CHAPTER 1

INTRODUCTION

Nowadays, in everyday life, we encounter with so many kinds of materials that being used in different types of applications. Some materials are developed from natural sources, but some are from the synthetic ones. The physical states of the materials are also in various forms depending on the practical usefulness of such materials. One form that has been widely used is a gel which is a semi-solid like jelly. A gel is a dispersion of molecules of a liquid within a solid in which the solid is the dispersing medium and the liquid is the dispersed phase. Thus, it forms a solid three-dimensional network that spans the volume of a liquid medium which exhibits no flow when in the steady-state. A gel can have properties ranging from soft and weak to hard and tough. It is the crosslinks within that determine the hardness of the gel and also its stickiness. However, the normal liquids that have been used as an extender may be either water (hydrogels) or oil. Therefore, no matter the compositions of the gels are regarded in terms of both by weight and volume, they are mostly fluid and have densities similar to those of their constituent liquids. For example, edible jelly, it is a hydrogel and its density is approximately about that of water [1].

1.1 Hydrogels

Hydrogels have been a topic of extensive research in the past decades and their properties as for example their high water content and the possible control over the

swelling kinetics make them very attractive for biomedical applications [2]. Hydrogels are composed of hydrophilic homopolymer or copolymer networks and can swell in the presence of water or physiological fluids. Chemical crosslinks (covalent bonds) or physical junctions (e.g. secondary forces, crystallite formation, chain entanglements) provide the hydrogels' unique swelling behavior and three-dimensional structure. Upon sufficient chemical or physical stabilization, hydrogels swell, but do not dissolve, in an aqueous environment [2-3]. Hydrogels can be prepared from either natural or synthetic polymers. The hydrogels formed from natural polymers such as proteins are very similar to natural tissue and often exhibit good biocompatibility. The characteristics of swelling and water imbibing have allowed hydrogels to be used in such as food additives, pharmaceuticals, cosmetics and biomaterials which widen its applicability [4-5].

1.1.1 Hydrogel formation

Hydrogel can be designed for a specific application by selecting proper starting materials and processing techniques [6]. By adapting the degree of crosslinking, the mechanical properties of the hydrogel can be adjusted. The higher the degree of crosslinking, the stronger and tighter the gel is, but also a more fragile structure is created. The challenge is to achieve the optimal degree of crosslinking, resulting in a strong, but still elastic hydrogel. Crosslinked hydrogels can be achieved through either physical interactions or chemical (mainly covalent) bonds. Chemical cross-linking involves a cross-linking agent, making the monomers polymerize and forming a strong cross-linked structure (**Fig. 1.1**) [7].

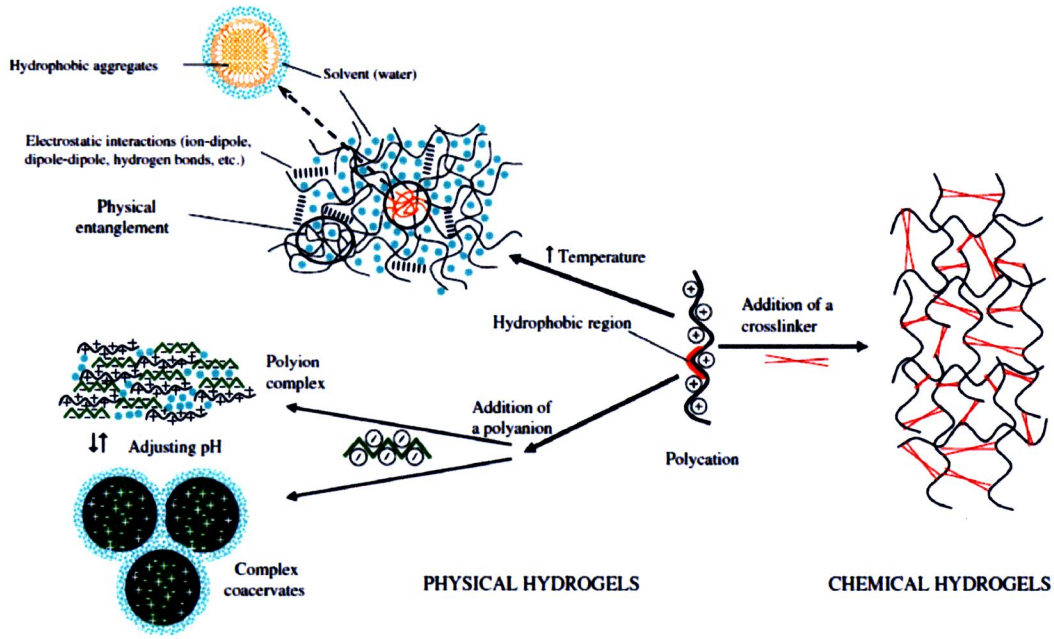

Fig. 1.1. Overview of mechanisms for formation of physical and chemical hydrogels [8]

The character of the water in hydrogel can determine the overall permeation of matters into and out of the gel. When a dry hydrogel begins to absorb water, the water molecules entering the matrix and the network can swell. After the polar and hydrophobic sites have interacted with bound water molecules, the network may imbibe additional water due to the osmotic driving force of the network chains towards infinite dilution. The additional swelling are opposed by the covalent or physical crosslinks leading to an elastic network retraction force. Therefore, the hydrogel can reach an equilibrium swelling level, thus the ionic, polar and hydrophobic groups become saturated with bound water. Moreover, the water can assume to fill the space between the network chains and/or the center of larger pores. When the network swells, the network chains or crosslinks tend to be degradable

resulting in the disintegration and dissolution at a rate which is dependent on its composition [9].

1.1.2 Preparation of hydrogels

Traditional methods of hydrogel synthesis include cross-linking copolymerization, cross-linking of reactive polymer precursors, and cross-linking via polymer–polymer reaction. Novel approaches in hydrogel design have revitalized this field of biomaterials research. New ideas on the design of hydrogels with substantially enhanced mechanical properties, superporous and comb-type grafted hydrogels with fast response times, self-assembling hydrogels from hybrid graft copolymers with property controlling protein domains and from genetically engineered triblock copolymers are just a few examples of hydrogel biomaterials with a smart future [10]. Common techniques for hydrogel preparation [5] are listed as follows:

1) Use of cross-linkers

Copolymerization of monomers using multifunctional co-monomer, which acts as cross linking agent and chemical initiator initiates the polymerization reaction which can be carried out in bulk solution or suspension. The hydrogels synthesized may contain weakly acidic groups like carboxylic acids or weakly basic groups like substituted amines or a strong acidic and basic group like sulfonic acid and quaternary ammonium compounds. Cross linkers incorporated are glutaraldehyde, calcium chloride and oxidized konjac glucomannan (DAK). They impart sufficient mechanical strength to the polymers and thus prevent burst release of the medicaments.

2) Isostatic ultra high pressure (IUHP)

Suspension of natural biopolymers (eg. starch) are subjected to ultra high pressure of 300-700 MPa for 5 or 20 minutes in a chamber which brings about changes in the morphology of the polymer (i.e. gelatinization of starch molecules occurs) and the temperature in the chamber is varied from 40 to 52°C.

3) Use of gelling agent

Gelling agents like glycophosphate 1-2-propanediol, glycerol, trehalose, mannitol etc., have been used in the preparation of hydrogels. However, presence of negatively charged moieties and turbidity are the problems associated with the method.

4) Use of irradiation and freeze thawing

Irradiation method is suitable as well as convenient but the processing is costly along with the poor mechanical strength of the product. Freeze thawing method imparts sufficient mechanical strength and stability to the hydrogels except that they are opaque in appearance with little swelling capacity. However, hydrogels prepared from microwave irradiation are more porous than conventional methods.

5) Synthesis of hydrogel in industry

Formulation of monomer along with initiators and additives lead to polymerization which forms the gel. The gel is dried, sieved and mixed with other additives and post treatment is done if needed.

1.1.3 Applications of hydrogels

Upon sufficient chemical or physical stabilization, the hydrogels swell, but do not dissolve, in an aqueous environment. Therefore, extensive uses of hydrogels in various applications [5] are as following:

1) **Wound healing**

Modified polysaccharide found in cartilage is used in formation of hydrogels to treat cartilage defects. For example, the hydrogel of gelatin and polyvinyl alcohol (PVA) together with blood coagulants are formulated.

2) **Soft contact lenses** (silicon hydrogels and polyacrylamides)

The first commercially available silicon hydrogels adopted two different approaches. First approach by Bausch and Lomb was a logical extension of its development of silicon monomers with enhanced compatibility in hydrogel forming monomers. The second by Ciba Vision was the development of siloxy monomers containing hydrophilic polyethylene oxide segments and oxygen permeable polysiloxane units.

3) **Industrial applicability**

Hydrogels are used as absorbents for industrial effluents like methylene blue dye. Another example is adsorption of dioxins by hydrogel beads.

4) **Tissue engineering**

Micronized hydrogels are used to deliver macromolecules (phagosomes) into cytoplasm of antigen-presenting cells. This property is also utilized in cartilage repairing. Natural hydrogel materials used for tissue engineering include agarose, methylcellulose and other naturally derived products.

5) Hydrogel for gene delivery

Modification of hydrogel composition leads to effective targeting and delivery of nucleic acids to specific cells for gene therapy. Hydrogel versatility has potential application in the treatment of many genetic or acquired diseases and conditions.

6) Cosmetology

Hydrogels when implanted into breast accentuate them for aesthetic reasons. These implants have silicon elastomer shell and are filled with hydroxyl propyl cellulose polysaccharide gel.

7) Topical drug delivery

Instead of conventional creams, hydrogel formulations are employed to deliver active components like Desonide, a synthetic corticosteroid used as an anti - inflammatory for better patient compliance.

8) Protein drug delivery

Interleukins conventionally administered as injection are now given as hydrogels which show better compliance and form *in-situ* polymeric network and release proteins slowly.

1.2 Silks

Silks are fibrous proteins with remarkable mechanical properties produced in fiber form by silkworms and spiders. Silk fibers in the form of sutures have been used for centuries. Recently regenerated silk solutions have been used to form a variety of biomaterials, such as gels, sponges and films, for medical applications.

Silks can be chemically modified through amino acid side chains to alter surface properties or to immobilize cellular growth factors [11].

1.2.1 Silk fibers

Silk fibers produced by cultivated *Bombyx mori* mulberry silkworm mainly consist of two proteins, sericin and fibroin; they also contain minor amounts of residues of other amino acids and various impurities: fats, waxes, dyes, and mineral salts [12]. As for the chemical composition, *Bombyx mori* fibers consist of residues of no less than 16 amino acids whose ratio varies between different areas of the supramolecular structure of fibroin (**Table 1.1**).

Table 1.1 Amino acid composition of fibroin and sericin [12]

Amino acid	Fibroin	Sericin	Amino acid	Fibroin	Sericin
Glycine	42.8	8.8	Glutamic acid	1.7	10.1
Alanine	32.4	4.0	Serine	14.7	30.1
Leucine	0.7	0.9	Threonine	1.2	8.5
Isoleucine	0.9	0.6	Phenylalanine	1.2	0.6
Valine	3.0	3.1	Tyrosine	11.8	4.9
Arginine	0.9	4.2	Proline	0.6	0.5
Histidine	0.3	1.4	Methionine	0.2	0.1
Lysine	0.5	5.5	Tryptophan	0.5	0.5

* Amino acid content (mol%)

Structural organization from protein sequence, through protein folding to the assembly of the fibrils appears to play a role in the toughness and elasticity of silk fibers. Relating structural features and patterns to physical properties is valuable as it could contribute to the ability to control the properties of future man-made silk analogous (for example, an ability to control mechanical properties by introducing a certain structural features to the thread during spinning could be beneficial). However, an understanding the structure of silk threads is still a challenge. Below is a review of the main structural features of silkworm and spider silks, starting at the overall thread organization and going down to the protein make up [13].

A similar structure has been observed in other silkworm's silk. *Bombyx mori* silk fiber has been shown to be composed of two protein-monofilaments (named brins) embedded in a glue-like sericin coating. The brins are fibroin filaments made up of bundles of nanofibrils, approx 5 nm in diameter, with a bundle diameter of around 100 nm. The nanofibrils are oriented parallel to the axis of the fiber, and are thought to interact strongly with each other [13]. A schematic representation of the structure of *Bombyx mori* thread is shown in **Fig. 1.2.**

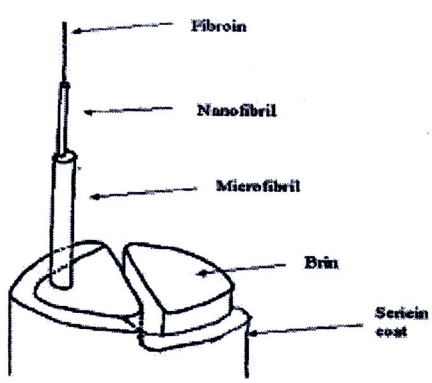

Fig. 1.2 Details of protein and other filament silk fiber [13]

1) Silk sericin

Sericin (SC) is a component of silk proteins in the silkworm cocoon. Silk protein consists of fibroin as the fiber and SC as the glue. The SC comprises 25-30% of the silkworm (*Bombyx mori*) cocoon. Although SC is composed of 18 different amino acids, it contains a high number of polar side chains with hydroxyl, carboxyl and amino groups. Isolation and characterization of SC components from the cocoon of *Bombyx mori* showed that SC primarily consists of three polypeptides with molecular weights of 150, 250 and 400 kDa [14]. Sericin is insoluble in cold water. However, it is easily hydrolyzed, where by the long protein molecules brakes down to smaller fractions, which are easily dispersed or solubilized in hot water [15].

Sericins are known to have several extraordinary properties: they resist oxidation, are antibacterial, UV resistant, and can absorb and release moisture easily. These properties are valuable in the protection of silk from microbial degradation, animal digestion, and other damaging processes. Recent studies show some surprising properties of sericin; dietary sericin has been suggested to suppress the development of colon tumors by reducing cell proliferation, and creating oxidative stress and nitric acid production. It has also been found, under certain conditions, to induce bone-like apatite deposition [15]. Thus because of its varied properties, sericin can be used as an additive in food, cosmetics, textiles and pharmaceutical products as indicated in **Fig. 1.3**.

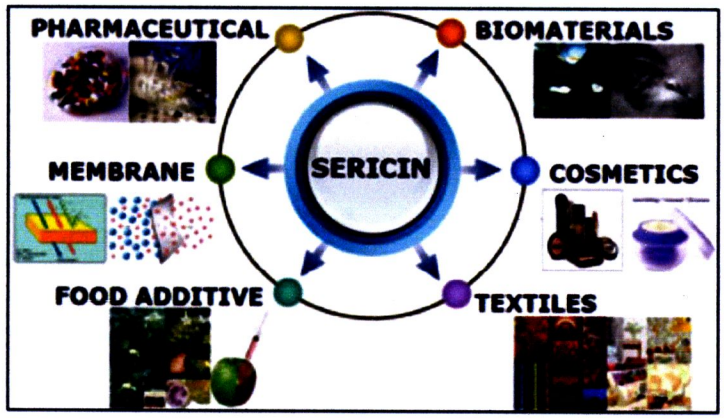

Fig. 1.3 Application of sericin in various industries [16]

2) Silk fibroin

Silk fibroin (SF) polymers consist of repetitive protein sequences and provide structural roles in cocoon formation, nest building, traps, web formation, safety lines and egg protection [11]. Fibroin is the structural protein of silk fibers and sericin is the water-soluble glue that binds the fibroin fibers together. SF is a linear polypeptide whose major amino acid composition consists of the Gly-Ala-Gly-Ala-Gly-Ser residue. The SF molecule consists of heavy and light chained polypeptides of ~350 kDa and ~25 kDa respectively, connected by a disulfide link. The SF derived from *Bombyx mori* cocoon is a macromolecular protein whose molecular weight is about 350 kD. It has been an important textile stock for a long time due to its unique tensile strength and elasticity, good thermal stability, hygroscopicity, and microbial resistance. Recently, natural fibroin has been developed and utilized for other purposes when dissolved in neutral salts at a high concentration and dialyzed against deionized water. The resulting aqueous fibroin is used in various forms such as gel, fiber, powder and membrane depending on the application [17]. The diagrammatic representation of attributes of SF is shown in **Fig. 1.4**.

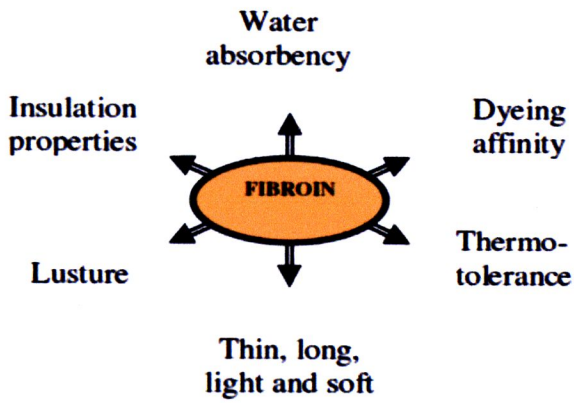

Fig 1.4 Diagrammatic representation of attributes of SF [18]

1.2.2 Silk proteins

Silk proteins were traditionally described as a matrix of rubbery polypeptide with small, rigid, inextensible β-sheets crystallites embedded in it. The crystalline regions have been suggested to be associated with the strength of silk, and the surrounding inter-phase with elasticity. However, other suggested models challenge the concept of the amorphous matrix, or suggested a model (for dragline spider silk) of cylindrical sub-units containing a crystalline poly (alanine) core capped by non-crystalline, glycine-rich regions. General structure of silk proteins are summarized as follows [13] and also depicted in **Fig 1.5**.

1) Primary structure

Silks have a highly repetitive primary structure, which gives rise to very regular conformations at the primary level. The repeating units are mostly poly glycine-alanine blocks in fibroin and poly-alanine with some glycine residues blocks in spidroin. High glycine content is thought to allow greater conformational variability. The central region of the protein is mostly hydrophobic and repetitive.

The N-terminal and the C-terminal domains are non repetitive and more hydrophilic. In fibroin, the hydrophilic spacer units are more complex and smaller (around 10%) than the hydrophobic repeating units. They also contain a much higher proportion of charge-carrying amino acids.

2) Secondary structure

Traditionally, silk proteins are thought to be two phased semi-crystalline polymers containing both crystalline and non-crystalline regions. Alternatively, silks are described as repetitive AB block copolymers, with alteration of hydrophobic (crystalline) and less hydrophobic (non-crystalline) blocks. The crystalline regions have been identified as rigid, tightly packed anti-parallel β-pleated sheets. The β-sheets are either poly-Ala, or poly-Gly-Ala, interlocking with adjacent chain via hydrogen bonds. The non crystalline domains are often described as poorly orientated, randomly coiled sections of the peptide, or 'amorphous'. However, new data indicate that those domains have a preferred secondary structure of glycine-rich 31-helix, and a specific orientation, with chains mostly parallel to the fiber. The ratio of crystalline to non-crystalline domains is known to vary between spider and silkworm silk, with spider silk containing less β-sheet and more helical regions. This is thought to be important for assembly and mechanical features.

An alternative hypothesis proposed for the secondary structure of spider silk proteins suggests the existence of three phases: an amorphous phase, highly oriented crystals, and a third, oriented non-crystalline phase.

3) Tertiary structure

The combination of extensive hydrogen bonding, high levels of crystallinity, and general hydrophobic nature are all thought to stabilize tertiary

structure. The hydrophobic nature of silk is essential to exclude water and produce the high packing density and β-crystallinity necessary for its mechanical function. Silk proteins are therefore insoluble in most solvents, including water and dilute acid and alkali. The crystalline regions of silk proteins are known to be well orientated along the silk fiber and hydrophobic interactions are thought to be responsible for the association between the chains, at least in lepidoptera.

4) Quaternary structure

The structure formed by several protein molecules (polypeptide chains), usually called protein subunits in this context, which function as a single protein complex.

It is apparent that the amino acids of spider and silkworm silks and their secondary structures are very different. However, the highly consistent design details, which are observed in both silk types, are likely to be important for the assembly of silk, and are of interest to evaluate and compare. A full, comparative and well-confirmed model of different silk types becomes a real requirement for understanding and in the future possibly controlling its properties.

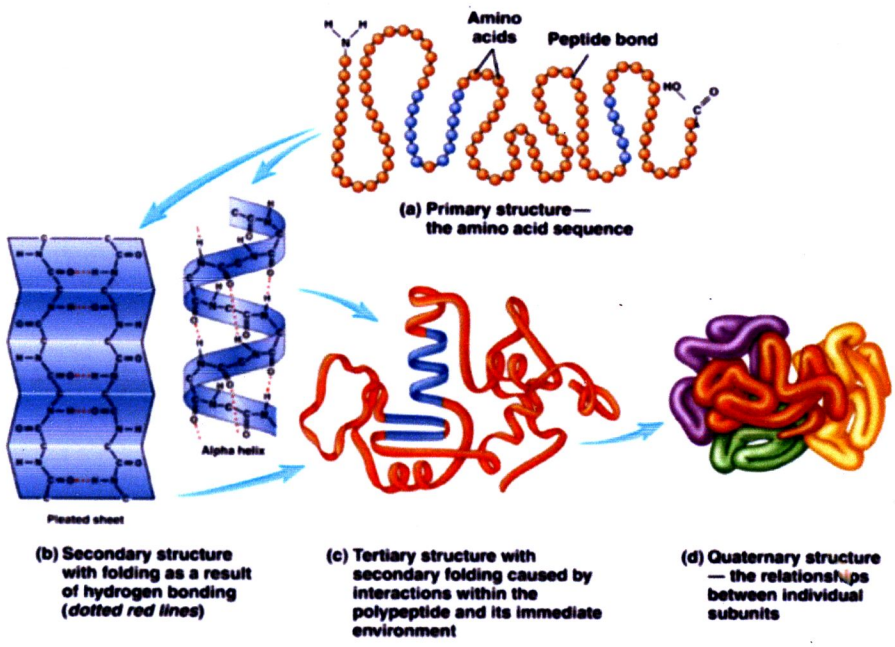

Fig. 1.5 Common structure of protein [19]

1.2.3 Silks in biomaterials

Silk fibers can be obtained by reeling from cocoons. Sutures braided from silk fibers have been used for centuries in gummed (virgin) and degummed (black braided silk) forms as sutures for surgical option. Due to the well-established sericulture process, 400,000 tons of dry silkworm cocoons are available worldwide per annum for the textile industry and thus for biomaterials applications. Several different material morphologies can be formed from aqueous or solvent formulations of the natural fiber form of silk for utilization in biomaterials for biomedical applications (**Fig. 1.6-1.7**.). The fibers must first be dissolved in aqueous systems, followed by reprocessing into desired material formats [11].

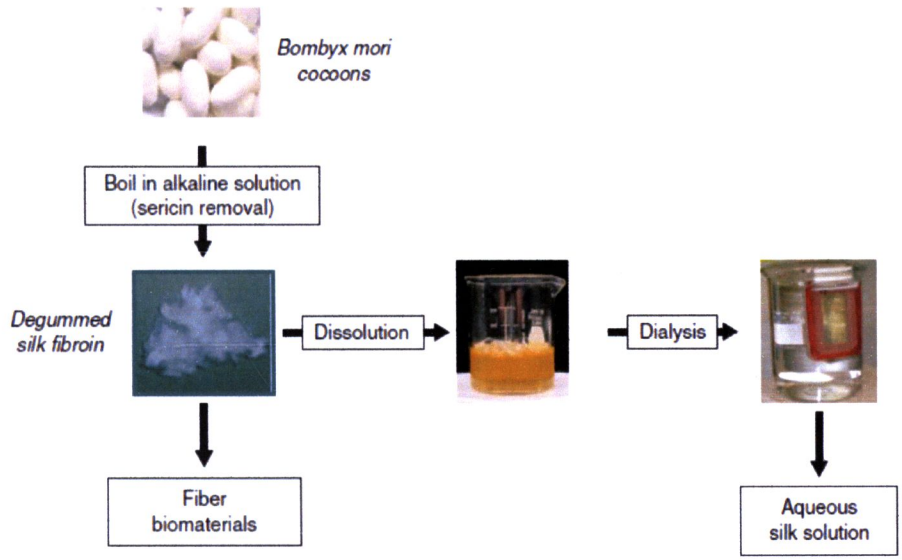

Fig.1.6 Purification of silk fibroin from silk fibroin-sericin mixtures [20]

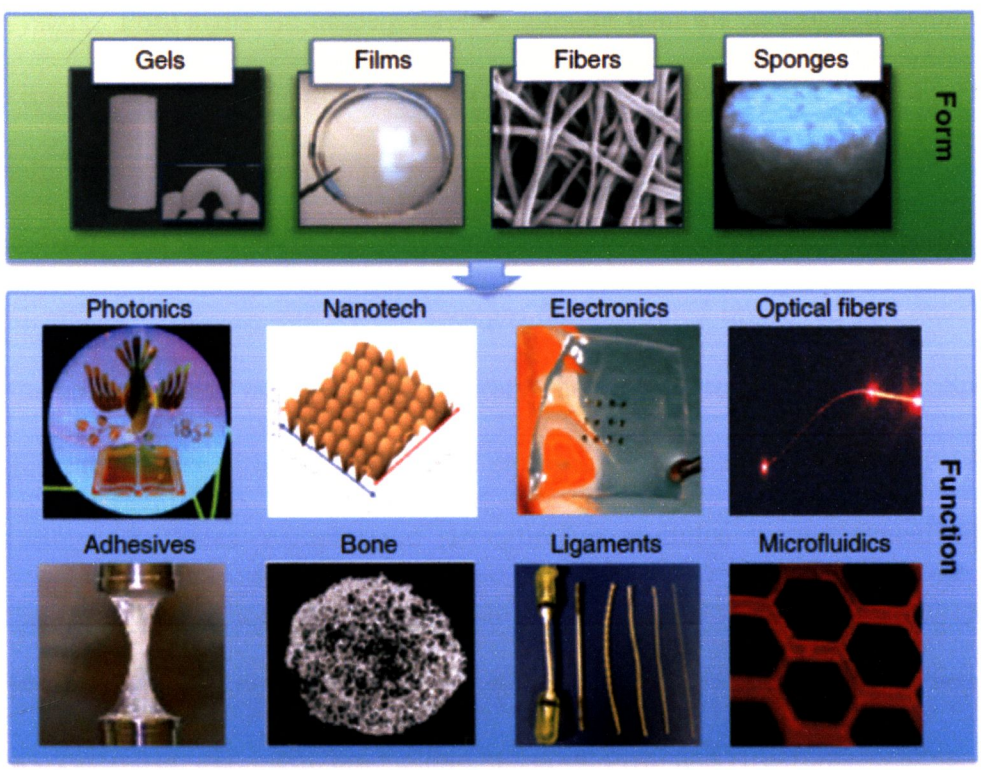

Fig. 1.7 A range of material generated from silks through processing into hydrogels, fibers, sponges, films and modification properties of silks [21]

Fibrous proteins such as silks exhibit impressive mechanical properties as well as biocompatibility; thus, these proteins are under study for biomaterials and scaffolds for tissue engineering. Depending on its application, SF can also be found in various forms [11] such as;

1) Silk fibroin porous sponges

Porous sponge scaffolds are important for tissue engineering applications for cell attachment, proliferation, and migration, as well as for nutrient and waste transport. Regenerated silk fibroin solutions, both aqueous and solvent, have been utilized in the preparation of porous sponges. Aqueous based porous silk sponges can be prepared using variable size salt crystals as porogen to control pore sizes from 490 to 940 mm by manipulating the percentage of silk solution and size of salt crystals. Pore sizes are usually 80-90% smaller than the size of salt crystals due to the limited solubilization of the surface of the crystals during supersaturation of the silk solution prior to solidification. Aqueous-based sponges have rougher surface morphology, based on SEM, than solvent-based sponges due to this partial solubilization. Aqueous silk fibroin sponges demonstrated improved cell attachment than the solvent-based porous sponges, likely due to these rougher surfaces. Sponges with high porosity and better mechanical strength were obtained with aqueous-based processing. Stiffness, compressive strength and modulus were elevated with an increase in percent silk fibroin solution utilized in the process. The concentration of silk fibroin solution and size of sodium chloride crystals (porogen) can be related to stable sponge formation with predictable morphological and structural features.

2) Silk fibroin films

Silk fibroin films have been casted from aqueous or organic solvent systems, as well as after blending with other polymers. Silk films prepared from aqueous silk fibroin solution had oxygen and water vapor permeability property depending on the content of silk I and silk II structures. Alteration of silk structure was induced by treatment with 50% methanol for varying times. Changes in silk structure resulted in differing mechanical and degradability properties of the films. Nanoscale silk fibroin films can also be formed from aqueous solution using a layer-by-layer technique. These ultrathin films were stable due to hydrophobic interactions and predictable film thickness could be obtained based on controlling solution conditions.

Microstructures in films, which are advantageous for increasing surface roughness for cell attachment, were formed via blending silk with PEO. The rough surfaces were exposed by extracting the PEO with water, after locking in the β-sheet crystallinity with methanol. The roughness was directly related to the content of PEO used in the process.

Fibroblast attachment to silk films has been shown to be as high as for collagen films. Other mammalian and insect cells also showed good attachment on silk fibroin films when compared with collagen films. Silk films, employed for healing full thickness skin wounds in rats, healed in seven days faster with a lower inflammatory response than traditional porcine-based wound dressings. Silk films have also been used for improved cell attachment and bone formation, particularly when chemically modified with RGD cell binding domains. Transparent films cast from a blend of silk and cellulose showed increased mechanical strength compared

with silk films alone. Films cast from blends of silk fibroin and recombinant human-like collagen were seeded with hepatocytes and showed higher cell viability than silk fibroin films alone. Silk fibroin solution, when coated on polyurethane and poly(carbonate) urethane films and scaffolds, increased the adhesion and proliferation of human fibroblasts. Films cast from silk fibroin and S-carboxymethyl kerateine (SCMK) showed decreased blood coagulation compared with silk fibroin or SCMK films alone.

3) Silk fibroin hydrogels

Hydrogels are three-dimensional polymer networks which are physically durable to swelling in aqueous solutions but do not dissolve in these solutions. Hydrogel biomaterials provide important options for the delivery of cells and cytokines. Silk fibroin hydrogels have been prepared from aqueous silk fibroin solution and are formed from β-sheet structures. The pH of the silk fibroin solution impacted the rate of solution gelation. Gelation of a 3% solution was obtained in two days at pH 3-4, compared with eight days as required from a solution with pH 5-12. Other factors important in gelation included silk polymer concentration and Ca^{2+}. An increase in silk fibroin concentration, increase in temperature, decrease in pH and an increase in Ca^{2+} concentration decreased the time of silk fibroin gelation. Hydrogel pore size was controllable based on silk fibroin concentration and temperature.

Based on the unique advantages of SF such as biocompatibility, biodegradability, high thermal stability, minimal inflammatory reaction and good water vapor permeability, it has been applied as biomaterials for many purposes such as enzyme immobilization, antithromboplastic materials, dialysis membranes and soft contact lenses. Moreover, the results of *in vivo* studies with fibroin membrane used as

a wound dressing showed that SF had no toxicity and irritation. However, the porous sponge form of SF is brittle, whereas its application to biomedical materials also requires sufficient mechanical strengths. Improvements in mechanical properties of SF-based materials have been sought by blending with other synthetic or natural polymers, including poly (sodium glutamate), sodium alginate, chitosan and cellulose [22].

1.3 Gel Formers

Hydrogels were prepared through the cross-linking between the polymeric units that form the backbone of the gel. These bridges can be chemical (covalent) or physical in nature (i.e., by ionic or hydrophobic interactions) [23]. Carbopol is an example of polymers that can be cross-linked by ionic interactions. One can attach hydrophobic units to hydrophilic polymers by Poloxamer and hydroxypropyl methylcellulose (HPMC). Therefore, gel formers such as Carbopol, Poloxamer and HPMC can be used as cross-linking agents in preparation method.

1.3.1 Carbopol

Carbopol is a cross-linked polymer of a high molecular weight acrylic acid that forms a hydrogel in aqueous solutions depending on the degree of hydration of the carboxyl group in carbopol. The chemical Structure of Carbopol is shown in **Fig 1.8**. Although Carbopol has many advantages as a candidate for an extended-release tablet matrix, e.g. a good gel-forming ability and muco-adhesive property, there are few reports on the application of Carbopol to the extended-release dosage forms.

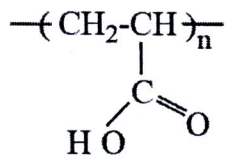

Fig. 1.8 Chemical structure of Carbopol [24]

This might be due to the ionic nature and high sensitivity of Carbopol to the pH of the medium. When Carbopol is exposed to water, the polymer begins to uncoil, generating an increase in viscosity and gel formation. In an alkaline environment, the carboxyl groups ionize, generating negative charge along the polymer backbone. The three dimensional nature of these polymers confers some unique characteristics, such as biological inertness, not found in similar linear polymers.

Gels were prepared by dispersing Carbopol in sufficient quantity of distilled water, being kept under magnetic stirring until homogeneous dispersion was formed. The dispersion was then neutralized and made viscous by the addition of triethanolamine (TEA). TEA is used as an intermediate in the manufacture of surface active agent, textile specialties, antirust compound, waxes, polishes, herbicides, petroleum emulsifier, cement additives and cutting oil. TEA is a chemical intermediate in manufacturing emulsifier of anionic and nonionic surfactants [24-27].

Table 1.2 Physical and chemical properties of Carbopol [24]

Appearance	Fluffy, white, mildly acidic polymer
Bulk Density	Approximately 208 kg/m3 (13 lbs. ft3) *
Specific gravity	1.41
Moisture content	2.0% maximum
Equilibrium moisture content	8-10% (at 50% relative humidity)
PKa	6.0 ± 0.5
pH of 1.0% water dispersion	2.5 - 3.0
pH of 0.5% water dispersion	2.7 - 3.5
Equivalent weight	76 ± 4
Ash content	0.009 ppm (average) **
Glass transition temperature	100-105 °C (212-221F)

* Polymers produced in co-solvent (a cyclohexane /ethyl acetate mixture) have a bulk density of 176 kg/m^3 (11 lbs/ft^3).

* * Polymers produced in ethyl acetate have an ash content (as potassium sulfate) of 1-3% on average.

Applications of Carbopol polymers:

The readily water-swellable Carbopol polymers are used in a diverse range of pharmaceutical applications to provide [24]:

- Controlled release in tablets

- Bioadhesion in buccal, ophthalmic, intestinal, nasal, vaginal and rectal applications
- Thickening at very low concentrations to produce a wide range of viscosities and flow properties in topical, lotions, creams and gels, oral suspensions and transdermal gel reservoirs.
- Permanent suspensions of insoluble ingredients in oral suspensions and topicals.
- Emulsifying topical oil-in-water systems permanently, even at elevated temperatures, with essentially no need for irritating surfactants

1.3.2 Poloxamer

Poloxamer, which consists of more than 30 different non-ionic surface-active agents, is ABA-type triblock copolymers composed of polyethylene oxide$_a$–polypropylene oxide$_b$–polyethylene oxide$_a$ [PEO_a–PPO_b–PEO_a] (**Fig. 1.9**) and is used as substitutes for skin in standardized third-degree thermal burns.

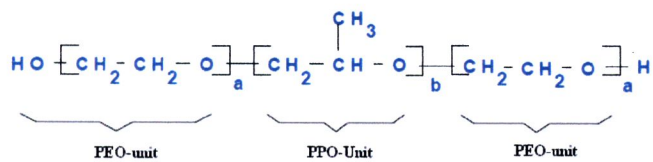

Fig. 1.9 Structure of Poloxamer [28]

They comprise a central block of relatively hydrophobic polypropylene oxide surrounded on both sides by the blocks of relatively hydrophilic poly-ethylene

oxide. Due to the PEO/PPO ratio of 2:1, when these molecules are immersed in the aqueous solvents, they form micellar structures above critical micellar concentration. Therefore, they are regarded as PEO-PPO-PEO copolymers. Chemically they are oxirane, methyl-, polymer with oxirane OR α-Hydro-ω-hydroxypoly(oxyethylene)a poly(oxypropylene)b poly(oxyethylene)a block copolymer. Generally, these are waxy, white granules of free-flowing nature and are practically odorless and tasteless. Aqueous solutions of pluronic in presence of acids, alkalis, and metal ions are very stable. The Poloxamers are readily soluble in aqueous, polar and non-polar organic solvents and due to this fact they have established themselves as a preferred molecule in the formulation techniques. The pluronic triblock copolymers are available in various grades differing in molecular weights and physical forms. Depending upon the physical designation for the grades are assigned, as F for flakes, P for paste, L for liquid. The general structure for the pluronic (**Table 1.3**) and specifications of Poloxamers (**Table 1.4**) can be stated as follows:

Table 1.3 Poloxamer grades and their chemical composition [28]

Pluronic ®	**Poloxamer**	**a**	**b**	**Content of Oxyethylene (percent)**	**Molecular Weight**
L 44 NF	124	12	20	44.8-48.6	2090-2360
F 68 NF	188	80	27	79.9-83.7	7680-9510
F 87 NF	237	64	37	70.5-74.3	6840-8830
F 108 NF	338	141	44	81.4-84.9	12700-17400
F 127 NF	407	101	56	71.5-74.9	9840-14600

Table 1.4 Specifications of Poloxamers [28]

Type of Poloxamer	124	188	237	338	407
Physical Form	Liquid	Solid	Solid	Solid	Solid
pH (2.5% in water)	5.0-7.5	5.0-7.5	5.0-7.5	5.0-7.5	5.0-7.5
Cloud point, 10%	71-75 C	> 100 C	> 100 C	> 100 C	> 100 C
APHA colour	50 max.	100 max.	100 max.	100 max.	120 max.
% H_2O	0.4 max.	Cast solid 0.4 max. Prill 0.75 max.	Cast solid 0.4 max. Prill 0.75 max.	Cast solid 0.4 max. Prill 0.75 max.	Cast solid 0.4 max. Prill 0.75 max.
BHT, ppm	-	50-125	50-125	50-125	50-125
Unsaturation mEq/g	0.020 ± 0.008	0.026 ± 0.008	0.034 ± 0.008	0.031 ± 0.008	0.048 ± 0.017
Ethylene Oxide, ppm	1 max.	1 max.	1 max.	1 max.	1 max.
Propylene Oxide, ppm	5 max.	5 max.	5 max.	5 max.	5 max.
1,4 dioxane, ppm	0.002 % max.	0.002 % max.	0.002 % max.	0.002 % max.	0.002 % max.

Poloxamers, like other surfactants when dispersed in the liquid, at low concentrations, exist individually as monomolecular micelles. This leads to decrease in the surface tension as well as surface free energy. But as the concentration of the

pluronic in the system increases, this results in the formation of multimolecular aggregates. PPO forms central hydrophobic core wherein methyl groups interact via van der Waals forces with substance undergoing solubilization. However, water solubility is believed to be due to the PEO block by hydrogen bonding interactions of ether oxygen with water molecules. Due to these interactions, pluronic are readily soluble in non-polar organic solvents and established themselves in formulation of dosage forms. In contrast to low molecular weight surfactants, 16 pluronic like block copolymers have shown different aggregate forms, depending on the molecular weight, block sizes, solvent composition, and temperature. Micellar behavior of different block copolymers is found to be dependent upon solvent composition and temperature. Micelle structures consist of hydrophobic PPO block lying in the micelle core and the PEO block in the corona.

Concentrated aqueous solutions of Poloxamer can form thermoreversible gels. The gelation mechanism of Poloxamer solutions has been investigated extensively, but is still being debated. Ultrasonic velocity, light-scattering and small-angle neutron scattering measurements of aqueous Poloxamer solutions have clearly indicated a micellar mode of association. Micelle formation occurs at the critical micellization temperature as a result of PPO block dehydration. With increasing temperature, micellization becomes more important, and at a definite point, micelles come into contact and no longer move. In addition, the formation of highly ordered structures, such as cubic crystalline phase, has been proposed as the driving force for gel formation, but this hypothesis has been questioned recently. Thus, packing of micelles and micelle entanglements may be possible mechanisms of Poloxamer

solution gelation with the increase of temperature. Furthermore, it has been suggested that intramolecular hydrogen bonds might promote gelation.

Poloxamer hydrogels have been used in a variety of biomedical fields such as medical, pharmaceutical, and cosmetic systems. The experimental modulation of wound healing suggested that the non-ionic Poloxamer could significantly enhance the rate of wound healing through yet unknown mechanism, possibly by stimulating the epithelial growth factor (EGF) [28-30].

1.3.3 Hydroxypropyl methylcellulose (HPMC)

HPMC is a non-ionic cellulose ether (**Fig. 1.10**), derived from alkali treated cellulose reacting with methyl chloride and propylene oxide through series of chemical changes.

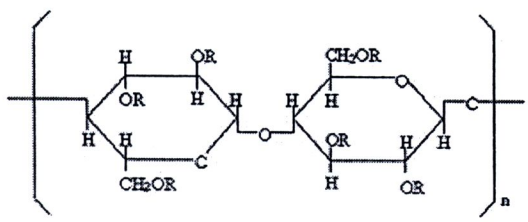

Fig. 1.10 Chemical structure of HPMC [31]

HPMC is characterized by the solubility in water, water retention, non-ionic type, stable pH value, surface activity, reversibility from gelling to solving at different temperature, thickening, binding, film-forming, lubricating and mold resistance. Due to all these special properties, it is widely applied for thickening, gelling, dispersing, stabilizing, water retaining and mixing improving in industries like building material, painting, synthetic resin, porcelain, medicine, food, textile,

agriculture, cosmetics, cosmetics and tobacco. In addition, it was used as an alternative to gelatin (animal based) capsules to be an ingredient of hard capsules used for encapsulating powdered herbs.

Gelation of methylcellulose or HPMC solutions is primarily caused by the hydrophobic interaction between molecules containing methoxy substitution. At low temperatures, the macromolecules are hydrated, and there is little polymer–polymer interaction other than simple entanglement. As the temperature is raised, the polymers gradually lose their water of hydration, which is reflected by a decline in relative viscosity. Eventually, when sufficient but not complete dehydration of the polymer occurs, polymer–polymer associations take place and the system approaches an infinite network structure, as reflected experimentally by a sharp rise in relative viscosity. This sol-gel transformation has been exploited to design *in situ* gelling systems. These systems exhibited low viscosity at 23 °C and formed soft gels at 37 °C [31-33].

In this study, the above mentioned gel formers will be used in mixing with silk fibroin to act as cross-linking agents in order to form silk fibroin hydrogels. These gel formers are expected to enhance mechanical strength of silk fibroin in different degrees providing that the resulting hydrogels will have appropriate gel properties for further applications.

1.4. Literature Review

Recently, some authors have processed silk fibroin in order to obtain different kinds of materials such as film, nets, powder and gels, which have been recommended for using in biomedical and surgical applications. For example, regenerated fibroin

films which display a high oxygen permeability, water vapour permeability, enzyme-immobilization ability, such properties make it be very compatible to mammalian cells and fibroin materials appear to stimulate osteoblast-based mineralization *in vitro* [34].

Samczewska *et al.* [35] investigated the effect of selected substances in neutralizing carboxyl groups of polyacrylic acid (sodium and potassium hydroxide, trietanoloamine, borax) on physicochemical parameters of the produced hydrogels with Carbopol 980 and Carbopol Ultrez 10. There were preparations of variant hydrogels with morphine sulfate on the base of Carbopol 980 and Carbopol Ultrez 10. Extensometric method was used to test spread-ability of the preparations, gravimetric method to determine the rate of volatile components loss, while viscosity parameters were determined with cone-plate digital rheometer. Potentiometric method was applied to measure pH of the produced hydrogels. The most beneficial applicative properties (including rheological ones) were obtained for model hydrogels containing Carbopol Ultrez 10, but the kind of the cross-linking base practically did not affect the rate of morphine sulfate diffusion from the produced hydrogels and the applied Carbopol for hydrogels in the prescription of which trietanoloamine was used.

Yoo *et al.* [22] reported that semi-interpenetrating polymer networks (SIPNs) prepared from silk fibroin and Poloxamer macromer showed enhanced mechanical properties. SIPNs are defined as a composition in which one or more polymers are cross-linked as a branched or linear co-network. These networks are generally formed from soluble polymers by cross-linking themselves through irradiation or chemical methods or by polymerizing hydrophilic monomers in the presence of a cross-linker. Poloxamer macromer, having acrylated-terminated PEOs, was chosen as

a blending material and cross-linked in the presence of chitosan (CS) to form the SIPNs [36]. Kim *et al.* [37] reported the possibility of using SIPNs, composed of CS and Poloxamer macromer, as a wound dressing. They introduced SIPNs system to provide the mechanical strength required for wound dressing application. The advantages of this system are the enhancement of mechanical strength of the network and increase in the compatibility of the polymer blends, which exhibit favorable properties of phase-separated materials. The CS/Poloxamer SIPNs sponge designed for wound dressing application was successfully prepared after UV irradiation with Poloxamer macromer in the presence of CS. The drawbacks of Poloxamer hydrogel, such as fast dissolution in aqueous solution, have limited its biomedical application. In order to improve the stability of hydrogel, a novel system was developed by combining the reversible thermo-sensitive property of Poloxamer 407 and the thiol-ene reactivity between the acrylate and thiol groups. Niu *et al.* [38] modified Poloxamer with acrylate and thiol groups. The behavior of sol-gel transition and the rheological properties of the modified P407 aqueous solution were compared with those of P407 aqueous solution. It was found that the sol-gel transition of the acrylate/thiol modified Poloxamer 407 mixture could be achieved at body temperature even with a low concentration of 17.5 wt.%. Meanwhile, the reaction between the acrylate and thiol modified Poloxamer 407s occurred spontaneously in mimic physiological conditions, thus the hydrogel with cross-linking structure was formed. As a result, the stability of the cross-linked hydrogel was enhanced remarkably. *In vitro* and *in vivo* experiments revealed that the biocompatibilities of the modified Poloxamer 407 hydrogel were similar to that of Poloxamer 407.

HPMC polymers are well-known for their ability to bind, retain water, thicken, form films, and provide many other performance benefits. An essential polymer, HPMC delivers the unique ability to enable controlled-release formulas and reverse thermal gelation [39]. Davaran *et al.* [40] investigated the effect of cross-linking agent on swelling behavior of HPMC-PEG hydrogels. The HPMC hydrogels contain poly ethylene glycol (PEG) as cross-links were prepared by reacting HPMC sodium salt with polyethylene glycol dichloride. Swelling parameters such as equilibrium degree of swelling, swelling ratio and network parameter such as molecular mass between cross-links (M_c) were determined. The cross-linking concentrations were 0.5%, 1%, 1.5%, and 2% (based on weight of HPMC). The equilibrium swelling ratio (Q) of cross-linked HPMC hydrogels increases from 13.2 to 27.1 as the cross-linker percentage increases from 0.5% to 2%. 5-aminosalicylic acid (5-ASA) was used as a model of an anti-inflammatory drug. Rodriguez-Tenreiro *et al.* [41] studied and characterized new hydrogels based on cyclodextrins cross-linked with ethyleneglycol diglycidylether (EGDE) under mild conditions. The cross-linking of hydroxypropyl-β-cyclodextrin (HPβCD) with EGDE, in the absence or presence of HPMC was optimized by applying oscillatory rheometry and Fourier transform infrared. Hydrogels were characterized regarding swelling in water. Solutions of HPβCD (14.28%), without or with HPMC (0.2-1.0%), provided firm and transparent hydrogels after cross-linking with EGDE (14.28%), in which around two thirds of the OH groups were cross-linked. The incorporation of HPMC progressively reduced the gel time and the swelling degree of hydrogels. The HPβCD hydrogels was good mechanical properties, it obtained by direct cross-linking with EGDE.

1.5 Experimental Techniques [42]

Many techniques have been used to characterize the structure and properties of SF for example; Fourier transform infrared (FTIR) spectroscopy, Differential Scanning Calorimetry (DSC), scanning electron microscopy (SEM), and UV-VIS spectroscopy.

1.5.1 Fourier transform infrared spectroscopy (FTIR)

Infrared spectrometry (IR) is a widely used technique in both research and industry. It is a simple and reliable technique for the identification of unknown materials, determination of the amount of components in a mixture as well as for quality control of materials. Infrared (IR) spectroscopy gives molecular structural information using the frequencies of the vibrational modes for a compound. IR is a type of energy absorption spectroscopy that uses the infrared region of the electromagnetic spectrum (**Figure 1.11**).

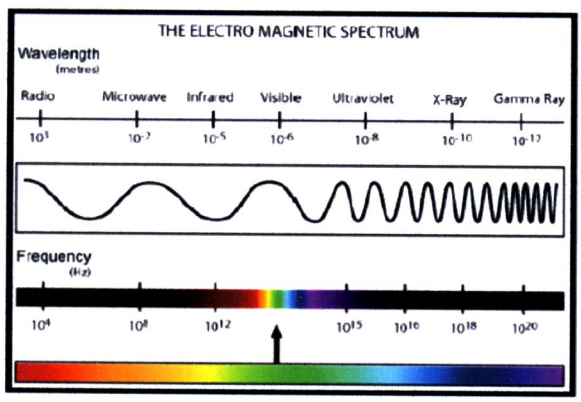

Fig. 1.11 Various regions of the electromagnetic spectrum [42]

Fourier Transform Infrared (FTIR) spectroscopy is a measurement technique whereby IR spectra are collected by using a time domain measurement. The original IR spectrometers are of the dispersive or filter types. They measure the amount of energy at each frequency of the IR spectra with the aid of a prism or grating. FTIR uses an interferometer which measures the signal and performs a Fourier transform on the data to provide an IR spectrum. There are several advantages for FTIR over dispersive IR methods such as:

- The measurement time is faster
- Simultaneous measurement can be performed
- It is a non-destructive technique
- It requires no external calibration
- It is inexpensive
- It provides improved sensitivity and resolution
- It has mechanical simplicity

Once a molecule absorbs IR radiation, each atom oscillates about its equilibrium position, e.g. simple harmonic motion, regarding as a normal vibrational mode. When the frequency of the IR radiation is the same as the vibrational frequency of a bond in a functional group, absorption of IR energy occurs. The absorption intensity depends on how efficiently the energy of an electromagnetic wave can be transferred to the atoms involved in the vibration. The greater the change in dipole moment during a vibration, the higher the intensity of absorption. Atoms in a functional group can vibrate in various ways, such as symmetrical and anti-symmetrical stretching, scissoring, rocking, wagging and twisting. An IR

spectrum is obtained when a sample absorbs energy which causes a transition between two vibrational energy levels, from ground state to excited state.

A basic FTIR spectrophotometer consists of an IR radiation source, the interferometer and the detector (**Figure 1.12**). Typically, an IR radiation source includes one of the following: Nernst glower, Glowbar source, incandescent wire source, mercury arc, tungsten filament lamp,and carbon dioxide laser. Interferometer works on the principle of superposition to combine separate waves together to have some meaningful properties. One widely used interferometer is the Michelson interferometer (**Figure 1.13**).

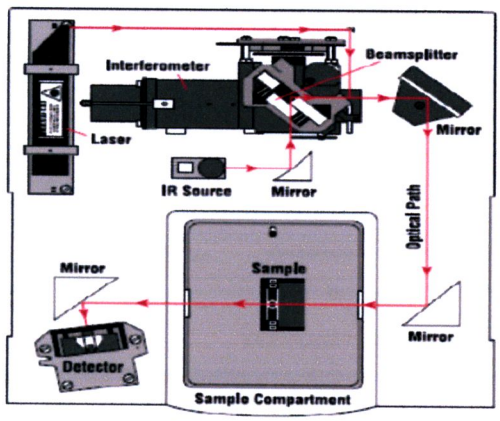

Fig. 1.12 Schematic presentation of a FTIR spectrophotometer [42]

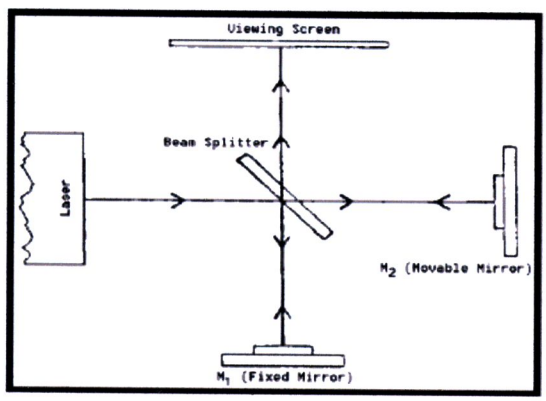

Fig. 1.13 A schematic diagram of Michelson interferometer [42]

Typical IR detectors which are used include: thermocouple, Bolometer, pyroelectric detector, or a photoconducting detector. The measured signal is transferred to the computer where the Fourier transformation of the data takes place and the typical percentage transmissions versus wave number plots are obtained. Various vibrational modes of the functional groups and atoms in organic compounds exhibit characteristic IR absorption peaks at a specified wave number range (**Fig. 1.14**). This helps to identify the nature of the functional groups and atoms present in a molecule.

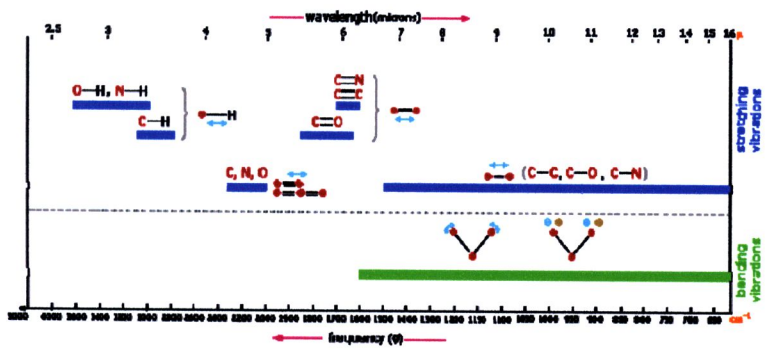

Fig. 1.14 General regions of IR spectrum showing various types of vibrational bands [42]

FTIR is very widely used for gases, liquids and solid samples. For gaseous samples, sample cells of sodium chloride (NaCl) or potassium bromide (KBr) are used. Liquids can be sandwiched between two IR transparent plates made up of NaCl or KBr. Solid samples can be prepared by four different methods. First, mixing with a mulling agent such as Nujol and applied onto salt plates. Second, mixing with potassium bromide and crushed in a mortar with a pestle. The mixture is then compacted to form a translucent pellet with the aid of a mechanical die press. The

third method is the cast film method in which a sample is dissolved in a non-hygroscopic solvent and a drop of the solution is deposited on the surface of NaCl or KBr plates; which is then evaporated to form a uniform thin film. The fourth method is microtomy in which thin films are cut from the solid sample.

1.5.2 Differential scanning calorimetry (DSC)

DSC is a thermoanalytical technique in which the difference in heat flow between a sample and reference material is measured as a function of time or temperature when they are subjected to the same heating rate in a controlled atmosphere. It is used to study thermal transitions involving energy or heat capacity changes, such as melting, glass transitions, recrystallization, mesomorphic transition temperature, corresponding entropy and enthalpy changes, and exothermic decompositions of various materials with great sensitivity. The technique was first developed by E.S. Watson and M.J. O'Neill in 1960.

The heat flux DSC belongs to the class of heat-exchanging calorimeters in which sample and reference pans are placed in a single furnace. A Platinum resistance thermometer or thermocouple is used to measure the temperature differential which is converted to energy flow via a mathematical equation. There are three major types of measuring systems used in heat flux DSC: disc type, turret type and cylinder type. The schematic diagram of the Power compensated and the Heat Flux DSC are shown in **Fig. 1.15**.

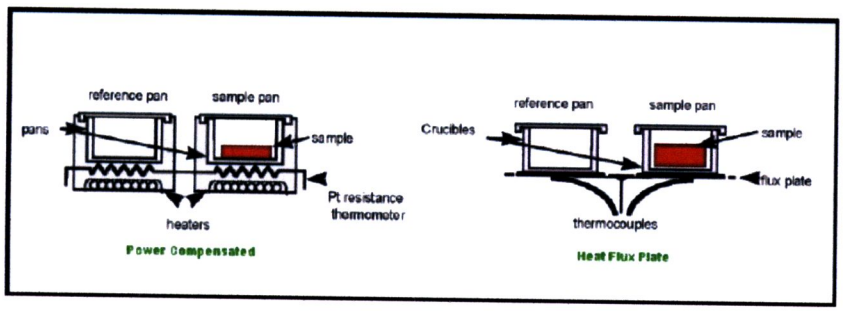

Fig. 1.15 Schematic diagram of the power compensated and the heat flux DSC [42]

Power compensated DSC belongs to the class of heat compensating calorimeters in which the heat to be measured is compensated with electric energy. The system is maintained in a thermal null state at all times by holding the sample and reference pans separate with its own heating element.

In order to ensure trustworthy and reproducible results for the DSC methods, the instrument needs to be routinely calibrated for quality assurance. The temperature and caloric calibration is recommended in the guidelines given by the International Confederation for Thermal Analysis and Calorimetry (ICTAC). Some of the common materials recommended for temperature calibration of DSCs are cyclopentane, water, gallium, indium, tin, lead, zinc, lithium sulfate, aluminum, silver and gold.

The analysis by DSC is normally displayed by DSC curves (known as thermograms) which are presented in the form of heat flow vs temperature or time. A typical DSC curve is characterized by the "baseline" (part of the curve obtained during steady state conditions when no reaction or transition occurred). The "peak" caused from transitions or reactions can be called differently as "interpolated baseline", "initial peak temperature (Ti)", "extrapolated peak onset temperature (Te)",

"peak maximum temperature (Tp)", "extrapolated peak offset temperature (Tc)", and "final peak temperature (Tf)" as depicted in **Figure 1.16**. Various thermal transitions of materials can be observed such as glass transition, melting, crystallization, oxidation, decomposition as seen in **Fig. 1.17**.

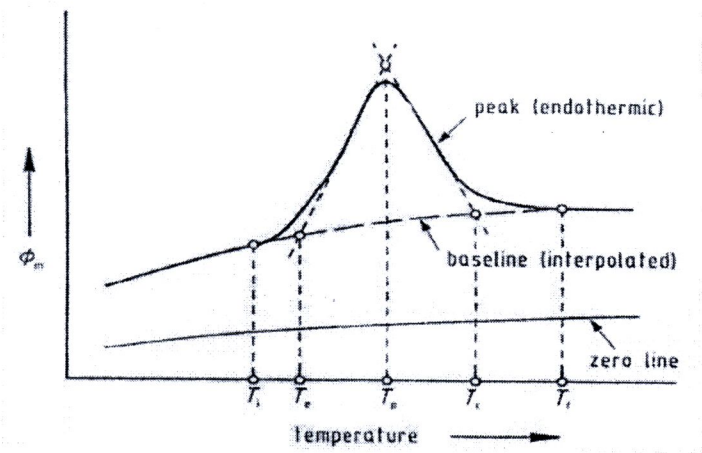

Fig. 1.16 Characteristics of the DSC curve [42]

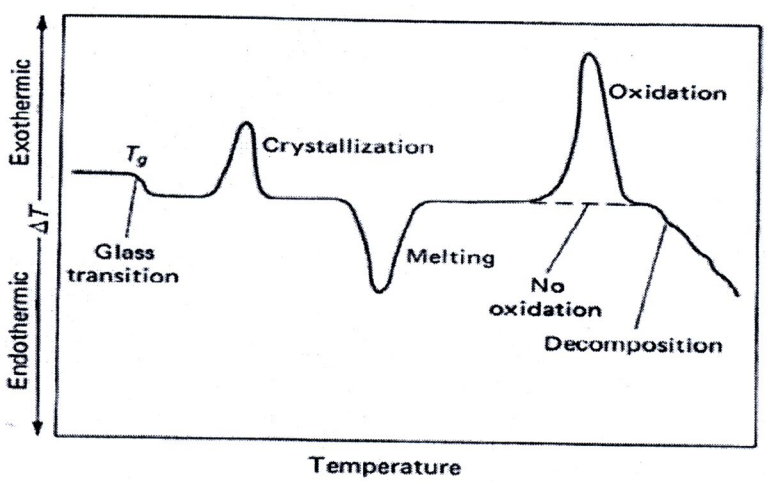

Fig. 1.17 Typical DSC thermogram showing commonly observed transitions [42]

DSC is routinely used in industry for raw material inspection (QA/QC), oxidative material stability, sub-ambient material classification; curing processes liquid crystals etc. Some of the common applications of DSC include:

- Measurement of Heat capacity
- Heats of reaction
- Kinetic investigation
- Glass transition process
- Thermal history/processing
- Crystallization temperature
- Degree of crystallinity
- Degree of cure
- Thermal safety/stability studies
- Protein denaturation
- Polymorphic transitions
- Porosity measurements

1.5.3 Scanning electron microscopy (SEM)

The scanning electron microscopy (SEM) is one of the most versatile techniques widely applied to surface microstructure imaging. SEM is a type of electron microscopy that images the sample surface of a solid specimen by using a focused beam of high-energy electrons. The signal contains information about surface topography, external morphology, chemical composition, crystallographic information and electrical conductivity.

An SEM instrument comprises major components including the electron gun, electron lenses, sample stage, detectors, data output devices, and the vacuum system. **Fig. 1.18** shows a structure of a conventional SEM. The electron gun produces a high current, small spot size, stable electron beam and accelerates at an energy level of about 0.1-0.3 keV. Tungesten, lanthanum hexaboride, and field emission are the types of electron guns widely used. The electron beams can be focused by electromagnets or electron lens and aperture. There are two types of

electron lenses: condenser lenses and objective lenses. Condenser lens converges and collimates the diverging electron beams into parallel beams. Objective lenses demagnify the electron beam into a probe point at the specimen surface.

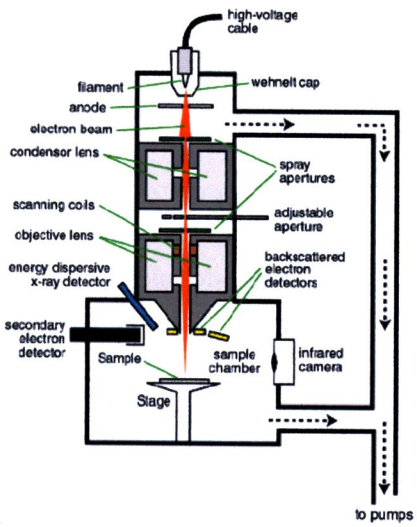

Fig. 1.18 Schematic diagram of a scanning electron microscope [42]

A stigmator consists of a series of coils surrounding the electron beam. It can be used to correct astigmatism and achieve an image with higher resolution. Interaction of the focused electrons on the specimen surface produces secondary electrons, back scattered electrons (BES), transmitted electrons, characteristic X-rays, and Auger electrons which are collected by detectors, processed and displayed in the output device. In order to avoid scattering of electrons by air molecules, and the contamination of the electron guns and other components a ultrahigh vacuum system is maintained using mechanical, diffusion, ion or turbo pumps.

Sample preparation for SEM analysis can be elaborate or minimal depending on the type of sample and image required. For conductive materials, the surface can be visualized directly by loading the sample onto carbon tape. For

nonconductive organic materials and biological samples, a thin layer of conductive materials such as carbon, gold, silver, platinum are coated by low vacuum sputter coating or high vacuum evaporation to make the surface conductive, increase signal and surface resolution, and prevent accumulation of static electric charge on the specimen. An alternative method for coating biological samples is to use OTO (Osmium Thiocarbohydrazie Osmium) stain.

Combination of large depth of field, high resolution, high magnification, minimal sample preparation, easy sample observation, and rapid data acquisition makes SEM one of the widely used instruments in a variety of research areas. SEM is routinely used to analyze shapes and surface topography of samples. It is used to analyze spatial variation in chemical compositions by using elemental maps, and spot chemical analysis. It is also used to identify the microfabric and crystalline orientation of materials. Though there are few limitations associated with SEM such as its applicability only for solid sample which are stable under vacuum, inability to detect very light elements (H, He, Li), and extensive sample preparation for nonconductive materials.

1.5.4 **Ultraviolet and visible spectrometry** (UV-VIS)

UV-VIS spectroscopy is a type of absorption spectroscopy which is carried out in the UV region (approximately 200-400 nm) and visible region (approximately 400-800 nm) of the electromagnetic spectrum. UV-VIS spectroscopy is very useful as an analytical technique in chemical and biochemical research for two reasons: to identify some functional groups in the molecules and to determine concentration and strength (assay) of a substance.

According to the Beer-Lambert law, the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. Thus, for a fixed path length, UV-VIS spectroscopy can be used to determine the concentration of the absorber in a solution by using the equation given below.

$$A = - \log (I/I_0) = \epsilon . c . L \qquad (1)$$

Where *(A)* is the measured absorbance, (I_0) is the intensity of the incident light at a given wavelength, *(I)* is the transmitted intensity, *(L)* the path length through the sample, and *(c)* the concentration of the absorbing species. For each species and wavelength, (ε) is a constant known as the molar absorptivity or extinction coefficient which is the fundamental molecular property in a given solvent, at a particular temperature and pressure.

There are a few terminologies used in UV-VIS spectroscopy which must be employed. A bathochromic shift is the change of absorption to a longer wavelength. A hypsochromic shift is the change of absorption to a lower wavelength. A hyperchromic shift is the increase in absorption intensity while a hypochromic shift refers to a decrease in absorption intensity.

A UV-VIS spectrophotometer consists of a light source (deuterium and tungstenhalogen lamps), dispersion element (prism, grating monochromator, interferometer), sample compartment and cells (cuvette, 96-well plate), detector (photomultiplier tubes, photodiode array, CCD cameras) and a data acquisition system (computer). The spectrophotometer may be of two types: single beam or dual beam **(Fig. 1.19-Fig. 1.20)**.

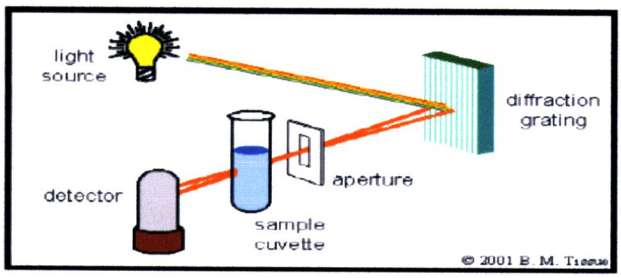

Fig. 1.19 Schematic of a single-beam UV-VIS spectrophotometer [42]

In a single-beam instrument, the transmittance of the sample and solvent (reference) at each wavelength is measured manually, whereas the double-beam measures the transmittance of the sample and solvent simultaneously by splitting the single beam of light into two halves. Complete absorption spectra for a compound can be obtained by scanning the sample in the desired wavelength range.

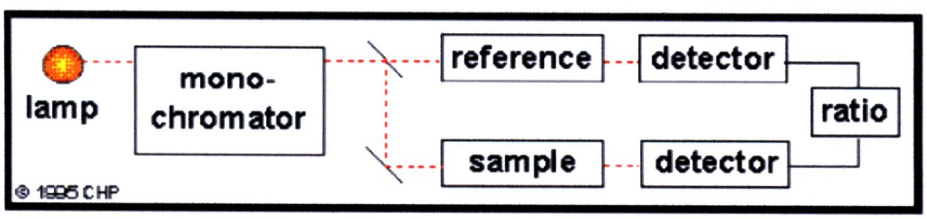

Fig. 1.20 Schematic of a dual-beam UV-VIS spectrophotometer [42]

Samples for UV-VIS spectroscopy are most often solutions. In special cases, gases and even solids can also be analyzed. Samples are typically placed in a transparent cell, known as a cuvette made of high quality fused silica, quartz glass or plastic. These materials are transparent throughout the UV, visible and near infrared regions. The solvents used should have negligible absorbance in the UV region. Commonly used solvents are acetonitrile (190 nm), water (191 nm), cyclohexane (195

nm), methanol (201 nm), ethanol (204 nm), ether (215 nm), methylene chloride (220 nm) etc.

UV-VIS spectroscopy is routinely used to for quantitative determination of wide range of samples, solutions, such as inorganic anions, transition metals, inorganic complexes, organic complexes, highly conjugated organic compounds including drugs, biomolecules such as proteins and nucleic acids. UV-VIS derivative spectroscopy is another analytical tool to study the resolution enhancement of overlapping peaks and elimination or reduction of background matrix absorption. The Woodward Fischer rule can be used to calculate the wavelength for maximum absorption (λ_{max}) for structural characterization of organic compounds containing conjugated systems.

1.6. Research Objectives

The aim of this research work is concentrating on the modification of SF with different gel formers for its property improvement of SF hydrogel. The main purposes of this study are;

1. To modify SF hydrogel by mixing with different gel formers, such as hydroxypropyl methylcellulose (HPMC), Carbopol and Poloxamer in order to obtain better hydrogel properties.
2. To characterize their products through the studies of IR absorption, swelling property and morphology of silk fibroin hydrogel in order to investigate and compare the features of the cross-linking of silk fibroin with different gel formers