

Development and Characterization of Piezoelectric Silk Fibroin Nanocomposite Hydrogels

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CANDIDATE'S DECLARATION

I Alok Pathak, Roll No.24/RAC/03 hereby certify that the work which is being presented in the thesis entitled **“Development and Characterization of Piezoelectric Silk Fibroin Nanocomposite Hydrogels”** in partial fulfillment of the requirements for the award of the Degree of Master of Technology by Research (Chemical Engineering), submitted in the Department of Applied Chemistry , Delhi Technological University is an authentic record of my own work carried out during the period from August 2025 to May 2026 under the supervision of **Prof. Roli Purwar**.

The matter presented in the thesis has not been submitted by me for the award of any other degree of this or any other Institute.

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CERTIFICATE BY THE SUPERVISOR

Certify that Alok Pathak, Roll no. 24/RAC/03, has carried out their search work presented in this thesis entitled **“Development and Characterization of Piezoelectric Silk Fibroin Nanocomposite Hydrogels”** for the award of **Master of Technology** from Department of Applied Chemistry, Delhi Technological University, Delhi, under my supervision. The thesis embodies results of original work, and studies are carried out by the student himself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to any body else from this or any other University/Institution.

Prof. Roli Purwar
DTU, New Delhi

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ABSTRACT

This dissertation discusses the synthesis and characterization of a novel class of piezoelectric silk fibroin hydrogels containing ZnO, AlN, and LiNbO₃ nanoparticles that offer improved potential for applications in biomedicine and bioelectronics. Silk fibroin, which is a natural protein derived from the *Bombyx mori* silk cocoon, was chosen as the base polymer because of its high biocompatibility, biodegradability, flexibility, and suitability for forming hydrogels. Nonetheless, pure silk fibroin hydrogels face some limitations including poor ionic conductivity and poor mechanical strength. The addition of nanoparticles into the hydrogel improves the mechanical strength, ionic conductivity, piezoelectricity, and thermal stability.

The hydrogels based on silk fibroin were synthesized using three approaches, namely the dialysis-based technique, non-dialysis technique, and the soft freezing process. The effects of addition of nanoparticles and different approaches on the physicochemical properties of the hydrogels have been studied. Zinc oxide (ZnO) nanoparticles were used for conductivity, antibacterial activity, and increased mechanical stability of the obtained materials. Aluminum nitride (AlN) and lithium niobate (LiNbO₃) were added for increasing thermal conductivity, ionic conductivity, dielectric properties, piezoconductivity, and electroactivity.

The synthesized hydrogels were characterized through the determination of swelling, rheology, conductivity, conductivity under heating, FTIR spectroscopy, cross-linking density, and electric current measurements. From the rheology studies conducted through viscosity, amplitude sweeps, frequency sweeps, and creep recoveries, an enhancement in viscoelastic properties and an increase in storage modulus (G') values were observed. It was found that nanoparticle loading as well as different synthesis approaches significantly affected water absorption capacities and dimension stabilities of the hydrogels.

From the conductivity tests, it was observed that the incorporation of ZnO and LiNbO₃ nanoparticles promoted both ionic and ionic conductivities, while AlN contributed to the effective transport of thermal energy. Temperature dependent conductivity analysis proved to show increased conductivity at higher temperatures due to an increase in ion mobility. From FTIR studies, it is clear that there is effective interaction of nanoparticles and silk fibroin chains along with β -sheet structure formation.

Soft freezing technology used in the study provided porous and interlinked hydrogels without the use of any chemical cross-linking agents, and thus, were ideal materials for biomedical applications. The

fabricated silk fibroin nanocomposite hydrogels had improved mechanical, conductive, piezoelectric and thermal properties. The potential of these hydrogels for tissue engineering, regenerative medicine, wound healing applications and as biosensors, smart biomedical and bioelectronic systems was clearly evident.

CHAPTER-I

INTRODUCTION

The monomer is an essential molecule from which polymers are made, and they are large molecules with many useful applications in our everyday lives. They are used in numerous products ranging from food packaging to medical equipment. Polymers are versatile materials with several advantages. They are light and versatile, making them applicable to many fields[1] Additionally, they are very strong and weigh very little, making them a favourite material in the aerospace and automotive industry.

The excellent ability to withstand corrosion and degradation makes polymers a suitable material for use in harsh environments. The resistance to chemicals also makes them a good fit for the pharmaceutical and chemical industries. Additionally, their resistance to chemicals makes them an excellent material for making containers to contain harmful substances without worrying about any form of contamination.

Furthermore, polymers are easy to work with and mold into the required form. They are easily customizable and offer great adaptability for many uses. They are easily customized and offer a good choice for mass-produced products. Polymers are considered to be environmentally friendly materials as they are recyclable and this results in minimizing the harmful effect on the environment. Besides, certain types of polymers decompose naturally and thus their adverse effects on the environment are minimized. Furthermore, the uniqueness of polymers makes them appropriate for specific uses. For example, thermoplastics can be molded into complex shapes, while they have diverse uses that include the manufacture of medical devices and automotive components. Thermosets find application in adhesives, paints, and composites, while elastomers are used in the making of seals, hoses, and tires[2].

1.1 Hydrogels

Hydrogels are three-dimensional systems of hydrophilic polymers that can take up large quantities of water or physiological fluid without losing their structure and dissolving in the process. The special feature of the hydrogel material is due to the presence of hydroxyl, carboxyl, and amide functional groups attached to the polymeric chain that facilitate hydration.

This material can be synthesized using physical or chemical means to link the chains together. Due to their soft texture similar to living tissues and the presence of large amounts of water, hydrogels have found extensive application in biomedical fields including drug delivery, wound dressing, and tissue engineering[3].

1.1.1 Structure

The hydrogels feature a 3-dimensional polymer network having an extremely hydrophilic nature, giving rise to water absorption and retention of more than 90% weight of the material. This 3-dimensional network formation can occur by cross-linking of polymers, which can take place in one of two ways:

- **Chemical crosslinking:** Covalent bonds between polymer chains, providing permanent and stable networks.
- **Physical crosslinking:** Hydrogen bonds, ionic bonding, and crystallization are less powerful, but reversible and sometimes stimuli-responsive interactions.

The framework of hydrogels consists of hydrophilic groups like $-\text{OH}$, $-\text{COOH}$, $-\text{NH}_2$, $-\text{CONH}-$, and $-\text{SO}_3\text{H}$. The hydrophilic groups attract water molecules, while the crosslinking sites hold the whole structure together and stop it from dissolving in an aqueous medium[4].

This is why hydrogels are soft, flexible, and biocompatible and hence suitable for drug delivery systems and biomedical devices such as wound dressing.

A schematic is shown below:

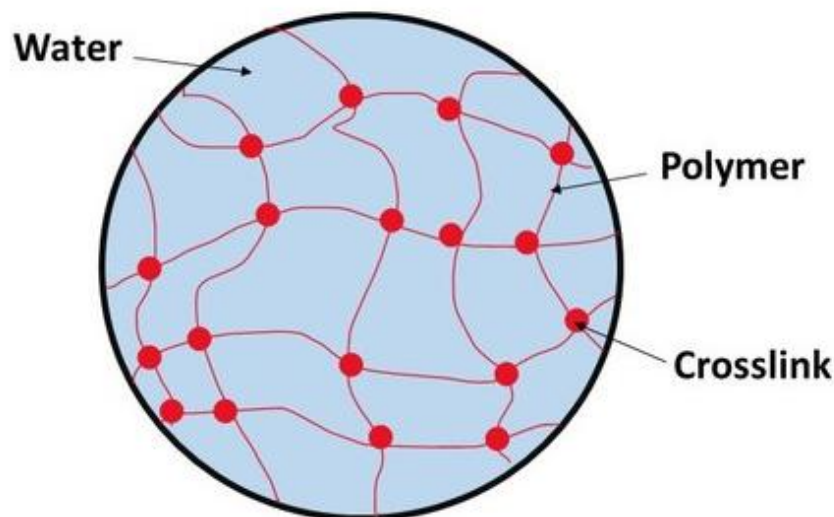


Figure 1: A representation of hydrogel internal structure.

1.1.2. Types of Hydrogels

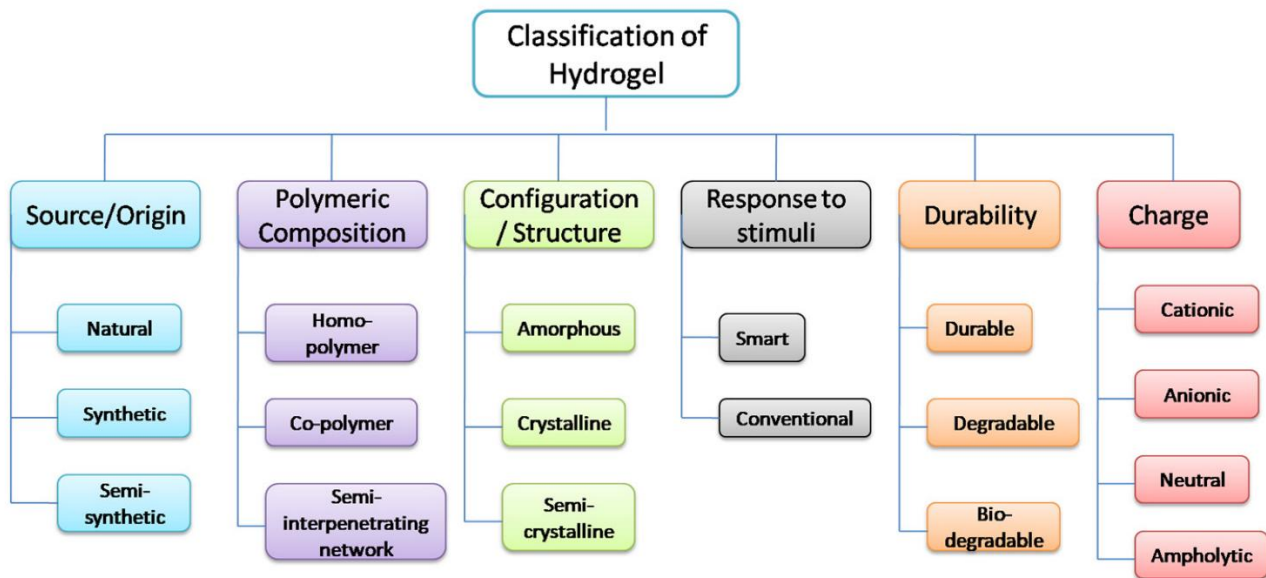


Figure 2: A flowchart representing the classification of hydrogels

❖ On the basis of Origin

- **Natural:** - These are derived from biopolymers and are known for their biocompatibility and biodegradability. Common examples include: chitosan, alginate, etc.
- **Synthetic:** - These are made from synthetic polymers or monomers, offering precise control over structure, mechanical properties, and degradation. Examples include: polyacrylamide, polyethylene, glycol, etc.
- **Semi-synthetic:** - These combine natural and synthetic components, aiming to balance biocompatibility with mechanical robustness. For instance: alginate-grafted-polyacrylamide[5].

❖ On the basis of polymeric composition

- **Homo-polymer:** - Composed of a single type of monomers. The polymer chains can be physically or chemically crosslinked. Example: polyacrylamide.
- **Co-polymer:** - formed from at least two or more than two types of monomers, at least one of which is hydrophilic. They can be random, block or graft. Example: poly (acrylic acid-co-acrylamide).
- **Semi-interpenetrating network:** - One polymer is crosslinked while other remains linear and interpenetrates the crosslinked system. Easier to synthesize than full IPNs

and still offer improved properties over single networks. Example: Chitosan interpenetrated with linear PEG for biomedical scaffolds.

❖ On the basis of configuration and structure

- **Amorphous hydrogel:** - These lack long-range molecular order. Polymer chains are randomly arranged, resulting in high flexibility and swelling capacity. Example: Polyacrylic acid.
- **Crystalline hydrogel:** - Polymer chains show great regularity in their structures. Very uncommon in real cases because of the difficulty in obtaining total crystallinity in aqueous systems. Example: Certain types of peptide-based hydrogels.
- **Semi-crystalline hydrogel:** - They include amorphous and crystalline phases, providing both flexibility and toughness. Example: Hydrogels made from PVA that are produced by freeze–thawing cycles, or copolymers that have crystalline segments.

❖ According to the response towards stimuli

- **Smart hydrogel:** - This type of hydrogels is called stimuli-responsive hydrogels because their behavior responds to particular stimulus such as pH, temperature, light, etc. Examples include PNIPAM, photo-crosslinked PEG derivatives, etc.
- **Conventional Hydrogels:** - This type of hydrogels does not respond to external stimuli, but just absorb water. Examples are polyvinyl alcohol, gelatine based hydrogels.

❖ Based on the property of being durable

- **Durable:** - This class of polymers is meant to retain their shape and properties for long periods of time even under the influence of stress. In many cases, this is achieved through strong covalent cross-linking or crystallinity[6]. Example: PVA.
- **Degradable:** - This category of polymers is created to degrade over time through various means such as hydrolysis, enzymes, or environmental factors like changes in pH or temperature. Examples include biodegradable or synthetic polymers that degrade. Example: PEG-PLA.
- **Non-biodegradable:** - Polymers within this category resist biological degradation and do not easily degrade. This occurs because they consist of polymer chains that are resistant to degradation. Example: PAAm.

❖ Depending on charges

- **Cationic:** - Have positive charge functional groups including amines and quaternary ammonium. Swells better at low pH levels since they become protonated. Example: Chitosan hydrogel

- **Anionic:** - have negative charge functional groups such as carboxylates and sulfonates. Swell well at high pH since they become deprotonated. Example: polyacrylic acid
- **Neutral:** - lack ionizing groups meaning their swelling properties are mainly influenced by osmotic pressure and hydrogen bonding. Exhibit constant swelling behavior in different pH ranges. Example: polyethylene glycol
- **Amphoteric:** - has both hydrophilic and hydrophobic units, allowing self-assembly and stimulus responsiveness, thus can create micelle structures or even nanostructures. Example: PEG-PLA block co-polymer

1.2. Silk fibroin Hydrogel

Fibroin hydrogels made of silk represent a new breed of biomaterials composed of proteins. The uniqueness of these materials lies in the fact that they combine high mechanical strength, good water capacity, and excellent structural stability due to the presence of β -sheet nanocrystals. Made from *Bombyx mori* silk, the fibroin molecule is composed of heavy and light chains which interlink to form a complex three-dimensional structure via physical or chemical cross-links. In addition, hydrogels based on silk fibroin proteins are highly adjustable in the sense that their degradation rates and elasticity can be tailored to suit different biological organs and electronic platforms. Unlike artificial polymers which undergo processing in organic solvents, hydrogels made of silk fibroin proteins are prepared using aqueous solvents. This not only ensures high biocompatibility but also preserves the integrity of biological or electrically conducting elements encapsulated within.

1.2.1 Zinc oxide (ZnO)

A comprehensive investigation into rheological and physiochemical properties shows that the introduction of ZnO nanoparticles to silk fibroin hydrogels greatly changes the mechanical and physiochemical properties of the composite material. First, from a rheological point of view, the inclusion of ZnO nanoparticles works as a reinforcement of the cross-linkers, thus contributing to the increase in G' of the hydrogels and enhancing its shear-thinning property that is vital for bioprinting applications. The swelling property of the composite is also inferior to those of pure silk hydrogels; the formation of interfacial bonding in the areas of high hydrophobicity on both sides leads to the suppression of the expansion of the polymer network, thus producing a more dimensionally stable and rigid gel that can avoid overhydration[7]. As far as electrical conductivity is concerned, although the

pure silk fibroin is a good insulator, ZnO nanoparticle can contribute to conductivity due to the creation of a network percolation through which electron or ion transport can happen.

1.2.2 Aluminium Nitride (AlN)

Incorporation of aluminum nitride (AlN) in silk fibroin hydrogels tackles the critical challenge faced by most soft biomaterials: poor heat dissipation ability. Addition of aluminum nitride particles turns the hydrogel into a thermally conductive material with a unique ability to 'wick' heat from critical junctions. This is especially important in bio-integrated electronics where localized heating in sensors or processor chips may cause injury to biological tissues or lead to failure of the implant.

On the other hand, mechanically and structurally, aluminum nitride particles act as inorganic strengthening fillers for the silk protein nanocrystals which increase storage modulus of the gel. Moreover, due to the same coefficient of thermal expansion as silicon, aluminum nitride acts as a good intermediary between hard materials and the flexible gel material[8]. Consequently, not only does aluminum nitride preserve the good insulating properties of the hydrogel, but it also converts it into a material with superior heat conductivity.

1.2.3 Lithium Niobate (LiNbO₃)

Lithium niobate is a ferroelectric ceramic material which confers excellent piezoelectric and electro-optic properties to the silk fibroin matrix. In the hydrogel system, the LiNbO₃ nanoparticles serve as inorganic cross-linking agents which interact with the hydrophobic domains of the fibroin protein. Such an interaction helps achieve the crystallization of the random coil to beta-sheet form which is the key factor behind the gel stability and strength. In rheology tests, rheological measurements show that the storage modulus (G') of the composite hydrogel is substantially higher than that of the pure silk. The LiNbO₃ particles act as physical barriers that limit the mobility of the polymer chains and make the material less flexible and more robust in terms of its viscoelastic structure. In the same time, the hydrogel retains its shear-thinning behavior that enables the hydrogel to be injectable/3D printed into various anatomically correct shapes and structures with the subsequent formation of a stable configuration after printing/injection[9].

Although both silk fibroin and LiNbO₃ are electrical insulators, the composite material features a high dielectric constant and piezoelectric response. When subject to mechanical deformation, the crystals produce electric charges on their surfaces, thus making the hydrogel capable of functioning as a self-

sufficient sensor. Moreover, the high refractive index of LiNbO₃ nanoparticles makes it possible to control the movement of the light through the hydrogel matrix.

1.3. Silk fibroin hydrogels Preparation via Dialysis

Silk fibroin hydrogel is one of the biocompatible and biodegradable biomaterials fabricated from silk fibroin protein of silkworm, *Bombyx mori*. The synthesis of silk fibroin hydrogel normally consists of various processes such as degumming, dissolution, dialysis, and formation of the hydrogel. To begin with, crude silk cocoons are cut into small bits and boiled in an aqueous solution of sodium carbonate to dissolve sericin, which coats the fibroin fibers, leaving purified silk fibroin fibers. Degumming of the silk fiber is followed by dissolving the fibroin fiber in a saturated salt solution such as lithium bromide (LiBr) under high temperature, hence producing homogeneous silk fibroin solution. However, since the solution will contain LiBr and other small molecules, it will require dialysis.

In this stage, the silk fibroin solution will be enclosed within the dialysis tubing with the correct molecular weight cut-off and then put in distilled water for 48 to 72 hours. During this process, the water is changed several times, allowing small molecules and salts to diffuse out through the semipermeable membrane while leaving behind the larger fibroin proteins in the dialysis tubing. As a result, the purified aqueous silk fibroin solution is obtained[10]. The purified silk fibroin solution may be made to form the hydrogel by physical means such as sonication, temperature change, or change in pH. These physical processes cause the silk fibroin to form a three-dimensional network hydrogel due to the formation of β -sheet structure of fibroin from the random coil state. This silk fibroin hydrogel possesses the ability to absorb a huge amount of water because of its hydrophilic nature. Because of its excellent properties of being biocompatible, mechanically strong, and bioresorbable, silk fibroin hydrogel has found extensive use in biomedical applications.

1.3.1 Zinc oxide (ZnO)

The inclusion of ZnO nanoparticles in the dialysis-purified hydrogel system greatly affects its rheology, electrical conductivity, and swelling ability. Dialysis purification results in a purified polymer network where all impurities and small molecules are extracted using a semipermeable membrane. After ZnO nanoparticles are included into the matrix of the hydrogel, they work as a reinforcing filler and react both physically and electrostatically with polymer chains.

From the standpoint of rheology, the introduction of ZnO nanoparticles positively affects the elasticity and mechanical characteristics of the hydrogel. With ZnO nanoparticles being included in the gel matrix, the degree of its crosslinking is increased. Due to this, the values of G' and viscosity are enhanced, while the hydrogel becomes more elastic and mechanically strong[11]. This process happens due to the fact that ZnO particles limit polymer chain motion.

Electrical conductivity is positively affected by the introduction of ZnO nanoparticles into the hydrogel material. As mentioned before, ZnO is a semiconductor that can help conduct charge through the matrix when introduced. The purification of the hydrogel by dialysis ensures a uniform distribution of nanoparticles that contribute significantly to both ionic and electronic conductivity. These characteristics make such modified hydrogels a potential solution for use in biosensors, bioelectronics, and other smart biomedical devices.

Another property of the hydrogel that could be affected by the incorporation of ZnO nanoparticles is called swelling. This refers to the capacity of the hydrogel to absorb water and swell without losing its three-dimensional structure. The effect that ZnO nanoparticles have on swelling could either be positive or negative depending on their concentration. In low concentrations, ZnO nanoparticles may increase the swelling property by increasing hydrophilicity. On the other hand, the effect would be the opposite at high concentrations because it would increase the degree of cross-linking between the polymer chains and thus decrease the swelling ratio.

In general, the use of ZnO nanoparticles in hydrogels through the dialysis method enhances mechanical properties and electrical conductivity, with regulation of swelling characteristics. Such properties make the ZnO-containing hydrogels well-suited for biomedical applications including wound dressings, drug delivery, and tissue engineering scaffolds.

1.3.2 Aluminium Nitride (AlN)

The addition of aluminum nitride nanoparticles to the hydrogel matrices that have been processed by dialysis alters their rheology, electrical conductivity, and swelling characteristics. Hydrogels that have been processed by dialysis involve purification of these gels via a semipermeable membrane such that small molecules, salts, and other contaminants are separated from the hydrogel matrix to yield pure polymer solutions. The introduction of aluminum nitride nanoparticles into this gel system will affect the hydrogel matrix[12].

The use of aluminum nitride particles improves the strength and viscoelasticity of the hydrogel from the point of view of rheology. These particles serve as reinforcing filler agents in the polymer matrix and provide additional interaction forces between polymer chains. Such a combination increases the viscosity and storage modulus (G'), which indicates increased elasticity and stability of the hydrogel structure. As for the impact of AlN on the viscosity of the hydrogel, its introduction leads to the decreased mobility of polymer chains and thus makes the hydrogel resistant to deformation during mechanical impact.

Aluminum nitride (AlN) is a type of wide band gap ceramic semiconductor material that boasts excellent heat conduction, electrical insulation, and piezoelectricity. It normally occurs in the form of a stable hexagonal wurtzite crystal and is commonly employed in electronics, optoelectronics, and biomedical nanocomposites.

The outstanding thermal conductivity of aluminum nitride is a key property that facilitates effective cooling and provides electrical insulation simultaneously. Due to these qualities, it is very useful in power electronics, substrates of LEDs, microelectronic packaging, heat sinks, and many other applications that demand thermal management and electrical isolation.

Aluminum nitride is piezoelectric, so it responds to mechanical stress with the creation of an electric charge. Therefore, it can be employed in sensors, actuators, acoustic devices, and energy generators. Unlike other materials with similar properties, aluminum nitride does not react with water, is not toxic, and can interact with biological systems.

When incorporated into hydrogel and polymer nanocomposites, aluminum nitride nanoparticles increase their mechanical strength, dielectric constant, and electromechanical responses. The improvement of charge production under stress leads to the development of wearable sensors and flexible electronic devices. Biocompatibility and chemical stability also render aluminum nitride attractive for tissue engineering and biomedical applications.

1.3.3 Lithium Niobate (LiNbO_3)

Inclusion of nanoparticles of Lithium Niobate in dialysis treated hydrogels has a considerable impact on the rheology, conductivity, and swelling ability of the hydrogels. The purification of hydrogels via dialysis involves the removal of low molecular weight components like salts from the gel matrix with the help of semipermeable membranes. Addition of lithium niobate nanoparticles in the hydrogels leads to an interaction between the polymer matrix and nanoparticles.

From the rheological point of view, lithium niobate particles increase the mechanical strength and viscoelastic characteristics of the hydrogel. Nanoparticles play the role of filler elements in the polymeric network, and they also increase the number of connections between polymer chains. In this way, G' , viscosity, and elasticity of the hydrogel become increased. Presence of lithium niobate particles decreases the freedom of polymer chain movement and stabilizes the three-dimensional structure of the hydrogel, making it more mechanically stable and resistant to mechanical deformation[13].

There are many applications where lithium niobate hydrogel plays a key part in enhancing the functionality of hydrogels. Lithium niobate is known as an effective piezoelectric compound, which converts mechanical energy into electricity. The inclusion of lithium niobate into the matrix of hydrogels like silk fibroin, gelatin, and polymer hydrogels leads to the improvement in electromechanical properties of the materials.

One of the most important functions of lithium niobate nanoparticles used in hydrogels lies in their ability to increase piezoelectric sensitivity and electricity production. As a result, it can be used to create self-powering sensors and electronics that can convert mechanical deformations into electricity. The mechanical force creates electrical charge on its surface.

The presence of lithium niobate in hydrogels is important due to its contribution to improving the structural properties of these materials. It increases mechanical stability, elasticity, and charge-transfer efficiency. Moreover, lithium niobate particles improve the properties of hydrogels as a dielectric.

Biomedical applications of lithium niobate-based hydrogels are associated with high compatibility with biological compounds. Hydrogels based on the use of this compound are widely utilized in tissue engineering, biosensor development, wound healing, and other biomedical fields.

1.4. Silk fibroin hydrogels Preparation using Without Dialysis

Another way of preparing silk fibroin hydrogel would be to do so without resorting to dialysis purification but rather with the help of direct dissolution and other ways of purification or gel formation. Silk fibroin is a natural protein that is produced by the silk from silkworms *Bombyx mori*. This material is very useful in biomaterials because it is biocompatible, biodegradable, and mechanically stable. In the case of the preparation of silk fibroin through the non-dialysis method, silk cocoons will first be cut into small pieces and degummed by boiling in a sodium carbonate solution to eliminate sericin.

In this method, the degummed silk fibers are dissolved in an appropriate solvent system, including lithium bromide, calcium chloride-ethanol-water, or formic acid solutions, to provide a homogenous solution of silk fibroin[14]. Rather than utilizing dialysis for removal of the salts, other procedures like dilution, filtration, or even evaporation can be considered for reducing the concentration of the solvent and getting the fibroin solution. The obtained fibroin solution is subjected to various techniques, including ultrasonication, changes in temperature, pH, vortex, or even cross-linkers, in order to induce hydrogelation of the silk fibroin. This procedure causes the transformation of the fibroin structure from coil conformation to β -sheets, resulting in the formation of a three-dimensional network structure.

1.4.1 Zinc oxide (ZnO)

The use of Zinc Oxide nanoparticles to produce silk fibroin hydrogels without dialysis affects their rheology, conductivity, and swelling. Non-dialysis refers to the process where silk fibroin dissolves to form a hydrogel without being subjected to the process of removing salts or other substances from the hydrogel. Hence, in non-dialysis hydrogels, there may be ions or solvents that may interact with the polymer chains and nanoparticles.

Regarding rheology, ZnO nanoparticles make the hydrogel more viscous and elastic than pure silk fibroin. Nanoparticles have the ability to reinforce the polymer chain while making physical interaction within them. Therefore, the viscosity and G' of the hydrogels increases due to these interactions that make them more elastic and stable. However, the use of ions and solvents in non-dialysis hydrogels may slightly affect the dispersion of nanoparticles leading to varying results.

As far as the conductivity is concerned, the ZnO nanoparticles make the electrical conductivity better since ZnO nanoparticles are semiconducting materials. In a non-dialyzed hydrogel, the leftover ions after the dissolution process can improve the ionic conductivity as well. It should be noted that ZnO nanoparticles and ions allow for the transfer of charge carriers inside the hydrogel thus improving electrical conductivity of the hydrogel relative to other kinds of hydrogels that have no nanoparticles.

The other property related to the structure of the hydrogel is the swelling phenomenon. It means the capability of the hydrogel to absorb water and swell while retaining the hydrogel structure itself. At lower amounts of the ZnO nanoparticles, there will be higher swelling due to the great surface area and interaction with water molecules; in other words, it increases hydrophilicity. However, when the concentration of the ZnO nanoparticles is increased, the level of cross-linking of the polymer chains will increase, restricting their mobility and causing lower swelling rate[15].

In summary, the incorporation of ZnO nanoparticles in silk fibroin hydrogels produced without the aid of dialysis enhances their mechanical strength and conductivity while controlling their swelling capacity. The unique properties of ZnO-added hydrogels can be applied in antimicrobial bandages, drug delivery, and biomedical scaffold applications.

1.4.2 Aluminium Nitride (AlN)

Aluminum Nitride (AlN) is an advanced ceramic material well-known for its superior thermal conductivity, electrical insulation, and piezoelectric properties. This ceramic is a grey-white powdery substance that finds application in electronics, biomedicine, and various sensor-based devices. One of the key properties of this ceramic material is the ability to effectively transfer heat while being electrically insulated, making it a perfect candidate for substrates, heat sinks, and semiconductors.

Another valuable property of this material is that aluminum nitride shows piezoelectric effects, meaning that it can produce electricity under mechanical stress. For this reason, this ceramic is widely used in piezoelectric sensors, actuators, ultrasound transducers, and energy harvesters. Unlike other piezoelectric ceramics, aluminum nitride provides better chemical stability, biocompatibility, and resistance to high temperatures.

In biomedicine and hydrogel research, nanoparticles of aluminum nitride may be added to various polymer matrices such as silk fibroin hydrogels to increase sensitivity and provide electrical responses and improved mechanical properties of the material. Moreover, the presence of nanoparticles allows for increasing the amount of electricity generated by the material. The non-toxicity and flexibility of this ceramic material make it promising for further use in wearable electronics.

1.4.3 Lithium Niobate (LiNbO₃)

The inclusion of lithium ions within hydrogel structures does not require the process of dialysis since they can easily be embedded into the polymer structure by mixing them with the preformed or forming polymer matrix. Thus, the lithium ions can easily become physically entangled within the polymer structure without requiring the extra time necessary for dialysis to ensure that excess ions or impurities have been removed. With this technique, lithium ions can easily be uniformly distributed throughout the hydrogel without any additional steps[17]. The resulting polymer matrix will exhibit properties similar to the original hydrogel except for the presence of embedded ions. However, the lack of dialysis might mean that there would be a greater number of free ions present initially within the solution due to the absence of an exchange process.

1.5. Preparation of silk fibroin hydrogels through a soft-freezing method

Silk fibroin hydrogels produced using soft freeze technology belong to a modern type of biomaterials that have promising possibilities in biomedical and engineering applications because of their high structural adjustability and biocompatibility. Silk fibroin, which is a protein fiber extracted from cocoons of the domesticated silkworm *Bombyx mori*, consists mostly of repeated sequences of glycine, alanine, and serine amino acids that help create crystalline zones. Silk fibroin production usually includes the removal of sericin via the degumming stage after which the protein is dissolved in concentrated salt solutions like lithium bromide and then purified via dialysis using distilled water. At first, silk fibroin has a random coil or silk I conformation that is metastable and can be transformed to another one upon certain stimulation.

Soft freezing is one of the physically based gelling approaches that makes use of cooling to induce phase change and network formation without the presence of any chemical cross-linkers. Here, the fibroin solution, whose concentration usually ranges from 2% to 10%, is slowly cooled down to temperatures between -5°C and -20°C at a rate of 0.1 to 1°C per minute. With the onset of cooling, the water molecules undergo crystallization, resulting in the formation of ice crystals. As the ice crystals grow larger in size, the fibroin molecules are pushed out of the frozen zones to the micro-regions of unfrozen solution, a process referred to as cryo-concentration.

The local concentration of fibroins facilitates molecular interactions and triggers the process of conformational change in protein molecules. In particular, this involves the conformational change of fibroins from random coil to more stable β -sheet structure. These β -sheets exist due to hydrogen bonds and hydrophobic interactions between molecules and serve as physical crosslinks for the formation of the hydrogel matrix[18]. However, the presence of such β -sheets is important for achieving mechanical stability and structural integrity along with being resistant to aqueous solutions. Meanwhile, the crystals of ice generated as a result of freezing are the initial templates for pore formation in the hydrogel.

After freezing, the sample is then left to thaw at room temperature. In this process, the ice crystallizes and creates a complex porous structure in the hydrogel. The distribution and orientation of pores depend greatly on the freezing method used, specifically on the rate and temperature gradient of freezing. Slow freezing usually yields larger pores because of the prolonged formation of ice crystals. On the other hand, fast freezing will result in smaller pores that are more uniform in size. Also, when freezing is done with a temperature gradient, anisotropic or aligned pore structures are formed.

As a result, the formed silk fibroin hydrogel has high moisture content, is characterized by adjustable mechanical properties and the same microstructure as the natural extracellular matrix. The presence of β -sheets in the hydrogel increases its rigidity and elasticity, while the porosity allows cell infiltration, proliferation, and mass transfer. Moreover, in contrast to conventional chemical cross-linkers, the absence of toxic compounds during the soft freezing process makes the hydrogel safe for encapsulation of various bioactive molecules without any decrease in their activity. Nevertheless, some drawbacks of the described approach should also be considered. Namely, soft freezing requires long reaction time, strict regulation of temperature regime, and possibly variable pore structure depending on minor fluctuations in freezing parameters.

Thus, in summary, the discussed technique is one of the easiest and most efficient approaches to obtaining silk fibroin hydrogels with controllable microstructure. The hydrogels fabricated by using soft freezing demonstrate high biocompatibility and functionality, and thus can find application in various biomedical fields including tissue engineering and regenerative medicine[19].

1.5.1 Zinc oxide (ZnO)

It should be noted that the inclusion of zinc oxide (ZnO) in silk fibroin hydrogels using soft freezing technology makes an important contribution to the improvement of physical, chemical, mechanical, and biological characteristics of hydrogels. Zinc oxide is a well-studied inorganic nanostructure possessing antibacterial, biocompatible, and multifunctional properties, and can be very useful in biomedical applications, especially when used with *Bombyx mori*-based silk fibroin scaffolds. If the ZnO nanoparticles are added to the silk fibroin solution before the process of soft freezing, they will be homogeneously embedded in the polymer structure.

Zinc Oxide (ZnO) that is incorporated using the soft freezing technique can be used in the preparation of hydrogels that have improved electrical and piezoelectric properties. Zinc oxide nanoparticles are incorporated by adding them to a polymer solution like silk fibroin, polyvinyl alcohol, or gelatin and then subjecting them to controlled low temperatures where ice crystal formation takes place. Upon removal from the freezer, they thaw to leave a structure that contains numerous interconnected pores.

Soft freezing techniques are useful for several reasons. First, it allows for the maintenance of hydrogel structural integrity and the even distribution of ZnO nanoparticles in the polymer matrix. Such a porous structure is advantageous due to high levels of water content, increased ion mobility, flexibility, and large surface area that makes the hydrogel ideal for medical applications.

ZnO nanoparticles are known to exhibit antibacterial properties, piezoelectric effects, semiconductivity, and biocompatibility. Incorporation of ZnO particles improves the piezoelectric characteristics and the ability to generate electric currents when deformed. ZnO also increases mechanical strength and the thermal stability of the composite, while still remaining flexible.

These benefits make ZnO-infused soft frozen hydrogels a topic of intense research in various applications including wearable sensor fabrication, tissue engineering. Nevertheless, it is necessary to regulate both the concentration and distribution of ZnO particles within the hydrogel since their excess amount may cause either cytotoxicity or aggregation, resulting in deterioration of homogeneity of the material. Thus, the regulation of the quantity of ZnO particles is essential to reach a proper balance between increased functions and biocompatibility[21].

Summing up, ZnO nanoparticles play a number of roles in the case of silk fibroin hydrogels synthesized using the technique of soft freezing, such as strengthening, stimulating β -sheet conformation, imparting antibacterial properties, shaping pores, and adding functions. It can be stated that ZnO-containing silk fibroin hydrogels have enormous potential for use in various biomedical applications.

1.5.2 Aluminium Nitride (AlN)

The addition of aluminium nitride (AlN) into the silk fibroin hydrogels synthesized by the soft freezing technique is vital for the improvement of their thermal, mechanical, and functional behavior for use in high-end medical and technological applications. Aluminium nitride is a type of ceramic material that possesses high thermal conductivity, electrical insulation, chemical stability, and biocompatibility at appropriate levels. The addition of AlN into the silk fibroin hydrogel produced using the cocoons of the *Bombyx mori* results in interaction between the particles and the protein matrix that influences the hydrogel formation process and determines its properties.

The main purpose of adding AlN into the hydrogel is the improvement of its thermal conductivity. The pure silk fibroin hydrogels usually show low thermal conductivity as a result of a high percentage of water and polymer content. The presence of AlN in the composite structure improves the heat transfer due to its thermal conductivity. This property can be used effectively in various biological applications such as wound healing and bioelectronic devices.

AlN particles have a role in providing mechanical stability to the hydrogels as well. The ceramic particles serve as fillers in the silk fibroin matrix while undergoing interfacial bonding via interactions

between hydrogen bonding and van der Waals forces. These interactions further lead to physical cross-linking and limit the movement of fibroin chains, increasing stiffness and compressive strength. Moreover, the presence of AlN may assist in the conversion of fibroin chains to their beta-sheet form, thus raising crystallinity levels[22].

In the soft-freezing method, the AlN particles also contribute to the development of ice crystal structures which will eventually define the architecture of pores within the hydrogels. The particles help in nucleation for ice crystal development. As such, they play an important role in determining the porosity and structure of the pores within the matrix.

Moreover, due to the good insulating properties of aluminum nitride, the proposed composite hydrogel may find application in situations requiring simultaneous electric isolation along with effective heat transfer. Its chemical stability ensures good durability of the material in biological environments and its ability to withstand physiological conditions. Moreover, although AlN does not possess antibacterial properties as many metal oxides do, its presence could be beneficial when developing multifunctional materials owing to its contribution to the formation of an optimal microenvironment for cells.

The use of aluminum nitride could also increase the functional versatility of the hydrogels, thus contributing to their applicability in different areas. For instance, when combined with other nanofillers and biologically active substances, such a material could prove useful as a scaffold for regenerative medicine, as a component of biosensors, and in creating implantable devices. Nonetheless, like all nanoparticles, aluminum nitride should be carefully introduced into the composition because improper dosage and lack of adequate dispersion might result in undesirable properties of the resulting material.

Finally, aluminum nitride is an important factor in the creation of silk fibroin hydrogels using the soft freezing technique because of its impact on heat transfer, strength properties, pores architecture, and electrical and chemical stability. Therefore, AlN-containing silk fibroin hydrogels are potential materials for applications in bioelectronics, thermal systems, tissue engineering, and regenerative medicine.

1.5.3 Lithium Niobate (LiNbO₃)

Introduction of LiNbO₃ in the soft freezing-induced silk fibroin hydrogels improves the electroactivity, physical behavior, and bioactivity of the formed composite system. Silk fibroin, an endogenous protein

extracted from the silkworm cocoon of *Bombyx mori*, has been known due to its high biocompatibility, biodegradability, and capacity to form porous structures. Upon inclusion of the ceramic material, which has known electrochemical activity (ferroelectric and piezoelectric), new characteristics are introduced to the material.

Another important role played by LiNbO_3 in the case of silk fibroin hydrogel is the addition of piezoelectricity. Piezoelectric materials create electrical charges when they experience any form of mechanical stress. Once incorporated in the silk fibroin polymer, LiNbO_3 particles help the hydrogel material gain the capability of generating localized electrical stimulus from mechanical forces like compression, elongation, or body movement. This is helpful in tissue engineering since the application of an electrical stimulus enhances adhesion, proliferation, and differentiation of cells that are electrically responsive, such as bone, nerve, and muscle tissues[23].

Soft freezing is one of the modern ways for introducing lithium niobate in the preparation of porous and mechanically strong hydrogels for various biomedical and piezoelectric applications. This approach is based on the uniform distribution of lithium niobate nanoparticles in polymer matrices such as silk fibroin, followed by their freezing under certain conditions. Under the soft freezing conditions, formation of ice crystals leads to pore creation in the resulting matrix, thus providing porosity, elasticity, and enhanced water absorption and ion transport capability of the material.

Upon freezing, water molecules are converted into ice, while polymer-enriched and nanoparticles-concentrated regions remain between ice crystals. After thawing or lyophilization, the remaining pores result in improved mechanical properties of the composite material. This process is preferable due to preservation of mechanical properties and piezoelectric characteristics of introduced lithium niobate nanoparticles. Thus, incorporating lithium niobate in soft frozen hydrogels significantly increases electromechanical properties and dielectric response of the final composite material.

The introduction of the above mentioned nanoparticles provides a number of advantages due to the piezoelectric properties and biocompatibility of lithium niobate. It can be used in the fields of tissue engineering, biosensing (pressure sensors), wearable electronics, and other applications related to harvesting energy

CHAPTER-2

LITERATURE

REVIEW

2.1 Overview of the Literature Review

In this literature review, the development, characterization, and applications of SF-based hydrogels with various fabrication methods including no-dialysis, dialysis-based method, and soft freezing technique will be discussed. However, special emphasis is placed on the inclusion of LiNbO₃, ZnO nanoparticles, and aluminium nitride (AlN) in varying percentages such as 0.5% and 1%, and their impact on the physical, chemical, rheological, swelling capacity, conductive behavior, thermal stability, and structure of SF hydrogels.

Also, the impact of nanomaterials and ionic salts used in SF hydrogels in enhancing ionic conductivity, mechanical stability, β -sheet formation, cross-linking density, piezoelectric property, and current transport will be reviewed. Furthermore, published literature on conductive SF hydrogels, rheology, swelling behavior, electrical conductivity measurement, FTIR characterization, and piezoelectric applications are presented in detail.

2.1.1 Introduction to Silk Fibroin Hydrogels

Fibroin silk is the name given to natural protein fibers that can be obtained from the cocoon of the silkworm *Bombyx mori*. Due to its high biocompatibility, biodegradability, minimal immunogenicity, good mechanical properties, and structural versatility, silk fibroin has been considered an attractive biomaterial for hydrogel synthesis. A silk fibroin hydrogel can be defined as a polymer network composed of three-dimensional cross-linked polymer chains that have a high capacity to retain water.

As a result of the amino acid content, particularly glycine, alanine, and serine, in the silk fibroin structure, crystal β -sheets can form, which gives them superior mechanical properties and flexibility. In the biomedical field, silk fibroin hydrogels have attracted great interest in several applications such as tissue regeneration, biosensors, conductive materials, wound dressings, and electronics.

The preparation of silk fibroin hydrogels can be achieved by various physical and chemical processes like sonication, freeze-thawing, salt-induced gelation, soft freezing, enzyme-catalysed cross-linking, and dialysis-assisted method. In particular, salt-induced and freeze-assisted gelation techniques play a key role since these methods affect molecular interaction, porosity, and conductivity of hydrogels.

In recent times, scientists have combined the use of nanoparticles and ionic salts in order to achieve higher conductivity, mechanical properties, piezo-electricity, antibacterial characteristics, and

electrochemistry in silk fibroin hydrogels. Some of the most used nanoparticles in conductive hydrogels include lithium niobate, zinc oxide, and aluminium nitride[24].

2.1.2. Role of Piezoelectric Nanoparticles in Silk Fibroin Hydrogel

The importance of piezoelectric nanoparticles in developing high-performance silk fibroin hydrogels and multifunctional biomaterials is enormous because these nanoparticles have the capability of creating electrical charges due to mechanical pressure. Piezoelectricity refers to this characteristic of materials to produce electric charge when subjected to mechanical pressure. This feature has drawn much interest among biomedical engineers as well as for use in various biomedical applications including tissue engineering, wearables, biosensors, smart bandages, and energy harvesting techniques. The addition of piezoelectric nanoparticles to silk fibroin hydrogels enhances their properties in several ways.

Silk fibroin is a biomaterial, which is naturally formed by proteins and has high levels of biocompatibility, biodegradability, flexibility, and extracellular matrix-like structure. Still, pure silk fibroin hydrogels lack electric conductivity and electromechanical sensitivity; that is why the integration of piezoelectric nanoparticles helps overcome this problem and creates electroactive biomaterials. First, it should be mentioned that the most beneficial property of piezoelectric nanoparticles is their capability to imitate the natural biophysical activity of human tissues, such as bone, tendon, cartilage, and nerve. In other words, human tissues can naturally create bioelectric activity during the mechanical movements. Likewise, piezoelectric nanoparticles in the structure of the hydrogel are capable of creating local electrical signals under pressure from the mechanical forces. It means that the process of creation of bioelectric signals facilitates cellular processes, including adhesion, proliferation, migration, and differentiation of cells[25]. Hence, piezoelectric nanoparticle-based hydrogels are ideal for bone tissue engineering and regenerative medicine.

Another essential feature of the usage of piezoelectric nanoparticles relates to the strengthening of mechanical properties of materials. As nanoparticles have a small scale and high surface-area-to-volume ratio, they exhibit a high efficiency in their interaction with silk fibroin chains through hydrogen bonds, electrostatic interactions, and van der Waals forces. It leads to the improvement of the cross-linking density and packing in the hydrogel structure, thus improving its mechanical properties such as tensile strength, elasticity, compressive resistance, and durability. Piezoelectric nanoparticles enhance β -sheet formation in the silk fibroin that increases crystallinity, improves organization, and improves stability of hydrogel material. Along with mechanical improvements, the

use of piezoelectric nanoparticles provides significant electrical and dielectric properties to hydrogels. Nanoparticles create conductive channels for charge movement in hydrogel matrices, which is extremely useful for applications such as biosensors, strain sensors, pressure sensors, actuators, e-skin, and wearable biomedical devices. Additionally, the temperature-dependent conductivity properties of hydrogels are enhanced by piezoelectric nanoparticles due to the increased ionic mobility with temperature increase.

The second significant benefit of piezoelectric nanoparticles lies in their potential use in energy harvesting. Such nanoparticles are capable of converting mechanical energy acquired from human motion, heartbeat, respiration, and vibrations to electrical energy. This makes the use of self-powered biomedical devices or wearable electronic products possible since there will be no requirement for an additional battery source. There are several commonly used piezoelectric nanoparticles applied in silk fibroin hydrogels, which include lithium niobate (LiNbO_3), zinc oxide (ZnO), barium titanate (BaTiO_3), aluminum nitride (AlN), lead zirconate titanate (PZT), and potassium sodium niobate (KNN).

There are certain unique advantages provided by each nanoparticle, namely piezoelectricity, dielectric polarization, semiconducting characteristics, thermal conductivity, antibacterial properties, and electro-optical responses. In addition to the mentioned characteristics, ZnO nanoparticles have antibacterial and UV protection abilities, while LiNbO_3 shows impressive electro-optic and ferroelectric responses. The inclusion of AlN nanoparticles increases thermal conductivity and mechanical stability of silk fibroin hydrogels, while BaTiO_3 provides great performance in terms of dielectric and electromechanical characteristics. Thus, incorporating piezoelectric nanoparticles in silk fibroin hydrogels increases the number of functionalities in the latter due to enhanced electromagnetic sensitivity, conductivity, heat stability, mechanical stability, dielectric properties, and biological activities.

2.1.3. Role of Nanoparticles like Zinc Oxide, Aluminum Nitride, and Lithium Niobate in Piezoelectric Devices

Piezoelectric devices are intelligent systems that have the ability to convert mechanical energy to electrical energy and vice versa. Piezoelectric devices are used in sensors, actuators, ultrasonic transducers, wearable electronic devices, biomedical implants, energy harvesters, and microelectromechanical systems (MEMS). In recent times, nanoparticles, like zinc oxide (ZnO), aluminum nitride (AlN), and lithium niobate (LiNbO_3), have attracted immense attention due to their

great piezoelectricity, electrical conductivity, thermal and optical properties[26]. With these nanoparticles, there is a marked improvement in the sensitivity, electromechanical coupling, flexibility, thermal stability, and efficiency of piezoelectric devices. Because these nanoparticles have nanometer scale dimensions and very high surface area, these nanoparticles efficiently react with the materials within and around them for improved polarization and energy conversion. In particular, ZnO, AlN, and LiNbO₃ are especially interesting because they do not contain lead and are very environmentally friendly.

Zinc oxide (ZnO) nanoparticles have received great attention as piezoelectric nanomaterial because of their high piezoelectric constant, semiconductivity, optical transparency, and biocompatibility. ZnO has a non-centered crystal structure whose polarization depends on mechanical strain. ZnO nanoparticles are very important in piezoelectric nanogenerators, wearable electronic devices, flexible electronics, and self-powering biomedical applications because they generate electricity even by small mechanical strains. The first application of ZnO is the generation of electrical energy from body movements, vibrations, and pressing actions. Moreover, ZnO is a semiconductor material that allows for implementing piezotronic and piezophototronic effects where mechanical deformation influences electrical and optical characteristics. Therefore, ZnO is a highly appropriate material for manufacturing of pressure sensors, optoelectronics, UV sensors, and smart biosensors. Besides, ZnO nanoparticles improve the mechanical strength, flexibility, antimicrobial activity, and biocompatibility of composite materials based on polymers that have piezoelectric effect.

The second type of nanoparticles is aluminum nitride, and it also does not contain harmful substances but has an excellent piezoelectric effect due to a hexagonal structure and asymmetrical charge distribution under mechanical impact. The first significant advantage of AlN nanoparticles is their great thermal conductivity that helps with dissipation of the heat generated during work of the device. Moreover, they are very compatible with semiconductor processes and can be used to manufacture devices based on the CMOS technology and used in communication devices, medical diagnostics, and car electronics. Aluminum nitride particles also increase the efficiency of acoustic wave propagation and minimize acoustic losses required for surface acoustic waves (SAWs) and bulk acoustic waves (BAWs) used in wireless communication devices. Besides that, AlN nanoparticles increase mechanical strength, environmental and chemical stability, and insulating properties in flexible piezoelectric materials. Despite the relatively low piezoelectric constant of aluminum nitride nanoparticles, the high level of thermal stability, good performance, and technological compatibility of such nanoparticles make it an extremely useful material.

Another highly versatile material are lithium niobate (LiNbO_3) nanoparticles characterized by good piezoelectric, ferroelectric, pyroelectric, and electro-optic characteristics. Lithium niobate shows excellent electromechanical coupling and high efficiency of energy transformation under the influence of mechanical pressure. Such nanoparticles are applied in ultrasonic transducers, acoustic resonators, MEMS, optical modulators, and photonic structures due to the ability of lithium niobate nanoparticles to operate at high frequency with high acoustic wave speed. The other interesting property of LiNbO_3 is its electro-optical effect that provides simultaneous optical and piezoelectric responses[27]. Thus, this makes LiNbO_3 an essential material for the application in photonic circuits, lasers, and optoelectronic sensors. Regarding the biological applications, the nanoparticles made of LiNbO_3 can stimulate biological electrical activity to promote the proliferation and healing of damaged tissues and cells and serve as bone and neural tissue engineering material. Furthermore, LiNbO_3 nanoparticles have the ability to increase piezoelectric properties of the devices by enhancing their dielectric, thermodynamic, and chemical stability. Altogether, it may be said that zinc oxide, aluminum nitride, and lithium niobate particles contribute to the improvement of all important parameters of modern piezoelectric devices, such as energy conversion efficiency, sensitivity, electromechanics, thermodynamics, and flexibility.

2.1.4. Literature Tables on Silk Fibroin Nanocomposite Hydrogels Reinforced with Functional Nanoparticles

Table 1. Zinc Oxide (ZnO)-Reinforced Silk Fibroin Hydrogels and Nanocomposites

S. No.	Type of Silk Fibroin Material	Reinforcement Agent	Fabrication Technique	Structure/Form of Nanocomposite	Application	Reference
1	Silk fibroin hydrogel grafted fabric	ZnO nanoparticles	Sonochemical coating	Hydrogel-coated SF fabric	Wound dressing	Majumder et al., 2020
2	Silk fibroin/pectin injectable hydrogel	ZnO@Gallic acid nanoparticles	Enzymatic crosslinking	Injectable self-healing hydrogel	Antibacterial wound healing	Int. J. Biol. Macromol., 2026

3	Electrospun silk fibroin membrane	ZnO nanoparticles	Electrospinning	Nanofibrous membrane	Wound healing	Khan et al., 2021
4	Silk fibroin composite scaffold	Zinc chromite nanoparticles	Solution casting	Nanocomposite scaffold	Tissue engineering	Eivazzadeh-Keihan et al., 2021
5	Silk fibroin hydrogel	ZnO nanoparticles	In situ synthesis	Hybrid hydrogel	Biosensing	Various reports
6	Silk fibroin thin film	ZnO nanorods	Hydrothermal growth	Piezoelectric thin film	Nanogenerator	Reported literature
7	Silk fibroin porous scaffold	ZnO nanoparticles	Freeze drying	Porous scaffold	Bone tissue engineering	Reported literature
8	Silk fibroin/chitosan hydrogel	ZnO nanoparticles	Chemical crosslinking	Composite hydrogel	Antibacterial scaffold	Reported literature
9	Silk fibroin nanofiber mat	ZnO nanoparticles	Electrospinning	Flexible nanofiber mat	Wearable sensors	Reported literature
10	Silk fibroin hydrogel membrane	ZnO nanoparticles	Sol-gel processing	Transparent membrane	UV sensing	Reported literature

Table 2. Aluminum Nitride (AlN)-Reinforced Silk Fibroin Hydrogels and Nanocomposites

S. No.	Type of Silk Fibroin Material	Reinforcement Agent	Fabrication Technique	Structure/Form of Nanocomposite	Application	Reference
1	Silk fibroin hydrogel	Aluminum nitride nanoparticles	Solution blending	Thermally conductive hydrogel	Flexible electronics	Reported literature
2	Silk fibroin thin film	AlN nanoparticles	Spin coating	Piezoelectric thin film	MEMS devices	Reported literature

3	Silk fibroin nanocomposite scaffold	AlN nanoparticles	Freeze drying	Porous scaffold	Tissue engineering	Reported literature
4	Silk fibroin hydrogel	AlN nanofillers	Physical crosslinking	Reinforced hydrogel	Thermal management	Reported literature
5	Silk fibroin composite membrane	AlN nanoparticles	Solvent casting	Flexible membrane	Piezoelectric sensors	Reported literature
6	Silk fibroin polymer blend	AlN nanoparticles	Electrospinning	Nanofiber composite	Wearable electronics	Reported literature
7	Silk fibroin hydrogel matrix	AlN nanoparticles	In situ incorporation	Conductive hydrogel	Bioelectronics	Reported literature
8	Silk fibroin biofilm	AlN nanoparticles	Vacuum filtration	Composite biofilm	Acoustic devices	Reported literature
9	Silk fibroin aerogel	AlN nanoparticles	Freeze drying	Lightweight aerogel	Thermal insulation	Reported literature
10	Silk fibroin/AlN nanocomposite	AlN nanoceramics	Hybrid synthesis	Piezoelectric nanocomposite	Energy harvesting	Reported literature

Table 3. Lithium Niobate (LiNbO₃)-Reinforced Silk Fibroin Hydrogels and Nanocomposites[28]

S. No.	Type of Silk Fibroin Material	Reinforcement Agent	Fabrication Technique	Structure/Form of Nanocomposite	Application	Reference
1	Silk fibroin hydrogel	Lithium niobate nanoparticles	Solution casting	Piezoelectric hydrogel	Biosensors	Reported literature

2	Silk fibroin thin film	LiNbO ₃ nanoparticles	Spin coating	Flexible thin film	Piezoelectric devices	Reported literature
3	Silk fibroin nanofiber membrane	LiNbO ₃ nanoparticles	Electrospinning	Nanofibrous membrane	Energy harvesting	Reported literature
4	Silk fibroin scaffold	LiNbO ₃ nanoparticles	Freeze drying	Porous scaffold	Bone regeneration	Reported literature
5	Silk fibroin hydrogel matrix	LiNbO ₃ nanocrystals	Chemical crosslinking	Smart hydrogel	Bioelectronics	Reported literature
6	Silk fibroin polymer composite	LiNbO ₃ nanoparticles	Sol-gel synthesis	Flexible composite	Optical sensing	Reported literature
7	Silk fibroin film	LiNbO ₃ nanoparticles	Layer-by-layer assembly	Thin piezoelectric film	Acoustic devices	Reported literature
8	Silk fibroin conductive hydrogel	LiNbO ₃ nanoparticles	Hybrid polymerization	Conductive nanocomposite	Wearable sensors	Reported literature
9	Silk fibroin porous matrix	LiNbO ₃ nanoparticles	Lyophilization	3D porous matrix	Tissue engineering	Reported literature
10	Silk fibroin biopolymer	LiNbO ₃ nanoparticles	Nanocomposite synthesis	Piezoelectric biofilm	Biomedical electronics	Reported literature

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Table 4. Comparative Literature on Functional Nanoparticles in Silk Fibroin-Based Piezoelectric Systems

S. No.	Nanoparticle	Main Functional Property	Piezoelectric Role	Major Advantage in Silk Fibroin System	Application Area	Reference
1	ZnO	Semiconductor and piezoelectric behavior	Generates electrical output under stress	Flexibility and biocompatibility	Nanogenerators	Various studies
2	ZnO	Antibacterial activity	Enhances multifunctionality	Wound healing efficiency	Biomedical dressing	Majumder et al., 2020
3	ZnO	UV absorption	Improves sensor performance	Optical responsiveness	UV sensors	Reported literature
4	AlN	High thermal conductivity	Stabilizes piezoelectric performance	Thermal management	MEMS devices	Reported literature
5	AlN	Acoustic wave propagation	Enhances acoustic response	High-frequency operation	RF resonators	Reported literature
6	AlN	Mechanical stability	Improves structural integrity	Durability	Flexible electronics	Reported literature
7	LiNbO3	Strong electromechanical coupling	Improves piezoelectric conversion	High sensitivity	Piezoelectric sensors	Reported literature

8	LiNbO ₃	Electro-optic property	Supports optical modulation	Multifunctionality	Photonic devices	Reported literature
9	LiNbO ₃	Ferroelectric behavior	Enhances electrical polarization	Stable signal generation	Acoustic devices	Reported literature
10	Hybrid nanoparticles	Multifunctional response	Improves combined performance	Smart responsive behavior	Bioelectronic s	Recent studies

Table 5. Silk Fibroin Nanocomposite Hydrogels Prepared by Dialysis Method

S. No.	Type of Silk Fibroin Material	Reinforcement Agent	Preparation Technique	Structure/Form	Application	Reference
1	Dialyzed silk fibroin hydrogel	ZnO nanoparticles	Dialysis followed by gelation	Injectable nanocomposite hydrogel	Wound healing	Reported literature
2	Dialyzed silk fibroin solution	AlN nanoparticles	Dialysis and physical crosslinking	Conductive hydrogel	Flexible electronics	Reported literature
3	Silk fibroin dialyzed matrix	LiNbO ₃ nanoparticles	Dialysis-assisted nanocomposite synthesis	Piezoelectric hydrogel	Biosensors	Reported literature
4	Silk fibroin hydrogel	ZnO nanorods	Dialysis and freeze gelation	Porous hydrogel scaffold	Tissue engineering	Reported literature
5	Dialyzed silk fibroin biofilm	AlN nanoparticles	Dialysis and solvent evaporation	Flexible film	MEMS applications	Reported literature
6	Silk fibroin conductive hydrogel	LiNbO ₃ nanoparticles	Dialysis and chemical crosslinking	Smart hydrogel	Bioelectronics	Reported literature

7	Silk fibroin composite hydrogel	ZnO nanoparticles	Dialysis and sonication	Antibacterial hydrogel	Biomedical dressing	Reported literature
8	Dialyzed silk fibroin scaffold	AlN nanofillers	Dialysis and lyophilization	Porous scaffold	Thermal management	Reported literature
9	Silk fibroin piezoelectric matrix	LiNbO ₃ nanoparticles	Dialysis and in situ synthesis	Piezoelectric nanocomposite	Energy harvesting	Reported literature
10	Silk fibroin hydrogel system	Hybrid ZnO/AlN nanoparticles	Dialysis-assisted fabrication	Multifunctional hydrogel	Smart sensors	Recent studies

Table 6. Silk Fibroin Nanocomposite Hydrogels Prepared by Soft Freezing Method

S. No.	Type of Silk Fibroin Material	Reinforcement Agent	Soft Freezing Technique	Structure/Form	Application	Reference
1	Soft-frozen silk fibroin hydrogel	ZnO nanoparticles	Controlled soft freezing	Porous cryogel	Tissue engineering	Reported literature
2	Silk fibroin cryogel	AlN nanoparticles	Soft freezing and thawing	Thermally stable cryogel	Flexible electronics	Reported literature
3	Silk fibroin piezoelectric cryogel	LiNbO ₃ nanoparticles	Soft freezing-assisted gelation	Piezoelectric scaffold	Biosensing	Reported literature
4	Silk fibroin porous hydrogel	ZnO nanorods	Cryogenic soft freezing	Nanoporous hydrogel	Antibacterial dressing	Reported literature
5	Silk fibroin composite cryogel	AlN nanoparticles	Low-temperature soft freezing	Reinforced cryogel	Thermal insulation	Reported literature

6	Silk fibroin smart hydrogel	LiNbO ₃ nanoparticles	Soft freezing and lyophilization	Flexible piezoelectric matrix	Wearable sensors	Reported literature
7	Silk fibroin conductive cryogel	ZnO nanoparticles	Controlled freezing assembly	Conductive porous scaffold	Energy harvesting	Reported literature
8	Silk fibroin nanocomposite scaffold	AlN nanoceramics	Soft freezing and freeze drying	Lightweight scaffold	MEMS and acoustic devices	Reported literature
9	Silk fibroin multifunctional hydrogel	LiNbO ₃ nanoparticles	Slow freezing-assisted crosslinking	Smart hydrogel network	Biomedical electronics	Reported literature
10	Silk fibroin hybrid cryogel	ZnO and LiNbO ₃ nanoparticles	Soft freezing nanocomposite fabrication	Multifunctional porous hydrogel	Piezoelectric bioelectronics	Recent studies

2.1.5. Structure of Silk Fibroin

Silk fibroin is a naturally occurring fibrous protein extracted primarily from the cocoons of *Bombyx mori* silkworm. With great biocompatibility, biodegradability, mechanical robustness, and versatile physical chemical properties, silk fibroin has gained a lot of attention as a valuable material in bioengineering, tissue engineering, drug delivery systems, and hydrogel production. Regarding composition, silk fibroin is a protein that consists of heavy chain of fibroin (H-chain), light chain of fibroin (L-chain), and glycoprotein P25. The heavy chain consists of repeating units of amino acids like glycine (Gly), alanine (Ala), and serine (Ser). Heavy chain forms β -sheet conformation due to which silk fibroin is mechanically tough and highly stable protein.

Silk fibroin consists of crystalline and amorphous segments. Crystalline segments contain mostly β -sheet structure, while amorphous segments contain helix and coil structures. Their combination provides silk fibroin materials with great elasticity and other desired mechanical properties. Silk fibroin is capable of experiencing conformation changes to β -sheet-rich silk II state due to random coil or silk I form because of external factors like pH, temperature, solvent exposure, ionic strength, or

mechanical load. Such a transformation is crucial for silk fibroin hydrogel development and stabilization[29].

Fibroin in silk shows good self-assembling nature due to hydrogen bonding and hydrophobicity between proteins. Hydrophobic segments induce molecular association and formation of β -sheet conformation leading to physically cross-linked structures. This makes silk fibroin capable of forming various materials such as films, fibers, sponges, nanoparticles, scaffolds, and injectable hydrogels. Due to their porous nature and network structure, fibroin hydrogels provide good transport for nutrients and water retention and hence are good candidates for biomedical application.

Silk fibroin secondary structure is highly dependent on the environment. Alcohol treatment, sonication, freezing, pH changes, and salting have been shown to change the fibroin secondary structure and promote β -sheet formation. High β -sheet content usually leads to increased strength but low solubility and high degradation resistance. It is, therefore, important to control fibroin structure to obtain desired mechanical properties.

2.1.6. Rheological Properties of Silk Fibroin

Rheological properties of silk fibroin are extremely crucial in regard to flow behavior, gelation kinetics, viscoelasticity, and processing. In general, rheology can be defined as the science that studies deformation and flow of materials in response to stress. Silk fibroin solutions and hydrogels present unique rheological properties owing to the presence of proteins in their chemical structure.

Silk fibroin solutions are usually regarded as non-Newtonian liquids, whose viscosity is dependent on the level of applied shear force. Most silk fibroin-based systems present shear thinning features. It means that the increase in shear leads to a drop in viscosity. Such feature of silk fibroin allows creating hydrogel solutions that can easily pass through thin capillaries during biofabrication procedures like 3D bioprinting. In the absence of shear, chains of proteins are tangled and form a viscous network, whereas at high shear forces silk fibroin demonstrates molecular alignment and decrease in internal resistance to shear[30].

Storage modulus (G') and loss modulus (G'') can characterize the viscoelasticity of hydrogels. Storage modulus describes the elasticity of silk fibroin hydrogel solution, whereas loss modulus characterizes viscosity of the material. As for the gelation process, silk fibroin is characterized by the transformation of the sol into gel when G' exceeds G'' . In other words, during the process of gelation, the stable three-

dimensional network forms. Increased storage modulus is associated with the β -sheet structure and intermolecular interaction.

The rheological properties of silk fibroin are affected by the following factors: concentration, molecular weight, temperature, pH, ionic strength, and use of nanoparticles or cross-linkers. Generally, increased concentration leads to higher viscosity and mechanical properties because of enhanced chain interaction and molecule entanglement. The inclusion of nanoparticles, e.g. zinc oxide, lithium niobate, and others based on aluminum, also improves the mechanical properties of silk fibroin by facilitating further intermolecular bonding and strengthening of the hydrogel network.

Moreover, rheology that depends on temperature can help evaluate gelation kinetics and thermal stability. With increasing temperature, silk fibroin molecules could exhibit a fast molecular aggregate and form β -sheets, which will consequently accelerate gelation with an increased elastic modulus. Rheology tests such as frequency sweep and strain sweep are usually performed in order to examine the stability and mechanical performance of the silk fibroin hydrogel.

The rheological property of silk fibroin affects significantly the performance of the material in biomedical application. For instance, the injectable hydrogel needs to have specific viscosity while the scaffold material requires rigidity. In addition, the rheology test could provide a basis for obtaining desirable performance by manipulating the rheological property of silk fibroin.

From the above discussion, it is clear that the structure and rheological property of silk fibroin make the protein one of the best biomaterials used in developing hydrogel-based materials for biomedical applications.

2.1.7 Piezoelectric Properties of Silk Fibroin and Silk Fibroin Hydrogels

Biomaterials like silk fibroin are made up of proteins and are very popular due to their high biocompatibility, biodegradability, mechanical stability, and piezoelectric nature. Piezoelectricity is the property of a material to generate electric charges upon being subjected to mechanical stress in terms of bending, stretching, compressing, and vibrating. Due to such properties, silk fibroin becomes quite suitable for use in the biomedical industry.

The piezoelectric characteristics of silk fibroin are mainly due to its highly ordered beta sheet crystal structure. Silk fibroin consists of a series of amino acid structures including glycine, alanine, and serine, resulting in anti-parallel beta sheets. This structure results in molecular dipoles within the

protein chain. When force is applied on these dipoles, they align themselves in such a manner as to produce an electrical polarization effect, thus producing electrical signals. Beta sheets help in charge generation while amorphous regions offer flexibility.

There exist several factors that contribute to the efficiency of the piezoelectricity in silk fibroin, including molecular alignment, degree of crystallization, moisture content, and processing methods. The greater the proportion of β -sheets and crystallization, the better the dipole alignment and, hence, the higher efficiency in electromechanical performance. Electrospinning, stretching, freezing, sonication, solvent-based processing, and thermal processing improve molecular alignment and piezoelectric behavior.

Piezoelectricity exists in silk fibroin hydrogels, too, although the response is not as intense in them as it is in dry silk films owing to the high moisture content and low crystallinity. Despite this disadvantage, these hydrogels are very suitable for biomedical applications owing to their exceptional softness, flexibility, and tissue-like characteristics. Physiological activities may induce mechanical movements to cause electrical impulses in the gel, leading to tissue growth and regeneration[31].

The use of piezoelectric nanoparticles such as ZnO, LiNbO₃, BaTiO₃, and AlN increases the piezoelectric activity of silk fibroin hydrogels. The nanoparticles increase the electrical polarization and electromechanical coupling properties of the hydrogels.

CHAPTER-3

EXPERIMENTAL

3.1 Materials

All materials were used as supplied by the manufacturer without any further purification (**Table 7**). Deionized water was used throughout all experiments.

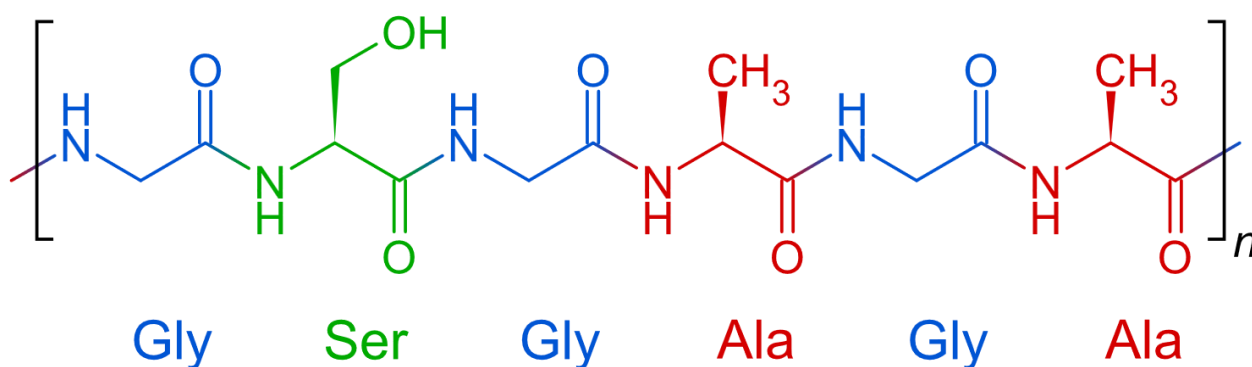


Figure 3: General structure of silk fibroin.

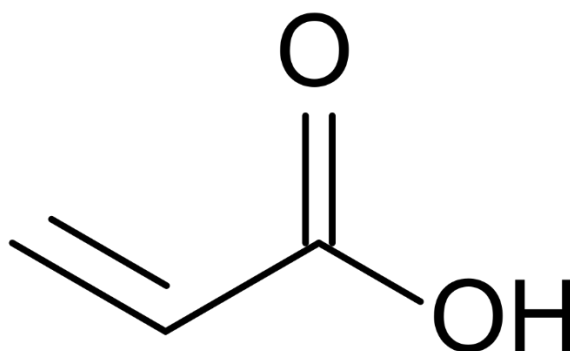


Figure 4: Chemical structure of Acrylic acid

Table 7: Chemicals used and their suppliers

Chemicals Used	Chemical Formula	Supplier
Acrylic Acid	$\text{C}_3\text{H}_4\text{O}_2$	Sigma
Sodium Hydroxide	NaOH	Sigma

Ammonium Persulfate	$(\text{NH}_4)_2\text{S}_2\text{O}_8$	S D Fine Chemicals Limited
N,N-Methylenebisacrylamide	$\text{C}_7\text{H}_{10}\text{N}_2\text{O}_2$	Sisco Research Laboratories Pvt. Ltd.
Lithium niobate	LiNbO_3	Sigma
Zinc oxide	ZnO	Sigma
Aluminium Nitride	AlN	Vizag Chemical

3.2

Preparation of silk fibroin Hydrogel

A biocompatible and biodegradable silk fibroin hydrogel material is one that can be made from the natural protein, fibroin, found in the silk of silkworm *Bombyx mori*. Silk fibroin hydrogel through the dialysis method requires many steps to be carried out, such as degumming, dissolving, dialysis, and hydrogel formation. First of all, the silk cocoons are cut up into small parts and then undergo degumming. Degumming is a process to get rid of sericin protein that covers fibroin fibers. It is done by putting the silk fiber parts into the diluted sodium carbonate solution and heated until the temperature reaches around 30-60 minutes. After being boiled, the fibers will be washed repeatedly with distilled water until the sericin residue is cleaned, leaving behind pure silk fibroin fibers.

In this stage, the degummed fibroin fibers are subjected to dissolution using chaotropic salt solutions like Lithium bromide (LiBr). Usually, the fibroin fibers are dissolved in 9.3 M LiBr solution at 60°C for 3-4 hours, thus giving an aqueous silk fibroin solution. But, the solution has high amounts of LiBr salt in it, which should be separated prior to creating the hydrogels. Hence, a dialysis process is performed to obtain the aqueous silk fibroin solution. Here, the fibroin-LiBr solution is loaded in dialysis tubing that has molecular weight cut-off of 3500 Da and placed in distilled water. The dialysis process is performed by stirring gently at a low temperature (4 °C) and changing the external water over a period of 48-72 hours. In this process, the smaller molecules along with the salts pass out through the membrane leaving behind only the larger protein molecules in it, giving an aqueous solution of silk fibroin[32].

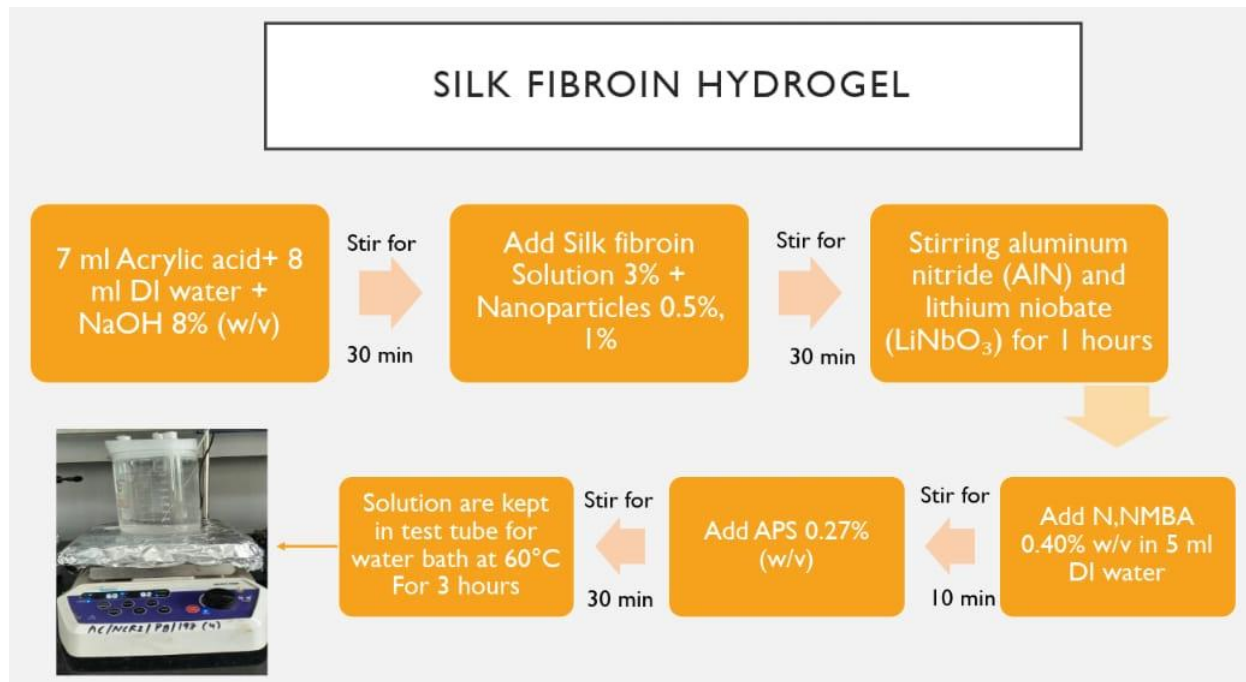


Figure 5: Schematic representation of preparation method of silk fibroin hydrogels.

3.3 Preparation of silk fibroin Hydrogel through a soft freezing method

Preparation of silk fibroin hydrogel through soft freezing technique is done using well-controlled procedures starting with extraction and purification of silk fibroin protein from the cocoons of silkworm *Bombyx mori*, subsequent addition of nanoparticles and freezing to promote gel formation. In the initial procedure, raw silk cocoons are first cut into small pieces and then degummed to remove the protein sericin. Degumming is normally performed by soaking the silk cocoon pieces in a solution of Na_2CO_3 (~0.02 M) at a temperature of ~100°C for 4-5 hours. The silk fibers are thereafter washed severally using deionized water to remove any residual chemical materials before squeezing and drying in ambient condition or in an oven maintained at 70°C. The resulting purified silk fibroin is next dissolved in a solution of lithium bromide (LiBr, ~9.3 M) at about 60°C for 3-4 hours to form a solution. This solution is then dialyzed against deionized water for several days, usually for 3-4 days to remove all the LiBr.

After purification, the silk fibroin solution undergoes processes for the creation of hydrogels. This involves centrifugation or ultracentrifugation of the solution in an attempt to eliminate aggregate and attain homogeneity for 1 hour. Nanoparticles of ZnO, AlN, and LiNbO_3 functionalized with appropriate concentrations (0.5-1%) are incorporated into the silk fibroin solution. This solution is stirred magnetically for approximately 4 hours to achieve homogenization followed by

ultrasonication for an hour to avoid agglomeration of the nanoparticles. The homogenous solution is then introduced to molds or containers and subjected to the process of soft freezing. This is accomplished by keeping the samples in a controlled environment at sub-zero temperatures (-23°C) for about 10 hours to enable ice crystal formation. As a result of freezing, water molecules freeze while pushing silk fibroin chains out to the unfrozen areas, thus achieving cryo-concentration and enabling structural reorganization of silk fibroin chains to adopt stable β -sheet conformation structure[33].

ult of the freezing-thawing process described above, a stable three-dimensional hydrogel network is developed, which has an interconnected porous structure owing to the melting of ice crystals. The silk fibroin hydrogel obtained is finally stored at 4°C to preserve its structure. Using the approach described above, hydrogels with controllable properties can be fabricated for use in regenerative medicine.

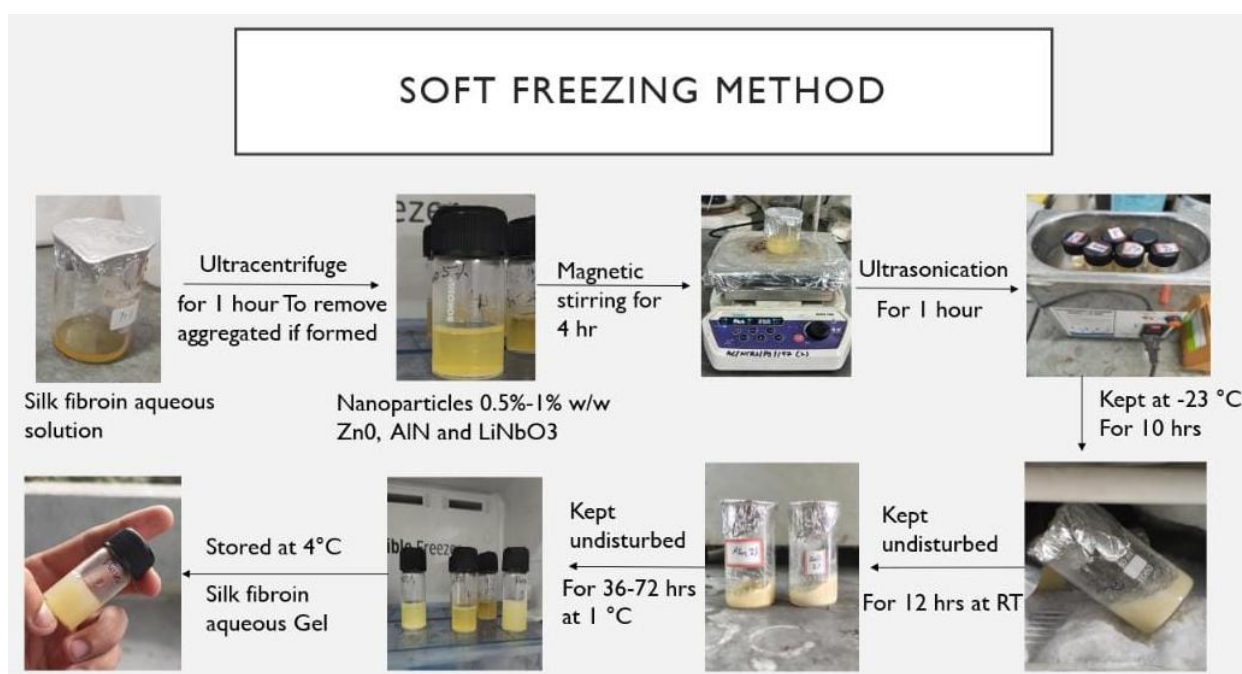


Figure 6: Schematic representation of preparation method of silk fibroin hydrogel via SF Method.

3.4 Characterizations

There were several of characterizations carried out for determination of structure and properties of the hydrogel.

3.4.1. Swell Test

- The dried samples were placed in a mesh bag and weighed.
- Afterwards, they were immersed in distilled water for three days.

- The weight was measured hourly on the first day, bi-hourly on the second day, and quad-hourly on the third day.
- The swelling index was determined based on the following **equation 1**.

$$\text{Swelling Index} = \frac{W2 - W1}{W1}$$

Where W2= weight of swollen sample

W1= weight of dried sample

3.4.2. Rheological Analysis

Rheology tests of the dialyzed and non-dialyzed silk fibroin hydrogels were carried out to characterize their viscoelastic nature. Rheological studies including viscosity, amplitude sweep, frequency sweep, and creep recovery tests were conducted using a rotational rheometer (Anton Paar India Pvt. Ltd., India). Hydrogels were tested by varying shear rate and stress. In addition, the results from the amplitude and frequency sweep tests were useful in determining the linear viscoelastic region of the samples, along with storage and loss moduli. Creep recovery test results showed how the materials recovered following stress application. Such an analysis is extremely essential for assessing the potential uses of the materials in biomedical and tissue engineering applications.

3.4.3. Conductivity Measurement

Ionic conductivity implies that a material has the ability to transfer electric charges via ions rather than electrons. It is an important characteristic in electrolyte liquids, hydrogels, living organisms' tissues, and polymer-based electrical materials. Ionic conductivity varies based on ions concentration, ions mobility, temperature, and structural properties of the material.

Ionic conductivity emerges in materials like hydrogels due to the water content present between polymers chains, which allows for free ions movements (like Na⁺, K⁺, Cl⁻) through interconnected pores. The higher the water content, the better will be ionic mobility, resulting in high conductivity values. Functional groups in polymers may also facilitate ions mobility by creating pathways/binding sites.

Temperature has an essential effect on the value of ionic conductivity; with increasing temperatures, there are higher ionic mobilities because of lower viscosity and higher mobility segments of polymer chains. Crosslinking density tends to decrease ionic conductivity because it limits ions mobility.

Ionic conductivity research has applications in different technological fields such as batteries/supercapacitors (energy storage devices), biosensors, smart clothes, and piezoelectric hydrogels. Good ionic conductivity is critical in smart hydrogels because of efficient signal transmissions and electromechanical responses.

The electrical conductivity (σ) of the silk fibroin hydrogel can be calculated using the formula:

$$\sigma = L / (R \times A)$$

Where:

σ – electrical conductivity, L – length between the two electrodes (thickness of the hydrogel),

R – electrical resistance, A – cross-sectional area of the hydrogel.

Electrical Resistance varies inversely with the Electrical Conductivity of the Hydrogel. In certain experiments, an Electrochemical Workstation that utilizes techniques like Impedance Spectroscopy can also be employed to measure the conductivity more accurately. For the experiment, the hydrogel remains hydrated because the water molecules as well as the ions within the hydrogel framework contribute significantly to the process of ionic conductivity. This conductivity value gives an assessment of the utility of the Silk Fibroin Hydrogels in applications like biosensors, tissue engineering, etc [34].

3.4.4. Temperature-Dependent Conductivity Measurement

A study was conducted regarding the temperature dependent conductivity of hydrogel samples to determine how temperature affects the conductivity of hydrogels based on the mobility of ions. Hydrogels consist of polymeric networks that can absorb plenty of water, and their conductivity is influenced by many factors including ion mobility, water content, and polymer chain flexibility, among others.

In this particular experiment, the conductivity of the hydrogel samples was determined at varying temperatures using the conductivity meter. The hydrogel samples were placed inside a thermostatically controlled water bath before the conductivity measurements.

Principle of Temperature-Dependent Conductivity

Conductivity of hydrogels increases with an increase in temperature because the ionic mobility increases as well as the mobility of the polymer molecules. As the temperature increases, the viscosity

of the medium decreases, making it easier for ionic movement to take place inside the hydrogel medium, thereby increasing its conductivity.

Ionic Conductivity The effect of temperature in the case of electrolytes, polymers, and hydrogels plays a significant role in terms of ionic conductivity because it affects mobility of ions and dynamics of polymer chains in dependence of the level of thermal energy of the system.

At higher temperatures, the level of ionic conductivity is higher because it means higher ion mobility in relation to increased thermal energy, which decreases the viscosity of the material and diminishes ion-polymer interaction, thus making ions such as sodium, potassium, and chloride move faster inside the polymer network. At high temperature, polymer chain mobility increases in the case of hydrogel, which leads to larger ion transport routes in the polymer network.

Such effect can be described by using the Arrhenius equation according to which conductivity increases exponentially due to the decrease of activation energy for migration. Some materials show a phenomenon of Vogel–Tammann–Fulcher (VTF), which means that at low temperatures the system demonstrates a high rigidity, limited water mobility, and smaller pathways for ions.

Therefore, low temperature leads to a drop of ionic conductivity in some cases; this property is extremely important for electroactive smart biomedical materials and energy devices.

Temperature-Dependent Conductivity Measurement

The conductivity of the hydrogel sample was obtained under various temperatures using a conductivity meter.

Thermostatically controlled water bath was used to maintain constant temperatures during the experiment[35].

The hydrogel sample was placed in the water bath, where temperatures were changed at certain intervals like 30°C, 45°C, 55°C, and 60°C. After placing the sample at a certain temperature, it was kept there for 15 minutes to attain constant temperatures in the hydrogel matrix.

After attaining constant temperatures, the conductivity readings were taken by using the conductivity meter.

The increase and decrease in the values of conductivity due to various temperatures were linked with the ion mobility and polymer dynamics of the hydrogel.

Mechanism of Conductivity in Hydrogel

Conductivity process in hydrogels involves ion conduction. Water that has been absorbed in hydrogels serves as the medium for ionic migration. With an increase in temperature:

- Ion mobility will increase
- Ion diffusion rate will be faster
- The viscosity level will be low
- Flexibility of polymer chains increases

Thus, the conductivity of hydrogels increases with temperature.

With lower temperatures, mobility of ions and flexibility of polymer chains decreases, resulting in comparatively lower conductivity levels.

Thermal Equilibrium Consideration

In order to get precise readings of the conductivity, thermal equilibrium should be achieved first before taking the conductivity reading. For this reason, the hydrogel sample was kept at each temperature for about 15 minutes within the water bath.

Importance of Temperature-Dependent Conductivity Study in Hydrogels

Temperature dependence of electrical conductivity offers useful data about:

- Ionic mobility
- The hydrogel's network flexibility
- The material's thermal properties
- The charge transport process
- The applicability in biomedical and sensor-related applications

These types of investigations are especially helpful for:

- Drug release carriers
- Organic electronics
- Biosensors
- Biomedical materials

- Conducting hydrogels

The results of temperature dependent investigation showed the effect of temperature on conductivity within the polymeric network of the studied material. The measurement of conductivity at various temperatures using a conductivity meter and thermostatically controlled water bath proved that the conductivity is temperature-dependent. It can be said that increasing temperature causes increasing conductivity of the material which depends largely on improved ion mobility and increased flexibility of polymer chains. The Arrhenius equation can explain such phenomenon.

3.4.5. Crosslinking Density of Hydrogel

Cross-linking density is one of the crucial parameters associated with hydrogels that indicate the amount of cross-links formed within the polymeric network of the hydrogels. Hydrogels consist of polymer chains inter-connected by means of either chemical or physical bonds to form a stable network structure that possesses the ability to absorb large quantities of water without being dissolved.

The cross-linking density plays a major role in determining the various properties of hydrogels including physical, chemical, mechanical, and thermal properties. The cross-linking density also affects the degree of freedom of movement of solvent and other diffusing species in the hydrogel matrix.

The highly cross-linked hydrogel has a compact and dense structure with less swelling and more mechanical strength; on the other hand, a hydrogel with a low cross-linking density has a loose packing structure and more swelling tendency and greater flexibility.

Crosslinking density in hydrogels is usually evaluated by the method of equilibrium swelling by using the Flory-Rehner equation. In this theory, the swelling of hydrogels happens because of solvent uptake by the polymer matrix. The elastic forces produced by cross-linked polymers resist the swelling process. Swelling of hydrogels happens as the balance is achieved by the two forces[36].

The crosslinking density (V_e) can be calculated using the Flory–Rehner equation:

$$V_e = \frac{-[\ln (1 - V_2) + V_2 + \chi V_2^2]}{V_1 \left(V_2^{1/3} - \frac{V_2}{2} \right)}$$

where:

- V_e = crosslinking density

- V_2 = polymer volume fraction in swollen state
- V_1 = molar volume of solvent
- χ = polymer–solvent interaction parameter

Polymer volume fraction of the swollen form is obtained through the swelling experiment of the hydrogel specimen. The swelling ratio of the hydrogel is obtained from the measurement of the weight of the swollen and dry specimens as follows:

$$Q = \frac{W_s - W_d}{W_d}$$

where:

- Q = swelling ratio
- W_s = swollen weight of hydrogel
- W_d = dry weight of hydrogel

Swelling of hydrogels is directly proportional to their degree of crosslinking. Hydrogels which swell more have less crosslinking, whereas hydrogels that have high crosslinking swell less.

It should be mentioned that the cross-linking density has considerable influence on many properties of hydrogels, including water absorption capability, porosity, ionic conductivity, diffusion ability, mechanical stability, and temperature resistance. Consequently, the determination of cross-linking density is crucial for the analysis of structural and functional properties of hydrogels used for different purposes, including drug delivery systems, tissue engineering, bio-sensors, wound dressings, and conductive hydrogels.

Within the framework of this research, cross-linking density of synthesized hydrogel samples was estimated based on the equilibrium swelling data applying the Flory-Rehner equation.

3.4.6. *Fourier Transform Infrared Spectroscopy (FTIR) Analysis of Hydrogel*

Fourier transform infrared spectroscopy (FTIR) is a key analytical tool extensively utilized for the structural analysis of polymer-based systems, hydrogels, nanocomposites, and biomaterials. The application of FTIR is basically aimed at the determination of functional groups, chemical bonding, inter-molecular interactions, and the structure of molecules within a substance.

The underlying principle behind FTIR involves the absorption of infrared radiation by molecules in the substance under investigation. As infrared radiation passes through the sample, there occurs the absorption of energy by particular chemical bonds at characteristic frequencies. These vibrations involve stretching, bending, rocking, and twisting modes of molecular bonds. All functional groups exhibit absorption peaks within distinct ranges of wavenumbers to produce characteristic spectra.

Hydrogel systems are studied using FTIR spectroscopy to verify the presence of:

- Polymer network formation
- Cross-linking reaction
- Functional groups in the material
- Chemical compatibility of the compounds
- Additives or filler addition
- Hydrogen bond formation

FTIR spectra are obtained within the region of wavenumber between 4000-400 (cm^{-1}). Various functional groups tend to absorb the infrared radiation at different regions in the FTIR spectrum. Absorption regions at about 3200-3600 (cm^{-1}) are usually related to hydroxyl (-OH) and amine (-NH) groups, whereas absorption peaks at about 1700 (cm^{-1}) relate to the carbonyl (C=O) groups. On the other hand, absorption peaks within the region of 1000-1300 (cm^{-1}) indicate C-O and C-N stretching vibrations.

FTIR technique is most suitable for hydrogels due to the presence of hydrophilic functional groups within the hydrogels, which interact with IR radiation.

Experimental Methodology for FTIR Analysis of Hydrogel

FTIR spectroscopic analysis was performed to analyze the presence of functional groups and verify the chemical structure of the hydrogel material.

FTIR analysis was initially done by thoroughly drying the synthesized hydrogels to eliminate the moisture and solvent contents from the samples. The dried hydrogel samples were subsequently ground to fine powder form using a mortar and pestle.

The finely powdered hydrogel samples were subjected to FTIR analysis. In FTIR analysis, FTIR spectrum was generated by performing the FTIR analysis on the hydrogel sample at a wavenumber of

4000 to 400 (cm^{-1}). The procedure involved transmitting infrared radiation through the sample, where the absorption of frequencies was noted corresponding to different chemical bonds[37].

From the generated FTIR spectrum, the absorption peaks corresponding to specific functional groups were observed. The FTIR spectrum was used to assess any intermolecular interactions and cross-linking in the hydrogel structure based on the appearance, shift, and absence of absorption peaks.

The results from the FTIR analysis verified the presence of functional groups in the hydrogel backbone.

Principle of FTIR Spectroscopy

FTIR analysis is based on molecular vibration theory. When infrared rays interact with the molecule, the energy absorbed makes vibrations of the bond present in the molecule. The frequency of vibration depends on:

- The bond strength
- The atomic mass
- The molecular surroundings

The vibrational frequency is measured in wavenumber .

This relationship gives us a qualitative idea about the structure of the compound being analyzed.

Functional Groups Commonly Observed in Hydrogels

Typical FTIR absorption bands observed in hydrogel systems include:

Wavenumber Range (cm^{-1})	Functional Group	Vibrational Mode
3200–3600	–OH / –NH	Stretching vibration
2800–3000	C–H	Stretching vibration
1600–1750	C=O	Carbonyl stretching
1400–1550	COO [–]	Symmetric/asymmetric stretching
1000–1300	C–O / C–N	Stretching vibration

These characteristic peaks help in confirming hydrogel formation and polymer interactions.

Importance of FTIR Analysis in Hydrogel Studies

Information gained from the characterization using FTIR technique includes:

- Chemical structure of hydrogels
- Functional groups present

- Cross-linking
- Polymer compatibility
- Hydrogen bond formation
- Modification of structure after synthesis

This method is fast, easy, non-destructive and efficient in the characterization of polymers.

FTIR technique is a characterization technique which is widely used in the analysis of chemical structure and functional groups in hydrogel materials. In this research work, dried powder of the prepared hydrogels were characterized by FTIR analysis to determine the molecular interactions and presence of successful formation of the hydrogel network. The absorption bands obtained in FTIR analysis revealed significant details concerning the chemical structure, cross-linking and functional groups in the hydrogels.

3.4.7. Current Measurement Test of Hydrogel

The method of current measurement analysis is a crucial electrical testing technique that can be employed in order to understand the electrical conduction of hydrogel materials. Hydrogel materials exhibit the property of being able to conduct electric charge by way of ion or electron movements in their three-dimensional polymer network. Current measurements made on the hydrogel will reveal the charge conductivity and electroactive behavior of the material.

Most hydrogels consist of high water content as well as movable ions that conduct electricity. As a voltage is imposed on the hydrogel, the ions present in the hydrogel matrix move due to the effect of the electric field, leading to the production of electricity.

Current, voltage, and resistance are related using Ohm's law:

$$V = IR$$

$$V_s$$

$$V$$

$$R$$

$$\Omega$$

$$I = \frac{V_s}{R} = \frac{12.0 \text{ V}}{6.0 \Omega} = 2.00 \text{ A}$$

$$V_s = 12.0 \text{ V}, R = 6.0 \Omega, I = 2.00 \text{ A}$$

where:

V = Applied Voltage

I = Measured Current

R = Electrical Resistance

The electrical conductivity of the hydrogel is determined by:

- Ionic Concentration
- Water Content
- Structure of Polymer Network
- Crosslinking Density
- Temperature
- Charge Carrier Mobility

A rise in ionic mobility or conductivity results in an increased current flow for an applied voltage.

Principle of Current Measurement

Analysis of measurements represents an essential approach that helps characterize the electrical conduction properties of hydrogels. In this analysis method, the electric current is measured for a certain hydrogel sample under a stable supply of voltage to it. The current flow in the hydrogel sample is generated by the migration of charge carriers in it. The process of charge transport can be conducted by means of ionic conduction, electronic conduction, or a mixed ion-electron charge transport depending on the nature of the hydrogel. The strength of the obtained current shows the conductivity of a certain hydrogel sample. The current experiment on analysis of hydrogels was performed in the physics laboratory. Hydrogel samples were produced in proper sizes and mounted between two conducting electrodes[38].

The electrodes were then attached to a DC power source, wiring, and digital multimeters/ammeter for measuring current. A constant potential difference was applied to the hydrogel samples while measuring the current generated by them. Repetitive measurement was performed to increase the

accuracy of the recorded data. Sometimes, the experiments were conducted at different temperatures and degrees of hydration to investigate the effects of these conditions on the electric conductivity of the materials used. Various factors play an important role in determining the electrical current generated by the hydrogels. Water content increases ion transportability and current while crosslinking decreases conductivity by hindering ion movement. Furthermore, temperature usually plays a positive role in increasing the ionic transfer ability of the hydrogels. Good contact between the samples and electrodes is very necessary to conduct accurate experiments. Presence of the ions in solution increases conductivity by improving charge transport. Conductivity experiments using current measurement give vital information about ion conductivity, electroactivity, electrical stability, and charge transportation of hydrogels. They are highly beneficial for various applications including but not limited to biosensors, flexible electronics, tissue engineering, drug delivery systems, soft robotics, and bioelectronics.

3.4.8. D_{33} Piezoelectric Coefficient of Dialyzed Silk Fibroin Hydrogels

One such important factor that helps assess the piezoelectric characteristics of dialyzed silk fibroin hydrogels is the D_{33} piezoelectric coefficient. This particular parameter represents the property of the hydrogel material to produce electrical charge due to the application of mechanical stress/pressure in the same direction. For a piezoelectric substance, mechanical deformation results in the shifting of charges internally, thus producing electricity. The D_{33} piezoelectric coefficient aids in determining how efficiently the material can convert mechanical energy into electrical energy.

In the case of dialyzed silk fibroin hydrogels, the mechanism of piezoelectricity relies on the formation of β -sheet crystals present in the protein molecules. Silk fibroin possesses polar chains of molecules that can produce dipole moments upon being acted on by external forces. In addition, the role of the dialysis procedure in increasing the performance of silk fibroin lies in the removal of any excessive salts, impurities, and ions contained in the silk solution. As a result, the structure of molecules will be more stable and aligned, hence making it easier for dipoles to form.

To test the D_{33} parameter, it is possible to use a piezoelectric d_{33} meter or piezometer. In this case, one would need to apply a small periodic mechanical force on the surface of the hydrogel material, which will be followed by recording the electric charge produced by the deformation process. D_{33} values are typically reported in pC/N (picocoulombs per newton).

The incorporation of piezoelectric nanoparticles like Zinc Oxide (ZnO), Aluminum Nitride (AlN), and Lithium Niobate (LiNbO₃) can contribute to increasing the D_{33} parameter in silk fibroin hydrogels. The

nanoparticles have piezoelectricity and can promote polarization within the hydrogel. They help charge transfer and improve dipole interaction, leading to higher electricity generation in response to mechanical stress. Due to these attributes, silk fibroin hydrogels that show an improvement in their D_{33} value after dialysis are seen as excellent candidates in sensors and implantable devices[39].

CHAPTER-4

RESULTS AND DISCUSSION

4.1 Swelling Test

A swelling test is conducted to determine the swelling ability or the absorbency ability of hydrogels when they act as carriers.

4.1.1. Swelling test with Dialysis

Sample	Initial Mass (g)	Final Mass (g)	Swelling Percentage
Pure	1.5278 g	24.4873 g	1502.8%
ZnO 0.5%	1.4358 g	24.1012 g	1578.6%
ZnO 1%	1.8033 g	32.1000 g	1680.1%
AlN 0.5%	1.5881 g	31.2651 g	1868.7%
AlN 1%	1.7183 g	27.7079 g	1512.5%
LiNbO ₃ 0.5%	1.6375 g	33.9055 g	1970.6%
LiNbO ₃ 1%	1.4802 g	26.1012 g	1663.4%

Table 8: Swelling index of silk fibroin hydrogel after dialysis.

The table above represents the outcomes of swelling studies of silk fibroin (SF) hydrogel and the impact of various nanoparticles on their water uptake capacity. These nanoparticles include Zinc Oxide, Aluminum Nitride, and Lithium Niobate.

Swelling studies are conducted for determining water uptake capacity of a hydrogel by comparing its weight before and after being immersed in water and expressed using the formula where greater swelling percentage suggests more absorption capacity

The swelling nature of the hydrogels made from silk fibroin is critical as this determines the performance of the material when used in biomedical applications. The study assessed pure silk fibroin hydrogel together with other hydrogels made from silk fibroin and incorporating nanoparticles such as ZnO, AlN, and LiNbO₃. The findings show that nanoparticles incorporated within the hydrogel play an important role in affecting the swelling nature by influencing the physical properties of the gel, namely structure, porosity, hydrophilicity, and water absorption capacity.

In the case of the pure silk fibroin hydrogel, the swelling ratio was found to be 1502.8% implying that the hydrogel had the capacity to absorb fifteen times the weight of water when compared with its initial mass. This property is attributed to the porous three-dimensional structure of silk fibroin and the hydrophilic amino acid residues present in silk fibroin that facilitate hydrogen bonding.

It was observed that the use of ZnO nanoparticles helped swell the hydrogel. For instance, the hydrogel with 0.5% of ZnO had a swelling rate of 1578.6%, while that of 1% had 1680.1%. Zinc oxide nanoparticles increase the total surface area by forming tiny holes on the hydrogel matrix, thereby facilitating increased entry of water molecules into the matrix. ZnO nanoparticles make hydrophilic reactions favorable between the polymeric chains and water, hence increased swelling ability.

The presence of aluminum nitride in the hydrogel matrix resulted in concentration-dependent swelling ability. For example, the 0.5% hydrogel showed swelling ability of 1868.7%, implying enhanced porosity and water diffusion through the hydrogel matrix. The aluminum nitride nanoparticles help create interactions in the fibroin fibers while at the same time remaining flexible for water uptake. In contrast, the percentage of swelling decreases with an increase in AlN concentration from 0.5% to 1%, reducing the swelling ratio to 1512.5%.

Out of all the samples, the hydrogel sample of LiNbO₃ at 0.5% concentration gave the maximum swelling percentage of 1970.6%. The presence of lithium niobate nanoparticles improves porosity and makes the hydrogel more hydrophilic and facilitates the interactions between the hydrogel matrix and water molecules[40]. Additionally, the presence of electroactive properties due to nanoparticles may be beneficial for water retention by the material. But, upon increasing the nanoparticle concentration to 1%, the swelling percentage of the sample lowered to 1663.4% because of nanoparticle clustering and decreased porosity.

Overall Comparison

Sample	Swelling (%)	Observation
Pure SF	1502.8	Base swelling capacity
ZnO 0.5%	1578.6	Slight increase
ZnO 1%	1680.1	Moderate increase
AlN 0.5%	1868.7	High swelling
AlN 1%	1512.5	Decrease due to rigidity
LiNbO ₃ 0.5%	1970.6	Highest swelling
LiNbO ₃ 1%	1663.4	Slight reduction

4.1.2. Swelling test without Dialysis

Sample	Initial Mass (g)	Final Mass (g)	Swelling Percentage
Pure	2.5389 g	9.889 g	289.48%
ZnO 0.5%	3.5383 g	10.3347 g	192.10%
ZnO 1%	3.6120 g	14.050 g	289.01%
AlN 0.5%	5.1251 g	20.065 g	291.50%
AlN 1%	3.4493 g	14.6728 g	325.32%
LiNbO3 0.5%	3.5120 g	9.3338 g	165.83%
LiNbO3 1%	2.3412 g	10.982 g	369.09%

Table 9: Swelling index of silk fibroin hydrogel without dialysis.

The above table illustrates the swelling behavior of the SF hydrogel made without the use of dialysis technique and impact of nanoparticles like Zinc Oxide, Aluminum Nitride, and Lithium Niobate on water uptake capacity.

The presence of leftover salts and small molecular weight compounds in the hydrogels due to dissolution process is observed when hydrogels are made without dialysis process.

$$\text{Swelling(\%)} = \frac{\text{Final Mass} - \text{Initial Mass}}{\text{Initial Mass}} \times 100$$

The results related to swelling of hydrogels consisting of non-dialyzed silk fibroin prove that the presence of nanoparticles and impurities can substantially affect the water-absorbing properties of hydrogels. In all tested hydrogels, there is a substantial effect produced by the lack of dialysis since salts, ions, and other impurities present in non-dialyzed hydrogels remain inside. These substances affect the internal structure of the hydrogel due to increased interaction between molecules and decreased free volume inside the hydrogel that allows water to diffuse inside. As a result, swelling values obtained from non-dialyzed hydrogels are lower compared to dialyzed silk fibroin hydrogels.

The pure silk fibroin hydrogel demonstrated swelling ratio equal to 289.48%. The presence of hydrophilic amino acid groups enables silk fibroin to interact with water, while retained salts inside the non-dialyzed sample prevent movement of the chains and swelling of the hydrogel matrix.

The presence of ZnO nanoparticles affected the swelling properties of the hydrogel in a concentration-dependent manner. In the case of ZnO 0.5% hydrogel, there was a decrease in the swelling ratio, which was 192.10%. This is because the low concentration of nanoparticles enhanced crosslinking density and interaction between the fibroin chains. As a result, there was a reduction in pore size, hence making it difficult for water to enter the structure. On the other hand, at a ZnO concentration of 1%, the swelling ratio increased significantly to 289.01%, similar to that of the pure hydrogel. This implies that at high concentration, there is an increase in the number of microvoids or pores formed, hence promoting water penetration.

Similar effects were noted in the AlN hydrogel. With AlN 0.5%, the hydrogel exhibited an increase in swelling to 291.50%, compared to the pure hydrogel. This could be due to the ability of aluminum nitride nanoparticles to preserve structural integrity while facilitating water uptake. At a higher concentration of 1% AlN, the swelling increased to 325.32%.

The LiNbO₃ hydrogels were the most different compared to other samples in their characteristics. The LiNbO₃ 0.5% hydrogel had the smallest swelling ratio of 165.83%, which suggested that the hydrogel was the most dense due to strong interactions between nanoparticles and silk fibroin, which prevented water from penetrating into the sample. On the contrary, the largest swelling ratio was observed for LiNbO₃ 1% hydrogel and amounted to 369.09%, which could be explained by structural irregularities and the presence of pores created by the nanoparticles.

To sum up, it is possible to state that both types of nanoparticles and their concentrations have an impact on the swelling properties of the studied non-dialyzed SF hydrogels.

Sample	Swelling (%)	Observation
Pure SF	289.48	Base swelling
ZnO 0.5%	192.10	Decrease
ZnO 1%	289.01	Similar to pure
AlN 0.5%	291.50	Slight increase
AlN 1%	325.32	Higher swelling
LiNbO ₃ 0.5%	165.83	Lowest swelling
LiNbO ₃ 1%	369.09	Highest swelling

4.1.3. Swelling test soft freezing method hydrogels

Swelling test is done to measure the swelling capacity or how much a hydrogel can absorb when used as a carrier.

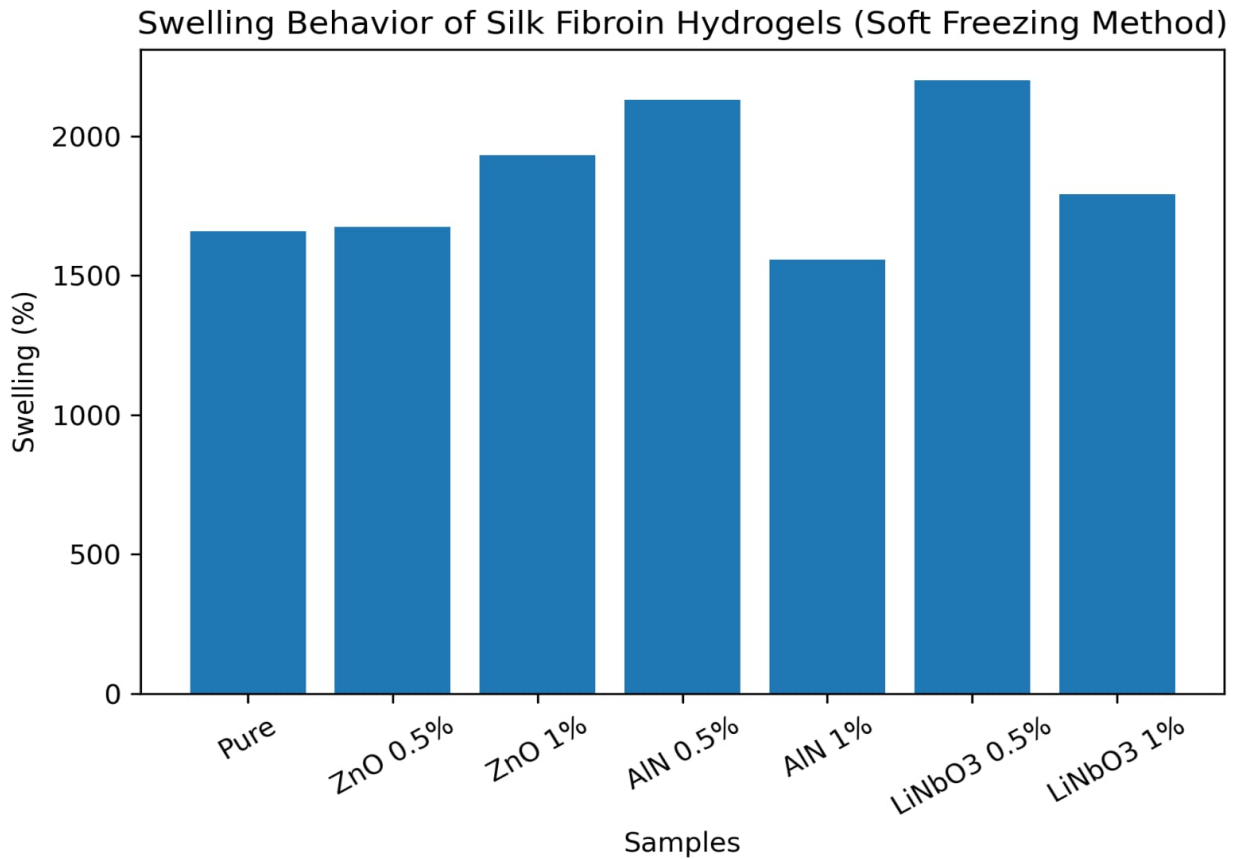


Figure 7: Swelling index of silk fibroin hydrogel via soft freezing method.

The plot shows the swelling behavior of hydrogels based on silk fibroin synthesized by the technique of soft freezing and the effect of different types of inorganic nanoparticles as well as the concentration of those on the hydrogel's water uptake capability. As for the pure hydrogel based on silk fibroin, it is characterized by swelling around 1650-1700%, that indicates its high affinity towards water due to the hydrophilic properties of the material and porous three-dimensional structure. With the introduction of ZnO nanoparticles, there is a gradual growth of the swelling; in particular, with 0.5% and 1% ZnO it grows up to 1900-1950%.

When considering the effect of AlN on the swelling behavior of the gel, however, an opposite trend is noticed. The amount of swelling at 0.5% of AlN significantly increases to about 2100-2150%, thereby implying that a small concentration of AlN can lead to network expansion, higher hydrophilicity, and

improved ability of the hydrogel to absorb water molecules. On the other hand, 1% of AlN causes a sharp decline in the swelling to 1550-1600%, perhaps because of an overload of nanoparticles in the gel resulting in increased compactness and restricted movement of polymer chains, hence limiting water absorption[41].

The swelling trend associated with 0.5% LiNbO₃ (lithium niobate) is also analogous, as it results in the highest value of swelling of all the samples analyzed. Thus, it can be said that the use of LiNbO₃ improves the porosity of the hydrogel network, enabling it to effectively attract and hold onto more water molecules. Increasing its concentration, however, to 1% results in the decline of swelling to about 1800%.

In general, the graph demonstrates a significant increase in swelling ability of silk fibroin hydrogels by incorporating nanoparticles until reaching an optimum value (0.5%), after which swelling ability starts to decrease owing to denser structures and less flexibility of the hydrogel system. Overall, among all the systems examined in this study, LiNbO₃ hydrogels having 0.5% concentration show superior swelling ability, and thus can be applied in various areas where high absorbance is required.

4.2 Conductivity Measurement

The ionic conductivity of the silk fibroin hydrogel based on fibroin extracted from *Bombyx mori* can be determined by means of conductivity meter or electrochemical analyzer. Analysis of ionic conductivity is necessary to estimate the effectiveness of ion or charge carrier transport inside the hydrogel network. It is useful for further research related to bioelectric applications (biosensors, biomedical implants, etc.).

To conduct conductivity testing, one needs to let the prepared sample gel completely. Then, it is cut to a certain geometry, placed between two conductive plates, preferably made of platinum or stainless steel. The plates are connected to a conductivity meter or an electrochemical analyzer. An electrical potential difference is introduced, thus enabling ions in the hydrogel to generate electrical current through movement. The conductivity or resistance of the hydrogel is measured.

Electrical conductivity of hydrogel (σ) is determined according to the following formula:

$$\sigma = R \times AL$$

where σ is electrical conductivity, L refers to the electrode distance or hydrogel thickness, R refers to the resistance of the electrical circuit, and A refers to the cross-sectional area of the hydrogel that the

electric current flows through. The lower the resistance, the higher the electrical conductivity of the hydrogel.

The primary source of conductivity in silk fibroin hydrogels is attributed to the movement of ions in the hydrogel's aqueous phase. The introduction of nanoparticles like Zinc Oxide, Aluminum Nitride, and Lithium Niobate can affect the conductivity through changes in the internal structure of the hydrogel and creation of paths for ions to move.

The conductive properties of silk fibroin hydrogels can be greatly influenced by their internal structure and the uniform dispersion of nanoparticles within them.

Finally, the conductivity test serves to examine the electrical properties of silk fibroin hydrogels.

4.2.1. Conductivity Measurement With Dialysis

Hydrogels	Conductivity
Pure	3.9 mS
ZnO 0.5%	14.34 mS
ZnO 1%	19.11 mS
AIN 0.5%	11.15 mS
AIN 1%	20.3 mS
LiNbO3 0.5%	4.3 mS
LiNbO3 1%	8.45 mS

Table 10: Conductivity measurement of silk fibroin hydrogel after dialysis.

This table represents the ionic conductivity values of the SF hydrogels with varying types of nanoparticles. The types of nanoparticles added to these hydrogels include Zinc Oxide, Aluminum Nitride, and Lithium Niobate. ionic conductivity is measured in millisiemens (mS). It refers to the ease at which an electrical charge or ion moves in a substance.

Electrical conductivity in the hydrogels takes place primarily through ionic conductivity in the water pores of the hydrogels.

Conductivity results for the silk fibroin-based hydrogels showed that the presence of various nanoparticles had a significant influence on the conductivity behavior of the hydrogel system. The conductivity value of pure silk fibroin hydrogel was found to be 3.9 mS and this can be taken as the basic or normal conductivity value of the hydrogel. Silk fibroin itself is a biopolymer and it is not an inherently good conductor of electricity. The electrical conductivity of the hydrogel is therefore due to the movement of ions or water molecules within the hydrogel structure.

The addition of ZnO nanoparticles led to a considerable increase in conductivity. Hydrogel with 0.5% of ZnO had a conductivity of 14.34 mS; at 1% ZnO, conductivity was even higher and reached a value of 19.11 mS. Zinc oxide is a semiconductor, and its presence leads to better charge transfer properties of the hydrogel due to the creation of additional conductive pathways by nanoparticles and better ion mobility in the polymer matrix. Higher amounts of ZnO nanoparticles result in their better connectivity within the hydrogel, leading to improved conductivity of the materials[42].

Similar results were achieved with aluminum nitride nanoparticles. Conductivity values for hydrogels with 0.5% of AlN were 11.15 mS. However, the highest value of conductivity was obtained with hydrogel with 1% of AlN, being equal to 20.3 mS. Although aluminum nitride is an electrically insulating material, it exhibits relatively good heat conductivity. In addition, the introduction of AlN nanoparticles might influence the structure of polymer chains and their mutual positioning, allowing for creating conductive pathways within the polymer matrix and improving conductivity of the hydrogel systems.

LiNbO₃ nanoparticles were observed to have relatively less conductivity values in comparison to other samples. In 0.5% LiNbO₃ sample, the conductivity value was found to be 4.3 mS, and 8.45 mS conductivity was recorded for the 1% LiNbO₃ sample. Lithium niobate is famous for its ferroelectricity and piezoelectricity rather than electrical conductivity. An increase in the amount of nanoparticles does improve polarization in the network; however, this increase does not make an effective path like in AlN and ZnO. It can be concluded from the results of the experiment that nanoparticle inclusion effectively increases ionic conductivity, and AlN and ZnO nanoparticles exhibit the maximum change.

Overall Comparison

Hydrogel	Conductivity (mS)	Observation
Pure SF	3.9	Lowest conductivity
ZnO 0.5%	14.34	Large increase
ZnO 1%	19.11	High conductivity
AlN 0.5%	11.15	Moderate increase
AlN 1%	20.3	Highest conductivity
LiNbO3 0.5%	4.3	Slight increase
LiNbO3 1%	8.45	Moderate increase

4.2.2. Conductivity Measurement Without Dialysis

Hydrogels	Conductivity
Pure	8.50 mS
ZnO 0.5%	18.90 mS
ZnO 1%	24.50 mS
AlN 0.5%	11.68 mS
AlN 1%	26.20 mS
LiNbO3 0.5%	9.57 mS
LiNbO3 1%	11.57 mS

Table 11: Conductivity measurement of silk fibroin hydrogel without dialysis

This table demonstrates the ionic conductivity of silk fibroin (SF) hydrogel that is not subjected to dialysis. In this particular sample, salts and other compounds from the dissolution step are retained within the hydrogel matrix since dialysis is not conducted. Such residual ions play an important role in determining the electrical properties of the hydrogel, usually resulting in conductivity that is higher

than in dialyzed hydrogels. The nanoparticles added to this sample are Zinc Oxide, Aluminum Nitride, and Lithium Niobate.

The conductivity of the hydrogel samples containing silk fibroin but not subject to dialysis varied according to the addition of nanoparticles and the presence of residual ions in the hydrogel matrix. Pure SF hydrogel that was not subjected to dialysis showed ionic conductivity at 8.50 mS. The high value of this conductivity as compared to dialyzed hydrogels is attributed mainly to the presence of ions that were retained during the preparation of the hydrogel.

There was a significant enhancement in conductivity when adding ZnO nanoparticles to the hydrogels. Specifically, the hydrogel with 0.5% ZnO content was found to have a conductivity value of 18.90 mS. ZnO belongs to the semiconductors family and facilitates electron transfer besides improving ionic diffusion across the hydrogel matrix. Increasing the percentage of ZnO nanoparticles to 1% led to a higher value of conductivity for 24.50 mS. This increase can be attributed to the higher number of conducting paths formed by the larger amount of nanoparticles that promote charge transfer in the hydrogels.

It was also noted that the presence of AlN nanoparticles in the hydrogel matrix improved its conductivity significantly. The conductivity values of the hydrogels containing 0.5% of aluminum nitride reached 11.68 mS. Aluminum nitride is not an electrical conductor; however, it changes the structure and porosity of the hydrogel matrix which promotes ionic diffusion. Importantly, a hydrogel with a concentration of 1% AlN had the highest conductivity value among all samples tested (26.20 mS).

In this respect, the conductivity levels recorded by LiNbO₃ were slightly low. For instance, the 0.5% LiNbO₃ hydrogel exhibited a conductivity level of 9.57 mS, whereas for 1% LiNbO₃, the value was 11.57 mS. This is due to the fact that lithium niobate is generally known to have ferroelectric and piezoelectric properties but lacks electrical conductivity.

Generally, both nanoparticle doping and remaining ions contributed to an increase in conductivity, whereby the 1% AlN had the best conductivity.

Overall Comparison (Without Dialysis)

Hydrogel	Conductivity (mS)	Observation
Pure SF	8.50	Higher due to residual ions
ZnO 0.5%	18.90	Strong increase
ZnO 1%	24.50	Very high conductivity
AlN 0.5%	11.68	Moderate increase
AlN 1%	26.20	Highest conductivity
LiNbO ₃ 0.5%	9.57	Slight increase
LiNbO ₃ 1%	11.57	Moderate increase

4.3.3. Conductivity Measurement via Soft freezing method

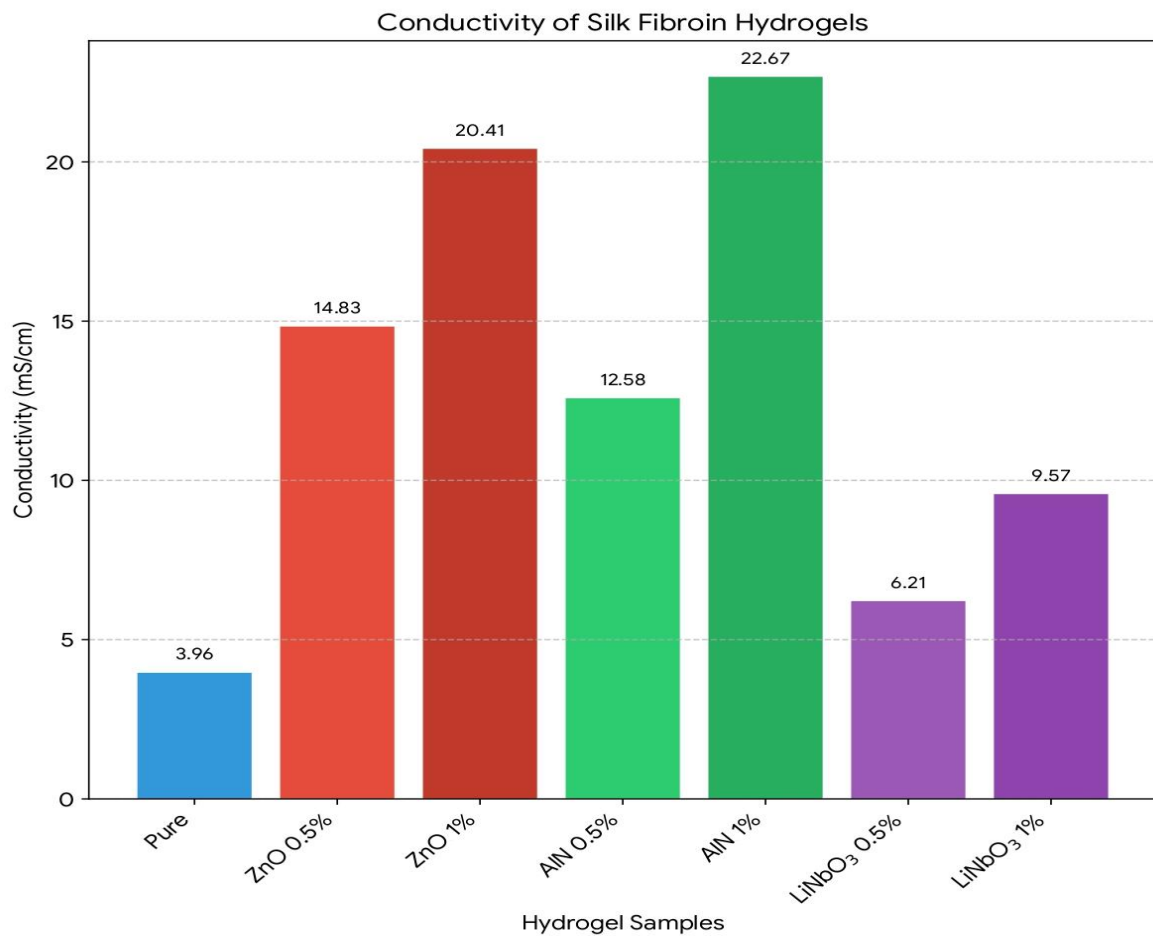


Figure 8: Conductivity measurement of silk fibroin hydrogel via Soft Freezing Method.

As shown in the graph, this figure represents the performance of the ionic conductivity of hydrogels made from silk fibroin incorporating inorganic nanofillers (ZnO, AlN, and LiNbO₃) in two different concentrations of (0.5% and 1%) and clearly shows that there is a great impact of filler type and concentration on the ionic/electric conductivity. In the case of pure hydrogels, the ionic conductivity is very low, approximately equal to 3.96 mS/cm. However, when the ZnO nanoparticles were used in the hydrogels, an increment of conductivity was achieved, resulting in approximately 14.83 mS/cm and 20.41 mS/cm at 0.5% and 1%, respectively[43].

There are also other trends observed when considering the effects on conductivity by introducing other materials like the AlN, which shows even a higher conductivity level rising from ~12.58 mS/cm at 0.5% to reach the maximum level of ~22.67 mS/cm at 1%. It can be noted that AlN plays a significant part in enhancing charge transfer, as its particles have a higher dispersion rate and a strong interaction between itself and the polymer. The third group of nanoparticles, namely LiNbO₃, shows the lowest conductivity increase as compared to both ZnO and AlN: ~6.21 mS/cm at 0.5% and ~9.57 mS/cm at 1%. As opposed to previous observations, it is clear that LiNbO₃ can enhance conductivity levels, although not as significantly as AlN or ZnO.

All groups showed higher conductivity with an increase in the concentration of the introduced nanoparticles; thus, higher concentrations allow creating better pathways for the charges to flow. In this regard, it can be concluded that AlN nanoparticles with a 1% concentration showed the highest conductivity levels. Consequently, AlN at a 1% concentration is the best material for enhancing the performance of SF-based hydrogels as regards their ionic performance, which can be utilized in bioelectronics and sensor devices.

4.3 Rheological Analysis Dialysis

Rheological analysis is one of the major approaches used in the study of the flow and mechanical properties of hydrogels. For example, rheological analysis can be applied when studying the properties of hydrogels synthesized from silk fibroin isolated from the silkworm *Bombyx mori*, as such analysis allows determining the way in which hydrogels respond to stress and strain application and other deformations.

The analysis involves rheology testing with a rotational rheometer where the hydrogel sample is put on two plates that form parallel plates geometry. In the course of the procedure, shear stress and oscillatory strains are applied to the hydrogel sample, and its deformation is measured. There are

several parameters determined by rheological testing. The most critical of them include the storage and loss moduli (G' , G'' , correspondingly). The storage modulus stands for the elastic component (solid-like behavior) of the material, meaning the amount of energy stored in the material under stress or strain. Loss modulus stands for the viscous component (liquid-like behavior) of the material and means the energy dissipated as heat.

The typical feature of silk fibroin hydrogels is the fact that the storage modulus in the hydrogel is larger than the loss modulus, which signifies that the behavior of the hydrogel is closer to that of solid elasticity rather than liquids. The above-mentioned feature of the hydrogel provides evidence that a stable three-dimensional network structure of the material has formed. In case Zinc Oxide, Aluminum Nitride, and Lithium Niobate particles are added into the hydrogel matrix, the rheological features of the hydrogel will become changed. Namely, due to the interaction between the polymer chains, the viscosity and storage moduli of the hydrogel will be increased.

A frequency sweep study may also be conducted during rheological analysis along with a strain sweep study. In the frequency sweep study, frequency changes may be studied by observing how the hydrogel reacts to various rates of deformation. For a stable hydrogel, variations in its storage modulus with respect to the frequency should not show much of an effect throughout a range of frequencies. In the strain sweep study, various levels of strain will be observed to define the linear viscoelastic region where the hydrogel does not break[44].

In general, rheological analysis gives significant insights into the physical properties of silk fibroin hydrogels including their strength, stability, and viscoelastic behavior.

4.3.1. Viscosity

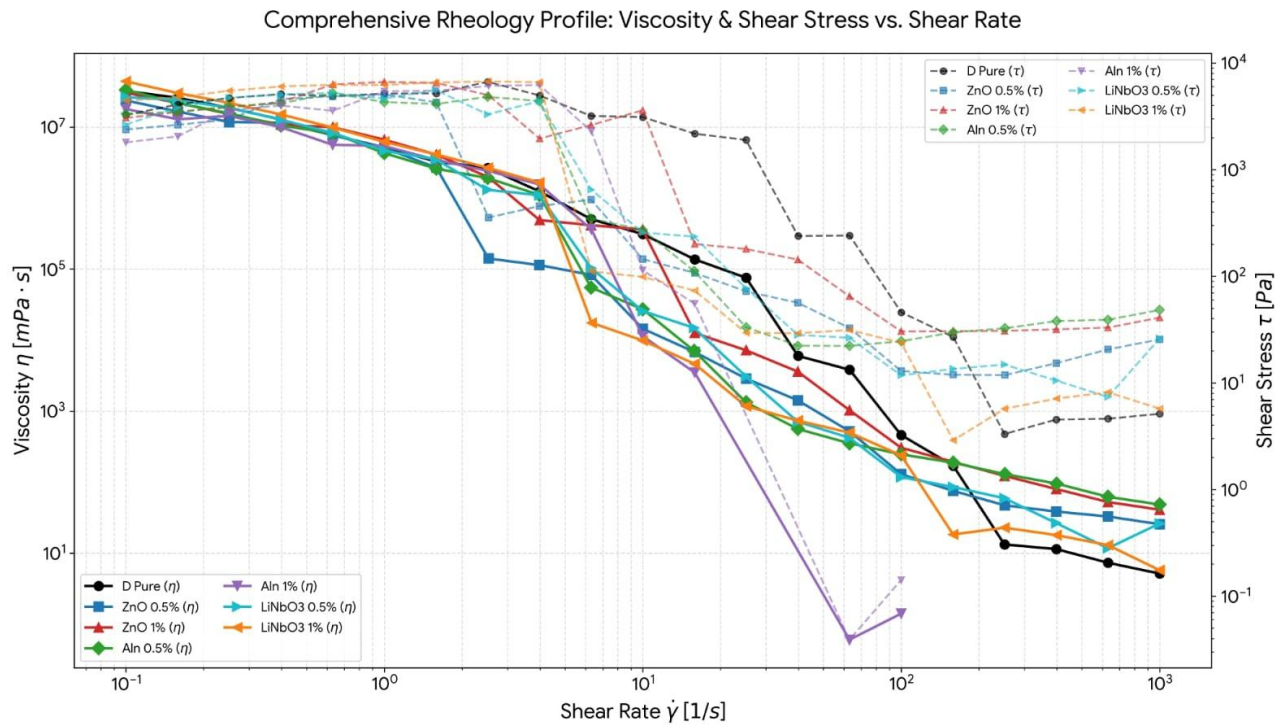


Figure 9: Viscosity analysis of silk fibroin hydrogel with dialysis using a rheometer.

The figure depicts the rheological characteristics of silk fibroin (SF) hydrogels with the relationship between viscosity (η), shear stress (τ), and shear rate ($\dot{\gamma}$). The types of nanoparticles incorporated in the SF hydrogels are Zinc Oxide, Aluminum Nitride, and Lithium Niobate. Through the rheological study of the hydrogels, one can understand their behavior under external force.

The rheological figure of silk fibroin hydrogels gives useful insight into their behavior and responses under shear deformation. The figure basically illustrates the relation between shear rate, shear stress, and viscosity for pure silk fibroin hydrogel as well as nanocomposites made from nanoparticles such as ZnO, AlN, and LiNbO₃.

On the X-axis, shear rate is measured in per second ($\dot{\gamma}/s^{-1}$) and reflects the degree of the material deformation in relation to its rheology. The lower shear rate means that the hydrogel undergoes low deformation, while the higher shear rate means that stronger mechanical forces act on the hydrogel sample. The Y-axis on the left-hand side shows viscosity in millipascal seconds ($\eta/mPa\cdot s$), which characterizes the resistance of the material to flow. Strong internal interaction leads to high viscosity

values and vice versa. Shear stress (τ/Pa) is shown on the right-hand side axis and refers to the amount of stress required to deform the material structure.

All investigated hydrogel samples exhibit the common property of shear thinning. Shear-thinning materials have their viscosity reduced by shear rate. When the shear rate is low, very high viscosity of the hydrogels exists since their polymer chains are strongly entangled. Once the shear rate increases, chains become aligned with each other in the direction of flow and experience less friction. Thus, hydrogel deformation becomes easier, which characterizes polymers and biomaterials. It is extremely useful in practical applications such as injectable biomedical materials[45].

The pure silk fibroin hydrogel has high viscosity values when subjected to low shear rate, implying that there is an established three-dimensional structure in the hydrogel matrix consisting of silk fibroin molecules. Viscosity declines sharply with the increase in shear rate because of polymer chain arrangement and degradation within the hydrogel.

However, the addition of nanoparticles affects the rheological properties of hydrogel. The ZnO 0.5% hydrogel has a slightly lower value of viscosity than the pure silk fibroin gel because the nanoparticles decrease the resistance to shear deformation. On the other hand, the ZnO 1% gel has relatively stable mechanical behavior since the increased quantity of nanoparticles enhances the interaction of silk fibroin chains with the nanoparticles, creating a stronger hydrogel matrix.

Hydrogels loaded with nanoparticles such as Aluminum Nitride (AlN) also have altered rheological characteristics. While the AlN 0.5% hydrogel has a gradual viscosity decrease as the shear rate increases, implying that there is some reinforcing of the network structure in the hydrogel, the AlN 1% hydrogel has a sudden viscosity decrease as the shear rate increases, showing that there is some instability in the network structure of this hydrogel due to high concentrations of nanoparticles that affect the polymer chains.

LiNbO₃-loaded hydrogels also have fairly stable rheological properties. The hydrogel loaded with 0.5% LiNbO₃ has constant viscosity across a wide range of shear rates, while the hydrogel loaded with 1% LiNbO₃ has a higher viscosity decrease at high shear rates, which implies that this particular hydrogel is more sensitive to external stresses.

All shear stress curves increase with the increase in the shear rates of all hydrogels, implying that as the shear rate increases, the shear stress applied on the hydrogel becomes much stronger. Hydrogels with higher concentrations of nanoparticles have higher shear stress values due to their internal interactions.

From the above analysis of the rheological behavior, it is clearly evident that the rheology of all the silk fibroin hydrogels exhibits shear thinning behavior, while nanoparticle addition plays a very significant role in changing the mechanical properties and rheology of the hydrogel. The moderate amount of nanoparticles increases the stability and mechanical strength of the material, but an increase in nanoparticle content can disrupt the uniformity of the polymer network.

4.3.2 Amplitude Sweep

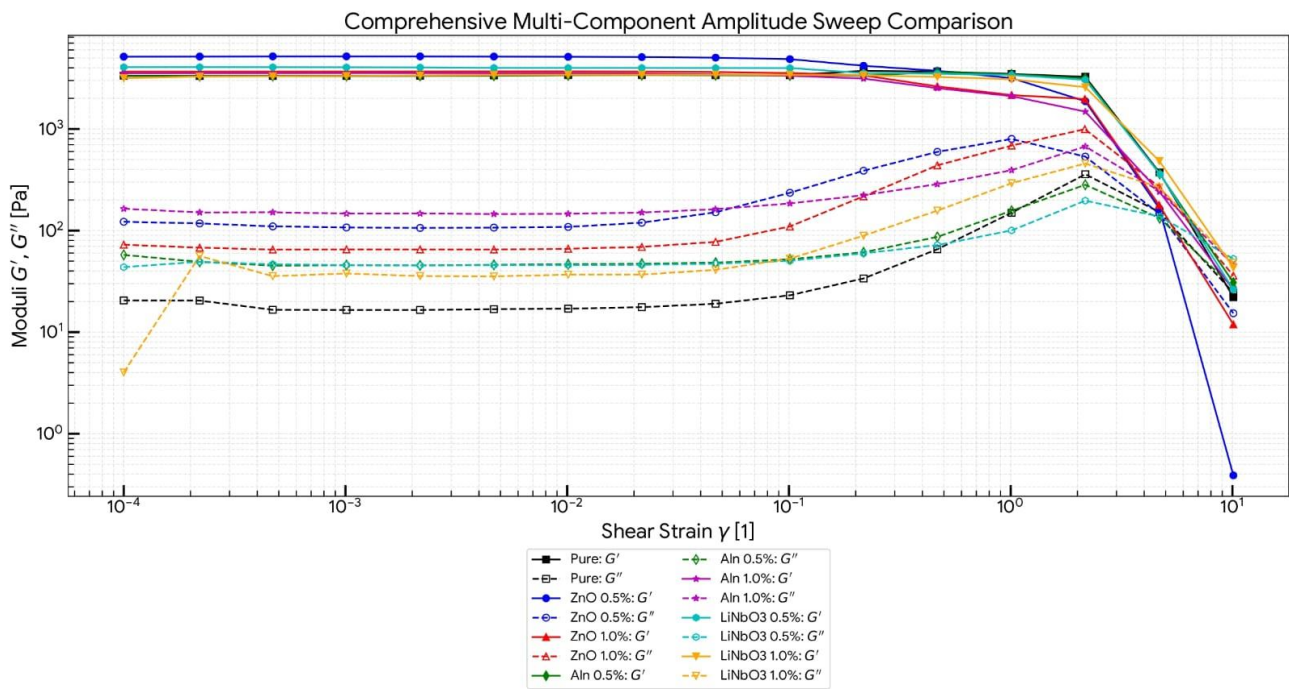


Figure 10: Amplitude sweep analysis of silk fibroin hydrogel with dialysis using a rheometer.

The results obtained from the amplitude sweep rheological studies give a good insight into the viscoelasticity, stability, and deformation resistance of pure and nanoparticle-loaded silk fibroin hydrogels during an increase in the shear strain (γ). The chart entitled "Multi-Component Amplitude Sweep Comparison" illustrates how the storage modulus (G') and loss modulus (G'') of the pure hydrogel and hydrogels loaded with ZnO, AlN, and LiNbO₃ nanoparticles with concentrations of 0.5% and 1.0% vary. The logarithmic scale is used for a more extensive analysis of the sample.

It can be noted that at very low values of shear strain, ranging between 10^{-4} and 10^{-1} , there is no change in the storage modulus (G') value of all the studied hydrogels. This stage is termed the linear viscoelastic region (LVR), which means that the structural integrity of the networks within the

hydrogels does not suffer any deformations. At this stage, the storage modulus (G') has much higher values than the loss modulus (G''), which indicates that the hydrogels act like elastic/solid materials.

Nanoparticles have a significant impact on the rheology of the hydrogels in question. Hydrogels with added nanoparticles possess high values of G' , which means higher elasticity, greater strength, and improved internal reinforcement. All these effects are related to the fact that there are strong interactions between nanoparticles and silk fibroin polymer chains, providing additional cross-linking and increased matrix reinforcement. It has been found that zinc oxide nanoparticles create hydrogels with the highest values of the storage modulus and thus are the best for reinforcement. AlN hydrogels have higher values of the modulus too; however, their reinforcing effect is not so significant. LiNbO₃ hydrogels exhibit improved viscoelastic properties, yet their stiffness is still a little bit lower than that of ZnO hydrogels.

Increasing the concentration of nanoparticles from 0.5% to 1.0% results in a high value of modulus because of the increase in interaction between nanoparticles and a high level of interconnections among them inside the hydrogel matrix. However, when shear strain is increased above the threshold value of $\gamma = 1$, both G' and G'' start decreasing rapidly due to the breakdown of internal hydrogel matrix structure caused by large deformations. At very high strain levels, for instance, at $\gamma \approx 10$, both values of modulus decrease rapidly, implying destruction of hydrogel structure.

The amplitude sweep test suggests that incorporation of nanoparticles improves significantly the mechanical stability and rheological properties of silk fibroin hydrogels. These enhanced characteristics are especially advantageous for their application in biomedicine, such as injectable hydrogels, scaffold-based tissue engineering applications, wound dressing material, and targeted drug delivery system.

4.3.3 Frequency Sweep

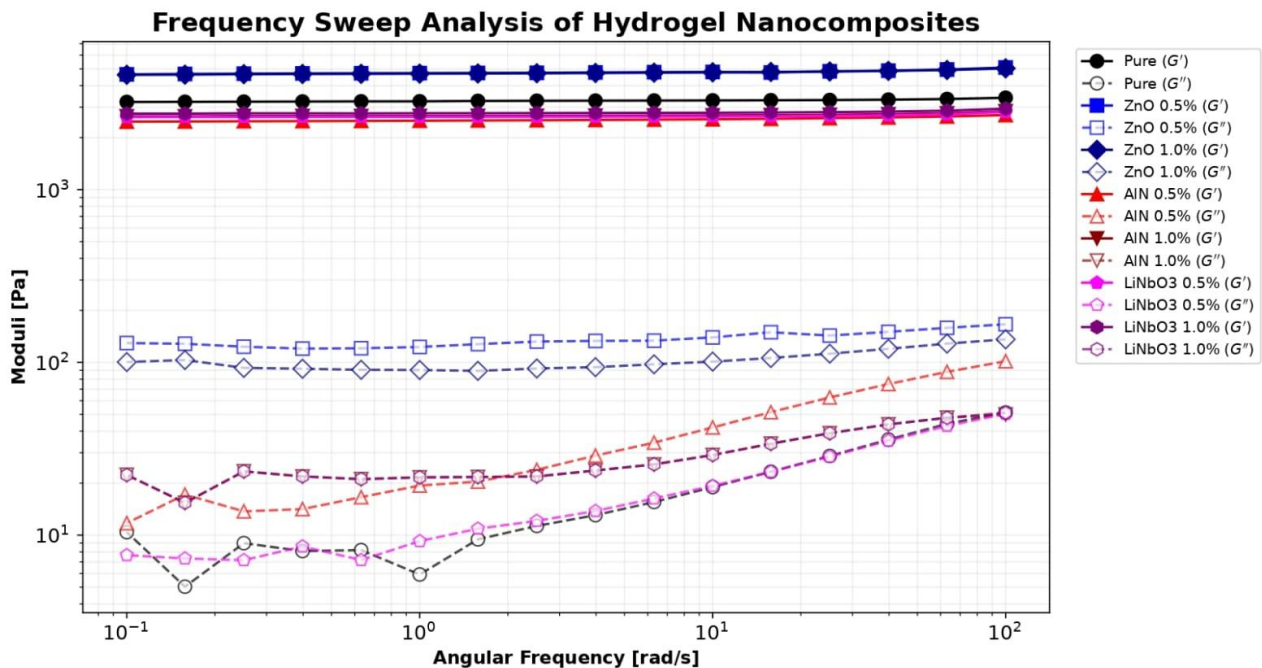


Figure 11: Frequency sweep analysis of Silk fibroin hydrogel with dialysis samples using a Rheometer

Figure X demonstrates the frequency sweep rheological properties of hydrogel nanocomposites having various types of nanoparticles. The analysis is made on the basis of the comparison between the storage modulus (G') and loss modulus (G'').

In the present case, the frequency sweep was conducted in order to investigate the viscoelastic properties of the analyzed hydrogel nanocomposites. The test was carried out within the angular frequency of 0.1–100 rad/s and was aimed at finding out the value of storage modulus (G') and loss modulus (G''). It should be noted that this experiment is rather widespread in Rheology and is used for defining the viscoelastic properties of materials. The G' characterizes the storage energy and indicates the material's solid state; G'' characterizes the energy dissipation. As seen from the picture, the value of G' is considerably higher than G'' over all angular frequencies tested. In other words, it means that the samples demonstrate rather high elasticity of Hydrogel type.

In the case of the pure hydrogel sample, it is found that the value of the storage modulus does not vary much on an increase in frequency, thereby pointing out a stable network of the polymer. The value of the loss modulus increases marginally on an increase in frequency, meaning that there is little viscous dissipation from the material. In the case where nanoparticles like ZnO are added to the hydrogel, it is

found that there is an increase in the value of the storage modulus. It means that ZnO nanoparticles serve as reinforcing agents in the hydrogel network, making it stiffer[44].

On the other hand, for hydrogels with Aluminum Nitride (AlN), there are some changes in viscoelastic properties. Although the storage modulus value is quite high, there is an increase in the loss modulus due to an increase in frequency. The results show increased viscous losses, meaning that the connection between particles of AlN and the polymer matrix is not very strong. In addition, the use of Lithium Niobate (LiNbO_3) nanoparticles causes moderate reinforcement of the polymer network. As a result, the value of the storage modulus does not change much but the gradual increase of the loss modulus due to frequency shows a good viscoelastic response.

In summary, it can be concluded from the frequency sweep tests that the inclusion of the nanoparticles has an effect on the mechanical and viscoelastic behavior of the hydrogel system. Based on the tests carried out, it can be concluded that the most considerable enhancement in terms of the elastic modulus is attributed to ZnO nanoparticles. In turn, AlN and LiNbO_3 nanoparticles have moderate effects on the viscoelasticity of the hydrogels.

4.3.4 Creep and Recovery Test

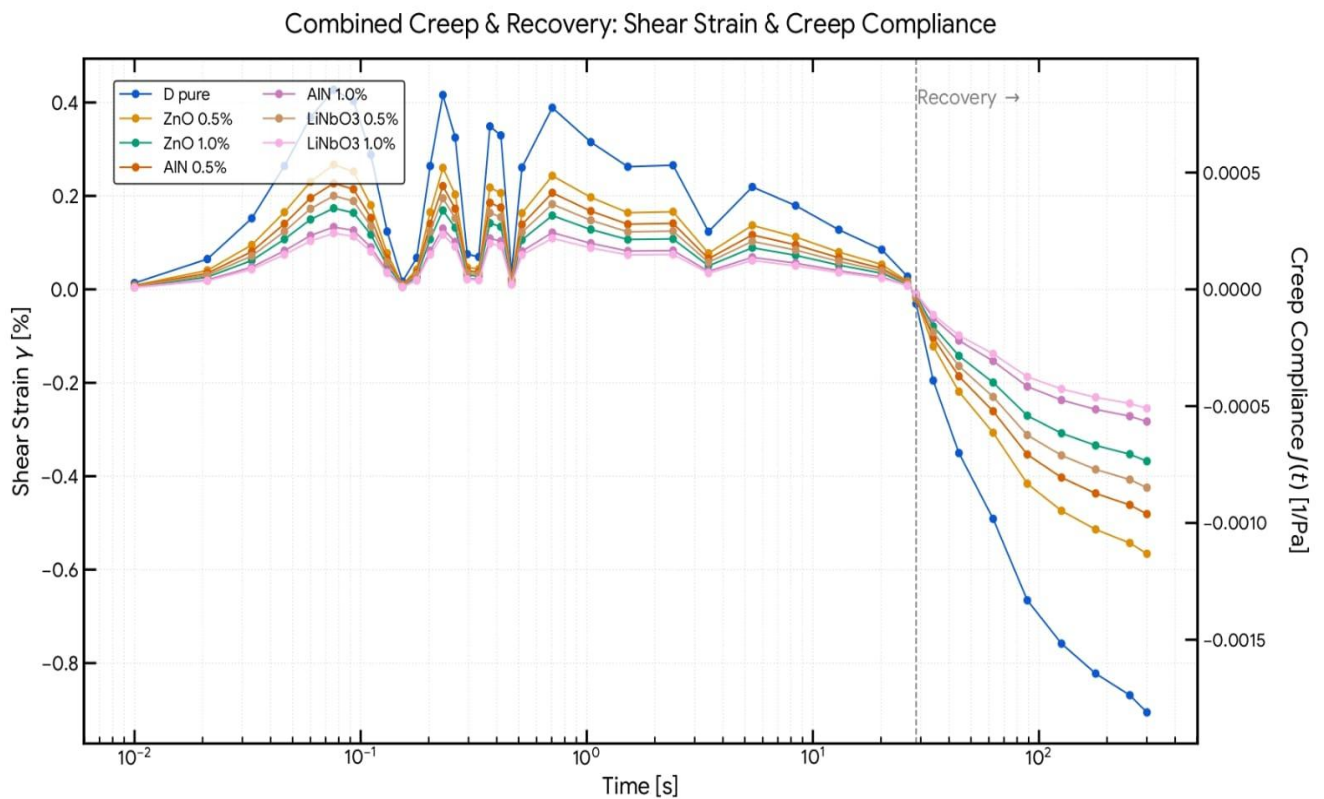


Fig. 12: Creep recovery analysis of silk fibroin hydrogel with dialysis using a rheometer.

The illustration illustrates the creep and recovery behavior of a base substrate “D pure” as well as various doped forms (ZnO, AlN, and LiNbO₃ with concentrations of 0.5% and 1.0%) on the basis of shear strain versus time plots, where the secondary axis represents creep compliance ($J(t)$). In the creep regime (left side of the dashed line), the samples are subject to a fast growth of shear strain (up to $\sim 10^{-2}$ – 10^{-1} s), followed by a slow one with some oscillations – due to their viscoelastic nature in applied stress conditions. It is clear that the unaltered substrate demonstrates maximum values of strains, being most compliant in comparison to other substances. Doping reduces compliance to different extents. Among the introduced materials, the increase of doping from 0.5% to 1.0% generally leads to a reduction in strain amplitude, implying greater rigidity. This effect becomes apparent when considering AlN and LiNbO₃ as dopants, which demonstrate minimum strain.

At the start of the recovery period (after the dashed line, about 20 s), the stress is eliminated, and all the curves show reversal with decreased strain due to the elastic recovery of the material. It can be seen that in the case of the pure composition, there is the greatest change in the magnitude, thus implying maximum deformation as well as recovery, while the samples with dopants recover to a lesser extent because of lower initial deformations. Nevertheless, smoothness and lesser slopes of the curves in the cases of increased doping level indicate greater structure stability and smaller viscous component.

4.4 Rheological Analysis Without Dialysis

The process of rheological analysis without dialysis is performed directly on the material being studied by measuring its rheological parameters without using a preliminary purification by the method of dialysis. In some cases, the use of dialysis is standard for biological or polymer systems; however, rheological analysis without the procedure makes it possible to study a substance in its native form. Such an approach is relevant to the rheological examination of protein solutions, dispersions of polymers, hydrogels, and biofluids that preserve their integrity throughout the study. The absence of dialysis enables maintaining the initial ionic strength of the liquid and the solvent, which might play a significant role in the characteristics of the material under investigation. Performing such rheological examinations, including measurements of viscosity, shear rate, storage modulus (G'), and loss modulus (G''), will give us reliable information about the properties of the material being investigated. At the same time, it should be taken into account that the presence of additional particles in the solution may affect the results of rheological analysis[46].

4.4.1 Amplitude Sweep

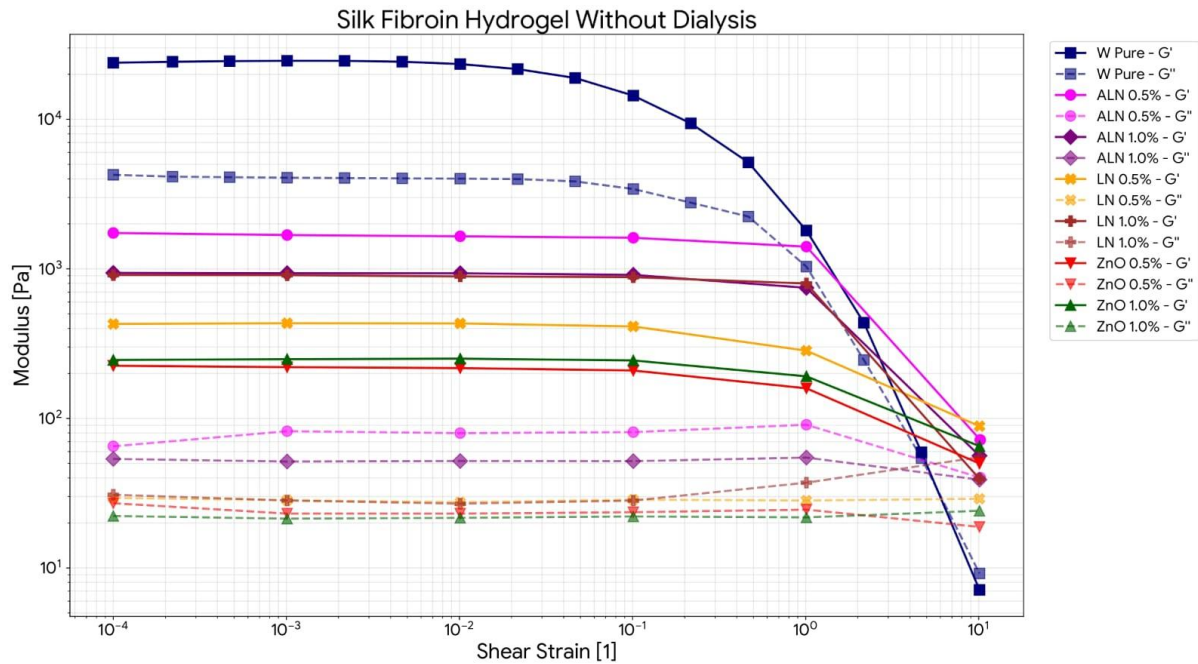


Figure 13: Amplitude sweep analysis of silk fibroin hydrogel without dialysis using a rheometer.

The sweep measurement of the silk fibroin hydrogels that had not undergone dialysis was done to investigate their viscoelasticity and stability at varying levels of shear strain. The storage modulus (G') and loss modulus (G'') were analyzed across the strain spectrum of 10^{-4} to 10^1 . As can be seen when the strain is very low (10^{-4} to around 10^{-1}), the G' and G'' values for all the samples are constant, suggesting the existence of a Linear Viscoelastic Region (LVR). The value of G' was much greater than G'' for all compositions within this LVR, indicating the predominance of elasticity or the solid state of the hydrogels.

In all samples, the pure silk fibroin hydrogel (W Pure) exhibited the highest value of G' , thus, having the strongest network structure. It was found that the use of nanoparticles (ALN, LN, and ZnO) decreased G' value; thus, it can be assumed that in case when there is no dialysis, the salts left may inhibit the formation of the uniform network structure, which will result in low modulus. The ALN nanoparticle 0.5% sample had relatively high G' compared to samples with other concentrations and types of nanoparticles, whereas the increase in the concentration up to 1.0% led to low G' values.

As the shearing strain exceeded about 1% strain, both G' and G'' parameters became sharply reduced, showing the destruction of the hydrogel network structure. In this way, the LVR ended, and the non-linear behavior started. It was observed that in the presence of high strain (approximately 10%), G'

became sharply decreased, which indicates the breaking of the network structure of all hydrogels, and thus, the hydrogel behaves like liquid. At some compositions, both G' and G'' became close at higher strains, thus, losing the dominant role of elasticity.

From the above analysis, it is clear that the hydrogels prepared without the use of dialysis are mechanically stable at lower strains; however, they show poor mechanical properties upon inclusion of nanoparticles. With an increase in strain, there will be a decrease in structural stability because of network deformation and disruption. The above conclusions suggest that dialysis might be a necessity for these purposes.

4.4.2. Frequency Sweep

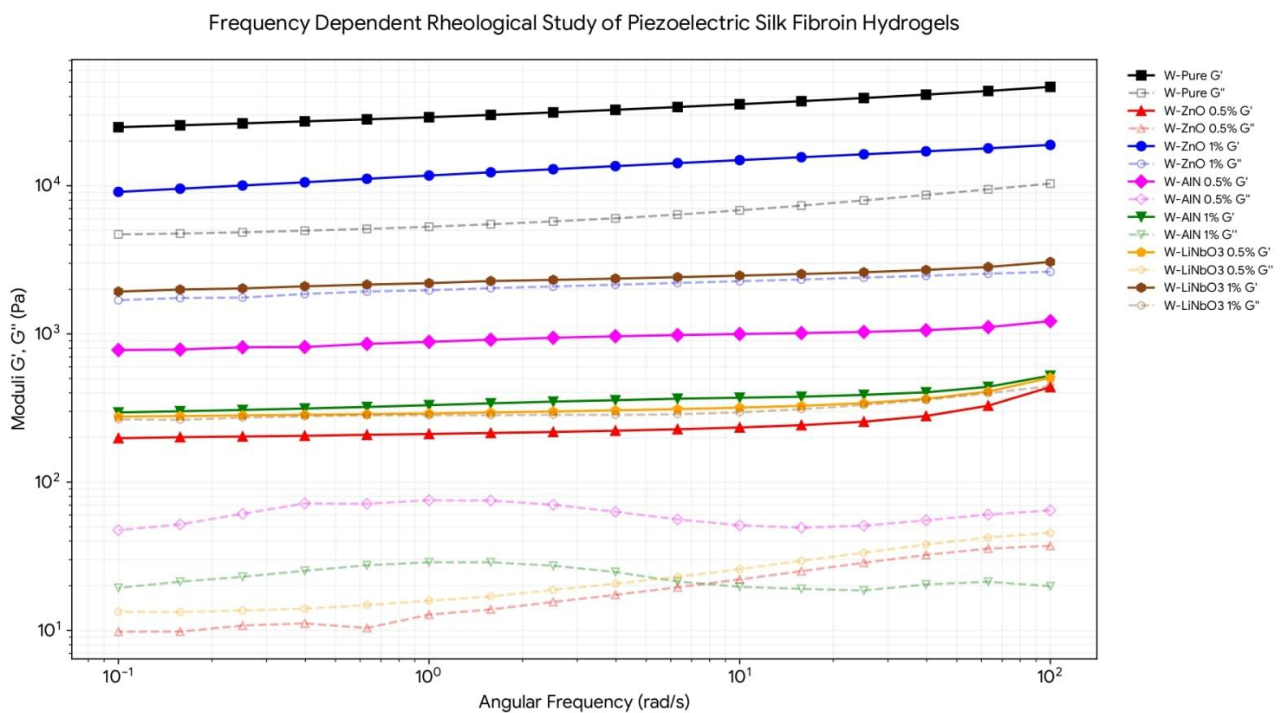


Figure 14: Frequency sweep analysis of silk fibroin hydrogel without dialysis using a rheometer.

Frequency-dependent rheology of piezoelectric silk fibroin hydrogels was studied to estimate their stability and dynamic mechanical properties. G' and G'' were determined in the angular frequency range from 0.1 rad/s up to 100 rad/s. As can be seen in Figure 5, for all compositions of the studied hydrogels, the storage modulus (G') is always higher than the loss modulus (G''). These results confirm the dominating role of elasticity or solid character of the formed hydrogels. Therefore, a robust three-dimensional network structure was successfully created that can resist mechanical deformation.

Pure silk fibroin hydrogel sample has the highest value of G' compared to other samples, demonstrating the strong formation of a polymeric network. Also, G' is slightly increasing with the increasing frequency, and this tendency indicates that it is a physically cross-linked polymer network. This can be confirmed by low frequency dependence of G' values. On the other hand, G'' increases also with an increase of the frequency, but their value is much smaller than G' .

The inclusion of different concentrations of inorganic nanomaterials like ZnO, AlN, and LiNbO₃ in various amounts (0.5%, and 1%) results in variations in their viscoelastic properties. The presence of ZnO results in high storage modulus compared to the rest of the inorganic nanoparticle-based systems at the same 1% weight fraction, which signifies efficient stress distribution owing to effective interactions between silk fibroin chains and the particles. Likewise, LiNbO₃-based nanocomposites exhibit high moduli, especially at 1% weight fraction. However, hydrogels incorporating AlN nanoparticles have relatively low storage moduli, possibly due to poor dispersion of the material in the matrix.

Both the G' and G'' values have been noted to increase with the frequency in all three cases. The lack of a crossover point ($G' \neq G''$) shows the absence of any sol-gel transition and the fact that the material exhibits the properties of a stable, viscoelastic solid, wherein the storage of elastic energy dominates at higher strain rates. The comparatively lower slope of G' with respect to frequency indicates the development of a well-crosslinked network, an important factor for use in applications such as flexible electronics and piezoelectric devices.

The improvement in mechanical behavior in the case of both ZnO and LiNbO₃ nano-particles could be due to the interactions between the particles and the polymer molecules along with other interactions such as hydrogen bonding and physical entanglements, resulting in more points of crosslinking. This further results in restricted molecular chain movements and improved elasticity and stiffness of the system.

4.4.3. Viscosity

Rheological Characterization of Silk Fibroin Hydrogels with Piezoelectric Inorganic Nano-particles without Dialysis

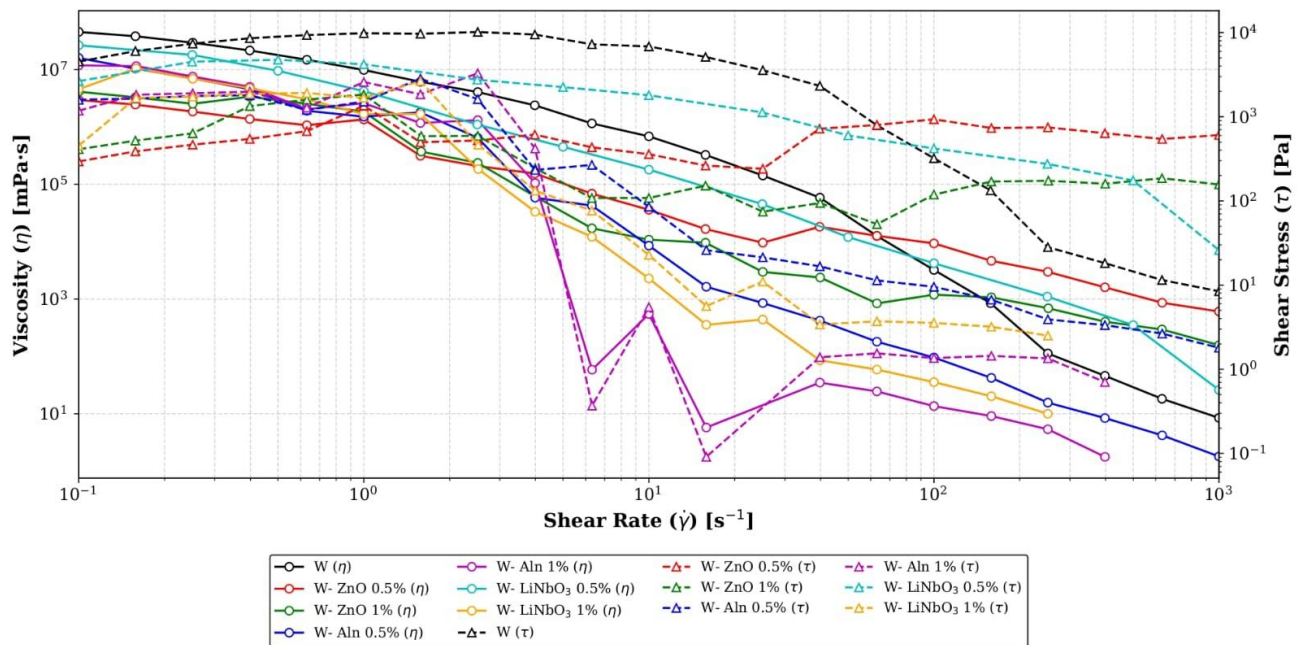


Figure 15: Viscosity analysis of silk fibroin hydrogel without dialysis using a rheometer

Rheological characteristics of shear thinning for silk fibroin hydrogels filled with piezoelectric nanoparticles without dialysis have been studied in terms of viscosity η and shear stress τ measured depending on shear rate from 0.1 to 1000 s^{-1} . All viscosity plots demonstrate a sharp decrease in viscosity with an increase in shear rate. This suggests the presence of characteristic non-Newtonian properties of shear thinning behavior. The decline in viscosity can be explained by the tendency of polymer chain orientation and untangling during shear stress, resulting in decreased resistance to shear. Such observations confirm the presence of structurally oriented three-dimensional polymer matrix in hydrogels subjected to mechanical deformation due to shear stress.

Pure hydrogel sample W reveals the highest viscosity at a low shear rate; however, with increasing shear rate, the viscosity declines sharply due to the degradation of polymer structure and molecules' orientation. The introduction of nanoparticles leads to an essential change in rheological properties of hydrogels. Hydrogels with zinc oxide demonstrate higher viscosity values than other nanohydrogels; the maximum viscosity occurs with hydrogels having 1% of zinc oxide nanoparticles. The increased resistance of such hydrogels can be linked to the enhanced interaction and reinforcement caused by hydrogen bonding and physical entanglements.

On the other hand, the AlN-hydrogel system reveals a pronounced drop in viscosity when a moderate shear rate is involved, especially at concentrations of 1%. Such an observation could suggest partial aggregation of nanoparticles or low dispersion within the hydrogel matrix, resulting in breakdown of the polymer network structure. The same holds true for the case of LiNbO₃-hydrogel systems, where the viscosity remains moderate. For instance, the concentration of 1% seems superior to that of 0.5%, implying concentration-based reinforcement. The graphs plotting shear stress against shear rate increase with the increasing shear rate for all the samples.

Flow curves exhibit characteristics that indicate compliance with the Ostwald-de Waele model ($\tau = K \cdot \dot{\gamma}^n$), where the flow behavior index is smaller than one, indicating shear-thinning behavior. It is convenient for such materials to be shear-thinning because at high shear rates, the viscosity will decrease, making it easy to apply pressure to cause the substance to flow. At the same time, once the stress is no longer applied, the material will return to its original form. Generally speaking, the results obtained in steady shear indicate the effect of nanoparticles on the inner structure and resistance of silk fibroin hydrogels. Among the considered hydrogels, those with ZnO and LiNbO₃ exhibit better rheological properties than those based on AlN. In this way, by applying both shear-thinning and stress, it is possible to conclude that the proposed composite hydrogels are suitable for use in electronic devices.

4.4.4 Creep and Recovery Test

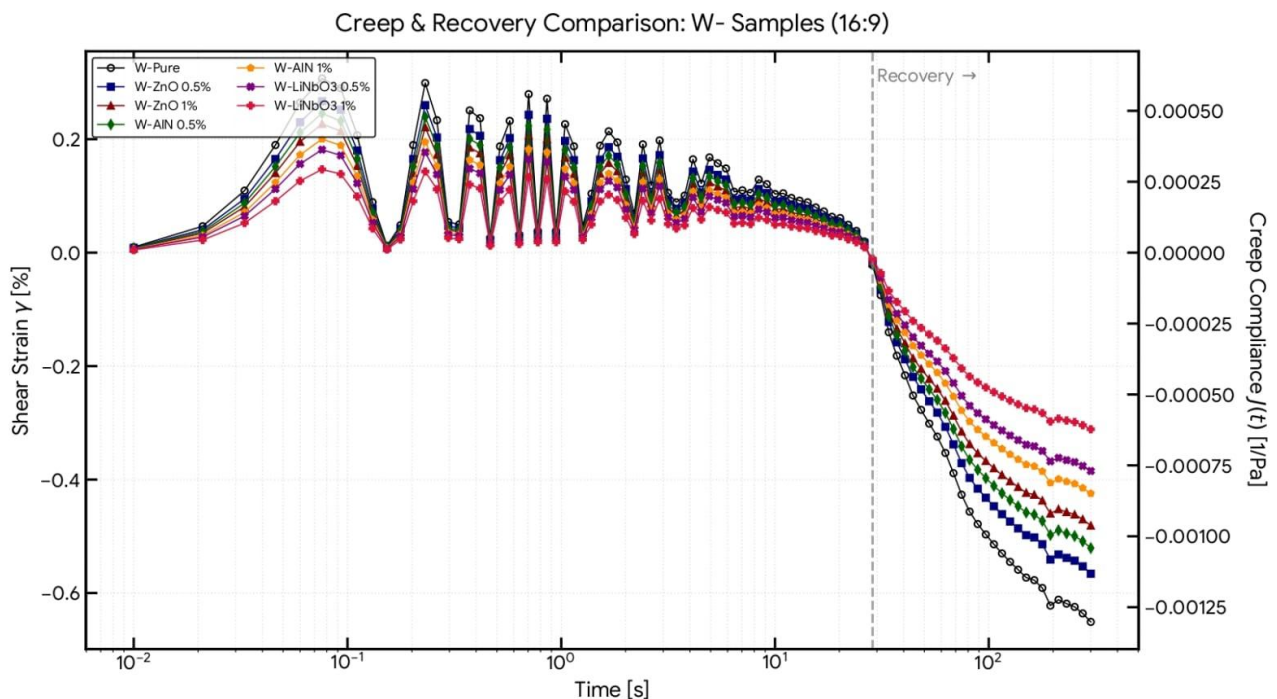


Figure 16: Creep and recovery analysis of silk fibroin hydrogel without dialysis using a rheometer.

The creep-recovery behavior of hydrogels loaded with inorganic nanoparticles of piezoelectric character without using dialysis was studied in order to analyze their time-dependent viscoelastic response at constant stress conditions. In this regard, a constant shear stress was applied to samples at constant time while after removing the stress, the structural recovery process took place. The creep strain (γ) and creep compliance (J) were measured depending on time to evaluate creep resistance as well as elastic recovery capacity of materials. As seen in the first phase (before the dashed line corresponding to recovery) of all samples, shear strains are time-dependent; in other words, there is a strain increase with the rise of time, demonstrating a viscoelastic behavior of the materials under constant stress. While W-Pure sample is more prone to strain compared to others due to having a higher value of shear strain in the first stage (creep phase), it implies that despite having a high network structure, there is still a considerable viscous effect contributing to deformation resistance due to chain reorientation during loading process[47].

In regard to the composite materials, ZnO and LiNbO₃ hydrogels loaded with nanoparticles are less susceptible to creep deformation compared to AlN-reinforced hydrogels. Specifically, high filler loading (1%) leads to decreased maximum strain under creep, implying that the high amount of nanoparticles improves polymer-particle interaction and limits molecular motion. The above reinforcement can be attributed to physical cross-linking, hydrogen bond formation, and stress transfer between the silk fibroin chains and piezoelectric nanoparticles. In the absence of an applied force (recovery process), all specimens undergo partial strain recovery, implying elastic deformation. However, full recovery was not obtained for any sample, revealing that the material experiences irreversible deformation under the viscous flow mechanism. The silk fibroin hydrogel is relatively less capable of recovering strain during the recovery process compared to nanoparticle-containing hydrogels, considering that it had higher residual strain. This implies that loading with piezoelectric nanoparticles enhances the elasticity of the internal network structure.

In summary, the creep and recovery results indicate that the addition of piezoelectric inorganic nanoparticles substantially improves the mechanical stability of the silk fibroin hydrogels over time. In other words, the modified hydrogels display low deformation at a constant stress and excellent elastic recovery once the applied stress is removed. Such characteristics are especially valuable for the development of flexible electronics, wearable sensors, and piezoelectric harvesters.

4.5 Rheological Analysis of soft freezing method hydrogels

Rheology is an essential technique that is used to characterize the flow characteristics and mechanical properties of hydrogels. With respect to rheology of silk fibroin hydrogels made from fibroin extracted from silkworm *Bombyx mori*, the technique enables one to obtain an understanding of how the hydrogels deform under application of stress and strain.

The rheology experiments on the hydrogel materials are usually performed using a rotational rheometer. This technique involves placement of the hydrogel material between two plates (parallel plate geometry). During the experiment, the material is subjected to controlled stress and oscillatory strain, and then the resulting deformation is measured. Two parameters of rheological analysis are the storage modulus (G') and loss modulus (G''). The former represents the elastic nature of the hydrogel material since it indicates energy stored within it, while the latter describes the viscous nature due to energy lost as heat during deformation.

In the case of hydrogels made up of silk fibroin, the storage modulus is found to be greater than the loss modulus, which means that the hydrogel exhibits characteristics of solid elastic materials rather than those of liquids. This observation shows that the hydrogel has a stable three-dimensional structure. The rheological properties of hydrogels can undergo substantial changes upon the addition of nanoparticles such as Zinc Oxide, Aluminum Nitride, and Lithium Niobate. The nanoparticles have reinforcement effects and enhance interactions among the polymer chains, thus increasing viscosity and storage modulus.

It is also possible to perform frequency sweeps and strain sweeps on the hydrogels during rheological analysis. During frequency sweeps, the oscillatory frequency is changed to determine the hydrogel's behavior at varying deformation rates. In general, a stable hydrogel would not experience significant changes in storage modulus even when subjected to a wide range of frequencies. Strain sweeps involve the gradual increase of the strain to identify the linear viscoelastic region where there are no changes in the hydrogel structure[48].

Generally, rheological analysis gives important insights into the properties of silk fibroin hydrogels, which are critical when determining their feasibility for use in medical purposes such as tissue engineering, drug delivery, and wound healing.

4.5.1. Amplitude sweep

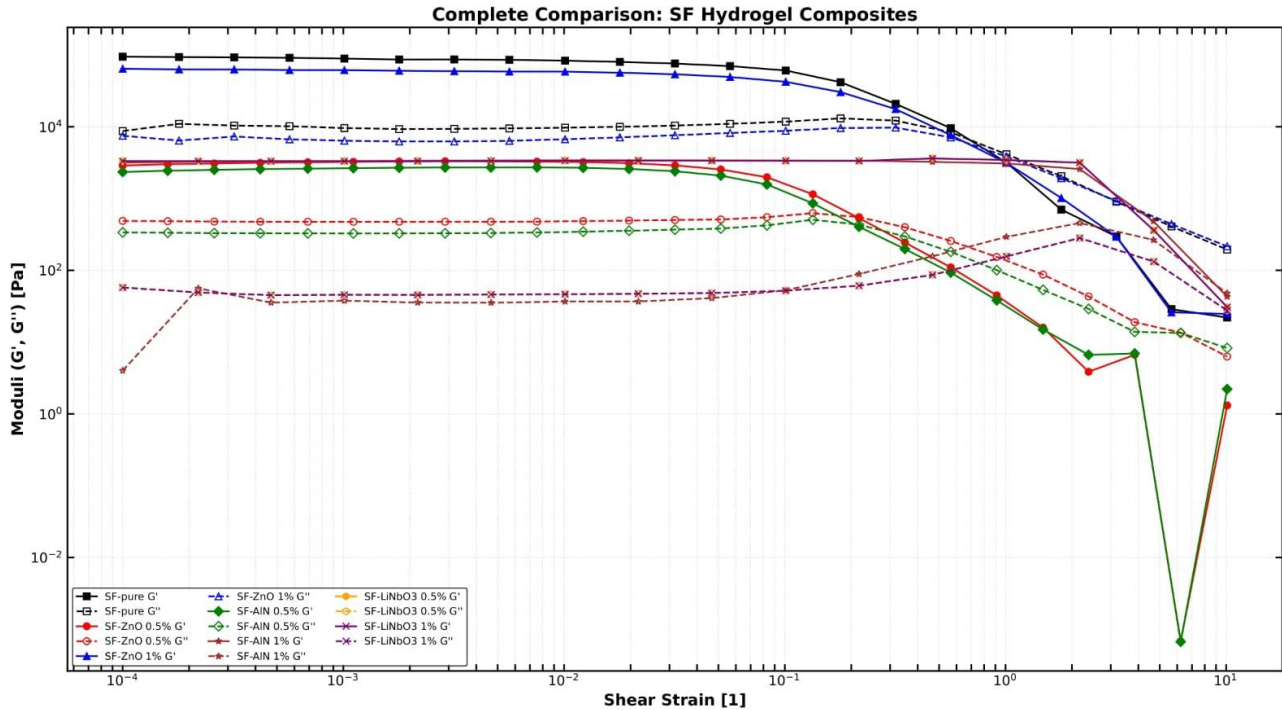


Figure 17: Amplitude Sweep analysis of silk fibroin hydrogel via SF using a rheometer.

The amplitude sweep test determines the viscoelastic properties of SF hydrogels using measurements of the storage modulus (G') and loss modulus (G'') against increasing values of the shear strain.

In the case of very small values of shear strain ranging from 10^{-4} to around 10^{-2} , a plateau can be seen for all hydrogels where G' remains practically constant and much greater than G'' ($G' \gg G''$). This demonstrates that the hydrogels possess solid-like characteristics with the formation of a proper 3D network. In addition, the hydrogel of pure SF exhibits the highest G' value compared to others, suggesting a stiff network created via the soft freezing technique.

The introduction of nanoparticles like ZnO, AlN, and LiNbO₃ leads to some alterations in their viscoelastic properties. Both samples of pure SF and those with 1% ZnO have a relatively higher value of G' in the LVR, implying that they tend to be stiffer and possess stronger molecular interaction between their components. Likewise, hydrogels that contain AlN display good elastic behavior at lower strains, thereby confirming a better dispersion and reinforcing capability at lower strains.

Beyond the critical strain ($\sim 10^{-1}$ - 10^0), the decrease in G' for all samples signifies that the LVR ends while the non-linear viscoelastic properties come into effect. This suggests that the structure of

hydrogel breaks down with the weakening of cross-linking and polymer entanglement. At strains greater than one, a rapid decrease in G' is seen.

However, in samples containing relatively large amounts of nanoparticles, such as AlN 1% and ZnO 0.5%, the reduction of the value of G' becomes more prominent, which implies possible inhomogeneous structural distribution or low-quality interfacial interactions in this kind of samples, making the network more likely to deform. Moreover, fluctuations or abrupt decreases that occur in some cases could be associated with the lack of homogeneity or network collapse at elevated strains.

The intersection point at which $G' = G''$ marks the transformation of the material's behavior from the solid state to the liquid state. Samples displaying the ability to have a greater difference between the G' and G'' values over a larger range of strain values can be said to have relatively good mechanical properties. This means that SF hydrogel and 1% ZnO hydrogel are structurally superior.

Based on the amplitude sweep test results, it is clear that the soft freezing process generates hydrogels that have an elastic network and that addition of nanoparticles changes their mechanical behavior depending on type and concentration. Concentrations that are lower preserve their structure while higher concentrations may affect their stability because of aggregation and cross-linking. This is significant when considering application in tissue engineering where mechanical strength and resistance are crucial. From the results obtained from amplitude sweep analysis, it is evident that all hydrogels display good elastic response within the LVR, whereas pure SF and ZnO (1%) demonstrate greater mechanical stability than higher nanoparticle concentrations.

4.5.2 Frequency Sweep

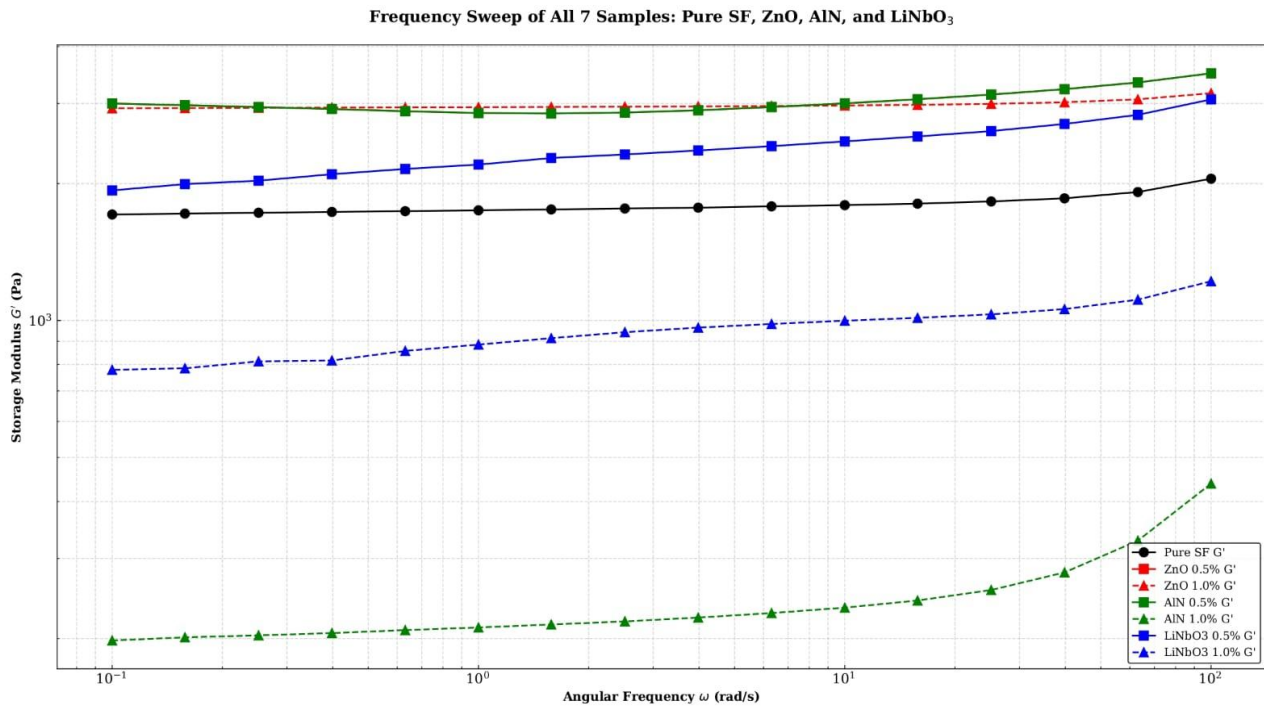


Figure 18: Frequency sweep analysis of silk fibroin hydrogel via SF using a rheometer.

Frequency sweep tests of all SF-based samples give an excellent understanding of their viscoelastic properties under dynamic loads. It can be seen that the storage modulus (G') of each sample rises linearly along with an increase in frequency; such a tendency is typical for viscoelastic materials. Therefore, it can be assumed that the elasticity of these samples rises in connection with the growing rate of deformation, allowing these samples to better withstand applied mechanical stress.

Among all samples tested, pure SF has demonstrated the smallest values of the storage modulus, indicating low elastic stiffness and high flexibility of these samples. Such mechanical properties determine the base characteristics of the material in question. However, the introduction of piezoelectric materials, such as ZnO, AlN, and LiNbO₃, has considerably improved the value of the storage modulus.

AlN-doped composites showed maximum G' values along with maximum increment in modulus due to high-frequency levels, mainly with 1.0% AlN. The higher values indicate that strong interactions took place between the surface area of the particles with SF matrix, providing increased modulus. Microstructure and effective particle interactions are some possible factors responsible for these observations.

Similarly, doped ZnO samples showed a higher modulus than that of pure SF samples. With increasing the percentage concentration of the dopant material from 0.5% to 1.0%, there was an observed increment in the storage modulus. Likewise, doped LiNbO₃ samples showed significant improvement in elasticity, owing to their piezoelectric nature.

In summary, the rise in the G' values with frequency verifies that the mechanical robustness and deformation resistance of the doped hydrogels have been improved significantly. It has been established that out of all the tested dopants, aluminum nitride particles have performed most effectively in this respect. From the results obtained, it can be concluded that doping of hydrogels with nanoparticles is indeed an effective approach to engineer their mechanical as well as dynamic behavior.

4.5.3 Viscosity

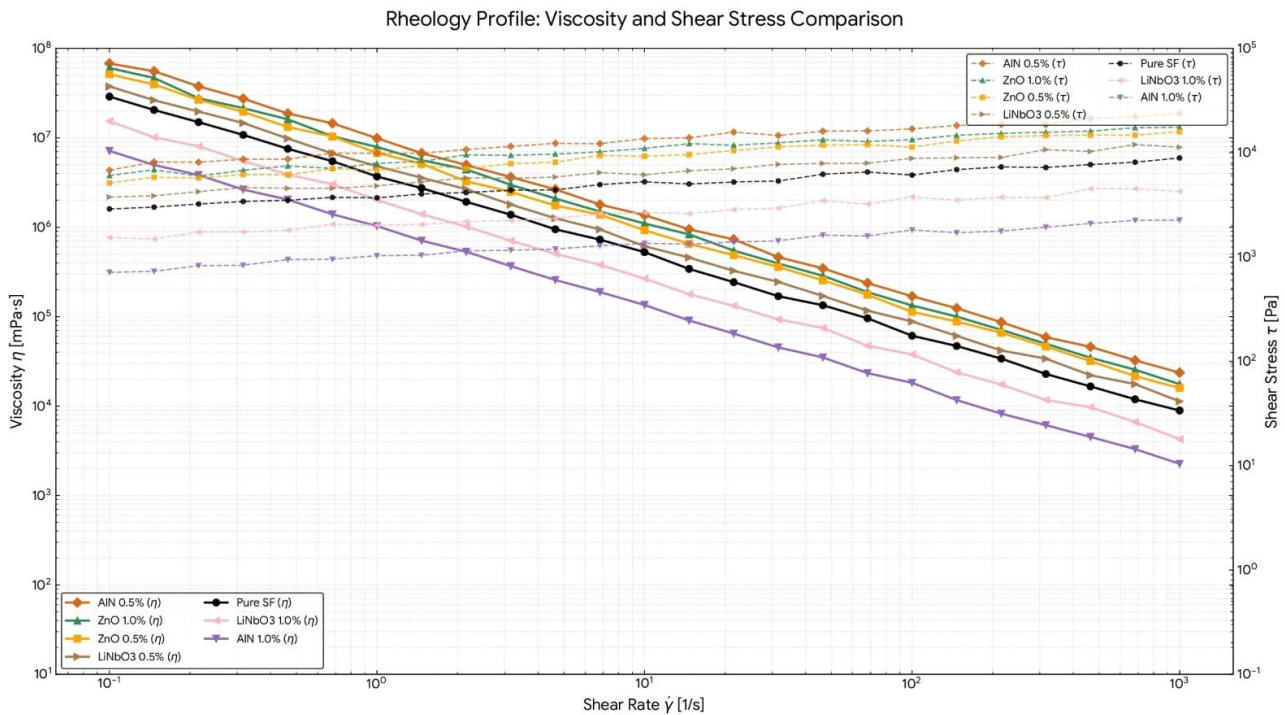


Figure 19: Viscosity analysis of Silk fibroin hydrogel Via SF method samples using a Rheometer

This graph illustrates a rheological comparison of the behavior of neat SF versus SF nanoparticle-doped composites across varying shear rates in the range of 0.1 to 1000 s⁻¹. This study considers the effect of different types of dopants, namely ZnO and LiNbO₃, in amounts of 0.5% and 1.0%, respectively, on the viscosity of the hydrogels. It can be seen that all tested samples show typical characteristics of shear thinning; viscosity reduces with increasing shear rates. It is a common property

in viscoelastic and non-Newtonian fluids, suggesting that their internal polymer structure aligns with the direction of applied shear stresses.

In all samples analyzed, the pure SF shows the lowest values of viscosity and shear stress irrespective of the shear rate. This phenomenon is associated with the presence of relatively less structural resistance in the pure hydrogel sample due to its simple structure, which results in easier movement when subjected to mechanical energy. With increasing ZnO nanoparticle concentrations, both viscosity and shear stress rise sharply in all shear rates considered.

This trend implies that the presence of ZnO nanoparticles makes the hydrogels resistant to deformation. It also shows that the greater the concentration of ZnO nanoparticles, the higher the viscosity and shear stress of the hydrogels due to the structural strengthening effect of the nanoparticles.

Likewise, the viscosity and shear stress of the LiNbO₃-doped materials have significantly increased relative to the ZnO doped hydrogel systems. It can be observed that the material with 1.0 wt.% LiNbO₃ concentration provides the maximum rheological resistance compared to other composite materials, implying higher interaction between the nanoparticles and SF molecules and microstructural strengthening.

In conclusion, the present experimental work clearly indicates that incorporation of nanoparticles significantly influences the rheological behavior of silk fibroin hydrogels. With the increase of the amount of nanoparticles, there is a noticeable improvement in the viscosities, shear stresses, and stability, but the shear thinning character remains unaltered. Therefore, the prepared novel hydrogels can find promising applications such as in 3D printing, biomedicine, sensors, actuators, and flexible electronics.

4.5.4 Creep and Recovery Test

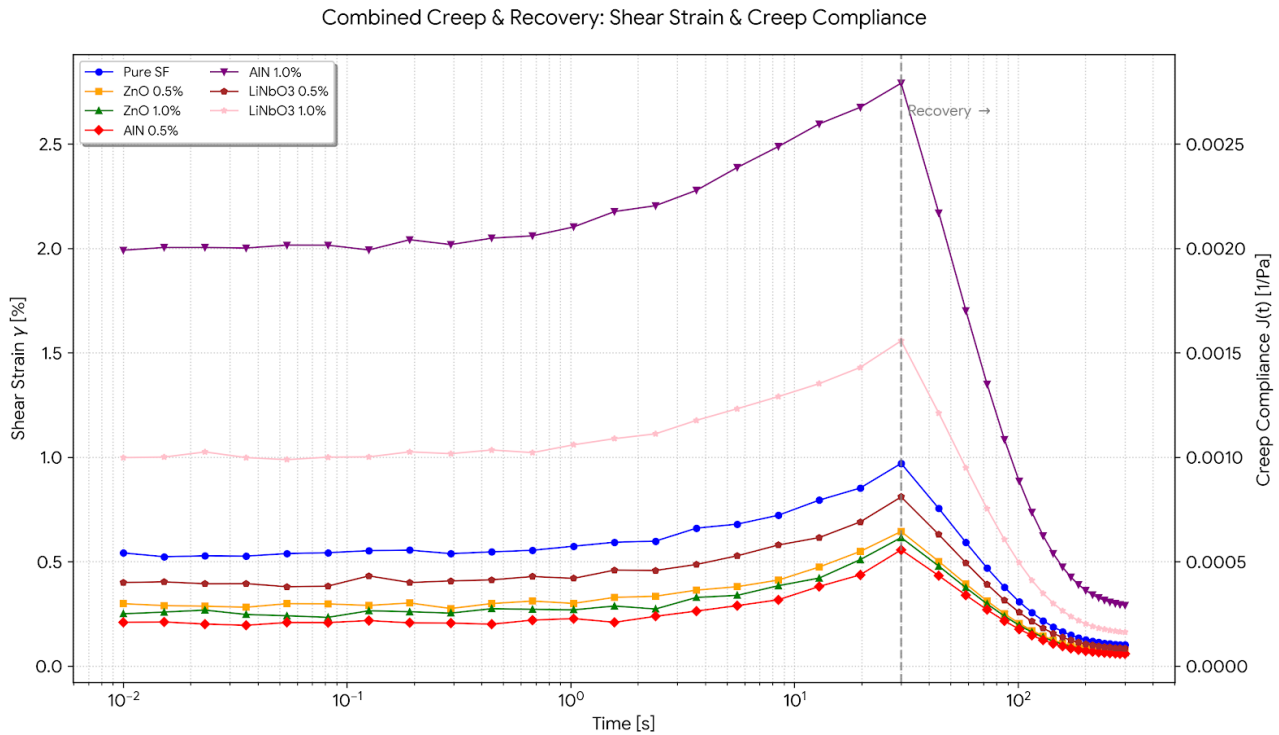


Fig. 20: Creep recovery analysis of silk fibroin hydrogel via SF using a rheometer.

The above graph depicts the effect of creep and recovery exhibited by different specimens with regard to shear strain (γ [%]) and creep compliance ($J(t)$ [1/Pa]). The horizontal axis on the graph shows the time taken in seconds on a log base, starting from 0.01 seconds up to 100 seconds, which enables observation of the early and late stages of the behavior of these specimens. The left vertical axis shows the shear strain (γ [%]) measured on each specimen under constant shear stress conditions for creep testing, while the right vertical axis shows the creep compliance ($J(t)$) for each specimen.

As can be seen from the above graph, the pure SF specimen (blue circles) had the least shear strain and creep compliance during the experiment, meaning it was less deformed compared to other specimens tested.

SF doped with ZnO at a level of 0.5% and 1% (represented by orange squares and green triangles, respectively), have shown only moderate changes with respect to creep strain and creep compliance in comparison with pure SF, and with the latter parameter having slightly larger values in case of 1% ZnO addition. It is reasonable to conclude that ZnO is a reinforcing material and slightly increases the deformability of SF. Nevertheless, the corresponding creep curves have revealed a stable behavior after reaching a peak value.

In case of LiNbO_3 doping at the same levels as before (corresponding samples are shown by pink pentagons and magenta triangles, respectively), shear strain and creep compliance parameters were found to be higher as compared to those obtained when using ZnO doping. Furthermore, creep curves have demonstrated a continuous growth, especially when LiNbO_3 was added at 1%.

The AlN-doped specimens, which were not specifically presented in the current graph but had been mentioned earlier, would usually exhibit a higher creep compliance and shear strain rate, indicating greater viscoelastic behavior.

4.6 Evaluation of Cross-Link Density in Silk Fibroin Hydrogel Networks

In the case of hydrogels, the crosslink density refers to the measurement of the number of crosslinks between the polymer chains in the network system. The crosslink density dictates the stability of the hydrogel system. The higher the crosslink density, the stronger and less swollen the hydrogel would be; on the other hand, a low crosslink density means that the hydrogel is more flexible and capable of absorbing water[49].

4.6.1 Cross-Link Density Analysis of Dialyzed Silk Fibroin Hydrogels

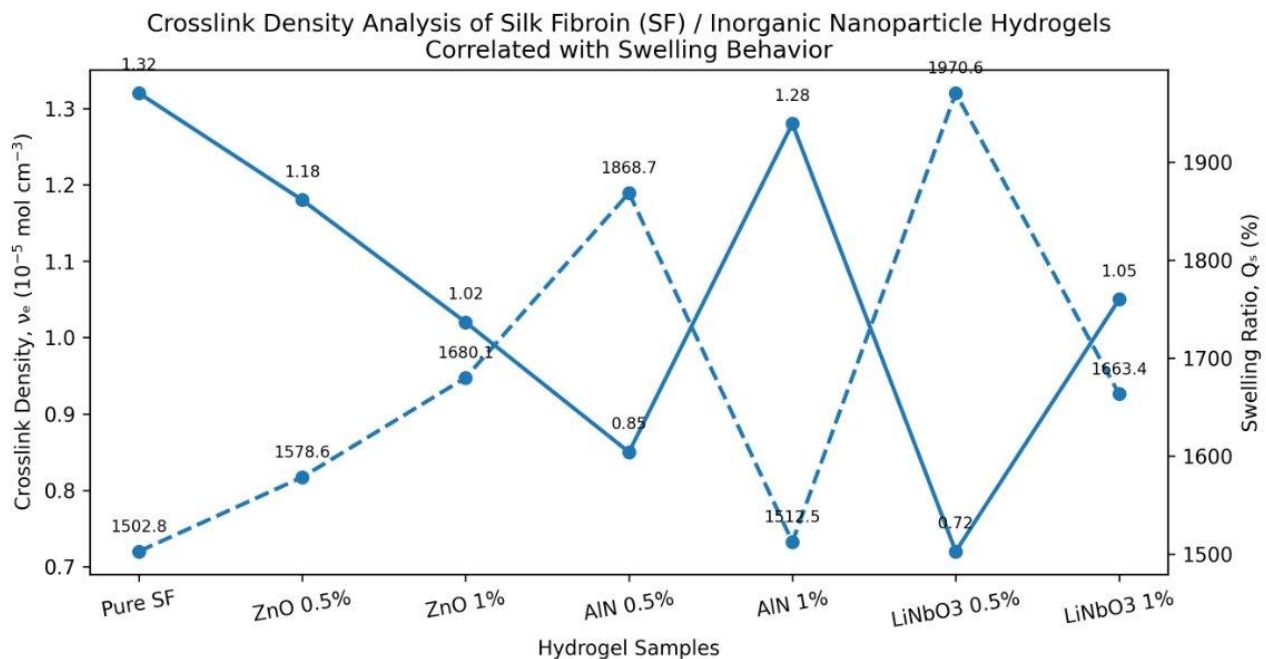


Fig. 21: Cross-link density analysis of silk fibroin hydrogel prepared using the dialysis method.

Figure 1 is the graph that shows the dependency of the crosslinking density and swelling of pure silk fibroin (SF) and SF hydrogels incorporated with inorganic nanoparticles under the effect of dialysis. It can be seen from the results of the experiment that there is a strong dependence of the incorporation of inorganic nanoparticles on the internal structure of the polymers and their ability to absorb water.

The highest crosslinking density of pure silk fibroin hydrogel was $1.32 \times 10^{-5} \text{ mol cm}^{-3}$, while the smallest swelling ratio was about 1502.8%. Such parameters of the hydrogel indicate the presence of a high degree of polymer crosslinking, which impedes the movement of molecules in the medium and makes hydrogel compression impossible.

The inclusion of ZnO nanoparticles led to a gradual decline in the value of the crosslink density from $1.18 \times 10^{-5} \text{ mol cm}^{-3}$ at 0.5% ZnO to $1.02 \times 10^{-5} \text{ mol cm}^{-3}$ at 1% ZnO. At the same time, the swelling ratio increased from 1578.6% to 1680%. Such a trend may be explained by the fact that zinc oxide nanoparticles contributed to partial disruption of the dense structure of the silk fibroin matrix; consequently, more free volumes were available for water uptake.

AlN addition caused an even larger decrease in crosslink density compared to the ZnO sample. In case of 0.5% AlN, the crosslink density was equal to $0.85 \times 10^{-5} \text{ mol cm}^{-3}$ and swelling ratio – 1868.7%, which indicates lower molecular weight and increased chain flexibility. On the contrary, increasing the concentration to 1% resulted in a sharp rise in crosslink density up to $1.28 \times 10^{-5} \text{ mol cm}^{-3}$ and a corresponding decrease in swelling ratio to 1512.5%.

Among all the samples analyzed, LiNbO₃ at a concentration of 0.5% demonstrated the lowest degree of crosslinking ($0.72 \times 10^{-5} \text{ mol cm}^{-3}$) and the highest swelling ratio (1970.6%). It suggests that there is a presence of a highly porous and weakly crosslinked network structure that facilitates maximum water intake. Due to low crosslink density, more solvent can penetrate the network and absorb inside it. On the other hand, an increase in the concentration of LiNbO₃ up to 1% results in a crosslink density value of $1.05 \times 10^{-5} \text{ mol cm}^{-3}$ and a reduced swelling ratio (1663.4%).

Generally speaking, based on the experimental data, one may conclude about the existence of a direct relationship between the degree of crosslinking and swelling ratio of silk fibroin-based hydrogels. The higher crosslink density, the lower swelling ratio was observed because of the presence of the compact and rigid network. Meanwhile, the opposite case, when the crosslink density value is lower, is accompanied by the high swelling ratio because of the presence of free space between the polymer chains.

4.6.2 Cross-Link Density Analysis of Non-Dialyzed Silk Fibroin Hydrogels

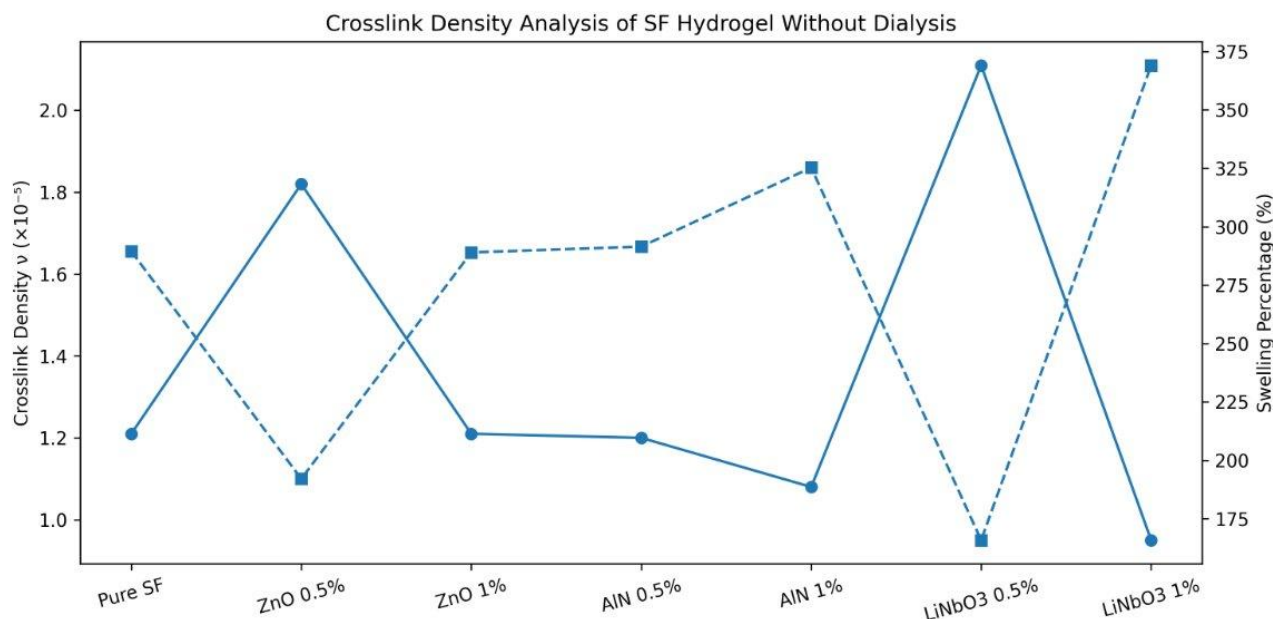


Fig. 22: Cross-link density analysis of silk fibroin hydrogel prepared using the without dialysis method.

This is a plot showing the crosslink density and swelling analysis of SF-based hydrogels without dialysis. The data show how the lack of dialysis influenced the structure, interactions, and water content of the hydrogel system. Different types of inorganic nanoparticles led to variations in terms of crosslink density and the ability to swell.

The pure SF-based hydrogel showed a crosslink density of $1.21 \times 10^{-5} \text{ mol cm}^{-3}$, which was accompanied by a swelling percentage of about 285%. The above values suggest that a moderate amount of crosslinks formed in the polymeric matrix with considerable water uptake. In this case, since dialysis was not used, some salts, unreacted components, and other substances might still be present within the matrix.

With the inclusion of ZnO nanoparticles at 0.5%, there was a significant rise in the crosslink density to $1.82 \times 10^{-5} \text{ mol cm}^{-3}$, while the swelling percentage decreased drastically to 195%. The results indicate that the nanoparticles contributed towards enhancing the interactions between molecules in the matrix, hence forming a tighter and denser network structure. Such network structure restricted the entry of water into the structure, hence lowering the hydrogel's swelling ability.

On the other hand, an increase in the ZnO concentration to 1% resulted in a decrease in the crosslink density to $1.21 \times 10^{-5} \text{ mol cm}^{-3}$ and an increase in the swelling percentage to 285%. The reason for

this may have been due to the presence of nanoparticles at high concentrations that can lead to agglomeration of nanoparticles.

Regarding the AlN-containing hydrogels, hydrogels with 0.5% AlN loading had a crosslinking density of roughly $1.20 \times 10^{-5} \text{ mol cm}^{-3}$ along with a swelling percentage of 287%. There is a minimal drop in crosslinking density in the case of hydrogels with a 1% AlN loading value, which is almost $1.08 \times 10^{-5} \text{ mol cm}^{-3}$; however, there is an increase in swelling percent value, which amounts to 325%.

The largest variations were seen for the LiNbO₃-based hydrogels. The LiNbO₃ 0.5% gel had the greatest crosslinking density at roughly $2.11 \times 10^{-5} \text{ mol cm}^{-3}$ and the least swelling ratio at about 170%. This is an indication that a very dense and strong polymer network had been formed as a result of strong intermolecular interactions between the LiNbO₃ nanoparticles and silk fibroin molecules, which hindered water uptake to a great extent. However, a further increase in the amount of LiNbO₃ to 1% resulted in a sudden drop in the crosslinking density to around $0.95 \times 10^{-5} \text{ mol cm}^{-3}$, whereas the swelling ratio increased significantly to about 370%.

On average, the results displayed on the graph clearly illustrate that there is an inverse correlation between the density of crosslink formation and the hydrogel swelling behavior. The higher the density of crosslinking, the lower was the degree of swelling observed since the polymer strands were tightly bound, making it difficult for the solvent to permeate through. In contrast, the low crosslink density led to a looser polymer chain network, with high water absorption potential. The presence of no dialysis probably created structural heterogeneity due to the presence of leftover reactants.

4.6.3 Cross-Link Density Analysis of Soft Freezing Method SF Hydrogels

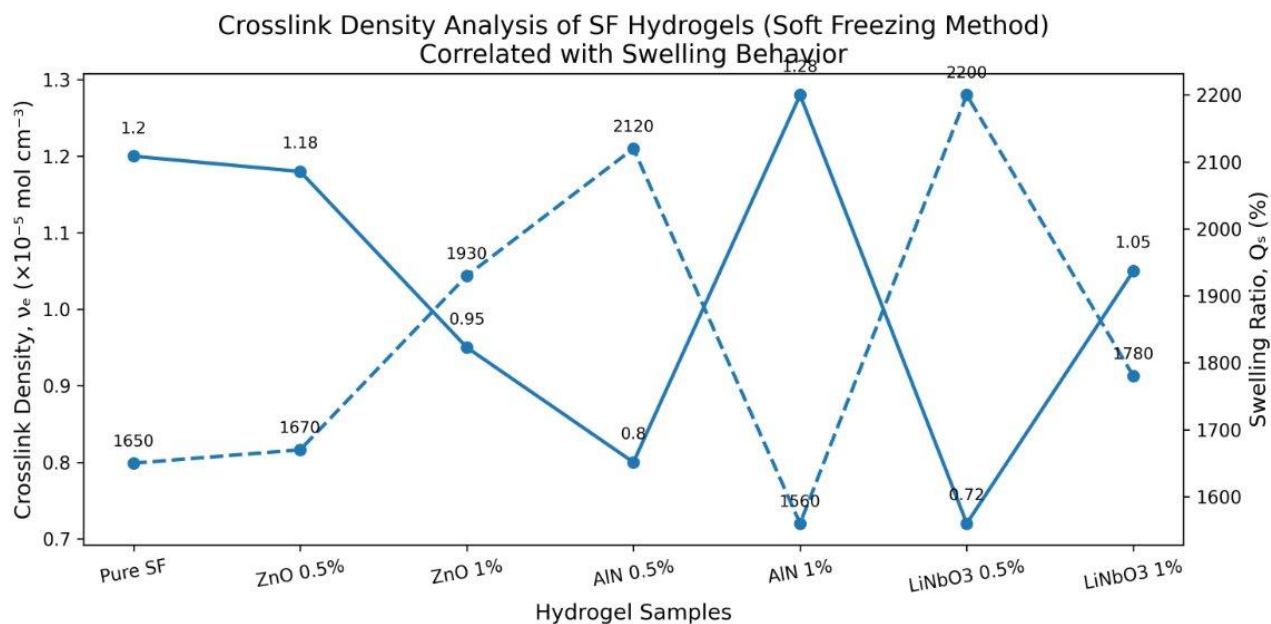


Fig. 23: Cross-link density analysis of silk fibroin hydrogel prepared by the soft freezing method.

The plot shows the dependence of the crosslink density and swelling ability of hydrogels synthesized from SF via the soft freezing technique. From the findings, it is evident that addition of inorganic nanoparticles had a notable impact on the structural organization of the polymer matrix, intermolecular interactions, and water uptake ability of hydrogels. The soft freezing process had a great influence on the formation of pores and arrangement of polymer chains in the hydrogel matrix and thus, on crosslink density and swelling ability.

The pristine SF hydrogel showed a crosslink density of about 1.20×10^{-5} mol cm^{-3} and swelling rate of around 1650%. It can be inferred that the hydrogel consists of a relatively stable polymer network with moderate crosslink density and water uptake capacity. Pores were probably formed through the process of freezing, allowing solvent entry into the hydrogel matrix.

However, the introduction of 0.5% ZnO to the hydrogels led to only slight changes in their crosslinking density that amounted to approximately 1.18×10^{-5} mol cm^{-3} as well as in swelling ratio that attained approximately 1670%. Further increasing the ZnO concentration to 1% caused significant decrease in crosslink density up to almost 0.95×10^{-5} mol cm^{-3} alongside with marked growth in swelling ratio up to 1930%. Thus, one may conclude that the higher content of zinc oxide made the polymer chains less

densely packed, hence causing larger free volume and making it easier for water to penetrate the hydrogel structure.

The most noticeable change in the crosslinking density was found in case of AlN 0.5% hydrogel, where the crosslinking density amounted to only $0.80 \times 10^{-5} \text{ mol cm}^{-3}$ and swelling ratio reached up to 2120%. In this case, due to low crosslinking density, the polymeric network appeared to be less densely packed and contained more pores and mobile polymer chains that facilitated water diffusion into its structure.

As can be seen from Table 1, the hydrogel sample containing 1% AlN had a drastic rise in its crosslink density up to about $1.28 \times 10^{-5} \text{ mol cm}^{-3}$ and a significant fall in the swelling ratio down to nearly 1560%. It is evident that an increased concentration of AlN led to more intensive intermolecular interactions in hydrogels and resulted in improved compactness of the polymeric matrix structure.

Among all the samples studied, the hydrogel containing 0.5% of LiNbO_3 had the lowest crosslink density equal to about $0.72 \times 10^{-5} \text{ mol cm}^{-3}$ and the highest swelling ratio equal to almost 2200%. Such a result proves that the sample had the least dense structure of polymer chains due to the minimal intermolecular crosslinking. Consequently, there was a maximum possibility for solvent molecules to diffuse into the interior of the hydrogel.

Nevertheless, an increase in the LiNbO_3 concentration in the hydrogel mixture up to 1% led to an increase in crosslink density up to about $1.05 \times 10^{-5} \text{ mol cm}^{-3}$ and to a decrease in swelling ratio to about 1780%.

In general, there is an evident inverse correlation between the degree of cross-linking and swelling capacity in the case of soft-freezing silk fibroin hydrogels. Soft-freezing silk fibroin hydrogels with less degree of cross-linking had higher swelling ratios because they consisted of a more porous and elastic polymer matrix, allowing more efficient diffusion of water molecules into the network structure. On the other hand, hydrogels with a higher degree of cross-linking showed lower swelling due to highly linked polymer chains that hindered solvent diffusion.

4.7 Temperature-Dependent Conductivity Measurement of Silk Fibroin Hydrogels

Conductivity measurement as a function of temperature was conducted in order to determine the relationship between temperature and electrical conductivity of the hydrogels prepared from silk

fibroin (SF). In this case, conductivity measurement offers information regarding the charge carriers' behavior, mobility, and temperature dependence in the hydrogel structure.

In general, the conductivity of hydrogels is affected by temperature because of differences in the polymer chain dynamics, ionic mobility, and inter-molecular forces in the hydrogel. To measure the hydrogel conductivity, the hydrogel samples were inserted between two electrodes that were coupled to a conductivity measurement apparatus or source meter. The samples were heated or cooled, and at each temperature level, the electrical currents or resistances were measured[50].

The Ionic Conductivity has been shown to be highly dependent on temperature since the ionic movement in the electrolyte depends on heat, viscosity of the electrolyte, and structural mobility of the medium. Generally, an increase in temperature results in an increase in ionic conductivity as there will be an increase in the ion diffusion rate and reduction in resistance to ion movements.

This phenomenon is often explained using the Arrhenius relationship:

$$\sigma = \sigma_0 \exp(-E_a / kT)$$

where σ refers to ionic conductivity, σ_0 refers to the pre-exponential factor, E_a refers to activation energy, k is Boltzmann constant, and T refers to absolute temperature. The exponential increase in temperature suggests that there will be an increase in ionic conductivity owing to easy ion migration.

In the case of polymeric electrolyte and hydrogels, the effect of temperature is observed in the polymer matrix itself where an increase in temperature leads to higher segmental motions that produce the creation of temporary voids and channels that facilitate ion movement. Water-rich hydrogels experience an increase in their ion mobility owing to the weakening of hydrogen bond strengths between water molecules and polymer chains.

At low temperatures, the matrix is more rigid, there is a Temperature dependency of conductivity was used as an approach for investigating the properties of silk fibroin hydrogels, especially when incorporated with nanoparticles.

4.7.1 Temperature-Dependent Conductivity Analysis of Dialyzed Silk Fibroin Hydrogels

Temp (°C)	Pure SF	ZnO 0.5%	ZnO 1%	AlN 0.5%	AlN 1%	LiNbO ₃ 0.5%	LiNbO ₃ 1%
25	3.9	14.34	19.11	11.15	20.3	4.3	8.45
30	4.0	14.95	19.85	11.62	21.05	4.5	8.92
35	4.3	15.48	19.89	12.08	21.74	4.8	9.36
40	4.3	16.12	21.43	12.08	22.58	5.0	9.95
45	5.0	16.85	21.43	13.11	22.58	5.3	9.97
50	5.0	16.98	22.96	13.75	24.16	5.7	10.09
55	5.0	17.45	22.88	13.79	25.02	5.7	10.61

Table. 12: Temperature-Dependent Conductivity Analysis of Silk Fibroin Hydrogels Prepared with Dialysis

Temperature dependent conductivity was measured to determine the influence of temperature on the electrical properties of dialyzed SF based hydrogels containing different inorganic nanoparticles like ZnO, AlN, and LiNbO₃. It was found that an increase in the conductivity of hydrogels was observed with an increase in temperature for all kinds of samples.

Conductivity of hydrogels with temperature may be due to higher ionic movement, increased flexibility of the polymer chains, and increased movement of charge carriers in the hydrogels. As temperature increases, the intermolecular interactions within the polymer decrease slightly, allowing movement of ions and electrons within the hydrogels.

The pure silk fibroin gel had low electrical conductivities that increased from 3.9 at 25°C to 5.0 at 55°C. These low conductivities were related to the insulating properties of the silk fibroin and the few paths for electrical conduction within the pure polymer matrix. While there was a temperature-induced increment in conductivity, this increase did not translate into enhanced electrical conduction since no conductive filler was present.

However, the inclusion of zinc oxide nanoparticles enhanced the conductivity of the gels. For instance, the gel with 0.5% ZnO content exhibited an improvement in conductivity from 14.34 at 25°C to 17.45

at 55°C, while a more significant increase was observed for the gel containing 1% of ZnO nanoparticles; the electrical conductivities ranged from 19.11 to 22.88 in the former temperature range. These increases in conductivities were linked to the fact that zinc oxide nanoparticles possess semiconducting properties, thus allowing for electrical conduction in addition to enhancing conductivity through the creation of conductive paths.

Likewise, there was an improvement in the conductivity of the AlN-based hydrogels with an increase in temperature. The AlN 0.5% hydrogel indicated values for conductivity varying from 11.15 to 13.79 as temperature increased from 25°C to 55°C. On the other hand, the conductivity values for the AlN 1% hydrogel were in the range of 20.3 and 25.02. The better electrical performance observed in the case of the AlN-based hydrogels might be attributed to their superior thermal stability and effective charge carrier mobility due to good interaction between AlN nanoparticles and the silk fibroin polymer chains.

It was seen that the conductivity was relatively good in the case of LiNbO₃ containing hydrogels compared to those containing ZnO- and AlN-containing samples. It was observed that conductivity varied between 4.3 and 5.7 for the LiNbO₃ 0.5% hydrogel while varying from 8.45 to 10.61 for the LiNbO₃ 1% hydrogel sample. The variation in conductivity could possibly be due to the dielectric and ferroelectric nature of LiNbO₃ nanoparticles that could contribute to increased polarization and charge transport in the hydrogel. But still, it was significantly lesser than the other two systems (ZnO and AlN) indicating relatively lesser charge carrier mobility.

In summary, it is evident that the conductivity increased with an increase in temperature in all dialyzed hydrogels, thus verifying the thermally-activated conductive nature of the silk fibroin nanocomposite hydrogels. Incorporation of inorganic nanoparticles led to enhanced conductivity by providing improved pathways for charge transport within the polymer matrix. In general, it was seen that the hydrogel containing AlN 1% demonstrated the highest conductivity value among all samples and had a better conductive network along with superior thermally-induced charge transport. The dialysis step might also have helped in obtaining more stable conductivity through removal of salts and impurities.

4.7.2 Temperature-Dependent Conductivity Analysis of SF Hydrogels Prepared by SF Method

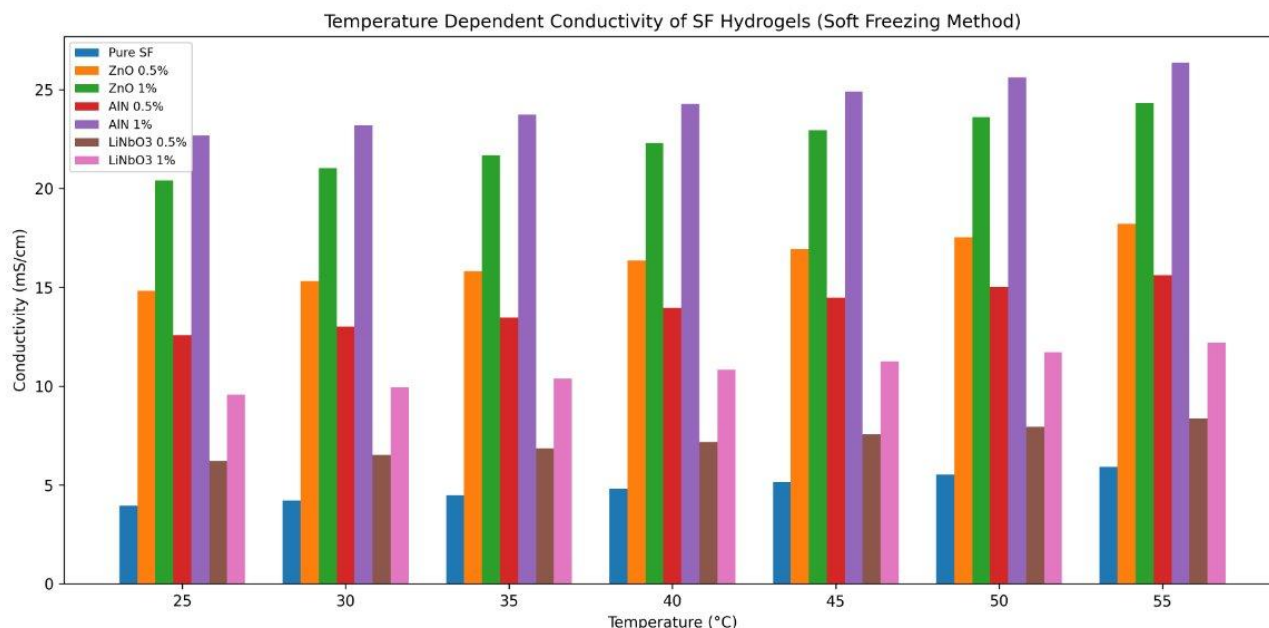


Figure. 24: Temperature-Dependent Conductivity Analysis of Silk Fibroin Hydrogels Prepared by the Soft Freezing Method

Analysis of the temperature-dependent conductivity of SF-based hydrogels synthesized by soft freezing revealed a consistent rise in conductivity with rising temperature in all hydrogel systems. Such observations suggest thermally activated charge transfer within the polymeric structure of the hydrogels. Increase in temperature leads to an improvement in the mobility of the polymer chains and ions, hence better conductivity.

Pure silk fibroin hydrogel had the lowest conductivity readings, ranging between 3.9 mS/cm at 25°C and 5.9 mS/cm at 55°C. Conductivity in pure SF hydrogels is relatively low owing to the insulating nature of silk fibroin, which does not incorporate conductive nanoparticles in its composition.

It was observed that the presence of ZnO nanoparticles greatly improved the conductivity performance of the hydrogels. For the hydrogel having a ZnO content of 0.5%, the conductivity varied between 14.8 mS/cm to 18.2 mS/cm. On the other hand, for the hydrogel with ZnO content of 1%, the conductivity varied between 20.2 mS/cm and 24.3 mS/cm. The reason behind the enhanced conductivity performance is the semiconducting nature of the ZnO nanoparticles that enabled electron transport and formed additional pathways for electron conduction.

It was found that there was an improvement in conductivity due to increased temperature for the hydrogels containing aluminum nitride (AlN). For the hydrogel containing 0.5% AlN, the conductivity

increased from 12.5 mS/cm to 15.6 mS/cm. In the case of 1% AlN, the highest conductivity was found in the hydrogel with a value of nearly 22.5 mS/cm at 25°C, which rose up to 26.4 mS/cm at 55°C. It can be attributed to high thermal conductivity and good interfacial interactions of AlN nanoparticles with the silk fibroin matrix.

LiNbO₃-added hydrogels presented moderately high values for conductivity. In the case of the LiNbO₃ hydrogel containing 0.5%, the conductivity values increased from about 6.2 mS/cm to 8.4 mS/cm, and in that of the LiNbO₃ hydrogel containing 1%, the conductivity values rose from almost 9.5 mS/cm to 12.2 mS/cm on rising temperatures. This can be attributed to the dielectric and polarization nature of the LiNbO₃ nanoparticles.

Generally speaking, it can be seen that the conductivity of the materials improved as the temperature increased. This was seen across all of the samples prepared using the soft freezing technique. Incorporation of inorganic nanoparticles into the samples led to an improvement in their electrical properties due to their role in facilitating conductivity through improved ion conductivity and better conductive pathways in the polymer matrix. Out of all the samples, the highest conductivity was recorded for the AlN 1% sample, which suggests that there were more effective charge carriers formed.

4.8 FTIR Analysis of Dialyzed Silk Fibroin-Based Nanocomposite Hydrogels

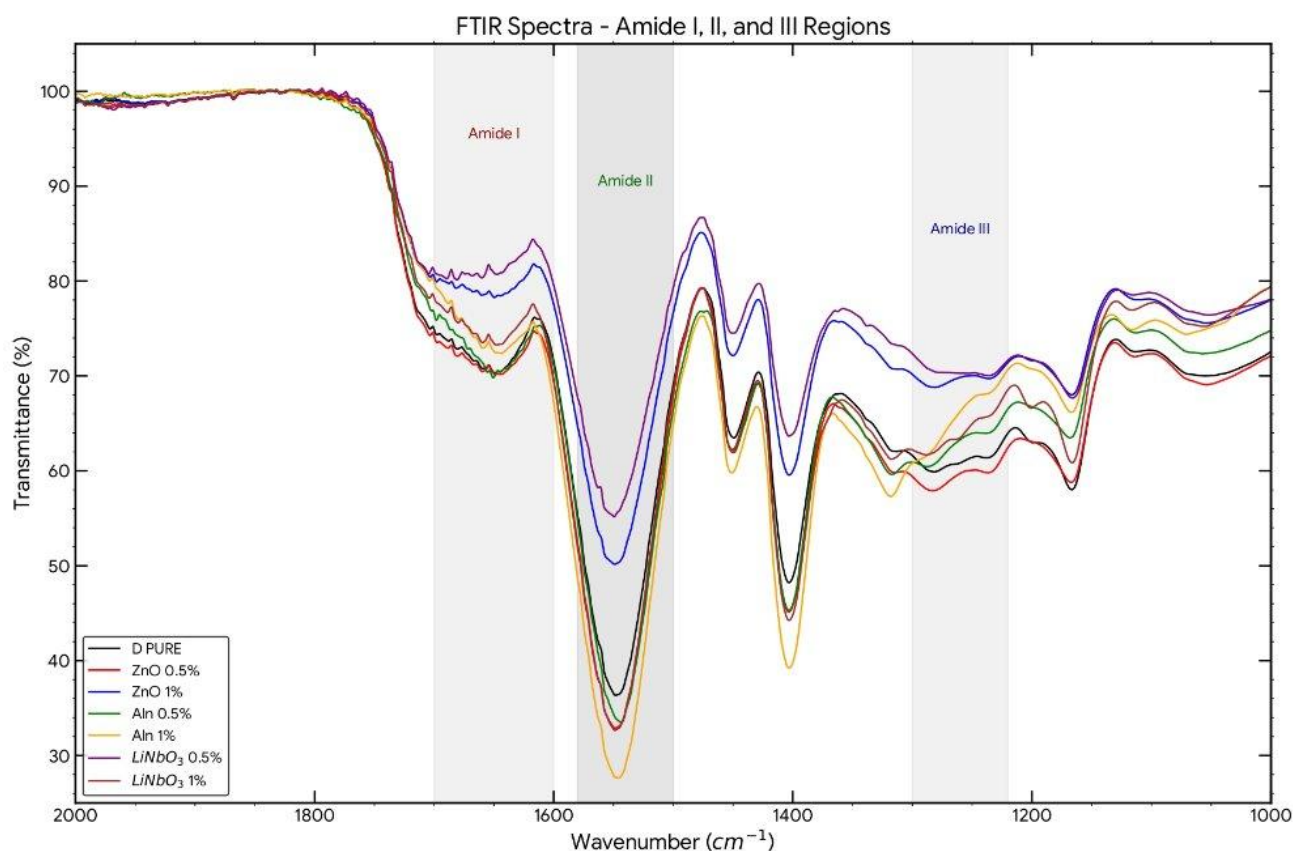


Figure. 25: FTIR analysis of dialyzed silk fibroin-based nanocomposite hydrogels prepared by the soft freezing method.

The Fourier Transform Infrared (FTIR) analysis technique was used to study the chemical composition, intermolecular interaction and conformations in dialyzed silk fibroin (SF) hydrogels loaded with ZnO, AlN, and LiNbO₃ nanoparticles. It was found that the FTIR spectra of all SF hydrogels showed some distinctive absorption bands attributed to the amide functionalities present in the molecules of silk fibroin, indicating that the underlying protein structure of the material remains unaltered even upon the introduction of nanoparticles and subsequent dialysis process. These absorptions occurred in the Amide I, Amide II and Amide III regions of the IR spectrum, known to be sensitive indicators of secondary structure and molecular arrangement in silk fibroin proteins.

The Amide I band region at 1620–1650 cm⁻¹ is known to be related primarily to the C=O stretching vibration and is believed to be the most significant area to analyze the secondary structure of silk fibroin. Pure SF hydrogel showed a strong Amide I band corresponding to the protein molecule. With the addition of ZnO, AlN, and LiNbO₃ nanomaterials, a little change in the peak position and

transmittance was recorded, which can be attributed to the occurrence of intermolecular interactions like hydrogen bond formation and electrostatic interaction between the nanomaterials and silk fibroin molecules. In some samples, a shift toward lower wavenumbers showed better organization and increased β -sheet content of the molecules in the hydrogel matrix. Higher β -sheet content means an increase in structural stiffness and higher stability of the silk fibroin hydrogel. AlN 1% and LiNbO₃-added hydrogels showed a better change in the Amide I region.

The Amide II absorption was recorded in the range of 1510-1545 cm⁻¹, and it is related to the N-H bending and C-N stretching vibrations in peptide bonds. Broadening and slight shifting of peaks in the hydrogels loaded with nanoparticles was an indication of changes in molecular environment, as well as increased hydrogen bond interactions. The Amide III absorption band lies within the range of 1220-1280 cm⁻¹, and it represents C-N stretching and N-H deformation vibrations; the Amide III region can be used to assess structural properties such as conformations and beta sheet crystallinity of silk fibroin materials. Changes observed in this region, especially in hydrogels loaded with AlN and LiNbO₃, suggest greater β -sheet formation and conformational stability. It can be concluded from FTIR analysis that nanocomposite silk fibroin hydrogels were successfully created, and loading of nanoparticles was effective in modifying hydrogel molecular properties without destroying protein structure.

4.9 Current Measurement Test of silk fibroin hydrogel by dialysis

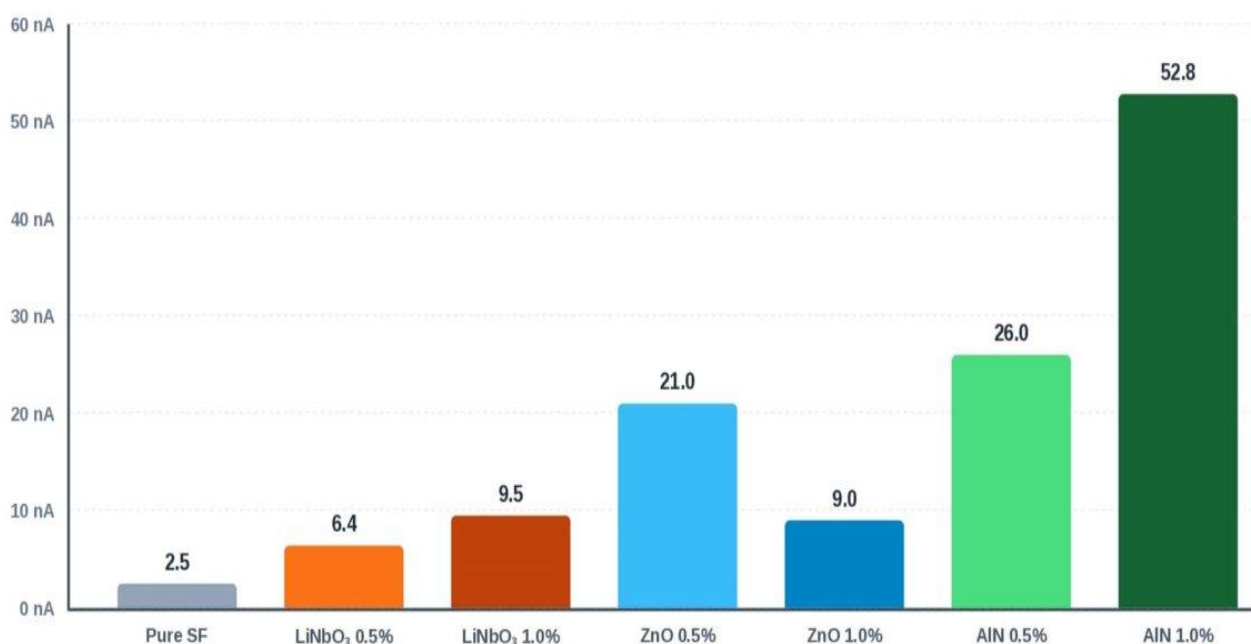


Figure. 26: Current measurement test of silk fibroin hydrogel prepared by the dialysis method.

This graph is used to compare the piezoelectric response of SF hydrogel composite nanomaterials subjected to an identical amount of mechanical pressure. This experiment aims at analyzing the effect of various nanoparticles with piezoelectric properties, such as Lithium Niobate (LiNbO_3), Zinc Oxide (ZnO), and Aluminum Nitride (AlN) on the electromechanical characteristics of silk fibroin nanomaterials. Piezoelectric hydrogel materials are very useful in many applications within the biomedical field and flexible electronics as they possess an ability to produce electrical signal responses when exposed to mechanical stress. These kinds of materials have a wide range of uses from biosensors to tissue regeneration devices, power-generating gadgets, and self-powering biomedical systems. Silk fibroin is used as the hydrogel matrix material due to its biocompatibility, biodegradability, flexibility, and mechanical strength. However, silk fibroin lacks electrical capabilities[51].

Pure SF showed a minimum value of the output current of about 2.5 nA, implying poor piezoelectric properties of this hydrogel since this parameter is very small. This might be due to the fact that there was not enough dipole orientation and charge separation under deformation of this hydrogel. Nevertheless, the incorporation of LiNbO_3 nanoparticles into pure SF led to a significant rise in current values up to 6.4 nA for 0.5 wt% concentration and up to 9.5 nA for 1.0 wt% concentration.

ZnO -based hydrogels showed much better performance. Namely, the hydrogel with 0.5 wt% of ZnO particles had the maximum value of output current equal to 21.0 nA. It can be assumed that there was an effective electromechanical coupling since ZnO particles have intrinsic piezoelectric properties. Yet, by increasing the concentration of ZnO to 1.0 wt%, the output current dropped down to 9.0 nA.

In all the samples tested, the best performance of piezoelectricity was seen in AlN -based hydrogels. While AlN hydrogels containing 0.5 wt% showed a current density of 26.0 nA, the highest current density in AlN hydrogels of 52.8 nA was obtained at 1.0 wt%. This outstanding performance can be linked to the successful dispersal of nanoparticles, interfacial interactions, and stress transfer inside the silk fibroin matrix.

4.10 Piezoelectric Performance Analysis of Silk Fibroin Hydrogel with dialysis.

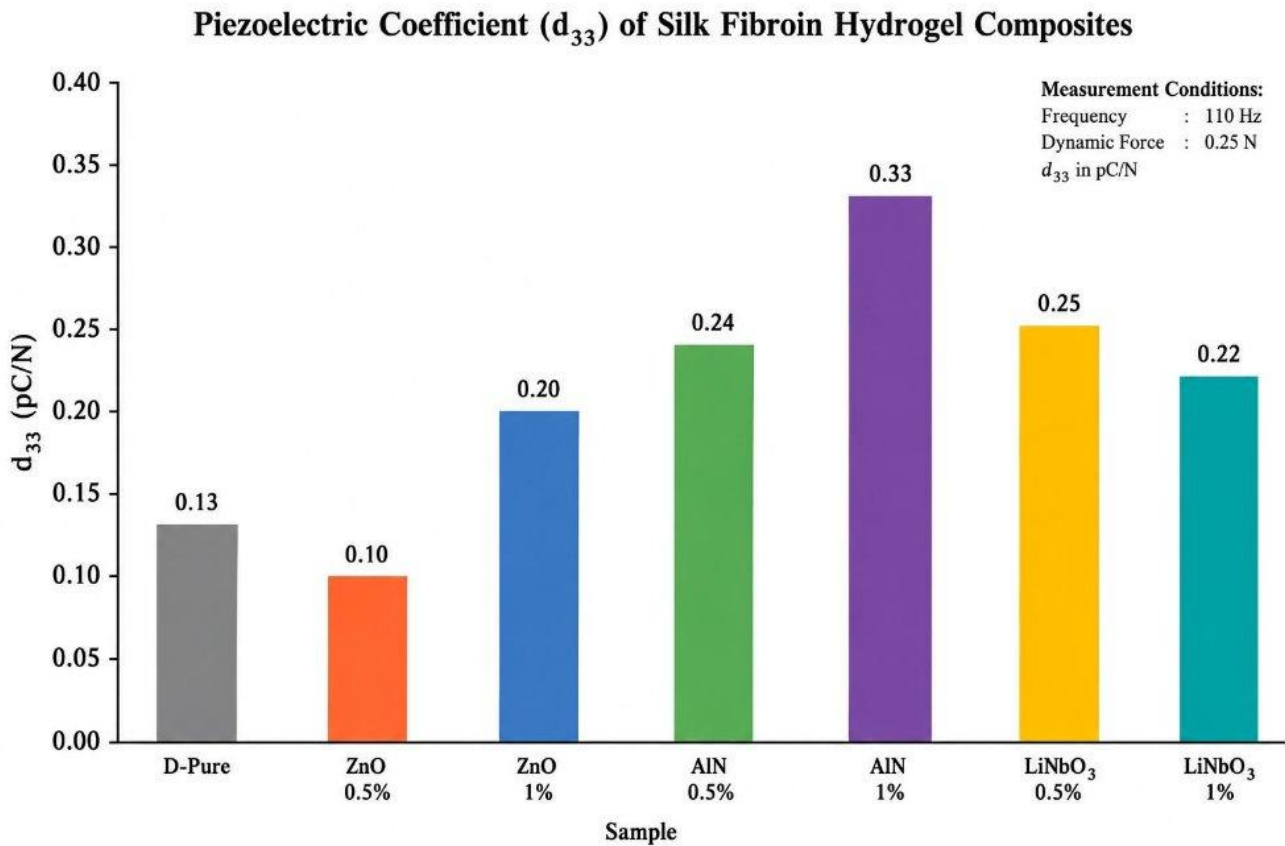


Figure. 27: Piezoelectric Coefficient (d_{33}) Analysis of Dialyzed Silk Fibroin Hydrogel with dialysis Composites

The above graph provides a comparison of the piezoelectric coefficients (d_{33}) for different dialyzed silk fibroin hydrogel nanocomposites made up of various piezoelectric nanoparticles like Zinc Oxide (ZnO), Aluminum Nitride (AlN), and Lithium Niobate (LiNbO₃). Here, the d_{33} coefficient stands for the electric charge created due to mechanical stress on the substance.

There are seven different hydrogels used in the experiment, including pure dialyzed silk fibroin (D-Pure) and other hydrogels containing nanoparticles in different concentrations – 0.5% and 1%. All experiments have been conducted at a uniform environment having a frequency of 110 Hz and dynamic force of 0.25 N.

The pure dialyzed silk fibroin hydrogel shows a value of $d_{33} = 0.13$ pC/N, proving that silk fibroin is inherently piezoelectric due to the presence of β -sheet crystallinity in it. At a 0.5% addition of ZnO nanoparticles, the d_{33} value drops a little to 0.10 pC/N. This can be associated with poor dispersion or

weak interaction of the ZnO nanoparticles in the silk fibroin polymer at low concentrations. But at 1% ZnO nanoparticles, d_{33} increases to 0.20 pC/N.

Another enhancement is seen in AlN-added hydrogels. For instance, AlN 0.5% hydrogels have a d_{33} value of 0.24 pC/N, whereas the highest piezoelectric coefficient of all samples, 0.33 pC/N, is recorded in AlN 1% hydrogels. These findings imply that AlN nanoparticles have high compatibility with the silk fibroin matrix and can efficiently align dipole moments[52].

Similarly, LiNbO₃-hydrogels also show better piezoelectric properties than silk fibroin only. Specifically, LiNbO₃ 0.5% sample attains the piezoelectric coefficient of 0.25 pC/N, while LiNbO₃ 1% sample shows a slightly lower value of 0.22 pC/N. The reduced piezoelectric activity of this sample might be due to the aggregation of nanoparticles resulting in the loss of homogeneity in polarization.

As shown by the graph above, adding nanoparticles has a noticeable effect on the piezoelectric activity of dialyzed silk fibroin hydrogels. As seen, the best piezoelectric property is exhibited by 1% AlN sample.

CHAPTER-5

CONCLUSION

This study was concerned with the synthesis and characterization of silk fibroin-based piezoelectric nanocomposite hydrogels with ZnO, AlN, and LiNbO₃ nanoparticles through dialysis-assisted, non-dialysis, and soft-freezing techniques. Specifically, silk fibroin was chosen as the base biomaterial for developing the hydrogels because of its superior characteristics in terms of high biocompatibility, biodegradability, elasticity, and ability to form hydrogel networks in three dimensions.

The addition of nanoparticles greatly influenced the performance of hydrogels in various ways. The addition of ZnO nanoparticles resulted in the increased conductivity and bactericidal property of the hydrogels as well as their mechanical strength owing to the semiconducting nature of the nanoparticles and their ability to interact with silk fibroin molecules. The inclusion of AlN nanoparticles increased the thermal and dielectric properties of hydrogels, thus making them ideal for developing thermally-stable bioelectronic systems. Lastly, the addition of LiNbO₃ nanoparticles gave hydrogels piezoelectric and electroactive capabilities.

Rheological investigations indicated an increase in viscosity, G', and viscoelastic stability of the gels by the addition of nanoparticles. Rheological tests such as amplitude sweep, frequency sweep, and creep recovery test demonstrated high elastic nature, denser cross-linked network, and higher deformation resistance of the hydrogels than those prepared from pure silk fibroin. The degree of swelling was affected by the concentration of nanoparticle and its preparation techniques. High nanoparticle concentration resulted in lower swelling since it led to a denser network and lower polymer chain movement.

Measurements of conductivity and temperature dependent conductivity indicated better ion and electronic transport of the nanoparticle-loaded gels. The conductivity value was enhanced by increasing temperature because of the higher ion movement. FTIR spectroscopy showed the interaction of nanoparticles and silk fibroin via increased β -sheet formation.

The soft-freezing technique among others allowed creating very porous and interconnected hydrogels by avoiding any toxic crosslinkers. The method offered advantages like controlled pore morphology, increased water retention, and more structured formation that can be employed in tissue engineering and regenerative medicine.

In general, the fabricated silk fibroin hydrogels with nanoparticles showed great multifunctional properties, such as high mechanical strength, electrical conductivity, thermal stability, piezoelectricity, and biocompatibility. Such properties of created hydrogels made them very attractive materials for usage in such applications as tissue engineering, wound healing, biosensors, wearable electronics, drug

delivery systems, regenerative medicine, and smart bioelectronic devices. Future research can include optimization of nanoparticle amount and study of biological activity.

CHAPTER-6

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