A REPORT ON

BOMBYX MORI SILK FIBROIN/ MODIFIED CLAY NANOCOMPOSITES FILMS: STUDIES ON ELECTRICAL CONDUCTIVITY & RHEOLOGICAL PROPERTIES.

M.TECH

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UTSAV BOSE

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Introduction

Silk, is a naturally occurring proteinaceous bio polymer known for its impressive mechanical strengthand lustre produced in fibre form by silkworms and spiders. Silk from worms have been used in high value textiles for thousands of years due to its excellent mechanical strength, vibrant lustre & light weight. Apart from its use in traditional textiles, silk has been used as suture materials for centuries.

Silkworm silk is a low-density structural protein, possesses a high content of β sheet or α helices with a core domain of random coils making it more crystalline and robust in nature. [2]. The Bombyx mori protein fiber is a composite material comprising a semi-crystalline silk core (i.e., silk fibroin), which is mainly responsible for the load-bearing capacity, and an outer layer of sericin, which functions as a gumming agent.[3]. Being nature's own gift, it is an excellent biocompatible material.

Recently, regenerated silk fibroin (RSF) solutions have been used to form a variety of biomaterials, in form of gels, sponges and films, for medical applications. The application includes wound healing and in tissue engineering of bone, cartilage, tendon and ligament tissues for its bioresorbable attributes. [4] It is also tuneable to biodegradation having least host immune response. As an intriguing and abundant biomaterial, silk also offers exquisite mechanical, optical, and electrical properties that are advantageous toward the development of biocompatible electronic devices, biomedical diagnosis, as well as human–machine interfaces. It also finds its application in high-performance bio-integrated electronics, computing technologies [5].

Montmorillonite nano clay are a family of sheet like nano fillers belonging to the family of 2:1 layered silicate consisting of two-dimensionallayers. A central octahedral sheet of alumina or magnesia is fused intotwo external silica tetrahedron so that the oxygen ions of octahedralsheet pertain to tetrahedral sheets. The thickness of layers is ca. 1 nm andthe lateral dimensions of the layers vary from several tens of nanometersto several micrometers and even larger. These layers will form stackswith van der Waals gaps between "interlayers" or "inter-galleries". Superior mechanical & physical properties of property of nano fillers may be attributed to their extremely high surface area.

Recently, ecological concerns have initiated a momentum of research towards the development and discovery of "Green" or "biodegradable" materials over synthetic ones.Bio nanocomposites are a class of composites made of nanosized materials. They contain the constituent of biological origin and particles with at least one dimension in the range of 1–100 nm.[6].

So, in this study, we have preparedSilk fibroin/ Montmorillonite nanocomposite films, where the nano clay is chemically modified to give it additional functionality. Silk films along with their robust mechanical properties, bio compatibility & optical transparency is a potential biomaterial which can integrate electronics, optics and human health monitoring.

Motivation of Research:

Mulberry silks are well researched fibrous proteins for biomedical application. However, a recent literature search revealed that there is no previous work done on Silk Fibroin/ Montmorillonite nanocomposite conducting films for next generation novel optoelectronics application and e-bandages.In India, the major mulberry-silk-producing states are Karnataka, AndhraPradesh, West Bengal, Tamil Nadu and Jammu and Kashmir, which together accountfor 92% of the country's total mulberry raw silk production [7]. So, there is an urgent need to develop, characterise and study these reverse engineered silk fibroin/ Montmorillonite biomaterialswith tailoredfunctionalities, potentially bioactive, electrically conductive films and find new applications. Further,Rheological studies of these silk fibroin/modified nanoclaysolutions is essential because it sheds insights on the microscopic and macroscopic structure of the nanocomposites. There is still a substantial lack of work on theoretical modelling of these novel modified layered-silicate silk-based nanocomposite systems.

Objectives:

- Preparation and characterization of *B. Mori* Silk Fibroin/ Montmorillonite nano composite films.
- Rheological analysis of SF/ MMT nano composites solutions.

Specific objectives:

- Preparation of modified Bioactive MMT clays using Copper, Silver & Quaternary Ammonium Salts.

- Preparation of B. Mori Silk/Bioactive clay Nano-composite films by solvent casting method.
- Characterization of the prepared Nanocomposite films using DSC, FE-SEM & XRD methods.
- Evaluation of Electrical conductivity & Optical properties of the films
- Evaluation of Rheological properties of the SF/MMt nanocomposite solutions.

II. Literature Review

Silks are primarily produced by the larvae of certain species in the class Insecta, including the order Lepidoptera i.e. moths and butterflies. Silkworms are classified as mulberry (e.g. Bombyx mori) and non-mulberry types (e.g. Antheraea mylitta, Antheraea assamensis, Philosamiaricini) depending on their food intake. Silks are produced by these insects for the purpose of cocoon formation, protection against external threats and reproduction. Compared to production of other man-made filaments of similar physical attributes, which are often, produced under extremely high temperatures or harsh solvent systems, silks are spun under relatively mild conditions. Though this process is not fully understood but it is believed that solid silk threads are spun from salivary glands of the silkworm. These glands are paired but two threads spun generally fuse near the pore. (Asakura, Umemura et al. 2007) Solid threads are extruded from an aqueous solution under low pressure and at room temperature. The changes in molecular interaction and orientation that transform water-soluble silk into an insoluble fibre is thought to be a combination of shear forces, dehydration, pH drop, and potassium and copper ions are also involved. (Hakimi, Knight *et al* 2007)

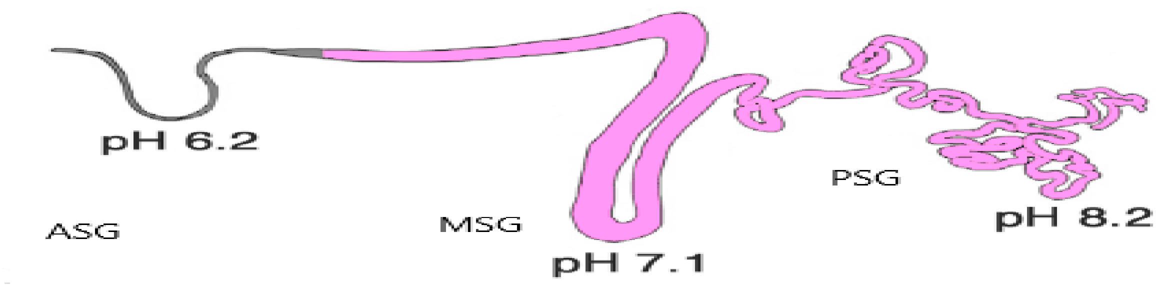
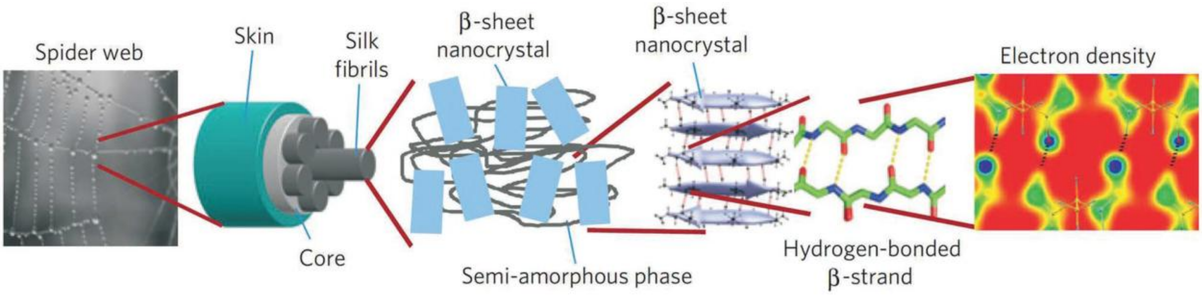


Figure showing pH drop across Posterior, middle & anterior (PSG, MSG & ASG respectively) silk gland in a *B.mori*silkworm



Diagrammatic representation of Silk Fibrils

SILK PROTEINS

Silk threads are natural composite of inner core consistingof two Fibroin filament and an outer protective layer of sericin, which forms the cocoon.

- **Fibroin**:

  Silk fibroin mainly consists of natural proteins made up of polypeptide chains with average molecular weights of 250-350kDa. They mainly consist of hydrophobic heavy chains (also called H-fibroin) of 390kDa and hydrophilic light chains (also called L-fibroin) of 26kDa with terminal C and N groups. These chains are bound by di-sulfide bond.[8]Silk fibroins are majorly block co-polymers composed of β-sheet forming blocks conjugated by spacers. These crystalline regions are amino acid sequences which are glycine-alanine-glycine-alanine-glycine-serine (GAGAGS) repeats, that are packed intohydrophobic antiparallel β-sheet structure. This is the primary reason behind silk's impeccable mechanical performance which surpasses that of steel or Kevlar. [9]

  Apart from it's mechanical performance, silk fibroin is also biodegradable and highly biocompatible which makes it an attractive candidate polymer for biomedical application. The in vivo degradation rate of these highly crystalline substances depends on factors such as type of processing used to prepare the bio materials, mechanical factors and site of implant. The rate of degradation can be precisely controlled with a well set of established processing protocols such as water vapor annealing, ultrasonication and methanol treatment. Proteolytic degradation mechanism has been pointed out by (Kundu, Rajkhowa et al) behind the degradability of silk materials, which can be altered by tuning the crystallinity and other processing factors.

Easy processability of silk fibroin from solutions have given rise to a number of material formats from nano-particles, films, sponges, powders, hydrogels and nano fibrous membranes has added advantage in the biomedical fields.Previous studies have pointed out silk proteins when subjected to alcohol treatment induces crystallinity and renders them insoluble.

- **Sericin:**

Protective outer layer of sericin (which wraps around the Fibroin core) is made up of glycoproteins and have been discarded by the textile industry for a long time. This is because the glue like sericin portion is removed by a process called degumming in order to extract out the fibroin which is in turn converted into traditional yarns and fabrics. Typically, 65-75% of the cocoon weight of a B. mori silk worm consists of fibroin while 15-25% is composed of sericin.

Sericin is a natural polypeptide, composed of 18 different types of amino acids with molecular weights of more than 250kDa, which is very similar to that of fibroin.The amino acid composition of SFis composed of two non-polar amino acids, glycine and alanine, which makes up for approximately 75% whilepolar amino acids, accounts only for approximately 25%. The amino acid composition of sericin is just the opposite; the majority is polar amino acids including serine and aspartic acid, with nonpolar amino acid accounting for only 23% of the protein. The chemical composition of the sericin is subject to differ depending on the variety of the silk worm.

Amino acid compositions of whole (S), outer (So), middle (Sm), inner (Si) sericin layers and fibroin (mol%) [1]

No.	Amino acids	S	So	Sm	Si	F
1.	Gly	17.85	16.29	16.35	17.87	42.62
2.	Ala	6.70	5.20	6.13	11.58	33.38
3.	Val*	4.05	3.77	4.27	5.43	2.58
4.	Leu*	1.49	1.21	1.77	4.06	0.54
5.	Ile*	1.02	0.79	1.17	3.76	0.72
6.	Phe*	0.67	0.64	0.66	2.49	0.81
7.	Met*	0.31	0	0	0.83	0.15
8.	Pro	0.81	0	0.64	0	0.47
9.	Tyr	3.10	2.87	3.98	4.09	5.84
10.	Cys	0.38	0.69	0.95	0.75	0.26
11.	Ser	25.50	28.00	25.57	13.32	7.65
12.	Thr*	7.47	7.78	8.13	5.66	0.85
13.	Asp	18.38	17.97	17.08	15.83	1.79
14.	Glu	5.74	6.25	4.65	7.34	1.36
15.	His	1.32	1.32	1.69	1.38	0.21
16.	Lys*	2.08	3.72	3.16	2.18	0.33
17.	Arg	3.12	3.52	3.83	3.41	0.44

Note: "*" denotes amino acids that are essential for the human body.

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Hydrophilic in nature, sericin is structurally a globular protein composed of random coils and β-sheet. It is readily soluble in hot water (50-60˚C) and shows sol-gel transition. The polar side chain and its structure allows cross-linking which impartssericin uniqueproperties such as antioxidant,antitumor, wound healing,antibacterial, antimicrobial protection against ultravioletradiation. It has been actively used in pharmaceutical, cosmetics and food industries.

PROPERTIES OF SILK FIBRES

Physical properties: Silk is the only natural fiber which is obtained in continuous filament form. It is important to note that cross-sectional morphology varies for mulberry and non-mulberry varieties.They usually exhibit triangular cross section with rounded corners with diameters ranging from 5-10µm, with a linear density of 1.1 dtex. The surface of silk fiber is smooth unlike many man-man fibres. Silk Being a poor conductor of electricity is susceptible to generating static charge.

Chemial properties: Silk manufactured by *B. Mori* silkworm constitutesof two main proteins, fibroin and sericin, fibr0in being the structural center 0f the silk, and serecin being the sticky cementing material surrounding it. Fibroin is made up of the amino acids Gly- Ser-Gly-Ala-Gly-Ala repeating sequence and forms anti parallel beta sheets.

Silk has been found to be resistant of most mineral acids except for sulphuric acid.

PROCESSINGS OF SILK PROTIENS:

Processing of silk cocoons before any biomedical or textile application, two steps are important: First, to reverse spin the cocoons which is known as reeling and secondly, to remove the sericin part either by a thermo-chemical or an enzymatic process. The chemical process of degumming in contrast to the enzymatic route typically involves heat treatment in an alkaline solution (e.g. sodium bicarbonate) for 20-60 minutes. [12] This degumming method can also be applied to shorter spans of time as low as 5 mins in order to keep damage imparted to the silk due to heat and alkali to its lowest.

It iswell reported in the literature that sericin can initiate anadverse immuneresponseswhen used in tandem with silk fibroin.[13–15] Therefore thecomplete removal of sericin is of great importance to the biocompatibilityof a silk fibroin-based medical device. Silk, before it can be value added, has to be processed in an alkaline solution to remove the gummy sericin part. This is termed as the process of degumming. The method adopted for degumming generally dictates the final quality of regenerated silk fibroin materials fabricated thereafter. After degumming the sericin less silk has to be dissolved in an aqueous or organic solvent to obtain regenerated protein solutions. Dissolution of non-mulberry silk is more cumbersome than mulberry silks due to their presence of highly crystalline &β-sheet structures due to the presence of poly al sequence in comparison to poly Gly- Aly- sequence in mulberry silks. Silk fibroin can be dissolved into a variety of compounds such as LiBr, LiSCN, Cacl2/ethanol/water, NaOH solution systems. [16-21].

Following this protocol, reversed engineered silk proteins can be transformed into nano to macro phase morphologies. These when further treated with 70% alcohol solution gives rise to β-sheet morphology and renders them insoluble in water.

Immune Response to Silk Proteins:

Human body is gifted with an immune system which by default can fight any invasion by foreign bodies that can potentially harm our systems. Foreign substances not only include pathogens, infectious viruses and bacteria but also include artificial implants. Whenever an implant is introduced into the host system, it activates an inflammatory response as it fails to recognize ‘as self’.

Immune response can be broken down into two main categories:innate immune response and adaptive immune response. Innate immune response has been briefly described before.Adaptive immune response refers to the immune response activated by previously detected specific foreign bodies which our immune system recognizes as harmful. A mild innate immune response is beneficial as it can accelerate wound healing process. On the contrary long term and severe innate immune response can have adverse side effects. The innate immune response is typically mediated by ‘macrophages’ which is found throughout healthy tissues in our body. These macrophages express pattern recognition receptors (PRR). When a PRR binds (sticks) to a foreign body it initiates various pathways such as release of inflammatorycytokines, initiation of phagocytosis, and recruitment ofadditional immune cells. [22]

Various studies have shown that silk is has minimal immunological response. [23, 22, 24]. The proof of excellent bio compatibility of silk is the sutures used in medical science for centuries. The role of sericin in inducing immune response is heavily debated. It is also reported that silk-based biomaterials induce foreign body giant cells (FBGCs). The main role of FBGG is to clear out foreign bodies which phagocytosis has failed to.

Degradation of silk:

Biodegradability and bioresorbable features are one of the may attractive properties of Silk biopolymer. Biodegradable polymers such as Poly-lactic acids(PLA) poly-glycolic acid(PGA) or their mixtures (PLGA) have been successfully used as implants and drug linkers over the years. But it comes with a cost. The degradation products of these esters which can disbalance the pH … For example, in recent years, there has been a number of studies done on silk, its processing and fundamental structure-property relationships which has led to the discovery of varied material formats and avenues to improve upon the purification of Silk fibroin from cementing sericin glycoproteins that can potentially trigger inflammatory response *in vivo.*Natural *B. mori* silk are reported to have hierarchical and dimorphic Silk I and Silk II structures. Silk I is said to be the structure exhibited by silk in the middle silk glands of native silk worms while silk II is said to be the structure contained in the aspun silk fibers.

The *in vivo* degradation behavior of silk depends on a number of parameters such a β-sheet content, material format, site of implant, FBGCs, macrophages and immune cell action. Earlier studies showed that sutures made out of silk had longer degradation time as the were coated with paraffin which inhibited the penetration of enzymes and proteases to the silk surface.

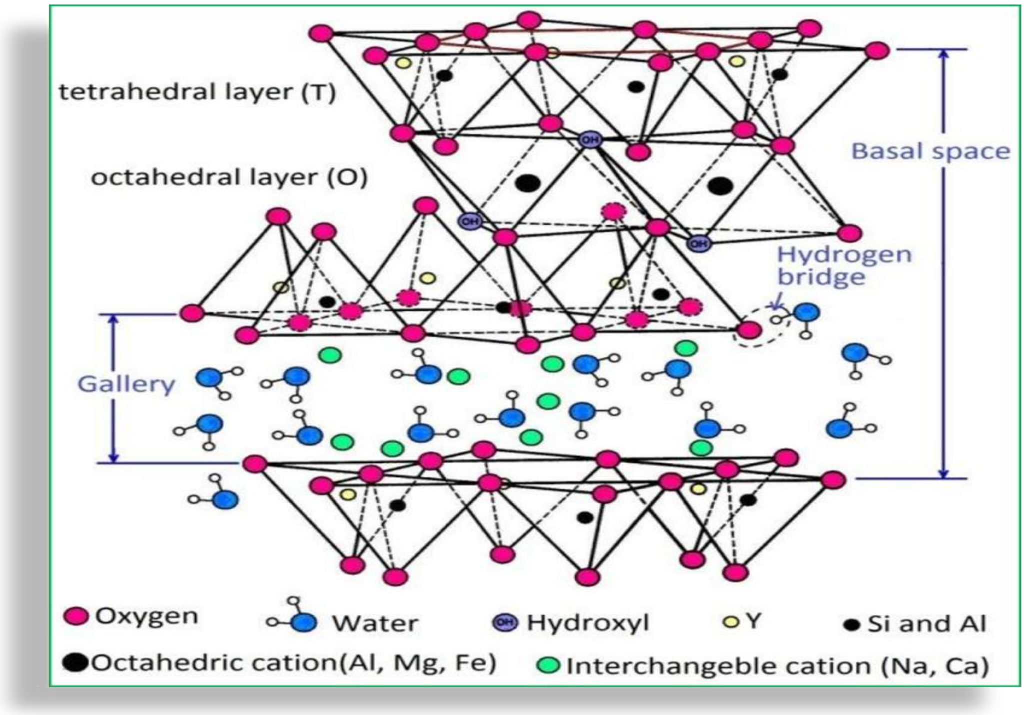
Coming to the morphology profile of silk-based bio materials it has been observed that materials with densely packed structures and low porosity (such as fibers and films) take longer time to degrade as the enzymes had more tortuous path to invade.

In vitro studies havedemonstrated that many proteases including protease K, collagenase,and alpha-chymotrypsin are able to cleave silk and cause adecrease in the material weight and strength over time.

Montmorillonite Clay:

MMT is the most common and widely studied clay, which has found extensive application as fillers in polymer nano-composite systems. Structurally MMT belongs to the class of 2:1 phyllosilicate and smectic clay group. Its layered structure consists of two tetrahedral silica layers separated by an octahedral alumina layer. Chemically, it can be defined as $Mx(Al_4{-}xMgx)Si_8O_20(OH)_4$, where M is a monovalent cation, x is the degree of isomorphous substitution ranging from 0.5 to 1.3.

Montmorillonite clays show tremendous intercalation properties, good water adsorption, ion exchange property due to its large surface area and so on. The unique properties of MMT clay results in great increase in tensile modulus, tensile strength, flexural modulus and flexural strength when used in 3-5% concentration in a polymer matrix.



Schematic Diagram of MMT-nano-clay

Nano-composites:

The basic approach of designing a nanocomposite is to incorporate either one of the components of the composites in nano range. When a polymer is incorporated with fillers of sizes reduced to nano meters, yields a hybrid material which shows much improved property performance as compared to virgin polymer. The nanoscale fillers have at least one characteristic, length scale, in the order of nanometers and vary essentially from isotropic to highly anisotropic sheet-like or needle-like morphologies.[18]

Since, its design and inception in late 1980's by researchers of Toyota group for the development of a composite cover for the timing belts in automotive engines, which reported to be nearly doubled modulus and increased strength by over 50% along with improved heat distortion temperatures in case of PA-6/layered silicate nano composites. [19,20]

Among various nano scale fillers, which are considered to be very important, include two dimensional (2D) layered silicates, Mxene,graphene, One dimensional (1D) nanotubes (mainly carbon nanotubes, CNTs), nanofibers, nano whiskers, and zero dimensional (0D) fullerenes, , SiO_2, metal oxides (e.g., TiO_2, Fe_2O_3, Al_2O_3), nanoparticles of metals (e.g., Au, Ag),Polyhedral oligomeric silsesquioxanes (POSS), semiconductors (e.g., PbS, CdS), carbon black, nanodiamonds, , Quantum dots . [Chrissafisa, Bikiaris 2011, 21, 22, 23]

Uniform dispersion of nanoparticles produces ultra-large interfacial area per volume between the nanoelement and host polymer. This immense internal interfacial area and the nanoscopic dimensions of nanoelements, fundamentally distinguish polymer/nanocomposites from traditional composites and filled polymers.[21]

Rheology of Nanocomposites:

Rheology is a branch of classical physics which deals with the correlation of flow behavior and deformation of materials. It is a powerful too which can be utilized to investigate a wide range of materials including that of polymer nano-composites for the following reasons:

- Rheology provides with valuable insights into the processability of these nano-composites.
- It provides deep understanding of the structure-property correlation ship of these nano-composites
- It also provides us with an understanding of the possible interaction of filler and matrix material at a nanoscale level.
- Time dependent characteristics such as creep, structural recovery and so on can be derived from rheological testing.

III. EXPERIMENTAL WORK

The following experimental section has been divided into three consecutive section for the convenience of the reader. Part 1 involves the modification Montmorillonite nano clay withcopper, silver and quaternary ammonium salts. Part two involves dissolution and subsequent regeneration of silk fibroin nano composite films through solvent casting method and its characterization. Finally, part 3 deals with the determination of rheological profile of the silk fibroin/MMT nano clay nanocomposite solution.

Part 1: Modification of montmorillonite nano clay

1. <u>Materials</u>

Montmorillonite nano clay with average particle size 80-150 nm was acquired from Sigma-Aldrich, India. Copper chloride, silver nitrate& quaternary ammonium (benzalkonium chloride) salts were also purchased from Sigma-Aldrich, India.

2. <u>Modification of Na-Montmorillonite nano-clay with copper, silver, & quaternary ammonium salts</u>

Initially 3g of Na-MMT clay was weighed and 50ml of 1mM solution of copper chloride was prepared and then 3g clay is mixed into beaker containing 30ml of above solution with continuous stirring for 7 days at temperature 35^{o}C. After 7days, the solution is dried in the oven at 60^{o}C for two days and then the clay agglomerate is crushed using mortar-pestle to make the clay particles uniform in size. Same procedure is followed for the modification of Na-MMT clay by silver nitrate and benzalkonium chloride salts to prepare 1%, 3% & 5% concentrations respectively.

<u>Part 2: Fabrication of montmorillonite nano clay Silk fibroin Films</u>

A. <u>Materials:</u>

Degummed non-mulberry *Bombyx mori* silk was purchased from Silk Development Department, Sonbhadra, Uttar Pradesh, India. Formic acid, methanol (99% purity) and glycerol were purchased from Sigma-Aldrich, India. Calcium chloride Fused LR was purchased from Thomas Baker (Chemicals).

B. Extraction of Silk fibroin from *Bombyx mori* silk cocoons*:*

Standard degumming method of Silk fibroin extraction was used which is a well-established protocol. The cocoons were cut into small pieces and put into a beaker containing Na_2CO_3 solution. The cocoons were boiled for 1 hour and then washed several times with distilled water to remove any trace amount of sericin present. Then the degummed silk was dried overnight in an oven at 60˚C.

C. Preparation of Silk fibroin Solution:

In a beaker containing 10ml of formic acid, 2% fused calcium chloride was added. The solution was stirred until the calcium chloride was completely dissolved. After that, the previously prepared modified MMt nano-clay was added to the solution and ultra-sonicated for 30mins. This solution was then transferred into a glass beaker placed on a magnetic heated stirrer. Finely cut pieces of silk fibroin (2gm) was then added slowly into the solution for dissolution. Formic acid was added dropwise (not exceeding 20ml volume in totality) until all the silk was successfully dissolved. At last, 10% glycerol by the weight of silk was added into the beaker. Finally, the dissolved silk fibroin solution in formic acid was ultra-sonicated for another 30 mins for uniform dispersion of the nano particles.

D. Preparation of cast silk fibroin/ MMt nanocomposite films:

The above prepared solution was cast on to a petri dish and allowed to dry completely. Then methanol was added to the films and left-over night at room temperature for 12 hrs. Finally, the cast films were extracted from the petri dish and wate vapour annealed for 30 mins to induce β-sheet formation.

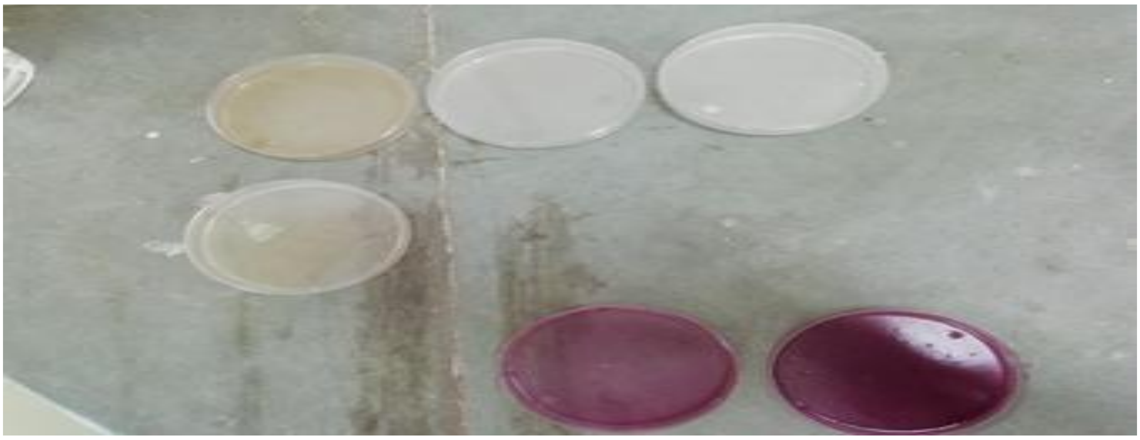


Fig. showing Regenerated Silk fibroin/Modified MMT nano-composite films

Characterization & Surface morphology analysis

1. **Thermal Analysis:** Differential Scanning calorimetry(TA Instruments Q100 DSC) was used for thermal analysis of Silk fibroin/ modified MMT nano-composite filmsunder a dry nitrogen gas flow rate of 50 ml/min. The silk fibroin nanocomposite film of approximately 5 mg was filled in aluminium pan and heated at 2 °C /min from 30 °C to 400 °C depending on types of samples.

2. **Optical Properties:** The optical properties of the prepared silk nanocomposite films were evaluated with the help of UV–Vis spectrophotometer of carry 300 Bio spectrophotometer (Agilent Technologies, Inc. U.S) in the wavelength range of 200–800 nm.

3. **FE-SEM & EDX Analysis:** The morphological and elemental analysis of the nano-composite films were carried out by Field Emission Electron Microscope with EDS (JEOL Model JSM-7900F Prime with Oxford-EDS system IE 250 X Max 150) at an accelerating voltage of 5 kV with the magnification level from 5000x up to 100000x. Before which the samples

were sputter coated with gold. The Elemental analysis of the same was done by EDX.

4. Electrical conductivity Analysis:

Electrical conductivity of the prepared 1%, 3% & 5% modified MMT/ silk fibroin solution was measured with the help of two-point probe method. The sample were cut in 2x2 cm^2 and place on a glass slide. The sheet resistivity was measured with the help of the following equation: [25]

$$\rho = \frac{\pi}{\ln(2)}\left(\frac{V}{I}\right)t = 4.532(R)t$$

$$\sigma = \frac{1}{\rho},$$

Where, ***R*** is the resistance (ohm), **V** is the voltage (V), ***I*** is the current (A), ***t*** is the thickness in cm, ***ρ*** is the sheet resistivity (*Ω*/square), **σ** is the conductivity (S cm^{-1}).

Part 3: Rheological Analysis of the Silk fibroin/ modified nano-clay nanocomposite solution

Raw degummed *B. mori* silk was dissolved in formic acid solution with pure silk as control & different percentages of clay loading i.e., 1%, 3% & 5% respectively. After clay loading the solution was ultrasonicated for 30 mins prior to all the measurements. Rheological studies were carried out on an Anton Parr MCR 301 compact rheometers. The obtained silk fibroin solution was carefully transferred to the rheometer fitted with a cone & plate measuring system of CP 40/2 (40 mm diameter with 2 ˚ opening angel). The rheometer was controlled using Rheocompass software which did the initial data processing. All the measurements were carried out at 25±0.5 ˚C.

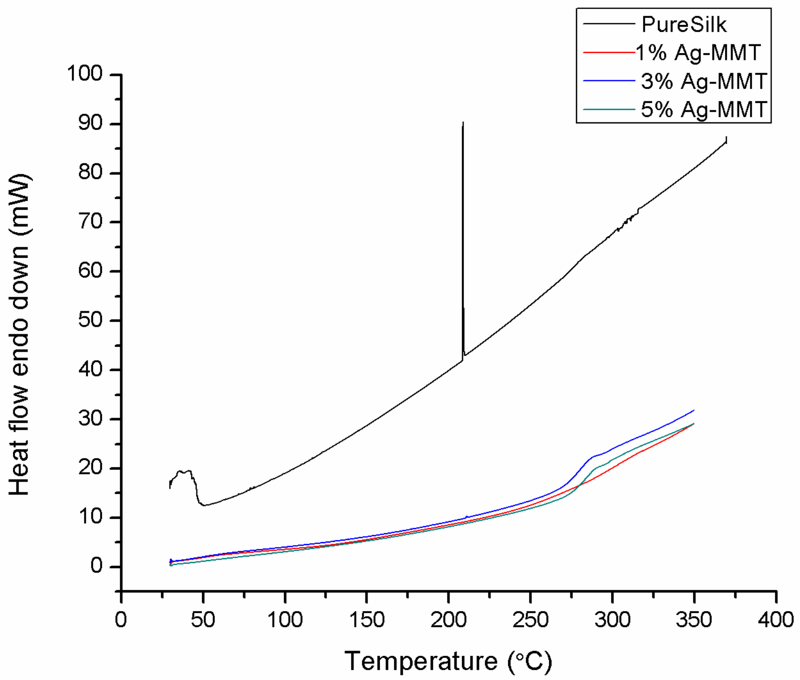
Structure recovery tests were performed using rotational shear by a series of shear rate 1-100-1/s to examine the recovery in structure from high shear to low shear.

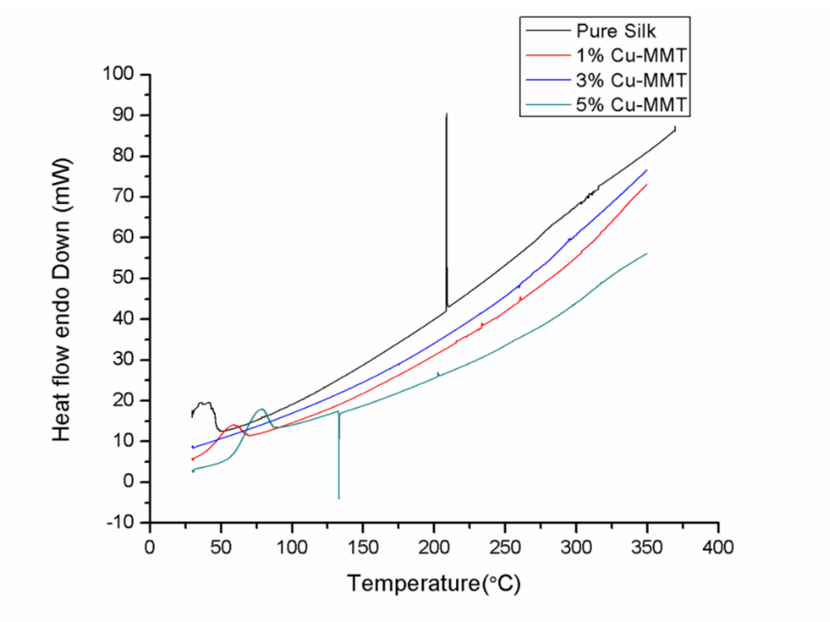
IV. Results & Discussion

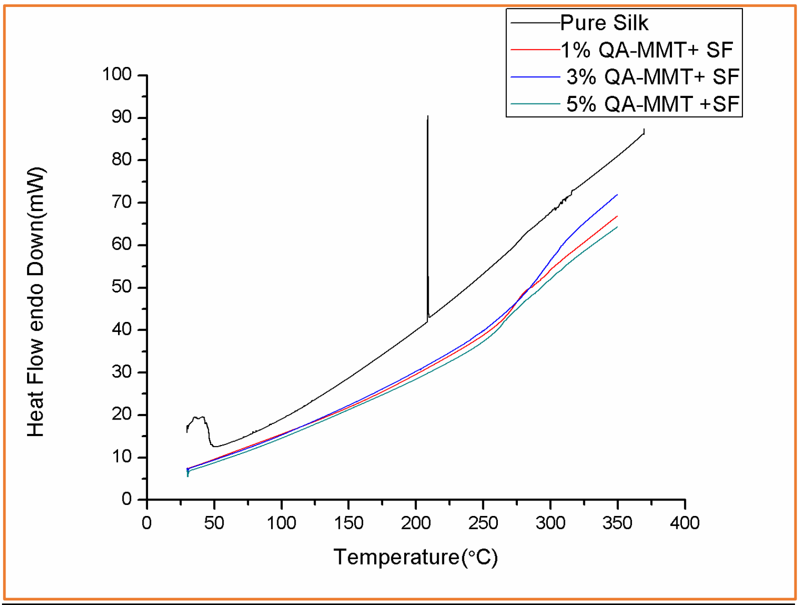
PART 1: Characterization of Silk Fibroin/ MMT nanocomposite films

a)DSC Analysis:

Curves of DSC runs are reported in the pictures below. All the samples were plotted against the control, which in this case is pure silk fibroin film. The wider peaks near 50-70˚c may be attributed to the water loss during the DSC run. The second endothermic peak at around 210˚c for Pure silk is the Tg. The modified Ag- MMT/ SF films how ever shows a wide endothermic peak at around 285˚C. This shift in Tg may be explained on the basis of induced β-sheet conformation and nano-composite formation.







For the case of Q.A modified-MMT/Silk fibroin films it shows a similar trend but the Tg shift is higher than that of Copper and silver modified MMT/SF nanocomposite films.

b) <u>SEM & EDX Analysis:</u>

To analyse the microstructure and homogeneity of the films FE-SEM was carried out. The FE-SEM micro graphs showed somewhat rough surface morphology with pores on it. This can be explained on the basis of surface etching caused by methanol treatment and subsequent evaporation o formic acid. Some films show agglomeration of nano particles which confirms the formation of nano-composite.

FE-SEM micrograph and EDX data of 1% Ag MMT/Silk Fibroin film

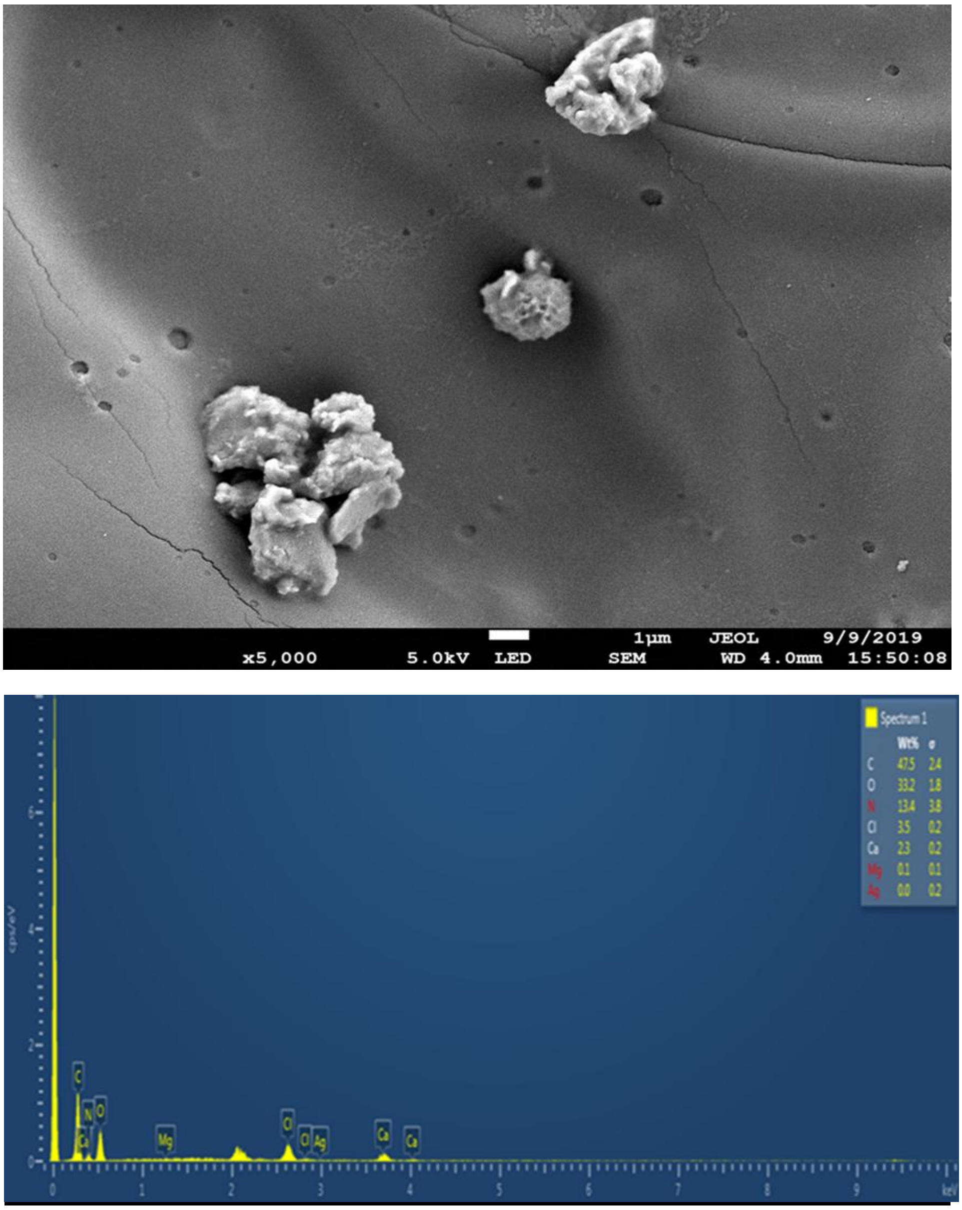
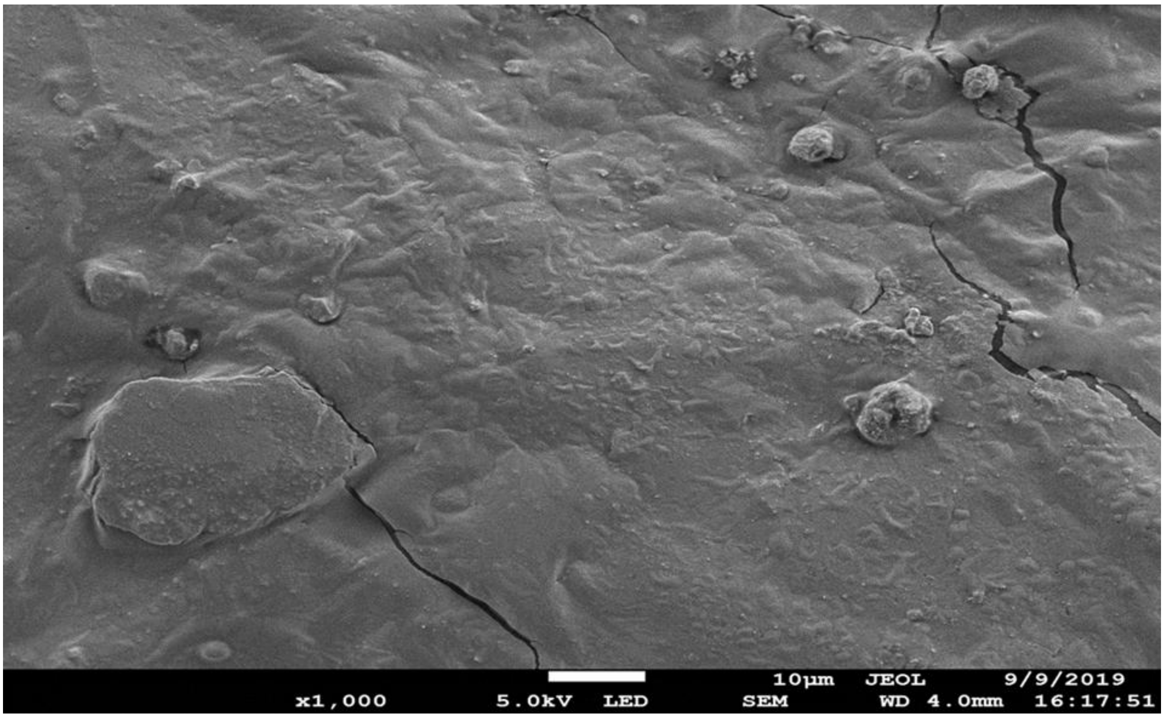


Fig : SEM & EDX of 1% Ag-MMT/Silk Fibroin Nanocomposite films

SEM & EDX of 1% Cu-MMT/Silk Fibroin nanocomposite films



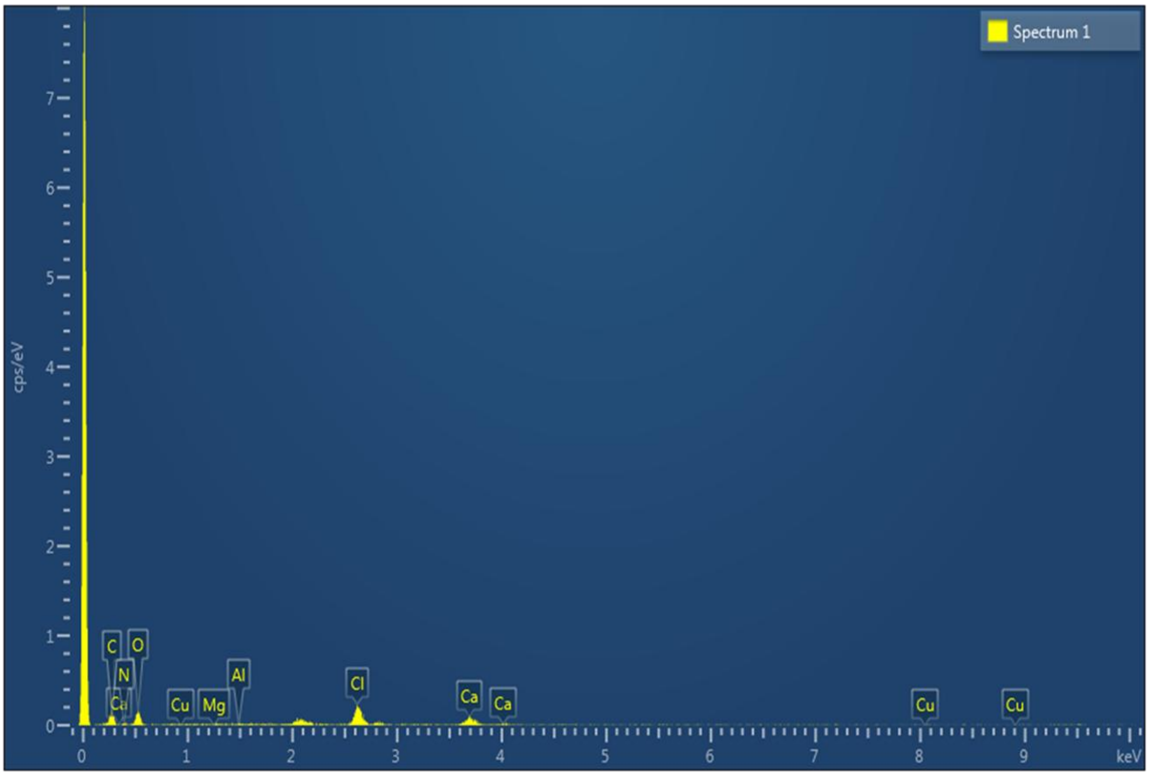
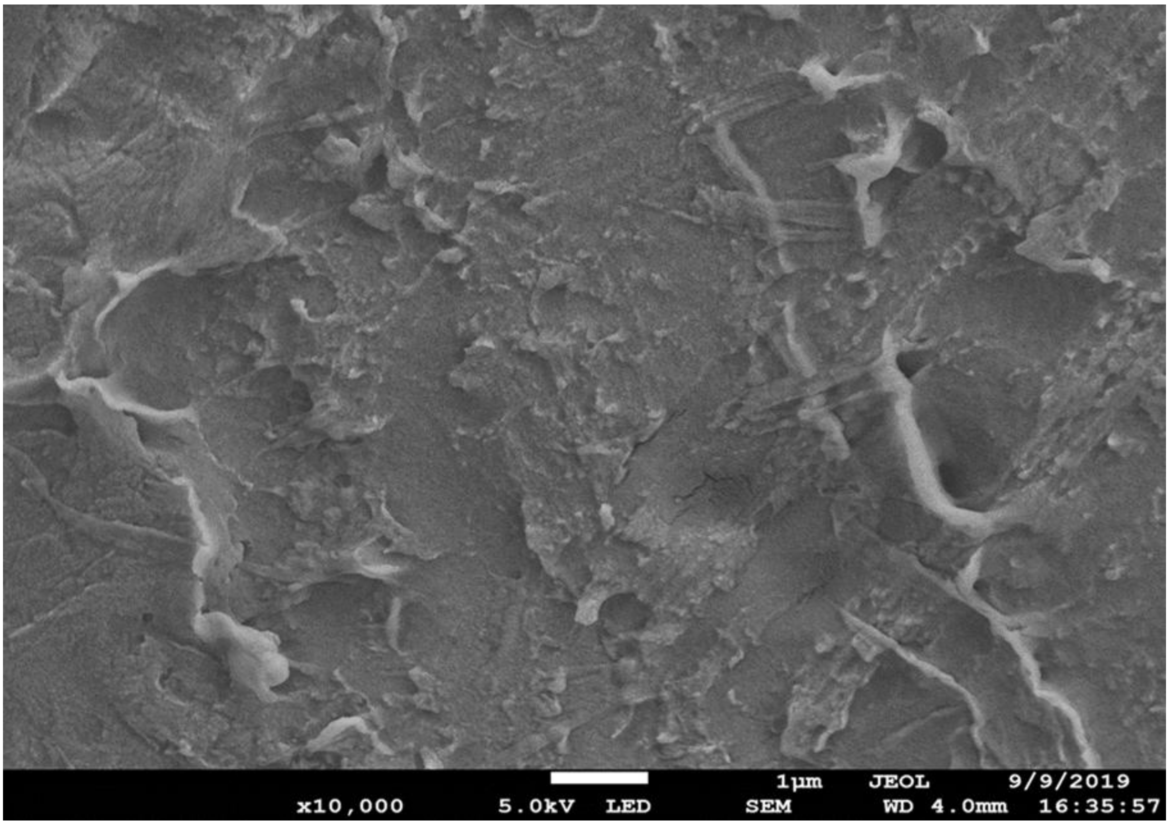
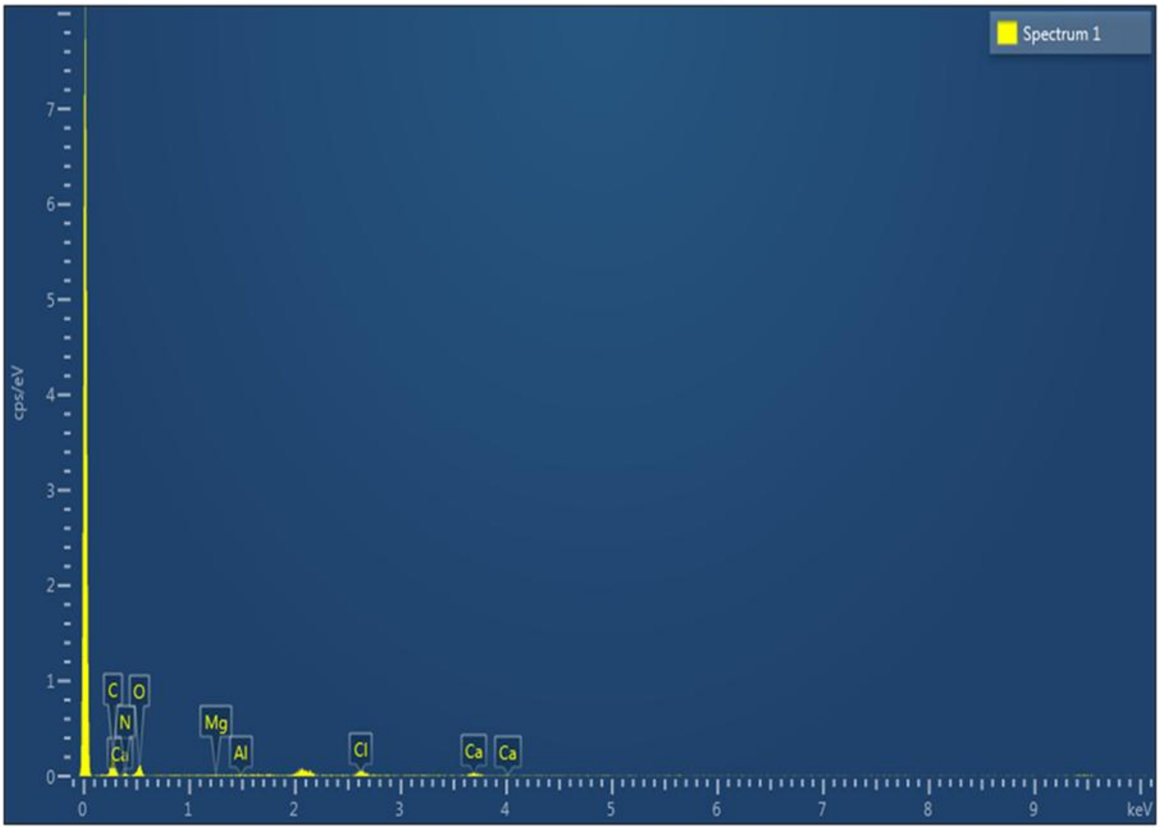


Fig : FE-SEM & EDX of 1%cu-MMT/SF nanocomposite films

FE-SEM and EDS of 1%QA-MMT/SF nanocomposite Film





c) UV-Transmittance of Modified MMT/SF nanocomposite films:

UV-Vis transmittance of Ag-Modified MMT/SF films showed a maximum 22 % transmittance at 5% concentration with increasing wave length. While 1% and 3% concentration showed less transmittance.

Compared to its Ag-MMt counterpart the Cu-MMT/SF films show much better with upto 80 % transmittance values with increasing wave length. The clay concentration had negligible effect on the result. The transmittance values when compared to the base line values for Quaternary ammonium modified Silk films shows highest transmittance at 5% concentration (60%) as wave length is increased. The values progressively decrease as the clay concentration decreases.

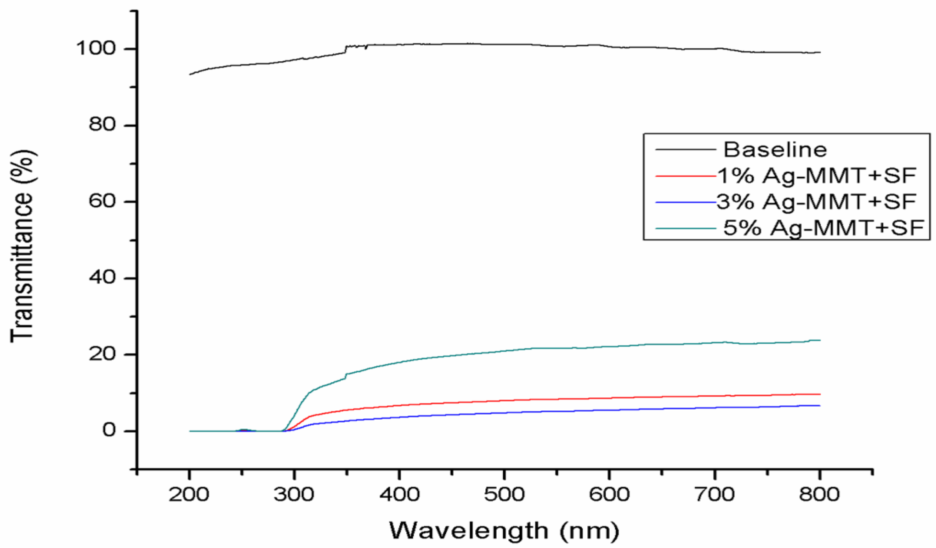


Fig Showing UV-Transmittance of Ag-MMT/SF nanocomposite films

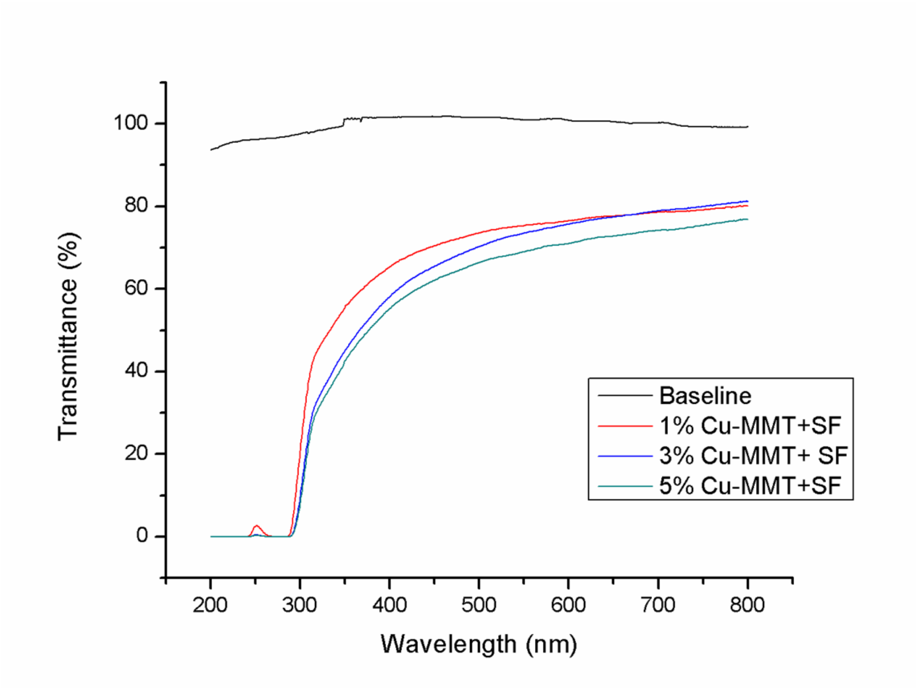


Fig Showing UV-Transmittance of Cu-MMT/SF nanocomposite films

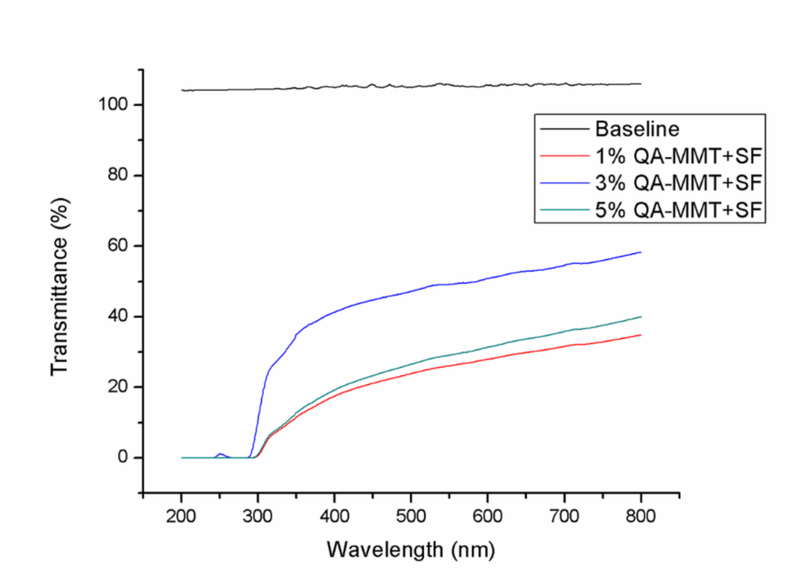


Fig Showing UV-Transmittance of Quaternary Ammonium-MMT/ SF films

d) Electrical property of Modified MMT/Silk Fibroin films

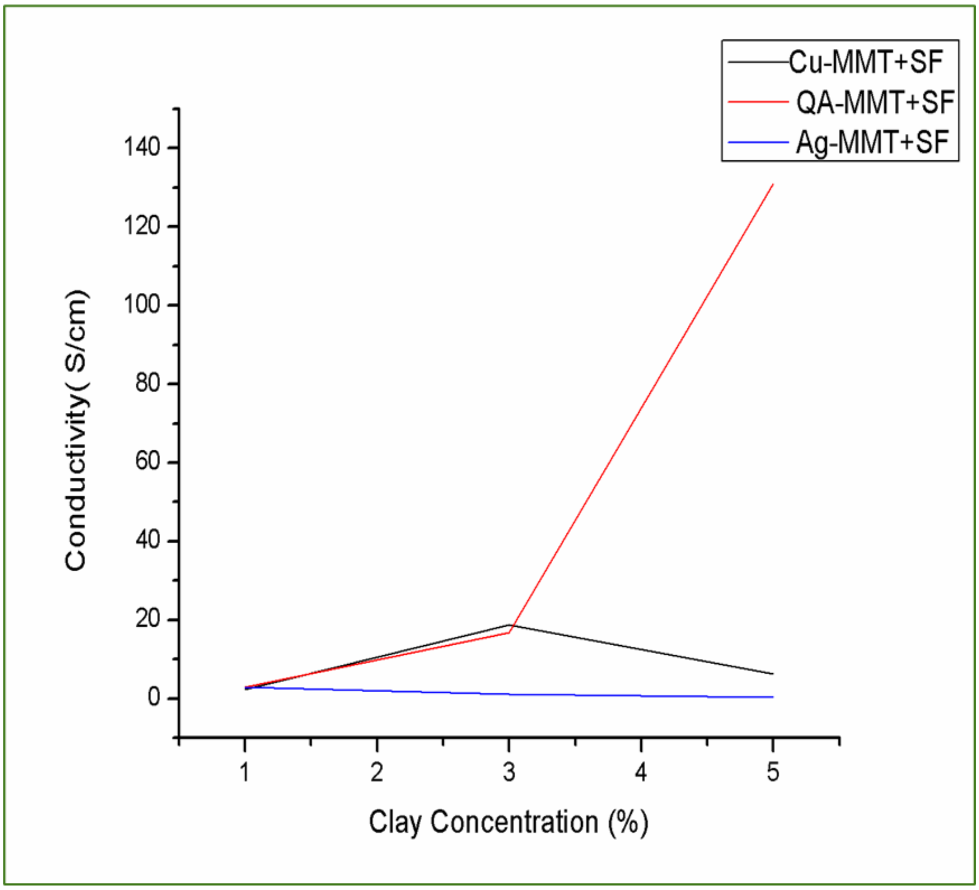


Fig showing electrical conductivity of Modified MMT/SF nano-composite films at varying concentrations.

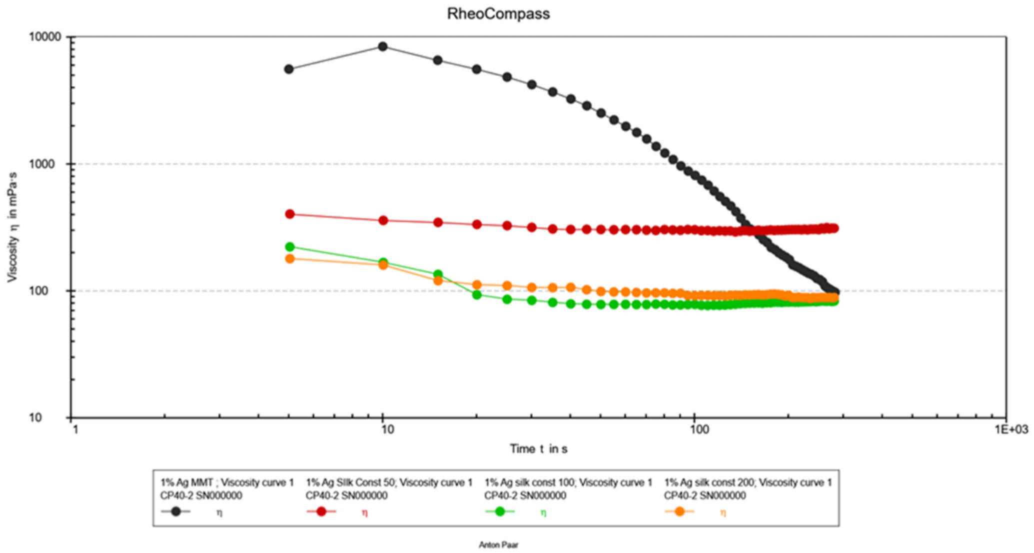
The electrical conductivity of the prepared nano composite films is established with the protocols described earlier. For Silver modified nano-clay silk fibroin films the electrical conductivity showed no result. However, with Copper modified MMT nanocomposite films the conductivity showed an upward trend with a peak at 3% concentration (20 S/cm) and decreased to zero at 5% concentration. With QA-MMT/SF nano-composite films there is an increasing trend in conductivity upto 3% concentration and there after is a sharp spike at 5% concentration. This can be attributed to the fat that at 5 % concentration agglomeration of nano-clay occurred which gave rise to the upward spike in conductivity.

Part 2: Characterization of Rheological Properties of Silk Fibroin clay nano composite solutions

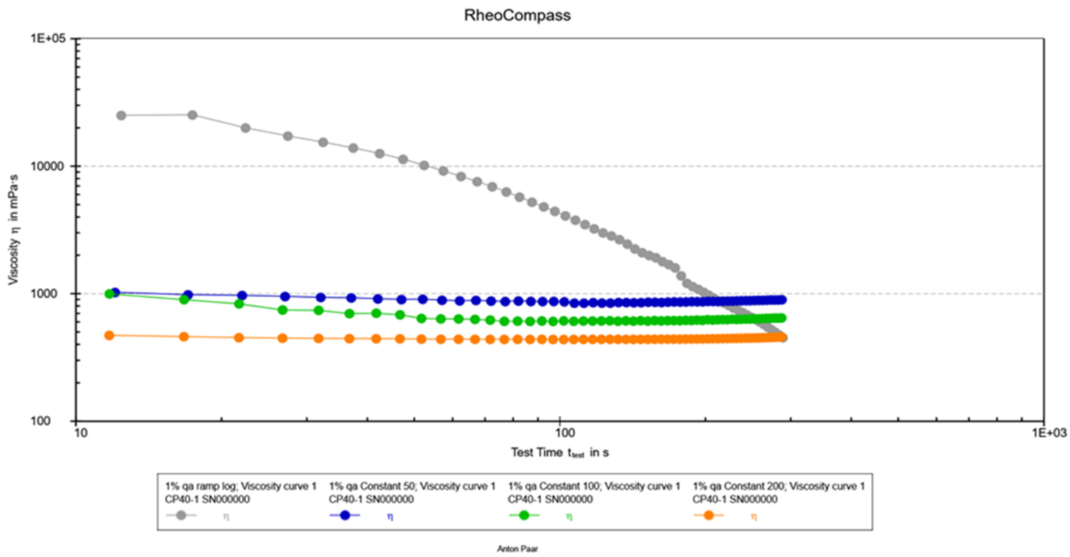
A) Viscosity Curves:

The viscosity curves when plotted against time shows shear thinning behaviour at low shear rates. At high shear rates viscosity reduces but at a lower rate than low shear rates. With addition of clay particles, the solution shows enhanced viscosity which may be attributed to the intercalation mechanism of polymer chains with clay particles which reduces the free volume of the chains. For low shear rate (ramp log) viscosity against time of pure silk and silk having increasing clay content shows a diminishing trend suggesting a non-Newtonian behavior with shear thinning effect of silk in formic acid solution. The drop in viscosity for pure silk is from 5202.1 Pa s to 144.1 Pa s when plotted against time. As the clay content is increased (1%, 3% & 5 %) the initial as well as the final viscosity increases as compared to pure silk solution. However, for high shear rates (measured at constant 200) the overall viscosity reduces several fold. This behavior can be explained by intercalation chemistry which results in increasing intergallery spacing hence increase in volume & volume decides viscosity. [Gupta et al. 2005, S. Thomas et al. 2020]

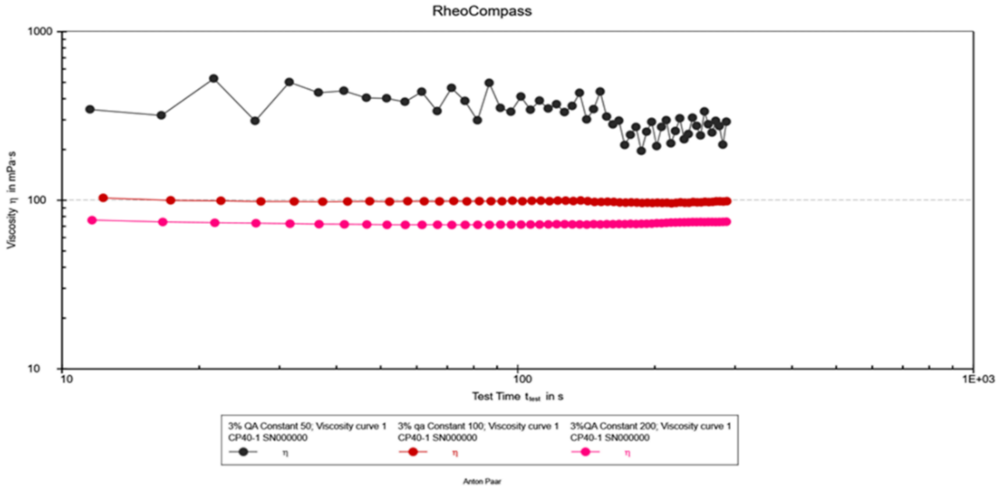
- **<u>Viscosity curve for 1% Ag-MMT/SF Solution</u>**



- **<u>Viscosity curve for 1% QA-MMT/SF solution</u>**



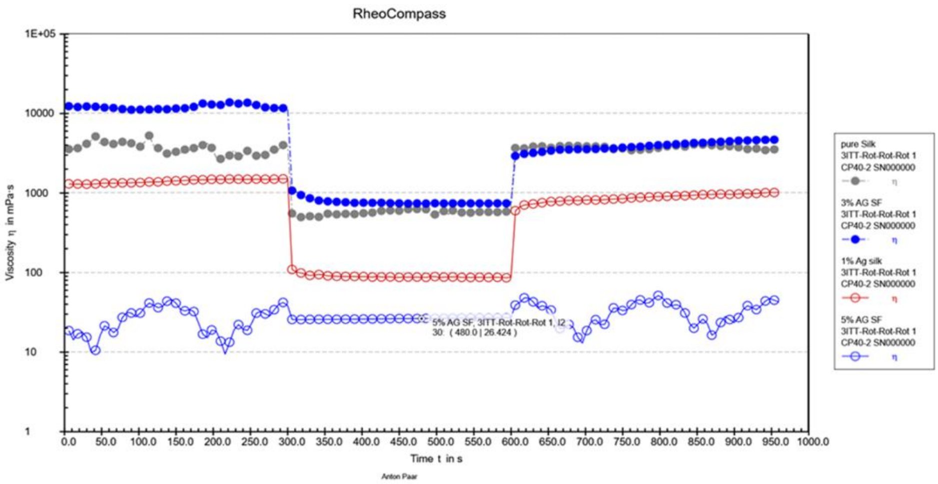
- **Viscosity vs Time curve for 3% QA-SF Solution**



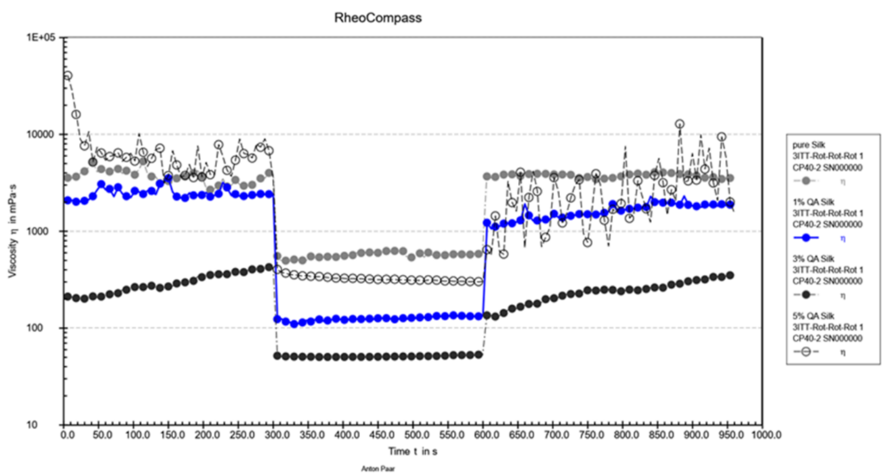
B) Structural Recovery Tests

Structural recovery tests reveal that in case of pure silk, complete structural recovery is observed which illustrates the elastic behaviour of silk polymer chains. As clay content is increased, structural recovery decreases, at 5% clay concentration it shows fully plastic behaviour. However, for 3 % clay concentration Rheopectic behaviour is observed as viscosity is seen to go up. For 1% & 5% concentration Thixotropic behaviour of the solution is seen. So we can conclude that with the addition of clay, structural recovery decreases which can be attributed to the fact that with increasing clay concentration, chain breakage occurs.

- **Viscosity Vs Time Curve for Ag-MMT/SF solution**



- **<u>Viscosity Vs time for QA-MM/SF Solution</u>**

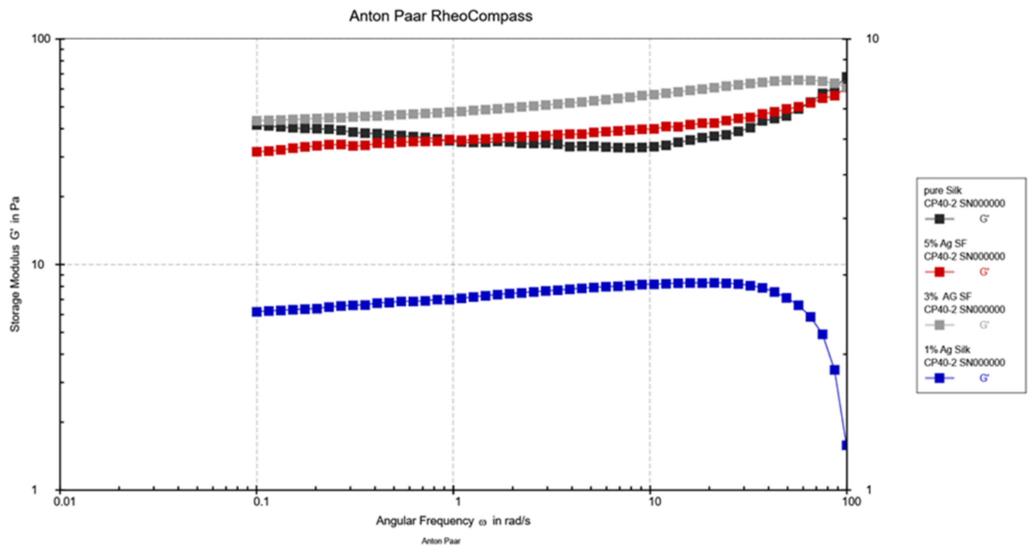


C) Frequency sweep Tests:

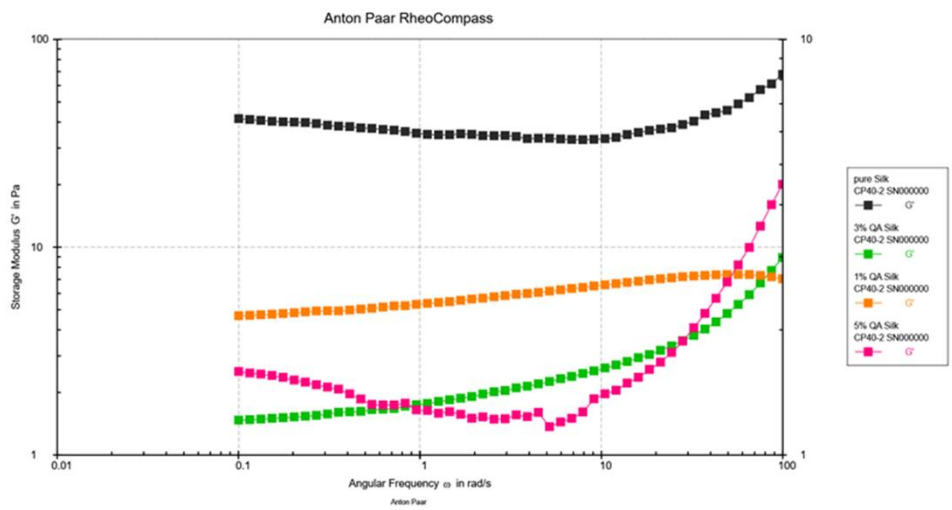
Frequency Sweep tests were carried out for Silver & Quaternary modified MMT/SF nano-composite solutions

• Storage Modulus Vs Angular frequency for Ag-MMT

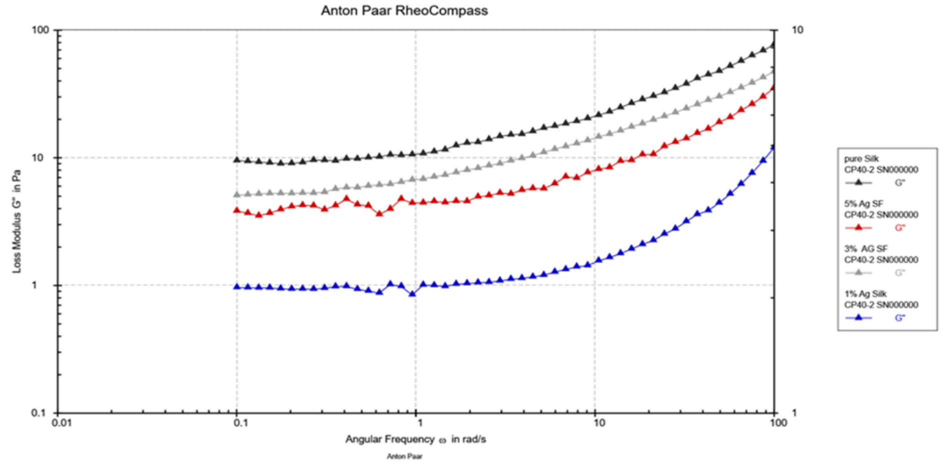
For this test G' & G" i.e., storage modulus and loss modulus values are plotted with respect to angular frequency. If the Storage modulus shows higher values than loss modulus the sample will show viscoelastic solid behaviour. While if the loss modulus is higher than storage modulus the sample will show viscoelastic liquid type behaviour. Low slope of storage modulus vs angular frequency indicates that the dispersion of clay in the solution system is very good. In case of 1%Ag-MMT/SF storage modulus is the lowest while for 3 & 5% its considerably higher.



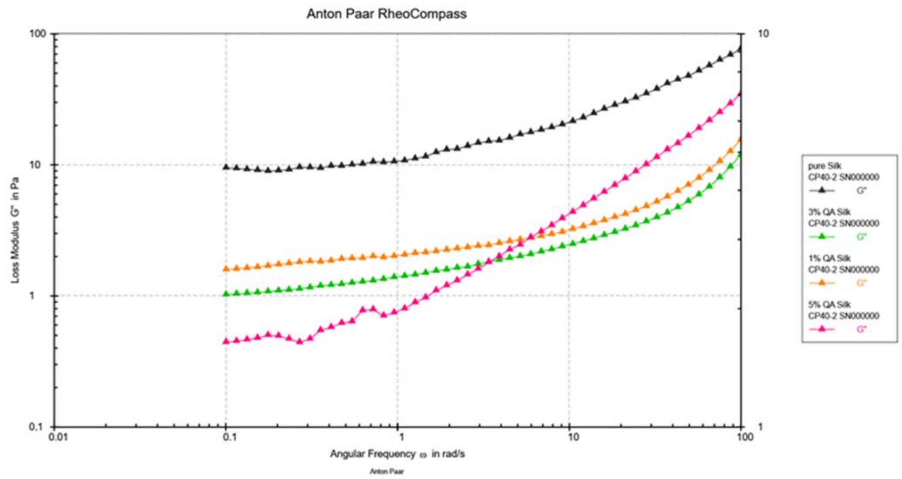
D) Storage Modulus Vs Angular frequency ofQ.A-MMT Silk Solution



E) Loss Modulus Vs Angular frequency for Ag-MMT/Silk Solution

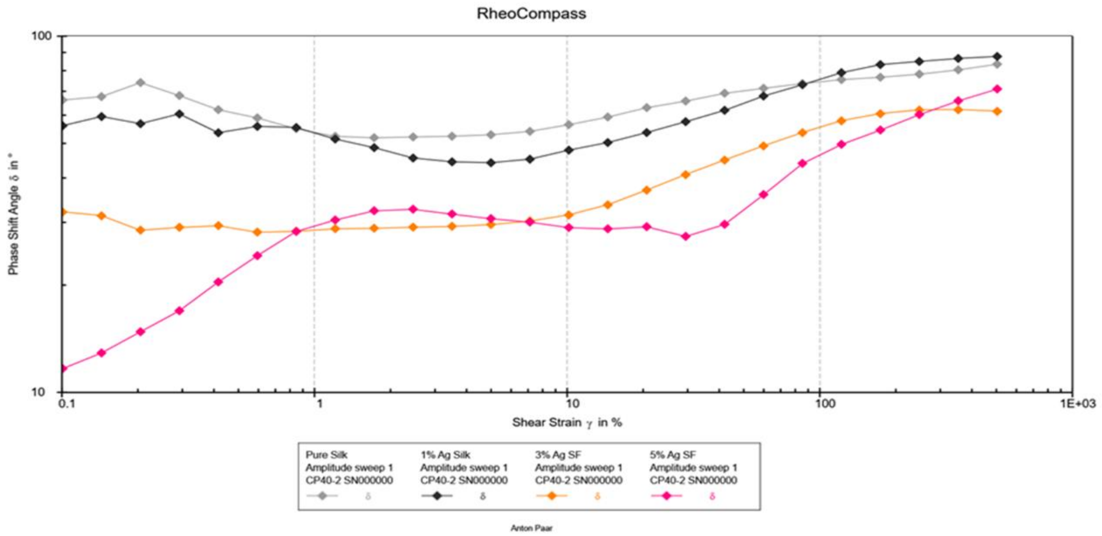


F) Loss Modulus Vs Angular Frequency For QA-SF solution



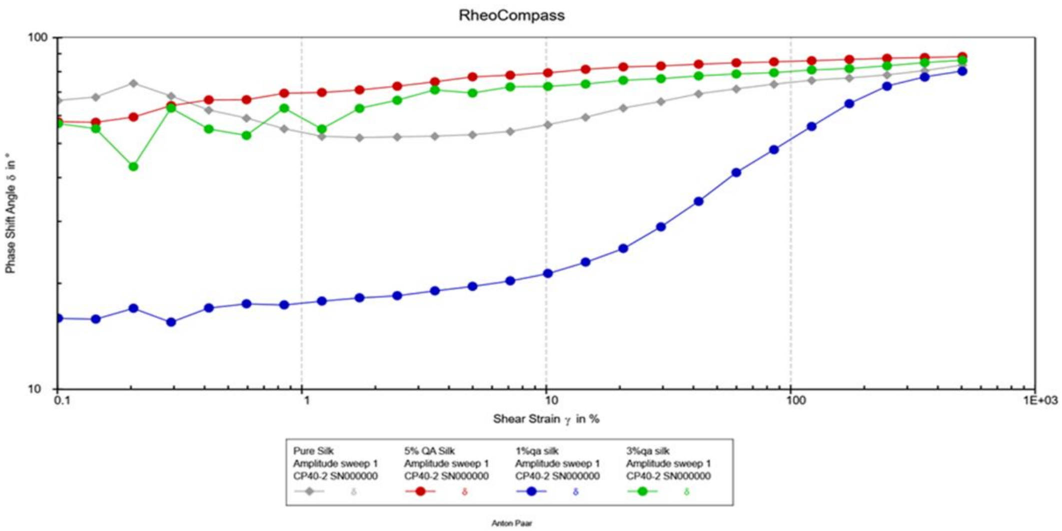
G) Phase Shift Angel Vs Shear Strain for Ag MMT+ SF solution

The phase shift angle for pure silk shows a very high (almost 90˚) value when compared to modified MMT/ SF solutions. The phase shift angle progressively decreases with increasing amount of clay loading into the solution



H) Phase Shift angle Vs Shear strain for QAMMT+ Sf QA-SF

Similarly, for QA-MMT/SF solutions 1% concentration of clay loading shows significant drop in phase shift angle while the 5 & 3% clay loaded samples do not show much variation from previously corroborated data.


RheoCompass
Phase Shift Angle δ in °
Shear Strain γ in %
100
10
0.1
1
10
100
1E+03
Pure Silk
Amplitude sweep 1
CP40-2 SN000000
5% QA Silk
Amplitude sweep 1
CP40-2 SN000000
1%qa silk
Amplitude sweep 1
CP40-2 SN000000
3%qa silk
Amplitude sweep 1
CP40-2 SN000000
Anton Paar

Conclusion

In this study, we have developed a novel nano-composite with modified Montmorillonite nano clay and regenerated Silk fibroin which has successfully showed electrical conductivity at a semi-conductor range. Its UV-transmittance has showed moderate transparency which severely impaired its potential as an optical material. The DSC curves indicate very good thermal stability and proof of nano-composite formation. The FE-SEM images of the films showed surface pores with a rough surface.

It is suggested that Silk being a natural protein and being bio degradable and biocompatible in nature, these films holds tremendous potential in futuristic electronics and implantable material formats. However, further Anti-microbial tests need to be carried out to stress upon confidently on its usage as a bio-material and future wound healing technology.

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