INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

UMI®
STUDY OF THE MICROSTRUCTURE OF SILK ARTIFACTS RECOVERED FROM A HISTORIC DEEP-OCEAN SITE

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

Rekha Srinivasan

****

The Ohio State University

2001

Dissertation Committee:
Professor Kathryn Jakes, Adviser
Professor Dennis Foreman
Professor Terry Gustafson
Professor Charles Noel

Approved by

Kathryn Jakes

Department of Consumer & Textile Sciences
College of Human Ecology
ABSTRACT

A comparative study of the microstructure of three marine silk textiles recovered from the site of the S.S. Central America, reference silk fabric, and three historic silks from the same era as the marine textiles was conducted. The analytical techniques used include optical microscopy, scanning electron microscopy, energy dispersive x-ray spectrometry, x-ray diffraction, infrared microspectroscopy, and differential scanning calorimetry.

Results indicate that the physical microstructure of two of the marine silk artifacts has been altered significantly. An increase in fiber diameter was observed in these two marine silks and their gross physical structure was considerably damaged. Crystallinity indices obtained from IR and XRD are lower in all of the marine and two of the historic silks in comparison with reference silk. Specimens vary in the extent to which short- and long-range order have been disturbed as a result of age and/or marine exposure. In all marine silks and two historic silks, the glass transition is suppressed and degradation temperature altered. Crystallite size perpendicular to the intersheet- and interchain-directions, and the unit cell dimensions, have not altered appreciably in any of the specimens. The primary effect of marine exposure appears to be conversion of some of the crystalline phase into an ordered-amorphous structure. Additionally, the amorphous
phase is partially dissolved or recrystallized to an ordered-amorphous phase. No qualitative alteration of the chemical microstructure of fibroin was observed. Organic and inorganic surface deposits are observed on the marine silks but these are not as extensive on the historic artifacts. The formation of biofilm is observed on both the historic and marine silk, but to varying extents.

Applicability of the analytical tools to analyze rare artifacts is addressed. While basic analytical studies are important in understanding the structure and degradation of rare artifacts, once understood, subsequent work should build on this research and employ techniques which require smaller samples. From a conservator’s perspective, the results indicate that the marine silks are more susceptible to degradation than are historic silks. Handling and storage treatments should focus on controlling the environment and on treating the artifacts to minimize subsequent degradation.
To

Will & our parents
ACKNOWLEDGMENTS

I would like to thank the members of my committee for their guidance and patience. Thanks go to Dr. Jakes for all her help and effort in spite of her very busy schedule. My thanks extend to Dr. Foreman for all he has taught me about x-ray diffraction, and for his help with writing the dissertation. I am grateful to Dr. Gustafson for what I have learned from him about vibrational spectroscopy. I also thank Dr. Noel for his help with the thermal analysis section of this dissertation.

I would like to thank Dr. John Mitchell and Sandy Jones in their help with scanning electron microscopy and x-ray diffraction respectively. Thanks Runying Chen both for her professional support and personal friendship. Her help with IR microspectroscopy is most appreciated. I am grateful to Bob Evans of the Columbus America Discovery Group for providing travel support to present parts of this research.

My deepest gratitude goes to my husband Will, without whose gentle and not-so-gentle urging, this would not have been possible. I am also grateful to him for his help with analyzing and understanding the x-ray diffraction data. I am deeply grateful to both our families whose support and love have helped us through the challenges of graduate school and living away from home.
VITA

1990.............................................................B.S. Jadavpur University

1993.............................................................M.S. The Ohio State University

1999.............................................................M.B.A. The Ohio State University

1992 – 1996..................................................Graduate Teaching and Research Associate, The Ohio State University

PUBLICATIONS


FIELD OF STUDY

Major Field: Consumer & Textile Sciences

Minor Field: Chemistry
# TABLE OF CONTENTS

ABSTRACT........................................................................................................... ii

DEDICATION ...................................................................................................... iv

ACKNOWLEDGMENT......................................................................................... v

VITA...................................................................................................................... vi

LIST OF TABLES.................................................................................................. x

LIST OF FIGURES............................................................................................ xiii

CHAPTER 1............................................................................................................ 1

INTRODUCTION .............................................................................................. 1

Problem statement ........................................................................................... 3

Purpose of the study ........................................................................................ 4

Significance..................................................................................................... 7

Limitations and assumptions........................................................................... 8

CHAPTER 2.......................................................................................................... 10

REVIEW OF LITERATURE.................................................................................. 10

vii
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Background of the S.S. Central America</td>
<td>11</td>
</tr>
<tr>
<td>Recovery operation</td>
<td>12</td>
</tr>
<tr>
<td>Site environment data</td>
<td>13</td>
</tr>
<tr>
<td>General deep-ocean environment</td>
<td>14</td>
</tr>
<tr>
<td>Investigations on marine textile artifacts</td>
<td>18</td>
</tr>
<tr>
<td>Silk fibers</td>
<td>20</td>
</tr>
<tr>
<td>Analytical techniques applied to the study of textile fibers</td>
<td>36</td>
</tr>
<tr>
<td>Summary</td>
<td>66</td>
</tr>
<tr>
<td>CHAPTER 3</td>
<td>68</td>
</tr>
<tr>
<td>METHODOLOGY</td>
<td>68</td>
</tr>
<tr>
<td>Experimental materials</td>
<td>69</td>
</tr>
<tr>
<td>Instrumentation and sample preparation</td>
<td>71</td>
</tr>
<tr>
<td>Data analysis</td>
<td>75</td>
</tr>
<tr>
<td>CHAPTER 4</td>
<td>87</td>
</tr>
<tr>
<td>PRESENTATION OF RESULTS</td>
<td>87</td>
</tr>
<tr>
<td>Optical microscopy</td>
<td>87</td>
</tr>
<tr>
<td>Scanning electron microscopy</td>
<td>90</td>
</tr>
<tr>
<td>Energy dispersive x-ray spectroscopy</td>
<td>102</td>
</tr>
<tr>
<td>FTIR microspectroscopy</td>
<td>112</td>
</tr>
<tr>
<td>X-ray diffraction</td>
<td>137</td>
</tr>
<tr>
<td>Differential scanning calorimetry</td>
<td>165</td>
</tr>
</tbody>
</table>
### LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Anticipated oceanographic parameters at the <em>S.S. Central America</em> wreck site.</td>
</tr>
<tr>
<td>2.2</td>
<td>Amino acid composition of <em>Bombyx mori</em> fiber sericin (mole %).</td>
</tr>
<tr>
<td>2.3</td>
<td>Amino acid composition of <em>Bombyx mori</em> silk fibroin (mole %).</td>
</tr>
<tr>
<td>2.4</td>
<td>Comparison of x-ray reflections of <em>Bombyx mori</em> fibroin observed by Warwicker and Marsh, Corey, and Pauling.</td>
</tr>
<tr>
<td>2.5</td>
<td>Frequency range of peptide bands in $\alpha$-, $\beta$-, and random coil conformations.</td>
</tr>
<tr>
<td>2.6</td>
<td>Summary of the characteristic infrared vibrations localized in the side chains.</td>
</tr>
<tr>
<td>3.1</td>
<td>Marine silk artifacts sampled.</td>
</tr>
<tr>
<td>3.2</td>
<td>Historic silk artifacts sampled.</td>
</tr>
<tr>
<td>4.1</td>
<td>Average and standard deviation values of fiber diameter measurements for Reference, Marine, and Historic Silk specimens.</td>
</tr>
<tr>
<td>4.2</td>
<td>ANOVA and Tukey’s pairwise comparison of fiber diameter measurements.</td>
</tr>
<tr>
<td>4.3</td>
<td>Summary of morphological characteristics observed in Reference, Historic, and Marine Silk fibers using scanning electron microscopy.</td>
</tr>
</tbody>
</table>
4.4 Summary of elemental composition of surface encrustations observed on Historic, and Marine Silk fibers ............................................................. 106

4.5 Infrared peak identification and assignment using standard and second derivative peak picking techniques ............................................. 125

4.6 Average and standard deviation values of IR crystallinity ratios for Reference, Marine, and Historic Silk specimens ........................................ 135

4.7 ANOVA and Tukey's pairwise comparison of IR crystallinity measurements . 136

4.8 Coefficients $a_0$ and $a_1$ for the background profile for Reference, Historic, and Marine Silk specimens ....................................................... 145

4.9 Coefficients $a_0$, $a_1$, $a_2$, $a_3$, $a_4$, and $a_5$ for the background profile for Reference, Historic, and Marine Silk specimens ........................................ 145

4.10 Peak parameters, estimation errors, experimental and reference interplanar spacings, and Miller indices for Reference Silk ...... 154

4.11 Peak parameters, estimation errors, experimental and reference interplanar spacings, and Miller indices for Marine Silk 29049 .............................. 154

4.12 Peak parameters, estimation errors, experimental and reference interplanar spacings, and Miller indices for Marine Silk 29054 ......................... 155

4.13 Peak parameters, estimation errors, experimental and reference interplanar spacings, and Miller indices for Marine Silk 33707 .............................. 155

4.14 Peak parameters, estimation errors, experimental and reference interplanar spacings, and Miller indices for Historic Silk 88b ............................... 156

4.15 Peak parameters, estimation errors, experimental and reference interplanar spacings, and Miller indices for Historic Silk 145b ................................. 156
4.16 Peak parameters, estimation errors, experimental and reference interplanar spacings, and Miller indices for Historic Silk 145c. ......................................................... 157

4.17 Total crystalline intensity ($I_{xt}$), amorphous intensity ($I_{am}$) and percent crystallinity ($%C$) for Reference, Marine, and Historic Silk specimens. ............ 158

4.18 Crystallite size ($\sigma$) and error in crystallite size ($s_\sigma$) estimations. .................. 160

4.19 Determination of the a-dimension of the fibroin unit cell. ................................. 163

4.20 Determination of the b-dimension of the fibroin unit cell. ................................. 163

4.21 Determination of the c-dimension of the fibroin unit cell. ................................. 164

4.22 Water evaporation, glass transition, and degradation temperatures in Reference, Historic, and Marine Silks................................................................. 169

5.1 Comparative summary of results from optical and scanning electron microscopy, x-ray diffraction, infrared microspectroscopy, and differential scanning calorimetry.......................................................... 177

5.2 Morphology and major elemental composition of deposits on Marine Silk fibers. ................................................................. 178

5.3 Crystallinity data for Reference, Marine, and Historic Silk specimens. .......... 188
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Diagrammatic view of α-helix.</td>
<td>27</td>
</tr>
<tr>
<td>2.2</td>
<td>Diagrammatic view of a single β-strand (A), and its incorporation into parallel (B) and antiparallel (C) pleated sheets.</td>
<td>28</td>
</tr>
<tr>
<td>2.3</td>
<td>Antiparallel β-pleated sheet structure of <em>B. mori</em> silk, with chain axis parallel to fiber axis.</td>
<td>31</td>
</tr>
<tr>
<td>4.1</td>
<td>Reference Silk fibers with smooth rounded surfaces and no surface defects, striations, or deposits.</td>
<td>95</td>
</tr>
<tr>
<td>4.2</td>
<td>Reference Silk fibers with smooth rounded surfaces and no surface defects, striations, or deposits.</td>
<td>95</td>
</tr>
<tr>
<td>4.3</td>
<td>Historic Silk 145c with smooth rounded surfaces and no surface defects, striations, or deposits.</td>
<td>95</td>
</tr>
<tr>
<td>4.4</td>
<td>Historic Silk 145b with rounded surfaces and initial signs of longitudinal surface degradation.</td>
<td>95</td>
</tr>
<tr>
<td>4.5</td>
<td>Historic Silk 88b with occasional surface deposits and surface pitting phenomenon.</td>
<td>96</td>
</tr>
<tr>
<td>4.6</td>
<td>Historic Silk 145c with surface deposits on some fiber surfaces.</td>
<td>96</td>
</tr>
</tbody>
</table>
4.7 Historic Silk 88b with occasional surface deposits. ............................................... 96
4.8 Historic Silk 145c with biofilms covering fiber surfaces, indicating microbial activity. ................................................................................................................. 96
4.9 Historic Silk 88b with detailed view of biofilm covering fiber surface. ............ 97
4.10 Historic Silk 145b with detailed view of biofilm covering fiber surface. .......... 97
4.11 Marine Silk 29054 displaying mechanical deformation of fibers. ................. 97
4.12 Marine Silk 29054 with occasional fiber flattening, and areas of fiber relatively free of surface deposits. ................................................................. 97
4.13 Marine Silk 29049 displaying irregularly spaced longitudinal striations along the fiber axis................................................................. 98
4.14 Marine Silk 33707 displaying regularly spaced longitudinal striations along the fiber axis and few surface deposits. .................................................. 98
4.15 Marine Silk 29054 displaying surface cracking patterns on the fiber surface. 98
4.16 Marine Silk 29049 displaying deep cracks of varying lengths in areas of fiber twisting................................................................. 98
4.17 Marine Silk 33707 displaying deep cracks along areas of fiber twisting. ........ 99
4.18 Marine Silk 29054 displaying deep cracks and extensive surface deposits. ..... 99
4.19 Marine Silk 33707 displaying deep cracks and extensive surface deposits. .... 99
4.20 Marine Silk 29049 displaying a deep crack and some surface deposits......... 99

xiv
4.21 Marine Silk 33707 showing the formation of fibrils ............................................. 100
4.22 Marine Silk 29049 showing the formation of fibrils ............................................. 100
4.23 Marine Silk 33707 displaying continuous and occasional irregular discrete deposits. ................................................................................................................ 100
4.24 Marine Silk 29049 displaying extensive continuous and discrete surface deposits. ................................................................................................................ 100
4.25 Marine Silk 29054 displaying irregular discrete surface deposits ......................... 101
4.26 Marine Silk 33707 exhibiting biofilm on fiber surface ........................................ 101
4.27 EDS of Reference Silk free of surface deposits and biofilms ................................. 102
4.28 EDS of Historic Silk 88b free of surface deposits and biofilms ............................ 103
4.29 EDS of Historic Silk 145b free of surface deposits and biofilms ......................... 103
4.30 EDS of Historic Silk 145c free of surface deposits and biofilms ......................... 104
4.31 EDS of Marine Silk 29054 free of surface deposits and biofilms .......................... 104
4.32 EDS of Marine Silk 29054 free of surface deposits and biofilms .......................... 105
4.33 EDS of Marine Silk 33707 free of surface deposits and biofilms .......................... 105
4.34 EDS of deposits on Historic Silk 88b ................................................................. 107
4.35 EDS of deposits on Historic Silk 88b ................................................................. 107
4.36  EDS of deposits on Historic Silk 145c ................................................................. 108
4.37  EDS of discrete cubic deposits on Marine Silk 29049 .............................................. 108
4.38  EDS of discrete cubic deposits on Marine Silk 29054 .............................................. 109
4.39  EDS of discrete irregular deposits on Marine Silk 33707 ......................................... 109
4.40  EDS of discrete irregular deposits on Marine Silk 33707 ......................................... 110
4.41  EDS of continuous paste-like deposits on Marine Silk 33707 ............................... 110
4.42  Average and standard deviation absorbance spectra of Reference Silk .................. 114
4.43  Average and standard deviation absorbance spectra of Historic Silk 88b ............... 115
4.44  Average and standard deviation absorbance spectra of Historic Silk 145b ............. 116
4.45  Average and standard deviation absorbance spectra of Historic Silk 145c ............ 117
4.46  Average and standard deviation absorbance spectra of Marine Silk 29049 .............. 118
4.47  Average and standard deviation absorbance spectra of Marine Silk 29054 .............. 119
4.48  Average and standard deviation absorbance spectra of Marine Silk 33707 .............. 120
4.49  Second derivative spectrum of Reference and Historic Silks (3400-2750cm$^{-1}$) .. 122
4.50  Second derivative spectrum of Reference and Marine Silks (3400-2750cm$^{-1}$) .... 122
4.51  Second derivative spectrum of Reference and Historic Silks (1800-1200cm$^{-1}$) .. 123
4.52 Second derivative spectrum of Reference and Marine Silks (1800-1200 cm\(^{-1}\))... 123
4.53 Second derivative spectrum of Reference and Historic Silks (1200-600 cm\(^{-1}\))... 124
4.54 Second derivative spectrum of Reference and Marine Silks (1200-600 cm\(^{-1}\))... 124
4.55 Original and deconvoluted spectra of Reference Silk between
1150-1350 cm\(^{-1}\).................................................................................................... 131
4.56 Original and deconvoluted spectra of Marine Silk 29049 between
1150-1350 cm\(^{-1}\).................................................................................................... 131
4.57 Original and deconvoluted spectra of Marine Silk 29054 between
1150-1350 cm\(^{-1}\).................................................................................................... 132
4.58 Original and deconvoluted spectra of Marine Silk 33707 between
1150-1350 cm\(^{-1}\).................................................................................................... 132
4.59 Original and deconvoluted spectra of Historic Silk 88b between
1150-1350 cm\(^{-1}\).................................................................................................... 133
4.60 Original and deconvoluted spectra of Historic Silk 145b between
1150-1350 cm\(^{-1}\).................................................................................................... 133
4.61 Original and deconvoluted spectra of Historic Silk 145c between
1150-1350 cm\(^{-1}\).................................................................................................... 134
4.62 Raw and smoothed diffractometric data and empirically determined
background and amorphous scattering profiles for Reference Silk. ............... 138
4.63 Raw and smoothed diffractometric data and empirically determined
background and amorphous scattering profiles for Marine Silk 29049. ............ 139

xvii
4.64 Raw and smoothed diffractometric data and empirically determined background and amorphous scattering profiles for Marine Silk 29054. .......... 140

4.65 Raw and smoothed diffractometric data and empirically determined background and amorphous scattering profiles for Marine Silk 33707. .......... 141

4.66 Raw and smoothed diffractometric data and empirically determined background and amorphous scattering profiles for Historic Silk 88b. .......... 142

4.67 Raw and smoothed diffractometric data and empirically determined background and amorphous scattering profiles for Historic Silk 145b. .......... 143

4.68 Raw and smoothed diffractometric data and empirically determined background and amorphous scattering profiles for Historic Silk 145b. .......... 144

4.69 Crystalline spectrum and Gaussian peak profiles for Reference Silk. .......... 147

4.70 Crystalline spectrum and Gaussian peak profiles for Marine Silk 29049. ....... 148

4.71 Crystalline spectrum and Gaussian peak profiles for Marine Silk 29054. ....... 149

4.72 Crystalline spectrum and Gaussian peak profiles for Marine Silk 33707. ....... 150

4.73 Crystalline spectrum and Gaussian peak profiles for Historic Silk 88b. ....... 151

4.74 Crystalline spectrum and Gaussian peak profiles for Historic Silk 145b. ....... 152

4.75 Crystalline spectrum and Gaussian peak profiles for Historic Silk 145c. ....... 153

4.76 Differential scanning calorimetric trace of Reference Silk. ....................... 165

4.77 Differential scanning calorimetric trace of Marine Silk 29049. ................. 166

xviii
4.78 Differential scanning calorimetric trace of Marine Silk 29054. .............................. 166
4.79 Differential scanning calorimetric trace of Marine Silk 33707. .............................. 167
4.80 Differential scanning calorimetric trace of Historic Silk 88b. .............................. 167
4.81 Differential scanning calorimetric trace of Historic Silk 145b. .............................. 168
4.82 Differential scanning calorimetric trace of Historic Silk 145c. .............................. 168

5.1 Schematic representation of three-phase structure of silk fibroin. ......................... 183
CHAPTER 1

INTRODUCTION

The recovery of historic and archaeological textiles from marine sites is uncommon. While the need for research concerning water-degraded archaeological textiles has been noted (1,2), the reports in the literature related to this topic predominantly address conservation methods, and only a few describe fabric structure or fiber identity. Additionally, limited sample availability precludes the use of some analytical techniques in examining these materials. Because of the paucity in research regarding the physico-chemical state of such materials and the operative degradative mechanisms, most conservation efforts have been experimental (2,3). There is therefore a need for fundamental studies of the chemical and physical structure of marine organic artifacts. Such studies are interdisciplinary and require expertise in fields like fiber and textile science, analytical chemistry, geochemistry, marine biology, and marine chemistry.

Trunks full of clothing and other artifacts recovered in 1990 from the ocean floor site of the shipwrecked S.S. Central America provide a unique opportunity to study materials exposed to a marine environment for 132 years. It was recognized that these textiles could be used to understand both cultural use in the Gold Rush era and the processes of material
degradation. Additionally, it was necessary to develop an appropriate protocol for the conservation of these marine textiles; and so an extensive study of the recovered artifacts was undertaken at The Ohio State University beginning in 1990. The project has involved the study of the functions and the structures of the textiles, as well as the examination of the physical and chemical characteristics of the fibers of which the textiles were made. Several previous publications have stemmed from this research. Jakes & Mitchell (4) describe appropriate methods for drying the waterlogged textiles. Jakes & Wang (5,6) conducted a microscopic examination of cotton, flax, wool, and silk fibers from this site and have described the physical and chemical structure of cellulosic fibers immersed for 3 months at the shipwreck site. Foreman & Jakes (7) have studied the changes in crystallinity of marine cellulosic fibers. Chen (8) studied the physical and chemical microstructure of dyed and undyed cotton fibers from the site using multiple analytical tools. Studies on the historical and cultural interpretation of the recovered clothing have also been have conducted (9,10).

The research reported here is directed toward determination of the physical and chemical microstructure of silk fibers from textiles in a recovered trunk identified as belonging to Ansel Ives Easton and Adeline Mills Easton (3). Because several silk artifacts were recovered from the trunks, and because apart from staining and surface deposits in some areas, they appeared to be in fairly good condition on recovery, these were chosen for study.

**Problem statement**

The study of textiles recovered from marine sites has so far been limited to conservation efforts and occasional microscopic fiber identification. Much remains to be
learned about the chemical and physical microstructure of such fibers. An in-depth study of the fibers from marine textiles will not only provide information that would determine conservation treatments, but will also help in understanding the mechanisms which caused the preservation or degradation of the textile artifacts during long term marine storage. Characterization of these artifacts also allows the historian to study these materials in the context of the time period from which they are recovered. Additionally, by using multiple techniques of fiber characterization, the effectiveness of these methods in monitoring fiber degradation can also be evaluated.

Several questions regarding fibers recovered from a marine site need to be addressed:

1. What is the physical and chemical microstructure of silk fibers from textiles exposed to particular conditions of the deep ocean environment for 132 years?

2. How does the microstructure of the marine silk fibers compare with that of modern silk fibers?

3. How does the microstructure of the marine silk fibers compare with that of historic silk fibers from the same period but not exposed to the marine environment?

Given the need for the fundamental study of organic artifacts from marine sites, and the opportunity to contribute to the overall research program being conducted on textile artifacts from the *S.S. Central America* at the Ohio State University, this study aimed to provide information on silk artifacts in particular.
Purpose of the study

Specifically, the purpose of the work reported herein was to examine the physical and chemical microstructure of selected silk textile fibers recovered from the deep-ocean site of the S.S. Central America, which sank off the coast of the Carolinas in 1857. A comparative study of the marine silks with modern silk reference and with historic silk fibers from the same era as the marine textiles, but not subjected to prolonged marine storage was conducted. Through such a comparative approach, an attempt was made to describe the effect of long-term storage in a deep-ocean environment. Additionally, because all historic artifacts are valuable and scarce, the analytical techniques employed were evaluated for effectiveness in studying micro-samples.

The specific objectives of this study follow:

a. To examine the physical and chemical microstructure of fibers from selected silk textiles recovered from the site of the S.S. Central America, from modern silk reference material, and from historic silk textiles dating from the second half of the 19th century, using multiple analytical techniques. Different techniques yield different types of information regarding fiber morphology, crystallinity, and chemical characteristics. Fiber morphology refers to microscopically observable features like fiber diameter, cracking and fibrillation patterns, and visual evidence of microbiological degradation. Crystallinity data are obtained from infrared spectroscopy, x-ray diffraction techniques, and differential scanning calorimetry. Chemical information includes secondary conformation of silk protein and functional group information obtained from infrared spectroscopy, as well as the elemental composition of deposits within and on the fibers obtained from energy dispersive x-ray spectrometry.
b. To compare the results obtained from the marine silk fibers to those obtained from a modern silk reference material.

c. To compare the results obtained from the marine silk fibers to those obtained from historic silk fibers from the second half of the 19th century.

d. To use the compilation of comparative data obtained to infer the preservative/degradative effect of prolonged marine storage on silk fibers.

e. To use the compilation of comparative data obtained from the different techniques to evaluate the effectiveness of each in assessing different aspects of fiber degradation.

In order to achieve a comprehensive picture of the physico-chemical microstructure of the silk artifacts a number of instrumental techniques were used in this research: optical microscopy, scanning electron microscopy, energy dispersive x-ray spectrometry, x-ray diffraction, Fourier transform infrared microspectroscopy, and differential scanning calorimetry.

The study of textile materials using optical techniques like optical microscopy and scanning electron microscopy are not only effective in revealing their structural characteristics, but can also provide insight into the source and nature of textile degradation (11). Techniques such as scanning electron microscopy (SEM) and energy dispersive x-ray spectrometry (EDS) are powerful techniques which allow the observation and characterization of heterogeneous organic and inorganic specimens on a micrometer scale(12). The primary advantages of using SEM for morphological characterization of fibers are the high resolution and the three dimensional image that the technique provides. When
SEM is used in combination with optical microscopy (OM), the two techniques provide a wide range of resolutions and observation modes for investigating fiber morphology.

The usefulness of the energy dispersive x-ray spectrometry (EDS) stems from the high-resolution compositional information and mapping that can be obtained. Apart from the fiber itself, the elemental composition of stains within and encrustations on the fibers provides valuable information regarding the chemical activity occurring in the deep ocean and interaction between textile fibers and the marine environment. By using SEM and EDS conjunctively, the appearance and the corresponding elemental composition of fibers and surface contaminants were studied.

Textile fibers like silk are composed of both crystalline and amorphous regions, and x-ray diffraction (XRD) can be used to obtain structural information about them. In this work XRD was used to study crystallite size, percent crystallinity, and interplanar spacing within the crystallites.

By using Fourier transform infrared (FTIR) microspectroscopy, spectra of very small areas of single fibers could be collected. FTIR spectroscopy of proteins such as silk provides information that is sensitive to changes in chemical composition and physical conformation. Silk has a unique IR spectrum due to its basic protein structure and its unique macromolecular conformation. Additionally, FTIR offers another way to study the crystallinity of the fiber by calculating specific peak ratios (13).

Differential scanning calorimetry (DSC) is a thermal analytical technique that allows one to accurately and rapidly measure such parameters as melting temperature, glass
transition, and crystallization, as well as to follow degradation processes in polymers (14). The glass transition and degradation temperatures of silk were studied using this technique.

Under the assumption that the 132 year exposure to the deep ocean environment had a much greater influence on fiber degradation compared to any other factor in the fibers’ history, the information obtained from each of these analytical techniques was compiled in order to determine the effect of the marine environment on silk.

**Significance**

Because of the interdisciplinary nature of this research, the results could potentially be useful to researchers in several disciplines. Since little research has been carried out on textile artifacts recovered from marine sites in general, and on silk artifacts from such sites in particular, this research is of value to the archaeologist, the conservation scientist and the textile historian. First, the characterization of the microstructure of silk fibers from the ocean can lead to new knowledge concerning the degradative and preservative effects of the marine environment on these materials. The 132 year exposure of these textiles to the environment of this site provided an unprecedented opportunity to study the reaction of silk fibers to a particular physical, chemical, and biological marine environment (2,15). Use of analytical tools which provide complementary information regarding different aspects of fiber structure results in a comprehensive multi-faceted view of the chemical and physical microstructure of the silk marine artifacts.
Second, understanding the condition of such artifacts is a crucial prerequisite to making recommendations for their treatment and conservation. As more marine sites are excavated, recommendations for handling such artifacts at the excavation site can be made to marine archaeologists based on this and other similar studies. In addition, preventive and restorative treatments can be recommended to conservators for handling such artifacts in a museum environment.

As mentioned above, in order to obtain a comprehensive picture of the condition of these materials multiple analytical tools need to be employed. The third implication of this study comes from the evaluation of these techniques with respect to their applicability in examining rare and valuable artifacts of any origin. Some issues to consider from this perspective are sample size required and whether the sample is destroyed in the analysis.

From a fiber science perspective, silk fibers have not been subject to the extent of investigation that other natural fibers like cotton and wool have been. Hence this study adds information to the body of literature on silk materials. Finally, the historian can use the information obtained from such a study to understand the artifacts in their technological, social, and cultural contexts.

Limitations and assumptions

The principal limitation of this study stems from sample size constraints inevitable with any destructive or invasive techniques used in the study of valuable and scarce historical artifacts. It is assumed that the marine specimens analyzed are representative of all silk artifacts exposed to the same deep marine environment for a protracted period of time.
Since historic silks not exposed to the marine environment have been studied for comparative purposes, the assumption is made that the chosen specimens are, in fact, from the second half of the nineteenth century. This assumption is based on assessment of garment style and construction, and from information concerning the garments provided when acquired.
CHAPTER 2

REVIEW OF LITERATURE

In this chapter the literature pertaining to the research objectives presented in the introduction is reviewed. As the focus of this study is the silk textiles recovered from the site of the S.S. Central America, this chapter starts with a brief background of the shipwreck and the recovery operation of the artifacts found on the site. This is followed by a description of the environmental data recorded at this particular site and a general description of deep-ocean environments. The next section focuses on the study of organic marine artifacts in general and of textile artifacts in particular, followed by a review of the chemical and physical microstructure of silk fibers. The last topic to be reviewed is the application of various analytical techniques to the study of silk fibrous materials. The following techniques are reviewed: optical and scanning electron microscopy, energy dispersive x-ray spectrometry, x-ray diffraction, infra-red microspectroscopy, and differential scanning calorimetry. The chapter closes with a summary of the reviewed literature and a restatement of the current research.
Background of the S.S. Central America

The discovery of gold in California in the middle of the 19th century fueled the need for efficient transportation between California and New York. The fastest route was the three- to four-week side-wheel steamer trip via Panama. The Panama route consisted of two steamship trips, one on the Pacific and the other on the Atlantic, connected by mule train, canoe or raft across the Isthmus (3).

The S.S. Central America was a steamship used for the Atlantic leg of the journey between the Isthmus of Panama and New York. In September of 1857, while she was making her 44th crossing, carrying 476 passengers, 102 crew members, a three-ton shipment of commercial gold, and additional passenger gold, she ran into a hurricane and sank. The sinking of the S.S. Central America was one of the greatest maritime disasters in the United States, and resulted in the loss of 425 lives and all of the ship's cargo (3).

The ship's remains stayed on the ocean floor for about 130 years, till in the mid-1980's a group of engineers, scientists, and entrepreneurs came together to form the Columbus-America Discovery Group and initiate the S.S. Central America Project. In their article on the S.S. Central America, Herdendorf et al. (3) mention that only an extremely small portion of the deep-ocean has so far been studied either through direct observation or with the aid of remotely operated submersibles.

The Project had several goals, including the location of the shipwreck; the recovery of gold, oceanographic specimens, and historic artifacts; the employment of new techniques for deep-ocean study; and the increased understanding of the ship, its era, and the deep-ocean environment. An Adjunct Science and Education Program was established by the Group in
1989 to encourage research on the artifacts from the wreck and specimens from this site. Over 150 scientists from several disciplines participated in the effort to collectively and systematically understand a myriad of components of this deep ocean site (3).

Artifacts from the ship, including the belongings of the passengers had been subjected to prolonged exposure to the unique environmental conditions of the deep ocean. The degradative and preservative effects of pressure, temperature, chemical environment, and biological processes on iron, wood, and textiles submerged for such a long time at this great depth have so far received little attention from the scientific community (3). A better understanding of the effects and the causes of deep-ocean degradation and preservation will allow more accurate treatment of other cultural artifacts found under similar circumstances in the future.

Recovery operation

Two trunks encased in Plexiglas containers were lifted from the shipwreck site by the research submersible Nemo in 1990 and 1991. The trunks were immersed in the seawater-filled Plexiglas containers and transported to Columbus, Ohio. In 1990, a few artifacts were removed from the top layer of the first trunk in order to assess their fragility. When they appeared to be contiguous, these items were reimmersed in water and the Department of Textiles and Clothing was contacted for advice concerning a treatment protocol for the textiles. Each trunk needed to be unpacked, and each item contained therein examined. A plan for flash freezing followed by slow drying was prescribed (4). The goals of this treatment
included stabilizing the waterlogged material, inhibiting microbial deterioration that would have proceeded in the wet state, and preparing the items for study as historic artifacts.

**Site environment data**

The site of the *S.S. Central America* was discovered off the coast of the Carolinas in the North Atlantic Ocean. Specifically, the wreck is located at the gradual slope of the largest sedimentary ridge in the Atlantic Ocean, the Blake-Bahama Outer Ridge, at a depth of 2200 meters. On-site observation and sample collection were conducted using an unmanned research submersible, *Nemo*. Water quality parameters at this site reported by Herdendorf et al. are: depth, 2200 m; solar radiation, nil; pressure, 220 atm; current 10 cm/s (mean); and visibility, 15 to 20 m. The authors also report anticipated values for other parameters extrapolated from literature (Table 2.1). National Oceanographic Data Center (NODC) data from 71 stations and accumulated over 34 years were also used to characterize deep-ocean parameters at the shipwreck site and these provided values comparable to the literature (3).

Herdendorf et al. also report that site observations indicate that the shipwreck served as a food source for several marine life forms. For example, they report that wooden parts were degraded by wood-boring bivalves, and iron parts were covered with rusticles formed by iron-eating bacteria. In microscopic examination of metal corrosion, they also report the presence of two types of fungi and yeast, and several non-filamentous and rod shaped bacteria.
Parameter Range
Depth (m) 1095 - 2500
Temperature (°C) 2.81 - 3.77
Salinity (0/00) 34.9 - 35.06
Dissolved oxygen (ml/l) 5.85 - 6.40
Inorganic phosphate (PO₄)³⁻ (μg-atoms/l) 1.20 - 1.82
Nitrates (NO₃⁻)¹ (μg-atoms/l) 17.0 - 23.9
Silicates (SiO₂) (μg-atoms/l) 12.0 - 25.9
Hydrogen ions (H⁺) (pH) 7.99 - 8.14

Taken from Herdendorf, C.E.; Thompson, T.G.; Evans, R.D. *Ohio J. Sci.* 1995, 95, p.76.

Table 2.1 Anticipated oceanographic parameters at the *S.S. Central America* wreck site.

**General deep-ocean environment**

Seawater contains more than 70 chemical elements. The predominant chemical species in seawater, which account for more than 99.5% of the dissolved materials, are Cl⁻, Na⁺, SO₄²⁻, Mg²⁺, Ca²⁺, and K⁺ (16). The Na and K are present predominantly in the form of free ions because of their high oxidation-reduction potentials. Divalent cations like Ca²⁺ and Mg²⁺ are capable of forming numerous complex ions and ion pairs with anions present in seawater.
Sulfate content is sensitive to biological activity. For example, sulfate can be reduced to \( \text{H}_2\text{S} \) by heterotrophic bacteria under anaerobic conditions. In the deep, stagnant water near the ocean floor the sulfate content is lower because of the sulfate reducing action of bacteria (17).

Florian (16) mentions that apart from the major constituents of seawater discussed above, there are several minor constituents. Of particular importance are iron and silicon. Iron is predominantly present in the ferrous (\( \text{Fe}^{2+} \)) and ferric (\( \text{Fe}^{3+} \)) forms. In oxidizing seawater the latter is present and in reducing conditions the former predominates. Silicon is present both in solution and as a suspension.

In terms of dissolved gases, oxygen and carbon dioxide are predominant. Oxygen is added to seawater mainly through absorption from the air and from photosynthetic activity where there is light penetration. It is depleted by atmospheric exchange, the respiration of aerobic aquatic life forms, and the decomposition of organic materials by aerobic bacteria. Some depletion also occurs because of reaction with inorganic materials in the reduced form. While the oxygen content is highest close to the surface and gradually decreases with depth, even at about 2000 meters there is a fair amount of dissolved oxygen. In the Atlantic Ocean, the oxygen concentration is between 5.0 and 5.5 ml/l at this depth (16).

In areas where there is more oxygen consumption than production, and where circulation is not adequate to ensure additional supply of oxygen, waters are anoxic. Under these circumstances the water chemistry is quite different, and sulfur plays the role usually taken by oxygen (17). Horne (17) discusses some consequences of anoxic waters that are relevant to the research. First, denitrification occurs and \( \text{NO}_2^- \) and \( \text{NO}_3^- \) disappear. Second, sulfate ions are reduced and \( \text{H}_2\text{S} \) is produced, and third, the redox potential is lowered.
resulting in reduced degradation of organic materials. In oxygenless conditions, the next biggest source of free energy, \( \text{NO}_3^- \), is used for oxidation. After this source has also been depleted, bacterial sulfate reduction occurs, with the production of \( \text{H}_2\text{S} \) and \( \text{NH}_3 \). The author notes that sulfide production under these circumstances results from sulfate reduction rather than from the decomposition of organic substrates.

The unique chemistry of anoxic marine environment lowers the redox potential, so that the oxidizing potential of the water is reduced (17). With respect to this work the implications of this fact are that organic artifacts like the silk specimens under study are less likely to be degraded when compared with similar material exposed to oxygen rich waters.

Carbon dioxide comes from the atmosphere and as a byproduct of anaerobic respiration, and is depleted by photosynthetic processes. \( \text{CO}_2 \) reacts with water to form carbonic acid (\( \text{H}_2\text{CO}_3 \)), which then dissociates to form \( \text{HCO}_3^- \) and \( \text{CO}_3^{2-} \). This equilibrium controls the alkalinity and pH of the ocean, as well as the formation of marine sediments, and thereby has a direct effect on the condition of marine artifacts (16).

Seawater is slightly alkaline, and has a pH range between 7.85 and 8.4. However, in anoxic, oxygen-consuming regions of the ocean, \( \text{H}_2\text{S} \) is produced and this reduces the pH to about 7.0 (16).

In terms of biological organisms in the marine environment, these are influenced by environmental parameters like depth, pressure, current, and oxygen concentration. The significance of understanding the effect of these organisms on artifacts is restricted to the physical and chemical effects that these organisms have had during marine storage, as they do not continue to grow after excavation. Marine fungi are found at all depths, but require
at least 0.30 ml/l of oxygen, i.e. they do not grow in anoxic conditions. Aerobic bacteria are dominant in oxygen-rich conditions and anaerobic bacteria are present in oxygen-depleted regions (16).

Florian (16) mentions that from the point of view of artifact degradation, sulfate-reducing anaerobic bacteria are the most important. Their role in the deterioration of metal artifacts from deep ocean sites and in the formation of metal sulfide deposits on marine artifacts has been extensively noted. These bacteria utilize the oxygen from the sulfate for oxidative metabolic activities by reducing the sulfate in the following reaction:

\[ 2\text{SO}_4^{2-} \rightarrow \text{S}^0 + \text{S}^{2-} + 4\text{O}_2 \]

The end product of the reduction is usually H\(_2\)S:

\[ 2\text{H}^+ + \text{S}^{2-} \rightarrow \text{H}_2\text{S} \]

H\(_2\)S has a detrimental effect on marine artifacts. The presence of H\(_2\)S alters marine chemistry drastically. It greatly increases the solubility of iron and manganese by reducing them, i.e. Fe\(^{3+}\) to Fe\(^{2+}\) and Mn\(^{4+}\) to Mn\(^{3+}\). As previously mentioned, in the mildly alkaline pH range of the ocean, H\(_2\)S ionizes and forms a weak acid that lowers the pH to about 7 (16).

While the preceding sections describe general marine environments and the ocean environment at the S.S. Central America shipwreck site, the environment that is most significant with respect to the degradation and preservation of artifacts is the immediate boundary between the artifact and its surroundings. Because of the increased concentration of reactants at these boundaries due to processes like absorption, adsorption, ion exchange, precipitation, and biological activities, reaction rates at the artifact-surroundings interfaces
are much higher than in the open sea (16). Florian (16) also mentions that while the major elemental composition remains constant because of normal circulation processes, the concept breaks down under local conditions where mixing is severely restricted. This is of significance to the present study, because the marine artifacts under study were recovered from within the restricted environment of a trunk.

**Investigations on marine textile artifacts**

While most archaeological environments are not conducive to the preservation of textile and other organic artifacts, those recovered from marine, terrestrial wet-sites and frozen sites possess microclimates which often lead to the remarkable preservation of this material (18). Despite this fact there has been very little study of how wet or frozen storage over prolonged periods has altered the basic physical and chemical structure of natural textile fibers, nor have systematic experimental studies to document these effects been conducted.

The study of textiles recovered from the *S.S. Central America* site by Jakes and collaborators at the Ohio State University has included a wide range of studies of marine textile materials. Fibers from the artifacts have been studied using multiple analytical techniques including optical and scanning electron microscopy, x-ray microanalysis, and IR microspectroscopy. Jakes and Wang (5) documented morphological characteristics of these fibers including fiber bulging, cracking, and fibrillation, and conducted elemental analysis both on fiber deposits and fibers. Foreman and Jakes (7) studied the fine structure of flax fibers from the site using an x-ray diffraction technique, and compare the crystallinity characteristics of the artifacts with that of modern flax. The results indicate lower overall crystallinity of the
historic material, and increased crystallite size compared to modern flax fibers. Unit cell
dimension was reported to be unchanged.

In an attempt to document the effect of the deep ocean environment on textile
materials, test materials have been immersed on the ocean floor for subsequent retrieval.
While one set of samples was recovered after a three month period, additional samples have
remained on the ocean floor since 1991 and are yet to be retrieved. The morphology of
cellulosic fibers immersed for a three-month period has been investigated (6, 19). Chen (8)
analyzed the physical and chemical structure of dyed and undyed cotton fibers from the site,
and compared it with modern cotton, and cotton immersed on the ocean floor for a three-
month period.

While the previous paragraphs refer primarily to studies performed on cellulosic
materials from the S.S. Central America site, results of preliminary analyses on silk fibers
using differential scanning calorimetry, energy dispersive x-ray spectoscopy, and scanning
electron microscopy, have been reported (20).

Apart from the research conducted on these materials at OSU, the analysis of textile
artifacts from marine sites and simulation studies have been limited. Though there have been
several reports on shipwrecks, only a few of these address the textiles recovered from these
sites. More pertinent, reports on the analysis and identification of textile fibers from these
sites are very limited. Wool and flax fibers from the Mary Rose and the Wasa have been
identified employing optical and scanning electron microscopy (21). Bengtsson (22) identifies
the fiber content of the sails of the Wasa as "vegetable" but the method for fiber identification
is not described. Morris and Seifert (23) describe treatment of textiles from the Defence with
oxalic acid but do not indicate how they identified the linen, hemp, and silk fibers that they report are present. Treatment of a silk cocade from the *Michault* is described in Jenssen (24).

Peacock (18,25) conducted experiments to study the effect of different soils on natural fiber degradation, including silk. With reference to silk degradation, she found that aerated soil at a higher pH (6.5) caused more degradation in silk than unaerated soil at a lower pH. She assessed the fibers under SEM and found surface pitting and fibrillation of the fibers as evidence of degradation. Peacock suggests that the greater availability of bacteria and fungi in aerated soil results in enzymatic hydrolysis of the silk fibroin. She also found that silk was more resistant to degradation than wool fibers. Peacock mentions that because of the paucity of research on microbiological degradation of silk, comparative experimental data was unavailable. However, she reports that analysis of water-degraded archaeological textiles showed evidence of fibrillation, erosion, perpendicular splitting, and pitting. She attributes the fibrillation observed in the experimental silk fibers to the beginning of very fine axial splitting called lousiness attributed to prolonged wetness, acid hydrolysis, or boiling.

In 1987 Florian (16) stated that there was no published data on the analysis of silk from a marine site, and the only analysis on silk artifacts recovered from wet or frozen sites reported since then has been that by Peacock in 1996 (18,25). However the researcher makes no reference to the source of the silk and other artifacts analyzed. Until the inception of the *S.S. Central America* project no study of the alterations in morphology and chemistry of silk due to exposure to the deep ocean environment had been reported in the literature.
Silk fibers

Silk filaments are produced by several species of Lepidoptera and Arthropoda (26), and the most commercially well-known one is that produced by the larvae of the Bombyx mori (27). Since this silk is the one used predominantly for garments and textiles the discussion which follows is restricted to Bombyx mori silk. Silk fiber in its original form is composed of two filaments or brins enclosed by a coating of gum. The filaments are made of the protein “fibroin”, while the gum is composed of the protein “sericin”. Since the silk fibers analyzed in this study are devoid of sericin, only a brief discussion of sericin follows in the paragraphs below, followed by a more detailed review of fibroin.

Silk Sericin

Sericin is a protein which gums together two filaments of fibroin. Generally sericin is removed to reveal the lustrous fibroin strands by a process known as degumming. The amino acid composition of Bombyx mori fiber sericin (27) is shown in Table 2.2. Since the side groups in the amino acids that comprise sericin are primarily hydrophilic, sericin is relatively easily solubilized during degumming.
<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Mole %</th>
<th>Amino Acid</th>
<th>Mole %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gly</td>
<td>12.7</td>
<td>Lys</td>
<td>3.26</td>
</tr>
<tr>
<td>Ala</td>
<td>5.51</td>
<td>Arg</td>
<td>2.86</td>
</tr>
<tr>
<td>Val</td>
<td>2.68</td>
<td>His</td>
<td>1.30</td>
</tr>
<tr>
<td>Leu</td>
<td>0.72</td>
<td>Tyr</td>
<td>3.40</td>
</tr>
<tr>
<td>Ile</td>
<td>0.55</td>
<td>Phe</td>
<td>0.43</td>
</tr>
<tr>
<td>Ser</td>
<td>31.97</td>
<td>Pro</td>
<td>0.57</td>
</tr>
<tr>
<td>Thr</td>
<td>8.25</td>
<td>Trp</td>
<td>0.00</td>
</tr>
<tr>
<td>Asp</td>
<td>13.84</td>
<td>Met</td>
<td>0.05</td>
</tr>
<tr>
<td>Glu</td>
<td>5.80</td>
<td>Cys</td>
<td>0.14</td>
</tr>
</tbody>
</table>


Table 2.2 Amino acid composition of *Bombyx mori* fiber sericin.

Silk Fibroin

This section reviews the physical and chemical characterization of silk fibroin and includes a discussion of the amino acid composition, molecular weight, primary, secondary, and tertiary structures, physical properties, and chemical properties of fibroin.

Amino Acid Composition

While the specific amino acid composition of fibroin depends on the source of the fibroin, analytical techniques including chromatographic separation, ninhydrin assay, and microbiological determinations, have yielded detailed results with very good consistency between researchers (28). The principal feature of the amino acid composition of fibroin is
that it contains a large percentage of the amino acids glycine, alanine, serine, and tyrosine.
The predominance of these simple amino acid residues, which can pack efficiently into a compact structure, is an essential feature of the chemical and physical behavior of fibroin (28).
Small amounts of other amino acids including valine, aspartic acid, glutamic acid, threonine, arginine, leucine, isoleucine, histidine, proline, tryptophan, and cystine are also reported in the literature (28). *Bombyx mori* fibroin consists of two polypeptide components, a heavy component known as the H-chain, and a light component, the L-chain, linked by a disulfide bond (29,30). Table 2.3 shows the amino acid composition of *B mori* fibroin as a whole as well as the composition of the H- and L-chains that constitute it.

Many properties of silk fibers derive from The predominance of small amino acid residues in fibroin and the fact that the number of acidic groups is about 2-3 times more than the number of basic groups. Takei et al. (29) and Shimura et al. (30) mention that though the amount of cystine in fibroin is small, it plays a significant role in its secondary structure by forming interchain disulfide linkages between the H- and L-chains.
<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Fiber</th>
<th>H-chain</th>
<th>L-chain</th>
<th>Amino Acid</th>
<th>Fiber</th>
<th>H-chain</th>
<th>L-chain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gly</td>
<td>44.60</td>
<td>49.40</td>
<td>9.20</td>
<td>Ile</td>
<td>0.66</td>
<td>0.14</td>
<td>7.8</td>
</tr>
<tr>
<td>Ala</td>
<td>29.40</td>
<td>29.80</td>
<td>14.20</td>
<td>Leu</td>
<td>0.53</td>
<td>0.09</td>
<td>7.5</td>
</tr>
<tr>
<td>Ser</td>
<td>12.10</td>
<td>11.30</td>
<td>9.00</td>
<td>Arg</td>
<td>0.47</td>
<td>0.18</td>
<td>4.5</td>
</tr>
<tr>
<td>Tyr</td>
<td>5.17</td>
<td>4.60</td>
<td>2.80</td>
<td>Lys</td>
<td>0.32</td>
<td>0.06</td>
<td>1.2</td>
</tr>
<tr>
<td>Val</td>
<td>2.20</td>
<td>2.00</td>
<td>6.40</td>
<td>Pro</td>
<td>0.36</td>
<td>0.31</td>
<td>3.2</td>
</tr>
<tr>
<td>Asp</td>
<td>1.30</td>
<td>0.65</td>
<td>14.80</td>
<td>His</td>
<td>0.14</td>
<td>0.09</td>
<td>2.3</td>
</tr>
<tr>
<td>Glu</td>
<td>1.02</td>
<td>0.70</td>
<td>9.20</td>
<td>Met</td>
<td>0.10</td>
<td>0.00</td>
<td>0.4</td>
</tr>
<tr>
<td>Thr</td>
<td>0.91</td>
<td>0.45</td>
<td>3.00</td>
<td>Trp</td>
<td>0.11</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Phe</td>
<td>0.63</td>
<td>0.39</td>
<td>2.70</td>
<td>Cys</td>
<td>0.20</td>
<td>0.00</td>
<td>1.4</td>
</tr>
</tbody>
</table>


Table 2.3 Amino acid composition of *Bombyx mori* silk fibroin (mole %).

**Molecular weight**

While earlier studies of the molecular weight of fibroin yielded a very wide range of values, more recently a fairly consistent range of values of about 350,000 daltons for the H-chain and 25,000 daltons for the L-chain have gained acceptance (27).

**Primary, Secondary, and Tertiary Structure**

The structure of protein material like silk can be organized into various levels, including the sequence of amino acids, the regular arrangement of the polypeptide backbone, and the three-dimensional arrangement of the polypeptide chains (31).
Primary Structure

The amino acid sequence of a protein is referred to as its primary structure. Site specific cleavage of fibroin using enzymes like chymotrypsin and trypsin, has provided detailed sequencing information on fibroin. Warwicker (32) studied the products of chymotrypsin hydrolysis of fibroin and reports two fractions: an insoluble fraction, $C_p$, that exhibited a high degree of crystallinity, and a soluble fraction, $C_s$. Analysis of the $C_p$ fraction by Lucas et al. (33) revealed that this fraction contained the peptide sequence Ser-Gly-Ala-Gly-Ala-Gly-Ala-Gly as the main structural element. Additionally these authors concluded that this fraction had the 59 residue sequence Gly-Ala-Gly-Ala-Gly-[Ser-Gly-(Ala-Gly)$_n$]$_8$-Ser-Gly-Ala-Ala-Gly-Tyr, where $n=2$. With regards to the $C_s$ fraction, the three dominant peptides found were: Gly-Ala-Gly-Ala-Gly-Ala-Gly-Tyr; Gly-(Gly$_3$-Ala$_2$-Val)-Tyr; and Gly-Ala-Gly-Tyr. The above researchers have attributed the $C_p$ and $C_s$ fractions to the H-chain of fibroin.

The amino acid sequence of the L-chain has been more recently elucidated by Yamaguchi et al. (34). The primary result of this study was that the L-chain contained 3 cystine residues. Two of these were present within the chain and have been suggested to form intramolecular disulfide bonds, while the third is present in the C-terminal position, and therefore considered to be the site of a disulfide linkage with the H-chain.

Secondary Structure

While the primary structure deals adequately with the chemical nature of the fibroin molecule, it does not address the conformation of the peptide chain. The secondary structure is concerned with a description of the conformation of the polypeptide backbone, and does not consider the nature and arrangement of the side chains of the amino acid residues. This
level of protein structure is effectively stabilized by hydrogen bonding between the N-H and the C=O of the amide linkages (35). There are different conformations that proteins can take at this structural level. The commonly encountered conformations include the α-helix, the parallel and anti-parallel β-pleated sheets, and the random coil conformations (31).

The α-helix conformation consists of a regular helical structure stabilized by hydrogen bonding almost parallel to the main axial dimension of the helix. Every peptide carbonyl oxygen is hydrogen bonded with the amide hydrogen of the fourth amino acid residue further along the helix (35). Figure 2.1 provides a diagrammatic view of the α-helix (36). The β-pleated sheet consists of extended polypeptide chains (31) aligned side by side, stabilized by extensive hydrogen bonding perpendicular to the direction of the main polypeptide chain. Figure 2.2 A provides a diagrammatic view of the β-pleated sheet (37). The alignment of the adjacent peptide chains may be parallel to one another or antiparallel (Figure 2.2 B & C). In the former, all chains run in the same direction from the N- to the C-terminal residue, while in the latter adjacent chains run in alternate directions.
Dimensions of an α-helix

5.1 Å rise per residue
26°
5.4 Å pitch
3.6 residues

Figure 2.1 Diagrammatic view of α-helix.

Figure 2.2 Diagrammatic view of a single β-strand (A), and its incorporation into parallel (B) and antiparallel (C) pleated sheets.
While the α- and β- forms are found in crystalline regions of proteins and are characterized by regular arrangement of the polypeptide chain, the random coil conformation corresponds to the amorphous regions and is not characterized by the regular packing of the main chain. Ishikawa et al. (38) investigated the amorphous and crystalline phases of B. mori and Tussah silk fibers by partial acid hydrolysis, and proposed a three phase model for explaining the secondary structure of fibroin. Apart from the well-packed, highly-ordered crystalline phase, they propose the presence of a transition phase with laterally ordered regions between the crystalline and amorphous phases. A similar model was also suggested by Shaw (39) who investigated the various fractions obtained after enzymatic hydrolysis of silk. From his findings he concludes that the intermediate phase of lateral order shows quite a high degree of order and orientation even though the chains are not well packed. Additionally he infers that this intermediate phase is more abundant in B. mori silk than in Tussah silk. More recently Tsukada and coworkers (40) have demonstrated this three phase structure in B. mori and Tussah silk using thermomechanical analysis, dynamic mechanical measurements, and x-ray diffraction.

The amorphous and crystalline structure of B mori fibroin has been extensively investigated by x-ray diffraction studies that will be discussed in detail in a subsequent section of this review. Marsh et al. (41,42) assigned the following unit cell dimensions for B. mori fibroin: a (interchain) = 0.94 nm; b (fiber axis) = 0.697 nm; c (inter-sheet) = 0.92 nm. The cell consists of amino acid residues arranged in an antiparallel β-pleated sheet structure. Several antiparallel chains group together to form pleated sheets parallel to the Marsh’s a-b plane.
Tertiary Structure

This level of protein structure refers to the arrangement of the amino acid side chains. The model shown in Figure 2.3 proposes that the smaller glycine side chain (H-) alternates with the bulkier side-chains of alanine (-CH₃) and serine (-OH) so that the glycine side groups on adjacent sheets face each other. This translates to an alternating inter-sheet separation, i.e. 0.35nm and 0.57 nm and is known as the polar antiparallel structure.

More recent x-ray studies by Takahashi et al. (43) elucidate that the side chains are arranged in an antipolar antim parallel manner. Namely, the methyl side chains of the alanine residues alternately point to either side of the sheet in the hydrogen bond direction.
Figure 2.3 Antiparallel β-pleated sheet structure of *B. mori* silk, with chain axis parallel to fiber axis.

Physical Properties

Because silk is a natural polymer, there is considerable variability in the properties from one filament to another. Some factors that affect its properties include species and growth conditions. While the properties outlined in this and following sections are typical, some variations can be expected between samples. This section outlines the microscopic and mechanical characteristics of B mori silk.

Microscopic Characteristics

The cross-sectional shape of the B mori fiber is that of an irregularly shaped triangle (27). In longitudinal view the fibers look like fine twisted filaments with few or no external markings. The average diameter of the individual filaments is between 8-13 μm. Though silk fibers appear as solid opaque rods under the microscope, swelling followed by mechanical maceration reveals the presence of the component fibrils. Often the ends of degraded silk fibers splay out into individual fibrils (28).

Microscopic observation of silk fibers degraded by chromic (44) and phosphoric (45) acids indicate that that the fibers first appear to be degraded at regularly spaced intervals. While Oku & Koide (44) report that the spacing of these initial signs of degradation were about 16μm or some multiple of this length, Schurz (45) reports a spacing of about 100μm. These results indicate that silk fiber is not completely homogeneous, but is composed of highly ordered, resistant regions, surrounded by more accessible regions of lower order.

Mechanical Properties

Of all the natural fibers, silk is most akin to synthetic fibers in its fairly high strength and extension at break. The typical tenacity of B mori silk is about 0.38 N/tex (27). Meredith
(46) has demonstrated the relationship between the fineness of silk fibers and their mechanical properties. His results point to an inverse relationship between the fineness of silk and its tenacity. In terms of elastic recovery, silk shows a moderately high elastic recovery from fairly large deformations. As the moisture content of the fiber increases, its recovery decreases when the applied stress is low (about 1%), but moisture content enhances elastic recovery at higher stresses (between 5 and 10%) (47). There is considerable evidence to show that water absorption occurs at definite amino acid sites in the protein and that these sites need not have hydrophilic side chains. This demonstrates that the peptide linkages are largely responsible for water absorption. The absorption at these sites greatly outweighs the significance of water absorption at the hydrophilic side chains (48). Valentine (49) found that 79% of the sorbed water in fibroin is associated with peptide linkages, and that fibroin has a lower accessibility to water than other fibrous proteins.

**Chemical Properties**

The chemical properties of silk fibroin fibers including hydrolysis, oxidation, and electrochemical properties are discussed in this section.

**Hydrolysis**

Acid and alkaline hydrolysis of fibroin occurs by the fission of the polypeptide chains. Simple physical tests like viscosity and extensibility indicate that the extent of hydrolysis is much greater with acid than with alkali, and is pH-dependent. Hydrolysis is minimal when the pH is between 4 and 8 (50,51). The very low concentration of cystine linkages is thought to account for silk's high alkali resistance. By plotting the relationship between strength loss and fluidity change as a result of acid and alkali hydrolysis, Cadwallader, Howitt, and Smith (52)
found evidence for the fact that the mechanisms of acid and alkaline hydrolysis are different. These researchers found that the fluidity increases were much greater under acid hydrolysis than under alkaline hydrolysis. However, they also found that the nitrogen content in alkaline liquors was much greater than in acid liquors as a result of silk hydrolysis. From these observations they concluded that while acids cause chain cleavage distributed widely throughout the polymer chain, alkaline hydrolysis takes place preferentially near the chain ends.

Other studies on fibroin hydrolysis include those by Gardner (53), and Nicolet and Shinn (54). While the former study demonstrated that the prolonged effect of mineral acids involves the serine and threonine residues, the latter concluded that alkaline treatment also causes the loss of these same residues with the liberation of ammonia. Yang (55) studied the IR spectra of untreated and sulfuric acid treated silk fibers. His results indicate that though upon initial inspection, the spectra are apparently similar, the difference spectrum shows a band at 1735 cm⁻¹, that indicates the formation of carbonyls.

Korchagin (56) studied the effect of salts on alkaline hydrolysis by NaOH and acid hydrolysis by HCl. Under alkaline conditions, the salt anions had little effect, while cations enhanced degradation in the following order of efficiency: Li > Na > K > Ca > Ba. Under acid conditions, the salt cations had little effect, while anions enhanced degradation in the following order of efficiency: Cl > Br > NO₃ > I.

Studies have also been conducted on the effect of hydrolysis on the crystalline and amorphous regions of silk. For example, Nadiger et al. (57) used infra-red spectroscopy to
study the partial acid hydrolysis of silk followed by deuteration, and demonstrated selective
dissolution of amorphous regions. Earlier, Sobue and Konishi (58) obtained similar results.

Finally, with respect to enzymatic hydrolysis, silk is fairly resistant to proteolytic
enzymes. This resistance has been attributed to the close packing of the polypeptide chains
over a large part of the fiber, making it difficult for large enzyme molecules to penetrate (28).

Oxidation

The oxidation reactions of fibroin are extremely complex, and there is little agreement
on the products of oxidation. However, Lucas et al. (28) present three possible modes of
oxidation: (i) oxidation of the side chains of certain amino acids; (ii) oxidation of the N-
terminal residues of the peptide chain; and (iii) breakage of the main chain peptide bonds.

Several researchers have also demonstrated that the amino acid tyrosine is involved
in fibroin oxidation. Nakanishi and Koboyashi (59) found that under the action of hydrogen
peroxide, peptide bond scission occurs at the linkages involving tyrosine residues. Similar
results were demonstrated by Sitch and Smith (60) using peracetic acid, and Akune and Koga
(61) using potassium permanganate.

Electrochemical Properties

The isoelectric point of Bombyx mori is defined by the pH of minimum movement of
the protein under the influence of an electric field. Early measurements of the isoelectric point
showed a large variation in results depending on the methods used, the starting silk material,
and even the type of the buffer material used (28). Current measurements put the value of the
isolectric point at a pH of 4.5 (62) or 5.0 (63). Some of the discrepancy between the
measurements results from the fact that molecules on the surface of a protein may have different salt-forming properties than bulk molecules due to different steric environments (28).

The isoelectric point is distinguished from the isoionic point, i.e., the point at which the bulk of the protein has neither basic nor acid properties. This is the pH at which there is minimal combination with acids and alkalis. The isoelectric point will lie within the isoionic range of silk. The isoionic range of silk spans the pH values from 4 to 8 (28).

**Analytical techniques applied to the study of textile fibers**

In the study of historic artifacts, the conflict between need to preserve the artifact without any destruction, and the need to analyze and understand the artifact often arises. However the optimal conservation protocol can only be determined if the material is understood. The compromise is therefore to use analytical techniques that are as minimally destructive as possible (64). Occasionally however, larger sample sizes and destructive analytical techniques need to be used in order to understand certain aspects of material structure. This section outlines some general principles of the analytical tools employed in this research, and reviews their use in the study of silk fibroin, and where relevant, to other protein materials. The following techniques are discussed: optical and scanning electron microscopy, energy dispersive x-ray spectrometry, x-ray diffraction, Fourier-transform infrared microspectroscopy, and differential scanning calorimetry.
Optical and Scanning Electron Microscopy

Morphological characterization of fibers not only enables their identification, but also provides information regarding their history. The structure and performance characteristics of textile fibers are directly impacted by fiber morphology, including factors like fiber diameter, surface characteristics, and defects. Microscopic techniques offer a wide array of methods to analyze these properties in fibers. Different microscopic techniques offer different modes of observation over a wide range of resolutions. Optical microscopy typically offers resolution in the $10^{-4} - 10^{0}$ cm range, scanning electron microscopy allows resolutions of $10^{-7}$ to $10^{0}$ cm, and resolutions of $10^{-8}$ to $10^{-4}$ cm are possible with transmission electron microscopy (65).

Optical and scanning electron microscopy are the most popular techniques used for investigating deterioration in textile products (66). This section will outline the relative merits and demerits of these two techniques and review the literature on their application to textile fibers in general and archaeological textile artifacts in particular.

Some advantages of optical microscopy include the ability to study surface and internal detail, the use of birefringence measurements to analyze average chain orientation, the ability to perceive color, contrast enhancement using techniques like phase and differential interference contrast, and ease and flexibility of sample preparation. Another advantage of this technique is the ability to study the morphological effects of chemical reactions as they occur. Optical microscopy however has several limitations including lower resolution, magnification limits, and small depth of focus that is exacerbated at higher magnifications (11,66,67).
Due to higher magnification and resolution of SEM in contrast to optical microscopy, surface features are better enhanced in comparison with optical techniques. In addition sample viewing facilities like tilting are possible. As the depth of focus is higher, excellent three-dimensional images are possible. Finally, combination of this technique with energy dispersive x-ray analysis allows for chemical analysis of fibers and occlusions. Despite these advantages, this method also has certain limitations. While optical microscopy allows surface characteristics, internal features, and color to be viewed, only surface characterization is possible with the electron image. Since the image is formed by electron scattering, color perception is not applicable.

While there has been extensive use of optical microscopic techniques in the investigation of textile materials in general, the use of these methods in studying historic artifacts is more limited. In one investigation, Körber-Grohne (68) investigated fibers from around 500 B.C. from a burial site in Germany using various microscopic techniques including light and scanning electron microscopy and stereomicroscopy and identified them as being of plant or animal origin. Ryder and Gabra-Sanders (69) have used this method to analyze archaeological plant fibers from Denmark, Turkey, and England. Fiber diameter distributions both in animal and plant fibers have also been studied by these authors (69,70).

Both historic and modern textiles have been studied using the SEM. Zeronian et al. (71) applied this analytical technique to the study of fabric failure. Cross-sectional studies of methacrylamide grafted silk fibers have been conducted by Tsukada et al. (72). Becker and Tuross (73) investigated the structure of silk fibers of First Ladies' Gowns using the SEM. Jakes and Wang (5,6,74) studied fibers recovered from the deep ocean site of the S.S. Central
America with SEM. The effect of different drying techniques on the morphology of waterlogged textiles were evaluated under the SEM (4).

While characteristics such as fiber shape, diameter, fiber failure characteristics, and structure of yarns and fabrics can be observed under a microscope, in order to study fibers completely, other techniques like x-ray diffraction and chemical spectroscopy should be used to complement microscopic investigations (11).

Energy Dispersive X-Ray Spectrometry

In energy dispersive x-ray spectrometry (EDS), compositional information with a spatial resolution on the order of 1 μm can be obtained from the analysis of characteristic x-ray radiation emitted by the sample. Compositional mapping can be conducted using this technique, and when used in conjunction with the SEM, elemental composition of observed morphological features can be determined (12).

Though EDS has proven to be useful in the analysis of a wide range of materials, it has not been extensively used in the analysis of fibrous materials (75). Only a few references in the literature use this analytical technique to study fibers and related phenomena. Jakes and Sibley (76) have analyzed partially mineralized textiles from Etowah using this method. Koestler, Indictor and Sheryll (77) have studied mordants on wool with EDS, while Ballard, Koestler, and Indictor (78) have examined the elemental composition of mordants and weighting agents on historic silk flags. As part of the S.S. Central America research project, Jakes and Wang (6) studied the elemental composition of surface encrustation on the
recovered textiles. Chen (8) used this method to study the differences in the elemental composition of dyed versus undyed cotton samples from the site.

X-Ray Diffraction

X-rays of known wavelength can be made to diffract from a material and this phenomenon is used to study the crystalline structure of the material. Structural studies using X-ray diffraction (XRD) can be carried out either by using a diffraction camera or a diffractometer. When the former method is used the output is a photograph, from which the intensity of the diffracted beam is measured as a function of the optical density of the film, and the position of the diffracted beam is measured as a function of distance from the center of the film. In diffractometric studies the output is a plot of position of the diffracted beam versus intensity. In this method, beam intensity is measured by an electronic counter, making the measurement more accurate than in the case of a photograph (79). Additionally, the diffractometer has the advantage that line position and intensity are measured simultaneously, while the use of the diffraction camera requires that each of the two steps be carried out separately.

XRD studies of fibrous polymers give a more complex picture than those of wholly crystalline materials because the former vary in properties from almost completely amorphous to highly crystalline (80). The literature on x-ray diffraction studies of silk fibroin can be broadly divided into two sections which will be discussed below: (i) work on structure determination and modification, and (ii) studies on crystallinity measurement and crystallite size determination. The fundamental information derived from x-ray diffraction studies
pertains to the determination of the crystal structure of materials, namely the density and dimensions of the unit cell, the position of individual atoms and groups of atoms within the cell, and the crystal symmetry displayed by the material. While extensive literature is available on this subject, it is not reviewed here, except as it pertains to the fundamental crystal structure of fibroin. The other focus of x-ray diffraction investigations is the study of unit cell aggregates in fibrous materials, particularly crystallite size and crystallinity determination. These parameters, which have been extensively studied using x-ray diffraction techniques, relate closely to fiber properties and can be altered by various treatments and environmental conditions to which fibers are exposed. Because of its relevance to the research reported herein, this aspect of x-ray diffraction studies is more generally reviewed.

**Structure Determination**

The earliest evidence of the presence of ordered molecular arrangement in fibroin came from the work of Herzog and Jancke (81) in 1920 on silk fibers. In 1923 Brill (82) studied the crystal structure of *B. mori* fibroin and concluded the presence of an orthorhombic unit cell of dimensions 7.00 Å along the fiber axis, 9.27 Å, and 10.4 Å. Based on the amino acid composition of fibroin, its density, and the volume of the unit cell, he proposed that the cell consisted of four alanylglycyl units. In subsequent work, Brill (83) refined the cell dimension along the fiber axis to 6.95 Å. Meyer and Mark (84) drew similar conclusions from their diffraction studies on silk fibroin. Kratky and Kuriyama (85) studied fibroin film prepared from silkworm gut, which had been doubly oriented by rolling and stretching. They found that the intensities of diffraction spots were a function of whether the incident x-ray radiation was applied to the edge or the face of the film. Based on these results, and a
consideration of the known density of the crystalline portion of fibroin, Kratky and Kuriyama (85) proposed six plausible monoclinic unit cells for fibroin.

In the 1950’s some of the most important investigations on the crystal structure of fibroin were conducted, and the early results were refined by several researchers. The most significant results are based on the work of Warwicker (32), and Marsh, Pauling and Corey (41,42). Warwicker (32) used the photographic method to compare the structure of native silk fiber with that of the enzyme resistant fraction of fiber. The latter was known to have a density of 1.398, and to be composed solely of glycine, alanine, and serine, with occasional tyrosine residues. He found that while the enzyme resistant fraction showed a strong resemblance to that of native fiber, the diffraction rings were sharper. Warwicker attributes the diffuse nature of the native fiber pattern to imperfections in the crystals caused by imperfect packing of the crystals, and to the bulky groups present at the periphery of the crystals. On the basis of his studies, Warwicker proposed an orthorhombic unit cell, with the dimensions a = 9.29Å, b = 9.44 Å, and c = 6.95 Å.

Studies conducted by Marsh, Corey, and Pauling (41,42) are also much quoted. By conducting extensive work on amino acids and simple peptides, these researchers concluded that: (i) the atoms in the amide group of peptides and proteins are coplanar; (ii) the optimal conformation of peptide chains are those that facilitate the formation of the maximum possible number of hydrogen bonds between the NH and C=O groups, and (iii) the atoms forming the hydrogen bonds are approximately linear. Based on this, they propose a chain structure in which the C=O and NH groups on successive amino acid residues protrude on either side of the main polypeptide chain, and adjacent chains lie side by side on pleated sheets so that
extensive hydrogen bonds are formed between the C=O and NH groups of adjacent chains. Depending on the orientation of adjacent chains in the sheet, the structure is either parallel or antiparallel. In the parallel form, the axis-identity distance was calculated to be 6.5 Å, while in the antiparallel arrangement it was 7.0 Å.

With respect to *Bombyx mori* fibroin structure determination using x-ray diffraction technique, these authors comment on the qualitative rather than quantitative nature of most XRD studies on fibroin, that have been based on photographs rather than on diffractometric techniques. Using a 2θ-range from 5 to 65°, and a combination of diffraction camera and diffractometer data, these authors conducted a thorough investigation of fibroin films made from doubly oriented silkworm gut to obtain an equatorial spectrum of fibroin. They obtain unit cell dimensions of \( a = 9.40 \text{ Å}, b = 6.97 \text{ Å}; \) and \( c = 9.20 \text{ Å} \), where the b-axis corresponds to the fiber axis. Based on the pleated sheet structure previously discussed, and the results from x-ray diffraction, the only compatible cell was an orthorhombic one. Such a cell could index all the observed reflections in fibroin, and except for one reflection observed at 9.70 Å, and calculated at 9.20 Å, produced good agreement between the calculated and observed reflections. In the proposed cell, the a-axis is parallel to the intra-sheet hydrogen bonds, and its identity distance is the distance between alternate chains. The b-axis is parallel to the main chain and its length corresponds to two residues along the chain. The c-axis corresponds to the inter-sheet packing distance between alternate sheets. Based on the fact that they found peaks at 3.5 Å and 5.7 Å, they concluded that adjacent sheets were packed alternately at these intervals. The amino acid side chains protrude perpendicularly from both sides of the sheet, with the side chains on adjacent residues along any given chain projecting in opposite
directions. If only hydrogen atoms from glycine residues protrude between adjacent sheets, the inter-sheet packing distance was calculated as 3.5 Å, whereas if the side chains from alanine and serine solely occupy the space between sheets, the packing distance is 5.7 Å. In contrast to Warwicker (32), these workers mention that while most of the pleated sheets pack in accordance with the alternate 3.5 and 5.7 Å distance between them, bulkier side groups of amino acids like tyrosine can also pack between the sheets in a crystalline conformation. This would result in a larger packing distance between sheets, and is supported by the observation of a reflection at 9.7 Å. Table 2.4 presents the x-ray reflection spacings and intensities as observed and classified by Warwicker (32) and Marsh, Corey, and Pauling (41,42).

More recent unit cell size determinations on B. mori silk have been made by Takahashi (86) following the indexing scheme of Warwicker (32) gives the following parameters: a = 9.38 Å, b = 9.49 Å; and c = 6.98 Å. As previously mentioned, Takahashi has elucidated that the side chains are arranged in an antipolar anti-parallel manner, namely, the methyl side chains of the alanine residues alternately point to either side of the sheet in the hydrogen bond direction, differing from the Marsh et al. (41,42) structure in which the methyl groups of alanine all point in the same direction.
<table>
<thead>
<tr>
<th>Spacing (Å)</th>
<th>Intensitya</th>
<th>Spacing (Å)</th>
<th>Intensitya</th>
<th>Marsh et alb</th>
<th>Warwickerc</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.7</td>
<td>90</td>
<td>9.26</td>
<td>m</td>
<td>001</td>
<td>100</td>
</tr>
<tr>
<td>4.7</td>
<td>450</td>
<td>4.68</td>
<td>s</td>
<td>002</td>
<td>200</td>
</tr>
<tr>
<td>4.24</td>
<td>900</td>
<td>4.33</td>
<td>vs</td>
<td>201, 201</td>
<td>120, 020, 200</td>
</tr>
<tr>
<td>3.59</td>
<td>001</td>
<td>3.26</td>
<td>vvw</td>
<td>021, 121, 201</td>
<td></td>
</tr>
<tr>
<td>3.05</td>
<td>180</td>
<td>3.06</td>
<td>ms</td>
<td>003</td>
<td>300</td>
</tr>
<tr>
<td>2.76</td>
<td>202, 222, 022</td>
<td></td>
<td>m</td>
<td>202, 122, 022</td>
<td></td>
</tr>
<tr>
<td>2.35</td>
<td>20</td>
<td>2.38</td>
<td>m</td>
<td>400</td>
<td>040, 400</td>
</tr>
<tr>
<td>2.10</td>
<td>2.04</td>
<td>2.26</td>
<td>m</td>
<td>013</td>
<td>103, 013</td>
</tr>
<tr>
<td>1.80</td>
<td>&lt;5</td>
<td>1.84</td>
<td>vw</td>
<td>005</td>
<td>303, 033</td>
</tr>
<tr>
<td>1.56</td>
<td>18</td>
<td>1.56</td>
<td>w</td>
<td>601, 601</td>
<td>060, 600, 160</td>
</tr>
<tr>
<td>1.20</td>
<td>&lt;5</td>
<td>1.32</td>
<td>w</td>
<td>800</td>
<td>063, 603, 163</td>
</tr>
<tr>
<td>1.15</td>
<td></td>
<td>1.15</td>
<td>m</td>
<td>006</td>
<td></td>
</tr>
</tbody>
</table>

aSymbols & intensities are those used by original authors. m= moderate, s= strong, v= very, w= weak; b= interchain direction, b= fiber axis, c= intersheet direction; c= a= intersheet direction, b= interchain direction, c= fiber axis.


Table 2.4 Comparison of x-ray reflections of Bombyx mori fibroin observed by Warwicker and Marsh, Corey, and Pauling.
More recently, work on XRD of silk fibroin reports results of the d-spacings of fibroin, and the effect of different treatments on the reflections displayed. For example, Bhat and Arirrao (87) conducted a study on fibroin films regenerated from lithium thiocyanate solution and subject to various treatments using XRD. They found that while the freshly regenerated fibroin film had a diffraction pattern with no peaks, subjecting it to storage and UV radiation brought about the random-coil \( \rightarrow \beta \) transformation. Bhat and Ahirrao report d-spacings of 4.73 and 4.76, 4.26 and 4.28, 3.46 and 3.62, and 3.11 Å for these four reflections for the UV irradiated and the stored films respectively. These spacings correspond to d-spacings of the \( \beta \)-sheet peaks reported by Warwicker (32). However the hkl indices reported by Bhat and Ahirrao for these four peaks: [002], [201], [300], and [003], do not correspond consistently with the indexing system of either Warwicker (32) or Marsh et al. (41,42). It is worth pointing out that there seems to be some confusion in the literature regarding the indexing of the peaks in fibroin diffraction patterns. In addition to the four peaks previously mentioned, Bhat and Ahirrao also report reflections with d-spacing of 3.76 and 3.92 Å in the UV irradiated and the stored film respectively, that they note have not been previously indexed in fibroin patterns.

In a study by Magoshi et al. (88), on undrawn and drawn fibroin, halos at 4.35 Å and 6.20 Å are attributed to the random coil conformation of fibroin, while peaks at 2.37, 3.26, 4.28, 4.85, and 9.76 Å, are assigned to the well-oriented \( \beta \)-conformation. These authors do not attempt to index the reflections. Tsukada, Freddi, and Minoura (89) investigated the changes in the fine structure of silk fibroin fibers following gamma irradiation, and note the
presence of only one major peak in the 3-35° 2θ range. This peak, which occurs at 20.5° 2θ, is reported to correspond to a d-spacing of 4.35, and is indexed as the [101] peak. No detail of the indexing procedure is presented.

**Crystallinity and crystallite size determination**

Several studies have focused on determining the percent crystalline component in silk fibroin based on XRD measurements. Most of the literature in this area claims to use one of two methods to estimate the amount of crystalline component in fibrous materials: that developed by Hermans and Weidinger (90), and that developed by Manjunath et al. (91). Occasionally (92) however, crystallinity measures are reported with no indication of the approach used. After briefly outlining the methods of Hermans and Weidinger, and Manjunath et al, the available literature on silk fibroin crystallinity estimations will be discussed.

The most often used method is that of Hermans and Weidinger (90), which was developed to estimate the crystalline component in various cellulosic materials. In preparing specimens for XRD analysis, these workers took great care to ensure that precisely the same weight of material was used for each sample, and that the dimensions of the pellets prepared for each sample were precisely the same. This allowed them to compare the intensities and peak heights across specimens. Their procedure for estimating crystallinity consists of measuring the integrated intensities of the crystalline peaks and the maximum height of the amorphous curve corrected for air and thermal scattering and Compton radiation for each specimen. The former is taken as a relative measure of the crystalline portion while the latter is considered to represent the amorphous portion of cellulose.
While these authors mention that the integrated intensity of the amorphous curve could have been used as an alternative measure of the amorphous component, they point out that doing so would require that amorphous contribution be estimated over a $180^\circ$ 2θ range, rather than the range over which crystalline peaks occur. However, these authors note that using the integrated amorphous intensity over the investigated two theta range of $7^\circ$ - $42^\circ$, yields results that are in good agreement with their computations using the corrected heights of the amorphous curve.

By computing the ratios of the measures of crystalline and amorphous components for different cellulosic materials, the relative amounts of crystalline and amorphous components are found. In order to estimate the absolute crystalline component for each specimen, the pair of equations obtained from the relative crystalline intensity ratio and the relative amorphous height ratio for each pair of samples is solved quadratically. If $x$ and $y$ are the percent crystalline components of samples $X$ and $Y$, $I_{xy}$ is the ratio of the integrated intensities of the crystalline peaks, and $A_{xy}$ is the ratio of the corrected maximum height of the amorphous curve, then $x/y = I_{xy}$ and $(1-x)/(1-y) = A_{xy}$. Measurement of $I_{xy}$ and $A_{xy}$, allow the determination of the percent crystallinity of both samples. The principal criticism of this method of crystallinity estimation arises from the time consuming nature of the technique, making it impractical for routine fiber analysis (93).

Hindeleh and Johnson (94,95) profiled the crystalline peaks in cellulosic fibers using a combined Cauchy-Gaussian function, and estimated crystallinity as the ratio of the integrated area under the crystalline peaks to the integrated area under the total diffraction
trace. Foreman and Jakes' (7) approach to crystallinity estimation based on x-ray diffraction analysis of cellulosic marine textiles is somewhat similar to Hindeleh and Johnson's peak fitting technique. These authors use simple Gaussian profiles to model the crystalline peaks after eliminating the background and amorphous contributions of the spectra by fitting a straight line and a polynomial function respectively. Crystallinity is estimated using integrated areas, similar to the method of Hindeleh and Johnson. Chen (96) has adopted Foreman & Jakes' methodology in her study of the crystallinity of mineralized plant fibers.

In comparison with evaluating the integrated intensity to determine crystallinity, the use of peak-height ratios in crystallinity determination is less time intensive. This second method of crystallinity estimation was developed by Manjunath et al. (91) for fibrous polymers. These workers compute a resolution factor (RF) defined as $RF = (m_1 + 2m_2 + 3m_3 + \ldots m_n - 1)/(m_1 + 2m_2 + 3m_3 + \ldots m_n)$, where $h_1, h_2, h_3, \ldots h_n$ are the peak heights of reflections in a sequence of increasing $2\theta$ values, and $m_1, m_2, m_3, \ldots m_n$ are the heights of the minima from the baseline. From the RF they find a lateral order factor, $L_0$, defined as $(1 - RF)$, which is the crystallinity index of the material.

While the last few paragraphs have addressed fiber crystallinity estimations in general, the following paragraphs discuss the literature on crystallinity estimation in silk. Hirabayashi et al. (92) examined the effect of UV irradiation on the crystallinity of silk using XRD. While these authors do not report any details of the method used to calculate crystallinity, they report a value of 39.3% for untreated silk fiber. On irradiating the sample with UV radiation for between 240 and 1700 hours, crystallinity values vary between 37.9% to 42%. However, these workers find no correlation between irradiation time and change in crystallinity.
Tsukada and coworkers (97) studied the effect of epoxide treatment on the microcrystalline properties of silk fibers using among other techniques, XRD measurements between 5 and 45° 2θ. They report the use of the method suggested by Hermans and Weidinger (90) to calculate the crystallinity of the fibers. While the crystallinity of the control fibers are not reported, these researchers report a crystallinity percent ranging from 20.6 to 24.6% in silk fibers treated with various epoxides. Additionally, they report a degree of orientation measure based on the half-widths of the x-ray diffraction curves, which ranges from 74.2 to 80.4% in various epoxide treated silks. In a more recent study (98) on the effects of various degrees of shrinking of silk in neutral salt solution, the percent crystallinity of untreated silk fiber is reported as 24%, while that of shrunk silk is 22%. The Hermans and Weidinger method of crystallinity estimation is also used in this work in the 5 - 35° 2θ range. From their results they conclude that the crystalline regions are inert to the shrinking treatment. They discuss a three phase structural model of silk, comprised of crystalline regions, laterally ordered regions, and amorphous regions, and attribute alterations in orientation and size that they find from other analytical techniques to the laterally ordered regions rather than the crystalline regions.

Bhat and Aahirao (99) investigated the fine structure and morphology of preswollen and untreated Bombyx mori silk fibers using XRD. Based on the method developed by Manjunath et al. (91) they report that the crystallinity index for untreated mulberry silk is 0.6571. Treatment with swelling agents like formic acid, lithium thiocyanate, and zinc chloride reduces this index to between 0.2865 and 0.5714. They also note that the higher the concentration of the swelling agent, the smaller the change in crystallinity. In another study,
Bhat & Nadiger (100) have investigated the effect of hydrolysis on the crystallinity of fibroin, and have computed a crystallinity index for control and hydrolyzed samples. They state that while different methods can be used to compute a crystallinity index, based on the relative peak heights or the integrated areas under the diffraction profile, these different methods all essentially result in the same numbers.

As discussed previously, there have been a fair number of references in the literature focussing on fibroin crystallinity estimation. However, studies on crystallite size estimations of fibroin are limited. What follows is a brief discussion on general techniques of crystallite size estimation, followed by a review of such studies on silk fibroin. Scherrer (101) first illustrated evaluation of crystallite size from spectral line breadth. He derived the relationship between the wavelength of incident radiation $\lambda$, total line breadth $B$, instrumental line broadening $B_i$, and the average crystallite dimension $\sigma$, perpendicular to the lattice plane of the reflection:

$$ B = \frac{K\lambda}{\beta \cos \theta} + B_i $$

where $K$ is nominally the constant 0.9, and $\bar{\theta}$ is the center position of the peak profile being evaluated.

Warren and Briscoe (102) further refined the Scherrer expression, which takes the following form:

$$ \sigma = \frac{K\lambda}{\sqrt{(B^2 - B_i^2) \cos \theta}} $$

51
Foreman and Jakes (7), Chen (96) and Chen (8) have used this approach in estimating crystallite size in cellulose fibers. These researchers have assumed that apart from the line broadening caused by instrumentation, crystallite size distribution is the sole contributor to broadening. However Hindeleh and Johnson (94,95) recognize that both crystallite size and crystallite strain contribute to line broadening.

Literature on crystallite size estimation in silk fibroin is extremely limited. Kawahara et al. use the [120] reflection to determine the crystallite size along this direction between 35 Å (103) and 40 Å (104) using the Scherrer equation and a value of 0.94 for the Scherrer constant. Bhat and Nadiger (100) measure a crystallite size of 10 Å along [200] and 19 Å along [120] using the Scherrer technique and a Scherrer constant of unity.

Using Fourier analysis of the intensity profiles of the [100] and [120] peaks Somashekar and Urs (105) obtain size estimates of 26 and 23 Å respectively. Somashekarappa et al. (106,107) also report crystallite sizes in silk based on Fourier analysis. In studying silk fibers treated with acid and metal complex dyes using the wide-angle x-ray diffraction profile of equatorial reflections, Somashekarappa et al. (106) use the peak at around 25° using Fe Kα radiation, which corresponds to Warwicker's [120] peak, and examine changes in crystal size perpendicular to this plane. They report a value of 33.9 Å for the volume-weighted crystallite dimension and 29.7 Å for the surface-weighted crystallite dimension in untreated silk. As a result of various dyeing treatments, the size drops to between 23.3 and 14.3 Å for the volume-weighted size, and between 21.5 and 11.3 Å for the surface-weighted size. The conclusion reached from these findings is that there is a rearrangement of the β-pleated structure of the fibroin molecules, which results in the
decrease in the crystallite sizes. The relation between decreased crystallite size and lower tenacity has also been pointed out. In an earlier study using the same technique to study the microcrystalline parameters of silk fibers, Somashekarappa et al. (107) report crystal size measurements based on Warwicker's [120] reflection. They assume a symmetric reflection in their Fourier analysis of this peak, and do not distinguish between the surface-weighted and volume-weighted crystallite size that they report in their subsequent work. The crystal size they report for untreated degummed silk is 10.69 Å.

Fourier Transform Infrared Microspectroscopy

While XRD techniques are useful in determining the crystal structure of materials, IR spectroscopy provides information about both the chemical and physical structure of materials. Chemical information regarding functional groups and the extent of hydrogen bonding and physical information regarding the conformation of molecular chains, chain orientation and crystallinity can be deduced using this analytical technique.

This section consists of a brief introduction about the FT-IR technique in protein characterization and a review of the literature on FT-IR studies on silk. Since the energy of the incident radiation, in the 770-1000 nm range, is of the same order as that of the vibrational transition of molecules, such vibrations can be studied using this method.

Infrared spectroscopy of proteins

Techniques like IR spectroscopy are often used in the study of proteins. Polymer molecules like proteins contain tens of thousands of atoms and may thus have tens to hundreds of thousands of normal modes of vibration. One might therefore expect the
vibrational spectra of such molecules to be extremely complicated. However, since polymers consist of chemically identical repeat units, each of which usually contains only tens of atoms or fewer, the spectra become considerably less complex (108).

To a first approximation, the vibrational spectrum of a polymer is that of its repeat unit. Such spectra can hence be used for qualitative analysis of the type of repeat units present in the polymer. The intensities of the vibrations depend on the concentration of the absorbing and scattering units, and so, the spectra can also be used for quantitative analysis (108).

The information obtained from vibrational spectra of proteins fall into three categories: (i) Absorption frequencies pertaining to particular chemical groups which can help identify the presence or absence of these groups; (ii) Information about physical structure of chemically identical polymers, which differ only in the physical arrangement of the polymer repeat units and often show obvious differences in their vibrational spectra; and (iii) Information about the orientation of molecular chains is obtained using polarized radiation (28,108). In examining large molecules using vibrational spectroscopy, chemical and structural information is arrived at by developing correlations between the observed spectral bands and known chemical and structural characteristics of other similar macromolecular systems (109). The research reported herein approaches vibrational spectral analysis through the assignment of group vibrational frequencies. The vibrational frequencies of certain submolecular groups of atoms consistently show up in a characteristic region of a vibrational spectrum. These are called group frequencies (109). Smith (110) classifies two kinds of group frequencies: those that have a constant frequency regardless of their molecular environment, and those that move according to their environment. Group frequency analysis is of
importance in polymer analysis, as it allows one to evaluate the presence or absence of particular groups in the polymer, and the orientation of such groups within the polymer (108). For example, the N-H bonds in the α-helix are nearly parallel to the axis of the helix, and are perpendicular to the axis of the β-pleated sheet. The infrared bands that result from the N–H group vibrations therefore display dichroic behavior according to the secondary conformation of the protein. Hence these bands are useful for distinguishing between the α-helix and β-pleated sheet structure of proteins (111). Vibrational spectroscopy is often used to obtain information about the secondary structure of protein.

**Assignment of IR absorption bands**

The vibrational modes of proteins can be broadly divided into three groups, (i) those that involve atomic motions of the main chain bonds; (ii) those that involve the highly localized side chains of the protein amino acids (112); and (iii) those that are characteristic of specific amino acid sequences (113).

**Main chain amide bands**

The spectra of polypeptides and proteins are dominated by the bands characteristic of the amide linkage. The principal peptide bonds are those associated with N-H stretching, C=O stretching (Amide I), and N-H deformation (Amide II). The positions of these three bands are particularly sensitive to the secondary conformation of the protein molecules and have been used extensively to study protein secondary structure. More recently (114), an FTIR method has been developed to analyze protein secondary structure by employing the amide III spectral region (1350-1200 cm⁻¹). The benefit of using this band is that the
interference from water vibration in the amide I region can be avoided. Additionally, this band is more easily resolved and better defined. Table 2.5 shows the frequency ranges of the amide bands as a function of the conformation of the protein molecule (112,114,115,115).

**Side-chain bands**

Despite the fact that amide bands predominate in the IR spectra of proteins, several bands ascribable to specific amino acid side-chains are described in the literature. Fraser and Suzuki (116) provide a summary of the characteristic vibrations localized in the side chains, which is reproduced in Table 2.6. Of particular importance to silk fibroin are the bands assigned to tyrosine and alanine. Bendit (117) reports a relatively strong band at 1515 cm$^{-1}$ accompanied by weaker bands at 1615, 1595, and 1440 cm$^{-1}$ for the tyrosine moiety. Elliot and Malcolm (118) assign bands at 1166, 1447, and 1453 cm$^{-1}$ to the CH$_3$ modes of alanine.

**Bands due to specific amino acid sequences**

Work in this area has been restricted to the work of early researchers (113,119). Asai et al. (113) studied the FTIR spectra of poly-glycine, poly-alanine, and various alanine-glycine copolymers. From the fact that a band at 1015 ± 20 cm$^{-1}$ is seen only in polypeptides containing the glycylglycine structure they conclude that it arises from this sequence. Their results are consistent with Blout and Lindsey's (119) observation of a strong band at 1015 ± 20 cm$^{-1}$ attributed to glycylglycine oligopeptides. Through similar reasoning, Asai et al. (113) also assign the band at 965 cm$^{-1}$ to the alanylalanine structure. In polypeptides that contain alanine-glycine or glycine-alanine moieties, two strong bands at 998 and 975 cm$^{-1}$ have been observed (113).
<table>
<thead>
<tr>
<th>Vibration</th>
<th>Designation</th>
<th>Frequency (cm(^{-1}))</th>
<th>(\alpha) helix</th>
<th>(\beta) sheet</th>
<th>Random coil</th>
</tr>
</thead>
<tbody>
<tr>
<td>C=O out-of-plane bending</td>
<td>Amide VI</td>
<td></td>
<td>660</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OCN out-of-plane bending</td>
<td>Amide IV</td>
<td></td>
<td>630</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH out-of-plane bending</td>
<td>Amide V</td>
<td></td>
<td>725</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CN stretching and NH in-plane bending</td>
<td>Amide III</td>
<td>1328-1289</td>
<td>1255-1224</td>
<td>1288-1256</td>
<td></td>
</tr>
<tr>
<td>NH in-plane bending (60%)</td>
<td>Amide II</td>
<td>1540-1550</td>
<td>1520-1525</td>
<td>1550</td>
<td></td>
</tr>
<tr>
<td>CN stretching (40%)</td>
<td>Amide I</td>
<td>1650-1660</td>
<td>1630</td>
<td>1680-1700</td>
<td></td>
</tr>
<tr>
<td>C=O stretching (80%)</td>
<td></td>
<td></td>
<td></td>
<td>1630</td>
<td></td>
</tr>
<tr>
<td>CN stretching (10%)</td>
<td></td>
<td></td>
<td></td>
<td>1680-1700</td>
<td></td>
</tr>
<tr>
<td>NH in-plane bending (60%)</td>
<td></td>
<td></td>
<td></td>
<td>1550</td>
<td></td>
</tr>
<tr>
<td>C-H stretching</td>
<td></td>
<td>3290-3300</td>
<td>3280-3300</td>
<td>2940</td>
<td>ca.3400</td>
</tr>
</tbody>
</table>


Table 2.5 Frequency range of peptide bands in \(\alpha\)-, \(\beta\)-, and random coil conformations.
<table>
<thead>
<tr>
<th>Side chain</th>
<th>Wave number (cm⁻¹)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cysteine</td>
<td>2600-2700</td>
<td>SH stretching</td>
</tr>
<tr>
<td>Cysteic acid</td>
<td>1040, 1175</td>
<td>SO₃⁻ stretching</td>
</tr>
<tr>
<td>Glutamine</td>
<td>3225, 3370</td>
<td>NH₂ stretching</td>
</tr>
<tr>
<td>Asparagine</td>
<td>3190,3430</td>
<td>NH₂ stretching</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>1710-1715</td>
<td>Carboxyl C=O stretching</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>1570, 1410</td>
<td>Ionized carboxyl COO⁻ stretching</td>
</tr>
<tr>
<td>Alanine</td>
<td>1166, 1447, 1453</td>
<td>CH₃ modes</td>
</tr>
<tr>
<td>Arginine</td>
<td>3350, 3140</td>
<td>NH₂ stretching</td>
</tr>
<tr>
<td></td>
<td>1680, 1625</td>
<td>Guanidium modes</td>
</tr>
<tr>
<td>Lysine</td>
<td>1600, 1495</td>
<td>NH₃⁺ deformation modes</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1515</td>
<td>Mode of p-disubstituted benzene ring</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1495</td>
<td>Mode of mono substituted benzene ring</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>3400</td>
<td>NH stretching (indole ring)</td>
</tr>
</tbody>
</table>

Table 2.6 Summary of the characteristic infrared vibrations localized in the side chains.

**Infrared Studies of Silk Fibroin**

The previous section discusses the vibrational modes of proteins in general. This section focuses on IR studies conducted on silk fibroin. The bulk of these studies have been done on fibroin in film and powder forms. For example, Miyawaza and Sonoyama (120) studied fibroin films using near IR radiation. Tsukada et al. (121) report on IR analysis of the structural changes induced by immersion of fibroin film in methanol solution. Lu, Akiyama and Hirobayashi (122) analyzed the properties of silk powder using IR spectroscopy. Only
a few references to the IR spectroscopic investigations of fibroin in fiber form are quoted in the literature. The literature on IR studies involving silk fibroin can be broadly classified into two categories: (i) Studies on IR band assignments for the secondary conformation of fibroin and (ii) Crystallinity assessment of fibroin using IR techniques.

IR band assignments

Early IR studies on fibroin focussed on assigning frequencies in the fibroin spectrum to specific vibrational motions and towards determining the secondary structure of fibroin. Studies (118) (13) on silk suture provided evidence of the β-structure for the material. These researchers found amide bands corresponding to those previously ascribed to the β-form of other proteins and polypeptides. In studying regenerated fibroin film, Lenormant (123) found frequencies for bands that were previously assigned to the random coil forms of other proteins. For example, he found the band at 1600 cm⁻¹ that corresponds to the C=O stretching frequency of the random coil form of proteins. Asai and associates (113) assign the bands between 3000 and 2800 cm⁻¹ to various CH stretching vibrations.

Since these early studies, several studies have focussed on the conformational changes induced in fibroin as a result of various treatments. Magoshi and coworkers studied the effect of inducing the random coil-β transition in fibroin film thermally (124) and by immersion in water (125). They assign the bands at 1660, 1540, 1235, and 650 cm⁻¹ to the amide I, amide II, amide III, and amide V bands of the random coil conformation respectively, and those at 1630, 1535, 1265, and 700 cm⁻¹ to these same modes in the β-crystalline form. Similar amide band assignments have been made by other researchers in studying various aspects of fibroin
structure. These studies include the effect of immersing fibroin film in methanol (126), structure of fibroin film regenerated from lithium thiocyanide solution (87) silk fibroin-poly(vinyl alcohol) blends (127), and silk fibroin-cellulose blends (128).

While the primary subject of study in the literature on IR spectroscopy of fibroin is the amide bands and their relation to the secondary fibroin conformation, a few studies focus on the identification of specific amino acids and amino acid sequences in fibroin. Asai et al. (113) studied the crystalline portion of fibroin and observed two strong bands at 996 and 976 cm\(^{-1}\). Based on the spectra of model polypeptides, they conclude that glycine and alanine alternate in the crystalline region of fibroin. These observations were confirmed more recently by Magoshi et al. (124) and Nadiger and Bhat (129). Additionally, Nadiger and Bhat found that these bands intensify on partial acid hydrolysis, indicating that the crystalline regions of fibroin have predominantly gly-ala linkages.

**Crystallinity studies**

Early estimations of the crystallinity in *B. mori* fibroin suture were made by Badger and associates (13) by comparing the relative intensities of the 1636 and 1664 cm\(^{-1}\) bands, attributed to the C=O stretching mode of \(\beta\)- and random coil form of fibroin, and the bands at 1528 and 1560 cm\(^{-1}\), attributed to the NH deformations of these secondary configurations. They obtained values ranging from 63-67% for the \(\beta\)-crystalline component of fibroin.

More commonly, the bands at 1265 and 1235 have been used in crystallinity estimations. The reasons for this are that these bands are better resolved in fibroin spectra and that there is no interference from water absorption at these frequencies (100). Freddi and coworkers (128,130) and Bhat and Nadiger (100) report values of about 66% for silk fibers
by computing the ratio between the bands at 1265 and 1235 cm\(^{-1}\). Bhat and Ahirrao (87) studied the effect of heat, alcohol, and UV-radiation treatments on fibroin film regenerated with lithium thiocyanide and obtained crystallinity indices ranging from 0.606 in freshly regenerated film to 0.6942 in film that had been heated to 190\(^\circ\)C. Nadiger and Bhat (129) found that plasma treatment of \textit{B. mori} silk fibers was accompanied by a decrease in crystallinity index from 0.62 to 0.56.

**FTIR Microspectroscopy of Single Fibers**

The desire to analyze microsamples and single fibers, as well as to carry out mapping studies has led to the development of special IR techniques. The principal technique involves restricting the microsample to a small area and using a beam condenser to obtain satisfactory signal. Some techniques developed include the preparation of a KBr microdisk with fibers dispersed in it; casting fibers into thin films; flattening fibers with a roller and then mounting on a KBr disk. Fibers have also been prepared by simply sandwiching between two surfaces in a diamond cell (75). In 1949, Barer, Cole, and Thomas (131) reported linking a microscope with an IR spectrometer. Visible light is used to focus on a magnified image of the sample, and then IR radiation is used to analyze the sample. They reported the ability to record spectra of fibers with a diameter of 20-50 \(\mu\)m over the full mid-IR range. With the development of FT techniques and advances in microspectrometry, spatial resolution of 10\(\mu\)m can be achieved with a conventional microspectrometer. Tungol, Bartick and Montaser (132) have developed a database for the identification of single fibers using IR microscopy. They
report that while highly reproducible spectra can be obtained for synthetic fibers, the results are less consistent for natural fibers. Fiber identification is not hindered by spectral variability.

**Mathematical procedures in the analysis of protein spectra**

IR spectra in general, and those of proteins in particular have intrinsically overlapping bands that can be better resolved by the application of mathematical procedures like Fourier self-deconvolution and second derivative analysis. This section discusses the applicability of these techniques to protein analysis.

The feasibility of Fourier self-deconvolution in analyzing infrared spectra has been pointed out (133). In practice, the application of this technique involves specifying the lineshape, bandwidth, and noise reduction factors to be used for deconvolution. Ideally the best lineshape is that which is intrinsically present in the spectra. However, the authors point out that a Lorentzian lineshape is adequate for condensed phase spectra. The optimal bandwidth can be achieved empirically by determining a series of spectra with different bandwidths. Underestimating this bandwidth produces little deconvolution, while overestimation leads to large negative side lobes in the peaks. By convolution of the spectra with an apodization function, noise contributions are diminished.

Since Kauppinen's work, a few studies on the analysis of protein spectra using this technique have been reported. The secondary structure of 21 globular proteins using the broad amide I band in the 1600-1700 cm\(^{-1}\) region has been analyzed (134) by deconvoluting the band using a Lorentzian lineshape with a bandwidth of 13 cm\(^{-1}\) and a noise reduction factor of 2.4. The deconvolution produced up to 11 different components in each protein, which were then assigned to the \(\alpha\)-helix, \(\beta\)-sheet, and random coil conformations. These researchers
have combined deconvolution with a peak-fitting procedure applied to the deconvoluted spectra to obtain quantitative results regarding the secondary conformations.

Yang and associates (135) analyzed the amide I and II regions in the diffuse reflectance IR spectra of lactoglobulin and cytochrome after deconvoluting them. Of particular significance is the fact that the deconvolution procedure was carried out independently in two different laboratories and produced similar band assignments and relative frequencies. The authors point this out as a counter-example to the often-made argument regarding the arbitrary nature of deconvolution.

The only instance in the literature of applying this data analysis technique to fibroin, is the amide I band analysis of fibroin film spectra (120). The broad band in the original spectra was deconvoluted into nine bands. Of these, eight were assigned to various \( \alpha \)-helix, random-coil, and \( \beta \)-sheet amide I modes, and the ninth at 1610\,cm\(^{-1}\) was attributed to the side chain of the tyrosine residues.

Second-derivative spectral analysis in wavenumber domain simply involves computing the rate of change of the slope of the original band directly from the spectrum (136). With respect to the applicability of this technique, a signal-to-noise ratio in the original spectrum of 100 or higher is necessary (133). It has been pointed out that using second-derivative analysis to independently determine peak positions is an effective way to support the results obtained from deconvolution (134). In studying the IR spectra of proteins in the solid state and in solution using Fourier self-deconvolution, Yang et al. (135) found good agreement between their results and those of Krimm and Bandekar (137) using second derivative analysis.
Differential Scanning Calorimetry

The International Confederation for Thermal Analysis and Calorimetry, defines thermal analysis as "as a group of techniques in which a property of the sample is monitored against time or temperature while the temperature of the sample, in a specified atmosphere, is programmed" (138). Thermal analysis affords structural fingerprinting of the fibers to be performed, and provides insight into the process-structure-property spectrum (65) of fibers. This section consists of a brief introduction about the thermal techniques in fiber characterization and a review of the literature on thermal analytical studies on silk.

The study of fibers using thermal techniques has three major applications: the analysis of physical transitions; the analysis of chemical phenomena; and the analysis of the kinetics of these chemical and physical changes (65). Physical transitions in polymers depend both on their molecular and supra-molecular structure. Such transitions include: the γ-transition which involves the rotation of the methylene groups in the amorphous region; the β or glass transition involving segmental motion of the main chain and alterations in specific heat, specific volume, and coefficient of expansion; crystallization; and melting. The location and shape of the transition interval are markedly influenced by sample history in general, and thermal history in particular (139) and these physical transformations can be efficiently studied using one or more thermal techniques. Chemical reactions such as the reaction, at elevated temperatures, between textile substrates and finishing treatments applied to them have also been studied by thermal analysis (139). Several different techniques fall under the category of thermal analysis, including thermo-mechanical analysis (TMA), thermo-gravimetric analysis (TGA), thermoluminescence, differential thermal analysis (DTA) and differential scanning
calorimetry (DSC), and the following paragraphs discuss the literature on the application and relevance of these techniques to the analysis of silk fibroin.

**Thermal analysis of silk**

The first thermal studies of protein fibers including silk fibers were conducted by Schwenker and Dusenbury (140). These researchers characterized silk, wool, and human hair over the 25-550° C temperature range. They found that while all three materials exhibit an endothermic peak between 108-116° C attributed to the loss of sorbed water, only wool and hair exhibit a second endothermic peak in the 160° C range, attributable to the loss of tightly bound water. On the other hand, silk shows a well-defined endothermic peak at 326° C that is not observed in the other proteins. Felix et al. (141) report similar findings on silk fibers.

Starting from the 1970's several researchers have analyzed the thermal behavior of silk in forms other than fiber. Magoshi and co-workers (125), studied the thermal behavior of fibroin film in the random coil conformation between 25 and 220° C using DSC and TMA. With the help of IR spectroscopy they established that inter- and intra-molecular hydrogen bonds break in the 150-180° C range, and this corresponded with the glass transition of 173°C that they report. The random coil to β transformation is observed at 180° C, and growth of β crystals is seen at 190° C. Other researchers (121,142) have studied the difference between crystalline and amorphous fibroin film using different thermal techniques and report findings similar to those reported by Magoshi and co-workers (125). Additionally they assign a peak at 212° C to cold crystallization of the random coil form to the β form, and a prominent endotherm at 280° C to the thermal decomposition of amorphous silk fibroin chains.
Crystalline film does not exhibit the glass and cold crystallization transitions at 180° C and 212° C respectively, and the decomposition temperature shifts to 284° C.

Thermal analytical tools have been used by several other researchers to investigate the effects of various treatments on silk. Tsukada and Hirabayashi (143) studied the effect of UV radiation on the degradation temperature of silk fibers using DSC. While the degradation temperature of untreated silk was 335° C, the corresponding temperature for UV irradiated silk fibers was determined to be 321° C. Sakaguchi et al. (144) utilized DSC in an investigation of weighted and unweighted silk fibers. They report that the endothermic decomposition peak at around 310° C in unweighted silk is shifted to higher temperatures as a result of weighting. A similar increase in decomposition temperature has been noted as a result of grafting methyl and ethyl methacrylate (145,146), methacrylonitrile (147) and epoxides (148) on silk. Tsukada and coworkers (149) investigated the effect of acylating silk using acid anhydrides. The effect of the treatment is manifest as an additional endothermic peak at 210° C, which has been attributed to the formation of cross-links between adjacent fibroin chains.

Summary

The subject of the present research are silk textiles recovered from the S. S. Central America, which sank about 140 years ago off the coast of the Carolinas. Recovery of the artifacts in the mid-eighties opened up the opportunity to study the effects and causes of deep-ocean degradation and preservation on these textiles. Such a systematic study of the chemical and physical microstructure of silk pursues three major goals: 1) understand the
preservative/degradative mechanisms operative in marine sites; 2) optimize the treatment and conservation of such artifacts; and 3) identify a set of analytical techniques that are optimally able to characterize artifacts.

Organic material like textiles in marine, terrestrial wet, or frozen sites often show a remarkable degree of preservation. The trunk interior contains a microclimate that is quite likely to be anoxic, mildly alkaline conditions with a pH less than 8. While the preserving character of certain marine, wet or frozen storage sites is known, no systematic study on how this type of storage affects the basic physical and chemical structure of silk fibers has been published. This gap in the current literature motivates the present research. The textiles studied in the present work were taken from a trunk belonging to Ansel and Adeline Easton.

The chemical microstructure of silk fibers is determined by simple amino acids which make up more than 80% of the amino acid content. Glycine, alanine, and serine have been identified as the main components present in the crystalline β-sheet phase of silk. Other phases of silk that are relevant for the present research are the amorphous or random-coil phase and the ordered amorphous phase. The ordered amorphous phase occurs at the interface between the crystalline and the amorphous phase and consists of amino acid chains that exhibit a high although not perfect, i.e., crystalline, degree of order and orientation.

The chemical and physical microstructure of Marine Silk in comparison to Reference Silk and Historic Silk has been examined in the present work by using a variety of techniques including optical and scanning electron microscopy, energy dispersive x-ray spectrometry, x-ray diffraction, Fourier-transform infrared spectroscopy, and differential scanning calorimetry.
CHAPTER 3

METHODOLOGY

The purpose of the research reported herein was to examine the physical and chemical microstructure of selected silk textile fibers recovered from the deep-ocean site of the S.S. Central America and compare these findings with the physical and chemical microstructure of modern silk reference material and with historic silk fibers from the same period. Such a comparative approach was taken in order to understand the effect of long term storage in a deep-ocean environment. Additionally, because of the nature of historic artifacts, the analytical techniques were evaluated for their effectiveness in studying micro-samples.

Several analytical techniques were used in this study to provide information about different aspects of the physical and chemical microstructure of the silk artifacts. The first section of this chapter discusses the test materials used in this work. The analytical instrumentation and sample preparation techniques are discussed in the next section. The third section consists of a discussion of the data analysis techniques for the different analytical methods.
Experimental materials

Three sets of silk samples were used in this research. Marine silk samples from three silk artifacts recovered from the *S.S. Central America*, samples from three historic silk artifacts attributed to the second half of the 19th century but not exposed to the marine environment, and reference silk fibers. The following paragraphs describe the specimens and the sampling technique.

Marine silk fibers

From a preliminary study of the entire collection of textile artifacts recovered from the *S.S. Central America*, items containing silk were identified. To avoid the confounding effect of dye, the three textiles selected for this study were those that appeared undyed. Small samples of fibers were taken from inconspicuous places in the artifacts (e.g., accessible unfinished seam allowances), so as not to affect the appearance of the garments in future display. For the marine silks, sample size ranged between 70 and 100 mm². Table 3.1 gives a description of the marine silks used in this study.

<table>
<thead>
<tr>
<th>Inv. #</th>
<th>Specimen desc.</th>
<th>Artifact desc.</th>
<th>Sampling area</th>
</tr>
</thead>
<tbody>
<tr>
<td>29049</td>
<td>Marine Silk 29049</td>
<td>Wool trousers with silk placket</td>
<td>Waist placket seam</td>
</tr>
<tr>
<td>29054</td>
<td>Marine Silk 29054</td>
<td>Wool undershirt with silk placket</td>
<td>Neck placket seam</td>
</tr>
<tr>
<td>33707</td>
<td>Marine Silk 33707</td>
<td>Wool undershirt with silk placket</td>
<td>Neck placket seam</td>
</tr>
</tbody>
</table>

Table 3.1 Marine silk artifacts sampled.

69
Historic silk fibers

For the purposes of comparison, three undyed historic silk textiles from the period 1860-1880 obtained from the Historic Costume and Textiles Collection, Department of Consumer and Textile Sciences, The Ohio State University, were also studied. Though the production and usage histories of the marine and historic silks from the collection are different, it was assumed that the principal difference between the two sets of silk fibers lay in the fact that the marine silk had been exposed to long term storage in a deep ocean environment, while the silks from the collection had not. The cumulative effects of 130 years in the two very different environments should be the overriding cause of the most significant differences between the marine and historic silks.

Small samples of fibers were taken from inconspicuous places in the artifacts (e.g., accessible unfinished seam allowances), so as not to affect the appearance of the garments in future display. Sample size ranged between 70 and 100 mm². Table 3.2 describes the historic silk artifacts used in this study.

<table>
<thead>
<tr>
<th>Inv. #</th>
<th>Specimen desc.</th>
<th>Artifact desc.</th>
<th>Sampling area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1986.145.1b</td>
<td>Historic Silk 145b</td>
<td>Cotton skirt with silk underskirt</td>
<td>Side seam</td>
</tr>
<tr>
<td>1986.145.1c</td>
<td>Historic Silk 145b</td>
<td>Wool undershirt with silk placket</td>
<td>Hem seam</td>
</tr>
<tr>
<td>1986.318.88b</td>
<td>Historic Silk 88b</td>
<td>Wool undershirt with silk placket</td>
<td>Pleat seam</td>
</tr>
</tbody>
</table>

Table 3.2 Historic silk artifacts sampled.
Reference silk fibers

Modern undyed and unfinished silk fibers (Testfabrics Style #601) obtained from Testfabrics Inc. Middlesex, NJ, was used in this work as a reference material and are referred to in this document as Reference Silk.

Instrumentation and sample preparation

The analytical methods used in this study are Optical Microscopy (OM), Scanning Electron Microscopy (SEM), Energy Dispersive X-Ray Spectroscopy (EDS), X-Ray Diffraction (XRD), Fourier Transform Infrared Microspectroscopy (FTIR), and Differential Scanning Calorimetry (DSC). This section describes the instrumentation and sample preparation techniques for each of the methods.

Optical Microscopy

A Zeiss Axioplan Research Microscope with bright-field, dark-field, differential interference contrast, phase contrast, and polarized light capabilities was used in this study. Nominal magnifications of 10x40 and 10x100 were used to examine the fibers. Width measurements were done on images that were digitally captured using an Optimas 5.2 image analysis system.

Between 10 and 15 fibers of each of the samples were mounted onto glass slides using Permount®, a histological mounting medium with refractive index 1.55, and allowed to dry for 24 hours before viewing under a microscope.
Scanning Electron Microscopy and Energy Dispersive X-ray Spectroscopy

In order to investigate fiber morphology, a JEOL JSM 820 scanning electron microscope at the Microscopic and Chemical Analysis Research Center (MARC) in the Department of Geological Sciences, The Ohio State University, was used. An accelerating voltage of 20 keV was used and the instrument was operated in the secondary electron mode. Analysis of the elemental composition of observed fiber morphological features and surface contaminants was performed using an Oxford Instruments Group Link Analytical eXL with a high resolution Pentafet detector and windowless capability attached to the above microscope. Operating conditions were the same as those used for SEM.

Fiber samples for both SEM and EDS were mounted on carbon planchettes with carbon tape and sputter-coated with carbon using a Denton Vacuum Desk II coater. Between 5 and 10 individual fibers of each sample were mounted for examination.

SEM photographs were taken under a variety of magnifications in order to elucidate various morphological characteristics. When the fibers themselves were being analyzed using EDS, spectra were collected at a magnification of <1000x so as to obtain the average elemental composition of the entire sampling area. On the other hand, in analyzing surface encrustations and biofilms, a more focussed spot sampling of the specific characteristic was performed at higher magnifications.

X-Ray Diffraction

Despite the larger sample size requirements of the diffractometric method of x-ray analysis, this method was chosen over the use of a diffraction camera due to better
measurement accuracy of the former (79) and due to sample packing constraints posed by the latter technique. The diffractometer, housed in the Department of Agronomy, The Ohio State University, consists of a Philips Electronics PW 1316/90 wide range goniometer with XRG 3100 x-ray generator, DMS-41 measuring system, theta compensating slit, graphite monochromator, and copper target. Intensity data was collected between 5 and 70° 2θ, at increments of 0.05° 2θ. The operating parameters were 35 KeV, 20 mA, and dwell time per data point was 10 seconds. The spectral data pairs (2θ versus relative intensities) were recorded on a computer disk for future data manipulation.

Fiber sample preparation for the diffractometer involved aligning approximately 0.15g of fiber manually, lacerating them with a scalpel oriented at about 40° to the fiber axis in order to obtain short segments, and thoroughly mixing them in a sliding disk mill to facilitate a random sample orientation. The sample was then packed tightly onto a holder for analysis.

Differential Scanning Calorimetry

The thermal behavior of the specimens was studied using differential scanning calorimetry. A TA Model 9100 DSC unit in the Department of Consumer and Textile Sciences, The Ohio State University, was used for thermal analysis of the fibers. Analyses were performed in non-hermetically sealed aluminum pans in an inert nitrogen atmosphere. The temperature range was from ambient to 375° C and the samples were heated at the rate of 5° per minute, and TA Instruments Universal Analysis program was used to analyze the data.
To maximize contact between the pan and the sample, small circular swatches of fabric were placed flat in the pan. Care was taken to ensure that the pan bottom stayed flat while closing the pan. Sample size ranged between 5 and 10 milligrams. Five thermograms were collected of the modern silk. Paucity of sample and the destructive testing procedure permitted only single thermograms to be collected for each Marine Silk specimen, and two for each Historic Silk.

**Fourier Transform Infra-Red Microspectroscopy**

Chemical composition of the fibers was analyzed using an FT-IR microspectrometer in the Department of Chemistry, The Ohio State University. The system consists of a Bruker Equinox 55 IR spectrophotometer, with microscope attachment. The instrument is equipped with a Mercury Cadmium Telluride detector, and a nitrogen purge to reduce carbon dioxide and moisture absorption. The instrument operation parameters were as follows: 4.0 cm\(^{-1}\) resolution, 520 scans per spectrum, Blackman-Harris apodization function, zero-filling factor of 2, and the gain switch turned on. Data collection was done using a magnification of 15x and an aperture size of 03.mm, which resulted in a spot size of about 20 \(\mu\)m. OPUS IR spectroscopic software, version 2.2 was used for data collection and data analysis was performed using the Grams 386 software.

About 15-20 single fibers each approximately 15mm in length were mounted to lay across a 4x20mm slit in a heavy-duty cardboard frame. The fibers were held in place with double-sided tape. The portions of the fiber in the slit were gently flattened using a roller. Flattening was required to ensure that the sample covered the aperture and to minimize
diffraction effects. However this method was chosen over other flattening techniques like using a KBr window or a diamond cell, because it was considered less disruptive than those techniques.

For each specimen 50 spectra were collected and analyzed. These spectra were collected on 10 different fibers for each specimen, by sampling 5 spots along each fiber. Care was taken to ensure that spectra were collected in spots that were relatively free of surface encrustations, and that fibers of varying thickness were sampled. As the technique is non-destructive, and the sample size small, five fibers from each specimen type were sampled, and five spectra were collected at different locations along the length of each fiber. All spectra were collected in transmission mode.

Data analysis

Data analysis involved a combination of qualitative and quantitative approaches. The following section describes the data analysis approach used with each of the analytical methods previously described.

Optical Microscopy

Because no distinctive features were observed with optical microscopy, this technique was used only to measure fiber diameters. Diameter measurements were done on 10 fibers per sample at 5 points along the fiber length. After confirming data normality for each sample, one way analysis of variance (ANOVA) was used to test the null hypothesis of no difference between the different sample means. Additionally, Tukey's pairwise comparison procedure
was performed to determine the differences between different pairs of means. All statistical analyses were performed using Minitab, version 11.0.

Scanning Electron Microscopy and Energy Dispersive X-ray Spectroscopy

For SEM analysis, a checklist was developed to investigate specimen morphology. With respect to the morphology of the fibers themselves, the following checklist of phenomena were investigated: smooth fibers, longitudinal striations, pits on fiber surface, fibrils, surface cracks, deep cracks, mechanical fiber deformation, fiber flattening, and the formation of fibrils. Smooth fiber surface refers to fibers that do not display any of the other morphological characteristics in the checklist. Longitudinal striations are defined as ridge-like surface characteristics in the direction of the fiber axis. The spacing between these striations has been qualitatively estimated as being regular or irregular, based on the distance between the ridges. Surface pitting phenomena refer to areas on the fiber that appear to have been eaten away. Cracks on the fibers are classified as being either surface cracks or deep cracks. Surface cracks are restricted to the fiber surface, while deep cracks penetrate the fiber bulk. Mechanical fiber deformation refers to fibers that appear to have been twisted and distorted. Fiber flattening indicates that the fiber is not rounded but rather has a flattened appearance.

In addition to the above characteristics that relate to the fibers themselves, two morphological characteristics extraneous to the fibers were investigated: surface deposits and biofilms. In the research reported herein, while both these characteristics are external to the fibers, deposits are not smooth surfaced, while biofilms are smooth.
Analysis of the EDS spectra involved a semi-quantitative approach. Based on the computer generated elemental composition data, the following arbitrary concentration definitions suggested by Goldstein et al. (12) were used to classify constituents: major (>10 weight %), minor (1-10 weight %), and trace (<1 weight %).

X-Ray Diffraction

Diffractometric intensity data in the 5 and 70° 2θ range were recorded directly from the instrument via the electronic counter. In order to reduce the effects of random noise, a 11-point smoothing algorithm was performed over the entire spectral range prior to data analysis. Data analysis was performed based on the approach developed by Foreman and Jakes (7). A Fortran program was written in order to analyze the data. This section describes the data modeling technique, followed by the procedure used to estimate crystallite size, sample crystallinity, and unit cell dimensions.

Data modeling

Because textile fibers are semi-crystalline, x-ray spectra produced by these materials are composed of three parts, attributed to crystalline scattering, amorphous scattering, and background scattering. The background profile was determined by sampling intensities in the range of 2θ ≈ 6.75° and 68.75°, and fitting a straight line, $y = a_0 + a_1x$, to the sampled points.

While the crystalline component in fibers causes characteristic peaks in the diffraction pattern, the amorphous portion produces a less coherent scattering whose intensity lies between the background and the crystalline scattering. The amorphous scattering profile was obtained by fitting selected data points to a fifth order polynomial, $y = a_0 + a_1x + a_2x^2 + a_3x^3$
+ a_4x^4 + a_5x^5. For each diffractometric profile, 129-131 data points were sampled in the following $2\theta$ ranges: 6.75-7.25°, 12.5-12.00°, 35.75-36.00°, 50.00-53.00°, and 66.00-68.00°. Data pairs were selected in $2\theta$ ranges that were clearly non-crystalline following Hermans and Weidinger's (90) method. The correlation coefficient ($r^2$) for each fit was calculated.

The residual spectrum obtained after background and amorphous scattering profiles were subtracted from the original spectra was attributable to crystalline scattering, and consist of a series of overlapping peak profiles. Individual peak profiles were fit to Gaussian distributions as described by Foreman and Jakes (7) using the method of least squares. Where peaks were reasonably resolved, peak parameters were determined by sampling data pairs on either side of the peak maxima and fitting them to Gaussian profiles. In the silk spectra two maxima in the 18-22° $2\theta$ range cannot be satisfactorily resolved. As Marsh, Corey, and Pauling (41,42) suggest, it is necessary to separate the maxima empirically into its two components. For these unresolved peaks, data sampling was done on the peak arm that did not overlap and mirroring the data for the overlapping side. It is probable that the analyses reported for these reflections are less reliable than those reported for other reflections. Somashekarappa et al. (106) have also used this approach of assuming symmetric peak profile, and sampling data from where overlap with neighboring reflections is minimum.

From the sampled data pairs for each reflection, the center of the reflection ($\bar{2}\theta$) was determined as the mean $2\theta$ value of the distribution of intensity $I$ over the sampled range:

$$
\bar{2}\theta = \frac{\sum_i I_i(2\theta_i)}{\sum_i I_i}
$$

78
The precision of the peak center determination \( s_{2\theta} \) was estimated from the standard deviation of the mean:

\[
s_{2\theta} = \sqrt{\frac{\sum I_i (2\theta_i - 2\bar{\theta})^2}{\sum I_i}}
\]

Based on the position and intensity of the peak center, the peak data is fit to a Gaussian profile:

\[
I = a' e^{-b(2\theta_i - 2\bar{\theta})^2}
\]

Taking the natural logarithm of this equation \( \ln I = \ln a' - b(2\theta_i - 2\bar{\theta})^2 \) allows the best-fit Gaussian parameters \( a \) and \( b \) to be determined using a linear least squares fitting procedure, assuming that there is negligible error in \( 2\theta \) measurement, and assigning an arbitrary and constant error, \( \sigma_i = \sigma_y \), in intensity measurement:

\[
a = \frac{\sum x_i^2 \sum y_i - \sum x_i \sum x_i y_i}{n \sum x_i^2 - (\sum x_i)^2}
\]

\[
b = \frac{\sum x_i \sum y_i - n \sum x_i y_i}{n \sum x_i^2 - (\sum x_i)^2}
\]

where \( a = \ln a' \), \( x_i = (2\theta_i - 2\bar{\theta})^2 \), and \( y_i = \ln I_i \).
Based on the computed best fit parameters, the error in intensity measurement, \( s_{y}^{2} \), is recomputed based on error propagation theory as:

\[
\begin{align*}
\sigma_{y}^{2} &= \frac{(\sum y_{i})^{2} \sum x_{i}^{2} - 2 \sum x_{i} y_{i} \sum x_{i} y_{i} + n (\sum x_{i} y_{i})^{2}}{(n-2)(n \sum x_{i}^{2} - (\sum x_{i})^{2})}
\end{align*}
\]

Probable uncertainties in the estimates of \( a \) and \( b \), \( s_{a}^{2} \) and \( s_{b}^{2} \) respectively, are further computed as:

\[
\begin{align*}
\sigma_{a}^{2} &= \frac{\sum x_{i}^{2}}{n \sum x_{i}^{2} - (\sum x_{i})^{2}} \sigma_{y}^{2} \\
\sigma_{b}^{2} &= \frac{n}{n \sum x_{i}^{2} - (\sum x_{i})^{2}} \sigma_{y}^{2}
\end{align*}
\]

In order to estimate how the data conforms to the model, Pearson’s product-moment correlation coefficient \( (r) \) is computed as:

\[
\begin{align*}
\rho &= \frac{\sum x_{i} y_{i} - \frac{1}{n} (\sum x_{i} \sum y_{i})}{\sqrt{\sum x_{i}^{2} - \frac{1}{n} (\sum x_{i})^{2}} \sqrt{\sum y_{i}^{2} - \frac{1}{n} (\sum y_{i})^{2}}}
\end{align*}
\]

The Gaussian slope parameter \( b \) obtained from the fitting procedure described above is related to the spectral line breadth \( B \), defined as the full-width-at-half-maximum:

\[
B = 2 \sqrt{\frac{\ln 2}{b}}
\]
Based on the error propagation theory, the error in line breadth determination, $s_B$, evaluates to:

$$s_B = \pm b^{-3/2} s_b \sqrt{3.0462 \times 10^{-4} \times \ln 2}$$

**Crystallite size determination**

As detailed in Chapter 2, crystallite dimension perpendicular to a chosen crystallographic plane can be determined based on the work of Scherrer (101) and Warren and Briscoe (102). In this work instrumental line broadening was ignored, and the following expression used to determine crystallite size $\sigma$:

$$\sigma = \frac{K\lambda}{B \cos \theta}$$

Klug and Alexander (150) point out that, for small crystallite size, this practical approach of substituting observed line width for the corrected line width, allows accurate crystallite size estimations. Error in size determination $s_\sigma$ was estimated as follows:

$$s_\sigma = \pm \frac{K\lambda \sec \theta}{B} \sqrt{\frac{s_B^2}{B^2} + \left(s_\theta \tan \theta\right)^2}$$

where $s_\theta = \frac{1}{4} \sum (2\theta_i - 2\bar{\theta})^2$

Crystallite sizes were estimated perpendicular to Warwicker’s [200] and [120] planes.
Crystallinity determination

Percent crystallinity (%C) was estimated using the integrated intensity approach used by Foreman & Jakes (7):

\[ \%C = 100 \times \frac{I_{xt}}{(I_{xt} + I_{am})} \]

Amorphous intensity (\(I_{am}\)) was calculated by integrating the amorphous polynomial over the appropriate 2\(\theta\) range. The sum of the areas under the all the Gaussians is the total crystalline intensity (\(I_{xt}\)). Using the individual Gaussian peak parameters (\(a\) and \(b\)) for the crystalline peaks, the area \(A\) under each Gaussian was computed for all samples as:

\[ A = e^a \sqrt{\frac{\pi}{b}} \]

Unit cell dimension determination

The dimensions of the orthorhombic fibroin unit cell were determined by combining Bragg’s Law:

\[ \lambda = 2d \sin \theta \]

with the plane-spacing equation applicable to orthorhombic crystals:

\[ \frac{1}{d^2} = \frac{h^2}{a^2} + \frac{k^2}{b^2} + \frac{l^2}{c^2} \]

where \(d\) is the interplanar spacing, \(\theta\) is the Bragg angle, \(\lambda\) is the wavelength of incident radiation, and \(a\), \(b\), and \(c\) are the unit cell dimensions. The \(d\)-value of each of the
crystalline reflections was first computed from Bragg’s Law, and then the cell dimensions were calculated based on the spacing equation above. The \( a \), \( b \), and \( c \) dimensions calculated in this work correspond to the dimensions of Warwicker (32) in which these dimensions correspond to the intersheet, interchain, and fiber axis respectively. The \( a \) dimension was computed from the \([200]\) and \([300]\) reflections, \( b \) dimension was computed from the \([040]\) reflection, and the \( c \) dimension was computed from the \([103]\) reflection.

**Fourier Transform Infra-Red Microspectroscopy**

As mentioned in the earlier sections of this chapter, 50 FT-IR spectra were collected for each specimen. This section discusses the qualitative and quantitative analysis of the data in the following order: qualitative comparison of spectra of a given specimen across different spots; band assignments and qualitative comparison of reference, marine, and historic silk specimens using standard absorbance and second derivative spectra for peak picking; and quantitative assessment of crystalline component using spectral deconvolution.

**Intra-specimen spectral comparison**

Similarity of spectra collected from a single fiber was visually estimated by superimposing the spectra. In order to estimate the intra-specimen spectral variation, average and standard deviation spectra were computed over the 50 spectra for each specimen.

**Band Assignment and Inter-specimen spectral comparison**

Two methods of peak picking were used in this work: the standard peak picking method and peak picking using the second derivative spectra. The standard peak picking method evaluates the \( x \)-values of the interpolated maxima of the absorbance spectra. An
absorbance threshold of 2% was used in this technique. Peak picking using the second derivative method evaluates absorbance maxima from peak minima in the second derivative spectra. This is particularly useful in the identification of strongly overlapping bands and weak shoulder bands. A threshold of 3% and a 9 data point smoothing value was used.

Peaks were assigned to atomic motions of the main chain bonds, the highly localized side chains of the protein amino acids, and to specific amino acid sequences based on reported literature. The presence or absence of these bands was qualitatively compared across specimens.

**Crystallinity measurement using infrared peak ratios**

The ratio of the absorbance intensities of the infrared bands at 1265 and 1235 cm\(^{-1}\) is most commonly used in fibroin crystallinity estimations (100,128,130). The band at 1235 cm\(^{-1}\) is assigned to the Amide III mode of the random coil conformation, and the corresponding band of the β-sheet conformation has been identified at 1265 cm\(^{-1}\) (116). Since these two peaks were not well resolved in the spectra under consideration, intensity measurement of the two peaks was difficult. In order to alleviate this problem, a Fourier-self-deconvolution was performed in the 1800-800 cm\(^{-1}\) region of the spectrum, which aided in resolving the two broad peaks into narrower ones. The boundaries of the range to be deconvoluted were chosen so that the intensities at the boundaries are close to zero (151). Deconvolution assumes that the measured spectrum is comprised of a series of narrow lines convolved by a common line-broadening factor (LBF) whose effect can be mathematically removed by multiplying the spectrum with a deconvolution function which is the inverse Fourier-transform of the LBF.
A Lorentzian LBF was used in the deconvolution as suggested by Kauppinen et al. (133). A deconvolution factor of 100 was chosen in order to achieve a net amplification of 3.4. This value was chosen after trials with deconvolution factors of 50, 100 and 1000 in the manner recommended by Kauppinen et al. (133). With a factor of 50, under-deconvolution is observed, while a factor of 1000 results in over-deconvolution and negative side-lobes. The deconvolution function amplifies the noise as well, and in order to reduce the noise, apodisation with a Blackman function is performed in conjunction with the deconvolution over the fraction of the interferogram specified by a noise reduction factor. A noise reduction factor of 0.5 was chosen. While the intensities of the deconvoluted peaks are higher than those in the original spectra, the relative peak intensities in the deconvoluted spectra remain the same as in the original (151).

Relative intensities of the peaks at 1235 and 1265 cm\(^{-1}\) were estimated by drawing a local baseline and measuring the peak heights on the deconvoluted spectra. The crystallinity ratio \(A_{1265 \text{ cm}^{-1}} / A_{1235 \text{ cm}^{-1}}\) was computed for each spectrum, and the average and standard deviation computed for each specimen. After confirming data normality for each sample, one way analysis of variance (ANOVA) was used to test the null hypothesis of no difference between the different sample means. Additionally, Tukey’s pairwise comparison procedure was performed to determine the differences between different pairs of means. All statistical analyses were performed using Minitab, version 11.0.
Differential Scanning Calorimetry

The glass transition temperature ($T_g$) and the degradation temperature ($T_d$) were studied using differential scanning calorimetry. The automatic transition analysis option of the TA Instruments Universal Analysis program was used to determine $T_g$. This option fits three tangents to the data curve within the limits specified by the operator. In measuring $T_d$, the onset temperature and the peak temperature of the degradation transition were determined using the TA Instruments Universal Analysis program.
CHAPTER 4

PRESENTATION OF RESULTS

This chapter presents the results of the analytical techniques employed in understanding the physical and chemical microstructure of the marine, historic, and reference silk textile artifacts. Morphological data based on Optical Microscopy (OM) and Scanning Electron Microscopy (SEM) are discussed in the first part of this chapter. The second section describes the elemental composition of the fibers and deposits on them obtained from Energy Dispersive X-Ray Spectroscopy (EDS). This is followed by the results obtained from x-ray diffraction (XRD), including crystallite size and percent crystalline composition. Glass transition temperature and decomposition temperature estimates obtained from Differential Scanning Calorimetry (DSC) are discussed in the fourth part of the chapter. Fourier Transform Infrared Microspectroscopic (FTIR) results are presented in the final section of the chapter.

Optical microscopy

Since optical microscopy offered little evidence of morphological changes between different specimens, this was not the technique of choice in investigating fiber morphology.
However, as mentioned in Chapter 3, optical microscopy was used in this work to investigate fiber diameters. Table 4.1 shows the average and standard deviation fiber diameters in the Reference, Historic, and Marine Silk specimens.

<table>
<thead>
<tr>
<th>Specimen ID</th>
<th>Average (µm)</th>
<th>Standard Deviation(µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>13.383</td>
<td>1.103</td>
</tr>
<tr>
<td>Marine 29049</td>
<td>14.035</td>
<td>1.172</td>
</tr>
<tr>
<td>Marine 29054</td>
<td>13.386</td>
<td>1.177</td>
</tr>
<tr>
<td>Marine 33707</td>
<td>14.048</td>
<td>1.140</td>
</tr>
<tr>
<td>Historic 88b</td>
<td>13.403</td>
<td>1.030</td>
</tr>
<tr>
<td>Historic 145b</td>
<td>13.368</td>
<td>1.060</td>
</tr>
<tr>
<td>Historic 145c</td>
<td>13.347</td>
<td>0.996</td>
</tr>
</tbody>
</table>

Table 4.1 Average and standard deviation values of fiber diameter measurements for Reference, Marine, and Historic Silk specimens.

After confirming data normality for each sample, one way analysis of variance (ANOVA) was used to test the null hypothesis of no difference between the different specimen means. Additionally, Tukey’s pairwise comparison procedure was performed to determine the differences between different pairs of means. Table 4.2 shows the results from the ANOVA and Tukey’s procedures. Based on the ANOVA p-value of 0.000 the null hypothesis of no difference between means is rejected. Based on Tukey’s pairwise comparisons procedure, the fiber diameters of the Marine Silk 29049 and 33707 do not differ
significantly from each other. However, these two specimens do differ significantly from the fiber diameters of the Reference specimen, Historic specimen 145b and 145c, and Marine Silk 29054. Additionally, 29049 and 33707 do not exhibit significant differences in fiber diameter as compared to Historic Silk 88b. No other significant fiber diameter differences are observed.

Table 4.2 ANOVA and Tukey’s pairwise comparison of fiber diameter measurements.
Scanning electron microscopy

While optical microscopy was useful for fiber diameter measurement, only limited morphological information was obtained from it. As scanning electron microscopy allows higher resolutions of $10^{-7}$ to $10^{-9}$ cm in comparison with optical techniques (65), this technique was used to investigate the morphology of fibers and surface deposits. Several features were observed in the different specimens studied, including smooth fiber surfaces, longitudinal striations on fiber surfaces, signs of severe mechanical damage as evidences by fiber distortion, fiber cracking, pitting, flattening, and fibrillation, external surface occlusions, and indications of microbiological activity. The following paragraphs describe the results of the morphological examination of fibers and extraneous deposits by SEM, which are summarized in Table 4.3.

Reference Silk

The fiber surfaces in Reference Silk fibers are smooth and rounded (Figure 4.1 and 4.2) and show none of the signs of degradation or morphological alteration like cracking, flattening, surface pitting, etc. displayed by the Historic and Marine Silk fibers. Fiber surfaces are clean of external encrustations and show no evidence of microbial activity. Examination of the fiber surface at high magnification did not reveal any evidence of longitudinal striations.
Table 4.3 Summary of morphological characteristics observed in Reference, Historic, and Marine Silk fibers using scanning electron microscopy.

Historic Silk

In the majority of fibers examined from Historic Silk 88b, Historic Silk 145b, and Historic Silk 145c, fiber surfaces are smooth and rounded (Figure 4.3) and show no signs of fiber damage under the SEM. These fibers resemble the Reference specimen closely. Occasionally however, the Historic Silk fibers do reveal some alteration in morphology. For example, Figure 4.4 shows signs of surface cracking Historic Silk 145c, and Figure 4.5 shows
surface pitting in Historic Silk 88b. Fiber morphology alteration is not consistently seen in all Historic Silk specimens. A small amount of surface deposit is seen in Historic specimens 88b and 145c. This deposit appears to be selective and is restricted to a few fiber surfaces (Figure 4.6 and 4.7). Morphologically, the deposits found on the Historic Silks appear to be flat-faceted and crystalline. The elemental composition of this feature is discussed in the following section. Specimen 145b did not have any surface deposits.

The most characteristic feature observed extensively on all Historic Silk specimens is the evidence of microbiological activity. While the nature of this activity is unknown, SEM examination reveals the presence of biofilms on individual fiber surfaces along the fiber length. Figures 4.8, 4.9, and 4.10 show evidence of microbial activity on Historic Silks. This feature appears like a smooth continuous covering on small sections along the fiber length. In some areas the formation closely follows the fiber dimensions (Figure 4.8), but in other places it appears as a swollen bulbous feature extending beyond the fiber diameter (Figure 4.9 and 4.10).

Marine Silk

In comparison with the other two sample types described above, these fibers reveal the most alteration in fiber morphology. However, the characteristics described in the following paragraphs are not present uniformly across the different Marine samples, or even in all areas of the same sample. While parts of the specimens examined appear very similar to the Reference Silk, some areas reveal considerable morphological alteration and degradation, along with extensive deposits on the fiber surface. A few fibers in all three Marine samples
show signs of severe mechanical deformation (Figure 4.11). In addition to this, some fibers appear considerably more twisted than Historic or Reference Silk fibers. Some fibers in Marine Silk 29054 are flattened (Figure 4.12). This characteristic was not observed in specimens 29049 and 33707.

In terms of fiber surface alterations, longitudinal striations are observed along the fiber length in all three Marine Silk specimens. These striations sometimes appear to have regular spacing between them (Figure 4.13), and sometimes consist of a set of irregularly spaced striations (Figure 4.14). Two different cracking patterns are observed in the Marine specimens. The first type of cracking, which appears restricted to the surface of the fibers, is observed only in Marine Silk 29054 (Figure 4.15). It is characterized by vertical and horizontal cracks with respect to the fiber axis. As this feature was observed only occasionally, no quantitative estimation of the crack spacing was made. The second type of cracking penetrates deeper into the fiber bulk. This type of cracking is observed in two areas of the specimens. First, this type of cracking is seen where fiber twisting occurs and appears to be a consequence of the twisting. This is observed in Marine silk 29049 (Figure 4.16) and 33707 (Figure 4.17). These cracks are approximately parallel to the fiber axis and are of varying lengths between 1-15 μm. The second area where this cracking phenomenon is observed in all three Marine samples (Figure 4.18, 4.19, and 4.20) is in areas that are characterized by heavy surface deposits. As previously pointed out, the second type of cracking phenomena penetrate the fiber more deeply than the first cracking pattern, which appears restricted to the fiber surface.
One feature observed in Marine Silk that is not seen in Historic specimens is the occurrence of fibrillation. While moderate amounts of this characteristic are observed in Marine Silk 33707 (Figure 4.21), this feature is seen only occasionally in Marine Silk 29049 (Figure 4.22) and Marine Silk 29054.

The extent of extraneous deposits on the Marine specimens varies widely. Some areas are almost completely free of deposits, others have moderate amounts of deposits, and some areas are so heavily covered that the fiber surface itself is not visible. Of the Marine Silks, 29049 and 33707 have the most deposits, and 29054 has fewer deposits. In comparison with the Historic Silks however, all Marine Silk have more deposits. These deposits can be categorized into three groups. The first is a continuous paste-like deposit with small beaded structures less than 0.5 μm in diameter on its surface (Figure 4.23). These deposits are similar to those reported by Chen (8) and Jakes and Wang (5) on cellulosic fibers from the SS Central America. Second, discrete cube shaped particles of varying size up to 1.5 μm in diameter that appear crystalline (Figure 4.24). Chen (8) also found this type of particle in cotton fibers from the same site. Third, irregular shaped discrete particles approximately 2 to 2.5 μm in diameter were observed occasionally (Figure 4.23 and 4.25).

Microbial growth as evidenced by biofilm formation similar to that described in the Historic Silks is observed in all the Marine Silks but to a lesser extent. Figure 4.26 shows biofilm formation on Marine Silk 33707.
Figure 4.1 Reference Silk fibers with smooth rounded surfaces and no surface defects, striations, or deposits.

Figure 4.2 Reference Silk fibers with smooth rounded surfaces and no surface defects, striations, or deposits.

Figure 4.3 Historic Silk 145c with smooth rounded surfaces and no surface defects, striations, or deposits.

Figure 4.4 Historic Silk 145b with rounded surfaces and initial signs of surface cracks.
Figure 4.5 Historic Silk 88b with occasional surface deposits and surface pitting phenomenon.

Figure 4.6 Historic Silk 145c with surface deposits on some fiber surfaces.

Figure 4.7 Historic Silk 88b with occasional surface deposits.

Figure 4.8 Historic Silk 145c with biofilms covering fiber surfaces, indicating microbial activity.
Figure 4.9 Historic Silk 88b with detailed view of biofilm covering fiber surface.

Figure 4.10 Historic Silk 145b with detailed view of biofilm covering fiber surface.

Figure 4.11 Marine Silk 29054 displaying mechanical deformation of fibers.

Figure 4.12 Marine Silk 29054 with occasional fiber flattening, and areas of fiber relatively free of surface deposits.
Figure 4.13 Marine Silk 29049 displaying irregularly spaced longitudinal striations along the fiber axis.

Figure 4.14 Marine Silk 33707 displaying regularly spaced longitudinal striations along the fiber axis and few surface deposits.

Figure 4.15 Marine Silk 29054 displaying surface cracking patterns on the fiber surface.

Figure 4.16 Marine Silk 29049 displaying deep cracks of varying lengths in areas of fiber twisting.
Figure 4.17 Marine Silk 33707 displaying deep cracks along areas of fiber twisting.

Figure 4.18 Marine Silk 29054 displaying deep cracks and extensive surface deposits.

Figure 4.19 Marine Silk 33707 displaying deep cracks and extensive surface deposits.

Figure 4.20 Marine Silk 29049 displaying a deep crack and some surface deposits.
Figure 4.21 Marine Silk 33707 showing the formation of fibrils.

Figure 4.22 Marine Silk 29049 showing the formation of fibrils.

Figure 4.23 Marine Silk 33707 displaying continuous and occasional irregular discrete deposits.

Figure 4.24 Marine Silk 29049 displaying extensive continuous and discrete surface deposits.
Figure 4.25 Marine Silk 29054 displaying irregular discrete surface deposits.

Figure 4.26 Marine Silk 33707 exhibiting biofilm on fiber surface.
Energy dispersive x-ray spectroscopy

When used in conjunction with scanning electron microscopy, energy dispersive x-ray spectroscopy provides compositional information regarding the observed morphological characteristics. This section discusses the results obtained from the EDS analysis of the morphological characteristics described in the previous section. EDS spectra of the fibers were collected at magnifications of 1000x in areas that were free of encrustations and biofilms. None of the fibers displayed any significant inorganic elemental composition (Figure 4.27 to Figure 4.33).

Figure 4.27 EDS of Reference Silk free of surface deposits and biofilms.
Figure 4.28 EDS of Historic Silk 88b free of surface deposits and biofilms.

Figure 4.29 EDS of Historic Silk 145b free of surface deposits and biofilms.
Figure 4.30 EDS of Historic Silk 145c free of surface deposits and biofilms.

Figure 4.31 EDS of Marine Silk 29054 free of surface deposits and biofilms.
Figure 4.32 EDS of Marine Silk 29054 free of surface deposits and biofilms.

Figure 4.33 EDS of Marine Silk 33707 free of surface deposits and biofilms.
EDS spot spectra of the discrete occlusions on the marine and historic fibers contained the following elements in different combinations and concentrations: Mg, Si, P, S, Cl, K, Mn, Fe, Sn, Al, Na, and Zn. Table 4.4 summarizes the elemental composition of the following morphological characteristics: encrustations on the historic silk fibers (Figure 4.34 to Figure 4.36); cube shaped discrete encrustations (Figure 4.37 and Figure 4.38), irregular shaped encrustations (Figure 4.39 and Figure 4.40), and continuous paste-like encrustation (Figure 4.41) on the marine fibers.

<table>
<thead>
<tr>
<th>Element</th>
<th>Specimen/ Morphological Characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>L</td>
</tr>
<tr>
<td>Ca</td>
<td>M</td>
</tr>
<tr>
<td>Cl</td>
<td>S</td>
</tr>
<tr>
<td>Fe</td>
<td>M</td>
</tr>
<tr>
<td>K</td>
<td>M</td>
</tr>
<tr>
<td>Mg</td>
<td>M</td>
</tr>
<tr>
<td>Na</td>
<td>L</td>
</tr>
<tr>
<td>P</td>
<td>S</td>
</tr>
<tr>
<td>S</td>
<td>M</td>
</tr>
<tr>
<td>Si</td>
<td>L</td>
</tr>
<tr>
<td>Sn</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>L</td>
</tr>
</tbody>
</table>

L indicates major constituent; M indicates minor constituent; S indicates trace constituent.

Table 4.4 Summary of elemental composition of surface encrustations observed on Historic, and Marine Silk fibers.
Figure 4.34 EDS of deposits on Historic Silk 88b.

Figure 4.35 EDS of deposits on Historic Silk 88b.
Figure 4.36 EDS of deposits on Historic Silk 145c.

Figure 4.37 EDS of discrete cubic deposits on Marine Silk 29049.
Figure 4.38 EDS of discrete cubic deposits on Marine Silk 29054.

Figure 4.39 EDS of discrete irregular deposits on Marine Silk 33707.
Figure 4.40 EDS of discrete irregular deposits on Marine Silk 33707.

Figure 4.41 EDS of continuous paste-like deposits on Marine Silk 33707.
As indicated in Table 4.4 and Figure 4.41, the continuous paste-like deposit observed on the marine specimen did not contain the mineral constituents that the other surface deposits are composed of. The cubic encrustations observed in Marine Silk 29049 and 29054 contain the following major constituents: Na, Mg, S, Zn. They both also contain Al, Cl, K, and Si in smaller proportions. The difference between the EDS spectra of these two specimens is that while 29049 contains Fe as a minor constituent, 29054 does not contain this element. The irregular discrete occlusions observed on Marine Silk 33707 is composed of Cl, Fe, Na, and S as major constituents, Mg as a minor constituent, and contains Al, P, and Sn in trace concentrations. These occlusions differ from the cubic encrustations in their high concentration of Fe, and the fact that they do not contain significant amounts of Zn. Additionally, of all the specimens examined these are the only encrustations that contain Sn, albeit in trace amounts.

The encrustations observed on Historic Silk 88b and 145c both contain major amounts of Al, Mg, Na, S, and Si, and trace concentrations of Cl. Additionally, Historic Silk 88b also contains minor concentrations of Ca, Fe, and K, not observed in Historic Silk 145c. In contrast to the cubic encrustations observed in Marine Silk 29049 and 29054, the encrustations on the Historic Silk specimens do not contain Zn. As mentioned in the previous paragraph, Zn is a major component of the cubic occlusions. While the relative amounts of each element differ between the encrustations observed on Historic Silk and the irregular discrete occlusions observed on Marine Silk 33707, both contain Al, Cl, Fe, Mg, Na, and S. The difference lies in the fact that Marine Silk 33707 contains trace amounts of Sn, which is
not present in the Historic Silk specimens. Also, Historic Silk 88b contains minor amounts of Ca and K, not present in Marine Silk 33707.

**FTIR microspectroscopy**

FT-IR microspectroscopy was employed to compare protein conformation of the fibers exposed to the marine environment with that of reference silk and historic silk not exposed to the deep ocean environment. Additionally, through the analysis of the IR absorption bands, changes in the chemistry of the fibers were monitored. This section presents the results of this technique in the following order: qualitative comparison of spectra of a given specimen across different spots; qualitative comparison of band assignments for reference, marine, and historic silk specimens using standard absorbance and second derivative spectra for peak picking; and quantitative assessment of crystalline component using spectral deconvolution.

**Intra-specimen spectral comparison**

Spectra of spots collected along the same fiber show little or no variation, both with respect to band positions and intensities. Similarity of spectra collected from a single fiber was visually estimated by superimposing the spectra. When spectra from different fibers for any given specimen were analyzed in a similar manner, they did show some variation with respect to peak intensities. Peak positions were fairly consistent across spectra for each specimen, though some variation in peak sharpness was noted. The peak sharpness was especially variable in the peak at 3298 cm⁻¹. In order to estimate the intra-specimen spectral variation,
average and standard deviation spectra were computed over the 50 spectra for each specimen. Figure 4.42 to 4.48 shows the average and standard deviation spectra for the Reference, Historic, and Marine Silks. The standard deviation for all samples is fairly low, indicating that spectral variability within a specimen is small. However, the extent of deviation varies across the spectral range, and is higher in specific areas of the spectra. In particular, the 3400-3200 cm\(^{-1}\) and 1700-1500 cm\(^{-1}\) wavenumber ranges show maximum variability.

Comparing the standard deviation spectra of the specimens indicates that two of the Marine Silk artifacts, 29054 and 33707, exhibit more variability than the others particularly in the 1500-880 cm\(^{-1}\) range. The third marine specimen, 29049, and all three Historic Silks, 145b, 145b, and 88b, exhibit the least variability across fibers. In these samples maximum variability was in the 3400-3200 cm\(^{-1}\) region, and there was little variability across the rest of the spectral range. The standard deviation spectrum of Reference Silk indicates intermediate variability in comparison with the other two groups.
Figure 4.42 Average and standard deviation absorbance spectra of Reference Silk.
Figure 4.43 Average and standard deviation absorbance spectra of Historic Silk 88b.
Figure 4.44 Average and standard deviation absorbance spectra of Historic Silk 145b.
Figure 4.45 Average and standard deviation absorbance spectra of Historic Silk 145c.
Figure 4.46 Average and standard deviation absorbance spectra of Marine Silk 29049.
Figure 4.47 Average and standard deviation absorbance spectra of Marine Silk 29054.
Figure 4.48 Average and standard deviation absorbance spectra of Marine Silk 33707.
Band Assignment and Inter-specimen spectral comparison

The two peak picking techniques used in this work are the standard peak picking method and peak picking using the second derivative spectra. All peaks identified in the standard peak picking technique were also identified with the second derivative method. Additional peaks were also identified using the second derivative technique. Figures 4.49 to 4.54 show the second derivative spectra of the Historic and Marine specimens in comparison to the Reference silk spectrum. Table 4.5 lists the results of both the standard and the second derivative peak picking method for the Reference, Marine, and Historic silk fibers. All peaks were consistently present in all three groups of spectra, and peak positions in the spectra of the Historic and Marine fibers varied from the peak positions in the Reference fibers over a ±10 cm$^{-1}$ wavenumber range. The variability between the spectra collected in this work and the peaks identified for silk fibroin in the literature was about ±5 cm$^{-1}$ wavenumbers. In comparing the peak positions with literature values for proteins other than fibroin, variability was higher, and differed up to ±6 cm$^{-1}$ wavenumbers in the standard peak picking method, and up to ±8 cm$^{-1}$ wavenumbers in the second derivative peak picking method. The following paragraphs discuss the bands observed in the spectra obtained from different specimens.
Figure 4.49 Second derivative spectrum of Reference and Historic Silks (3400-2750cm⁻¹).

Figure 4.50 Second derivative spectrum of Reference and Marine Silks (3400-2750cm⁻¹).
Figure 4.51 Second derivative spectrum of Reference and Historic Silks (1800-1200 cm\(^{-1}\)).

Figure 4.52 Second derivative spectrum of Reference and Marine Silks (1800-1200 cm\(^{-1}\)).
Figure 4.53 Second derivative spectrum of Reference and Historic Silks (1200-600cm\(^{-1}\)).

Figure 4.54 Second derivative spectrum of Reference and Marine Silks (1200-600cm\(^{-1}\)).
Table 4.5 Infrared peak identification and assignment using standard and second derivative peak picking techniques.

<table>
<thead>
<tr>
<th>Band Assignment</th>
<th>Reference Silk 29049</th>
<th>Marine Silk 29054</th>
<th>Marine Silk 33707</th>
<th>Historic Silk 88b</th>
<th>Historic Silk 145b</th>
<th>Historic Silk 145c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S (cm(^{-1}))</td>
<td>SD (cm(^{-1}))</td>
<td>S (cm(^{-1}))</td>
<td>S (cm(^{-1}))</td>
<td>S (cm(^{-1}))</td>
<td>S (cm(^{-1}))</td>
</tr>
<tr>
<td>NH stretching (\beta) sheet</td>
<td>3296</td>
<td>3286</td>
<td>3296</td>
<td>3284</td>
<td>3295</td>
<td>3292</td>
</tr>
<tr>
<td></td>
<td>3296</td>
<td>3286</td>
<td>3296</td>
<td>3284</td>
<td>3295</td>
<td>3292</td>
</tr>
<tr>
<td>NH stretching (\beta) sheet</td>
<td>3080</td>
<td>3079</td>
<td>3081</td>
<td>3076</td>
<td>3083</td>
<td>3072</td>
</tr>
<tr>
<td></td>
<td>3080</td>
<td>3079</td>
<td>3081</td>
<td>3076</td>
<td>3083</td>
<td>3072</td>
</tr>
<tr>
<td>CH stretching</td>
<td>2979</td>
<td>2981</td>
<td>2980</td>
<td>2983</td>
<td>2980</td>
<td>2983</td>
</tr>
<tr>
<td></td>
<td>2979</td>
<td>2981</td>
<td>2980</td>
<td>2983</td>
<td>2980</td>
<td>2983</td>
</tr>
<tr>
<td>CH stretching</td>
<td>2935</td>
<td>2934</td>
<td>2935</td>
<td>2935</td>
<td>2935</td>
<td>2935</td>
</tr>
<tr>
<td></td>
<td>2935</td>
<td>2934</td>
<td>2935</td>
<td>2935</td>
<td>2935</td>
<td>2935</td>
</tr>
<tr>
<td>CH stretching</td>
<td>2875</td>
<td>2875</td>
<td>2875</td>
<td>2875</td>
<td>2875</td>
<td>2875</td>
</tr>
<tr>
<td>Amide I random coil</td>
<td>1700</td>
<td>1699</td>
<td>1699</td>
<td>1699</td>
<td>1699</td>
<td>1699</td>
</tr>
<tr>
<td>Amide I (\beta)-sheet</td>
<td>1663</td>
<td>1663</td>
<td>1663</td>
<td>1663</td>
<td>1663</td>
<td>1663</td>
</tr>
<tr>
<td>Amide I (\beta)-sheet</td>
<td>1648</td>
<td>1644</td>
<td>1644</td>
<td>1644</td>
<td>1645</td>
<td>1645</td>
</tr>
<tr>
<td>Amide I (\beta)-sheet</td>
<td>1633</td>
<td>1629</td>
<td>1629</td>
<td>1630</td>
<td>1631</td>
<td>1630</td>
</tr>
<tr>
<td>Amide II (\beta)-sheet</td>
<td>1555</td>
<td>1553</td>
<td>1554</td>
<td>1554</td>
<td>1555</td>
<td>1554</td>
</tr>
<tr>
<td>Amide II random coil</td>
<td>1539</td>
<td>1535</td>
<td>1536</td>
<td>1537</td>
<td>1538</td>
<td>1537</td>
</tr>
<tr>
<td>Amide II random coil</td>
<td>1519</td>
<td>1519</td>
<td>1520</td>
<td>1520</td>
<td>1519</td>
<td>1520</td>
</tr>
<tr>
<td>Mode of (p)-disubstituted benzene ring (tyrosine)</td>
<td>1519</td>
<td>1519</td>
<td>1519</td>
<td>1519</td>
<td>1519</td>
<td>1519</td>
</tr>
<tr>
<td>CH(_{3}) modes of alanine</td>
<td>1448</td>
<td>1440</td>
<td>1449</td>
<td>1450</td>
<td>1449</td>
<td>1450</td>
</tr>
<tr>
<td>CH(_{3}) modes of alanine</td>
<td>1410</td>
<td>1409</td>
<td>1411</td>
<td>1412</td>
<td>1412</td>
<td>1411</td>
</tr>
<tr>
<td>Amide III (\beta)-sheet</td>
<td>1266</td>
<td>1265</td>
<td>1266</td>
<td>1265</td>
<td>1265</td>
<td>1265</td>
</tr>
<tr>
<td>Amide III (\beta)-sheet</td>
<td>1233</td>
<td>1223</td>
<td>1234</td>
<td>1232</td>
<td>1232</td>
<td>1232</td>
</tr>
<tr>
<td>CH(_{3}) modes of alanine</td>
<td>1168</td>
<td>1169</td>
<td>1168</td>
<td>1170</td>
<td>1168</td>
<td>1169</td>
</tr>
<tr>
<td>CH(_{3}) modes of alanine</td>
<td>1069</td>
<td>1071</td>
<td>1070</td>
<td>1072</td>
<td>1070</td>
<td>1073</td>
</tr>
<tr>
<td>Ala-gly and gly-ala</td>
<td>997</td>
<td>997</td>
<td>997</td>
<td>997</td>
<td>997</td>
<td>996</td>
</tr>
<tr>
<td>Ala-gly and gly-ala</td>
<td>975</td>
<td>975</td>
<td>975</td>
<td>976</td>
<td>976</td>
<td>975</td>
</tr>
<tr>
<td>Amide IV (\beta)-sheet</td>
<td>701</td>
<td>701</td>
<td>698</td>
<td>700</td>
<td>697</td>
<td>702</td>
</tr>
<tr>
<td>Amide IV random coil</td>
<td>634</td>
<td>635</td>
<td>633</td>
<td>632</td>
<td>634</td>
<td>634</td>
</tr>
</tbody>
</table>

\(^{1}\)S = Standard spectrum; \(^{2}\)SD = Second derivative spectrum.
Band at 3300 cm\(^{-1}\). This band is observed in all specimens both in the absorbance and the second derivative spectra. In the absorbance spectra this band is located in the 3295-3296 cm\(^{-1}\) region, while in the second derivative spectra it is located in the 3286-3292 cm\(^{-1}\) region. This peak arises from the NH stretching vibration of the peptide bond which is involved in hydrogen bonding (113), and is the most intense vibrational mode for all the specimens. For all the observed spectra, there is a broad shoulder on the higher wavenumber side of the band at 3300 cm\(^{-1}\), which has been attributed to adsorbed water (113). In spite of prolonged desiccation, prior to collecting the spectra, this shoulder was present. Asai et al. (113) comment that this bound water could not be removed from the poly-\(\beta\)-alanine in their work even after dehydration over concentrated sulfuric acid.

Band at 3080 cm\(^{-1}\). This band, which is also attributed to the hydrogen-bonded NH stretching vibration, is located at 3079-3082 cm\(^{-1}\) in the standard absorbance spectra, and at 3072-3080 cm\(^{-1}\) in the second derivative spectra. The band is observed in all the specimens.

Bands at 3000-2800 cm\(^{-1}\). The CH stretching vibrations contribute to the bands in this region (113). Bands at 2979-2980 cm\(^{-1}\) and 2934-2936 cm\(^{-1}\) are observed in the standard absorbance spectra of the Reference, Marine, and Historic specimens. When peak picking is done using the second derivative spectra, a peak at 2875-2876 cm\(^{-1}\) is observed in addition to the two peaks observed with the standard peak picking technique. This peak is also observed in all the spectra.

Bands at 1700-1630 cm\(^{-1}\). The spectra for the Reference, Historic, and Marine specimen showed no differences in this region. While the bands in this region are attributed to the Amide I vibrations, their exact position is sensitive to protein conformation. The Amide
I bands arise from the CO stretching, NH in-plane bending, and CN stretching modes (112). While standard peak picking yields only one peak in this region, 1644-1648 cm\(^{-1}\) for all the specimens investigated, the second derivative technique resolves three peaks in this region. The peak in the 1700-1699 cm\(^{-1}\) region identified in all the spectra, arises from the non hydrogen-bonded Amide I vibrations associated with the random coil conformation. Two additional peaks at 1663 cm\(^{-1}\) and between 1629-1633 cm\(^{-1}\) are also identified in all spectra, and arise from the hydrogen-bonded Amide I vibrations of the β-sheet conformation. Since the effect of hydrogen bonding is to decrease the stretching frequencies and increase the bending frequencies (112), the observed positions of the random coil and β-sheet Amide I vibrations indicate a greater contribution from the CO and CN stretching modes, in comparison with the NH bending vibration. The primary vibrational contribution to this band comes from CO stretching (116).

Bands at 1560-1515 cm\(^{-1}\). The standard peak picking technique identifies only one band in this region, located at 1519-1520 cm\(^{-1}\) for all the specimens. However, three peaks are identified in this region when the second derivative technique is used. Two peaks, centered at 1555-1553 cm\(^{-1}\) and at 1539-1535 cm\(^{-1}\), are associated with the Amide II vibrations of the β-sheet and the random coil secondary conformations respectively. These bands, resulting primarily from NH in-plane bending, and with some contribution from CH stretching (112) are observed in all specimens.

The third peak identified using the second derivative peak picking technique is located at 1514 cm\(^{-1}\) in all the specimens. This peak is assigned to the stretching mode of the p-
disubstituted benzene ring in tyrosine (117). Bendit (117) reports a relatively strong band at 1515 cm\(^{-1}\) accompanied by weaker bands at 1615, 1595, and 1440 cm\(^{-1}\) for the tyrosine moiety. In the specimens studied however, the band at 1515 cm\(^{-1}\) is not discernable in the absorbance spectra, while the other bands attributed to tyrosine are not identified in either the absorbance or the second derivative spectra.

**Bands at 1450 and 1166 cm\(^{-1}\).** The standard absorbance and second derivative spectra of all the spectra exhibit a band between 1448 and 1451 cm\(^{-1}\), and another between 1168 and 1170 cm\(^{-1}\). These bands have been assigned to the CH\(_3\) modes of alanine (152). While Elliot and Malcolm (152) identify two absorbance bands in the 1447 to 1453 cm\(^{-1}\) region, only one band is observed in this region in the spectra collected in this work.

**Band at 1410 cm\(^{-1}\).** This band is identified in all specimens using both the standard and second derivative peak picking methods. In the standard method the peak is located between 1410 and 1412 cm\(^{-1}\), while in the second derivative method it is located between 1409 and 1413 cm\(^{-1}\). Researchers have assigned the band in this region to two different vibrational modes in proteins. Asai et al. (113) assign this and other bands in the 1480-1340 cm\(^{-1}\) region of fibroin and other proteins and polypeptides to various CH deformations. Fraser and Suzuki (116) assign a band at 1410 cm\(^{-1}\) to the ionized carboxyl COO\(^{-}\) stretching mode of aspartic acid. However, because of the low concentration of aspartic acid in fibroin (27) it is not likely that the carboxyl vibration produces the moderately intense peak seen in the fibroin spectra. Therefore the peak observed at 1410 cm\(^{-1}\) is assigned to the CH deformation suggested by Asai (113).
Bands at 1265 and 1235 cm⁻¹. A band in the 1231 to 1234 cm⁻¹ region is identified in both the standard absorbance as well as the second derivative spectra of all the specimens. This is attributed to the Amide III modes of the random coil conformation which arises primarily from the CN stretching mode, with minor contribution from the CH in plane bending mode. The corresponding band of the β-sheet conformation has been identified at 1265 cm⁻¹ (116) and is seen in the absorbance spectra of the specimens as a shoulder peak in the peak at 1235 cm⁻¹. However, this band is not identified using the standard peak picking method. Visual examination of this region shows that the shoulder is most pronounced in the spectra of the Reference Silk and the Historic Silk specimen 88b. Second derivative peak picking identifies this peak between 1265 and 1266 cm⁻¹ in all the specimens.

Band at 1068-1073 cm⁻¹. This band was identified in all the specimens using the second derivative technique, and in all but the Historic Silk 88b specimen using the standard peak picking method. While the literature does not assign this band in proteins and polypeptides to a specific vibration, Asai et al. (113) indicate that this band is present in fibroin and in the spectra of alanine-containing polypeptides. It is therefore possible that this band is related to the alanine moiety.

Bands at 996 and 976 cm⁻¹. These two bands are characteristic of the alanine-glycine and glycine-alanine structures (113), and are identified in the second derivative spectra of all the specimens.

Band at 700 cm⁻¹. This band, which is observed in the 697-702 cm⁻¹ region of both the standard and second derivative spectra of all the specimen, has been assigned to the Amide
Vibration of the β-sheet conformation of fibroin (124,125). The Amide V vibration is a result of the NH out of plane bending vibration (112).

Band at 630 cm⁻¹. The Amide IV band in the 630 cm⁻¹ region is attributed to the OCN out of plane bending (112). This band is not discernible in the standard absorbance spectra, but is identified in the second derivative spectra of all specimens in the 635-633 cm⁻¹ range.

Crystallinity measurement using infrared peak ratios

The ratio of the absorbance intensities of the infrared bands at 1265 and 1235 cm⁻¹ is most commonly used in fibroin crystallinity estimations (100,128,130). As mentioned in the previous section, the band at 1235 cm⁻¹ is assigned to the Amide III mode of the random coil conformation, and the corresponding band of the β-sheet conformation has been identified at 1265 cm⁻¹ (116).

In all the spectra, the peak at 1265 cm⁻¹ appears as a weak shoulder in the band at 1235 cm⁻¹. This made intensity measurement of the two peaks difficult. In order to alleviate this problem, a Fourier-self-deconvolution was performed in the 1800-800 cm⁻¹ region of the spectrum. Figure 4.55 to Figure 4.61 show the original absorbance spectra superimposed with the deconvoluted spectra of the Reference, Historic, and Marine specimens.
Figure 4.55 Original and deconvoluted spectra of Reference Silk between 1150-1350 cm\(^{-1}\).

Figure 4.56 Original and deconvoluted spectra of Marine Silk 29049 between 1150-1350 cm\(^{-1}\).
Figure 4.57 Original and deconvoluted spectra of Marine Silk 29054 between 1150-1350 cm$^{-1}$.

Figure 4.58 Original and deconvoluted spectra of Marine Silk 33707 between 1150-1350 cm$^{-1}$.
Figure 4.59 Original and deconvoluted spectra of Historic Silk 88b between 1150-1350 cm$^{-1}$.

Figure 4.60 Original and deconvoluted spectra of Historic Silk 145b between 1150-1350 cm$^{-1}$. 
Intensity measurements were performed using the method previously described on the 50 spectra collected for each specimen, and the ratio $A_{1265\text{cm}^{-1}}/A_{1235\text{cm}^{-1}}$ was computed for each spectrum. The average crystallinity ratio for the Reference silk specimen was calculated to be 65.215%, and this compares closely with the IR crystallinity values reported in the literature for *Bombyx mori* silk fibroin. For example, Freddi and coworkers (128,130) and Bhat and Nadiger (100) report values of about 66% for silk fibers by computing the ratio between the bands at 1265 and 1235 cm$^{-1}$. Average and standard deviation values of the crystallinity ratio for all samples is shown in Table 4.6.
Table 4.6 Average and standard deviation values of IR crystallinity ratios for Reference, Marine, and Historic Silk specimens.

<table>
<thead>
<tr>
<th>Specimen ID</th>
<th>Average</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>0.65215</td>
<td>0.07993</td>
</tr>
<tr>
<td>Marine 29049</td>
<td>0.59240</td>
<td>0.07559</td>
</tr>
<tr>
<td>Marine 29054</td>
<td>0.56033</td>
<td>0.06825</td>
</tr>
<tr>
<td>Marine 33707</td>
<td>0.57307</td>
<td>0.07196</td>
</tr>
<tr>
<td>Historic 88b</td>
<td>0.62593</td>
<td>0.06862</td>
</tr>
<tr>
<td>Historic 145b</td>
<td>0.57243</td>
<td>0.07283</td>
</tr>
<tr>
<td>Historic 145c</td>
<td>0.58227</td>
<td>0.07291</td>
</tr>
</tbody>
</table>

After confirming data normality for each sample, one way analysis of variance (ANOVA) was used to test the null hypothesis of no difference between the sample means. Additionally, Tukey’s pairwise comparison was performed to determine differences between different pairs of means. Table 4.7 shows results from the ANOVA and Tukey’s procedures. Based on the ANOVA p-value of 0.000 the null hypothesis of no difference between means is rejected. Based on Tukey’s pairwise comparisons procedure, the crystallinity of Reference Silk specimen differs significantly from the crystallinity of all Marine Silks and Historic Silk 145b and 145c, but does not differ significantly from the crystallinity of Historic Silk 88b. The crystallinity of all Marine Silks and Historic Silk 145b and 145c is significantly lower than that of Reference Silk. The crystallinity of Historic Silk 88b is significantly higher than that of Historic Silk 145b and 145c, and Marine Silk 29054 and 33707. The crystallinity of Historic Silk 88b does not differ significantly from Marine Silk 29049.
Table 4.7 ANOVA and Tukey's pairwise comparison of IR crystallinity measurements.
X-ray diffraction

In this section, XRD data will be presented with the results of spectral analysis. Warwicker's (32) indexing notation (in which \(a\), \(b\), and \(c\) dimensions correspond to intersheet, interchain, and fiber axis directions respectively) is used in this work. The section is divided into the following sections: presentation of raw and smoothed data and empirical determination of background and amorphous scattering; fitting of crystalline peak profiles and determination of crystallographic parameters of spectra; crystallinity determination; crystallite size determination; and unit cell parameter estimation.

Raw and smoothed data and empirical determination of background and amorphous scattering

Figures 4.62 to 4.68 present raw diffractometric data, superimposed with the smoothed data, and the empirically determined background and amorphous scattering profiles.

Table 4.8 shows the coefficients \(a_0\) and \(a_1\) for the straight-line background profile for Reference, Historic, and Marine specimens. The coefficients in Table 4.9 represents the amorphous polynomial profile for Reference, Historic, and Marine specimens. The coefficient of determination (\(r^2\)) is also presented for each polynomial fit.
Figure 4.62 Raw and smoothed diffractometric data and empirically determined background and amorphous scattering profiles for Reference Silk.
Figure 4.63 Raw and smoothed diffractometric data and empirically determined background and amorphous scattering profiles for Marine Silk 29049.
Figure 4.64 Raw and smoothed diffractometric data and empirically determined background and amorphous scattering profiles for Marine Silk 29054.
Marine Silk 33707

Figure 4.65 Raw and smoothed diffractometric data and empirically determined background and amorphous scattering profiles for Marine Silk 33707.
Figure 4.66 Raw and smoothed diffractometric data and empirically determined background and amorphous scattering profiles for Historic Silk 88b.
Figure 4.67 Raw and smoothed diffractometric data and empirically determined background and amorphous scattering profiles for Historic Silk 145b.
Figure 4.68 Raw and smoothed diffractometric data and empirically determined background and amorphous scattering profiles for Historic Silk 145C.
### Table 4.8 Coefficients $a_0$ and $a_1$ for the background profile for Reference, Historic, and Marine Silk specimens.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Coeff. for bkg contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$a_0$</td>
</tr>
<tr>
<td>Reference Silk</td>
<td>420</td>
</tr>
<tr>
<td>Historic Silk 145B</td>
<td>422</td>
</tr>
<tr>
<td>Historic Silk 145C</td>
<td>381</td>
</tr>
<tr>
<td>Historic Silk 88B</td>
<td>431</td>
</tr>
<tr>
<td>Marine 29049</td>
<td>657</td>
</tr>
<tr>
<td>Marine 29054</td>
<td>544</td>
</tr>
<tr>
<td>Marine 33707</td>
<td>683</td>
</tr>
</tbody>
</table>

### Table 4.9 Coefficients $a_0$, $a_1$, $a_2$, $a_3$, $a_4$, and $a_5$ for the background profile for Reference, Historic, and Marine Silk specimens.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Coefficients for amorphous contribution</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$a_0$</td>
<td>$a_1$</td>
</tr>
<tr>
<td>Reference Silk</td>
<td>-86.9988</td>
<td>10.4004</td>
</tr>
<tr>
<td>Historic Silk 145b</td>
<td>53.3829</td>
<td>-18.6664</td>
</tr>
<tr>
<td>Historic Silk 145c</td>
<td>58.5998</td>
<td>-20.7779</td>
</tr>
<tr>
<td>Historic Silk 88b</td>
<td>20.5087</td>
<td>-15.9706</td>
</tr>
<tr>
<td>Marine 29049</td>
<td>237.8495</td>
<td>-87.6173</td>
</tr>
<tr>
<td>Marine 29054</td>
<td>311.5675</td>
<td>-101.8353</td>
</tr>
<tr>
<td>Marine 33707</td>
<td>204.1212</td>
<td>-80.5081</td>
</tr>
</tbody>
</table>
Crystalline peak profiles and crystallographic parameters of spectra

After the background and amorphous scattering profiles were subtracted, pure crystalline spectra consisting of a series of overlapping peak profiles were obtained and decomposed into Gaussian peak profiles following the method of Foreman and Jakes (7). Figures 4.69 to 4.75 show the pure crystalline spectrum for each sample, overlaid with the individual Gaussian peak profiles. Table 4.10 to Table 4.16 present the following peak parameters for all the peaks in the reference, historic, and marine silk samples: peak centroid (2θ), Gaussian intercept (a) and slope (b), full width at half maxima (B), and crystallite size (c) perpendicular to the direction of the plane under consideration. The standard deviations of these parameters (s_2θ, s_a, s_b, s_B, s_c, respectively) and the correlation of coefficient (r) of the modeled Gaussians versus the raw spectral data in the same 2θ range are also shown. Finally, the experimental inter-planar spacing (d), and the d-spacings and Miller indices reported by Warwicker (32) are also summarized in these tables.
Figure 4.69 Crystalline spectrum and Gaussian peak profiles for Reference Silk.
Figure 4.70 Crystalline spectrum and Gaussian peak profiles for Marine Silk 29049.
Figure 4.71 Crystalline spectrum and Gaussian peak profiles for Marine Silk 29054.
Figure 4.72 Crystalline spectrum and Gaussian peak profiles for Marine Silk 33707.
Figure 4.73 Crystalline spectrum and Gaussian peak profiles for Historic Silk 88b.
Figure 4.74 Crystalline spectrum and Gaussian peak profiles for Historic Silk 145b.
Figure 4.75 Crystalline spectrum and Gaussian peak profiles for Historic Silk 145c.
<table>
<thead>
<tr>
<th>Pk</th>
<th>2θ (deg)</th>
<th>S_{2θ} (cps)</th>
<th>a (deg^2)</th>
<th>b (deg)</th>
<th>r (deg)</th>
<th>B (Å)</th>
<th>S_{B} (Å)</th>
<th>d (Å)</th>
<th>Warwicker, 1954</th>
<th>hkl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.29</td>
<td>0.49</td>
<td>4.35</td>
<td>0.04</td>
<td>0.52</td>
<td>0.02</td>
<td>0.96</td>
<td>0.0403</td>
<td>9.52</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>18.81</td>
<td>0.83</td>
<td>6.60</td>
<td>0.05</td>
<td>0.13</td>
<td>0.01</td>
<td>0.77</td>
<td>0.0806</td>
<td>4.72</td>
<td>468</td>
</tr>
<tr>
<td>3</td>
<td>20.83</td>
<td>0.39</td>
<td>7.34</td>
<td>0.00</td>
<td>0.32</td>
<td>0.00</td>
<td>1.00</td>
<td>0.0510</td>
<td>4.27</td>
<td>430</td>
</tr>
<tr>
<td>4</td>
<td>24.21</td>
<td>0.45</td>
<td>6.46</td>
<td>0.03</td>
<td>0.39</td>
<td>0.02</td>
<td>0.93</td>
<td>0.0466</td>
<td>3.68</td>
<td>301</td>
</tr>
<tr>
<td>5</td>
<td>28.73</td>
<td>0.95</td>
<td>5.91</td>
<td>0.03</td>
<td>0.11</td>
<td>0.00</td>
<td>0.91</td>
<td>0.0889</td>
<td>3.11</td>
<td>300</td>
</tr>
<tr>
<td>6</td>
<td>34.53</td>
<td>0.13</td>
<td>3.51</td>
<td>0.09</td>
<td>4.90</td>
<td>0.59</td>
<td>0.89</td>
<td>0.0131</td>
<td>2.60</td>
<td>202</td>
</tr>
<tr>
<td>7</td>
<td>36.34</td>
<td>0.09</td>
<td>2.94</td>
<td>0.18</td>
<td>16.09</td>
<td>2.27</td>
<td>0.89</td>
<td>0.0072</td>
<td>2.47</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>37.82</td>
<td>0.18</td>
<td>4.23</td>
<td>0.06</td>
<td>1.54</td>
<td>0.27</td>
<td>0.76</td>
<td>0.0234</td>
<td>2.38</td>
<td>040</td>
</tr>
<tr>
<td>9</td>
<td>40.11</td>
<td>0.29</td>
<td>4.68</td>
<td>0.01</td>
<td>0.34</td>
<td>0.01</td>
<td>0.98</td>
<td>0.0497</td>
<td>2.25</td>
<td>103</td>
</tr>
<tr>
<td>10</td>
<td>43.87</td>
<td>0.30</td>
<td>4.12</td>
<td>0.01</td>
<td>0.46</td>
<td>0.02</td>
<td>0.94</td>
<td>0.0427</td>
<td>2.06</td>
<td>203</td>
</tr>
<tr>
<td>11</td>
<td>46.22</td>
<td>0.13</td>
<td>3.57</td>
<td>0.03</td>
<td>1.16</td>
<td>0.27</td>
<td>0.72</td>
<td>0.0269</td>
<td>1.96</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>46.98</td>
<td>0.09</td>
<td>3.41</td>
<td>0.07</td>
<td>5.09</td>
<td>1.17</td>
<td>0.78</td>
<td>0.0129</td>
<td>1.93</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>59.55</td>
<td>0.94</td>
<td>3.62</td>
<td>0.02</td>
<td>0.12</td>
<td>0.00</td>
<td>0.97</td>
<td>0.0841</td>
<td>1.55</td>
<td>304</td>
</tr>
</tbody>
</table>

Table 4.10 Peak parameters, estimation errors, experimental and reference interplanar spacings, and Miller indices for Reference Silk.

<table>
<thead>
<tr>
<th>Pk</th>
<th>2θ (deg)</th>
<th>S_{2θ} (cps)</th>
<th>a (deg^2)</th>
<th>b (deg)</th>
<th>r (deg)</th>
<th>B (Å)</th>
<th>S_{B} (Å)</th>
<th>d (Å)</th>
<th>Warwicker, 1954</th>
<th>hkl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.91</td>
<td>0.38</td>
<td>3.84</td>
<td>0.03</td>
<td>0.63</td>
<td>0.03</td>
<td>0.94</td>
<td>0.0367</td>
<td>9.92</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>18.28</td>
<td>0.63</td>
<td>6.94</td>
<td>0.01</td>
<td>0.21</td>
<td>0.00</td>
<td>0.99</td>
<td>0.0628</td>
<td>4.85</td>
<td>468</td>
</tr>
<tr>
<td>3</td>
<td>21.07</td>
<td>0.38</td>
<td>7.50</td>
<td>0.02</td>
<td>0.34</td>
<td>0.03</td>
<td>0.86</td>
<td>0.0501</td>
<td>4.22</td>
<td>430</td>
</tr>
<tr>
<td>4</td>
<td>24.54</td>
<td>0.45</td>
<td>6.39</td>
<td>0.01</td>
<td>0.13</td>
<td>0.01</td>
<td>0.90</td>
<td>0.0808</td>
<td>3.63</td>
<td>370</td>
</tr>
<tr>
<td>5</td>
<td>29.57</td>
<td>0.79</td>
<td>5.95</td>
<td>0.01</td>
<td>0.12</td>
<td>0.00</td>
<td>0.97</td>
<td>0.0840</td>
<td>3.02</td>
<td>300</td>
</tr>
<tr>
<td>6</td>
<td>38.06</td>
<td>0.16</td>
<td>4.38</td>
<td>0.01</td>
<td>1.12</td>
<td>0.07</td>
<td>0.97</td>
<td>0.0274</td>
<td>2.36</td>
<td>040</td>
</tr>
<tr>
<td>7</td>
<td>40.63</td>
<td>0.23</td>
<td>4.35</td>
<td>0.03</td>
<td>0.67</td>
<td>0.11</td>
<td>0.74</td>
<td>0.0354</td>
<td>2.22</td>
<td>203</td>
</tr>
<tr>
<td>8</td>
<td>43.97</td>
<td>0.31</td>
<td>4.44</td>
<td>0.03</td>
<td>0.48</td>
<td>0.05</td>
<td>0.84</td>
<td>0.0419</td>
<td>2.06</td>
<td>203</td>
</tr>
<tr>
<td>9</td>
<td>60.30</td>
<td>0.82</td>
<td>3.84</td>
<td>0.02</td>
<td>0.12</td>
<td>0.00</td>
<td>0.97</td>
<td>0.0822</td>
<td>1.53</td>
<td>304</td>
</tr>
</tbody>
</table>

Table 4.11 Peak parameters, estimation errors, experimental and reference interplanar spacings, and Miller indices for Marine Silk 29049.
Table 4.12 Peak parameters, estimation errors, experimental and reference interplanar spacings, and Miller indices for Marine Silk 29054.

<table>
<thead>
<tr>
<th>Pk</th>
<th>2θ</th>
<th>S₂θ</th>
<th>a</th>
<th>Sₐ</th>
<th>b</th>
<th>Sₜ</th>
<th>r</th>
<th>B</th>
<th>Sₕ</th>
<th>d</th>
<th>Warwicker, 1954</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(deg)</td>
<td>(cps)</td>
<td>(deg²)</td>
<td>(deg)</td>
<td>(deg)</td>
<td>(deg)</td>
<td>(Å)</td>
<td>d (Å)</td>
<td>hkl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>8.15</td>
<td>0.27</td>
<td>3.05</td>
<td>0.05</td>
<td>1.40</td>
<td>0.09</td>
<td>0.92</td>
<td>0.0246</td>
<td>0.001</td>
<td>10.85</td>
<td>9.29</td>
</tr>
<tr>
<td>2</td>
<td>18.24</td>
<td>0.68</td>
<td>6.80</td>
<td>0.01</td>
<td>0.20</td>
<td>0.00</td>
<td>0.99</td>
<td>0.0653</td>
<td>0.000</td>
<td>4.86</td>
<td>4.68</td>
</tr>
<tr>
<td>3</td>
<td>20.87</td>
<td>0.40</td>
<td>7.49</td>
<td>0.01</td>
<td>0.28</td>
<td>0.01</td>
<td>0.99</td>
<td>0.0549</td>
<td>0.001</td>
<td>4.26</td>
<td>4.30</td>
</tr>
<tr>
<td>4</td>
<td>24.88</td>
<td>0.51</td>
<td>6.53</td>
<td>0.01</td>
<td>0.10</td>
<td>0.01</td>
<td>0.88</td>
<td>0.0912</td>
<td>0.003</td>
<td>3.58</td>
<td>3.70</td>
</tr>
<tr>
<td>5</td>
<td>30.61</td>
<td>0.89</td>
<td>5.96</td>
<td>0.01</td>
<td>0.08</td>
<td>0.00</td>
<td>0.95</td>
<td>0.1031</td>
<td>0.001</td>
<td>2.92</td>
<td>3.06</td>
</tr>
<tr>
<td>6</td>
<td>38.57</td>
<td>0.27</td>
<td>4.58</td>
<td>0.01</td>
<td>0.46</td>
<td>0.02</td>
<td>0.95</td>
<td>0.0427</td>
<td>0.001</td>
<td>2.33</td>
<td>2.38</td>
</tr>
<tr>
<td>7</td>
<td>40.52</td>
<td>0.16</td>
<td>4.22</td>
<td>0.03</td>
<td>1.09</td>
<td>0.22</td>
<td>0.74</td>
<td>0.0278</td>
<td>0.003</td>
<td>2.23</td>
<td>2.27</td>
</tr>
<tr>
<td>8</td>
<td>44.05</td>
<td>0.42</td>
<td>4.16</td>
<td>0.03</td>
<td>0.33</td>
<td>0.03</td>
<td>0.83</td>
<td>0.0504</td>
<td>0.002</td>
<td>2.06</td>
<td>2.10</td>
</tr>
<tr>
<td>9</td>
<td>59.91</td>
<td>0.89</td>
<td>3.43</td>
<td>0.03</td>
<td>0.90</td>
<td>0.00</td>
<td>0.83</td>
<td>0.0994</td>
<td>0.003</td>
<td>1.54</td>
<td>1.57</td>
</tr>
</tbody>
</table>

Table 4.13 Peak parameters, estimation errors, experimental and reference interplanar spacings, and Miller indices for Marine Silk 33707.

<table>
<thead>
<tr>
<th>Pk</th>
<th>2θ</th>
<th>S₂θ</th>
<th>a</th>
<th>Sₐ</th>
<th>b</th>
<th>Sₜ</th>
<th>r</th>
<th>B</th>
<th>Sₕ</th>
<th>d</th>
<th>Warwicker, 1954</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(deg)</td>
<td>(cps)</td>
<td>(deg²)</td>
<td>(deg)</td>
<td>(deg)</td>
<td>(deg)</td>
<td>(Å)</td>
<td>d (Å)</td>
<td>hkl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>8.92</td>
<td>0.36</td>
<td>3.88</td>
<td>0.03</td>
<td>0.74</td>
<td>0.03</td>
<td>0.95</td>
<td>0.0338</td>
<td>0.001</td>
<td>9.91</td>
<td>9.29</td>
</tr>
<tr>
<td>2</td>
<td>18.20</td>
<td>0.61</td>
<td>6.83</td>
<td>0.00</td>
<td>0.21</td>
<td>0.00</td>
<td>1.00</td>
<td>0.0636</td>
<td>0.000</td>
<td>4.88</td>
<td>4.68</td>
</tr>
<tr>
<td>3</td>
<td>20.63</td>
<td>0.37</td>
<td>7.55</td>
<td>0.00</td>
<td>0.31</td>
<td>0.00</td>
<td>0.99</td>
<td>0.0519</td>
<td>0.000</td>
<td>4.31</td>
<td>4.30</td>
</tr>
<tr>
<td>4</td>
<td>24.10</td>
<td>0.49</td>
<td>6.42</td>
<td>0.02</td>
<td>0.22</td>
<td>0.01</td>
<td>0.90</td>
<td>0.0620</td>
<td>0.002</td>
<td>3.69</td>
<td>3.70</td>
</tr>
<tr>
<td>5</td>
<td>28.90</td>
<td>0.82</td>
<td>6.07</td>
<td>0.01</td>
<td>0.11</td>
<td>0.00</td>
<td>0.98</td>
<td>0.0878</td>
<td>0.001</td>
<td>3.09</td>
<td>3.06</td>
</tr>
<tr>
<td>6</td>
<td>37.89</td>
<td>0.19</td>
<td>4.46</td>
<td>0.05</td>
<td>2.62</td>
<td>0.16</td>
<td>0.95</td>
<td>0.0180</td>
<td>0.001</td>
<td>2.37</td>
<td>2.38</td>
</tr>
<tr>
<td>7</td>
<td>40.49</td>
<td>0.31</td>
<td>4.41</td>
<td>0.01</td>
<td>0.28</td>
<td>0.02</td>
<td>0.86</td>
<td>0.0554</td>
<td>0.003</td>
<td>2.23</td>
<td>2.27</td>
</tr>
<tr>
<td>8</td>
<td>44.00</td>
<td>0.46</td>
<td>4.37</td>
<td>0.02</td>
<td>0.23</td>
<td>0.02</td>
<td>0.85</td>
<td>0.0603</td>
<td>0.002</td>
<td>2.06</td>
<td>2.10</td>
</tr>
<tr>
<td>9</td>
<td>60.34</td>
<td>0.89</td>
<td>4.08</td>
<td>0.02</td>
<td>0.10</td>
<td>0.00</td>
<td>0.91</td>
<td>0.0926</td>
<td>0.002</td>
<td>1.53</td>
<td>1.57</td>
</tr>
</tbody>
</table>

155
Table 4.14 Peak parameters, estimation errors, experimental and reference interplanar spacings, and Miller indices for Historic Silk 88b.

<table>
<thead>
<tr>
<th>Pk</th>
<th>2θ</th>
<th>S_2θ</th>
<th>a</th>
<th>S_a</th>
<th>b</th>
<th>S_b</th>
<th>r</th>
<th>B</th>
<th>S_B</th>
<th>d</th>
<th>Warwicker, 1954</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(deg)</td>
<td>(cps)</td>
<td>(deg')</td>
<td>(deg)</td>
<td>(deg)</td>
<td>(deg)</td>
<td></td>
<td></td>
<td>(Å)</td>
<td>d (Å)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>8.91</td>
<td>0.38</td>
<td>3.84</td>
<td>0.03</td>
<td>0.63</td>
<td>0.03</td>
<td>0.94</td>
<td>0.037</td>
<td>0.001</td>
<td>9.92</td>
<td>9.29 100</td>
</tr>
<tr>
<td>2</td>
<td>18.28</td>
<td>0.63</td>
<td>6.94</td>
<td>0.01</td>
<td>0.21</td>
<td>0.00</td>
<td>0.99</td>
<td>0.0628</td>
<td>0.000</td>
<td>4.85</td>
<td>4.68 200</td>
</tr>
<tr>
<td>3</td>
<td>21.07</td>
<td>0.38</td>
<td>7.50</td>
<td>0.02</td>
<td>0.34</td>
<td>0.03</td>
<td>0.86</td>
<td>0.0501</td>
<td>0.002</td>
<td>4.22</td>
<td>4.30 120, 020, 200</td>
</tr>
<tr>
<td>4</td>
<td>24.54</td>
<td>0.45</td>
<td>6.39</td>
<td>0.01</td>
<td>0.13</td>
<td>0.01</td>
<td>0.90</td>
<td>0.0808</td>
<td>0.002</td>
<td>3.63</td>
<td>3.70 211, 121, 201</td>
</tr>
<tr>
<td>5</td>
<td>29.57</td>
<td>0.79</td>
<td>5.95</td>
<td>0.01</td>
<td>0.12</td>
<td>0.00</td>
<td>0.97</td>
<td>0.0840</td>
<td>0.001</td>
<td>3.02</td>
<td>3.06 300</td>
</tr>
<tr>
<td>6</td>
<td>38.06</td>
<td>0.16</td>
<td>4.38</td>
<td>0.01</td>
<td>1.12</td>
<td>0.07</td>
<td>0.97</td>
<td>0.0274</td>
<td>0.001</td>
<td>2.36</td>
<td>2.38 040, 400</td>
</tr>
<tr>
<td>7</td>
<td>40.63</td>
<td>0.23</td>
<td>4.35</td>
<td>0.03</td>
<td>0.67</td>
<td>0.11</td>
<td>0.74</td>
<td>0.0354</td>
<td>0.003</td>
<td>2.22</td>
<td>2.27 103, 013</td>
</tr>
<tr>
<td>8</td>
<td>43.97</td>
<td>0.31</td>
<td>4.44</td>
<td>0.03</td>
<td>0.48</td>
<td>0.05</td>
<td>0.84</td>
<td>0.0419</td>
<td>0.002</td>
<td>2.06</td>
<td>2.10 203, 023</td>
</tr>
<tr>
<td>9</td>
<td>60.30</td>
<td>0.82</td>
<td>3.84</td>
<td>0.02</td>
<td>0.12</td>
<td>0.00</td>
<td>0.97</td>
<td>0.0822</td>
<td>0.001</td>
<td>1.53</td>
<td>1.57 304, 034</td>
</tr>
</tbody>
</table>

Table 4.15 Peak parameters, estimation errors, experimental and reference interplanar spacings, and Miller indices for Historic Silk 145b.

<table>
<thead>
<tr>
<th>Pk</th>
<th>2θ</th>
<th>S_2θ</th>
<th>a</th>
<th>S_a</th>
<th>b</th>
<th>S_b</th>
<th>r</th>
<th>B</th>
<th>S_B</th>
<th>d</th>
<th>Warwicker, 1954</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(deg)</td>
<td>(cps)</td>
<td>(deg')</td>
<td>(deg)</td>
<td>(deg)</td>
<td>(deg)</td>
<td></td>
<td></td>
<td>(Å)</td>
<td>d (Å)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9.65</td>
<td>0.47</td>
<td>3.64</td>
<td>0.04</td>
<td>0.51</td>
<td>0.02</td>
<td>0.93</td>
<td>0.0405</td>
<td>0.001</td>
<td>9.17</td>
<td>9.29 100</td>
</tr>
<tr>
<td>2</td>
<td>17.83</td>
<td>0.48</td>
<td>6.01</td>
<td>0.01</td>
<td>0.24</td>
<td>0.00</td>
<td>0.98</td>
<td>0.0599</td>
<td>0.001</td>
<td>4.97</td>
<td>4.68 200</td>
</tr>
<tr>
<td>3</td>
<td>20.82</td>
<td>0.32</td>
<td>6.95</td>
<td>0.02</td>
<td>0.31</td>
<td>0.03</td>
<td>0.85</td>
<td>0.0526</td>
<td>0.002</td>
<td>4.27</td>
<td>4.30 120, 020, 200</td>
</tr>
<tr>
<td>4</td>
<td>24.51</td>
<td>0.37</td>
<td>6.14</td>
<td>0.00</td>
<td>0.24</td>
<td>0.24</td>
<td>0.99</td>
<td>0.0590</td>
<td>0.001</td>
<td>3.63</td>
<td>3.70 211, 121, 201</td>
</tr>
<tr>
<td>5</td>
<td>29.21</td>
<td>0.78</td>
<td>5.49</td>
<td>0.02</td>
<td>0.11</td>
<td>0.00</td>
<td>0.94</td>
<td>0.0883</td>
<td>0.001</td>
<td>3.06</td>
<td>3.06 300</td>
</tr>
<tr>
<td>6</td>
<td>37.79</td>
<td>0.19</td>
<td>3.90</td>
<td>0.07</td>
<td>2.26</td>
<td>0.20</td>
<td>0.91</td>
<td>0.0193</td>
<td>0.001</td>
<td>2.38</td>
<td>2.38 040, 400</td>
</tr>
<tr>
<td>7</td>
<td>39.99</td>
<td>0.28</td>
<td>4.33</td>
<td>0.01</td>
<td>0.42</td>
<td>0.01</td>
<td>0.99</td>
<td>0.0447</td>
<td>0.001</td>
<td>2.25</td>
<td>2.27 103, 013</td>
</tr>
<tr>
<td>8</td>
<td>44.10</td>
<td>0.34</td>
<td>3.86</td>
<td>0.01</td>
<td>0.31</td>
<td>0.01</td>
<td>0.97</td>
<td>0.0518</td>
<td>0.001</td>
<td>2.05</td>
<td>2.10 203, 023</td>
</tr>
<tr>
<td>9</td>
<td>59.00</td>
<td>0.59</td>
<td>3.48</td>
<td>0.02</td>
<td>0.29</td>
<td>0.01</td>
<td>0.97</td>
<td>0.0538</td>
<td>0.001</td>
<td>1.57</td>
<td>1.57 304, 034</td>
</tr>
</tbody>
</table>
Table 4.16 Peak parameters, estimation errors, experimental and reference interplanar spacings, and Miller indices for Historic Silk 145c.

<table>
<thead>
<tr>
<th>Pk</th>
<th>$2\theta$ (deg)</th>
<th>$S_{2\theta}$ (cps)</th>
<th>$a$ (deg$^\circ$)</th>
<th>$S_a$</th>
<th>$b$ (deg$^\circ$)</th>
<th>$S_b$</th>
<th>r (deg)</th>
<th>B (A)</th>
<th>$S_{B}$ (A)</th>
<th>d (A)</th>
<th>$d_{B}$ (A)</th>
<th>Warwicker, 1954 hkl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.67</td>
<td>0.46</td>
<td>3.67</td>
<td>0.03</td>
<td>0.42</td>
<td>0.02</td>
<td>0.94</td>
<td>0.0451</td>
<td>0.001</td>
<td>9.15</td>
<td>9.29</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>17.73</td>
<td>0.54</td>
<td>6.21</td>
<td>0.02</td>
<td>0.24</td>
<td>0.01</td>
<td>0.94</td>
<td>0.0598</td>
<td>0.001</td>
<td>5.00</td>
<td>4.68</td>
<td>200</td>
</tr>
<tr>
<td>3</td>
<td>21.05</td>
<td>0.33</td>
<td>7.04</td>
<td>0.03</td>
<td>0.37</td>
<td>0.05</td>
<td>0.76</td>
<td>0.0476</td>
<td>0.003</td>
<td>4.22</td>
<td>4.30</td>
<td>120, 020, 200</td>
</tr>
<tr>
<td>4</td>
<td>24.51</td>
<td>0.37</td>
<td>6.14</td>
<td>0.00</td>
<td>0.24</td>
<td>0.01</td>
<td>0.99</td>
<td>0.0590</td>
<td>0.001</td>
<td>3.63</td>
<td>3.70</td>
<td>211, 121, 201</td>
</tr>
<tr>
<td>5</td>
<td>29.21</td>
<td>0.78</td>
<td>5.49</td>
<td>0.02</td>
<td>0.11</td>
<td>0.00</td>
<td>0.94</td>
<td>0.0883</td>
<td>0.001</td>
<td>3.06</td>
<td>3.06</td>
<td>200</td>
</tr>
<tr>
<td>6</td>
<td>37.97</td>
<td>0.24</td>
<td>4.12</td>
<td>0.06</td>
<td>1.68</td>
<td>0.11</td>
<td>0.93</td>
<td>0.0225</td>
<td>0.001</td>
<td>2.37</td>
<td>2.38</td>
<td>200, 1040, 400</td>
</tr>
<tr>
<td>7</td>
<td>40.19</td>
<td>0.35</td>
<td>4.43</td>
<td>0.02</td>
<td>0.44</td>
<td>0.03</td>
<td>0.91</td>
<td>0.0437</td>
<td>0.001</td>
<td>2.24</td>
<td>2.27</td>
<td>103, 013</td>
</tr>
<tr>
<td>8</td>
<td>44.23</td>
<td>0.40</td>
<td>3.96</td>
<td>0.01</td>
<td>0.31</td>
<td>0.01</td>
<td>0.95</td>
<td>0.0522</td>
<td>0.001</td>
<td>2.05</td>
<td>2.10</td>
<td>203, 023</td>
</tr>
<tr>
<td>9</td>
<td>58.99</td>
<td>0.57</td>
<td>3.56</td>
<td>0.02</td>
<td>0.27</td>
<td>0.01</td>
<td>0.97</td>
<td>0.0562</td>
<td>0.001</td>
<td>1.57</td>
<td>1.57</td>
<td>304, 034</td>
</tr>
</tbody>
</table>

While thirteen peaks were identified in the Reference silk specimen, only nine were seen in the Marine and Historic silks. Ten of the thirteen peaks exhibited d-spacings that corresponded to silk fibroin peaks identified in the literature (32). However the remaining three, with interplanar spacings of 2.47, 1.96, and 1.93 are not cited in the literature. Since these are minor and overlap with other peaks it is unclear whether they are real peaks or artifacts. Additionally, it is these three peaks along with the peak with a d-spacing of 2.60, that are not present in any of the Marine and Historic Silk. This last peak is also a minor peak, but corresponds to Warwicker’s (32) [202] peak. All Historic and Marine Silk specimens exhibit the same peaks, indicating that from an x-ray diffraction perspective, the crystal structure of Marine Silk is not qualitatively altered in comparison with Historic Silk.
Crystallinity Determination

Table 4.17 shows the total crystalline and amorphous intensities and the percent crystallinity calculations for all specimens. The Reference Silk had the highest percent crystallinity of 59.56%. Historic Silk 88b had a very similar crystallinity, with an index of 59.52%. The other two Historic Silk specimens, 145b and 145c, had slightly lower crystallinity values of 56.09% and 54.59% respectively. Of the Marine Silks, specimen 29054 had a crystallinity of 53.99%, close to that of the latter two Historic Silk specimens. Marine Silk 29049 and 33707 displayed crystallinities of 46.56% and 46.35% respectively, considerably lower than that of the Reference and Historic Silks.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>$I_{xtl}$</th>
<th>$I_{am}$</th>
<th>%C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference Silk</td>
<td>13308.03</td>
<td>9034.43</td>
<td>59.56</td>
</tr>
<tr>
<td>Marine Silk 29049</td>
<td>15272.25</td>
<td>17531.18</td>
<td>46.56</td>
</tr>
<tr>
<td>Marine Silk 29054</td>
<td>16567.64</td>
<td>14118.61</td>
<td>53.99</td>
</tr>
<tr>
<td>Marine Silk 33707</td>
<td>15326.54</td>
<td>17737.08</td>
<td>46.35</td>
</tr>
<tr>
<td>Historic Silk 88b</td>
<td>13456.64</td>
<td>9152.53</td>
<td>59.52</td>
</tr>
<tr>
<td>Historic Silk 145b</td>
<td>8425.99</td>
<td>6595.81</td>
<td>56.09</td>
</tr>
<tr>
<td>Historic Silk 145c</td>
<td>8816.04</td>
<td>7333.15</td>
<td>54.59</td>
</tr>
</tbody>
</table>

Table 4.17 Total crystalline intensity ($I_{xtl}$), amorphous intensity ($I_{am}$) and percent crystallinity (%C) for Reference, Marine, and Historic Silk specimens.
Crystallite Size Determination

Mean crystallite dimension ($\sigma$) and the standard deviation in the dimension ($s_n$) perpendicular to Warwicker's [200] and [120] crystallographic planes are found in Table 4.18. Crystallite size estimated from the [200] reflection gives the crystallite dimension along the $a$ direction. The results for crystallite size in the $b$ direction have been obtained from those for the [120] direction by multiplication with $\sin 27^\circ$, where $27^\circ$ is the angle between the [120] direction and the $a$-axis (153,154). This assumes that since the unit cell is orthorhombic, fibroin crystallites are also orthorhombic. The following paragraphs describe the crystallite dimensions perpendicular to each of these planes in the Reference, Marine, and Historic silk specimens.
<table>
<thead>
<tr>
<th></th>
<th>$[200]$</th>
<th>$[120]$</th>
<th>$b$-dimension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference Silk</td>
<td>17.2</td>
<td>27.3</td>
<td>24.3</td>
</tr>
<tr>
<td></td>
<td>2.4</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Historic Silk 88b</td>
<td>22.1</td>
<td>27.8</td>
<td>23.7</td>
</tr>
<tr>
<td></td>
<td>2.4</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Historic Silk 145b</td>
<td>21.3</td>
<td>25.4</td>
<td>23.6</td>
</tr>
<tr>
<td></td>
<td>2.3</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>Historic Silk 145c</td>
<td>21.8</td>
<td>26.9</td>
<td>26.1</td>
</tr>
<tr>
<td></td>
<td>2.1</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>Marine Silk 29049</td>
<td>21.2</td>
<td>26.7</td>
<td>24.7</td>
</tr>
<tr>
<td></td>
<td>2.2</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Marine Silk 29054</td>
<td>23.2</td>
<td>26.5</td>
<td>22.6</td>
</tr>
<tr>
<td></td>
<td>1.8</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Marine Silk 33707</td>
<td>23.2</td>
<td>29.3</td>
<td>23.9</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>2.6</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.18 Crystallite size ($\sigma$) and error in crystallite size ($s_\sigma$) estimations.
Crystallite size perpendicular to the [200] plane

This represents the crystallite size in the intersheet direction is represented by [200]. This crystallite dimension ranges from 17.23 to 23.23 Å in the different specimens, and the standard deviation ranges from 1.75 to 2.44 Å. The dimension perpendicular to this plane therefore does not vary much between samples.

Crystallite size perpendicular to the [120] plane

This represents the crystallite dimension at an angle of 27° to the intersheet direction. Like the dimension perpendicular to the [200] plane, this crystallite dimension does not vary much between the specimens. The dimension ranges between 25.39 and 29.30 Å, and the standard deviation between 1.83 and 2.55 Å. Somashekarappa et al. (106, 107) have reported on the crystallite size perpendicular to this plane based on the Fourier analysis of the intensity profile of this peak. While in their earlier work (107) they report a value of 10.96 Å, in 1998, they report values in the 29.7 to 33.9 Å range for untreated silk. These latter results correspond reasonably well with the dimensions reported in the current study. However, while Somashekarappa et al. (106) also report that as a result of various wet treatments, this dimension drops as low as 11.3 Å, our analysis does not find any significant alteration in this crystallite dimension as a result of prolonged marine exposure. The crystallite dimension in the b-direction, (i.e. interchain direction) varies between 22.6 and 26.1 Å, and not much interspecimen variation is observed.
Unit Cell Parameter Estimation

Tables 4.19, 4.20, and 4.21 present the $a$, $b$, and $c$ dimensions of the unit cell based on the equation presented in Chapter 3 for the orthorhombic silk fibroin unit cell.

The structure of silk remains under discussion. While in 1954 Warwicker (32) suggested a polar-antiparallel structure, more recent work by Takahashi (86) suggests an antipolar-antiparallel structure. Correspondingly, the unit cell parameters between the two experiments vary by as much as 0.1 Å. For example, Warwicker reports an intersheet dimension of 9.29 Å compared to 9.38 Å for Takahashi. The values for the intersheet, interchain, and fiber axis dimensions reported in this work agree generally to within 0.1-0.2 Å with the values reported in the literature. An influence of long-term marine storage or age on the unit cell structure cannot be identified. Generally, agreement of the present data with Takahashi’s work is better than with Warwicker’s work. Variations between the current work and the work of Warwicker and Takahashi are to be expected since this work does not do an extensive least square fit of the x-ray intensity based on structure and atomic form factors but uses the equations given in Chapter 3.
<table>
<thead>
<tr>
<th>Specimen</th>
<th>( a , (\text{\AA}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warwicker (32)</td>
<td>9.29</td>
</tr>
<tr>
<td>Takahashi (86)</td>
<td>9.38</td>
</tr>
<tr>
<td>Reference</td>
<td>9.39</td>
</tr>
<tr>
<td>Historic 88b</td>
<td>9.50</td>
</tr>
<tr>
<td>Historic 145b</td>
<td>9.56</td>
</tr>
<tr>
<td>Historic 145c</td>
<td>9.59</td>
</tr>
<tr>
<td>Marine 29049</td>
<td>9.38</td>
</tr>
<tr>
<td>Marine 29054</td>
<td>9.24</td>
</tr>
<tr>
<td>Marine 33707</td>
<td>9.52</td>
</tr>
</tbody>
</table>

Table 4.19 Determination of the \( a \)-dimension of the fibroin unit cell.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>( b , (\text{\AA}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warwicker (32)</td>
<td>9.44</td>
</tr>
<tr>
<td>Takahashi (86)</td>
<td>9.49</td>
</tr>
<tr>
<td>Reference</td>
<td>9.52</td>
</tr>
<tr>
<td>Historic 88b</td>
<td>9.48</td>
</tr>
<tr>
<td>Historic 145b</td>
<td>9.52</td>
</tr>
<tr>
<td>Historic 145c</td>
<td>9.48</td>
</tr>
<tr>
<td>Marine 29049</td>
<td>9.44</td>
</tr>
<tr>
<td>Marine 29054</td>
<td>9.32</td>
</tr>
<tr>
<td>Marine 33707</td>
<td>9.48</td>
</tr>
</tbody>
</table>

Table 4.20 Determination of the \( b \)-dimension of the fibroin unit cell.
<table>
<thead>
<tr>
<th>Specimen</th>
<th>$c$ ($\text{Å}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warwicker (32)</td>
<td>6.95</td>
</tr>
<tr>
<td>Takahashi (86)</td>
<td>6.98</td>
</tr>
<tr>
<td>Reference</td>
<td>6.95</td>
</tr>
<tr>
<td>Historic 88b</td>
<td>6.95</td>
</tr>
<tr>
<td>Historic 145b</td>
<td>6.95</td>
</tr>
<tr>
<td>Historic 145c</td>
<td>6.91</td>
</tr>
<tr>
<td>Marine 29049</td>
<td>6.85</td>
</tr>
<tr>
<td>Marine 29054</td>
<td>7.22</td>
</tr>
<tr>
<td>Marine 33707</td>
<td>6.88</td>
</tr>
</tbody>
</table>

Table 4.21 Determination of the c-dimension of the fibroin unit cell.
Differential scanning calorimetry

Figures 4.76 to 4.82 show the thermograms of the Reference, Historic, and Marine Silks. Table 4.22 presents the water evaporation temperature, the glass transition temperature, and the degradation temperature for the samples.

Figure 4.76 Differential scanning calorimetric trace of Reference Silk.
Figure 4.77 Differential scanning calorimetric trace of Marine Silk 29049.

Figure 4.78 Differential scanning calorimetric trace of Marine Silk 29054.
Figure 4.79 Differential scanning calorimetric trace of Marine Silk 33707.

Figure 4.80 Differential scanning calorimetric trace of Historic Silk 88b.
Figure 4.81 Differential scanning calorimetric trace of Historic Silk 145b.

Figure 4.82 Differential scanning calorimetric trace of Historic Silk 145c.
<table>
<thead>
<tr>
<th>Specimen</th>
<th>Water evap. temp. (°C)</th>
<th>$T_g$ (°C)</th>
<th>$T_d$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference Silk</td>
<td>88.50 - 90.20$^1$</td>
<td>210.38 - 213.95</td>
<td>301.24 - 303.52</td>
</tr>
<tr>
<td>Marine Silk 29049</td>
<td>86.93</td>
<td>n/o$^2$</td>
<td>308.94</td>
</tr>
<tr>
<td>Marine Silk 29054</td>
<td>88.64</td>
<td>n/o$^2$</td>
<td>308.94</td>
</tr>
<tr>
<td>Marine Silk 33707</td>
<td>86.09</td>
<td>n/o$^2$</td>
<td>307.44</td>
</tr>
<tr>
<td>Historic Silk 88b</td>
<td>99.40 - 102.80</td>
<td>203.00 - 203.13</td>
<td>303.90 - 304.80</td>
</tr>
<tr>
<td>Historic Silk 145b</td>
<td>94.40 - 96.40</td>
<td>n/o$^2$</td>
<td>310.60 - 311.78</td>
</tr>
<tr>
<td>Historic Silk 145c</td>
<td>74.40 - 84.40</td>
<td>n/o$^2$</td>
<td>308.80 - 308.90</td>
</tr>
</tbody>
</table>

$^1$ranges represent the data from multiple samples of a given specimen.

$^2$indicates that transition is not observed.

Table 4.22 Water evaporation, glass transition, and degradation temperatures in Reference, Historic, and Marine Silks.

The first transition observed is an endothermic peak attributed to the loss of sorbed water (140,141). In Reference Silk, and all three Marine Silks this endotherm is seen in the 87 to 90°C range. In Historic Silk 145c this transition occurs between 74 and 84°C, while Historic Silk 88b and 145b show this transition at between 96 and 103°C. Because of the limited number of data points, no statistical testing of differences in the temperatures could be performed.

Magoshi and co-workers (125) and Tsukada et al. (121) attribute the endothermic shift in the DSC curve of *Bombyx mori* fibroin film in the random coil conformation at about 180°C to the glass transition in the amorphous silk fibroin chains. This glass transition is only
observed in the Reference Silk and Historic Silk 88b. However in comparison with the results of Magoshi et al. (125) and Tsukada et al. (121) the glass transition temperature in Reference and Historic Silk 88b is higher. Reference Silk exhibits this transition in the 209 to 214°C range, while in Historic Silk 88b the transition is manifest at 203°C. Additionally, the baseline shift that occurs in the two mentioned specimens is small compared to the baseline shift shown by Magoshi et al. (125) and Tsukada et al. (121). As previously mentioned, these researchers used fibroin film which was entirely in the random coil form, making the glass transition more pronounced. However, in the current research, DSC is performed on specimens in the fiber form. Since silk fiber consists of fibroin in both β-crystalline and random coil forms, it is reasonable that the transition is less pronounced as compared to non-crystalline fibroin film. The suppression of this transition in other remaining specimens can be attributed to a reduction in the amount of amorphous component. Magoshi and Nakamura (142) and Tsukada et al. (121) conducted DSC analysis on crystalline films, and report that the glass transition was not observed.

The third transition observed in the specimens is a prominent endotherm in the 301-312°C range, which is attributed to the decomposition of fibroin. Magoshi and Nakamura (142) and Tsukada et al. (121) report the occurrence of this endotherm at 280°C in fibroin film in the random-coil conformation. Additionally, they report that in crystalline fibroin film this transition shifts higher to 284°C. As mentioned in Chapter 2, Tsukada and Hirabayashi (143) studied the effect of UV radiation on the silk fibers. While the degradation temperature of untreated silk was 335°C, the corresponding temperature for UV irradiated silk fibers was determined to be 321°C.
In the Reference Silk and Historic Silk 88b, decomposition occurs in the 301-305° C range. In Historic Silk 145b, 145c, and all three Marine Silks, the decomposition temperature shifts higher to between 308 and 312° C.

**Summary**

This section summarizes the results obtained from the various analytical methods employed in this research. Morphological data on Reference, Marine, and Historic Silks are based on optical microscopy and scanning electron microscopy. These techniques were able to establish that the morphological changes and surface deposits are much more pronounced in the Marine than in the Historic Silk specimens. Microbiological activity was more extensively found on the Historic Silk samples in comparison with Marine Silk. Fiber diameters of two Marine Silk samples are about 5% larger than those of all other samples. Coarser fibers are known to exhibit a lower degree of crystalline and molecular orientation than finer fibers. X-ray crystallinity indices for these two samples are significantly lower than the corresponding indices for all of the other silks studied in the present work.

The elemental compositions of the fibers, biofilms, and fiber deposit were determined using EDS. None of the fibers displayed any significant mineral content indicating that the minerals were unable to penetrate the inner part of the fiber. Discrete encrustations were shown to contain inorganic elements while continuous paste-like encrustations are organic in nature.

Structural information about the silk samples including their crystallite size, crystallinity, and unit cell parameters was obtained from x-ray crystallography. The evaluation
of the x-ray spectra required a decomposition of the spectra into a crystalline, an amorphous, and a background component. The amorphous component contains contributions from the random-coil and the ordered amorphous silk. This leads to lower x-ray crystallinity values compared to those determined via Fourier-transform infrared spectroscopy. While no qualitative differences between the Reference, Marine, and Historic Silks regarding peak positions could be determined, the crystallinity of the Reference Silk was determined as 60% compared to 60%, 56%, and 54% for Historic Silk 88b, 145b, and 145c respectively. The corresponding values for Marine Silk 29054, 29049, and 33707 were 54%, 47%, and 46% respectively. The crystallite sizes along the [200] and [120] directions were estimated using Scherrer’s equation. In the [200] or intersheet direction, the Reference Silk crystallites measure 17 Å while those of Marine and Historic Silk are slightly larger with 21-22 Å and 21-23 Å, respectively. The crystallite size in the b or interchain direction can be derived from the [120] crystallite dimension and amounts to about 11-13 Å for all samples. Unit cell sizes are not influenced by long-term storage or the combined effect of long-term storage and marine environment. Values of a = 9.4 Å, b = 9.5 Å, and c = 7 Å were obtained in good agreement with results by Warwicker and more recent results by Takahashi.

Further chemical and structural information about the silk samples was gathered using FTIR. All FTIR peaks present in Reference Silk are also observed in Marine and Historic spectra. Main differences of the Historic and Marine artifacts with respect to the Reference Silk are therefore not chemical in nature but consist of minor shifts in positions and relative intensities of bands that are sensitive to conformation. The average FTIR crystallinity measures short-range effects and is generally higher than crystallinity values obtained using
x-ray diffraction data. The average FTIR crystallinity values for Reference Silk, Marine Silks, and Historic Silks are 65%, 56-59%, and 57-63% respectively.

The present study uses DSC to determine the glass transition temperature, the loss temperature for sorbed water, and the sample degradation temperature. The glass transition was only weakly observable in Reference and Historic 88b Silk and not observed in any of the other specimens. This indicates a loss of the amorphous component in all other samples either due to preferential degradation or due to recrystallization, i.e., the transformation of random-coil silk into ordered amorphous silk.
CHAPTER 5

DISCUSSION

The purpose of this work was to examine the physical and chemical microstructure of selected silk textile fibers recovered from the deep-ocean site of the S.S. Central America. For comparative purposes, modern silk reference and historic silk fibers from the same era as the marine textiles, but not subjected to marine storage were also analyzed. Through such a comparative approach, an attempt was made to infer the preservative and/or degradative effects of prolonged marine storage on silk fibers and to understand the implications for artifact conservation. Additionally, because of sample paucity, the multiple analytical techniques used in this research were evaluated for their effectiveness in studying microsamples.

This chapter discusses the results obtained from the different analytical techniques in the context of the stated research problems. The results of the individual techniques discussed in Chapter 4 give complementary information regarding the chemical and physical state of the materials under study and are compiled in Table 5.1. The first section of this chapter comprehensively discusses these results both individually and in conjunction with each other, and compares and contrasts the results obtained from the Reference, Historic, and Marine Silk.
specimens. This section is divided into the following subsections: surface deposits, fiber morphology, physical microstructure, chemical microstructure, and microbiological activity.

The results reported herein indicate that the marine environment did not appear to have had the same effect on the three marine artifacts under study. These findings support the view that the environment that is most significant with respect to the degradation and preservation of artifacts is the immediate boundary between the artifact and its surroundings (16). Because the normal circulation processes of the ocean are severely restricted in closed environments like the trunk, the elemental composition in these microenvironments are significantly different from the relatively constant composition of the open ocean. The varying effects of the marine environment on the different Marine Silks are also discussed in the first section.

Since it was also the goal of this work to evaluate the different analytical techniques with respect to their effectiveness in studying historic artifacts, the second section of this chapter is devoted to this end. By analyzing the compiled data, inferences regarding the effectiveness of the different analytical techniques in detecting different degrees and types of degradation/preservation can also be made. For example, morphological changes observed under the SEM may not necessarily be accompanied by chemical changes reflected in FT-IR investigations. Similarly, while crystallinity changes observed using XRD are indicative of long-range order, changes in IR crystallinity are indicative of short-range order. In addition, the sample requirements of the techniques are discussed in the second section.

The third section of this chapter focuses on the conservation considerations that emerge from the findings of this work. The task of the conservator is to reduce or arrest the
rate of degradation processes. This can be achieved by several techniques. Inhibiting the artifact from absorbing the activation energy required for deterioration can be achieved by controlling the storage conditions. For example, exclusion of light is particularly important in the conservation of silk artifacts, as are controlling the oxygen, pH, and relative humidity levels in the storage atmosphere.
Table 5.1 Comparative summary of results from optical & scanning electron microscopy, x-ray diffraction, infrared microspectroscopy, and differential scanning calorimetry.
Surface deposits

Florian mentions that one of the most common characteristics of artifacts from marine wreck sites is the ubiquitous presence of surface deposits (16). Surface deposits of three different morphologies and elemental compositions were observed on the Marine Silk specimens. Table 5.2 summarizes the morphology and main elemental compositions of these deposits.

<table>
<thead>
<tr>
<th>Morphology</th>
<th>Major elemental composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous, paste-like deposit</td>
<td>Organic in nature</td>
</tr>
<tr>
<td>Discrete, irregular shaped deposit</td>
<td>Mainly Cl, Fe, Na, and S; some Mg</td>
</tr>
<tr>
<td>Discrete, cube-shaped deposit</td>
<td>Mainly Mg, Na, S, and Zn; some Fe and Si</td>
</tr>
</tbody>
</table>

Table 5.2 Morphology and major elemental composition of deposits on Marine Silk fibers.

The continuous paste-like deposit observed on the Marine Silk specimens has been reported on cellulosic artifacts from this site (8). EDS results indicate that these deposits are organic in nature. Florian (16) mentions that organic sediments are often formed either from the settling out process of suspended particulate and colloidal matter or the remains of plankton. The discrete deposits of irregular morphology correspond to material that Chen (8) reports on cotton artifacts. In addition to Na and Cl, these deposits are rich in Fe and S. The importance of sulfate reducing bacteria in the marine environment in producing H₂S, and the
subsequent reaction of $\text{H}_2\text{S}$ with the iron in the seawater to produce FeS has been documented (16). Florian (16) mentions that FeS residue is extensively found on marine artifacts including pottery and wood. The iron and sulfur rich deposits found on Marine Silks in this research are quite possibly the insoluble reduction products formed by the reaction of $\text{H}_2\text{S}$ produced by the sulfide reducing bacteria with $\text{Fe}^{2+}$ and $\text{Fe}^{3+}$ species in the seawater. The growth of such bacteria requires an anoxic environment with suitable organic nourishment (155). The implications of such an environment in the vicinity of the silk artifacts plays a significant role in their chemical stability in the marine environment, and this will be discussed in a subsequent section of this chapter. Chen (8) observed Fe- and S- rich black deposits, which she proposes are a consequence of the $\text{H}_2\text{S}$ produced by the action of sulfate reducing bacteria. However, while she did observe the presence of rod and cocci bacteria that caused degradation in the cotton artifacts she studied, no correspondence was found between areas where there are black deposits and areas where artifact biodegradation was observed. She therefore speculates that some other microorganisms are responsible for biodegradation in cotton materials in a marine environment. In this research, no evidence of biodeterioration of the silk fibers was observed, so it likely that the sulfate reducing bacteria are not proteolytic. It has been noted that wood is often a source of nourishment for sulfate reducing bacteria (155).

The third type of deposit, which has been classified as discrete cube-shaped deposit, was found to contain Zn in addition to Mg, Na, Si, S, and Fe, which one expects to find in the marine environment. Mg and Na are major constituents of seawater and exist both in the ionic form and as complexes (16). The source of Fe and S has been discussed in the previous
paragraph. Si is an important constituent of the sea, and has a low solubility below a pH of 8.5. Florian points out that when silica in solution encounters a surface of lower pH it precipitates out (16). Since the pH in the trunk is probably lower than that of the surrounding seawater due to the action of the sulfate reducing bacteria, it is likely that Si is precipitated out from solution in the microenvironment of the trunk. While Zn is not typically found either in ionic form in the water and or as a constituent of artifact deposits, it is a constituent of marine sediments (16).

**Fiber morphology**

Fiber diameter measurements using OM and observations of fiber morphology using SEM will be discussed in this section. The fiber diameters for Marine Silk 29049 and 33707 are significantly higher than the fiber diameters of all other specimens under study (Table 5.1). The diameter of Marine Silk 29054 is not significantly different from that of the Reference Silk or any of the Historic Silks. This could be indicative of the fact that because of its position within the trunk, 29054 is not exposed to the marine environment to the same extent that the remaining marine specimens are. Water and aqueous solutions of chemical agents are known to cause swelling and dissolution in silk fibers. The critical level of water content in a solution beyond which only fiber swelling rather than dissolution occurs was found to be between 20-21%. Nadiger (156) reports on the presence of microfibrils in *Bombyx mori* fibroin that vary between 40 and 2000 Å in width and additionally mention the presence of microvoids in fibroin. Kawahara and Shioya (157) suggest that water and other swelling agents penetrate microvoids in silk fibers and cause swelling due to the dissociation of the
hydrogen bonds between the silk fibrils. An increase of 18% in fiber cross-sectional area has been reported (103). Immersion in water has been shown to increase fiber compliance (158), and it is proposed that on immersion in water inter- and intra-sheet protein-protein hydrogen bonds are replaced by water-protein bonds in the amorphous regions. This leaves weaker van der Waals forces as the dominant non-covalent protein-protein interaction, making silk more compliant. The inverse relationship between filament size and molecular orientation has been demonstrated (159) and will be discussed in a subsequent section.

Fibers in the Reference Silk were smooth and showed no signs of degradation. In comparison with Reference silk, the Historic and Marine specimens showed successively increasing signs of degradation. Historic silk exhibits occasional surface pitting phenomena, and some cracking which appears to be restricted to the surface. On the other hand, Marine Silks show more extensive morphological damage. For example, Marine Silks exhibit cracking phenomena that penetrates deep into the fiber bulk, surface striations, fibrillation, and fiber flattening. These changes in morphology observed with SEM and the increased diameter of two of the Marine Silks are supramolecular changes that are indicative of changes in the physical microstructure of the artifacts.

Physical microstructure

In this work, information regarding the physical microstructure of the materials under investigation is obtained from IR, XRD, and DSC techniques. This section begins with a discussion of a three-phase view of silk fibroin microstructure, and is followed by a discussion
of crystalline-amorphous configurations, crystallite size estimates, and unit cell parameters of the different specimens under investigation.

Three-phase structure of silk fibroin

While semicrystalline polymers are often considered to be composed of two phases, crystalline and amorphous, the inadequacy of this two-phase model in describing polymer behavior has been noted (160,161). A three-phase model is needed to explain the behavior of such polymers more accurately and the third phase is often referred to as the interphase or oriented-amorphous phase(162). This interphase is not a thermodynamically distinct phase, but rather one based on morphology and geometry.

Based on enzymatic (39) and acid (38) hydrolysis, researchers have proposed such a three-phase structure of silk fibers. The three phases include a highly ordered well packed crystalline region, an amorphous phase, and an intermediate phase of laterally ordered material with a fair degree of order and orientation. It has been shown that this laterally ordered phase acts as a transition phase between the crystalline and amorphous phases. Based on hydrolysis kinetics (39) this intermediate phase is reported to be more abundant in B. mori silk in comparison with wild silk.

A similar multi-component phase structure has also been proposed for spider silk (163). Based on NMR results it is concluded that 40% of the methyl groups of alanine are present in a classical crystalline phase, in which the β-sheets are tightly packed and highly oriented with respect to the fiber axis. The remaining 60% are in “protocrystals”, namely a weakly oriented β-sheet type configuration. Based on the results of TEM imaging and
diffraction studies, Thiel et al. (164) conclude that only part of the volume of high contrast domains seen under a TEM diffracts as crystals. This observation can be accounted for by crystallites surrounded by oriented amorphous material. Figure 5.1 shows a schematic picture of silk protein chains in the crystalline $\beta$-sheet, ordered amorphous and the amorphous random coil configuration.

![Schematic representation of three-phase structure of silk fibroin.](image)

**Figure 5.1** Schematic representation of three-phase structure of silk fibroin.
Crystalline-amorphous conformation

Crystallinity estimates obtained from IR and XRD techniques are sensitive to short and long range order respectively (165). The classical picture of infrared spectroscopy assumes that the incident radiation causes a distortion of the molecule and sets the molecule into a vibrating motion. If the frequency of this motion corresponds to the frequency of a normal mode, the vibration is resonant and radiation will be absorbed. The frequency of localized vibrations is determined by the immediate environment of the vibrating molecular unit and hence depends only on short-range order. For example, the vibrational frequency of the Amide III vibration in proteins is dominated by a localized CN stretching mode and has a minor contribution of NH in-plane bending. As a consequence, the difference between this vibration frequency in a crystalline β-sheet environment and an amorphous random-coil configuration is about 30 cm⁻¹.

It is to be expected that even if the long-range order in fibroin is disturbed, as long as the short-range order is relatively undisturbed, the IR crystallinity index will remain fairly unchanged. As can be inferred from Figure 5.1, the short-range order of silk in the crystalline configuration and the ordered amorphous configuration is very similar. Both these structures exhibit the β-pleated sheet structure in the short-range. However, from a short-range perspective, the difference between crystalline and ordered amorphous configurations on the one hand, and the random coil configuration on the other hand is pronounced. As a consequence, the intensity of localized vibrations will not significantly change if the secondary structure of fibroin changes from a crystalline to an ordered amorphous environment or vice versa. From a peak position perspective, while a rearrangement of material between the
ordered amorphous and crystalline phases could lead to a frequency shift, the magnitude of this shift is such that at most it manifests in a broadening of the crystalline peak and a slight shift in peak position. Some of the reported variation in peak position between the Reference versus the Historic and Marine Silks could therefore be caused by the minor alterations in the local environments of the atoms participating in the vibration. However, the conformation sensitive vibrational frequencies of the crystalline and ordered amorphous conformations are not different enough to be resolved in the IR spectra.

While IR provides information about the short-range order in a material, information about the long-range order needs to be obtained from a complementary technique that is sensitive to such order. X-ray crystallography is such a technique. In a simplified picture, when the electrons in an atom are subjected to a monochromatic beam of x-rays, the electrons vibrate with a frequency equal to that of the incident beam. As a result they emit radiation of the same wavelength in all directions, i.e. diffraction occurs. Diffraction is possible since x-rays used in diffraction work have a wavelength of the same order of magnitude as the atomic diameter (about 1 Å). Thus, in an atom, all electrons contribute to the scattering of x-rays in this fashion. While all individual atoms in a regular solid scatter radiation in all directions, there are a few directions in which the radiation emitted by all atoms reinforce each other. Positive interference requires regular, i.e. crystalline, order over dimensions that are large compared to a typical atomic distance in the solid under consideration.

As discussed in the results section, if the crystalline and amorphous components in the x-ray spectrum are separated following the technique of Hermans and Weidinger (90), the crystalline contribution pertains to material that exhibits long-range order. The amorphous
contribution obtained from this method pertains to material that does not exhibit long-range order, regardless of the short-range order of the material. With respect to the three-phase model of fibroin, the crystalline contribution relates to the crystalline phase, while the amorphous contribution obtained from the Hermans and Weidinger approach is attributed to both the amorphous and ordered amorphous phases.

In order to distinguish between the amorphous and ordered amorphous phases using x-ray diffraction, Tsukada et al. (40) suggest that the area between Hermans and Weidinger’s amorphous profile and the scattering profile of a completely amorphous material can be attributed to the ordered amorphous material. While their approach is applicable for fibroin films that can be generated in completely amorphous form and then imparted crystallinity by treatment, this approach is not applicable in the current research on fibrous historic artifacts. The separation of the amorphous phase from the ordered amorphous phase therefore cannot be achieved from x-ray diffraction analysis of the samples under investigation. Fossey and Tripathy (160) mention that it is likely that the order present in the ordered amorphous material will not be detected in WAXD.

The third piece of evidence regarding the crystalline state of the material is obtained from DSC results. The glass transition is associated with the long-range segmental mobility in the amorphous phase of semi-crystalline polymers. As the amorphous content decreases, the effect on the glass transition is to decrease the intensity of the transition, to widen the temperature range over which the transition occurs, and to shift the transition to a higher temperature. Often, the first two effects are more pronounced than the third (166). Hydrogen bonding is the principal factor influencing the glass transition temperature. For example it has
been shown that in aliphatic polyamides, as the hydrogen-bonding index increases, as evidenced by the concentration of NH groups, glass transition temperature increases (167).

A two-phase structural model is not adequate to describe the effect of crystallinity on the glass transition. A more appropriate model is one that depicts a semicrystalline polymer as composed of a crystalline phase, a mobile amorphous phase and a rigid amorphous phase. The rigid amorphous phase exists at the crystalline interfaces and is subject to stresses that inhibit segmental mobility (166). This rigid amorphous phase is similar to the laterally ordered region demonstrated in silk.

The decomposition temperature is also influenced by the amount of ordered material in a specimen. As the degree of order and orientation in a semicrystalline material like silk increases, the decomposition temperature has been shown to increase (89,159,168) because of the degree of mobility of the fibroin molecules. While increased decomposition temperature of silk is usually accompanied by a corresponding increase in crystallinity, it also been shown (97) that increase in thermal stability as evidenced by increase in decomposition temperature is not necessarily accompanied by a corresponding increase in XRD crystallinity. This was taken to indicate that change in order occurs in the amorphous rather than the crystalline regions of fibroin (97). Table 5.3 summarizes the crystallinity information obtained from IR, XRD, and DSC techniques.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>0.65</td>
<td>0.60</td>
<td>210-214°C</td>
<td>301-304°C</td>
</tr>
<tr>
<td>Marine 29049</td>
<td>0.59</td>
<td>0.47</td>
<td>n/o</td>
<td>309°C</td>
</tr>
<tr>
<td>Marine 29054</td>
<td>0.56</td>
<td>0.54</td>
<td>n/o</td>
<td>309°C</td>
</tr>
<tr>
<td>Marine 33707</td>
<td>0.57</td>
<td>0.46</td>
<td>n/o</td>
<td>307°C</td>
</tr>
<tr>
<td>Historic 88b</td>
<td>0.63</td>
<td>0.60</td>
<td>203°C</td>
<td>304-305°C</td>
</tr>
<tr>
<td>Historic 145b</td>
<td>0.57</td>
<td>0.56</td>
<td>n/o</td>
<td>311-312°C</td>
</tr>
<tr>
<td>Historic 145c</td>
<td>0.58</td>
<td>0.55</td>
<td>n/o</td>
<td>309°C</td>
</tr>
</tbody>
</table>

Table 5.3 Crystallinity data for Reference, Marine, and Historic Silk specimens.

The IR crystallinity indexes of the Reference and Historic Silk 88b are significantly higher than those of the Marine Silks, and Historic Silk 145b and 145c. This suggests that the short-range order in all Marine Silk specimens, and Historic Silk 145b and 145c is disrupted in comparison with the Reference Silk and Historic Silk 88b. Additional qualitative indications of the change in short-range order based on the slight shifts in the conformation sensitive IR bands has also been observed. In Chapter 4 it was reported that peak positions in the spectra of the Historic and Marine Silks varied up to ±10 cm⁻¹ from the peak positions in the Reference Silks. As previously discussed, it is possible that slight variations in the local environment of the atoms are responsible for this shift. While the observed shifts are not large enough to indicate a transition from the β-crystalline to the random-coil conformations or
vice-versa, it could be interpreted as a change from β-crystalline or random-coil conformations of fibroin to an ordered amorphous structure.

With respect to XRD crystallinity, the materials under investigation fall into three categories. Reference Silk and Historic Silk 88b have the highest crystallinity index, Historic Silk 145b, 145c, and Marine Silk 29054 fall into an intermediate category, and Marine Silk 29049 and 33707 have the lowest crystallinity index. This suggests that while long-range order in silk is altered in all three Marine Silks, and Historic Silk 145b and 145c, this disruption is more pronounced in Marine Silk 29049 and 33707. When these results are considered in conjunction with the crystallinity information obtained from IR measurements, it can be surmised that in the case of Historic Silk 88b, disruption of neither short- nor long-range order occurs to any appreciable extent. In the case of Historic Silk 145b, Historic Silk 145c, and Marine Silk 29054, the alteration in short-range order occurs to the same extent that disruption of long-range order occurs. The implications of this result will be discussed shortly in conjunction with the crystallinity implications of the thermal behavior of the artifacts.

The most interesting result from IR and XRD analysis is seen in Marine Silk 29049 and 33707. While some disruption of the short range order can be inferred from the lower IR crystallinity index, the disruption of the long-range order of these specimens appears to be much more pronounced based on the much greater reduction in the XRD crystallinity measure in comparison with the IR crystallinity measure. It is therefore proposed that in these two specimens, parts of the ordered β-crystalline material have been transformed to a less-ordered material that still maintains a large part of its short-range β-sheet structure. Such a
conformation corresponds to the laterally-ordered or ordered-amorphous phase of silk fibroin previously postulated by several researchers (38,39,162-164).

While quantitative measures of crystallinity obtained from IR and XRD indicate that the crystalline content of all Marine Silks and Historic Silk 145b and 145c are lower than those of Reference and Historic Silk 88b, seemingly contradictory results are obtained from the qualitative crystallinity implications obtained from DSC. Earlier in this section the dependence of the glass transition and degradation temperature on amorphous content has been discussed. The glass transition is only observed in the Reference Silk and Historic Silk 88b. As previously discussed, the observance or suppression of the glass transition in a semicrystalline polymer is an indication of the amount of amorphous material in the polymer. When the amorphous content decreases, this transition may be suppressed. As shown in Table 5.2 the degradation temperature of Reference Silk and Historic Silk 88b are the lowest of all the specimens under study. The degradation temperatures of the Marine Silks and Historic Silk 145b and 145c are somewhat higher, and this also indicates a decreased amorphous content.

DSC results indicate therefore that there is selective alteration of the amorphous material in all Marine Silks, and Historic Silk 145b and 145c. Two possible operative mechanisms for this alteration are selective degradation of the amorphous material and alteration of the physical microstructure of the amorphous material. Based on chemical microstructure investigation using IR spectroscopy, no qualitative change in the chemical character of fibroin has been established in this work and this will be discussed in a following section. Quantitative chemical characterization of the artifacts by amino acid analysis needs
to be performed to establish selective chemical alteration of the amorphous region. The other explanation for lower amorphous content based on DSC data is that part of the amorphous material has been altered into ordered amorphous material. Tsukada et al. (121) and Magoshi et al. (125) both report recrystallization of amorphous silk fibroin under immersion of the fibroin in water.

The reduction in both amorphous and crystalline contents can be explained in the context of the three-phase structural model of silk fibroin discussed previously. While the amorphous component is decreasing either due to selective dissolution or alteration of conformation to an ordered amorphous form, parts of the crystalline component are also undergoing conformational relaxation into the ordered amorphous state. A simple picture of the action of water on silk corroborates our results. The main effect of water absorption is the fiber swelling due to the penetration of water into the more accessible amorphous regions of silk. This penetration is aided by the fact that the amorphous component of silk contains many of the amino acids with polar and bulky side-chains which are predisposed to the formation of hydrogen bonds. Interchain crosslinks are broken and replaced with hydrogen bonds between the protein and water. The insertion of water molecules between adjacent fibroin chains weakens their mutual interaction and increases their mobility (40). There are three consequences of the swelling and the increased mobility. First, it is easier for solvents to reach the ordered amorphous and crystalline regions of the sample. Once solvents reach those regions they can start replacing protein-protein hydrogen bonds by solvent-hydrogen bonds and hence transform, for example, crystalline silk into ordered amorphous silk. Second, strain in the silk samples is released. Besides a distribution of crystal sizes, strain is a very
important parameter determining the width of x-ray diffraction profiles (169). Strain in silk has been determined by Somashekar and collaborators to be about 2-8% depending on the sample (105,107). Releasing strain should lead to sharper x-ray peaks unless the release in strain is countered by other effects such as a broader distribution of crystallite size as will be discussed below. The third effect of water is related to molecular alignment and orientation. The relationship between fiber diameter and degree of order in silk fibers has been demonstrated. The finer the fiber, the greater the degree of order (159). Increased mobility in swollen fibers could lead to a reduction of long-range order in the fiber. This correlates well with the much reduced x-ray crystallinity of the Marine Silk 29049 and 33707 which have an about 5% larger diameter than all other silk samples considered in this work.

Unit cell parameters and crystallite size estimation

Tables 4.19 to 4.21 list the unit cell dimensions of the Reference, Marine, and Historic Silks as well as the lattice parameters given by Warwicker (32) and Takahashi (86). Note that this work follows Warwicker’s (32) notation rather than Robson’s (27) notation shown in Fig. 2.3. Robson’s \(a\), \(b\), and \(c\) parameters correspond to Warwicker’s \(b\), \(c\), and \(a\) directions respectively. This means that \(b\) measures the interchain unit cell dimension, \(a\) measures the intersheet unit cell dimension, and \(c\) measures the dimension along the fiber axis.

As mentioned earlier, the detailed structure of \(\beta\)-silk is still under discussion. In particular, Takashi favors an antipolar-antiparallel structure compared with the polar-antiparallel structure of Marsh et al. (41,42). As a consequence, Takahashi’s unit cell size differs by about 0.1 Å from the results of Warwicker (32) and Marsh et al. (41,42). Similar
differences are seen between the unit cell parameters determined in this work and the results given in the literature (32). These differences result from the following approximations: 1) use of the plane-spacing equation for orthorhombic unit cells to determine cell parameters rather than a rigorous least-squares fitting of the x-ray intensity data based on structure and form factors, 2) inability to use the [100] reflection for the determination of the size of the unit cell parameter \(a\), 3) empirical separation of overlapping reflections such as the [200] and the [120] reflections, and 4) neglect of strain and temperature effects. In particular, high-accuracy results for unit cell dimensions require a detailed and cumbersome calculation of the x-ray spectra based on assumptions about the unit cell geometry (incorporated in the structure factor) and the scattering power of the unit cell atoms (incorporated in the atomic form factors). Since the focus of the current work is a comparison of Reference, Marine, and Historic Silk, such a procedure is not warranted. Moreover, the [100] peak in the present work has too much background and overlaps with other reflections such that it is not possible to record a clear reflection profile. This problem is common in x-ray studies of silk (170) (106). The difficulty of separating the strongly overlapping [200] and [120] peaks has been described earlier in this work. Temperature and strain effects have been included by Takahashi (86) to improve the accuracy of his unit cell size determination but are neglected in the current work.

Taking the above approximations into account, the unit cell dimensions of this work agree with published literature data for Reference Silk to within 0.1 Å. Agreement with the results of Takahashi (86) is better than with the Warwicker (32) results. No significant change in the unit cell dimensions between the Reference, Marine, and Historic Silks is found. This
indicates that whatever degradation mechanisms occur in the Marine and Historic Silks, they have a negligible influence on the unit cell dimensions of the crystalline silk regions. This observation is in agreement with literature (158). While the action of water on silk has been shown to have an effect on molecular orientation, no effect on unit cell parameters is observed in this work.

Crystallite sizes along the \(a\) and \(b\) direction are estimated using the Scherrer equation from the data for the [200] and [120] reflections. The crystallite size obtained in this work for Reference Silk in the [200] direction and [120] direction are 17.2 Å and 27.3 Å respectively. The crystallite size estimated from the [200] reflection gives the crystallite dimension along the \(a\) direction. The results for crystallite size in the \(b\) direction have been obtained from those for the [120] direction based on the fact that the angle between the [120] and \(b\) direction directions is 27° (153,154), and assuming that the crystallites in silk fibroin are orthorhombic. It should be noted that crystallite size along the \(c\) direction, i.e. along the fiber axis, has not been computed here. Measurements of this dimension from x-ray data have not been reported in the reviewed literature.

While the crystal sizes from the [200] and the [120] directions agree well with some reports in the literature (103-105), there is considerable discrepancy with other reports (100,106,107). It is important to note that differences in technique and parameter value assumptions could be partially responsible for differences in estimated size between literature values and those reported here. For example, researchers (103,104) have used different values for the Scherrer constant versus the 0.90 that has been used in this work. In addition, some researchers use Fourier analysis of the intensity profile of the peak to compute crystallite size.
Given these limitations it is to be expected that there will be considerable variations between the results obtained by different workers.

As shown in Table 4.18, a slight increase of crystallite size along the [200] direction is noticeable between the Reference and the Marine and Historic Silks. This may indicate a certain amount of recrystallization that occurs in this direction. However, the results for the Marine and Historic Silks are within three standard deviations of crystallite size for the Reference Silk. The results of the Marine and Historic Silks are identical within one standard deviation of each other. Further studies on additional samples are therefore needed to confirm whether the differences in crystallite size are statistically significant. This indicates that while the degradation mechanisms of Marine and Historic Silk are not identical their effect on the crystalline silk component is similar and small if existent at all. No changes along the interchain direction can be observed.

Since the overall crystallinity of the Marine and Historic Silks is reduced with respect to the Reference and since the average crystallite sizes in the a and b direction do not change, increase of disorder must occur at the ends of the crystallites along the fiber axis. Schematic representations of the crystalline, ordered amorphous, and amorphous components of silk found in the literature (40,98,163) indicate that the transition between the crystalline conformation and the ordered amorphous conformation occurs approximately in the direction of the fiber axis. The same is true for the ordered amorphous to amorphous transition. In Marine Silk for example, polar solvents such as water most easily attack the ends of the crystalline region. Water molecules are channeled through the amorphous and ordered amorphous regions to the ends of the crystalline region where they can substitute protein-
protein hydrogen bonds by protein-water hydrogen bonds and transform crystalline silk into ordered amorphous silk. Attack of crystalline silk along the $a$ and $b$ directions is much harder since no similar channels exists.

Based on the above argument if one assumes that the main reduction in the crystallinity index comes from the transformation of crystalline silk to ordered amorphous silk at the extreme ends of the crystallites along the fiber axis, it is unlikely that crystallites are totally unaffected by aging along the $a$ and $b$ directions. While no conclusive evidence for such an attack can be obtained from the crystallite size measurements along these dimensions, some insight can be gained from the following consideration. As mentioned previously, water releases strain in silk samples. This should lead to sharper x-ray reflections, but the full widths at half maximum of the [200] and [120] reflections are not sharper in comparison with Reference Silk (Table 4.10 – 4.16). Hence, strain release must be compensated by another effect such as a broader distribution of crystallite sizes along $a$ and $b$ even though the average crystallite size does not change much.

**Chemical microstructure**

Gross elemental compositions of the various silk specimens were obtained from energy dispersive spectroscopic analysis. Chemical microstructures of the Marine, Historic, and Reference Silk materials were investigated with infrared microspectroscopy.

As reported in Chapter 3, none of the silk artifacts contained significant inorganic content when the fibers themselves were examined using EDS. This indicates that surface deposits had not penetrated the fiber substrate. Based on the EDS results it was additionally
concluded that none of the artifacts had been subjected to weighting treatments often applied to silk. This would be expected since undyed samples were selected for study.

Variations in the infrared spectra within and between specimens were studied in this work. Intra-specimen variation for spectra collected along a given fiber is minimal with respect to band positions and intensities. When spectra from different fibers for any given specimen were compared, peak positions were fairly consistent but there was variation with respect to peak intensities. Being a natural polymer, silk exhibits considerable variation in properties due to several factors including rearing conditions and fiber spinning process (159). Such variations have been observed by numerous researchers (159,171-173). Because these variations in spectra are unavoidable, it is particularly important to minimize variations that could arise from inconsistent sample preparation and data-collection techniques, so as to obtain quality spectra.

The infrared spectra of Reference Silk were consistent with spectral data of Bombyx mori silk reported in the literature. All characteristic fibroin peaks identified in the Reference Silk were also identified in the Historic and Marine Silks. Additionally, no new peaks were identified in either the Historic or the Marine specimens. Based on infrared spectroscopic evidence, neither the age of the Historic Silks nor the combined effect of age and the deep-ocean environment appear to have chemically altered the structure of silk fibroin. Literature reports of carbonyl formation due to oxidation resulting in an infrared band at 1735 cm$^{-1}$ in silk fibroin (55) were investigated, and no evidence of this band was found in Historic and Marine Silks. Degradation of fibroin both as a result of exposure to light (73,92,174) and chemical oxidants (59-61) has been shown to notably alter the tyrosine content of fibroin.
However no evidence of alteration of the infrared band at 1515 cm\(^{-1}\) attributed to the tyrosine moiety (116) was found in the Historic and Marine Silks versus the Reference Silk.

There are several complementary effects that could contribute to the chemical stability of the Historic and Marine Silks. Firstly, all specimens came from relatively unexposed areas of the artifacts, i.e. from seam allowances. It has been shown that chemical- and photo-oxidation occur maximally at exposed surfaces of the material (175), and it quite likely that limited exposure to surrounding chemical agents is partially responsible for the preservation of the chemical character of the Historic and Marine Silks. While light exposure is not a factor in the marine environment, for Historic Silks light has been shown to cause substantial chemical degradation and consequent yellowing. Limited light exposure decreases susceptibility to chemical degradation in the Historic Silks.

As discussed in the literature review, the isoionic point is important in controlling the chemical reactivity of silk fibroin. The pH of the marine environment at the S.S. Central America site is in the 7.99 to 8.14 range (3), and if the previously discussed anoxic conditions and resulting lower pH within the trunk are considered, the local environment in the vicinity of the artifacts falls within the isoionic range of silk. In this range, the degradation of silk fibers due to acidic or alkaline hydrolysis is minimal. With respect to the Historic Silks, ambient pH also falls well within the silk’s isoionic range, and inhibits acidic and alkaline attack.

Thirdly, the catalytic effect of heavy metals like tin, lead, and zinc used in weighting silks on fibroin degradation has been well-established (176). Based on EDS results of the fibers themselves neither the Historic nor the Marine Silks appear to have been weighted,
making them less susceptible to chemical attack. The fourth reason for chemical stability in
the marine environment can be attributed to the anoxic conditions that are thought to persist
within the confined environment of the trunks in which the artifacts were stored. Egerton
(177) reports that negligible chemical damage was observed on dyed and undyed silk when
oxygen is absent from the surrounding environment. Lastly, the cold temperature and the lack
of light at the site also contribute to chemical stability of these marine silk artifacts.

From a chemical perspective therefore, it appears that several factors including limited
accessibility to the surroundings, an isoionic environment, lack of weighting agents, oxygen
exclusion, low temperature, and dark conditions act in tandem to promote chemical
preservation of the artifacts. Despite these indications of artifact preservation, further
quantitative evidence is needed to confirm the stability of silk to chemical degradation.

Microbiological activity

While deep-sea environments are rich in microbial population, the slow rate of
decomposition of organic materials not readily available to microorganisms in this
environment has been noted (178). Herdendorf et al. (3) mention that these observations in
the deep-sea environment have been validated at the S.S. Central America site. They note that
the reason for this is perhaps the physical separation of the organic matter from the
microorganisms.

The microorganisms found at the site include large numbers of non-filamentous
bacteria and two types of fungi. While the fungi were isolated only from the sediment in the
area of the shipwreck, bacteria were found both in the sediment and the shipwreck artifacts
(3). The presence of rod and cocci bacteria on linen artifacts from the *S.S. Central America* has been previously reported (3). Chen (8) observed the digestive action of rod-shaped microorganisms on cotton artifacts from this site. Neither the presence of such bacteria nor any indication of microbiological degradation in the marine environment has been observed on the silk specimens. It is concluded that the bacterial strains isolated from the site are cellulolytic rather than proteolytic in nature.

The fact that biofilms observed on the silk artifacts in this work have not been reported on any other artifacts from the *S.S. Central America* site (3), and the fact that this feature is more extensively observed on the Historic Silk artifacts in comparison with the Marine Silks, indicates that this feature is not the result of marine exposure. Rather, microbial attachment on the Marine Silks appears to be a post-exavation consequence of storage. Additionally, though in absolute terms this characteristic is more extensively observed in Historic Silks, given the differences in time of exposure to ambient conditions between the Marine and Historic Silk artifacts, it is proposed that Marine Silks are more susceptible substrates for such microorganisms. Two possible explanations for the increased susceptibility are the residual effects of the slightly alkaline marine environment and the change in the physical microstructure of Marine Silks.

Since the deep-ocean environment at the *S.S. Central America* site has been shown to be mildly alkaline in nature (3), it is assumed that even after removal from that environment into deionized water and subsequent freeze-drying (4), the residual, internally adsorbed moisture that is not removed on freezing could be alkaline. For instance, the difficulty in completely neutralizing silk without prolonged dialysis against distilled water has been noted.
Since the silk specimens in this research came from the relatively well-protected seam-allowances of the neck- and waist-plackets of the artifacts, the residual alkalinity could be more pronounced in comparison with other artifacts that were more directly exposed to the deionizing and freeze-drying treatments.

Since silk fibroin is amphoteric in nature, i.e. containing both acidic and basic groups, its charge density varies as a function of the pH of the solution around it. When the pH of the solution is in the vicinity of the isoelectric point of silk, namely in the 4.5 to 5.0 pH range, the fibroin becomes neutral with respect to electric charge. As the pH deviates positively from the isoelectric point the negative charge residing on the fiber increases and vice versa (62).

The importance of the ionization state of silk fibers as a function of pH has been demonstrated with respect to fiber behavior, such as dyeing (159). When silk is subjected to an aqueous solution the net charge that develops on the surface is a function of the ionization of its amino and carboxyl groups, which in turn is pH dependent (159).

The electrostatic attraction of the charged fibroin substrate and oppositely charged pharmaceutical penetrants has been demonstrated (62). The close relationship between the positive charge on a fibroin substrate and fibroblast cell attachment has also been shown (179,180). With respect to microbial attachment, the importance of electrostatic interactions between substrates and microbial cells has been extensively studied (181-187). Since the Historic Silks have been in an environment where the pH is higher than the isoelectric range, this indicates that there is a net negative charge on the fibers. Additionally, since microbial attachment has been observed on the Historic artifacts, it is assumed that if electrostatic attraction is the cause for such attachment, the attached cells must be positively charged. It
is proposed that because of the relatively more alkaline character of the Marine Silks as compared to the Historic Silks, a greater negative charge develops on the Marine Silks. Therefore the Marine Silks are more likely substrates for this type of microorganism.

The second possible cause for increased microbial susceptibility of the Marine Silks is attributed to the change in the physical microstructure of the Marine Silks. Degummed silk fibers are extremely resistant to microbial attack mainly due to the compact packing of the polypeptide chains in the fiber, which prevents easy access of large enzyme molecules into the internal structure of fibroin (28,188). Because of this inaccessibility, even if microbes grow on the fiber substrate, they depend on extraneous digestible material for their nourishment rather than cause enzymatic degradation of the protein. However they could produce metabolic wastes like acids that cause damage to the fibroin. In the previous sections, exposure to the deep-ocean environment has been shown to cause considerable swelling and alteration to the crystalline character of two of the Marine Silk artifacts in comparison with the Historic Silks and the Reference Silk. The effect of these changes microstructure is twofold. First, it is possible that the enzyme molecules are now able to penetrate the less densely packed and oriented fine structure of the silk, making it more susceptible to enzymatic attack. Second, the more accessible structure increases susceptibility to degradation by the metabolic waste products produced by the microbes. While no signs of microbial degradation were noted in the areas of microbial activity in either the Marine or Historic Silk specimens under investigation, this could be a function of the exposure time necessary for such degradation to occur. It is also possible that the analytical tools employed in this work are not sensitive to the nature and extent of microbial degradation that has occurred.
Analytical technique evaluation

The principal issue concerning the analytical study of historic artifacts stems from the fact that such materials are rare and large amounts of sample are not available. The dilemma lies in the tradeoff between the necessity for physical and chemical microcharacterization of artifacts on the one hand, and sample consumption on the other. The challenge is to use the right combination of analytical characterization techniques so as to be able to understand fully the degradative/preservative state of the material and speculate on the possible physical and chemical processes that result in this state. Armed with this knowledge, the conservator can make informed recommendations regarding treatments to optimize the longevity of artifacts, rather than take an experimental approach to artifact conservation. This section discusses the sampling requirements of the different techniques used in this research and evaluates the techniques in terms of the kinds of information they provide regarding the state of the material.

Sample consumption

Before evaluating the different techniques with respect to sample consumption considerations, it is necessary to point out that the issue with limiting sample size and sampling area is that the sample will not necessarily be representative of the physico-chemical state of the entire artifact. In this work, samples of Historic and Marine Silks were obtained from the seam allowances of the artifacts. Such areas are more shielded from external chemical and physical agents that cause degradation. It is therefore quite likely that areas that
are more open are more degraded than the findings reported herein. However, this issue is unavoidable, as sampling from areas of the artifact that affect their display is not viable.

Two parameters of importance in evaluating the applicability of analytical techniques to the study of historic artifacts are the amount of sample needed and the destructiveness of the technique on the sample, namely, sample requirement and sample consumption. These two considerations are not only crucial in determining whether or not a particular technique will be used, but should also drive the order in which the analyses are performed (189).

In this work, DSC and XRD analysis needed more sample than the other techniques. Sample requirements for DSC ranged between 5 to 10 mg per scan. XRD consumed the maximum amount of sample. While initial XRD trials on Reference: Silk using a standard holder necessitated about 400 mg of silk fibroin sample to be used for a good quality spectrum to be obtained, subsequent use of a holder with a smaller cell size allowed quality spectra to be obtained with about 75 mg of sample. Because of the large sample requirements, multiple diffraction patterns for each specimen were not collected. The principal drawback of this is that statistical tests could not be performed to test for significant differences in unit cell dimensions and crystallite sizes. Attempts were also made to use the Debye-Scherrer camera method due to the much smaller sample requirements of this technique versus the powder diffractometric technique reported herein. However, resilience of silk fibroin prevented satisfactory sample packing into thin bore capillary tubes, resulting in poor quality spectra. The diffractometric technique was therefore chosen over the camera method.

In terms of the sample requirement, IR, OM, SEM, and EDS analyses could be performed on single fibers. While multiple fibers were used in order to be able to get
statistically reliable results, from a qualitative perspective single fiber analysis is possible with these techniques. As previously mentioned, to ensure that data is representative of the entire artifact sampling multiple fibers is advisable. For both IR crystallinity and fiber diameter measurement, multiple measurements were performed on at least 10 fibers for each specimen.

As opposed to traditional IR spectrometry which requires about 0.2 mg of specimen to be able to obtain good quality spectra using a 0.7mm diameter pellet (8), the IR microspectroscopic technique used in this work permitted quality spectra to be collected on single fibers. Additionally because the sample preparation involved fixing the single fibers across a slit in a cardboard template, the sample was not destroyed and could be reused. Sample preparation for SEM and EDS involved fixing the samples on stubs with carbon paste and carbon-coating to improve conductivity. While the samples could therefore not be reused for other analyses, the same samples were used for both these techniques. With these techniques too, in the interest of obtaining representative data, multiple fibers were mounted and analyzed.

While specimen consumption and destructibility are crucial factors in determining whether or not a given technique is suitable for the study of historic artifacts, the importance of understanding the physical and chemical microstructure are equally important as they directly impact conservation and storage recommendations. This in turn determines the subsequent survival of artifacts. In light of the fact that the conservation of organic marine artifacts is still in its infancy (2), the implications of the previous statement are particularly serious with textile artifacts. Also since little or no work has been published on marine silk artifacts (16), exploration of the action of the ocean on silk needs to be established. Once
established, investigations based on the use of less-destructive techniques that do not have relatively large sample requirements can be used, to understand the state of silk artifacts recovered from marine burials.

Structural information

The methods used in this research provided complementary information regarding the physical and chemical structure of the artifacts at various levels of molecular arrangement. SEM and OM provided information regarding the gross fiber morphology of the fibers and aided in the investigation of deposits on the fiber surfaces. Additionally, microbiological activity that was not apparent to the naked eye or under examination with OM could be inferred from the use of SEM. Since SEM was used in conjunction with EDS, elemental composition of the fibers, microbial growth, and of the surface deposits could be determined. It is extremely important to understand the composition of surface deposits before making recommendations regarding the possible role of these deposits in accelerating further deterioration. Clues regarding the considerable detrimental effects on the physical structure of silk fibroin under prolonged marine influence can be inferred. For example, fiber diameter increases in two of the three Marine Silk specimens provide information at a macro-level regarding possible microstructural decrease in order caused by the marine environment. Similarly, changes in fiber gross morphology observed under the SEM such as cracking and fibrillation are indicators of alterations in the chemical and physical microstructure of materials. Such alterations can be confirmed with the other techniques used in this work.
Single fiber IR microspectroscopy provides information regarding both the chemical and physical microstructure of silk. While this technique provides quantitative information regarding the short-range order in the specimens investigated, chemical information obtained is qualitative in nature. Quantitative comparisons of peak intensities to assess chemical composition differences between specimens cannot be made. From a chemical perspective, qualitative comparisons of the presence or absence of IR sensitive bands in fibroin are useful in understanding degradation mechanisms or lack thereof. For a better understanding of the chemical changes in fibroin as a result of aging and deep-ocean exposure, it is recommended that quantitative amino acid analysis be performed. This will provide information about the selective chemical alterations in the amorphous and crystalline regions.

As previously mentioned, IR provides quantitative information about the short-range order in the artifacts. In contrast, XRD results are indicative of the artifact's long-range order. Information regarding molecular mobility and thermal stability are obtained from DSC results. When the results from these three techniques are considered in conjunction with other, not only is a more complete understanding of the physical microstructural characteristics of the specimens possible, but information regarding the changes to the physical microstructure in the Historic and Marine Silk artifacts can also be obtained. For example, in this work, based on the fact that in two of the marine specimens, long-range order is found to be much more altered than the short-range order, molecular rearrangement from the crystalline to the ordered amorphous configuration is hypothesized.

To summarize, the need for the use of multiple techniques in understanding the state of the material and possible mechanisms of alteration has been emphasized. While sampling
considerations are important, the need for fundamental information regarding the state of silk artifacts is needed, given the paucity of such work particularly from a deep-sea context. Without such information, the subsequent lifetime of such artifacts can be drastically reduced.

Conservation implications

The task of the conservator is to arrest and reduce the rate of degradation processes. This can be achieved by several techniques. Inhibiting the artifact from absorbing the activation energy required for deterioration can be achieved by controlling the storage conditions. For example, exclusion of light is particularly important in the conservation of silk artifacts, as are controlling the oxygen, pH, and relative humidity levels in the storage atmosphere. This is important not only to inhibit detrimental chemical alterations in fibroin, but also in controlling microbial attack. Mechanical deterioration can be controlled by resting the silk artifacts on a flat surface.

Based on the results of the analytical techniques reported herein, the following characteristics of Marine Silk should be considered in determining conservation treatments. First, Marine Silks have a more accessible structure as evidenced by their lower crystallinity and larger fiber diameter in comparison with Reference Silk. Additional evidence of alteration to the physical structure of Marine Silk comes from striations, cracking, and flattening phenomena observed in these artifacts using SEM. Second, extensive mineral deposits on the surface of Marine Silks could also exacerbate subsequent degradation of these materials. Third, the residual alkalinity in the Marine Silks could increase their susceptibility to chemical agents and microbiological activity.
Conservation and storage efforts can focus on treating the artifacts themselves and/or controlling the environment in which they are stored. Vacuuming with a low powered vacuum unit is suggested in order to remove surface contaminants including loose mineral deposits. Wet cleaning of textile artifacts with deionized water has been shown to successfully alter the pH of such artifacts (189). Such a treatment is suggested on the Marine Silk artifacts to remove the residual seawater salts and alter the pH of the artifacts. This could reduce the susceptibility of the artifacts to attack by microorganisms and chemical degradation agents. Additionally, wet cleaning could remove some surface deposits. For example, the use of a mercaptoacetic acid bath to remove iron compounds on the surface of silk textiles from the Machault has been reported to be successful (24). Prior to using such a technique however, systematic testing of the treatment on small samples should be conducted.

In terms of controlling the storage environment the importance of protecting textiles from light, dust, handling, and physical stresses has been noted (24). Because of the sensitivity of silk to light, storage should ideally be in the dark. If this is not possible due to display requirements, low light levels should be used. The use of an inert atmosphere like nitrogen can minimize the exacerbating effects of oxygen. Atmospheric pH control is important because of the amphoteric nature of silk and from the perspective of microbial enzymatic attack. Maintaining conditions that lie within both the isoionic and isoelectric range for fibroin can help control both chemical attack and microbiological activity.
CHAPTER 6

SUMMARY AND CONCLUSIONS

The purpose of this work was to investigate the physical and chemical microstructure of Marine Silk textiles in comparison to modern Reference Silk and Historic Silk, develop an understanding of the mechanisms which cause preservation or degradation of silk artifacts during long term marine storage, initiate a more scientific approach to conservation problems, and assess the effectiveness of specific analytical techniques in characterizing textile artifacts. In particular, the present study was conducted to close gaps in the current literature both regarding the research on historic silk textiles in a marine environment and regarding the systematic nature of the analytical approach adopted.

Physical and chemical microstructure of silk

The first goal of the present work was attained by using a diverse set of physical and chemical micro-characterization techniques. Optical and scanning electron microscopy were used to determine the fiber diameter, fiber surface characteristics, deposits, and defects. The elemental composition of fibers and surface deposits was analyzed using energy dispersive x-ray spectrometry. X-ray diffraction provided useful information on crystallite size, unit cell
dimensions, and degree of crystallinity. Fourier-transform infrared spectrometry was used to contrast the long-range order measured using x-rays with the short-range order measured in FTIR. Differential scanning calorimetry provided additional information regarding the crystalline state of the material through characterization of the thermal transitions.

Compilation of results from all the above techniques provides experimental support for the three-phase structure of silk and the alteration of this structure in the Historic and Marine Silks under investigation. In the three-phase model, the crystalline β-sheet structure of silk is linked to the amorphous or random-coil phase via a phase in which molecular chains are not perfectly arranged but nevertheless exhibit a high degree of lateral order. To substantiate the existence of the ordered amorphous phase, and the alteration of the physical microstructure of Historic and Marine Silks, results of four complementary techniques have been used: optical microscopy, x-ray diffraction, FTIR microspectroscopy, and differential scanning calorimetry. Optical microscopy was used to identify significant differences in fiber diameter between two of the Marine Silk samples, 29049 and 33707, and the other samples. Since it is known that coarser fibers exhibit a lower degree of molecular and crystalline order than finer fibers (159) this measurement indicates that the degree of crystallinity of these two Marine Silk samples may be lower than the degree of crystallinity of all other samples. Crystallinity measurements based on x-ray diffraction and FTIR spectroscopy, show a decrease in crystallinity in all Marine silks and Historic Silk 145b and 145c in comparison with Reference Silk and Historic Silk 88b. In particular, the two Marine Silks with the larger fiber diameters, i.e. 29049 and 33707, have x-ray crystallinity values that are significantly lower than those of Historic Silk 145b, Historic Silk 145c, and Marine Silk 29054. However, FTIR
crystallinity ratios in these two specimens are not significantly different from FTIR
crystallinity ratios of Historic Silk 145b, Historic Silk 145c, and Marine Silk 29054.  
Crystallinity measures using FTIR spectroscopy are sensitive to short range order. Based on  
the results of these two techniques, the long-range order appears to be more disrupted than  
the short-range order as a result of prolonged exposure to the deep ocean environment. This  
suggests that part of the crystalline phase has been transformed into ordered amorphous silk.  
Ordered amorphous silk has a similar short-range order as crystalline silk, but exhibits  
significant long-range disorder.

The x-ray and FTIR crystallinities of all Marine Silks and Historic Silk 145b and 145c  
are lower than that of the Reference Silk. This indicates that crystalline silk content decreases  
relative to amorphous silk content. This implies a transition from the crystalline phase to the  
ordered amorphous and amorphous phases of fibroin. However, DSC measurements show  
no glass transition for all Marine Silk specimens and Historic Silk 145b and 145c. The  
intensity of the glass transition, which is very weak in the Reference Silk and the Historic Silk  
88b, is proportional to the amount of amorphous material present. Since the amorphous  
materials contains amino acids with bulky and polar side chains, water and other solvents are  
known to attack the amorphous silk first and substitute protein-protein hydrogen bonds with  
protein-water hydrogen bonds. This will lead to preferential dissolution of amorphous silk and  
hence to a suppression of the glass transition temperature.

The crystallite size in the interchain and intersheet directions were determined in the  
Reference, Marine, and Historic Silk specimens and were found to be relatively unchanged  
as a result of age and prolonged marine exposure. This, in conjunction with the reduced
crystallinity of the Historic and Marine Silk artifacts, and the fact that crystallite size did not change appreciably in the interchain and intersheet directions, suggests that reduction in crystallinity occurs at the ends of the crystallites along the fiber axis. At these ends the crystalline silk is transformed into ordered amorphous silk. Since water and other agents are preferentially adsorbed in the amorphous region these agents have a natural channel to attack the ordered amorphous and crystalline region.

In comparison with Reference Silk, the fibroin unit cell parameters were not altered in the Historic and Marine Silk specimens. Results for the unit cell parameters agree well with literature, in particular, with the recent results of Takahasahi (86) that support a antipolar-antiparallel β-pleated sheet structure of silk.

SEM analysis established that the morphological changes and surface deposits are much more pronounced in the Marine than in the Historic Silk specimens. The consequences of microbiological activity were however found to be more extensive on the Historic Silk specimens in comparison with Marine Silk. It is suggested that the more accessible physical microstructure of the Marine Silks, along with any residual alkalinity in these specimens, make Marine Silks more susceptible to attack by microorganisms. Since their recovery from the seafloor, these silks may well degrade more quickly than their counterparts that remained in a terrestrial environment.

The elemental compositions of the fibers, biofilms, and fiber deposits were determined using energy dispersive x-ray spectrometry. The fibers themselves displayed the composition of fibroin alone with no elemental composition attributable to contamination from the seawater. Rather, only discrete deposits on the surfaces of the Historic and Marine Silks were
shown to contain inorganic elements while continuous paste-like deposits on the surface of Marine Silks were organic. Differences in the elemental composition of the deposits on the Historic and Marine Silks were attributed to the environments to which they were exposed.

Comparison of the IR bands between the different specimens investigated does not provide evidence for chemical alterations in the Historic and Marine Silks in comparison with Reference Silk and with each other. While this provides confirmation of the chemical robustness of silk, additional investigations of the chemical composition are suggested. This is discussed in a later section of this chapter. The chemical stability in silk is usually attributed to the compact physical microstructure of fibroin, and apart from hydrolysis of silk on immersion in alkali or acid solutions, light is the primary cause for chemical degradation of silk. Since Historic Silk does not appear to be chemically altered in comparison with Reference Silk, it is quite likely that these specimens have not been stored under conditions of overexposure to light. Given the prolonged exposure of the Marine Silks to a slightly alkaline aqueous environment, the chemical stability of the Marine Silks is somewhat surprising. However the immediate microenvironment in the vicinity of the artifacts, i.e. anoxic conditions, pH within the isoionic range of silk, darkness, and low temperature are probably responsible for this stability in the marine environment.

**Evaluation of analytical techniques**

The main challenge in the characterization of historic artifacts lies in the scarcity of sample available for systematic research. In general, techniques that use little sample or that are non-invasive, non-destructive, and allow sample reuse are preferred. Additionally analysis
costs constrain the range of tools available to the conservator. At the same time complete
colorization of such artifacts is required to make informed conservation and storage
decisions. This is especially true when information regarding similar artifacts exposed to
comparable conditions is not available as a reference against which to compare new finds. In
the current context of artifacts recovered from a deep-ocean site, the effects of exposure of
different artifacts to this environment have first to be established. Subsequently recovered
artifacts can then be understood and handled by drawing on the information obtained from
prior characterization studies.

One goal of the current study was to establish the efficiency and usefulness of the
chosen analytical techniques in inferring the physical and chemical state of the artifacts. In
particular, it is important to know if the use of techniques with fairly large sample
requirements such as differential scanning calorimetry or x-ray crystallography is warranted.

Optical microscopy and scanning electron microscopy are techniques that can be
applied to single fibers and that give a good overview over the gross morphology of the
artifacts. While these techniques do not provide information regarding the physical and
chemical nature of the artifacts at a molecular level, they can be indicative of the molecular
microstructure and can direct subsequent work to investigate this structure.

When scanning electron microscopy is used in conjunction with energy dispersive x-
ray spectrometry, elemental information regarding characteristics observed with scanning
electron microscopy can be obtained. For example, through energy dispersive x-ray
spectrometry, the surface deposits observed with scanning electron microscopy can be
examined to determine their elemental composition and to establish which are inorganic
deposits and which are organic in nature. Also, in the context of the current work, energy dispersive x-ray spectrometry established that inorganic elements had not appreciably penetrated the fibers themselves, an indication of the integrity and resilience of silk in a marine environment.

Apart from the elemental information obtained from energy dispersive x-ray spectrometry, FTIR microspectroscopy is the only other technique used in this work that provides information regarding the chemical character of the artifacts. This non-destructive technique allows good quality spectra to be collected using single fibers. FTIR spectroscopy demonstrated that neither age nor the combination of a marine environment and age appear to have qualitatively altered the chemical structure of the silk specimens under consideration. For example, no oxidation bands are observed in any of the artifacts investigated. However, more subtle quantitative alterations in chemical structure, such as the selective dissolution of the amino acid tyrosine, are difficult to investigate using FTIR due to variations in path length and concentrations between samples. It is suggested that further chemical investigation using amino acid analysis be employed for this purpose.

Fiber diameter measurements using optical microscopy indicated that two marine specimens may have a different physical microstructure than the other specimens due to their increased fiber diameters. While this difference could not be identified in FTIR measurements, it was clearly recognizable in x-ray diffraction measurements. Therefore in the current context, the comparison of FTIR and x-ray crystallinity values pointed to the central role that the ordered-amorphous phase in fibroin plays in the degradation process of Marine Silk. Despite the sample requirements of x-ray diffraction, the use of this technique in conjunction
with IR spectroscopy is needed to understand the physical alteration in the crystalline phase of fibroin. Finally, in order to understand the physical changes involving the amorphous phase of silk, a technique like differential scanning calorimetry was necessary to establish an overall decline in amorphous material either due to recrystallization or due to preferential dissolution.

Based on the above discussion it is concluded that while the multiple techniques used in this work are needed for the basic characterization of marine silk artifacts. Once established, further characterization of other silk artifacts from marine sites can be restricted to techniques that are less sample-intensive. For example, changes in fiber diameter may be enough to indicate disruption to long range order, established in this work via the x-ray diffraction technique. Additionally, the necessity for the use of a technique such as amino acid analysis that is sensitive to quantitative changes in chemical structure has also been suggested.

**Conservation considerations**

The conservation of marine artifacts is still in its infancy. A thorough understanding of the physical and chemical microstructure of marine and historic artifacts is important for the future conservation efforts. Since little or no work has been published on marine silk artifacts, exploration of the action of marine conditions on silk needs to be established. Once established, investigations based on the use of less-destructive techniques that do not have relatively large sample requirements can be employed to infer the state of silk artifacts recovered from marine sites.

Based on the results of the research reported herein, conservators must be aware of the increased frailty of Marine Silk compared to Historic and Reference Silk. Some possible
causes of this frailty include: increased accessibility to degradation agents due to the change in the physical microstructure of silk; increased susceptibility to microorganisms due to the increased alkalinity of Marine Silk and more open microstructure; and high concentration of mineral surface deposits on the Marine Silk specimens. Conservation and storage efforts can focus on treating the artifacts and/or controlling the environment they are exposed to. Artifact treatment can include vacuuming to remove surface deposits, and wet cleaning to modify pH and to remove deposits. Environmental control can focus on pH control, control of oxygen and water vapor in the atmosphere, and limiting exposure to light.

Suggestions for future research

While the present work attempts to study marine silk artifacts in a comprehensive and systematic manner, several questions regarding these artifacts remain to be answered. One of the basic assumptions of this research was that the chosen silk samples are representative of the artifact as a whole. Additionally, it is assumed that results obtained from these specimens can be generalised to other silk artifacts recovered from marine sites. Based on the results, which show that one Marine Silk specimen is different from the other two, it is obvious that this assumption is not necessarily valid. For Marine Silks, the immediate environment plays a crucial role with respect to the degradation or preservation of artifacts. This indicates that our results are valid for material taken from protected areas of the garment and cannot be trivially extended to the full artifact or to all other silk artifacts. A more systematic study of the influence of the local environment of silk specimens, e.g., via the comparison of exposed areas of the same garment with the results presented here, is necessary. In particular, it is
important to establish if more exposed garment areas exhibit different or more pronounced degradation and to establish the consequences of these differences for conservation.

A more thorough understanding of the mechanisms by which surface deposits are formed on marine silk artifacts and of the implication of the presence of these deposits on the degradation of these artifacts must be established. Measurements that systematically compare the properties of areas that have large amount of deposits versus areas that are free of surface deposits can establish the consequence of deposits on marine silks.

The link between long-term marine storage and an increased vulnerability to microbial attack once the marine silk artifact is stored in an ambient environment deserves further attention. Correlations between swelling, crystalline order, alkaline environment and the rate of microbial attack should be studied. For example, pH measurements of the Historic versus the Marine Silks can determine whether there is a difference between the two sets of artifacts. A process for eliminating the threat of microbial attack to marine silk needs to be put in place. Microbial activity in the different artifacts should also be monitored over time in order to establish whether Marine Silks are more susceptible to subsequent microbial attack.

Finally, while FTIR measurements demonstrated that the silk fibers did not undergo chemical modifications in a qualitative sense, it is desirable to perform a quantitative amino acid analysis. This will allow an accurate, quantitative estimation of the chemical composition of Marine Silk as compared to Reference and Historic Silk from which further conclusions regarding denaturation processes can be drawn.
LIST OF REFERENCES


    Fiori, C.; Lifshin, E. Scanning Electron Microscopy and X-Ray Microanalysis; 2nd

13. Badger, R. M.; Pullin, A. D. E.; Rubalcava, H. Research Rept. No. 8; Gates and
    Crellin Laboratory: University of California, 1953.

14. Gallagher, P. K. Thermoanalytical Instrumentation, Techniques, and Methodology,
in Thermal Characterization of Polymeric Materials, Volume 1, 2nd Edition ed.;

    1994, 266 1027-1029.


18. Peacock, E. E. International Biodeterioration and Biodegradation 1996, 38(1), 49-
    59.

    and Chemical Characteristics of Linen Fabrics. M.S. Thesis: The Ohio State
    University, Columbus, 1992.

20. Srinivasan, R.; Jakes, K. A. Examination of silk fibers from a deep ocean site: SEM,
    EDS, and DSC.; Vandiver, P. B.; Druzik, J. R.; Merkel, J. F.; Stewart, J., editors;
    Materials Research Society Symposia ProceedingsMaterials Issues in Art and


230


231


