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EFFECTS OF PROCESSING PARAMETERS ON MORPHOLOGY AND DIAMETER OF ELECTROSPUN GELATIN NANOFIBERS

Miss Nuanchan Choktaweesap

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By	Miss Nuanchan Choktaweesap
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Thesis Advisor	Assistant Professor Duangdao Aht-ong, Ph.D.
Thesis Co-advisor	Assistant Professor Pitt Supaphol, Ph.D.

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……………………………………………..Dean of the Faculty of Science
(Professor Piamsak Menasveta, Ph.D.)

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……………………………………………..Thesis Co-advisor
(Assistant Professor Pitt Supaphol, Ph.D.)

……………………………………………..Member
(Associate Professor Paiparn Santisuk)

…………………………………………….. Member
(Assistant Professor Pranut Potiyaraj, Ph.D.)

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เจลาทินเป็นพอลิเมอร์ชีวภาพซึ่งมีความเป็นไปได้ที่จะนำไปประยุกต์กับงานทางด้านการแพทย์ในอนาคตอันใกล้ งานวิจัยนี้จึงเลือกเจลาทินมาผลิตเส้นใยนาโนโดยใช้กระบวนการปั่นเส้นใยด้วยไฟฟ้าสถิตโดยการเตรียมสารละลายเจลาทินในระบบตัวทำละลายเดี่ยวและตัวทำละลายผสมที่อัตราส่วนต่างๆ ก่อนที่จะปั่นเป็นเส้นใยนาโนเจลาทินด้วยกระบวนการปั่นเส้นใยด้วยไฟฟ้าสถิต ตัวทำละลายเดี่ยวที่ใช้คือ กรดแอซิติก และตัวทำละลายผสมที่ใช้ได้แก่ กรดแอซิติก/ไดเมทิลซัลฟอกไซด์ กรดแอซิติก/เอทิลีนไกลคอล กรดแอซิติก/ฟอร์มัลไมด์ และกรดแอซิติก/ไตรฟลูออโรเอทานอล จากนั้นจึงศึกษาผลของพารามิเตอร์ต่างๆ ของสารละลายเจลาทิน ได้แก่ ความเข้มข้นของสารละลายเจลาทิน ชนิดของตัวทำละลาย และสมบัติของสารละลาย (ความหนืด แรงตึงผิว และความนำไฟฟ้า) ซึ่งส่งผลต่อสัณฐานวิทยาและขนาดเส้นผ่านศูนย์กลางของเส้นใยเจลาทิน จากการตรวจสอบสัณฐานวิทยาของเส้นใยที่เตรียมได้ในระบบตัวทำละลายเดี่ยวโดยอาศัยเทคนิคการส่องด้วยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราดพบว่า เมื่อความเข้มข้นของสารละลายเจลาทินในกรดแอซิติกเพิ่มขึ้นจาก 15 ถึง 29 เปอร์เซ็นต์โดยน้ำหนักต่อปริมาตร สามารถผลิตเส้นใยเจลาทินที่มีขนาดเส้นผ่านศูนย์กลางของเส้นใยเพิ่มขึ้นจาก 214 นาโนเมตร เป็น 840 นาโนเมตร ในขณะที่ในระบบตัวทำละลายผสมพบว่าสมบัติของตัวทำละลายมีผลอย่างมากต่อสัณฐานวิทยาและขนาดเส้นผ่านศูนย์กลางของเส้นใยที่ผลิตได้โดยเฉพาะเส้นใยเจลาทินที่เตรียมจากสารละลายผสมระหว่างกรดแอซิติก/ไดเมทิลซัลฟอกไซด์ ที่อัตราส่วนระหว่างกรดแอซิติกต่อไดเมทิลซัลฟอกไซด์เป็น 97:3 ถึง 91:9 จะทำให้เส้นใยเจลาทินที่เตรียมได้มีความเรียบ ปราศจากปม และมีขนาดเส้นผ่านศูนย์กลางเพิ่มขึ้นเมื่อปริมาณไดเมทิลซัลฟอกไซด์ในตัวทำละลายผสมเพิ่มมากขึ้น

ภาควิชา วัสดุศาสตร์ ลายมือชื่อนิสิต……………………..................

สาขาวิชาวิทยาศาสตร์พอลิเมอร์ประยุกต์และเทคโนโลยีสิ่งทอลายมือชื่ออาจารย์ที่ปรึกษา..............................

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4672517723 : MAJOR APPLIED POLYMER SCIENCE AND TEXTILE TECHNOLOGY
KEYWORD: ELECTROSPINNING / GELATIN / NANOFIBERS / BIOPOLYMER

MISS NUANCHAN CHOKTAWEESAP : EFFECTS OF PROCESSING PARAMETERS ON MORPHOLOGY AND DIAMETER OF ELECTROSPUN GELATIN NANOFIBERS. THESIS ADVISOR : ASSISTANT PROFESSOR DUANGDAO AHT-ONG, Ph.D., THESIS CO-ADVISOR : ASSISTANT PROFESSOR PITT SUPAPHOL, Ph.D., 79 pp. ISBN 974-53-1595-8.

Gelatin is a natural biopolymer which has a potential to apply in biomedical field in the near future. Therefore, this research was focused on the preparation of electrospun gelatin nanofibers. Gelatin solutions were prepared in single (acetic acid) and mixed solvent (acetic acid/dimethylsulfoxide; Ac/DMSO, acetic acid/ethylene glycol; Ac/EG, acetic acid/formamide; Ac/F, and acetic acid/trifluoroethanol; Ac/TFE) systems prior to electrospinning. The effects of solution parameters, including gelatin concentration, solvent system and solution properties (e.g. viscosity, surface tension, and conductivity) on appearance of the electrospun webs and diameter of gelatin fibers were examined. The morphological appearance of the as-spun fibers were carefully investigated using scanning electron microscopy. From the results, based on single solvent system, it was found that the average diameter of the as-spun fibers was increased from about 214 nm to about 840 nm upon increasing gelatin concentration from 15%w/v to 29%w/v, respectively. Moreover, it was also found that, for the mixed solvent systems, the morphological appearance of the fibers and the electrospun webs depended very much on the properties of the solvents, and hence of the resulting solutions. Particulary, for the gelatin nanofiber electrospun from Ac/DMSO system at various ratios ranging from 97:3 to 91:9, the obtained fibers were smooth without any beads and their diameter were increased with an increase in the amount of DMSO in the mixed solvent.

Department of Materials Science Student's signature…………………………....
Field of study Applied Polymer Science and Textile Technology Advisor's signature………………………...
Academic year 2004 Co-advisor's signature……………………..

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CHAPTER I

INTRODUCTION

One of the methods of producing fibers with average diameters in the nanometer range is through the process of electrospinning. Electrospinning is a process that uses an electric field to control the formation and deposition of polymers. This process is remarkably efficient, rapid, and inexpensive. In electrospinning, a polymer solution or melt is injected with an electrical potential to create a charge imbalance on surface of polymer source that has a hemispherical shape of pendent drop at the end of capillary tip and placed in proximity to grounded collection plate. At a critical voltage, the charge imbalance on surface begins to overcome the surface tension of the polymer source, forming an electrically charged jet. As the charged jet comes out from the polymer source, it moves as a straight jet for some distance, and then travels in a spiral path, which is proved to be triggered by the electrically-driven bending instability alternatively referred to as whipping instability. The electric field is directed towards a grounded collection plate, during which time the solvent evaporates and fibers are formed.

Gelatin is widely used in food processing. It is a mixture of simple proteins derived from collagen of animal connective tissues through a series of degradation or hydrolytic steps. Collagen, one of the most abundant proteins found in the animal body, is the major component of the white fibers in the connective tissue and is present in skin, sinews, hides, and ossein, the connective tissue protein of bones. Gelatin is a complex mixture of collagen degradative products with a wide range of molecular weights (being about 30,000 to 80,000 Da or greater). The exact sequence of amino acids in the molecular chain is ill-defined, but it has been found that nearly half of the gelatin molecules are comprised of glycine, proline, and hydroxyproline. The chemically-crosslinked gelatin will not dissolve in water when heated. Due to the natural origin, gelatin is useful as a biodegradable polymeric material. Gelatin fibers find specific use as biodegradable sutures, but the properties of the resulting fibers were not good. To explore new possibilities, electrospinning technique can be used to fabricate gelatin into ultrafine fibers, which could be useful for various biomedical applications in the future.

This work can be divided into two parts. In the first part, single solvent systems were explored for electrospinning of gelatin to study the effects of gelatin concentration on the morphology and average diameter of electrospun gelatin nanofiber. For the second part, the effects of mixed solvent systems on the morphology of electrospun gelatin nanofiber were studied. Moreover, in both systems, the ratios of voltage per distance between the needle tip to the collection plate were varied in order to investigate the effect of voltage per distance on the morphology of electrospun gelatin nanofibers.

Therefore, the objectives of this research are to investigate the effect of solvent parameters, including the gelatin concentration and solution properties and processing parameter (the ratio of voltage per distance or electric field) on morphology of the gelatin electrospun webs.

CHAPTER II

THEORY AND LITERATURE SURVEY

2.1 Gelatin [1]

Gelatin is a protein obtained by partial hydrolysis of collagen, the chief protein component in skin, bones, hides, and white connective tissues of the animal body. Type A gelatin is produced by acid processing of collagenous raw material; type B is produced by alkaline or lime processing. Because it is obtained from collagen by a controlled partial hydrolysis and does not exist in nature, gelatin is classified as a derived protein. Animal glue and gelatin hydrolysate, sometimes referred to as liquid protein, are products obtained by a more complete hydrolysis of collagen and thus can be considered as containing lower molecular-weight fractions of gelatin.

Use of animal glues was first recorded ca 4000 BC in ancient Egypt. Throughout subsequent centuries, glue and crude gelatin extracts with poor organoleptic properties were prepared by boiling bone and hide pieces and allowing the solution to cool and gel. Late in the seventeenth century, the first commercial gelatin manufacturing began. At the beginning of the nineteenth century, commercial production methods were gradually improved to achieve the manufacture of high molecular weight collagen extracts with good quality that form characteristic gelatin gels.

Uses of gelatin are based on its combination of properties; reversible gel-to-sol transition of aqueous solution; viscosity of warm aqueous solutions; ability to act as a protective colloid; water permeability; and insolubility to cold water, but complete solubility in hot water. It is also nutritious. These properties are utilized in the food, phramaceutical, and photographic industries. In addition, gelatin forms strong, uniform, clear, moderately flexible coatings which readily swell and absorb water and are ideal for the manufacture of photographic films and pharmaceutical capsules.

2.1.1 Chemical Composition and Structure

Gelatin is not a single chemical substance. The main constituents of gelatin are large and complex polypeptide molecules of the same amino acid composition as parent collagen, covering a broad molecular weight distribution range. In the parent collagen, the 18 different amino acids are arranged in orderd, long chains, each having ~95,000 mol wt. These chains are arranged in a rod-like, triple-helix structure consisting of two identical chains, called α_1, and one slightly different chain called α_2. These chains are partially seperated and broken, i.e., hydrolyzed, in the gelatin manufacturing process. Different grades of gelatin have average molecular weight ranging from ~20,000 to 250,000. Molecular weight distribution studies have been carried out by fraction precipitation with ethanol or 2-propanol and by complexing with anionic detergent molecules. The coacervates are isolated and recovered as gelatin fractions.

Analysis shows the presence of amino acids from 0.2% tyrosine to 30.5% glycine. The five most common amino acids are glycine, 26.4-30.5%; proline, 14.8-18%; hydroxyproline, 13.3-14.5%, glutamic acid, 11.1-11.7%; and alanine, 8.6-11.3%. The remaining amino acids in decreasing order are arginine, aspartic acid, lysine, serine, leucine, valine, phenylalanine, threonine, isoleucine, hydroxylysine, histidine, methionine, and tyrosine. Table 2.1 shows name and chemical structure of 18 different kinds of amino acids in gelatin. Warm gelatin solutions are more levorotatory than expected on the basis of the amino acid composition, indicating additional order in the molecule, which probably results from Gly-Pro-Pro and Gly-Pro-Hypro sequences as show in Figure 2.1. The α-chain form of gelatin behaves in solution like a random-coil polymer, whereas the gel form may contain as much as 70% helical conformation. The remaining molecules in nonhelical conformation link helical regions together to form the gel matrix. Helical regions are thought to contain both inter- and intramolecular associations of chain segments.

Table 2.1 Name and chemical structure of amino acids in gelatin

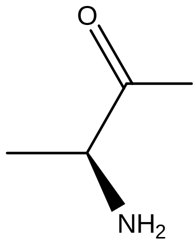
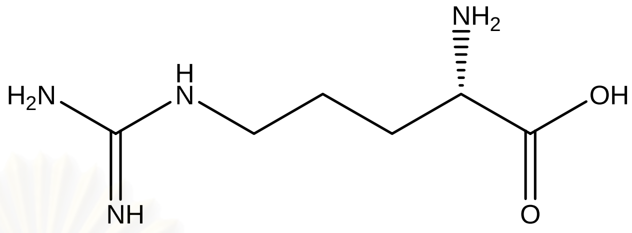
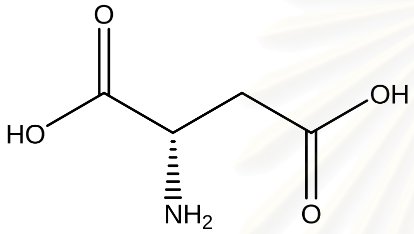
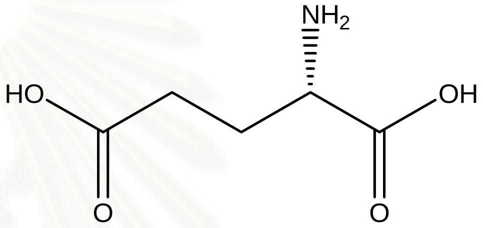
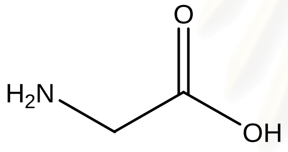
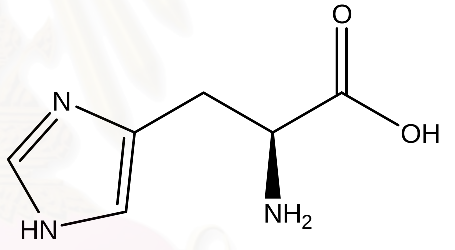
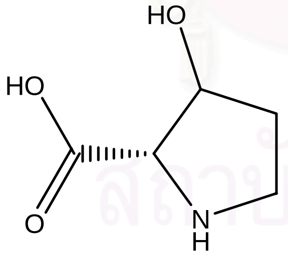
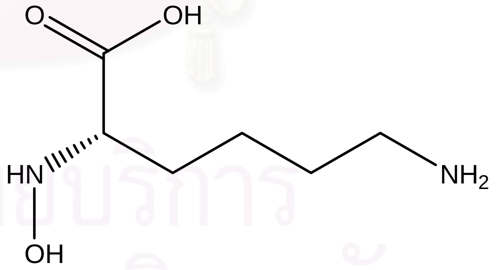
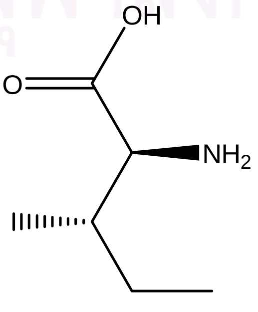
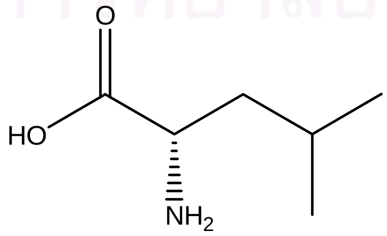
Structure of amino acids	Structure of amino acids
 alanine	 arginine
 aspartic acid	 glutamic acid
 glycine	 histidine
 hydroxyproline	 hydroxylysine
 isoleucine	 leucine

Table 2.1 (cont.) Name and chemical structure of amino acids in gelatin

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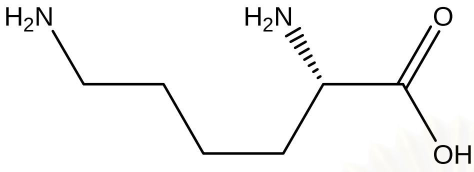
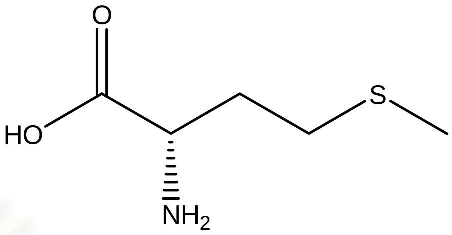
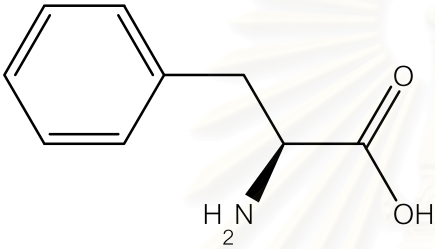
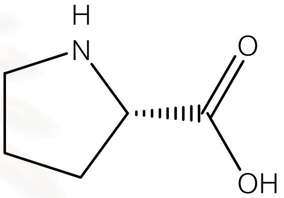
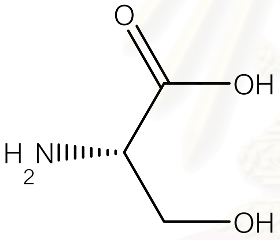
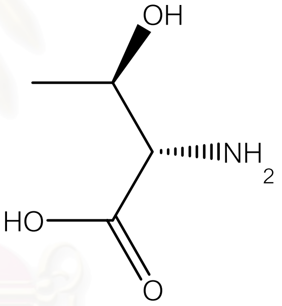
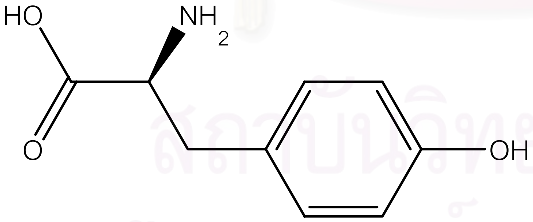
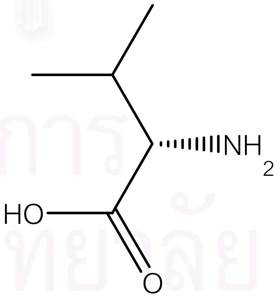
 lysine	 methionine
 phenylalanine	 proline
 serine	 threonine
 tyrosine	 valine

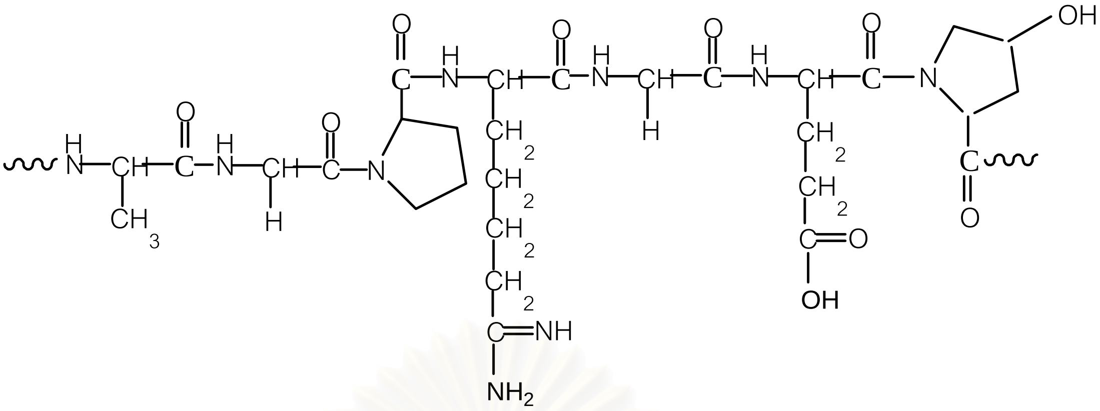


Figure 2.1 The general structure of gelatin is -Ala-Gly-Pro-Arg-Gly-Glu-4Hyp-Gly-

Gelatin structures have been studied with the aid of an electron microscope. The structure of the gel is a combination of fine and coarse interchain networks; the ratio depends on the temperature during the polymer-polymer and polymer-solvent interaction leading to bond formation. The rigidity of the gel is approximately proportional to the square of the gelatin concentration. Crystallites, indicated by x-ray diffraction pattern, are believed to be at the junctions of the polypeptide chains.

Homogeneous α-chain gelatin has been prepared by pretreating collagen with pronase in the presence of 0.4 M $CaCl_2$, and extracting the gelatin with hot water at 80°C and pH 7.0 after inactivating the enzyme and removing the salts.

2.1.2 Physical and Chemical Properties

Commercial gelatin is produced in mesh sizes ranging from coarse granules to fine powder. In Europe, gelatin is also produced in thin sheets for use in cooking. It is a vitreous, brittle solid, faintly yellow in color. Dry commercial gelatin contains about 9-13% moisture and is essentially tasteless and odorless with specific gravity between 1.3 and 1.4. Most physical and chemical properties of gelatin are measured on aqueous solution and are functions of the source of collogen, method of manufacture, conditions during extraction and concentration, thermal history, pH, and chemical nature of impurities or additives.

2.1.2.1 Gelatin. Perhaps the most useful property of gelatin solution is its capability to form heat reversible gel-sols. When an aqueous solutionof gelatin with a concentration greater than about 0.5% is cooled to about 35 to 40°C, it first increases in viscosity, then forms a gel. The gelation process is thought to proceed through three stages:

a) rearrangement of individual molecular chains into ordered, helical arrangement, or collagen fold;
b) association of two or three ordered segments to create crystallites; and
c) stabilization of the structure by lateral interchain hydrogen bonding within the helical regions.

The rigidity or jelly strength of the gel depends on the concentration, the intrinsic strength of the gelatin sample, pH, temperature, and additives.

Because the economic value of gelatin is commonly determined by jelly strength, the test procedure for its determination is of great importance. Commercially, gelatin jelly strength is determined by standard tests which measure the force required to depress the surface of a carefully prepared gel by a distance of 4 mm using a flat-bottomed plunger 12.7 mm in diameter. The force applied may be measured in the form of the quantity of fine lead shot required to depress the plunger and is recorded in grams. The measurement is termed the bloom strength after the inventor of the lead shot device. In the early 1990s, sophisticated testing equipment utilizing sensitive load cells for the measurement are commonly used.

The conversion temperature for gelatin is determined as setting point, i.e., sol to gel, or melting point, i.e., gel to sol. Commercial gelatins melt between 23 and 30°C, with the setting point being lower by 2-5°C. Melting point determination utilizes test tubes filled with gelatin solution that are gently chilled to form a gel. The tubes are tilted and colored carbon tetrachloride solution is placed on the gelatin surface. The tube is gradually warmed and the end point is determined when the descent of the colored solution is observed. Several methods have been used to determine the setting point of gelatin. Properties of gelatin are shown in Table 2.2.

Table 2.2 Typical property values of gelatin

Properties	Value
Density,dry (g/cm^3)	1.35
Refractive index,dry at 546.1 nm	1.54
Weight average MW, M_w	$1x10^5$ to $5x10^5$
Number average MW, M_n	$5x10^4$ to $5x10^5$
Viscosity 6.67 wt%, 60°C, mPa.s	5-10
Gel rigidity, g Bloom	75-330
Specific optical rotation at 589 nm, degrees	(-134)-(-140)
Refractive increment at 300 nm, dn/dv	0.187
Glass transition, dry, °C	217
Moisture, wt% at 50% RH	9-15
Ash, wt%	0-3
Isoionic pH -Type B	4.8-5.2
-Type A	7-9
Nitrogen, wt% -Type B	18.15
-Type A	18.30
Hexose sugars, wt%	0.45-0.65

2.1.2.2 Solubility. In most commercial applications, gelatin is used as a solution. Gelatin is soluble in water and in aqueous solutions of polyhydric alcohols such as glycerol and propylene glycol. Examples of highly polar, hydrogen-bonding organic solvents in which gelatin dissolves are acetic acid, trifluoroethanol, and formamide. Gelatin is practically insoluble in less polar organic solvents such as acetone, carbon tetrachloride, ethanol, ether benzene, dimethylformamide, and most other nonpolar organic solvents. Many water-soluble organic solvents are compatible with gelatin, but interfere with gelling properties. Dry gelatin absorbs water exothermally. The rate and degree of swelling is a characteristic of the particular gelatin. Swelled gelatin granules dissolve rapidly in water above 35°C. The crosslinking of gelatin matrix by chemical means is used extensively in photographic products, and this so-called hardening permanently reduces the solubility of gelatin.

2.1.2.3 Amphoteric Character. The amphoteric character of gelatin is due to the functional groups of the amino acids and the terminal amino and carboxyl groups created during hydrolysis. In strongly acidic solution, the gelatin is positively charged and migrates as a cation in an electric field. In strongly alkaline solution, it is negatively charged and migrates as an anion. The intermediate point, where net charge is zero and no migration occurs, is known as the isoelectric point (IEP) and is designated in pH units. A related property, the isoionic point, can be determined by utilizing a mixed-bed ion-exchange resin to remove all nongelatin cations and anions. The resulting pH of the gelatin solution is the isoionic point and is expressed in pH units. The isoionic point is reproducible, whereas the isoelectric point depends on the salts present. Type A gelatin has a broad isoionic region between pH 7 and pH 10; type B is in a lower, more reproducible region, reaching an isoionic point of 5.2 after 4 weeks of liming, which drops to 4.8 after prolonged or more vigorous liming processes. The isoelectric point can also be estimated by determining a pH value at which a gelatin solution exhibits maximum turbidity. Many isoionic point references are recorded as isoelectric points even though the letter is defined as a pH at which gelatin has net charge of zero and thus shows no movement in the electric field.

2.1.2.4 Viscosity. The viscosity of gelatin solutions is affected by gelatin concentration, temperature, molecular weight of the gelatin sample, pH, additives, and impurities. In aqueous solution above 40°C, gelatin exhibits Newtonian behavior. Standard testing methods employ use of a capillary viscometer at 60°C and gelatin solutions at 6.67 or 12.5% solids. The viscosity of gelatin solutions increases with increasing gelation concentration and with decreasing temperature. For a given gelatin, viscosity is at a minimum at the isoionic point and reaches maximum at pH values near 3 and 10.5. At temperatures between 30 and 40°C, non-Newtonian behavior is observed, propably due to linking together of gelatin molecules to form aggregates. Addition of salts decreases the viscosity of gelatin solutions. This effect is most evident for concentrated gelatin solutions.

2.1.2.5 Colloid and Emulsifying Properties. Gelatin is an effective protective colloid that can prevent crystal, or particle, aggregation, thereby stabilizing a heterogeneous suspension. It acts as an emulsifying agent in cosmetics and pharmaceuticals involving oil-in-water dispersions. The anionic or cationic behavior of gelatin is important when used in conjunction with other ionic materials. The protective colloid property is important in photographic applications where it stabilizes and protects silver halide crystals while still allowing for their normal growth and sensitization during physical and chemical ripening processes.

Coacervation. A phenomenon associated with colloids wherein dispersed particles separate from solution to form a second liquid phase is termed coacervation. Gelatin solutions form coacervates with the addition of salt such as sodium sulfate, especially at pH below the isoionic point. In addition, gelatin solutions coacervate with solutions of oppositely charged polymers or macromolecules such as acacia. This property is useful for microencapsulation and photographic applications.

Stability. Dry gelatin stored in airtight containers at room temperature has a shelf life of many years. However, it decomposes above 100°C. For complete combustion, temperatures above 500°C are required. When dry gelatin is heated in air at relatively high humidity, ≥60% rh, and at moderate temperatures, i.e., above 45°C, it gradually loses its ability to swell and dissolve. Aqueous solutions or gels of gelatin are highly susceptible to microbial growth and breakdown by proteolytic enzymes. Stability is function of pH and electrolytes and decreases with increasing temperature because of hydrolysis.

Swelling. The swelling property of gelatin is not only important in its solvation but also in photographic film processing and the dissolution of pharmaceutical capsules. That pH and electrolyte content affect swelling has been explained by the sample Donnan equilibrium theory, treating gelatin as a semipermeable membrane. This explains why gelatin exhibits the lowest swelling at its isoelectric pH. At pH below the isoelectric point, proper choice of anions can control swelling, whereas above the isoelectric point, cations primarily affect swelling. These effects probably involve breaking hydrogen bonds, resulting in increased swelling. The rate of swelling follows approximately a second-order equation. In photographic products, the swelling of the gelatin layer is controlled by coating conditions, drying conditions, chemical cross-linking, and the composition of the processing solutions. Conditioning at 90% rh and 20°C for 24 h greatly reduces swelling of hot dried film coatings. The ratio of lateral to vertical swelling is of great concern in the photographic industry since it can cause curling of photographic papers or films when changes in humidity or general moisture content take place.

2.1.3 Manufacture and Processing

Although new methods for processing gelatin, including ion exchange and crossflow membrane filtration, have been introduced since 1960, the basic technology for modern gelatin manufacture was developed in the early 1920s. Acid and lime processes have seperate facilities and are not interchangeable. In the past, bones and ossein, i.e., decalcified bone, have been supplied by India and South America. In the 1990s, slaughterhouses and meat-packing houses are an important source of bones. The supply of bones has been greatly increased since the meat-packing industry introduced packaged and fabricated meats, assisted by the growth of fast-food restaurants. Dried and rendered bones yield about 14-18% gelatin, whereas pork skins yield about 18-22%.

Most type A gelatin made from pork skins yielding grease as a marketable by-product. The process includes macerating of skins, washing to remove extraneous matter, and swelling for 10-30 hours in 1-5% hydrochloric, phosphoric, or sulfuric acid. Then four to five extractions are made at temperatures increasing from 55-65°C for the first extract to 95-100°C for the last extract. Each extraction lasts about 4-8 hours. Grease is then removed, the gelatin solution filtered, and, for most applications, deionized. Concentration to 20-40% solids is carried out in several stages by continuous vacuum evaporation. The viscous solution is chilled, extruded into thin noodles, and dried at 30-60°C on a continuous wire-mesh belt. Drying is completed by passing the noodles through zones of successive temperature changes wherein conditioned air blows across the surface and through the noodle mass. The dry gelatin is then ground and blended to specification.

Type B gelatin is made mostly from bones, but also from bovine hides and pork skins. The bones for type B gelatin are crushed and degreased at the rendering facilities, which are usually located at a meat-packing plant. Rendered bone pieces, 0.5-4 cm, with less than 3% fat, are treated with cool, 4-7% hydrochloric acid from 4 to 14 days to remove the mineral content. An important by-product, dibasic calcium phosphate, is precipitated and recovered from the spent liquor. The demineralized bones, i.e., ossein, are washed and transferred to large tanks where they are stored in a lime slurry with gentle daily agitation for 3-16 weeks. During the liming process, some deamination of the collagen occurs with evolution of ammonia. This is the primary process that results in low isoelectric ranges for type B gelatin. After washing for 15-30 hours to remove the lime, the ossein is acidified to pH 5-7 with an appropriate acid. Then the extraction processing for type A gelatin is followed. Throughout the manufacturing process, cleanliness is important to avoid contamination by bacteria or proteolytic enzymes.

Bovine hides and skins are substantial sources of raw material for type B gelatin and are supplied in the form of splits, trimmings of dehaired hide, raw hide pieces, or salted hide pieces. Like pork skins, the hides are cut to smaller pieces before being processed. Sometimes the term calfskin gelatin is used to describe hide gelatin. The liming of hides usually takes a little longer than the liming of ossein from bone.

Most manufacturing equipment should be made of stainless steel. The liming tanks, however, can be either concrete or wood. Properly lined iron tanks are often used for the washing and acidification, i.e., souring, operations. Most gelatin plants achieve efficient processes by operating around the clock. The product is tested in batches and again as blends to confirm conformance to customer specifications.

2.1.4 Economic Aspects

World gelatin production in 1993 was believed to be about 200,000 ton. The United States produced about 30,000 ton, followed by France, Germany, Japan, Brazil, and Mexico. Of the gelatin produced in the United States, 55% is acid processed, i.e., type A. The U.S. food industry consumes about 20,000 t/yr; with an annual growth rate of 0.5%; the pharmaceutical industry consumes about 10,000 t/yr;and the photographic industry about 7,000 ton/year. In the United States, the pharmaceutical gelatin market is expected to grow on the average of 2.5% per year. The photographic gelatin market has been stable or growing slightly. Color paper and X-ray products use over 55% of the photographic gelatin in the United States, with graphic arts and instant films using an additional 30%.

2.1.5 Analytical Test Methods and Quality Standards

Gelatin is identified by a positive test for hydroxyproline, turbidity with tannic acid, or a yellow precipitate with acidic potassium dichromate or trinitrophenol. An 5% aqueous solution exhibits reversible gel-to-sol formation between 10 and 60°C. Gelatin gives a positive color test for aldehydes and sugars that are considered undesirable impurities in photographic gelatin; nucleic acids are considered restrainers in photographic gelatins and their concentration is monitored closely for this application. Elemental analysis of commercial gelatin is reported as carbon, 50.5%; hydrogen, 6.8%; nitrogen, 17%; and oxygen 25.2%; a purer sample analyzed for 18.2-18.4% nitrogen. Regulations for quality standards vary from country to country, but generally include specifications for ash content, SO_2, heavy metals, chromium, lead, fluoride, arsenic, odor, and for the color or clarity of solutions. In addition, certain bacteriological standards, including *E. coli* and *Salmonella*, are specified. Restrictions on certain additives and preservatives are also listed. In United States, the *Food Chemicals Codex* has been considering a new specification for food-grade gelatin; a final version should be issued soon. Standard testing procedures for viscosity, pH, ash, moisture, heavy metals, arsenic, bacteria, and jelly strength are described. Additional test procedures have been published by the photographic and gelatin industries including the Japanese PAGI method. Specific tests for photographic gelatin have been devised by the International Working Group for Photographic Gelatin (IAG) in Fribourg, Switzerland, and by individual photographic companies and gelatin companies.

2.1.6 Used

2.1.6.1 Food Products. Gelatin formulations in the food industry use almost exclusively water or aqueous polyhydric alcohols as solvents for candy, marshmallow, or dessert preparations. In dairy products and frozen foods, gelatin's protective colloid property prevents crystallization of ice and sugar. Gelatin products having a wide range of bloom and viscosity values are utilized in the manufacture of food products, specific properties being selected depending on the needs of the application. For example, a 250-bloom gelatin may be utilized at concentrations ranging from 0.25% in frozen pies to 0.5% in ice cream; the use of gelatin in ice cream has greatly diminished. In sour cream and cottage cheese, gelatin inhibits water seperation, i.e., syneresis. Marshmallows contain as much as 1.5% gelatin to restrain the crystallization of sugar, thereby keeping the marshmallows soft and plastic; gelatin also increases viscosity and stabilizes the foam in the manufacturing process. Many lozenges, wafers and candy coatings contain up to 1% gelatin. In these instances, gelatin decreases the dissolution rate. In meat products, such as canned hams, various luncheon meats, corned beef, chicken rolls, jellied beef, and other similar products, gelatin in 1-5% concentration helps to retain the natural juices and enhance texture and flavor. Use of gelatin to form soft, chewy candies, so-called gummi candies, has increased worldwide gelatin demand significantly. Gelatin has also found new uses as an emulsifier and extender in the production of reduced-fat margarine products. The largest use of edible gelatin in the United States, however, is in the preparation of gelatin desserts in 1.5-2.5% concentrations. For this use, gelatin is sold either premixed with sugar and flavorings or as unflavored gelatin packets. Most edible gelatin is type A, but type B is also used.

2.1.6.2 Pharmaceutical Products. Gelatin is used in the pharmaceutical industry for the manufacture of soft and hard capsules. The formulations are made with water or aqueous polyhydric alcohols. Capsules are usually preferred over tablets in administering medicine. Elastic or soft capsules are made with a rotary die from two plasticized gelatin sheets which form a sealed capsule around the material being encapsulated. Methods have been developed to encapsulate dry powders and water-soluble materials which may first be mixed with oil. The gelatin for soft capsules is low bloom type A, 170-180 g; type B, 150-175 g; or a mixture of type A and B. Hard capsules consisting of two parts are first formed and then filled. The manufacturing process is highly mechanized and sophisticated in order to produce capsules of uniform capacity and thickness. Medium-to-high bloom type A, 250-280 g; type B, 225-250 g; or the combination of type A and B gelatin are used for hard capsules. Usage of gelatin as a coating for tablets has increased dramatically. In a process similar to formation of gelatin capsules, tablets are coated by dipping in colored gelatin solutions, thereby giving the appearance and appeal of a capsule, but with some protection from adulteration of the medication. The use of glycerinated gelatin as a base for suppositories offers adventages over carbowax or cocoa butter base. Coated or cross-linked gelatin is used for enteric capsules. Gelatin is used as a carrier or binder in tablets, pastilles, and troches.

For arresting hemorrhage during surgery, a special sterile gelatin sponge known as absorbable gelatin sponge or Gelfoam is used. The gelatin is partially insolubilized by a crosslinking process. When moistened with a thrombin or sterile physiological salt solution, the gelatin sponge, left in place after bleeding stops, is slowly dissolved by tissue enzymes. Special fractionated and prepared type B gelatin can be used as a plasma expander.

Gelatin can be a source of essential amino acids when used as a diet supplement and therapeutic agent. As such, it has been widely used in muscular disorders, peptic ulcers, and infant feeding, and to spur nail growth. Gelatin is not a complete protein for mammalian nutrition, however, since it is lacking in the essential amino acid tryptophan and is deficient in sulfur-containing amino acids.

2.1.6.3 Photographic Products. Gelatin has been used for over 100 years as a binder in light-sensitive products. The useful functions of gelatin in photographic film manufacture are a result of its protective colloidal properties during the precipitation and chemical ripening of silver halide crystals, setting and film-forming properties during coating, and swelling properties during processing of exposed film or paper. Quality requirements of photographic gelatin may be very elaborate and can include over 40 chemical and physical tests, in addition to photographic evaluation. Most chemical impurities are limited to less than 10 ppm. Aqueous solutions are employed for emulsions. Photographic gelatins are manufactured to standard specifications since the testing is time-consuming and costly. A new gelatin product may require 6-12 months of testing, including extensive field testing prior to commercialization. Photographic products may have up to twenty gelatin layers grouped into three categories:

a) light-sensitive silver halide-bearing layers of 2-10 μm thickness, referred to as emulsion layers;
b) surface, spacer, filter, or protective layers of 1-2 μm thickness; and
c) backing antihalo, or noncurl layers coated on the opposite side of the film substrate from the emulsion layer.

The quality and uniformity standards are highest for emulsion gelatin because it controls silver halide nucleation, crystal growth, chemical sensitization, latent image stability, and numerous other factors affecting the total photographic response. Since the early 1970s, the photographic industry has switched from so-called active gelatins derived from hides to inert types derived from bones. The latter are very low or void of natural restrainers, reduction, and sulfur sensitizers. Other changes in techniques have been brought about by abandoning the lengthy noodle wash technique used to remove salts after silver halide precipitation in favor of precipitating, coagulating, or derivatizing gelatin and washing the precipitate by decanting or utilizing ultrafiltration techniques; by new coating techniques that allow simultaneous coating of several layers at one time at speeds 10 times as fast as before; and by short-time high temperature processing which may require new crosslinking agents unlike the aldehydes and metal salts previously used. Many new hardeners are extremely fast-acting and are metered into the solution during the coating operation. It is quite common to use a derivatized gelatin, such as phthalated gelatin, to precipitate silver halide. These materials with a low pH isoionic point form a coacervate at pH <4.0. Precipitation in this case is accomplished by lowering the pH, washing at low pH, and then increasing pH to above 6.5 to dissolve and redisperse the emulsion before reconstituting it with gelatin. Gelatin used in the auxiliary layers must be able to withstand high temperature processing and allow high speed coating.

Gelatin is also used in so-called subbing formulations to prepare film bases such as polyester, cellulose acetate, cellulose butyrate, and polyethylene-coated paper base for coating by aqueous formulations. Solvents such as methanol, acetone, or chlorinated solvents are used with small amounts of water. Gelatin containing low ash, low grease, and having good solubility in mixed solvents is required for these applications. In certain lithographic printing, light-sensitive dichromate; gelatin is used. Light causes permanent crosslinking of gelatin in the present of the dichromate; this phenomenon is used to make relief images for printing. Dichromated gelatin coatings are commonly used in production of high quality holographic images. In this application, the light sensitivity of the image-receiving medium is less important than the image-resolving power. Gelatin coatings in photographic products are further tested for brittleness, scratch resistance, friction, swelling rate, drying rate, curling tendency, dry adhesion, wet adhesion, and pressure sensitivity. These properties are becoming more critical with the development of more sophisticated cameras and printing and processing equipment. Photographic technology offers a rapidly changing, highly sophisticated, very competitive market for photographic gelatin manufacturers.

2.1.6.4 Derivatized Gelatin. Chemically active groups in gelatin molecules are either the chain terminal groups or side-chain groups. In the process of modifying gelatin properties, some groups can be removed, e.g., deamination of amino groups by nitrous acid, or removal of guanidine groups from arginine by hypobromite oxidation; the latter destroys the protective colloid properties of gelatin. Commercially successful derivatized gelatins are made mostly for the photographic gelatin and microencapsulation markets. In both instances, the amino groups are acylated. Protein detergent is made by lauroylating gelatin. Phthalated gelatin is now widely used in the photographic industry. Arylsulfonylated gelatin has been patented for microencapsulation. Carbamoylated gelatin, made by treating gelatin with cyanate or nitrourea in neutral aqueous solution, is also used by the photographic industry. Active double bonds react with the amino groups in gelatin, and acrylic polymers have been grafted to gelatin. Gelatin has been derivatized by epoxides, cyclic sulfones, and cyanamide. Crosslinking or hardening of gelatin attacks the same active groups, but an agent with two active sites is needed, e.g., divinylsulfone, bis(isomaleimide), aziridines, bisepoxides, epichlorohydrin, polyisocyanates, and dichlorotriazine. Aldehydes such as formaldehyde and glyoxal are still used and to a small extent even potassium chromium alum, $Cr_2K_2(SO_4)_4 \bullet 24H_2O$, and potassium aluminum alum, $Al_2K_2(SO_4)_4 \bullet 24H_2O$.

2.2 Electrospinning Theory [2]

In electrospinning process a high voltage is used to create an electrically charged jet of polymer solution or melt out of the pipette. One electrode is placed into the spinning solution or melt and the other attached to the collector. A pendent droplet of polymer solution was supported by surface tension at the tip of the spinneret. When the electrical potential difference between the spinneret and the grounded collector was increased, the motion of ions through the liquid charged the surface of the liquid. The electrical forces at the surface overcame the forces associated with surface tension. A liquid jet emerged from a conical protrusion that formed on the surface of the pendent droplet. The jet was electrically charged. It carried away the ions that were attracted to the surface when the potential was applied. Increasing the potential increased both the charge density on the jet and the flow rate of the jet. From Figure 2.2 shows a sequence of droplet shapes taken at a frame rate of 500 frames per second and a shutter speed 2 ms.

The solution flowed through a hole in the bottom of the bowl of a metal spoon that was shown in Figure 2.3. The electric force was high enough to overcome both surface tension and viscoelastic forces, and a fluid jet was ejected. Jet diameters near the droplet were in the range from micro to nanometer. After the charged jet was ejected, the conical protrusion relaxed to a rounded shape, reaching a steady shape in a few milliseconds. A jet can also be initiated, at a lower potential, by mechanically pulling a jet out of the pendent droplet, since the voltage required for initiation was higher than that required for maintaining the jet flow. The observed semi-vertex angle as the jet emerged from the droplet was less than the 49.3° that Taylor calculated. Sometimes the jet current and the shape of the droplet pulsated while the applied voltage was constant. A steady current was associated with a droplet that had a constant shape.

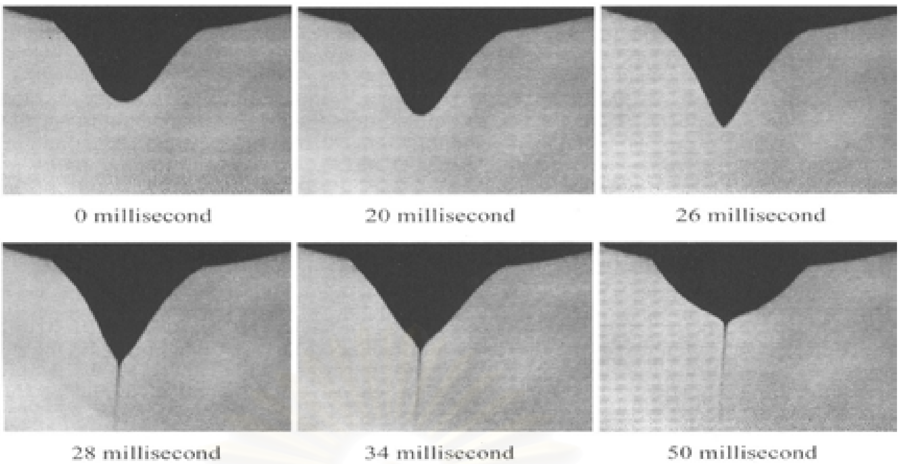
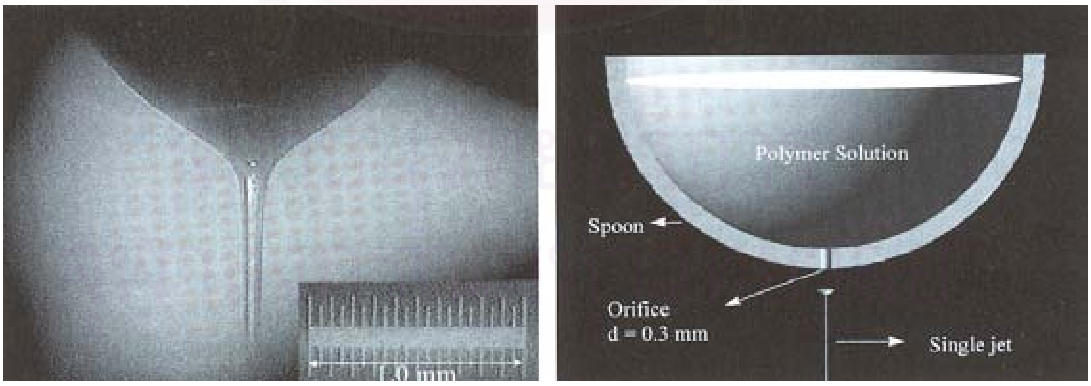


Figure 2.2 Photographs of the pendent droplet and jet, near the time the jet wasejected

The jet diameter decreased with the distance from the orifice. Higher electric field and a lower surface tension coefficient favored the formation of a thicker jet. Addition of salt to the solution, with other parameters held constant, reduced the diameter of the jet. Increasing the viscosity of the solution did not always increase the jet diameter. The largest jet diameter occurred when the solution viscosity was in a medium range. Both higher and lower viscosity favored a thinner jet.



(a) (b)

Figure 2.3 (a) Magnified image of the shape of the jet near the spoon
(b) Schematic drawing of a jet flowing from a hole in the bowl of a spoon.

After initiation, the path of the jet was straight for a certain distance. Then, an electrically driven bending instability grew at the bottom end of the straight segment. The bending allowed a large elongation to occurred in self-similar cycles. Each cycle had three steps and was smaller in scale than the proceeding cycle.

The three steps in each cycle were:

Step 1. A smooth segment that was straight or slightly curved suddenly developed an array of bends.

Step 2. As the segment of the jet in each bend elongated, the linear array of bends became a series of spiraling loops with growing diameters.

Step 3. As the perimeter of each loop increased, the cross-sectional diameter of the jet forming the loop grew smaller, and the conditions for Step 1 were established everywhere along the loop.

After the first cycle, the axis of a particular segment might lie in any direction. The continuous elongation of each segment was most strongly influenced by the repulsion between the charges carried by adjacent segments of the jet. The externally applied field, acting on the charged jet, caused the entire jet to drift towards the collector, which was maintained at an attractive potential.

From Figure 2.4 shows two cycles of bending instability. The jet entered the image near the end of the straight segment, where the first electrically driven bending instability produced an array of helical bends. While the jet ran continuously, it shifted through a series of similar but changing paths. Most loops moved downward at a velocity of about 1 m/s, but some loops with larger diameters remained in the field of view for long time. The slightly curved thin segment that runs horizontally cross the left image in Figure 2.4 is part of such a loop that remained in view for long time.

The slightly curved thin segment that runs horizontally across the lift image in Figure 2.4 is part of such a loop that remained in view for over 15ms. This segment was smooth unit, in a time interval of only one ms, the bends and loops shown in the right image of Figure 2.4 eveloped. During this 15 ms period, many bends and loops of the first cycle of bending instability formed and moved downward through the field of view. The diameter of every segment of the jet became smaller, and the length of every segment increased. The loops grew larger. Bending instabilities developed and grew.

It was often possible to follow the evolution of the shape of segments, such as those shown in the right part of Figure 2.4 back to the time at which they entered the upper left corner of image, by stepping backwards in time through the image files created by the electronic camera. It was more difficult to follow the evolution of the jet into the third cycle of bending because the images of the path grew fainter as the jet became thinner, and soon were ambiguous. The elongation and the associated thinning of the jet continued as long as the charge on the jet supplied enough force to overcome the surface tension and viscoelastic forces. Meanwhile, the elongational viscosity increased as the solvent evaporated and eventually the elongation stopped. Details of the evolution of the solidification process remain to be investigated.

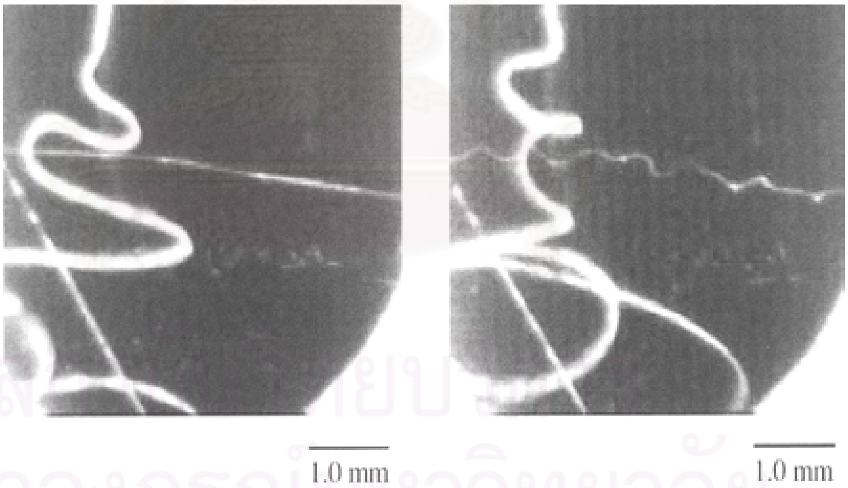


Figure 2.4 The development of the second cycle of bending instability. The time interval between these two images was 15 ms. The camera shutter speed was 0.25 ms.

The “area reduction ratio”, which was defined as the ratio of the cross-sectional area of the upper end of a segment to the cross-sectional area at the lower end of the same segment, was equal to the draw ratio if the volume of material in the segment was conserved. Consider a jet, with a 6% concentration of polymer in a volatile solvent, which went from a jet diameter of 50 μm to dry nanofibers with a relatively large diameter of 0.5 μm. The area reduction ratio was 2500/0.25 = 10000. The drying process accounted for a factor of 16, and the elongation of the initial straight part of the jet contributed an additional factor of 5. The remaining area reduction ratio, 125, occurred in the parts of the jet affected by the bending instabilities.

Many nanofibers as thin as 0.05 μm (50nm) in diameter were observed. The corresponding total area reduction ratio was 1,000,000, for an initial jet diameter of 50 μm. If, as above, evaporation of solvent contributed as factor of 16 and the elongation of the straight segment contributed a factor of 5, in this case , the bending and looping part of the jet provided the remaining the factor of 12,5000 to the area reduction ratio.

While 12,5000 is a high area reduction ratio, it occurred as many segments of the jet were drawn in different directions at the same time, in expanding loops. If the jet were drawn in a straight line to a ratio of 12,5000, the velocity required at the nanofiber end of the jet would be much faster than the speed of sound in most solids. The actual path achieved very high elongation without such an unreasonably high velocity.

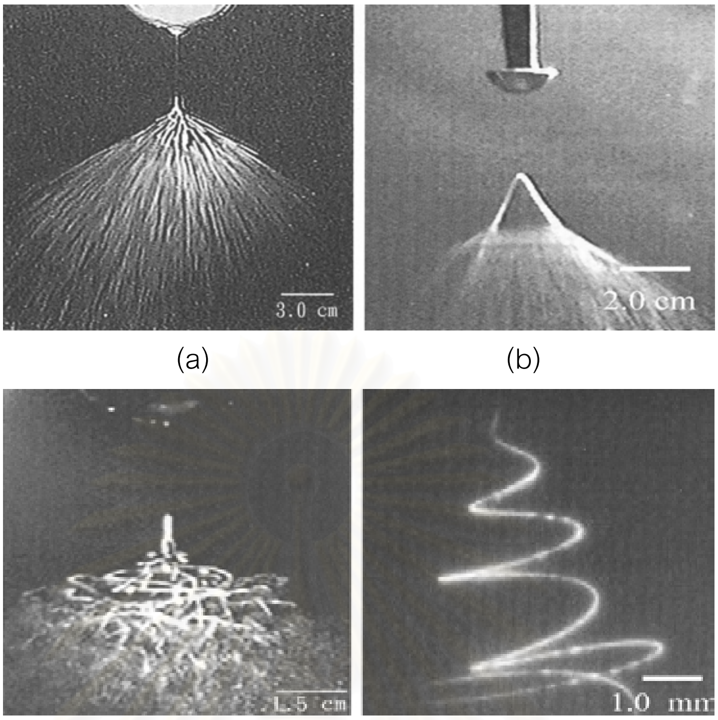
The longitudinal strain rate could be estimated as follows, by using the area reduction ratio and the time of flight. The time that a typical segment of the jet was in flight (δt = 0.2 s) could be estimated as the distance between the pendent droplet and the collector (20 cm) divided by the downward velocity of the jet (1 m/s). The longitudinal strain rate was ($\delta\zeta/(\delta t*\zeta)$) where ζ is the initial segment length, and $\delta\zeta$ is the growth in length. The draw ratio $\delta\zeta/\zeta$ was, assuming no solvent loss, around 125 to 12,5000, so that the longitudinal strain rate was around 625 to 62500 s^{-1} for the two cases described above.

Theory suggests that the transformation from a random coil to an elongated macromolecule occurs when the strain rate multiplied by the conformational relaxation time of the molecule is greater than 0.5. If the relaxation time of the polymer solution were longer than 0.01 s, which is a conservative estimate, the macromolecules are expected to be elongated and axially oriented during electrospinning.

Before high frame-rate, short exposure time cameras were available, visual observations and viseo images of electrically driven jets were interpreted as evidence for a process that splayed the primary jet into many smaller jets. The splaying jets were supposed to emerge from the region at the end of the straight segment. Figure 2.5(a) and 2.5(b) show images from a video frame with an exposure time of 16.7 ms. The jet path was illuminated with a single bright incandescent light that projected a narrow beam through the path of the jet. Figure 2.5(c) shows a jet similar to that shown in Figure 2.5(a), illuminated with light from a broader source, and photographed at 30 frames per second. A shorter exposure time of 1 ms was used. Several loops were visible. The parts of the jet nearer the beginning of the bending instability appeared only as bright, short and unconnected lines. Specular reflections of the narrow beam of light, off nearly horizontal segments of downward during the longer exposure and created the lines that are prominent.

At 30 frames per second for any particular frame, the preceding and the following frames showed loops and spirals in completely different positions. After the illumination was obvious that the envelope cone was occupied by one long, looping, spiraling, continuous, and gradually thinner jet as shown in Figure 2.5(d).

Although the elongation of the jet during the bending instability is considered as the major cause for the decrease in the diameter of the jet in the experiments described in period, splitting and splaying of the jet are seen in some experiments with other materials. These processes provide viable alternative mechanisms for the production of nanofibers.



(a) (b)

(c) (d)

Figure 2.5 Images of electrospinning jet with different exposure times.
(a), (b), and (c), shutter speed was 16.7 ms
(d) shutter speed was 1.0 ms.

2.3 Literature Reviews

Presently, because of merits of nanofibers which have trend to apply in biomedical material. Many researchers investigated electrospinning nanofibers to study the effects of various parameters on morphology and diameter of obtained nanofiber. Based on their researches, although synthetic polymers can be easily spun into nanofiber but sometime they cannot be used in biomedical application. Therefore, the natural polymers which have biocompatible and biodegradable properties have drawn a lot of attention in order to apply in biomedical field.

In 2002, J. A. Matthews et.al. [3] described how electrospinning can be adapted to produce tissue-engineering scaffolds composed of collagen nanofibers. Type I and type III collagen from different sources as polymer were dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP). Electrospinning is a rapid and efficient process that can be used to selectively deposit polymers in a random fashion or along a predetermined and defined axis. The inherent properties of the electrospinning process make it possible to fabricate complex and seamless three-dimensional shapes. Electrospun collagen promotes cell growth and the penetration of cells into the engineered matrix. The structural, material, and biological properties of electrospun collagen suggest that this material may represent a nearly ideal tissue engineering scaffold.

Later in 2004, Z. M. Huang and coworkers [4] investigated electrospinning of a natural biopolymer, gelatin, and mass concentration-mechanical property relationship of gelatin nanofibers. 2,2,2-Trifluoroethanol (TFE) was used as a solvent to dissolve gelatin. The resulting solution with a mass concentration in between 5 and 12.5 % can be successfully electrospun into nanofibers of a diameter ranging from 100 to 340 nm. Furthermore lower or higher mass concentration was inapplicable in electrospinning at ambient conditions. They have found that the highest mechanical behavior did not occur to the nanofibrous nonwoven mats electrospun from either lowest or the highest mass concentration solutions. Instead, the nanofiber mat that had the finest fiber structure and no beads on surface obtained from the 7.5% mass concentration exhibited the largest tensile modulus and ultimate tensile strength, which are respectively 40 and 60% greater than those produced from the remaining mass concentration, i.e., 5, 10, and 12.5%, solutions.

Within the same year, D. S. Katti, et.al. [5] also studied the effect of frabication parameters such as polumer solution concentration and voltage per distance on morphology and diameter of electrospun poly(lactide-co-glycolide) (PLAGA) nanofiber. The range of polymer solution concentration studied was from 0.10 g/ml to 0.30 g/ml. The results indicated that the diameter of nanofibers decreased with an increase in the concentration of polymer solution. The voltage study demonstrated that the average diameter of the nanofibers decreased with an increase in voltage. However, the effect of voltage on fiber diameter was less pronounced as compared to polymer solution concentration.

In 2002, H. J. Jin and co-workers [6] explored electrospinning for protein-based biomaterial to fabricate scaffolds and membranes from regenerated silkworm *Bombyx mori* silk solutions. To improve processability of protein solution, poly(ethylene oxide) (PEO) was blended with the silk fibroin. The obtained fiber diameters were uniform and less than 800 nm.

CHAPTER III

EXPERIMENT

3.1 Materials and Chemicals

Materials and chemicals used in this research are listed as follow

1. Gelatin powder was purchased from Ajax Finechem, Australia.
2. Glacial acetic acid (Ac) was purchased from Lab Scan, Thailand.
3. Dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich, Germany.
4. Ethylene glycol (EG) was purchased from Fluka, USA.
5. Formamide was purchased from Asia Pacific Specially Chemicals Limited, Germany.
6. 2,2,2-Trifluoroethanol (TFE) was purchased from Lab Scan, Thailand.

3.2 Instruments

For this experiment have 6 instuments as the follow Table 3.1

Table 3.1 Summary of instruments used in this research

Instrument	Model
1. High Voltage Power Supply	Gamma High Voltage Reaserch D-ES30PN/M692
2. Viscometer	Brookfield DV-III
3. Tensiometer	KrÜss K10T
4. Conductivity Meter	Orion 160
5. Scanning Electron Microscope (SEM)	JEOL JSM-6400
6. Ion Sputtering	Balzers Union SCD 040

3.3 Experimental Procedures

3.3.1 Single Solvent System

Figure 3.1 displays the entire process for the electrospinning of gelatin nanofibers in single solvent system. The details for each procedure are as follows

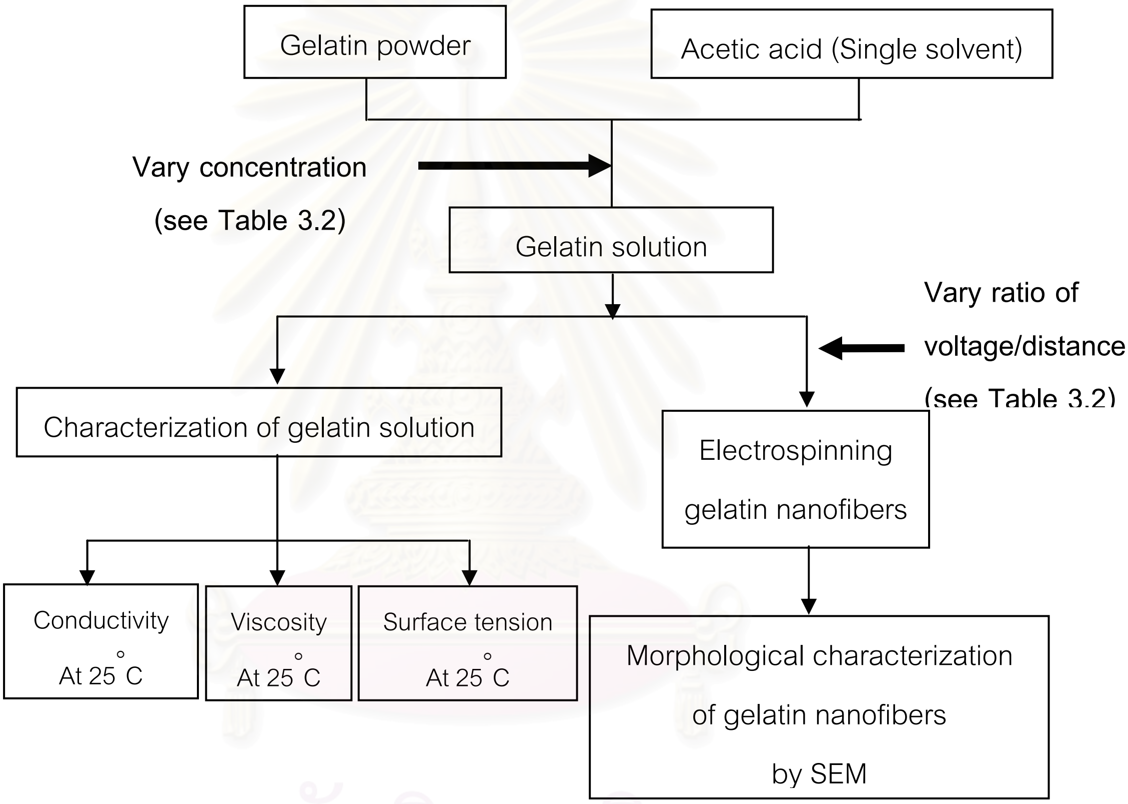


Figure 3.1 Flow chart of electrospinning process for single solvent system

3.3.1.1 Preparation of Gelatin Solution

Gelatin solution was prepared by dissolving gelatin powder in acetic acid at various concentrations as shown in Table 3.2. The gelatin solution was stirred for 24 hours at room temperature in order to obtain homogeneous gelatin solution.

3.3.1.2 Electrospinning Process

The different concentrations of gelatin solution were placed into 3 ml glass syringe and then electrospun into gelatin nanofiber using a Gamma High Voltage Research D-ES30PN/M692 DC High Voltage Power Supply. These solutions were electrospun for 10 minutes at various ratios of voltage per distance between needle tip to collection plate (aluminium foil) as shown in Table 3.2.

Table 3.2 The formular of gelatin solution in single solvent system of acetic acid

Concentration of gelatin solution (%w/v)	Ratio of voltage per distance	Voltage (kV)	Distance (cm)
15	1 kV/cm	7.5	7.5
		15	15
		22.5	22.5
	2 kV/cm	15	7.5
	3 kV/cm	22.5	7.5
17	1 kV/cm	7.5	7.5
		15	15
		22.5	22.5
	2 kV/cm	15	7.5
	3 kV/cm	22.5	7.5
19	1 kV/cm	7.5	7.5
		15	15
		22.5	22.5
	2 kV/cm	15	7.5
	3 kV/cm	22.5	7.5
21	1 kV/cm	7.5	7.5
		15	15
		22.5	22.5
	2 kV/cm	15	7.5
	3 kV/cm	22.5	7.5

Table 3.2 (cont.) The formular of gelatin solution in single solvent system of acetic acid

Concentration of gelatin solution (%w/v)	Ratio of voltage per distance	Voltage (kV)	Distance (cm)
23	1 kV/cm	7.5	7.5
		15	15
		22.5	22.5
	2 kV/cm	15	7.5
	3 kV/cm	22.5	7.5
25	1 kV/cm	7.5	7.5
		15	15
		22.5	22.5
	2 kV/cm	15	7.5
	3 kV/cm	22.5	7.5
27	1 kV/cm	7.5	7.5
		15	15
		22.5	22.5
	2 kV/cm	15	7.5
	3 kV/cm	22.5	7.5
29	1 kV/cm	7.5	7.5
		15	15
		22.5	22.5
	2 kV/cm	15	7.5
	3 kV/cm	22.5	7.5

Figure 3.2 displays the schematic of electrospinning set-up used in this research. The positive lead from a high voltage supply was attached via an alligator clip to the external surface of the metal syringe needle. Grounded counter electrode was connected to aluminium foil, used as a collector.

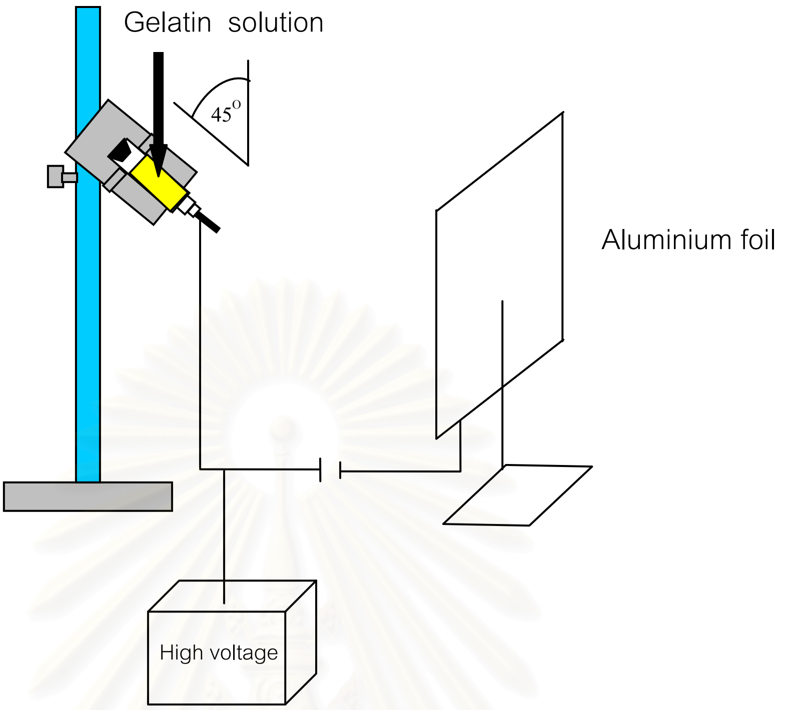


Figure 3.2 Electrospinning set-up

3.3.1.3 Characterization of Gelatin Solution

The viscosity (η) of gelatin solution was determined by a Brookfield DV-III Programmable Rheometer at 25 °C. In addition, the conductivity of gelatin solution was measured by an Orion 160 Conductivity Meter at 25 °C, whereas, the surface tension of gelatin solution was determined at 25 °C using a Krüss K10T Tensiometer.

3.3.1.4 Characterization of Gelatin Nanofibers

The gelatin nanofibers were coated with gold by a Balzers Union SCD040 Ion Sputtering and then their morphologies were characterized by a JEOL JSM-6400 Scanning Electron Microscope (SEM).

3.3.2 Mixed Solvent Systems

Mixed solvent systems were prepared by mixing acetic acid to different solvents, i.e., DMSO, EG, Formamide, and TFE at various volumetric ratios prior to dissolving gelatin powder to prepare 19%w/v of gelatin solution. The ratios of mixed solvents are summarized in Table 3.3.

The gelatin solutions in each mixed solvent systems were stirred for 12 hours at room temperature and then electrospun for 10 minutes. The electrospinning was carried out with applied voltage being between 7.5 to 22.5 kV and the collection distance being between 7.5 to 22.5 cm. In other words, similar to single solvent system, various ratios of voltage/collection distance were also applied. The same procedures for characterization of both gelatin solutions and gelatin nanofibers produced from single solvent system were performed, as shown in Figure 3.3.

Table 3.3 Ratios of mixed solvent systems (Acetic acid : Adjusting liquid)

Ratio of Ac:TFE (%v/v)	Ratio of Ac:DMSO (%v/v)	Ratio of Ac:EG (%v/v)	Ratio of Ac:F (%v/v)
100:0	100:0	100:0	100:0
90:10	97:3	97:3	97:3
80:20	95:5	95:5	95:5
70:30	93:7	93:7	93:7
60:40	91:9	91:9	91:9
50:50	-	-	-
40:60	-	-	-

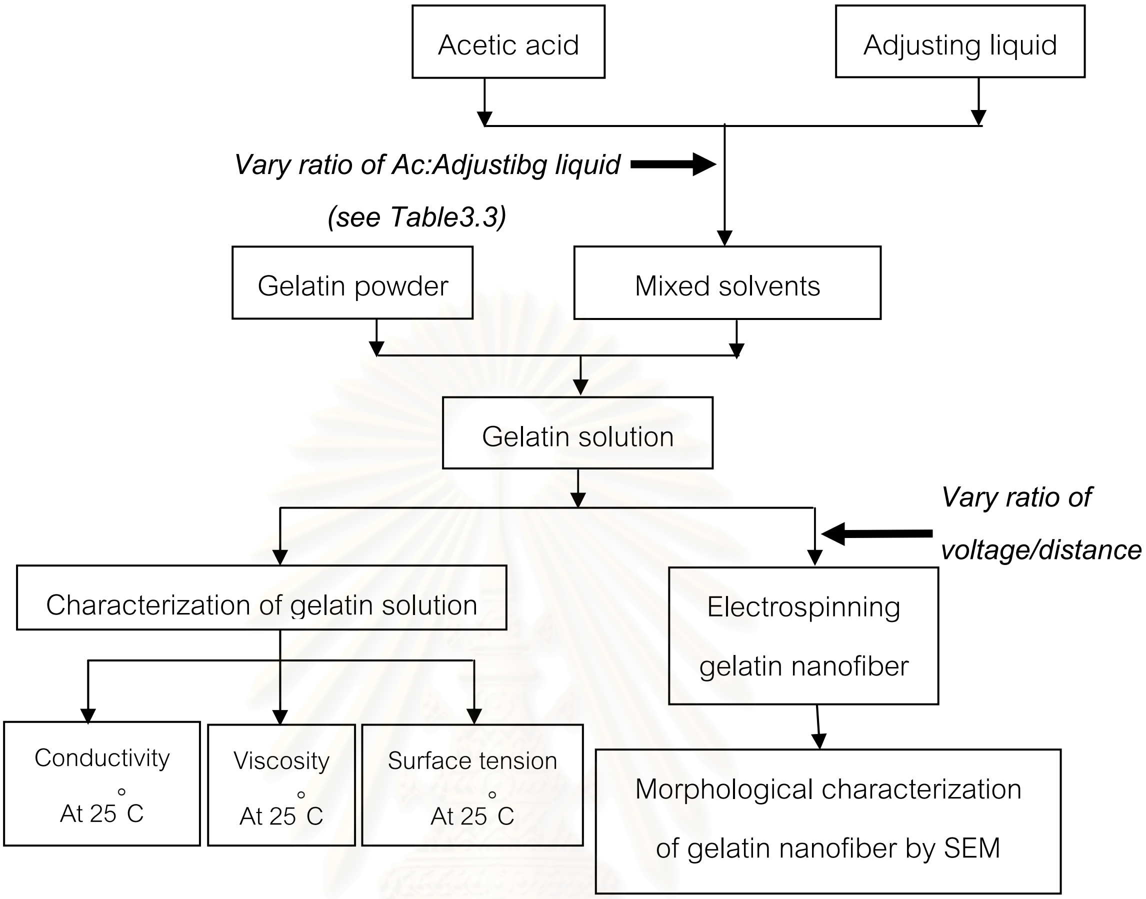


Figure 3.3 Flow chart of electrospinning process for mixed solvent systems

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Single Solvent System

4.1.1 Effect of Gelatin Solution Concentration

Gelatin solutions were prepared in acetic acid at various concentrations ranging from 15 to 29% w/v. Table 4.1 summarizes the properties of obtained gelatin solutions, e.g. viscosity, conductivity, and surface tension, and the average diameter of the as-spun gelatin nanofibers. The applied electrostatic potential used 7.5kV/7.5cm. Both acetic acid and trifluoroethanol (TFE) can dissolve gelatin powder into homogeneous gelatin solutions, while other solvents such as dimethyl sulfoxide (DMSO), ethylene glycol (EG), and formamide (F) cannot. Since TFE has already been studied recently [4], acetic acid was used as the single solvent to study the effect of the solution concentration on the morphology of the obtained fiber.

Table 4.1 The effect of gelatin concentration on the gelatin solution properties and average gelatin fiber diameter (7.5kV/7.5cm)

Concentration (%w/v)	Properties of solution			Average fiber diameter (nm)
	Viscosity (cP)	Surface tension (mN/m)	Conductivity (μS/cm)	
15	114	26.70 ± 0.04	167	214 ± 0.06
17	180	26.22 ± 0.15	205	220 ± 0.06
19	250	26.75 ± 0.72	224	271 ± 0.11
21	400	26.00 ± 0.05	254	337 ± 0.09
23	594	25.92 ± 0.06	296	413 ± 0.08
25	699	25.80 ± 0.05	304	502 ± 0.06
27	1,217	25.72 ± 0.05	317	616 ± 0.05
29	1,586	24.68 ± 0.09	342	839 ± 0.09

According to Table 4.1, it can be seen that the viscosity and conductivity of gelatin solution increased and the surface tension decreased when the gelatin concentration increased. Specifically, the viscosity of the gelatin solutions was found to increase from about 114 cP at 15% w/v to about 1,586 cP at 29% w/v. On the other hand, the surface tension of the gelatin solutions decreased slightly from about 26.7 mN/m at 15% w/v to about 24.7 mN/m at 29% w/v. Interestedly, the average fiber diameter was found to increase monotonically with increasing gelatin concentration from about 214 nm at 15% w/v to about 839 nm at 29% w/v. The viscosity and the surface tension were graphically displayed as a function of the concentration in Figure 4.1.

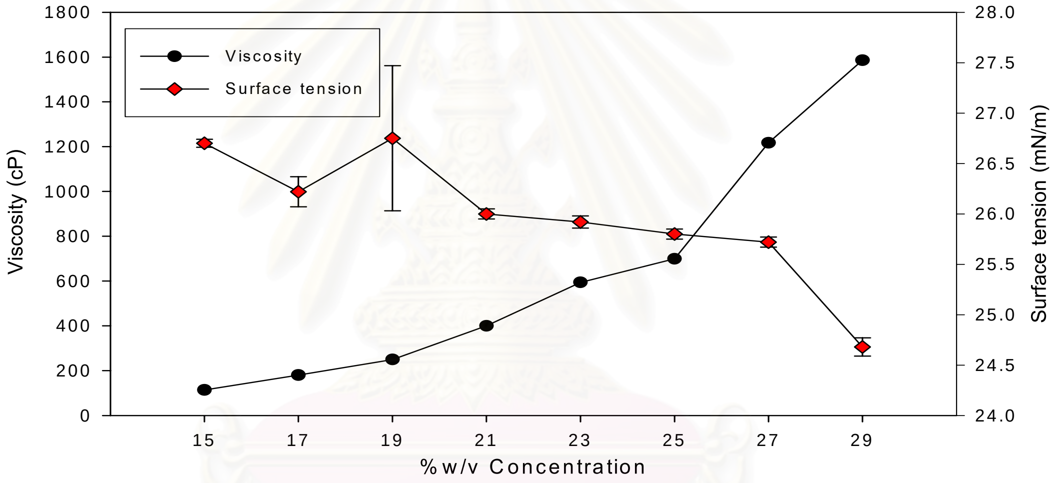
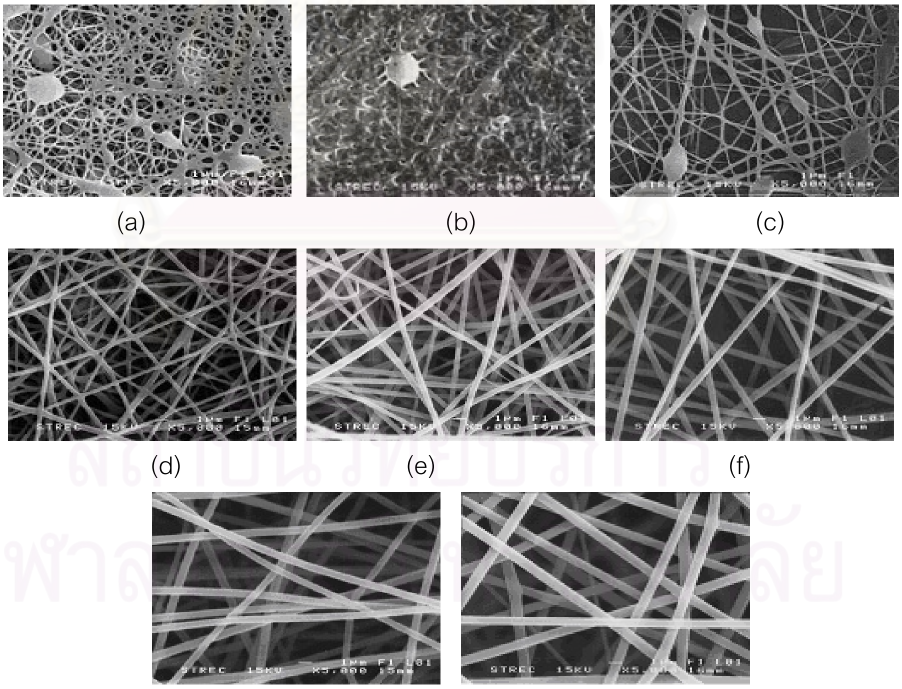


Figure 4.1 Viscosity and surface tension of the gelatin solutions at various concentrations in acetic acid

Figure 4.2 shows selected SEM images of as-spun gelatin fibers. According to Figure 4.2, at low gelatin concentrations (i.e. between about 15 and 19% w/v), the presence of beads on the as-spun fibers was evident and the amount of beads decreased and disappeared altogether when the gelatin concentration was between about 21 and 29% w/v. Obviously, the diameters of the as-spun fibers increased monotonically with increasing gelatin concentration and, interestingly, as the gelatin concentration increased, the number of the as-spun fibers per unit area was found to decrease. The formation of beaded and smooth fibers could be explained based on the interplay between the three major forces, that are Coulombic force, viscoelastic force, and surface tension [7]. At low gelatin concentrations, the low viscosity of the solutions, hence the low viscoelastic force, was not enough to withstand the Coulombic repulsion force. The overstretching of the ejected, charged jet could result in the partial breakup of the jet and, as a result of the surface tension, the beads were formed. At high concentrations, only smooth fibers were observed because the viscoelastic force was high enough to withstand the Coulombic repulsion force from the electrical charges present within the charged jet. Mit-uppatham et al. [7] showed that an increase in the polyamide-6 solution concentration caused a marked increase in the solution viscosity. In addition, they reported that the relationship between the solution viscosity and the solution concentration could be approximated by an exponential growth equation. Further increasing in the solution viscosity resulted in a reduced number of beads and increased fiber diameters.



(a) (b) (c)

(d) (e) (f)

(g) (h)

Figure 4.2 SEM micrographs of gelatin fibers at various concentrations of gelatin solutions in acetic acid at the spinning condition of voltage/distance 7.5kV/7.5cm (a) 15%w/v (b) 17%w/v (c) 19%w/v (d) 21%w/v (e) 23%w/v (f) 25%w/v (g) 27%w/v (h) 29%w/v

Moreover, the results showed that the average diameter of the obtained nanofibers increased with increasing solution concentration. This results can be explained that the higher viscosity of the solution resulted in marked increase in the viscoelastic force that could withstand both the Coulombic repulsion and the force incurred during the bending instability. These results are in good agreement with those reported by Fong and coworkers [8] who investigated the bead formation along electrospun poly(ethylene oxide) (PEO) nanofibers by relating with the properties of the PEO solutions. They found that the number of beads decreased with increasing viscosity of the solutions, but with decreasing surface tension of the solutions.

4.2 Mixed Solvent Systems

4.2.1 Effect of Mixed Solvent

The purpose of this part is to study the relationship between the ratios of mixed solvent-solvent properties and the resulting gelatin nanofibers. In order to investigate the effect of mixed solvent on appearance of the as-spun gelatin fibers, various types of solvent listed in Table 4.2 were used. From this table, it was shown that acetic acid has the lowest viscosity, dipole moment, and dielectric constant values, whereas trifluoroethanol has the lowest boiling point. Dimethyl sulfoxide, ethylene glycol, and formamide all have higher boiling point, dipole moment, and dielectric constant values than those of acetic acid and trifluoroethanol.

The gelatin solutions were prepared by dissolving gelatin powder in mixed solvent systems, such as acetic acid/trifluoroethanol (Ac/TFE), acetic acid/dimethyl sulfoxide (Ac/DMSO), acetic acid/ethylene glycol (Ac/EG), and acetic acid/formamide (Ac/F) in various compositional ratios. Based on the results obtaiend on the single solvent system, 19% w/v of gelatin concentration was used to study the effect of mixed solvent systems because the viscosity of the resulting gelatin solution was high enough to result in the as-spun fibers with less formation of beads and acetic acid (Ac) was used as the main solvent to prepare the various mixed solvents because acetic acid could dissolve the gelatin powder into homogeous gelatin solutions. Recently, TFE was reported as a good solvent for gelatin and was used a the spinning solvent for producing as-electrospun gelatin fibers [4].

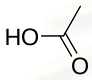
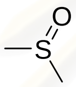
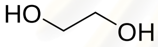
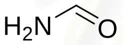
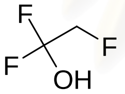
The volumetric ratio of various mixed solvent systems was varied as follows: 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, and 40:60 (for Ac/TFE) and 100:0, 97:3, 95:5, 93:7, and 91:9 (for Ac/DMSO, Ac/EG, and Ac/F, respectively). The rather high volumetric ratio of TFE in the mixed solvent system of Ac/TFE was because TFE was a good solvent for gelatin as previously noted. On the other hand, DMSO, EG, and F were not as good a solvent; therefore, their content in the mixed solvent systems was not that high. The properties of the obtained gelatin solution in each mixed solvent were shown in Table 4.3. As shown, the viscosity and the conductivity values of the gelatin solutions in mixed solvent as Ac/TFE increased and the surface tension values decreased with increasing the amount of TFE in Ac/TFE system. The viscosity of gelatin solution in Ac/TFE increased because TFE is a good solvent of gelatin that gelatin molecules can expand well, resulting in the observed increase in the viscosity of the gelatin solution. In addition, because TFE has a high conductivity, the conductivity of the obtained gelatin solutions increased.

For the other three mixed solvent systems, it was shown that their viscosity values increased with increasing the amount of dimethyl sulfoxide (DMSO), ethylene glycol (EG), and formamide (F) in mixed solvent systems. This is because dimethyl sulfoxide, ethylene glycol, and formamide all have a viscosity value higher than that of acetic acid. Likewise, all of these liquids exhibited a conductivity value greater than that of acetic acid, so the viscosity of the mixed solvent increased, and since the conductivity value of these solvents is greater than that of acetic acid, the conductivity of the resulting solutions was greater as well.

Acetic acid has a high boiling point and TFE has a low boiling point. Both of them are good solvents for gelatin. Figure 4.3(a) shows electrospun webs at 100:0 v/v which have beads. Figures 4.3(b), (c), and (d) show electrospun webs of fused fibers from gelatin solutions having a high content of acetic acid ranging between

70 and 90, while Figures 4.3(e), (f), and (g) show electrospun webs of discrete round-shaped fibers from gelatin solutions having a lower content of acetic acid ranging between 40 and 60.

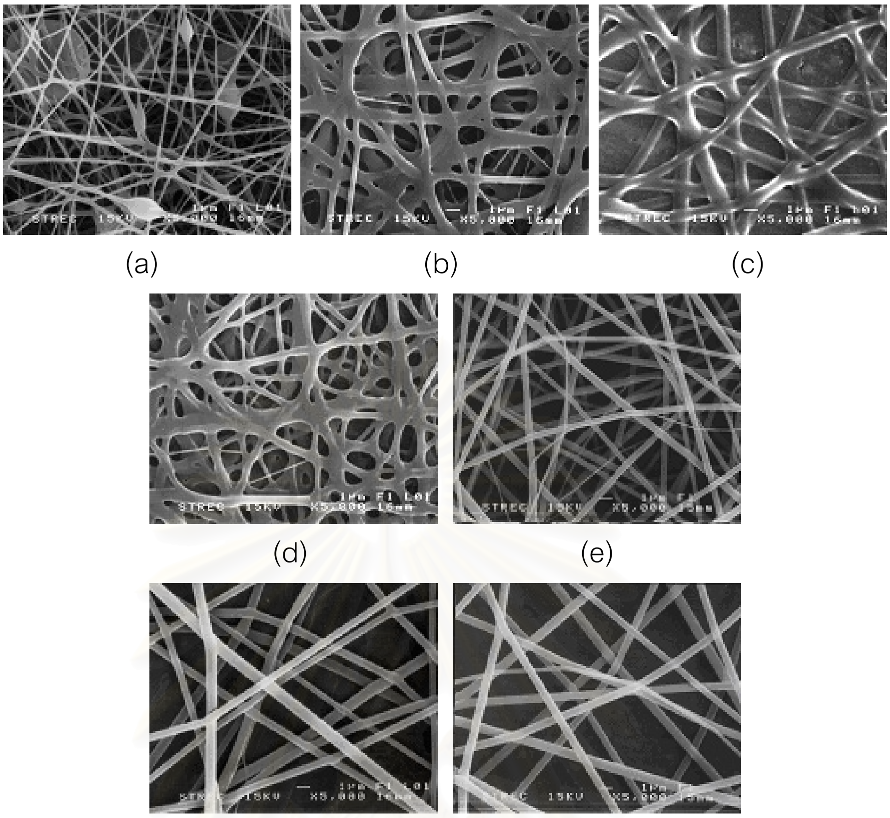
Table 4.2 The important properties of solvents used in this research

Solvent	Structure	Density (g/cm^3)	Viscosity (cP)	Boiling point (^{o}C)	Dipole moment (D)	Dielectric constant	Surface tension (mN/m)
Acetic acid (Ac)		1.05	1.12	118	1.68	6.15	26.90
Dimethyl sulfoxide (DMSO)		1.10	2.00	189	3.90	46.70	43.00
Ethylene glycol (EG)		1.11	16.13	197	2.20	37.70	47.00
Formamide (F)		1.13	3.30	211	3.37	111.00	59.10
Trifluoroethanol (TFE)		1.38	1.24	74	2.52	8.55	-

The average diameter of the as-electrospun gelatin fibers was 282, 634, and 500 nm for gelatin solutions in 60:40, 50:50, and 40:60 mixed solvents, respectively. Table 4.4 summarizes the average diameter and morphology of the as-spun gelatin nanofibers prepared from gelatin solutions in Ac/TFE solvent. Interestingly, no beads were presented on these webs, due perhaps to the higher dielectric constant of TFE that resulted in a higher conductivity of the resulting solutions (see Tables 4.2 and 4.3).

Table 4.3 The important properties of gelatin solution in mixed solvent

Ratio of mixed solvent (%v/v)	Properties of solution		
	Viscosity (cP)	Surface tension (mN/m)	Conductivity (μS/cm)
Single solvent Acetic acid (Ac)			
100:0 (19%gelatin)	250	26.75±0.72	224
Mixed solvent			
Ac/TFE			
90:10	294	24.24±0.02	350
80:20	423	22.41±0.04	411
70:30	469	21.23±0.04	476
60:40	510	20.02±0.03	491
50:50	543	19.02±0.03	502
40:60	560	18.42±0.03	537
Ac/DMSO			
97:3	290	24.79±0.29	256
95:5	317	25.64±0.15	301
93:7	397	25.99±0.21	347
91:9	408	26.54±0.04	383
Ac/EG			
97:3	287	27.37±0.02	287
95:5	317	27.64±0.02	277
93:7	357	27.84±0.02	250
91:9	365	28.08±0.09	210
Ac/F			
97:3	340	27.70±0.02	426
95:5	400	27.98±0.03	457
93:7	465	28.17±0.02	480
91:9	515	28.35±0.07	490



(a) (b) (c)

(d) (e)

(f) (g)

Figure 4.3 SEM micrographs of as-spun gelatin fiber from gelatin solution in various ratios of Ac/TFE solvent electrospun at the ratios of voltage/distance of spun fiber as 22.5kV/22.5cm (a)100:0%v/v (b) 90:10%v/v (c) 80:20%v/v (d) 70:30%v/v (e) 60:40%v/v (f) 50:50%v/v (g) 40:60%v/v

Table 4.4 The average diameter of the as-spun gelatin in Ac:TFE

Sample	Average diameter (nm)	Morphological nanofiber
Ac:TFE 100:0	277±0.08	fiber+beads
Ac:TFE 90:10	430±0.10	fused
Ac:TFE 80:20	-	fused
Ac:TFE 70:30	-	fused
Ac:TFE 60:40	282±0.06	smooth fiber
Ac:TFE 50:50	634±0.13	smooth fiber
Ac:TFE 40:60	500±0.07	smooth fiber

With an extremely high dielectric constant, addition of DMSO resulted in marked increase in the conductivity of the resulting gelatin solutions (see Tables 4.2 and 4.3). Moreover, addition of DMSO also resulted in an increase in the viscosity of the resulting solutions because DMSO has a quite high viscosity. Figure 4.4 shows some selected SEM images of as-spun gelatin fibers as a function of DMSO content, from which the average fiber diameter was found to increase with increasing DMSO content, as shown in Table 4.5. Obviously, the obtained gelatin fibers were smooth and no beads were observed, which could be a result of the high dielectric constant of DMSO that could be responsible for the increase in the charge repulsion force in gelatin solutions.

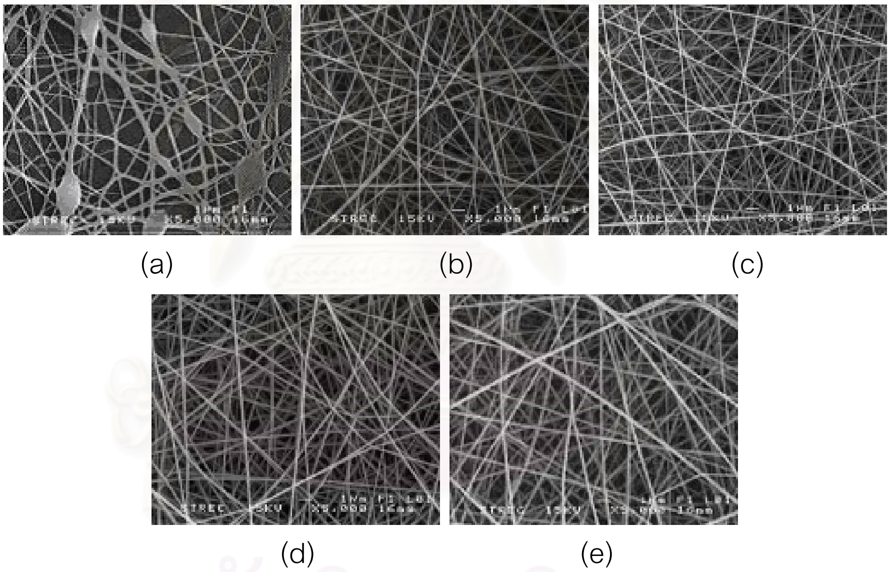


Figure 4.4 SEM micrographs of as-spun gelatin fiber from gelatin solution in various ratios of Ac/DMSO solvent electrospun at the ratios of voltage/distance of spun fiber as 7.5kV/7.5cm (a) 100:0%v/v (b) 97:3%v/v (c) 95:5%v/v (d) 93:7%v/v (e) 91:9%v/v

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For Ac/EG mixed solvents, the results obtained showed that when the amount of EG increased the viscosity and the surface tension of the resulting solutions increased whereas the conductivity decreased, as show in Table 4.3. Morphology of the electrospun gelatin nanofibers obtained at lower EG concentrations (97:3, 95:5, and

93:7 v/v) was smooth without any beads, while, at a higher EG concentration (91:9 v/v), fused gelatin nanofibers was obtained, as shown in Fugure 4.5.

Table 4.5 The average diameter of the as-spun gelatin in Ac:DMSO

Sample	Average diameter (nm)	Morphological nanofiber
Ac:DMSO 100:0	271±0.11	fiber+beads
Ac:DMSO 97:3	185±0.03	smooth fiber
Ac:DMSO 95:5	187±0.03	smooth fiber
Ac:DMSO 93:7	230±0.03	smooth fiber
Ac:DMSO 91:9	225±0.06	smooth fiber

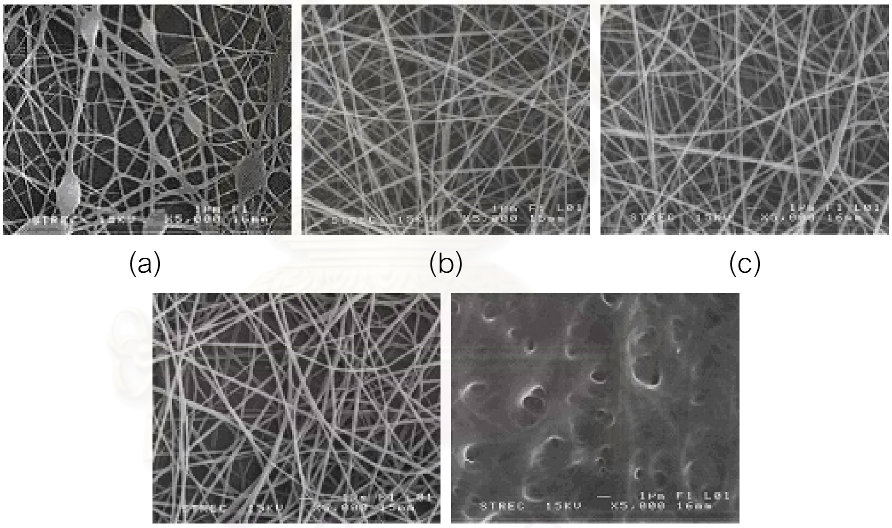

(a) (b) (c)
(d) (e)

Figure 4.5 SEM micrographs of as-spun gelatin fiber from gelatin in various ratios of Ac/EG solvent electrospun at the ratios of voltage per distance of spun Fiber as 7.5kV/7.5cm (a) 100:0%v/v (b) 97:3%v/v (c) 95:5%v/v (d) 93:7%v/v (e) 91:9%v/v

The average diameter of gelatin nanofibers from solutions in Ac/EG mixed solvents is listed in Table 4.6, but the as-spun product from the solution in 91:9 Ac/EG mixed solvent could not be determined. Obviously, the fibers obtained from gelatin solutions in 97:3, 95:5, and 93:7 Ac/EG mixed solvents were smoother that those obtained from corresponding gelatin solution in Ac. The reason should be based on the viscosity and conductivity value of the resulting solutions in these mixed solvents. The increased viscosity and conductivity resulted in greater viscoelastic and Coulombic stretching force acting on a jet segment. Furthermore, a possible explanation for the fused fibers observed from the solution in 91:9 Ac/EG mixed solvent could be based on the result of the competitive evaporation of the two solvents. Since the boiling point of Ac is lower than that of EG, evaporation of Ac from the as-spun products should happen in a much faster rate, causing the volumetric ratio between EG and Ac content to increase over time. As a result, at 91:9 of the mixed solvent, the ratio between the two components was just right that the amount of EG that was left in the fibers resulted in the fusing of the deposited fibers.

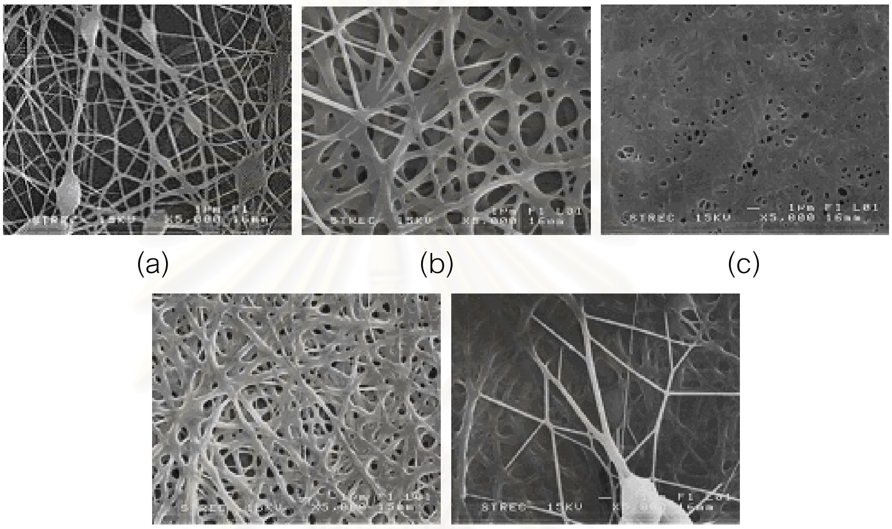
Table 4.6 The average diameter of the as-spun gelatin in Ac:EG

Sample	Average diameter (nm)	Morphological nanofiber
Ac:EG 100:0	271±0.11	fiber+beads
Ac:EG 97:3	225±0.04	smooth fiber
Ac:EG 95:5	264±0.04	smooth fiber
Ac:EG 93:7	228±0.07	smooth fiber
Ac:EG 91:9	fuse	fuse

For the Ac/F mixed solvents, all of the viscosity, surface tension, and conductivity of the resulting gelatin solutions were found to increase with increasing F content, as shown in Table 4.3. Figure 4.6 shows morphology of gelatin fibers electrospun from solution in Ac/F mixed solvent solvent under an applied electrostatic field strength of 7.5kV/7.5cm. Evidently, only fused as-spun fibers were observed. Since the boiling point of F was much greater than that of Ac. During the transport of the ejected, charged jet to the target, compatitive evaporation of the solvents resulted in the increase in the actual content of F (since Ac could evaporate at a much faster rate).

Because of this, the solvents in the ejected, charged jet could completely evaporate between being deposite on the target, resulting in the formation of fused fibers.

Interestly, according to the results obtained on these mixed solvents, the observed increase in both the viscosity and the conductivity of the resulting solutions caused the as-spun to be bead-free.



(a) (b) (c)

(d) (e)

Figure 4.6 SEM micrographs of as-spun gelatin fiber from gelatin in various ratios of Ac/F solvent electrospun at the ratios of voltage/distance of spun fiber as 7.5kV/7.5cm (a) 100:0%v/v (b) 97:3%v/v (c) 95:5%v/v (d) 93:7%v/v (e) 91:9%v/v

4.3 Effect of Voltage per Distance

The effect of the ratios between the applied voltage and the collection distance, i.e. the electrostatic field strength, of 7.5 kV/7.5 cm, 15 kV/7.5 cm, and 22.5 kV/ 7.5cm, respectively, on the morphological appearance of the resulting gelatin fibers.

Based on the results shown in Figure 4.7, the different electrostatic field strengths were not found to affect the diameters of the resulting gelatin fibers as much.

Obviously, the average diameter of the as-spun fibers for all of the electrostatic field strengths investigated, ranged between about 210 and 900 nm.

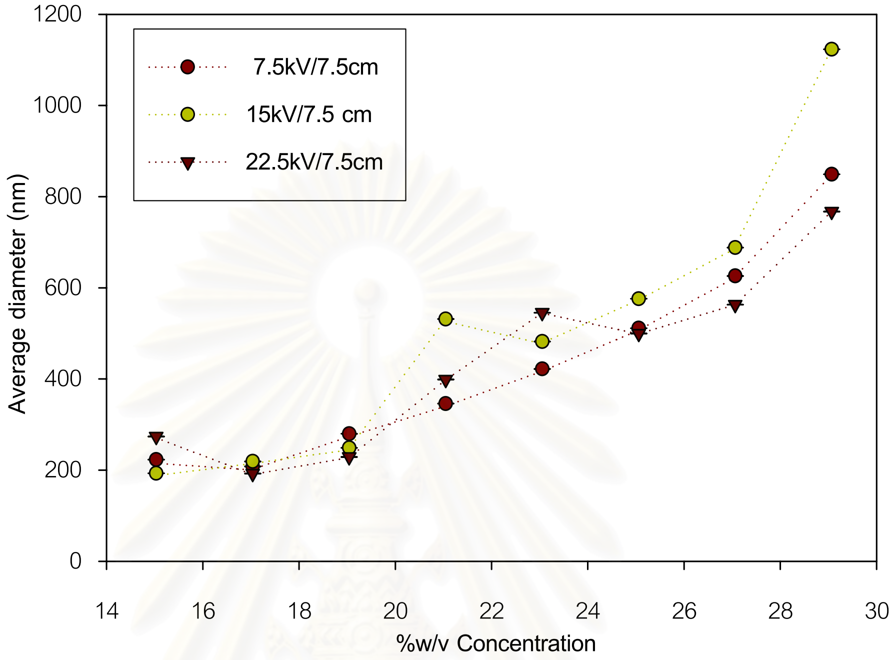
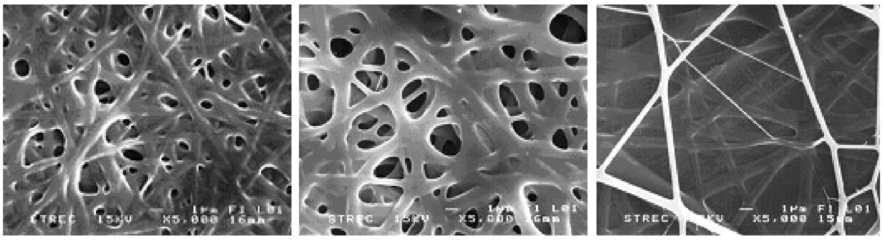


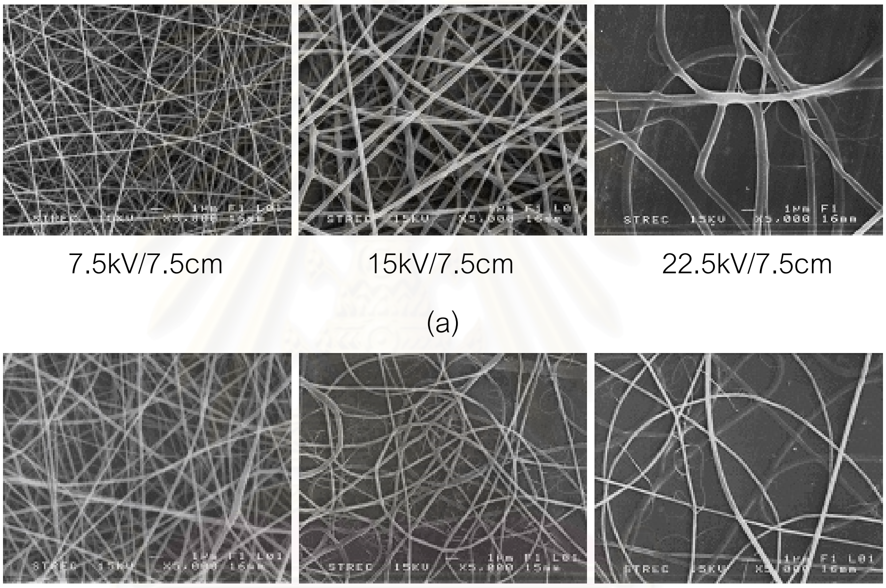
Figure 4.7 The relationship between the gelatin concentration in acetic acid and the average diameter of the electrospun gelatin nanofiber at various ratios of voltage/distance

The morphological structure of the resulting fibers electrospun from 19% w/v gelatin solution in 70:30 Ac:TFE mixed solvent system and in 95:5 Ac:DMSO and Ac:EG mix solvent systems is illustrated in Figure 4.8. From the results shown, it can be inferred that increasing electrostatic field strength caused the as-spun fibers to be larger in their size. Moreover, the number of as-spun fibers per unit area was also found to decrease with increasing electrostatic field strength. The increase in the size of the fibers could be a result of the increased electrostatic force, hence increased the mass throughput of the charged jet, while the decrease in the number of the as-spun fibers per unit are could be due to the increased number of charges within a jet segment that resulted in higher extent of repulsion between adjacents fibers during deposition on the target.



7.5kV/7.5cm 15kV/7.5cm 22.5kV/7.5cm

Figure 4.8 Effect of increasing ratios of voltage/distance on the gelatin electrospun nanofibers at 19%gelatin in 70:30%v/v of Ac:TFE



7.5kV/7.5cm 15kV/7.5cm 22.5kV/7.5cm

(a)

7.5kV/7.5cm 15kV/7.5cm 22.5kV/7.5cm

(b)

Figure 4.9 SEM micrographs of gelatin nanofibers from (a) Ac/DMSO 95:5%v/v and (b) Ac/EG 95:5%v/v at various ratios of voltage/distance

CHAPTER V

CONCLUSIONS

This work demonstrated that electrospinning is a sound technique for the fabrication of nanofibers from biodegradable polymers such as gelatin. Gelatin nanofibers were electrospun from the single solvent (i.e. acetic acid, Ac) and various mixed solvent systems [i.e. Ac/dimethyl sulfoxide (DMSO), Ac/ethylene glycol (EG), Ac/formamide (F), Ac/trifloroethanol (TFE)] to investigate the effects of the solution concentration and solvent properties on the morphology and diameters of the as-spun gelatin fibers.

In the single solvent system (acetic acid), the gelatin solutions of various concentrations were electrospun. The results showed that when the gelatin concentration increased the viscosity increased, causing the diameters of the as-spun fibers to increase. The average diameter of the as-spun fibers increased from about 214 nm at the concentration of 15% w/v to about 840 nm at the concentration of 29% w/v.

In the mixed solvent systems, the morphological appearance of the fibers and the electrospun webs depended very much on the properties of the solvents, and hence of the resulting solutions. In the mixed solvent systems, when the amount of the solvent having a high dielectric constant increased, the amount of beads on the gelatin electrospun nanofibers decreased. Obviously, gelatin nanofibers electrospun from the solutions in Ac/DMSO systems were smooth. It should be noted that when the solvent with a higher boiling point was added, the obtained fibers were smooth and fused together as a web-like structure because the solvent with a lower boiling could not completely evaporate before the ejected, charged jet deposited on the target.

In the single solvent system, the electrostatic field strength was not found to affect the diameters of the as-spun gelatin nanofibers, while, in the mixed solvent systems, increasing electrostatic field strength resulted in as-spun fibers of larger diameters and the as-spun fibers appeared to be more losely packed.

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APPENDICES

Appendix A

The importance properties of gelatin solution in both single and mixed solvent system

Table A1 The effect of gelatin concentration on the gelatin solution properties in acetic acid single solvent

Concentration %w/v	Properties of solution		
	Viscosity(cP)	Surface tension(mN/m)	Conductivity(μS/cm)
15	114	26.70±0.04	167
17	180	26.22±0.15	205
19	250	26.75±0.72	224
21	400	26.00±0.05	254
23	594	25.92±0.06	296
25	699	25.80±0.05	304
27	1,217	25.72±0.05	317
29	1,586	24.68±0.09	342

Table A2 The important properties of gelatin solution in Acetic acid/TFE mixed solvent

Ratio of mixed solvent (%v/v)	Properties of solution		
	Viscosity (cP)	Surface tension (mN/m)	Conductivity (μS/cm)
100:0	250	26.75±0.72	224
90:10	294	24.24±0.02	350
80:20	423	22.41±0.04	411
70:30	469	21.23±0.04	476
60:40	510	20.02±0.03	491
50:50	543	19.02±0.03	502
40:60	560	18.42±0.03	537

Table A3 The important properties of gelatin solution in Acetic acid/DMSO mixed solvent

Ratio of mixed solvent (%v/v)	Properties of solution		
	Viscosity (cP)	Surface tension (mN/m)	Conductivity (µS/cm)
100:0	250	26.75±0.72	224
97:3	290	24.79±0.29	256
95:5	317	25.64±0.15	301
93:7	397	25.99±0.21	347
91:9	408	26.54±0.04	383

Table A4 The important properties of gelatin solution in Acetic acid/EG mixed solvent

Ratio of mixed solvent (%v/v)	Properties of solution		
	Viscosity (cP)	Surface tension (mN/m)	Conductivity (µS/cm)
100:0	250	26.75±0.72	224
97:3	287	27.37±0.02	287
95:5	317	27.64±0.02	277
93:7	357	27.84±0.02	250
91:9	365	28.08±0.09	210

Table A5 The important properties of gelatin solution in Acetic acid/F mixed solvent

Ratio of mixed solvent (%v/v)	Properties of solution		
	Viscosity (cP)	Surface tension (mN/m)	Conductivity (µS/cm)
100:0	250	26.75±0.72	224
97:3	340	27.70±0.02	426
95:5	400	27.98±0.03	457
93:7	465	28.17±0.02	480
91:9	515	28.35±0.07	490

Appendix B

The average diameter of electrospun gelatin fiber

Table B1 The average diameter of gelatin nanofibers from gelatin solution in acetic acid at various ratio of voltage/distance

Sample	Voltage/Distance	Average diameter (nm)
15%w/v	1:1; 7.5kV/7.5cm	214±0.06
	1:1; 15kV/15cm	181±0.06
	1:1; 22.5kV/22.5cm	172±0.05
	2:1; 15kV/7.5cm	185±0.07
	3:1; 22.5kV/7.5cm	265±0.01
17%w/v	1:1; 7.5kV/7.5cm	220±0.06
	1:1; 15kV/15cm	194±0.06
	1:1; 22.5kV/22.5cm	217±0.07
	2:1; 15kV/7.5cm	210±0.08
	3:1; 22.5kV/7.5cm	184±0.08
19%w/v	1:1; 7.5kV/7.5cm	271±0.11
	1:1; 15kV/15cm	265±0.09
	1:1; 22.5kV/22.5cm	277±0.08
	2:1; 15kV/7.5cm	240±0.07
	3:1; 22.5kV/7.5cm	220±0.07
21%w/v	1:1; 7.5kV/7.5cm	337±0.09
	1:1; 15kV/15cm	414±0.11
	1:1; 22.5kV/22.5cm	328±0.10
	2:1; 15kV/7.5cm	522±0.09
	3:1; 22.5kV/7.5cm	390±0.10

Table B1(cont.) The average diameter of gelatin nanofibers from gelatin solution in acetic acid at various ratio of voltage/distance

Sample	Voltage/Distance	Average diameter (nm)
23%w/v	1:1; 7.5kV/7.5cm	413±0.08
	1:1; 15kV/15cm	644±0.14
	1:1; 22.5kV/22.5cm	817±0.20
	2:1; 15kV/7.5cm	473±0.11
	3:1; 22.5kV/7.5cm	536±0.19
25%w/v	1:1; 7.5kV/7.5cm	502±0.06
	1:1; 15kV/15cm	566±0.09
	1:1; 22.5kV/22.5cm	647±0.16
	2:1; 15kV/7.5cm	567±0.08
	3:1; 22.5kV/7.5cm	490±0.04
27%w/v	1:1; 7.5kV/7.5cm	616±0.05
	1:1; 15kV/15cm	876 ±0.15
	1:1; 22.5kV/22.5cm	551± 0.05
	2:1; 15kV/7.5cm	678±0.16
	3:1; 22.5kV/7.5cm	554±0.13
29%w/v	1:1; 7.5kV/7.5cm	839±0.09
	1:1; 15kV/15cm	1319±0.29
	1:1; 22.5kV/22.5cm	1673±0.75
	2:1; 15kV/7.5cm	1112±0.27
	3:1; 22.5kV/7.5cm	757±0.24

Table B2 The average diameter of gelatin nanofibers from gelatin solution in acetic acid/TFE at various ratio of voltage/distance

Ratio of Ac:TFE	Voltage/Distance	Average diameter (nm)
100:0	1:1; 7.5kV/7.5cm	271±0.11
	1:1; 15kV/15cm	265±0.09
	1:1; 22.5kV/22.5cm	277±0.08
	2:1; 15kV/7.5cm	240±0.07
	3:1; 22.5kV/7.5cm	220±0.07
90:10	1:1; 7.5kV/7.5cm	fused
	1:1; 15kV/15cm	505±0.13
	1:1; 22.5kV/22.5cm	430±0.10
	2:1; 15kV/7.5cm	fused
	3:1; 22.5kV/7.5cm	551±0.17
80:20	1:1; 7.5kV/7.5cm	fused
	1:1; 15kV/15cm	fused
	1:1; 22.5kV/22.5cm	fused
	2:1; 15kV/7.5cm	fused
	3:1; 22.5kV/7.5cm	fused
70:30	1:1; 7.5kV/7.5cm	fused
	1:1; 15kV/15cm	fused
	1:1; 22.5kV/22.5cm	fused
	2:1; 15kV/7.5cm	fused
	3:1; 22.5kV/7.5cm	fused

Table B2 (cont.) The average diameter of gelatin nanofibers from gelatin solution in acetic acid/TFE at various ratio of voltage/distance

Ratio of Ac:TFE	Voltage/Distance	Average diameter (nm)
60:40	1:1; 7.5kV/7.5cm	269±0.04
	1:1; 15kV/15cm	367±0.07
	1:1; 22.5kV/22.5cm	282±0.06
	2:1; 15kV/7.5cm	387±0.10
	3:1; 22.5kV/7.5cm	331±0.12
50:50	1:1; 7.5kV/7.5cm	443±0.06
	1:1; 15kV/15cm	569±0.09
	1:1; 22.5kV/22.5cm	634±0.13
	2:1; 15kV/7.5cm	845±0.25
	3:1; 22.5kV/7.5cm	479±0.11
40:60	1:1; 7.5kV/7.5cm	372±0.06
	1:1; 15kV/15cm	333±0.06
	1:1; 22.5kV/22.5cm	500±0.07
	2:1; 15kV/7.5cm	525±0.09
	3:1; 22.5kV/7.5cm	580±0.20

Table B3 The average diameter of gelatin nanofibers from gelatin solution in acetic acid/DMSO at various ratio of voltage/distance

Ratio of Ac:DMSO	Voltage/Distance	Average diameter (nm)
100:0	1:1; 7.5kV/7.5cm	271±0.11
	1:1; 15kV/15cm	265±0.09
	1:1; 22.5kV/22.5cm	277±0.08
	2:1; 15kV/7.5cm	240±0.07
	3:1; 22.5kV/7.5cm	220±0.07
97:3	1:1; 7.5kV/7.5cm	185±0.03
	1:1; 15kV/15cm	350±0.09
	1:1; 22.5kV/22.5cm	407±0.11
	2:1; 15kV/7.5cm	348±0.08
	3:1; 22.5kV/7.5cm	312±0.06
95:5	1:1; 7.5kV/7.5cm	187±0.03
	1:1; 15kV/15cm	413±0.06
	1:1; 22.5kV/22.5cm	428±0.10
	2:1; 15kV/7.5cm	336±0.06
	3:1; 22.5kV/7.5cm	272±0.06

Table B3 (cont.) The average diameter of gelatin nanofibers from gelatin solution in acetic acid/DMSO at various ratio of voltage/distance

Ratio of Ac:DMSO	Voltage/Distance	Average diameter (nm)
93:7	1:1; 7.5kV/7.5cm	230±0.03
	1:1; 15kV/15cm	511±0.12
	1:1; 22.5kV/22.5cm	504±0.12
	2:1; 15kV/7.5cm	fuse
	3:1; 22.5kV/7.5cm	434±0.09
91:9	1:1; 7.5kV/7.5cm	225±0.06
	1:1; 15kV/15cm	367±0.05
	1:1; 22.5kV/22.5cm	394±0.10
	2:1; 15kV/7.5cm	fuse
	3:1; 22.5kV/7.5cm	417±0.11

Table B4 The average diameter of gelatin nanofibers from gelatin solution in acetic acid/EG at various ratio of voltage/distance

Ratio of Ac:EG	Voltage/Distance	Average diameter (nm)
100:0	1:1; 7.5kV/7.5cm	271±0.11
	1:1; 15kV/15cm	265±0.09
	1:1; 22.5kV/22.5cm	277±0.07
	2:1; 15kV/7.5cm	240±0.07
	3:1; 22.5kV/7.5cm	220±0.07
97:3	1:1; 7.5kV/7.5cm	225±0.04
	1:1; 15kV/15cm	266±0.07
	1:1; 22.5kV/22.5cm	230±0.05
	2:1; 15kV/7.5cm	216±0.06
	3:1; 22.5kV/7.5cm	fuse
95:5	1:1; 7.5kV/7.5cm	264±0.04
	1:1; 15kV/15cm	238±0.07
	1:1; 22.5kV/22.5cm	223±0.04
	2:1; 15kV/7.5cm	229±0.04
	3:1; 22.5kV/7.5cm	285±0.09

Table B4 (cont.) The average diameter of gelatin nanofibers from gelatin solution in acetic acid/EG at various ratio of voltage/distance

Ratio of Ac:EG	Voltage/Distance	Average diameter (nm)
93:7	1:1; 7.5kV/7.5cm	228±0.07
	1:1; 15kV/15cm	224±0.04
	1:1; 22.5kV/22.5cm	fuse
	2:1; 15kV/7.5cm	fuse
	3:1; 22.5kV/7.5cm	fuse
91:9	1:1; 7.5kV/7.5cm	fuse
	1:1; 15kV/15cm	fuse
	1:1;22.5kV/22.5cm	152±0.04
	2:1; 15kV/7.5cm	fuse
	3:1; 22.5kV/7.5cm	fuse

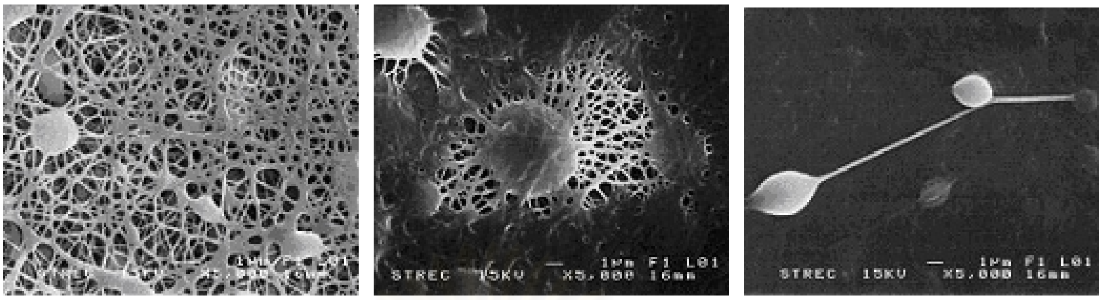
Table B5 The average diameter of gelatin nanofibers from gelatin solution in acetic acid/Formamide at various ratio of voltage/distance

Ratio of Ac:F	Voltage/Distance	Average diameter (nm)
100:0	1:1; 7.5kV/7.5cm	271±0.11
	1:1; 15kV/15cm	265±0.09
	1:1; 22.5kV/22.5cm	277±0.08
	2:1; 15kV/7.5cm	240±0.07
	3:1; 22.5kV/7.5cm	220±0.07
97:3	1:1; 7.5kV/7.5cm	324±0.08
	1:1; 15kV/15cm	352±0.08
	1:1; 22.5kV/22.5cm	351±0.07
	2:1; 15kV/7.5cm	2085±1.56
	3:1; 22.5kV/7.5cm	374±0.15
95:5	1:1; 7.5kV/7.5cm	fuse
	1:1; 15kV/15cm	fuse
	1:1; 22.5kV/22.5cm	fuse
	2:1; 15kV/7.5cm	fuse
	3:1; 22.5kV/7.5cm	fuse

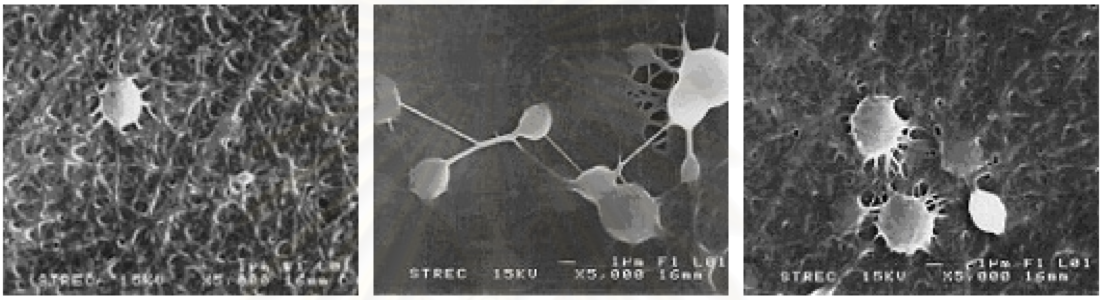
Table B5 (cont.) The average diameter of gelatin nanofibers from gelatin solution in acetic acid/Formamide at various ratio of voltage/distance

Ratio of Ac:F	Voltage/Distance	Average diameter (nm)
93:7	1:1; 7.5kV/7.5cm	278±0.11
	1:1; 15kV/15cm	fuse
	1:1; 22.5kV/22.5cm	fuse
	2:1; 15kV/7.5cm	fuse
	3:1; 22.5kV/7.5cm	fuse
91:9	1:1; 7.5kV/7.5cm	237±0.07
	1:1; 15kV/15cm	fuse
	1:1; 22.5kV/22.5cm	fuse
	2:1; 15kV/7.5cm	fuse
	3:1; 22.5kV/7.5cm	fuse

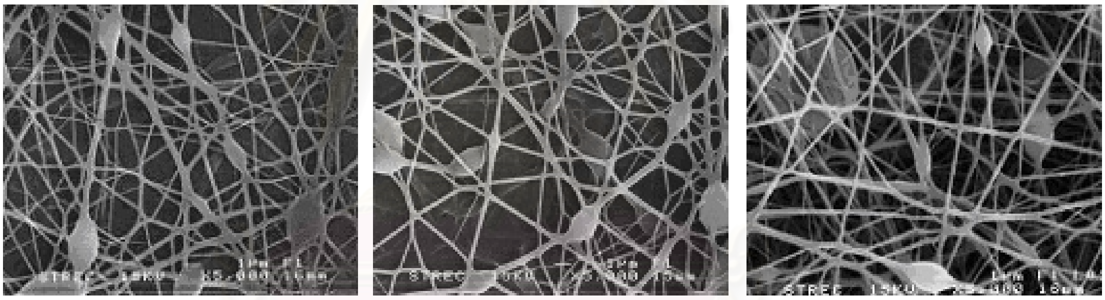
Appendix C



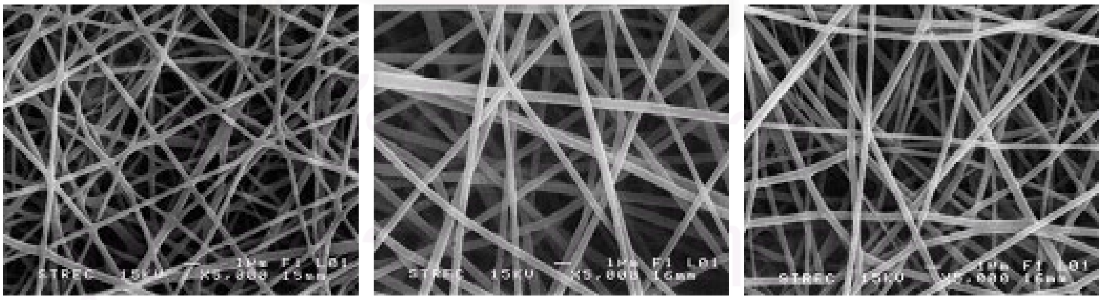
15%w/v; 7.5kV/7.5cm 15%w/v; 15kV/7.5cm 15%w/v; 22.5kV/7.5cm



17%w/v; 7.5kV/7.5cm 17%w/v; 15kV/7.5cm 17%w/v; 22.5kV/7.5cm

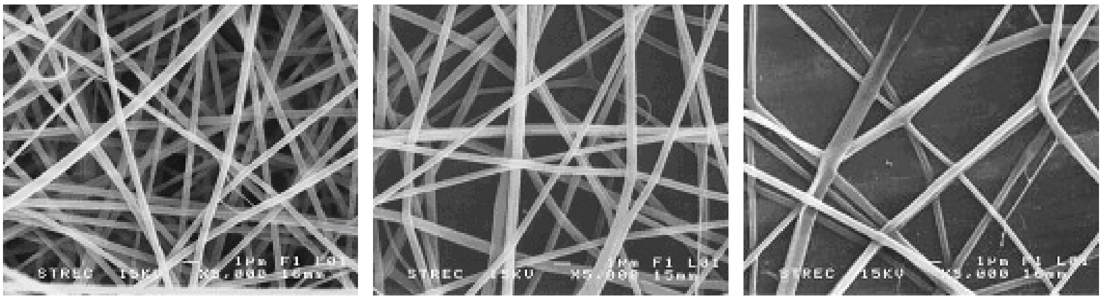


19%w/v; 7.5kV/7.5cm 19%w/v; 15kV/7.5cm 19%w/v; 22.5kV/7.5cm

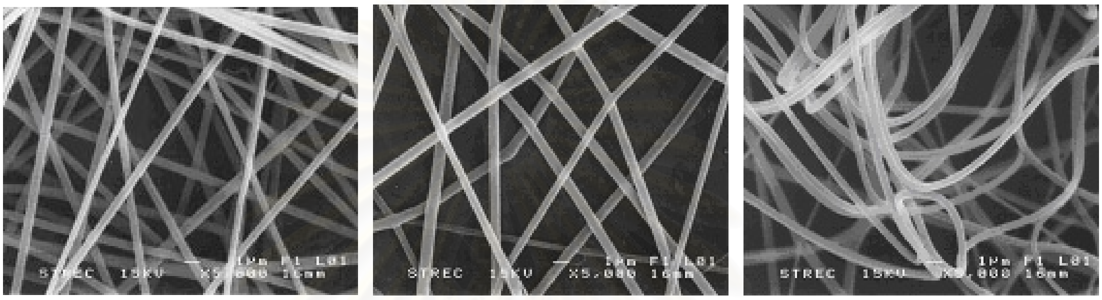


21%w/v; 7.5kV/7.5cm 21%w/v; 15kV/7.5cm 21%w/v; 22.5kV/7.5cm

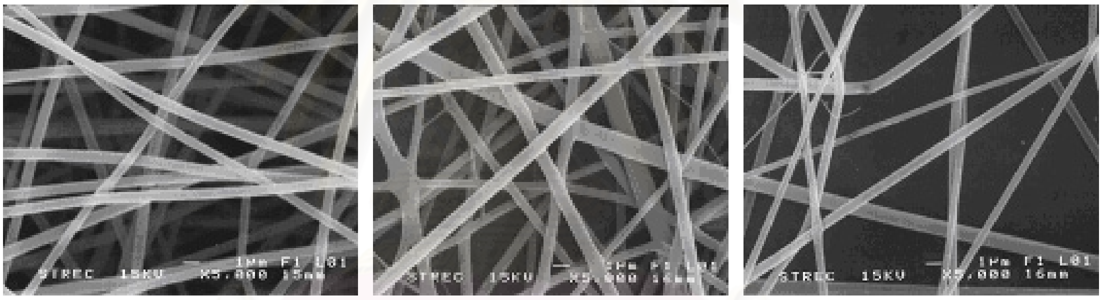
Figure C1 The morphology of electrospun nanofibers were obtained from gelatin solution in acetic acid at various ratio of voltage/distance



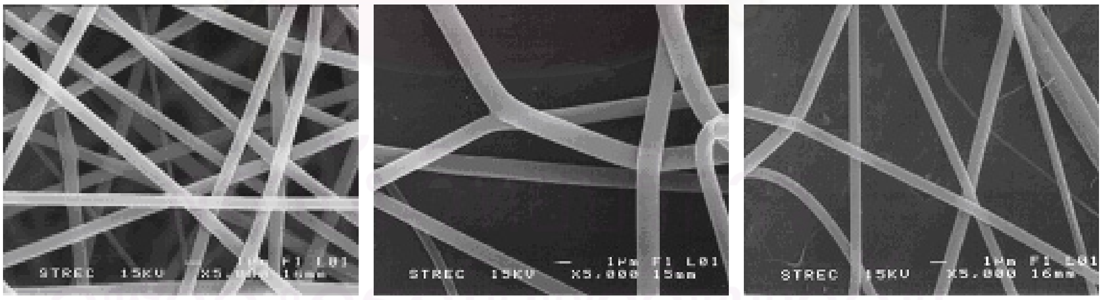
23%w/v; 7.5kV/7.5cm 23%w/v; 15kV/7.5cm 23%w/v; 22.5kV/7.5cm



25%w/v; 7.5kV/7.5cm 25%w/v; 15kV/7.5cm 25%w/v; 22.5kV/7.5cm

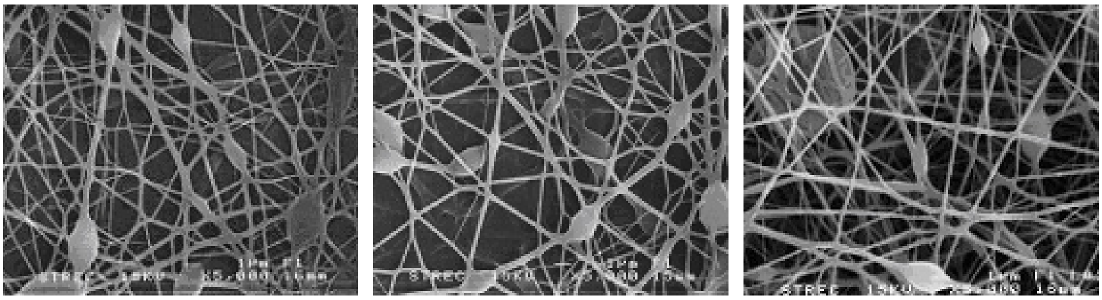


27%w/v; 7.5kV/7.5cm 27%w/v; 15kV/7.5cm 27%w/v; 22.5kV/7.5cm

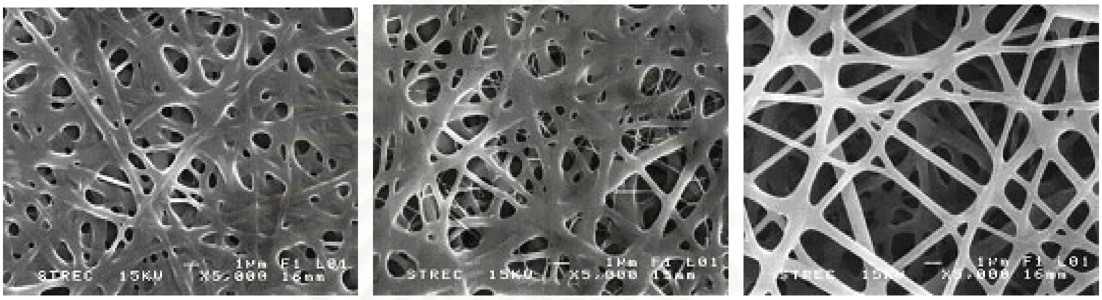


29%w/v; 7.5kV/7.5cm 29%w/v; 15kV/7.5cm 29%w/v; 22.5kV/7.5cm

Figure C1 (cont.) The morphology of electrospun nanofibers were obtained from gelatin solution in acetic acid at various ratio of voltage/distance

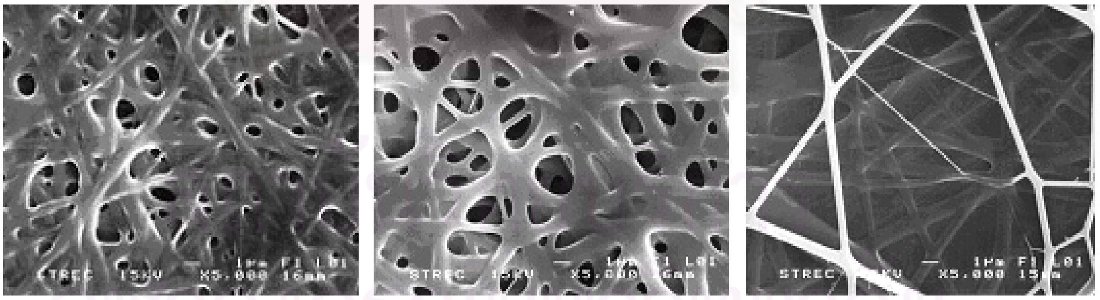


100:0; 7.5kV/7.5cm 100:0; 15kV/7.5cm 100:0; 22.5kV/7.5cm



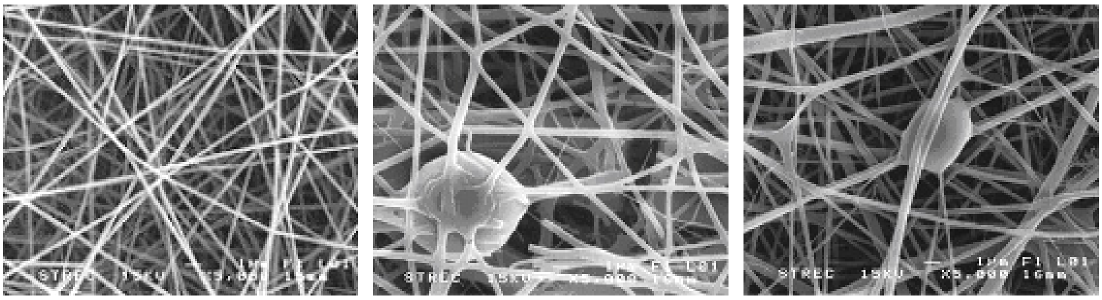
90:10; 7.5kV/7.5cm 90:10; 15kV/7.5cm 90:10; 22.5kV/7.5cm

80:20; 7.5kV/7.5cm 80:20; 15kV/7.5cm 80:20; 22.5kV/7.5cm

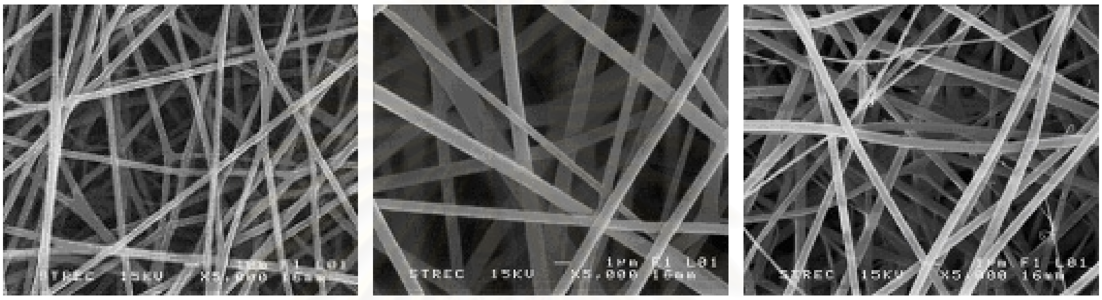


70:30; 7.5kV/7.5cm 70:30; 15kV/7.5cm 70:30; 22.5kV/7.5cm

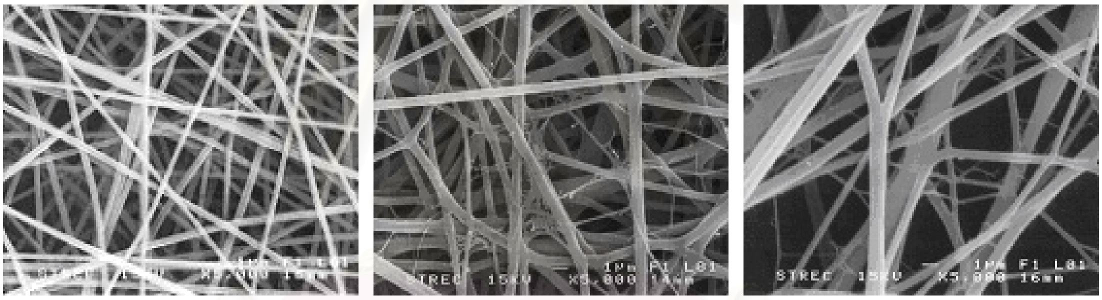
Figure C2 The morphology of electrospun nanofibers were obtained from gelatin solution in acetic acid/TFE at various ratio of voltage/distance



60:40; 7.5kV/7.5cm 60:40; 15kV/7.5cm 60:40; 22.5kV/7.5cm

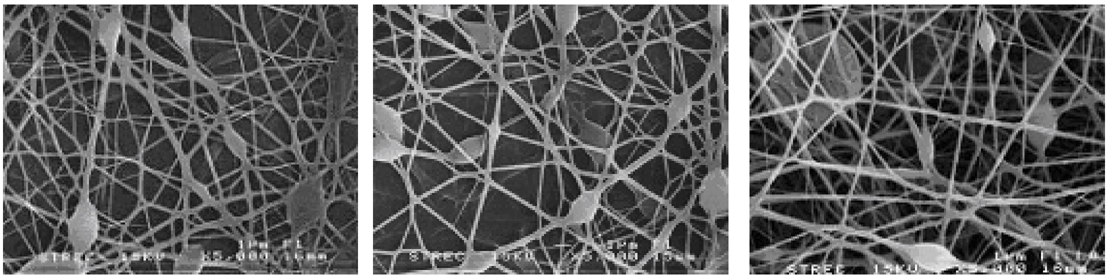


50:50; 7.5kV/7.5cm 50:50; 15kV/7.5cm 50:50; 22.5kV/7.5cm

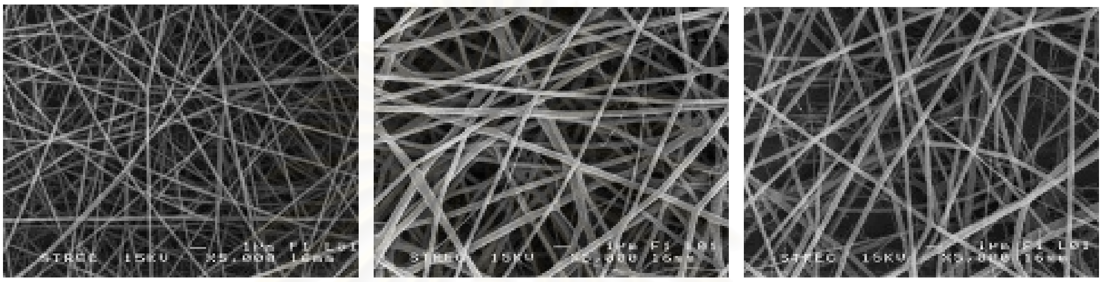


40:60; 7.5kV/7.5cm 40:60; 15kV/7.5cm 40:60; 22.5kV/7.5cm

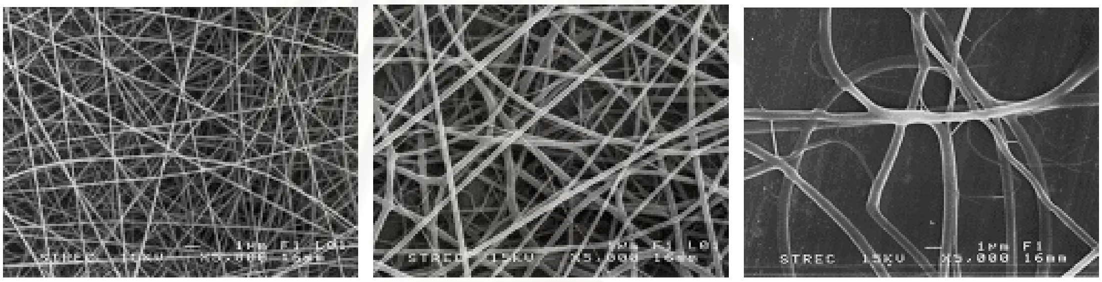
Figure C2 (cont.) The morphology of electrospun nanofibers were obtained from gelatin solution in acetic acid/TFE at various ratio of voltage/distance



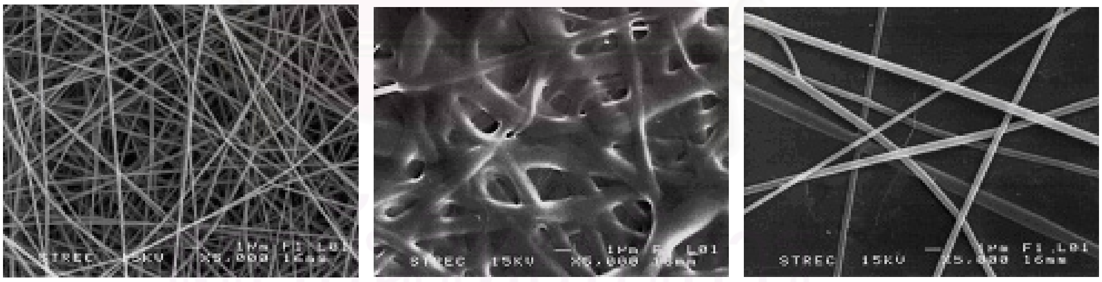
100:0; 7.5kV/7.5cm 100:0; 15kV/7.5cm 100:0; 22.5kV/7.5cm



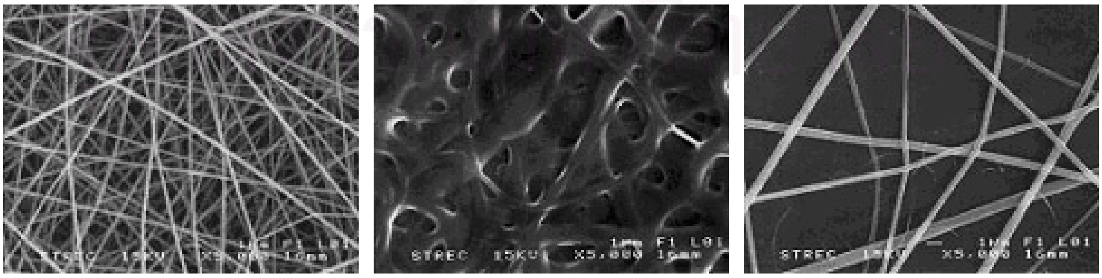
97:3; 7.5kV/7.5cm 97:3; 15kV/7.5cm 97:3; 22.5kV/7.5cm



95:5; 7.5kV/7.5cm 95:5; 15kV/7.5cm 95:5; 22.5kV/7.5cm

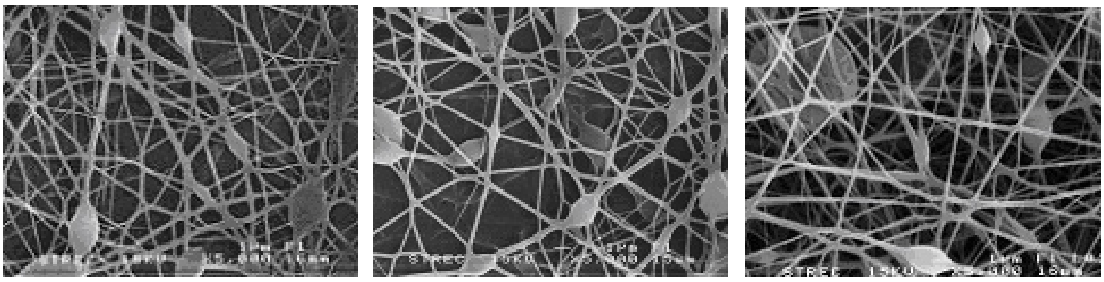


93:7; 7.5kV/7.5cm 93:7; 15kV/7.5cm 93:7; 22.5kV/7.5cm

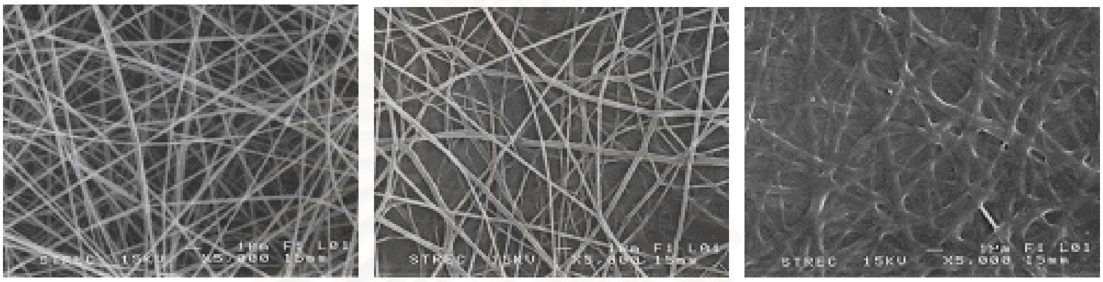


91:9; 7.5kV/7.5cm 91:9; 15kV/7.5cm 91:9; 22.5kV/7.5cm

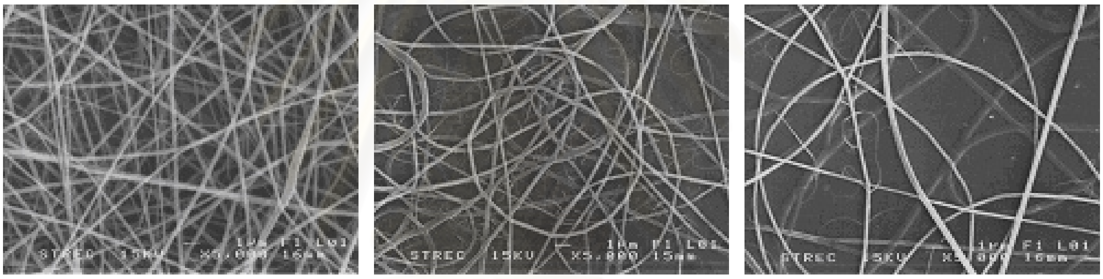
Figure C3 The morphology of electrospun nanofibers were obtained from gelatin solution in acetic acid/DMSO at various ratio of voltage/distance



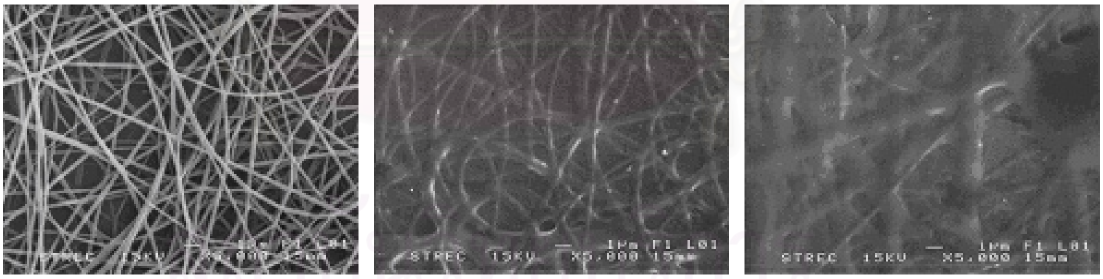
100:0; 7.5kV/7.5cm 100:0; 15kV/7.5cm 100:0; 22.5kV/7.5cm



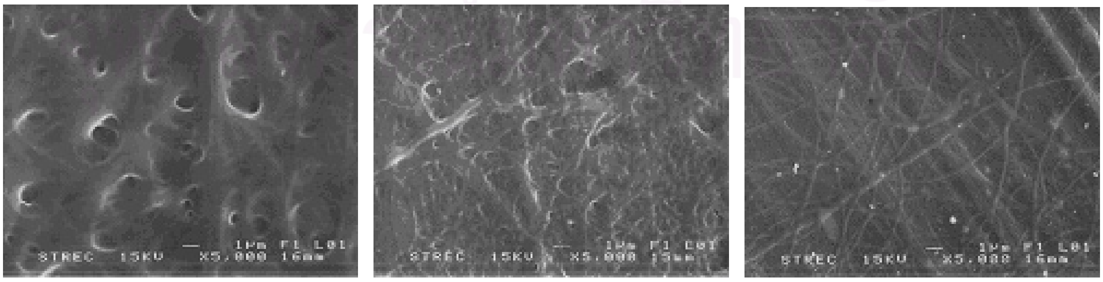
97:3; 7.5kV/7.5cm 97:3; 15kV/7.5cm 97:3; 22.5kV/7.5cm



95:5; 7.5kV/7.5cm 95:5; 15kV/7.5cm 95:5; 22.5kV/7.5cm

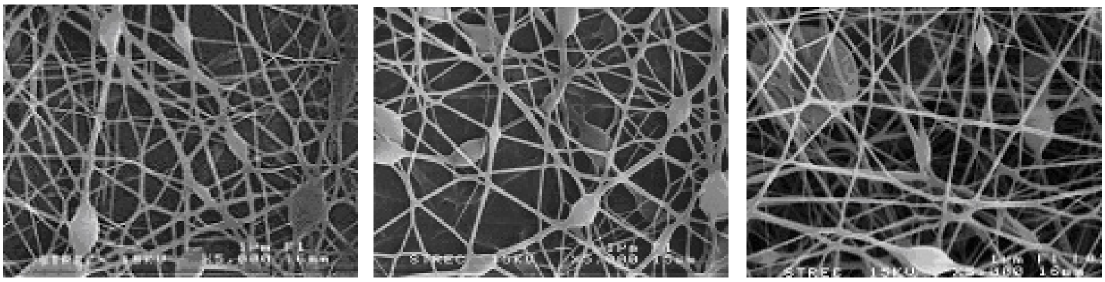


93:7; 7.5kV/7.5cm 93:7; 15kV/7.5cm 93:7; 22.5kV/7.5cm

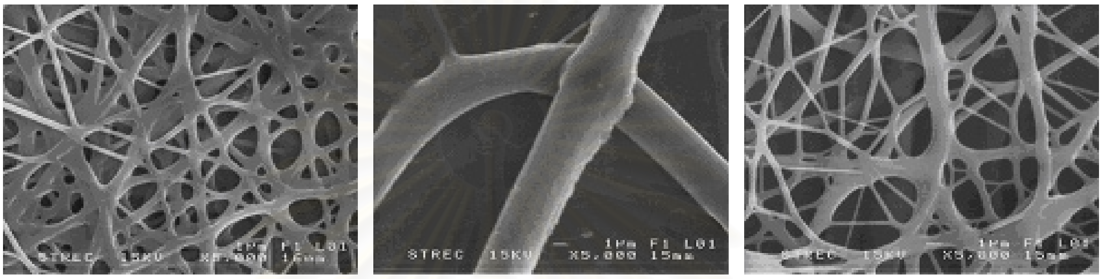


91:9; 7.5kV/7.5cm 91:9; 15kV/7.5cm 91:9; 22.5kV/7.5cm

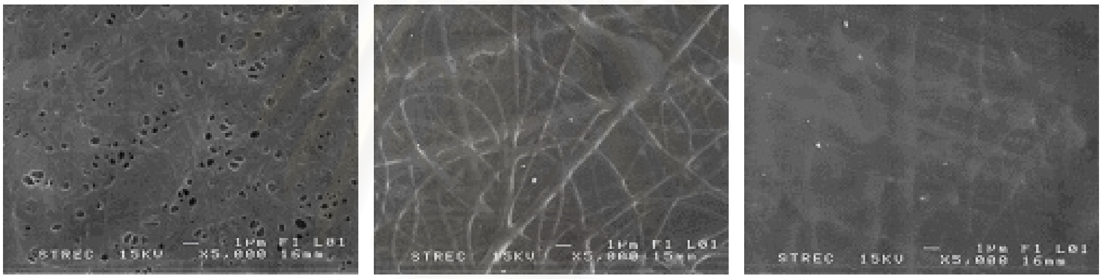
Figure C4 The morphology of electrospun nanofibers were obtained from gelatin solution in acetic acid/EG at various ratio of voltage/distance



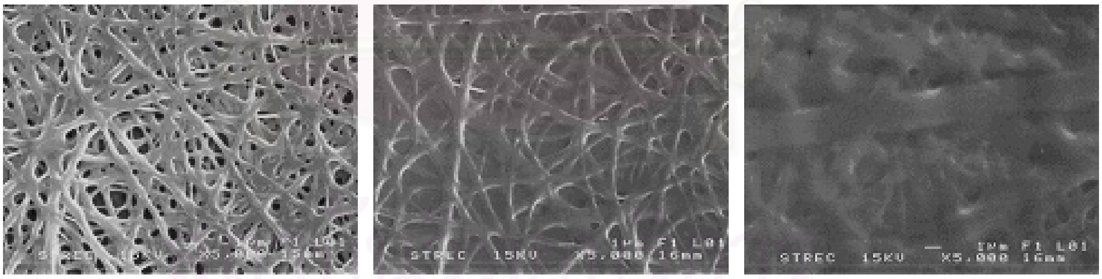
100:0; 7.5kV/7.5cm 100:0; 15kV/7.5cm 100:0; 22.5kV/7.5cm



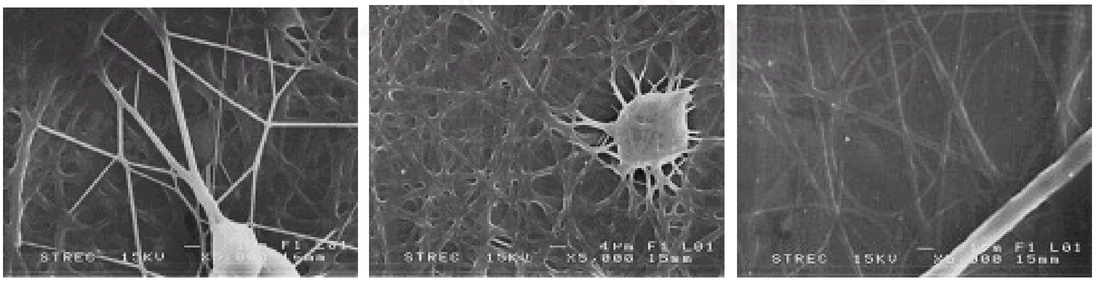
97:3 7.5kV/7.5cm 97:3 15kV/7.5cm 97:3 22.5kV/7.5cm



95:5 7.5kV/7.5cm 95:5 15kV/7.5cm 95:5 22.5kV/7.5cm

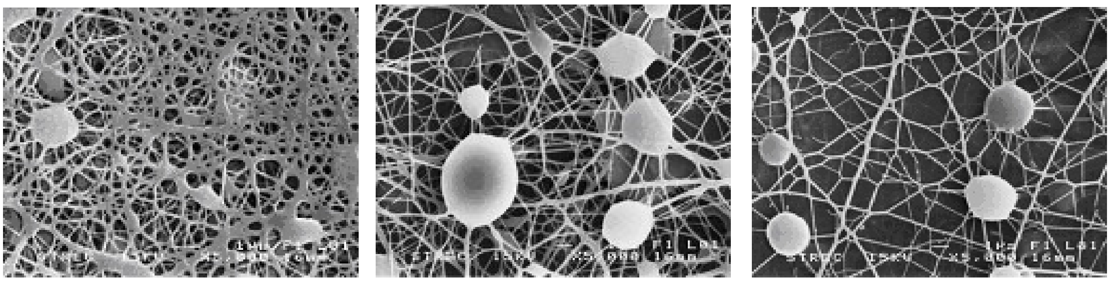


93:7; 7.5kV/7.5cm 93:7; 15kV/7.5cm 93:7; 22.5kV/7.5cm

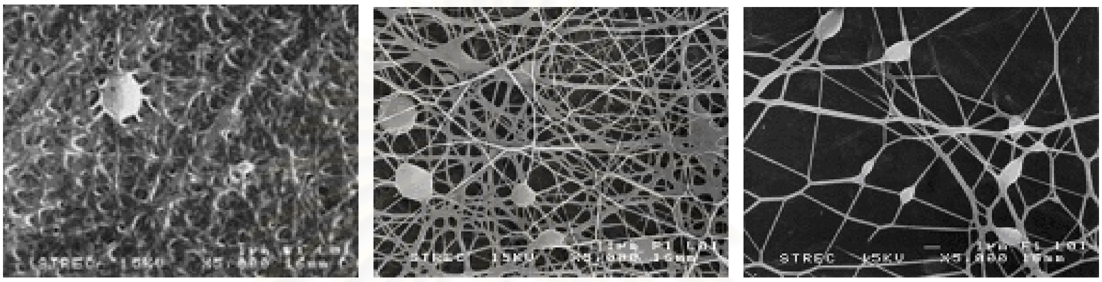


91:9; 7.5kV/7.5cm 91:9; 15kV/7.5cm 91:9; 22.5kV/7.5cm

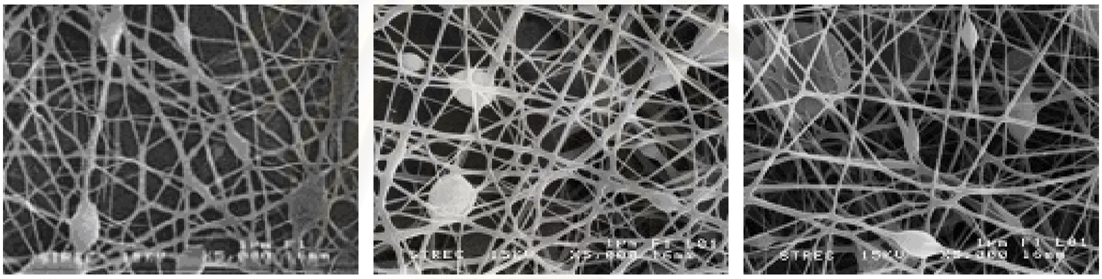
Figure C5 The morphology of electrospun nanofibers were obtained from gelatin solution in acetic acid/Formamide at various ratio of voltage/distance



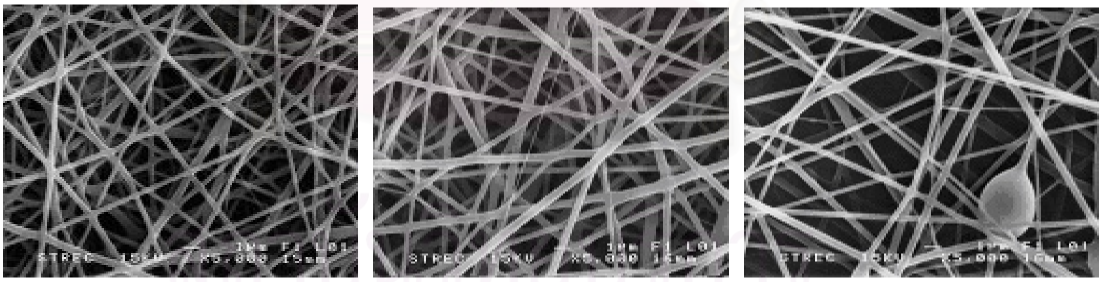
15%w/v; 7.5kV/7.5cm 15%w/v; 15kV/15cm 15%w/v; 22.5kV/22.5cm



17%w/v; 7.5kV/7.5cm 17%w/v; 15kV/15cm 17%w/v; 22.5kV/22.5cm

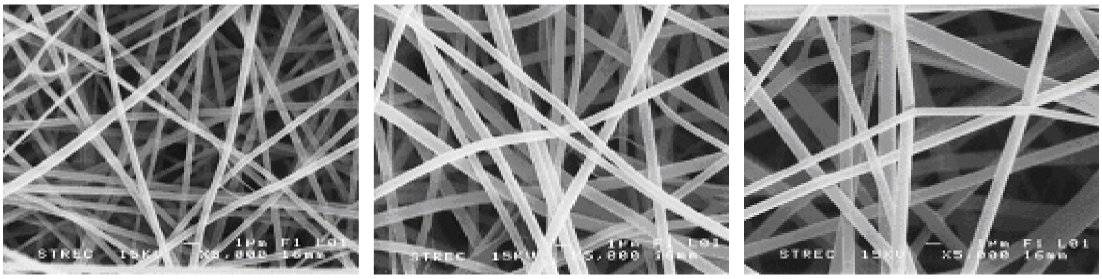


19%w/v; 7.5kV/7.5cm 19%w/v; 15kV/15cm 19%w/v; 22.5kV/22.5cm

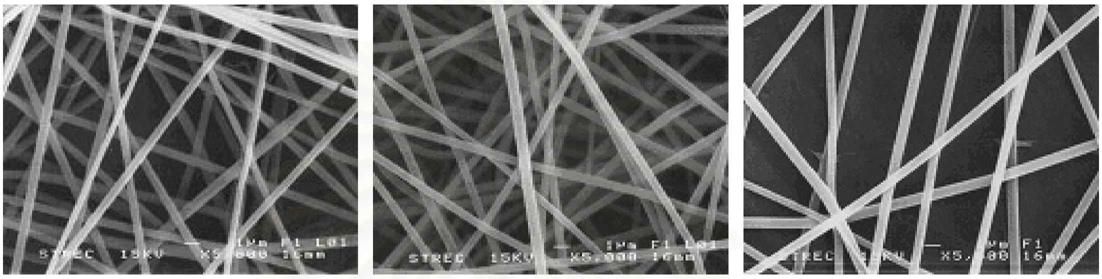


21%w/v; 7.5kV/7.5cm 21%w/v; 15kV/15cm 21%w/v; 22.5kV/22.5cm

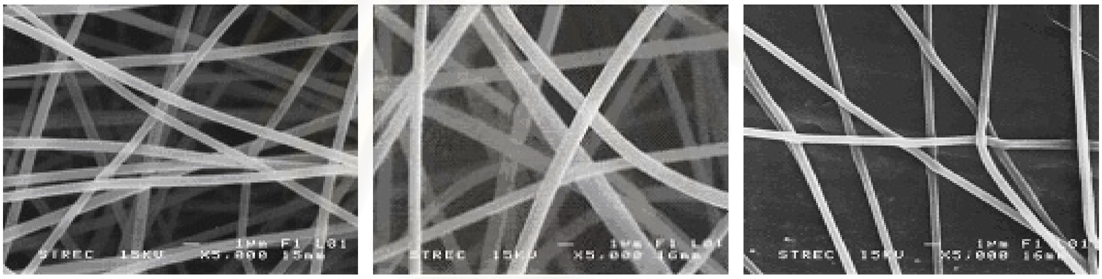
Figure C6 The morphology of electrospun nanofibers were obtained from gelatin solution in acetic acid at various ratio of voltage/distance



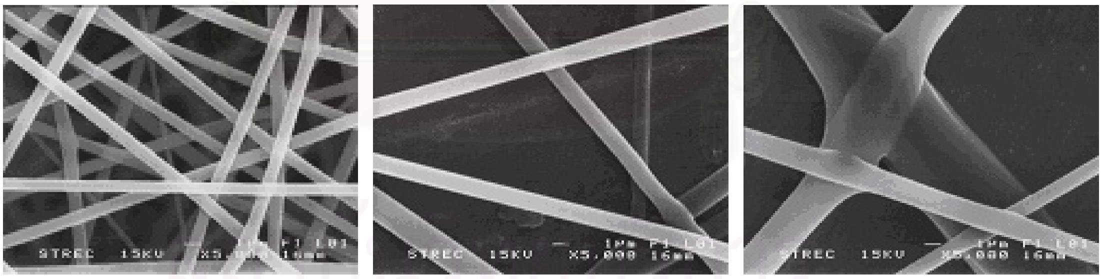
23%w/v; 7.5kV/7.5cm 23%w/v; 15kV/15cm 23%w/v; 22.5kV/22.5cm



25%w/v; 7.5kV/7.5cm 25%w/v; 15kV/15cm 25%w/v; 22.5kV/22.5cm

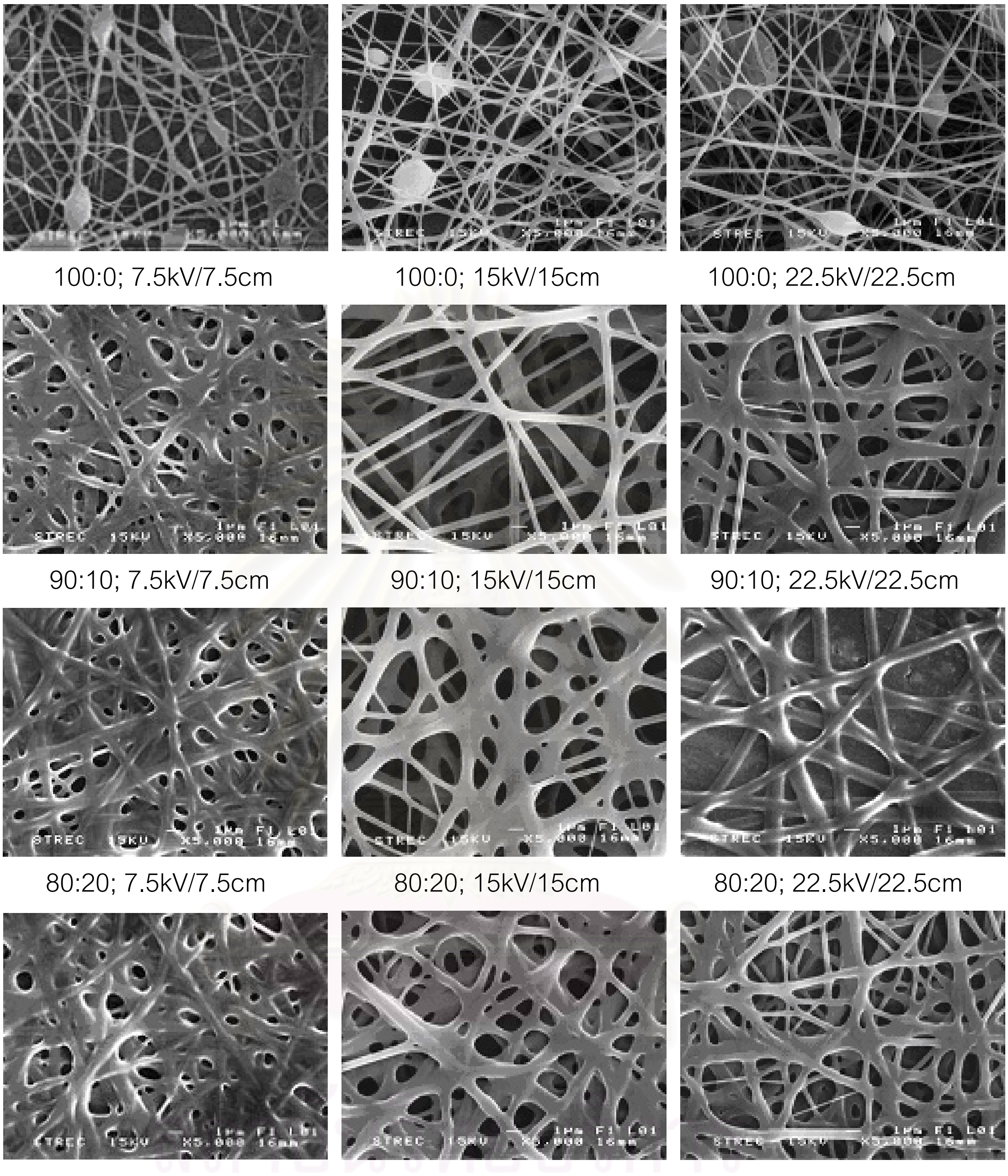


27%w/v; 7.5kV/7.5cm 27%w/v; 15kV/15cm 27%w/v; 22.5kV/22.5cm



29%w/v; 7.5kV/7.5cm 29%w/v; 15kV/15cm 29%w/v; 22.5kV/22.5cm

Figure C6 (cont.) The morphology of electrospun nanofibers were obtained from gelatin solution in acetic acid at various ratio of voltage/distance



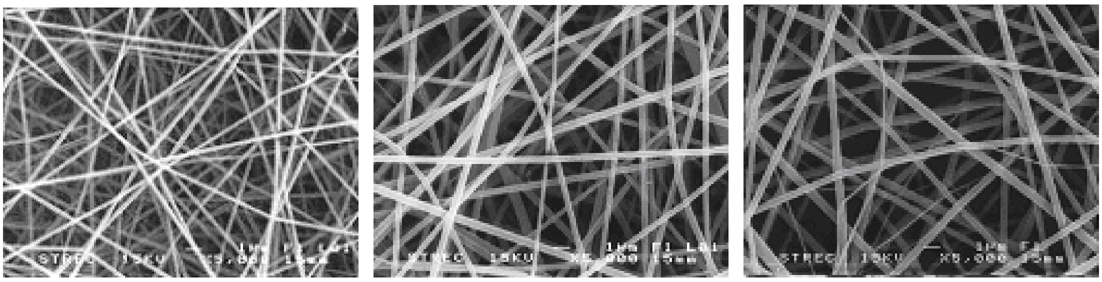
100:0; 7.5kV/7.5cm
100:0; 15kV/15cm
100:0; 22.5kV/22.5cm

90:10; 7.5kV/7.5cm
90:10; 15kV/15cm
90:10; 22.5kV/22.5cm

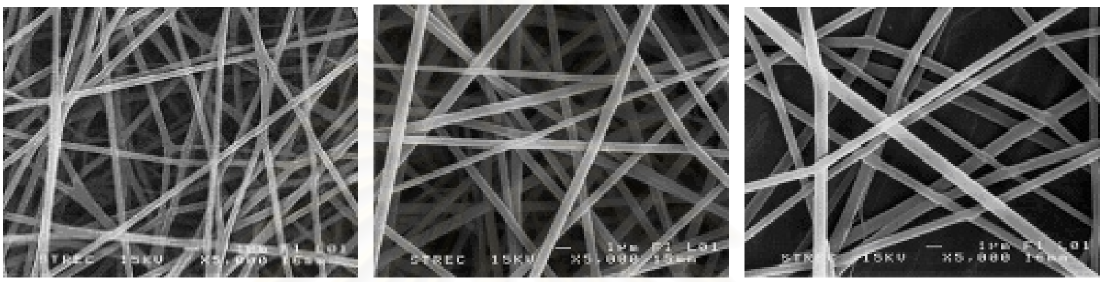
80:20; 7.5kV/7.5cm
80:20; 15kV/15cm
80:20; 22.5kV/22.5cm

70:30; 7.5kV/7.5cm
70:30; 15kV/15cm
70:30; 22.5kV/22.5cm

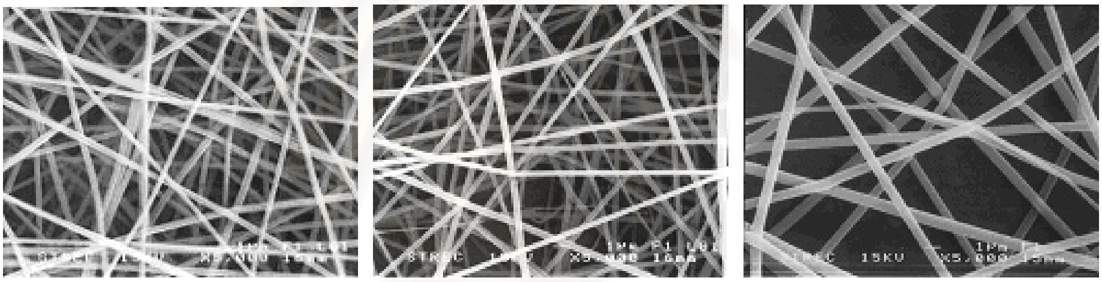
Figure C7 The morphology of electrospun nanofibers were obtained from gelatin solution in acetic acid/TFE at various ratio of voltage/distance



60:40; 7.5kV/7.5cm 60:40; 15kV/15cm 60:40; 22.5kV/22.5cm

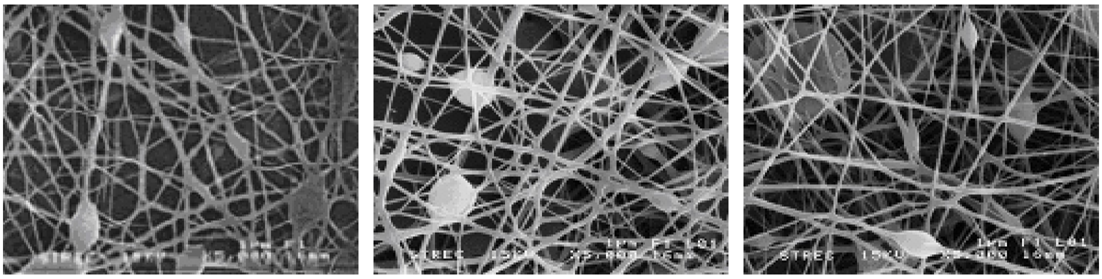


50:50; 7.5kV/7.5cm 50:50; 15kV/15cm 50:50; 22.5kV/22.5cm

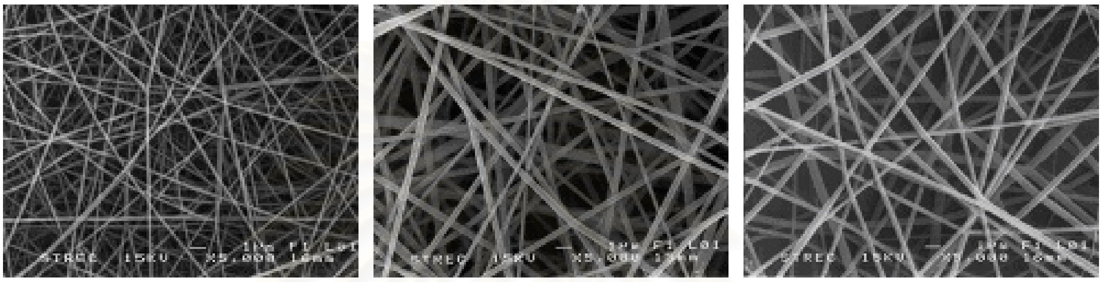


40:60; 7.5kV/7.5cm 40:60; 15kV/15cm 40:60; 22.5kV/22.5cm

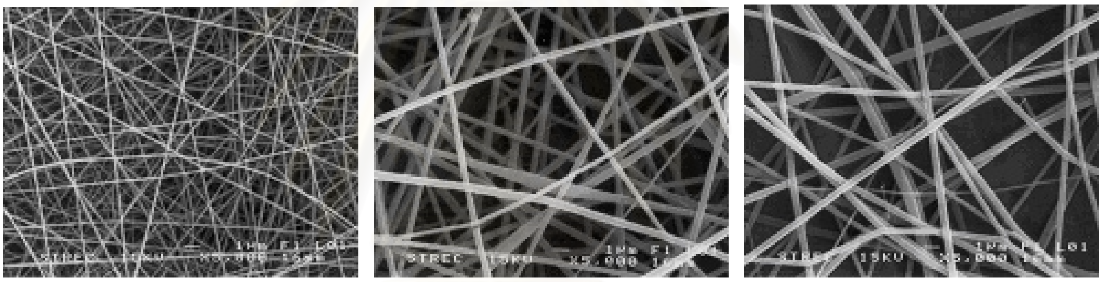
Figure C7 (cont.) The morphology of electrospun nanofibers were obtained from gelatin solution in acetic acid/TFE at various ratio of voltage/distance



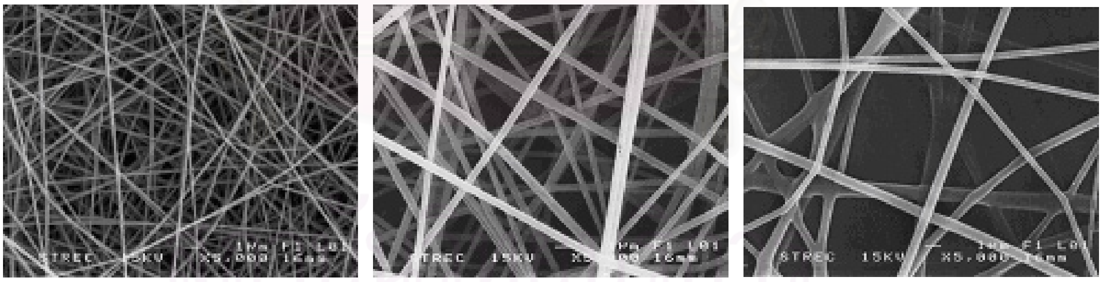
100:0; 7.5kV/7.5cm 100:0; 15kV/15cm 100:0; 22.5kV/22.5cm



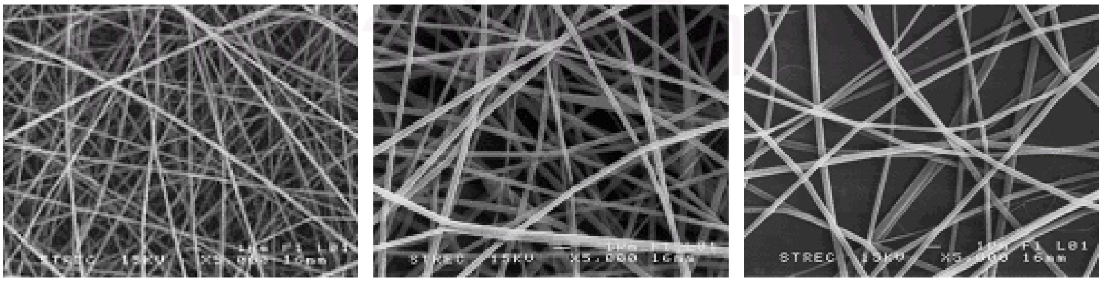
97:3; 7.5kV/7.5cm 97:3; 15kV/15cm 97:3; 22.5kV/22.5cm



95:5; 7.5kV/7.5cm 95:5; 15kV/15cm 95:5; 22.5kV/22.5cm

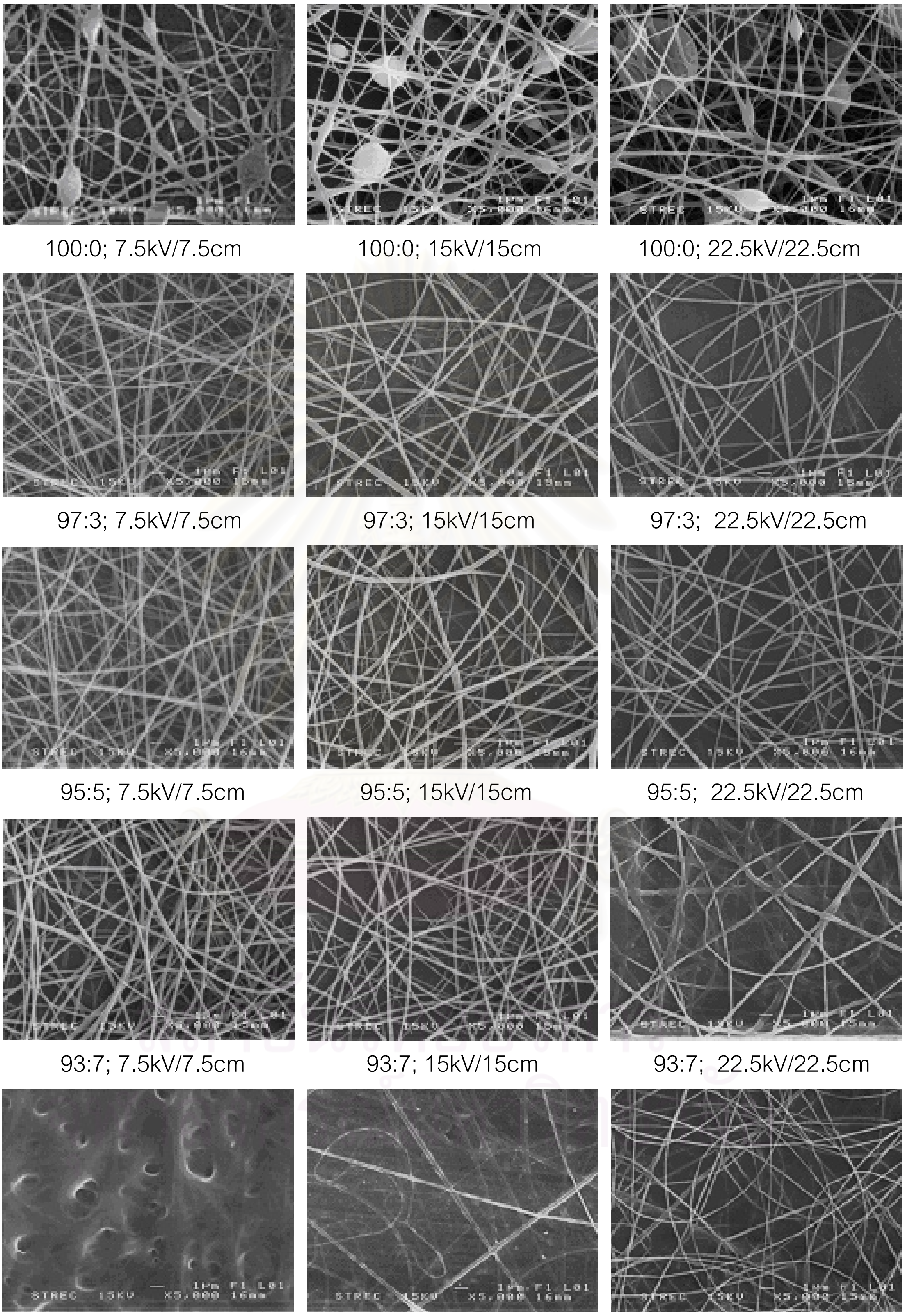


93:7; 7.5kV/7.5cm 93:7; 15kV/15cm 93:7; 22.5kV/22.5cm



91:9; 7.5kV/7.5cm 91:9; 15kV/15cm 91:9; 22.5kV/22.5cm

Figure C8 The morphology of electrospun nanofibers were obtained from gelatin solution in acetic acid/DMSO at various ratio of voltage/distance



100:0; 7.5kV/7.5cm
100:0; 15kV/15cm
100:0; 22.5kV/22.5cm

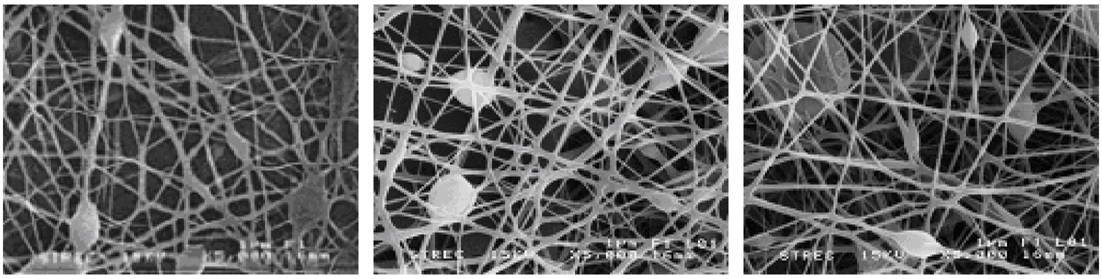
97:3; 7.5kV/7.5cm
97:3; 15kV/15cm
97:3; 22.5kV/22.5cm

95:5; 7.5kV/7.5cm
95:5; 15kV/15cm
95:5; 22.5kV/22.5cm

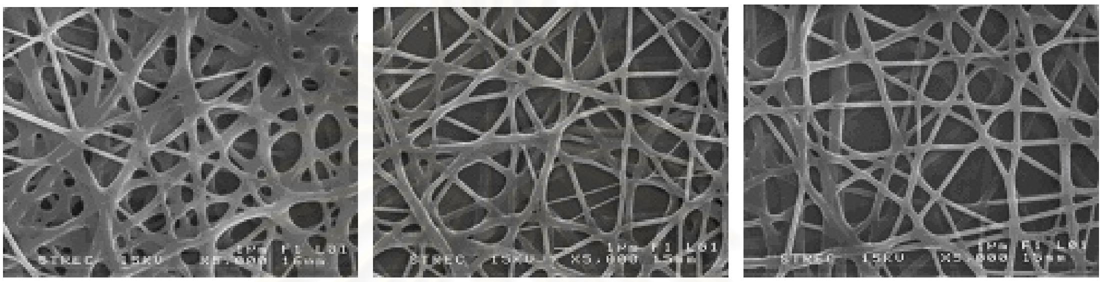
93:7; 7.5kV/7.5cm
93:7; 15kV/15cm
93:7; 22.5kV/22.5cm

91:9; 7.5kV/7.5cm
91:9; 15kV/15cm
91:9; 22.5kV/22.5cm

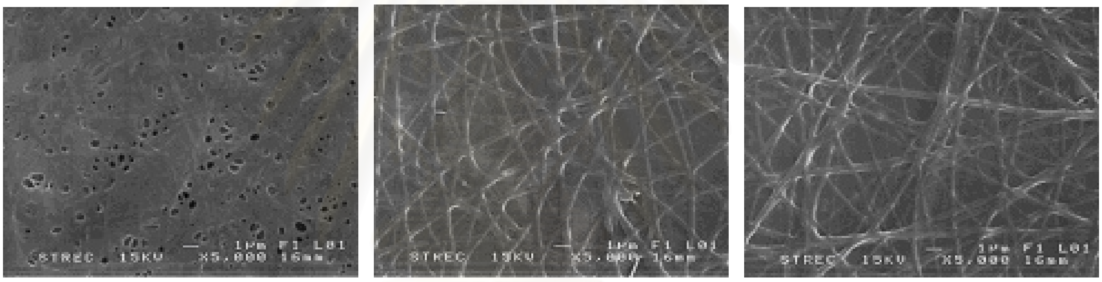
Figure C9 The morphology of electrospun nanofibers were obtained from gelatin solution in acetic acid/EG at various ratio of voltage/distance



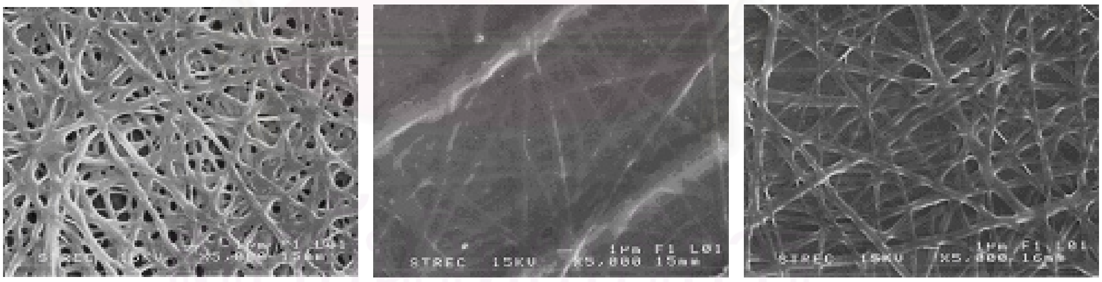
100:0; 7.5kV/7.5cm   100:0; 15kV/15cm   100:0; 22.5kV/22.5cm



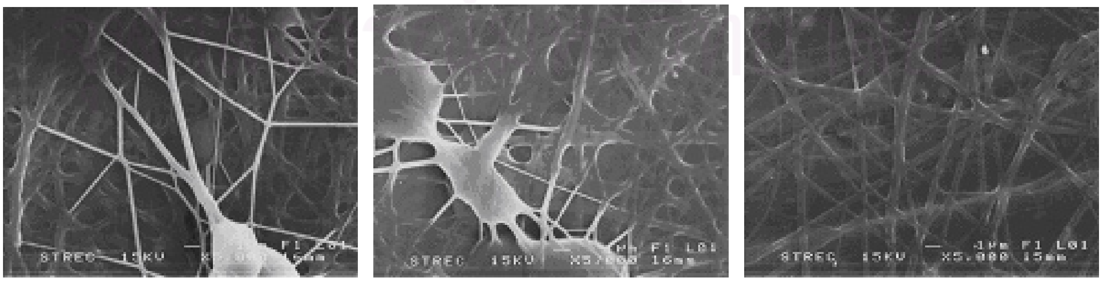
97:3; 7.5kV/7.5cm   97:3; 15kV/15cm   97:3; 22.5kV/22.5cm



95:5; 7.5kV/7.5cm   95:5; 15kV/15cm   95:5; 22.5kV/22.5cm



93:7; 7.5kV/7.5cm   93:7; 15kV/15cm   93:7; 22.5kV/22.5cm



91:9; 7.5kV/7.5cm   91:9; 15kV/15cm   91:9; 22.5kV/22.5cm

Figure C10 The morphology of electrospun nanofibers were obtained from gelatin solution in acetic acid/Formamide at various ratio of voltage/distance

CURRICULUM VITAE

Name : Miss Nuanchan Choktaweesap

Date of birth : 3 August 1981

Place of birth : 58/1-2 Ratsadonyindee Road, Muang, Nhamuang, Ratchaburi,
Thailand 70000

Education :-

Year	Degree	University
2002	Bachelor of Science (Industrial Chemistry)	King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand
2004	Master of Science (Applied Polymer Science and Textile Technology)	Chulalongkorn University, Bangkok, Thailand

Summer Trainee : 2 months Internship in Research and Development Zone
at Union Footware Company, Chacherngsow, Thailand.

Publications and Presentations :

1. Choktaweesap, N., Aht-Ong, D., and Supaphol, P., (2004). "Electrostatic spinning of Gelatin", Abstract Book of The International Conference on Smart Materials 2004, Chiang Mai, Thailand.
2. Choktaweesap, N., Aht-Ong, D., and Supaphol, P., (2005). "Effects of Processing Parameters on Morphology and Diameter of Electrospun Gelatin Nanofibers".

Award :

1. The First Prize Poster Presentration in Applied Science and Technology of The 13th Academic Conference at Faculty of Science, Chulalongkorn University, Bangkok, Thailand.