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เอทีอาร์เอฟที-ไออาร์ไมโครสเปกโทรสโคปี

นางสาวณิชากร ปทุมรังสรรค์

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EFFECTS OF HEAT ON SECONDARY STRUCTURES OF SILK FIBER STUDIED BY ATR FT-IR MICROSPECTROSCOPY

Miss Nichakorn Pathumrangsan

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Petrochemistry and Polymer Science
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Thesis Title	EFFECTS OF HEAT ON SECONDARY STRUCTURES OF SILK FIBER STUDIED BY ATR FT-IR MICROSPECTROSCOPY
By	Miss Nichakorn Pathumrangsan
Field of Study	Petrochemistry and Polymer Science
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ณิ ากร ปทุมรังสรรค์: ผลของความร้อนต่อโครงสร้างทุติยภูมิของเส้นไหมศึกษาโดยเอทีอาร์ เอฟที - ไออาร์ไมโครสเปกโทรสโกปี (EFFECTS OF HEAT ON SECONDARY STRUCTURES OF SILK FIBER STUDIED BY ATR FT-IR MICROSPECTROSCOPY) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ชูชาติ ธรรมเจริญ , 130 หน้า.

ได้นำเอทีอาร์เอฟทีไออาร์ไมโครสเปกโทรสโกปีมาใช้ในการวิเคราะห์เส้นไหม โดยสเปกตรัมของเส้นไหมประกอบด้วยสี่ยอดหลัก ได้แก่ เอไมด์เอ, เอไมด์วัน, เอไมด์ทู และ เอไมด์ทรี ความแตกต่างของสเปกตรัมระหว่างเส้นไหมที่ยังไม่ได้ลอกกาวและเส้นไหมที่ลอกกาวแล้วคือไหล่ของแถบเอไมด์วัน ที่ตำแหน่ง 1679 cm^{-1} ถูกกำหนดให้เป็นแผ่นบีตาแบบขนานสวน แผ่นบีตาเป็นโครงสร้างทุติยภูมิชนิดหนึ่งที่แสดงความเป็นผลึก โดยความเป็นผลึกจะเกี่ยวข้องกับสมบัติของเส้นไหม เช่น ความอ่อนนุ่มและความสามารถในการติดสี ดังนั้นสมบัติของเส้นไหมจะเกี่ยวพันกับโครงสร้างทุติยภูมิ โครงสร้างทุติยภูมิที่ประกอบในเส้นไหมนั้นอธิบายด้วยการวิเคราะห์เดอร์ฟฟิททิง แถบเอไมด์วันของสเปกตรัมใช้สำหรับหาปริมาณของโครงสร้างทุติยภูมิแต่ละชนิด ในการศึกษานี้สภาวะที่ใช้ควบคุมคือ อุณหภูมิ, เวลา และ วิธีการระงับ จากความหลากหลายของภาวะที่ใช้ ปริมาณของโครงสร้างทุติยภูมิจะถูกคำนวณจากพื้นที่ใต้ยอดแต่ละยอดมีความผันแปร จึงอนุมานว่าการเปลี่ยนแปลงที่เกิดขึ้นไม่มีนัยสำคัญ

สาขาวิชา.ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ ลายมือชื่อนิสิต...ณิชากร ปทุมรังสรรค์...

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NICHAKORN PATHUMRANGSAN: EFFECTS OF HEAT ON SECONDARY STRUCTURES OF SILK FIBER STUDIED BY ATR FT-IR MICROSPECTROSCOPY. THESIS ADVISOR: ASSOC. PROF. CHUCHAAT THAMMACHAROEN, 130 pp.

Attenuated total reflection Fourier transform infrared (ATR FT-IR) microspectroscopy has been employed to characterize the silk fiber. The character of silk spectrum contents of the four major peaks: amide A, amide I, amide II and amide III. The difference of spectra between the un-degummed and degummed silk fiber is the shoulder of amide I band at 1697 cm^{-1}. It was assigned to the anti-parallel β-sheet. The β-sheet is one type of secondary structure that shows the crystallinity. The crystallinity is related to the properties of silk fibers such as the softness and dyeability. Consequently, the properties of silk fiber are associated with the secondary structure. The secondary structure contained in the silk fiber could elucidate by curve fitting analysis. The amide I band of spectrum was used to determine the quantity of individual secondary structure. In this study, the controlled conditions are temperatures, times and quenching methods. According to the varied conditions, the quantity of secondary structure that was calculated from individual peak area has fluctuated. It is imply that it does not have significantly changes.

Field of study Petrochemistry and Polymer Science Student's signature. Nich Path

Academic year.....2009..... Advisor's signature. C. Thammacharoen

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Whatever shortcomings in the thesis remain, they are the sole responsibility of the author.

Above all, I am profoundly grateful to my wonderful parents and the endearing family for their patient love, perpetual encouragement, and overwhelming support.

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LIST OF ABBREVIATIONS

B. mori	: *Bombyx mori*
Ala	: Alanine
ATR	: Attenuated total reflection
FSD	: Fourier self-deconvolution
FT-IR	: Fourier transform infrared
Ge	: Germanium
Gly	: Glycine
IR	: Infrared
TIR	: Total internal reflection
IRE	: Internal reflection element
MCT	: Mercury-cadmium-telluride
μm	: Micrometer (10^{-6} m)
mL	: Milliliter (10^{-3} L)
cm^{-1}	: Per cemtimeter
^{o}C	: Degree of Celsius
min	: Minute
M	: Molar
SEM	: Scanning electron microscopy
Ser	: Serine
$NaHCO_3$	: Sodium hydrogen carbonate
TEM	: Transmission electron microscopy
vdW	: van der Waals

LIST OF SYMBOLS

A	: Absorbance
ε	: Absorption coefficient
α	: Alpha
α_1	: Angle of incidence
α_2	: Angle of reflection
θ	: Angle of incidence
β	: Beta
^{o}C	: Celsius
θ_c	: Critical angle
c	: Concentration
l	: Film thickness
I_0	: Intensity of incident beam
I_R	: Intensity of reflected beam
I_S	: Intensity of scattered beam
I_T	: Intensity of transmitted beam
I_A	: Intensity of absorbed beam
I	: Light intensity
μ	: Micron (10^{-6} m)
d_p	: Penetration depth
n_1	: Refractive index of the dense medium (IRE)
n_2	: Refractive index of the sample
ν	: Wavenumber
λ	: Wavelength

CHAPTER I

INTRODUCTION

The fibers of textile industry can be divided into two types, natural and synthetic fibers. Natural fibers either from animals or from plants are remarkable biopolymer. Silk worm is one of natural fiber from animals; it produces silk fiber to form the silk cocoon. *Bombyx mori* silk fibers, natural protein fibers, have been widely used for the production of textile goods for centuries due to their character (i.e. luster, moisture absorbance, strength, etc.). Normally, a silk fiber consists of two types of protein: ***fibroin*** and ***sericin***. Fibroin protein constitutes over 70% of the cocoon. Sericin, the glue, constitutes 20-30% of the cocoon. The structure of silk fiber is two cores of fibroin surrounded by a cementing layer of sericin. When sericin is coated, silk fibers are unsmooth and hard. Therefore the producing of commercial goods will removed the sericin protein; the removal sericin process is called **silk degumming process** [1]. The production procedure of commercial silk fibers has numerous factors that affect the mechanical properties. Thermal treatment is one of factors, which makes impact on molecular conformation of silk fiber. According to protein silk fibers, the structure of protein have four types: ***α-helix***, ***β-strand***, ***random coil*** and ***turn***. Secondary structure of protein is the most interesting thing to study the changes of molecular structure of silk fibers. The quantity of beta strand is related to the crystallinity of the silk fibers. Most of silk fiber consists of the beta strand-this means that silk fiber has greater crystallinity. Due to various applications of silk fiber, such as drug released, anti-bacterial, and antioxidant, so the researcher is interested in silk fiber.

For past decades, many researchers studied the properties of silk fiber. The heat treatment is one of the most popular conditions to testing because it is used frequently in production line of silk goods. In 1976, Hirabayashi et al. [2] studied the changes of molecular conformation by heat treatment. The liquid silk fibroin in silk gland of domestic silk worm changed the random-coil to β-form at about 56^{o}C, and the solid-state silk fibroin changed its structure at about 200^{o}C. For tussah silk fibroin, the α-β transition temperature is 230^{o}C and its degradation temperature is 370-380^{o}C. In 2000, Kweon et al. [3] studied the effect of heat treatment on conformational changes.

The molecular conformation of regenerated *Antheraea pernyi* silk fibroin film was changes above 230^{o}C. The β-sheet structure could be transformed from a random coil conformation on heat treatment. The contents of secondary structure were calculated by IR absorption band of amide V region. In 2002, Zhang et al. [4] used thermogravimetric, differential thermal analysis, and Fourier transform infrared absorption spectroscopy to study the color, size, and shape of cocoon shell after heat treatment and increasing temperature. The size of cocoon decreased with an increasing in temperature and weight was lost from the cocoon shell.

One of the most important properties of silk fiber is the molecular conformation. The molecular orientation and molecular vibration of protein is characterized by FT-IR spectroscopy. This technique is the measurement of the wavelength and intensity of the absorption of infrared light interacting by the functional groups of sample. Many modes of FT-IR spectroscopy can be performed such as transmission and reflection. ATR technique is one of the reflection modes. This technique is the surface analysis, which was used to investigate the secondary structure. In 2004, Shao et al. [5] investigated the structure of silk fibroin by FT-IR spectroscopy. FT-IR absorbance spectra showed the doublet at 1230 and 1263 cm^{-1} (amide III). The doublet band was used to determine the crystallinity of silk fiber. In 2003, Hu et al. [6] determined β-sheet crystallinity in fibrous protein by IR spectroscopy. When IR spectra were collected, the crystallinity of fibrous was determined by mean of the Fourier self-deconvolution (FSD).

For textile industry, degumming is a key process for removing sericin by thermo-chemical treatment of the cocoon. In 2005, Jiang et al. [7] studied the effect of degumming on tensile behavior and mechanical properties of silk fiber. Five different solutions for degumming are distilled water, boracic acid-sodium borate buffer, sodium carbonate, urea, and succinic acid. It was shown that borate buffer degumming is the best among other solutions in term of silk mechanical properties and sericin removal.

According to literatures, it is interesting to study the changes of secondary structure by heat treatment. The molecular structure of silk fibers was investigated by mean of Attenuated Total Reflection Fourier Tranform Infrared (ATR FT-IR) spectroscopy. ATR FT-IR spectroscopy is one of FT-IR sampling techniques, which can provide molecular information of sample surface. Due to the limitation of the commercial ATR accessory, a homemade Ge μIRE were developed by Sensor

Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University. The advantages of this technique are required minute amount of sample and non-destructive technique.

1.1 The objectives of this research

The objective of this research is to study the effect of thermal treatment on the secondary structure by ATR FT-IR microspectroscopy for basic guideline of other applications.

1.2 The scopes of this research

1. To investigate the characteristic of silk fiber (un-degummed silk fibers, degummed silk fibers, silk fibers from each layer of silk cocoon) with ATR FT-IR microspectroscopy.
2. To determine the quantity of secondary structure of non-varied condition and varied condition fibers.
3. To elucidate the relationship of the quantity of secondary structure and crystallinity of silk fibers.

CHAPTER II

THEORETICAL BACKGROUND

Silk fiber is a continuous protein fiber produced by the silkworm so as to form its cocoon. The silk fiber can divide to two main types of silkworms: ***mulberry silk*** (cultivated silk) and ***tussah silk*** (wild silk). Thai silk is one of the mulberry silkworm (*Bombyx mori*) silks [8]. The mulberry silk is produced by silkworm larvae cultivated in providing habitats and fed with the mulberry leaves. It has yellow in color and more silk gum than normal mulberry silk.

2.1 *Bombyx mori* silkworm

The life cycle of silkworm runs for 55 to 60 days. The organism of silkworm passes through four different developmental stages: (i) Egg; the female moth lays many tiny eggs that would be hatched silkworm caterpillars. (ii) Larva; the caterpillar worm eats mulberry leaves and grows bigger. It goes through 4 molts. During the fifth larva period before molting to pupa, silk production occurs. (iii) Pupa; the silkworm extrudes silk fiber from spinneret in the head of the caterpillar worm. When silk fiber is extruded, silkworm draws silk fiber around its body to form a silk cocoon. (iv) Moth; the pupa changes to a moth and comes out of the cocoon. After that, the male and female moths breed. The female moth lays eggs that will continue the next life cycle [9]. The silkworm life cycle is shown in Figure 2.1.

2.2 Silk gland

The silk worm has a gland for synthesizing the silk protein. The gland can be divided into three parts: posterior, middle, and anterior part as shown in Figure 2.2. The posterior part is long about 15 cm. It is used to synthesize silk fibroin that is the pre-spin silk protein called silk I. The middle silk gland produces silk sericin and stores it until spinning. The silk sericin is used to cement the fibroin filament of the cocoon. At the anterior part has a duct, which is called **spinneret**, for spinning the silk

fiber. After extruding, the silk protein would have solid state (similar filament) and more stable than silk I**.** Silk fiber or **silk II** (water insoluble) is drawn and spinned to form silk cocoon [10].

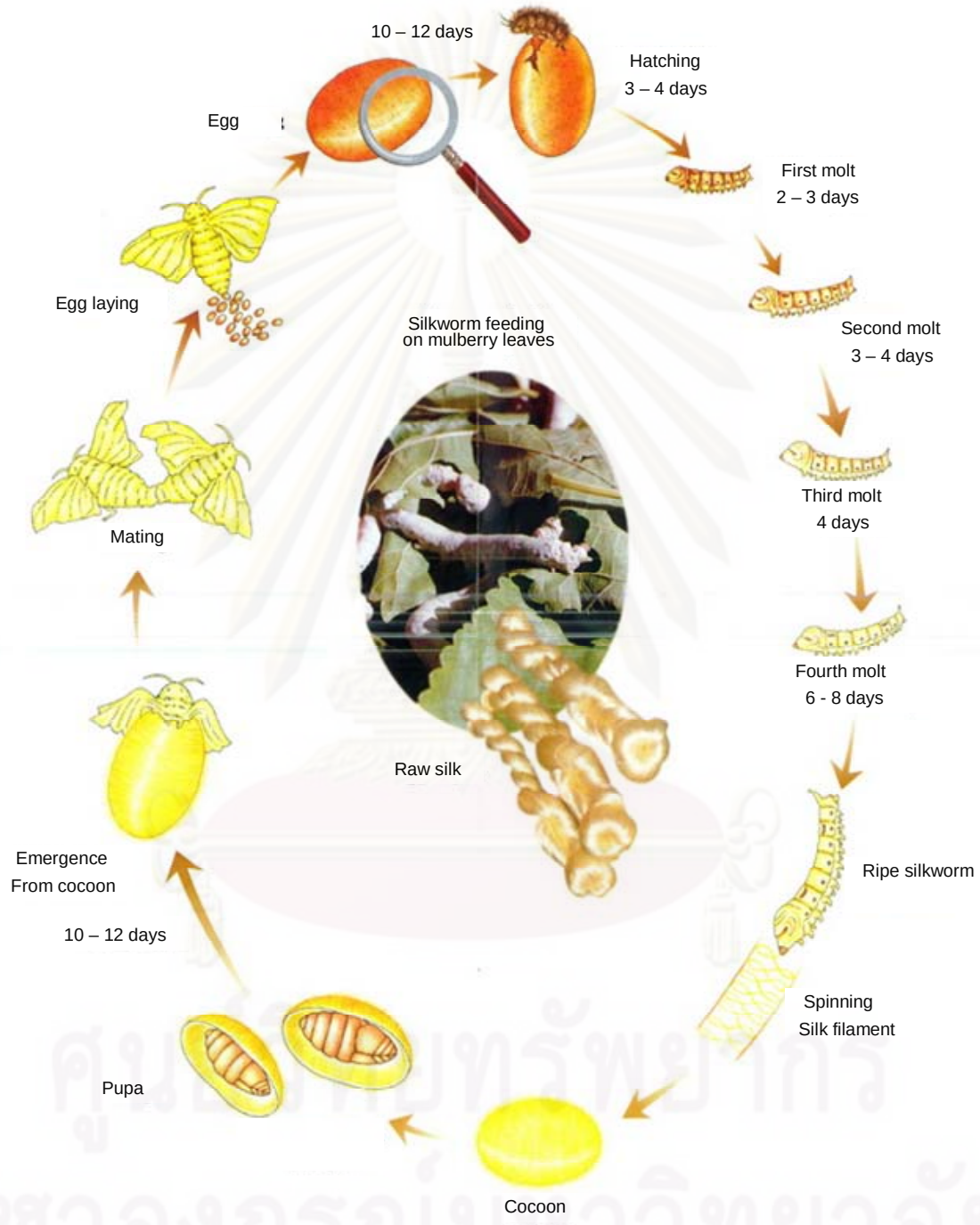

Figure 2.1 The life cycle of *Bombyx mori* silkworm [11].

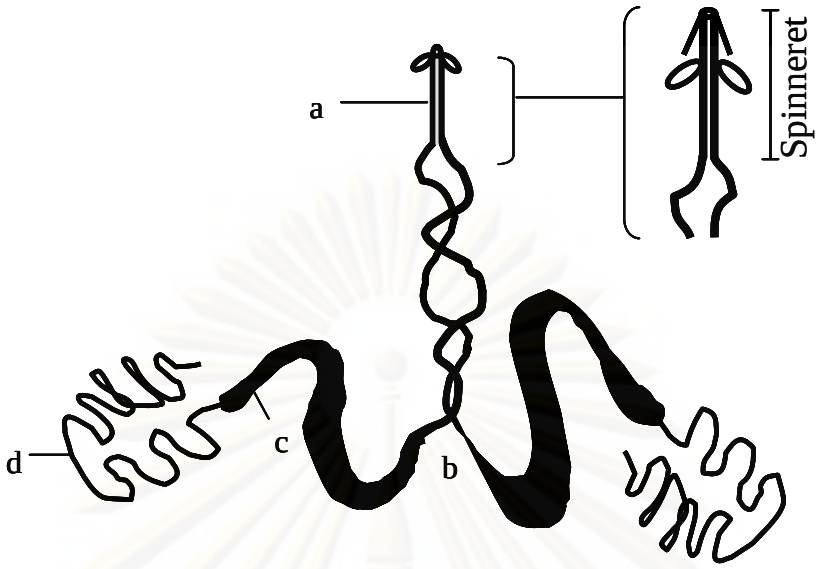

Figure 2.2 Silk gland of *Bombyx mori* silkworm. The length of anterior part (a-b), middle part (b-c), and posterior part (c-d) is about 4, 8, and 20 cm, respectively [10].

2.3 Silk fiber

Silk fiber consists of two types of protein that are ***fibroin*** and ***sericin***. The two brins of fibroin were cemented by sericin silk protein as shown in Figure 2.3. Sericin contains other natural impurity such as fat, wax, inorganic matters, and coloring matters. The properties of sericin are water soluble, oxidative resistant, antibacterial, UV resistant, etc. After degumming, the leftover is fibroin that has different properties from sericin such as water insolubility, dyeing affinity, thermo tolerances and luster. The properties of fibroin are appropriate to produce clothes or many products. The fibroin is formed from amino acids. The major amino acids are glycine (Gly, 43%), alanine (Ala, 30%), and serine (Ser, 12%). The forming of amino acid has an effect on conformation of secondary structure that relates to the crystallinity of silk fiber. The crystallinity can expose the soft and hard property of silk fiber [12-13]. The percentages of amino acid in silk fiber are shown in Table 2.2.

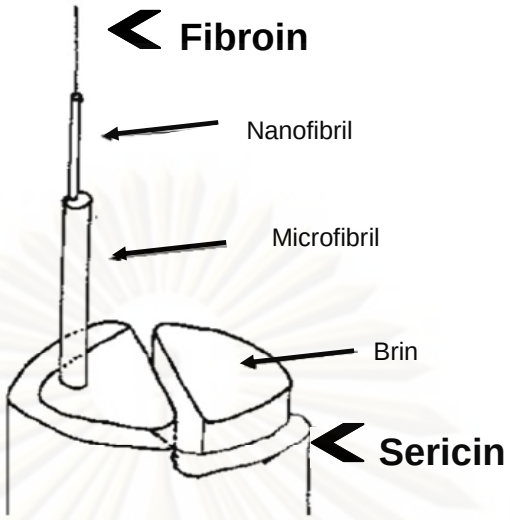

Figure 2.3 The structure of silk fiber. The silk fibroin filament coated by sericin [14].

Table 2.1 The composition of silk fiber [15].

Component	%
Fibroin	70-80
Sericin	20-30
Wax mater	0.4-0.8
Carbohydrates	1.2-1.6
Inorganic matter	0.7
Pigment	0.2
Total	100

Table 2.2 Amino acid composition of silks [8].

Amino acid	Domestic silkworm *B.mori*	
	fibroin	sericin
Glycine	42.9	13.5
Alanine	30.0	5.8
Serine	12.2	34.0
Tyrosine	4.8	3.6
Aspartic Acid/Asparagine	1.9	14.6
Arginine	0.5	3.1
Histidine	0.2	1.4
Glutamic Acid/Glutamine	1.4	6.2
Lysine	0.4	3.5
Valine	2.5	2.9
Leucine	0.6	0.7
Isoleucine	0.6	0.7
Phenylalanine	0.7	0.4
Proline	0.5	0.6
Threonine	0.9	8.8
Metionine	0.1	0.1
Custeine	trace	0.1

2.4 Protein structure

Proteins are biological polymers which the monomer units are amino acid. When amino acids are linked, ***peptide bond*** is formed by linking between the amino group on one amino acid and the carboxyl group on another amino acid. The three dimensional structures of protein are classified to four structures; primary, secondary, tertiary and quaternary structures as shown in Figure 2.4.

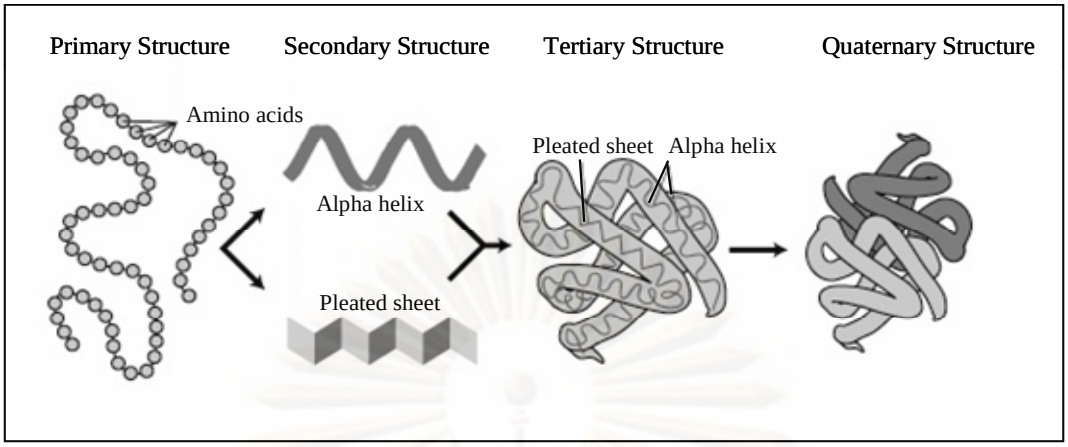

Figure 2.4 The four levels of secondary structure of protein [16].

2.4.1 Primary structure

The linear sequence of amino acid residues along the polypeptide chain is called the primary structure. Individual amino acids are linked via peptide bond.

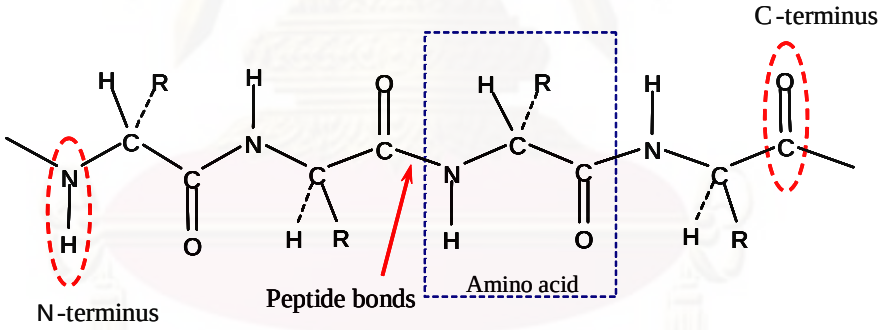

Figure 2.5 The model primary structure of protein [17].

2.4.2 Secondary structure

The secondary structure is a spatial relationship of amino acid residues that is formed by hydrogen bond linking with other. Four common secondary structures in proteins are α-helix, the β-sheet, β-turns, and random coil.

2.4.2.1 α-helix

In α-helix, the polypeptide backbone is coiled at ***right-handed helix*** that looks like rod structure. The α-helix is stabilized by hydrogen bond between the carbonyl group and the amide nitrogen that is the forth residues ahead in the linear sequence. Thus, one full turn of the peptide backbone requires 3.6 residues and the displacement of α-helix per turn is 5.4 A^o as shown in Figure 2.6.

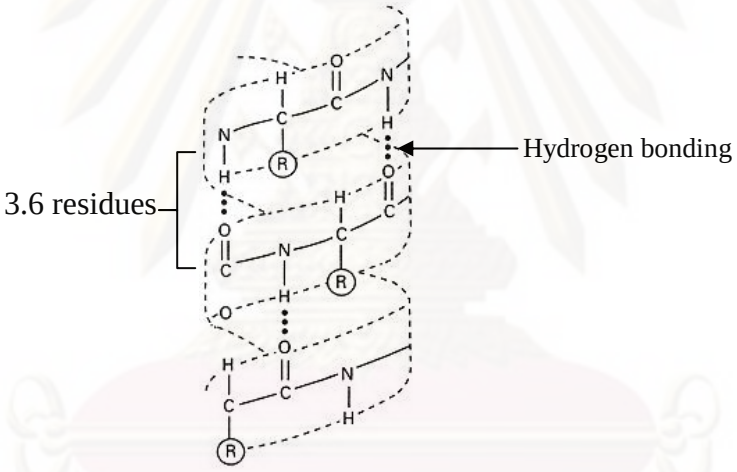

Figure 2.6 The arrangement of hydrogen bonds of α-helix [18].

2.4.2.2 β-sheet

β-sheet is different from α-helix: it is not in the form of rod but sheet. The β-sheet is stabilized by hydrogen bond between the carbonyl and the amide nitrogen group in different polypeptide chains. The β-sheet can be classified to two types that are parallel and anti-parallel β-sheet. Differentiation of two types β-sheets is the direction of the polypeptide chain from the N- to C- terminal. The polypeptide chains are aligned in the same direction from one terminus (N or C) to the other in

parallel β-sheet. On the contrary, the polypeptide chains in anti-parallel β-sheet is aligned in the opposite direction.

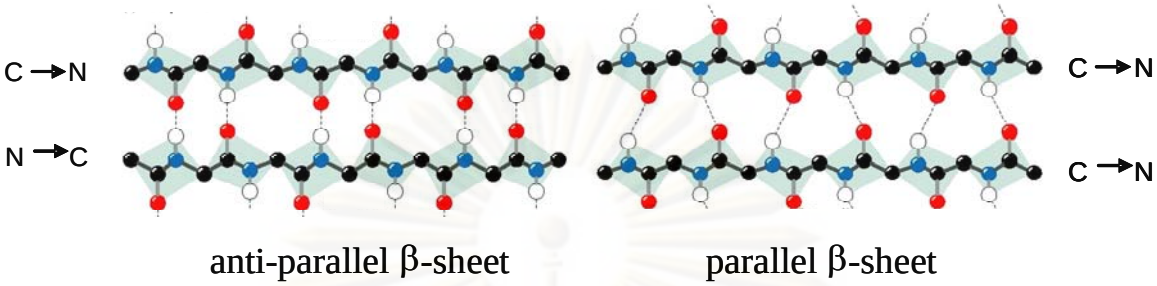

Figure 2.7 The different direction of polypeptide chains in parallel and anti-parallel β-sheet [19].

2.4.2.3 β-turn

A β-turn is U-shaped. Generally, β-turn is found to link two strands of anti-parallel beta-sheet which folds back nearly 180 degrees upon forming of hydrogen bonds within the same chain. Two main types of β-turn are Type I and II which are mirror images of each other.

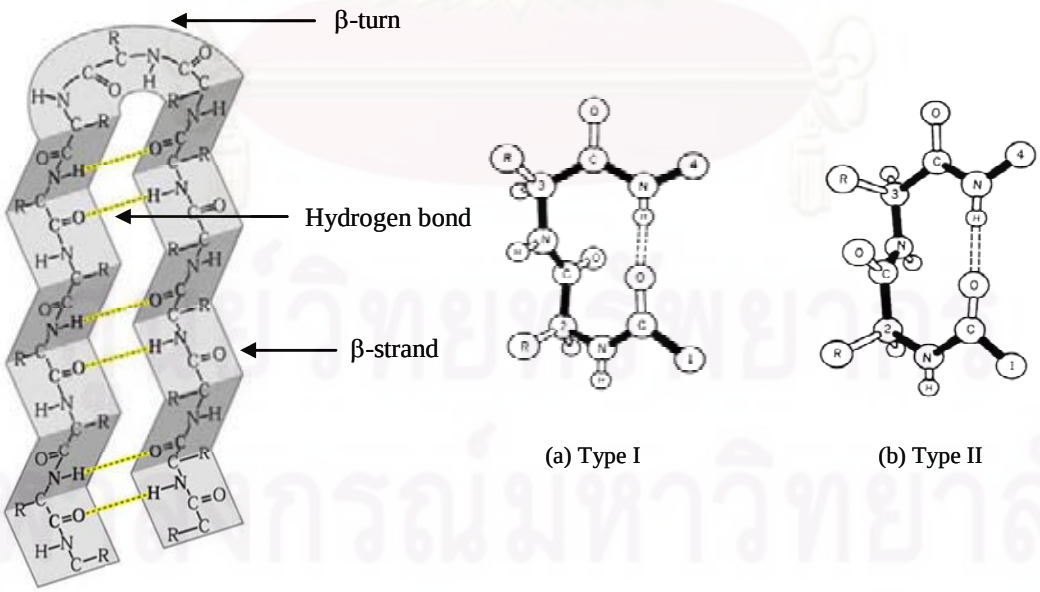

Figure 2.8 The arrangement of molecule of β-turn can form type I (a) and type II (b) [20].

2.4.2.4 Random coil

Random coil is a random conformation of proteins. It does not have the specific shape. Nevertheless, it is a statistical distribution of shapes for all the chains in a population of macromolecules.

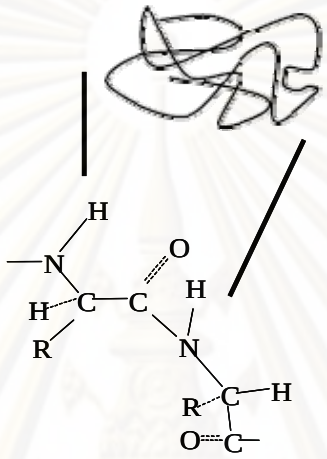

Figure 2.9 Arrangement of random coil structure

2.4.3 Tertiary structure

Tertiary structure can be formed by the folded-polypeptide chain to a compact globular molecule. The tertiary structure occurs from the interactions of secondary structure such as hydrophobic, electrostatic, van der Waals (vdW), hydrogen bonds, and also depends on the forming of disulfide (S-S) bridges [21].

2.4.4 Quaternary structure

The character of quaternary structure is the affiliation of two or more polypeptide chains. The interactions between these chains are exactly the same as those responsible for tertiary structure. The term “subunit” is usually used instead of polypeptide chains.

2.5 Infrared spectroscopy

Infrared spectroscopy is one of the most common spectroscopic techniques for chemical analysis. The chemical functional groups in a sample can be determined by this technique. Since various sampling accessories of this technique, the wide range of sample, such as gases, liquids, and solids, can be analyzed. Hence, Infrared spectroscopy is the most popular technique for elucidating structure and identifying composition of the compound.

The infrared region can be divided into three regions; near, mid, and far infrared regions. The range of infrared region is presented from 12,800 to 10 cm^{-1}. The mid infrared region is the most interest for analyzing chemical structure. The atoms in molecules will vibrate with specific frequency which depending on the atomic weight, bond length, and bond strength.

The molecular vibrations have two major types which are stretching and bending. The various types of vibration of molecules are illustrated in Figure 2.10. The change of dipole moment during vibration called **infrared active** has the effect on the vibration of molecules. Homo-nuclear diatomic molecule such as hydrogen (H_2), nitrogen (N_2), and oxygen (O_2) are not observed the infrared absorption because all of these molecules have zero dipole moment as stretching. For hetero-nuclear diatomic molecules, such as carbon monoxide (CO), has a permanent dipole moment and infrared activity because the stretching of this bond changes the dipole moment.

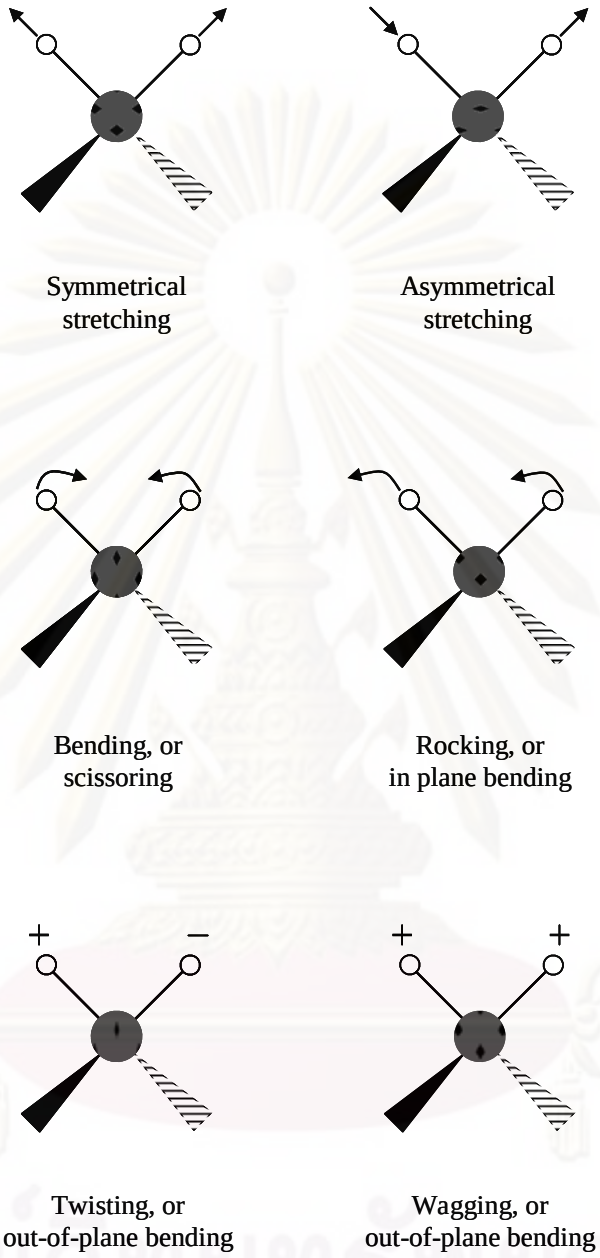

Figure 2.10 Illustration of various vibration modes of CH_2.

2.5.1 Fundamental of infrared spectroscopy

Infrared spectroscopy is one of the most useful scientific techniques that uses to study the interaction of infrared light with matter. When infrared light or electromagnetic radiation impinges with a specimen, the incident beam can be reflected, scattered, transmitted, or absorbed as expressed by the following relationship [22].

$$I_0 = I_R + I_S + I_T + I_A \tag{2.1}$$

where I_0 is the intensity of the incident beam. Respectively, I_R, I_S, I_T, and I_A are the reflected, scattered, transmitted, and absorbed beams. If a sample interpolates between a source of light and a detector as shown in Figure 2.11, the incident beam will be absorbed by a sample. Transmitted beam is detected, but reflected and scattered beam are not detected.

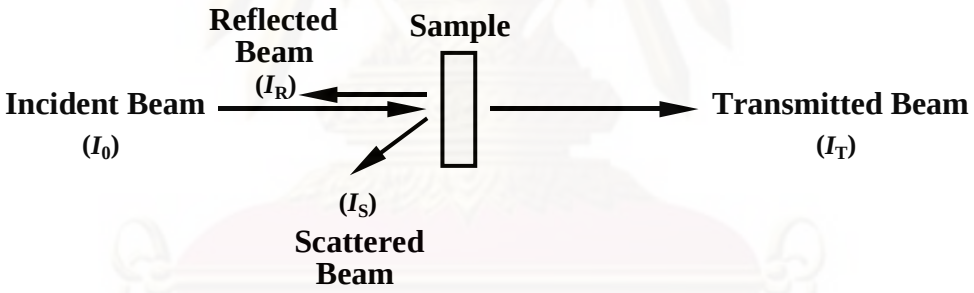

Figure 2.11 The position of beam after incident beam impinges on a sample.

After the transmitted beam is detected, the proportion of transmittance of sample is known by measuring the ratio of the sample attenuated (I_o) and non-attenuated (I) intensities [23].

$$I / I_0 = e^{-A(\bar{\nu})} = e^{-c_2 \varepsilon(\bar{\nu}) l} \tag{2.2}$$

where $A(\bar{\nu})$ is the absorbance at a given wavenumber $\bar{\nu}$. c_2 is the concentration of the absorbing functional group of sample. $\varepsilon(\bar{\nu})$ is the wavenumber dependent

absorption coefficient. l is the film thickness for the IR beam at a normal incidence to the sample surface.

2.6 Analysis secondary structural information from Fourier transform infrared spectrum

The spectra of silk collected by Fourier Transform infrared (FT-IR) spectroscopy have four absorption bands: amide A, amide I, and amide II and amide III. The absorption associated with amide I band leads to stretching vibrations of the C=O bond of amide that is different from other bands because it does not interfere with another functional group. Shape of amide I has been used to correlate the secondary structure content. However, many individual peaks are severely overlapped in this region. The commonly enhancement technique is Fourier self-deconvolution that used to identify the component band. After deconvolution of amide I band, curve fitting method is used to estimate the number of peaks, their locations, and their bandwidths. The advantage of curve fitting resolution has no effect on relative component fraction. Gaussian, Lorentzian, and also a combination of the Gaussian and Lorentzian functions are used to predict the quantity of secondary structure.

2.7 Attenuated Total Reflection Fourier Transform Infrared (ATR FT-IR) Spectroscopy

ATR FT-IR spectroscopy is the technique that is used to obtain the spectra of solids, liquids, semi-solids, and thin film. This technique is developed due to the studied surface arrangement, and resolved the problem of transmission technique (i.e. too thick sample). In this technique, the sample is placed in contact with the internal reflection element (IRE), the light is totally reflected and the sample interacts with the ***evanescent wave*** which resulting in the absorption of radiation by the sample [24]. The ATR method is suitable for this research more than another method such as regular reflection and diffuse reflection method. The regular reflection method is suitable for polish (mirror-like) surface, not silk fiber surface. The too thickness of silk fiber is one reason for unsuitable because it will give over-absorbed spectrum.

2.7.1 Principles of light reflection and refraction

Refraction and reflection occur when electromagnetic radiation thrusts a boundary between two media with different refractive indices. The reflection process requires equality of the incidence angle and reflection angle. In this case, reflection is specula. Passed electromagnetic radiation from one medium to another will suddenly change the direction of beam and it will be detected because of the difference in propagation velocity through two media. If light propagates through a medium with refractive index n_1 and enters a medium with refractive index n_2 (Figure 2.12), the light path will be changed; the extent of refraction is given by Snell's law [22].

$$\frac{\sin \alpha_1}{\sin \alpha_2} = \frac{n_2(\bar{\nu})}{n_1(\bar{\nu})} \tag{2.3}$$

where α_1 is the incidence and α_2 is the refraction angle.

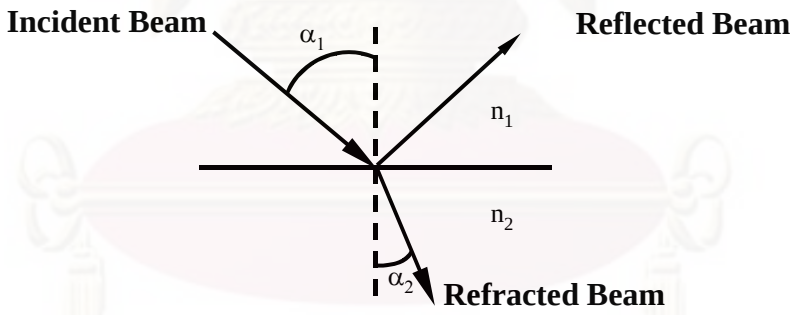

Figure 2.12 Reflection and refraction of a plane wave at a dielectric based on Snell's Law.

Snell's Law is an important phenomenon in application of total internal reflection. Total internal reflection is the travelling light through a medium of high refractive index to a lower index (i.e., $n_1 > n_2$) with an incident angle greater than the critical angle. The critical angle can be derived from Snell's law as shown in Equation 2.4 [25].

$$\theta_c = \sin^{-1}(n_2(\bar{\nu}) / n_1(\bar{\nu})). \quad (2.4)$$

According to Figure 2.13, the refracted angle equals 90^o when the angle of incidence equals the critical angle (θ_c). The total reflection phenomenon is un-travelling of light from the optically denser medium across the interface into the optically rarer medium. All of the reflected incidence radiation is occurred by non-absorbing rarer medium. For absorbing rarer medium, incident light are absorbed and reduced at particular wavelength. Then the internal reflection is attenuated. This attenuation of the total reflection produces ATR spectra.

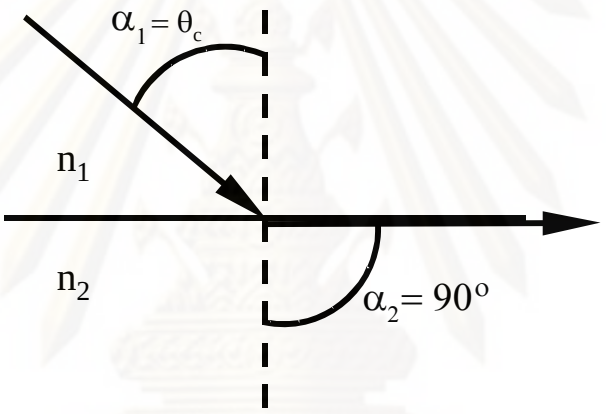

Figure 2.13 The total internal reflection occurs when light travels from an optically denser medium and impinges at the surface of the optically rarer medium ($n_1 > n_2$) with angle of incidence equals the critical angle.

Accordingly, total internal reflection spectroscopy is the technique of recording the optical spectrum of a sample material that contact with an optically denser medium. The wavelength dependence of the reflectivity of this interface can be measured introducing light into the denser medium. The reflectivity of this technique is a measure of the interaction of the electric field with the material.

2.7.2 Internal reflection element (IRE)

The internal reflection element (IRE) is made of a high reflective index material and transparent throughout the mid-infrared spectral region [24]. Typical IREs are zinc selenide (ZnSe), silicon (Si), germanium (Ge), and diamond as shown in Table 2.3. The IRE material has an effect on the ATR measurement because the refractive index has affected the depth of penetration.

Table 2.3 IRE materials used in ATR application.

Material	Reflective index at 1000 cm^{-1}	ATR spectral range (cm^{-1})	Hardness (kg mm^{-2})	Depth of penetration (μm) (at 45°,1000 cm^{-1})
Germanium	4.0	5500-675	550	0.66
Silicon	3.4	8900-1500, 360-120	1150	0.85
Zinc selenide	2.4	15000-650	120	2.01
Diamond	2.4	25000-100	5700	2.01

Two groups of IRE are single-reflection and multiple-reflection IRE as shown in Figure 2.14. The materials that have adequately strong or contrast absorption would be recorded the spectra by single-reflection IRE [25]. On the other hand, the multiple-reflection can be used to enhance the contrast that cannot be obtained with single-reflection. Designed IRE shapes have been developed such as hemi-cylinder, microhemicylinder, and hemisphere depending on simply instrumentation and nature of samples.

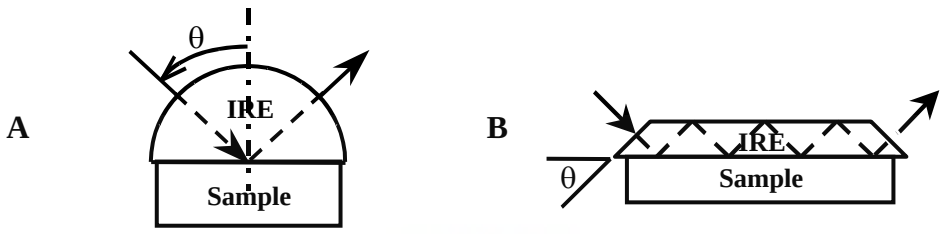

Figure 2.14 Illustration of IRE configurations: (A) Single reflection hemispherical crystal, and (B) Multiple reflections.

2.7.3 ATR spectral intensity

According to the critical angle (θ_c) of Snell's law, the real portion of complex refractive index is the ratio of refractivity from the high refractive index medium ($n_1(\nu)$) (i.e., IRE) and the lower refractive index medium ($n_2(\nu)$) (i.e., sample) at frequency ν. The complex refractive index of a medium consists of the real and imaginary part as follows [26]:

$$\hat{n}(\nu) = n(\nu) + ik(\nu) \tag{2.5}$$

where $\hat{n}(\nu)$ is the complex refractive index of a medium, $n(\nu)$ is the refractive index, i is equal to $\sqrt{-1}$, and $k(\nu)$ is the absorption index at frequency ν.

Two important phenomenon of ATR technique are ***total reflection phenomenon*** and ***attenuated total reflection (ATR) phenomenon.*** These phenomena will occur when incident light travels from IRE and impinges at the interface between the IRE and sample with incident angle greater than the critical angle. The difference between these phenomena is the absorption capability of sample (lower refractive index medium). The interface of absorbing medium has a strong electric field because no light travels across the boundary. It is interesting to note that the magnitudes of the interaction between light and the sample can be expressed in term of absorbance. The relationship between absorbed and reflected intensity in an ATR spectrum is given by:

$$A(\theta,\nu) = 1 - R(\theta,\nu) \tag{2.6}$$

where $A(\theta, \nu)$ and $R(\theta, \nu)$ are absorbance and reflectance, respectively.

Generally, absorbance in ATR can be expressed in terms of experimental parameters and material characteristic by the following expression [27-28]:

$$A_l(\theta,\nu) = \frac{4\pi\nu}{n_1 \cos\theta} \int_0^\infty n_2(\nu) k_2(\nu) \left\langle E_{zl}^2(\theta,\nu) \right\rangle dz \tag{2.7}$$

where $A(\theta, \nu)$ is absorbance and l indicates the polarization of the incident beam. $\left\langle E_{zl}^2(\theta,\nu) \right\rangle$ is the mean square electric field (MSEF) at depth z, n_1 is the refractive index of the IRE, $n_2(\nu)$ and $k_2(\nu)$ are the refractive index and absorption index of the sample, respectively.

The MSEF is a function of both the material properties (e.g., refractive index of the two media) and the experimental parameters (e.g., angle of incidence, frequency, and polarization of the incident beam. The strength and decay characteristic of the MSEF are varied as the absorption strength as shown in Figure 2.8

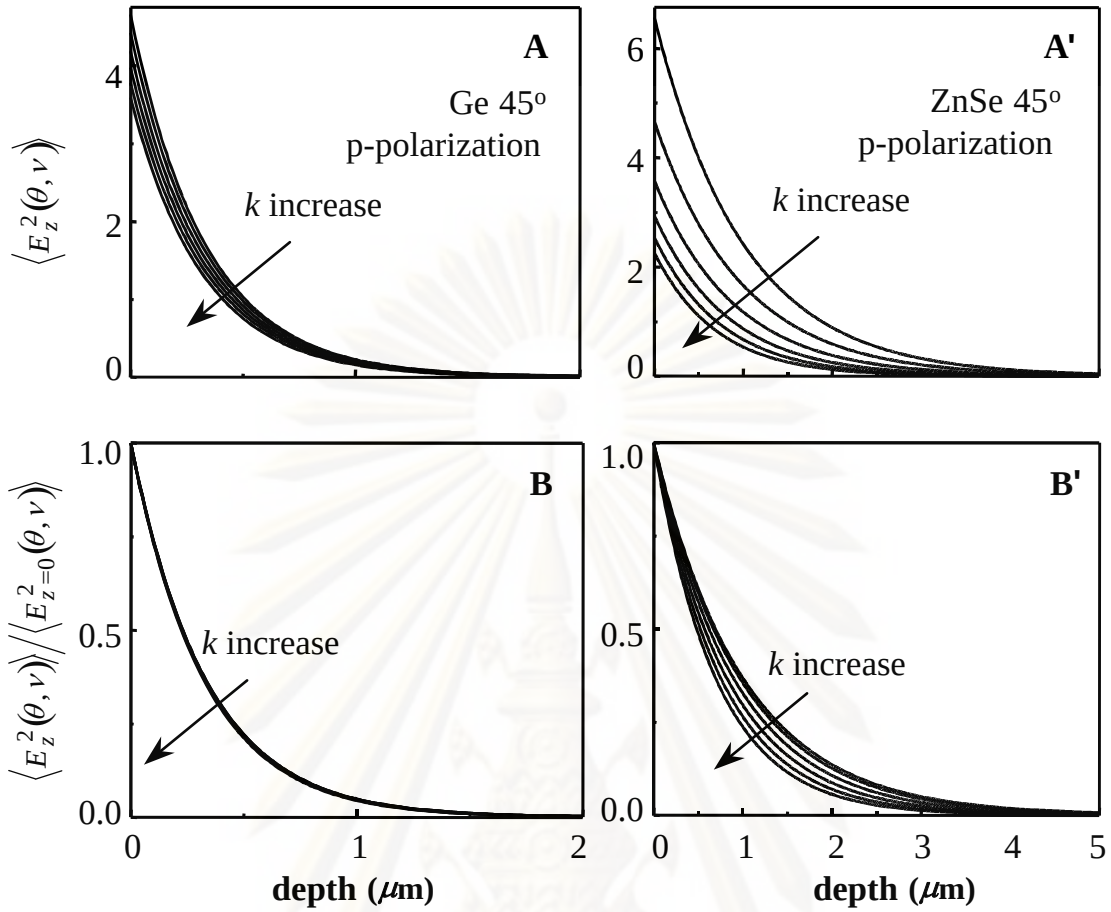

Figure 2.15 The MSEF at various experimental conditions (A, A′) and its decay characteristics (B, B′). The simulation parameters are $n_0 = 4.00$ for Ge, $n_0 = 2.40$ for ZnSe, $\nu = 1000\ cm^{-1}$, $n_1(\nu) = 1.50$, $k_1(\nu) = 0.0$, 0.1, 0.2, 0.3, 0.4 and 0.5, respectively.

2.7.4 Depth profiling using ATR FT-IR spectroscopy

Depth profiling can be determined by ATR FT-IR spectroscopy that has MSEF under total internal reflection condition. The power of MSEF at interface decays as an exponential function of distance from the interface of IRE and sample. The decay pattern of MSEF can be expressed by the following equation [28]:

$$\left\langle E_z^2(\theta,\nu)\right\rangle = \left\langle E_0^2(\theta,\nu)\right\rangle e^{-2z/d_p(\theta,\nu)} \qquad (2.8)$$

where $\left\langle E_0^2(\theta,\nu)\right\rangle$ and $\left\langle E_z^2(\theta,\nu)\right\rangle$ are the MSEF at the interface and the depth *z*, respectively. $d_p(\theta,\nu)$ is the penetration depth. The characteristic of evanescent field at the boundary is shown in Figure 2.16 [22].

The penetrated depth of light into the sample can be determined by the decaying of the MSEF value to 1/e of its. The experimental parameter of penetration depth is given by:

$$d_p(\theta,\nu) = \frac{1}{2\pi\nu n_1\left(\sin^2\theta - (n_2/n_1)^2\right)^{1/2}} \qquad (2.9)$$

where $d_p(\theta,\nu)$ is the penetration depth, n_1 and n_2 are the refractive index of the IRE and sample, respectively. The factors (i.e. changing the angle of incidence, type of IRE, frequency, etc.) can vary the penetration depth.

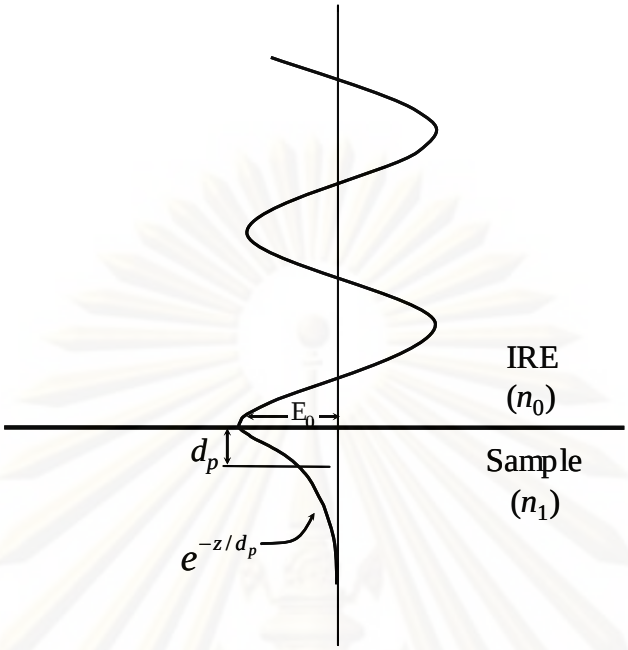

Figure 2.16 The evanescent field at the boundary between the IRE and the sample.

2.7.5 Limitations of ATR FT-IR spectroscopy

The efficiency of ATR analysis depends on the uniform sample-IRE contact. The IRE of traditional ATR spectroscopy (macro method) is designed to achieve the contact over the entire surface (approximately 5x5 mm). It has a good contact with liquid samples, conversely solid sample. Moreover, large contacts are also results in average molecular information over a large sampling area. The change of molecular information in small area cannot be investigated.

2.7.6 Principle of light entering the Ge μIRE

The hemispherical dome of the miniature cone-shaped Ge IRE facilitates the coupling of the focused radiation travelling into the IRE by minimizing the reflection loss at the air/Ge interface. If a nearly perfect coupling is assumed, the radiation will transmit through the air/Ge interface of the dome and impinge the Ge/air interface of the tip without a significant change in the angle of incidence [29]. For ensuring a good contact, the circular tip of the IRE should be a hemispherical surface. Since the contact area is small (~100 μm in diameter or less than), a good contact was achieved with a minimal force exerted on the tip. For the Ge μIRE (n_{Ge} = 4.0), the critical angle for the total internal reflection (TIR) at the interface with air (n_{air} = 1.0) and an organic medium ($n_{organic}$ = 1.5), respectively, are 14.48^{o} and 22.02^{o}. As a result, parts of the coupled radiation can be employed for ATR FT-IR investigation of a material having an optical contact with the tip of the miniature IRE. To eliminate interference from the internal reflection associated with the radiation having an angle of incidence smaller than the critical angle, an opaque circular adhesive tape is placed on the center of the hemispherical dome. Due to an effective condensation of the coupled radiation and an efficient light-matter interaction under the ATR condition at the tip of the IRE, ATR FT-IR spectra of a small specimen or a small area can be acquired with superb spectral quality.

The Ge IRE for using in this experiment is designed the optical focusing radiation in range from 15^{o} to 35^{o}. The cause is that the coupling of these focused the radiations will make the angle of incidence at the sampling surface greater than the critical angle.

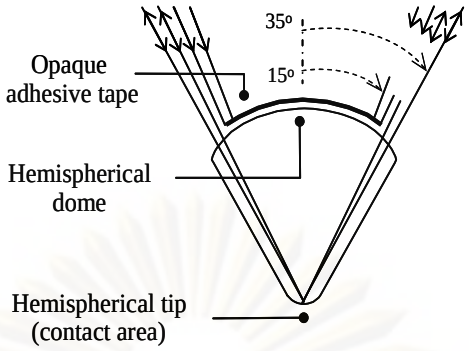

Figure 2.17 Schematic illustration of ray tracing within the infrared objective focusing radiation travelling within the Ge μIRE.

2.7.7 Homemade Slide-on Ge IRE Accessory

The homemade slide-on Germanium μATR accessory supplied with a cone-shaped Ge μIRE. One of all advantage of this accessory is a small sampling area (50x50 μm^2). Moreover, samples can be analyzed without additional sample preparation.

The homemade slide-on Ge μATR accessory provided by ***Sensor Research Unit, SRU, Department of Chemistry, Faculty of Science, Chulalongkorn University, Thailand***, consists of two parts as shown in Figures 2.18. The first component is the slide-on housing which is designed for placing the slide-on Ge μIRE into the Continuμm infrared microscope. The second component is the slide-on Ge μIRE which is designed for alignment adjusted to obtain high energy throughput. In Figure 2.19, the slide-on Ge μIRE is slid into the position and locked by knob of the slide-on housing which is located on the built in 15x Schwarazschild-Cassegrain infrared objective. The incident radiation from the infrared microscope is coupled into the dome-shaped Ge μIRE. The incident radiation impinges on the tip when total internal reflection occurrs at the Ge tip. The couple radiation can be employed for ATR FT-IR spectra acquisition as the tip of the Ge μIRE contact the ink sample.

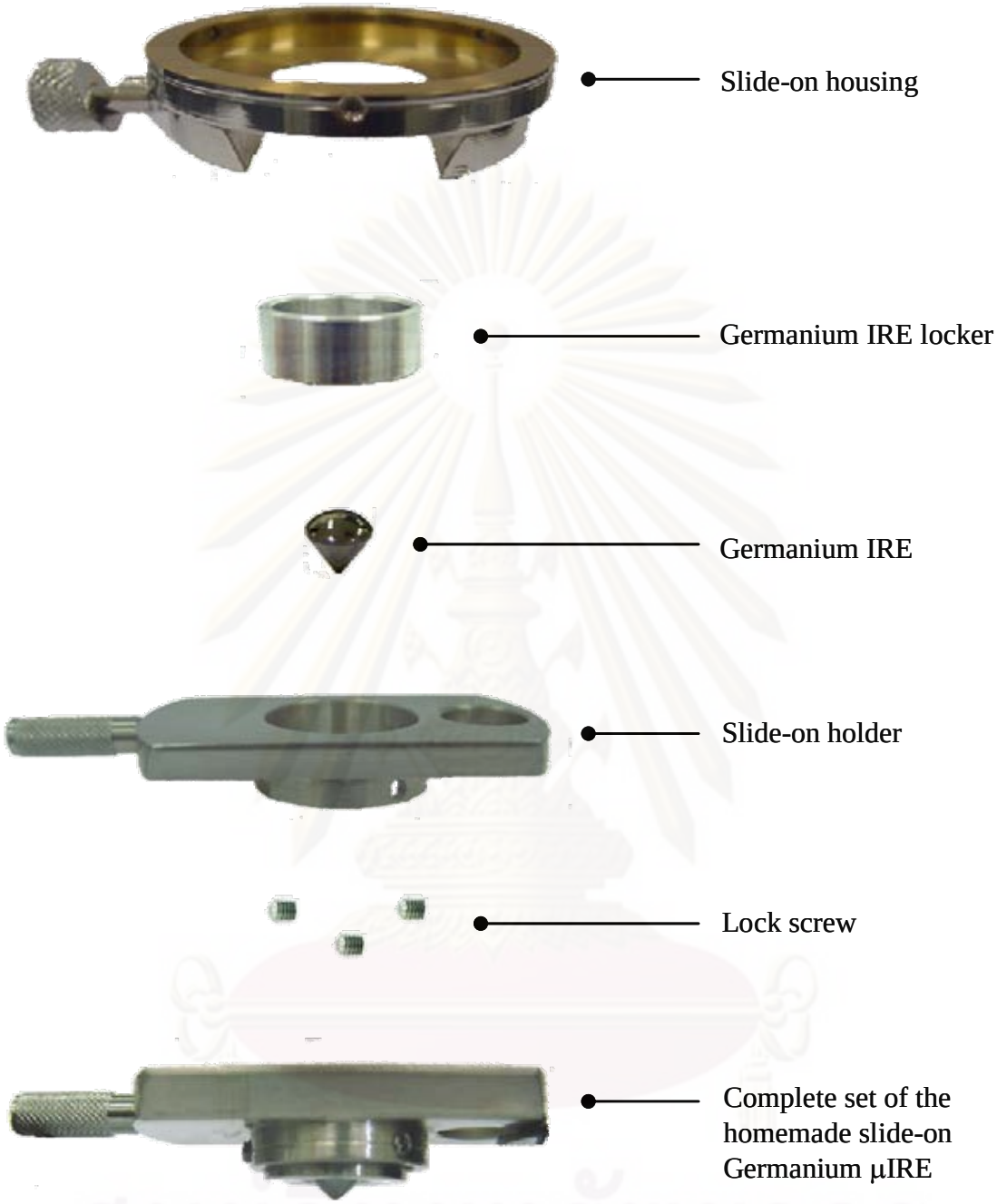

Figure 2.18 The slide-on housing and compositions of the homemade Germanium μATR accessory.

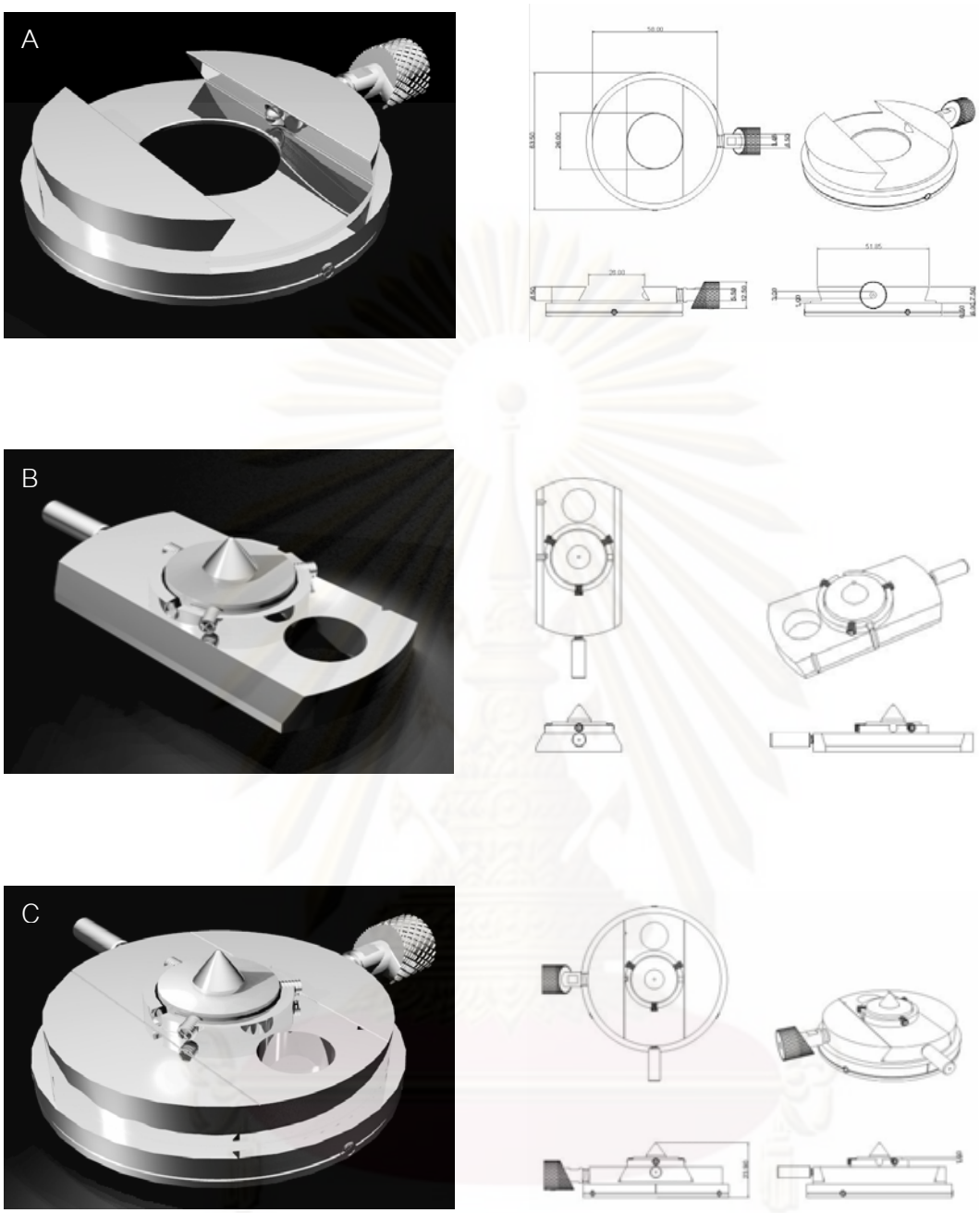

Figure 2.19 Detailed drawings of the homemade miniature ATR accessory with Germanium μIRE. (A) the housing attached to the objective, (B) the slide-on set with the Ge μIRE, and (C) the complete accessory.

CHAPTER III

EXPERIMENTAL SECTION

Thermal treatment is generally applied to commercial silk fibers for degumming, dyeing, *etc*. In this study, the degummed silk fibers were prepared by the common commercial procedure [30-31]. The molecular conformations of the silk fibers were studied by ATR FT-IR microspectroscopy. Owing to a single silk fiber with 10-15 μm in diameter, homemade Ge μIRE was employed to investigate ATR FT-IR spectra from silk fibers.

3.1 Materials and

3.1.1 Materials

1. Silk fiber from cocoon of *Bombyx mori*, reared locally in Queen Sirikit Sericulture (Northern Part: Phrae).
2. Sodium hydrogencarbonate ($NaHCO_3$) was purchased from Fisher Scientific UK Limited**,** Thailand
3. Deionized water (DI water)

3.1.2 Instruments

1. Nicolet 6700 FT-IR spectrometer equipped with a mercury-cadmium-telluride (MCT) detector.
2. Continuμm^{TM} infrared microscope with 15X Cassegrain infrared objective and 10X glass objective.
3. Homemade slide-on germanium (Ge) μIRE

3.2 Default Spectral Acquisition Parameter

Nicolet 6700 FT-IR Spectrometer

Instrumental Setup

Source	Standard Globar™ Infrared Light Source
Detector	MCT
Beam splitter	Ge-coated KBr

Acquisition Parameters

Spectral resolution	4 cm^{-1}
Number of scans	128 scans
Spectral format	Absorbance
Mid-infrared range	4000-750 cm^{-1}

Advanced Parameters

Zero filing	none
Apodization	Happ-Genzel
Phase correction	Mertz

Continuμm™ Infrared Microscope

Instrumental Setup

Detector	MCT
Objective	15X Schwarazschild-Cassegrain
Aperture size	150 μm x 150 μm

3.3 Experimental section

3.3.1 Preparation of degummed silk fiber

The native silk fibers were heated 3 times in 0.02 M $NaHCO_3$ at 60 ^oC for 30 min. Then, the degummed silk fibers were boiled 3 times in deionized water.

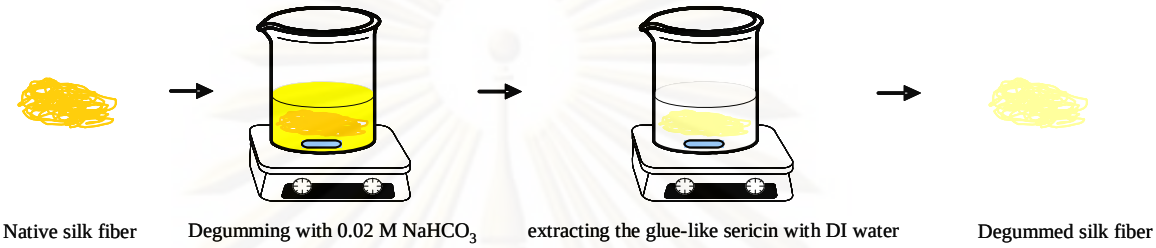

Figure 3.1 The degummed procedure of native silk fiber

3.3.2 Thermal treatment of silk fiber

In this work, thermal treatment was chosen to treat degummed silk fiber. The treatment temperature, treatment interval, and quenching method were varied to study their effects on molecular conformation. Degummed silk fibers were heated at different temperatures (50^o, 60^o, 70^o, 80^o, 90^o, and 100^{o}C) in water at different times (10, 20, 30, 40, 50, and 60 min). Then, they were quenched by one of three quenching methods, exposing into the air, soaking into water, or dipping into ice-cold water. After that, each treated silk fiber was analyzed by ATR FT-IR microspectroscopy.

3.3.3 Alkaline treatment of silk fiber

The alkaline treatment was used to investigate the changes of the secondary structure of silk fibers. Sodium hydrogen carbonate ($NaHCO_3$) was used as soft base. The prepared concentrations were 0.1, 0.5 and 1 M of $NaHCO_3$ solution at 50 mL. The degummed silk fibers were heated in each prepared solution at 60^{o}C for 30 min. Afterward the degummed silk fibers in each condition were divided into two parts. The first part was soaked in methanol for 30 min while the second one remained un-soaked. All of the degummed silk fibers, which were controlled condition, were collected the spectrum by ATR FT-IR microspectroscopy.

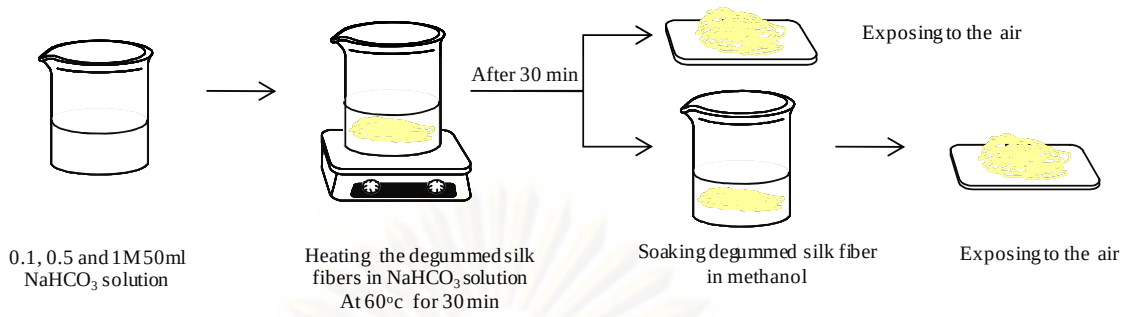

Figure 3.2 The procedure of alkaline treatment of silk fiber

3.4 Characterization of silk fiber

3.4.1 Attenuated Total Reflection Fourier Transform Infrared (ATR FT-IR) microspectroscopy

The molecular conformation of silk fiber samples was acquired by ATR FT-IR microspectroscopy. The spectra of the silk fiber samples were recorded in the frequency ranging from 750 to 4000 cm^{-1} on a Nicolet 6700 FT-IR spectrometer with a mercury-cadmium-tellurium (MCT) detector at resolution of 4 cm^{-1}. All samples and backgrounds were collected at 128 co-addition times. Ge μIRE was placed on the objective microscope and a silk fiber on glass slide was positioned on the microscope stage. After that, the stage of microscope was raised in order to contact the silk fiber with the Ge μIRE. The reflection mode of infrared microscope was used to record the spectra.

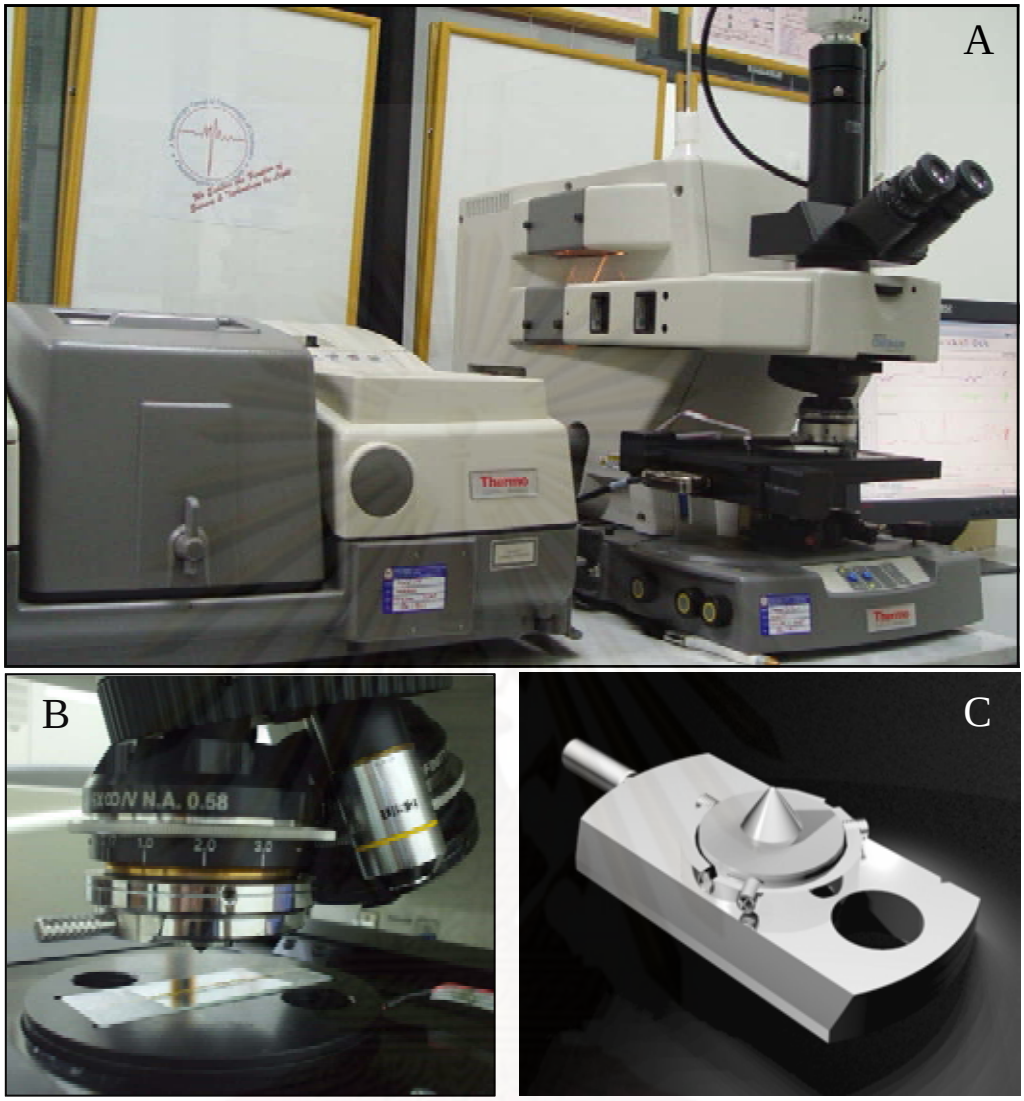

Figure 3.3 ATR FT-IR microspectroscope: (A) Continuμm® infrared microscope coupled with the Nicolet 6700 FT-IR spectrometer, (B) the slide-on Ge μIRE is fixed on the position of slide-on housing on the infrared objective, and (C) Homemade slide-on germanium (Ge) μIRE.

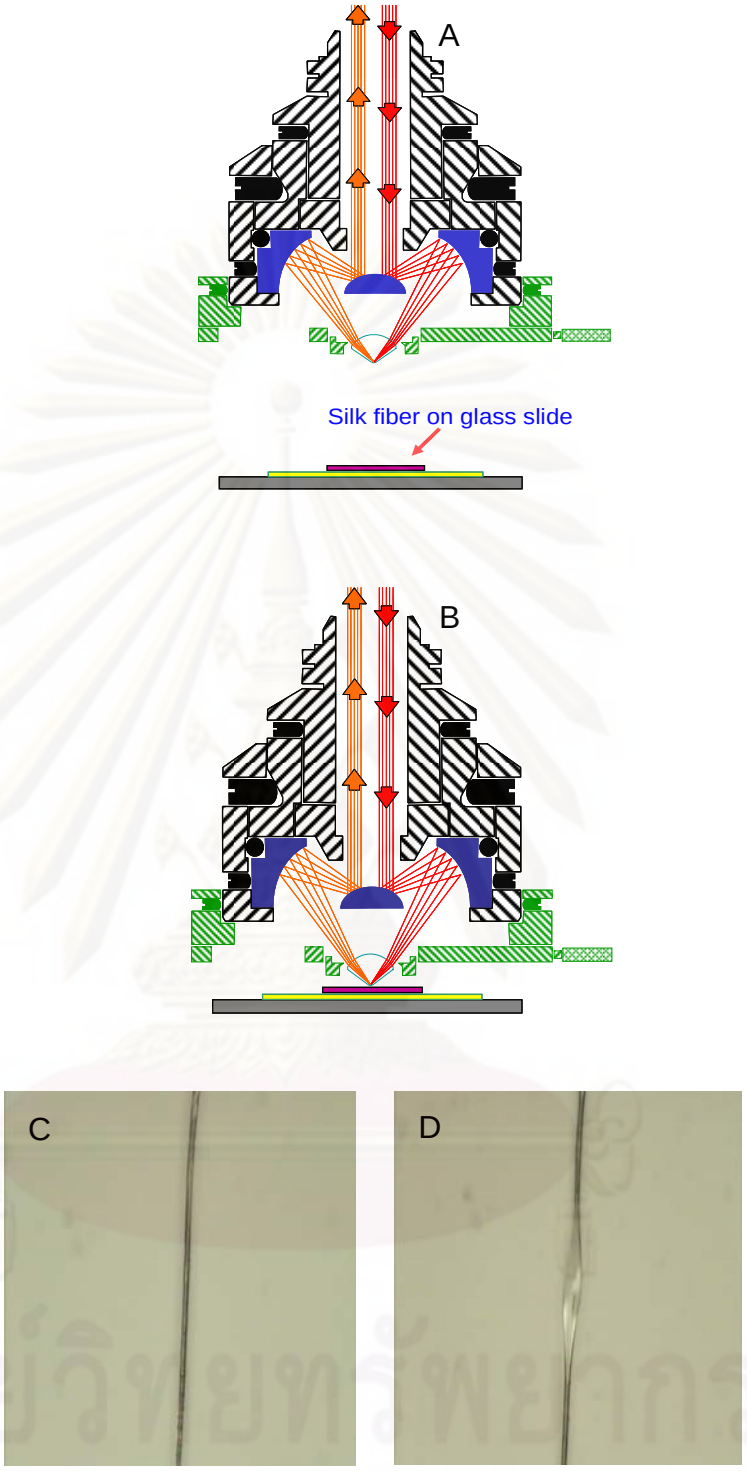

Figure 3.4 The finished preparation of sample before (A) and after (B) contacting with Ge μIRE probe. The probe pressed on a single silk fiber and expanded the width of single silk fiber. (C) and (D) showed the single silk fiber before and after expanding, respectively.

3.4.2 Curve fitting

The spectra were collected by ATR FT-IR microspectroscopy that would be determined the quantitative of secondary structure. The procedures for determining the secondary structure of silk fiber are as follows: (i) Baseline correction was applied to the collected spectra for flattening spectra baseline. This step is the most important step than other step because it has the effect on peak area, as shown in Figure 3.4 (ii) The amide I band that assigned to C=O stretching functional group (non-interfere from other functional group) was used to determine the quantitative of the secondary structure. A straight baseline tangent to spectra around 1733 to 1574 cm^{-1} was performed. (iii) The overlapping component band were calculated by OMNIC 7.3 with the related *Gaussian and Lorentzian functions* and were identified the position by referring the second order derivertive. When the curve was fitted, the width and the height of peak were varied. The band positions were changed within the limited region. The quality of fitted curve is determined by comparing the reconstituted overall curve to the original spectrum. The fitted band areas were estimated to the proportions of the secondary structure of silk fiber.

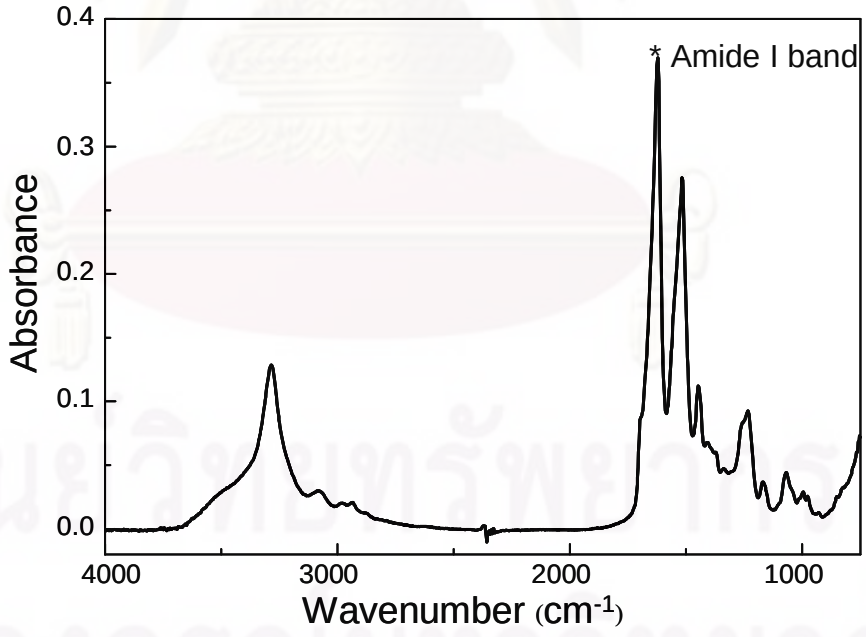

Figure 3.5 The spectrum of degummed silk fiber after subtracting and correcting.

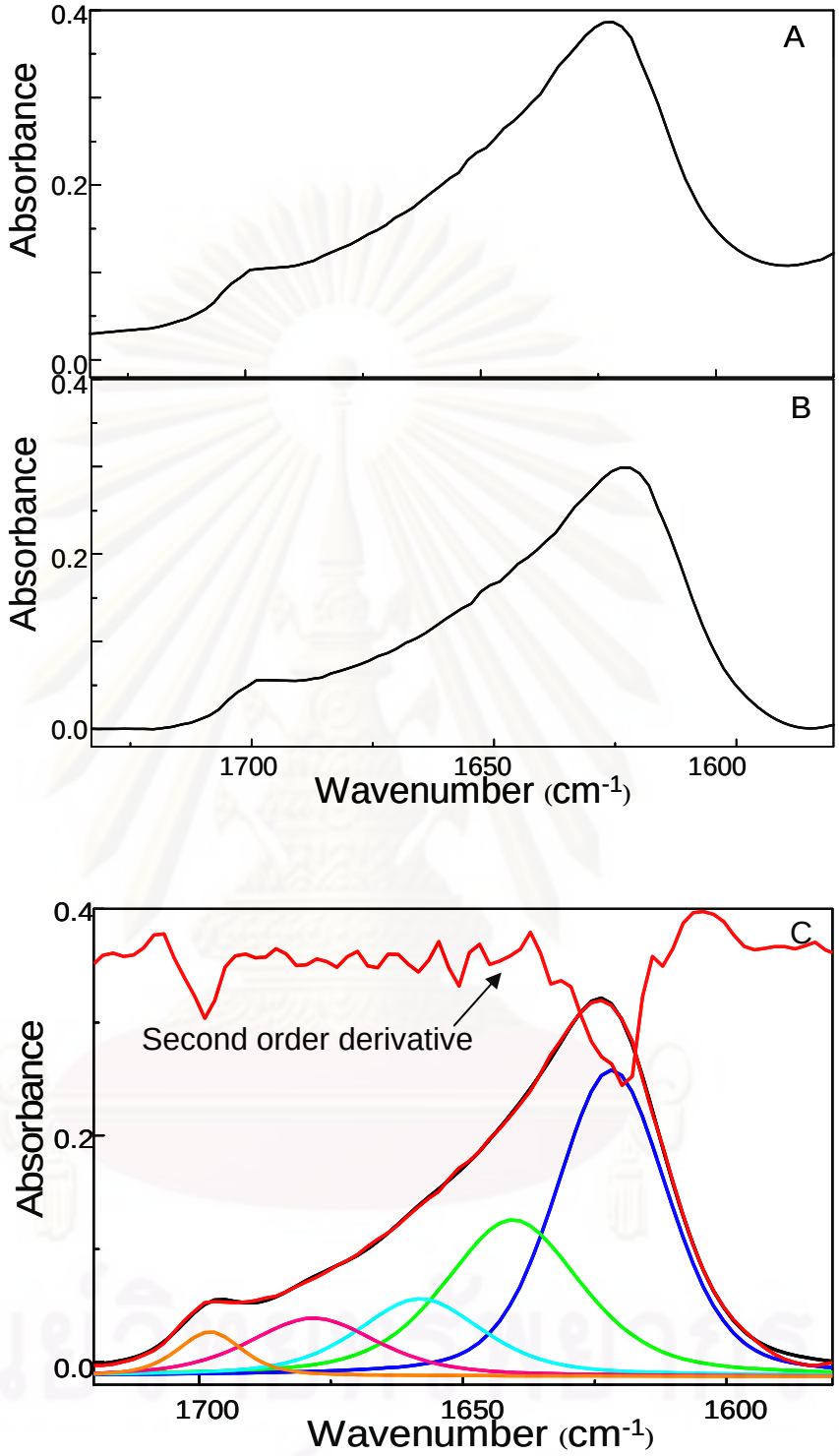

Figure 3.6 Before using the amide I band to determine the secondary structure, the amide I band should be corrected baseline around 1733 to 1574 cm^{-1}. (A) and (B) are amide I band before and after correcting baseline. After that, amide I band was fitted by the second order derivative to identify the individual secondary structure, as shown in (C)

3.4.3 Calculated the percentage of secondary structure element

The percentage of secondary structure element can be evaluated from the peak area at the resolved peak. Their percentage value can be used to define the crystallinity of the secondary structure. More β-sheet structures imply more crystallinity of silk fiber. For example, the percentage of individual secondary structure elements was determined in corelationship with the total secondary structure of silk fiber by the following equation (3.3).

$$\% \text{ of individual secondary structure} = \frac{\text{Peak area of individual secondary structure}}{\text{Total of peak area of secondary structure}} \times 100$$

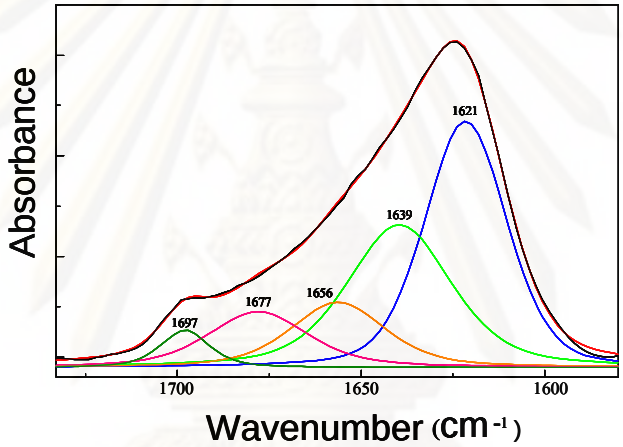

Figure 3.7 The resolved peak shows the secondary structure that content in silk fiber

Table 3.1 The percentage values were calculated from peak area.

Wavenumber (cm^{-1})	Peak area	Percentage value
1621	7.77	39.72
1639	6.11	31.23
1656	2.59	13.23
1677	2.33	11.89
1697	0.77	3.91

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CHAPTER IV

RESULTS AND DISCUSSION

ATR FT-IR microspectroscopy is well-known for its spectral information related to molecular structure and chemical composition. The spectrum of silk fiber can be elucidated the signature of sericin and fibroin protein and the quantity of secondary structure element by curve fitting method. The quantity structures were determined the relationship with the crystallinity of silk fiber.

4.1 Characterization of silk fiber

4.1.1 ATR FT-IR spectra of un-degummed and degummed silk fibers

The un-degummed silk fiber composes of protein, water, lipid, and wax. The two major proteins of silk fibers are sericin and fibroin. The differences of silk fibers before and after degumming were shown in Figure 4.1. Before degumming, the silk fibers are yellow, hard and rough. Conversely, degummed silk fibers are creamy-white, tender, smooth and lustrous. The cause of these differences is sericin that coated on fibroin.

ATR FT-IR spectrum of un-degummed silk fiber and degummed silk fiber are shown Figure 4.1 (A) and (B), respectively. The spectra of silk fibers have four major peaks: amide A, amide I, amide II and amide III. Amide A band is assigned to N-H stretching vibration at 3450 to 3160 cm^{-1}. Degummed silk has sharper amide A band than that of un-degummed silk. This is because sericin is a highly hydrophilic protein and it can absorb more water. The peak at 1700 to1600 cm^{-1} is a band of amide I that primarily represents a C=O stretching vibration of the amide group. The remarkable shoulder at amide I band of degummed fiber at 1696 cm^{-1} is assigned to anti-parallel β-sheet [32-39]. Other regions are fingerprint region at 1540 to1510 cm^{-1} and 1300 to 1225 cm^{-1} that are assigned to amide II and amide III of protein. Amide II band contains a contributions of N-H bending and C-N stretching vibrations. Amide II bands of un-degummed and degummed silk fiber are centered at about the same frequencies. At the amide III region, an information of N-H bending plus C-N

stretching and a contribution of O=C-N bending are obtained. Amide III band of un-degummed silk at 1235 cm^{-1} is assigned to unordered structure. Contrarily speaking, the amide III band of degummed silk showed a center at 1230 cm^{-1} and a weak shoulder at 1262 cm^{-1}.These are assigned to the crystalline structure.

The difference of peak frequencies of both spectra can be related to the different amino acid composition between un-degummed and degummed silk fiber. The weak band at 1398 cm^{-1} is assigned to O-H bending vibration of serine amino acid. This peak does not appear in the degummed silk fiber spectrum because it composes of serine amino acid only 12.5%. For un-degummed silk fiber, the serine amino acid containing is approximately 31.97%. The spectrum of degummed silk fiber has the weak band at 998 and 976 cm^{-1}. These bands are assigned to the Gly-Ala sequence. Degummed silk fiber which is the mainly composition of non-polar amino acid such as glycine and alanine are approximately 42.9% and 30.0%, respectively. On the other hand, the un-degummed silk fiber does not show these bands because it composes of glycine and alanine approximately 13.5% and 5.8%, respectively. According to both spectra, degummed silk fiber is pure fibroin protein filaments but un-degummed silk fiber shows sericin character. The band assignments of silk fiber associated with the functional group of protein are summarized in Table 4.1.

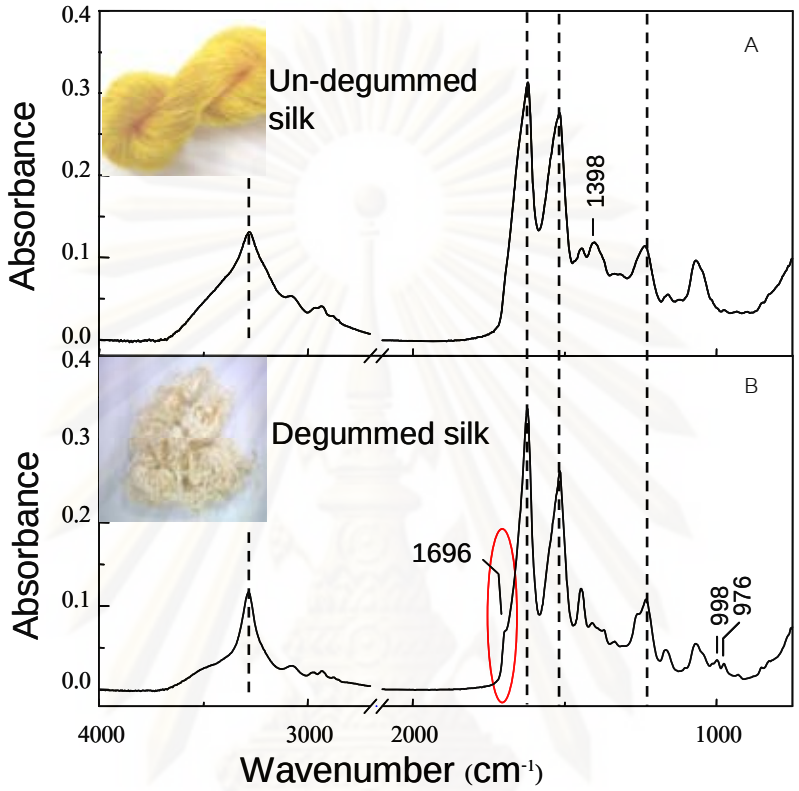

Figure 4.1 ATR FT-IR Spectrum of un-degummed (A) and degummed (B) silk fiber. The clearly difference of these spectrum is the shoulder of amide I band at 1696 cm^{-1}. The dash line is shown the position of amide A, amide I, amide II and amide III from higher to lower wavenumber, respectively.

Table 4.1 Band assignments of silk fiber [32-39].

Wavenumber (cm^{-1})	**Band Assignments**
3450-3160	**Amide A** N-H stretching
~3100	Overtone of Amide II
2975-2950	Asymmetric C-H stretching of CH_3
2940-2915	Symmetric C-H stretching of CH_3
2885-2865	Asymmetric C-H stretching of CH_2
1698-1690	Anti-parallel β -sheet
1700-1600	**Amide I** C=O stretching
1540-1510	**Amide II** N-H bending (wagging) plus C-N stretching
1480-1440	C-H bending (scissoring) of CH_2
1410-1350	C-H bending (wagging) of CH_3
1440-1260	C-H and O-H bending vibration
1300-1225	**Amide III** N-H bending (twisting) plus C-N stretching and contribution from O=C-N bending
1175-1165	O-H bending of phenolic residue in Tyr
1100-1050	C-N stretching of RCH_2-NH_2, R_2CH-NH
1090-1000	C-OH stretching vibration
1000-945	C-C skeletal of Gly-Ala sequences

4.1.2 ATR FT-IR spectra of silk fiber of each layer of silk cocoon

The silk cocoon was separated to many layers to study the character of silk fiber. In this study, the silk cocoon could be separated to eight layers, which are shown in Figure 4.2. The amount of layers depends on the species of silk worm. The outer layer has dark yellow compared with the most inner layer. After that, single silk fiber from each layers were collected the spectra. The spectrum of each layers have the differences of peak frequencies. (i) Amide A band of outer layer is the broadest because outer layer has more amount of coated sericin. Serine amino acid in sericin has OH group in the side chain position. Consequently, an abundance of serine and threonine residues cause a difficulty on obtaining structural information from N-H stretching vibration because the absorption band overlapped with a strong O-H stretching at 3500-3200 cm^{-1}. (ii) Amide I band of inner layer is shaper than other layer and the shoulder at 1697 cm^{-1} is clearly. (iii) At 1400 cm^{-1}, the band in this position is assigned to CH bending of alanine amino acid side chain. The intensity had increased from outer to inner layer. (iv) The intensity of peak at 1350 cm^{-1} that is assigned to OH bending of serine amino acid had decreased from outer to inner layer. (v) The peak intensity at 1150 cm^{-1}(OH bending of tyrosine amino acid) of inner layer was higher than other layers. The differences of each spectrum occur from the quantity of sericin. The inner layer has minimum sericin compared with other layers. All of the difference was shown in Figure 4.3.

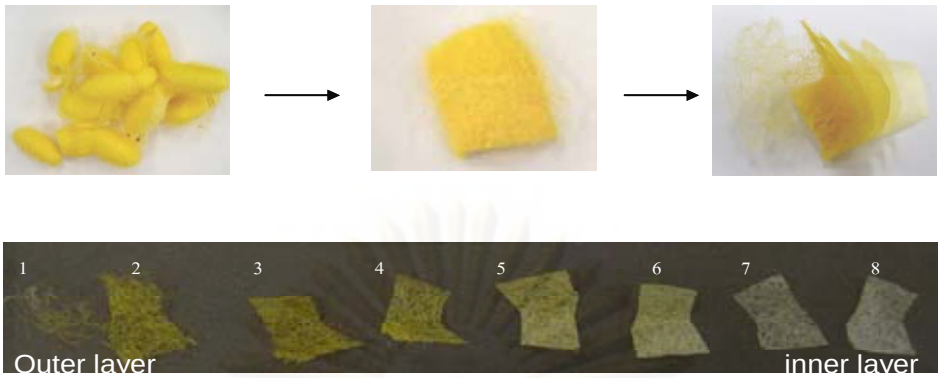

Figure 4.2 The silk cocoon was separated to eight layers. Number 1 to 8 show the layer of silk cocoon from outer to inner.

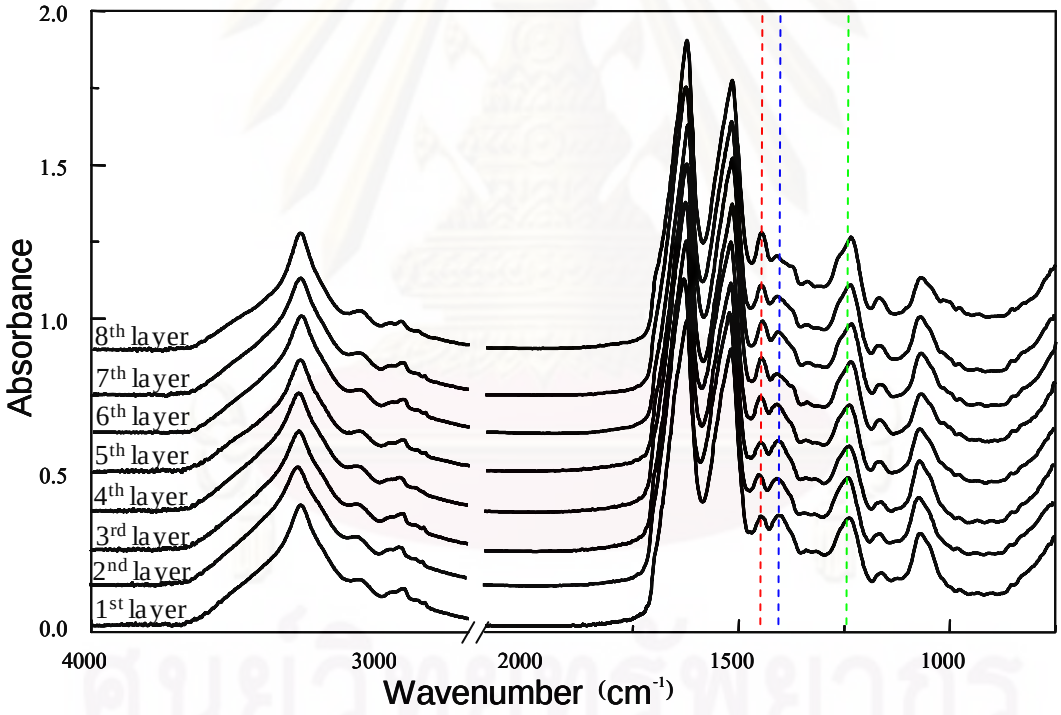

Figure 4.3 Illustration of the difference of each spectrum. Red, blue and green dash line show the position of peak at 1400 cm^{-1} (CH bending of CH_3 of alanine amino acid), 1350 cm^{-1} (OH bending of serine amino acid) and 1150 cm^{-1} (OH bending of tyrosine amino acid), respectively.

4.2 Secondary structure of silk fiber analyzed by ATR FT-IR spectroscopy

Generally, protein contains more than one secondary structure, which is correlated to the amide groups of protein spectra. Most of secondary structure of protein can be classified as ***α-helix, β-sheet, β-turns, and random coil***. Analysis of secondary structure of protein by mean of FT-IR spectroscopy is an established technique. Since secondary structures are associated with a characteristic pattern of hydrogen bonding between amide C=O and N-H groups. Amide I absorption found in the 1700-1600 cm^{-1} region was the most useful evidence for determining protein secondary structures. The amide I band was used to monitor the structural change of protein because it was predominantly from C=O stretching vibration of the protein backbone. However, overlapping of the bands in this region is an obstacle to extract the information of secondary structure. The second derivative and deconvolved method have been applied to distinguish the overlapped peaks. The assignments of the vibrational bands are shown in Table 4.2.

Table 4.2 Vibrational band assignments in amide I region of silk fiber [7].

Range of wavenumber (cm^{-1})	Band assignment
1605-1615	(Tyr) side chain/aggregate strands
1616-1621	Aggregate β-strand/ β-sheet (weak)[a]
1622-1627	β-sheet (strong)[a]
1628-1637	β-sheet (strong)[b]
1638-1646	Random coils/extended chains
1647-1655	Random coils
1656-1662	α-helices
1663-1670	Turns
1671-1685	Turns
1686-1696	Turns
1697-1703	β-sheet (weak)[a]

[a] Intermolecular β-sheets, [b] Intramolecular β-sheets

4.2.1 Fitted curve of amide I band of un-degummed and degummed silk fiber

The spectral envelop of degummed silk fiber is sharper than that of un-degummed silk fiber and the shoulder at 1697 $cm^{-1}$of degummed silk fiber shows clearly. Un-degummed silk fiber contains aggregate β-sheet that is the disorder assemblage of β-strands. On the other hand, the degummed silk fiber contains the anti-parallel β-sheet. The anti-parallel β-sheet is the order assemblage of β-strands. The other secondary structure containing in these fibers are random coils, α-helices and turns. The secondary structure that can define of un-degummed silk fiber is the characteristic of sericin protein. Conversely, the secondary structure of degummed silk fiber shows the characteristic of fibroin protein. The spectral envelope, amide I band shape, of un-degummed silk is broader than that of the degummed silk fiber because it has more amount of random coil structure. Then, the spectral envelope depends on the amount of individual secondary structure containing in silk fibers. The crystallinity of silk fiber can refer from the quantity of anti-parallel β-sheet. Hence the fibroin protein has the more crytallinity than the sericin protein which was shown in Figure 4.4.

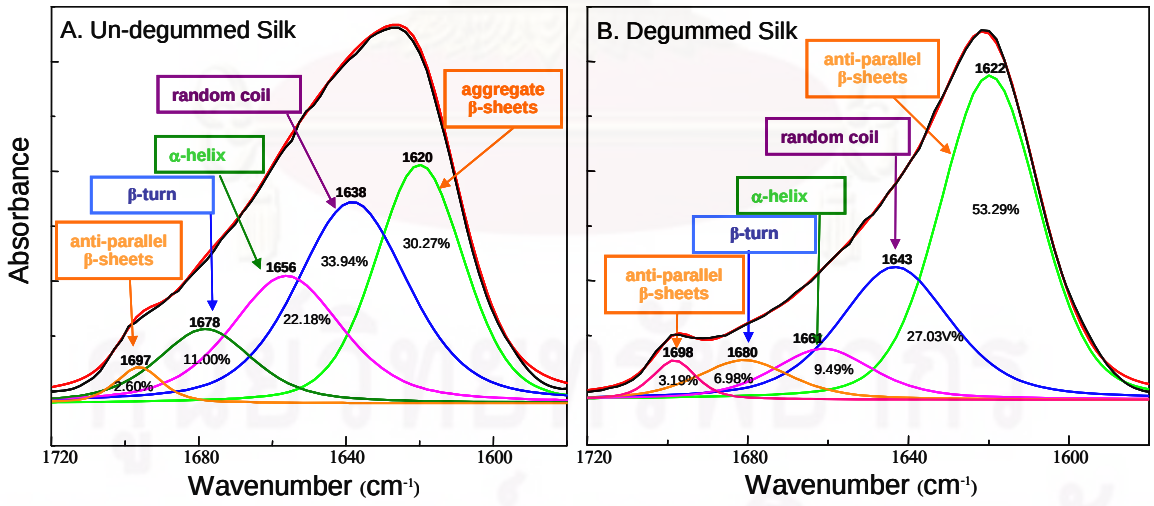

Figure 4.4 Fitted curve of amide I band of un-degummed (A) and degummed (B) silk fiber.

For the silk fibers of each layer of silk cocoon, the amide I band of all spectra was fitted. After that, the similarity silk fiber of each layer is the secondary structure that consists of aggregate β-sheet, α-helices, random coils and turns. The remarkable of the difference is the broadest spectral envelope of amide I of outer layer or first layer and the shoulder at ~1690 cm^{-1} of inner layer or eighth layer is clearly than other layer. The spectral envelope of inner layer of amide I band has similarity in shape with the spectral envelope of amide I band of fibroin when it was compared with the spectral envelope of amide I of each layer as shown in Figure 4.5. The result from this section can apply to consider the complete degumming process. If the degumming process is complete, the curve fitting of amide I will show the dominant curve of β-sheet structure (blue curve in Figure 4.5).

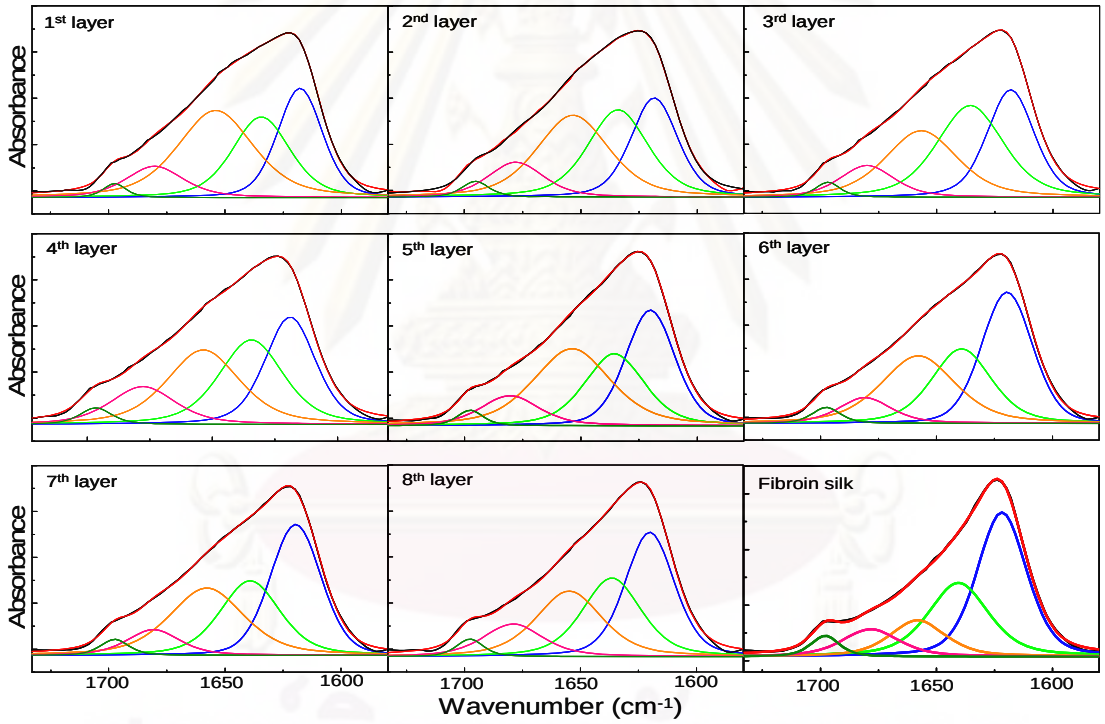

Figure 4.5 The fitted curve of Amide I band of silk fibers.

The amount of secondary structure element of sericin can be calculated by peak area. The inner layer has more quantity of β-aggregate than other layers. The quantity and the position of deconvolved peak are shown in Table 4.3.

Table 4.3 The secondary structure analysis of silk fiber cocoon (first to forth layer)

Literatures	Assignments	Current work							
		1st layer		2nd layer		3rd layer		4th layer	
		Wavenumber (cm^{-1})	Area (%)	Wavenumber (cm^{-1})	Area (%)	Wavenumber (cm^{-1})	Area (%)	Wavenumber (cm^{-1})	Area (%)
1605-1621	Aggregate β-strand	1618	26.59	1618	25.89	1618	27.25	1618	27.37
1638-1655	Random coil	1638	25.77	1638	30.34	1638	33..88	1638	28.19
1656-1662	α-helices	1660	35.89	1658	34.07	1656	26.98	1660	28.64
1663-1696	β-turn	1678	9.89	1677	7.05	1680	9.77	1677	12.95
1697-1703	β-sheets (Intermolecular)	1697	1.86	1697	2.55	1697	2.12	1697	2.85

Table 4.3 (continued) The secondary structure analysis of silk fiber cocoon (fifth to eighth layer)

Literatures	Assignments	Current work							
		5^{th} layer		6^{th} layer		7^{th} layer		8^{th} layer	
		Wavenumber (cm^{-1})	Area (%)	Wavenumber (cm^{-1})	Area (%)	Wavenumber (cm^{-1})	Area (%)	Wavenumber (cm^{-1})	Area (%)
1605-1621	Aggregate β-strand	1620	29.99	1619	30.82	1619	31.72	1620	33.65
1638-1655	Random coil	1638	23.91	1639	29.43	1639	30..53	1638	26.54
1656-1662	α-helices	1659	33.74	1658	29.12	1656	23.42	1656	25.45
1663-1696	β-turn	1680	10.03	1680	7.91	1678	11.35	1679	11.50
1697-1703	β-sheets (Intermolecular)	1697	2.33	1697	2.70	1697	2.96	1699	2.86

4.3 Characterization of the original degummed silk fiber

The original degummed silk fiber was used to compare with the thermal treatment and alkaline treatment of silk fibers. The specimen of original degummed silk fiber was analyzed at five positions by controlling the height of amide I band. All of amide I bands of the specimen was fitted curve and determined the percentage of each secondary structure element of silk fiber. The percentage of individual secondary structure element of five position would be calculated the average value of them. The mean and SD values of individual secondary structure that are the reference values were shown in Figure 4.6.

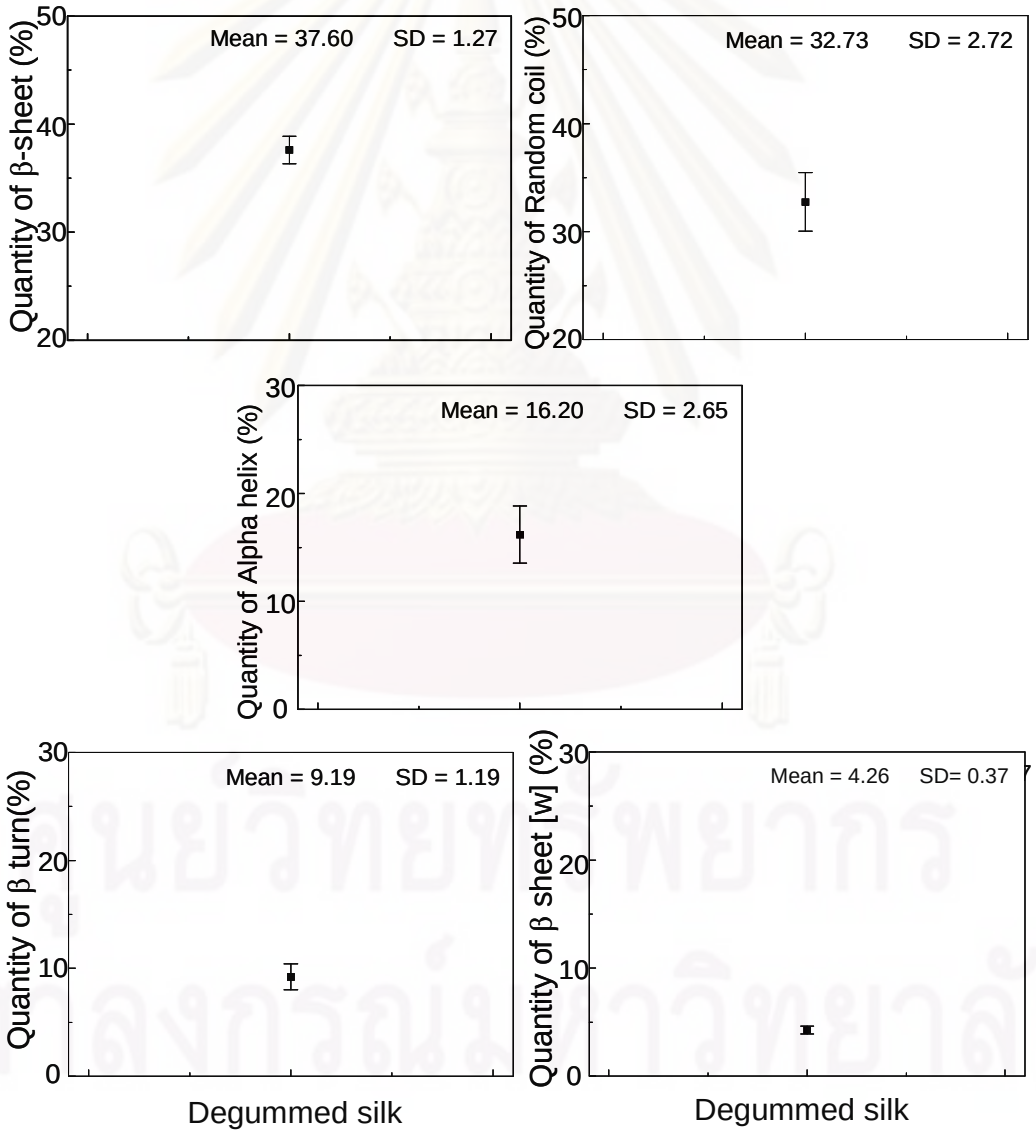

Figure 4.6 The average value of each secondary structure element of original degummed silk fiber

4.4 Analysis of the thermal treatment of silk fiber

In this study, it is interesting to study the effect of heat on the secondary structure of silk fiber. Therefore the silk fibers were heated at different temperatures and different times. The quenching method such as exposing into the air, soaking into the water and dipping into the ice cold-water were used to stop the changes of molecular conformation of silk fiber. The secondary structure of the varied condition of silk fiber was acquired by curve fitting analysis.

At different temperature, we calculated the average percentage value of secondary structure (β-sheet, α-helix, turn, random-coil and β-sheet (intermolecular)). Its mean and SD value showed the fluctuation. Range of the average percentage value of β-sheet, α-helix, turn, random-coil and β-sheet (intermolecular) are 30-45, 25-35, 15-25, 5-15 and 0-5, respectively. After that, we determined the average percentage value of secondary structure at different time and quenching method. It gave similar result with the different temperature condition. The average percentage values that can calculate comparing with the average percentage value of standard degummed silk fiber. They did not give the difference of the average percentage values of individual secondary structure. Consequently, the different temperature, time and quenching method have no significant to change the secondary structure in silk fibers. The causes of this phenomenon are the original silk fiber from silk worm that is the semi-crystalline materials. It has the fluctuation value of secondary structure element that contains in silk fiber. Accordingly, the thermal treatment that is a mild condition method cannot change the secondary structure of silk fiber.

4.5 Analysis of the alkaline treatment of silk fibers

The structure of fibroin fiber (or degummed fiber) can be divided to two regions: crystalline and amorphous regions. The crystalline region has a good orientation because it contains more β-sheet structure than other structure. The hydrogen bond both within and between molecular chain plays a crucial role in the fibroin fiber. If the hydrogen bond was destroyed, the amorphous region would increase. Alkali is one of all agents that can damage the hydrogen bond.

In this section, the alkaline solution was used to change the secondary structure by destroying some hydrogen bond. We chose $NaHCO_3$ as the alkaline solution because it could terminate the sericin protein by breaking the hydrogen bond. The $NaHCO_3$ solution was prepared to 0.1, 0.5 and 1M. After soaking fibroin fiber in $NaHCO_3$ solution, the fibroin fiber was divided into two portions for soaking and un-soaking with methanol. This method was used after heating in $NaHCO_3$ solution since the changes of secondary structure can observe clearly. The methanol, the crystallization reagent, was used to change the secondary structure from random coil to β-sheet structure. The graphs in Figure 4.22 are shown the changes of secondary structure. The mean and SD values of the percentage of individual secondary structure has fluctuated. The range of mean value of individual secondary structure is 35-45 (β-sheet), 25-35 (random-coil), 15-25 (α-helix) and 5-15 (turn) and 0-5 (β-sheet (intermolecular)). All types of secondary structure have nearly the same range of the average percentage value comparable to the original degummed silk fibers. The cause is according to the fluctuation of the amount of secondary structure in original silk fiber. Although the alkaline treatment is more vigorous condition than thermal treatment, it could not change the secondary structure of silk fiber. Consequently, the varying condition of $NaHCO_3$ solution has no significant effect on the secondary structure of fibroin fiber.

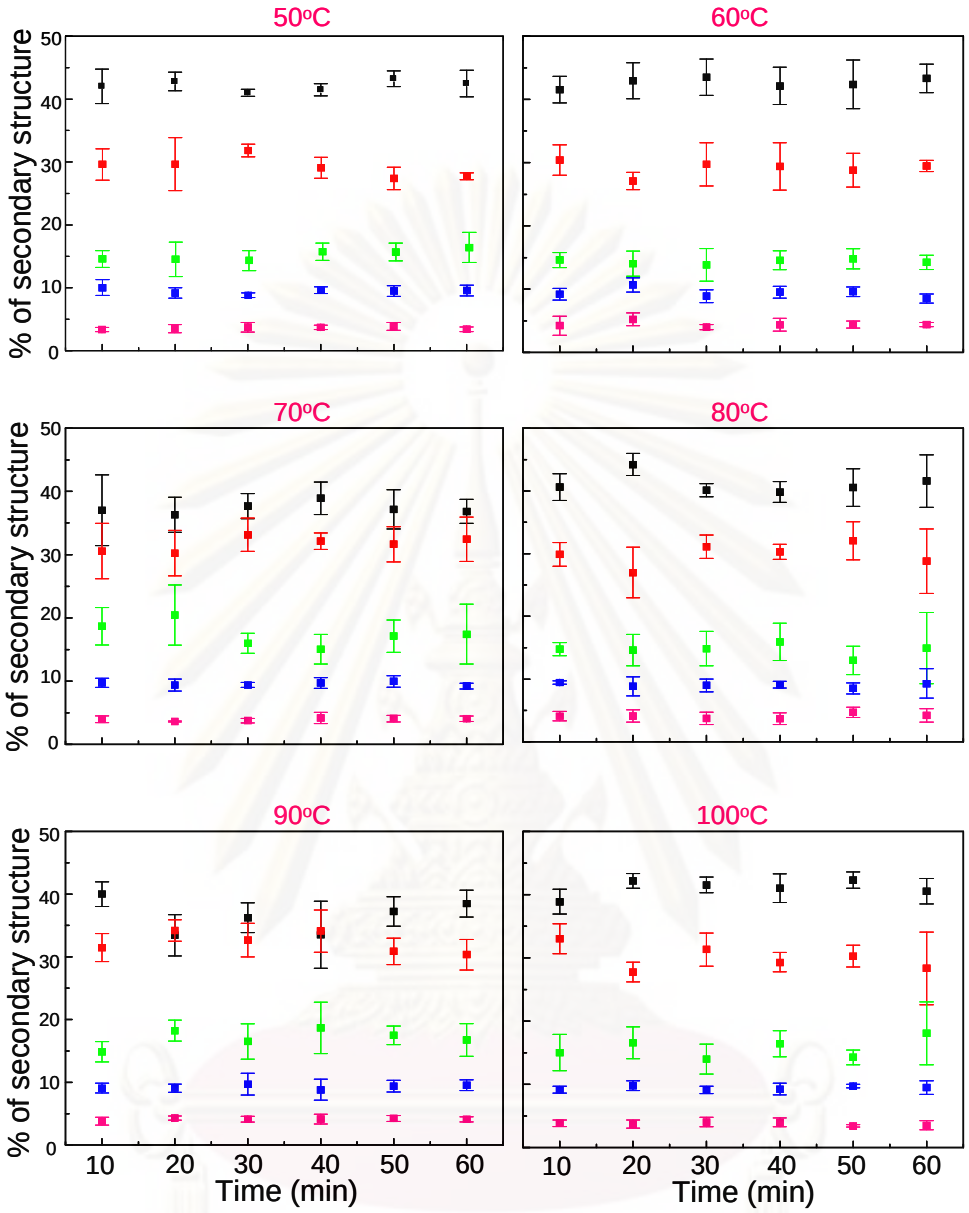

Figure 4.7 The average value of individual secondary structure in silk fiber: β-sheet (black), random coils (red), α-helix (green), turn (blue) and β-sheet (intermolecular) (pink).The silk fibers, which were varied as temperatures and times, were exposed to the air.

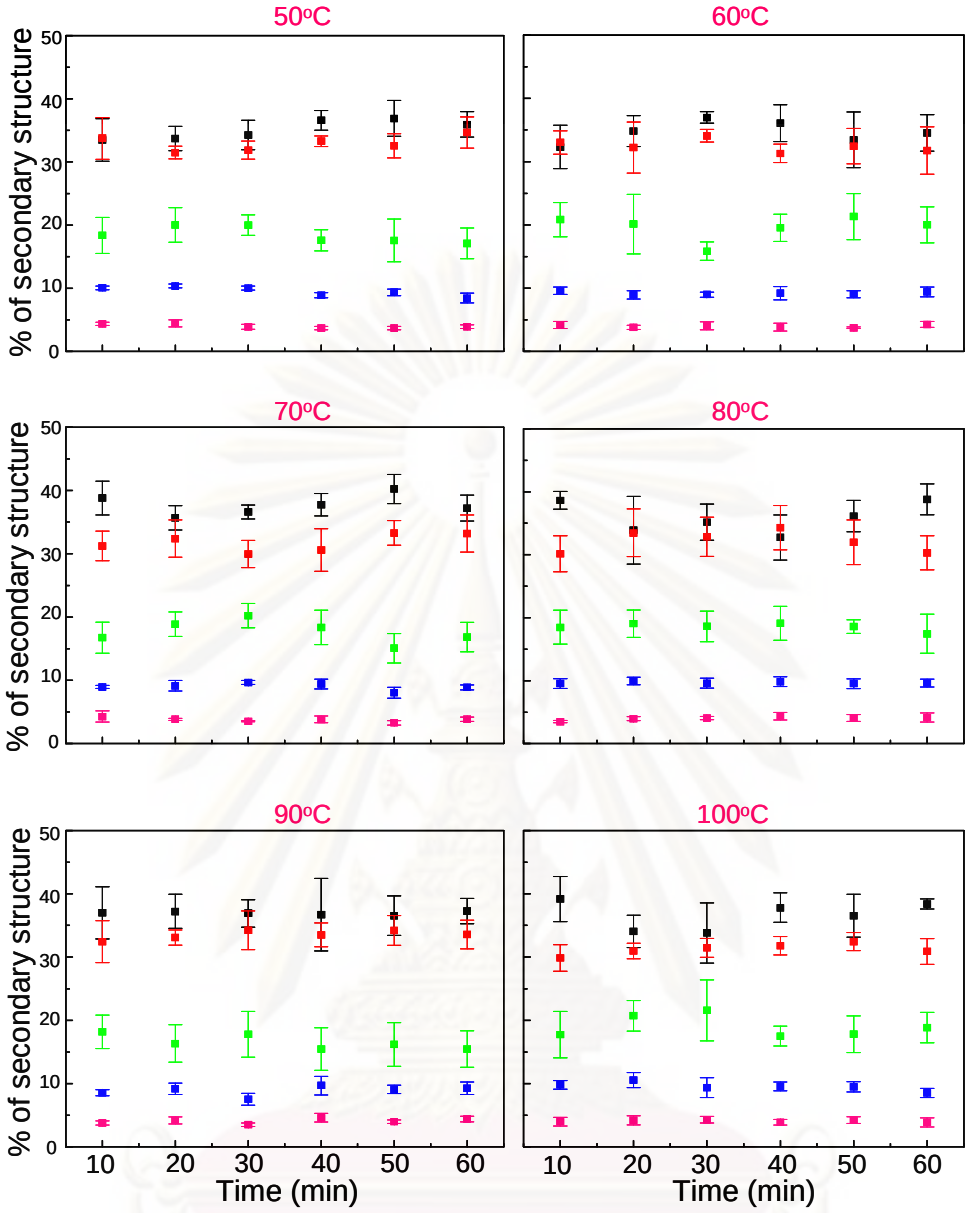

Figure 4.8 The average value of individual secondary structure in silk fiber: β-sheet (black), random coils (red), α-helix (green), turn (blue) and β-sheet (intermolecular) pink. The silk fibers, which were varied as temperatures and times, were soaked into the water.

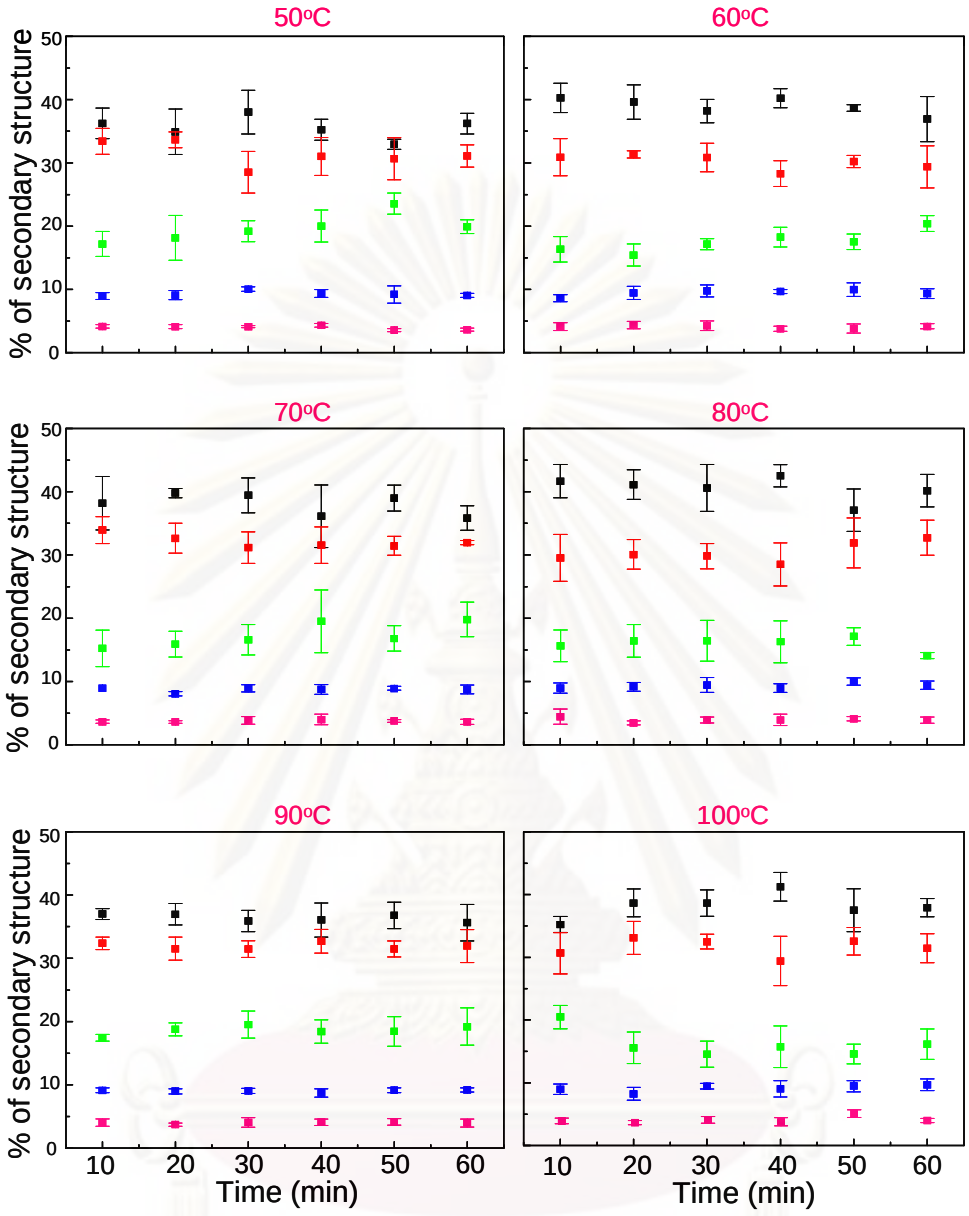

Figure 4.9 The average value of individual secondary structure in silk fiber: β-sheet (black), random coils (red), α-helix (green), turn (blue) and β-sheet (intermolecular) pink. The silk fibers, which were varied as temperatures and times, were dipped into the ice cold-water.

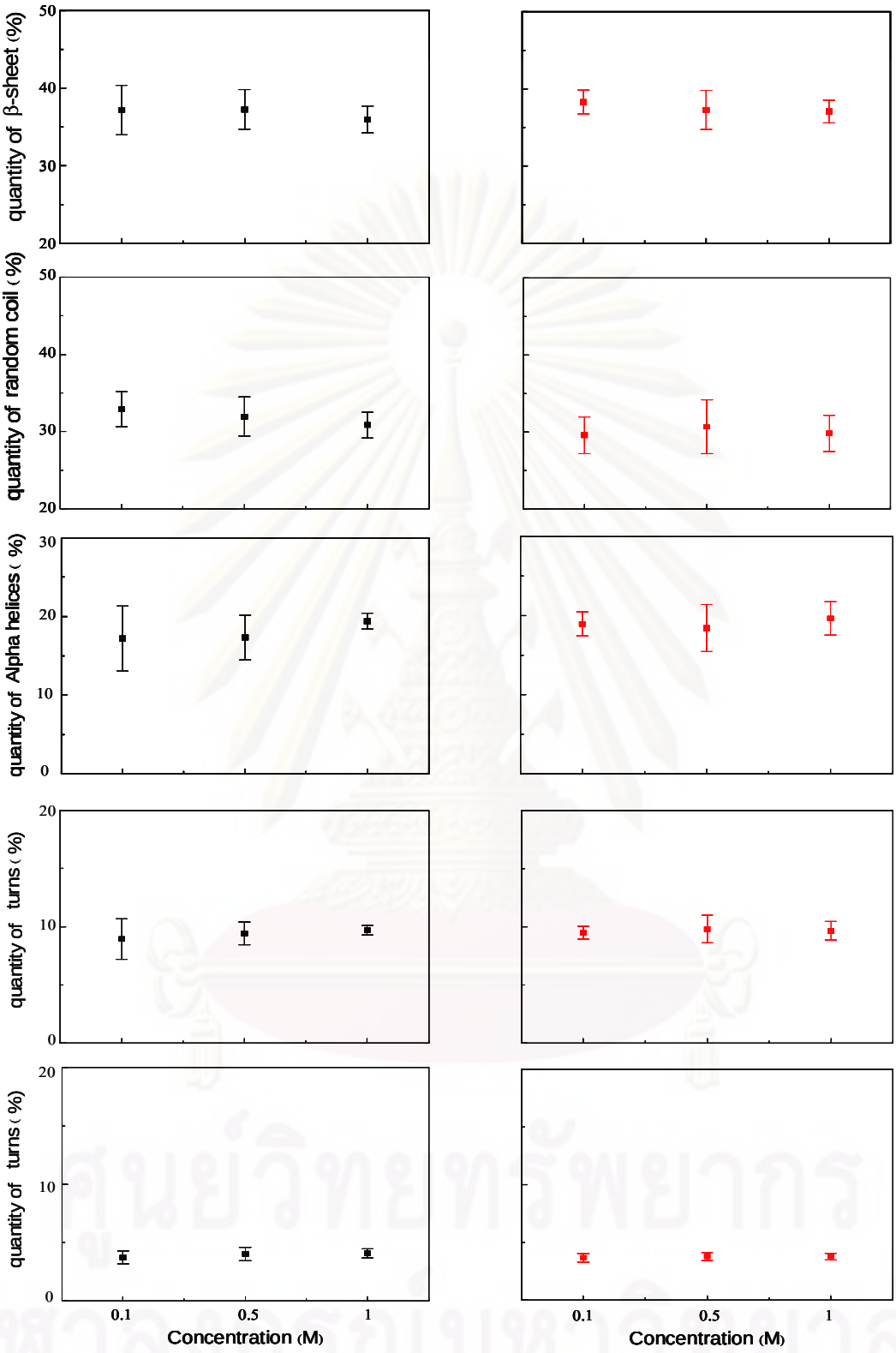

Figure 4.10 The percentage of α-helices, turn and β-sheet (intermolecular) structure of silk fiber with (left) and without (right) treated with methanol was calculated from peak area. The concentrations of $NaHCO_3$ are 0.1, 0.5, 1M.

CHAPTER V

CONCLUSIONS

The homemade μIRE accessories and the slide-on Ge μIRE were employed for ATR FT-IR spectral acquisition by mean of an infrared microscope. Regarding to the small sampling area of Ge tip, the small size of silk fiber (~ 10μm) can be analyzed. The spectrum acquired by the μIRE shows unique characteristic of silk fiber. Since the contact area of the IREs is small, the characterization process is non-destructive, and there is virtually no noticeable change in the original sample, the specimen can be employed for further analysis.

In this study, un-degummed silk fiber of silk cocoon could be characterized by means of ATR FT-IR microspectroscopy. The silk cocoon was divided to eight layers. The amount of layer depends on species of silkworm. The eighth layer (inner layer) showed the more quantity of β-sheet than other layer because of the decrease coated sericin. After that, the character of un-degummed silk fiber was compared with that of degummed silk fiber. The difference of these silk fibers was the shoulder of amide I band at 1697 cm^{-1}. It was assigned to anti-parallel β-sheet. The β-sheet is one type of secondary structures that contained in silk fiber. The amount of secondary structure such as β-sheet, random-coil, α-helix and turn could determine by the curve fitting method. It has relationship with crystallinity of silk fiber. And the heat treatment has effect on the secondary structure. So, the silk fiber was treated by controlling the different temperature, time and quenching method. After that, the treated silk was fitted curve and evaluated. The average mean values after calculating from area of deconvolved peak were fluctuated. This result does not depend on the distance of measurement. The end of right-hand, the middle and the end of left-hand of silk fiber give the similarity fluctuation value. It is concluded that the changes of secondary structure were insignificant. The causes of this phenomenon are (i) original silk fibers before heating have fluctuated. (ii) the thermal method is the mild condition, so it is not vigorous condition for changing the secondary structure.

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APPENDICES

APPENDICES

1. Amino structure

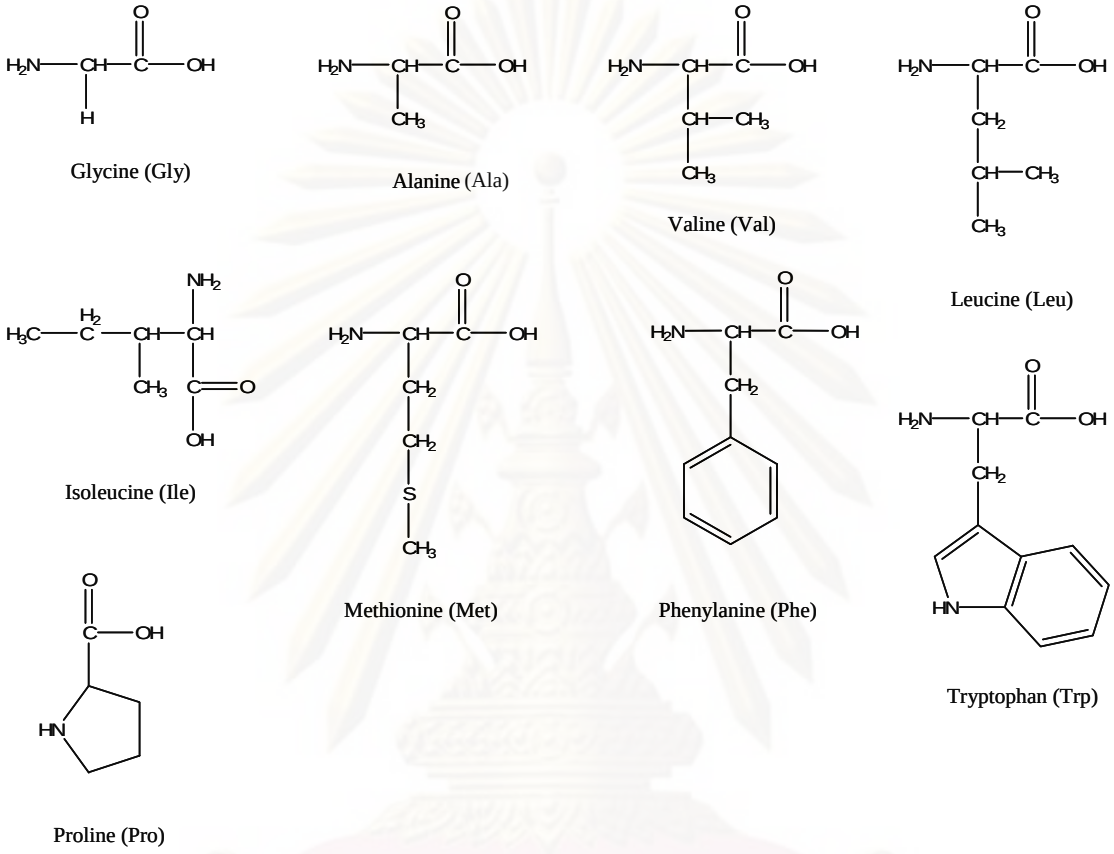
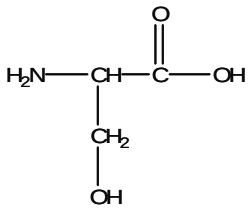
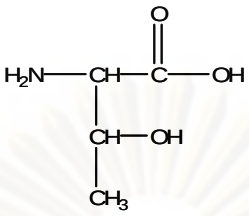


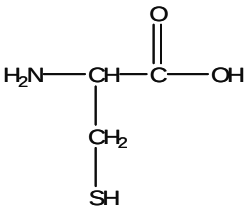
Figure A1. Non-polar amino acid.



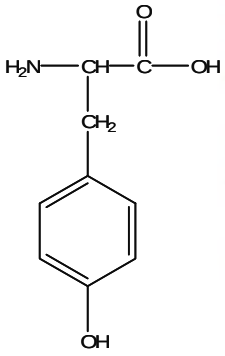
Serine (Ser)



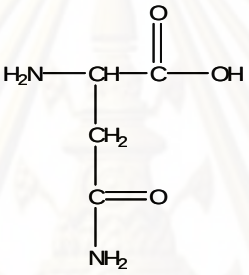
Threonine (Thr)



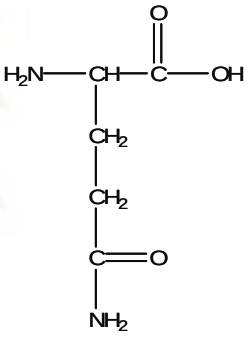
Cysteine (Cys)



Tyrosine (Tyr)



Asparagine (Asn)



Glutamine (Gln)

Figure A2. Polar amino acid.

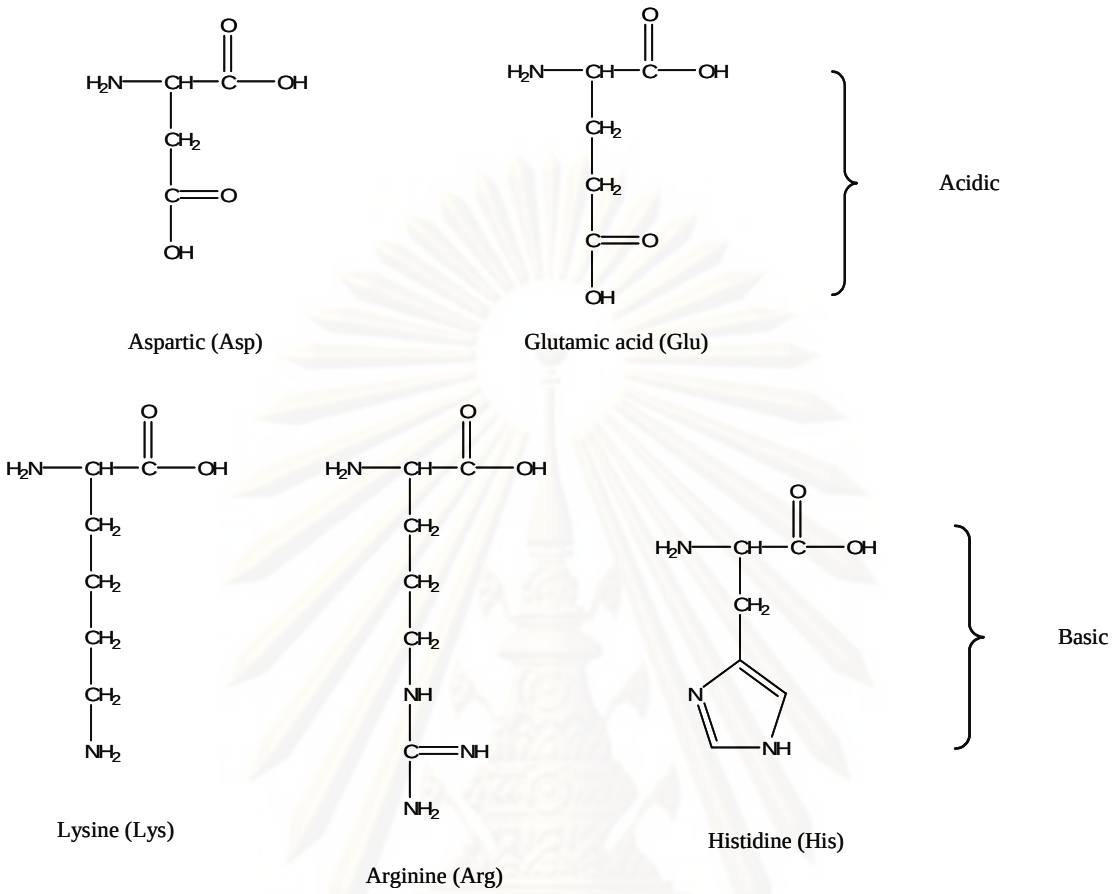
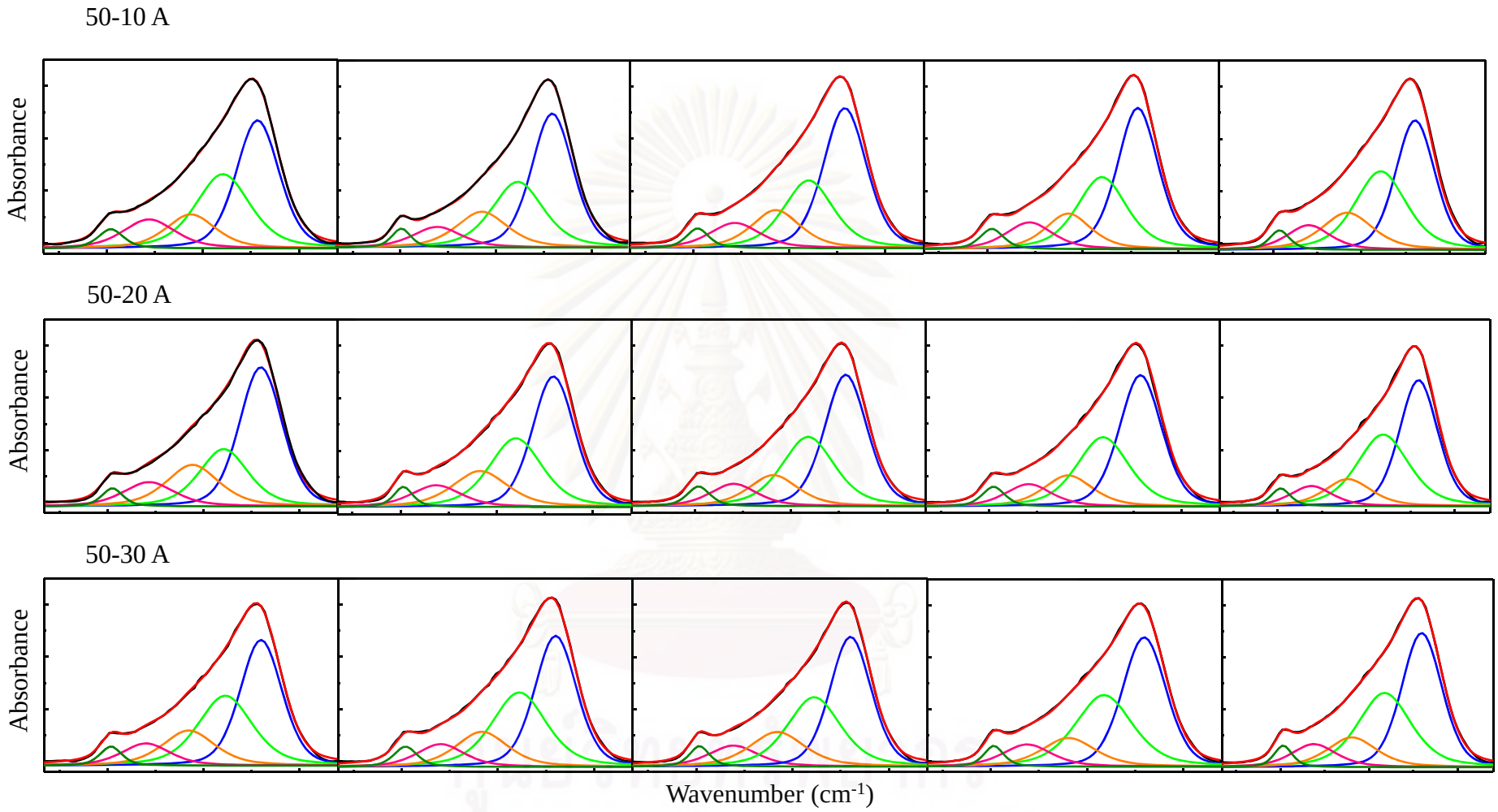


Figure A3. Electrically charged amino acid.

2. Polarity of solvent

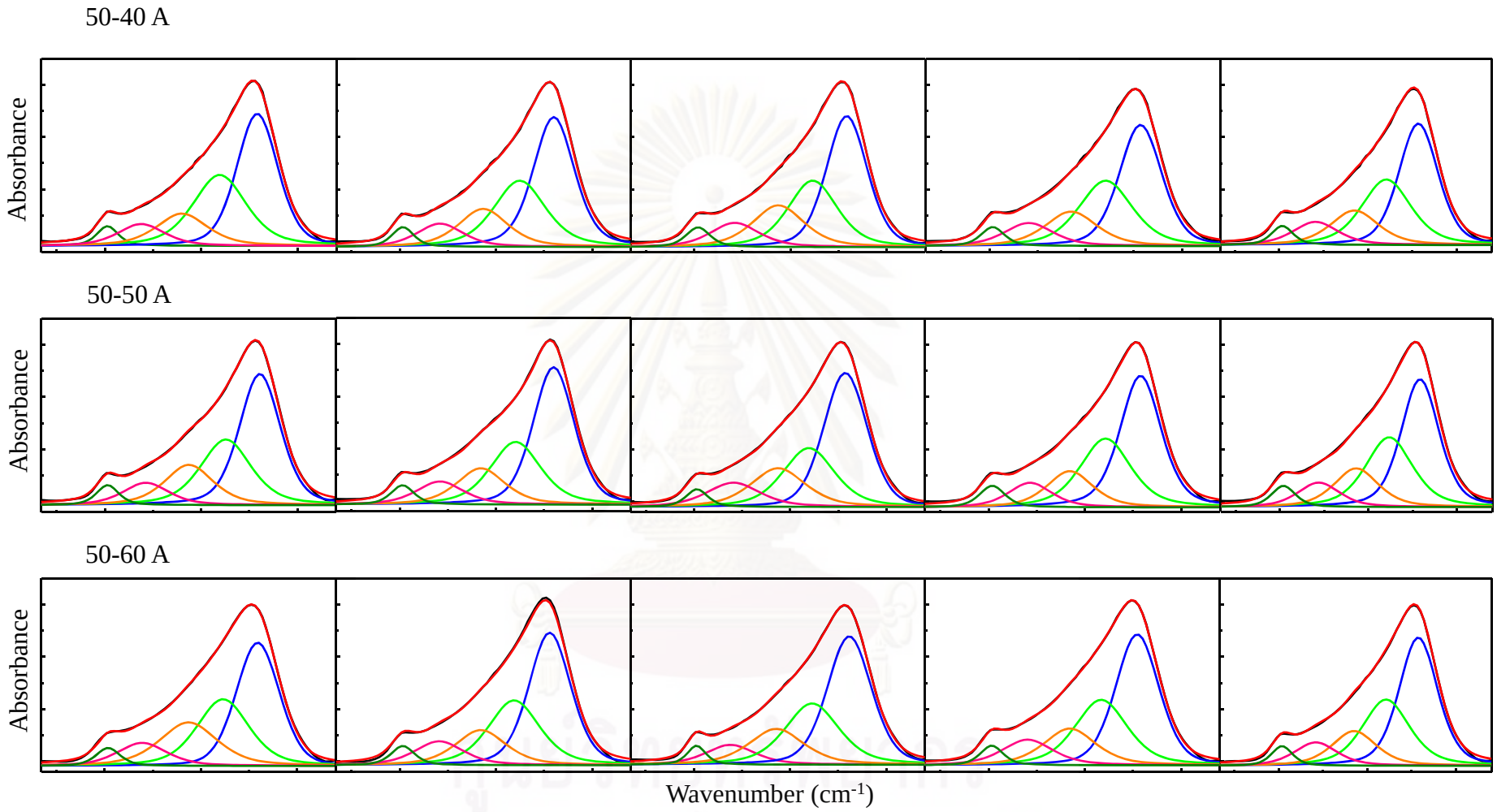
3. The percentage of individual secondary structure calculated from peak area of deconvolve peak after curve fitting.



Note : 50-10 A, 50-20 A and 50-30 A (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

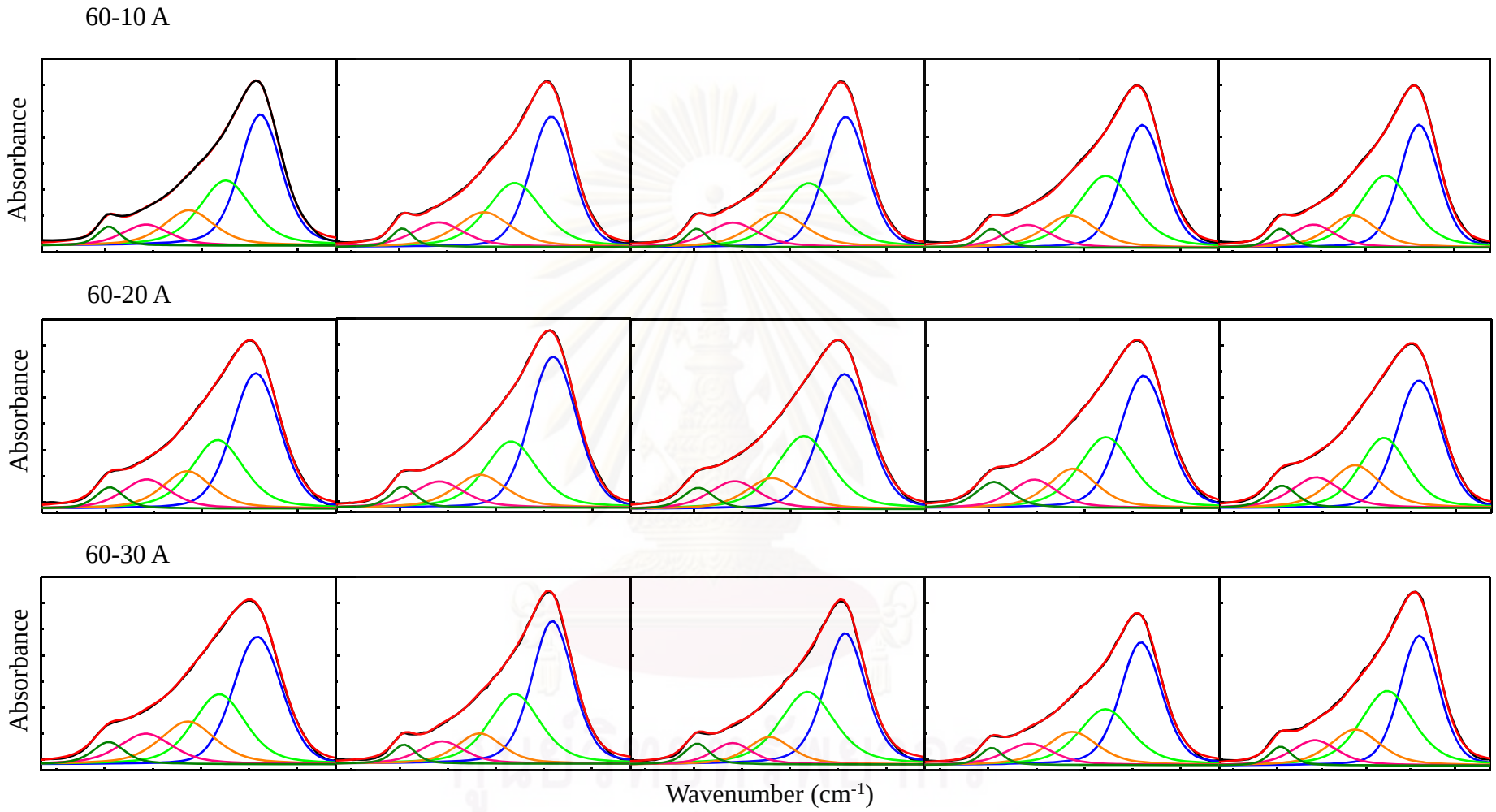
Figure A4. Curve fitting of treated silk fiber at 50°C by varying time (10, 20 and 30 min) and exposing into the air.



Note : 50-40 A, 50-50 A and 50-60 A (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

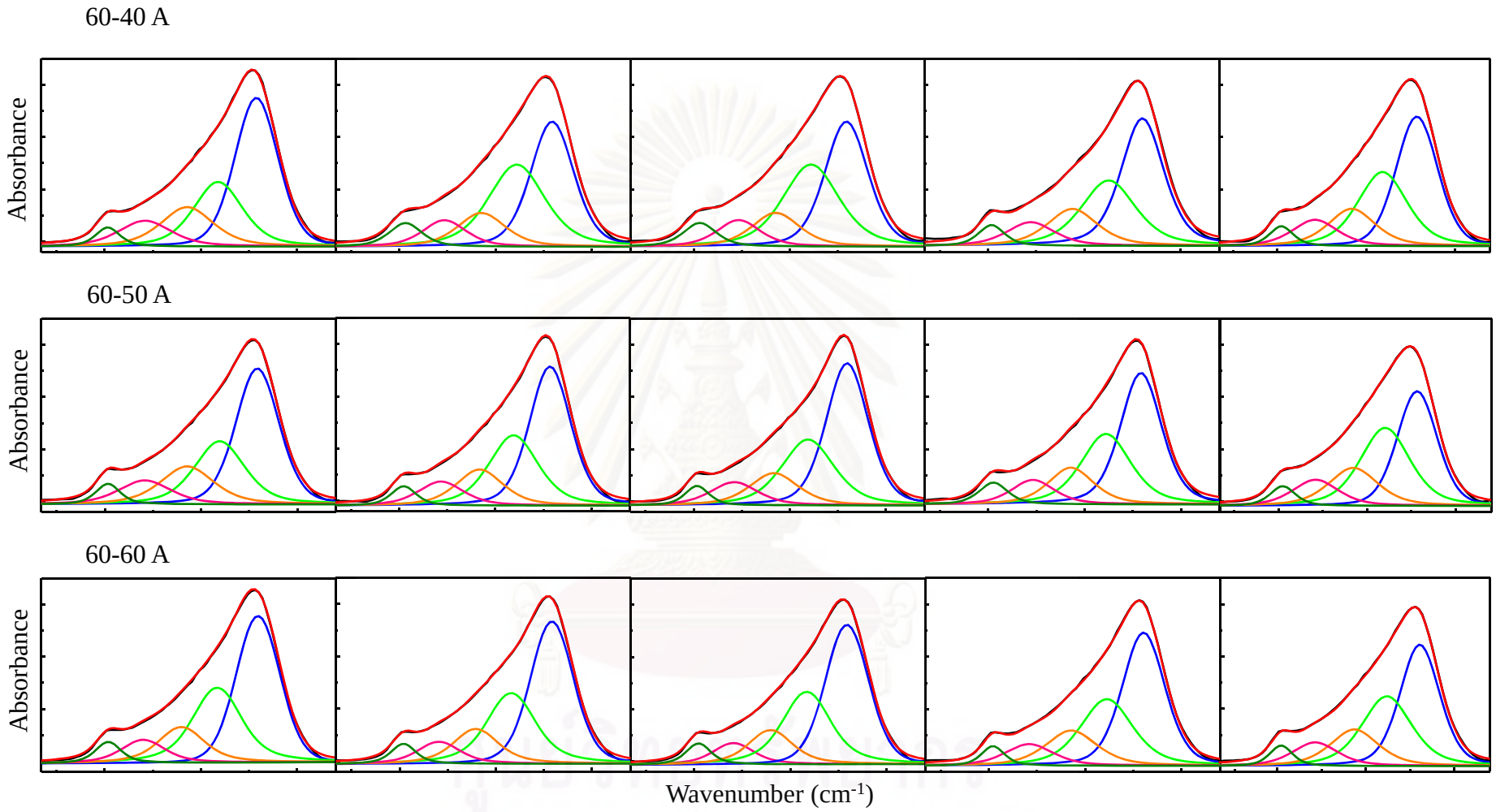
Figure A5. Curve fitting of treated silk fiber at 50°C by varying time (40, 50 and 60 min) and exposing into the air.



Note : 60-10 A, 60-20 A and 60-30 A (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

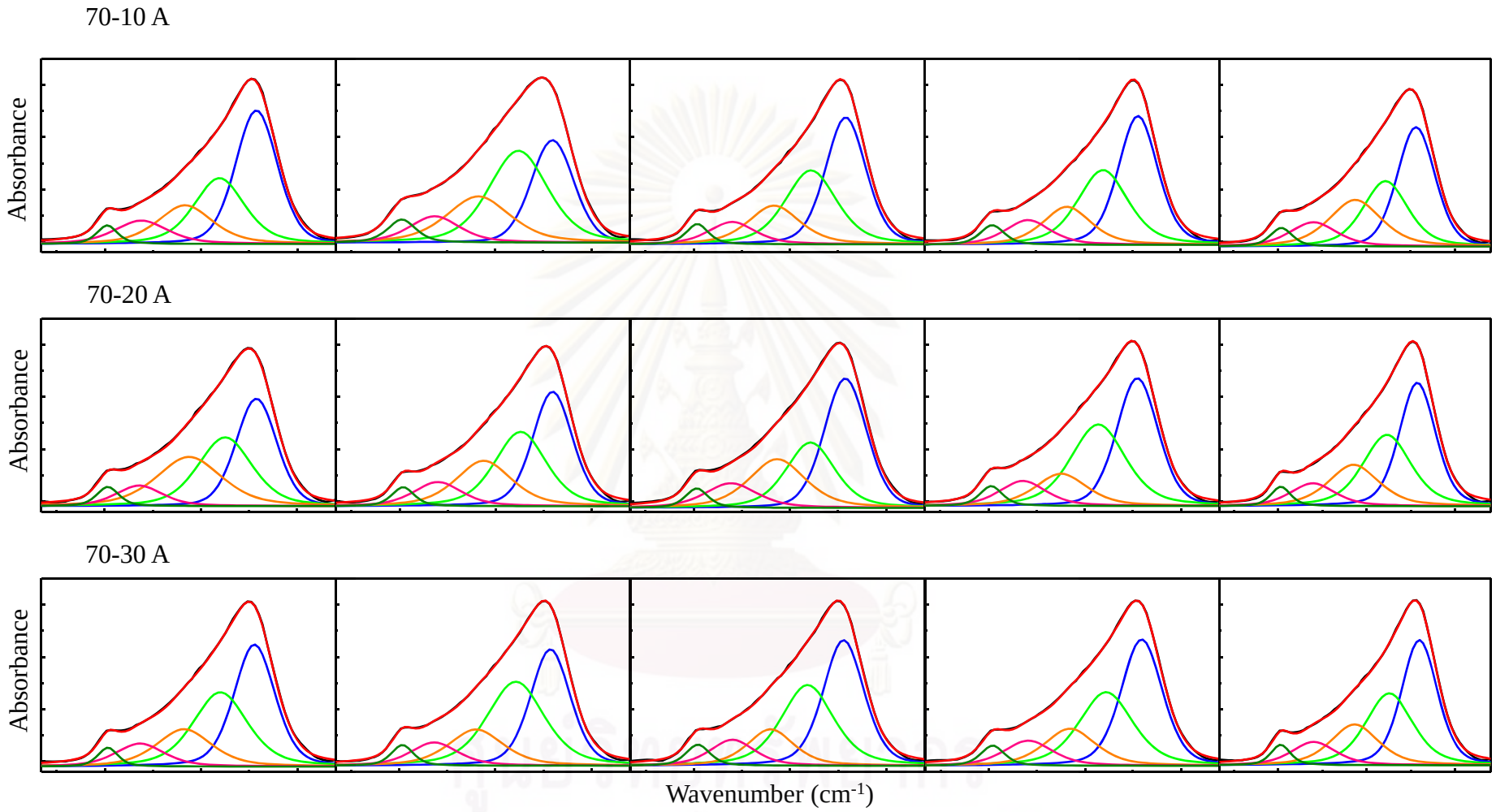
Figure A6. Curve fitting of treated silk fiber at 60°C by varying time (10, 20 and 30 min) and exposing into the air.



Note : 60-40 A, 60-50 A and 60-60 A (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

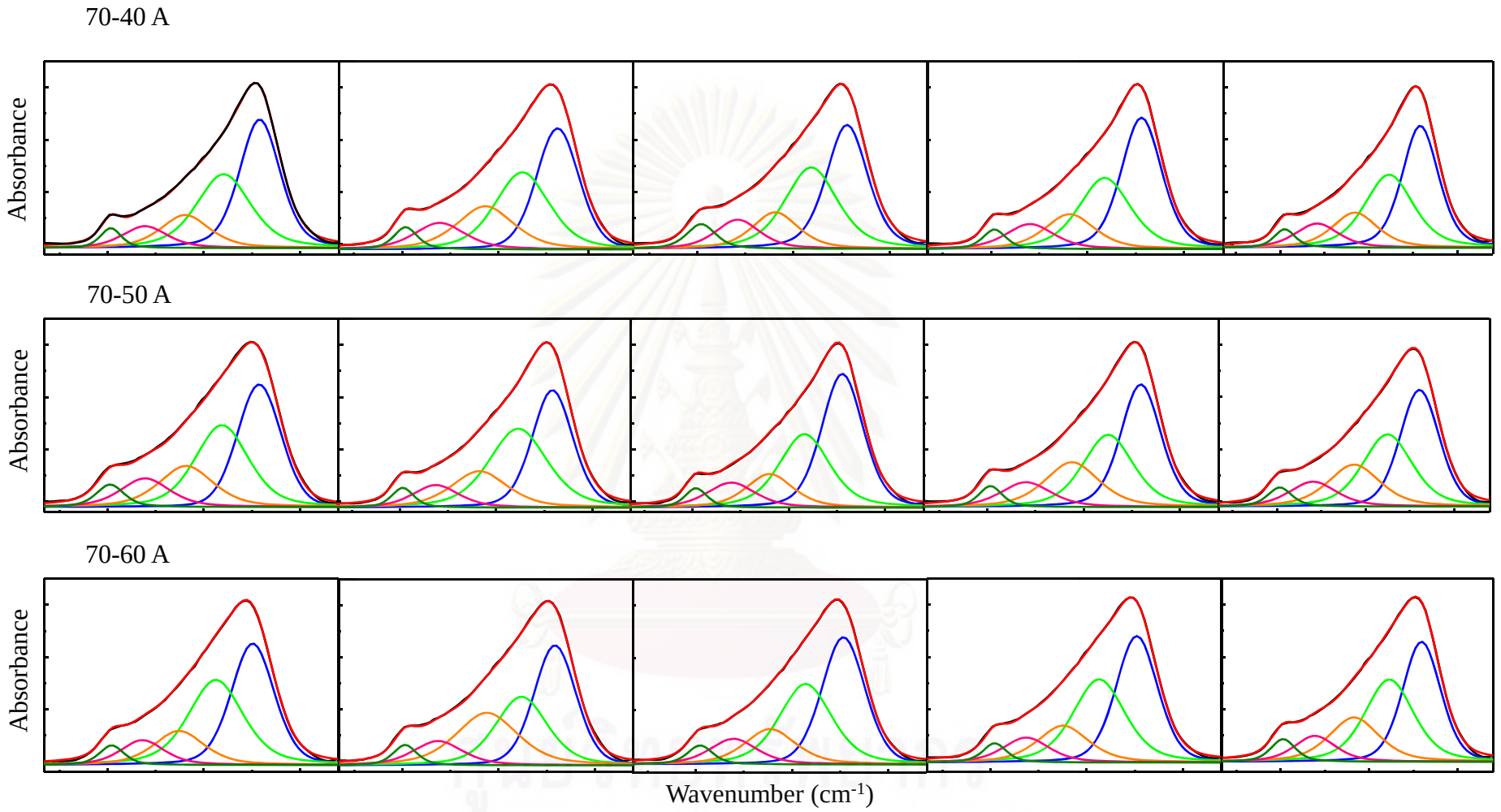
Figure A7. Curve fitting of treated silk fiber at 60°C by varying time (40, 50 and 60 min) and exposing into the air.



Note : 70-10 A, 70-20 A and 70-30 A (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

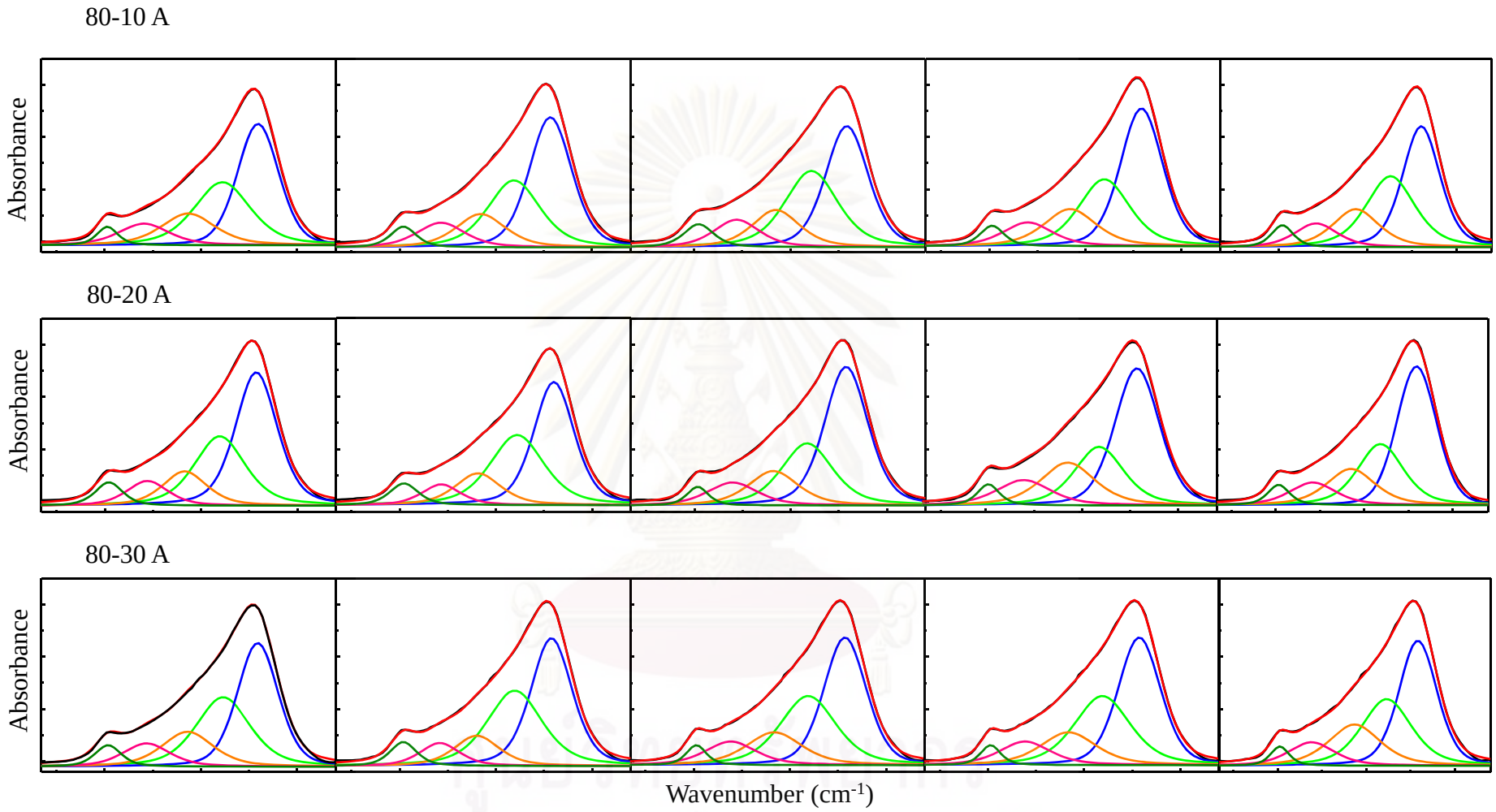
Figure A8. Curve fitting of treated silk fiber at 70°C by varying time (10, 20 and 30 min) and exposing into the air.



Note : 70-40 A, 70-50 A and 70-60 A (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

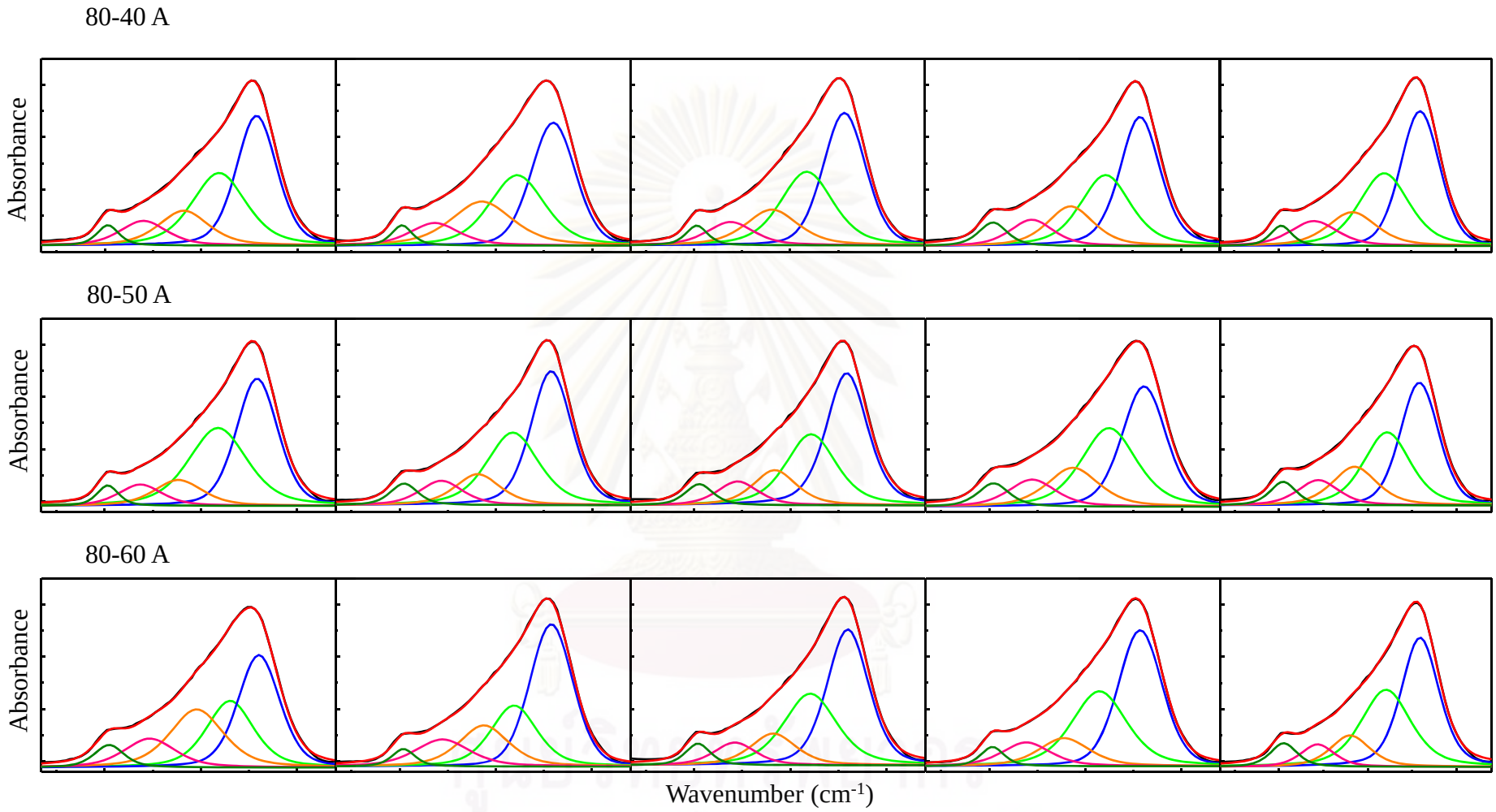
Figure A9. Curve fitting of treated silk fiber at 70°C by varying time (40, 50 and 60 min) and exposing into the air.



Note : 80-10 A, 80-20 A and 80-30 A (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

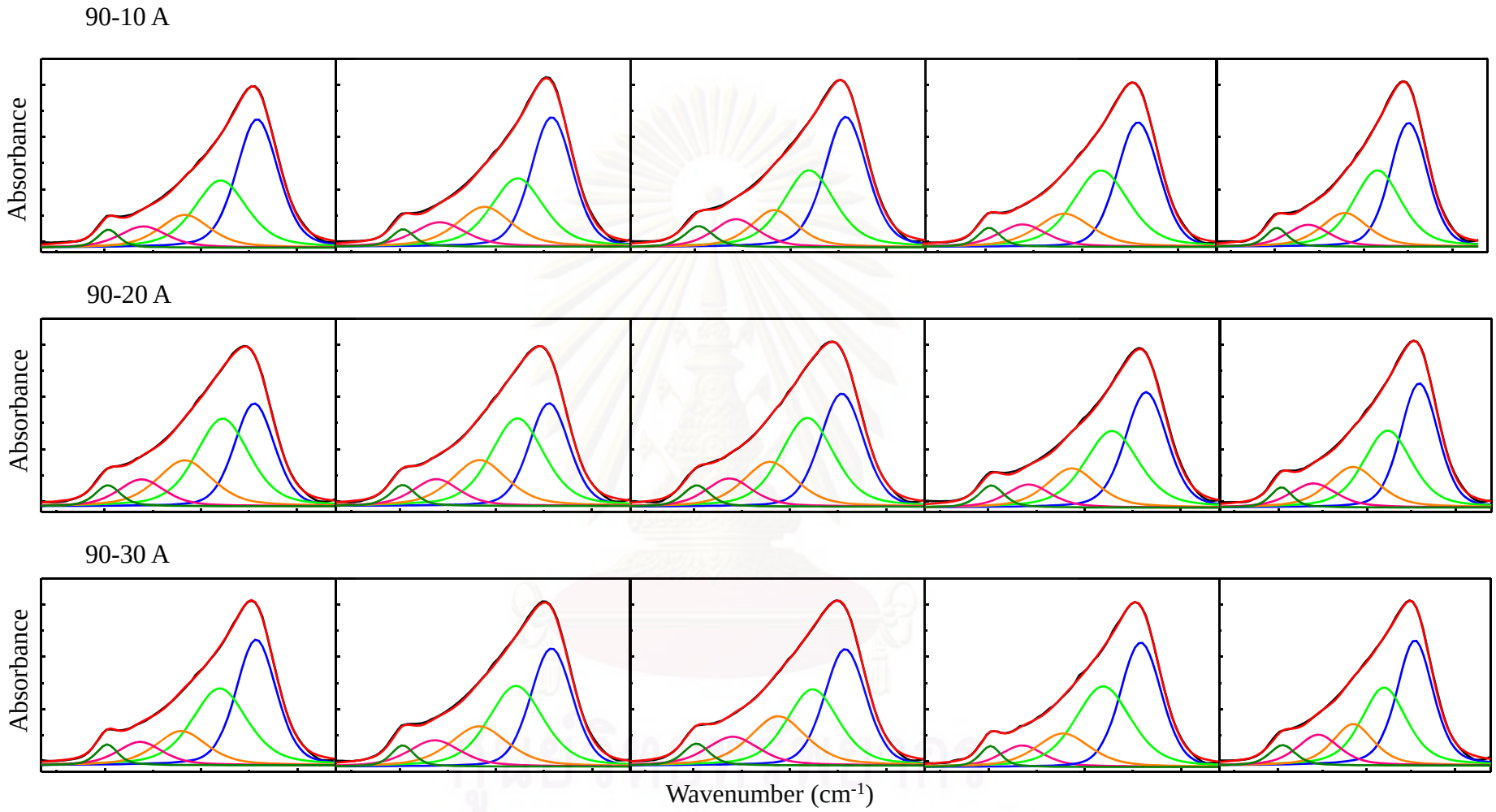
Figure A10. Curve fitting of treated silk fiber at 80°C by varying time (10, 20 and 30 min) and exposing into the air.



Note : 80-40 A, 80-50 A and 80-60 A (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

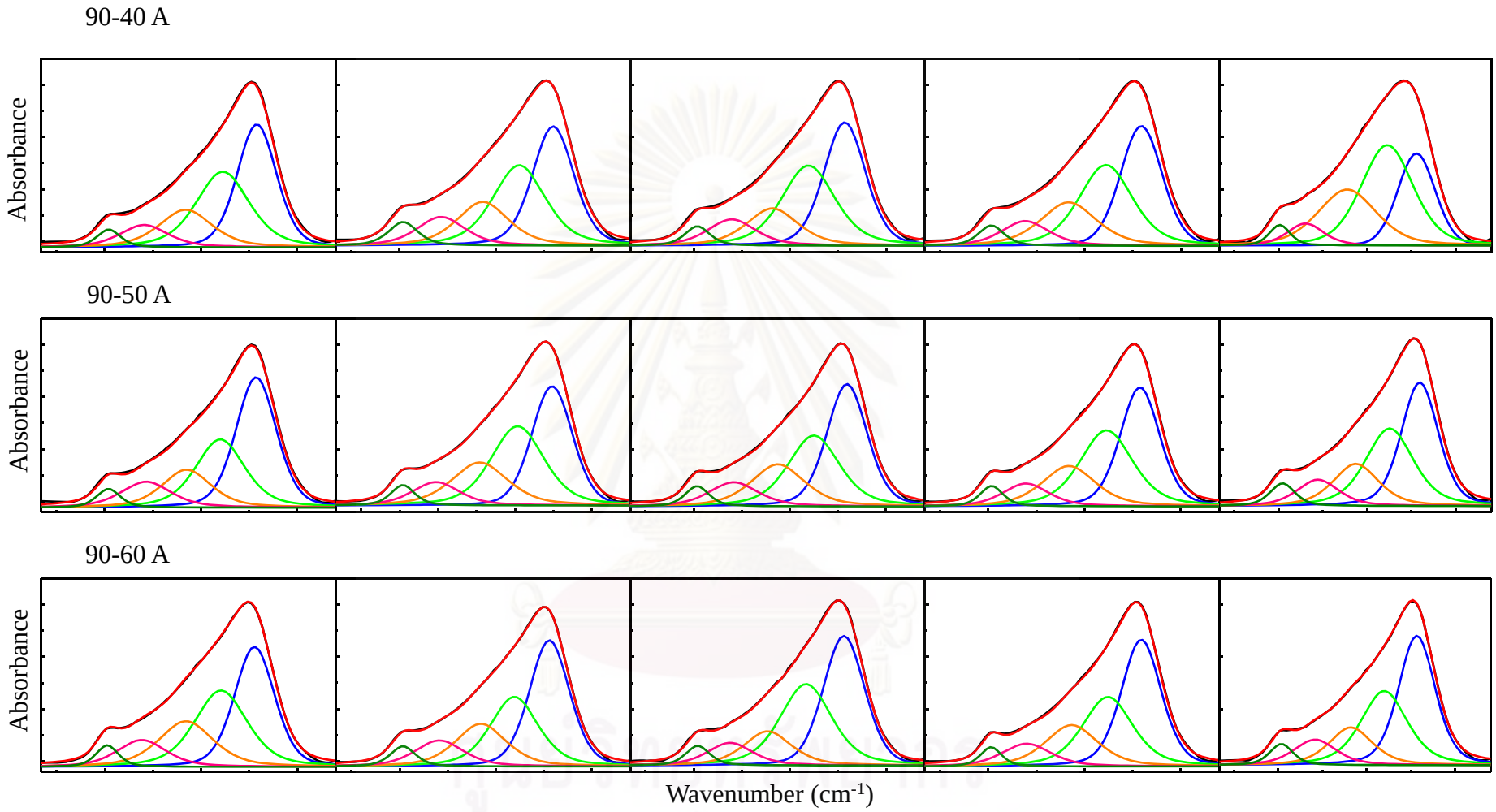
Figure A11. Curve fitting of treated silk fiber at 80°C by varying time (40, 50 and 60 min) and exposing into the air.



Note : 90-10 A, 90-20 A and 90-30 A (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

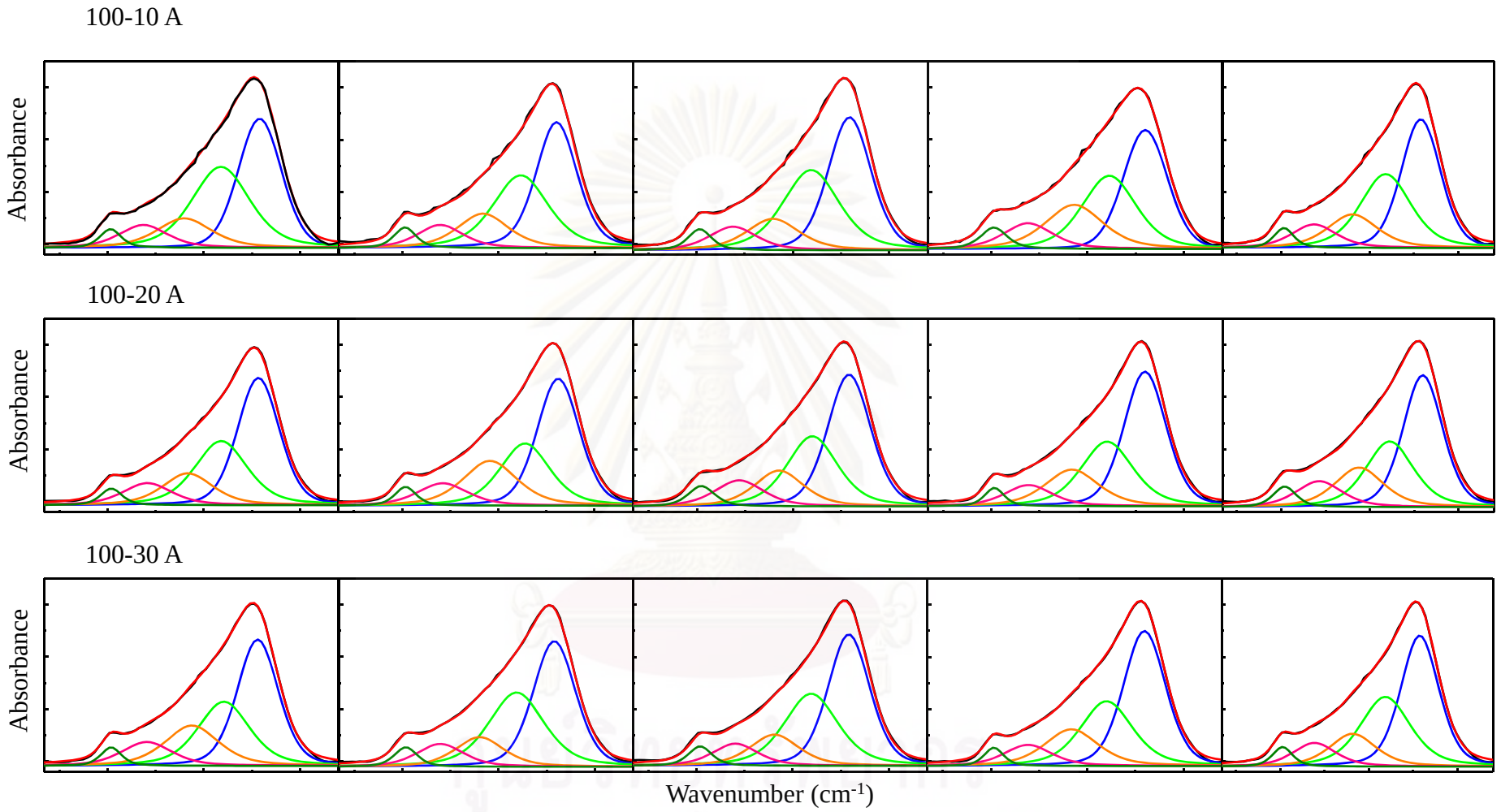
Figure 12. Curve fitting of treated silk fiber at 90°C by varying time (10, 20 and 30 min) and exposing into the air.



Note : 90-40 A, 90-50 A and 90-60 A (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

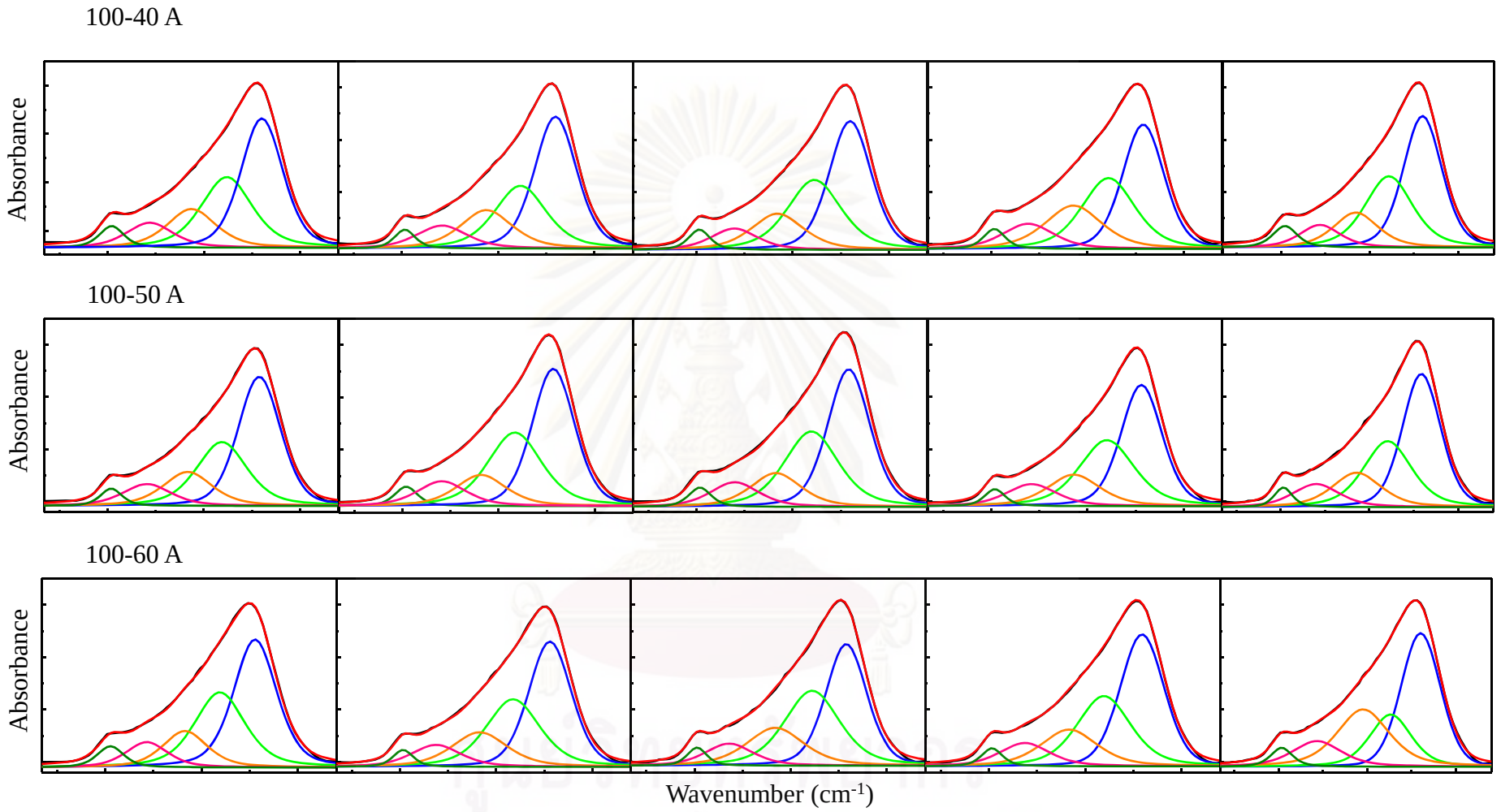
Figure 13. Curve fitting of treated silk fiber at 90°C by varying time (40, 50 and 60 min) and exposing into the air.



Note : 100-10 A, 100-20 A and 100-30 A (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

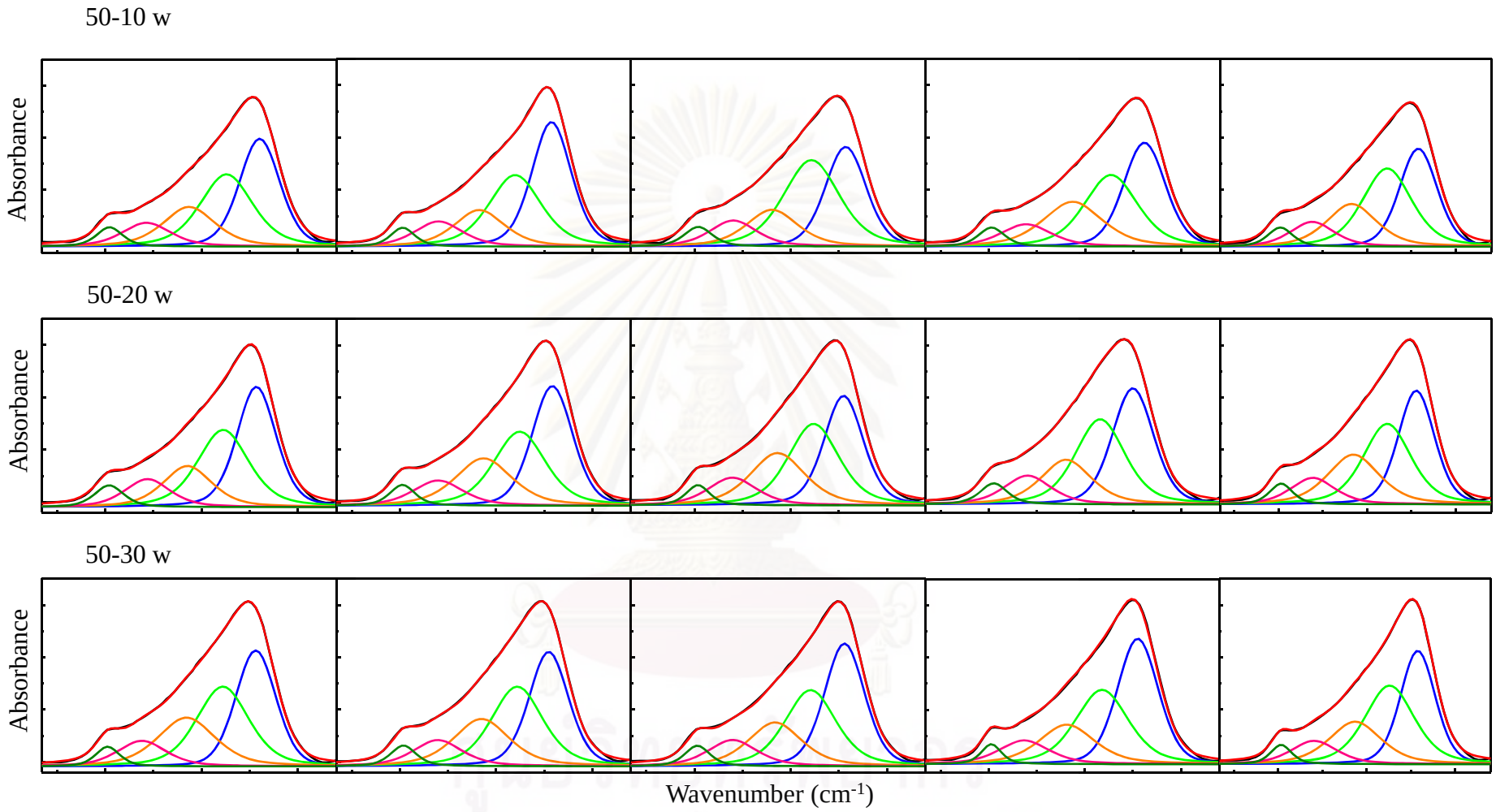
Figure A14. Curve fitting of treated silk fiber at 100°C by varying time (10, 20 and 30 min) and exposing into the air.



Note : 100-40 A, 100-50 A and 100-60 A (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

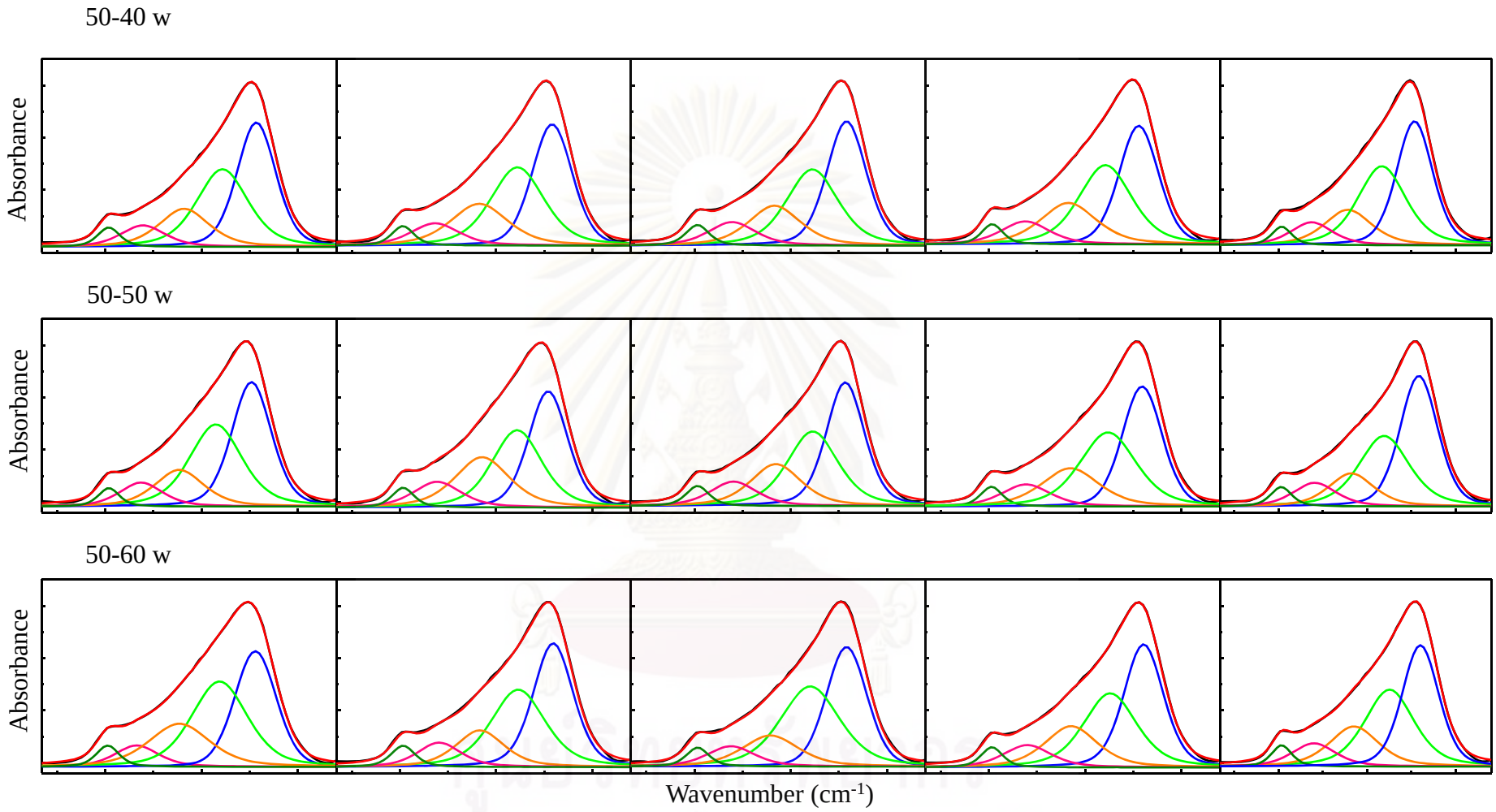
Figure A15. Curve fitting of treated silk fiber at 100°C by varying time (40, 50 and 60 min) and exposing into the air.



Note : 50-10 w, 50-20 w and 50-30 w (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

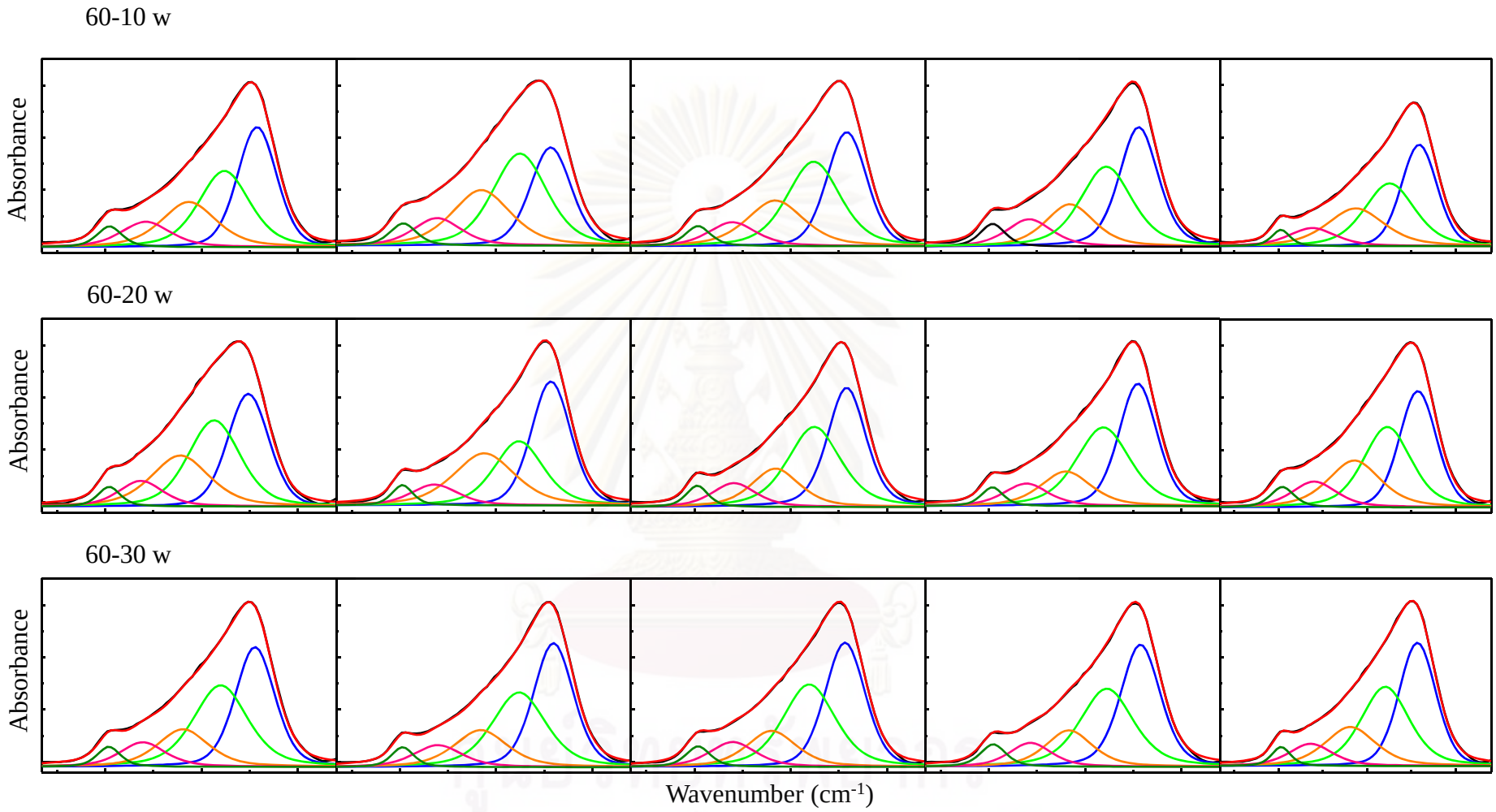
Figure A16. Curve fitting of treated silk fiber at 50°C by varying time (10, 20 and 30 min) and soaking into the water.



Note : 50-40 w, 50-50 w and 50-60 w (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

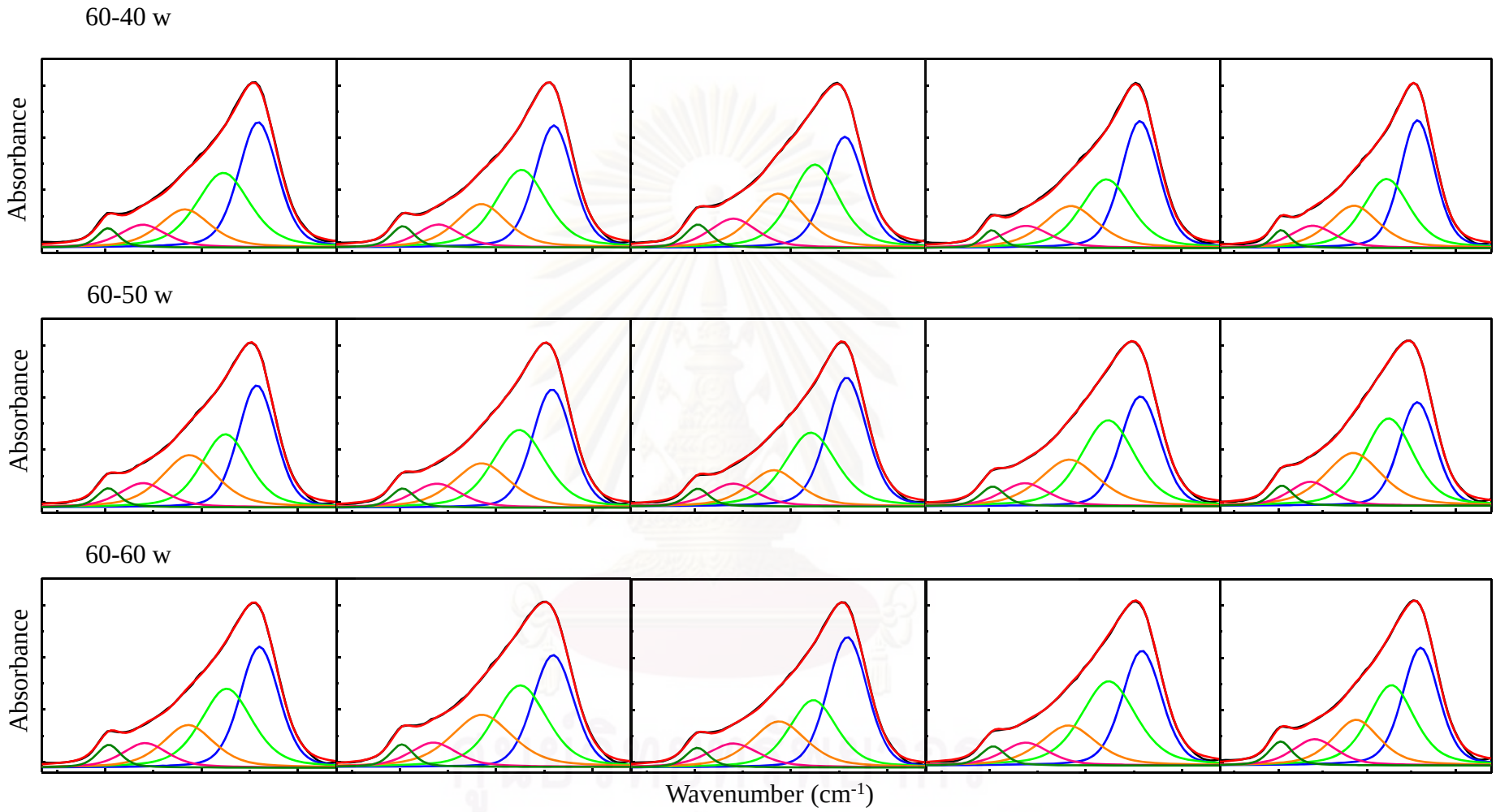
Figure A17. Curve fitting of treated silk fiber at 50°C by varying time (40, 50 and 60 min) and soaking into the water.



Note : 60-10 w, 60-20 w and 60-30 w (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

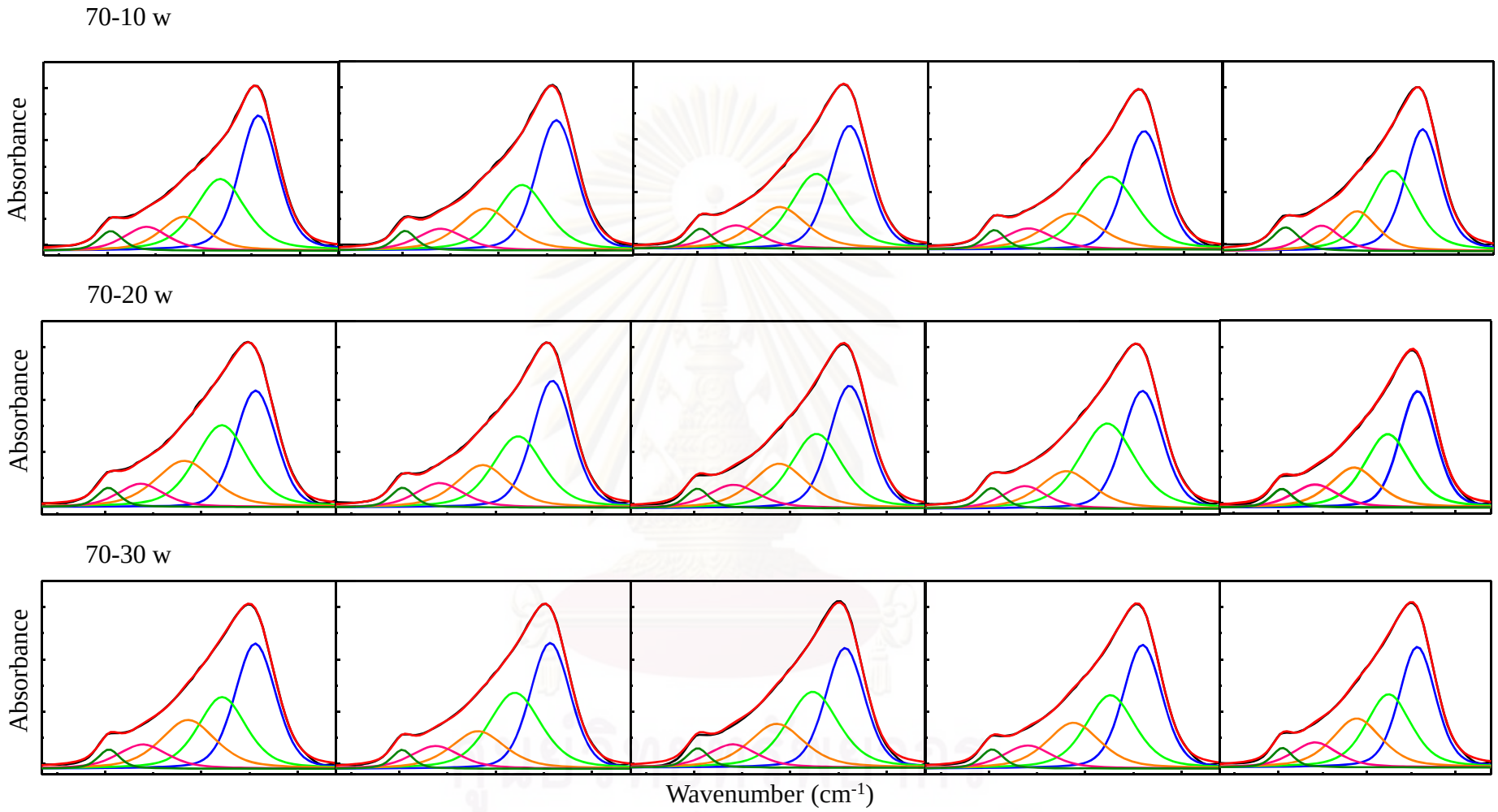
Figure A18. Curve fitting of treated silk fiber at 60°C by varying time (10, 20 and 30 min) and soaking into the water.



Note : 60-40 w, 60-50 w and 60-60 w (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

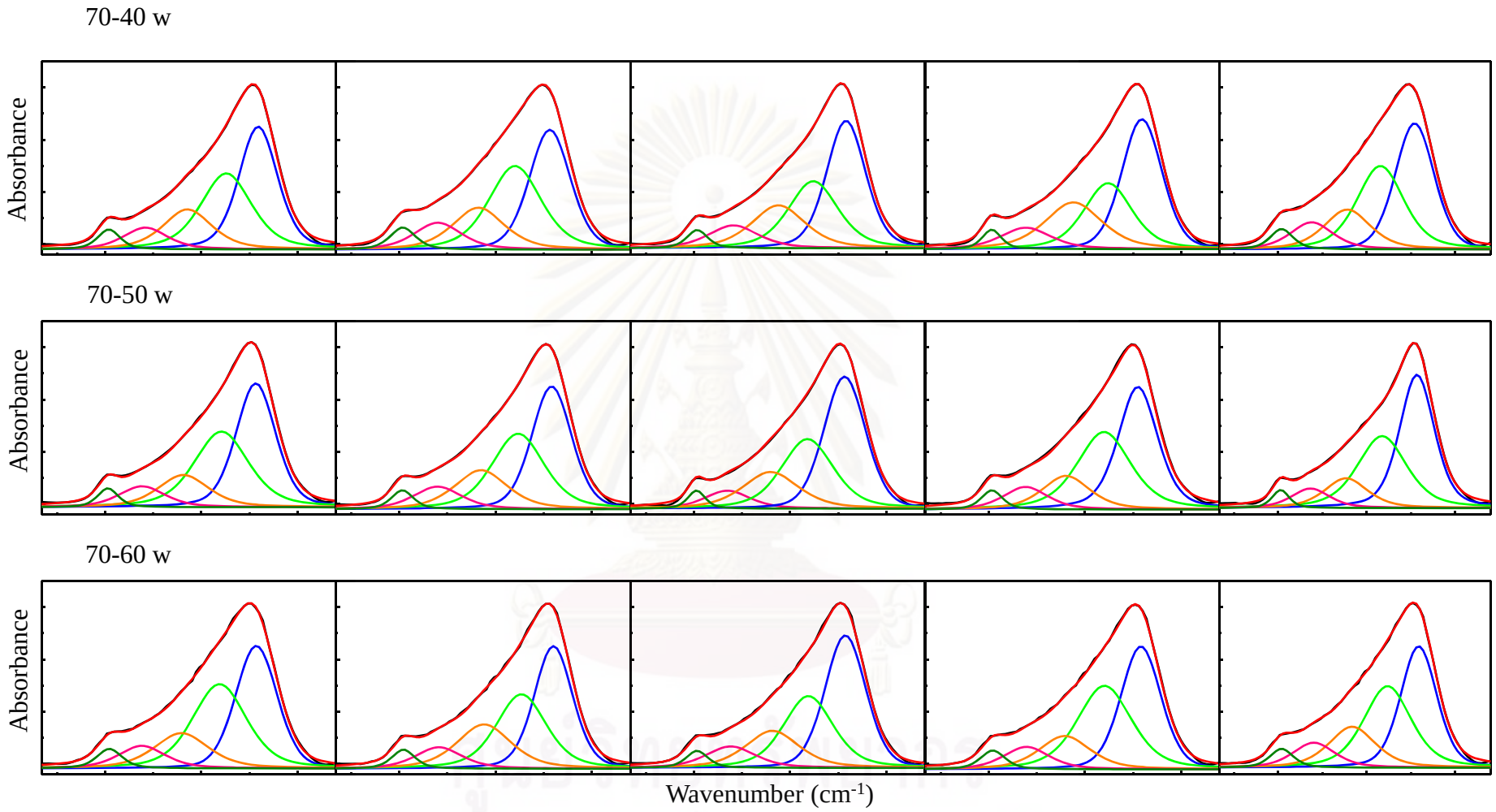
Figure A19. Curve fitting of treated silk fiber at 60°C by varying time (40, 50 and 60 min) and soaking into the water.



Note : 70-10 w, 70-20 w and 70-30 w (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

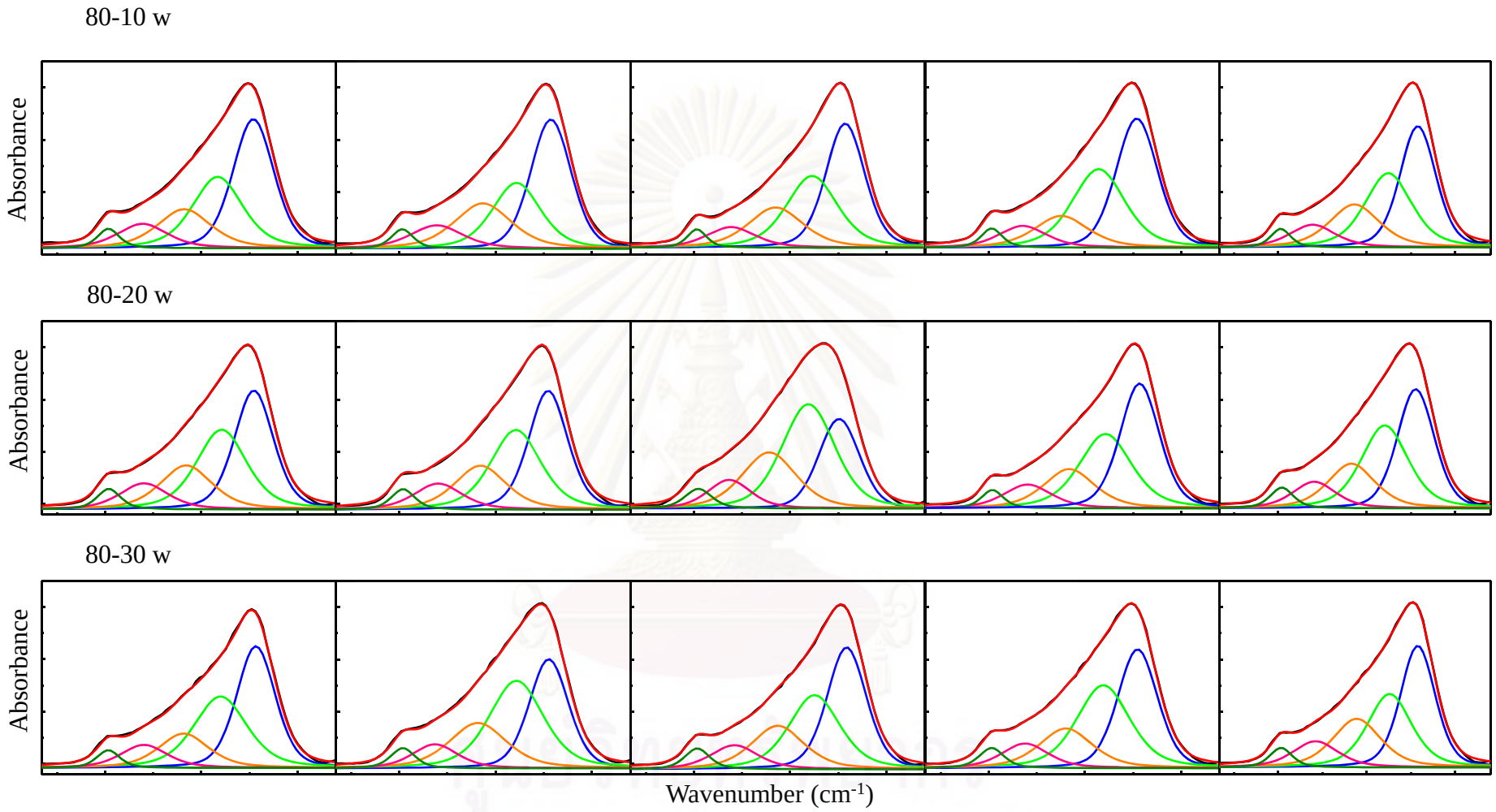
Figure A20. Curve fitting of treated silk fiber at 70°C by varying time (10, 20 and 30 min) and soaking into the water.



Note : 70-40 w, 70-50 w and 70-60 w (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

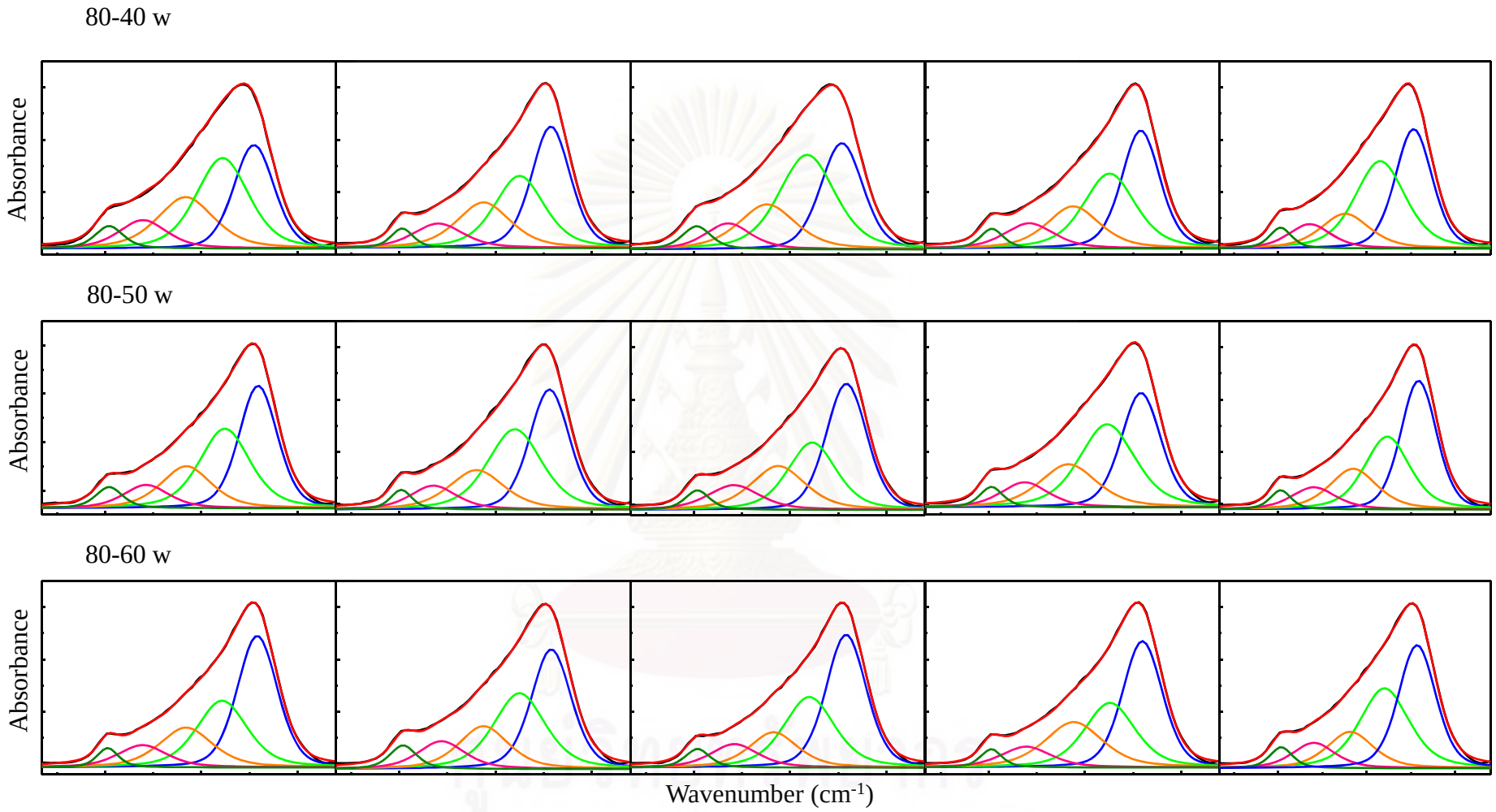
Figure A21. Curve fitting of treated silk fiber at 70°C by varying time (40, 50 and 60 min) and soaking into the water.



Note : 80-10 w, 80-20 w and 80-30 w (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

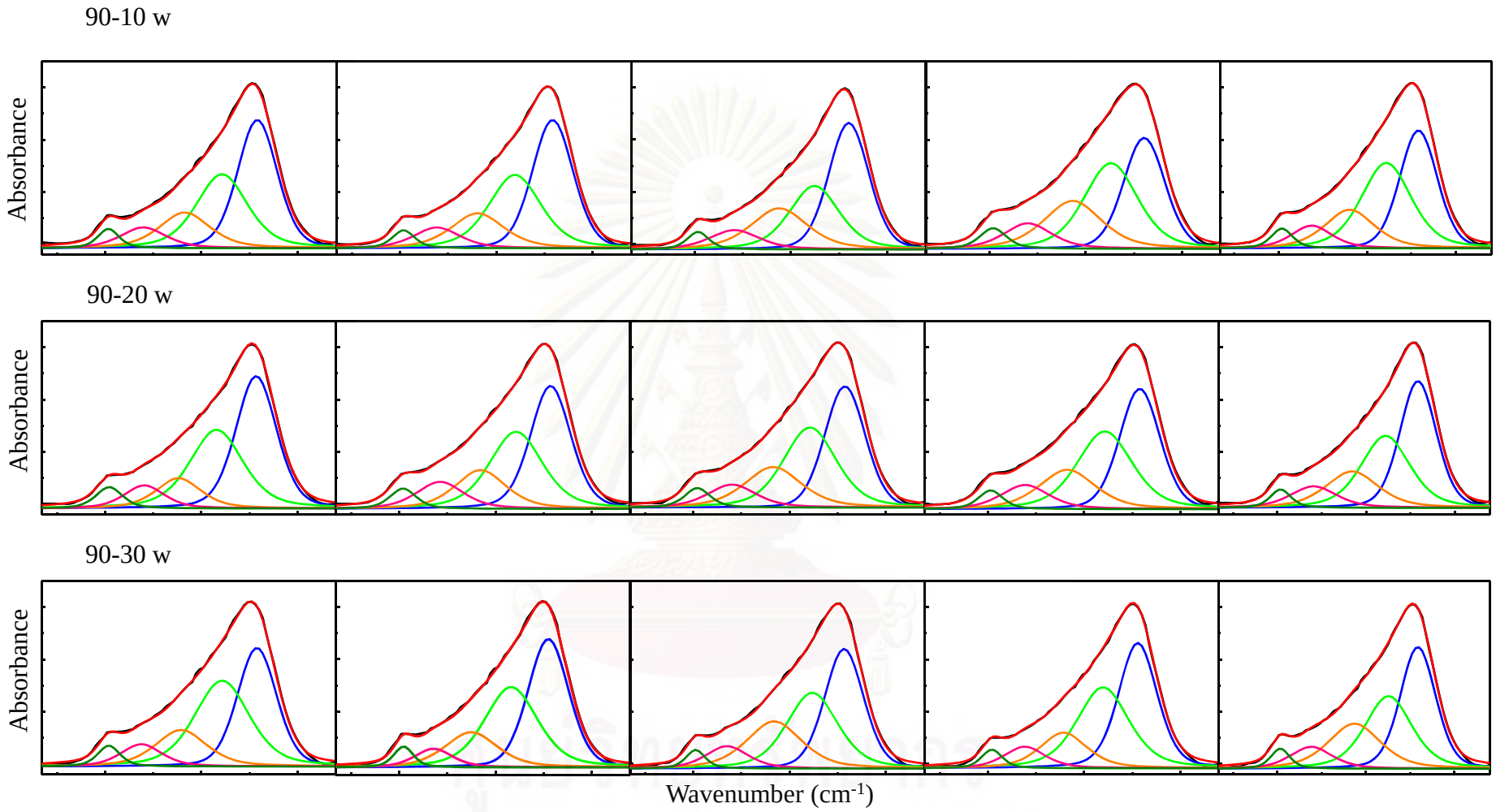
Figure A22. Curve fitting of treated silk fiber at 80°C by varying time (10, 20 and 30 min) and soaking into the water.



Note : 80-40 w, 80-50 w and 80-60 w (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

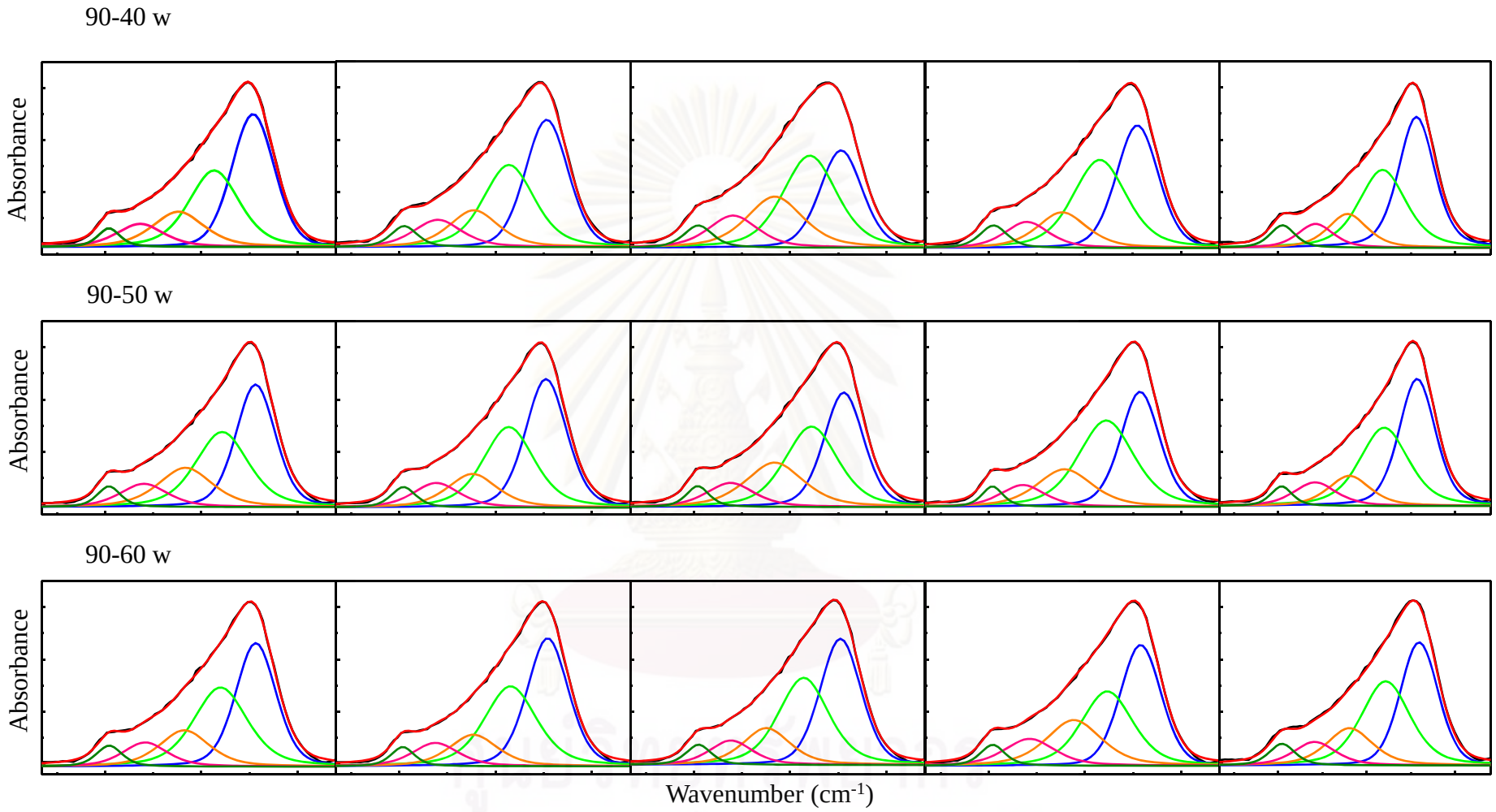
Figure A23. Curve fitting of treated silk fiber at 80°C by varying time (40, 50 and 60 min) and soaking into the water.



Note : 90-10 w, 90-20 w and 90-30 w (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

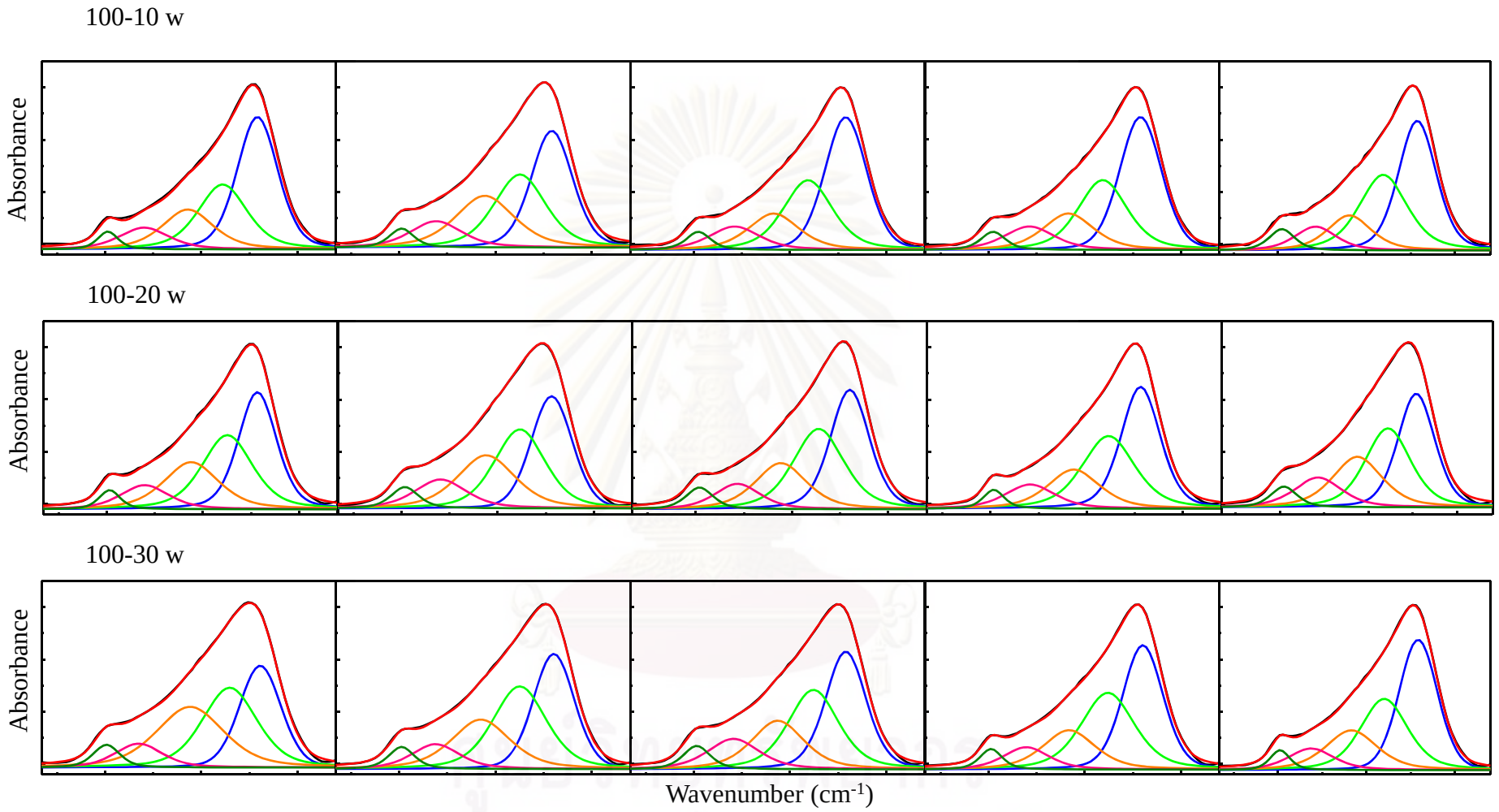
Figure A24. Curve fitting of treated silk fiber at 90°C by varying time (10, 20 and 30 min) and soaking into the water.



Note : 90-40 w, 90-50 w and 90-60 w (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

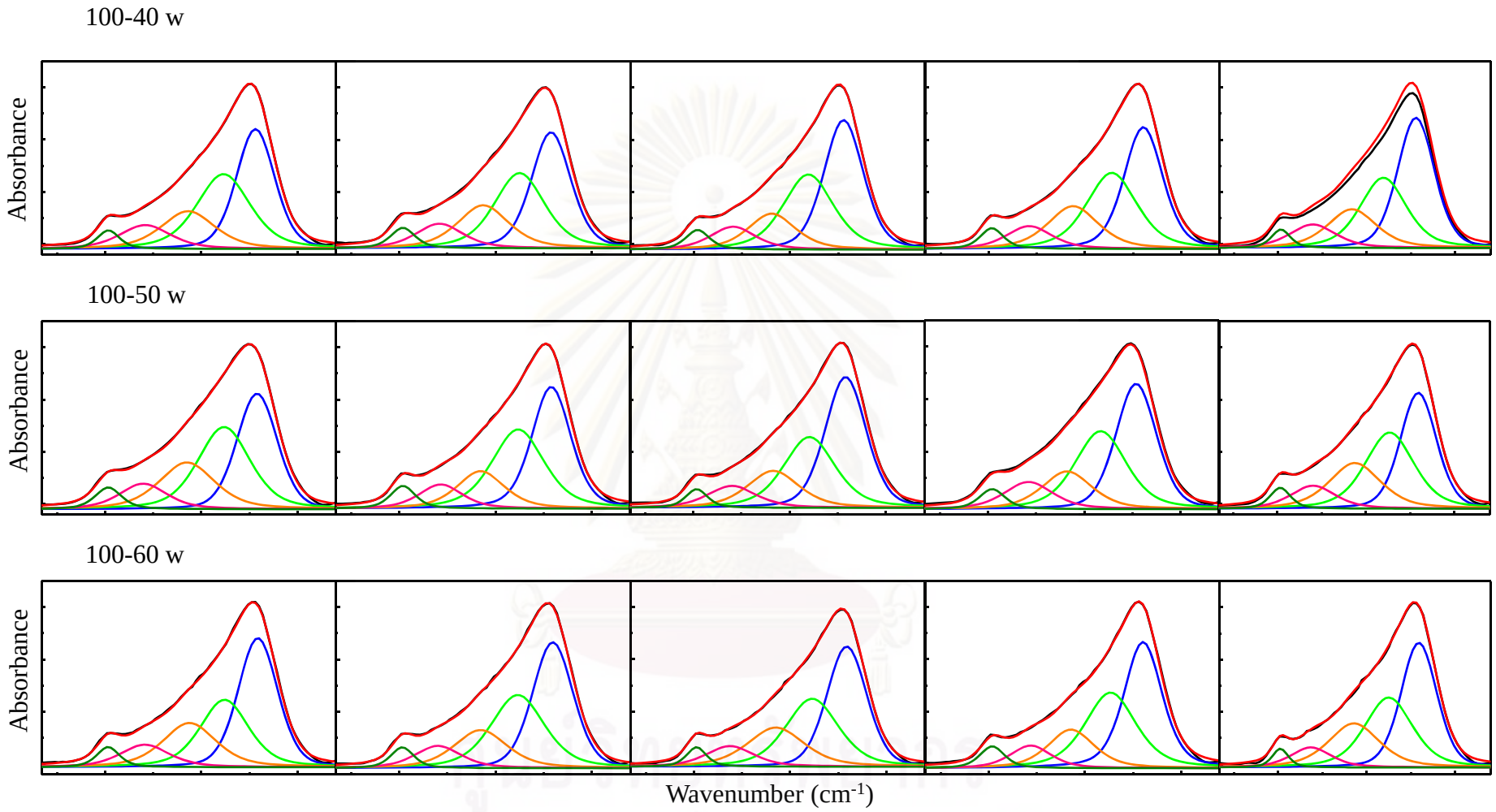
Figure A25. Curve fitting of treated silk fiber at 90°C by varying time (40, 50 and 60 min) and soaking into the water.



Note : 100-10 w, 100-20 w and 100-30 w (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

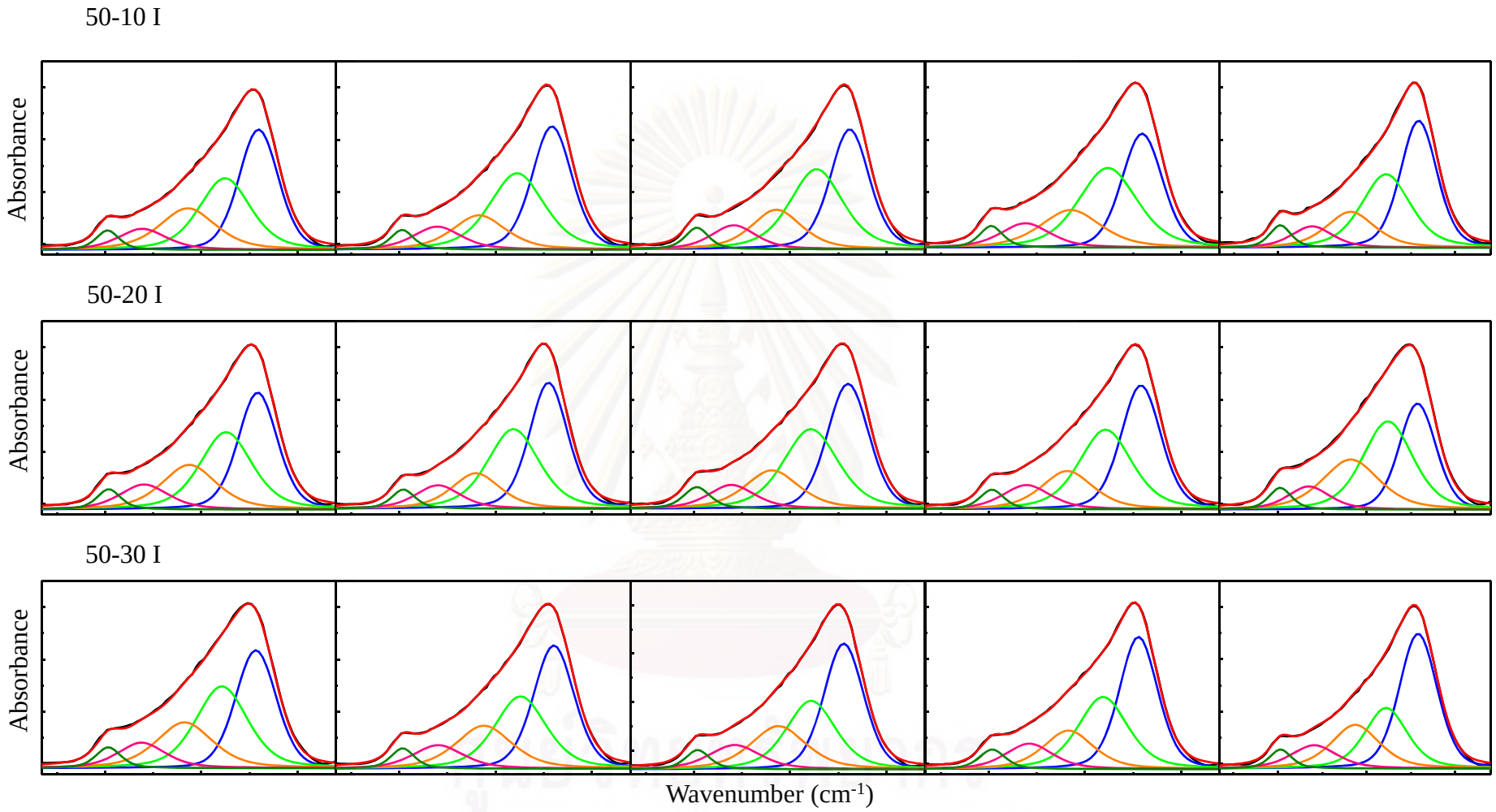
Figure A26. Curve fitting of treated silk fiber at 100°C by varying time (10, 20 and 30 min) and soaking into the water.



Note : 100-40 w, 100-50 w and 100-60 w (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

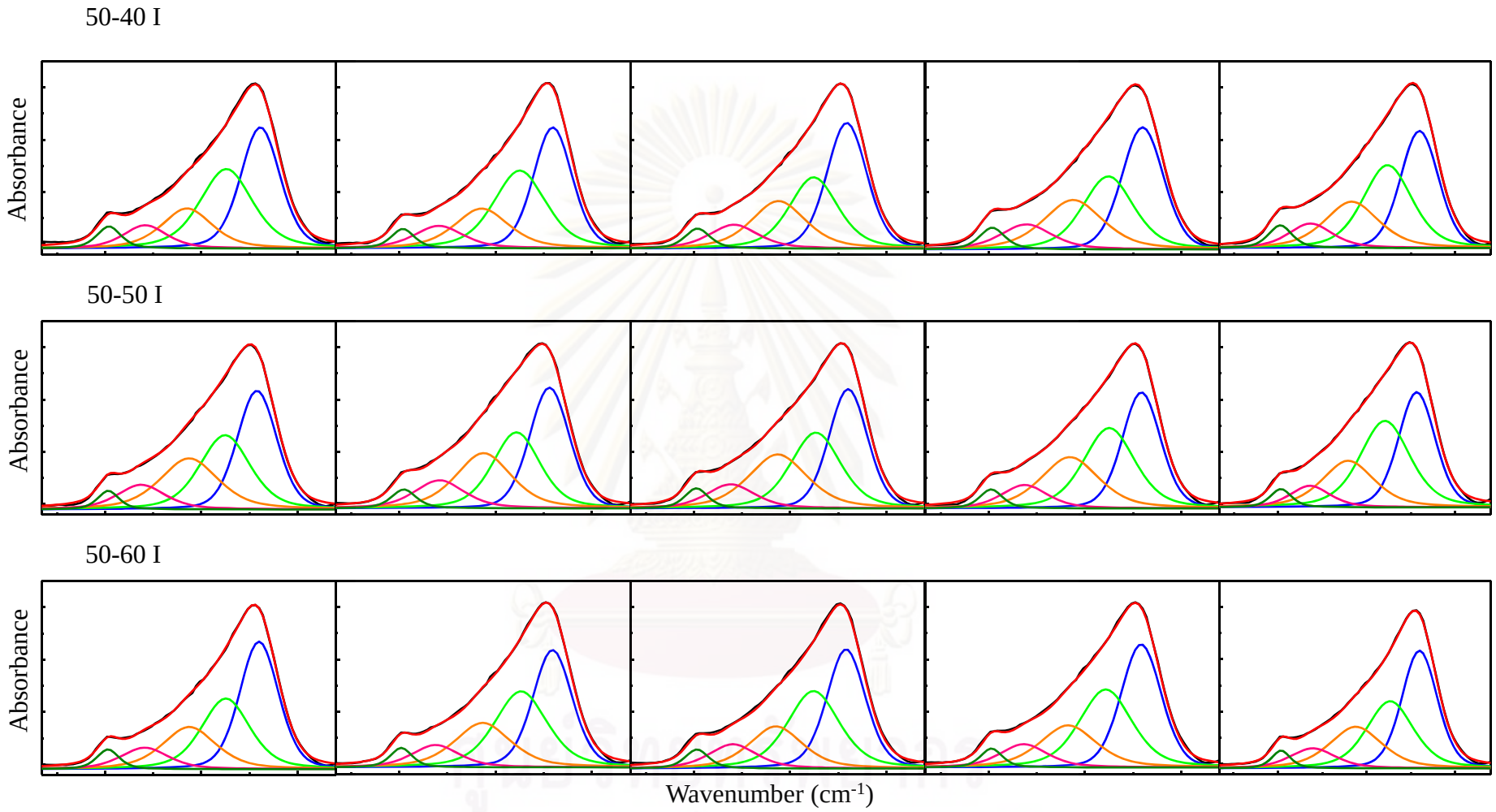
Figure A27. Curve fitting of treated silk fiber at 100°C by varying time (40, 50 and 60 min) and soaking into the water.



Note : 50-10 I, 50-20 I and 50-30 I (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

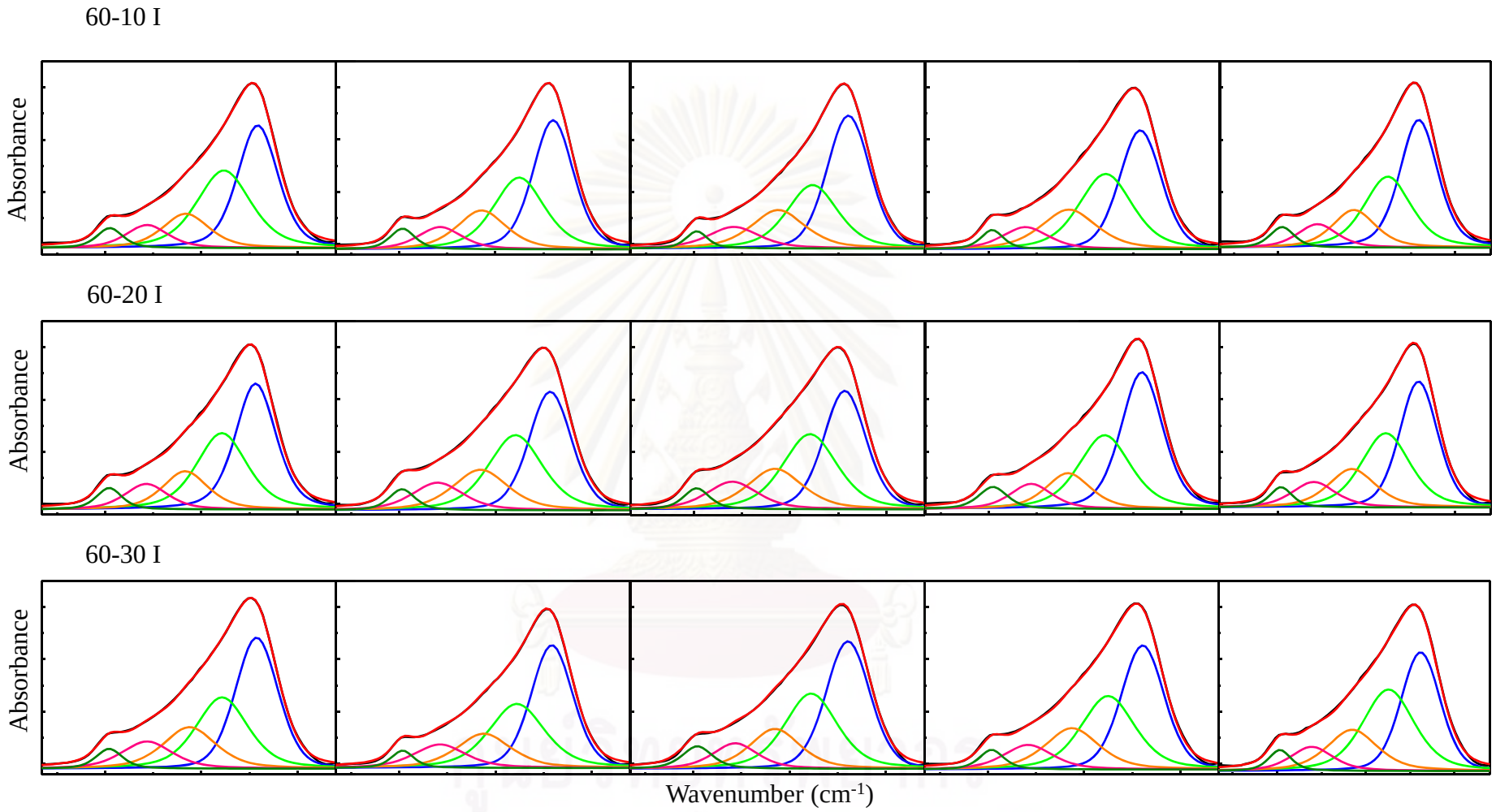
Figure A28. Curve fitting of treated silk fiber at 50°C by varying time (10, 20 and 30 min) and dipping into the ice cold-water.



Note : 50-40 I, 50-50 I and 50-60 I (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

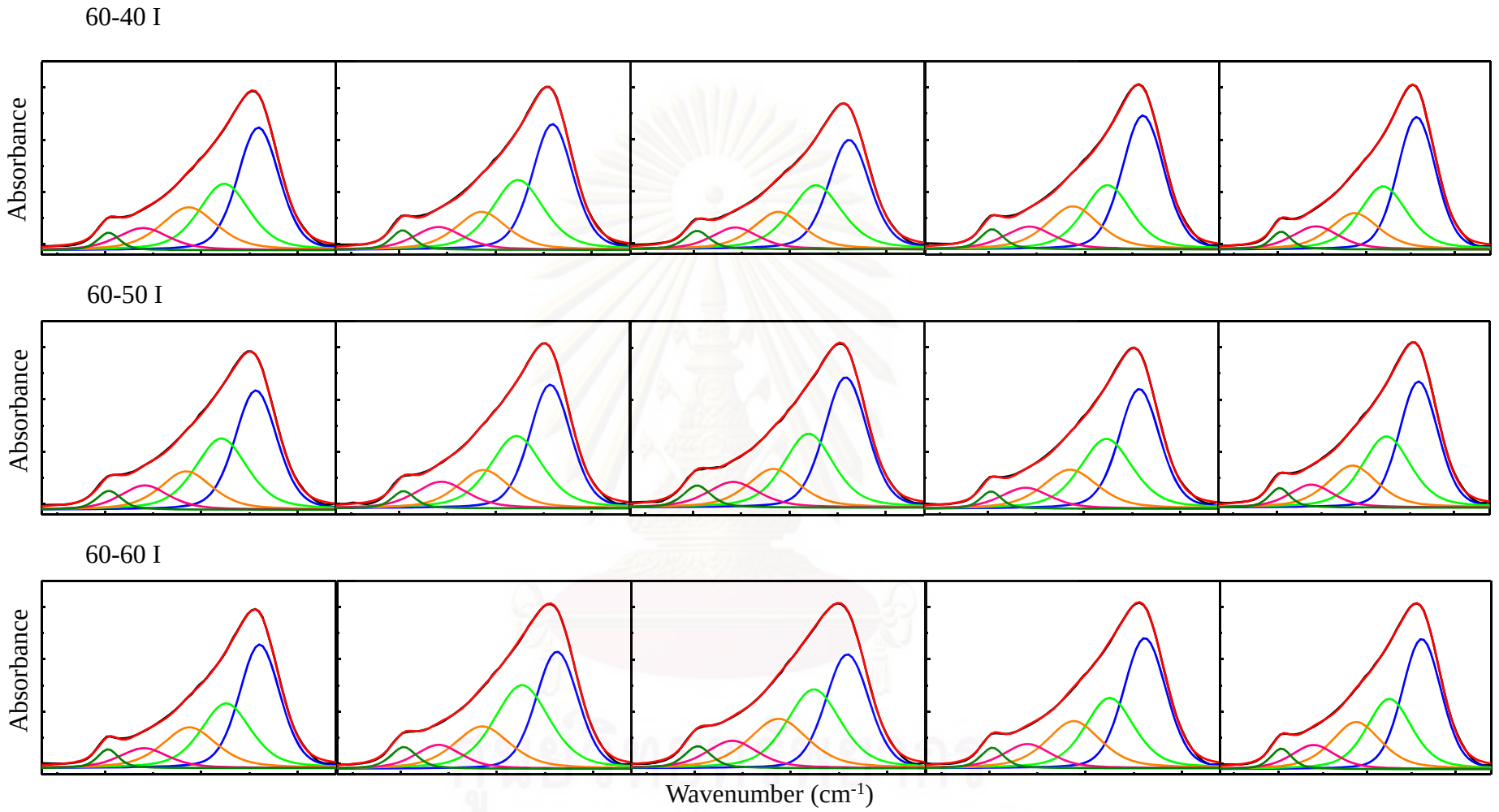
Figure A29. Curve fitting of treated silk fiber at 50°C by varying time (40, 50 and 60 min) and dipping into the ice cold-water.



Note : 60-10 I, 60-20 I and 60-30 I (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

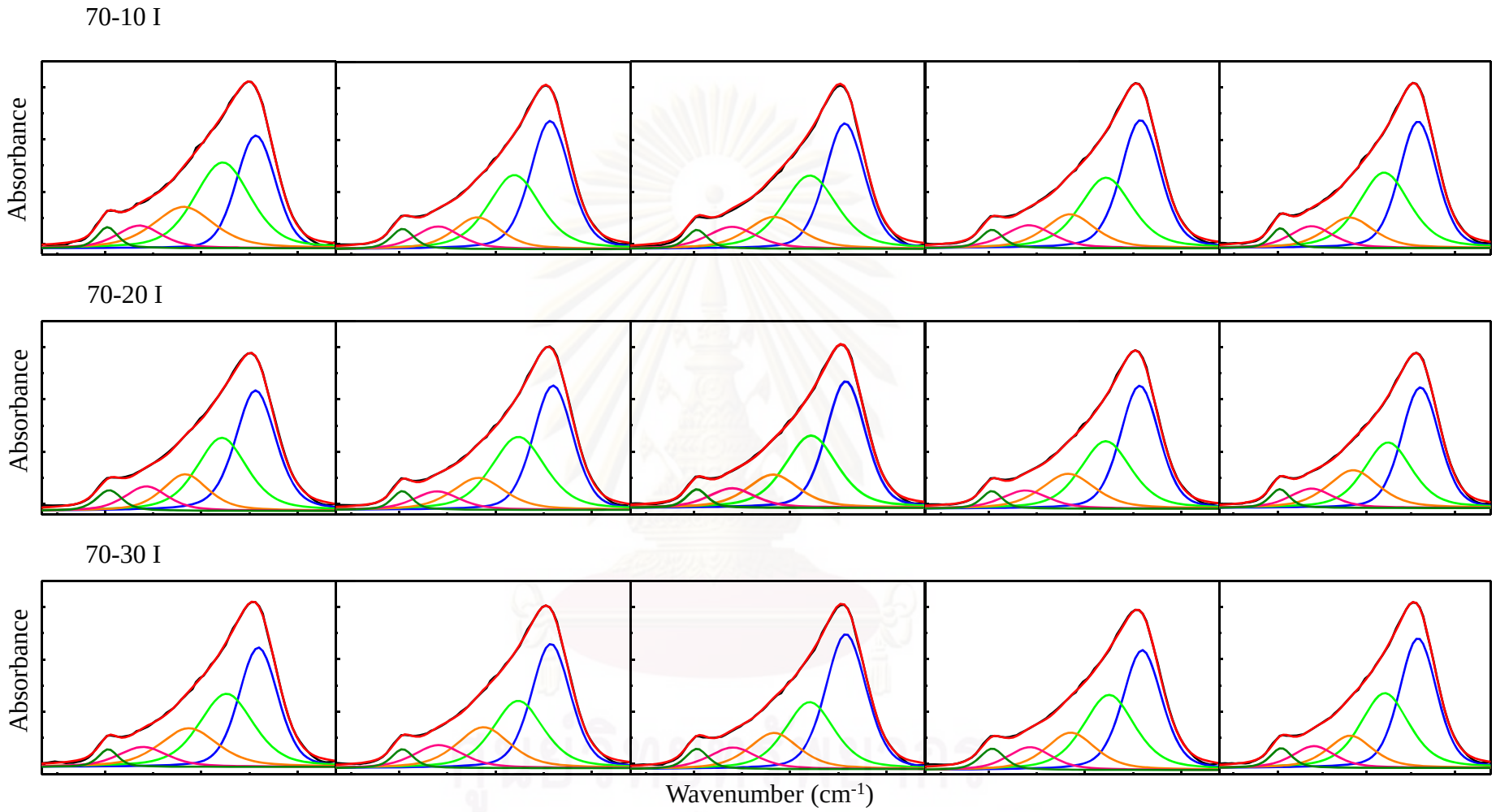
Figure A30. Curve fitting of treated silk fiber at 60°C by varying time (10, 20 and 30 min) and dipping into the ice cold-water.



Note : 60-40 I, 60-50 I and 60-60 I (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

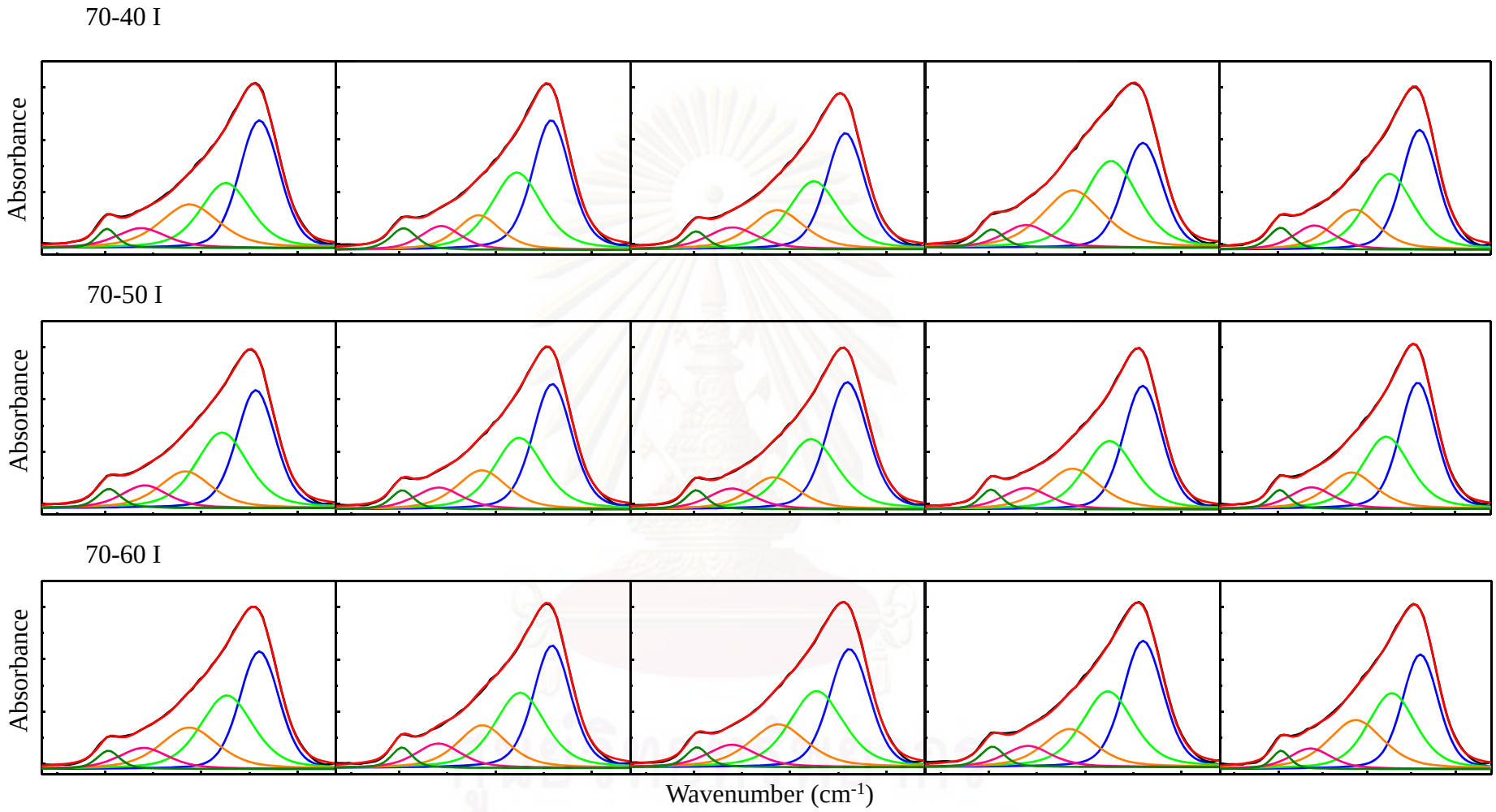
Figure A31. Curve fitting of treated silk fiber at 60°C by varying time (40, 50 and 60 min) and dipping into the ice cold-water.



Note : 70-10 I, 70-20 I and 70-30 I (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

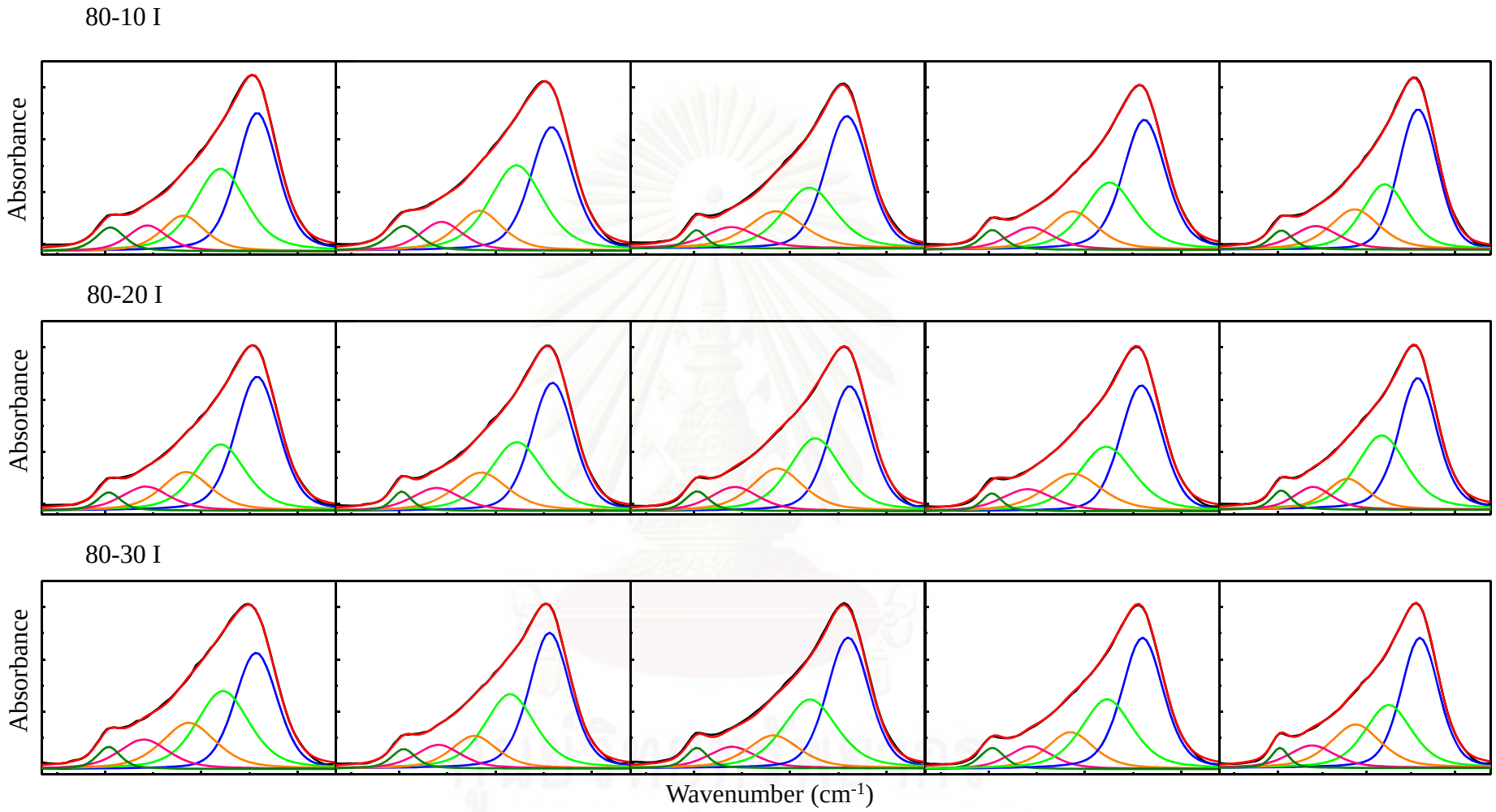
Figure A32. Curve fitting of treated silk fiber at 70°C by varying time (10, 20 and 30 min) and dipping into the ice cold-water.



Note : 70-40 I, 70-50 I and 70-60 I (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

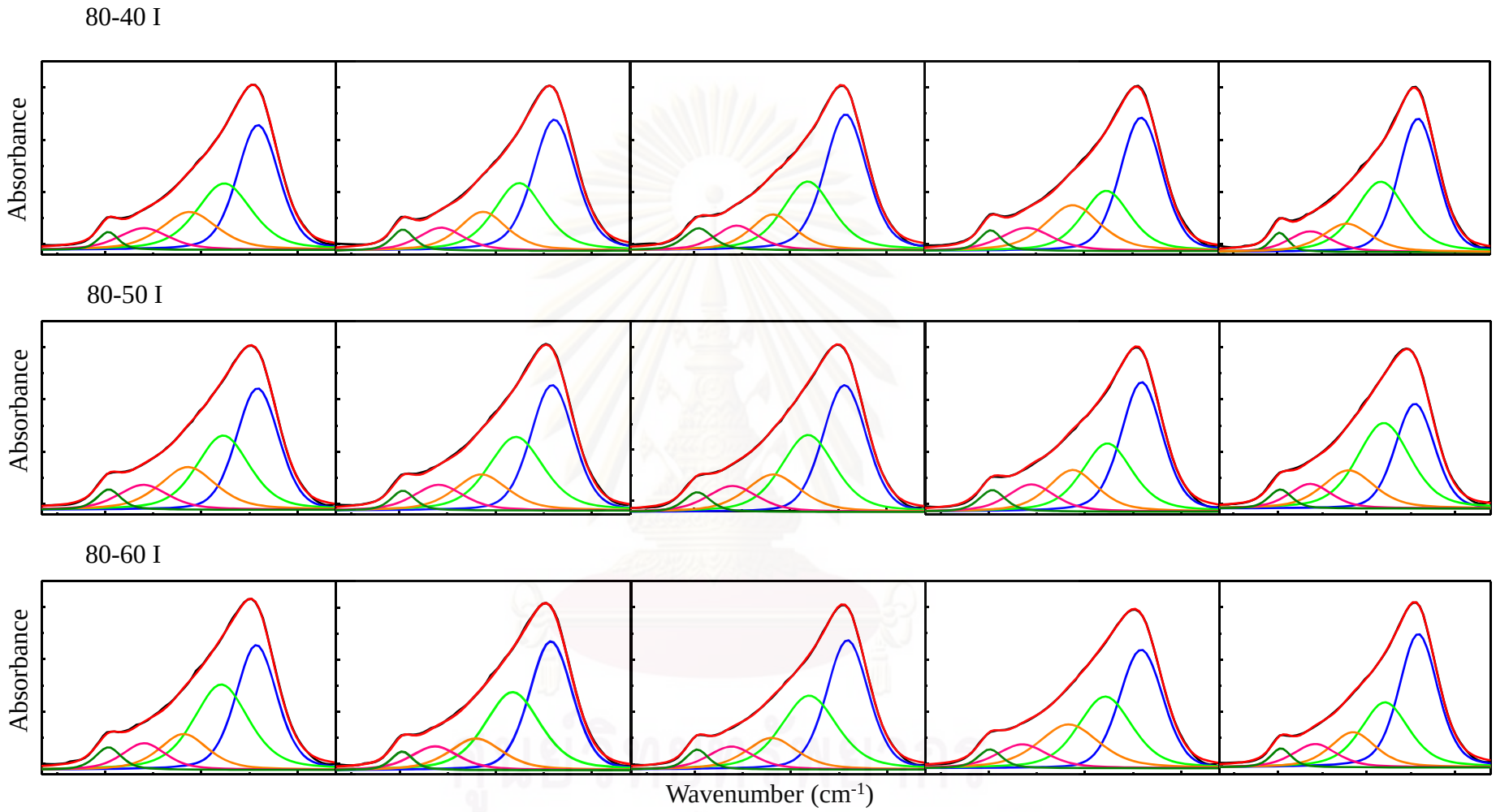
Figure A33. Curve fitting of treated silk fiber at 70°C by varying time (40, 50 and 60 min) and dipping into the ice cold-water.



Note : 80-10 I, 80-20 I and 80-30 I (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

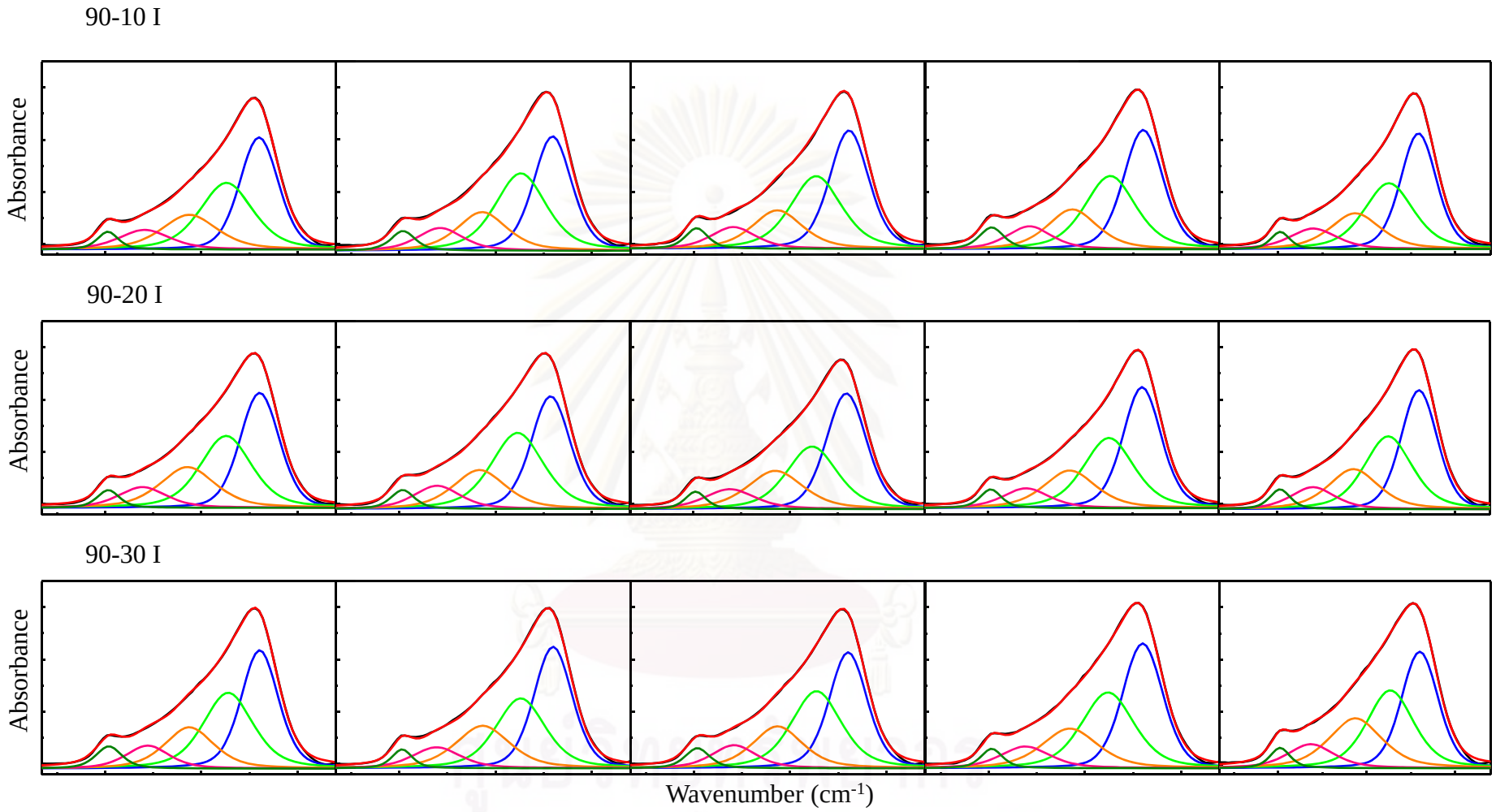
Figure A34. Curve fitting of treated silk fiber at 80°C by varying time (10, 20 and 30 min) and dipping into the ice cold-water.



Note : 80-40 I, 80-50 I and 80-60 I (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

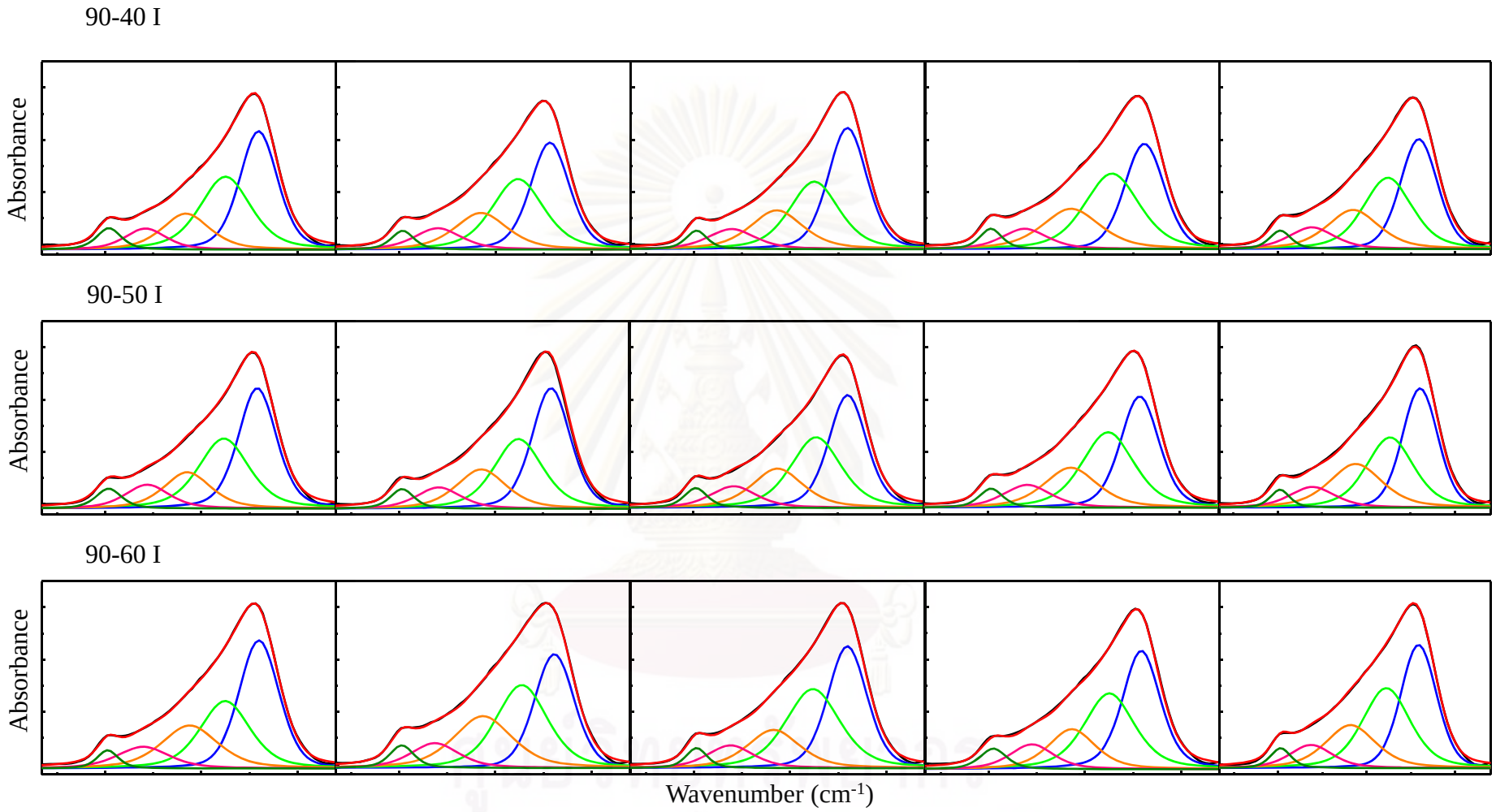
Figure A35. Curve fitting of treated silk fiber at 80°C by varying time (40, 50 and 60 min) and dipping into the ice cold-water.



Note : 90-10 I, 90-20 I and 90-30 I (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

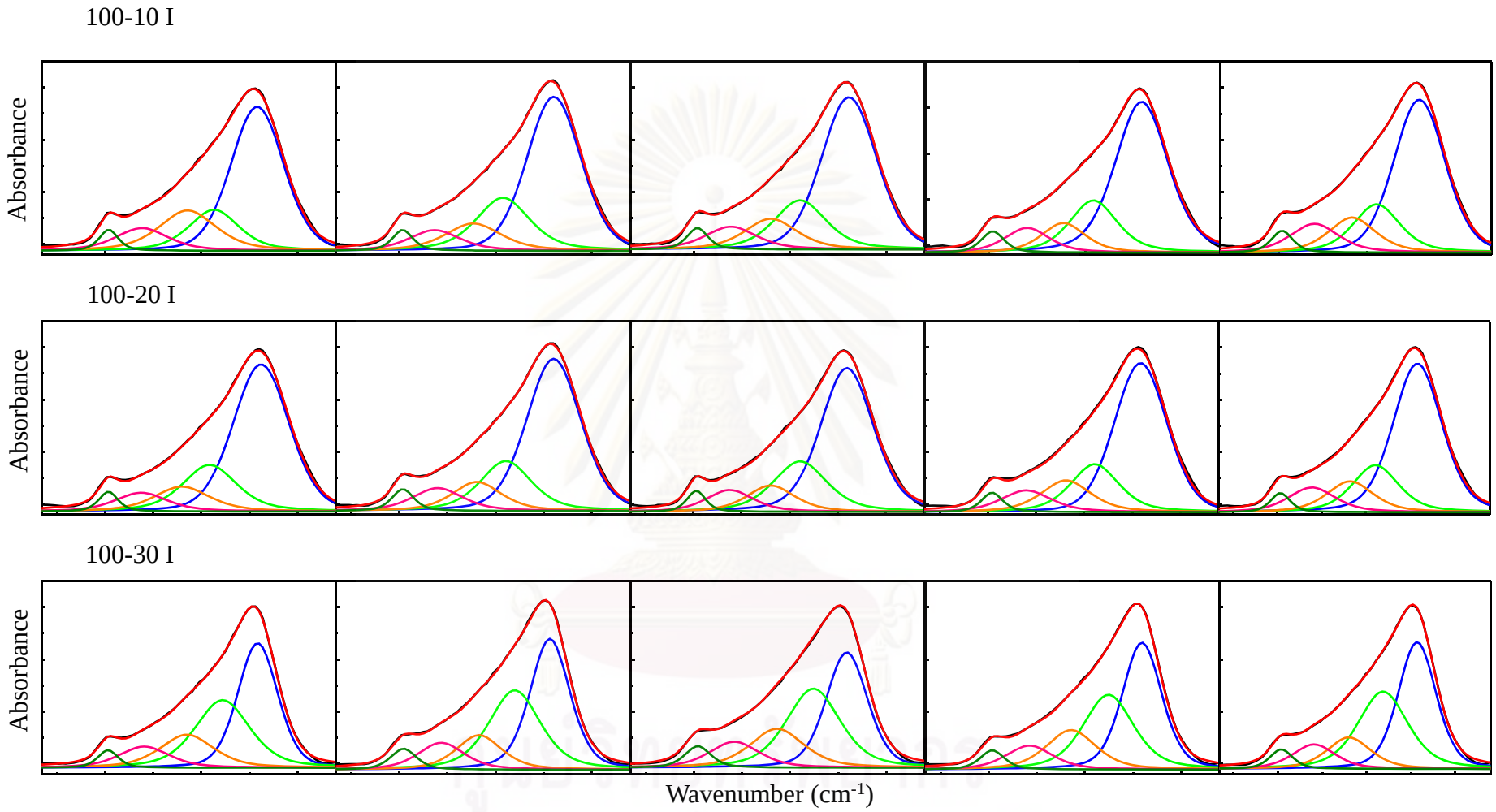
Figure A36. Curve fitting of treated silk fiber at 90°C by varying time (10, 20 and 30 min) and dipping into the ice cold-water.



Note : 90-40 I, 90-50 I and 90-60 I (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

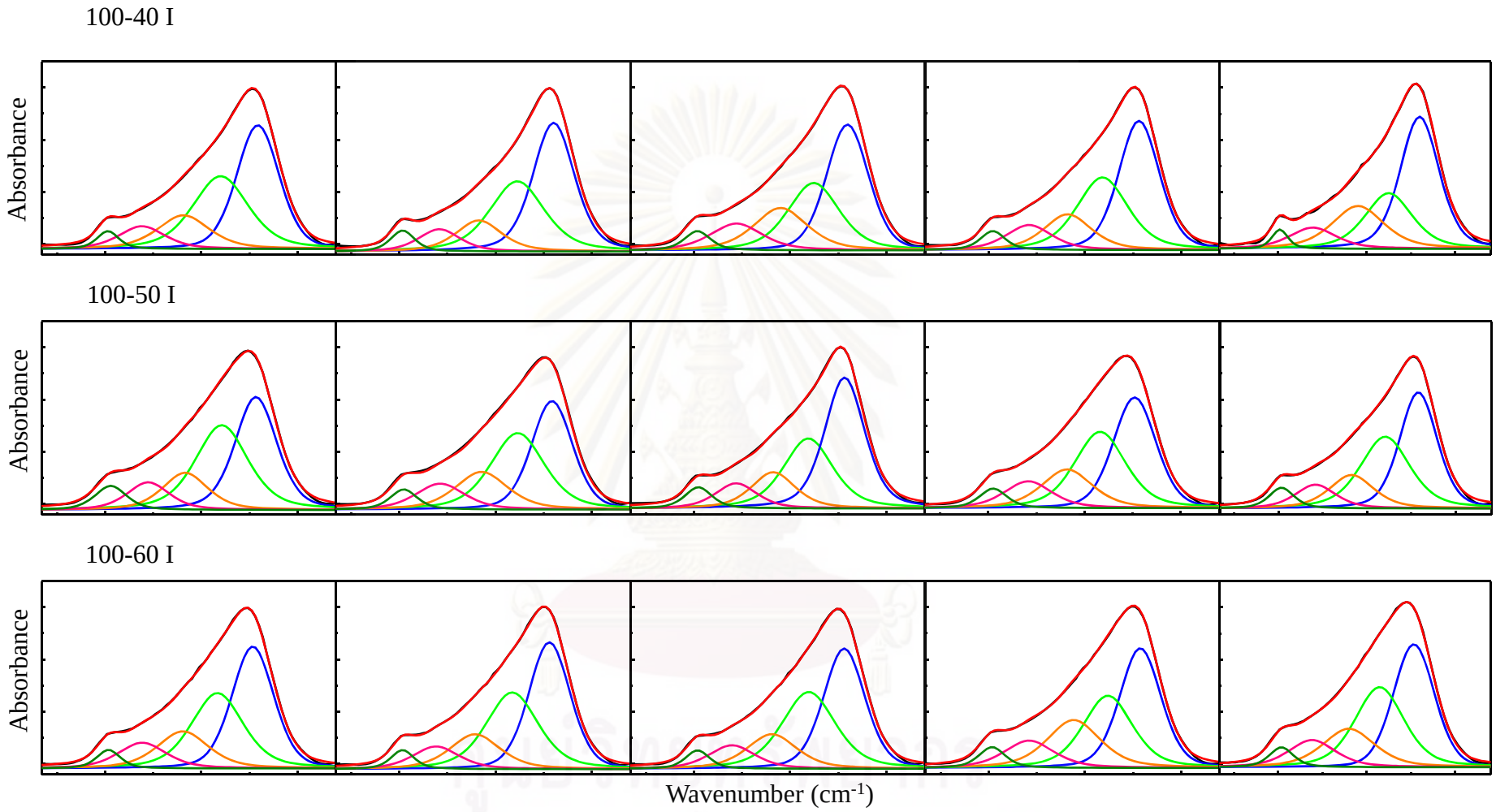
Figure A37. Curve fitting of treated silk fiber at 90°C by varying time (40, 50 and 60 min) and dipping into the ice cold-water.



Note : 100-10 I, 100-20 I and 100-30 I (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

Figure A38. Curve fitting of treated silk fiber at 100°C by varying time (10, 20 and 30 min) and dipping into the ice cold-water.



Note : 100-40 I, 100-50 I and 100-60 I (temperature-time quenching method) is the label of the condition that used to treat silk fiber.

(Collected spectrum 5 times per specimen)

Figure A39. Curve fitting of treated silk fiber at 100°C by varying time (40, 50 and 60 min) and dipping into the ice cold-water.

temp.-time method*	β-sheet	random coil	α-helix	turn	β-sheet(w)	SUM	%β-sheet	%random coil	%alpha	%turn	%β-sheet(w)
50-10 A	7.773	6.1122	2.5887	2.328	0.7661	19.568	39.72302	31.23569092	13.22925	11.89697	3.915065413
50-10 A	7.2212	4.723	2.5846	1.4239	0.5161	16.4688	43.84776	28.67847081	15.69392	8.646046	3.133804527
50-10 A	7.103	4.2836	2.3996	1.6417	0.5839	16.0118	44.36103	26.75276983	14.98645	10.25306	3.646685569
50-10 A	8.3677	5.4993	2.5174	1.9479	0.7549	19.0872	43.83933	28.8114548	13.18894	10.20527	3.955006497
50-10 A	7.3698	6.3494	3.0764	1.7306	0.6462	19.1724	38.43963	33.11739793	16.04598	9.026517	3.370470051
50-20 A	6.8484	3.5243	2.7876	1.6032	0.4964	15.2599	44.87841	23.095171	18.26749	10.50597	3.252970203
50-20 A	6.5107	4.684	2.6539	1.4599	0.5243	15.8328	41.1216	29.58415441	16.76204	9.220732	3.311479966
50-20 A	7.5476	5.3127	2.2795	1.5569	0.6843	17.381	43.42443	30.56613544	13.1149	8.957482	3.937057707
50-20 A	6.7554	4.8925	1.98	1.4127	0.7578	15.7984	42.76003	30.96832591	12.53291	8.942045	4.796688272
50-20 A	7.3458	6.0926	2.1361	1.4279	0.5955	17.5979	41.74248	34.62117639	12.13838	8.114036	3.383926491
50-30 A	7.3796	5.7131	2.8366	1.6554	0.6562	18.2409	40.45634	31.32027477	15.55077	9.07521	3.597410216
50-30 A	7.7067	6.1181	2.8179	1.6739	0.7492	19.0658	40.42159	32.08939567	14.77987	8.779595	3.929549245
50-30 A	6.8812	5.1559	2.72	1.4884	0.6003	16.8458	40.84816	30.60644196	16.14646	8.835437	3.563499507
50-30 A	6.8014	5.4223	2.0862	1.4825	0.5719	16.3643	41.56243	33.13493397	12.74848	9.059355	3.494802711
50-30 A	7.988	6.249	2.4256	1.5676	1.0217	19.2519	41.49201	32.4591339	12.59928	8.142573	5.307008659
50-40 A	6.9093	5.1263	2.3036	1.4419	0.5969	16.378	42.18647	31.29991452	14.06521	8.803883	3.644523141
50-40 A	7.1825	4.8051	2.654	1.574	0.6237	16.8393	42.6532	28.53503412	15.76075	9.347182	3.70383567
50-40 A	7.5922	4.9516	3.3186	1.8356	0.7505	18.4485	41.15348	26.8401225	17.98845	9.94986	4.068081416
50-40 A	7.3589	5.3227	2.7657	1.7526	0.7008	17.9007	41.10957	29.73459138	15.45023	9.790679	3.914930701
50-40 A	7.0277	5.176	2.7328	1.7719	0.7838	17.4922	40.17619	29.59033169	15.62296	10.12966	4.480854324
50-50 A	6.8069	4.4097	2.6372	1.3847	0.6088	15.8473	42.95306	27.82619121	16.64132	8.737766	3.84166388
50-50 A	7.6783	4.5777	2.5805	1.6038	0.6689	17.1092	44.87819	26.75578052	15.08253	9.373904	3.9095925
50-50 A	7.7024	4.4225	3.135	1.9456	0.5883	17.7938	43.28699	24.85416269	17.6185	10.93415	3.30620778
50-50 A	7.3423	4.8639	2.3504	1.5099	0.7897	16.8562	43.55845	28.85525801	13.94383	8.957535	4.684923055
50-50 A	7.0994	4.9946	2.6433	1.5892	0.8086	17.1351	41.43191	29.1483563	15.42623	9.27453	4.718968667
50-60 A	7.2753	5.1704	3.833	1.7548	0.6805	18.714	38.87624	27.62851341	20.48199	9.376937	3.636315058
50-60 A	8.1691	5.1955	2.8275	1.8609	0.7365	18.7895	43.47694	27.65108172	15.0483	9.903936	3.919742409
50-60 A	6.432	4.0257	2.4385	1.2177	0.4471	14.561	44.17279	27.64713962	16.74679	8.36275	3.07053087
50-60 A	7.134	4.6417	2.643	1.8077	0.6324	16.8588	42.31618	27.53280186	15.67727	10.72259	3.751156666
50-60 A	7.8375	5.1882	2.5917	1.6835	0.7138	18.0147	43.50614	28.79981349	14.38658	9.345146	3.962319661

* temp.-time method is varied temperature (°C) - varied time (min) exposed silk fiber into the air (quenching method).

Table A1. The calculated percentage of individual secondary structure from peak area.

temp.-time method*	β-sheet	random coil	α-helix	turn	β-sheet(w)	SUM	%β-sheet	%random coil	%alpha	%turn	%β-sheet(w)
60-10 A	6.7158	4.4163	2.3188	1.2975	0.5715	15.3199	43.8371	28.82721167	15.13587	8.469376	3.730442105
60-10 A	7.5085	5.3063	2.8985	1.9283	0.5987	18.2403	41.16434	29.09107855	15.89064	10.57165	3.282292506
60-10 A	8.1284	6.5029	2.5924	1.6842	1.4017	20.3096	40.02245	32.01884823	12.76441	8.29263	6.901662268
60-10 A	7.1668	6.1988	2.5978	1.6587	0.7009	18.323	39.11368	33.83070458	14.17781	9.052557	3.825246957
60-10 A	7.5583	4.8987	2.6012	1.6631	0.5867	17.308	43.6694	28.30309683	15.02889	9.608851	3.38976196
60-20 A	8.8225	5.4639	2.8516	2.1722	0.9273	20.2375	43.59481	26.9988882	14.09067	10.73354	4.582087708
60-20 A	9.521	5.2494	2.6377	2.0646	0.8685	20.3412	46.80648	25.80673707	12.96728	10.14984	4.269659607
60-20 A	9.1066	6.12	2.4894	2.183	1.0488	20.9478	43.47282	29.21547848	11.88383	10.42114	5.006731017
60-20 A	8.8514	5.8047	2.9604	2.012	1.4593	21.0878	41.97403	27.52634225	14.03845	9.541062	6.920114948
60-20 A	8.2812	5.5105	3.6555	2.6562	1.1409	21.2443	38.98081	25.93872239	17.20697	12.50312	5.370381702
60-30 A	7.6546	4.3879	2.7546	1.5251	0.7433	17.0655	44.85424	25.71210923	16.14134	8.936744	4.355571182
60-30 A	8.5119	5.4707	2.1617	1.5881	0.7055	18.4379	46.16524	29.67094951	11.72422	8.613237	3.826357665
60-30 A	8.0048	6.103	1.8878	1.3288	0.8099	18.1343	44.14176	33.65445592	10.41011	7.327551	4.46612221
60-30 A	7.3117	4.5154	2.6227	1.612	0.6007	16.6625	43.88117	27.09917479	15.74014	9.674419	3.605101275
60-30 A	7.6393	6.4463	3.0167	1.9571	0.7209	19.7803	38.62075	32.58949561	15.25103	9.894188	3.644535219
60-40 A	8.774	4.7566	3.1968	2.1027	0.7351	19.5652	44.84493	24.31153272	16.33921	10.74714	3.757181117
60-40 A	7.9824	5.1645	2.5107	1.5189	0.6199	17.7964	44.85402	29.01991414	14.10791	8.534872	3.483288755
60-40 A	7.6871	7.0323	2.4942	1.7851	1.2266	20.2253	38.00735	34.769818	12.33208	8.826074	6.064681364
60-40 A	6.2641	4.2223	2.2191	1.3663	0.6602	14.732	42.52036	28.66073853	15.06313	9.274369	4.481401032
60-40 A	7.9509	5.9448	2.946	2.0027	0.8044	19.6488	40.46507	30.25528277	14.99328	10.19248	4.093888685
60-50 A	7.6	4.468	2.9127	1.8165	0.7349	17.5321	43.34906	25.48468238	16.61353	10.36099	4.191739723
60-50 A	8.1679	5.2479	2.6072	1.6897	0.7635	18.4762	44.20768	28.40356783	14.11113	9.145279	4.132343231
60-50 A	8.5134	5.252	2.4292	1.7063	0.7277	18.6286	45.7007	28.19320829	13.04016	9.159572	3.906359039
60-50 A	7.38	4.9933	2.4035	1.5241	0.919	17.2199	42.85739	28.99726479	13.95769	8.850806	5.336848646
60-50 A	6.8363	6.2916	3.1199	2.0052	0.8508	19.1038	35.78503	32.93376187	16.33131	10.49634	4.453564212
60-60 A	8.347	5.6062	2.5946	1.5965	0.8784	19.0227	43.87915	29.47110557	13.63949	8.392605	4.617641029
60-60 A	8.2933	5.3155	2.4879	1.4904	0.8175	18.4046	45.06102	28.88136661	13.51782	8.097976	4.441824327
60-60 A	7.5273	5.0086	2.228	1.2969	0.7974	16.8582	44.65067	29.71017072	13.21612	7.692992	4.730042353
60-60 A	7.2517	4.7519	2.5223	1.4649	0.6447	16.6355	43.59172	28.56481621	15.16215	8.805867	3.875447086
60-60 A	7.413	5.799	2.9582	1.8095	0.8118	18.7915	39.44869	30.8596972	15.74222	9.629354	4.320038315

* temp.-time method is varied temperature (°C) - varied time (min) exposed silk fiber into the air (quenching method).

Table A2. The calculated percentage of individual secondary structure from peak area.

temp.-time method*	β-sheet	random coil	α-helix	turn	β-sheet(w)	SUM	%β-sheet	%random coil	%alpha	%turn	%β-sheet(w)
70-10 A	6.659	4.3133	2.8193	1.6522	0.5199	15.9637	41.71339	27.01942532	17.66069	10.34973	3.256763783
70-10 A	4.9768	6.7271	3.8173	1.7241	0.8662	18.1115	27.47867	37.14269939	21.07666	9.519366	4.782596693
70-10 A	6.4323	5.1374	2.7623	1.4401	0.6296	16.4017	39.21728	31.32236293	16.84155	8.780187	3.838626484
70-10 A	7.0302	5.5314	2.729	1.6828	0.7268	17.7002	39.7182	31.25049434	15.4179	9.507237	4.106168292
70-10 A	6.6928	4.7185	4.0568	1.9062	0.6993	18.0736	37.03081	26.1071397	22.446	10.54688	3.869179356
70-20 A	5.9415	5.4549	4.8684	1.4499	0.6598	18.3745	32.33557	29.68733843	26.49541	7.890827	3.590846009
70-20 A	6.3799	5.7215	3.8948	1.7992	0.6707	18.4661	34.54926	30.98380275	21.09162	9.743259	3.632060912
70-20 A	6.5368	4.175	3.7094	1.7513	0.6147	16.7872	38.93919	24.87013915	22.0966	10.43235	3.661718452
70-20 A	6.9149	6.3089	2.4396	1.7197	0.6752	18.0583	38.29209	34.93628968	13.50958	9.523045	3.739000903
70-20 A	6.296	5.1481	3.1927	1.5709	0.5965	16.8042	37.46682	30.63579343	18.99942	9.348258	3.549707811
70-30 A	6.5505	5.6074	2.9179	1.5936	0.5494	17.2188	38.04272	32.56556787	16.94601	9.255	3.190698539
70-30 A	6.2274	6.8053	2.8922	1.6097	0.6817	18.2163	34.18587	37.35829998	15.87699	8.836591	3.742252818
70-30 A	6.9189	6.025	2.4485	1.679	0.7442	17.8156	38.83619	33.81867577	13.74357	9.424325	4.177237926
70-30 A	7.1164	5.7534	2.8325	1.8159	0.7247	18.2429	39.00915	31.53774893	15.52659	9.95401	3.972504372
70-30 A	6.6925	5.3239	3.1215	1.652	0.6498	17.4397	38.37509	30.52747467	17.89882	9.47264	3.725981525
70-40 A	7.0275	5.6507	2.3159	1.4062	0.6068	17.0071	41.32098	33.22553522	13.61725	8.268311	3.567921633
70-40 A	6.4164	5.696	3.4762	1.9089	0.7596	18.2571	35.14468	31.19882128	19.04026	10.45566	4.160573147
70-40 A	7.0673	6.189	2.4554	1.8771	1.061	18.6498	37.89478	33.18534247	13.16582	10.06499	5.689069052
70-40 A	7.1239	5.2382	2.5205	1.7432	0.6619	17.2877	41.20791	30.3001556	14.57973	10.08347	3.828733724
70-40 A	7.2009	6.0089	2.7147	1.7805	0.6767	18.3817	39.17429	32.68957713	14.76849	9.686264	3.681378763
70-50 A	6.652	6.0569	3.2118	2.0434	0.9312	18.8953	35.20452	32.05506131	16.99788	10.81433	4.928209661
70-50 A	6.6644	6.88	3.1434	1.6014	0.6836	18.9728	35.12608	36.26243886	16.56793	8.440504	3.603052791
70-50 A	6.771	4.85	2.1029	1.6101	0.5828	15.9168	42.53996	30.47094893	13.21183	10.11573	3.661540008
70-50 A	6.7685	5.2955	3.6119	1.858	0.7443	18.2782	37.03045	28.97167117	19.7607	10.16511	4.072063989
70-50 A	6.8363	5.813	3.6282	1.9694	0.7863	19.0332	35.91776	30.54136982	19.06248	10.34718	4.13120232
70-60 A	6.6686	6.3975	2.4035	1.5147	0.6834	17.6677	37.74458	36.2101462	13.60392	8.573272	3.868075641
70-60 A	6.3697	4.8885	4.6103	1.7104	0.7081	18.287	34.83185	26.73210477	25.21081	9.353092	3.872149614
70-60 A	7.0177	5.9374	2.6277	1.7681	0.7153	18.0662	38.84436	32.86468654	14.54484	9.786784	3.959327363
70-60 A	6.914	6.1333	2.7566	1.6853	0.6831	18.1723	38.04692	33.75081855	15.16924	9.274005	3.759017846
70-60 A	6.5917	6.1948	3.5254	1.7232	0.9076	18.9427	34.7981	32.70283539	18.61086	9.096908	4.791291632

* temp.-time method is varied temperature (°C) - varied time (min) exposed silk fiber into the air (quenching method).

Table A3. The calculated percentage of individual secondary structure from peak area.

temp.-time method*	β-sheet	random coil	α-helix	turn	β-sheet(w)	SUM	%β-sheet	%random coil	%alpha	%turn	%β-sheet(w)
80-10 A	6.8488	5.3241	2.6304	1.6882	0.6084	17.0999	40.0517	31.1352698	15.38255	9.872572	3.557915543
80-10 A	7.8641	5.331	2.5472	1.8477	0.8511	18.4411	42.64442	28.90825385	13.81263	10.01947	4.615234449
80-10 A	7.4215	6.1334	2.7305	1.9332	1.0774	19.296	38.46134	31.78586235	14.1506	10.01866	5.58354063
80-10 A	8.1585	5.1667	2.9993	1.8275	0.7758	18.9278	43.10327	27.29688606	15.846	9.65511	4.09873308
80-10 A	7.1499	5.7182	2.9675	1.7257	0.8283	18.3896	38.88013	31.09474921	16.13684	9.384108	4.504176274
80-20 A	7.6314	5.0283	2.1858	1.474	0.9436	17.2631	44.20643	29.12744524	12.66169	8.538443	5.465993941
80-20 A	7.2864	5.7519	2.2403	1.235	0.9737	17.4873	41.66681	32.8918701	12.81101	7.062268	5.568040807
80-20 A	6.5589	3.6747	2.0121	1.3453	0.4721	14.0631	46.63908	26.13008512	14.30766	9.56617	3.357012323
80-20 A	5.222	2.6611	2.1848	1.3082	0.45	11.8261	44.15657	22.50192371	18.47439	11.06197	3.805142862
80-20 A	7.1757	4.0794	2.6348	1.5725	0.6847	16.1471	44.43956	25.26397929	16.31748	9.738591	4.240389915
80-30 A	7.1413	5.6534	2.7616	1.6934	0.8931	18.1428	39.36162	31.16057058	15.22147	9.33373	4.922613929
80-30 A	7.1316	5.8371	1.9436	1.3057	0.9278	17.1458	41.59386	34.0439058	11.33572	7.615276	5.411237738
80-30 A	6.8657	5.3141	2.5748	1.6927	0.6183	17.0656	40.23123	31.13925089	15.08766	9.918784	3.623078005
80-30 A	6.4503	4.9427	2.4073	1.5934	0.5363	15.93	40.49153	31.02762084	15.11174	10.00251	3.366603892
80-30 A	6.3085	4.6935	3.0949	1.5837	0.5367	16.2173	38.89982	28.94131576	19.08394	9.765497	3.30942882
80-40 A	6.3164	5.0135	2.2735	1.5073	0.5934	15.7041	40.22134	31.92478397	14.47711	9.59813	3.778631058
80-40 A	5.736	4.5646	3.3313	1.3791	0.5303	15.5413	36.90811	29.37077336	21.43514	8.873775	3.412198465
80-40 A	7.6574	5.786	2.8903	1.678	0.6733	18.685	40.98154	30.96601552	15.46856	8.980466	3.603425207
80-40 A	7.0426	5.0658	2.6542	1.6528	0.9871	17.4025	40.4689	29.10961069	15.25183	9.497486	5.672173538
80-40 A	7.7662	5.9029	2.7647	1.9606	0.7381	19.1325	40.59166	30.85273749	14.45028	10.24748	3.857833529
80-50 A	7.4396	6.847	1.9344	1.4293	0.6901	18.3404	40.564	37.3328826	10.54721	7.793178	3.762731456
80-50 A	8.3256	6.1056	2.3183	1.7264	0.9956	19.4715	42.75788	31.3565981	11.90612	8.866292	5.113114038
80-50 A	8.1141	5.4649	2.363	1.5166	0.9226	18.3812	44.14347	29.73092072	12.85553	8.250821	5.019258808
80-50 A	7.5129	6.5843	3.1389	2.0118	1.1696	20.4175	36.79638	32.2483164	15.37358	9.853312	5.728419248
80-50 A	7.0914	5.5917	2.8957	1.7503	1.0666	18.3957	38.54923	30.39677751	15.74118	9.514724	5.79809412
80-60 A	6.7294	4.7495	4.7778	2.2798	1.0568	19.5933	34.34541	24.24042913	24.38487	11.63561	5.393680493
80-60 A	8.2592	4.2363	3.1346	2.284	0.629	18.5431	44.54056	22.84569462	16.9044	12.31725	3.39209733
80-60 A	8.1338	5.7664	2.2219	1.46	0.8407	18.4228	44.15073	31.30034522	12.0606	7.924963	4.563367132
80-60 A	8.2006	6.1498	2.1985	1.7839	0.7108	19.0436	43.06224	32.29326388	11.54456	9.367452	3.732487555
80-60 A	8.1596	6.6637	2.1846	1.3367	1.1387	19.4833	41.87997	34.20211155	11.21268	6.860747	5.844492463

* temp.-time method is varied temperature (°C) - varied time (min) exposed silk fiber into the air (quenching method).

Table A4. The calculated percentage of individual secondary structure from peak area.

temp.-time method*	β-sheet	random coil	α-helix	turn	β-sheet(w)	SUM	%β-sheet	%random coil	%alpha	%turn	%β-sheet(w)
90-10 A	7.6613	5.3981	2.55	1.5536	0.5985	17.7615	43.13431	30.3921403	14.3569	8.747009	3.369647834
90-10 A	7.3629	5.3985	3.3255	1.9381	0.5991	18.6241	39.53426	28.9866356	17.8559	10.40641	3.216799738
90-10 A	7.9646	6.0867	2.6775	1.9816	0.9453	19.6557	40.52056	30.96658984	13.622	10.08155	4.809291961
90-10 A	6.9923	6.3013	2.7899	1.6535	0.6658	18.4028	37.99585	34.24098507	15.16019	8.985046	3.617927707
90-10 A	7.4417	6.4066	2.8299	1.6908	0.7419	19.1109	38.93956	33.52327729	14.80778	8.847307	3.882077767
90-20 A	5.6741	6.9399	3.8776	1.9089	0.8333	19.2338	29.50067	36.08179351	20.16034	9.924716	4.332477202
90-20 A	6.0224	6.8841	3.9196	1.7351	0.7964	19.3576	31.11129	35.56277638	20.24838	8.963405	4.114146382
90-20 A	5.7389	6.0107	3.1144	1.7282	0.7558	17.348	33.08105	34.64779802	17.9525	9.961955	4.356698178
90-20 A	6.8594	6.2909	3.0664	1.6002	0.8916	18.7085	36.66462	33.62589197	16.39041	8.553331	4.765748189
90-20 A	7.0205	6.0522	3.3806	1.8459	0.7358	19.035	36.88206	31.79511426	17.75992	9.6974	3.865510901
90-30 A	6.4723	5.7528	2.4477	1.478	0.6338	16.7846	38.56094	34.27427523	14.58301	8.805691	3.776080455
90-30 A	6.3012	6.1822	3.3428	1.9776	0.7134	18.5172	34.0289	33.3862571	18.05241	10.6798	3.852634308
90-30 A	5.8974	5.21	3.7339	2.0369	0.7822	17.6604	33.39335	29.50103055	21.14278	11.53371	4.429118253
90-30 A	6.7182	6.4949	2.7288	1.3867	0.6835	18.0121	37.29826	36.05853843	15.14982	7.698714	3.79467136
90-30 A	6.6286	5.377	2.6183	1.9932	0.8238	17.4409	38.00607	30.82983103	15.01241	11.42831	4.723380101
90-40 A	6.7311	6.0248	3.0537	1.6691	0.6632	18.1419	37.10251	33.20931104	16.83231	9.200249	3.655625927
90-40 A	6.224	5.6857	3.1092	1.9062	0.9753	17.9004	34.77017	31.76297736	17.36944	10.64892	5.448481598
90-40 A	6.7876	6.1202	2.8342	1.8691	0.7272	18.3383	37.01325	33.37386781	15.45509	10.19233	3.965471172
90-40 A	6.2655	5.9582	3.4926	1.6868	0.7631	18.1662	34.48988	32.79827372	19.22581	9.285376	4.200658366
90-40 A	4.1825	6.9329	4.4486	1.1122	0.599	17.2752	24.21101	40.13209688	25.75137	6.438131	3.467398351
90-50 A	7.3845	5.0706	2.8732	2.0082	0.6963	18.0328	40.95038	28.11876137	15.93319	11.13637	3.861297192
90-50 A	6.5168	6.1815	3.6841	1.7009	0.7619	18.8452	34.58069	32.80145607	19.54928	9.02564	4.042939316
90-50 A	6.9615	5.4948	3.455	1.8451	0.7587	18.5151	37.59904	29.67739845	18.66044	9.96538	4.097736442
90-50 A	6.6234	6.0124	3.2995	1.62	0.736	18.2913	36.21066	32.87027166	18.03863	8.85667	4.023770864
90-50 A	7.0875	6.0904	3.1883	1.8419	0.9659	19.174	36.96412	31.76384688	16.62825	9.606238	5.03755085
90-60 A	6.2025	5.541	3.5187	1.8753	0.6994	17.8369	34.77342	31.06481507	19.72708	10.5136	3.921084942
90-60 A	7.0508	5.0155	3.3246	1.9577	0.8091	18.1577	38.83091	27.62189044	18.30959	10.78165	4.455960832
90-60 A	6.6741	5.7432	2.3098	1.4531	0.6774	16.8576	39.59104	34.06890661	13.70183	8.619851	4.018365604
90-60 A	7.1442	5.2713	3.3457	1.7324	0.6448	18.1384	39.38716	29.06154898	18.4454	9.551008	3.554889075
90-60 A	6.6167	5.0891	2.4641	1.6285	0.7556	16.554	39.9704	30.74241875	14.88522	9.837502	4.564455721

* temp.-time method is varied temperature (°C) - varied time (min) exposed silk fiber into the air (quenching method).

Table A5. The calculated percentage of individual secondary structure from peak area.

temp.-time method*	β-sheet	random coil	α-helix	turn	β-sheet(w)	SUM	%β-sheet	%random coil	%alpha	%turn	%β-sheet(w)
100-10 A	7.4053	6.916	2.4831	1.7007	0.6201	19.1252	38.72012	36.16171334	12.98339	8.892456	3.242319035
100-10 A	7.4467	6.1025	2.7278	1.6443	0.7174	18.6387	39.95289	32.74101735	14.63514	8.821967	3.848980884
100-10 A	8.0065	6.8181	2.5544	1.7274	0.779	19.8854	40.26321	34.28696431	12.84561	8.686775	3.917446971
100-10 A	6.9668	5.844	3.9019	1.9629	0.9014	19.577	35.58666	29.85135618	19.93104	10.02656	4.604382694
100-10 A	6.9983	5.6159	2.6016	1.6539	0.6287	17.4984	39.99394	32.09379143	14.86765	9.451721	3.592899922
100-20 A	7.3069	5.0606	2.4634	1.6637	0.5511	17.0457	42.86653	29.68842582	14.45174	9.760233	3.233073444
100-20 A	7.4398	4.6822	3.749	1.7635	0.6301	18.2646	40.73344	25.63538211	20.52604	9.655289	3.449842865
100-20 A	8.2136	5.587	2.8193	2.0427	0.8806	19.5432	42.02792	28.58794875	14.42599	10.45223	4.505915101
100-20 A	7.2016	4.6135	2.7428	1.4165	0.5081	16.4825	43.6924	27.99029273	16.64068	8.593963	3.082663431
100-20 A	7.9942	5.1774	3.2433	2.0191	0.8157	19.2497	41.52896	26.89600357	16.84857	10.48899	4.237468636
100-30 A	7.3188	4.9915	3.3435	1.8578	0.985	18.4966	39.56835	26.98604068	18.0763	10.04401	5.325303029
100-30 A	7.5165	6.2013	2.2128	1.6573	0.7081	18.296	41.08275	33.89429383	12.09445	9.058264	3.870244862
100-30 A	8.1719	6.1926	2.4865	1.6446	0.7585	19.2541	42.44239	32.16250045	12.91413	8.541557	3.939420695
100-30 A	7.8363	5.9813	2.4037	1.608	0.6239	18.4532	42.46581	32.41334836	13.02593	8.713936	3.380985412
100-30 A	7.3222	5.4101	2.4082	1.6129	0.6369	17.3903	42.10508	31.1098716	13.84795	9.274711	3.662386503
100-40 A	7.462	5.2909	2.8318	1.7494	0.8182	18.1523	41.10774	29.14727059	15.60023	9.637346	4.507417793
100-40 A	6.9761	4.3466	2.7287	1.587	0.5437	16.1821	43.10998	26.86054344	16.86246	9.807133	3.359885305
100-40 A	7.1069	5.2755	2.7014	1.4149	0.6272	17.1259	41.49797	30.80422051	15.77377	8.261756	3.662289281
100-40 A	6.8352	5.3944	3.6144	1.8936	0.642	18.3796	37.18906	29.34993145	19.66528	10.30273	3.493003112
100-40 A	8.0745	5.8076	2.7101	1.5948	0.9388	19.1258	42.21784	30.36526577	14.16986	8.338475	4.908552845
100-50 A	7.6487	4.8742	2.5772	1.6362	0.5898	17.3261	44.14554	28.13212437	14.87467	9.443556	3.40411287
100-50 A	8.1467	6.0046	2.4355	1.8931	0.699	19.1789	42.47741	31.30836492	12.69885	9.870743	3.644630297
100-50 A	8.2374	6.1943	2.6443	1.849	0.6873	19.6123	42.00119	31.58375101	13.48287	9.427757	3.504433442
100-50 A	7.026	5.4884	2.5546	1.7178	0.5459	17.3327	40.5361	31.66500314	14.73862	9.910747	3.149538156
100-50 A	7.6935	5.2007	2.8252	1.8174	0.6146	18.1514	42.38516	28.65178444	15.56464	10.01245	3.385964719
100-60 A	8.1334	5.7841	2.5066	1.6408	0.8868	18.9517	42.91647	30.52021718	13.22625	8.657799	4.679263602
100-60 A	7.4149	5.4499	2.9419	1.6842	0.5408	18.0317	41.12147	30.22399441	16.31516	9.340218	2.999162586
100-60 A	6.9649	6.1755	3.3214	1.6287	0.5544	18.6449	37.35552	33.12165793	17.81399	8.735365	2.973467275
100-60 A	7.8311	5.6134	3.1677	1.8071	0.5945	19.0138	41.1864	29.52276767	16.66	9.50415	3.126676414
100-60 A	7.6055	3.4818	5.0124	2.1357	0.7192	18.9546	40.12482	18.36915577	26.44424	11.26745	3.794329609

* temp.-time method is varied temperature (°C) - varied time (min) exposed silk fiber into the air (quenching method).

Table A6. The calculated percentage of individual secondary structure from peak area.

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temp.-time method*	β-sheet	random coil	α-helix	turn	β-sheet(w)	SUM	%β-sheet	%random coil	%alpha	%turn	%β-sheet(w)
50-10 W	6.1058	5.6973	3.1941	1.7857	0.7894	17.5723	34.74673	32.42205061	18.1769	10.16202	4.492297536
50-10 W	7.0009	5.6728	2.8419	1.8644	0.7173	18.0973	38.68478	31.3461124	15.70345	10.30209	3.963574677
50-10 W	5.6067	7.1775	2.8871	1.8694	0.8523	18.393	30.48279	39.02299788	15.69673	10.16365	4.633828087
50-10 W	5.977	5.6673	4.0789	1.7425	0.7808	18.2465	32.75697	31.05965528	22.35442	9.549777	4.279176828
50-10 W	5.5148	6.1935	3.546	1.8155	0.8066	17.8764	30.84961	34.6462375	19.83621	10.15585	4.512094158
50-20 W	6.7938	5.9821	3.091	1.9718	0.9965	18.8352	36.0697	31.76021492	16.41076	10.4687	5.290626062
50-20 W	6.3086	5.4025	3.8712	1.8263	0.7797	18.1883	34.68493	29.70316082	21.28401	10.04107	4.286821748
50-20 W	5.4915	5.5177	3.9374	1.8393	0.6609	17.4468	31.47569	31.62585689	22.56804	10.54233	3.788087214
50-20 W	5.9278	5.5685	3.0778	1.8503	0.797	17.2214	34.42113	32.33476953	17.87195	10.74419	4.627962883
50-20 W	5.7039	5.6724	3.9135	1.7855	0.7384	17.8137	32.01974	31.84290743	21.96905	10.02318	4.145124258
50-30 W	5.3863	5.2548	3.6544	1.6319	0.6299	16.5573	32.53127	31.73705858	22.07123	9.856076	3.804364238
50-30 W	5.5784	5.3439	3.5464	1.7397	0.7352	16.9436	32.92335	31.53934229	20.93062	10.26759	4.33910149
50-30 W	5.9763	5.059	3.1287	1.7357	0.7037	16.6034	35.99443	30.46966284	18.84373	10.45388	4.238288543
50-30 W	5.2442	4.3656	2.5266	1.3781	0.4695	13.984	37.50143	31.21853547	18.06779	9.854834	3.357408467
50-30 W	5.3744	5.6903	3.3279	1.6124	0.6203	16.6253	32.32663	34.2267508	20.01708	9.698472	3.731060492
50-40 W	6.4583	5.6736	2.7967	1.4054	0.6478	16.9818	38.03072	33.40988588	16.46881	8.275919	3.814672178
50-40 W	5.8637	5.485	3.2253	1.4531	0.5869	16.614	35.29373	33.01432527	19.41315	8.746238	3.532562899
50-40 W	6.1199	5.2618	2.851	1.5362	0.6727	16.4416	37.22205	32.00296808	17.34016	9.343373	4.091450954
50-40 W	5.1971	5.033	2.8655	1.3778	0.5424	15.0158	34.61088	33.51802768	19.08323	9.175668	3.612195154
50-40 W	5.863	5.321	2.4125	1.3884	0.539	15.5239	37.76757	34.2761806	15.54055	8.943629	3.472065654
50-50 W	5.8971	5.4664	2.3404	1.3485	0.5134	15.5658	37.88498	35.11801514	15.03553	8.663223	3.298256434
50-50 W	5.4658	4.9439	3.6731	1.6387	0.5919	16.3134	33.50497	30.30576091	22.51585	10.04512	3.628305565
50-50 W	6.322	5.3563	3.0825	1.6506	0.6795	17.0909	36.99045	31.34006986	18.03591	9.657771	3.975799987
50-50 W	5.8616	5.6286	3.0689	1.5118	0.6173	16.6882	35.12422	33.72802339	18.38964	9.059096	3.699020865
50-50 W	6.7695	5.2942	2.2757	1.5505	0.6405	16.5304	40.95182	32.02705319	13.76676	9.379688	3.874679379
50-60 W	5.5951	6.0861	3.48	1.2391	0.6626	17.0629	32.79103	35.66861436	20.39513	7.261954	3.883278927
50-60 W	6.4634	5.7994	2.5047	1.5105	0.7463	17.0243	37.96573	34.06542413	14.7125	8.872612	4.383733839
50-60 W	6.0605	6.5448	2.4952	1.3781	0.6227	17.1013	35.43883	38.27077474	14.5907	8.058452	3.641243648
50-60 W	6.3862	5.4815	3.1034	1.5207	0.6392	17.131	37.27862	31.9975483	18.1157	8.87689	3.731247446
50-60 W	6.3521	5.8023	3.0824	1.5966	0.6942	17.5276	36.24056	33.10379059	17.58598	9.109062	3.960610694

* temp.-time method is varied temperature (°C) - varied time (min) soaked silk fiber into the water (quenching method).

Table A7. The calculated percentage of individual secondary structure from peak area.

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temp.-time method*	β-sheet	random coil	α-helix	turn	β-sheet(w)	SUM	%β-sheet	%random coil	%alpha	%turn	%β-sheet(w)
60-10 W	5.9574	5.3379	3.5155	1.7794	0.7533	17.3435	34.34947	30.77752472	20.26984	10.25975	4.343413959
60-10 W	4.4078	5.841	3.9467	1.5961	0.731	16.5226	26.6774	35.35157905	23.88668	9.660102	4.424243158
60-10 W	5.5174	5.9411	3.5643	1.5595	0.7076	17.2899	31.91112	34.36167936	20.61493	9.019717	4.09256271
60-10 W	5.5924	5.1722	2.6473	1.5962	0.7442	15.7523	35.50212	32.83457019	16.8058	10.13312	4.724389454
60-10 W	5.2721	5.0758	3.6076	1.4285	0.5119	15.8959	33.16641	31.93150435	22.69516	8.986594	3.220327254
60-20 W	4.8193	5.0063	3.4541	1.3325	0.5275	15.1397	31.8322	33.06736593	22.81485	8.801363	3.484216992
60-20 W	5.352	3.7523	3.8083	1.2171	0.5314	14.6611	36.50476	25.59357756	25.97554	8.30156	3.624557503
60-20 W	5.402	5.2228	2.3158	1.3202	0.6005	14.8613	36.34944	35.14362808	15.58276	8.883476	4.040696305
60-20 W	5.6562	5.4587	2.3121	1.3395	0.5525	15.319	36.92278	35.63352699	15.09302	8.744043	3.606632287
60-20 W	5.4276	5.2944	3.5388	1.6708	0.7135	16.6451	32.60779	31.80755898	21.26031	10.03779	4.286546792
60-30 W	6.1335	6.0569	2.7011	1.5764	0.6676	17.1355	35.79411	35.34708646	15.76318	9.199615	3.896005369
60-30 W	6.4605	5.6139	2.8343	1.5267	0.6518	17.0872	37.809	32.85441734	16.58727	8.934758	3.814551243
60-30 W	6.4584	6.0852	2.5883	1.6745	0.7423	17.5487	36.80273	34.67607287	14.74924	9.542017	4.229942959
60-30 W	6.065	5.4699	2.2821	1.354	0.797	15.968	37.98221	34.25538577	14.29171	8.479459	4.991232465
60-30 W	6.3779	5.8031	3.1242	1.5358	0.5813	17.4223	36.60768	33.308461	17.93219	8.815139	3.336528472
60-40 W	6.1939	5.3106	2.6632	1.4409	0.5792	16.1878	38.26277	32.80618738	16.4519	8.901148	3.578003188
60-40 W	5.9689	5.4112	3.0816	1.4031	0.7008	16.5656	36.0319	32.66528227	18.6024	8.469962	4.23045347
60-40 W	5.147	5.1568	3.6267	1.8138	0.7653	16.5096	31.1758	31.23516015	21.96722	10.98634	4.635484809
60-40 W	6.0594	5.0098	3.4523	1.3907	0.6092	16.5214	36.67607	30.32309611	20.89593	8.417568	3.687338845
60-40 W	6.1008	4.692	3.1678	1.4897	0.4804	15.9307	38.29587	29.45256643	19.88488	9.351127	3.015561149
60-50 W	6.0225	4.9521	4.208	1.6606	0.6509	17.4941	34.42589	28.30725788	24.05382	9.492343	3.720682973
60-50 W	5.9795	5.7381	3.6285	1.6896	0.6662	17.7019	33.77886	32.41516447	20.4978	9.544738	3.763437823
60-50 W	7.0688	5.593	2.7674	1.6352	0.6217	17.6861	39.96811	31.62370449	15.64732	9.245679	3.515189895
60-50 W	5.4237	6.1886	3.7939	1.5073	0.6878	17.6013	30.8142	35.15990296	21.55466	8.563572	3.907665911
60-50 W	4.9385	6.0954	4.3582	1.4669	0.6535	17.5125	28.19986	34.80599572	24.88622	8.376303	3.731620271
60-60 W	6.1826	5.7121	3.1381	1.6142	0.8012	17.4482	35.43403	32.73747435	17.98524	9.251384	4.591877672
60-60 W	5.2648	5.5042	4.2158	1.5184	0.7055	17.2087	30.59383	31.98498434	24.49807	8.823444	4.099670515
60-60 W	6.6042	4.4534	3.6423	1.7858	0.6819	17.1676	38.46898	25.94072555	21.21613	10.40215	3.972017055
60-60 W	5.7357	6.2569	3.065	1.4793	0.651	17.1879	33.37057	36.40293462	17.83231	8.606636	3.787548217
60-60 W	5.8489	5.3605	3.1311	1.6911	0.8192	16.8508	34.70992	31.81154604	18.58131	10.03573	4.861490256

* temp.-time method is varied temperature (°C) - varied time (min) soaked silk fiber into the water (quenching method).

Table A8. The calculated percentage of individual secondary structure from peak area.

temp.-time method*	β-sheet	random coil	α-helix	turn	β-sheet(w)	SUM	%β-sheet	%random coil	%alpha	%turn	%β-sheet(w)
70-10 W	7.2184	5.27	2.3048	1.5513	0.6952	17.0397	42.36225	30.92777455	13.52606	9.104034	4.079884036
70-10 W	7.1085	4.7999	3.3024	1.5428	0.6484	17.402	40.84875	27.58246179	18.97713	8.865648	3.726008505
70-10 W	6.84	5.7208	3.4292	1.7019	0.7335	18.4254	37.12267	31.048444	18.61126	9.236706	3.980917646
70-10 W	6.3606	5.9233	3.1526	1.5794	0.6626	17.6785	35.9793	33.50567073	17.83296	8.934016	3.748055548
70-10 W	7.0384	6.1758	2.748	1.6061	1.0886	18.6569	37.72545	33.10196228	14.72914	8.608611	5.834838585
70-20 W	5.824	5.9046	3.7702	1.4892	0.6277	17.6157	33.06142	33.5189632	21.4025	8.453822	3.563298648
70-20 W	6.5748	5.0843	3.2008	1.6823	0.6718	17.214	38.19449	29.53584292	18.59417	9.772859	3.902637388
70-20 W	6.578	5.4964	3.6112	1.7479	0.6889	18.1224	36.29762	30.32931621	19.92672	9.64497	3.801372887
70-20 W	6.0688	6.4544	2.8338	1.4126	0.7034	17.473	34.73244	36.93927774	16.21817	8.084473	4.025639558
70-20 W	6.3961	5.6098	3.2366	1.7308	0.6871	17.6604	36.21719	31.76485244	18.32688	9.800458	3.890625354
70-30 W	6.1876	4.5024	3.6191	1.6244	0.5725	16.506	37.48697	27.27735369	21.92597	9.84127	3.46843572
70-30 W	6.1978	5.331	2.8052	1.5394	0.5525	16.4259	37.73187	32.45484266	17.07791	9.371785	3.363590427
70-30 W	5.9997	5.4305	3.4255	1.6295	0.6143	17.0995	35.08699	31.75823854	20.03275	9.529518	3.592502705
70-30 W	6.5092	5.2154	3.619	1.662	0.6433	17.6489	36.88162	29.55085019	20.50553	9.417017	3.644986373
70-30 W	6.0221	4.8394	3.6275	1.6929	0.6082	16.7901	35.86697	28.82293733	21.60499	10.08273	3.622372708
70-40 W	6.0488	5.1524	2.6553	1.2976	0.6174	15.7715	38.35272	32.66905494	16.83607	8.227499	3.914656184
70-40 W	5.7606	5.5364	2.8122	1.66	0.7502	16.5194	34.87173	33.51453443	17.02362	10.04879	4.541327167
70-40 W	6.2845	4.4958	3.1644	1.5829	0.5337	16.0613	39.12822	27.99150754	19.70202	9.855367	3.322894162
70-40 W	6.1268	4.0843	3.5257	1.4273	0.4999	15.664	39.11389	26.0744382	22.5083	9.111977	3.19139428
70-40 W	6.2584	5.5057	2.6586	1.6687	0.6862	16.7776	37.30212	32.81577818	15.84613	9.945999	4.089977112
70-50 W	6.7724	6.0077	2.4459	1.4451	0.5925	17.2636	39.22936	34.79981	14.16796	8.370792	3.432076739
70-50 W	6.4458	5.5972	2.9937	1.5687	0.6165	17.2219	37.42793	32.50047904	17.3831	9.108751	3.579744395
70-50 W	6.438	4.6334	2.7109	1.0765	0.4256	15.2844	42.12138	30.3145691	17.73638	7.043129	2.784538484
70-50 W	6.29	5.557	2.1772	1.3507	0.5626	15.9375	39.46667	34.86745098	13.66086	8.47498	3.530039216
70-50 W	6.9468	5.4932	2.0204	1.1723	0.4913	16.124	43.0836	34.06846936	12.53039	7.270528	3.047010667
70-60 W	6.3819	6.2356	2.7513	1.525	0.7235	17.6173	36.22519	35.39475402	15.61704	8.656264	4.106758697
70-60 W	6.416	5.4241	3.5967	1.5132	0.6755	17.6255	36.4018	30.77416244	20.40623	8.585288	3.83251539
70-60 W	6.5339	4.7433	2.7206	1.4689	0.5231	15.9898	40.86293	29.66453614	17.0146	9.186481	3.271460556
70-60 W	6.6212	6.5816	2.5565	1.5407	0.7129	18.0129	36.7581	36.53825869	14.19261	8.553315	3.95771919
70-60 W	6.2705	5.8931	2.9817	1.6508	0.7049	17.501	35.82938	33.67293298	17.03731	9.432604	4.027769842

* temp.-time method is varied temperature (°C) - varied time (min) soaked silk fiber into the water (quenching method).

Table A9. The calculated percentage of individual secondary structure from peak area.

temp.-time method*	β-sheet	random coil	-helix	turn	β-sheet(w)	SUM	%β-sheet	%random coil	%alpha	%turn	%β-sheet(w)
80-10 W	6.0597	4.4155	2.6414	1.5467	0.5514	15.2147	39.82793	29.02127548	17.36084	10.16583	3.624126667
80-10 W	6.1695	4.1078	3.4337	1.6543	0.5839	15.9492	38.68219	25.75552379	21.52898	10.37231	3.660998671
80-10 W	5.9353	4.8085	2.9529	1.3489	0.4887	15.5343	38.20771	30.95408226	19.0089	8.683365	3.145941562
80-10 W	6.2506	5.2118	2.2515	1.374	0.5432	15.6311	39.98823	33.34250309	14.40398	8.790168	3.475123312
80-10 W	5.8569	4.9677	3.1393	1.5138	0.5466	16.0243	36.55011	31.00104217	19.59087	9.446903	3.411069438
80-20 W	6.1145	4.9275	3.052	1.7591	0.639	16.4921	37.07533	29.87794156	18.50583	10.66632	3.874582376
80-20 W	5.5355	5.0408	2.9144	1.6134	0.6287	15.7328	35.18446	32.04006915	18.52436	10.25501	3.996110038
80-20 W	4.1287	6.6762	3.8006	1.5282	0.6713	16.805	24.56828	39.72746206	22.61589	9.093722	3.994644451
80-20 W	5.7833	4.8923	2.5695	1.4611	0.522	15.2282	37.97757	32.12658095	16.8733	9.594699	3.427850961
80-20 W	5.904	5.5687	3.1001	1.6925	0.723	16.9883	34.75333	32.77961891	18.24844	9.962739	4.255870217
80-30 W	6.6641	5.7059	2.6229	1.648	0.632	17.2729	38.58125	33.03382756	15.18506	9.540957	3.658910779
80-30 W	5.2991	6.2857	3.5039	1.4671	0.6763	17.2321	30.75133	36.47669176	20.33356	8.513762	3.924652248
80-30 W	6.5398	5.5009	3.3919	1.7041	0.7847	17.9214	36.49157	30.69458859	18.92653	9.508744	4.378564175
80-30 W	6.2686	6.2298	3.0737	1.6794	0.744	17.9955	34.83426	34.61865466	17.08038	9.332333	4.134366925
80-30 W	6.1045	4.9541	3.6497	1.856	0.7038	17.2681	35.35131	28.6893173	21.1355	10.74814	4.075723444
80-40 W	4.653	5.6901	3.6395	1.7133	0.8081	16.504	28.19317	34.47709646	22.05223	10.38112	4.896388754
80-40 W	5.7997	4.7126	3.2839	1.6438	0.5946	16.0346	36.16991	29.39019371	20.48009	10.25158	3.708230951
80-40 W	4.5618	5.7899	2.9113	1.3548	0.7705	15.3883	29.6446	37.62533873	18.91892	8.804091	5.007050811
80-40 W	6.1827	5.6549	3.3168	1.8475	0.6842	17.6861	34.95796	31.97369686	18.75371	10.44606	3.868574756
80-40 W	5.6694	6.0637	2.4186	1.4719	0.6687	16.2923	34.79803	37.2181951	14.84505	9.034329	4.104392873
80-50 W	6.4545	5.8721	3.1667	1.6036	0.8787	17.9756	35.90701	32.66705979	17.61666	8.920982	4.888293019
80-50 W	6.207	6.0771	3.1114	1.5959	0.6179	17.6093	35.24842	34.51074148	17.66907	9.062825	3.508941298
80-50 W	7.0122	4.8646	3.6579	1.9806	0.75	18.2653	38.39083	26.63301451	20.0265	10.84351	4.106146628
80-50 W	5.7077	6.1773	3.3582	1.6218	0.6622	17.5272	32.56481	35.24407778	19.15993	9.253047	3.77812771
80-50 W	6.6111	5.1555	3.0824	1.585	0.6592	17.0932	38.67678	30.1611167	18.0329	9.272693	3.856504341
80-60 W	6.2855	4.3528	2.7499	1.4399	0.5546	15.3827	40.86084	28.29672294	17.87658	9.360515	3.605348866
80-60 W	5.8276	5.0666	2.8125	1.7195	0.8502	16.2764	35.80399	31.12850507	17.27962	10.56438	5.223513799
80-60 W	6.3875	4.6157	2.2386	1.4706	0.611	15.3234	41.68461	30.12190506	14.60903	9.597087	3.987365728
80-60 W	6.6313	4.68	3.8321	1.525	0.5831	17.2515	38.43898	27.12807582	22.21314	8.839811	3.379995942
80-60 W	5.9167	5.4098	2.3458	1.5108	0.6807	15.8638	37.29686	34.10153935	14.78713	9.523569	4.290901297

* temp.-time method is varied temperature (°C) - varied time (min) soaked silk fiber into the water (quenching method).

Table A10. The calculated percentage of individual secondary structure from peak area.

temp.-time method*	β-sheet	random coil	-helix	turn	β-sheet(w)	SUM	%β-sheet	%random coil	%alpha	%turn	%β-sheet(w)
90-10 W	6.3416	5.1209	2.5188	1.316	0.5838	15.8811	39.93174	32.2452475	15.86036	8.28658	3.676067779
90-10 W	7.0296	5.6812	2.8322	1.4806	0.6291	17.6527	39.82167	32.18317878	16.044	8.387385	3.563760784
90-10 W	6.8269	4.6904	3.5498	1.4742	0.5908	17.1321	39.84859	27.37784627	20.72017	8.6049	3.448497265
90-10 W	5.516	6.0603	3.8103	1.683	0.7739	17.8435	30.91322	33.96362821	21.35399	9.432006	4.337153585
90-10 W	6.1518	6.4797	3.0244	1.4579	0.6821	17.7959	34.56864	36.41119584	16.99493	8.192336	3.832905332
90-20 W	7.1579	5.9718	1.9799	1.3552	0.8481	17.3129	41.34432	34.49335467	11.43598	7.827689	4.898659381
90-20 W	6.2849	5.5218	2.7208	1.7614	0.7568	17.0457	36.87088	32.39409353	15.9618	10.3334	4.4398294
90-20 W	5.9552	5.7178	3.2391	1.5687	0.7156	17.1964	34.6305	33.24998255	18.83592	9.122258	4.161336094
90-20 W	6.3227	6.0927	3.2203	1.687	0.6802	18.0029	35.12045	33.84288087	17.88767	9.370712	3.778280166
90-20 W	6.575	5.4184	3.0353	1.5946	0.618	17.2413	38.13518	31.42686456	17.60482	9.248723	3.584416488
90-30 W	5.655	6.1584	2.5245	1.2299	0.6357	16.2035	34.89987	38.00660351	15.57997	7.590335	3.923226463
90-30 W	6.1029	5.4361	2.4003	0.9164	0.5434	15.3991	39.63154	35.30141372	15.58727	5.950997	3.528777656
90-30 W	5.8646	5.2733	3.6458	1.3354	0.5207	16.6398	35.24441	31.69088571	21.91012	8.025337	3.129244342
90-30 W	6.0497	5.5695	2.2429	1.1888	0.5248	15.5757	38.84063	35.75762245	14.39999	7.632402	3.369350976
90-30 W	6.0995	5.177	3.6486	1.4281	0.6062	16.9594	35.96531	30.52584407	21.51373	8.420699	3.574418906
90-40 W	6.9698	5.3111	2.6155	1.562	0.5961	17.0545	40.86781	31.14192735	15.33613	9.158873	3.495265179
90-40 W	6.6411	5.6347	2.5289	1.8393	0.7899	17.4339	38.09303	32.32036435	14.50565	10.55014	4.53082787
90-40 W	4.8134	6.2255	3.7524	2.0713	0.85	17.7126	27.17501	35.14729627	21.18492	11.69394	4.798843761
90-40 W	6.1963	6.1263	2.4309	1.5799	0.852	17.1854	36.05561	35.64828284	14.14515	9.193269	4.957696649
90-40 W	7.2073	5.8085	2.1296	1.3621	0.9188	17.4263	41.35875	33.33180308	12.22061	7.816347	5.272490431
90-50 W	6.3326	5.5499	3.0582	1.5965	0.7082	17.2454	36.72052	32.18191518	17.73342	9.257541	4.10660234
90-50 W	6.7752	5.6815	2.3888	1.6306	0.7396	17.2157	39.35478	33.00185296	13.87571	9.471587	4.296078579
90-50 W	5.9061	5.882	3.6826	1.6691	0.6996	17.8394	33.10706	32.97196094	20.64307	9.356256	3.921656558
90-50 W	6.0797	6.8589	3.0422	1.4223	0.64	18.0431	33.69543	38.01397764	16.86074	7.882792	3.547062312
90-50 W	6.8325	5.9792	2.0254	1.6143	0.6903	17.1417	39.85894	34.88102114	11.81563	9.417386	4.027021824
90-60 W	6.5288	5.9646	2.6093	1.5584	0.7964	17.4575	37.39825	34.16640412	14.94658	8.926822	4.561936131
90-60 W	6.5852	5.6218	2.0999	1.4715	0.6326	16.411	40.12674	34.25629151	12.79569	8.966547	3.854731582
90-60 W	6.3321	5.8381	2.3871	1.4453	0.7058	16.7084	37.8977	34.94110747	14.28683	8.65014	4.224222547
90-60 W	6.031	5.122	3.5291	1.9136	0.7409	17.3366	34.78767	29.54443201	20.35636	11.03792	4.273617664
90-60 W	6.4502	6.214	2.6878	1.5586	0.8999	17.8105	36.21572	34.88953146	15.0911	8.751018	5.052637489

* temp.-time method is varied temperature (°C) - varied time (min) soaked silk fiber into the water (quenching method).

Table A11. The calculated percentage of individual secondary structure from peak area.

temp.-time method*	β-sheet	random coil	α-helix	turn	β-sheet(w)	SUM	%β-sheet	%random coil	%alpha	%turn	%β-sheet(w)
100-10 W	7.2674	4.6349	3.0185	1.5995	0.5472	17.0675	42.58034	27.1562912	17.68566	9.371613	3.206093452
100-10 W	6.455	5.4375	4.5142	2.0148	0.6996	19.1211	33.75852	28.4371715	23.60847	10.53705	3.658785321
100-10 W	7.4925	5.1286	2.8386	1.8199	0.7012	17.9808	41.66945	28.52264638	15.78684	10.12135	3.899715252
100-10 W	6.9479	5.849	3.0619	1.8722	0.7507	18.4817	37.5934	31.6475216	16.5672	10.13002	4.061855782
100-10 W	7.049	5.5898	2.4069	1.5554	0.8805	17.4816	40.3224	31.97533407	13.76819	8.897355	5.036724327
100-20 W	6.4208	5.6648	3.9773	1.7978	0.6351	18.4958	34.71491	30.62749381	21.5038	9.720045	3.433752528
100-20 W	5.7463	5.5	4.3917	2.1573	0.8647	18.66	30.79475	29.47481243	23.53537	11.56109	4.63397642
100-20 W	6.7087	6.1826	3.6642	1.7972	0.9403	19.293	34.77272	32.04581973	18.99238	9.315296	4.873788421
100-20 W	6.3335	5.3613	2.8998	1.716	0.5691	16.8797	37.5214	31.76182041	17.17922	10.16606	3.371505418
100-20 W	6.0724	5.4903	3.9614	2.2434	0.8807	18.6482	32.56293	29.44144743	21.2428	12.03012	4.722707822
100-30 W	5.1357	5.8076	5.5318	1.5259	0.829	18.83	27.27403	30.84227297	29.37759	8.103558	4.402549124
100-30 W	5.9437	6.0804	4.1632	1.7285	0.8432	18.759	31.68452	32.41324164	22.19308	9.214244	4.49490911
100-30 W	6.2033	5.5674	3.6194	2.2425	0.9204	18.553	33.43556	30.00808495	19.50844	12.08699	4.960922762
100-30 W	6.53	5.7626	2.9933	1.4841	0.6842	17.4542	37.4122	33.01554927	17.14945	8.502825	3.919973416
100-30 W	6.8281	5.1328	3.2224	1.5596	0.6365	17.3794	39.28847	29.5338159	18.54149	8.973843	3.6623819
100-40 W	6.4357	5.9853	3.1045	1.8667	0.6399	18.0321	35.69024	33.19247342	17.21652	10.35209	3.548671536
100-40 W	6.4979	5.703	3.3952	1.787	0.7898	18.1729	35.75599	31.38189282	18.68276	9.833323	4.346031729
100-40 W	6.9065	5.4482	2.5017	1.5209	0.6995	17.0768	40.44376	31.90410381	14.6497	8.906235	4.096200693
100-40 W	6.5003	5.589	3.2023	1.533	0.7268	17.5514	37.03579	31.84361361	18.24527	8.734346	4.140980207
100-40 W	6.9239	5.0382	3.0006	1.7268	0.5659	17.2554	40.12599	29.19781634	17.38934	10.0073	3.279553067
100-50 W	6.0897	6.0764	3.8095	1.7915	0.8701	18.6372	32.67497	32.60360998	20.4403	9.612495	4.66861975
100-50 W	6.0007	5.4656	2.3835	1.4045	0.7699	16.0242	37.44774	34.10841103	14.87438	8.764868	4.80460803
100-50 W	6.4744	4.7368	2.5456	1.4301	0.5529	15.7398	41.13394	30.09441035	16.17301	9.085884	3.512751115
100-50 W	6.634	5.62	2.7023	1.906	0.7549	17.6172	37.65638	31.90064255	15.33899	10.81897	4.285016915
100-50 W	5.7326	5.4529	3.549	1.5598	0.6633	16.9576	33.80549	32.15608341	20.92867	9.198236	3.911520498
100-60 W	6.6905	4.668	3.4509	1.6231	0.6863	17.1188	39.08276	27.26826647	20.15854	9.481389	4.00904269
100-60 W	6.5324	5.4406	2.8793	1.4844	0.7034	17.0401	38.33546	31.92821638	16.8972	8.711216	4.127910047
100-60 W	6.5166	5.3219	3.4253	1.5175	0.61	17.3913	37.47046	30.60093265	19.69548	8.725627	3.507500877
100-60 W	7.1051	5.9021	2.7639	1.4389	0.8614	18.0714	39.31682	32.65989353	15.29433	7.962305	4.766647852
100-60 W	5.9608	4.8075	3.3175	1.2076	0.4589	15.7523	37.84082	30.51935273	21.06042	7.666182	2.91322537

* temp.-time method is varied temperature (°C) - varied time (min) soaked silk fiber into the water (quenching method).

Table A12. The calculated percentage of individual secondary structure from peak area.

temp.-time method*	β-sheet	random coil	α-helix	turn	β-sheet(w)	SUM	%β-sheet	%random coil	%alpha	%turn	%β-sheet(w)
50-10 I	6.5474	5.4937	3.6334	1.585	0.709	17.9685	36.43821	30.57406016	20.22094	8.820992	3.945794028
50-10 I	6.5621	5.9973	2.6735	1.6251	0.6755	17.5335	37.42607	34.20480794	15.24795	9.268543	3.852624975
50-10 I	6.5975	6.2733	2.983	1.6129	0.7714	18.2381	36.17427	34.39667509	16.35587	8.843575	4.229607251
50-10 I	5.7306	6.3397	3.1855	1.7063	0.7311	17.6932	32.38871	35.83127981	18.00409	9.643818	4.132095946
50-10 I	6.4458	5.3542	2.6604	1.3567	0.7587	16.5758	38.88681	32.30130672	16.0499	8.184824	4.577154647
50-20 I	6.0271	5.6031	3.492	1.7538	0.7053	17.5813	34.28131	31.86965697	19.86201	9.975372	4.01164874
50-20 I	6.5026	5.857	2.5114	1.5424	0.6684	17.0818	38.06742	34.2879556	14.7022	9.029493	3.912936576
50-20 I	6.4618	5.9707	2.9277	1.583	0.8279	17.7711	36.36128	33.59780768	16.4745	8.907721	4.658687419
50-20 I	6.6102	6.017	2.9082	1.7007	0.7053	17.9414	36.84328	33.53695921	16.20944	9.479193	3.93113135
50-20 I	5.1678	6.3029	4.1987	1.4371	0.7423	17.8488	28.95321	35.31273811	23.52371	8.051522	4.158823002
50-30 I	5.9517	5.7144	3.6122	1.7267	0.7473	17.7523	33.52636	32.18963177	20.34779	9.726627	4.209595376
50-30 I	6.6498	5.3314	3.5331	1.7494	0.719	17.9827	36.97887	29.64738332	19.64722	9.728239	3.998287243
50-30 I	6.5139	4.8345	3.4959	1.8399	0.7034	17.3876	37.4629	27.80429732	20.10571	10.58168	4.045411673
50-30 I	7.1743	5.4424	2.9714	1.8643	0.8027	18.2551	39.30025	29.81303855	16.2771	10.21249	4.397127378
50-30 I	7.7065	4.1955	3.5163	1.8016	0.7134	17.9333	42.97313	23.3950249	19.60766	10.04612	3.978074309
50-40 I	6.2809	5.8466	2.893	1.4621	0.7818	17.2644	36.38064	33.86506337	16.75703	8.468872	4.528393689
50-40 I	6.0124	5.6528	3.0713	1.5801	0.655	16.9716	35.42624	33.30740767	18.0967	9.310259	3.859388626
50-40 I	6.4935	4.8641	3.7512	1.7109	0.7417	17.5614	36.97598	27.69767786	21.36048	9.74239	4.223467377
50-40 I	6.1597	4.9502	4.0665	1.7913	0.7834	17.7511	34.70038	27.88672251	22.90844	10.09121	4.41324763
50-40 I	5.8361	5.8227	3.746	1.6268	0.8224	17.854	32.68791	32.61285986	20.98129	9.111684	4.6062507
50-50 I	5.9842	5.1255	4.2019	1.6881	0.5741	17.5738	34.05183	29.16557603	23.91003	9.605777	3.266794888
50-50 I	5.784	4.7248	4.1797	1.9206	0.6549	17.264	33.50324	27.36793327	24.2105	11.12488	3.793443003
50-50 I	4.8946	4.3945	3.8004	1.4039	0.574	15.0674	32.4847	29.16561583	25.22267	9.317467	3.809549093
50-50 I	5.0756	5.066	3.7403	1.3611	0.561	15.804	32.11592	32.0551759	23.66679	8.612377	3.549734244
50-50 I	5.7535	6.3289	3.6604	1.3056	0.6001	17.6485	32.6005	35.86083803	20.74057	7.397796	3.400288976
50-60 I	6.8789	5.0972	3.4677	1.5786	0.695	17.7174	38.82567	28.76945827	19.57228	8.909885	3.922697461
50-60 I	5.8505	5.4303	3.4995	1.488	0.6074	16.8757	34.66819	32.17822076	20.73692	8.817412	3.599258105
50-60 I	6.0862	5.636	3.1912	1.6612	0.6558	17.2304	35.32245	32.70962949	18.52075	9.641099	3.806063701
50-60 I	6.0367	5.4749	3.3019	1.5434	0.5853	16.9422	35.63115	32.31516568	19.4892	9.109797	3.454687113
50-60 I	6.2573	5.0925	3.632	1.5145	0.5698	17.0661	36.66508	29.83985796	21.28196	8.874318	3.338782733

* temp.-time method is varied temperature (°C) - varied time (min) dipped silk fiber into the ice cold-water (quenching method).

Table A13. The calculated percentage of individual secondary structure from peak area.

temp.-time method*	β-sheet	random coil	α-helix	turn	β-sheet(w)	SUM	%β-sheet	%random coil	%alpha	%turn	%β-sheet(w)
60-10 I	7.6354	6.7429	2.6101	1.5966	0.8683	19.4533	39.2499	34.66198537	13.41726	8.207348	4.463510047
60-10 I	7.4858	5.5466	2.9409	1.5003	0.8046	18.2782	40.9548	30.34543883	16.08966	8.208139	4.401965183
60-10 I	8.1645	5.1247	3.3647	1.8309	0.6189	19.1037	42.73779	26.82569345	17.61282	9.584007	3.239686553
60-10 I	6.7509	5.9473	3.3686	1.5704	0.6834	18.3206	36.84868	32.46236477	18.38695	8.571772	3.730227176
60-10 I	7.2676	5.2672	2.582	1.4854	0.8155	17.4177	41.72537	30.24050248	14.824	8.528106	4.682018866
60-20 I	7.2282	5.6566	2.5616	1.6154	0.8261	17.8879	40.40832	31.62249342	14.32029	9.030686	4.618205603
60-20 I	6.9334	5.4694	2.5711	1.5539	0.6158	17.1436	40.44308	31.90345085	14.99743	9.064024	3.592011013
60-20 I	7.0233	6.1425	3.4375	2.1693	0.8555	19.6281	35.78186	31.29441973	17.51316	11.05201	4.358547185
60-20 I	8.2958	5.8532	2.4801	1.6136	1.0007	19.2434	43.10985	30.41666234	12.88806	8.385213	5.200224493
60-20 I	7.2473	5.9416	3.0313	1.8631	0.7807	18.864	38.41868	31.49703138	16.06923	9.876484	4.138570823
60-30 I	7.681	5.5644	3.3812	2.1319	0.8241	19.5826	39.2236	28.4150215	17.26635	10.88671	4.208327801
60-30 I	6.9976	5.3214	2.8316	1.8651	0.6124	17.6281	39.69571	30.18703093	16.06299	10.58027	3.473998899
60-30 I	7.5965	5.9333	3.0781	1.7607	1.071	19.4396	39.07745	30.52171855	15.83417	9.057285	5.509372621
60-30 I	7.3034	5.8909	3.4152	1.8618	0.7682	19.2395	37.96045	30.61877907	17.75098	9.676967	3.992827256
60-30 I	6.925	6.8084	3.4633	1.7135	0.8109	19.7211	35.11467	34.52342922	17.56139	8.688663	4.111839603
60-40 I	7.1193	5.0813	3.6815	1.7686	0.6018	18.2525	39.00452	27.83892617	20.16984	9.689632	3.297082591
60-40 I	7.3426	5.524	3.0499	1.7537	0.6956	18.3658	39.97974	30.07764432	16.60641	9.548726	3.787474545
60-40 I	6.5284	5.1427	2.9268	1.5847	0.7247	16.9073	38.61291	30.41703879	17.31087	9.372874	4.286314196
60-40 I	6.339	3.8592	2.8889	1.4559	0.6099	15.1529	41.83358	25.46839219	19.065	9.608062	4.024972118
60-40 I	6.6247	4.3998	2.6606	1.6171	0.5448	15.847	41.80413	27.7642456	16.7893	10.20446	3.437874677
60-50 I	7.0393	5.6144	3.0718	1.8251	0.7311	18.2817	38.50462	30.71049191	16.80259	9.983207	3.999081048
60-50 I	6.7213	5.4451	2.865	2.0026	0.5793	17.6133	38.16037	30.9147065	16.26612	11.36982	3.288991841
60-50 I	7.3116	5.3072	2.9774	1.9791	0.9174	18.4927	39.53776	28.698892	16.10041	10.70206	4.960876454
60-50 I	6.8841	5.5277	3.306	1.593	0.5658	17.8766	38.509	30.92142801	18.49345	8.91109	3.165031382
60-50 I	6.4615	4.9885	3.1307	1.5074	0.6186	16.7067	38.6761	29.85927801	18.73919	9.022727	3.7027061
60-60 I	6.9832	4.8535	3.4091	1.4997	0.6173	17.3628	40.21932	27.95344069	19.63451	8.637432	3.55530214
60-60 I	6.6796	6.8264	3.6312	1.7112	0.9065	19.7549	33.81237	34.55547738	18.38126	8.662155	4.588734947
60-60 I	6.2566	5.9479	4.2037	2.0258	0.8684	19.3024	32.41359	30.81430288	21.77812	10.49507	4.498922414
60-60 I	7.4759	5.1302	3.9091	1.7945	0.7465	19.0562	39.2308	26.9214219	20.51353	9.416883	3.917360229
60-60 I	7.1447	4.8958	3.7602	1.7595	0.7776	18.3378	38.9616	26.69785907	20.50519	9.594935	4.240421425

* temp.-time method is varied temperature (°C) - varied time (min) dipped silk fiber into the ice cold-water (quenching method).

Table A14. The calculated percentage of individual secondary structure from peak area.

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temp.-time method*	β-sheet	random coil	α-helix	turn	β-sheet(w)	SUM	%β-sheet	%random coil	%alpha	%turn	%β-sheet(w)
70-10 I	4.7407	5.7061	3.1115	1.2743	0.5491	15.3817	30.82039	37.09667982	20.22858	8.28452	3.569826482
70-10 I	7.0282	5.6626	2.2305	1.4988	0.7004	17.1205	41.05137	33.0749686	13.02824	8.754417	4.091002015
70-10 I	6.5415	5.687	2.4868	1.5867	0.5986	16.9006	38.70573	33.64969291	14.71427	9.388424	3.541886087
70-10 I	6.9701	5.3822	2.5262	1.6554	0.6159	17.1498	40.64246	31.38345637	14.7302	9.652591	3.591295525
70-10 I	6.9876	6.0773	2.3882	1.5265	0.6087	17.5883	39.72868	34.55308358	13.57834	8.679065	3.460823388
70-20 I	6.4789	5.9727	2.267	1.4237	0.6634	16.8057	38.5518	35.53972759	13.48947	8.47153	3.947470204
70-20 I	6.8957	5.9201	2.5897	1.3143	0.6018	17.3216	39.80983	34.17755866	14.9507	7.587636	3.474274894
70-20 I	7.0135	5.6933	2.6311	1.401	0.6073	17.3462	40.43249	32.82159781	15.16816	8.076697	3.501054986
70-20 I	6.7114	5.1583	2.8656	1.3123	0.5615	16.6091	40.40797	31.05707112	17.25319	7.90109	3.380676858
70-20 I	6.8179	5.0863	3.1972	1.4104	0.6303	17.1421	39.77284	29.67139382	18.65116	8.227697	3.676912397
70-30 I	6.0489	5.5264	3.2074	1.4093	0.499	16.691	36.24049	33.11005931	19.21634	8.443473	2.989635133
70-30 I	6.905	5.1407	3.3498	1.7506	0.6343	17.7804	38.8349	28.91217295	18.83985	9.845673	3.567411307
70-30 I	6.0594	3.9224	2.1931	1.2653	0.5631	14.0033	43.27123	28.01054037	15.66131	9.035727	4.021195004
70-30 I	6.7868	5.8804	2.8471	1.5755	0.835	17.9248	37.86263	32.80594484	15.88358	8.789498	4.658350442
70-30 I	7.3706	5.9412	2.4236	1.5292	0.7231	17.9877	40.97578	33.02923665	13.47365	8.501365	4.019969201
70-40 I	6.5828	4.5765	3.6953	1.4453	0.552	16.8519	39.06266	27.15717516	21.92809	8.576481	3.275595037
70-40 I	7.3096	5.9743	2.3772	1.4977	0.898	18.0568	40.48115	33.08615037	13.16512	8.294382	4.973195694
70-40 I	6.3463	5.1643	3.2276	1.685	0.6226	17.0458	37.23087	30.29661266	18.93487	9.885133	3.652512642
70-40 I	4.5358	5.6193	4.2942	1.2895	0.5414	16.2802	27.86084	34.51616074	26.37683	7.920664	3.325511972
70-40 I	6.6728	6.0434	3.1881	1.6795	0.8856	18.4694	36.12895	32.72114958	17.26152	9.093419	4.794958147
70-50 I	6.4396	5.909	2.957	1.6436	0.7187	17.6679	36.44802	33.44483498	16.73657	9.302747	4.067829227
70-50 I	7.0202	5.3885	3.0512	1.5927	0.6742	17.7268	39.60218	30.39747727	17.21236	8.984701	3.803280908
70-50 I	7.4031	5.6629	2.4754	1.5048	0.6392	17.6854	41.85995	32.02019745	13.99686	8.508713	3.614280706
70-50 I	6.5832	5.1562	3.4241	1.5772	0.6654	17.4061	37.82122	29.62294828	19.67184	9.061191	3.822797755
70-50 I	6.5817	5.3242	2.758	1.4745	0.5857	16.7241	39.35458	31.83549488	16.49117	8.816618	3.502131654
70-60 I	6.3558	5.7722	3.6587	1.617	0.6529	18.0566	35.19932	31.96725851	20.2624	8.955174	3.615852375
70-60 I	6.1223	5.3013	3.0219	1.5854	0.6172	16.6481	36.77477	31.84327341	18.15162	9.523009	3.707329966
70-60 I	6.0998	5.6898	3.4941	1.5997	0.6387	17.5221	34.81204	32.47213519	19.9411	9.129613	3.645111031
70-60 I	6.6688	5.5164	2.8835	1.4464	0.7137	17.2288	38.70728	32.01848068	16.73651	8.395245	4.142482355
70-60 I	5.6617	5.2942	4.0375	1.301	0.5028	16.7972	33.70621	31.5183483	24.03674	7.745339	2.993356036

* temp.-time method is varied temperature (°C) - varied time (min) dipped silk fiber into the ice cold-water (quenching method).

Table A15. The calculated percentage of individual secondary structure from peak area.

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temp.-time method*	β-sheet	random coil	α-helix	turn	β-sheet(w)	SUM	%β-sheet	%random coil	%alpha	%turn	%β-sheet(w)
80-10 I	8.3949	6.5631	2.3771	1.5635	1.0818	19.9804	42.01568	32.84769074	11.89716	7.825169	5.41430602
80-10 I	7.6463	7.01	2.8566	1.892	1.2265	20.6314	37.06147	33.97733552	13.84589	9.170488	5.94482197
80-10 I	5.822	3.5883	2.3717	1.2637	0.4055	13.4512	43.28238	26.67643036	17.63188	9.394701	3.014600928
80-10 I	6.491	4.2711	2.3313	1.2855	0.5861	14.965	43.37454	28.54059472	15.57835	8.590043	3.916471767
80-10 I	7.983	4.7999	3.3329	1.859	0.7392	18.714	42.6579	25.64871219	17.80966	9.933739	3.949983969
80-20 I	6.8231	4.2069	2.4525	1.4954	0.5563	15.5342	43.92309	27.08153622	15.78775	9.626502	3.581130667
80-20 I	6.7279	4.9635	2.829	1.5431	0.5264	16.5899	40.55419	29.91880602	17.05254	9.301442	3.173014907
80-20 I	6.0845	4.7954	2.8387	1.4946	0.6113	15.8245	38.44987	30.30364309	17.93864	9.444848	3.862997251
80-20 I	6.5749	4.9046	3.0137	1.5971	0.5091	16.5994	39.60926	29.54685109	18.15548	9.621432	3.066978325
80-20 I	6.4182	4.9956	1.748	1.1799	0.5171	14.8588	43.19461	33.62048079	11.76407	7.940749	3.480092605
80-30 I	6.131	5.5565	3.458	2.0483	0.7409	17.9347	34.18513	30.98183967	19.28106	11.42088	4.131097816
80-30 I	7.0495	5.0562	2.0455	1.4625	0.6528	16.2665	43.33753	31.0835152	12.57492	8.990871	4.013155872
80-30 I	7.1201	5.2802	2.4609	1.4728	0.6495	16.9835	41.92363	31.09017576	14.48995	8.671946	3.824300056
80-30 I	6.6823	4.6271	2.2843	1.3352	0.6924	15.6213	42.77685	29.62045412	14.62298	8.547304	4.432409595
80-30 I	6.5735	4.2572	3.2212	1.5469	0.5157	16.1145	40.79245	26.41844302	19.98945	9.599429	3.200223401
80-40 I	6.7427	5.088	2.9774	1.5505	0.5193	16.8779	39.94988	30.14593048	17.64082	9.186569	3.076804579
80-40 I	6.2063	3.9812	2.192	1.2277	0.5699	14.1771	43.77694	28.08190674	15.46155	8.65974	4.019863019
80-40 I	7.0295	4.4931	2.1204	1.3761	0.8573	15.8764	44.27641	28.30049633	13.35567	8.667582	5.399838754
80-40 I	5.8683	3.2889	2.9402	1.4119	0.5478	14.0571	41.74616	23.39671767	20.91612	10.04403	3.8969631
80-40 I	6.8556	5.2044	2.0236	1.3237	0.5186	15.9259	43.04686	32.6788439	12.70635	8.311618	3.256330882
80-50 I	7.0407	5.8542	3.6845	1.9411	0.7714	19.2919	36.49563	30.34537811	19.09869	10.06174	3.998569348
80-50 I	7.4193	6.1043	2.9641	1.9698	0.7371	19.1946	38.65306	31.80217353	15.44236	10.26226	3.84014254
80-50 I	7.6594	6.2901	3.1936	2.0533	0.8257	20.0221	38.25473	31.41578556	15.95037	10.25517	4.123943043
80-50 I	7.2025	4.9206	2.9705	1.8697	0.8427	17.806	40.44985	27.63450522	16.68258	10.50039	4.732674379
80-50 I	6.0508	7.3253	3.269	1.722	0.7335	19.1006	31.67859	38.35115127	17.11465	9.015424	3.840193502
80-60 I	7.378	7.1095	2.6975	1.8041	0.9325	19.9216	37.03518	35.68739459	13.54058	9.056	4.680848928
80-60 I	7.6468	6.4923	2.5961	1.7711	0.6346	19.1409	39.95005	33.91846778	13.5631	9.252961	3.315413591
80-60 I	6.999	5.7454	2.2939	1.5158	0.6489	17.203	40.68476	33.3976632	13.3343	8.811254	3.772016509
80-60 I	6.8864	5.6757	2.4558	1.854	0.7058	17.5777	39.17691	32.28920735	13.97111	10.54746	4.015314859
80-60 I	7.3195	4.6997	2.4207	1.5579	0.6093	16.6071	44.07452	28.29934185	14.5763	9.380927	3.668912694

* temp.-time method is varied temperature (°C) - varied time (min) dipped silk fiber into the ice cold-water (quenching method).

Table A16. The calculated percentage of individual secondary structure from peak area.

temp.-time method*	β-sheet	random coil	-helix	turn	β-sheet(w)	SUM	%β-sheet	%random coil	%alpha	%turn	%β-sheet(w)
90-10 l	5.9763	5.2881	2.955	1.5492	0.5823	16.3509	36.55028	32.34133901	18.0724	9.474708	3.561271857
90-10 l	6.2844	5.9867	2.9465	1.598	0.7557	17.5713	35.76514	34.07089971	16.76882	9.094375	4.300763176
90-10 l	6.613	5.6079	2.9872	1.5147	0.7339	17.4567	37.8823	32.12462837	17.11205	8.676898	4.204116471
90-10 l	6.6426	5.6577	3.0786	1.5861	0.8538	17.8188	37.2786	31.75129638	17.27726	8.901273	4.791568456
90-10 l	6.3458	5.3398	3.0258	1.6234	0.555	16.8898	37.57179	31.61553127	17.91495	9.611718	3.286006939
90-20 l	6.1905	5.4935	3.4448	1.5298	0.6149	17.2735	35.83813	31.80305092	19.94269	8.856341	3.559788115
90-20 l	6.217	5.9858	3.1387	1.6536	0.6991	17.6942	35.13581	33.82916436	17.73858	9.345435	3.951012196
90-20 l	6.4443	4.7532	3.2578	1.5395	0.563	16.5578	38.92003	28.70671224	19.67532	9.297733	3.400210173
90-20 l	6.5655	5.3636	3.0192	1.4219	0.6259	16.9961	38.62945	31.55782797	17.76408	8.366037	3.68260954
90-20 l	6.499	5.6801	3.3579	1.6297	0.7058	17.8725	36.36313	31.78122814	18.78808	9.118478	3.949083788
90-30 l	6.3575	5.7456	3.1376	1.5442	0.925	17.7099	35.898	32.44287094	17.71664	8.719417	5.223067324
90-30 l	6.5306	5.1733	3.5207	1.5378	0.5989	17.3613	37.61585	29.7978838	20.27901	8.857632	3.449626468
90-30 l	6.2834	5.8176	3.2394	1.5826	0.7761	17.6991	35.50124	32.86946794	18.30263	8.941698	4.384968727
90-30 l	6.4561	5.5245	3.1962	1.5559	0.6187	17.3514	37.20795	31.83892942	18.42042	8.967	3.565706514
90-30 l	5.7336	5.2359	3.9469	1.6773	0.6237	17.2174	33.3012	30.41051494	22.9239	9.741889	3.62249817
90-40 l	6.4824	5.6241	2.6445	1.3434	0.8392	16.9336	38.28129	33.21266594	15.61688	7.93334	4.955827467
90-40 l	5.9146	5.5028	2.9651	1.5388	0.6617	16.583	35.66665	33.18338057	17.88036	9.279383	3.990230959
90-40 l	6.598	5.031	3.1558	1.4764	0.617	16.8782	39.09185	29.80768091	18.69749	8.747378	3.655603086
90-40 l	5.6676	6.1226	3.5759	1.4224	0.7057	17.4942	32.39702	34.99788501	20.44049	8.130695	4.033908381
90-40 l	6.0687	5.6169	3.3787	1.6452	0.6637	17.3732	34.93139	32.3308314	19.44777	9.469758	3.820251882
90-50 l	6.7011	5.3998	2.6497	1.6285	0.812	17.1911	38.98005	31.41043912	15.41321	9.472925	4.723374304
90-50 l	6.6508	5.1681	3.031	1.4735	0.7383	17.0617	38.98088	30.29065099	17.76494	8.636302	4.327235856
90-50 l	6.1473	5.3988	3.1148	1.5654	0.7273	16.9536	36.25956	31.84456399	18.3725	9.233437	4.289944319
90-50 l	6.0728	5.8999	3.3016	1.6764	0.7131	17.6638	34.37992	33.40108018	18.69133	9.490597	4.037070166
90-50 l	6.2212	5.365	3.8755	1.575	0.5908	17.6275	35.29258	30.43539923	21.98553	8.934903	3.351581336
90-60 l	6.7953	4.833	3.5668	1.6737	0.5767	17.4455	38.95159	27.70341922	20.44539	9.593878	3.305723539
90-60 l	5.3726	5.501	4.0517	1.6049	0.7521	17.2823	31.0873	31.83025407	23.44422	9.28638	4.351851316
90-60 l	6.236	5.9782	2.8576	1.5071	0.6199	17.1988	36.25834	34.75940182	16.61511	8.762821	3.604321232
90-60 l	6.6062	5.8326	2.9108	1.6816	0.8758	17.907	36.89172	32.57162004	16.2551	9.390741	4.890824817
90-60 l	5.9906	5.6236	3.3073	1.5292	0.6409	17.0916	35.04997	32.90271244	19.35044	8.947085	3.749795221

* temp.-time method is varied temperature (°C) - varied time (min) dipped silk fiber into the ice cold-water (quenching method).

Table A17. The calculated percentage of individual secondary structure from peak area.

temp.-time method*	β-sheet	random coil	α-helix	turn	β-sheet(w)	SUM	%β-sheet	%random coil	%alpha	%turn	%β-sheet(w)
100-10 I	6.3743	5.4862	3.8055	1.68	0.6584	18.0044	35.40412	30.47144031	21.1365	9.331052	3.656883873
100-10 I	6.4652	5.5183	3.7649	1.5823	0.5822	17.9129	36.09243	30.80629044	21.01781	8.833299	3.250171664
100-10 I	6.4676	4.6366	3.895	1.8654	0.7453	17.6099	36.72707	26.32950783	22.11824	10.59291	4.232278434
100-10 I	5.9048	5.4546	3.8477	1.6497	0.7692	17.626	33.50051	30.94632929	21.82968	9.359469	4.364007716
100-10 I	6.3234	6.5324	3.2221	1.5441	0.7481	18.3701	34.42224	35.55995885	17.53992	8.405507	4.072378485
100-20 I	6.7054	6.1686	2.7822	1.3007	0.6472	17.6041	38.08999	35.04070075	15.80427	7.38862	3.676416289
100-20 I	7.1743	5.5626	2.2008	1.4705	0.5914	16.9996	42.20276	32.7219464	12.94619	8.650204	3.478905386
100-20 I	6.4949	6.3172	2.591	1.5749	0.6717	17.6497	36.79893	35.79210978	14.68014	8.923098	3.805730409
100-20 I	6.1484	4.8503	3.2797	1.6807	0.6907	16.6498	36.92777	29.13128086	19.69813	10.09442	4.148398179
100-20 I	6.7313	5.7424	2.762	1.3515	0.5539	17.1411	39.26994	33.50076716	16.11332	7.884558	3.231414553
100-30 I	6.8919	5.5654	2.7333	1.649	0.5654	17.405	39.59724	31.975869	15.70411	9.474289	3.248491813
100-30 I	7.5567	6.2123	2.4354	1.9349	0.8538	18.9931	39.78655	32.70819403	12.82255	10.18738	4.49531672
100-30 I	6.741	6.4293	3.2288	2.0041	0.8874	19.2906	34.94448	33.32866785	16.73769	10.389	4.600167957
100-30 I	7.1641	5.7179	3.0256	1.7372	0.7369	18.3817	38.97409	31.1064809	16.45985	9.450704	4.008878395
100-30 I	7.4147	6.3363	2.3176	1.7585	0.7631	18.5902	39.88499	34.08408731	12.46678	9.459285	4.104850943
100-40 I	7.2644	6.0718	2.6886	1.615	0.6061	18.2459	39.81388	33.27761305	14.73537	8.851304	3.321842167
100-40 I	7.8181	5.8479	2.2041	1.4319	0.7995	18.1015	43.19034	32.30616247	12.17634	7.910394	4.416761042
100-40 I	7.2894	5.2181	3.4951	2.16	0.7895	18.9521	38.46223	27.5330966	18.44176	11.39715	4.165765271
100-40 I	7.4637	5.6355	2.6191	1.7687	0.7348	18.2218	40.96028	30.927241	14.37344	9.706505	4.032532461
100-40 I	5.8116	3.1447	2.6928	1.1993	0.3813	13.2297	43.92843	23.77000234	20.3542	9.065209	2.882151523
100-50 I	6.9586	6.8134	2.6075	1.8131	1.1935	19.3861	35.89479	35.14580034	13.45036	9.352577	6.156472937
100-50 I	6.2472	6.1652	3.023	1.952	0.8528	18.2402	34.24962	33.80006798	16.57328	10.70164	4.675387331
100-50 I	7.7743	5.3025	2.468	1.62	0.9048	18.0696	43.0242	29.34486652	13.6583	8.965334	5.007305087
100-50 I	6.5192	5.8412	3.0088	1.9678	0.8371	18.1741	35.87083	32.14024353	16.55543	10.8275	4.606005249
100-50 I	6.7814	5.8437	2.5079	1.6261	0.8915	17.6506	38.42022	33.1076564	14.20858	9.212718	5.050819802
100-60 I	7.1022	5.8443	2.9152	1.9054	0.6853	18.4524	38.4893	31.67230279	15.79849	10.32603	3.713880037
100-60 I	7.2482	5.9965	2.6929	1.6242	0.6956	18.2574	39.70007	32.84421659	14.74964	8.896119	3.809961988
100-60 I	7.0157	6.1804	2.6555	1.674	0.6868	18.2124	38.52156	33.93512113	14.58073	9.19154	3.771057082
100-60 I	6.9173	5.3361	3.9182	2.0975	0.8643	19.1334	36.15301	27.88892722	20.47833	10.96251	4.517231647
100-60 I	6.6441	5.7142	3.0182	1.9329	0.7468	18.0562	36.79678	31.64674738	16.71559	10.70491	4.135975454

* temp.-time method is varied temperature (°C) - varied time (min) dipped silk fiber into the ice cold-water (quenching method).

Table A18. The calculated percentage of individual secondary structure from peak area.

type of condition	β-sheet	random coil	α-helix	turn	β-sheet(w)	SUM	%β-sheet	%random coil	%alpha	%turn	%β-sheet(w)
0.1 untreated 1*	6.4742	5.7807	2.565	1.3465	0.596	16.7624	38.62335	34.48611177	15.3021	8.032859	3.555576767
0.1 untreated 2	5.5316	6.0505	4.008	1.0895	0.5013	17.1809	32.19622	35.2164322	23.32823	6.341344	2.917774971
0.1 untreated 3	6.4236	5.0617	3.3607	1.7577	0.6695	17.2732	37.18825	29.30377695	19.45615	10.17588	3.875946553
0.1 untreated 4	6.1699	5.5715	2.4813	1.751	0.7436	16.7173	36.90728	33.32775029	14.84271	10.47418	4.448086713
0.1 untreated 5	6.9265	5.4694	2.2275	1.6428	0.6492	16.9154	40.94789	32.33384963	13.16847	9.71186	3.83792284
0.5 untreated 1	6.0986	5.1065	2.9815	1.7251	0.6832	16.5949	36.74984	30.77150209	17.96636	10.39536	4.11692749
0.5 untreated 2	5.5614	5.841	2.9478	1.3723	0.5618	16.2843	34.15191	35.8689044	18.1021	8.427135	3.449948724
0.5 untreated 3	6.59	5.367	2.765	1.6097	0.6404	16.9721	38.82843	31.62248632	16.29144	9.484389	3.773251395
0.5 untreated 4	6.8311	5.4676	2.2264	1.4211	0.836	16.7822	40.70444	32.57975712	13.26644	8.467901	4.981468461
0.5 untreated 5	6.042	4.8917	3.5438	1.7521	0.6423	16.8719	35.81102	28.99317801	21.00415	10.38472	3.806921568
1 untreated 1	5.5671	5.609	3.3382	1.6561	0.6876	16.858	33.02349	33.27203702	19.80187	9.823823	4.078775655
1 untreated 2	6.0101	4.7827	3.2905	1.4557	0.5674	16.1064	37.31498	29.69440719	20.42977	9.038022	3.522823226
1 untreated 3	5.8795	4.7613	3.2785	1.6545	0.7377	16.3115	36.04512	29.18983539	20.09932	10.14315	4.522576097
1 untreated 4	5.7721	4.7301	2.8728	1.4896	0.681	15.5456	37.13012	30.42725916	18.47983	9.582133	4.380660766
1 untreated 5	5.774	5.0706	2.8937	1.5779	0.609	15.9252	36.257	31.84010248	18.17057	9.908196	3.824127797

* 0.1 untreated 1 can defind to the concentration of NaOH solution, untreated with methanol and time of collected spectrum.

Table A19. The calculated percentage of individual secondary structure from peak area.

type of condition	β-sheet	random coil	α-helix	turn	β-sheet(w)	SUM	%β-sheet	%random coil	%alpha	%turn	%β-sheet(w)
0.1 treated 1*	6.1978	5.5976	3.0154	1.6626	0.6594	17.1328	36.17506	32.67183414	17.60016	9.704193	3.848757938
0.1 treated 2	6.7335	4.7949	3.4391	1.4923	0.6284	17.0882	39.40438	28.05971372	20.12558	8.732927	3.677391416
0.1 treated 3	6.6015	4.6683	3.0539	1.6957	0.502	16.5214	39.95727	28.25607999	18.48451	10.26366	3.038483422
0.1 treated 4	6.6693	4.719	3.6176	1.6318	0.6618	17.2995	38.55198	27.27824504	20.91159	9.432643	3.825544091
0.1 treated 5	6.4924	5.4755	3.0556	1.6154	0.6879	17.3268	37.47028	31.60133435	17.63511	9.323129	3.970150287
0.5 treated 1	6.0725	4.4055	2.9984	1.5572	0.6101	15.6437	38.81754	28.16149632	19.16682	9.954167	3.899972513
0.5 treated 2	5.9488	4.1507	2.6321	1.6161	0.5223	14.87	40.00538	27.91324815	17.70074	10.86819	3.512441157
0.5 treated 3	5.0097	4.2529	3.345	1.5613	0.5701	14.739	33.98942	28.85473913	22.69489	10.59298	3.867969333
0.5 treated 4	5.7965	5.3498	2.9586	1.6161	0.7034	16.4244	35.292	32.57227052	18.01344	9.839629	4.282652639
0.5 treated 5	5.4895	5.1596	2.0686	1.1206	0.4938	14.3321	38.30213	36.000307	14.43333	7.818812	3.445412745
1 treated 1	5.9275	4.5594	3.1502	1.449	0.5724	15.6585	37.85484	29.11773158	20.11815	9.25376	3.65552256
1 treated 2	6.1497	4.6582	3.0782	1.4077	0.53	15.8238	38.86361	29.43793526	19.45298	8.896093	3.349385103
1 treated 3	5.6655	4.4339	3.6704	1.6301	0.6484	16.0483	35.3028	27.62847155	22.87096	10.15746	4.040303334
1 treated 4	5.9755	5.6439	2.8765	1.5326	0.6483	16.6768	35.83121	33.84282356	17.24851	9.190012	3.887436439
1 treated 5	6.5313	5.0547	3.238	1.8741	0.6953	17.3934	37.55045	29.06102315	18.61626	10.77478	3.997493302

* 0.1 treated 1 can defind to the concentration of NaOH solution, treated with methanol and time of collected spectrum.

Table A20. The calculated percentage of individual secondary structure from peak area.

time of collected*	β-sheet	random coil	α-helix	turn	β-sheet(w)	SUM	%β-sheet	%random coil	%alpha	%turn	%β-sheet(w)
1	6.6156	6.0155	2.7059	1.5665	0.7136	17.6171	37.55215	34.14580152	15.35951	8.891929	4.050609919
2	6.6585	6.0953	2.6279	1.4474	0.6698	17.4989	38.05096	34.83247518	15.01752	8.271377	3.827669168
3	6.1755	5.0655	3.5153	1.9038	0.7349	17.395	35.50158	29.12043691	20.20868	10.94452	4.224777235
4	6.7838	5.4516	3.0737	1.7558	0.7871	17.852	38.00022	30.53775487	17.21768	9.835313	4.409029801
5	6.5459	5.8937	2.2212	1.3533	0.8078	16.8219	38.91296	35.03587585	13.20422	8.04487	4.802073488

* time of collected can defind to time of collected spectrum of original degummed silk fiber

Table A21. The calculated percentage of individual secondary structure from peak area.

4. Average mean values of the percentage of secondary structure

Average mean values of 50°C					
Time (min)	% β-sheet	% random coil	% α-helix	% turn	% β-sheet (intermolecular)
10	42.04215	29.71916	14.62891	10.00557	3.604206
20	42.78539	29.76699	14.56314	9.148052	3.736425
30	40.95611	31.92204	14.36497	8.778434	3.978454
40	41.45578	29.2	15.77752	9.604252	3.962445
50	43.22172	27.48795	15.74248	9.455576	4.092271
60	42.46966	27.85187	16.46819	9.542272	3.668013

Average mean values of 60°C					
Time (min)	% β-sheet	% random coil	% α-helix	% turn	% β-sheet (intermolecular)
10	41.5614	30.41419	14.59952	9.199012	4.225881
20	42.96579	27.09723	14.03744	10.66974	5.229795
30	43.53263	29.74524	13.85337	8.889227	3.979538
40	42.13834	29.40346	14.56712	9.514988	4.376088
50	42.37997	28.8025	14.81076	9.602599	4.404171
60	43.32625	29.49743	14.25556	8.523759	4.396999

Average mean values of 70°C					
Time (min)	% β-sheet	% random coil	% α-helix	% turn	% β-sheet (intermolecular)
10	37.03167	30.56842	18.68856	9.740679	3.970667
20	36.31659	30.22267	20.43853	9.387548	3.634667
30	37.6898	33.16155	15.9984	9.388513	3.761735
40	38.94853	32.11989	15.03431	9.711738	4.185535
50	37.16375	31.6603	17.12016	9.976572	4.079214
60	36.85316	32.45212	17.42793	9.216812	4.049972

Average mean values of 80°C					
Time (min)	% β-sheet	% random coil	% α-helix	% turn	% β-sheet (intermolecular)
10	40.62817	30.0442	15.06572	9.789983	4.47192
20	44.22169	27.18306	14.91445	9.193489	4.487316
30	40.11561	31.26253	15.16811	9.32716	4.126592
40	39.83431	30.44478	16.21659	9.439468	4.064852
50	40.56219	32.2131	13.28472	8.855665	5.084324
60	41.59578	28.97637	15.22142	9.621204	4.585225

Average mean values of 90°C					
Time (min)	% β-sheet	% random coil	% α-helix	% turn	% β-sheet (intermolecular)
10	40.02491	31.62193	15.16055	9.413465	3.779149
20	33.44794	34.34267	18.50231	9.420161	4.286916
30	36.25751	32.80999	16.78809	10.02925	4.115177
40	33.51736	34.25531	18.9268	9.153002	4.147527
50	37.26098	31.04635	17.76196	9.71806	4.212659
60	38.51059	30.51192	17.01382	9.860722	4.102951

Average mean values of 100°C					
Time (min)	% β-sheet	% random coil	% α-helix	% turn	% β-sheet (intermolecular)
10	38.90336	33.02697	15.05257	9.175896	3.841206
20	42.16985	27.75961	16.57861	9.790142	3.701793
30	41.53288	31.31321	13.99175	9.126495	4.035668
40	41.02452	29.30545	16.41432	9.269487	3.98623
50	42.30908	30.26821	14.27193	9.733051	3.417736
60	40.54094	28.35156	18.09193	9.500996	3.51458

Table A. The mean values of exposing into the air by varying the temperature (50, 60, 70, 80, 90 and 100) and time (10, 20, 30, 40, 50, 60 min).

Average mean values of 50°C					
Time (min)	% β-sheet	% random coil	% α-helix	% turn	% β-sheet (intermolecular)
10	33.50418	33.69941	18.35354	10.06668	4.376194
20	33.73424	31.45338	20.02076	10.3639	4.427724
30	34.25542	31.83827	19.98609	10.02617	3.894045
40	36.58499	33.24428	17.56918	8.896965	3.704589
50	36.89129	32.50378	17.54874	9.360979	3.695212
60	35.94295	34.62123	17.08	8.435794	3.920023

Average mean values of 60°C					
Time (min)	% β-sheet	% random coil	% α-helix	% turn	% β-sheet (intermolecular)
10	32.3213	33.05137	20.85448	9.611858	4.160987
20	34.8434	32.24913	20.1453	8.953646	3.80853
30	36.99915	34.08828	15.86472	8.994198	4.053652
40	36.08848	31.29646	19.56047	9.225228	3.829368
50	33.43738	32.46241	21.32796	9.044527	3.727719
60	34.51547	31.77553	20.02261	9.423868	4.262521

Average mean values of 70°C					
Time (min)	% β-sheet	% random coil	% α-helix	% turn	% β-sheet (intermolecular)
10	38.80768	31.23326	16.73531	8.949803	4.273941
20	35.70063	32.41765	18.89369	9.151316	3.836715
30	36.61089	29.97284	20.22943	9.648464	3.538378
40	37.75374	30.61306	18.38323	9.437927	3.81205
50	40.26579	33.31016	15.09574	8.053636	3.274682
60	37.21548	33.20893	16.85356	8.88279	3.839245

Average mean values of 80°C					
Time (min)	% β-sheet	% random coil	% α-helix	% turn	% β-sheet (intermolecular)
10	38.65123	30.01489	18.37871	9.491714	3.463452
20	33.91179	33.31033	18.95356	9.914498	3.909812
30	35.20194	32.70262	18.53221	9.528788	4.034444
40	32.75273	34.1369	19.01	9.783436	4.316928
50	36.15757	31.8432	18.50101	9.470612	4.027603
60	38.81706	30.15535	17.3531	9.577072	4.097425

Average mean values of 90°C					
Time (min)	% β-sheet	% random coil	% α-helix	% turn	% β-sheet (intermolecular)
10	37.01677	32.43622	18.19469	8.580641	3.771677
20	37.22027	33.08144	16.34524	9.180556	4.172504
30	36.91635	34.25647	17.79822	7.523954	3.505004
40	36.71004	33.51793	15.47849	9.682512	4.611025
50	36.54734	34.21015	16.18571	9.077112	3.979684
60	37.28522	33.55955	15.49531	9.266489	4.393429

Average mean values of 100°C					
Time (min)	% β-sheet	% random coil	% α-helix	% turn	% β-sheet (intermolecular)
10	39.18482	29.54779	17.48327	9.811478	3.972635
20	34.07334	30.67028	20.49071	10.55852	4.207146
30	33.81896	31.16259	21.35401	9.376293	4.288147
40	37.81035	31.50398	17.23672	9.56666	3.882287
50	36.5437	32.17263	17.55107	9.496091	4.236503
60	38.40926	30.59533	18.62119	8.509344	3.864865

Figure A5. The mean values of soaking into the water by varying the temperature (50, 60, 70, 80, 90 and 100) and time (10, 20, 30, 40, 50, 60 min).

Average mean values of 50°C					
Time (min)	% β-sheet	% random coil	% α-helix	% turn	% β-sheet (intermolecular)
10	36.26282	33.46163	17.17575	8.95235	4.147455
20	34.9013	33.72102	18.15437	9.08866	4.134645
30	38.0483	28.56988	19.19709	10.05903	4.125699
40	35.23423	31.07395	20.02079	9.344882	4.32615
50	32.95124	30.72303	23.55011	9.21166	3.563962
60	36.22251	31.16247	19.92022	9.070502	3.624298

Average mean values of 60°C					
Time (min)	% β-sheet	% random coil	% α-helix	% turn	% β-sheet (intermolecular)
10	40.30331	30.9072	16.06614	8.619874	4.103482
20	39.63236	31.34681	15.15763	9.481684	4.381512
30	38.21438	30.8532	16.89518	9.777977	4.259273
40	40.24698	28.31325	17.98828	9.68475	3.766744
50	38.67757	30.22096	17.28035	9.997781	3.823337
60	36.92754	29.3885	20.16252	9.361294	4.160148

Average mean values of 70°C					
Time (min)	% β-sheet	% random coil	% α-helix	% turn	% β-sheet (intermolecular)
10	38.18973	33.95158	15.25593	8.951803	3.650967
20	39.79499	32.65347	15.90254	8.05293	3.596078
30	39.437	31.17359	16.61495	8.923147	3.851312
40	36.15289	31.55545	19.53329	8.754016	4.004355
50	39.01719	31.46419	16.82176	8.934794	3.762064
60	35.83992	31.9639	19.82568	8.749676	3.620826

Average mean values of 80°C					
Time (min)	% β-sheet	% random coil	% α-helix	% turn	% β-sheet (intermolecular)
10	37.00962	32.38074	17.4291	9.151794	4.028745
20	36.97731	31.5356	18.78175	8.996805	3.708541
30	35.90485	31.47193	19.52852	9.045527	4.049173
40	36.07364	32.70649	18.4166	8.712111	4.091164
50	36.7786	31.47643	18.4455	9.153633	4.145841
60	35.64778	31.95348	19.22205	9.196181	3.980503

Average mean values of 90°C					
Time (min)	% β-sheet	% random coil	% α-helix	% turn	% β-sheet (intermolecular)
10	37.00962	32.38074	17.4291	9.151794	4.028745
20	36.97731	31.5356	18.78175	8.996805	3.708541
30	35.90485	31.47193	19.52852	9.045527	4.049173
40	36.07364	32.70649	18.4166	8.712111	4.091164
50	36.7786	31.47643	18.4455	9.153633	4.145841
60	35.64778	31.95348	19.22205	9.196181	3.980503

Average mean values of 100°C					
Time (min)	% β-sheet	% random coil	% α-helix	% turn	% β-sheet (intermolecular)
10	35.22927	30.82271	20.72843	9.304446	3.915144
20	38.65788	33.23736	15.84841	8.588179	3.668173
30	38.63747	32.64066	14.8382	9.792132	4.091541
40	41.27103	29.56282	16.01622	9.386113	3.76381
50	37.49193	32.70773	14.88919	9.811953	5.099198
60	37.93214	31.59746	16.46455	10.01622	3.989621

Figure A6. The mean values of dipping into the ice cold-water by varying the temperature (50, 60, 70, 80, 90 and 100) and time (10, 20, 30, 40, 50, 60 min).

Average mean values of treated silk fiber with methanol					
Conc.	% β-sheet	% random coil	% α-helix	% turn	% β-sheet (intermolecular)
0.1	37.1726	32.93358	17.21953	8.947224	3.727062
0.5	37.24913	31.96717	17.3261	9.431902	4.025704
1	35.95414	30.88473	19.39627	9.699065	4.065793

Average mean values of un-treated silk fiber with methanol					
Conc.	% β-sheet	% random coil	% α-helix	% turn	% β-sheet (intermolecular)
0.1	38.31179	29.57344	18.95139	9.49131	3.672065
0.5	37.2813	30.70041	18.40185	9.814757	3.80169
1	37.08058	29.8176	19.66137	9.654421	3.786028

Figure A.7 Methanol treatment of silk fiber (0.1, 0.5 , 1M of NaOH solution, at 60°C, 30 min).

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