

RHEOLOGICAL STUDY OF NON- MULBERRY SILK (TASAR) AND GELATIN BLEND SOLUTION

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Submitted by:

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2K19/PTE/03

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CANDIDATE'S DECLARATION

I Priti, 2K19/PTE/03 student of M.Tech Polymer Technology hereby declare that the project dissertation title “**RHEOLOGICAL STUDY OF NON-MULBERRY SILK (TASAR) AND GELATIN BLEND SOLUTION**” which is submitted by me to the Department of Applied Chemistry, Delhi Technological University, Delhi in the partial fulfilment of the requirement for the award of the degree of Master of Technology in Polymer Technology in the Department of Applied Chemistry, Delhi Technological University, Delhi – 110042, under the supervision of **Prof. Roli Purwar**, is original and not copied from any source without proper citation. To the best of my knowledge, this research work has not been submitted in part or full for the award of any degree or diploma of Delhi Technological University or any other University/Institution.

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CERTIFICATE

I hereby certify that the project dissertation title “**RHEOLOGICAL STUDY OF NON-MULBERRY SILK (TASAR) AND GELATIN BLEND SOLUTION**” which is submitted by PRITI, 2K19/PTE/03, Department of Applied Chemistry, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Master of Technology, is a record of the project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

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Priti

ABSTRACT

I studied the impacts of blending *Antheraea mylitta* silk fibroin solution (10% w/v) with gelatin solution (20% w/v) using formic acid as a solvent in this study. The viscoelastic properties of blends of different ratios such as S0G10, S2G8, S3G7, S5G5, and S10G0 were investigated. To confirm miscibility of pure polymeric components in blend solutions, optical microscopy, thermogravimetric analysis and differential scanning calorimetry were used to analyze surface morphology and thermal properties of the blend solution. Blend solutions with varying shear rates in the range of 0.01-500 s⁻¹ were studied for steady shear behaviour. The dynamic rheological experiment including amplitude and frequency sweeps were performed. Blending gelatin with silk fibroin reduced overall viscosity when compared to pure silk fibroin solution, demonstrating shear thinning behaviour in the applied shear range, according to studies. All of the solutions deviated from the Cox-Merz rule, with the exception of pure gelatin, which obeyed it. A frequency sweep research was carried out with a specific proportion of strain applied. The steady and oscillatory experiments revealed a shift in the solution's behaviour from viscous fluid (as in gelatin) and solid (as in silk fibroin) to viscoelastic. The samples were studied to time-dependent tests such as structural recovery, creep recovery, and stress relaxation. It was discovered that the amount of gelatin in a blend effects its structural recovery. The samples were studied to time-dependent tests such as structural recovery, creep recovery, and stress relaxation. It was discovered that the amount of gelatin in a blend effects its structural recovery. The properties of blend solutions can be tailored by changing parameters such as time, shear rate, angular frequency, and blend ratios to achieve desired features for specific end use applications such as packaging, tissue engineering, medical textile, and filtration, according to such rheological analysis.

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LIST OF ABBREVIATIONS

Symbol	Notation
SF	Silk fibroin
Gel	Gelatin
PVA	Polyvinyl alcohol
BMIMAc	1-butyl-3-methylimidazolium acetate
PBS	Phosphate-buffered saline
CaCl ₂	Calcium chloride
NaSCN	Sodium thiocyanate
LiBr	Lithium bromide
RGD	Arginine-glycine-aspartate
DSC	Differential Scanning Calorimetry
TGA	Thermal gravimetric analysis
T _g	Glass transition temperature
T _m	Melting temperature
T _c	Crystallization temperature
Pa	Pascal
Pa.s	Pascal second
mPa.s	Milli pascal second
cP	Centipoise
SI	International system
US	Standard units
ASTM	American society for testing and materials
w/v	Weight per litre
w/w	Weight per weight
°C	Degree Celsius
η	Viscosity
η^*	Complex viscosity

η_0	Zero shear viscosity
γ	Strain
$\dot{\gamma}$	Shear rate
σ	Stress
ω	Angular frequency
t	Time
λ	Relaxation time
n	Power law exponent
a	Rate index of transition
δ	Phase angle
Tan δ	Loss factor
G'	Storage modulus
G''	Loss modulus
LVE	Linear viscoelastic
J(t)	Creep compliance

CHAPTER 1. INTRODUCTION

1.1 Introduction

Polymer solutions behave eccentrically under variable stress or strain conditions, so it is critical to assess their rheological properties in a way that is representative of the procedure, which might be carried out at the industrial, commercial, or research level. Processing parameters can be optimized with respect to polymers or their blend solutions using rheological study, as the need be.

Silk is a natural biopolymer derived from silkworms and can be divided into two types. Mulberry (*Bombyx mori*) is one, and non-mulberry is the other (*A. mylitta*, *A. assamensis*, *P. ricini* etc.). Because of the existence of arginine-glycine-aspartate (RGD) sequences, non-mulberry silk fibroin has an advantage over mulberry silk fibroin in terms of cell adhesion and proliferation [1]. However, unlike mulberry silk, non-mulberry silk obtained from silkworm cocoons is difficult to dissolve in conventional solvents which are being used for mulberry silk e.g., calcium chloride (CaCl_2), sodium thiocyanate (NaSCN) and lithium bromide (LiBr) etc [2]. After several attempts to dissolve non-mulberry silk fibroin in the above-mentioned standard solvents, Silva et al discovered a few additional solvents, such as ionic liquids, to make a blend solution of non-mulberry silk fibroin [3]. These solvents assist in the breaking of non-mulberry silk hydrogen bonds, rendering them soluble at the cost of disturbing its structural arrangements, which alters its physiological qualities. In order to improve physiological features such as mechanical strength, flexibility, and biodegradability etc., researchers have developed a method for blending silk fibroin with other polymers [4].

Various blends like Silk fibroin/PVA [5], Silk fibroin/Cellulose[6], silk fibroin/ wool keratose [7], Silk fibroin/Chitosan [8], [9],Silk fibroin/Nylon 66[10], Silk fibroin/Nylon 6 [11], Silk fibroin/S carboxymethyl kerateine [12] have been reported in the literature as of now. Only the mulberry type of silk has been explored in the majority of the published research, leaving the non-mulberry type unexplored despite its enormous potential. Researchers' attention has recently moved as a variety of blend materials are being prepared with non-mulberry silk fibroin and polymers like poly (L-lactic acid-co-caprolactone) [13], chitosan [14] and polyvinyl alcohol [15] etc. for a wide range of applications.

Gelatin is biocompatible, biodegradable, and affordable, so it is a viable option for blending with non-mulberry silk fibroin. Collagen is found in animal tissues like skin, muscle, and bone

and is used to produce it [16]. When silk and gelatin are blended, they can create a new class of material with better properties than either pure polymer on its own. However, before using these blends to make a material, it's important to check their solution properties, such as miscibility and viscoelasticity. In order to build materials like films and fibres with tailorable properties, a level of coherence between the solid and fluid like properties of solutions must be obtained while studying viscoelastic behaviour. Rheology is one of the tools used to do such research.

In this research work, steady state rheological curves were obtained to examine the rheological behaviour of silk fibroin, gelatin, and their blend solutions. These data showed the relationship between shear viscosity and shear rate of blend solutions. Due to the limited rheological tests, multiple rheological models were developed to precisely estimate the additional rheological properties of the solutions. When the viscose performance of solutions is dependent on the shear rate, Cross or Carreau model [17], Carreau-yasuda model [18], Power law model [19], and Sisko model [20] are used to characterize viscose properties such as theoretical viscosity at zero and infinite shear rates, relaxation time, and power exponent. Rheological models offer the advantage of explaining the shape and curvature of a flow curve with a small number of fitting parameters while also predicting behaviour at unmeasured shear rates. These models depict a more detailed picture of the molecular structure as it changes during processing, as well as the change in viscoelastic behaviour when mechanical shear is applied. The term viscoelastic refers to a material that combines viscosity and elasticity at the same time. Because polymers' microstructure is composed of long molecular chains, polymers display this behaviour more prominently [21]. Short-term viscoelastic properties like storage modulus (G') and loss modulus (G''), as well as long-term viscoelastic properties like stress relaxation and creep recovery, were studied under dynamic rheological studies. The long-term properties are strain dependent properties that cause the material to soften due to chain scission, viscous flow, bond interchange, and molecular relaxation. [22]. Besides that, the structural recovery as a function of time was monitored to investigate material behaviour at low, high, and low shear rates. Complex viscosity (η^*) and loss factor ($\tan \delta$) were also obtained from dynamic rheological measurements.

As a result, the material's chemical and physical properties were studied as a function of time and mechanical shear. Steady state and dynamic state rheological measurements on all blending solutions, as well as pure polymer solutions, were performed to thoroughly analyze the influence of blending ratios on rheological properties.

1.2 Research gap

1. Almost negligible work has been reported on rheological study of any polymer solution.
2. A review of the literature revealed that, despite its enormous potential, there is very little research on non-mulberry silk fibroin.
3. Rheology is mandatory for processing of any polymeric material; thus, needs to be studied.

1.3 Objective of research work

The following research objectives were determined for this study based on the literature review.

1. Preparation and characterization of several blend solutions of regenerated *Antheraea Mylitta* (Tasar) silk fibroin/gelatin.
2. Determine the miscibility of silk fibroin/gelatin blend solutions in different proportion through optical microscopy, Differential Scanning Calorimetry (DSC).
3. Determine the steady rheological characteristics of silk fibroin/gelatin solutions under variable as well as constant steady shear.
4. Determine the dynamic oscillation behaviors of silk fibroin/gelatin blend solutions through amplitude, frequency sweep, loss factor, complex viscosity, time dependent structure recovery, creep recovery, stress relaxation etc.
5. Fit the rheological model and determine its parameters.

CHAPTER 2. LITERATURE REVIEW

2.1 Rheology

Rheology is the study of the flow of any Newtonian and non-Newtonian material under the influence of an applied force or stress. Rheology is an important characterisation technique for designing materials with desired physical properties and managing manufacturing process parameters to assure end product quality. Rheology is suitable for polymer characterization because it can detect even minor changes in polymer structures. Rheometry is experimental technology to determine rheological properties of polymeric materials viz. steady shear flow, dynamic viscoelastic and time dependent properties of polymeric materials.

2.1.1 Steady shear rheological properties

Viscosity is a characteristic that may be measured at a range of shear rates and different constant shear rates. Viscosity represents the dissipation of deformation energy during flow and assesses how the material can resist flow. In the rheological study, deformational force is expressed in terms of stress, defined as the ratio of applied force per unit area of the sample. Deformation is expressed as a strain, which is the ratio of sample dimensions post and pre deformation. Shear rate is a simple ratio of the velocity of the moving surface after applying force to thickness (or gap between moving and stationary surfaces) of the sample. The mathematical formula of viscosity is a ratio of shear stress and shear rate at a constant temperature, as written below[23]

$$\text{Viscosity} = \text{Shear stress} / \text{Shear rate}$$

The Greek symbol of viscosity is η , is used to denote

The SI unit for viscosity is Pa.s

The US unit for viscosity is centipoise(cP), which is more commonly used

$$1 \text{ cP} = 1 \text{ mPa.s}$$

2.1.2 Dynamic oscillatory rheological properties

The rheometer induces sinusoidal shear deformation in the sample and measures the stress response; the time scale study is determined by the frequency of the shear deformation oscillation. In a typical experiment, the sample is placed on a stationary plate (bottom plate). While a motor rotates the top plate, a time-dependent strain is imposed on the sample. A typical experiment involves placing the sample on a stationary plate (bottom plate). A time dependent strain

$\gamma(t) = \gamma \cdot \sin(\omega t)$ is imposed on the sample. Simultaneously, the time dependent stress $\sigma(t) = \sigma \cdot \sin(\omega t + \delta)$ is measured by measuring the torque imposed on the bottom plate by the sample, as shown in fig 1(a)



Fig 2.1 Rotational rheometer Anton Paar MCR 302

Measuring this time-dependent stress at a specific angular frequency reveals the distinction between materials, namely ideal elastic solids, purely viscous fluids, and viscoelastic materials. The value of $\delta=0$ indicates that stress and applied sinusoidal strain deformation are in the same phase in the case of an ideal elastic solid. The sample stress is proportional to the strain deformation, and their proportionality constant is the shear modulus, as shown in top graph of fig 1(b). In contrast, if the material is a purely viscous fluid, the stress in the sample is proportional to the rate of strain deformation, with the proportionality constant being the viscosity of fluid. The applied strain and measured stress are out of phase, with a phase angle of $\delta=\pi/2$, as shown in the middle graph in fig 1(b). As shown in the bottom graph of fig1(b), viscoelastic materials exhibit a response that includes both in-phase and out-of-phase

contributions; these contributions reveal the extents of solid-like (red line) and liquid-like (blue dotted line) behaviour. As a result, the total stress response (purple line) shows a phase shift between solids and liquids in reaction to applied strain deformation. $0 < \delta < \pi/2$. The storage modulus, $G'(\omega)$, and the loss modulus, $G''(\omega)$, which characterise the solid-like and fluid-like contributions to the measured stress response, respectively, characterize the viscoelastic behaviour of the system at angular frequency ω . The stress response of a viscoelastic material is given by $\sigma(t) = G'(\omega)\gamma_0 \sin(\omega t) + G''(\omega)\gamma_0 \cos(\omega t)$ for a sinusoidal strain deformation $\gamma(t) = \gamma \cdot \sin(\omega t)$ [24].

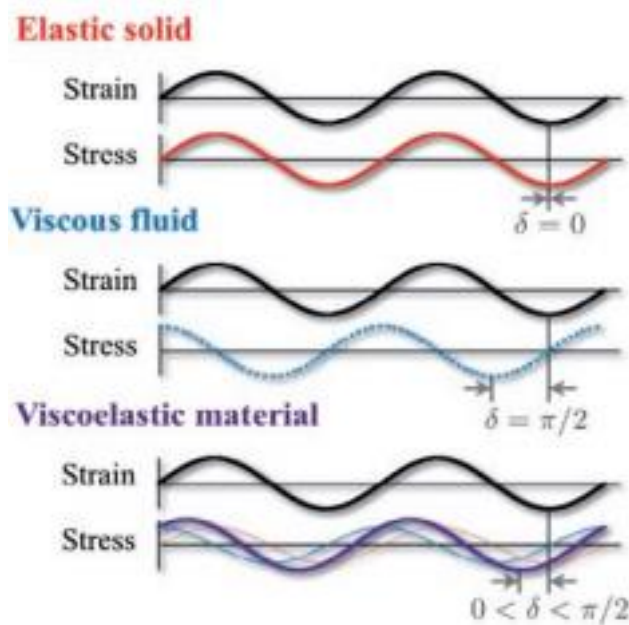


Fig 2.2 The stress response to oscillatory strain deformation of an elastic solid, a viscose fluid, and viscoelastic materials is investigated.

2.1.2.1 Storage modulus

G' denotes the storage modulus, which is pronounced "G prime." The G' -value represents the amount of deformation energy stored by the sample during the shear process. After the load is removed, this energy is completely free to act as the driving force for the reformation process, which will partially or completely compensate for the previously obtained structural deformation. Materials that store the entire deformation energy applied exhibit completely reversible deformation behaviour because, at the end of a load cycle, they have the same shape. As a result, G' represents elastic behaviour of a material. The unit of storage modulus is Pa.

In ASTM D4092, G' is defined as “storage modulus, measured in shear,” as opposed to E' , which is defined as “storage modulus, measured in tension or flexure”[25].

2.1.2.2 Loss modulus

G'' denotes the loss modulus, which is pronounced "G double prime." The G'' -value is a measure of the deformation energy consumed by the sample during the shear process and thus lost for the sample. This energy is expended during the process of changing the structure of the material, i.e. when the sample flows partially or completely.

Flow, also known as viscoelastic flow, denotes relative motion between molecules, particles, aggregates, or other components. Frictional forces are then generated between these components, resulting in frictional heat. This is also known as "viscous heating." This friction process consumes and dissipates energy. A portion of this energy may be used to heat the sample, while another portion may be lost as heat to the surrounding environment. Energy-losing materials exhibit irreversible deformation behaviour because, at the end of a load cycle, they have a different shape. Thus, G'' represents a test material's viscous behaviour. The unit of storage modulus is Pa. In ASTM D4092, G'' is defined as “loss modulus, measured in shear,” as opposed to E'' , which is defined as “loss modulus, measured in tension or flexure”[25].

2.1.2.3 Loss factor

The loss factor is calculated as the ratio of the loss modulus to the storage modulus; it is denoted by $\tan \delta$ and pronounced -tan delta. It has no units. It reveals the viscoelastic and viscoelastic components of viscoelastic materials.

The numerical value of $\tan \delta$ always lies between 0 and infinite. $\tan \delta=0$ implies $\delta=0$ degree in ideal elastic materials, and storage modulus completely dominates loss modulus. In contrast, the ideal viscose fluid has a value of infinite or $=90$ degree, and the loss modulus completely dominates the elastic modulus. In the case of viscoelastic materials, the loss factor value is somewhere in the middle.

2.1.2.4 Linear viscoelastic region

The LVE region denotes the specific range within which the test can be undertaken without disrupting the sample's structure. The curves of the G' and G'' are parallel in the LVE region, and their values are constant, resulting in the so-called plateau value. Furthermore, the values of G' and G'' in the LVE region are frequently assessed. This represents the sample's viscoelastic properties. The sample has a gel-like or solid structure, and is characterized as a

viscoelastic solid substance when $G' > G''$. The sample has a fluid structure and is characterised as a viscoelastic liquid if $G'' > G'$. The measurements are normally required to be taken at strain or stress levels inside the LVE region. When doing an oscillatory test on an unknown sample, an amplitude sweep must be performed first to identify the LVE region's limit.

2.1.2.5 Crossover point

This is the point in a frequency sweep experiment where the storage and loss moduli curves intersect when operated at constant strain, as determined by an amplitude sweep experiment. The loss factor is equal to one at the crossover point.

2.1.2.6 Complex viscosity

A Complex Viscosity is the viscosity determined in an oscillatory experiment. The Complex viscosity is defined as:

$$\eta^* = \eta' - i \eta''$$

Complex viscosity can be expressed in terms of modulus, as written below:

$$\eta^* = G^*/\omega$$

2.1.3 Time dependent properties

2.1.3.1. Structure recovery

This experiment determines the viscosity of a polymer solution as a function of time. In this study, a specific amount of shear is applied for a set amount of time, then increased to a higher value and then returned to the initial low shear value, and structural recovery in terms of viscosity is measured [23]. This type of viscosity change is known as thixotropic behaviour or rheopectic behaviour[25]. Thixotropy is the restoration of initial viscosity following the removal of high shear, which caused the viscosity to decrease in the first place. Such viscosity recovery can be linked to structural recovery in polymer solutions.

2.1.3.2. Creep recovery test

The entire deformation energy that was previously stored by the distorted material during the creep phase can now be recovered and utilised for the reformation process in this test. Because it reveals their time-dependent deformation behaviour, the creep test has the potential to evaluate polymer mechanical responses[26].

2.1.3.3. Stress relaxation test

Under constant strain, stress relaxation refers to a time-dependent reduction in stress. The load or stress required to maintain a fixed quantity of deformation or strain is measured as a function of time to study the polymer's typical behaviour[27].

2.2 Silk

Silk is an example of polypeptide biopolymer. India is one of the world's leading silk producers. Insects make silk in the form of continuous fine strands of fibres that are connected by a glue-like protein called sericin and enclosed in cocoons. It is divided into two types: domestic (mulberry) and wild (non-mulberry). *Bombyx mori* is the example of mulberry type, while *tasar*, *muga* and *eri* etc. are examples of non-mulberry types.

2.2.1 Molecular structure of silk fibroin

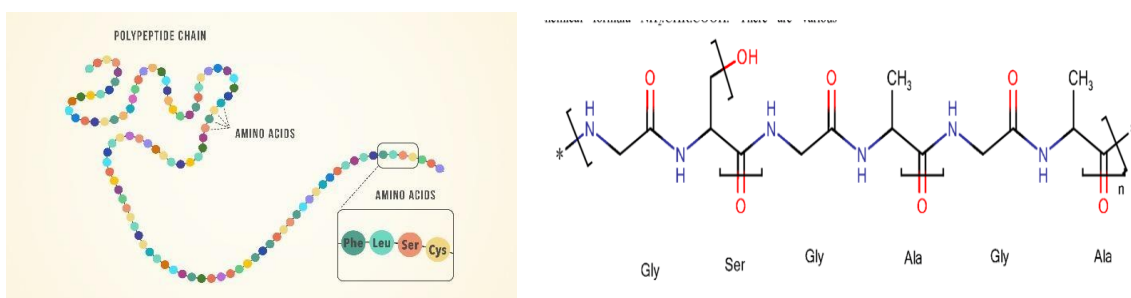
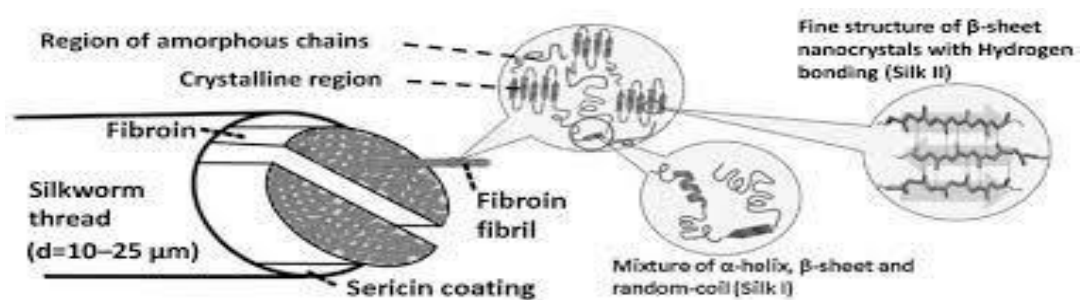


Fig 2.3 Molecular structure of silk fibroin

Amorphous region comprises a combination of α -helix, β -sheet random coil, whereas crystalline region has fine structure of β -sheet nano crystals with hydrogen bonding. Sericin and fibroin are the two most common proteins found in silk. Sericin, the sticky material that

surrounds fibroin and holds the fibrils together, is the structural centre. Fibroin protein is made up of 16 different amino acids.

2.2.2 Crystal structure of silk fibroin

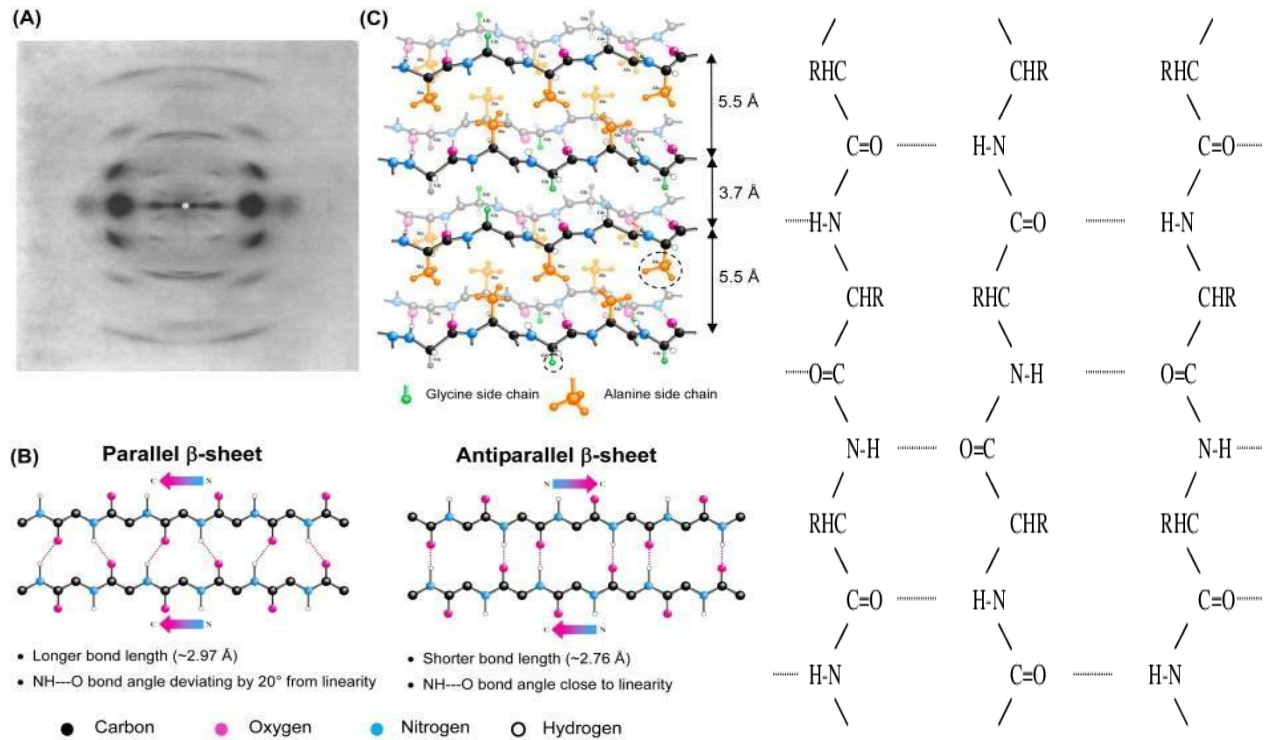


Fig 2.4 Crystal structure of silk fibroin

2.2.3 Category of silk

1. Mulberry Silk (*Bombyx mori*)



2. Tasar Silk (*Antheraea mylitta*)



3. Muga Silk (*Antheraea assamensis*)



4. Eri (*Philosamia ricini*)



Fig 2.5 Category of silk fibroin

2.2.4 Composition of silk

Fibroin	70-75%
Sericin	25-30%
Waxy substances	2-3%
Natural Colours	1-1.5%
Mineral matter	0.5-1%

Silk Composition

Different amino acids present in fibroin (mulberry silk)

Glycine	36.8 %	Valine	2.7 %
Alanine	22.2 %	Phenylalanine	1.5 %
Serine	11.4%	Histidine	0.1 %
Tyrosine	11.1 %	Lysine	0.2 %
Leucine	1.5 %	Threonine	1.3 %
Proline	1%.	Arginine	0.9 %

Different amino acids present in sericin

	Mulberry silk	Wild silk
Lysine	20-30 %	20-30 %
Serine	16-20 %	7-16 %
Glycine	8-10 %	10-20 %
Aspartic acid	8-12 %	7-10 %
Glutamic acid	-	4-6 %
Arginine	-	5-15 %
Threonine	4-6 %	3-10 %
Histidine	4-6 %	3-6 %
Tyrosine	-	4-6 %

2.2.5 Properties of silk

1. Silk is the one of the strongest fiber available.
2. It is highly crystalline (70-80%)
3. Silk possesses elastic-plastic nature but not as elastic as wool due to high crystallinity.
4. It shows 20-25% elongation under normal conditions.
5. It shows very good mechanical strength.
6. Non-mulberry variety possesses cell recognition sites in its amino acid sequence, which helps in tissue growth.
7. Silk sericin is antibacterial, antioxidant, UV resistant and has moisture absorbing properties.
8. It is a biocompatible and biodegradable polymer.
9. It has thermal degradation temperature around 250 C.
10. Silk degraded in around one year.
11. Silk polymer can be degraded faster in “protease solution” than “PBS solution”
12. Its degradation rate (time) can be increased (decreased) by reducing its crystallinity-by blending some other fast degradable polymer like gelatin.
13. Non-Immunogenic.
14. Utilization of cocoons discarded by textile industry
15. Easily available

2.2.6 Application of silk

1. Textile Industry
2. Biomedical Constructs- Surgical sutures, surgical tape etc.
3. Cosmetics
4. Biosensors
5. Tissue engineering- Bone tissue engineering, skin tissue engineering, nerve tissue regeneration, drug delivery etc.

2.2.7 Advantages of non-mulberry silk over mulberry silk

1. Non mulberry is biocompatible
2. Arginine-glycine-aspartate (RGD) sequences are present, which promote cell adhesion and proliferation.

2.3 Gelatin

Gelatin is a biodegradable biopolymer. It is a biopolymer that belongs to the peptide family.

Collagen is partially hydrolysed to produce it. It has extraordinary physical properties.

Collagen is partially hydrolysed from the skin, bones, and tissues of domesticated cattle, fowl, pigs, horses, and fish to make gelatin.

2.3.1 Chemical structure of gelatin

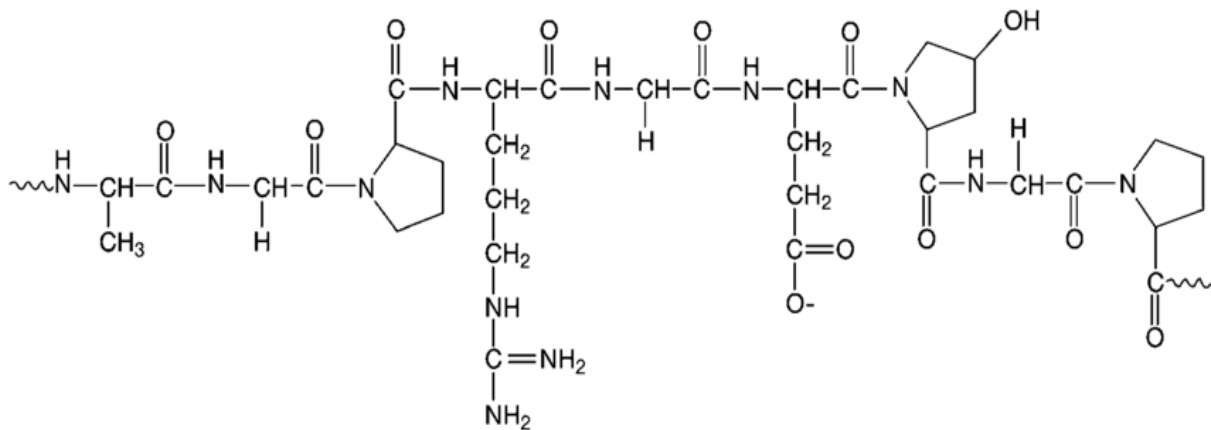


Fig 2.6 Chemical structure of gelatin

2.3.2 Category of gelatin

1) Hard gelatin: Gelatin derived from bones produces tough, forming films that are hard and brittle.

2) Soft gelatin: This is made from pork skin and provides soft and flexible films.

Commercial gelatins are offered in two varieties:

Type A: a product of acid treatment with an isoelectric point of roughly pH-9. It is primarily made from animal skin.

Type B: isoelectric point at pH 4.7, derived after alkali treatment. It is primarily made from animal bones.

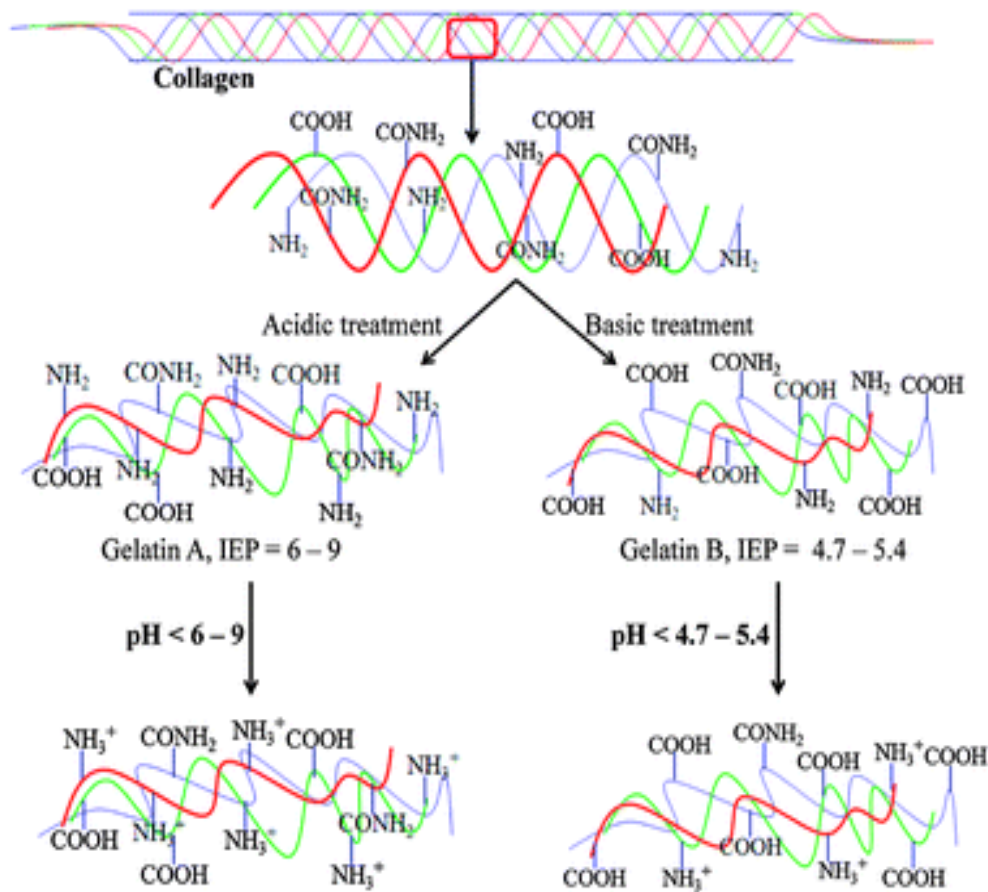


Fig 2.7 Commercial grade of gelatin

2.3.3 Properties of gelatin

1. It is amorphous type.
2. Gelatin is colorless and translucent in physical appearance
3. The mechanical properties of gelatin are very sensitive towards temperature variations.
4. The upper melting point of gelatin is lower than that of the human body.
5. Gelatin readily dissolves in hot water, and sets to a gel on cooling and gel can be transformed into liquid on heating again.
6. It has initial thermal degradation temperature around 250 C.
7. Gelatin can be degraded in a day.
8. It is irreversibly hydrolysed form of collagen.

2.3.4 Hydrolysis of Collagen

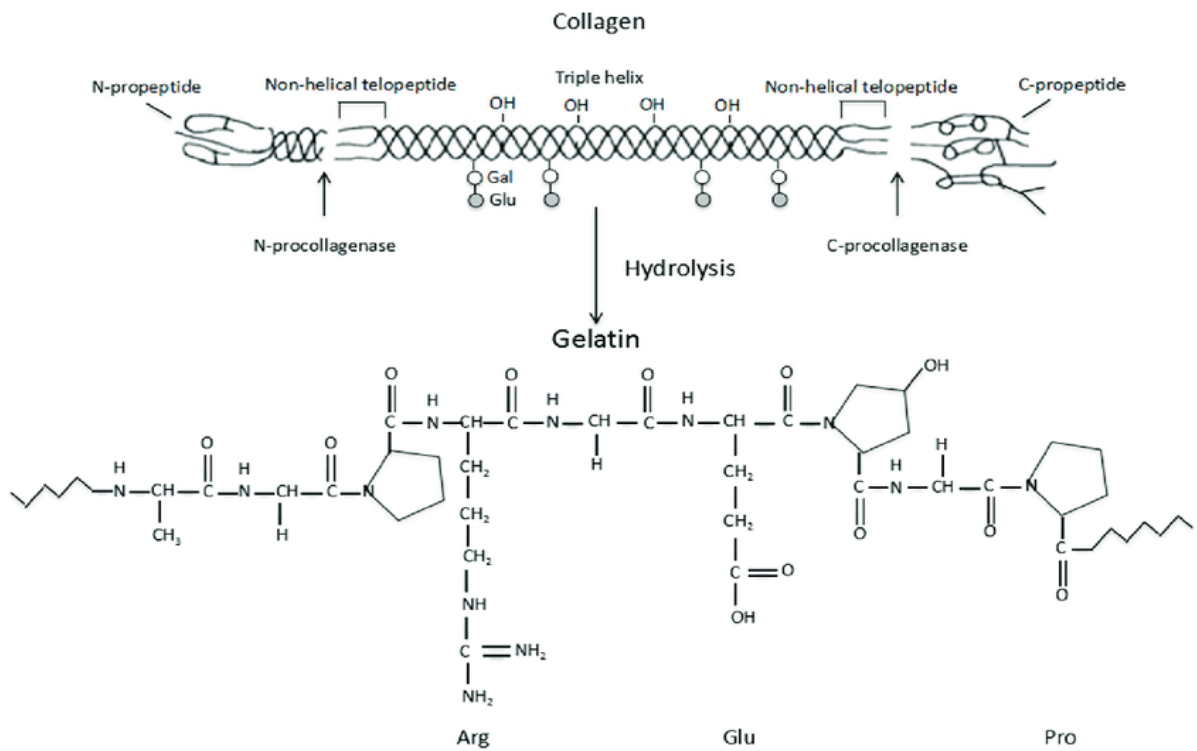


Fig 2.8 Hydrolysis of Collagen

2.3.5 Sol-gel transition of gelatin

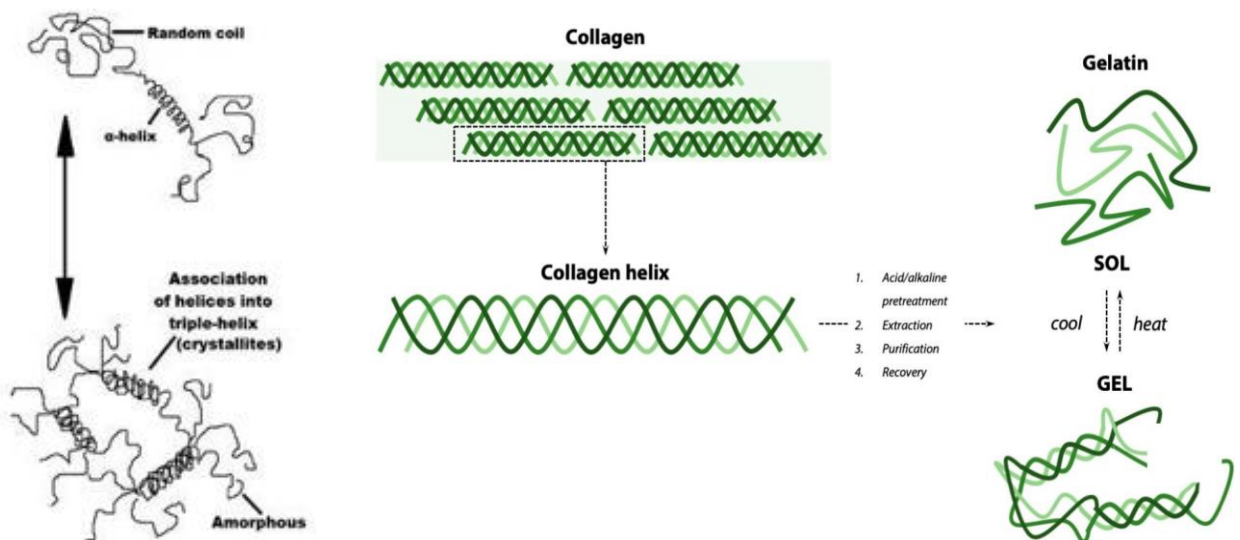


Fig 2.9 Sol-gel transition of gelatin

2.3.6 Application of gelatin

1. Food and packaging industry

- 1.1 Gelatinous desserts, gummy candy, a variety of yoghurts, and certain marshmallows
- 1.2 Agents for thickening, emulsification, binding, and adhesion
- 1.3 Attract and clear contaminants in fruit juices, wines, and vinegar
- 1.4 Frequently added to low-fat dairy products such as yoghurt, ice cream, and buttermilk to simulate the taste of fat.
- 1.5 Food items are protected, maintained, or extended using gelatin-based edible films and coatings.
- 1.6 For packaging applications, active gelatin-based films with possible antibacterial and antioxidant benefits due to additives such as essential oils, metallic nanofillers, and green tea extracts are utilised.

2. Biomedical

- 2.1 In bone tissue engineering, microcarriers are used.
- 2.2 Wound dressings are made of gelatin-based hydrogel films and sponges.
- 2.3 Soft tissue engineering applications benefit from gelatin blended with other polymers.
- 2.4 As a biological adhesive for soft tissues, gelatin and poly-L-glutamic acid hydrogels are utilised.
- 2.5 Drug carriers are made of gelatin nanoparticles.
- 2.6 Contact lenses are made with gelatin.

3. Cosmetics

- 3.1 Skin creams and lotions, face masks, shampoos, hair conditioners, hair sprays, nail polishes, and lipsticks all include gelatin.
- 3.2 Gelatin draws moisture to the product and thickens it, giving it a creamy texture.

4. Pharmaceutical

- 4.1 Gelatin is also used to enclose medication and vitamins in hard and soft capsules
- 4.2 A stainless-steel mould is dipped into a heated gelatin solution to create hard capsules, which are made up of two portions. After that, the capsules are filled with a drug.
- 4.3 Soft capsules, often known as soft gels, are one-part capsules constructed of gelatin sheets. As they are manufactured, they are filled with a drug.

2.4 Significance of polymer blend solutions

Because of the existence of arginine-glycine-aspartate (RGD) sequences, non-mulberry silk fibroin has an advantage over mulberry silk fibroin in terms of cell adhesion and proliferation[1]. Non-mulberry silk derived from silkworm cocoons, on the other hand, is difficult to dissolve in standard solvents used for mulberry silk, such as calcium chloride (CaCl_2), sodium thiocyanate (NaSCN), and lithium bromide (LiBr)[2]. After several attempts to dissolve non-mulberry silk fibroin in the above-mentioned standard solvents, Silva et al discovered a few other solvents, such as ionic liquids, to make a blend solution of non-mulberry silk fibroin[3]. These solvents aid in the breaking of non-mulberry silk hydrogen bonds, rendering it soluble at the cost of disturbing its structural arrangements, which alters its physiological qualities. In order to improve physiological features such as mechanical strength, flexibility, and biodegradability, it was developed, researchers have taken a novel technique to blending silk fibroin with other polymers[28].

A various mixture of blends such as Silk fibroin/PVA[5], Silk fibroin/Cellulose[6], silk fibroin/wool keratose[7], Silk fibroin/Chitosan [8], [9],Silk fibroin/Nylon 66[10], Silk fibroin/Nylon 6 [11], Silk fibroin/S carboxymethyl kerateine [12] have been reported in the literature. Only the mulberry type of silk has been investigated in the majority of the published research work, leaving the non-mulberry type unexplored despite its enormous potential. Researchers' attention has recently switched to the preparation of non-mulberry silk fibroin blend materials with polymers such as poly (L-lactic acid-co-caprolactone)[13], chitosan[14], and polyvinyl alcohol[29] for a variety of applications. Because it is biocompatible, biodegradable, and affordable, gelatin is one of the feasible options for blending with non-mulberry silk fibroin. When silk and gelatin are blended, they can create a new class of material with better properties than either pure polymer on its own. However, before using these blends to make a material, it's vital to examine their solution properties, such as miscibility and viscoelasticity. In order to build materials like films and fibres with tailorable qualities, a level of coherence between the solid and fluid like properties of solutions must be obtained while studying viscoelastic behaviour. Rheology is one of the tools used to do such research.

CHAPTER 3. EXPERIMENTAL

3.1 Material

Central Silk Board, Son Bhadra, Uttar Pradesh, India provided *Antheraea mylitta* silkworm cocoons directly. From Sigma Aldrich, we got the ionic liquid BMIMAc (1-butyl-3-methylimidazolium acetate). Thermo Scientific, India, provided gelatin bacteriological (average M.W. 20000 g mol⁻¹) as well as a Slide-A-Lyzer dialysis flask (20K MWCO, 250 mL). Loba Chemie Pvt. Ltd., India, supplied formic acid with a purity of 98 percent. In the dialysis of silk fibroin ionic solution, Milli-Q water was employed.

3.2 Methodology

Antheraea mylitta silkworm cocoons were degummed using a process previously published [30]. Under nitrogen environment, degummed silk fibres were dissolved in BMIMAc ionic liquid at 80-100°C. This silk fibroin ionic liquid solution was dialyzed for 5 days against miliQ water, with the water being changed every 12 hours. The water in the resultant aqueous silk fibroin solution was evaporated in a hot air oven. This dried silk powder was then dissolved in formic acid to generate silk fibroin stock solution (10% w/v), which was then blended with gelatin stock solution (20% w/v) in formic acid to make blends. Both solutions were agitated for one hour in a magnetic stirrer. The produced stock solutions of pure silk fibroin and pure gelatin were blended in particular ratios (w/w) to obtain the appropriate silk fibroin/gelatin blend solutions.

3.2.1. Pictorial presentation of preparation of regenerated *Antheraea Mylitta* (Tasar) silk fibroin stock solution

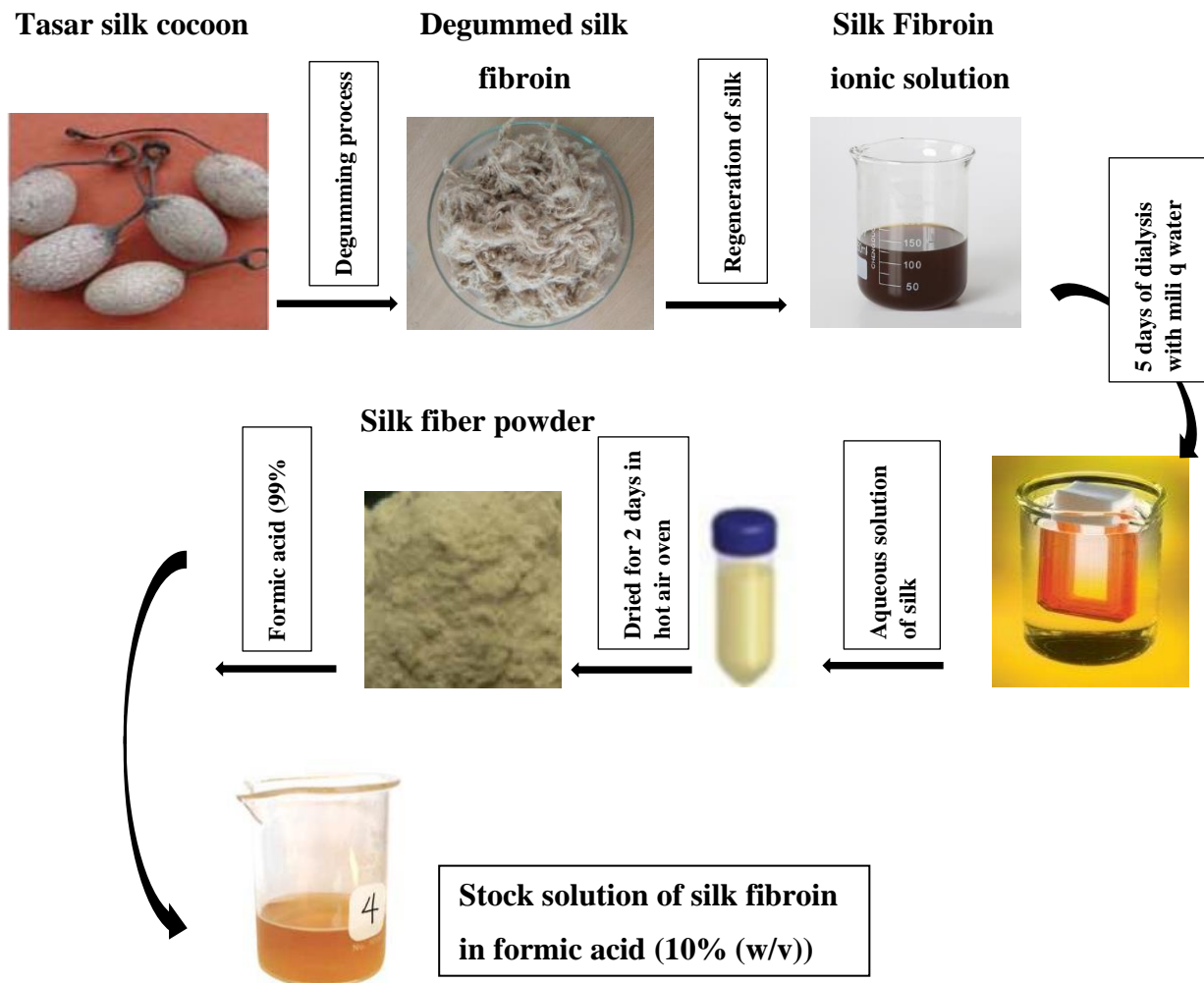


Fig 3.1 Pictorial presentation of preparation of regenerated *Antheraea Mylitta* (Tasar) silk fibroin stock solution

3.2.2. Pictorial presentation of preparation of gelatin stock solution

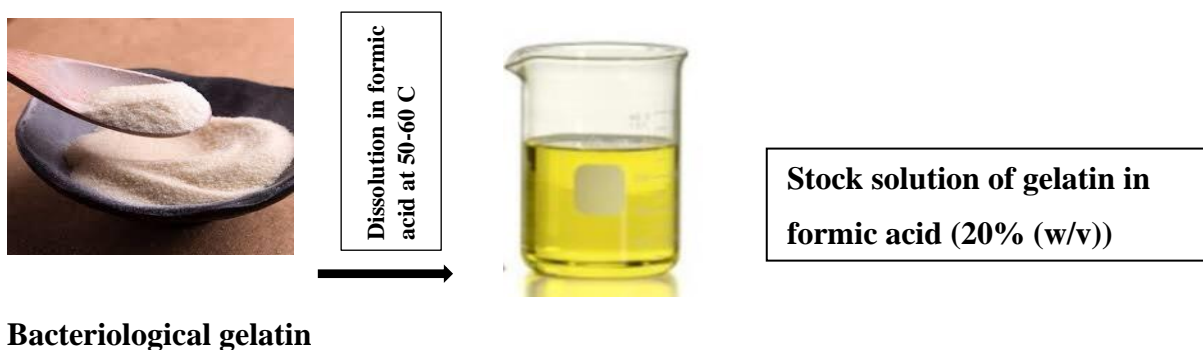


Fig 3.2 Pictorial presentation of preparation of gelatin stock solution

Table 3.1 Silk fibroin/gelatin blend solutions in formic acid abbreviations

S.No.	Silk fibroin 10% (w/v)	Gelatin 20% (w/v)	Sample code	% Solid content of polymer (w/v)
1	100	0	S10G0	10%
2	50	50	S5G5	15%
3	30	70	S3G7	17%
4	20	80	S2G8	18%
5	0	100	S0G10	20%

3.2.3. Pictorial presentation of preparation of silk fibroin/gelatin blend solutions

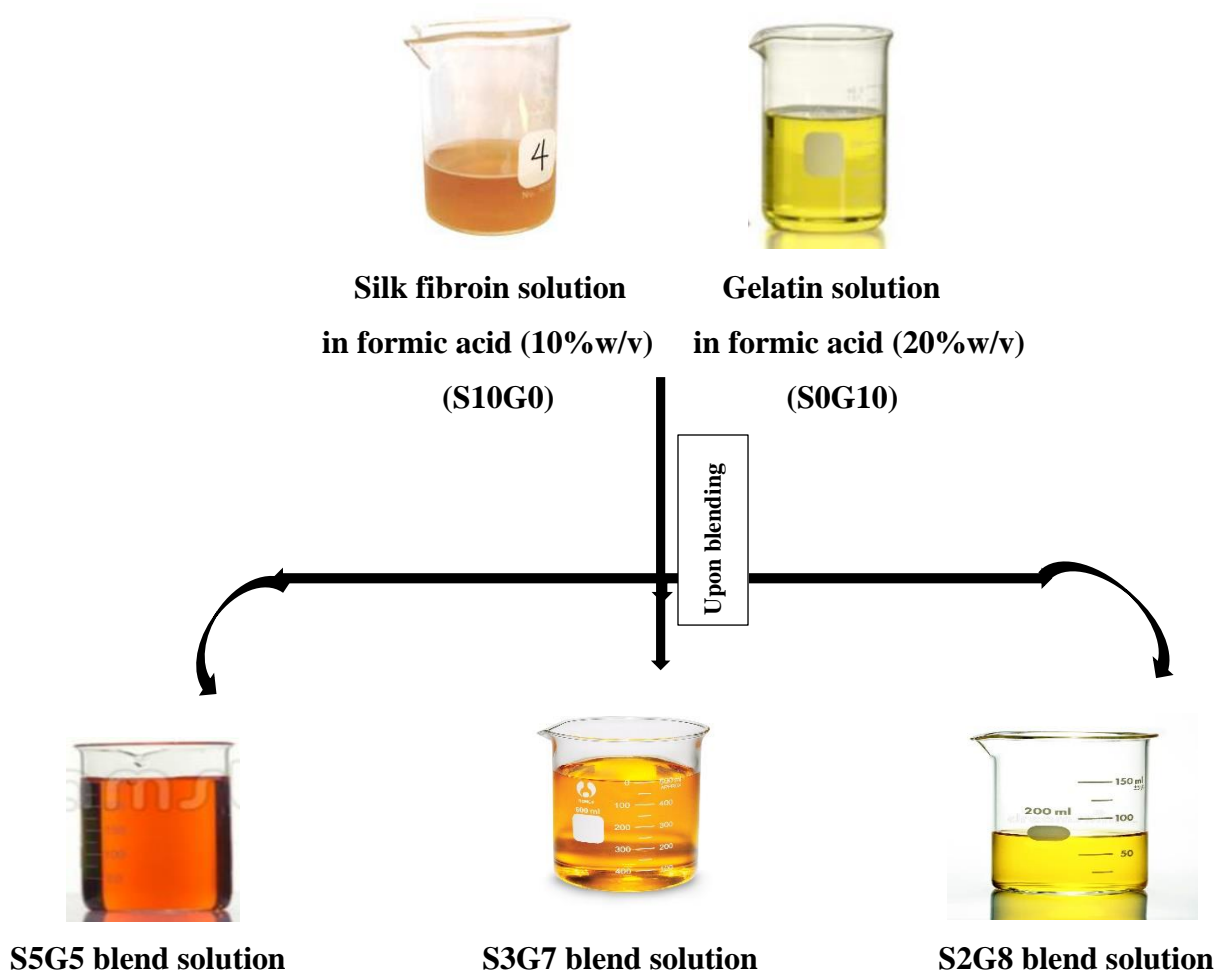


Fig 3.3 Pictorial presentation of preparation of silk and gelatin blend solutions

3.3. Characterization Techniques

3.3.1. Optical microscopy

The optical microscope, also known as a light microscope, is a type of microscope that employs visible light and a lens system to magnify images of small objects that are unable to be seen without a magnifying glass.

1. Putting the sample to be viewed on the microscope slide and covering it with a coverslip.
2. Setting the resolution power of the objective lens to the lowest possible.
3. Position the microscope slide containing the sample to be viewed on the stage and secure it.
4. Raising the stage to get it as close to the objective as possible while avoiding contact between the lens and the sample.
5. Adjusting the focus knob to bring the image into focus while seeing the sample through the eyepiece.
6. Increasing the contrast of the image by changing the condenser and light intensity.
7. Rotate the microscope slide such that the sample's part of interest is in the centre of the field of view.
8. To improve image clarity, adjust the focus knob, condenser, and light intensity once more.
9. Switching to the next objective lens and fine-tuning the focus, condenser, and light as needed to see the image clearly.

The stage should be lowered after using an optical microscope so that the microscope slide may be easily removed.

The surface morphology of the five liquid solutions was examined using a microscope at magnifications of 4X, 10X, 20X, and 40X to validate the maximum miscible blend solution among the five samples.



Fig 3.4 Motic BA410E optical microscopy

3.3.2 Rheology

Rheology is the study of material flow and deformation, as well as how stresses, strains, and time affect the flow. Rheology describes the relationship between a material's characteristics, structure, and processing. The material's response qualities as a function of the following factors:

Shear rate, the method and the magnitude of the imposing stress, the physical and chemical structure and measurement method, external environmental factor such as temperature.

A rotational rheometer was used to examine the rheological properties of all five blend solutions, including steady shear, dynamic oscillatory, and time sweep rheological experiments.



Fig 3.5 Rotational rheometer Anton Paar MCR 302

3.3.3 Thermogravimetric analysis (TGA)

Thermogravimetric analysis studies the amount and rate of change in mass of a sample as a function of temperature or time in a controlled environment. The measurements are primarily used to determine a material's thermal and/or oxidative stabilities, as well as to analyse its composition. The procedure was carried out in a N₂ gas environment. The compositional analysis can be performed using auto stepwise TGA, which heats the sample at 3°C/min and then retains it under isothermal conditions when the instrument detects a considerable weight loss. Thermal stability of silk fibroin and gelatin blends, as well as its pure form, may be determined by Thermogravimetric analysis. When no weight change is seen in a polymeric sample throughout a specified temperature range, the polymer is said to be thermally stable. TGA is used to determine the temperature of thermal degradation, which is required to conduct DSC. So, before running the samples for glass transition temperature (T_g) detection using DSC, the thermal degradation temperature must be determined using TGA. A Perkin Elmer TGA 4000 thermal analysis equipment was used to perform Thermogravimetric analysis (TGA) of silk fibroin and gelatin blend solutions, as well as pure forms of both polymeric solutions. Under nitrogen atmosphere, all five solutions were thermogravimetrically analysed at a rate of 10°C/min between temperatures ranging from 30 to 900°C.



Fig 3.6 Perkin Elmer TGA 4000

3.3.4 Differential Scanning Calorimetry (DSC)

Measurements of transitions such as the glass transition, melting, and crystallization are possible with DSC. Thermal curing, specific heat capacity, and purity analysis are examples of chemical reactions that can be measured. DSC is a technique to determine enthalpy changes as a function of temperature or time induced by changes in a material's physical and chemical properties. The process allows materials to be identified and characterized. Differential scanning calorimetry is quick, accurate, and simple to use. The energy transferred to or from a sample undergoing a physical or chemical change is measured using differential scanning calorimetry (DSC). In a N₂ gas environment, the technique was carried out. DSC may be used to determine the glass transition temperature (T_g), crystallization temperature (T_c) and melting temperature (T_m) of silk fibroin and gelatin blends, as well as their pure form of solutions. Differential Scanning Calorimetry was performed on silk fibroin and gelatin blend solutions, as well as pure forms of both polymeric solutions, using Perkin Elmer DSC 8000 equipment. T_g can occur as an amorphous material's temperature increases. These transitions manifest as an endothermic peak in the DSC signal curve, with the lowest temperature corresponding to the initial exothermic peak. As the temperature rises, an amorphous substance loses its viscosity. The molecules may finally get enough freedom of motion to spontaneously arrange themselves into a crystalline structure, at which point the temperature is known as the crystallization temperature (T_c) and a second exothermic peak is produced. The amorphous to crystalline transition occurs. As the temperature rises, the sample approaches melting temperature (T_m). The melting process causes the endothermic peak in the DSC curve. Differential scanning calorimetry (DSC) was performed using thermal analysis instrument with a scanning speed of 10°C/min and a nitrogen gas flow of 50ml/min.



Fig 3.7 Perkin Elmer DSC 8000

CHAPTER 4: RESULT AND DISCUSSION

4.1 Rheology

4.1.1 Steady shear rheological behaviors of silk fibroin and gelatin blend solutions

4.1.1.1 Effect of variable shear rate on shear viscosity

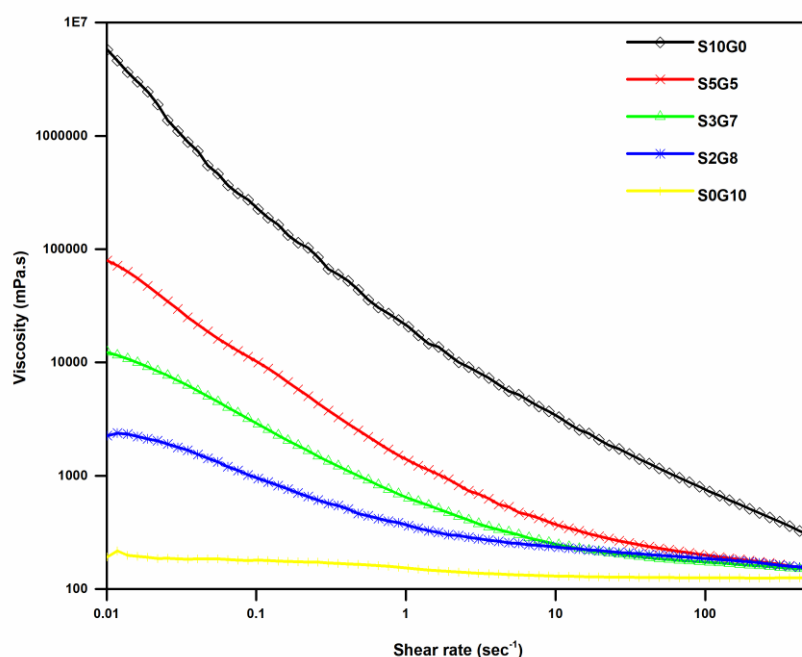


Fig 4.1 Steady shear rheological curves of the silk fibroin/gelatin blend solution with different blending ratios at 25 °C.

At 25 °C, the steady shear profiles of silk fibroin/gelatin blend solutions were investigated, as shown in Fig. 4.1. Because of differences in macromolecular chain entanglement, the viscosities of polymeric solutions varied with changes in polymer content. In the applied range of shear rate (0.01-500 s⁻¹), the low concentration of polymer in S10G0 (10%) showed the highest viscosity at low shear rate, suggesting a highly entangled polymer structure in the solution, which subsequently displayed shear thinning behaviour when high shear was applied. In S0G10, however, the high polymer concentration (20%) resulted in lower viscosity at low shear rates and virtually little shear thinning as the shear rate was increased. This behaviour showed that polymer chains in the solution were less entangled molecularly. Their viscosities changed with respect to the polymeric component when these two were blended in various

ratios. The blend solution with the highest silk fibroin concentration (S5G5) had a higher viscosity than the blend solutions with the highest gelatin content (S3G7, S2G8). Even though its polymer concentration is lower (15%) than the other two blends (17% and 18% for S3G7 and S2G8 respectively) owing to the higher entanglement of molecular chains. As indicated by the shear thinning behaviour of these solutions, the application of high shear rate resulted in disentanglement and further alignment of molecular chains in the blend solutions. In the literature, mulberry silk fibroin solution in formic acid exhibited similar shear thinning behaviour [30].

4.1.1.2 Carreau-Yasuda model fitting for silk fibroin and gelatin blend solutions

A comparison of the apparent viscosity at shear rates 1, 50, and 100 s⁻¹ was done to further investigate the link between viscosity and shear rates at varied blend ratios. The values of viscosities at various shear rates can be derived directly from experimental data; however, zero shear viscosity can only be acquired through rheological model fitting.

The three-parameter Carreau-Yasuda model [31] was used to conduct one such fitting, and the fitted curve is shown in Fig. 4.2.

$$(\eta - \eta_{\infty})/(\eta_0 - \eta_{\infty}) = [1 + (\lambda\dot{\gamma})^a]^{\frac{n-1}{a}} \quad (1)$$

Where η represents the viscosity in mPa.s, η_{∞} means the infinite shear-rate viscosity in mPa.s, η_0 denotes the zero shear-rate viscosity in mPa.s, λ is the relaxation time in sec, $\dot{\gamma}$ represents the shear rate in s⁻¹, n denotes the power-law exponent and a is known as the rate index of transition.

The usual rheological parameters such as η_{∞} , η_0 , λ , n , a and linearity R^2 of all five solutions were found using curve fitting, as shown in Table 4.1. Because Carreau's model is commonly applicable to non-Newtonian solutions, the rheological parameters obtained by fitting this model might not be as reliable for pure gelatin solution as they are for the other four solutions. The flowability of the solutions was investigated in relation to plateau length using a plot of $\log \eta$ vs $\log \dot{\gamma}$ as shown in Fig. 4.2. In general, in polymer solutions, a longer plateau is linked with Newtonian behaviour. Throughout the applied range of shear rate, pure silk fibroin solution showed no plateau, indicating Non-Newtonian behaviour, whereas pure gelatin solution was determined to be Newtonian, displaying the longest plateau. At high shear rates,

the effect of blending silk fibroin with gelatin was visible as a small plateau. Furthermore, when the gelatin concentration in blend solutions increases, the plateau length increases, reflecting greater Newtonian behaviour at a higher shear rate. Researchers earlier discovered a similar shift in plateau length for gelatin solution in ionic liquids [6][32]

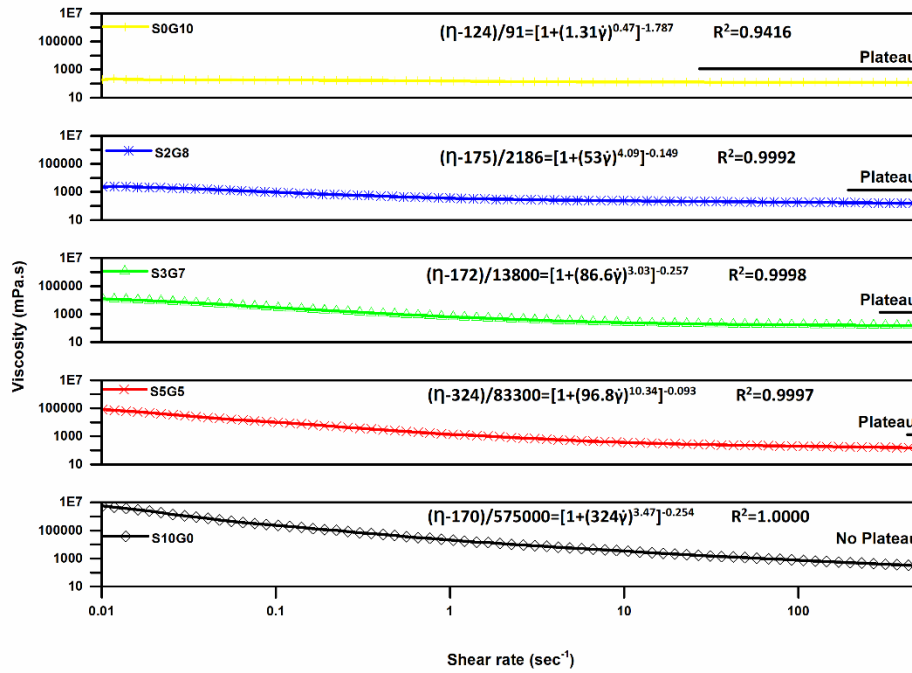


Fig 4.2 Carreau-Yasuda model fitting for silk fibroin/gelatin blend solutions with various blending ratios

Table 4.1 Model fitting was used to get rheological parameters.

S.No.	Sample code	η_0 (mPa.s)	η_∞ (mPa.s)	λ (sec)	n	a	R^2
1	S0G10	215	124	1.31	0.160	0.47	0.9416
2	S2G8	2361	175	53	0.391	4.09	0.9992
3	S3G7	13972	172	86.6	0.221	3.03	0.9998
4	S5G5	83624	324	96.8	0.038	10.34	0.9997
5	S10G0	575170	170	324	0.119	3.47	1.000

4.1.1.3 Comparison of estimated values vs experimental values of silk fibroin and gelatin blend solutions

For the purpose of comparing the estimated and experimental viscosities of blend solutions at the shear rate of 0 s^{-1} (η_0), 1 s^{-1} (η_1), 50 s^{-1} (η_{50}) and 100 s^{-1} (η_{100}) were analysed. η_1, η_{50} and η_{100} of pure solutions was determined using experimental data, whereas η_0 was determined via rheological model fitting.

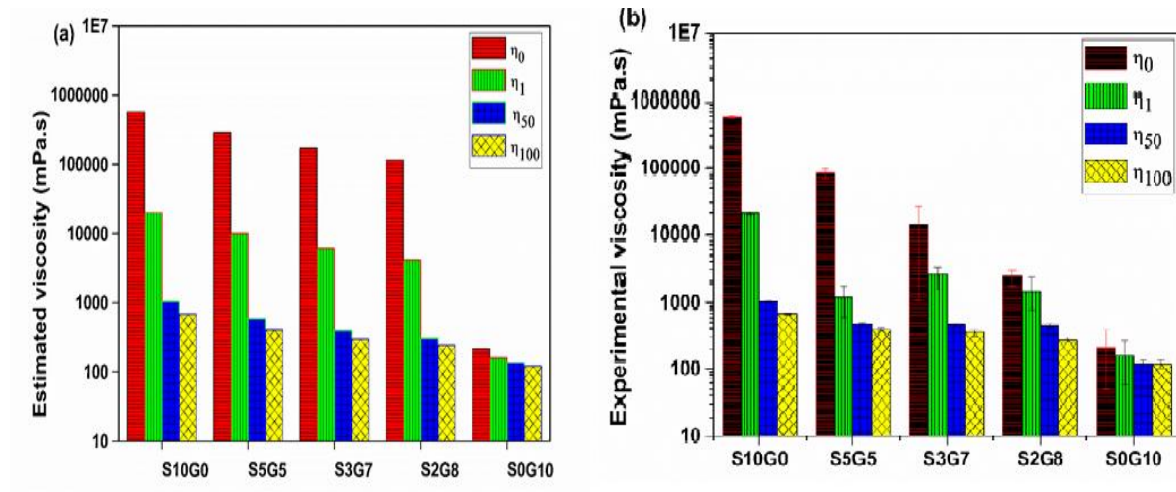


Fig 4.3(a) Estimated viscosity values, **Fig 4.3(b)** Experimental viscosity values at shear rates of 0, 1, 50, and 100 s⁻¹, the viscosity of all silk fibroin/gelatin blend solutions was measured.

4.1.1.4 Log-additivity rule for silk fibroin and gelatin blend solutions

While studying the solution properties in blends, miscibility is a parameter of concern as it affects their properties drastically. To check the compatibility of silk fibroin with gelatin in blend solutions, viscoelastic functions can be calculated from the log-additivity rule. In literature, this rule has previously been applied to study the blend miscibility of polymer solutions by several researchers. Zhang and Pan studied the miscibility of silk/PVA blend solutions by analysing the deviation behaviour of viscoelastic functions[5]. Yao and co-workers analysed the miscibility in silk/cellulose blend solution with the help of log-additivity rule[6].

Miscibility is a criterion to consider while investigating the solution properties of blends because it has a significant impact on their properties. The log-additivity rule can be used to calculate viscoelastic functions to see if silk fibroin and gelatin are compatible in blend

solutions. Assuming Silk fibroin and gelatin are entirely miscible, equation 2 can be used to calculate viscosity and dynamic modulus.

$$\log F = W_{SF} \log F_{SF} + W_{gel} \log F_{gel} \quad (2)$$

Where F : Viscoelastic function like viscosity, dynamic modulus etc.

W_{SF}, W_{gel} : Weight fraction of silk fibroin and gelatin in blend solution respectively.

F_{SF}, F_{gel} : Viscoelastic function of pure silk fibroin and gelatin respectively.

Fig 4.3(a) and 4.3(b) show a comparison of estimated viscosity values obtained using the additivity rule as per equation 2 with experimental viscosity values of pure and blended solutions at shear rates of 0, 1, 50, and 100 s^{-1} . Based on the $\eta_0, \eta_1, \eta_{50}$, and η_{100} results presented in Fig 4.3, the discrepancy between actual values (bold lines) and estimated values (dotted lines) of apparent viscosities of blend solutions was depicted in a new Fig. 4.4.

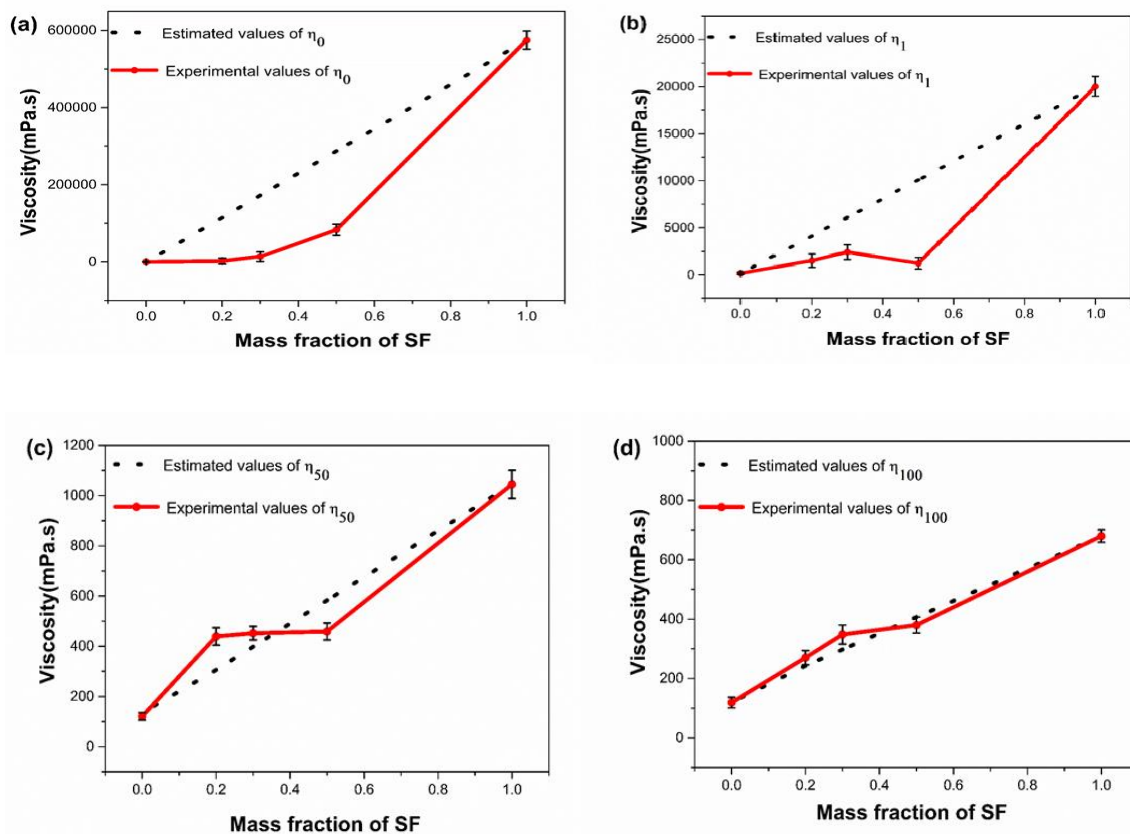


Figure 4.4 Plots of the estimated values (the blue dotted lines) and experimental values (the red bold lines) of the viscosities at a shear rate of (a) 0 s^{-1} , (b) 1 s^{-1} , (c) 50 s^{-1} , (d) 100 s^{-1} for silk fibroin/gelatin blend solutions with a different mass fraction of silk fibroin

The obtained experimental values deviated from the estimated parameters, showing partial miscibility of the polymeric components with a continuous-discrete phase morphology. Positive deviation is generally related to good interface interaction between the two polymeric components (homogeneity) and negative deviation indicates the poor compatibility or uneven mixing (heterogeneity) of the polymers[6]. At zero shear, the apparent viscosities showed a negative deviation for all blend ratios examined, as can be seen. This is due to the incompatibility of silk fibroin and gelatin in steady state, as their viscosities are significantly different. As the shear rate was raised, the apparent viscosity deviation from the estimated values varied, and phase morphology was observed to change for particular blend compositions. Fig 4.5 depicts the likely phase morphology of these solutions. When the silk component in the blends was decreased, the apparent viscosities approached the estimated values at low shear rates (1 s^{-1}), but negative deviation remained. S2G8 and S3G7 showed inversion of phase morphology with positive deviation in viscosities at high shear rates (50, 100 s^{-1}), however S5G5 showed only negative deviation.

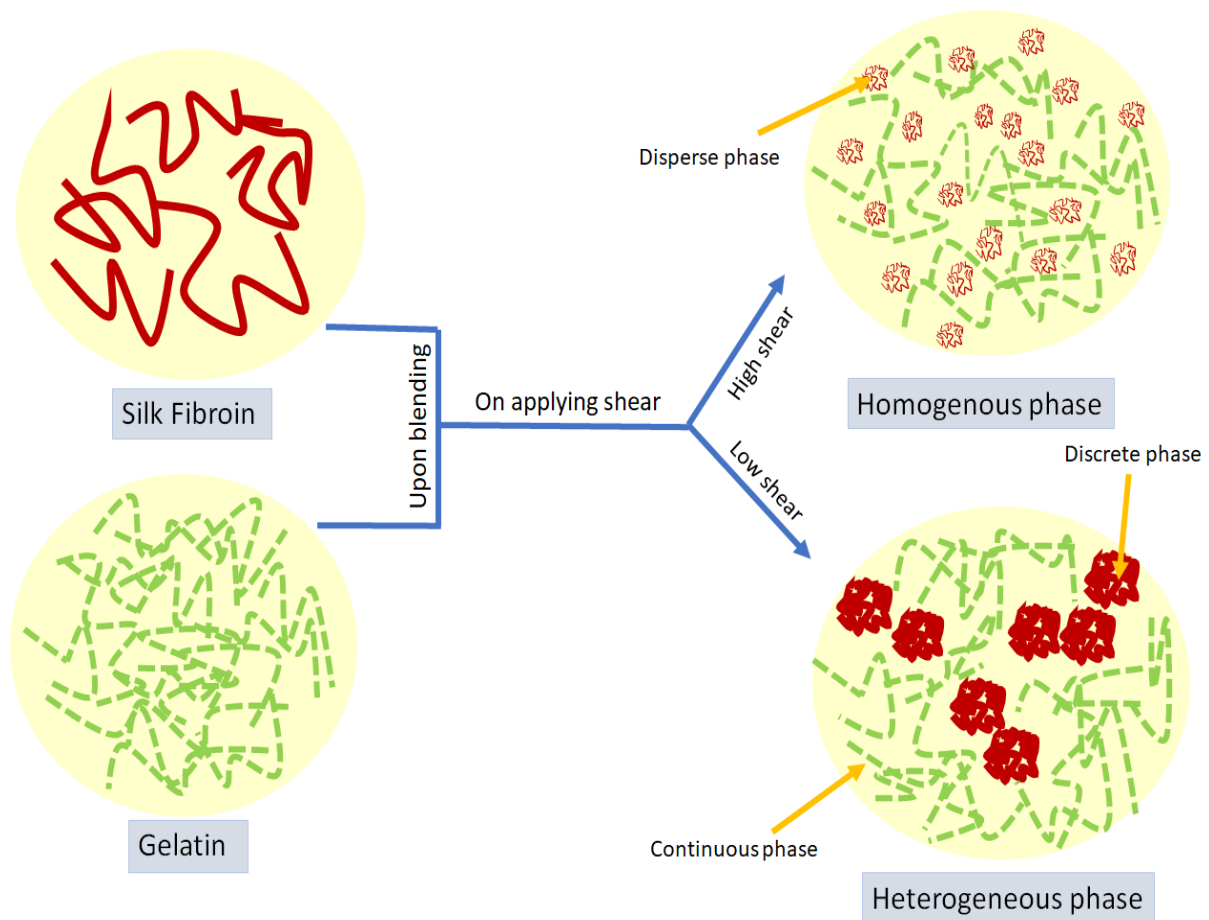


Figure 4.5. A schematic figure depicting the likely phase morphology of silk fibroin/gelatin blend solutions when shear rates are applied at low and high levels.

It's possible that because the concentration of gelatin in the blend solutions is higher (20%), it exists as a continuous phase while silk fibroin exists as a dispersed phase. The size of the continuous and dispersed phases, on the other hand, is variable and depends on the polymeric content as well as the relative viscosities of the two phases. Figure 4.12 shows the size variation of the dispersed silk fibroin phase in the continuous gelatin phase at high shear rate as shown using an optical microscope. Because the concentration of highly viscous silk fibroin is low in S2G8 and S3G7, the size of the dispersed phase is smaller than in S5G5, resulting in the formation of a homogeneous blend morphology characterised by positive deviation in apparent viscosities with increased shear rate. Due to the higher concentration of highly viscous silk fibroin in S5G5, the size of the dispersed phase is large enough even after applying a high shear rate, producing a heterogeneous blend morphology with negative apparent viscosities. The phase morphology of polymer blend solutions has been shown to be influenced by applied shear rate and polymer composition in the literature [5], [6]. Yao et al. found that applying high shear increased the interface interaction of silk and cellulose, which is similar to our results. High shear can be utilised to generate homogeneous blend solutions for smooth processability while electrospinning or 3D printing because it can improve the dispersion of silk fibroin in gelatin.

To conclude, all blend solutions were unstable at zero shear rate, but as shear rate increased, different behaviour was appeared, with blend solutions with more than 17% polymer content, such as S2G8 and S3G7, approaching the estimated value at shear rate 1 s^{-1} , even with negative deviation. Positive deviation began to appear as the shear rate was increased, i.e., 50 s^{-1} and 100 s^{-1} blend solutions with more than 17% polymer content, as shown in Fig. 4.4 (b), (c), and (d).

4.1.2 Dynamic oscillatory behaviors of silk fibroin and gelatin blend solutions

4.1.2.1 Amplitude sweep

The amplitude of the deformation or shear strain is altered while the frequency remains constant in amplitude sweep. The frequency was kept at 10 rad/sec in this current research. To investigate the linear viscoelastic region (LVER) of solutions, the storage modulus (G') and loss modulus (G'') were plotted versus deformation or % shear strain, as shown in Fig 4.6.

LVER is the region until G' and G'' remain constant, indicating that blend solutions are stable and their structures are unaffected. When G' is more than G'' , the sample acts more like a viscoelastic solid; when G' is less than G'' , the sample behaves more like a viscoelastic fluid [33][34]. As demonstrated in Fig. 4.6(a), the S10G0 solution exhibited viscoelastic solid behaviour up to 70% shear strain and viscoelastic fluid-like behaviour above that. Surprisingly, the behaviour of S0G10 is viscoelastic fluid across the shear strain range, as seen in Fig 4.6(e). Furthermore, as illustrated in Figs 4.6(b), (c), and (d), all blend solutions behaved as viscoelastic solids at low shear strain and viscoelastic fluids at high shear strain. This is in agreement with the steady shear analysis we did before.

The frequency sweep analysis will be performed using the LVE region generated in Fig. 4.6 by applying precise strain within this region.

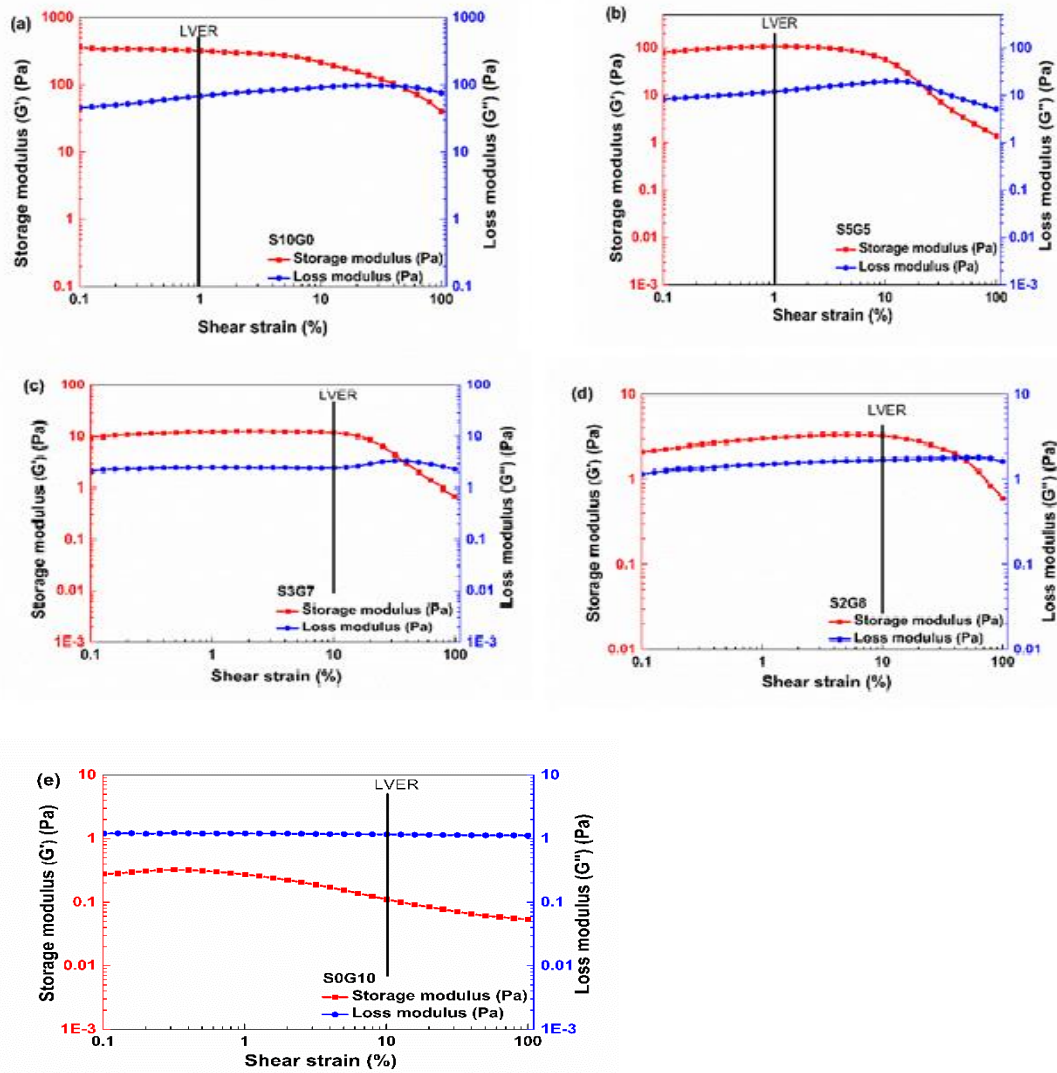


Fig 4.6 Amplitude sweep indicating linear viscoelastic (LVE) region (a) S10G0, (b) S5G5, (c) S3G7, (d) S2G8, and (e) S0G10.

4.1.2.2 Frequency sweep

The frequency was varied from 0.1 to 100 rad/sec in frequency sweep, and the percent shear strain of each blend (S10G0=1%, S5G5=1%, S3G7=10%, S2G8=10%, and S0G10=10% shear strain) was held constant inside the LVE region as determined by amplitude sweep. As illustrated in Fig 4.6, G' and G'' were plotted versus angular frequency for analysis.

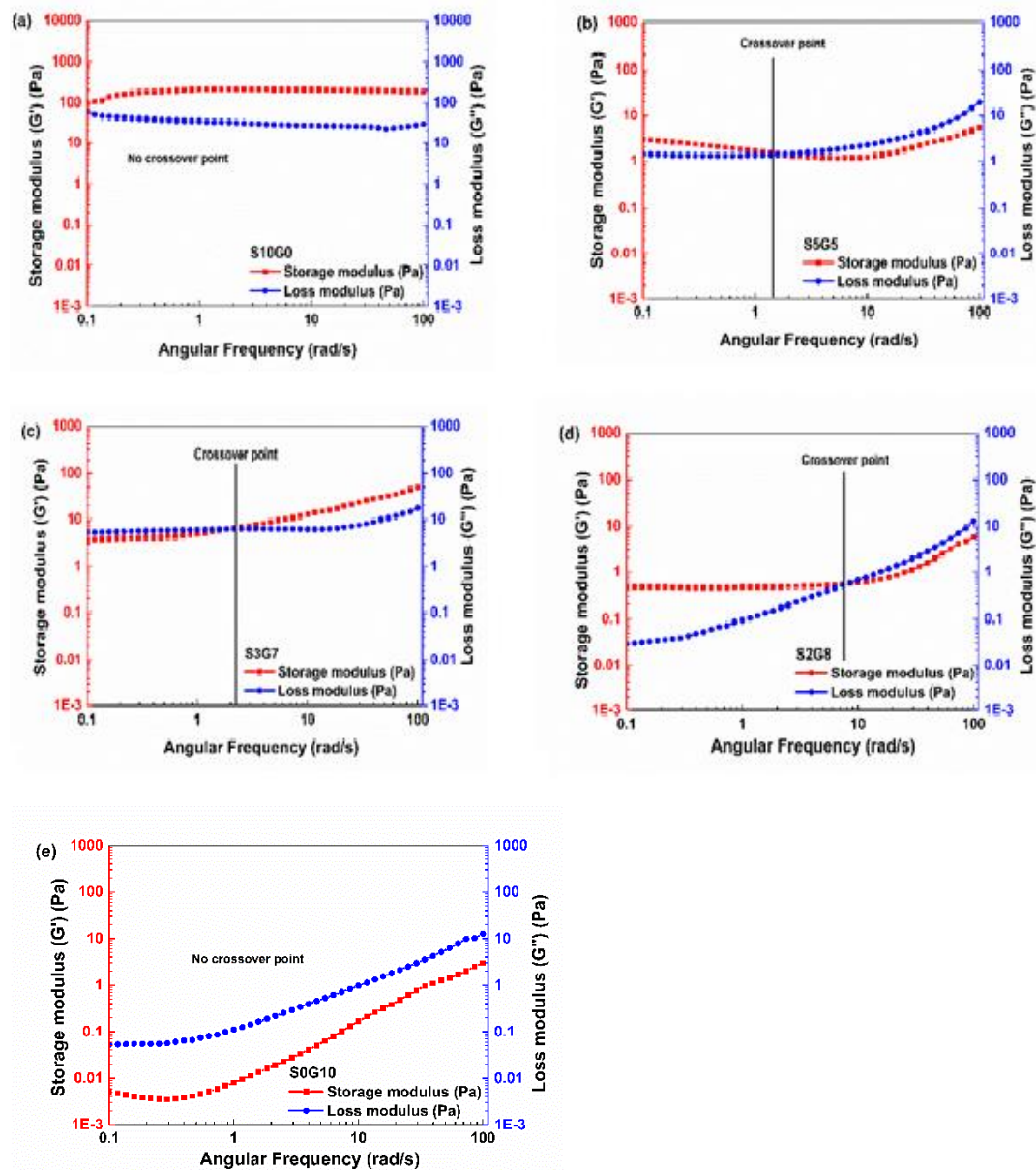


Fig 4.7 Frequency sweep of silk fibroin and gelatin solutions (a) S10G0 (b) S5G5, (c) S3G7, (d) S2G8, and (e) S0G10.

G' dominates the angular frequency range representing viscoelastic solid behaviour for S10G0, as seen in Fig 4.7(a). G'' was prominent over the angular frequency range in pure gelatin (Fig 4.7(e)), implying viscous fluid behaviour. The viscoelastic behaviour of both the native was seen to change after blending. In the case of S5G5 and S2G8 (Fig 4.7(b) and 4.7(d)), G'' is higher than G' at high frequencies, indicating viscoelastic fluid behaviour, however at lower frequencies, a crossover point is found, after which the trend of G'' and G' reverses, indicating viscoelastic solid behaviour. However, as seen in Table 4.2, the position of crossover points differed between such two blends. Surprisingly, the viscoelastic behaviour of the S3G7 solution (Fig 4.7(c)) differed from that of the other two blends, which could be due to the intermolecular interactions of silk fibroin and gelatin, but more research is needed to be sure. Overall, blending silk fibroin with gelatin resulted in solutions that behaved at different frequencies like viscoelastic solids as well as fluids. This means that such changes in the behaviour of polymeric solutions can help them process more easily. Silk fibroin solution has previously been shown to have similar frequency-dependent viscoelastic properties[34]. By altering the oscillation frequency, Mu et al were able to interconvert gel and fibrils using silk's viscoelastic characteristic[35]

Table 4.2 Frequency sweep data of pure silk fibroin / pure gelatin and their blend solutions.

Sample code	At low frequency			Crossover point	At high frequency			Moduli at crossover point
	G'	G''	Loss factor (Tan δ)	Angular frequency in rad/sec	G'	G''	Loss factor (Tan δ)	
S0G10	0.004	0.028	6.62	No crossover	0.97	3.75	3.88	-
S2G8	0.085	0.03	0.53	8.04	1.28	6.02	4.71	6.32
S3G7	1.29	2.41	1.38	2.37	16.21	9.11	0.62	1.4
S5G5	5.51	2.61	0.54	1.44	17.60	25.51	2.63	-
S10G0	120.89	48.72	0.16	No crossover	186.28	26.29	0.67	-

4.1.2.3 Loss factor

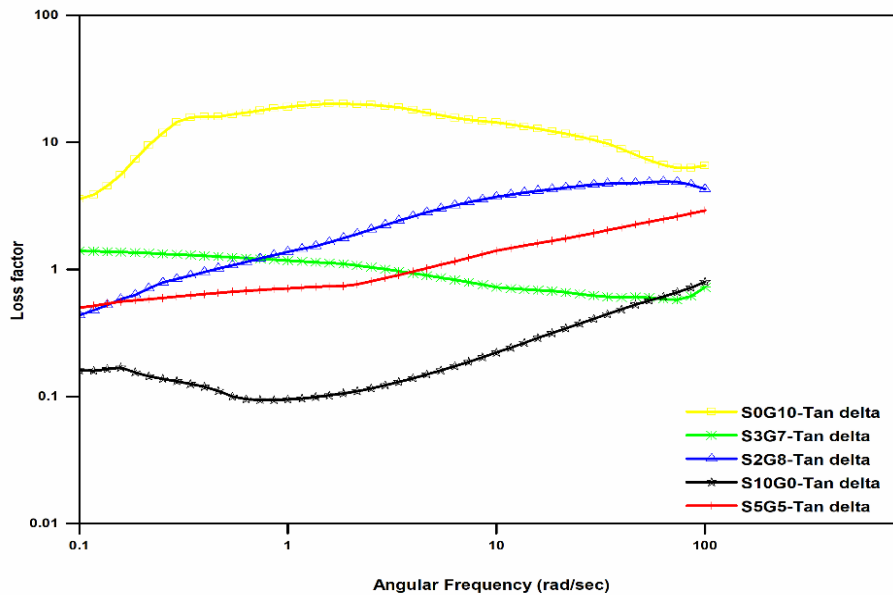


Fig 4.8 Loss factor (Tan δ) for silk fibroin/gelatin blending solutions with various blending ratios as a function of angular frequency

Tan δ denotes the loss factor as the ratio of G'' and G' . This is a crucial criterion for determining the viscoelasticity of polymer solutions. When Tan δ is more than one, the fluid property of the viscoelastic material takes precedence, while when Tan δ is less than one, the solid property takes precedence. Fig 4.8 shows that in the whole frequency range, pure silk fibroin solution had a Tan δ value less than one, but pure gelatin solution had a Tan δ value more than one. This indicates that solid-like behaviour dominates in silk fibroin solution, but fluid-like behaviour dominates in gelatin solution. Changes in frequency were found to alter the loss factor in blend solutions, as shown in Table 4.2. Tan $\delta > 1$ was seen at high frequency in S2G8 and S5G5, however it was less than one in S3G7. The blend solutions revealed the exact opposite at low frequencies.

4.1.2.4 Complex viscosity

As illustrated in Fig 4.9, comparison plots of complex and shear viscosity investigating the viscous properties of pure and blend solutions were generated. Despite being measured under steady and dynamic conditions, the viscosity and complex viscosity of pure silk fibroin and blend solutions showed a nearly identical trend but numerically different values, contradicting

the well-known Cox-Merz rule that states that these two viscosities should be numerically equal. However, depending on the type of entanglement among polymeric chains in the solution, the values of complex and shear viscosities can differ from one another, according to the literature [36].

Shear viscosity of blend solutions decreases with increasing gelatin content in blend solutions, as shown in Table 4.3, and follows the trend

S10G0>S5G5>S3G7>S2G8>S0G10 at low shear rate and

S10G0>S5G5>S2G8>S3G7>S0G10 at high shear rate.

With increasing gelatin content, the complex viscosity of the blend solutions decreases, with S10G0>S3G7>S5G5>S2G8>S0G10 at low angular frequency and

S10G0>S3G7>S5G5>S0G10>S2G8 at high angular frequency.

The structural disruptions that may have happened as a result of uncoiling on applying shear are responsible for the observed discrepancy in shear viscosity and complex viscosity values in Table 4.3

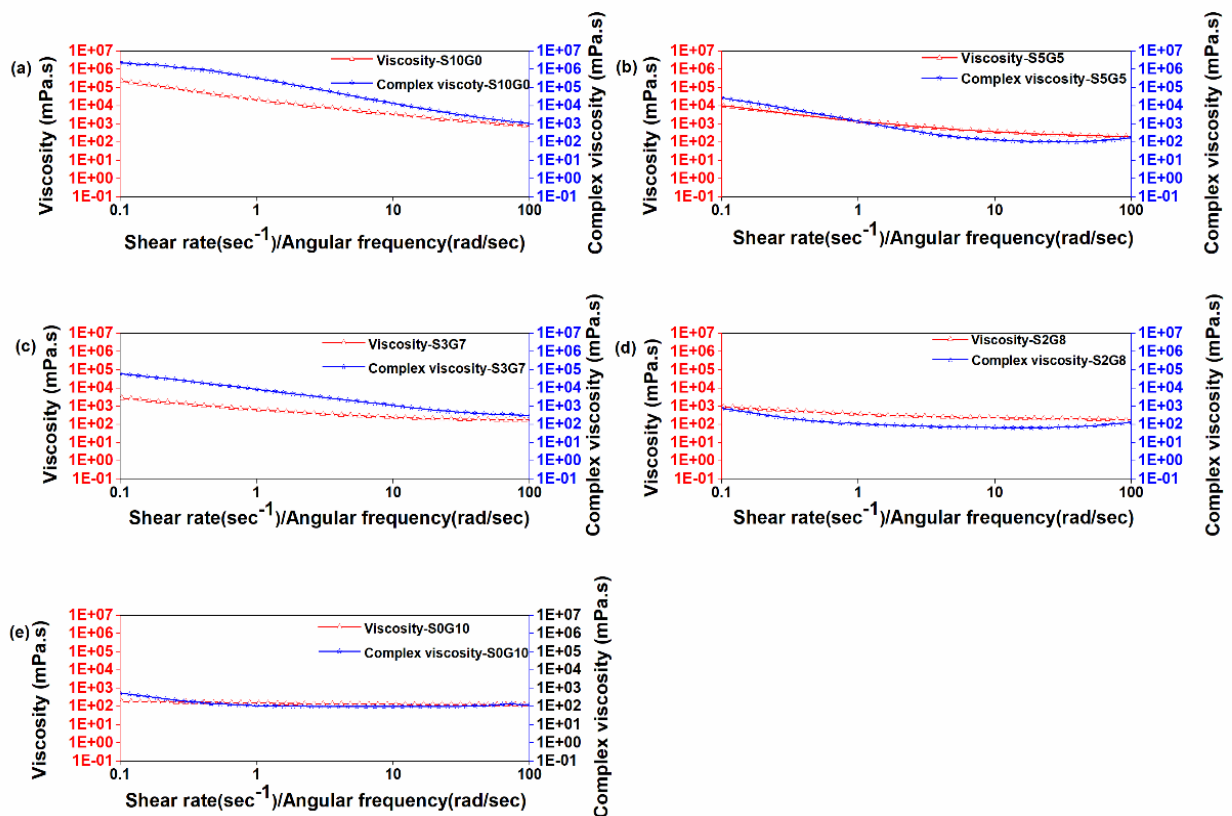


Fig 4.9 Comparison diagram of η and η^* for different blending ratios of silk fibroin/gelatin blend solutions (a) S10G0 (b) S5G5, (c) S3G7, (d) S2G8, and (e) S0G10.

Table 4.3 Data on the viscosity and complex viscosity of pure silk, pure gelatin, and various blend solutions.

Sample code	At low shear rate	At high shear rate	At low angular frequency	At high angular frequency
	Viscosity (mPa.s)	Viscosity (mPa.s)	Complex viscosity (mPa.s)	Complex viscosity (mPa.s)
S10G0	165258	883.98	1985260	1417.44
S5G5	7807.92	208.99	19454.6	142.76
S3G7	2311.904	176.75	48746.01	342.47
S2G8	823.35	190.51	556.54	109.23
S0G10	177.18	126.05	416.87	125.58

4.1.3 Time-dependent behaviors of silk fibroin and gelatin blend solutions

4.1.3.1 Structure recovery behavior of silk fibroin and gelatin blend solutions

The structural behaviour of polymeric solutions is investigated using a rotational shear test with respect to time, which reveals polymer viscosity that is time dependent. In this study, a certain amount of shear is applied for a set period of time, then increased to a higher value, then reduced to the initial low shear value, and structural recovery in terms of viscosity is assessed [23]. Thixotropic or Rheopectic behaviour is the term used to describe this type of viscosity change in behaviour [37]. Thixotropy is the return of initial viscosity after removing the strong shear that caused it to decrease in the first place. The return of viscosity in polymer solutions can be linked to structural recovery. As shown in Fig.4.10, because of its entangled structure, pure silk fibroin solution displayed the highest viscosity at low shear rates [38][39][40], and after applying high shear rates, viscosity decreased, reflecting a change in structure owing to uncoiling of molecular chains during the high shear rate stage.

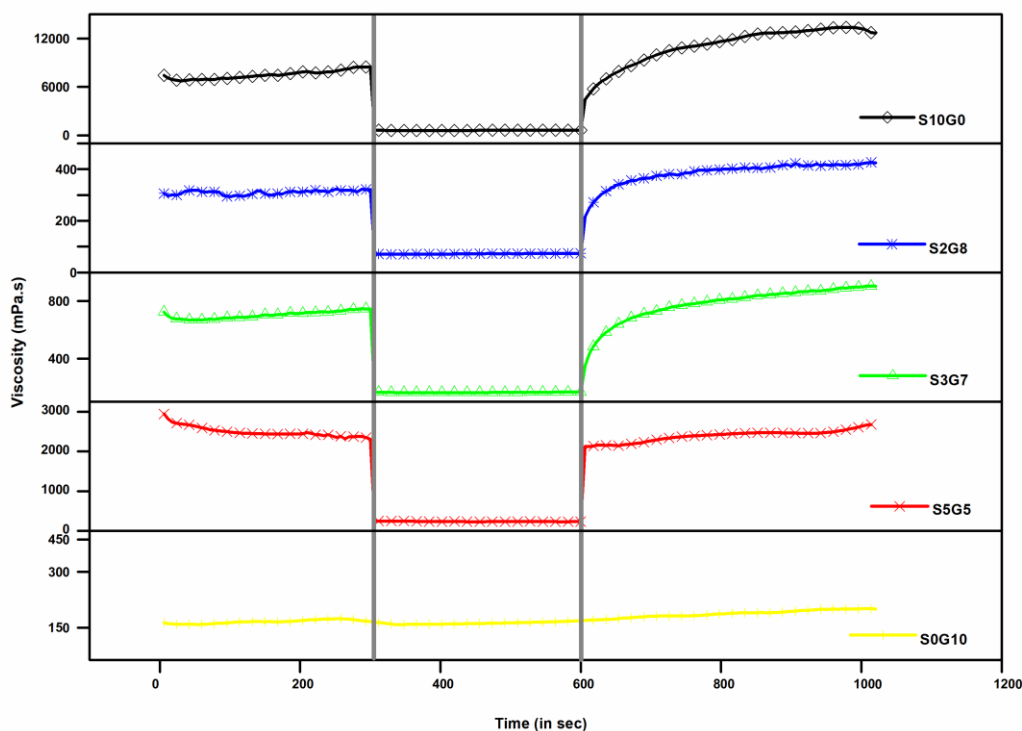


Fig 4.10 Structure recovery of silk and gelatin blend solutions by measuring shear viscosity in three intervals of low-high-low shear rates under time dependent rotational shear behaviour.

After the high shear was removed, viscosity returned to thixotropic behaviour, with a structural recovery of 78.9%. Partially recovering the structure could be due to breakdown between some molecular chains rather than disentanglement, which could have occurred as a result of high shear application. In our previous investigation[41], we observed similar thixotropic activity in mulberry silk fibroin solution with 11 percent structural recovery. As a viscous fluid, pure gelatin solution demonstrated perfect structural recovery. All of the blend solutions showed similar thixotropic behaviour; however, the proportion of structural recovery was found to be different, with 70 percent, 74.7 percent, and 89.4 percent for S2G8, S3G7, and S5G5, respectively. It is important to note that, in contrast to pure silk solutions, the percentage structural recovery improved as the silk content in blend solutions increased. This can be explained by our findings in the frequency sweep test, which showed that blending produced solutions with viscoelastic. When high shear was applied to these blend solutions, molecular disentanglement occurred without any disruption of chain-chain interactions, allowing them to regain their structure and move closer to complete recovery.

4.1.3.2 Creep recovery and compliance behavior of silk fibroin and gelatin blend solutions

Because it offers time dependent deformation behaviour, the creep test has the ability to evaluate the mechanical responses of polymers[26]. The creep and creep recovery results from experiments done on blend solutions are shown in Figure 4.11 (a). Deformation of pure silk fibroin solution S10G0 occurs at around 26% strain, and after the load is released, it shows delayed permanent deformation at around 20% strain, showing viscoelastic solid like behaviour. However, S0G10 showed persistent deformation upon loading and no reformation was detected in the solution after the load was released, implying perfect viscous behaviour, which is consistent with our frequency sweep analysis findings. After the load was released, the blend solutions S2G8, S3G7, and S5G5 showed no reformation, indicating that adding gelatin to the silk solution changed its own viscoelastic solid behaviour, comparable to our frequency sweep results. When a load of 5 Pa is applied to S5G5, S3G7, and S2G8, the extent of the deformation increases on increasing the gelatin content in silk solution i.e., S5G5, S3G7, and S2G8 have 543000, 1640000, and 3230000 percent deformation, respectively. Since pure gelatin is deformed (around 2370000 percent) much more than pure silk solution (around=25 percent), the behaviour of gelatin is found to be dominant in blend solution. The creep compliance $J(t)$ is defined as the change in deformation with time when a constant load is applied instantly. As illustrated in Fig. 4.11(b), the creep compliance behaviour of blend solutions has been investigated. The creep compliance of pure silk fibroin solution increased at first and then remained constant at 0.052 Pa^{-1} throughout time. Pure gelatin solution, on the other hand, showed a steady increase with time up to 4735 Pa^{-1} , while blend solutions with compliance values of 6596, 3296, and 1080 Pa^{-1} of S2G8, S3G7, and S5G5, respectively, showed the same pattern. When gelatin is added to a pure silk fibroin solution and a high load is applied, the viscoelastic solid transforms into an ideal viscous solution [26].

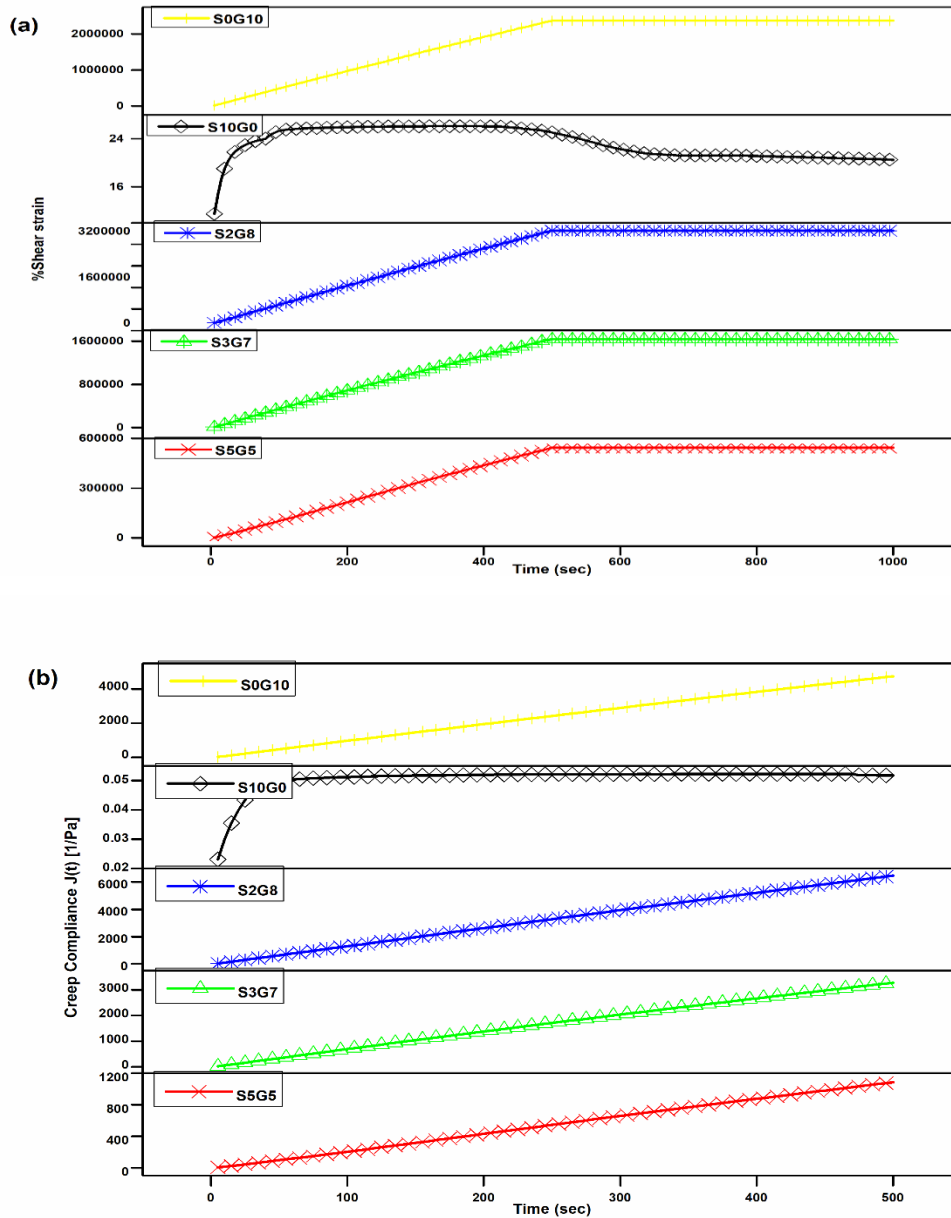


Fig 4.11 Creep recovery (a) and creep compliance (b) of silk fibroin/gelatin blend solutions with various blending ratios.

4.1.3.3 Stress relaxation behavior of silk fibroin and gelatin blend solutions

Under constant strain, stress relaxation is a time-dependent decrease in stress. The load required to sustain a fixed quantity of deformation or strain as a function of time is used to investigate the polymer's characteristic behaviour[27]. In the stress relaxation test, silk fibroin/gelatin blend solutions were tested by applying a percent pre strain value of 1, 0.5, 1, 0.5 for S0G10, S10G0, S2G8, S3G7, and S5G5, respectively, and keeping this pre strain value constant for

100 seconds for each solution. Following that, relaxation strains of 10, 1, 10, 10 and 1 were applied to S0G10, S10G0, S2G8, S3G7, and S5G5, respectively and for the next 1000 seconds, this strain value was kept constant. In Fig 4.12, the permissible maximum deformation was chosen within the LVE region of each individual solution, and time-dependent stress relaxation curves with stress on the vertical axis and time on the horizontal axis are shown

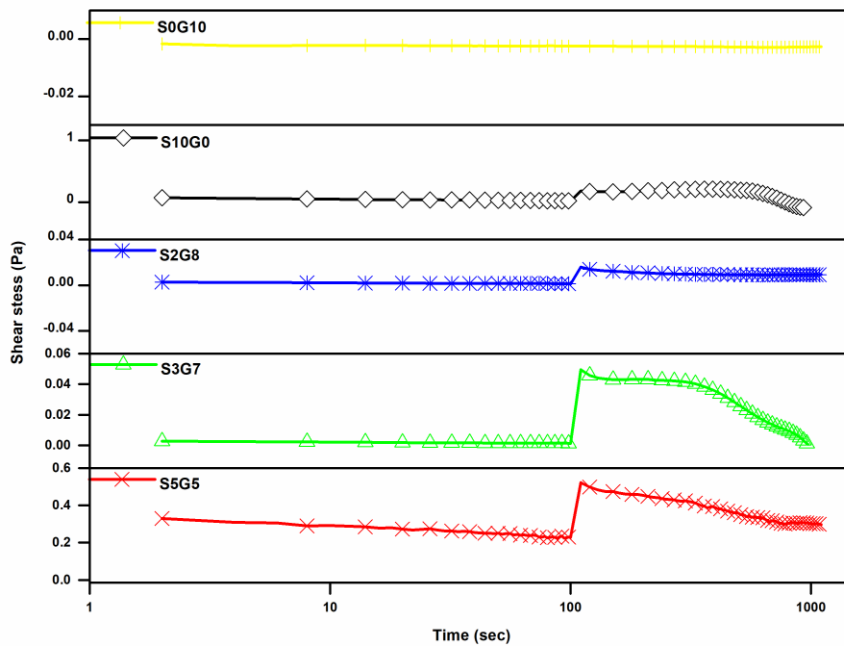


Fig 4.12 Stress relaxation curve of silk fibroin and gelatin blend solutions.

S0G10 solution showed no stress relaxation throughout the whole test time interval, implying that its polymer chains entirely absorbed the stress. This behaviour is common in highly viscous polymer solutions, and it is consistent with our amplitude sweep results. However, due of its viscoelastic solid-like behaviour, the S10G0 solution demonstrated stress relaxation towards the ending of the test period, which was also seen by the creep recovery test. Kothari, Rajkhowa, and Gupta reported similar relaxation behaviour in non-mulberry silk fibroin, indicating that it has a more viscoelastic characteristic[42]. The blends with higher gelatin content, such as S2G8, exhibited minimal stress relaxation, but the blends with higher silk content, such as S3G7 and S5G5, showed consistent stress relaxation throughout the study period. This behaviour indicates to viscoelastic properties in the blend solutions, which were confirmed by other dynamic oscillatory rheological experiments carried out in this investigation.

4.2 Optical microscopy

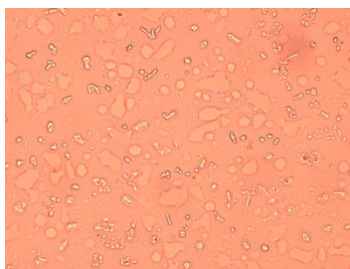


Fig 4.13(a) S0G10_40X

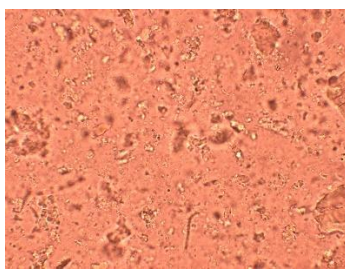


Fig 4.13(b) S2G8_40X

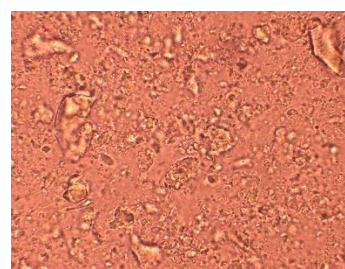


Fig 4.13(c) S3G7_40X

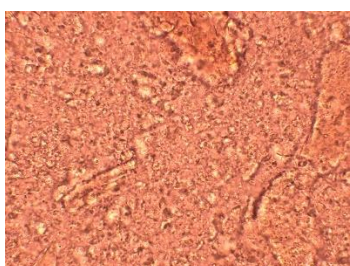


Fig 4.13(d) S5G5_40X

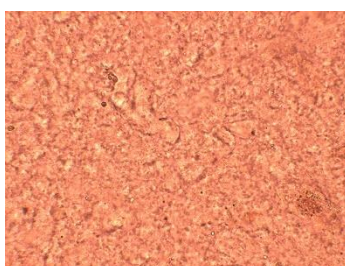


Fig 4.13(e) S10G0_40X

Fig 4.13 In an optical microscope, the surface roughness of silk, gelatin, and their blend solutions were studied.

The optical microscope was used to evaluate the miscibility of silk and gelatin solution in terms of surface roughness in various proportions under 40X resolution. The surface of S2G8, S3G7, and S5G5 blend solutions was found to be rough under an optical microscope, as illustrated in Fig. 4.13, and this roughness is linked to the continuous-discrete phase of the silk and gelatin blend solution. Due to the larger concentration of gelatin in all blend solutions, it was presumed that gelatin was present as a continuous phase, while silk was present as a discrete or dispersed phase due to the low concentration. The miscibility of the blend solutions was assessed by the size of dispersed silk in gelatin. The small size of silk in S2G8 and S3G7 indicates a homogeneous solution. The high size of silk in S5G5 solution, on the other hand, made the solution heterogeneous. This was corroborated by other dynamic oscillatory rheological studies conducted in this study.

4.3 Thermogravimetric Analysis (TGA)

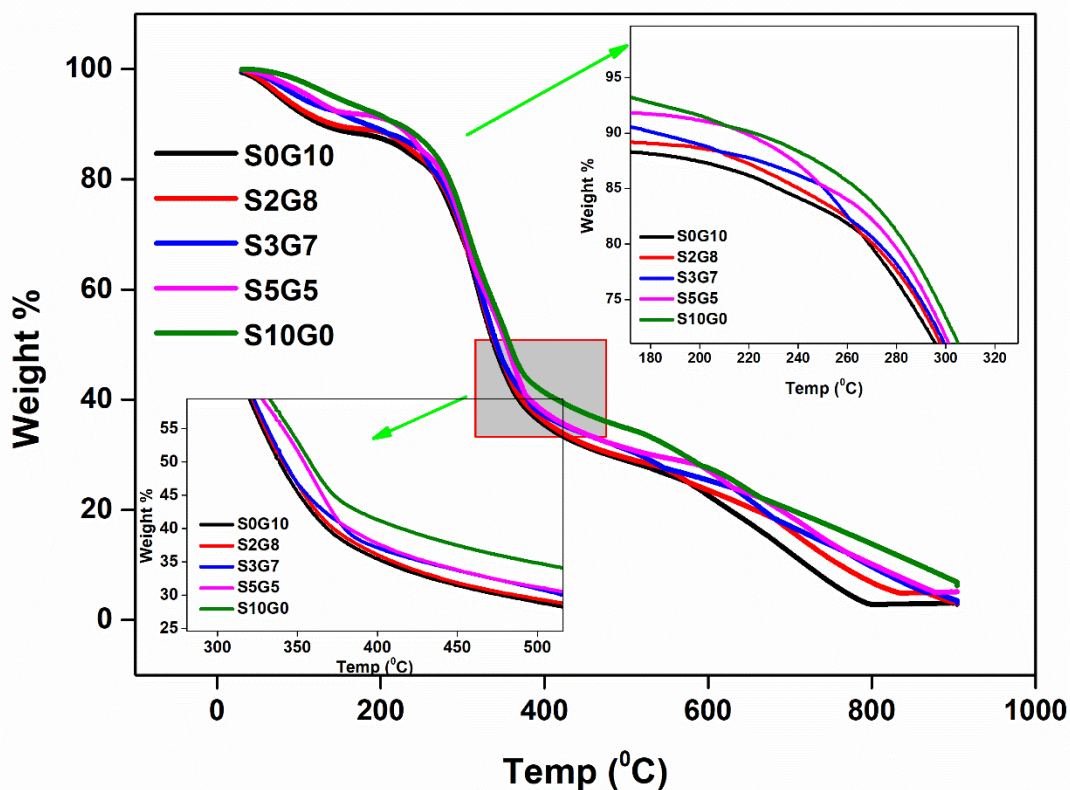


Fig 4.14 TGA of pure silk, gelatin and their blend solutions was studied.

Thermogravimetric analysis was used for thermal analysis. Study was done under inert environment. Fig 4.14 shows how the percentage weight of pure and blended silk and gelatin solutions decreases as the temperature rises. Evaporation of water or other volatile substances causes the initial weight loss in all of the samples at roughly 100°C. The second weight loss is found at temperatures between 300 and 400°C, which can be attributed to the thermal degradation of amino-acid side chain groups as well as the protein backbone, which includes peptide linkages. Andiappan, Kumari, Sundaramoorthy, Meiyazhagan, Manoharan, and Venkataraman observed similar results for tasar silk fibroin scaffolds, with one weight loss at 100°C and the other at 370°C[43]. In addition, by retaining the addition of gelatin in silk fibroin, the weight loss curve in the blend solution shifted towards lower temperatures, as seen in Fig 4.14. At final temperature that is 900°C, all the blend solutions demonstrated a lower final percentage weight loss than pure silk fibroin but a higher final percentage weight loss than pure gelatin film as anticipated. Similar behaviour

was reported by Tsukada, Freddi, and Kasai in A. Pernyi/ B. Mori SF blend films, which exhibited intermediate thermal behaviour of the two pure polymeric components[44]. Blending silk polymer with gelatin polymer improves the thermal stability of gelatin polymer, however gelatin accelerates the thermal degradation of silk polymer.

4.4 Differential Scanning Calorimetry (DSC)

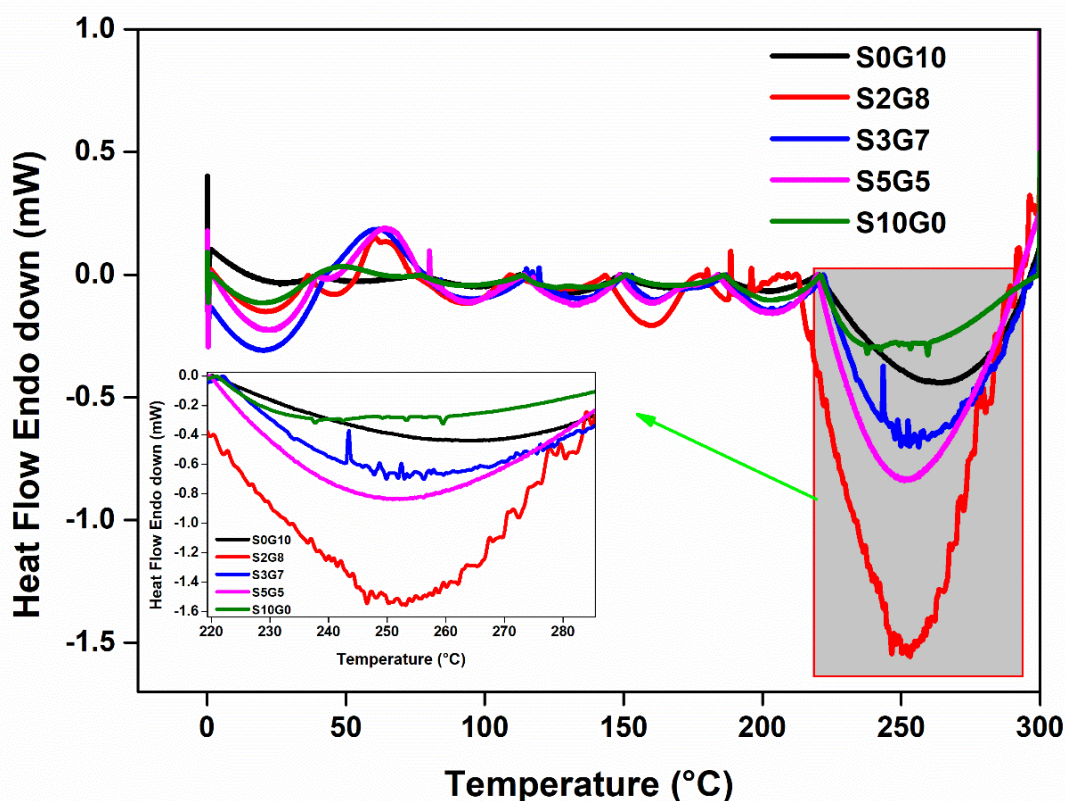


Fig 4.15 DSC of pure silk, gelatin and their blend solutions was studied.

Differential Scanning Calorimetry was used for thermal analysis. Study was done under inert environment. The DSC thermogram of pure gelatin solution shown major endotherm at temperature around 270°C, as illustrated in Fig 4.15. The pure silk fibroin solution shown endotherm at around 240°C. As seen in the DSC thermograms, blending silk fibroin with gelatin results in a considerable change in the peaks. There was no crystallisation peak due to the solvent treatment of silk with methanol during the degumming process. T_g and T_c disappeared from the DSC thermogram of methanol treated film samples, as reported by

Gil, Frankowski, Bowman, Gozen, Hudson, and Spontak for *B. mori* silk fibroin/gelatin blend film [45]

Taddei and co-workers earlier reported on the effects of blending *B. mori* silk fibroin and gelatin, observing significant changes in the DSC thermogram of blends with the absence of T_g and T_c [46]. The primary endotherm peaks for the blend solutions S2G8, S3G7, and S5G5 were 255°C, 248°C, and 243°C, respectively. Furthermore, the observed endotherm peak shift in S2G8, S3G7, and S5G5 blend solutions when compared to pure silk can be attributed to partial miscibility of two pure polymeric components when they are blended. DSC endotherm shifts in silk-PVA blends have been associated to polymer blending by Lee and co-workers [47]. When *B. mori* silk fibroin was blended with S-carboxymethyl keratine, Lee and Ha observed a shift in the decomposing endotherm [12].

CHAPTER 5: CONCLUSION

Steady and dynamic shear experiments were used to investigate the rheological behaviour of pure silk fibroin, pure gelatin, and their blend solutions in various compositions. At rest, the viscosity of the S10G0 solution was the highest, while the viscosity of the S0G10 solution was the lowest, which decreased with increasing shear rates. The viscosity of the blend solutions was intermediate to that of the pure solutions, and shear thinning was seen in all of them. When the Carreau yasuda model was fitted to the log viscosity vs log shear rate curves, it was discovered that when Silk fibroin solution was blended with gelatin, which was Newtonian in pure form at high shear rate, the non-Newtonian behaviour of silk fibroin solution became Newtonian. The log additivity rule revealed low miscibility of silk fibroin and gelatin at zero shear when analyzing blend compatibility. High shear induced homogeneity in blend solutions, resulting in increased interface contacts. The longest LVE region was recorded in in amplitude sweep test of S2G8 solution confirming the system's highest stability. In general, blend solutions with less than 11% silk were shown to be more miscible than those with higher silk. The frequency sweep test revealed that pure silk fibroin solution, which behaved like a viscous solid, and pure gelatin solution, which behaved like a viscous fluid, changed the behaviour of blend solutions to viscoelastic, and it was found that this behaviour was frequency dependent. Blend solution behaviour was also validated by loss factor data. Except for the pure gelatin solution, all other solutions deviated from the Cox-Merz rule, possibly due to chain entanglement variations. Gelatin showed complete structural recovery, with the level of recovery varying depending on the amount of gelatin in the blend. In a creep recovery test, the application of a high load over a period of time changed the behaviour of blends from solid like to ideal viscous solution. The data on stress relaxation was quite helpful in generating viscoelastic behaviour in blend solutions. In general, specific factors could change the viscoelastic behaviour of solutions. The ability to modify the conditions at the solution level can be beneficial in the development of materials with tailorable qualities. Thermal investigation of all solutions using DSC revealed partial miscibility of the pure components in the blend, which was not visible under optical microscopy. When gelatin was blended with silk fibroin, TGA showed that the thermal stability of the gelatin was improved.

REFERENCES

- [1] S. Jasmine and B. B. Mandal, *Types and properties of non-mulberry silk biomaterials for tissue engineering applications*. Woodhead Publishing Limited, 2014.
- [2] H. Y. Wang, Z. G. Wei, and Y. Q. Zhang, “Dissolution and regeneration of silk from silkworm *Bombyx mori* in ionic liquids and its application to medical biomaterials,” *Int. J. Biol. Macromol.*, vol. 143, pp. 594–601, 2020, doi: 10.1016/j.ijbiomac.2019.12.066.
- [3] S. S. Silva, J. M. Gomes, A. C. Vale, S. Lu, R. L. Reis, and S. C. Kundu, “Green Pathway for Processing Non-mulberry *Antheraea pernyi* Silk Fibroin/Chitin-Based Sponges: Biophysical and Biochemical Characterization,” *Front. Mater.*, vol. 7, no. May, 2020, doi: 10.3389/fmats.2020.00135.
- [4] A. Sionkowska, “Current research on the blends of natural and synthetic polymers as new biomaterials: Review,” *Prog. Polym. Sci.*, vol. 36, no. 9, pp. 1254–1276, 2011, doi: 10.1016/j.progpolymsci.2011.05.003.
- [5] X. Zhang and Z. Pan, “Rheological behavior of regenerated silk fibroin/polyvinyl alcohol blended solutions in steady and dynamic state and the effect of temperature,” *J. Mater. Sci.*, vol. 55, no. 31, pp. 15350–15363, 2020, doi: 10.1007/s10853-020-05086-4.
- [6] Y. Yao, K. S. Mukuze, Y. Zhang, and H. Wang, “Rheological behavior of cellulose/silk fibroin blend solutions with ionic liquid as solvent,” *Cellulose*, vol. 21, no. 1, pp. 675–684, 2014, doi: 10.1007/s10570-013-0117-y.
- [7] D. H. Baek, C. S. Ki, I. C. Um, and Y. H. Park, “Metal ion adsorbability of electrospun wool keratose/silk fibroin blend nanofiber mats,” *Fibers Polym.*, vol. 8, no. 3, pp. 271–277, 2007, doi: 10.1007/BF02877269.
- [8] N. Bhardwaj and S. C. Kundu, “Silk fibroin protein and chitosan polyelectrolyte complex porous scaffolds for tissue engineering applications,” *Carbohydr. Polym.*, vol. 85, no. 2, pp. 325–333, 2011, doi: 10.1016/j.carbpol.2011.02.027.
- [9] H. Kweon, I. C. Um, and Y. H. Park, “Structural and thermal characteristics of *Antheraea pernyi* silk fibroin/chitosan blend film,” *Polymer (Guildf)*, vol. 42, no. 15, pp. 6651–6656, 2001, doi: 10.1016/S0032-3861(01)00104-5.
- [10] Y. Liu, Z. Shao, P. Zhou, and X. Chen, “Thermal and crystalline behaviour of silk

- fibroin/nylon 66 blend films,” *Polymer (Guildf)*., vol. 45, no. 22, pp. 7705–7710, 2004, doi: 10.1016/j.polymer.2004.09.005.
- [11] H. Chen, X. Hu, and P. Cebe, “Nylon-6 With Silk Fibroin,” *J Therm Anal Calorim*, vol. 93, pp. 201–206, 2008.
- [12] K. Y. Lee and W. S. Ha, “DSC studies on bound water in silk fibroin/S-carboxymethyl kerateine blend films,” *Polymer (Guildf)*., vol. 40, no. 14, pp. 4131–4134, 1999, doi: 10.1016/S0032-3861(98)00611-9.
- [13] J. Wang *et al.*, “Fabrication and characterization of *Antheraea pernyi* silk fibroin-blended P(LLA-CL) nanofibrous scaffolds for peripheral nerve tissue engineering,” *Front. Mater. Sci.*, vol. 11, no. 1, pp. 22–32, 2017, doi: 10.1007/s11706-017-0368-x.
- [14] H. Kweon, H. C. Ha, I. N. C. Um, and Y. H. Park, “Physical Properties of Silk Fibroin / Chitosan Blend Films,” *J. Appl. Polym. Sci.*, vol. 80, no. 7, pp. 928–934, 2001.
- [15] D. Chouhan, B. Chakraborty, S. K. Nandi, and B. B. Mandal, “Role of non-mulberry silk fibroin in deposition and regulation of extracellular matrix towards accelerated wound healing,” *Acta Biomater.*, vol. 48, pp. 157–174, 2017, doi: 10.1016/j.actbio.2016.10.019.
- [16] C. S. Ki, D. H. Baek, K. D. Gang, K. H. Lee, I. C. Um, and Y. H. Park, “Characterization of gelatin nanofiber prepared from gelatin-formic acid solution,” *Polymer (Guildf)*., vol. 46, no. 14, pp. 5094–5102, 2005, doi: 10.1016/j.polymer.2005.04.040.
- [17] I. C. F. Moraes, L. H. Fasolin, R. L. Cunha, and F. C. Menegalli, “Dynamic and steady-shear rheological properties of xanthan and guar gums dispersed in yellow passion fruit pulp (*Passiflora edulis f. flavicarpa*),” *Brazilian J. Chem. Eng.*, vol. 28, no. 3, pp. 483–494, 2011, doi: 10.1590/S0104-66322011000300014.
- [18] Y. Zare, S. P. Park, and K. Y. Rhee, “Analysis of complex viscosity and shear thinning behavior in poly (lactic acid)/poly (ethylene oxide)/carbon nanotubes biosensor based on Carreau–Yasuda model,” *Results Phys.*, vol. 13, no. April, p. 102245, 2019, doi: 10.1016/j.rinp.2019.102245.
- [19] S. W. Jeong, S. Leroueil, and J. Locat, “Applicability of power law for describing the rheology of soils of different origins and characteristics,” *Can. Geotech. J.*, vol. 46, no. 9, pp. 1011–1023, 2009, doi: 10.1139/T09-031.
- [20] W. J. Bailey and I. S. Weir, “Investigation of methods for direct rheological model parameters estimation,” *J. Pet. Sci. Eng.*, vol. 21, no. 1–2, pp. 1–13, 1998, doi: 10.1016/S0920-4105(98)00040-0.

- [21] *Polymer Physics*. .
- [22] D. Şerban, H. Hanson, L. Marşavina, and V. V Silberschmidt, “Viscoelastic properties of semi-crystalline thermoplastic polymers : dynamic analysis and creep,” vol. 188, pp. 211–218, 2012, doi: 10.4028/www.scientific.net/SSP.188.211.
- [23] G. Paroline, “Basics of Applied Rheology,” pp. 21–29, 2016, [Online]. Available: <http://web.engr.oregonstate.edu/~rochefow/Polymer Course Notes 2016/Applied Rheology by Anton Paar.pdf>.
- [24] R. Larsen, “Oscillatory Rheology,” pp. 68–70, 2007.
- [25] T. G. Mezger, *Italy’ s dilemma*, vol. 59, no. 4. 2010.
- [26] D. Chotpattananont, A. Sirivat, and A. M. Jamieson, “Creep and recovery behaviors of a polythiophene-based electrorheological fluid,” *Polymer (Guildf)*., vol. 47, no. 10, pp. 3568–3575, 2006, doi: 10.1016/j.polymer.2006.03.061.
- [27] N. D. Polychronopoulos and J. Vlachopoulos, “Polymer Processing and Rheology,” 2019, pp. 133–180.
- [28] A. Sionkowska, “Current research on the blends of natural and synthetic polymers as new biomaterials: Review,” *Prog. Polym. Sci.*, vol. 36, no. 9, pp. 1254–1276, 2011, doi: 10.1016/j.progpolymsci.2011.05.003.
- [29] D. Chouhan, B. Chakraborty, S. K. Nandi, and B. B. Mandal, *Role of non-mulberry silk fibroin in deposition and regulation of extracellular matrix towards accelerated wound healing*. 2016.
- [30] B. N. Singh, N. N. Panda, R. Mund, and K. Pramanik, “Carboxymethyl cellulose enables silk fibroin nanofibrous scaffold with enhanced biomimetic potential for bone tissue engineering application,” *Carbohydr. Polym.*, vol. 151, pp. 335–347, 2016, doi: 10.1016/j.carbpol.2016.05.088.
- [31] J. Boyd, J. M. Buick, and S. Green, “Analysis of the Casson and Carreau-Yasuda non-Newtonian blood models in steady and oscillatory flows using the lattice Boltzmann method,” *Phys. Fluids*, vol. 19, no. 9, 2007, doi: 10.1063/1.2772250.
- [32] C. Qiao, T. Li, L. Zhang, X. Yang, and J. Xu, “Rheology and viscosity scaling of gelatin/1-allyl-3-methylimidazolium chloride solution,” *Korea Aust. Rheol. J.*, vol. 26, no. 2, pp. 169–175, 2014, doi: 10.1007/s13367-014-0017-1.
- [33] T. Mezger, “8. Oscillatory tests,” *Rheol. Handb.*, pp. 153–247, 2020, doi: 10.1515/9783748603702-009.
- [34] K. Kaewprasit, T. Kobayashi, and S. Damrongsakkul, “Thai silk fibroin gelation process enhancing by monohydric and polyhydric alcohols,” *Int. J. Biol. Macromol.*,

- vol. 118, pp. 1726–1735, 2018, doi: 10.1016/j.ijbiomac.2018.07.017.
- [35] X. Mu, V. Fitzpatrick, and D. L. Kaplan, “From Silk Spinning to 3D Printing : Polymer Manufacturing using Directed Hierarchical Molecular Assembly,” vol. 1901552, pp. 1–17, 2020, doi: 10.1002/adhm.201901552.
- [36] K. Yasuda, R. C. Armstrong, and R. E. Cohen, “Shear flow properties of concentrated solutions of linear and star branched polystyrenes,” *Rheol. Acta*, vol. 20, no. 2, pp. 163–178, 1981, doi: 10.1007/BF01513059.
- [37] L. A. Utracki and M. R. Kanial, “Melt rheology of polymer blends,” *Polym. Eng. Sci.*, vol. 22, no. 2, pp. 96–114, 1982, doi: 10.1002/pen.760220211.
- [38] A. Matsumoto, A. Lindsay, B. Abedian, and D. L. Kaplan, “Silk fibroin solution properties related to assembly and structure,” *Macromol. Biosci.*, vol. 8, no. 11, pp. 1006–1018, 2008, doi: 10.1002/mabi.200800020.
- [39] J. Zhu, Y. Zhang, H. Shao, and X. Hu, “Electrospinning and rheology of regenerated Bombyx mori silk fibroin aqueous solutions: The effects of pH and concentration,” *Polymer (Guildf)*, vol. 49, no. 12, pp. 2880–2885, 2008, doi: 10.1016/j.polymer.2008.04.049.
- [40] S. B. Ross-Murphy, “Structure and rheology of gelatin gels,” *Imaging Sci. J.*, vol. 45, no. 3–4, pp. 205–209, 1997, doi: 10.1080/13682199.1997.11736407.
- [41] R. Yadav and R. Purwar, “Influence of metal oxide nanoparticles on morphological, structural, rheological and conductive properties of mulberry silk fibroin nanocomposite solutions,” *Polym. Test.*, vol. 93, no. July 2020, p. 106916, 2021, doi: 10.1016/j.polymertesting.2020.106916.
- [42] V. K. Kothari, R. Rajkhowa, and V. B. Gupta, “Stress relaxation and inverse stress relaxation in silk fibers,” *J. Appl. Polym. Sci.*, vol. 82, no. 5, pp. 1147–1154, 2001, doi: 10.1002/app.1949.
- [43] M. Andiappan, T. Kumari, S. Sundaramoorthy, G. Meiyazhagan, P. Manoharan, and G. Venkataraman, “Comparison of eri and tasar silk fibroin scaffolds for biomedical applications,” *Prog. Biomater.*, vol. 5, no. 2, pp. 81–91, 2016, doi: 10.1007/s40204-016-0047-5.
- [44] M. Tsukada, G. Freddi, and N. Kasai, “Physical properties and phase separation structure of *Antheraea pernyi*/*Bombyx mori* silk fibroin blend films,” *J. Polym. Sci. Part B Polym. Phys.*, vol. 32, no. 7, pp. 1175–1182, 1994, doi: 10.1002/polb.1994.090320705.
- [45] E. S. Gil, D. J. Frankowski, M. K. Bowman, A. O. Gozen, S. M. Hudson, and R. J.

- Spontak, "Mixed protein blends composed of gelatin and Bombyx mori silk fibroin: Effects of solvent-induced crystallization and composition," *Biomacromolecules*, vol. 7, no. 3, pp. 728–735, 2006, doi: 10.1021/bm050622i.
- [46] P. Taddei, V. Chiono, A. Anghileri, G. Vozzi, G. Freddi, and G. Ciardelli, "Silk fibroin/gelatin blend films crosslinked with enzymes for biomedical applications," *Macromol. Biosci.*, vol. 13, no. 11, pp. 1492–1510, 2013, doi: 10.1002/mabi.201300156.
- [47] K. H. Lee, D. H. Baek, C. S. Ki, and Y. H. Park, "Preparation and characterization of wet spun silk fibroin/poly(vinyl alcohol) blend filaments," *Int. J. Biol. Macromol.*, vol. 41, no. 2, pp. 168–172, 2007, doi: 10.1016/j.ijbiomac.2007.01.011.