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A STUDY OF THE MECHANICAL BEHAVIOR OF SPIDER SILKS

by

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Collaborative Research, Inc.
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Natick, Massachusetts 01760

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FOREWORD

This Study of the Mechanical Behavior of Spider Silks was conducted under Contract No. DAAG17-67-C-0135 during the period 15 April 1967 to 15 April 1968. The program was under the overall direction of Dr. O. M. Friedman of Collaborative Research, Inc.

The importance of the study lies in the confirmation of the existence of very high tenacity, natural polypeptide fibers complemented by relatively large elongations to break. Equally important is the fact that the principal amino acids making up the polypeptides are the relatively simple glycine, alanine, glutamic acid, and proline. This points to the future possibility of synthesizing a polypeptide fiber with properties derived for a specific application.

The chemical part of this work was carried out by Mrs. I. E. Holleck under the supervision of Dr. N. A. Starkovsky at Collaborative Research, Inc. Physical and mechanical investigations were performed by Miss S.D. Hoenshell under the direction of Mr. G. A. M. Butterworth at Fabric Research Laboratories, Inc. X-ray diffraction studies were by Mr. A. King of the U.S. Army Natick Laboratories. Amino acid analyses were by Dr. E. W. Westhead, courtesy of Prof. E. W. Lenz at the University of Massachusetts.

The spider silk used in these studies was collected and identified under the direction of Prof. H. W. Levi of the Harvard Museum of Comparative Zoology.

Mr. Roy C. Laible was Project Officer for the U.S. Army Natick Laboratories and Mr. Anthony Alesi was alternate Project Officer.

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ABSTRACT

A preliminary physical and chemical examination of a selected group of spider silks was made. The program's ultimate goal is the synthesis of "super tenacity" protein fibers, and the work reported here was designed to:

1. Confirm the existence of very strong, natural protein fibers, specifically, of spider silks.
2. Determine some of the physical properties of such fibers.
3. Obtain information on the chemical makeup of strong protein fibers and compare them with weaker protein fibers.

Collection techniques were streamlined and large quantities of spider silk were obtained. It was shown that a variety of these spider silks have average rupture tenacities exceeding 10 gms per denier and that a few show tenacities exceeding 15 gms per denier. These strengths class these fibers among the strongest organic fibers known. Equally remarkable, however, are the elongations before rupture - amounting to 15 percent and more.

Fractionation studies on spider silk indicate that it is almost completely of the fibrous form with little or no soluble (globular) components. Amino acid assays show that the silk contains primarily the simpler amino acids and has a possible relation to a collagen structure.

A STUDY OF THE MECHANICAL BEHAVIOR OF SPIDER SILKS

Introduction

The work reported here is a continuation of a previous program (1) to explore the possibility of synthesizing "super tenacity" protein fibers using high-strength spider silk as a model. Specifically, it was planned to:

- a. Broaden and refine techniques for collecting spiders and their silks.
- b. Conduct a more detailed and statistically reliable physical evaluation of the various silks obtained and attempt to confirm the isolated reports of very high strength in some silks.
- c. Examine some facets of the chemical makeup of a few spider silks to look for obvious chemical differences between "strong" and "weak" silks.

Collection of Silk

In the previous work (1) it was found that silks from the species Nephila clavipes taken in Florida had proven to be very strong -- averaging 12.2 gpd rupture tenacity. It was therefore decided to concentrate on this species and on similar species of "orb spinning" spiders. It was decided to concentrate on spiders from Brazil, Panama, and Florida. It was thought not worthwhile to obtain the frequently mentioned Nephila madagascariensis.

Over 40 spiders of several species were collected in Brazil. From a number of these, silk was drawn at time of collection. Also collected were the webs corresponding to several of the specimens. All of the spiders were brought back to the U.S. in apparent good health, and they were then silked under controlled conditions. The details of silk spinning and the identification of the specimens are given in Appendix A.

Over 70 specimens of Nephila clavipes and Argiope aurantia were collected in Florida. Also obtained were samples and silk from spiders at the point of collection. All live specimens were successfully brought back to the U.S. where silk was again drawn from them under controlled conditions. Identification of the specimens and silks is again reported in Appendix A.

The abundant supplies of silk gathered in both Brazil and Florida obviated the need for a collection trip to Panama. A total of about 3000 feet of spider silk was obtained from these trips.

Chemical Studies

Samples of spider silk were submitted to fractionation as follows: a) degumming with hot water and Castile soap solution; b) partial dissolution in specially chosen solvents. Before and after each treatment of the fiber (for example, degumming with hot water), the fibers were kept in vacuo over phosphorous pentoxide for 24 hours. It was found that simple degumming removed about 5 percent of the weight of the silk. The remainder was kept soaked in a 40 percent lithium bromide solution. This solvent is known to dissolve most of silkworm silk (2). However, in the case of the spider silks tested, not more than 10 percent of the silk went into solution, and the fiber structure was not modified as judged visually and under a low-power microscope. The fiber thus treated with lithium bromide solution was then dissolved in cupric ethylene diamine solution*. Only some 5-10 percent remained as a solid residue. During dialysis (for removing the cupric salt), the dissolved protein re-precipitated out almost completely (another distinction from silkworm silk). Only a very small amount of protein (less than 1 percent of the original fiber) remained in solution and was isolated by lyophilization of the filtrate.

The small amounts of materials to be dealt with (usually of the order of 0.1-1 mg) required the development of special handling procedures and the use of miniaturized equipment. Estimations of proteins that went into solution at different stages of the degumming and fractionation procedures were done by ultraviolet spectrophotometric techniques.

The preliminary experiments were carried out on samples of Brazilian spider silk that were not used for mechanical testing and analysis, as the first thing necessary was to establish the techniques of handling the fibers (without disrupting them) as long as possible (to permit further x-ray work) and to obtain powders from solubilized protein fractions that could be used for powder x-ray diffraction work. These techniques have now been established and the work planned for the future includes working on fibers of predetermined strength and correlating the results of chemical work with x-ray diffraction and amino acid analysis results.

Results show that spider silk can be converted into a water-soluble form by the use of certain solubilizing salts. The aqueous solution obtained upon removal of the solubilizing salts is unstable. The fibroin is precipitated by the addition of denaturing agents or by the subsequent removal of the salt (such as lithium bromide or copper ethylene diamine) which was used to achieve solubilization. If, as it has been suggested (3) (4), the water

* Prepared by dissolving 6g of cupric hydroxide and 8g of ethylene diamine in water to make 100 ml (procedure of D. Coleman and F. O. Howitt, Proc. Roy. Soc. (London), A 190, 145-169 (1946)).

soluble and insoluble forms of silk fibroin are the globular and fibrous forms, respectively, then spider silk is almost totally in the fibrous form.

Experimental

1) Spider silk (0-5 mg) was wound on small nylon spools (ca 0.1 mg per spool) and submitted to the following treatments: a) boiling water; b) 4 percent aqueous ammonium hydroxide at 70°C; c) 4 percent sodium carbonate solution at 70°C; d) first boiling water, and then 5 percent sodium bicarbonate at 70°C). The fibers were then washed in succession, with water, ethanol, and ether, and dried in vacuo. Their x-ray diffraction patterns were recorded. However, the patterns were too weak to allow useful interpretation. Washing with ethyl alcohol and diethyl ether, then drying 24 hours at room temperature in vacuo was a standard procedure followed in each of the following experiments.

2) Spider silk (ca. 2 mg) (previously degummed with hot water and soap) was kept in 5 ml of 40 percent lithium bromide solution for 24 hours at room temperature. Only a small amount of protein and some colored material dissolved upon this treatment. Upon removal of lithium bromide from this solution by dialysis against running water, most of the protein separated out as flocculent masses, which were isolated by centrifugation. The weight of this material was ca. 0.2 mg. Protein estimation of the centrifuged solution showed that less than 0.02 mg of material was present in solution*.

3) The above sample (0.19 mg), after treatment with lithium bromide solution, was stirred with 3 ml of cupriethylene diamine hydroxide solution (6/8 strength, by Coleman and Howitt's nomenclature (4)). After 1 hour, almost all the protein was dissolved. The solution was centrifuged and neutralized with acetic acid (? ml). The cupric salt was removed by dialysis. At the start of the dialysis, the solution was clear, but as it gradually became colorless while the copper salt was being removed, a flocculent precipitate was formed. At the end of the dialysis (24 hours), the precipitated protein was centrifuged off. It amounted to ca. 0.17 mg of material. The fresh centrifugate still containing some dissolved protein was subjected to the following: a) shaking, or introducing air bubbles, b) treatment with twice its volume of alcohol; c) treatment with 0.1N sulfuric acid; d) bringing to 0.8 saturation with ammonium sulfate. In all these cases, a small amount of precipitate was formed (representing denatured soluble fibroin). However, the amounts of material produced were very small and were not useful for further study (e.g., powder x-ray diffraction).

Amino Acid Analyses of Spider Silk

Three spider silks were analyzed for their amino acid content. The method used for the amino acid analyses is described in Appendix B. One of the three

* Protein was estimated spectrophotometrically.

samples (Paneiras GB, Spider No. 3) was analyzed under two different conditions of hydrolysis to check on the reliability of the results. The results were found to be fairly concordant (see columns 1 and 2, Table III). As for the other spider silk so far analyzed, data were obtained for a strong sample of Nephila cruentata silk (30-1) and a weak sample of Nephila clavipes silk (30-2) (see columns 3 and 4, Table III).

In order to compare these results with the literature data on silkworm silk and on some spider silks, relevant information is collected in Tables I, II, and IV.

The amino acid composition of silks of different origins has been repeatedly discussed in the literature (5) (6). A general feeling is that no precise relations exist between the amino acid composition of the various fibroins and their biological or x-ray classification (7). However, the data in this study represent the first attempt at relating the physical properties of the fibers (mainly their strength) to their amino acid composition.

By comparing data of Table I (silkworm silk and its components) with those of Table II (spider silk), it can be seen (see also Table IV) that spider silk of different origins has widely different compositions and that, in general, spider silk, as compared to silkworm fibroin, is characterized by a high dicarboxylic acid content (Asp + Glu) and in certain, but not all, the cases by a high proline content. Of the two samples #30-1 (strong) and #30-2 (weak), the stronger was characterized by a higher Ala + Gly or Ala + Gly + Glu ratio, and lower ratios for Arg + Lys and Ser + Thr. The stronger of the two samples had a Gly:Ala:Glu ratio approaching 4:3:1. The comparison between these two different samples of spider silk is not absolute, however, as the samples belong to two different species of Nephila (cruentata and clavipes, resp.). The absence of cysteine is noteworthy. However, the relatively high tyrosine content could suggest the possibility of cross-linking occurring via this phenolic amino acid.

The absence of cysteine (or cystine) in silk fibroin was considered to be one of the important characteristics of this fiber until it was demonstrated that S-containing amino acids were present in silkworm fibroin, but they escaped detection by the analytical procedures formerly used. More refined analytical techniques showed the presence of S-S linkages (Comp. F. Lucas, Nature, 210, 952 (1966)). It is noteworthy that spider silk contains methionine and tyrosine. The latter may lead to cross-linking between protein chains (two tyrosyl units coupled by a biphenyl linkage). It was observed in these experiments that some samples of spider silk were colored brown and remained brown even after degumming and partial hydrolysis processes. The origin of this brown color merits investigation (compare setting and hardening of cockroach egg-cases which occur with considerable browning. P. C. J. Brunet and P. W. Kent, Proc. Royal Soc. B, 144, 259 (1955)).

The presence of a significant amount of proline in the samples so far analyzed (especially in the Paneiras GB sample where it is as high as 9-10 percent) calls for a comparison with Nematus ribesii silk (the so-called gooseberry sawfly). It has been noted by Rudal (6) that the fiber spun by

this insect cannot be properly classified as a silk, but rather as a collagen which has also the properties of silk. X-ray examination of this collagenous silk confirmed the fact that its 10 percent content in proline endows it with a typical collagen structure.

It would be of considerable interest to submit spider silk containing a high amount of proline (and showing at the same time outstanding resiliency, elasticity, and tenacity) to detailed x-ray analysis, as it is possible that some of the useful properties of spider silk could be related to its degree of "collagenicity".

The amino acid composition data under consideration refer only to the "drag line" obtained naturally or the "pulled silk" produced by "milking" spiders. It is known that the drag line (and also the "frame fibers") has more outstanding mechanical properties than other types of silk (such as swathing silk or cocoon silk). However, caution must be exercised in comparing these data with those of the literature, as different authors handled the fibers in different ways, and variations can be attributed to the presence of extraneous matter, to inaccuracies in the analytical methods, and to the analyses themselves, even to wrong identification of the spiders.

Future Chemical Plans

The next step in our program is to repeat the work described above on samples of spider silk that have shown excellent mechanical properties and to prepare, by gradual degumming and dissolution, pure spider silk fibroin. Amino acid analysis and x-ray diffraction patterns of such high-tenacity fibroin will be invaluable for a better understanding of the structure of spider silk.

It will also be desirable to submit spider silk fibroin to fractionation with such reagents as rivanol and ammonium sulfate in order to check on the existence of fractions corresponding to silkworm plastic and fibrin (8) (9). In this way knowledge can be gained of the basic structures of spider silk, and comparison with silkworm silk might bring revealing results. These plastic and fibrin structures will also be examined by x-ray methods in collaboration with the U.S. Army Natick Laboratories. It would be interesting to determine whether high-tenacity silk containing a relative high proportion of proline shows some similarity to collagen in its crystal structure (see results of amino acid analysis).

Finally, molecular weight estimations can be carried out on samples of solubilized spider silk by the gel filtration techniques. Sephadex columns, if properly designed and used, can provide an excellent way for determining molecular weights and molecular weight distribution. One can foresee that the small amount of material at hand will render other techniques (light scattering, viscosity determinations...) difficult to apply, and that gel chromatography will be the most rational approach to molecular weight determination.

Still further work will involve the stepwise digestion of spider silk with enzymes. The fractionation of the fibroin of B. mori by Shaw (10) with

trypsin can be taken as an example, as it has yielded important information on the regions of high and low crystallinity of silkworm silk.

Silkworm silk is coated with "sericin", a protein which differs from the fibroin proper both in properties and composition (Table I). In the preliminary work, samples of spider silk were not analyzed before and after submitting them to treatments known to dissolve sericin-like proteins away and to leave the bare fibroin. There is no a priori reason for saying that spider silk is pure fibroin and that it does not possess a sericin-like counterpart. One of the important experiments to be included in a plan for future work would consist in analyzing a good strong fiber for its amino acid content both before and after a degumming treatment, or a procedure such as the one used to fractionate silkworm silk into sericin and fibroin, and then to separate fibroin into fibrin and plastin (Table I). In other words, future work should involve the determination of amino acids in different soluble and insoluble fractions of a given sample of spider silk, chosen from among those which have shown outstanding mechanical properties in the testing program.

Table I
 AMINO ACID COMPOSITION OF
 VARIOUS FRACTIONS OF SILKWORM SILK^a

Sericin ^b		Fibroin ^c		Fibrin ^d		Plastin ^d	
Ser	37.3	Gly	44.5	Gly	46.4	Gly	18.3
Asp	14.8	Ala	29.4	Ala	30.4	Ala	16.5
Gly	14.7	Ser	12.1	Ser	11.0	Asp	10.8
Thr	8.6	Tyr	5.2	Tyr	4.9	Ser	10.4
Ala	4.3	Val	2.2	Val	1.9	Glu	6.5
Arg	3.5	Asp	1.3	Asp	1.1	Leu	5.5
Val	3.5	Glu	1.0	Glu	0.8	Val	5.4
Glu	3.4	Thr	0.9	Thr	0.8	Tyr	4.8
Tyr	2.5	Ileu	0.7	Phe	0.6	Arg	4.1
Lys	2.4	Phe	0.6	Pro	0.4	Pro	3.1
Leu	1.4	Arg	0.5	Ileu	0.3	Thr	2.6
His	1.1	Lys	0.3	Lys	0.2	Phe	2.5
Ileu	0.8	Pro	0.3			Lys	1.6
Pro	0.8						

a) Residues per 100 total residues.

b) From Seifter and Gallop (1966) (ref. 5)

c) From Lukas et al. (1958) (ref. 11)

d) From Shaw (1964) (ref. 9)

Table II
 AMINO ACID COMPOSITION OF
 VARIOUS SPIDER SILKS (LITERATURE VALUES)^a

Araneus diadematus ^b		Araneus diadematus ^c		Nephila madagascariensis ^d		Nephila senegalensis ^d		Nematus ribesii ^f	
Ala	32.7	Gly	21.8	Gly	40.6	Ala	28.7	Gly	26.2
Gly	24.3	Ala	17.7	Ala	32.1	Ser	22.6	Ala	14.0
Glu	17.9	Glu	17.3	Glu	11.6	Gly	11.9	Ser	10.8
Ser	6.3	Pro	16.9	Ser	4.2	Glu	9.8	Pro	9.8
Arg	3.2	Ser	8.4	Tyr	3.2	Leu	6.5	Glu	9.7
Pro	3.1	Tyr	6.4	Leu	2.9 ^e	Thr	4.3	Asp	5.8
Thr	2.1	Val	2.2	Arg	2.4	Phe	3.7	Arg	4.5
Leu	2.1	Lys	1.8	Asp	0.9	Asp	3.2	Phe	3.8
Val	1.9	Leu	1.6	Val	0.9	Ileu	2.5	Val	3.7
Try	1.8	Thr	1.3	Thr	0.6	Val	2.3	Thr	3.7
Lys	1.8	Ileu	1.0	Phe	0.6	Tyr	2.0	Tyr	2.6
Ileu	1.7	Asp	0.9			Arg	1.9	Leu	2.2
Asp	1.1	Cy(SO ₃ H)	0.4			Lys	0.6	Lys-OH	2.1
		Phe	<0.2			His	0.1	Ileu	2.1
								His	0.6

a) Residues per 100 total residues unless specified otherwise.

b) From Peakall (1964) (in g/100g) (ref. 12).

c) From Fisher and Brander (19) (in g/100g) (ref. 13).

d) From Lucas et al (1960) (ref.7).

e) Comprising Ileu.

f) From Seifter and Gallop (1966) (ref. 5). Nematus ribesii is not a spider, but a fly (gooseberry sawfly). Data for its silk are included for comparison.

Table III
 AMINO ACID COMPOSITION OF SPIDER SILK
 SAMPLES AS DETERMINED UNDER PRESENT CONTRACT^a

Spider Silk Paneiras GB, Spider No. 3 hydrolysis: 49 hr		Nephila cruentata (strong sample) 30-1		Nephila clavipes (weak sample) 30-2			
Gly	38.8	Gly	39.7	Gly	43.3	Gly	41.5
Ala	20.2	Ala	22.6	Ala	30.4	Ala	27.0
Glu	16.3	Glu	15.7	Glu	10.0	Glu	9.0
Pro	8.6	Pro	9.6	Tyr	4.0	Ser	6.9
Ser	4.7	Ser	4.8	Ser	3.0	Tyr	2.7
Tyr	2.7	Leu	2.2	Pro	2.6	Asp	2.5
Leu	2.1	Tyr	1.7	Leu	1.8	Arg	2.0
Val	1.5	Val	1.5	Asp	1.3	Leu	2.0
Asp	1.1	Asp	1.1	Arg	1.1	Thr	1.9
Thr	1.0	Thr	1.0	Thr	0.7	Val	1.2
Arg	0.8	Arg	0.9	Ileu	0.5	Pro	1.1
Ileu	0.6	Ileu	0.6	Lys	0.3	Lys	0.8
Phe	0.5	Phe	0.4	Val	0.1	His	0.7
Lys	0.4	Lys	0.4	Phe	(0.04)	Ileu	0.6
Met	0.2	Met	0.2	Met	(0.01)	Phe	0.5
His	0.1	His	0.0	His	(0.03)	Met	trace
Cys	0.0	Cys	0.0	Cys	0.0	Cys	0.0

a) Residues per 100 total residues.

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APPENDIX A
PHYSICAL CHARACTERIZATION AND
INFRARED SPECTRA ANALYSIS OF
SPIDER SILKS

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APPENDIX A
PHYSICAL TESTING
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INTRODUCTION

This appendix summarizes the results of studies concerned with the characterization of certain physical and mechanical properties of spider silk filaments. Earlier preliminary studies had indicated that single or multi-filament spider silk strands could have tenacities as high as 12 to 13 grams per denier, i.e., 160 kilograms per square millimeter, rupture elongations of approximately 20 percent and initial tensile moduli in excess of 100 grams per denier. Maximum individual test results showed a rupture tenacity of 17.8 grams per denier, i.e., 215 kilograms per square millimeter, an initial modulus of 150 grams per denier and a rupture elongation of 26 percent, each for different specimens. The purpose of the work reported herein was to verify these and other data reported in literature and, to the extent possible, determine filament anisotropy.

SAMPLE MATERIALS

Spider silks were obtained from five different types of spider. These were:-

Nephila Clavipes
Argiope Aurantia
Nephila Cruentata
Parawixla Audax
Argiope Argentata

One series of spider silks was obtained from Brazil in August 1967 (series 1) and another series obtained from Florida in October 1967 (series 2). Each series did not necessarily include silk from each of the five types of spider listed. Samples of main frame and trailing thread were obtained for each spider in its natural habitat. Spiders were collected, silked immediately after capture, shipped to the United States and, following acclimatization, silked again. The artificially induced spinning of spider silk, referred to herein as "silking," is illustrated in Figure 1. The spider is held lightly between thumb and forefinger with its spinneret pointing away from the operator and aligned with the edge of the winding spool. The distance between the spider spinneret and the winding spool is about three inches. The thread is started by applying the sticky surface of a small piece of adhesive tape to the spinneret of the spider, holding it in place for about ten seconds and then pulling gently away from the spider. By using this procedure, it was found possible to pull away a continuous strand of spider silk. Induced extrusion of the silk thread material could be continued until the spinning fluids were exhausted or the spider broke the thread. The thread was taped to one end of the winding spool and the spool driven at constant surface speed by means of a variable speed electric motor. Approximately 20 to 30 feet of spider silk were taken from each spider. In certain instances spiders were silked until the thread line broke due to lack of fiber-forming materials within the spider's spinneret.

The samples of main frame fiber removed from freshly spun webs made in the spider's natural habitat were extremely poor in quality. This lack of quality was due to the fact that the filaments were coalesced and that it was physically difficult to sample the webs for the required filaments. In addition, the natural accumulation of airborne detritus loaded the filaments, making diameter measurements extremely inaccurate. Most of the filament samples obtained from naturally spun webs were multifilament rather than monofilament, suggesting that the spider has intentionally constructed its web that way or that some coalescence had occurred during sample collection and shipment. In almost every case it was impossible to isolate lengths of main frame thread suitable for tensile testing. Consequently, the data reported herein describe the physical characteristics of trailing silk and spider silk silked under controlled conditions after acclimatization.

Trailing silk and silk which was artificially silked from spiders were infinitely superior in quality, usually composed of one or two coalesced filaments and free of sticky residues. A total of 87 different spider silk sample materials produced by five different species of spider under a variety of spinning speeds were evaluated. The lowest spinning speed was 3.7 feet per minute while the highest speed was 120 feet per minute.

Determinations of filament birefringence and torsional modulus were made. Birefringence and the relationship between torsion and tensile modulus have been used to estimate the degree of anisotropy of each of the five spider silk materials.

Attempts were made to mechanically draw two samples of spider silk in an effort to alter filament morphology and hence improve tensile rupture and/or energy absorption characteristics. Unfortunately, while considerable time and effort were expended in this endeavor, test results were unobtainable because of repeated failure of test filaments during mechanical drawing and setting.

PHYSICAL PROPERTY CHARACTERIZATION.

Each of the spider silk materials received was included in an initial screening of tensile properties. This screening was intended to eliminate spider silk samples with inferior tensile strength, i. e., tenacities less than 2 grams per denier unless this was characteristic of a spider silk species. Silk samples which evidenced high strength or rupture elongation were included in a much more exhaustive evaluation of tensile properties. Tensile testing was performed on an Instron Tensile Tester using the following test conditions:-

Test Environment	65% relative humidity 70 °F
Test Gauge Length	2 inches
Strain Rate	100% per minute
Full Scale Load	2 grams.

Instron fiber jaws with rubber faces were used to grip the test lengths of the spider silk. Approximately 6 inches of filament were required for each tensile test; the center three inches were used for the actual test while the 1-1/2 inch lengths to either side of the test length were measured with a microscope to determine the number of filaments present and their diameter. These diameter measurements were used as a basis for computing the tensile strength of each test specimen. During the investigation this procedure was changed somewhat. Test filaments were mounted on rectangular strips of card across a 2-inch diameter hole cut in the center of the card. The test filament was aligned parallel to the longest dimension of the card, along the diameter of the circular aperture, and was then attached to the card by adhesive tape applied to the fiber and the supporting card at each side of the circular aperture. The card frame made it easier and less hazardous to handle the test filament and also facilitated measuring of the number and diameter of silk filaments within the test gauge length rather than immediately adjacent to it. After the number and diameter of the test filaments in a particular silk specimen had been noted, the card frame was mounted in the Instron with the fiber clamps holding the card and the fiber at each end of the circular aperture. The card frame connecting top and bottom jaws was then cut and the tensile test made. A careful examination of tensile test data obtained for a variety of spider silks in which the tensile strength and modulus were based upon (a) specimen dimensions adjacent to the test length and (b) specimen dimensions within the test length showed both sets of data to be in good agreement. However, due to the variability of the filament tensile properties, it is impossible to comment further upon the preferred technique judged on the basis of increased accuracy or the reduction in test data variability. The preferred method is obviously the card frame mounting technique in which tensile properties are based upon fiber dimensions within the test length. The card frame technique makes it easier to handle test filaments and reduced the number of test samples inadvertently damaged prior to tensile testing.

Typical load elongation curves obtained for the five different types of spider silk studied are shown in Figures 2-6. At least 5 individual filament tests were made for each spider silk. Rupture tenacity, rupture elongation and initial modulus were computed for each load elongation curve obtained and averaged for each silk sample. Rupture tenacity and initial modulus have been expressed in grams per denier, where the filament denier, or the linear density, is the weight in grams of 9,000 meters of the filament. Some of the spider silk materials had a spiral crimp. The effect of this crimp can be noted in Figures 4 and 5; the low modulus elongation to 5 percent is a decrimping modulus.

DENIER COMPUTATION

The number and diameter of individual filaments in each test length were determined microscopically prior to testing. By assuming a specific gravity for spider silk of 1.25, it was possible to compute fiber denier from the relationship

$$\text{denier} = (\mu)^2 \times 0.00707 \times \rho$$

where

μ = the filament diameter in microns and
 ρ = the filament density.

Quite obviously this relationship assumes that the filaments are perfectly circular in cross section. If tensile data are based upon this computed denier, then a further assumption is made that the section of test silk viewed microscopically is truly representative of the silk strand at all points along the test length. If one or more strands in a multifilament strand are broken or nonexistent at any point in the test length, then of course the experimental data will reflect primarily the properties of the intact through-going filaments with subsequent calculations being based upon an incorrect (high) denier value. Test filaments were examined as carefully as possible to insure that the test specimen was uniform both in diameter and also in the number of filaments over the test gauge length and that there were no broken filaments.

Perhaps the most critical parameter in the computation of denier from the transverse filament diameter is the accuracy of the diameter measurements. Most of the silk filaments examined had diameters in the range of 1-8 microns. At this level of fiber fineness, particularly in the 1-4 micron range, the limits of useful optical magnification are being approached and the Becke line makes it particularly difficult to resolve the true boundary of the silk filament. It is suspected that in the 1-4 micron filament diameter range, the accuracy of the diameter measurements is ± 1 micron, -2 microns. For a silk filament of nominally 4 microns, measured to an accuracy of ± 1 micron, this would indicate that the particular filament could have a linear density anywhere in the range of 0.08 to 0.22 denier compared with 0.14 denier computed on the basis of the nominal 4 micron diameter determination. Some caution should, therefore, be exercised in the examination of the tensile test data. While there is reason to suspect that the finer silk filaments could be intrinsically stronger than the coarser ones, the diameter error increases with decrease

in filament diameter, leading to optimistically high values of tensile modulus and tensile strength. Taking the example cited, a 4-micron thread which would break at a 2-gram loading would have a tenacity of 14.3 grams per denier. If, however, the true filament diameter was 5 microns, then the true fiber tenacity would be 9.1 grams per denier. If the filament diameter had been 5 microns and the tenacity measured had been based upon a 4-micron filament diameter, then the error in filament tenacity would be 58 percent.

DETERMINATION OF THE SPECIFIC GRAVITY OF SPIDER SILK

The specific gravity of the two samples of spider silk was measured by means of a density gradient column using a procedure similar to that described in ASTM Test Method D 1505-63T. Spider silk samples were conditioned to 65 percent R. H., 70° F and cut into small lengths prior to being placed into the density gradient column. No attempt was made to remove any of the surface impurities. The density gradient column consisted of mixtures of carbon tetrachloride and xylene prepared by the double siphon method. The column was calibrated with certified glass filaments. All tests were conducted at 23° C. In view of the rather small size of the available samples of spider silk, it was necessary to allow them to come to buoyancy equilibrium in the density gradient column over a period of 24 hours. Three samples of each type of spider silk were characterized. The specific gravity of *Argiope Aurantia* was 1.347 while that of *Nephila Clavipes* was 1.35. These values are somewhat higher than the assumed specific gravity of 1.25 accepted for silkworm's silk. All the tensile data in this work have been computed on the basis of a filament specific gravity of 1.25. On this basis, therefore, the reported filament deniers are 8 percent too low and the computed tensile data (tenacity and modulus) about 8 percent too high.

DISCUSSION OF TENSILE PROPERTIES

The tensile test data obtained from the Brazilian spider silk materials are summarized in Table A-1. The reel or frame number indicates the sample number and this is identified with the particular spider which produced the silk sample. Certain of the submitted samples were unidentified; they have been included because one or two of them possess particularly good tensile properties. Of the identifiable samples, the filament rupture tenacity ranged from 1.50 grams per denier for a sample of *Argiope Argentata* to 14.91 for a sample *Nephila Cruentata*. One unidentifiable sample had an average rupture tenacity of 17.07 grams per denier. In the case of the

Nephila Clavipes, data were obtained indicating average filament tenacities of the order of 17.8 grams per denier and tensile moduli as high as 231 grams per denier. Two samples of Argiope Aurantia had average tenacities of 13.5 grams per denier and average tensile moduli of the order of 100 grams per denier. Rupture elongations ranged from a low of 10 percent to a high of approximately 40 percent while the statistically sound tensile modulus data covered the range of 11.91 gpd to 180 gpd. An unidentifiable sample had a calculated modulus of 288 grams per denier. If the average filament denier data are examined, the coarsest filament denier was 0.72 while the finest was 0.05. While the low tenacity and low modulus filaments were not necessarily the coarsest filaments, the high tenacity and high modulus filaments were invariably the lower denier filaments. While ultrafine filaments can be stronger than coarse filaments of the same material, there is a distinct possibility of a directed bias error in the computation of denier for the finer spider silk filament materials. This has already been discussed and, consequently, a skepticism arises, although apparently high values of average filament tenacity and tensile modulus noted are intriguing.

The tensile properties of the second series of spider silk materials obtained from Florida are summarized in Table A-II. Computed tenacities ranged from a low of 4.25 grams per denier to a high of 15.2 grams per denier. Rupture elongations were anywhere from 9.8 to 32.1 percent. Tensile modulus values covered the range of 46.4 grams per denier to 250 grams per denier. Here again it was noted that while low filament tenacities and tensile moduli were not necessarily found with coarse denier filaments, high tenacity and tensile modulus values were invariably associated with fine (very low) filament deniers.

Table A-III summarizes all the tensile test data by species. Nephila Clavipes has the highest average tenacity (approximately 8 grams per denier) of the five spider silk species examined. This compares very favorably with the measured tenacity values of commercially available textile fibers. Argiope Aurantia silk filaments had an average tenacity of 7.23 grams per denier while the Nephila Cruentata, Parawixia Audax and Argiope Argentata were somewhat weaker. Both the Nephila Clavipes and Argiope Aurantia had high values of initial modulus (98.6 grams per denier and 89.7 grams per denier, respectively), substantially higher than the other three silk samples.

EFFECT OF SPINNING VELOCITY UPON SPIDER SILK TENSILE PROPERTIES

Table A-IV contains tensile test data for *Nephila Clavipes* and *Argiope Aurantia* spider silk, which had been artificially silked in the laboratory at three different spinning speeds, viz., 22, 66 and 120 feet per minute.

For the three silking speeds employed there is slight evidence to suggest that higher filament tenacities are obtained at lower silking speeds, while modulus and rupture elongation properties are essentially unaffected. Filament denier appears to be quite sensitive to silking speeds. According to the data in Table A-IV, an increase in silking speed results in an increase in filament denier. This would tend to suggest that the spider's ability to control the morphological orientation and filament denier is perturbed by the increase in filament spinning tension which results from an increase in silking speed. At the lowest silking speed of 22 feet per minute, the spider is probably able to meet the demands for fiber forming protein by applying muscular pressure to the spinning liquid. This will probably result in extrusion at a low filament tension downstream of the spinneret. As the spinning speed is increased, one will reach a spinning velocity where there is a negative pressure in the fluid substance which manifolds to the spider's spinning apparatus. It is quite likely that under artificial conditions of silking, the spider is unable to stop the spinning process until the thread breaks due to exhaustion of its spinning fluids. As the silking speed is increased the fluid shear within the spider's spinneret will increase, thereby increasing the normal pressure between the nascent element of filament in the region of filament solidification and the surface of the spider's spinneret muscle. This pressure increase will cause the spinneret muscle to relax, leading to the production of increased denier, probably less oriented, silk filament. For a given spinning speed, the lower the viscosity of the spinning fluid at the region of the spinneret where the filament is formed, the greater the degree of filament orientation.

Examination of the denier and spinning rate data in Table A-IV indicates that as the spinning velocity is increased, filament denier increases as does the absolute mass of fiber spun per unit time. In the conventional spinning of synthetic fibers an increase in spin draw (filament velocity away from the spinneret face) produces finer denier filaments, usually highly anisotropic without necessarily increasing the mass of fiber spun per unit time.

INFRARED SPECTRA ANALYSIS

Infrared spectra were obtained for Bombyx Mori and Tussah silkworm silk and Nephila Clavipes and Miranda Aurantia spider silk, using a Perkin Elmer No. 337 grating infrared spectrophotometer. Samples of silk were placed in a Wilkes Mini-cell, which, in turn, was placed in one of the instrument's two beams. No attempt was made to parallelize the silk filament in the Mini-cell. Solvents of like refractive index were used with the samples and placed in both sample and reference cells to facilitate the acquisition of a better spectrum. Unfortunately, the most convenient solvents, e. g., bromoform and carbon bisulfide, both obscure important parts of the spectra. Carbon bisulfide is rather infrared inactive except in the 1400-2400 cm^{-1} region and at 850 cm^{-1} . For these regions, bromoform is relatively inactive except for one peak at 1300 cm^{-1} . In one instance, a sample of Nephila Clavipes was run as a KBr disc. The same peaks are present, but the spectrum was not as clear as those obtained with equivalent refractive index solvents.

All proteins absorb in the 3300, 1600, and 1500 cm^{-1} regions of an IR spectra. Additional absorption is largely dependent upon the nature of the amino acids present and their subsequent arrangement. It should be noted that amide groups are very sensitive to molecular environment, namely, the hydrogen bonding, coupling and structural configurations which are present in proteins. This, in addition to the fact that the characteristic band of the repeating backbone unit tends to mask the weaker side chain absorptions, makes it unreasonable to expect that the IR spectra will afford much information describing the specific identification or arrangement of amino acids in silk materials. The spectra obtained and included at the end of this report should be examined in conjunction with the text following.

The main NH stretching absorption appears at 3280 cm^{-1} in all of the silk spectra. This is expected for secondary amides. Several studies have correlated the various peaks in the 3270-3300 cm^{-1} region with crystalline amorphous and alpha-beta Keratin structures. However, these studies have not gone into such depth. The broad peak and the shoulder at 3400-3500 cm^{-1} suggest both O-H and N-H hydrogen bonding. The peak at 3080 cm^{-1} is always found in protein spectra. Although the source is not known with any certainty, there are several possible explanations. For example, it could be another N-H mode, possibly an aromatic mode, an overtone of the amide II band or even a combination of these.

The set of peaks in the 2800-3000 cm^{-1} range are C-H stretching modes belonging to CH_3 and CH_2 groups. Unfortunately, individual peak assignment is not as clear-cut as textbooks would indicate.

The two most prominent peaks in all protein spectra are the amide I and II bands. The amide I band has been attributed to the amide carbonyl stretching and is found in the 1680-1630 cm^{-1} region. The amide II band, 1570-1515 cm^{-1} is produced by C-N stretching and N-H in-plane bending. These two bands are also greatly affected by all the structural variations and environments of the molecule. The existing literature concludes that one cannot deduce the nature of the amide environment from the exact position of these rather broad bands.

There are also weaker amide bands found in most proteins. These are:

- Amide III - 1305-1240 cm^{-1} , mixed vibrations of OCN and NH modes
- Amide IV - 630 cm^{-1} , N-C = O, in-plane bending
- Amide V - 700-650 cm^{-1} , N-H, out-of-plane bending
- Amide VI - 600 cm^{-1} , C = O, out-of-plane bending.

The amide III is the strongest of these and shows marked shifts as a result of state change. The apparent shift of this band in the spider silk spectra may have interesting implications. The rest of these amide bands are ill-defined and of little value in this study. The amide IV band has been correlated with a helical protein molecular configuration but this cannot be deduced from the spectra obtained here.

There are several bands in the 1300-1450 cm^{-1} region which are produced by the CH_3 , CH_2 and C-H deformations. Some of these bands could be helpful in the identification of the amino groups; however, the infrared techniques must be refined before any definite conclusions can be formed.

Although in protein spectra the amide absorptions predominate, there are weak absorption bands in the 900-1080 cm^{-1} region, which can somewhat ambiguously be assigned to the various amines. The comparison of this region in the five attached spectra shows that the three spider silks are identical and that the Bombyx Mori and Tussah silk are identical, but that these silkworm silks and spider silks are definitely different. The spectra of Nephila Cruentata were the best of the three spiders examined and, consequently, should be used for any further infrared analyses that might be required.

Approximately 75-85 percent of natural silk is glycine, alanine and serine. Of this fraction, Bombyx Mori is half glycine, Tussah is half alanine and spider silk half serine. For a variety of reasons, there can be no direct correlation between simple amino acids and the amine groups in a polypeptide or protein. However, a few frequency assignments have been made that may be useful in interpreting these spectra:

<u>Assignment</u>	<u>Frequency (cm⁻¹)</u>
alanine	1453, 1447, 1166
glycine - glycine sequence	1015 \pm 10
glycine - alanine sequence	998, 975

Although there is a slight increase in the 1166 cm⁻¹ region of the Tussah spectra, one would have expected a much greater change. It is felt that little value can be placed upon noted changes in intensity in these spectra. In the spectrum of Nephila Cruentata, the alanine peak has shifted to 1170 cm⁻¹ and is definitely present.

The pair of peaks at 998 and 975 cm⁻¹ are present in both the Bombyx Mori and Tussah silk spectra. This confirms other research findings which have indicated the presence of glycine-alanine copolymers in silk fibroin. The absence of these two peaks in the spider silk spectra is just as significant. The only other peak that can currently be identified in this important region of the spectra is the glycine-glycine copolymer at 1015 \pm 10 cm⁻¹. The spider silk spectra do show an absorption at 1025 cm⁻¹ which could be explained as a glycine-glycine repeat. This peak is not evident in the spectra of the two silkworm fibroins.

There are two principal obstacles impeding complete infrared spectra interpretation. One is the present inability to identify each of the major peaks in this 900-1080 cm⁻¹ region. The other is the lack of infrared spectra data pertaining to polypeptide identification. In protein materials, band absorption is significantly influenced by the molecular environment, making it technically impossible to assign all spectral peaks and characteristics.

EXAMINATION OF FILAMENT ANISOTROPY

An attempt was made to determine filament anisotropy by comparing tensile and torsional moduli. Torsional properties of spider silk samples were determined by means of a torsion pendulum. In this device, the test specimen is the torsional element which is hung vertically in a draft-proof enclosure supporting a torsion bob at one end. The period of this torsion bob

as it oscillates about an axis coincident with the longitudinal axis of the test specimen permits one to calculate the torsional rigidity of the test specimen. The oscillations of the torsion bob must be of small amplitude to minimize inelastic torsional behavior; the torsion bob dimensions and mass had to be selected with care to insure that the combined effects of tension and torsion did not induce yielding prior to or during oscillation. On the other hand, the torsion bob had to be heavy enough to insure that the filament was twisted without buckling. Due to the fragile nature of the test filaments and the rather low permissible mass of the torsion bob, it was physically impossible to make torsion tests in air at 65 percent relative humidity and 70°F. This was because the air damped out the torsional vibrations in less than a complete oscillation. Consequently, these torsional modulus determinations had to be made in a vacuum (50 millimicrons). No attempt has been made to correct the test data for the increased rigidity anticipated by virtue of the reduction of fiber moisture regain in vacuum. A sample of silkworm silk has been included for comparison.

For a circular cross section which has been assumed to be the case for the silk studied, the shear modulus G was derived from the expression

$$G = \frac{39.5 I \ell}{\tau^2 J}$$

where

G = filament shear modulus (psi)

I = moment of inertia of torsion bob (inch-pound sec²)

ℓ = test filament length (inches)

J = the filament polar moment of inertia (inches⁴), and

τ = the period for one oscillation (seconds)

Alternatively, and in more detail, the filament torsional modulus can be computed in grams per denier from the following relationship

$$G = \frac{8.139 \times 10^7 \times W \times \ell}{\rho d^4 \tau^2} \left(\frac{r^2}{4} + \frac{L^2}{12} \right)$$

where

G = shear modulus (grams per denier)

W = the weight of pendulum bob (pounds)

ℓ = filament length (inches)

r = radius of the pendulum bob wire (inches)

L = length of pendulum bob wire (inches)

ρ = spider filament density (grams per cc)

d = spider silk filament diameter (thousandths of an inch)

τ = the period for one oscillation (seconds).

MEASUREMENT OF SPIDER SILK FILAMENT BIREFRINGENCE

The birefringence of a filament is the difference in refractive index between the axial direction and the direction perpendicular to the filament axis and is an index of filament anisotropy. A convenient method for measuring birefringence is by means of the Berek compensator in a polarizing microscope. This instrument measures optical retardation differences between transverse and lengthwise directions. Filament birefringence is computed by dividing the optical retardation by the filament thickness. Retardation and thickness must be in identical units; birefringence is a dimensionless parameter.

The thickness of each filament tested was obtained by measuring the fiber width with a calibrated filar micrometer. Here again the accuracy of the diameter measurements was subject to the same limitations already discussed for projection microscope filament diameter measurements.

DISCUSSION OF FILAMENT ANISOTROPY DETERMINATIONS

At least five separate determinations of birefringence, torsional modulus and tensile modulus were made for each of the five species of spider silk available. A sample of Japanese yellow silk was included for comparison. The results of these experimental determinations have been averaged for each spider species and are given in Table A-V.

The birefringence data are in accordance with published data on silkworm silk and suggest that spider silk filaments are in general about as anisotropic as silkworm silk. Examination of the tensile and torsional data would tend to indicate that comparisons between them are questionable. According to the property relationships already discussed, for most isotropic materials E/G lies between 2.5 and 3.0: i. e., the tensile modulus is 2.5 to 3.0 times as large as the torsion modulus. However, in four instances, the average torsional modulus is larger than the measured tensile modulus. It is suspected that this incompatibility is attributable both to the different moisture contents for tension and torsion and also to errors in the determination of the filament diameter. In the computation of the torsional modulus, G is proportional to the inverse of the filament diameter raised to the fourth power. Hence, any error in the determination of filament diameter will inevitably lead to large errors in the computed value of torsional modulus.

FILAMENT APPEARANCE CHARACTERISTICS

Longitudinal and cross section photomicrographs have been made of typical samples of each of the five species of spider silk and of the comparative sample of Japanese silk. (Fig. 7-12.)

The filament specimens used for the longitudinal views were metal shadowed to enhance filament surface detail. The larger fibers present in the cross section photomicrographs are wool and rayon used to pack the spider silk samples in the sectioning microtome.

TABLE A-1

AVERAGE TENACITY, MODULUS AND ELONGATION DATA OF
SERIES 1 SPIDER SILK SAMPLES
BRAZILIAN SPIDERS

Reel or Frame	Spider No.*	Species	Rupture Load (gms)	Rupture Tenacity (gpd)	Rupture Elong (%)	Initial Modulus (gpd)	Denier
30-1	41 L	Nephila Cruentata	0.90	9.32	27.5	54.6	0.16
30-2	35 L	Nephila Clavipes	1.42	2.37	16.0	11.9	0.60
30-3	4 L	Parawixla Audax	0.66	4.33	30.2	17.6	0.17
30-5	42 L	Nephila Cruentata	0.85	3.33	17.9	29.1	0.27
30-6	35 L	Nephila Clavipes	0.65	3.02	19.9	30.9	0.25
30-7	32 L	Unknown	0.55	3.05	17.9	42.8	0.14
30-8	27 L	Argiope Argentata	0.06	1.50	10.1	25.0	0.04
30-9	17 L	Nephila Cruentata	2.19	3.77	12.8	35.1	0.60
30-11	37 L	Unknown	0.31	4.37	22.0	28.6	0.07
30-12	38 L	Nephila Clavipes	1.01	2.79	15.6	31.0	0.34
30-14	34 L	Unknown	0.66	4.13	19.0	40.6	0.16
30-15	35 L	Nephila Clavipes	0.71	2.28	15.6	40.7	0.31
30-16	36 L	Unknown	1.49	8.15	29.4	28.6	0.09
30-17	41 L	Nephila Cruentata	0.79	5.19	15.9	26.4	0.24
30-18	18 L	Nephila Cruentata	0.94	5.46	17.0	31.7	0.20
30-19	17 L	Nephila Cruentata	2.27	3.28	12.3	26.9	0.72
30-20	42 L	Nephila Cruentata	1.11	4.88	23.9	49.9	0.23
30-21	4 L	Parawixla Audax	0.46	2.92	26.3	19.4	0.15
30-23	13 T	Parawixla Audax	0.36	7.20	22.1	20.0	0.05
32-24	19 T	Parawixla Audax	0.08	2.00	20.5	25.0	0.04
32-25	43 T	Parawixla Audax	0.27	5.58	25.3	25.0	0.05
32-26	17 T	Nephila Cruentata	0.94	1.71	12.8	24.3	0.55
32-27	24 T	Argiope Argentata	0.29	6.46	24.3	46.9	0.05
32-29	16 T	Nephila Cruentata	0.60	4.25	39.3	18.3	0.14
32-31	25 L	Parawixla Audax	0.24	2.15	30.2	44.0	0.11
36	1 L	Unknown	0.36	6.14	21.1	25.3	0.28
37	2 T	Parawixla Audax	0.34	6.43	32.5	39.2	0.06
38	3 T	Parawixla Audax	1.12	6.35	21.8	33.6	0.25
39	4 T	Parawixla Audax	0.73	4.15	35.5	32.9	0.10
40	6 T	Unknown	0.85	4.71	39.7	33.1	0.18
41	7 T	Nephila Cruentata	0.80	6.31	10.1	110.5	0.14
42	8 T	Nephila Cruentata	1.31	14.91	18.0	179.3	0.18
43	9 T	Nephila Cruentata	1.88	5.89	14.2	39.6	0.32
44	10 T	Nephila Clavipes	0.76	13.91	21.7	77.6	0.14
45	11 T	Nephila Clavipes	2.13	9.32	13.2	119.7	0.28
53	31 T	Unknown	0.48	13.53	20.0	55.0	0.05
54	32 T	Unknown	1.85	18.51	22.7	124.7	0.09
56	32 T	Unknown	0.46	10.03	24.3	45.0	0.05
33	32 T	Unknown	1.05	7.03	16.9	107.3	0.16
35	32 T	Unknown	1.00	17.07	12.9	288.2	0.10

* T = Trailing Silk; L = Acclimated Laboratory Product.

TABLE A-II
AVERAGE TENACITY, MODULUS AND ELONGATION DATA OF
SERIES 2 SPIDER SILK SAMPLES
FLORIDA SPIDERS

Reel or Frame	Spider No. *	Species	Rupture Load (gms)	Rupture Tenacity (gpd)	Rupture Elong (%)	Initial Modulus (gpd)	Denier
6-1	6L	Nephila Clavipes	1.59	9.91	30.2	89.0	0.18
11-1	11L	Nephila Clavipes	1.39	9.10	18.4	95.0	0.18
12-1	12L	Nephila Clavipes	3.00	5.55	11.4	61.5	0.54
31-1E	31L	Nephila Clavipes	0.74	13.69	17.9	75.0	0.06
32-1	32T	Nephila Clavipes	0.91	14.20	12.8	108.0	0.09
33-1E	33L	Nephila Clavipes	0.97	6.91	11.0	139.0	0.14
33-3	3L	Nephila Clavipes	1.38	7.20	16.9	90.6	0.19
34-1	34L	Nephila Clavipes	1.17	6.29	13.4	103.0	0.19
36-1	36L	Nephila Clavipes	3.60	11.43	15.8	156.0	0.32
37-1E	37L	Nephila Clavipes	0.92	10.25	21.1	107.0	0.09
38-2	38L	Nephila Clavipes	0.76	5.44	13.6	71.4	0.14
40-1	40L	Nephila Clavipes	0.85	6.06	9.8	97.1	0.14
41-1	41L	Nephila Clavipes	0.76	5.46	32.1	78.6	0.14
42-1	42L	Nephila Clavipes	1.66	6.62	12.6	115.0	0.26
43-1	43L	Nephila Clavipes	0.78	6.01	22.6	89.0	0.14
43-1E	43L	Nephila Clavipes	1.01	5.83	13.0	108.0	0.19
43-2	43T	Nephila Clavipes	1.54	9.65	15.0	140.0	0.16
44-1	44L	Nephila Clavipes	1.46	5.82	12.8	59.7	0.28
45-1	45L	Nephila Clavipes	0.90	7.93	20.6	94.1	0.12
46-1	46L	Nephila Clavipes	0.95	9.48	19.0	92.5	0.10
46-2	46L	Nephila Clavipes	0.23	2.88	10.5	50.0	0.08
48-2	48L	Nephila Clavipes	1.73	5.92	24.4	67.4	0.30
48-3	48L	Nephila Clavipes	1.28	6.00	21.5	89.0	0.25
49-2E	49L*	Nephila Clavipes	1.60	8.23	17.5	98.4	0.20
49-1	44L	Nephila Clavipes	0.94	8.30	14.5	135.0	0.15
52-2	52L	Nephila Clavipes	1.23	15.10	13.2	135.2	0.10
53-1	53L	Nephila Clavipes	1.26	10.94	25.1	115.0	0.12
54-3	54L	Nephila Clavipes	0.52	6.45	8.1	80.0	0.08
54-2E	54L	Nephila Clavipes	1.53	9.92	19.4	199.0	0.18
56-1	56L	Nephila Clavipes	0.82	9.87	16.1	132.0	0.08
58-1	58L	Nephila Clavipes	2.73	9.18	24.3	118.0	0.35
62-1E	62L	Nephila Clavipes	0.97	7.90	13.2	134.0	0.13
64-1	64L	Nephila Clavipes	2.01	10.20	19.2	104.0	0.20
65-1E	65L	Nephila Clavipes	1.68	9.21	13.0	141.0	0.20
66-1	66AT	Argiope Aurantia	1.78	4.25	21.5	53.9	0.44
66-2	66AL	Argiope Aurantia	1.75	6.04	18.3	70.4	0.33
67-3	67AL	Argiope Aurantia	0.78	8.91	19.8	93.6	0.09
68-1E	68AL	Argiope Aurantia	1.78	7.18	21.4	203.0	0.22
68-2E	68AL	Argiope Aurantia	0.75	6.05	21.5	46.4	0.13
68-3	68AL	Argiope Aurantia	0.97	10.92	21.3	70.7	0.09
70-1	70L	Nephila Clavipes	1.49	5.91	22.4	56.7	0.25
70-2	70L	Nephila Clavipes	1.38	6.25	11.0	80.0	0.22
70-3	70L	Nephila Clavipes	1.34	7.05	13.2	85.5	0.20
71-1E	71L	Nephila Clavipes	1.03	7.77	14.9	83.9	0.13
72-1	72AL	Nephila Clavipes	1.31	5.95	11.0	72.7	0.22
72-2	72L	Nephila Clavipes	0.57	7.76	10.1	126.0	0.10
73-1	73L	Nephila Clavipes	6.61	15.25	17.4	250.0	0.04

* T = Trailing Silk; L = Acclimated Laboratory Product.

TABLE A-III

SUMMARY OF MEAN VALUES OF EACH SPECIES TESTED

<u>Species</u>	<u>No. Reels Tested</u>	<u>Rupture Load (gms)</u>	<u>Rupture Tenacity (gpd)</u>	<u>Rupture Elong (%)</u>	<u>Initial Modulus (gpd)</u>	<u>Denier</u>
Nephila Ciavipes	47	1.26	7.93	16.7	98.6	0.19
Argiope Aurantia	6	1.30	7.23	20.6	89.7	0.22
Nephila Cruentata	9	1.18	4.58	19.9	32.9	0.35
Parawixla Audax	9	0.47	4.57	21.2	28.5	0.11
Argiope Argentata	2	0.18	3.98	17.2	35.9	0.05

Tested at 65% RH
 70 °F
 100% Strain Rate

TABLE A-V
SUMMARY OF FILAMENT ANISOTROPY DATA

Sample No.	Species	Number Determinations	Retardation $M\mu$	Thickness μ	Δn Birefringence	Torsional Modulus (gpd)	Tensile Modulus (gpd)
30-3	Parawixla Audax	6	127.42	5.00	0.0248	173.0	76
30-5	Nephila Cruentata	4	175.85	4.47	0.0396	30.7	*
32-27	Argiope Argentata	5	81.30	2.54	0.0323	364.0	90
44-1	Nephila Clavipes	6	234.68	8.59	0.0297	9.87	42
52-2	Nephila Clavipes	6	202.15	4.14	0.0495	58.1	*
53-1	Nephila Clavipes	5	161.00	2.97	0.0543	431.0	198
66-2	Argiope Aurantia	5	122.85	5.51	0.0225	25.2	64
67-3	Argiope Aurantia	5	142.24	4.73	0.0304	121.0	54
68-2E	Argiope Aurantia	5	9.07	2.85	0.0347	103.0	*
Silk. A-108-3	Japanese Yellow	5	639.0	16.24	0.0394	8.84	57

* Samples broken prior to testing.

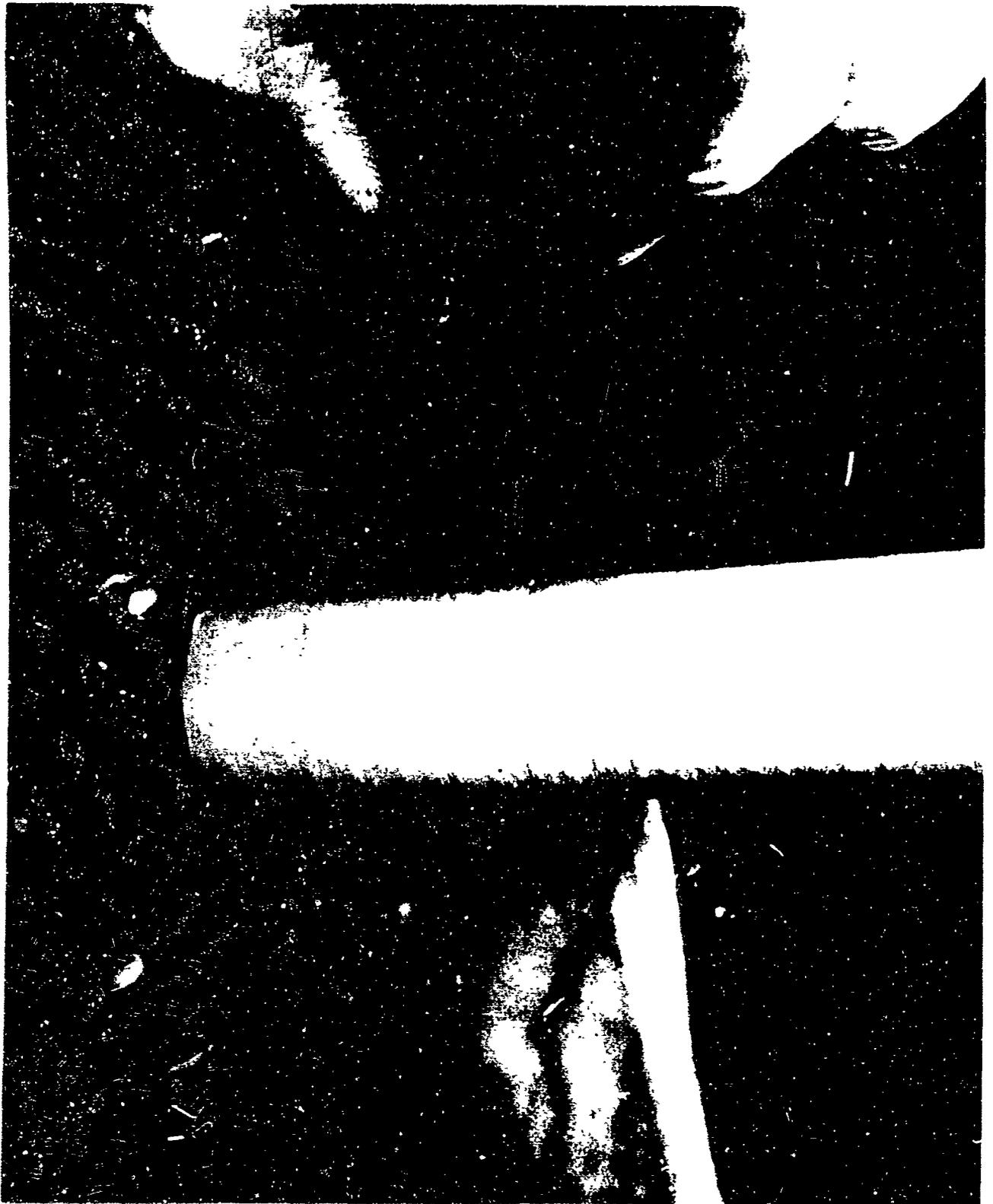


FIGURE 1

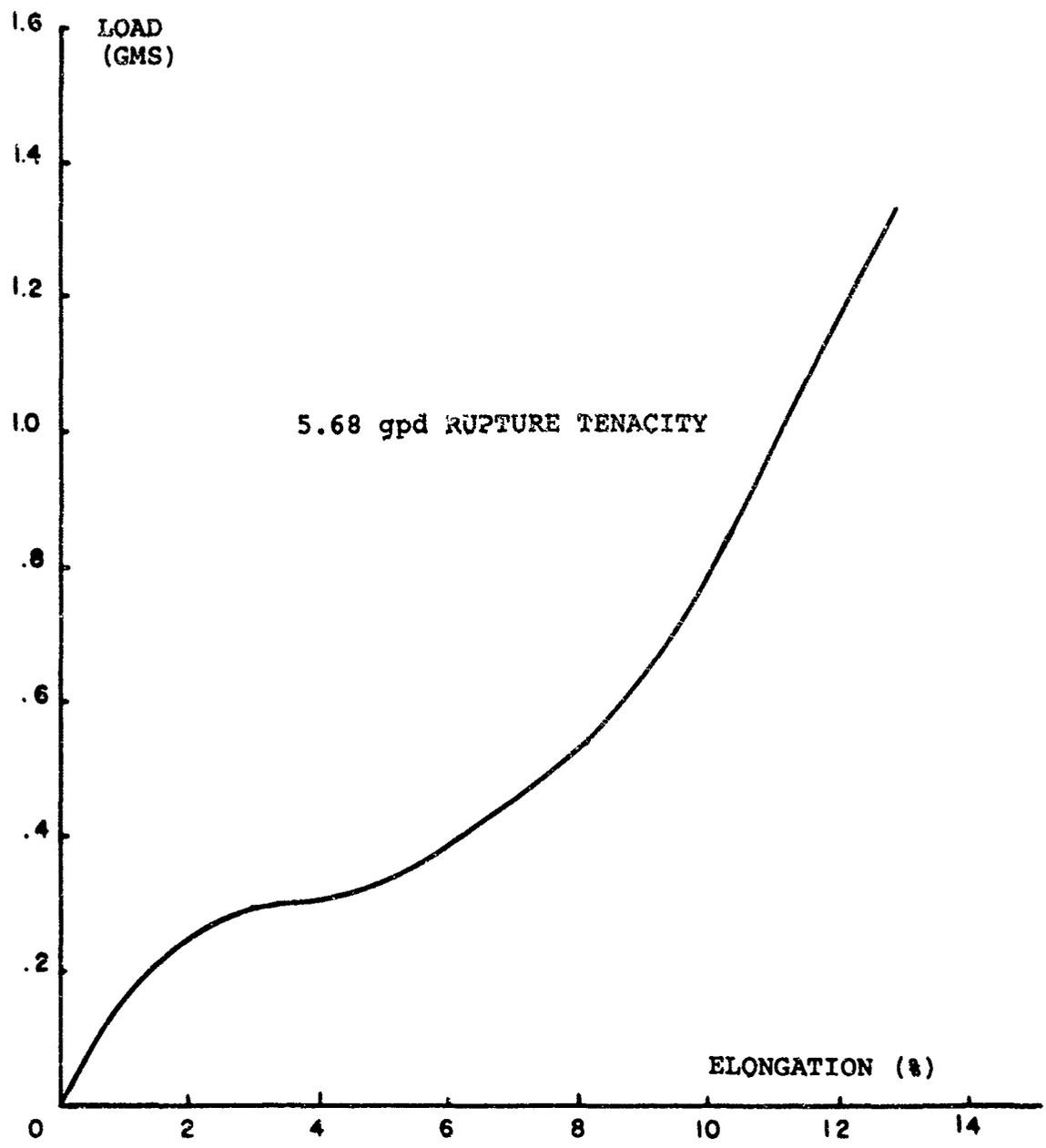


Figure 2. Typical Load-Elongation Curve
Nephila Clavipes

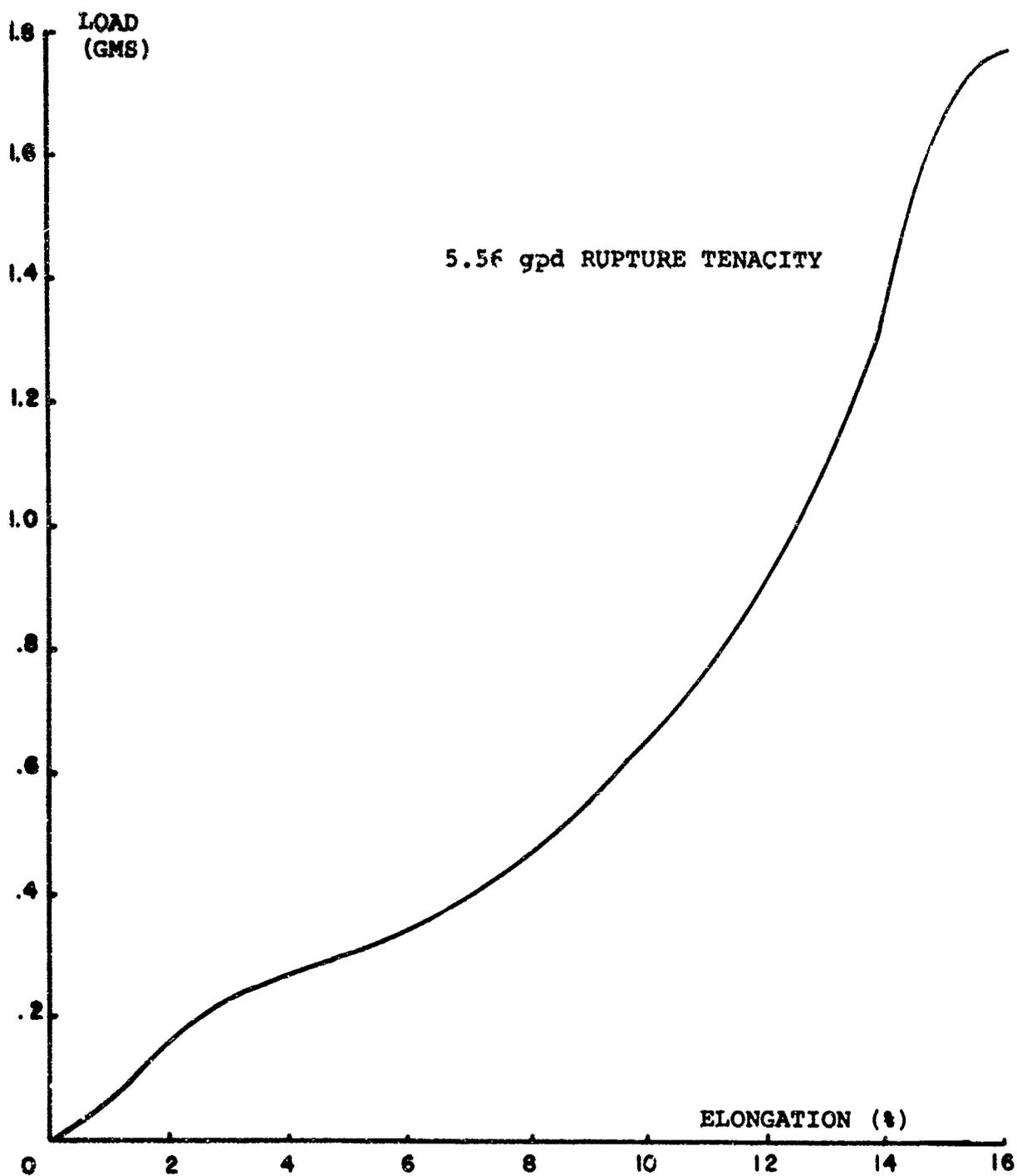


Figure 3. Typical Load-Elongation Curve
Argiope Aurantia

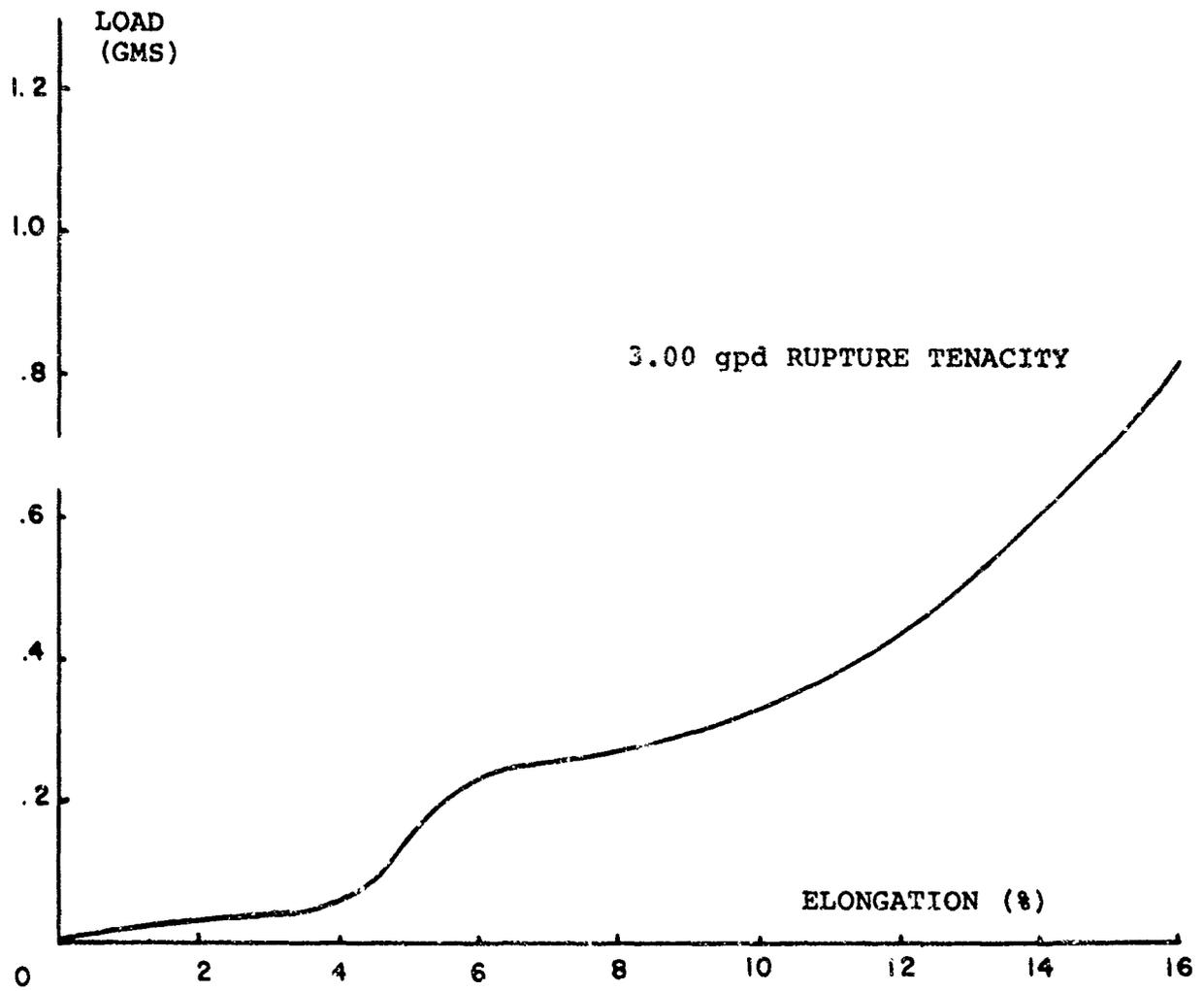


Figure 4. Typical Load-Elongation Curve
Nephila Cruentata

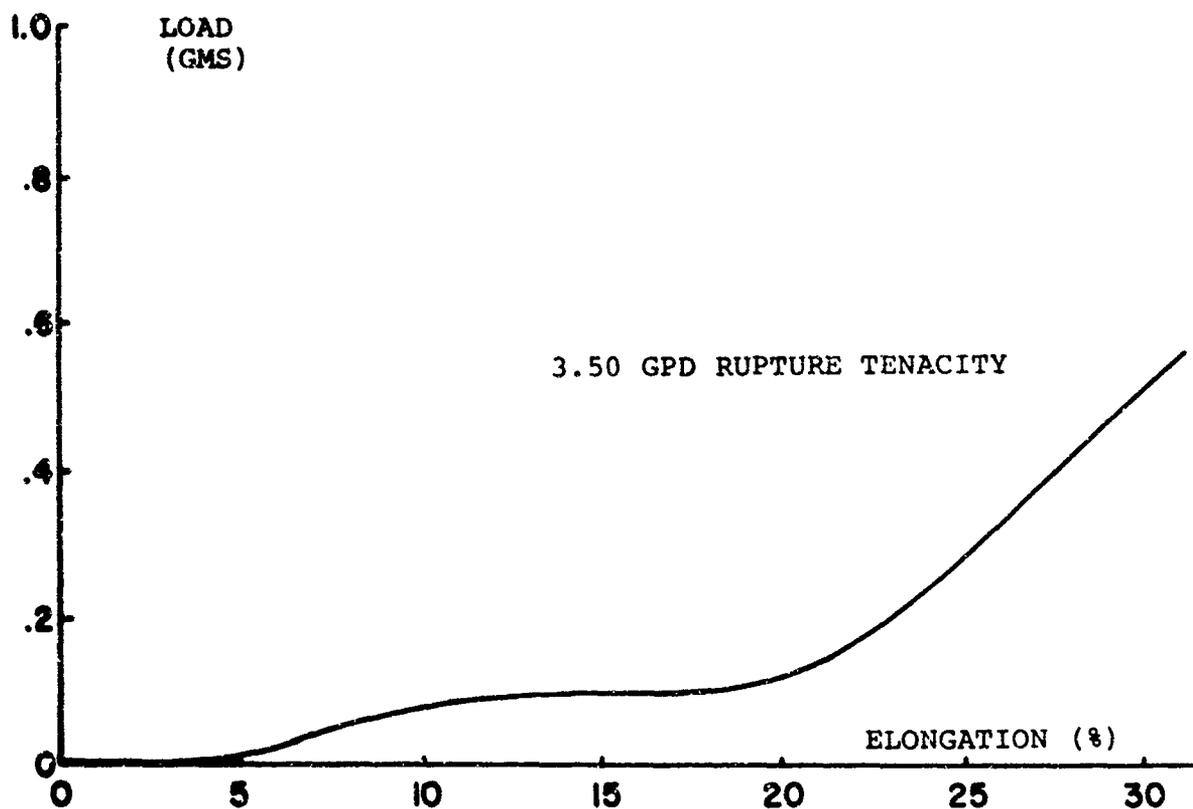


Figure 5. Typical Load-Elongation Curve
Parawixla Audax

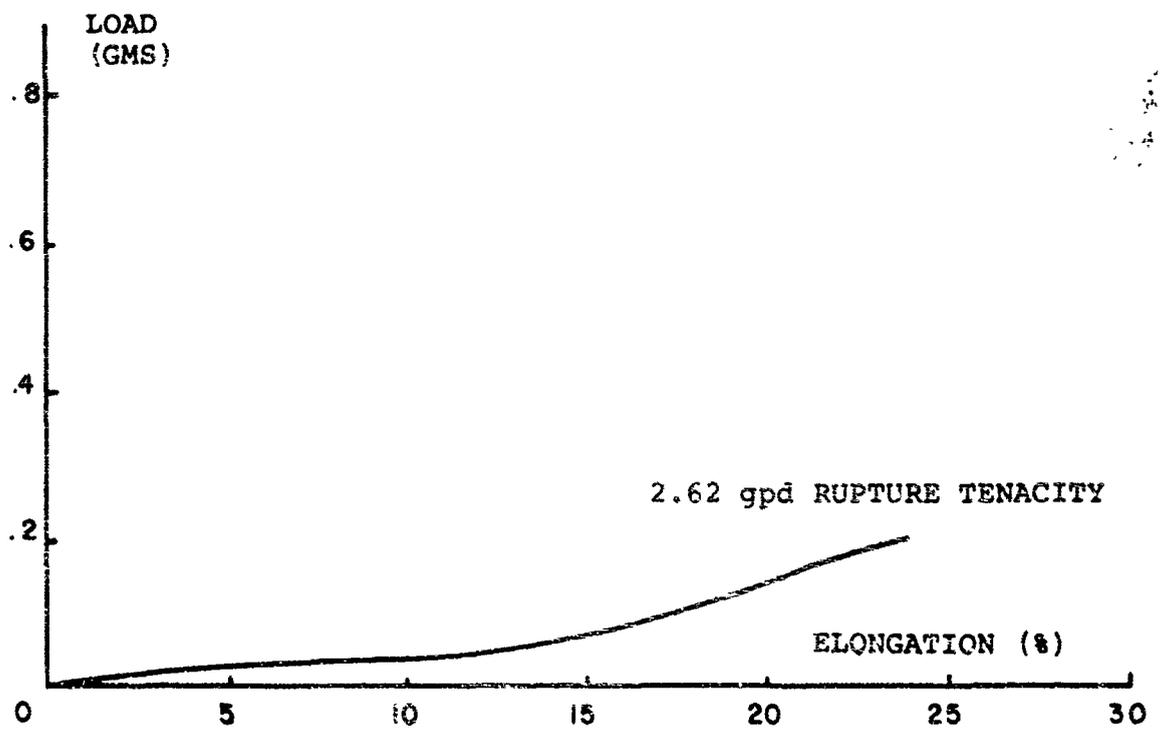
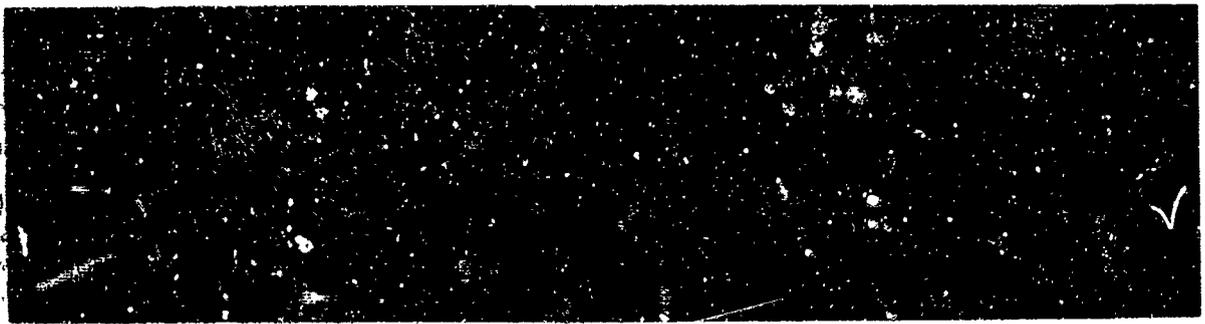
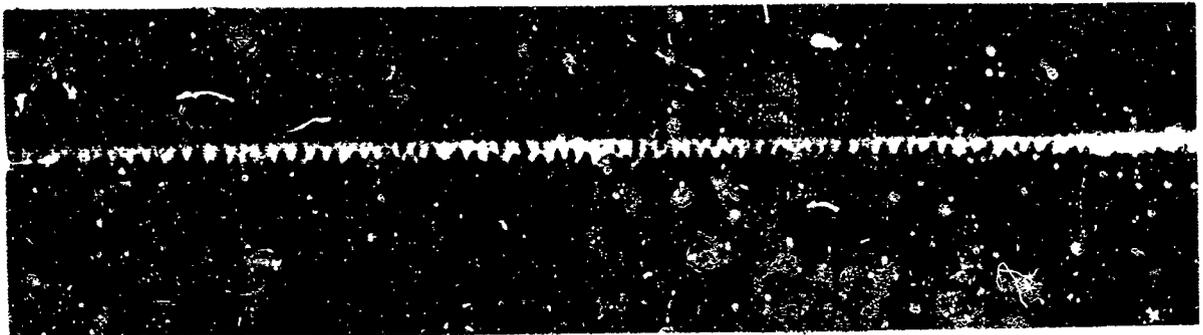


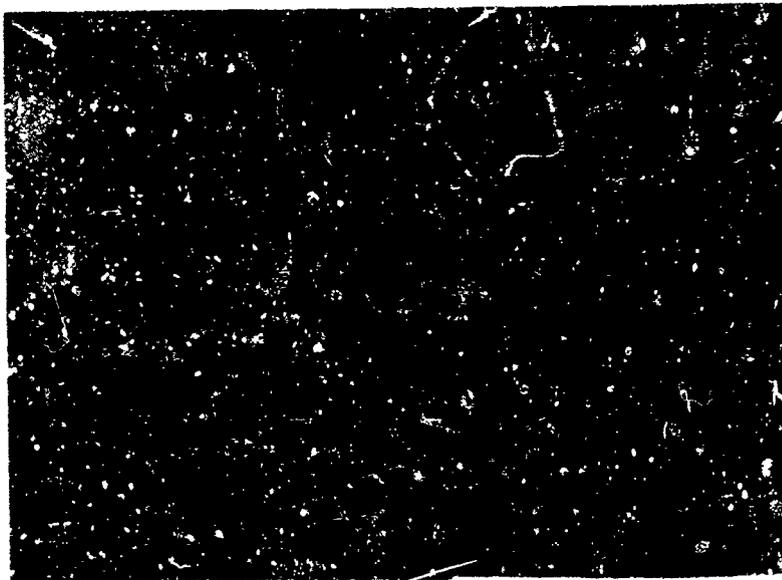
Figure 6. Typical Load-Elongation Curve
Argiope Argentata



MAGNIFICATION X1770



MAGNIFICATION X1770



FILAMENT CROSS SECTION
MAGNIFICATION X1610

Figure 7. Photomicrographs
of Nephila Clavipes Spider
Silk Filaments



MAGNIFICATION X1770

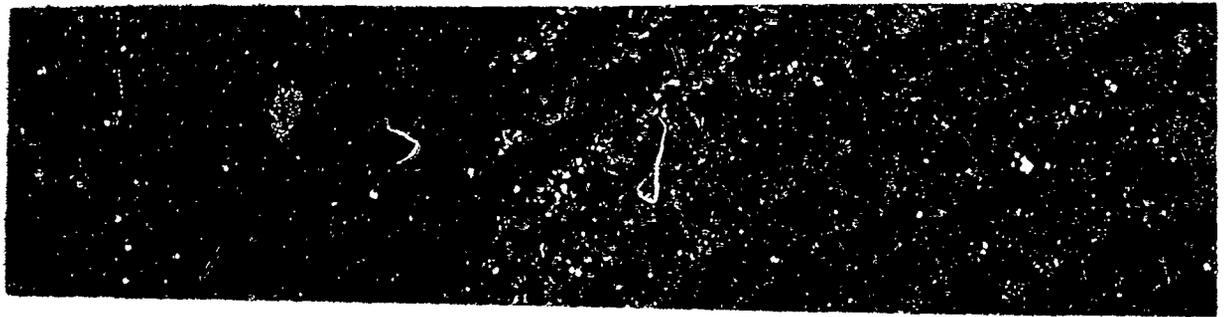


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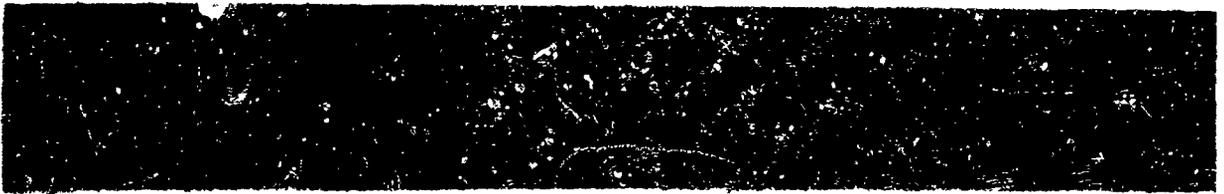


FILAMENT CROSS SECTION
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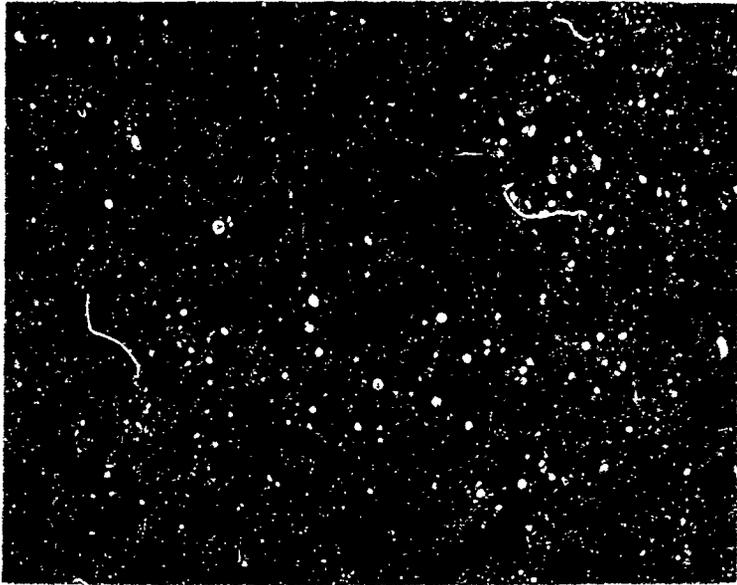
Figure 8. Photomicrographs of Argiope Aurantia Spider Silk Filaments



MAGNIFICATION X1770

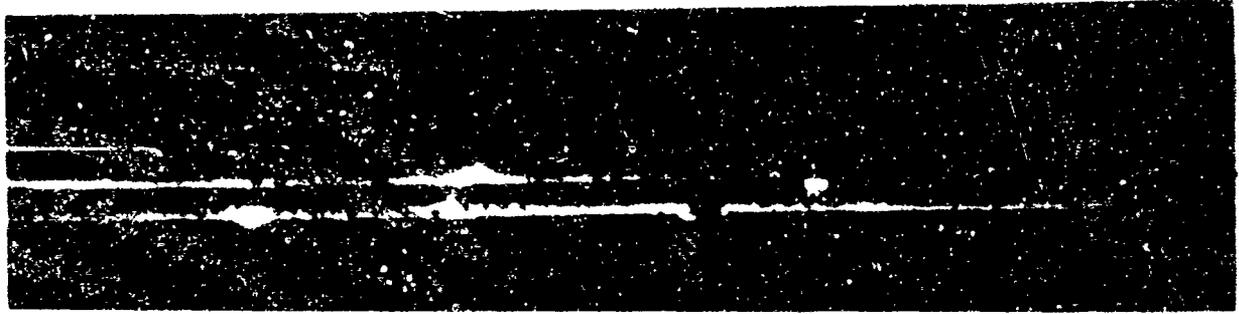


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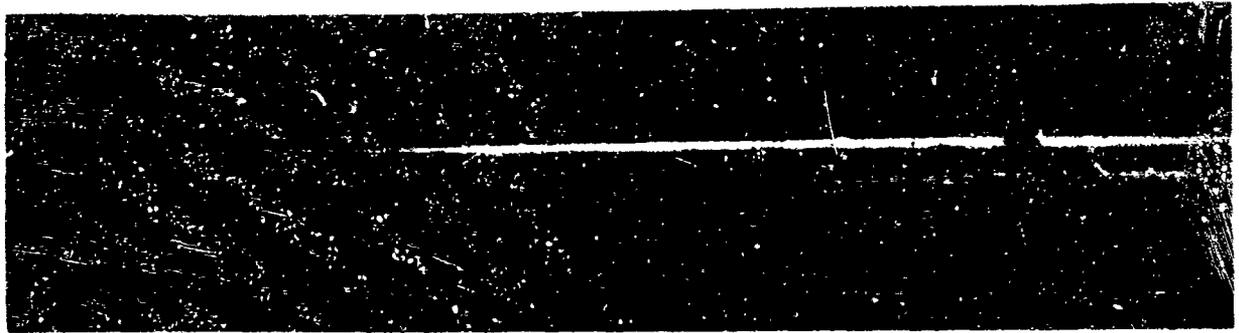


FILAMENT CROSS SECTION
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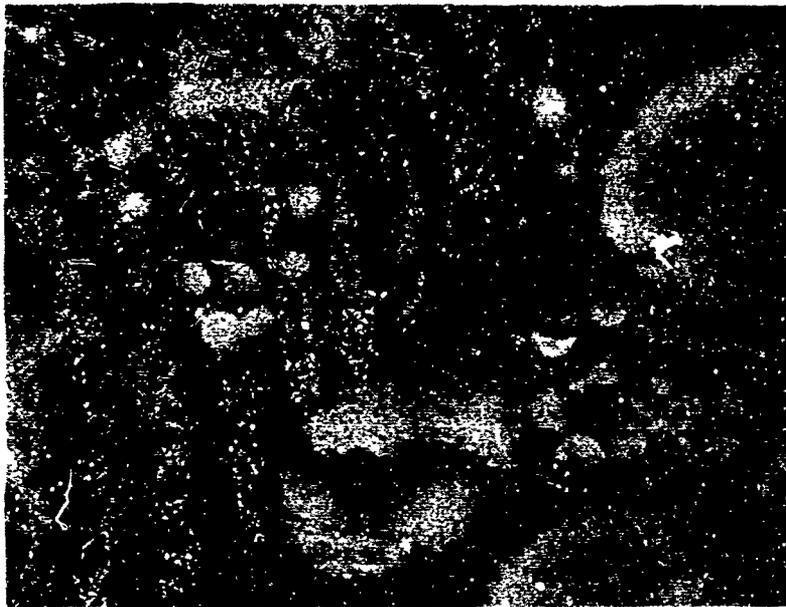
Figure 9. Photomicrographs
of Nephila Cruentata Spider
Silk Filaments



MAGNIFICATION X170

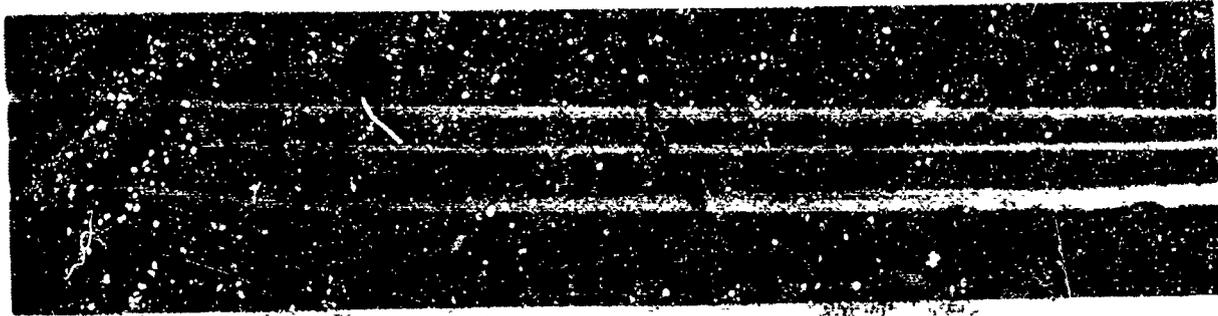


MAGNIFICATION X170



FILAMENT CROSS SECTION
MAGNIFICATION X1619

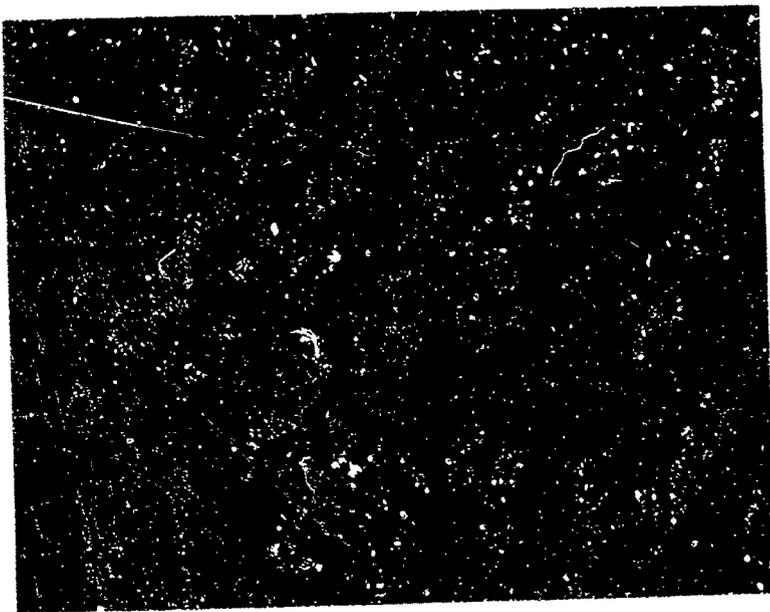
Figure 10. Photomicrographs
of Parawixla Audax Spider
Silk Filaments



MAGNIFICATION X1770

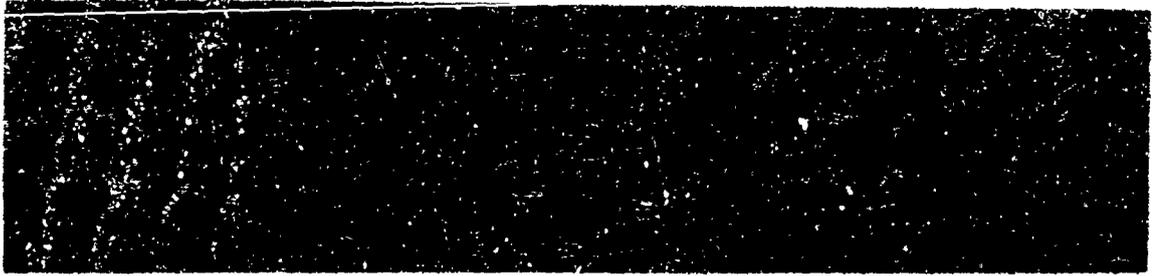


MAGNIFICATION X1770



FILAMENT CROSS SECTION
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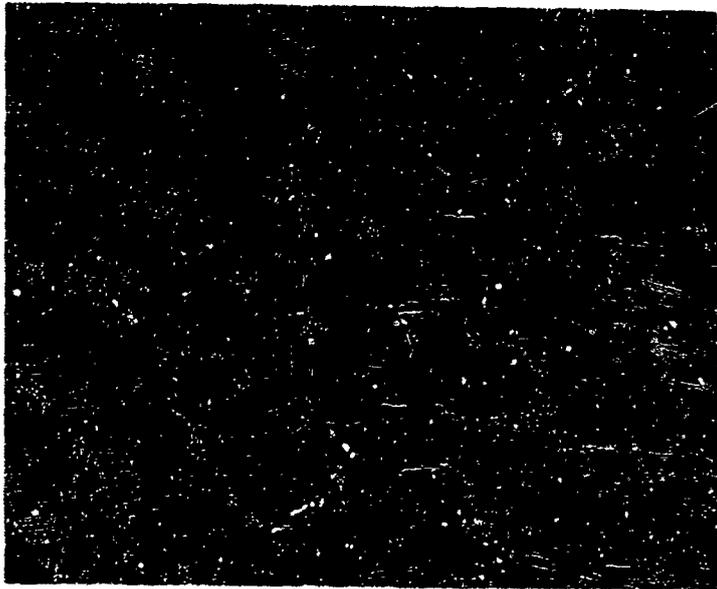
Figure 11. Photomicrographs
of Argiope Argentata Spider
Silk Filaments



MAGNIFICATION X1770



MAGNIFICATION X1770



FILAMENT CROSS SECTION
MAGNIFICATION X1610

Figure 12. Photomicrographs
of Japanese Yellow Silk
Filaments

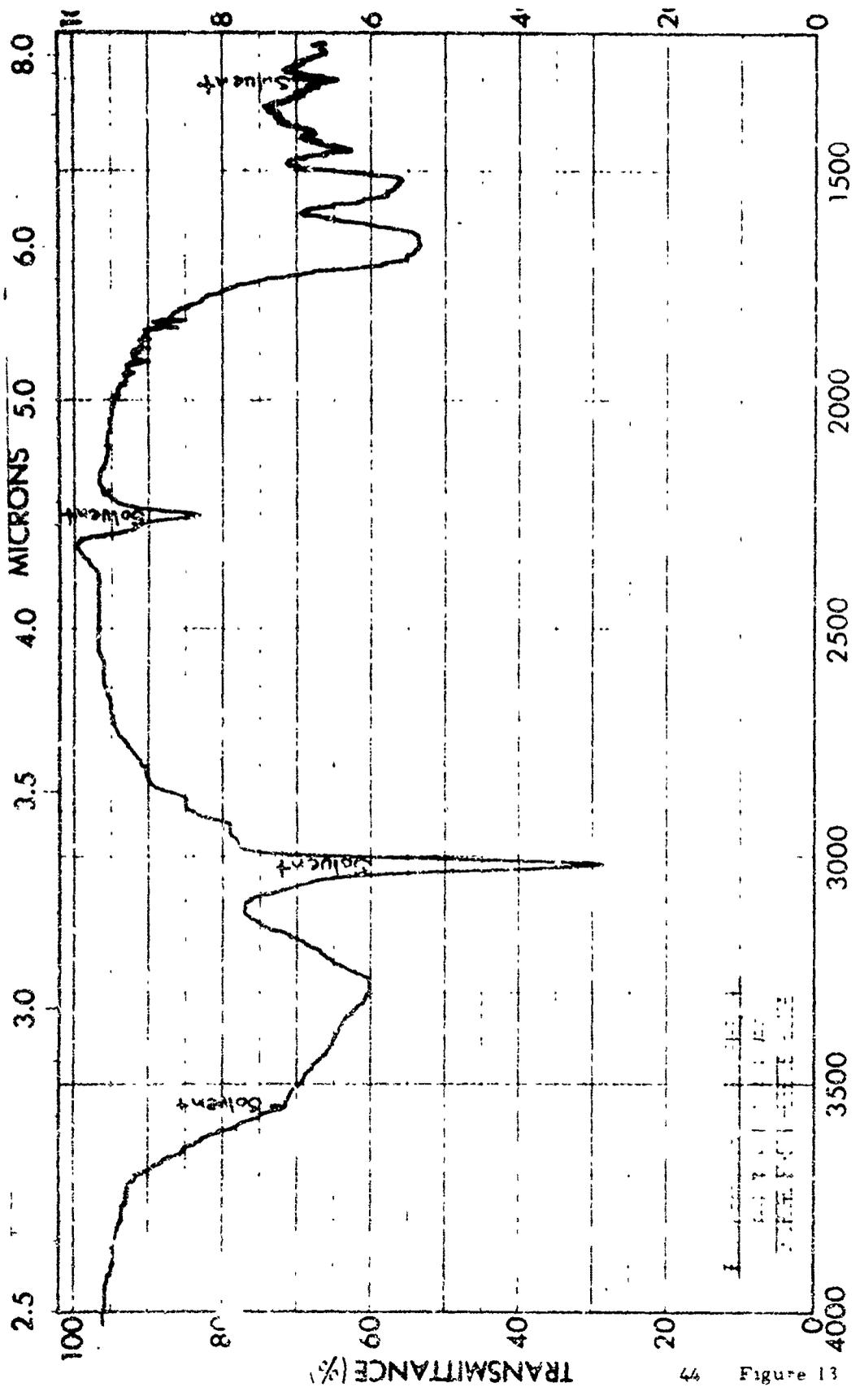


Figure 13

SAMPLE Golden Garden Spider (Miranda Aurantia)	CURVE NO. 1	SCAN SPEED Fast	OPERATOR PMI
ORIGIN	CELL PATH MIRC-CELL	SIT Normal	DATE 1-25-68
SOLVENT Ethanol	REFERENCE B (solvent)	REMARKS PR: (05003-1-2)	

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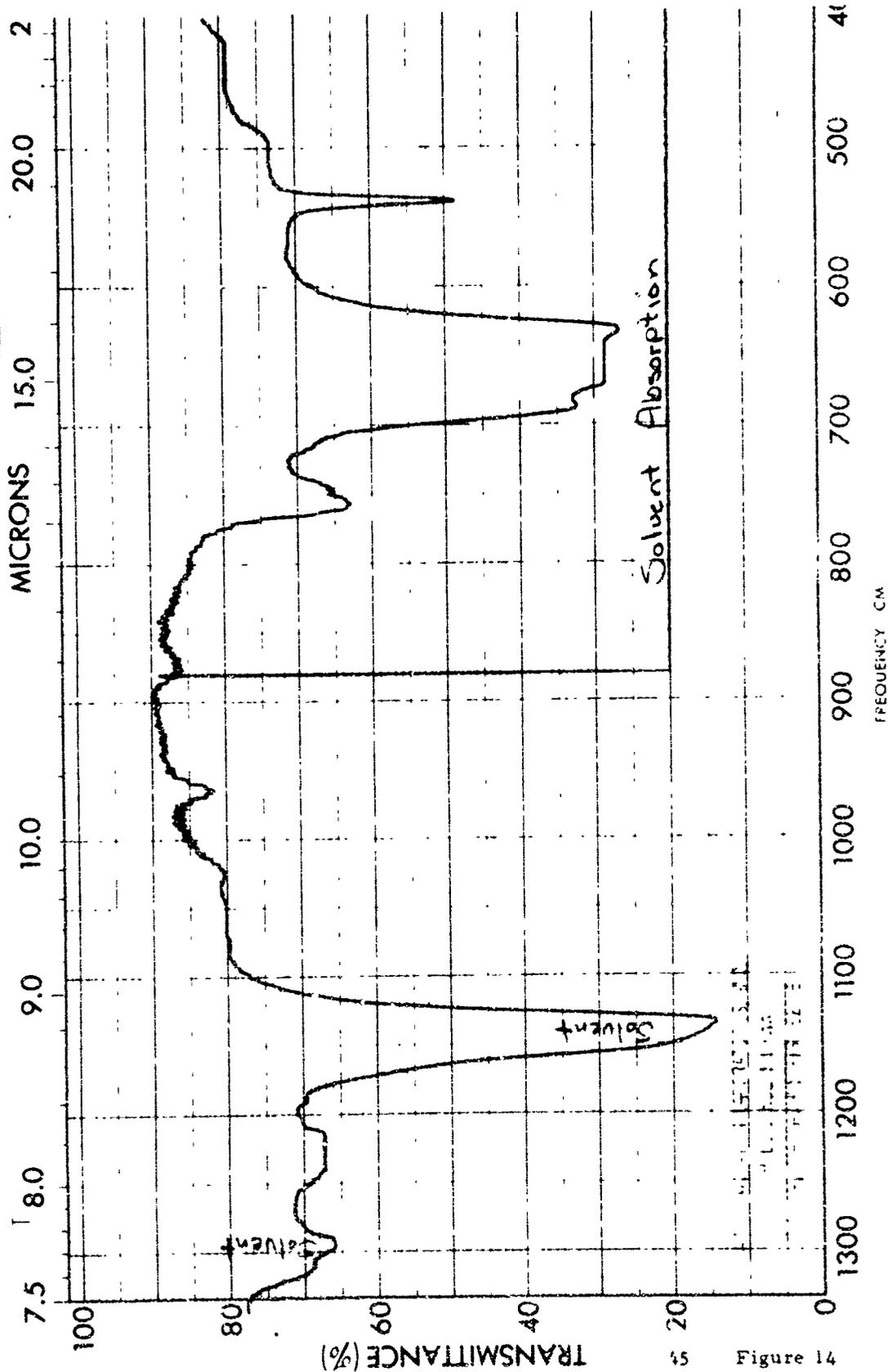
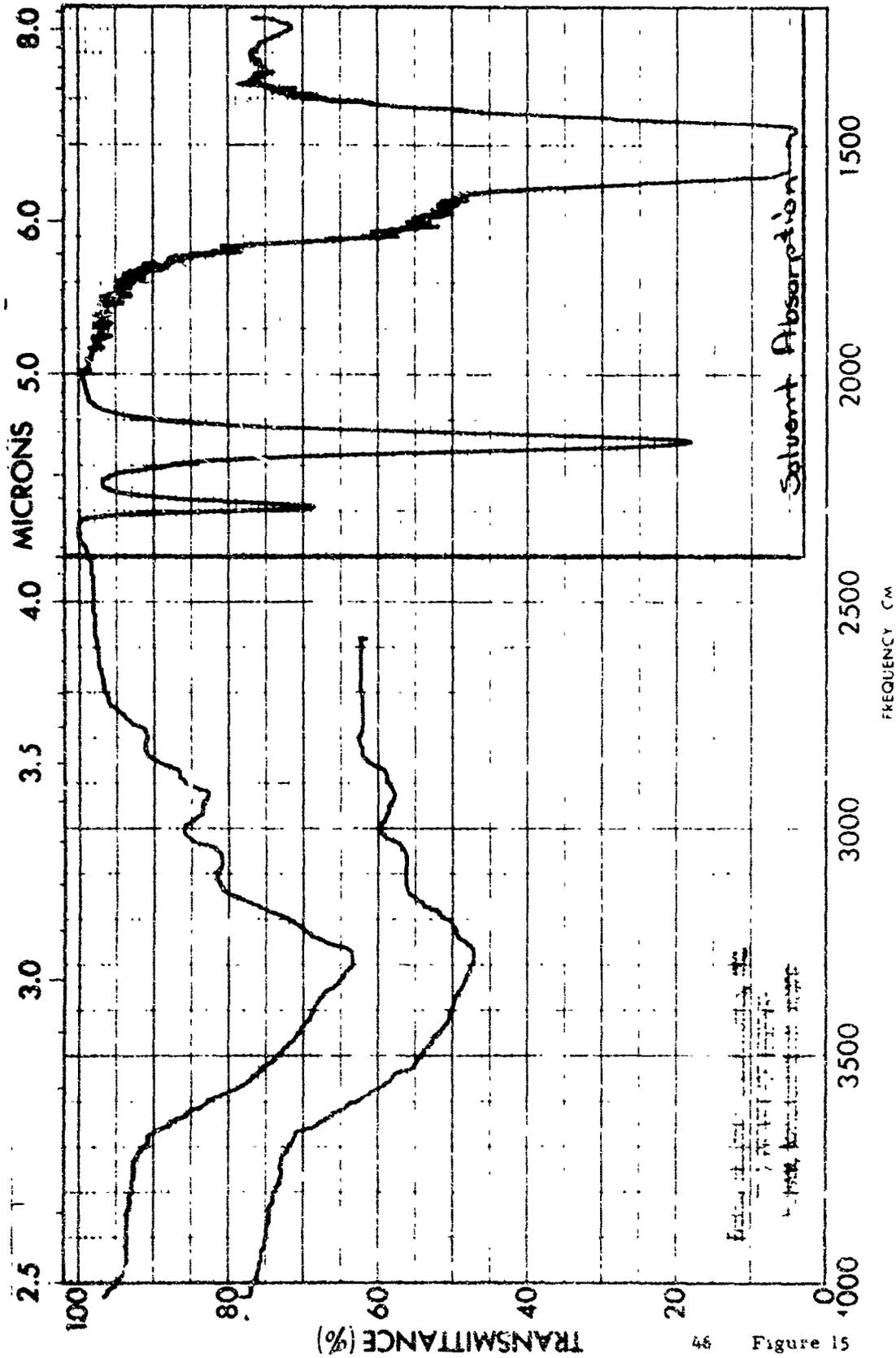


Figure 14

SAMPLE <u>Golden Garden Spider</u>	CURVE NO	SCAN SPEED <u>Fast</u>	OPERATOR <u>PM</u>
<u>(Miranda Aurantia)</u>	CONC	<u>Normal</u>	DATE <u>1-23-68</u>
ORIGIN	CELL PATH <u>Mini-Cell</u>	REMARKS <u>FRL Co5593-1-2</u>	
SOLVENT <u>Bromoform</u>	REFERENCE <u>Bromoform</u>	<u>Co Laboratory Research, L. S. Army</u>	

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PART NO 337 1203

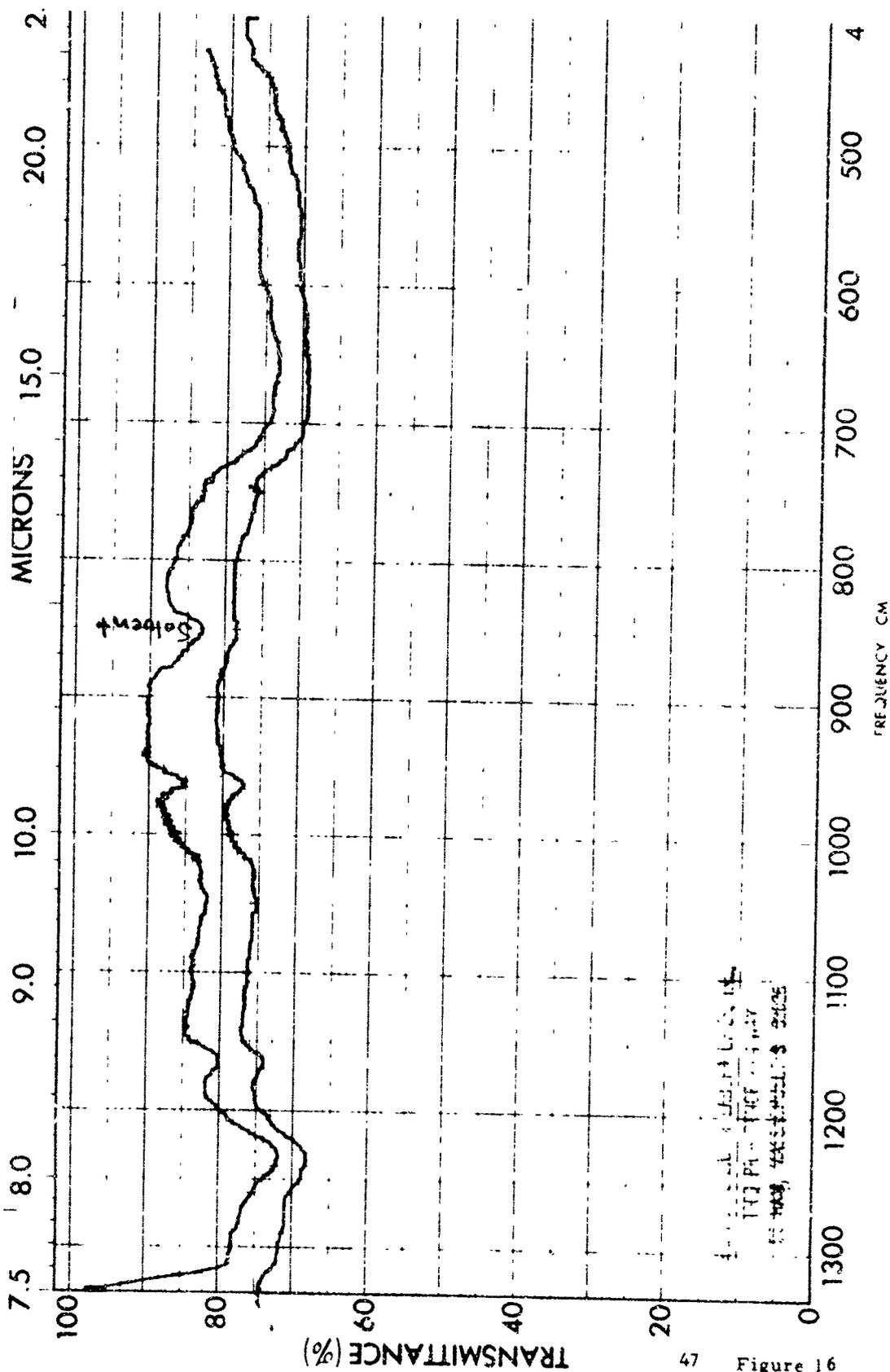


46 Figure 15

SAMPLE <u>Golden Garden Spider</u>	CURVE NO	SCAN SPEED <u>Fast</u>	OPERATOR <u>PM</u>
<u>(Miranda Aurantia)</u>	CONC	<u>500 Normal</u>	DATE <u>1-23-68</u>
ORIGIN	CELL PATH <u>Mini-Cell</u>	REMARKS <u>FRL Co559-1-2</u>	
SOLVENT <u>CS₂</u>	REFERENCE <u>Biobolony</u>	<u>300 Comparative Research</u>	

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Golden Garden Spider
 117 PM - 1/23/68
 50 mg, 4x5 mm KBr disc

SAMPLE <u>Golden Garden Spider</u>	CURVE NO	SCAN SPEED <u>Fast</u>	OPERATOR <u>PM</u>
<u>(Miranda Aurantia)</u>	<u>100</u>	SPLIT <u>Normal</u>	DATE <u>1-23-68</u>
ORIGIN	CELL PATH <u>Miranda Cell</u>	REMARKS <u>FRL C055001-2</u>	
SOLVENT <u>CS₂</u>	REFERENCE <u>Bromoforn</u>	Cooperatively Research U.S. Army	

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Figure 16

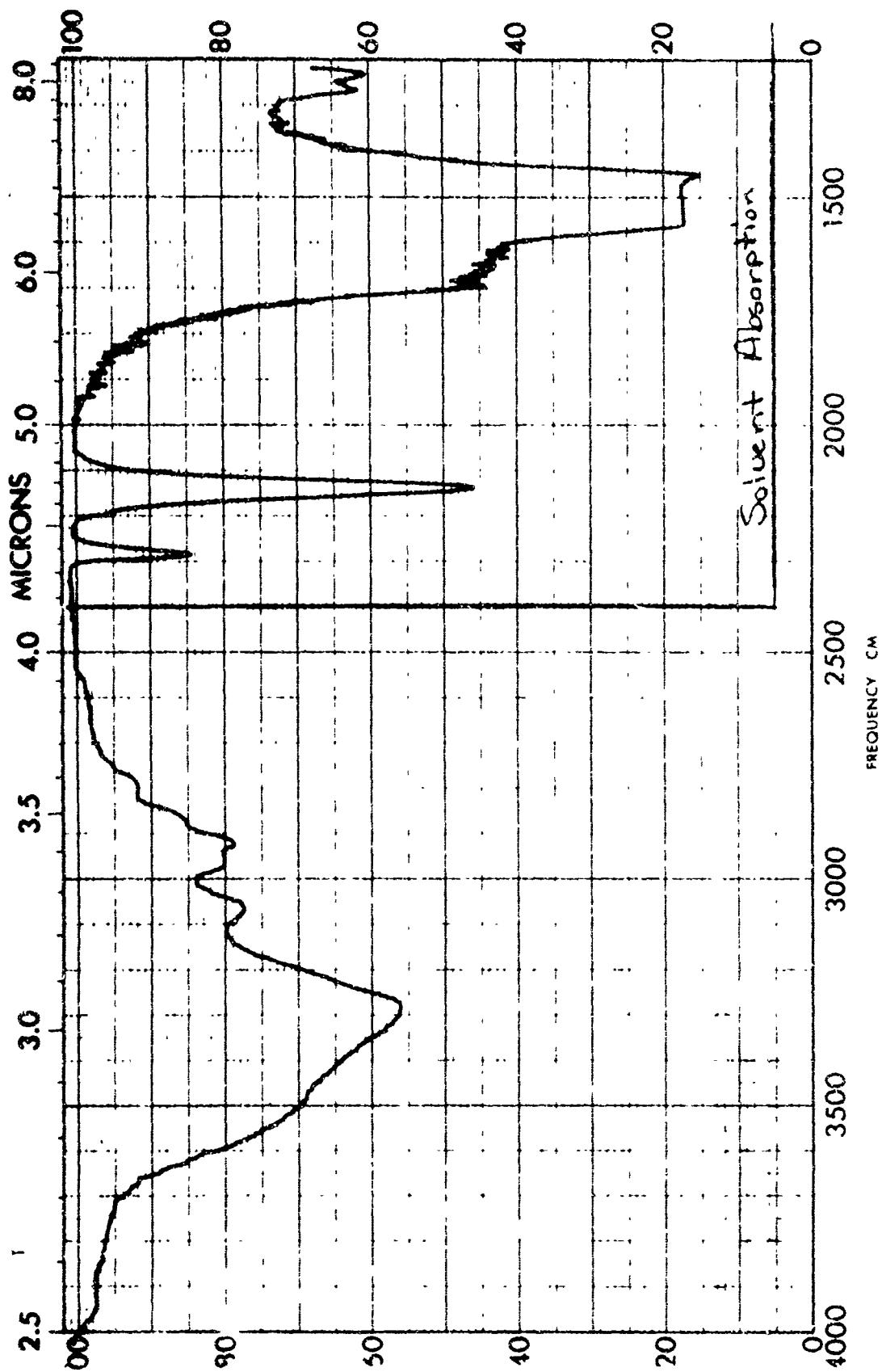
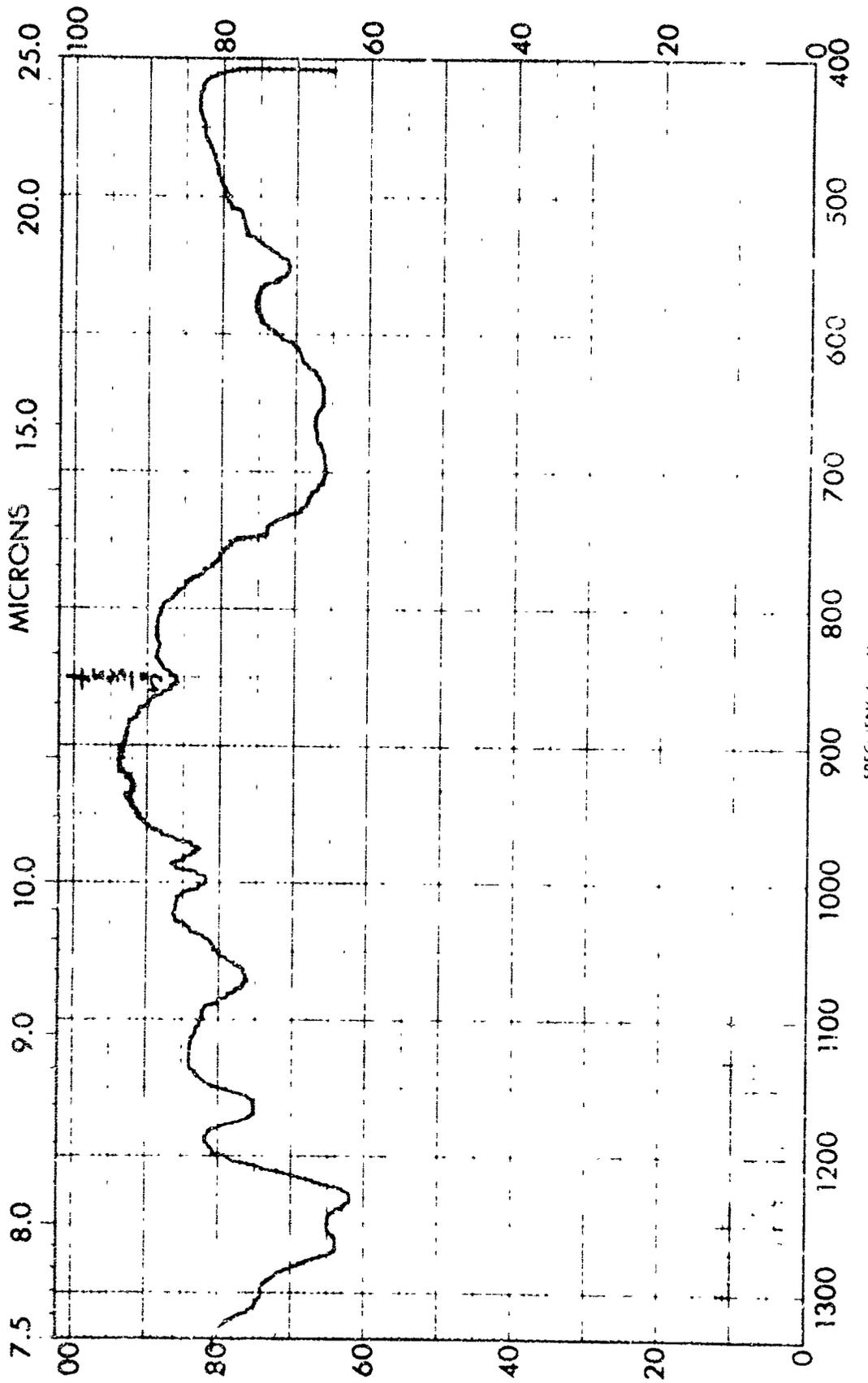


Figure 17

SAMPLE Silk Fiber - Degummed	CURVE NO	SCAN SPEED Fast	OPERATOR PM
ORIGIN Fiber Collection A-118	CONC	SIT Normal	DATE 1-23-64
SOLVENT CS ₂	CELL PATH Mini-Cell	REMARKS FRI 65503-1-2	
	REFERENCE CS ₂	Col'd. Univ. Research U.S. Army	



SAMPLE <u>Silk Fiber - Degummed</u>	CURVE NO. _____	SCAN SPEED <u>Fast</u>	OPERATOR <u>PNI</u>
ORIGIN <u>Fiber Collection A-108 5</u>	CONC. _____	SLT. <u>Normal</u>	GATE <u>1-2-3-4-5</u>
SOLVENT <u>C₂H₅</u>	CELL PATH <u>MIR-Cell</u>	REMARKS <u>ERI 0.55</u>	
	REFERENCE <u>C₂H₅</u>		

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PART NO 337 1293

Figure 18

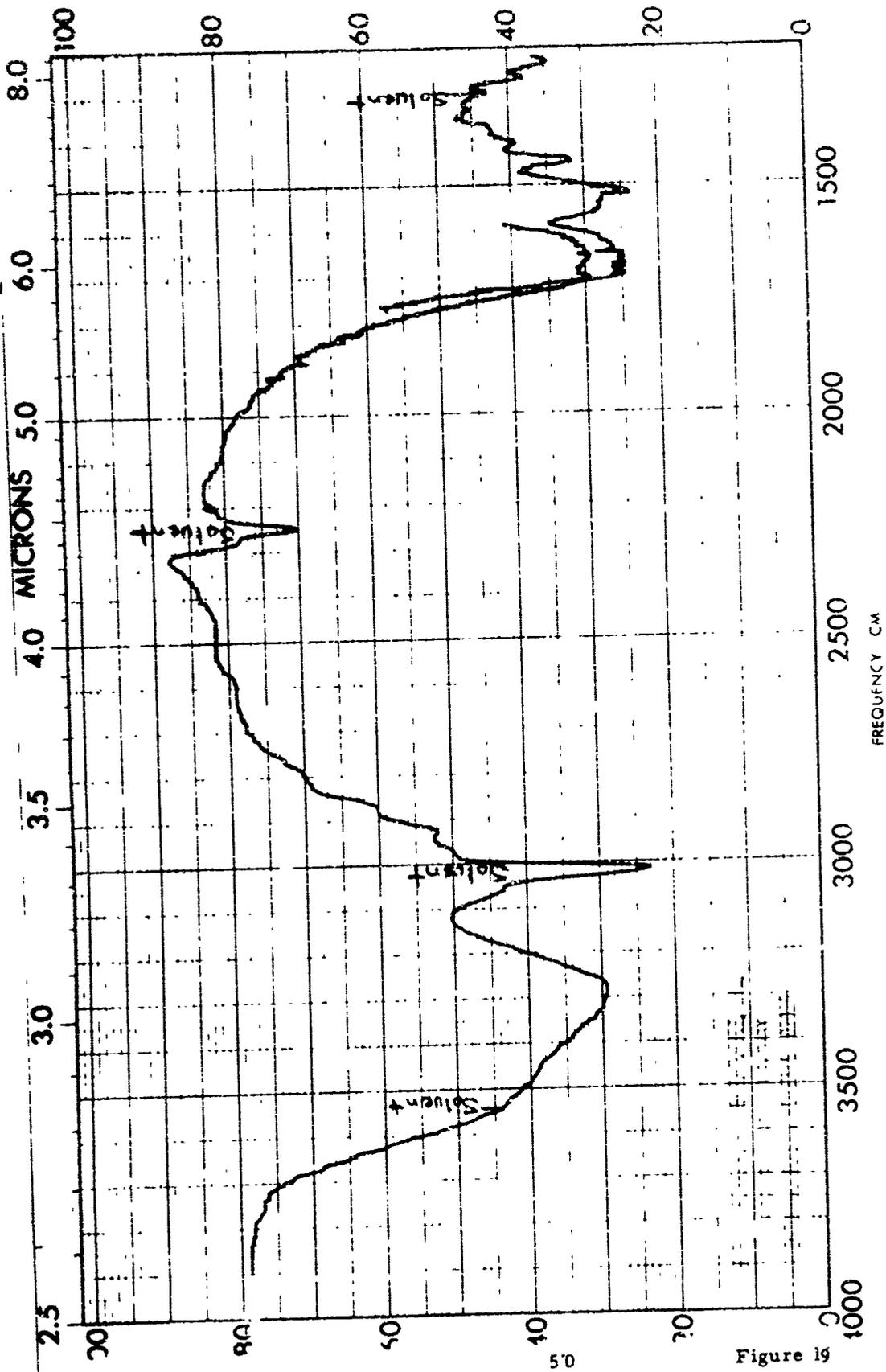


Figure 19

SAMPLE <u>Silk Fiber - Degummed</u>	CURVE NO	SCAN SPEED <u>Fast</u>	OPERATOR <u>PM</u>
ORIGIN	CONC	SAT <u>Normal</u>	DATE <u>1-23-68</u>
SOLVENT <u>Bromoform</u>	CELL PATH <u>Mini-Cell</u>	REMARK <u>FRL 6559-1-2</u>	
	REFERENCE <u>Bromoform</u>	(Continued on Research U.S.A.)	

PERKIN ELMER

PART NO 337 1203

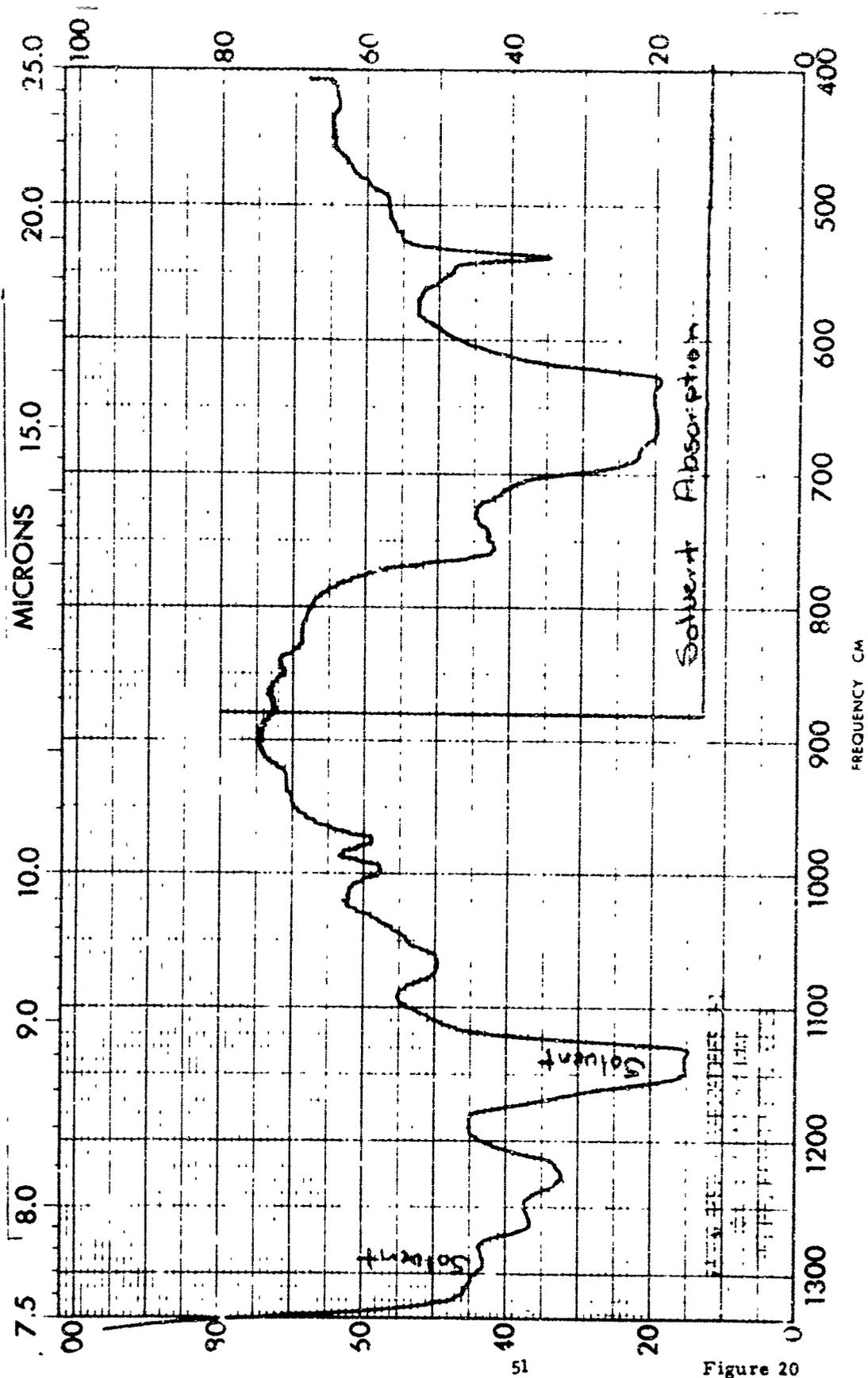
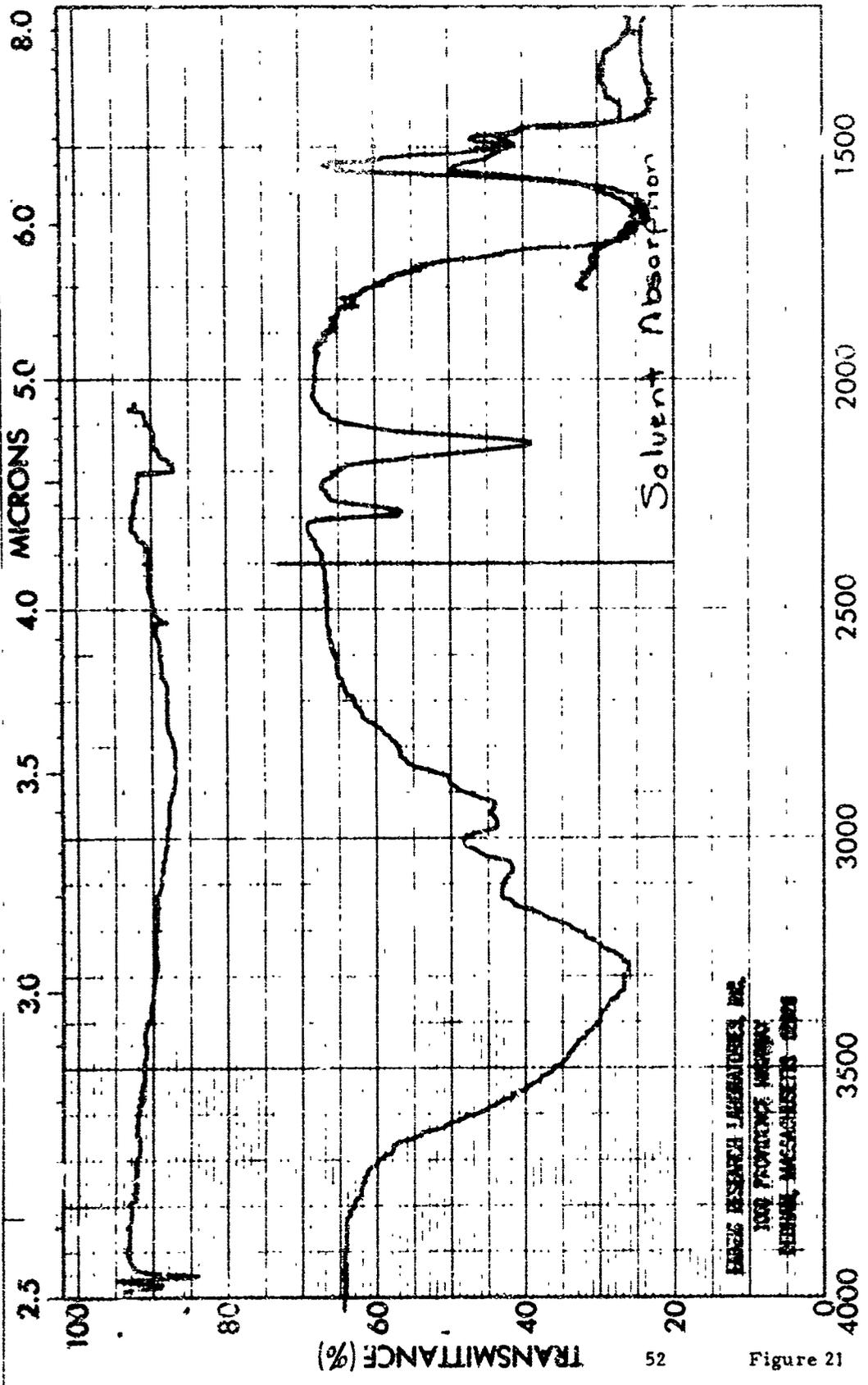


Figure 20

SAMPLE Silk Fiber - Degummed	CURVE NO	SCAN SPEED Fast	OPERATOR PM
ORIGIN Fiber Collection A-108-5	CONC	SUT Normal	DATE 1-23-68
SOLVENT Bromoform	CELL PATH Mini-Cell	REMARKS F.R.L. C65593-1, 2	
	REFERENCE Bromoform	Collaborative Research U.S. Army	

PART NO 337-1203

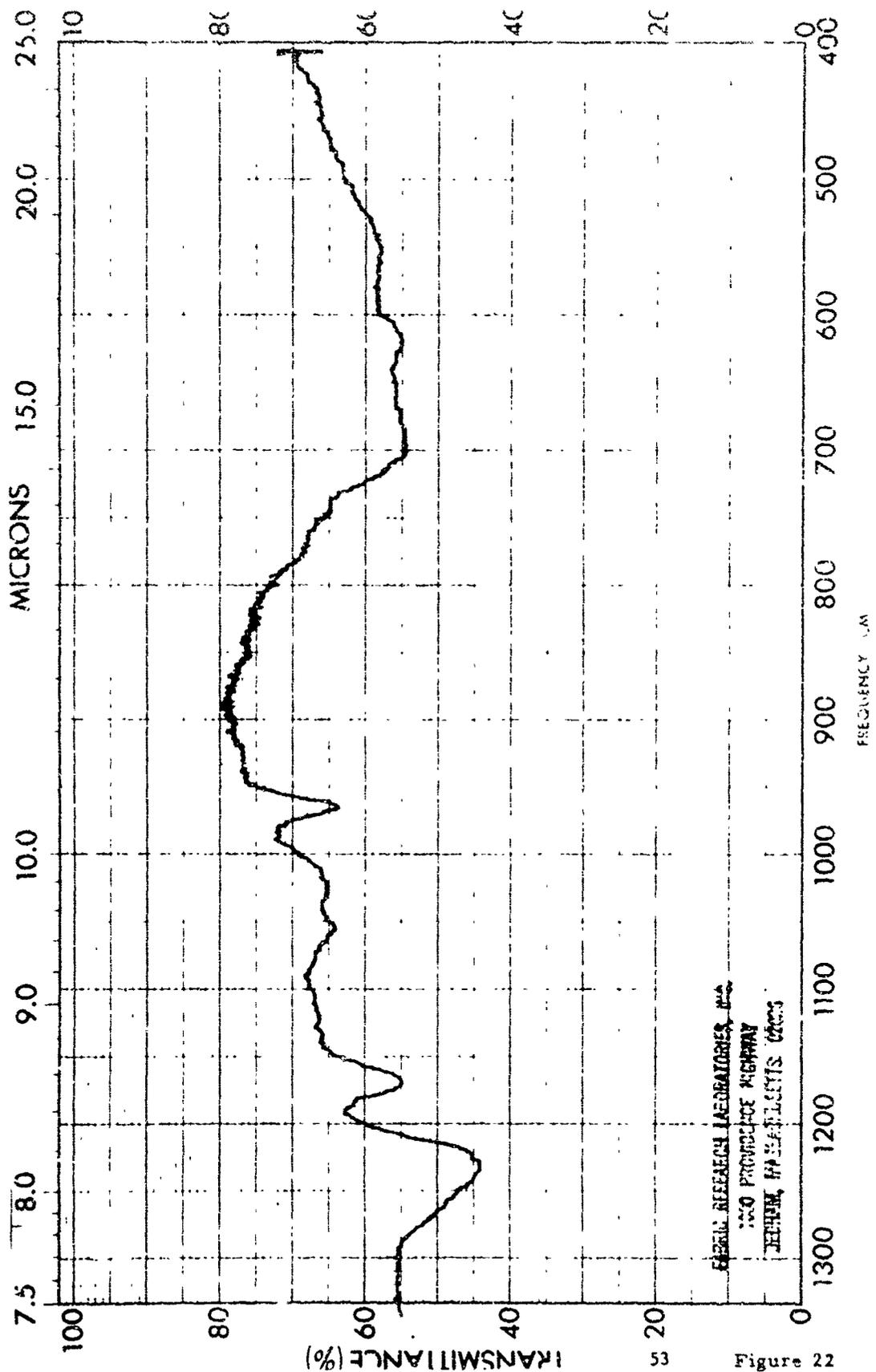
PERKIN-ELMER



SAMPLE N. Cuentata - 4 spots	CURVE NO	SCAN SPEED Fast	OPERATOR
Fine Web	CONC	SUIT Normal	DATE 1-23-57
ORIGIN	CELL PATH Mini-Cells	REMARKS PERL, Case 101	
SOLVENT CS2	REPETITION		

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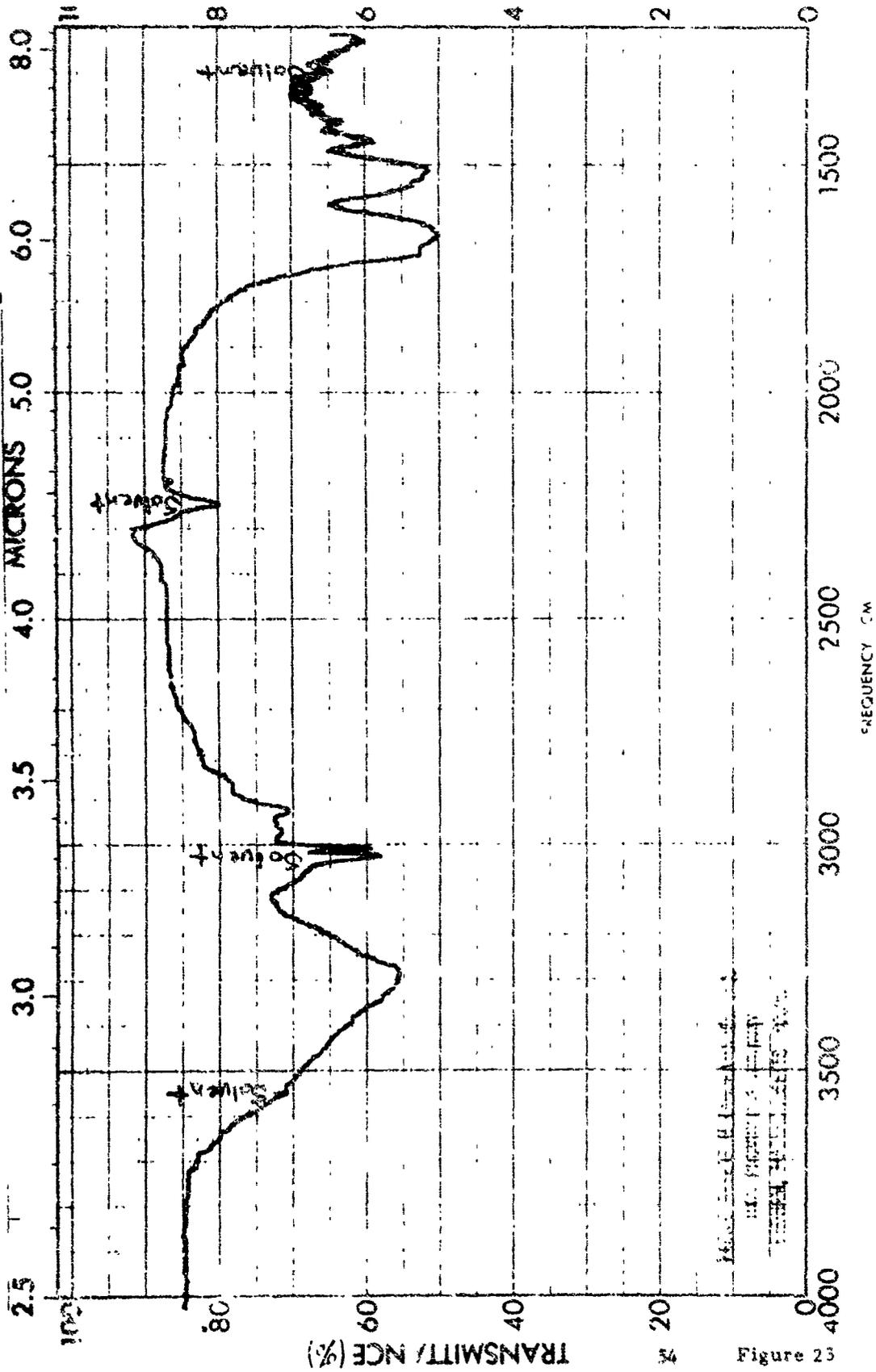
PART NO 337 120J



SAMPLE No. <u>N. Cruentata - 4 spots</u>	CURVE NO. _____	SCAN SPEED <u>Fast</u>	OPERATOR <u>PAJ</u>
<u>Fine Web</u>	CONC. _____	SUIT <u>Normal</u>	DATE <u>1-2-55</u>
ORIGIN _____	CELL PATH <u>Mini-Cells</u>	REMARKS <u>FRU' C655 B-JA-2</u>	
SOLVENT <u>CS2</u>	REFERENCE <u>CS2</u>	C. LABORATORY RESEARCH	

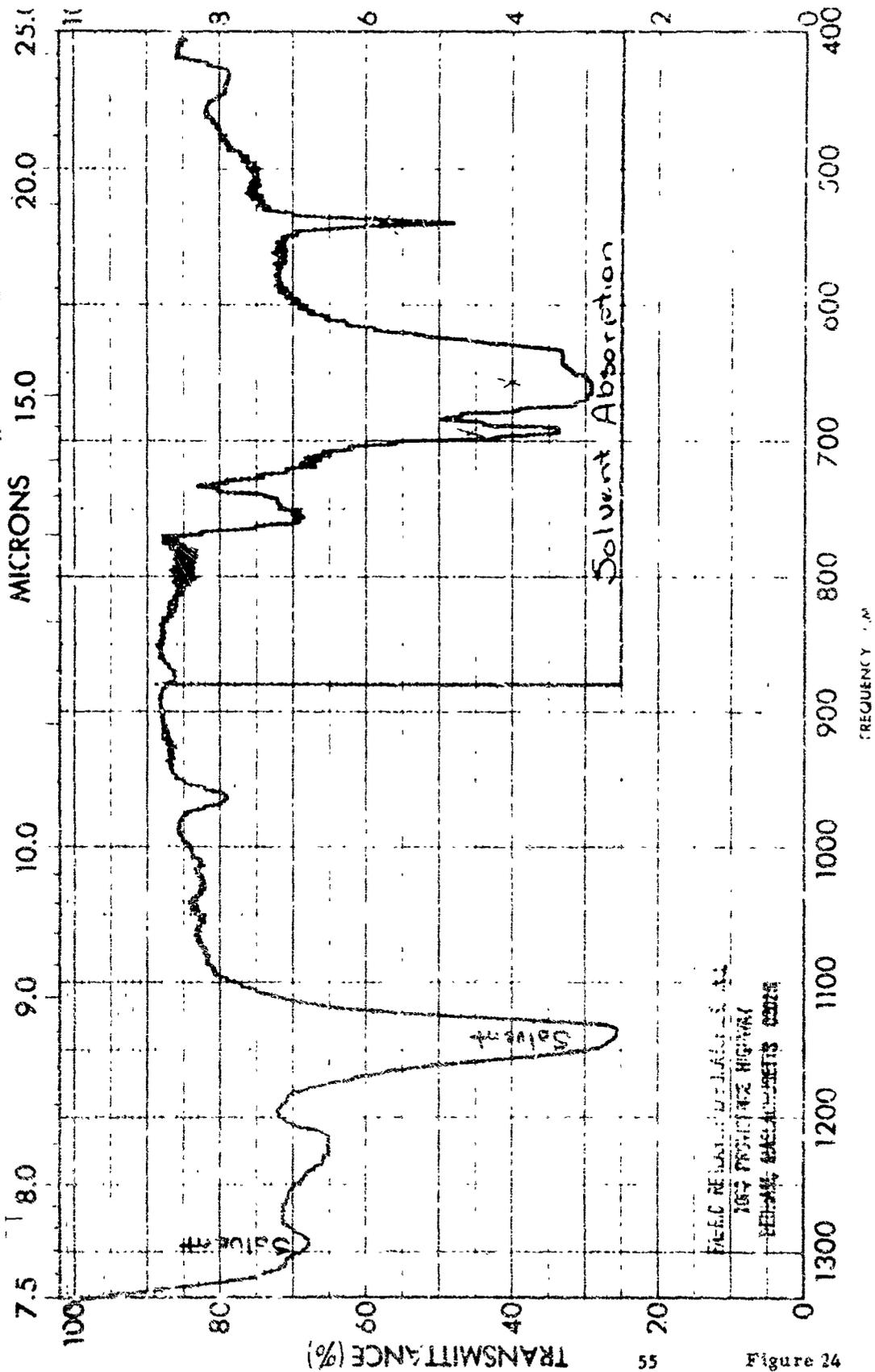
PERKIN ELMER

PART NO 337 1203



SAMPLE <u>N. Cruchata - J spols</u>	CURVE NO. <u>1</u>	SCAN SPEED <u>Fast</u>	OPERATOR <u>EM</u>
<u>Fine Web</u>	<u>CM</u>	<u>Normal</u>	DATE <u>1-2-58</u>
ORIGIN <u>PerkinElmer</u>	CELL <u>Mini-Cells</u>	REMARKS <u>FRL Co5531-2</u>	
SOLVENT <u>Formic acid</u>	REFERENCE <u>PerkinElmer</u>	PERKINELMER	

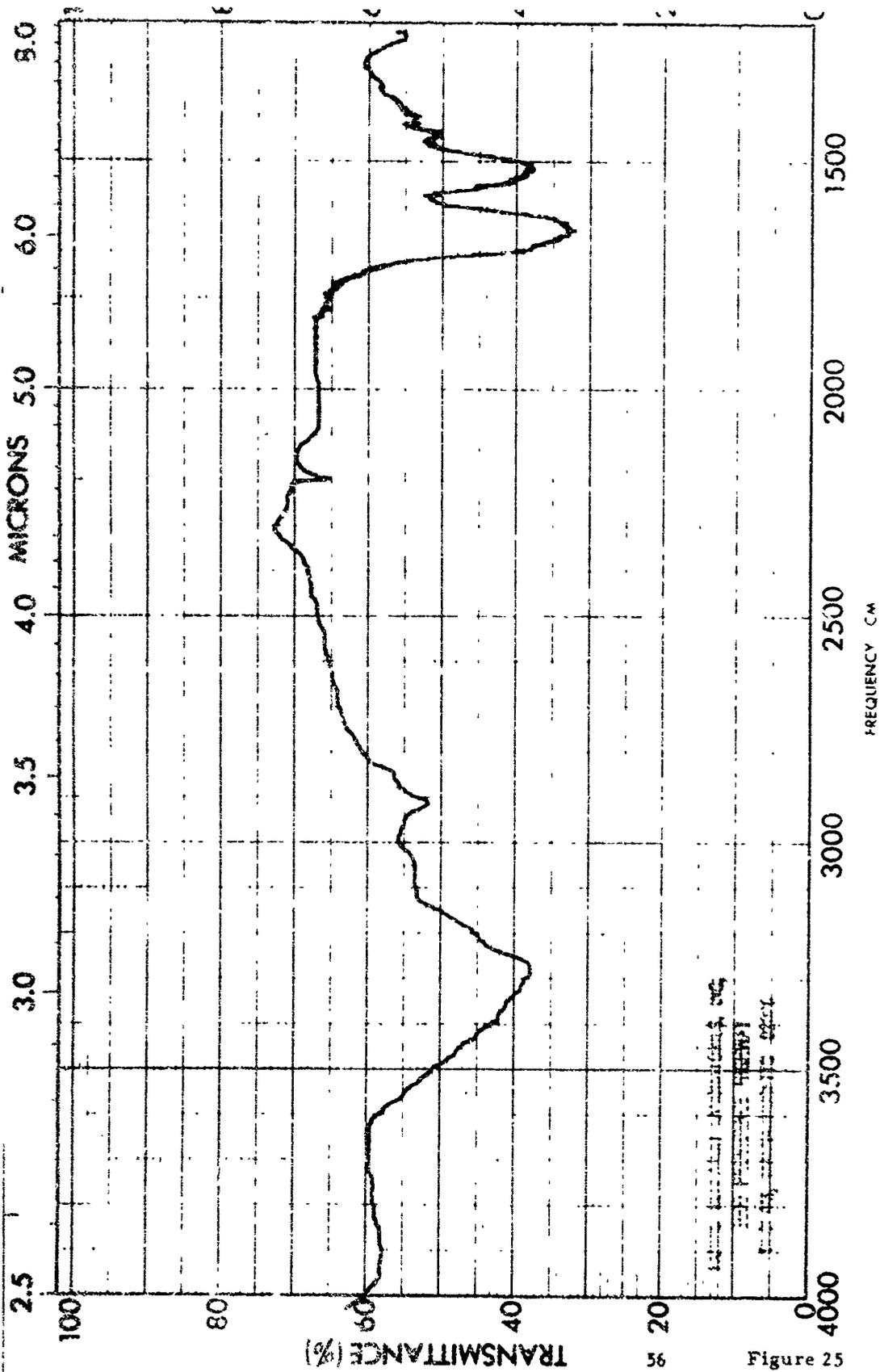
PART NO 317 1703



SAMPLE <u>N. Cruentata - 4 spots</u>	CURVE NO	SCAN SPEED <u>Fast</u>	OPERATOR <u>EM</u>
<u>Fine Web</u>	<u>COAC</u>	SIF <u>Normal</u>	DATE <u>1-23-68</u>
ORIGIN	<u>CELL PATH Mini-Cells</u>	REMARKS <u>IRL COSY-1-1-68</u>	
SOLVENT <u>Bromobenz</u>	REFERENCE <u>Bromobenz</u>		

PERKIN ELMER

PART NO 337 120



SAMPLE <u>Spider Silk 33-3</u>	CURVE NO _____	SCAN SPEED <u>Fast</u>	OPERATOR <u>PVI</u>
<u>Nephila Clavipes</u>	CONC _____	SUI <u>Normal</u>	DATE <u>2-10-68</u>
ORIGIN _____	CELL <u>ATH KBr Disc</u>	REMARKS <u>FRI. Co5503-1, 2</u>	
SOLVENT _____	REFERENCE _____	<u>Comparative Research</u>	<u>S. Vep</u>

PERKIN ELMER

PART NO 137 1203

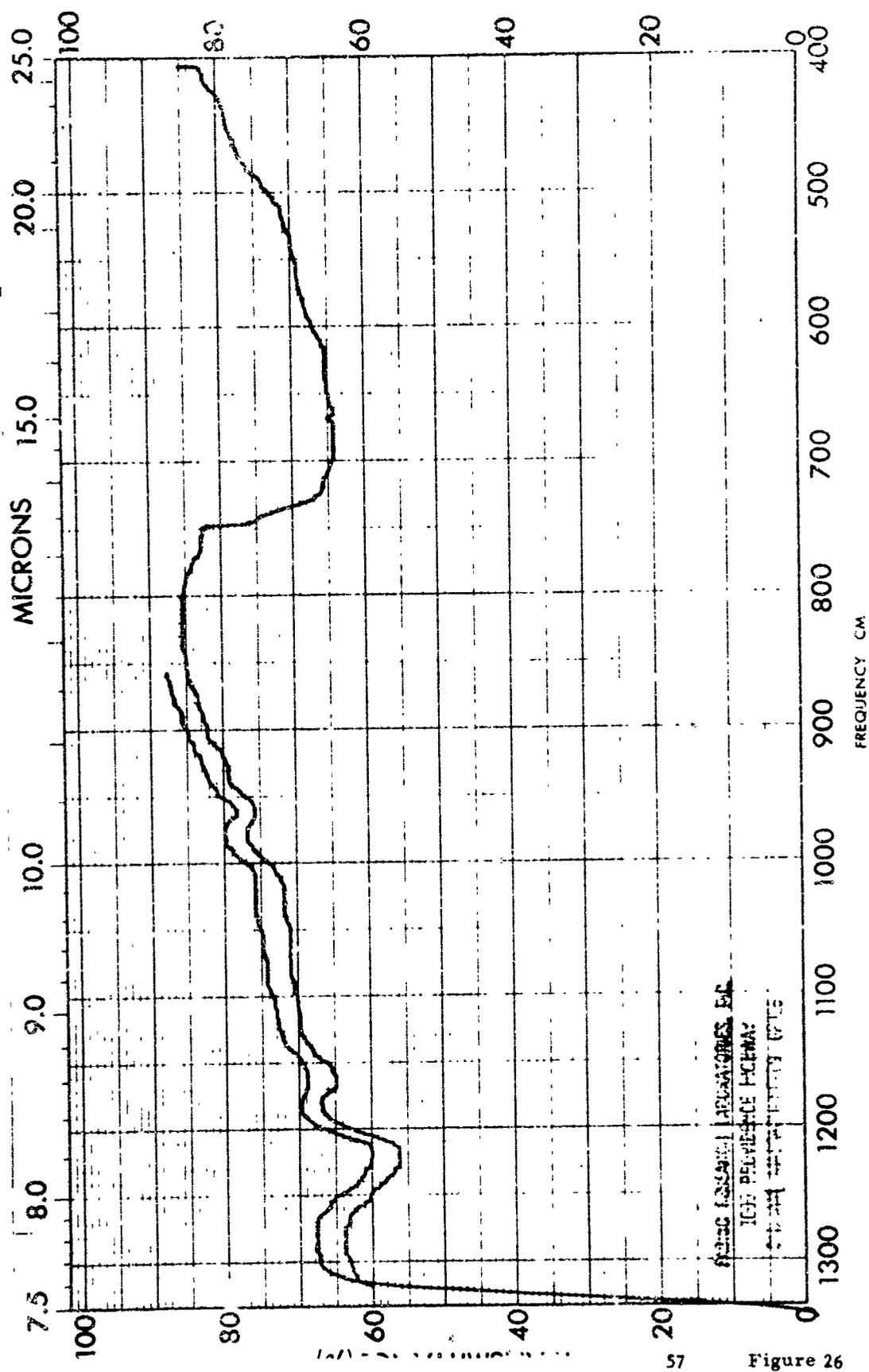


Figure 26

SAMPLE <u>Spider Silk 33-3</u>	CURVE NO. _____	SCAN SPEED <u>Fast</u>	OPERATOR <u>PMI</u>
<u>Nephila clavipes</u>	CON. _____	<u>Normal</u>	DATE <u>2-16-68</u>
ORIGIN _____	CELL PATH <u>KBr Disc</u>	REMARKS <u>FRL: C65593-1-2</u>	
SOLVENT _____	REFERENCE _____	<u>Collaborative Research Co., Inc.</u>	

PERKIN-ELMER

PART NO 337-1201

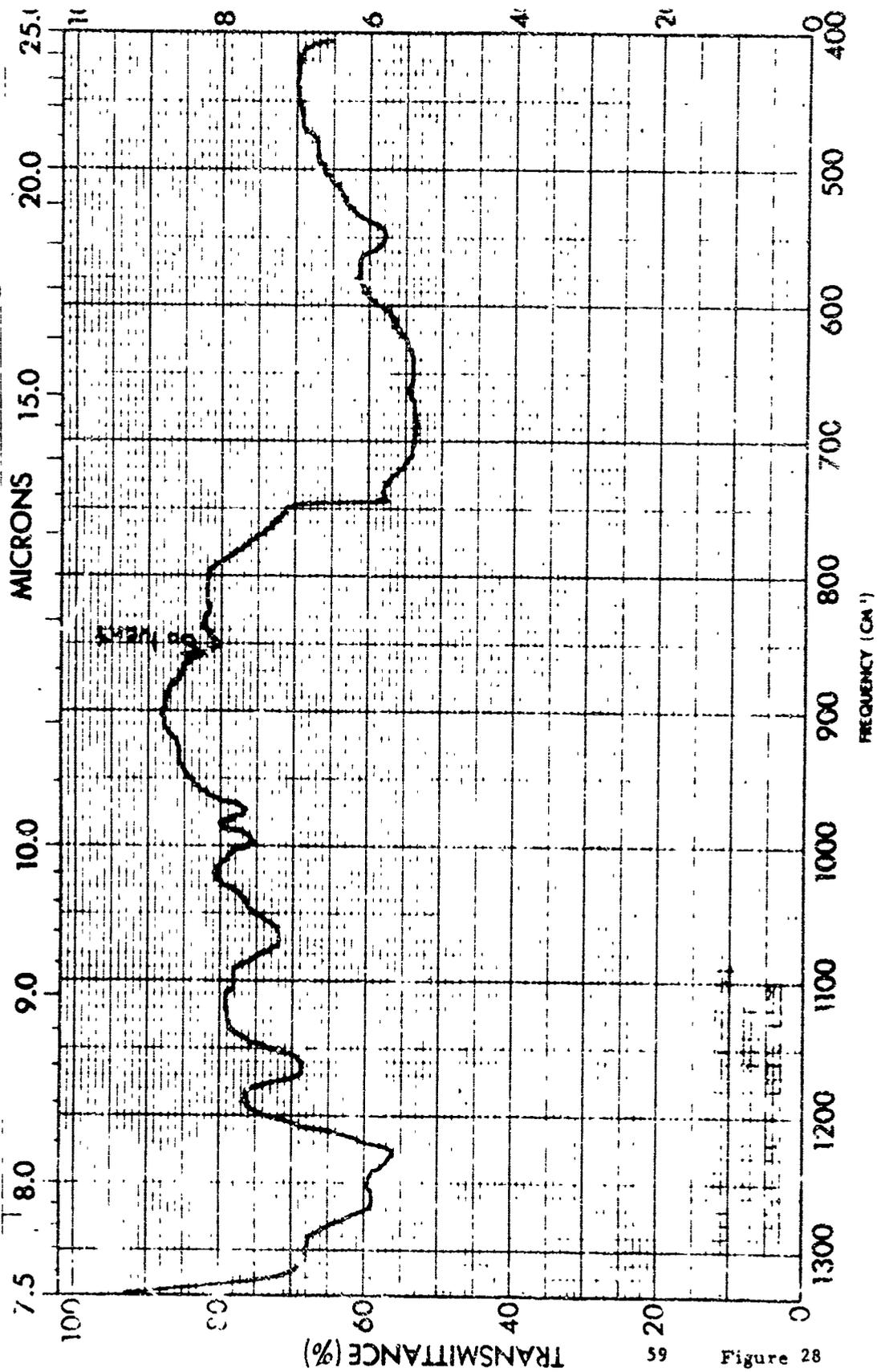
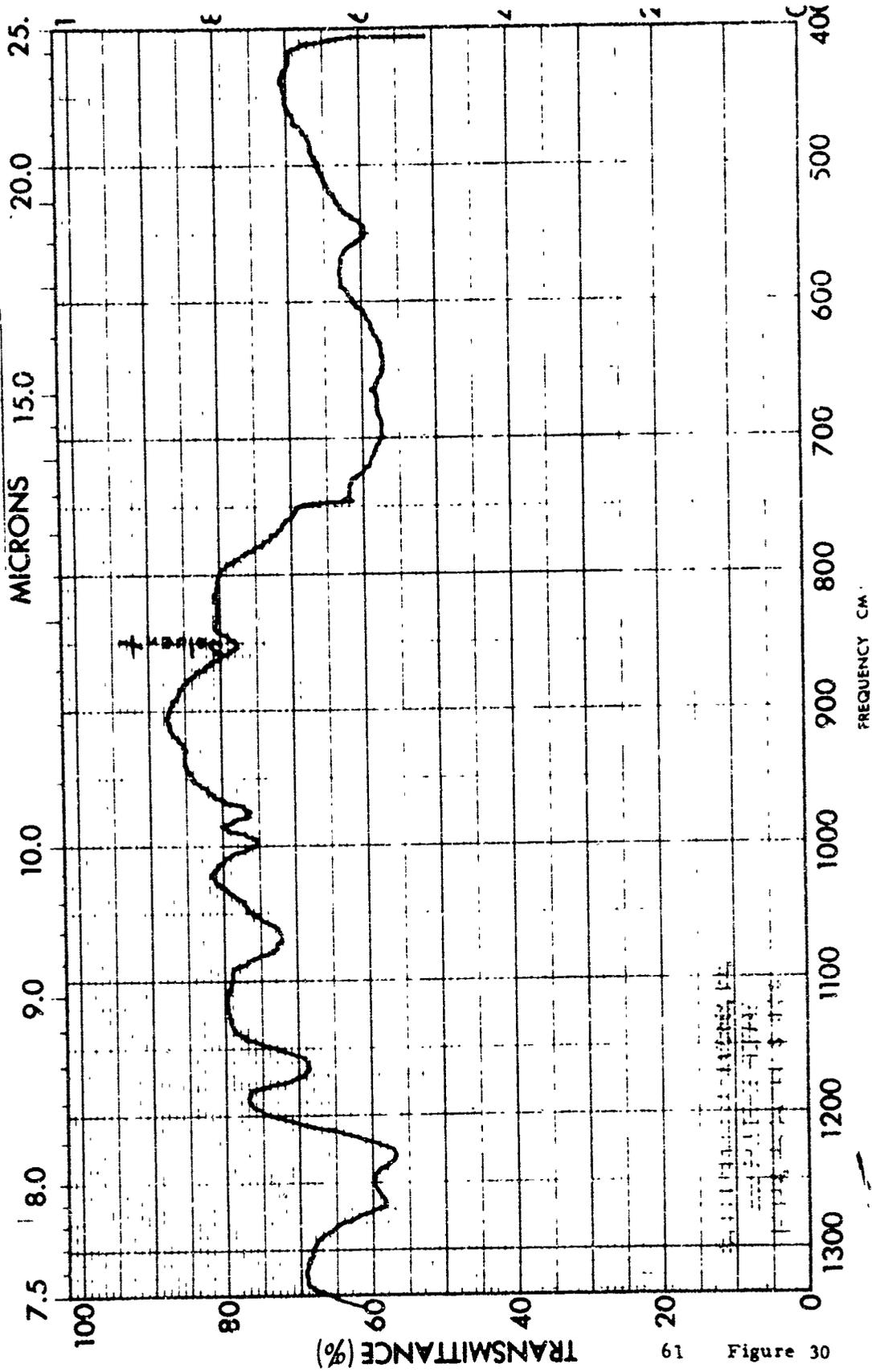


Figure 28

SAMPLE <u>Wild Turquoise Silk</u>	CURVE NO. _____	SCAN SPEED <u>Fast</u>	OPERATOR <u>PM</u>
ORIGIN <u>Fiber Coll. A-102</u>	CONC. _____	SIT <u>Normal</u>	DATE <u>1-24-68</u>
SOLVENT <u>CS₂</u>	CELL PATH <u>Mini-Cell</u>	REMARKS <u>F.R.® C65593-1, -2</u>	
	REFERENCE <u>C52</u>	Collaborative Research/U.S. Army	

PART NO. 337-1203

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61 Figure 30

SAMPLE <u>Wild Turrah Silk</u>	CURVE NO. _____	SCAN SPEED <u>Fast</u>	OPERATOR <u>PM</u>
ORIGIN <u>Fiber Coll. A-108-t</u>	CONC. _____	SUT <u>Normal</u>	DATE <u>1-24-68</u>
SOLVENT <u>CS2</u>	CELL PATH <u>Mini-Cell</u>	REMARKS <u>FRL C65593-1.1-2</u>	
	REFERENCE <u>CS2</u>	Collaborative Research U. S. Army	

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APPENDIX B

NOTES TO ACCOMPANY AMINO ACID ANALYSES

The acid and neutral amino acids are determined using a longer column of lower pH. In order to analyze samples too small to weigh, a portion of the hydrolyzed sample was placed onto the short column for observation of the early peaks, which come off before the basic amino acids and contain the unresolved neutral and acidic amino acids. The amount of color in these peaks gives some indication of the sample size so that the amount of the remaining sample to be placed onto the long column is known.

Although the analyzer is fairly new and in excellent condition, there is always a possibility that a mechanical or electronic failure will occur during an analysis. It is preferred to have a small portion of the sample in reserve so that useful results may still be obtained in the event that the primary analysis is a failure.

RESULTS

Table B-I
Amino Acid Assay - Paneiras CB

49 hour hydrolysate

Sample: Spider Silk, Spider #3 Paineiras GB IV-21-67, tenacity 6.35 gpd.

	<u>Micromoles</u> <u>per sample</u> <u>sample=200ul</u>	<u>Mole</u> <u>Percent</u>	<u>Notes</u>
Lysine	0.0047	0.35	
Histidine	0.0006	0.05	
Ammonia	0.29	21.7	
Arginine	0.0103	0.77	
Aspartic acid	0.0147	1.10	Not included in total; calculated for comparison only
Threonine	0.0131	0.98	
Serine	0.0627	4.70	
Glutamic acid	0.217	16.3	
Proline	0.115	8.62	
Glycine	0.518	38.8	
Alanine	0.269	20.2	
Valine	0.0197	1.48	
			Buffer change under early side of peak; value calculated from 1/2 peak and doubled; error would give high value here, but see 25 hour hydrolysate
Methionine	0.0031	0.23	
Isoleucine	0.0076	0.57	
Leucine	0.0278	2.08	
Tyrosine	0.0365	2.74	
Phenylalanine	0.0062	0.46	
Total	1.33		

- 1.) Total hydrolysate column 4.00 ml; 1 ml dried and dissolved in 0.500 ml citrate buffer.
- 2.) Same hydrolysate as 24 hour hydrolysate, but after removal of 1.00 ml for first sample, solution was again flushed to remove O₂ and returned to 110° for an additional 25 hours. Note particularly the increase in tyrosine content, which is certainly real. See general notes for discussion of other smaller differences.

TABLE B-II

Amino Acid Assay - *Nephila cruentata*

Sample: Spider Silk, Reel 30-1, Tenacity 9.32 gpd.

	<u>Micromoles per sample sample=200ul</u>	<u>Corrected to same sample size</u>	<u>Mole Percent</u>	<u>Notes</u>
Lysine	0.0023	0.0046	0.32	
Histidine	trace	trace	0.03	
Ammonia	0.176	0.353	24.9	not included in total; mole percent calculated for comparison
Arginine	0.008	0.016	1.13	
<u>sample=400ul</u>				
Aspartic acid		0.018	1.27	
Threonine		0.010	0.71	
Serine		0.042	2.96	
Glutamic acid		0.141	10.0	
Proline		0.037	2.61	
Glycine		0.613	43.3	
Alanine		0.430	30.4	
Half Cystine		none detected		
Valine		0.008	0.056	
Methionine		0.002	0.014	
Isoleucine		0.007	0.49	
Leucine		0.026	1.83	
Tyrosine		0.057	4.02	
Phenylalanine		0.006	0.043	
Total micromoles excluding ammonia		1.418		

- 1.) Silk sample too small to be weighed. Hydrolysed at 110°
± 0.3 for 36 hours in 6N HCl. Total sample volume after
hydrolysis, removal of HCl and re-solution = 1.00 ml;
therefore, original sample calculated to be about 0.17 mg.
- 2.) High ammonia more likely due to contaminations of hydrolysate
from atmosphere than to any other reason.

Table B-111
Amino Acid Assay - Paneiras GB

24 hour hydrolysate

Sample: Spider Silk, Spider #3 Paineiras GB IV-21-67, tenacity 6.35 gpd.

	<u>Micromoles per sample sample=200ul</u>	<u>Mole Percent</u>	<u>Notes</u>
Lysine	0.0047	0.37	
Histidine	faint trace	0.00	
Ammonia	0.189	15.7	Not included in total; mole percent for comparison only
Arginine	0.0109	0.91	
Aspartic acid	0.0133	1.11	
Threonine	0.0119	1.00	
Serine	0.0589	4.79*	Previous calculation found in error on checking
Glutamic acid	0.1880	15.7	
Proline	0.1150	9.60	
Glycine	0.4760	39.7	
Alanine	0.2710	22.6	
Valine	0.0176	1.46	
Methionine	0.002	0.17	Detected as methionine sulfone, a result of oxidation during storage of hydrolysate
Isoleucine	0.0075	0.62	
Leucine	0.0267	2.22	
Tyrosine	0.0203	1.70	
Phenylalanine	0.0049	0.41	
Total	1.20		

- 1.) Silk sample approximately 1.2 mg hydrolysed at $110^{\circ} \pm 0.3$ for 24 hours.
- 2.) No detectable cystine, cysteine, or cysteic acid.
- 3.) No difficulties with analysis.

TABLE B-IV

Amino Acid Assay - *Nephila clavipes*Sample: Spider Silk, Reel 30-2, Low Tenacity *Nephilas*

	<u>Micromoles per sample sample=200ul</u>	<u>Corrected to same sample size</u>	<u>Mole Percent</u>	<u>Notes</u>
Lysine	0.0025	0.0050	0.75	
Histidine	0.0022	0.0044	0.66	
Ammonia	0.0631	0.126	18.9	Not included in total; calculated for comparison only.
Arginine	0.0067	0.0133	2.00	
<u>sample=400ul</u>				
Aspartic acid		0.0169	2.53	
Threonine		0.0124	1.86	
Serine		0.0457	6.85	
Glutamic acid		0.0603	9.04	
Proline		0.0072	1.09	
Glycine		0.277	41.5	
Alanine		0.180	27.0	
Valine		0.0078	1.17	
Methionine		trace		
Isoleucine		0.0039	0.58	
Leucine		0.0130	2.00	
Tyrosine		0.0182	2.71	
Phenylalanine		0.00324	0.49	

- 1.) Hydrolyzed at $110^{\circ} \pm 0.3$ for 25 hours in 4.00 ml 6N HCl sample too small to weigh. One ml taken to dryness and dissolved in 1.00 ml citrate buffer. Basics column run with 200ul and 0.500 ml applied to second column. Column cracked during run, before any amino acids had been eluted.

An additional 0.50 ml of the original hydrolysate was dried, dissolved in 0.50 ml of citrate buffer, and 400 ul was applied to another long column.

- 2.) This sample contained material that was soluble in the 6N HCl but which did not redissolve in pH 2.2 citrate buffer. The cloudy solution was applied to the resin columns to avoid further manipulations that might lead to differential loss of amino acids. Back pressure was raised on the columns by this material but fell to normal upon regenerations of columns with 2N NaOH. There was no evidence of poor elution, or trailing of any amino acids, nor were there any new peaks apparent due to foreign ninhydrin-positive materials.

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13. ABSTRACT A preliminary physical and chemical examination of a selected group of spider silks was made. The program's ultimate goal was the synthesis of "super tenacity" protein fibers, and the work reported here was designed to: <ol style="list-style-type: none">1. Confirm the existence of very strong, natural protein fibers, specifically, spider silks.2. Determine some of the physical properties of such fibers.3. Obtain information on the chemical make-up of strong protein fibers and compare them with weaker protein fibers. <p>During this study, collection techniques were streamlined and large quantities of spider silk were obtained. It was shown that a variety of these spider silks have average rupture tenacities exceeding 10 gms per denier and that a few show tenacities exceeding 15 gms per denier. These strengths class these fibers among the strongest organic fibers known. Equally remarkable, however, are the elongations before rupture - amounting to 15 percent or more.</p> <p>Fractionation studies on spider silk indicate that it is almost completely of the fibrous form with little or no soluble (globular) components. Amino acid assays show that the silk contains primarily the simpler amino acids and has a possible relation to a collagen structure.</p>		

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Physical properties	8					