STUDIES ON STRUCTURE AND PROPERTIES OF NEPHILA-SPIDER SILK DRAGLINE

Raju Seenivasan Rengasamy, Manjeet Jassal and Chidambaram Rameshkumar

Department of Textile Technology
Indian Institute of Technology, Delhi-110 016
E-mail: rsr60@hotmail.com. manjeetjassal@hotmail.com. crktex@rediffmail.com

Abstract

Spider dragline silk is an extremely strong biopolymer and has unique combination of desirable mechanical properties. In the present paper dragline of Golden Nephila spider was studied for dimensional, structural, physical and tensile properties. The test results established significant variability in diameter and denier of dragline filaments. The filaments possessed nearly circular cross-section and were found to be sensitive to moisture. The draglines exhibited super contraction in water. It has high strength and large elongation to break (45.9 cN/tex and 38.7 %, respectively). X-ray crystallinity of 17.5 % was obtained. The fibres were also subjected to thermo- mechanical and dynamic mechanical analysis.

Key words:

Spider silk, Dragline, Golden Nephila, Characterization, Mechanical properties

Introduction

In the world of natural fibres, spider filament has been recognized as the wonder fibre for its unique combination of high strength and elongation at break. Unlike B.Mori silk, spider silk has not been domesticated for textile applications. This is due to the difficulty in raising dense population of spiders due to their solitary and predatory nature. In addition, orb webs are not reel able as a single fibre, unlike the fibroin from the cocoon of the silk worm. Furthermore, in comparison to the silkworm cocoon silk, spider generates only small quantities of silk and function as prey capture, reproduction, vibrational sensors, safety lines and dispersion tools [1].

The spiders produce wide range of silk fibres for many different purposes with surprisingly varied mechanical properties. However, the most outstanding properties are found in the dragline and radial threads of some giant spiders such as Nephila. Although they are not as strong as some synthetic fibres such as Kevlar, but it is more elastic. This allows it to absorb more energy prior to breaking than any commonly used synthetic material. These fibres combine the advantages of a protein structure, including hydrophilic properties, biodegradability, biocompatibility with high strength and high modulus, comparable to some synthetic high performance fibres but with an extremely high extension of break [2].

Sullivan [3] cited that the breaking stress of the dragline silk for three species of spiders range from 1420 to 1550 million N/m^2 with elongation at break ranging from 16 to 30%. Together these result in a specific toughness comparable to the best synthetic material. The breaking stress of Kevlar 49, high-tenacity nylon and Carbon fibers are 2000, 1600 and 1750 million N/m^2, with the elongation at break of 4.0%, 16% and 1.0% respectively.

In the past one decade, a great deal of progress has been made in understanding the silk genetics and protein structure. Beard [4] reported that only 50 % of protein chemistry of silk is understood, and work is in progress to understand the protein building blocks and the process of turning them into a crystalline polymer and curing of this polymer into a tough insoluble fibre. Gosline et al [5] found that when a dragline is immersed in water it absorbs water and contracts to about 55 % of its dry length, accompanied by a drastic reduction in elastic modulus from ~10^{10} to 10^{7} Nm^{-2} and a large increase in extensibility. X-ray diffraction results have shown that crystalline regions are relatively not affected by this contraction. This super contraction has been attributed to the breaking of the hydrogen bonds and coiling and disorientation of molecular chains [6].

A better understanding of the relationship between microstructure and mechanical properties will lead to an improved understanding of the behavior of polymeric fibres in general. This can potentially result in the development of new synthetic fibres for structural applications spun from solutions. In this study we report physical, structural, and mechanical properties of Nephila spider dragline.

Evaluation of structural properties

Effect of treatment with solvents

A known weight of dried dragline sample was treated with excess of solvent at room temperature and from change in weight, the weight loss after solvent treatment was determined. The surface characteristics of the fibre were studied using scanning electron microscope.

Wide-angle x-ray diffraction

Untreated, toluene treated and stretched draglines were tested for crystallinity. Wide-angle X-ray diffraction (WAXD) measurements were obtained on Philips X-ray diffractometer model PW1732/10 using nickel filter Cu radiator. Intensity of scan was in the range of 10-35°. The crystallinity index was calculated as:

$$X_c = \frac{A_c}{A_c + A_a} \times 100\%$$

Where A_c and A_a are the area of the crystalline and amorphous portion of the X-ray diffractograms respectively.

Fourier transform infrared spectroscopy analysis

Perkin Elmer FTIR Spectrum BX was used to record FTIR of fibre samples. The KBr pellets or fibre sample were used for the test. The stretched and contracted draglines were also tested.

Differential scanning calorimetry

Thermogrames were recorded using a Perkin Elmer –DSC-7. Samples were dried in a desiccator for 24 hours before the test. For each run ~5mg of samples was used, and the thermogram was recorded from -20°C to 280°C at a heating rate of 20°C/min., under nitrogen atmosphere.

Thermo gravimetric analysis

Thermo gravimetric analysis (TGA) was carried out using dried sample under nitrogen atmosphere at a heating rate of 20˚C/min up to 900° C using Perkin Elmer TGA7.

Thermal mechanical analysis

Thermal mechanical analysis (TMA) of the spider silk dragline was carried using Perkin Elmer TMA-7 instrument from 50° C to 250° C, at a force of 10 mN under nitrogen atmosphere.

Dynamic mechanical analysis

The Dynamic mechanical analysis (DMA) was carried out from -70˚C to 240˚C with static load range 10-500mN and the dynamic load ranges 9-500mN.

Evaluation of dimensional properties

Measurement of diameter and its variations of single filaments

Filaments each of length 6 cm were separated from the dragline using a fine pin without stretching and were cut by a sharp knife. Each end of the filament was pasted on to a glass slide in a tensionless state and the diameter was measured using Leica Microscope at a magnification of 100 x. An average of ten readings was taken for each filament. For measuring inter filament diameter variation, 5 filaments from each dragline were selected.

Measurement of filament denier

The denier of the filaments was measured with Textechno H vibromat ME-41066. This instrument uses acoustic oscillation excitation and electronic amplitude detection principle. For intra filament denier variation, deniers were recorded for every 1 cm length of a filament and an average of 16 readings was taken. For inter filament denier variations, 120 filaments from different draglines were used.

Swelling and moisture absorption studies

Filaments were conditioned for two days in desiccators under various RH from 0 to 97%. The temperature of the environment was kept at 22° C. Diameters of the filaments and weights of the dragline were measured corresponding to various RH values.

Testing of contraction of dragline

A dragline was kept straight and from which a specimen of 15 cm length was cut. The specimen was placed in water for 12 hours, taken out, dried at room temperature for 24 hours and the length was measured. An average of 10 draglines was recorded.

Testing of tensile properties

Tensile testing of dragline

A dragline was tested with the Statemate ME tensile tester. An extension rate of 50 mm/minute and a gauge length of 50 mm were used. The test was carried out after conditioning the dragline under standard test condition for one day.

Tensile testing of filaments

Single filaments were separated from the dragline, before the tensile test. The tensile test was performed using Instron tensile tester. The filaments were pasted on to a paper window. A 25 mm gauge length and an extension rate of 50 mm/min were used. To study the effect of contraction on tensile behavior, filaments were wetted in water for 10 min. and then allowed to dry. The filaments before and after contraction were tested for tensile properties.

Results and discussion

Effect of solvents on weight loss of dragline

The filaments were stable with non-polar solvents and no dimensional changes were observed. As shown in Table 1, a very small fraction (2-4 %) of gum impurities was removed.

Table 1. Weight loss on treatment with non-polar solvents

Solvent	Weight Loss %
Carbon Tetra Chloride	4
Toluene	4
Xylene	2

Figure 1.a and 1.b show SEM micrographs of spider dragline and toluene treated draglines, respectively. The untreated dragline exhibits a slightly rough surface, while the surface of toluene treated sample appears to be smooth with very little gum deposit. The micrograph shows fibrils are enclosed within the fibre core. In some layers, nano-fibrils seem point straight along with the fibre axis, while in the others they coil around the fibres. This arrangement may help the fibre to absorb huge amount of energy before it breaks.

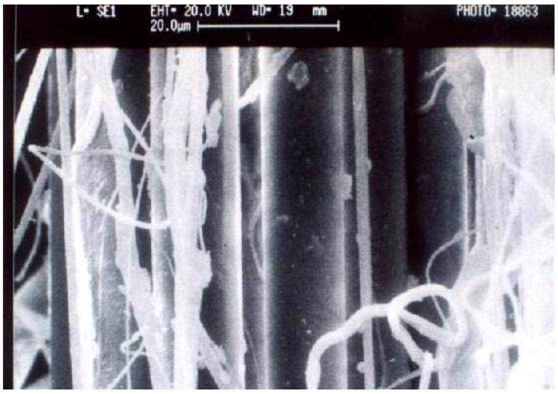

Figure 1a. SEM micrograph of untreated dragline silk with gummy part

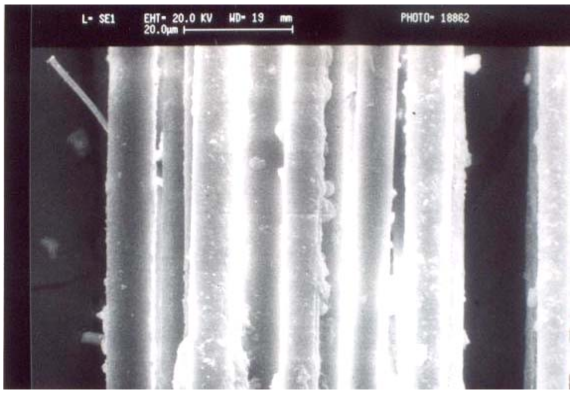

Figure 1b. SEM micrograph of dragline treated with toluene

Fourier transform infrared spectroscopy (FTIR) Analysis

FTIR of dragline samples were recorded before and after toluene treatment. The FTIR of contracted dragline was also compared with the stretched dragline sample. As detailed in Table 2, a number of well-defined peaks are obtained in the range of 600-1800cm^{-1}. The spider silk draglines show a distinct CO amide-I band in the range of 1610-1660 cm^{-1}. In untreated, toluene treated and contracted samples this absorption appears at lower frequency value while for stretched dragline the absorption shifts to higher frequency. Higher frequency peaks (in stretched sample) is characteristics of β sheets configuration of polypeptide back bone and is in agreement with some proteins that contains high proportion of this configuration. The lower wave number peak might indicate the random coil structure.

Table 2. FTIR absorption peak values (major) for different spider silk samples

Peak range cm-1	Assignm -ents	Spider silk				Remarks
		untreated	Toluene treated	contracted	stretched	
530-584	N-H	537			545 (s,I)	N-H plane wagging
643-967		643		698(s)	698,620	Absorption due to bending / vibrations of alpha helix structure
900		907	907	907	907	C-CH_3 Stretching
1015-1032		1038	1032	1022	1021(s,I)	Absorption due to bending / vibrations
1230	C-O	1222	1244	1273	1231(s,I)	C-O single bond amide III
1140-1492	N-H, amide-II	1429		1447	1449(s,I)	-N-H-Deformation of sec. amide
1612-1633	Protein amide	1612	1633	1633(s), 1669 (w)	1656(s,I), 1669	Higher frequency peaks due to β structure
2941-2946	O-H, C-H	2941	2941	2925		O-H bond formation/ salt formation and C-H stretching
3297-3300	O-H, N-H	3290, 3437 (b)	3304 (b)	3291	3292(s,I)	Hydroxyl groups/N-H stretching

(s): strong, (I): intense, (b): broad, (w): weak

Below 1200 cm $^{-1}$, spider silk exhibits distinct bands at 1170, 1100 and 1020 cm^{-1} for absorption due to bending vibrations. If the polyypeptide chains are in the form of α helix, it appears at 1100cm^{-1} and if they are in the β helix, it appears at 1080 cm^{-1}. In addition spider silk exhibits a sharp peak at

907cm^{-1}, which is associated with C-CH_3 stretching. This band is known to appear in the spectrum of poly (L–alanine) but not in the spectra of Poly (alanine–glysine) and Poly (glysin–serine). Therefore this particular peak confirms that the spider silk dragline has units of $(alanine)_n$ where n = 4 to 7.

On contraction of the spider silk, the ratio of the intensity of 1669 to 1652 peaks reduced in amide I region, suggesting that the proportion of β conformation decreased significantly on the contraction. In amide III, 1230 cm^{-1} peak-β sheets shifted to higher wave number at 1273cm^{-1} (assigned to α helix).

Differential Scanning Calorimetry (DSC)

Figure 2 shows DSC trace for spider silk dragline. A broad endotherm with a peak at ~90-95 ˚C was observed. This broad endothermic peak may be associated with water loss. This is consistent with the TGA studies. The spider silk samples are known to contain bound water even after drying in the desiccator.

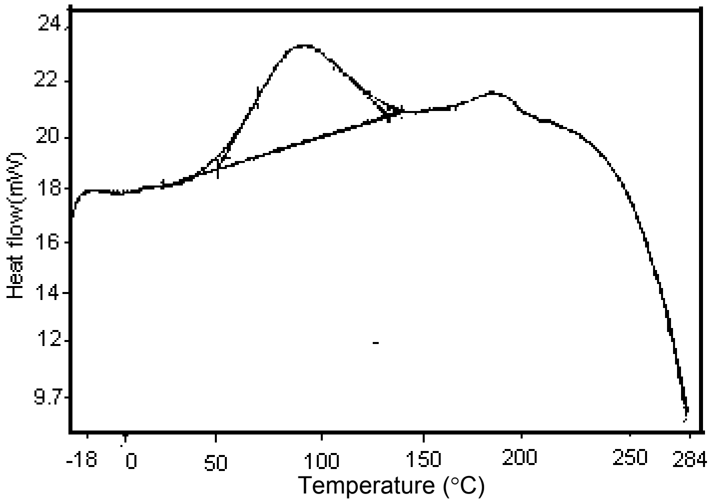

Figure 2. DSC Thermogram of spider silk dragline

Thermo Gravimetric Analysis (TGA)

The Thermo gravimetric and differential thermo gravimetric (DTG) curves of spider silk dragline are shown in Figure 3. Thermo gravimetric analysis (TGA) was carried out using dried dragline sample under nitrogen atmosphere at a heating rate of 20˚C/min. Below 150˚C the TG curves of sample 1 and 2 exhibit a weight loss of 5.68% and 6.99 %, respectively. This weight loss was attributed to the entrapment of water as observed from DSC studies. Above 150˚C, the TGA curves show two-step degradation of the fibre as detailed in Table 3. The first step of weight loss observed in the temperature range 200 to 501˚C is probably associated with the breakdown of side chain group's in the amino acids and residues [7].

Table 3. Thermo Gravimetric Analysis (TGA)

Dragline sample	Degradation temperature and % Weight loss						
	Ist step			IInd step			Ultimate Residue
	Temp. range (˚C)	T_{pk1} Temp (˚C)	Wt. loss %	Temp. range (˚C)	T_{pk1} Temp (˚C)	Wt. loss %	
1	200-501	342	47.28	501-895	655	41.8	3.7
2	200-501	334	47.60	501-895	659	44.8	1.2

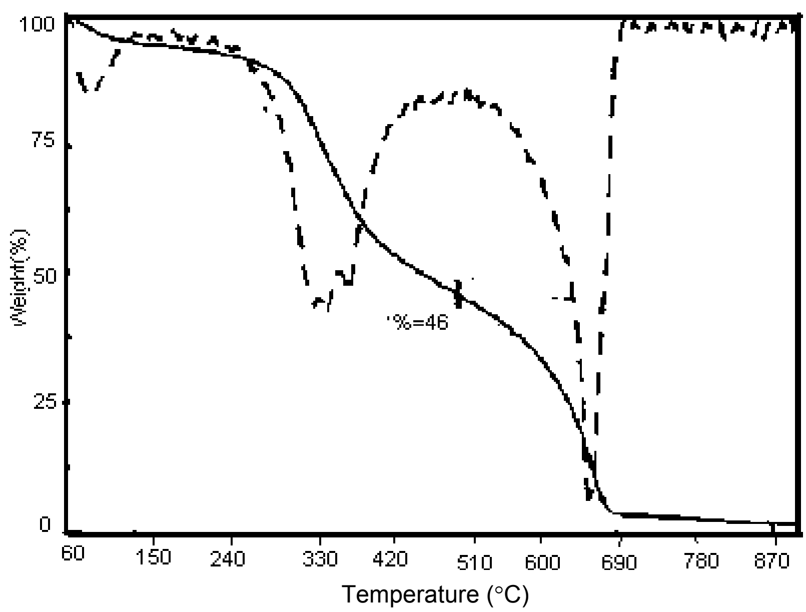

Figure 3. TGA curves in nitrogen for a Nephila spider dragline sample

The second major degradation from 501 to 896 ˚C with a weight loss of 42% - 44% can be attributed to the degradation of main chain amino acids. An ultimate residue of 1.2% to 3.7% indicates almost the total decomposition of the sample at about 900˚C.

Thermal Mechanical Analysis (TMA)

TMA of the spider silk Dragline was carried with Perkin Elmer TMA-7 instrument from 50˚to 250˚C at a force of 10 mN under nitrogen atmosphere. A typical plot is shown in Figure 4. A marked change in the gradient (thermal expansion coefficient α) at 186.4˚C from -6.59 x 10^{-4} to -8.2 x10^{-3} corresponds to the transition temperature. This characteristic provides a sensitive way of detecting the glass transition temperature in small samples as thermal expansion data is not dependent upon accurate measurement of sample diameter and are also insensitive to the moisture content of the sample.

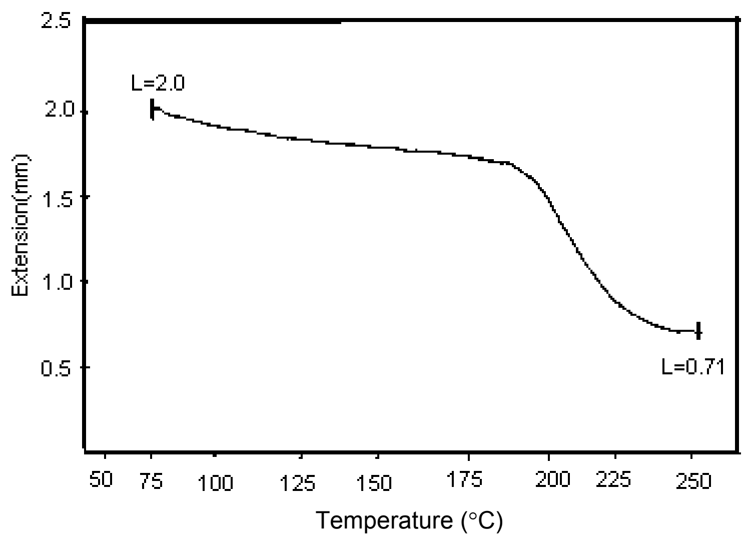

Figure 4. Typical plot of dragline length versus temperature

Dynamic mechanical analysis (DMA)

The DMA analysis was carried out with and with out nitrogen purging. Temperature was varied from -70˚C to 240˚C with static load range 10-500 mN and the dynamic load ranges 9-500 mN. The plots of storage and loss moduli as a function of temperature are noisy and have poor reproducibility. The storage modulus (E^1) fluctuated with temperature and reached a maximum at 60 - 65˚C, which is associated with moisture content. The fibre thickness also affects the ability of samples to loose bound moisture. The noisy profile of this storage modulus (E^1) versus temperature plots may also be due to variability in cross-section or diameter of dragline. The spider silk dragline exhibits a gradual decrease in storage modulus near the Tg and suggests its semi crystalline condition. The main softening point of spider silk is at 186.4˚C.

X-ray diffraction study

The crystallinity of the samples was determined for draglines including stretched and toluene treated ones. The un-stretched and toluene treated sample showed a very broad x-ray diffraction peak at 2θ = 10-35°. However, the stretched spider dragline exhibited a relatively sharper peak at 2θ = 20°, accounting for % crystallinity of 17.5. This value is much lower than the reported value of 30 % [8]. This difference may be attributed the difference in the dragline collection mechanism.

Physical properties of spider silk

The Nephila spider filament has uniform circular cross-section. The diameter variations in the filament appeared over length as short as few milli meters [9]. Between the draglines the filament diameter varies from 5.0 to 13.0 microns. Within a dragline out of 18 filaments were tested, the diameter varies from 9.14 to 12.85 microns. In a single filament the C.V% of diameter at 1 cm. interval was found between 1.05 and 2.41. Garrido et al [10] reported that the cross section anisotropy of spider filament was 1.05 and the forcibly silked fibres were having more variation in diameter.

Effect of humidity on spider filament

Table 4 shows the effect of RH on regain and diameter change of the filaments. It is observed that the regain goes up to 39.3 % when the RH is 97%. The diameter of the filament increases by 80% when the RH increases from 0 to 97 %. The longitudinal views of filaments conditioned at 0% and 97% RH are shown in the Figures 5a and 5b, respectively.

Table 4. Influence of RH on regain and swelling of the filaments

Saturated solutions of chemicals	RH %	Regain %	% increase in diameter
Conc. Sulphuric Acid	0	0	0
Potassium Acetate	22	6.3	6.1
Sodium nitrite	66	10.9	59.9
Ammonium Sulphate	81	18.3	69.7
Potassium Sulphate	97	39.3	81.8

Figure 5a. Nephila spider filament at 0% RH

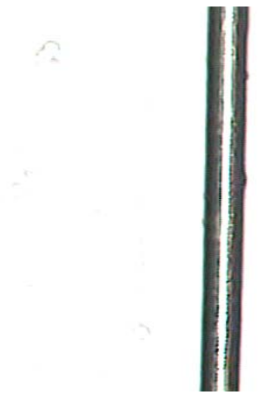

Figure 5b. Nephila spider filament at 97% RH

Effect of wetting on contraction and tensile properties of dragline

When spider silk is placed in water, wetting causes the fibre to swell and extensibility increases considerably accompanied by about 1000-fold drop in initial stiffness of the fibre [11]. Average contraction of dragline after wetting and drying is found to be 28.5% with a coefficient of variation of 7.9%. Since there is a disorder in the arrangement of individual filaments in a dragline the contraction of individual filaments would be much higher than this value. Water treated filaments have lower tenacity and higher breaking extension compared to normal filaments. The reduction in tenacity and increase in breaking extension of water treated filaments is 40 and 45% respectively from those for normal filaments.

Tensile properties of dragline

A typical load-extension curve of a dragline is shown in Figure 6. It exhibits non-catastrophic failure. Each filament of the dragline breaks one after another. It shows that the filaments are not parallel to each other. The average tenacity of a dragline is 18.2 cN/tex and extension at peak load is 20.8%.

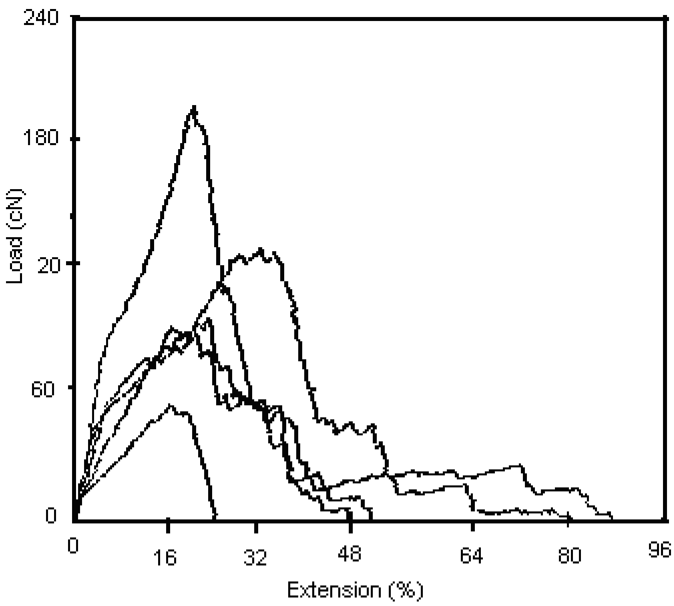

Figure 6. Load- extension curve of Nephila Spider Dragline

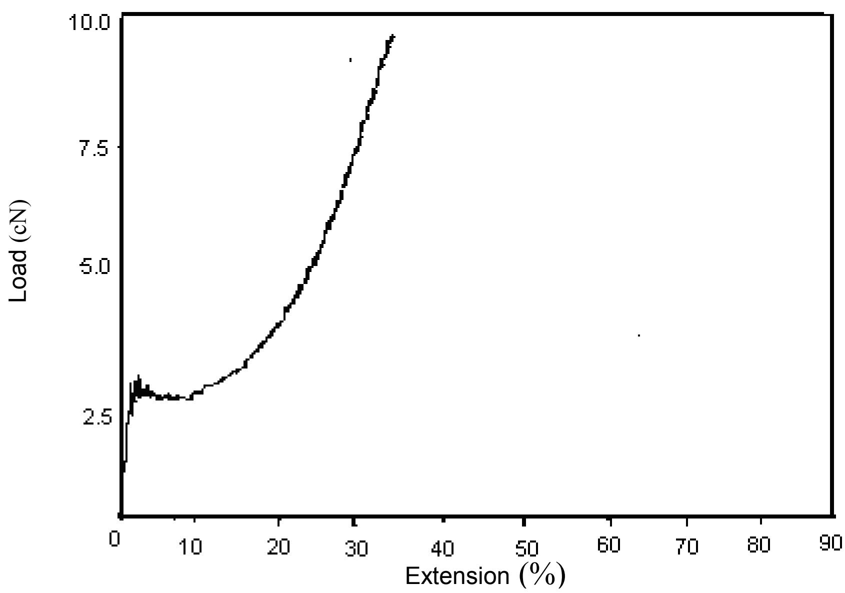

Figure 7. Load- extension curve of a Nephila spider filament

Tensile properties of single filaments

The properties of the spider silk depend on the chemical composition and the conditions under which the bulk silk secretions are converted into fibre [12]. The load-extension diagram for single a filament is depicted in Figure.7. It shows a yield region, strain-hardening region (flattening region) followed by plastic deformation. Filaments of a dragline have an average tenacity of 45.9 cN/tex and breaking extension of 38.7 %. Tenacity of the coarse filaments is generally found to be lower than the fine ones. SEM Photo of a filament under tensile load shows a ductile failure (Figure. 8)

Figure 8. SEM Photo of broken filament

Conclusions

The studies conducted on Nephila Golden spider silk established the following facts: The filaments from major ampullate gland have variability in their diameter, denier. Dragline has multiple filaments and has around 4.0% gummy part. The filaments have circular cross section and are cylindrical. Broken filament during the tensile test shows a ductile failure. Filaments are very sensitive to moisture and swell at higher moisture regain. The dragline shows a super contraction in water (28%). The filaments have a tenacity of 45.9cN/tex along with large extension of 38.74%. The water treated filaments have very large breaking extension of 56%. The X-ray diffraction of stretched filaments shows 17.5% crystallinity. X-ray analysis and Birefringence methods do not show clearly the molecular orientation. FTIR analysis confirms the presence of protein amine in dragline silk. TMA test revealed negative thermal expansion coefficient. Tg is likely to occur at 186.5˚C. The dragline shows good stability up to 250˚C. DMA study on dragline silk has shown noisy storage modulus and Tg-peaks are not clear.

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