

# FTIR Spectroscopic Characterization of Spider Silk of Family *Lycosidae* and *Erasidae*.

Mangle Vijay S<sup>1</sup> and Vairale Amit<sup>2</sup>

<sup>1</sup>Department of Environmental Science, Arts, Science and Commerce College, Chikhaldara, Dist. Amravati.

<sup>2</sup>Department of Zoology, Gulab Nabi Azad Arts, Commerce and Science College, Barshitakli, Dist. Akola.

\*Corresponding author Email: [malhar.1698@rediffmail.com](mailto:malhar.1698@rediffmail.com)

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## ABSTRACT

Spiders are unique in their ability to synthesize and utilize silk for various purposes. The primary structure of silk is its amino acid sequence, mainly consisting of highly repetitive blocks, which is why silks are often referred to as a block co-polymer. This attempt was made to characterize silk of *Lycosidae* and *Eresidae* for the functional group amino acids by FTIR spectroscopy. The test results show that, the silk of *Lycosidae* contain strong -COOH asymmetrical vibration and -NH<sub>3</sub> symmetrical bending vibration for Glutamic acid and Lysine, while in *Eresidae*, Arginine amino acid and with -NH<sub>2</sub> medium bending vibrations. Both the silk samples show presence of Beta - sheet secondary protein structure. In *Lycosidae*, strong vibrations in Amide-I region, while in *Eresidae*, medium vibrations get observed. Amide-II band are absent in *Eresidae*. Amide -B band are observed in both the samples.

**Keywords-** amide, amino acid, *eresidae*, *lycosidae*, silk, spider

## INTRODUCTION

The term silk normally refers to a wide range of continuous filament spun by the spider, use for building structures for various purposes including prey capture. Spiders are unique in their ability to synthesize and utilize silk for various purposes. Many species of spider have different glands to produce silk with different properties for different purposes, including housing, web construction, defence, capturing and detaining prey, egg protection and mobility gossamer for ballooning or for a strand allowing the spider to drop down as silk extruded. Different specialized silk have evolved with properties suitable for different uses. The orb-web spinners are equipped with 5-7 different types of silk secreting glands, each synthesizing its own type of silk to be utilized for a specific purpose, e.g., construction of dry and sticky parts of the web, construction of egg-sac and swathing silk of captured prey (Lombardi, *et al.*, 1990).

Silk is made up of two primary proteins; a fibrous protein known as fibroin and a sticky protein s are sericin with two comprising 70-80% and 20-30% of silk, respectively. Silk as well as many other biomaterials have a hierarchical structure (e.g., cellulose, hair etc.). The primary structure is its amino acid sequence mainly consists of highly repetitive glycine and alanine blocks, which is why silks are often referred to as a block co-polymer. The proteins largely made from non-essential amino acids. Most spiders produce a variety of silks from different specialized glands. The polygenetic divergence is reflected in the variability of silk properties in response to varying environmental conditions (Madsen *et al.*, 1999). Various compound other than protein is used to enhance the fibre's properties, these are sugars, lipids, ions and pigments that might affects the aggregation behaviour and act as a protective layer in the final fibres.

The mechanical properties of spider silk originate from the core of the fibre, which is protected by a series of 'skin' layers. The outer core ( $\approx 15\%$  of the radius) is thought to consist mainly of the highly ordered protein Spidroin I, with the inner core ( $\approx 80\%$  of the radius) consisting of both Spidroin I and the less-ordered Spidroin II<sub>1,2</sub>. The intrinsic disorder in Spidroin II occurs due to the presence of proline, which twists away from simple, ordered configurations 3. This combination of proteins results in a distribution of approximately 40% ordered domains (two hydrogen bonds per amide group), 15% permanently disordered domains (one hydrogen bond per amide group) and 45% intrinsically disordered domains but with potential for order 3 (Brown., C.P, *et al.* 2011)

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. This fibres combine the advantages of protein structure, including hydrophilic properties, biodegradability, biocompatibility with high strength and high module, comparable to some synthetic high performance fibres but with an extremely high extension of break (Rangaswamy, *et al.*, 2005).

Amino acid composition of many ampullate gland silk (dragline) produced by the mature, male golden orb

weaving spider, *Nephilla clavipes* was 4. Although the amino acid composition is known for the seven silks from one animal (Anderson, 1970), only two of the seven types have been investigated in any detail. *Nephilla clavipes* is a large orb weaving spider, dispersed in the tropical and subtropical areas of the western hemisphere (Lombardi, S.J., *et al.*, 1900). The design principles of dragline silk spider and nature's high-performance fibre are still largely unknown in particular for the non-crystalline glycine rich domains, which form the bulk of the material. The alanine residues are predominantly found in a  $\beta$  sheet conformation. The glycine residues are partly incorporated into the  $\beta$  sheets and otherwise form helical structures with an approximately 3-fold symmetry (Beek, J.D., *et al.*, 2002).

*Lycosidae* is a worldwide family of ground living spiders. Most are free living and are the likely to be encountered in the field. The funnel web is a sheet web built under a ground, litter or rocks. It ends in tubular section. The spider stays hidden in the funnel and emerges the moments it detects prey struggling on its web. The victim is pulled into the funnel from where escape is impossible.

*Eresidae* family, all the species of *Stegodyphus* are plant-living either solitary or living in community nests, e.g., *S. dumicola* and *S. mimosarum*. A retreat is made by the solitary species usually in grass heads with trip lines extending outwards (South African National Survey of Arachnida, Technical Report, 2010, version.Pp.244). Present attempt is made to compare the protein and functional group characteristics of silk of family *Lycosidae* and *Eresidae*. The infrared spectra usually have sharp features that are characteristics of specific types of molecular vibrations making the spectra useful for sample indication.

## MATERIAL AND METHODS

The clean silk samples were collected from the natural habitat of the spiders of *Lycosidae* and *Eresidae* family from basin of Chhatri Tank region near Amravati locality. The silk samples carried to Central Instrumentation Cell of Arts, Science and Commerce College, Kiran Nager, Amravati, then clean fibres were cut into small pieces and analysed on Agilent Technology Model CARY & 630 FTIR.

## RESULTS

Fourier Transform Infra Red Spectroscopic results for silk of *Lycosidae* sample (Fig.-1) shows that, very strong N-H stretching vibration at 3282  $\text{cm}^{-1}$  for Amide -A group and medium stretching vibrations at 1625  $\text{cm}^{-1}$  for C=O (70-85%) and C-N (10-20 %) for Amide -I group. Strong N-H bending vibrations (40-60 %) at 1625  $\text{cm}^{-1}$  for C-N (18-40

%) and C-C (10 %) occurred. Medium vibrations occur at 1337  $\text{cm}^{-1}$  for Amide-III group. Strong absorption at 1560  $\text{cm}^{-1}$  for - COOH asymmetrical stretching vibration for glutamic amino acid was observed while -NH<sub>3</sub> symmetrical bending vibration at 1622  $\text{cm}^{-1}$  explains  $\beta$ -sheet secondary structure of protein. Strong vibrations in 3923-3282  $\text{cm}^{-1}$  indicates Amide-B group.

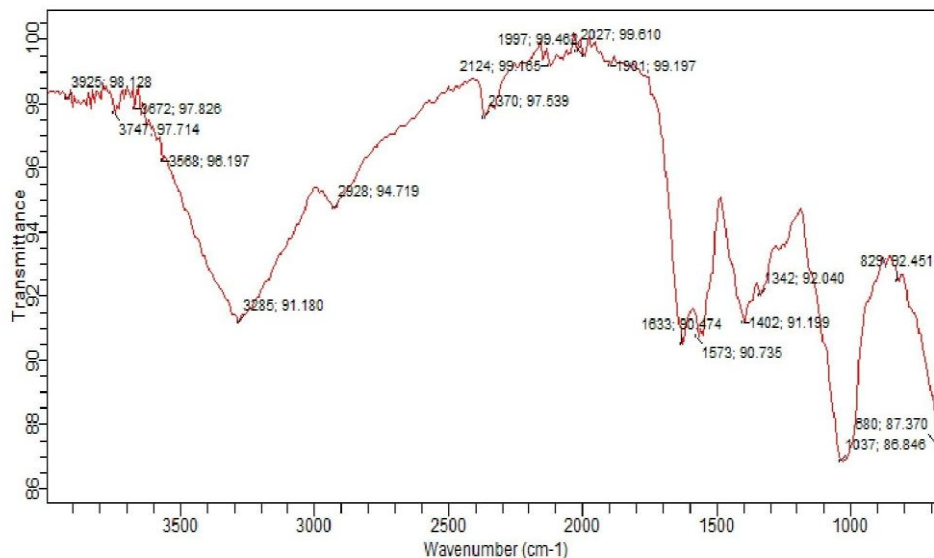


Figure 1- FTIR spectra of *Lycosidae*

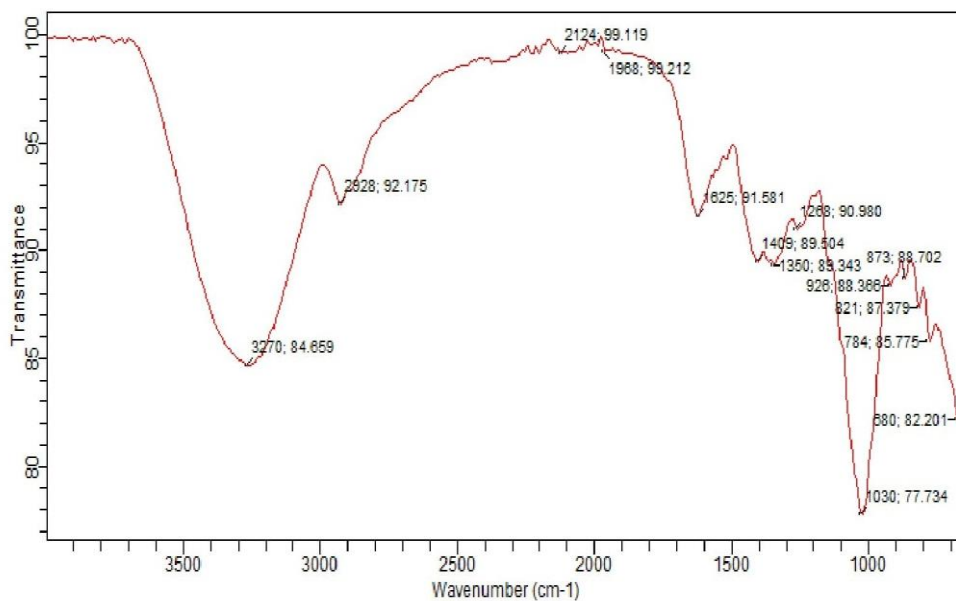


Figure 2- FTIR spectra of *Eresidae*

Fourier Transform Infra-Red Spectroscopic results for silk of *Eresidae* sample (Fig.-2) shows that, a strong N-H stretching vibration (95%) at 3265  $\text{cm}^{-1}$  for Amide -A group and C=O stretching (70-80 %) and C-N stretching (10-20 %) vibration at 1622  $\text{cm}^{-1}$  indicate Amide-I group. Strong  $-\text{NH}_2$  bending vibration for arginine protein at 1622  $\text{cm}^{-1}$  was observed and it also indicates  $\beta$ - sheet secondary structure of protein. Presence of Amide-B absorbance at 2926-3265  $\text{cm}^{-1}$  was observed.

## DISCUSSION

The FTIR spectrum showed number of peaks that are characteristics of specific types of molecular vibrations. Silk protein or spidroin largely consist of repeated sequence of amino acids. These repetitive regions of the spidroin are confined in highly organized secondary structure which is responsible for semi crystalline nature of spider silk. The presence of bulky glutamine residues limit the growth of  $\beta$ -sheet and force the loops, tie chains and the surrounding amorphous matrix (Saravanan, 2016). It is however generally concluded that spider silk is made from different motif; (1) an elastic  $\beta$ -sheet spiral that is composed of multiple GPGXX, X varies in protein, (2) crystalline  $\beta$ -sheet, (3) tight amino acid repeats form a helical structure and (4) space region (Gosline *et al.*, 1999).

Beard (1998) reported that the spider silk drag lines show a distinct CO amide -I band in the range of 1610-1660  $\text{cm}^{-1}$ . Below 1200  $\text{cm}^{-1}$ , spider silk exhibits distinct bands at 1170  $\text{cm}^{-1}$ , 1100  $\text{cm}^{-1}$  and 1020  $\text{cm}^{-1}$  for absorption due to bending vibrations. If the polypeptide chains are in the form of  $\alpha$  helix, it appears at 1100  $\text{cm}^{-1}$  and if they are in  $\beta$  helix, it appears at 1080  $\text{cm}^{-1}$ . Mid IR spectra from all types of spider silk showed protein peaks in the amide I and II regions. The widow silk had a peak around the 3250  $\text{cm}^{-1}$  wave number that was not present in other silk types. There was also a reversal of alpha helix and  $\beta$  sheet content between two structurally different types of silk from the orb weaver (Normandeau, 2014).

Female spiders in super family *Araneoidea* (orb- spinning spiders and their close relatives) spin six different kinds of silk (three fibroins and three fibre protein glues) that differ in amino acid content and protein structure. In addition to this diversity in silk produced by different

glands, we found that individual spiders of the same species can spin dragline silk (drawn from the spider's ampullate gland) that vary in content as well (Catherine,*et al.*, 2000).

## CONCLUSION

After FTIR spectroscopic characterization of silk samples of families Lycosidae and Eresidae it is to conclude that, Amide-I and Amide-A groups are present in both samples. Amide-II is present in *Lycosidae* while absent in sample of *Eresidae*. In sample *Lycosidae*, glutamic amino acid and lysine is present while asparagine amino acid present in *Eresidae*. The  $\beta$ - sheet secondary structure of protein is present in both the samples.

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

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