CHAPTER I
INTRODUCTION

Biopolymer is a macromolecule that is formed by linking several small molecules together. Biopolymers are produced by micro-organisms or plant or animal materials. In micro-organisms, bacteria cells can produce biopolymers such as polyhydroxyalkanoate (PHA), polyhydroxybutyrate (PHB), and polylactic acid (PLA) which are based on fermentation processes by using various materials as feedstock materials. Plants can produce starch which is found in the roots, seeds, and stems of corn, wheat and potatoes, etc. Moreover, plant tissues consist of PHB which is a completely biodegradable material. In animal, biopolymers are proteins from amino acids or DNA from nucleotides. Most of biopolymer is blended with synthetic polymers to produce materials with improved properties. In this study, biopolymer such as sericin is focused because it is mostly discarded in silk processing wastewater. In Thailand, silk is very famous and produced in many provinces. If this sericin is recovered and recycled, it can represent a significant economic and social benefit. The sericin protein is antibacterial, antioxidation and UV resistant. It absorbs and releases moisture easily (Zhang, 2002; Sarovart *et al.*, 2003).

Nanoparticles are colloidal particles of sizes below 1 μm. Their applications have some advantages including easy purification and sterilization, drug targeting possibilities, and sustained release action. There are several methods to prepare nanoparticles such as diafiltration (Cho *et al.*, 2003), nanoprecipitation (Lee *et al.*, 2003; Peltonen *et al.*, 2004), and electrospraying method. Electrospray technique has many advantages such as achieving tiny particle size, no-aggregation, high yield and using a short period of time.

Nanofibers are non-woven fibers with diameters between 50 nm and 10 μm. They are large surface area per unit mass and small pore size These fibers are generated by electrospinning process and widely used in cosmetics, clothing, sensor, filtration, reinforcement, medicals, and electric device. Various synthetic polymers and biopolymers have been spun by electrospinning (Huang *et al.*, 2004; Park *et al.*, 2004; Kim *et al.*, 2005).

Until now, there is no report on electrospraying and electrospinning of sericin because it is easy to form gel in water. On the other hand, there are many reports on electrospinning of silk fibroin. Modification of silk sericin with synthetic polymer can help to generate sericin nanoparticles and nanofibers. In this research work, polyacrylamide (PAM) is chosen to modify with silk sericin such as by solution blending and chemical modification as graft copolymer. These mixtures are made into nanoparticles and nanofibers by eletrospraying and electrospinning technique, respectively.

Sericin

In the formation of silk filament, the cocoon shell consists primarily of the proteins "sericin" and "fibroin". The bulk is fibroin or fiber, which is held together by sericin or silk gum. Sericin is removed through a degumming treatment of cocoon silk. Most of the sericin must be removed during raw silk production at the reeling mill and the other stages of silk processing so sericin is mostly discarded in silk processing wastewater. The amount of sericin ranges from 19 to 28 percent according to the type of cocoon, usually the sericin content of the cocoon shell is the maximum at the outside layer 1 and becomes progressively lower at the middle layers 2 and 3 and the minimum at the inside layer 4. (Becker *et al.*, 1995)

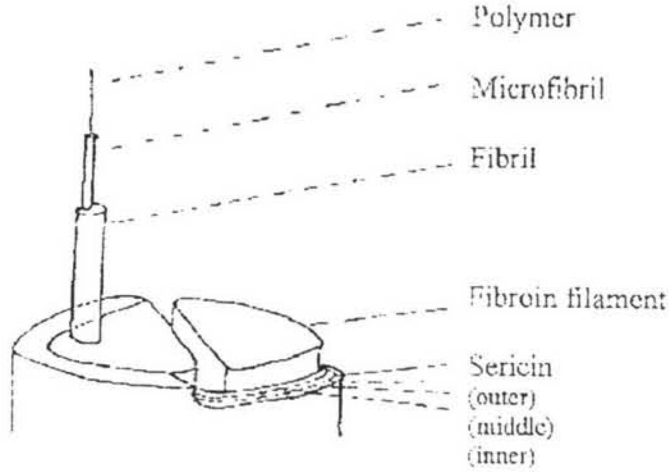

Figure 1.1 Three layers of sericin.

Sericin is a macromolecular protein. Its molecular weight ranges widely from about 10 to over 300 kDa. The sericin protein is made of 18 amino acids most of which have strongly polar side groups such as hydroxyl, carboxyl, and amino groups. In addition, the amino acids e.g. serine and aspartic acid constitute approximately 33.4% wt and 16.7% wt of sericin, respectively.

$$H\text{-}(HN\text{-}CHR\text{-}CO\text{-})_n\text{-}OH$$

Figure 1.2 Chemical structure of protein.

Table 1.1 Amount of the key amino acids in sericin

Amino acid	R	per 100 grams of protein
Serine	CH_2OH	30.1 g
Aspartic acid	CH_2COOH	16.8 g
Glutamic acid	CH_2CH_2COOH	10.1 g
Threonine	$CH(OH)\ CH_3$	8.5 g

Sericin is a water-soluble protein. When sericin is dissolved in a polar solvent, hydrolyzed in acid or alkaline solutions, or degraded by a protease, the size of the resulting sericin molecules depends on factors such as temperature, pH, and the processing time. Low-molecular weight sericin peptides or sericin hydrolysates are used in cosmetics including skincare and haircare products, health products, and medications. High-molecular weight sericin peptides are mostly used as medical biomaterials, degradable biomaterials, compound polymers, functional biomembranes, hydrogels, and functional fibers and fabrics (Zhang, 2002).

This protein can be crosslinked, copolymerized, and blended with other macromolecular materials to produce materials with improved property. Especially synthetic polymers such as polyester, polyamide, polyalcohol (Ishikawa *et al.,* 1987; Wang *et al.*, 1998), polyacrylic acid (Ahn *et al.*, 2001), polyacrylonitrile (Yamada and Fuwa, 1993) and polyurethane (Zhang, 2002) or natural polymers such as chitosan (Nabeshima *et al.*, 1997).

Freeze drying

Freeze drying (also known as Lyophilization) is a dehydration process typically used to preserve a perishable material or make the material more convenient for transport. Freeze drying works by freezing the material and then reducing the surrounding pressure to allow the frozen water in the material to sublimate directly from the solid phase to gas. The process of freeze drying is devided into 3 steps including pre-freezing, primary drying, secondary drying. Pre-freezing is to suddenly reduce temperature to produce ices in material. The material is frozen by internal or external freezing and the thickness of material is about 10 mm. Primary drying changed the frozen material to gas phase under vacuum and high pressure condition. The moisture residues are removed by secondary drying process. The chemical property of freeze drying material is not changed and easy to reconstitute.

Polyacrylamide

Polyacrylamide (PAM) is a synthetic water-soluble polymer. PAM is low toxicity so PAM is a polymer of biomedical and pharmaceutical interest. It is widely studied as hydrogel for blood compatible applications. Moreover, PAM is used in reducing irrigation-induced erosion, treatment waste water, food packaging, adhesives, a boiler water additive, film former in imprinting of soft-shell gelatin capsules, and adjuvants in the manufacturing of paper and paperboard.

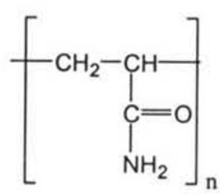

Figure 1.3 Chemical structure of polyacrylamide.

Electrospraying

Electrospray is a method of generating a very fine liquid aerosol through a strong electric field. A liquid is passed through a capillary which is held at high potential. The effect of the high electric field as the solution emerges is to generate a mist of highly charged droplets. During that transition, the droplets reduce in size by evaporation of the solvent. The high charge of droplet leads to disintegration at the "Rayleigh limit" where Columb repulsion overcomes surface tension, modifying the droplet size distribution. These tiny droplets are less than 10 μm in diameter and fly about searching for a potential surface with opposited charge to land on that is opposite in their charge.

The most common electrospray apparatus, a high-voltage power supply is connected to the outlet of the capillary. The capillary is positioned in front of a plate, called a counter-electrode, commonly held at ground potential. When the power supply is turned on and adjusted for the proper voltage, the liquid being pumped through the tube transforms into a fine continuous mist of droplets that fly rapidly toward the counter-electrode. (Gaskell, 1997)

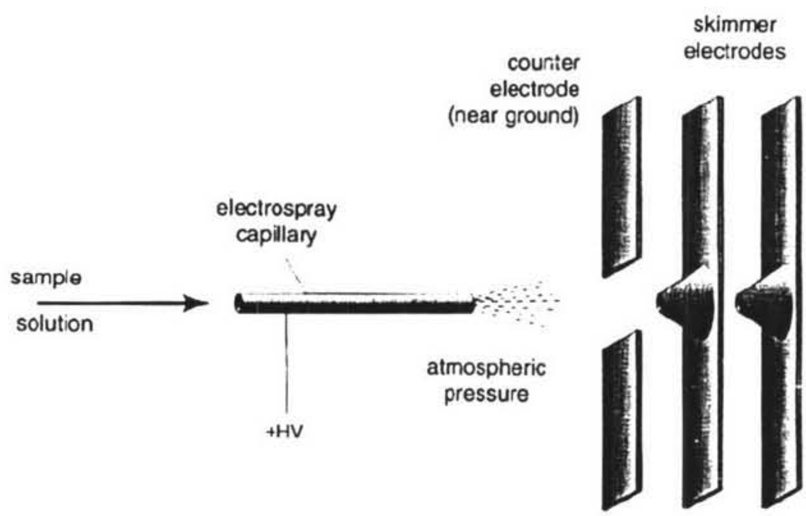

Figure 1.4 Essential features of the electrospray interface.

In general, as the liquid begins to exit the needle, it charges up and assumes a conical shape, referred to as the Taylor cone. At a high enough field, the cone is drawn to a filament as the jet via a budding process. However, this jet becomes unstable, breaking up into the mist of fine droplets. The diameter of the droplet formed is influenced by a number of parameters including the applied potential, the solution flow rate, and solvent properties. Since these droplets are all highly charged with the same electrical charge they repel each other very strongly. Thus the droplets fly apart from each other and cover a wide surface area. If the liquid consists of a polymer melt or a polymer in solution and the concentration of that polymer is sufficiently high to cause molecular chain entanglement, a fiber, rather than a droplet, is drawn from the tip of the Taylor cone.

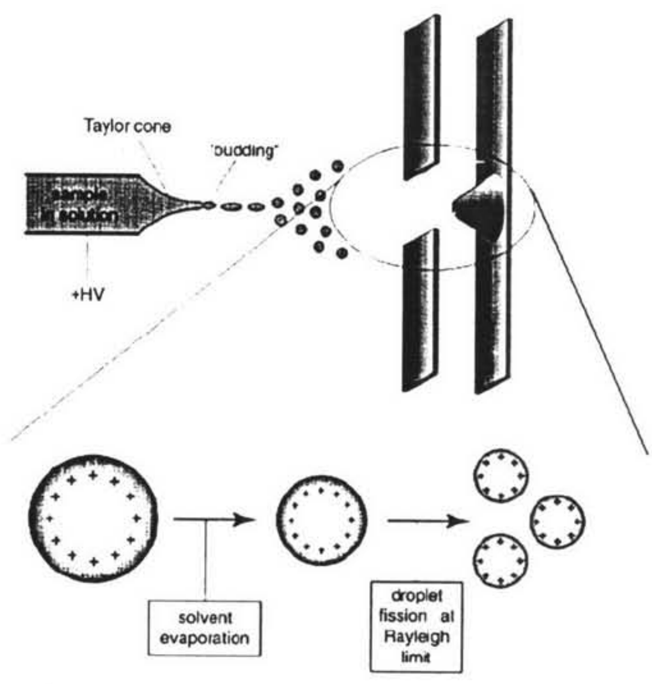

Figure 1.5 Droplet production in the electrospray interface.

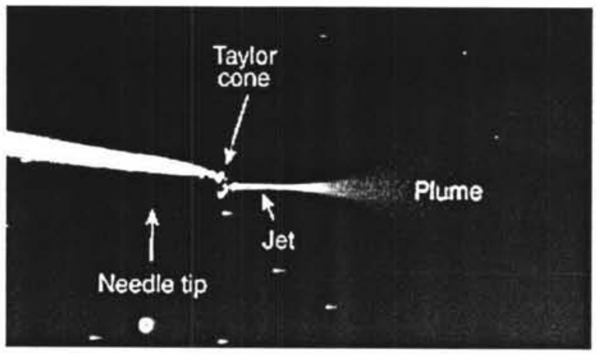

Figure 1.6 The droplets formation of electrospray process.

On the industrial scale, electrospray is used in the application of paints and coatings to metal surfaces. The fine spray results in very smooth even films, with the paint actually attracted to the metal, so the paint material is used more efficiently. This lowers the cost, cuts down on the amount of organic solvents required, and reduces environmental impact.

Electrospinning

Electrospinning is a simple technique for generating nanofibers. A basic electrospinning system consists of a charged polymer solution (or melt) that is fed through a small opening or nozzle (usually a needle or pipette tip). Because of its charge, the solution is drawn toward a grounded collecting plate (usually a metal screen, plate, or rotating mandrel), typically 5–30 cm away, as a jet. During the jet's travel, the solvent gradually evaporates, and a charged polymer fiber is left to accumulate on the grounded target. The charge on the fibers eventually dissipates into the surrounding environment. The resulting product is a non-woven fiber mat that is composed of tiny fibers with diameters between 50 nanometers and 10 microns. The properties of the fibers synthesized by electrospinning was controlled by varying some parameters such as (i) solution parameters including polymer properties (i.e. molecular weight, molecular weight distribution, and architecture of the polymer such as branched or linear chain), solvent properties (i.e. boiling point, density, and dipole moment), and solution properties (viscosity, conductivity, and surface tension), (ii) processing parameters (i.e. applied voltage, collection distance, emitting electrode polarity, and needle size), and (iii) ambient parameters (i.e. temperature and humidity).

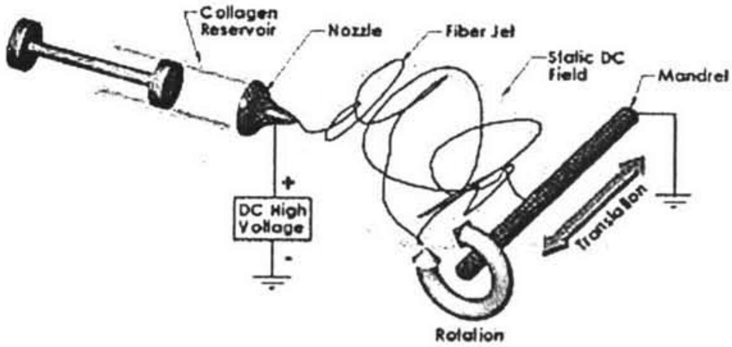

Figure 1.7 Schematic of an electrospinning device.

Typically, electrospinning is applicable to a wide range of polymers like those used in conventional spinning, i.e. polyolefin, polyamides, polyester, aramide, acrylic as well as biopolymers like proteins, DNA, polypeptides, or others like electric conducting and photonic polymers.

Nonwoven mats of nanofibers have found applications in areas such as biomedical structural elements (scaffolding used in tissue engineering, wound dressing, drug delivery, artificial organs, vascular grafts), catalysis and sensing, in fabrication of composite materials, supercapacitors, lithium-ion batteries, protective shields in special fabrics, and filter media for submicron particles in separation industry.