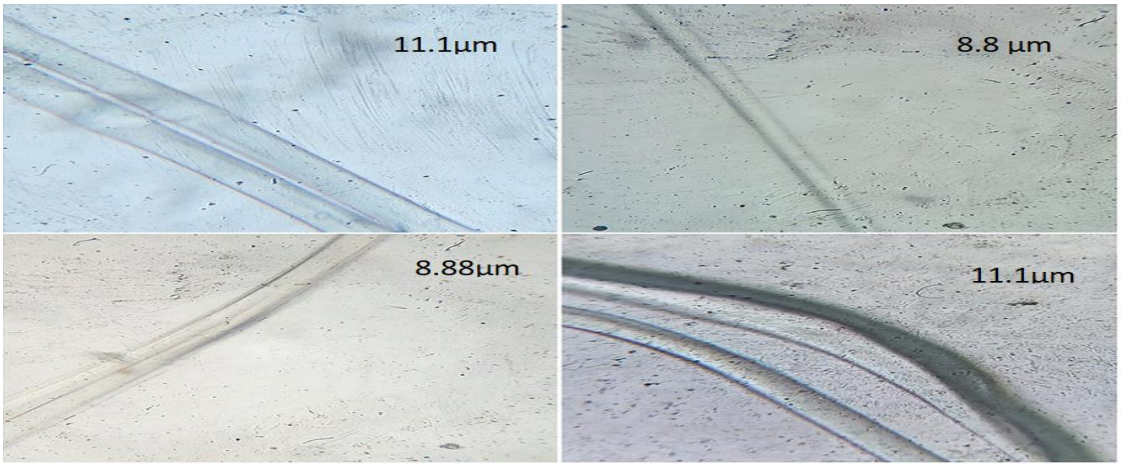


Figure: 3(c) - At temperature 90^0 C for four different times.

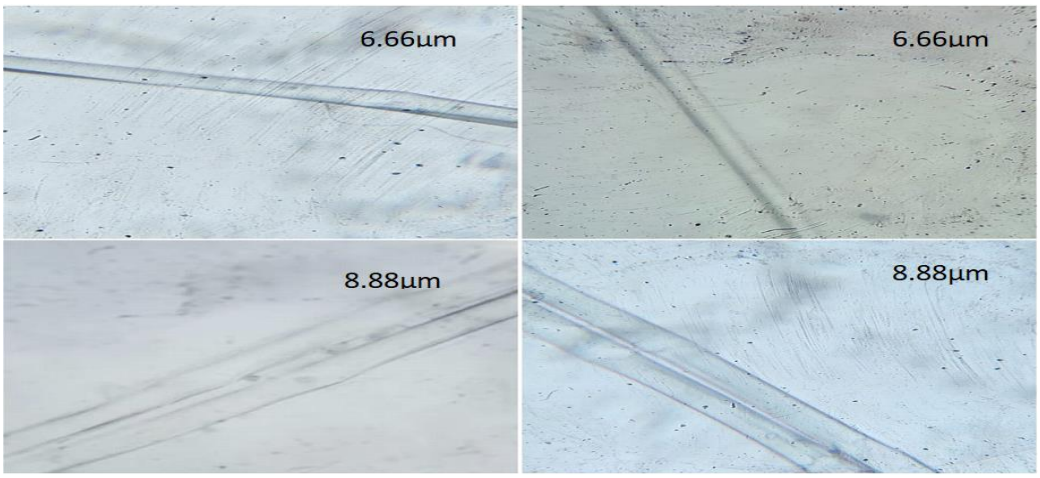


Figure: 3(d) - At temperature 100^0 C for four different times.

Table: 1 Efficiency of degumming silk cocoons with four different temperature and time.

Sr.No	Methods	Temperature	Time	Silk fibroin weight(gm)	Sericin weight(gm)	Efficiency (%)
1	traditional Method – 1 gm silk cocoons+40 ml distilled water	70°C	50 Min	0.94	0.06	94%
			60 Min	0.98	0.02	98%
			70 Min	0.98	0.02	98%
			80 Min	0.98	0.02	98%
		80°C	50 Min	0.95	0.05	95%
			60 Min	0.94	0.06	94%
			70 Min	0.92	0.08	92%
			80 Min	0.90	0.10	90%
		90°C	50 Min	0.91	0.09	91%
			60 Min	0.89	0.11	89%
			70 Min	0.87	0.13	87%
			80 Min	0.86	0.14	86%
		100°C	50 Min	0.90	0.10	90%
			60 Min	0.91	0.09	91%
			70 Min	0.91	0.09	91%
			80 Min	0.93	0.07	93%

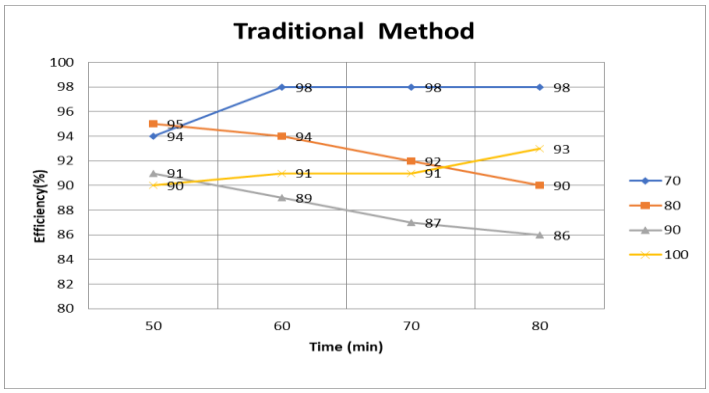


Figure: 4 – Variation of efficiency percentage with time of four different temperatures for traditional method.

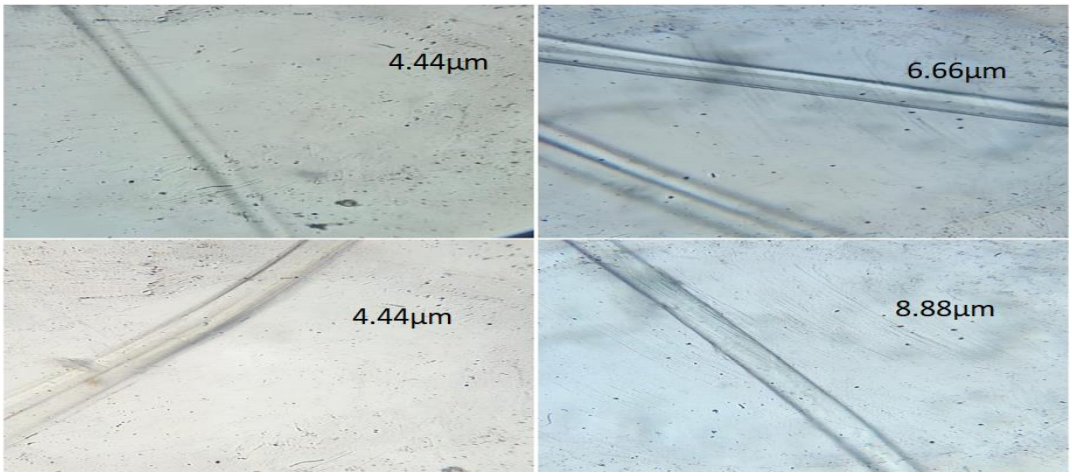


Figure :5(a)- At temperature 70^0C for four different times

Figure :5(b)- At temperature 80^0C for four different times.

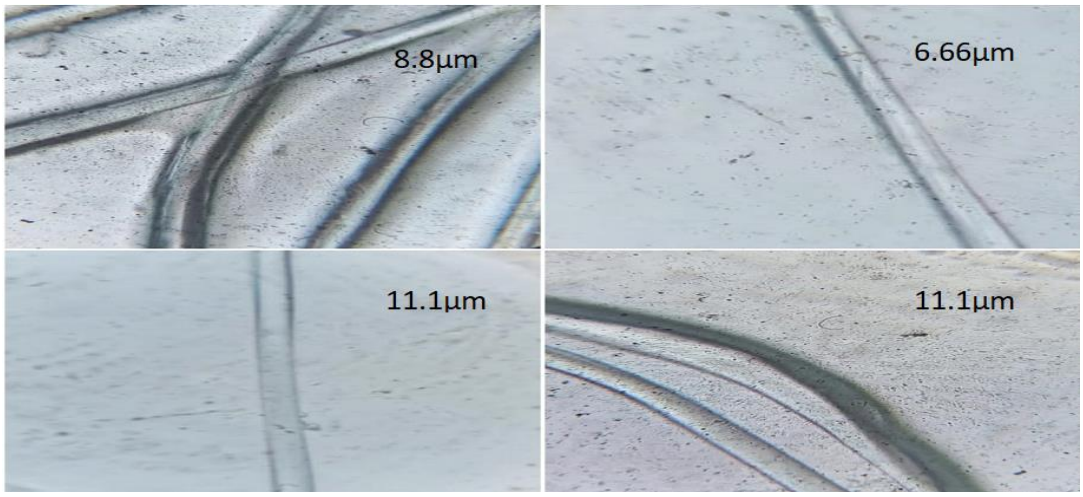


Figure :5(c)- At temperature 90^0C for four different times.

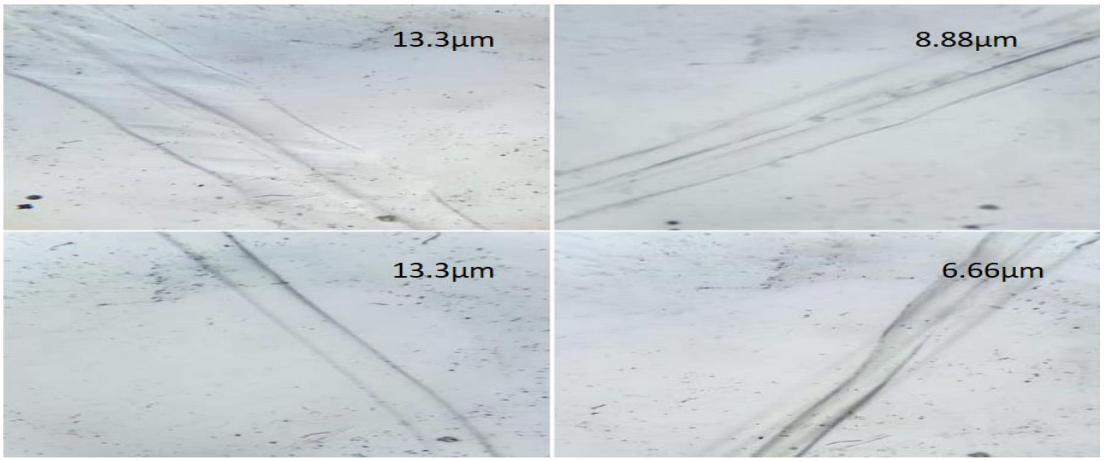


Figure :5(d)- At temperature 100^{0}C for four different times

Table: 2 – Efficiency of degumming silk cocoons with four different temperature and time by standard method.

Sr. No	Methods	Temperature	Time	Silk fibroin weight(gm)	Sericin weight(gm)	Efficiency (%)
1	Standard Method – 1 gm silk cocoons+0.02 M Na2CO3 (sodium carbonate)	70°C	10 Min	0.94	0.06	94%
			15 Min	0.81	0.19	81%
			20 Min	0.84	0.16	84%
			25 Min	0.82	0.18	82%
		80°C	10 Min	0.65	0.35	65%
			15 Min	0.70	0.30	70%
			20 Min	0.71	0.29	71%
			25 Min	0.72	0.28	72%
		90°C	10 Min	0.68	0.32	68%
			15 Min	0.66	0.34	66%
			20 Min	0.64	0.36	64%
			25 Min	0.61	0.39	61%
		100°C	10 Min	0.95	0.05	95%
			15 Min	0.87	0.13	87%
			20 Min	0.83	0.17	83%
			25 Min	0.78	0.22	78%

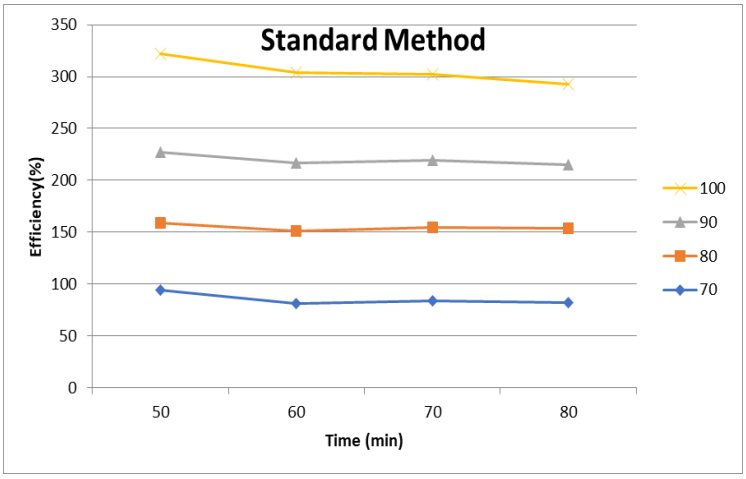


Figure: 6 – Variation of efficiency percentage with time of four different temperatures for standard method.

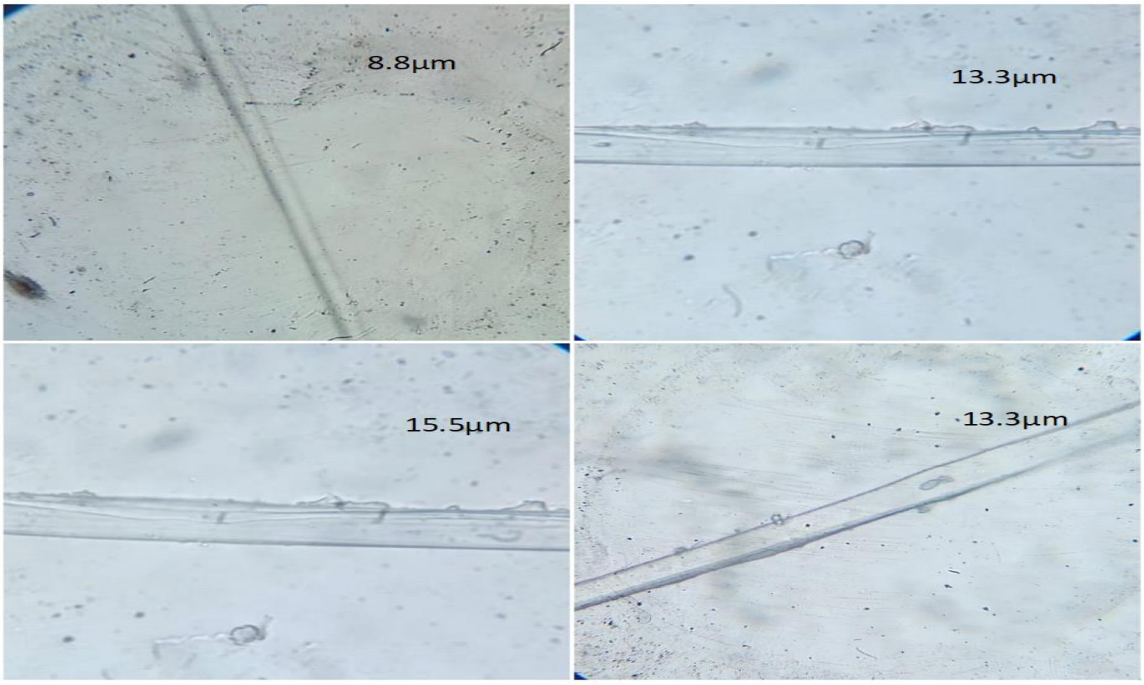


Figure 7- At temperature 187^{0}C for four different times.

Table: 3 – Efficiency of degumming silk cocoons with four different times with constant temperature by microwave method.

Sr.No	Methods	Temperature	Time	Silk fibroin weight(gm)	Sericin weight(gm)	Efficiency (%)
3	Microwave Method	187°C	1.30 Min	0.80	0.20	80%
			2Min	0.72	0.28	72%
			2.30 Min	0.75	0.25	75%
			3Min	0.72	0.28	72%

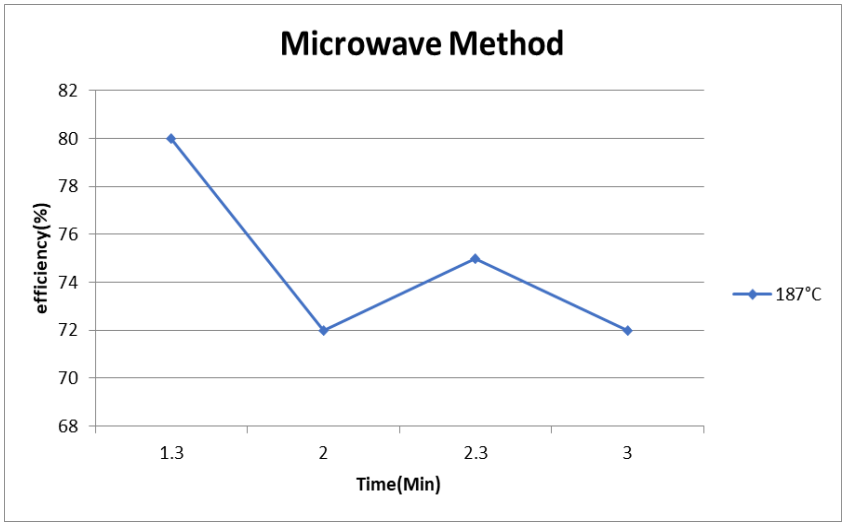
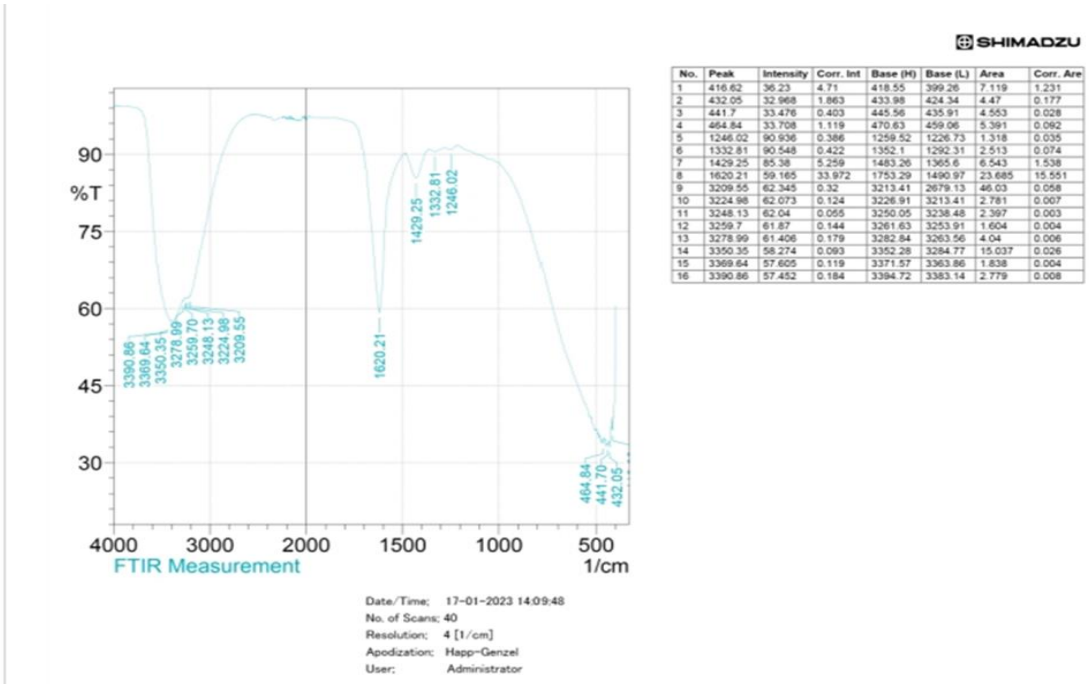


Figure: 8 – Variation of efficiency percentage of four different times with constant temperatures for microwave method.



No.	Peak	Intensity	Corr. Int	Base (H)	Base (L)	Area	Corr. Are
1	416.62	36.23	4.71	418.55	399.26	7.119	1.231
2	432.05	32.968	1.863	433.98	424.34	4.47	0.177
3	441.7	33.476	0.403	445.56	435.91	4.553	0.028
4	464.84	33.708	1.119	470.63	459.06	5.391	0.092
5	1246.02	90.936	0.386	1259.52	1226.73	1.318	0.035
6	1332.81	90.548	0.422	1352.1	1292.31	2.513	0.074
7	1429.25	85.38	5.259	1483.26	1365.6	6.543	1.538
8	1620.21	59.165	33.972	1753.29	1490.97	23.685	15.551
9	3209.55	62.345	0.32	3213.41	2679.13	46.03	0.058
10	3224.98	62.073	0.124	3226.91	3213.41	2.781	0.007
11	3248.13	62.04	0.055	3250.05	3238.48	2.397	0.003
12	3259.7	61.87	0.144	3261.63	3253.91	1.604	0.004
13	3278.99	61.406	0.179	3282.84	3263.56	4.04	0.006
14	3350.35	58.274	0.093	3352.28	3284.77	15.037	0.026
15	3369.64	57.605	0.119	3371.57	3363.86	1.838	0.004
16	3390.86	57.452	0.184	3394.72	3383.14	2.779	0.008

Figure: 9 – FTIR (dissolution of SF in $ZnCl_2$ 56% w/v)

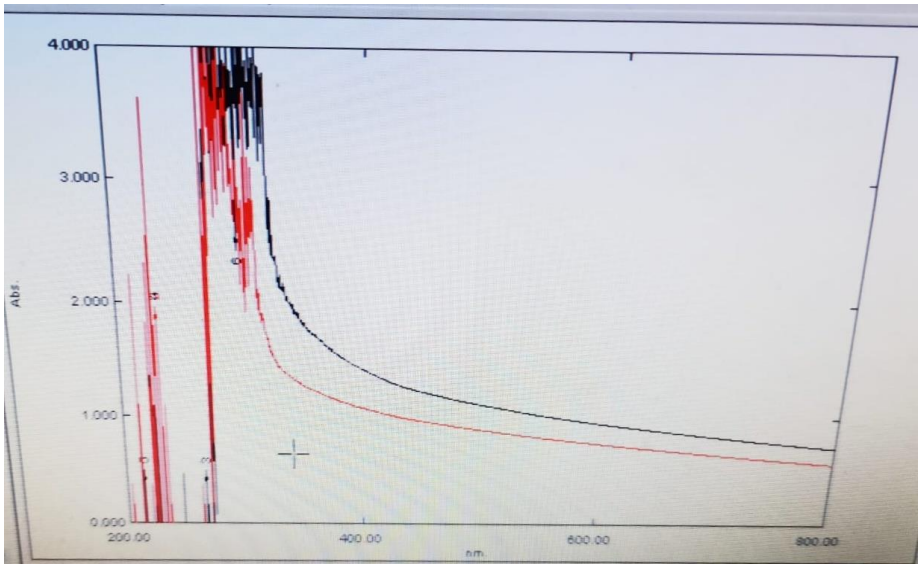


Figure: 10-UV-VISIBLE for SF dissolved in $ZnCl_2$ (56%) w/v.

3.2 Chemical Characterization –

3.2.1- Fourier Transform Infra-Red Spectroscopy (FTIR)-

FTIR analysis is used to identify molecular compounds. It works by measuring absorbance of infrared radiation by a sample. The resulting spectrum can then be used to identify the functional groups present in the compounds.

In above figure: 9, 3200cm^{-1} to 3400cm^{-1} frequency represents primary and secondary amines and amides. I.e. our solution contains primary or secondary amines /amides (N-H Bond) are present.

The characteristic vibration bands around 1620 cm^{-1} were assigned to the absorption peak of the peptide backbone of amide I (C = O stretching).1429 cm^{-1} frequency indicates O-H bond stretching .All these characteristic absorbance peaks indicate the existence of a hydrogen-bonded NH group .The molecular conformation of B. mori silk fibroin is characterized by β-sheet absorption peak[8,9].

3.2.2- UV- Visible spectroscopy-

Silk fibroin was dissolved in ZnCl2 (56% w/v), then the UV-Visible absorbance peak are observed at 214.50 nm wavelength, i.e. may be in general Tryptophan, Histidine, Phenylalanine and Tryosine are strong contributors to absorbance at 241 nm, the value reported for these amino acids agree rather well with each other [8,9].

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Conflicts of interest: The authors stated that no conflicts of interest.

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