CHAPTER 2

LITERATURE REVIEW

2.1 Gelatin

Gelatin is water-soluble, hydrophilic, derived colloidal proteins produced by controlled hydrolysis of water-insoluble collagen, which is the major protein component in skin, bones, hides, and white connective tissue of animal body (Ockerman and Hansen, 1988). Although the relative proportions and sequences of the constituent amino acids in collagen and gelatin are substantially the same, the physical and chemical properties of the two proteins differ markedly (Ledward, 2000). Gelatins are produced on a large scale from skin and bones of mammalian origin (mainly beef and pork) by alkaline or acidic extraction (Veis, 1964). Some fish gelatins are commercially available, although they are not well characterized. One of the most important differences between mammalian and fish gelatins are that the latter have normally low gelling and melting temperatures and also lower gel strength (Norland, 1990). In order to obtain gelatin, a pre-treatment process is required to convert the tissue collagen into a suitable form for extraction. There are two main types of gelatin. Type A gelatin is derived from collagen with exclusively acid pretreatment. Type B gelatin is the result of an alkaline pretreatment of the collagen.

2.2 Collagen

The term collagen derives from the Greek word for glue and was defined in the 1893 edition of Oxford Dictionary as "that constituent of connective tissue which yields gelatin on boiling" (Branden and Tooze, 1999).

Collagen, which is a fibrous protein, is present in all tissues and organs where it provides the framework that gives the tissues their form and structural strength. Collagens and their denatured forms, gelatins, are composed of long chains of amino

acids connected by peptide bonds (Ockerman and Hansen, 1988). Free amino acids are joined in a peptide chain which twists itself into a left-handed helix (called α-chain). Each collagen polypeptide chain contains about 1000 amino acid residues. Three of these interlink to form a right-handed superhelix (Figure 2.1) (De Man, 1990). This is the tropocollagen molecule, a rod-shaped protein about 15 °A in diameter and 3000 °A long (Wong, 1989). The three chains are held together by hydrogen bonding. Tropocollagen molecules line up in a staggered array to overlap by one-quarter of their length to form a fibril (De Man, 1990). Generally, it contains 33% glycine, 12% proline, 11% hydroxyproline, and alanine and is almost completely lacking in tryptophan and is low in methionine, cystine, and tyrosine (Xiong, 1997; Jamilah and Harvinder, 2002). The protein is comprised of peptide triplets, glycine – X – Y, where X and Y can be any one of the amino acids but proline has a preference for the X position and hydroxyproline the Y position (Baily and Light, 1989). The amino acid sequence shows that most of the polypeptide chain makes up of glycine-led triplets of the following distribution:

Triplet	Proportion
Gly-X-X	0.44
Gly-X-I	0.20
Gly-I-X	0.27
Gly-I-I	0.09

where I = imino acid residue (proline or hydroxyproline) and X = other amino acid residues. The segment of the polypeptide chain consisting of repeating triplets with imino acid residues are the nonpolar regions, and the segments containing Gly-X-X triplets are mostly polar (Wong, 1989).

Imino acids impart a significant degree of rigidity to the molecule encouraging the stability of long-range order. It is also probable that interstitial water molecules play a crucial role, acting as hydrogen-bond bridges in order to increase the stability of the helix (Johnston-Banks, 1990).
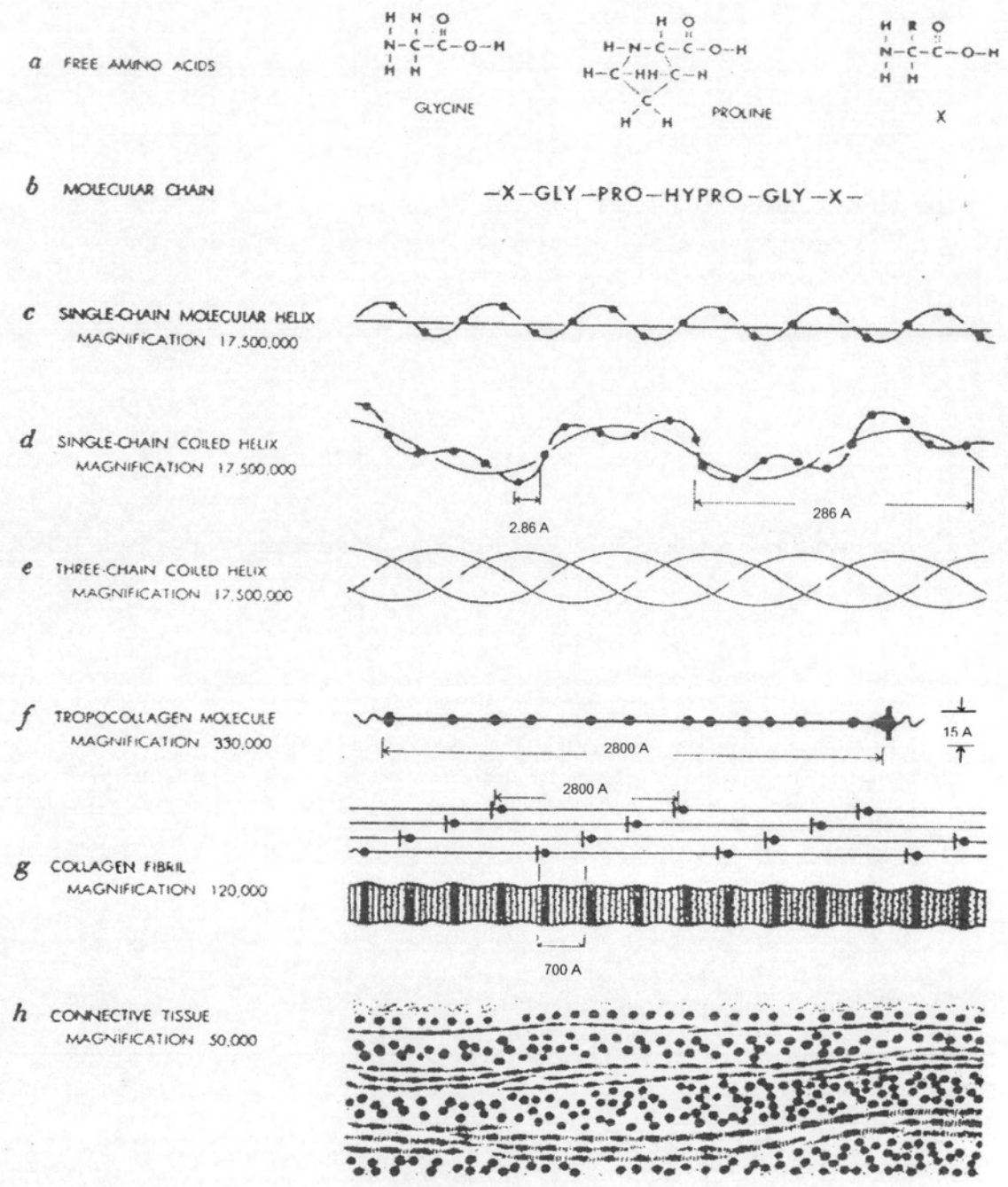
a FREE AMINO ACIDS

H H O
| | ‖
N—C—C—O—H
| |
H H

GLYCINE

H O
‖
H—N—C—C—O—H
|
H—C   C—H
\   /
C
/ \
H   H

PROLINE

H R O
| | ‖
N—C—C—O—H
| |
H H

X

b MOLECULAR CHAIN

—X—GLY—PRO—HYPRO—GLY—X—

c SINGLE-CHAIN MOLECULAR HELIX
MAGNIFICATION 17,500,000

d SINGLE-CHAIN COILED HELIX
MAGNIFICATION 17,500,000

2.86 A   286 A

e THREE-CHAIN COILED HELIX
MAGNIFICATION 17,500,000

f TROPOCOLLAGEN MOLECULE
MAGNIFICATION 330,000

2800 A   15 A

g COLLAGEN FIBRIL
MAGNIFICATION 120,000

2800 A

700 A

h CONNECTIVE TISSUE
MAGNIFICATION 50,000

Figure 2.1 Stages in the formation of collagen.

Source: De Man (1990)

2.3 Gelatin structure

2.3.1 Primary structure

The primary structure of gelatin closely resembles the parent collagen. Small differences are due to raw material sources and the pretreatment and extraction procedures. These can be summarized as follows (Johnston-Banks, 1990).

(I) Partial removal of amide groups of asparagine and glutamine results in an increase in the aspartic and glutamic acid contents. The number of carboxyl groups in the gelatin increases and thus lowers the isoelectric point. The degree of conversion is related to the severity of the pretreatment process. The mildly acid pretreatment will remove only a few amide groups, while liming treatment will result in almost complete conversion of amide.

(II) Conversion of arginine to ornithine occurs during long or extended liming processes. This occurs by removal of a urea group from the arginine side-chain. This has been proposed as one explanation of the greater surface active properties found in pigskin gelatins, as the guanidine groups of arginine seem to be related to these functional properties.

(III) There is a tendency for trace amino acids, such as cysteine, tyrosine, isoleucine, serine, etc., to be found in gelatin in lower proportions than in their parent collagens. This is due to the unavoidable removal of some telopeptide during cross-link cleavage, which is then lost in the pretreatment solutions.

2.3.2 Secondary structure and molecular weight

The molecular weight has been referred to explain the various aspects of gelatin behaviour in solutions and in gels. Relating physical properties to the average molecular weight of a polydisperse colloid is difficult. Gelatin is not completely polydisperse, but has a definite molecular weight distribution pattern corresponding to the α-chain and its oligomers. One to eight oligermers may be detected in solution, but it is possible that higher numbers exist. Doublets, known as β-chains, are formed from both α1- and α2 –chains, giving rise to β11- and β12-molecules. Oligermers of three

α-chains will mainly exist as intact triple helices, but a certain proportion will exist as extended α-polymers bonded randomly by end-to-end or side-to-side bonds. The structure of oligomers of greater than four α-chain units obviously becomes increasingly more complex and difficult to interpret (Johnston-Banks, 1990).

Polyacrylamide gel electrophoresis (PAGE) can be used to obtain highly accurate molecular weight spectra, giving quantitative separation. The study of these spectra can relate physical properties to molecular structure. Gelatin structures are shown in Figure 2.2 (Johnston-Banks, 1990).
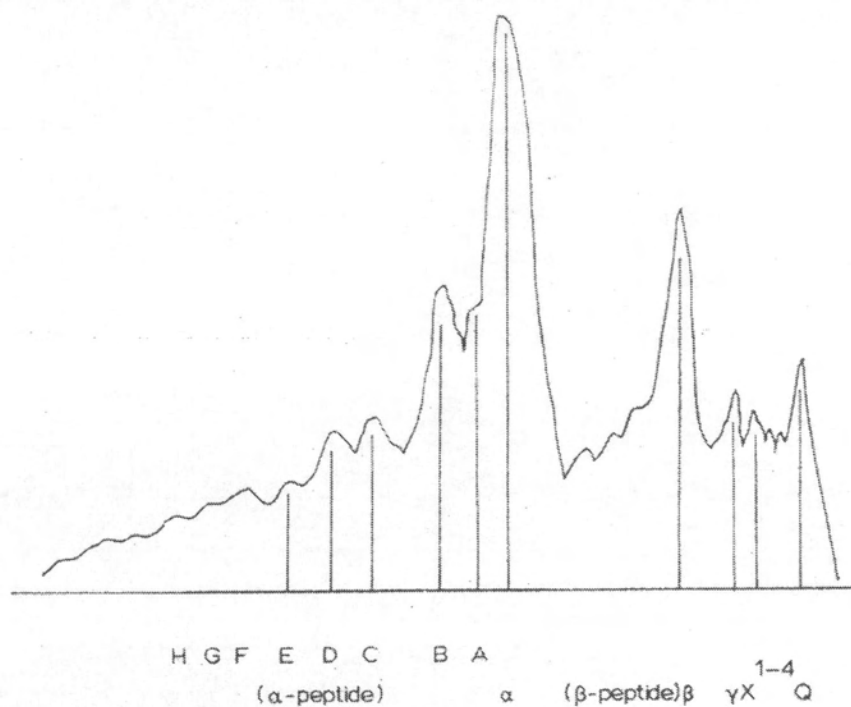

Figure 2.2 Molecular weight distribution showing the major structural components of gelatin (see Table 2.1).

Source: Johnston-Banks (1990)

Table 2.1 The major molecular fractions of gelatin as seen in Figure 2.2.

Molecular fraction	Description
Q	Very high molecular weights, of 15-20 $\times$ 10^6 daltons and thought to be branched in character owing to their inability to penetrate the gel successfully
1-4	Oligomers of α-chains, levels of five to eight
X	Oligomers of four α-chains
γ	285000 daltons, i.e. 3 $\times$ α-chain
β	190000 daltons, i.e. 2 $\times$ α-chain
α	95000 daltons
A-peptide	86000 daltons
α-, β- and γ- peptide	Seen as tailing their parent peaks

Source: Johnston-Banks (1990)

Differences can be detected between commercial gelatins from different raw materials. The sum of the α- and β- fractions, together with their larger peptides, is related to gel strength, and the amount of higher molecular weight material is related to viscosity. The setting time increases with an increase in peptide fractions that have molecular weight lower than α-chain fractions, but decreases as a certain proportion of very high molecular weight 'Q' fraction increases. The melting point of gelatin also increases in line with higher molecular weight peptide content (Johnston-Banks, 1990).

2.4 Manufacturing of gelatin

The aim of gelatin prodution is to control the hydrolysis of collagen from raw material and to convert the resulting product into a soluble material with desirable physical and chemical properties, such as gel strength and colour (Ockerman and Hansen, 1988). The three changes that are involved in transforming collagen into gelatin are: (De man, 1990)

a) Rupture of a limited number of peptide bonds to reduce the length of the chain.

b) Rupture or disorganization of a number of the lateral bonds between chains.

c) Change in chain configuration.

Production of gelatin includes three major steps that are 1) preparation for extraction, removal of non-collagenous compounds from material, 2) extraction, heating of collagen fibers in hot water and 3) recovery and drying of the gelatin powder (Ockerman and Hansen, 1988).

1. Preparation for extraction

Two processes are in common use for the preparation of raw material for extraction. The processes are described as following:

1.1 Acid pretreatment for the extraction of type A gelatin

Most type A gelatin is extracted from pig skins and bone. This process is particularly desirable if the raw material contains bones or cartilages. Inorganic acids such as hydrochloric, sulphurous, phosphoric, or sulphuric, which results in a pH of approximately 4 can be used. The extraction at high acid concentration causes the collagen to swell and be suitable for extraction (Ockerman and Hansen, 1988). Pretreatment depolymerizes the collagen by breaking down the inter- and intramolecular cross-linkages in order to enable the release of free α-chains. The purpose of this process is also to remove other organic substances, such as proteoglycan, blood,

mucins, sugars, etc., that occur naturally in the raw material (Wong, 1989; Johnston-Banks, 1990). Most of the non-collagenous proteins have an isoelectric point of pH 4-5 and consequently are least soluble and rapidly coagulated during acid pretreatment (Ockerman and Hansen, 1988).

The isoelectric point of type A gelatin lies in the pH 6.0 to 9.5 range, unless the raw material has been pretreated in very mild acid condition for a short time (Wainewright, 1977). The amino acid of gelatins prepared by the acid process contains lower hydroxyproline and higher tyrosine contents (Eastoe and Leach, 1977).

The acid treatment usually lasts from 10-72 hours (24-48 hours for sinews, 48-72 hours for swim bladders) and is often replaced with fresh acid at 24-36 hours. Continual acid soaking may increase yield but may also lower gel strength and viscosity of the gelatin (Ockerman and Hansen, 1988). Acid pretreatment, especially when using high acid concentration, is less effective than alkali pretreatment in that it could damage the raw material by excessive hydrolysis (Wong, 1989; Johnston-Banks, 1990).

1.2 Alkaline pretreatment for the extraction of type B gelatin

Alkaline processes are normally applied to bovine hide and ossein. Lime is most commonly used for this purpose, because it is relatively mild and doses not cause significant damage to the raw material by excessive hydrolysis (Johnston-Banks, 1990). Any water-soluble bases could be used but lime is normally preferred because its solubility as a saturated solution will regulate the desired alkalinity and because it dose not cause collagen to swell as much as other alkaline hydroxides would do at the same pH. This pretreatment causes the non-collagen material such as keratin, globulins, mucopolysaccharides, elastin, mucins, albumins, and sometimes mucus to be changed to a more soluble product and some of the fats to be converted into polar products so that they can be removed by subsequent washing (Ockerman and Hansen, 1988).

Lime or alkaline processed gelatins have an isoelectric point that can vary from 4.8 to 5.2 (Wainewright, 1977). The amino acid of type B gelatins possess higher hydroxyproline and lower tyrosine contents than either the type A gelatins or the raw materials (Eastoe and Leach, 1977).

The length of liming depends upon raw material type and pretreatment temperature and the final product that is being produced, but often requires from 7 days to 3 months (Ockerman and Hansen, 1988).

2. Extraction

Gelatin is produced from pretreated raw materials by extraction with water under suitable temperature condition. The extraction process is one of the most significant processing steps in gelatin production because it influences the duration of the process as well as the quality of extracted gelatins. The ease of extraction varies according to type and age of the animals as well as the tissue involved (Hinterwaldner, 1977).

The conversion of pretreated raw material into gelatin takes place in five basic steps: (I) washing, (II) extraction, (III) purification, (IV) concentration, and (V) drying. The extraction process is designed to obtain the maximum yield in combination with the most economic of physical properties, i.e. to optimize the balance between pH, temperature and the extraction time. The relationship between these parameters are shown in Figure 2.3 (Johnston-Banks, 1990).
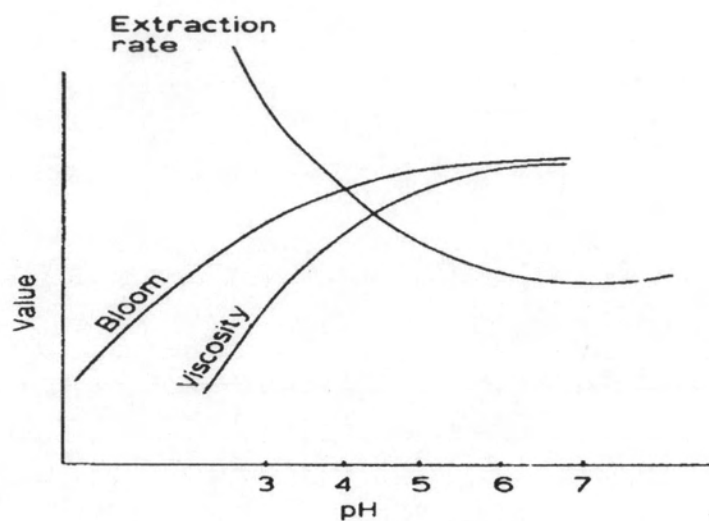
Figure 2.3 Factors determining optimum gelatin extraction conditions. Abscissa represents the pH value of extracting collagen; ordinate represents the bloom or viscosity value of the resultant gelatin, or the extraction rate as weight/h.

Source: Johnston-Banks (1990)

The easiest path of conversion is the denaturation of soluble collagen. It takes place in mild conditions either by heating at neutrality to about 40 °C or by adding hydrogen bond breakers. During conversion of collagen to gelatin, the inter- and intra-molecular bonds linking collagen chains as well as some peptide bonds are broken to produce one, two or three random chain gelatin molecules (Johns and Courts, 1977; Muyonga, Cole, and Duodu, 2004). Three basic types of new chains result after cleavage: the alpha-chain consisting of one peptide chain, the beta-chain made of two peptide chains still connected, and the gamma-chain consisting of three connected peptide chains. Therefore a single gelatin sample contains peptide chains with various molecular weights (Ockerman and Hansen, 1988).

Under these mild conditions no covalent linkages are destroyed in the times required. The more severe the extraction condition (e.g. from 60°C upwards), the greater the extent of hydrolysis of covalent bonds and therefore the higher the

proportion of peptides with molecular weight less than than of α-peptide. (Johns and Courts, 1977; Muyonga, Cole, and Duodu, 2004).

The final step in the conversion of collagen into gelatin involves the breakage of hydrogen bonds and hydrophobic bonds which are a key stabilizing factor in collagen structure. This activity is needed even though the pretreated raw material has already adequately disrupted the crosslinks and, to some extent, broken peptide bonds. Two methods are available for breaking the hydrogen bonds in gelatin extraction, namely,

(I) Raising the temperature of the collagen until the shrinkage point is reached;

(II) Treating the collagen in the concentrated solution of a hydrogen bond breaking agent at room temperature.

The first method is generally used in the gelatin production industry, the second having only theoretical interest (Hinterwaldner, 1977).

The pH of extraction can be selected either for the maximum extraction rate (low pH) or for the maximum in physical properties (neutral pH). More efficient pretreatment conditions also allow the manufacture to use lower extraction temperatures, resulting in gelatins of greater gel strength. Shorter treatments commonly require higher extraction temperatures if neutral pH levels are chosen, resulting in gelatins of lower gel strength (Johnston-Banks, 1990).

3. Recovery of gelatin in the final form

The extracted gelatins are filtered to remove suspended insolubles such as fat or unextracted collagen fibers (Johnston-Banks, 1990). Filtration and clarification of colloidal solutions ranks among the most difficult process in industrial practice. Chemical clarification of gelatin liquors is today relatively unimportant, not only because the processes are expensive, but also because gelatin quality may be degraded in the

process (Hinterwaldner, 1977). The clarification is usually performed using materials such as diatomaceous earth to give solutions of high clarity (Johnston-Banks, 1990).

Following filtration, the dilute gelatin liquors are evaporated to obtain the high concentrated gelatin liquor. Reduction of the temperature is effected by the application of a vacuum evaporator. The boiling temperature range for the evaporation of gelatin liquor must lie between 80°C and 40°C. The temperature must be above the melting point of the gelatin and below temperatures at which degradation is rapid (Hinterwaldner, 1977).

The final stage is drying. For most practical purposes, dry gelatin is obtained from concentrated sols by cooling to the solid gelatin gel and drying the gel. Removal of water from the liquid gelatin solution can only be achieved by spray drying or roller drying. Freeze drying can enable the instantly resoluble "sol" configuration of the gelatin molecule to be obtained in the dry state but the process is expensive (Hinterwaldner, 1977). These processes are performed as quickly as possible to minimize loss of gelatin properties (Johnston-Banks, 1990).

2.5 Formation of the gelatin gel

The thermal stability of collagen is related to the content of imino acids, proline and hydroxyproline (Wong, 1989). The imino acid content is also implicated in the gelling mechanism. The sites most likely to be involved in the formation of junction zones being those that are rich in imino acids. (Johnston-Banks, 1990).

Collagen denatures at temperature above 40°C to a mixture of random-coil single, double, and triple strands. The reformation of the helical form occurs when controlled cooling below the melting temperature (T_m). The energy barrier for refolding is ~4 kJ / mole. The initial reforming is rapid and involves the –gly–I–I– regions of the polypeptide chain, forming a single turn of a left-handed helix. This nucleation along the polypeptide chain is structurally stabilized by hydrogen bonding (Wong, 1989). Hydrogen bonding

takes place between the imino acids and nearby C–H groups, e.g. on glycine found on adjacent chains (Johnston-Banks, 1990). The nucleated polypeptide then (1) folds back into loops, with the nucleated regions aligned to form triple strands, and (2) has its nucleated region aligned with that of the other nucleated polypeptide chain (Figure 2.4). At high enough concentrations, interchain arrangement becomes possible and association of polypeptide chains to form triple-helical collagen molecules can occur. In both cases, once the nucleated regions are aligned, the remainder of the chain starts to renaturate. The rate of which depends on the cooling temperature. Rapid cooling which large ΔT would cause rapid renaturation, resulting in areas unavailable for the formation of helical structures. Various processes contribute to the formation of a gelatin gel network (Wong, 1989).
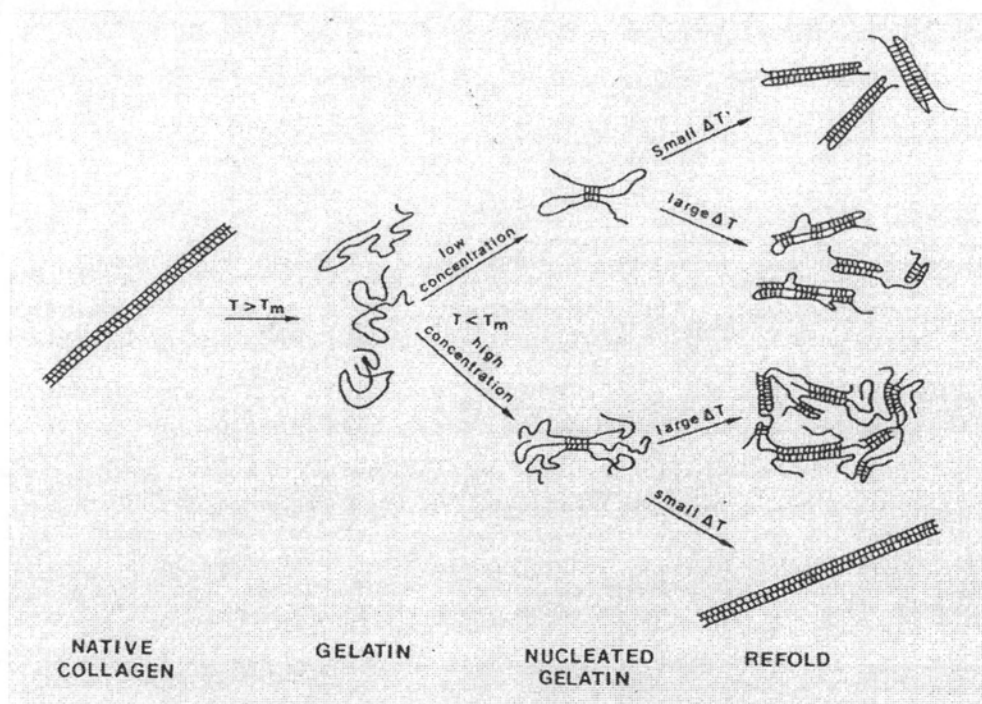

Figure 2.4 Scheme for the concentration- and temperature-dependent pathways for helix formation in α chains derived from collagen.

Source: Wong (1989)

2.6 Physico-chemical properties

Gelatin is almost tasteless and odourless. It is a glassy brittle solid with a relative density of 1.3-1.4 kg/L (Ockerman and Hansen, 1988). It, although insoluble in cold water and other liquids such as milk, will swell and absorb water up to 10 times its weight. The rate of which is dependent on the surface area of the gelatin per unit weight. The swelling characteristics in cold water are determined by temperature and the salt or sugar content of liquid, all of which can affect the rate of water uptake (Johnston-Banks, 1990). Gelatin is also soluble in polyhydric alcohols (e.g. glycerol or propylene glycol) but is not soluble in organic solvents (e.g. benzene, ether, acetone, and carbon tetrachloride). Gelatin is comprised of 50.5% carbon, 6.8% hydrogen, 17% nitrogen and 25.2% oxygen (Ockerman and Hansen, 1988). Gelatin is an amphoteric. The amphoteric character of gelatin is due to the functional groups of the amino acids and terminal amino and carboxyl groups created during hydrolysis. It can act as either an acid or base depending on the pH of a water solution of gelatin and can also undergo reactions such as acylation, esterification, deaminization, cross-linking, and polymerization (Ockerman and Hansen, 1988).

Gel strength is the most commercially important physical property of gelatins. It is a measure of hardness, stiffness, strength, firmness, and compressibility of a gel at a particular temperature. Gel strength properties are related to the α- and β- chain component (Johnston-Banks, 1990). Gel strength is tested on a Bloom gelometer which measures the resistance to depression of the jelly surface by a plunger under standard conditions. The conditions for the Bloom gelometer consist of a 6.67% solution chilled to $10\pm1°C$ for 17 hours. A 12.7 mm diameter circular plunger is loaded with shot until it depresses the jelly surface 4 mm (Ockerman and Hansen, 1988). Table 2.2 shows bloom strength ranges for type A and type B gelatins.

Table 2.2 Bloom strength ranges for type A and type B gelatins

Type	Bloom strength	Viscosity (mpoise)	Remarks
High bloom	250-300	30-70	limed hide/ossein
		30-60	acid pigskin/ossein
		70-130	specialized hide/ossein
Medium bloom	150-200	20-80	limed hide/ossein
		20-40	acid pigskin/ossein
Low bloom	50-100	15-30	limed hide/ossein
		15-30	limed hide/ossein

Source: Johnston-Banks (1990)

Viscosity is the second most commercially important physical property of gelatins. Molecular weight seems to be more important in viscosity measurements than it is in gel strength measurements (Wainewright, 1977). Gelatin solution viscosity is at minimum at its isoelectric point, and increases to a maximum at pH~3 and 10.5 (Stainsby, 1977). An addition of salt reduced the viscosity of gelatin solution. This is due to both inter- and intrachain repulsion effects (Johnston-Banks, 1990). The viscosity of gelatin is evaluated by the time required for a standard concentration (usually 6.67%; viscosity increases with concentration of gelatin) of solution to flow from a standard viscosity pipette such as a U-tube viscometer and a standard orifice from a cup. Viscosities of gelatin solution are commonly measured at 60°C (Ockerman and Hansen, 1988).

Gelatin colour in a dilute solution should be colourless to light amber or faintly yellow. Lower grade gelatin has an orange-brown colour. Clarity is checked by looking at print through a breaker of solution or observing a solution in a strong light. High quality gelatin should be clear and sparkling and have only a trace sediment of foreign material. The lower quality one is opalescent to cloudy. The turbidity of gelatin solution is a function of pH. Maximum turbidity occurs at the isoelectric point in a salt-free solution

(Finch and Jobling, 1977). In general, colour does not influence other properties or usefulness of gelatin (Ockerman and Hansen, 1988).

Standard microbial techniques are used to evaluate the bacterial, mold, and yeast quality of gelatin. Most food gelatins contain less than 3000 bacteria per gram and these are not pathogenic. The United States Pharmacopeia (U.S.P.) maximums are 1000/g and *Salmonella* and *Escherichia coli* must be absent. If the gelatin has a pH value below 4, bacteria growth will be suppressed, but moulds and yeast will continue to grow. If the pH value is above 5, proteolytic bacteria can be present (Ockerman and Hansen, 1988).

Chemical test for gelatin include moisture, ash and pH analysis. Moisture is normally between 9 and 13% (range 7-15%) and will vary, not only with the extent of drying but also with the humidity of storage and the moisture permeability of the packaging material. Ash content has a U.S.P. maximum level of 2%. However, high-quality gelatin should have no more than 0.5% ash. The U.S.P limit for heavy metals is 50 ppm. In particular, the standard limit for copper is ≤ 30 ppm. High quality gelatin has less than 5 ppm copper. The standard limit for zinc is ≤ 2.57 ppm (Ockerman and Hansen, 1988). Most commercial gelatin has pH in the 5.0-5.8 range. An exception to this is found in certain pigskin gelatins, where no deionization takes place and the extraction pH (4.0-4.5) is the same as the pH of sale (Johnston-Banks, 1990).

2.7 Applications of gelatin

Food products The applications of gelatin in foods vary extensively. Gelatin is used as a gelling agent, stabilizer, emulsifier, thickener, foaming agent, water binder, crystal growth modifier, glaze, adhesive, binder, and fining agent (Jones, 1977). Gelatin products having a wide range of bloom and viscosity values are utilized in the manufacture of food products. Specific properties being selected depend on the needs of the application. Edible gelatin is used as an ingredient in food industry owing to the following functional properties (Johnston-Banks, 1990).

(I) It forms high quality gels in dilute solution with typical clean melt-in-the- mouth textures.

(II) It forms elastic gum-type texture in the concentrated gel that slowly dissolves in the mouth.

(III) It emulsifies and stabilizes immiscible liquid-liquid, liquid-air, or liquid-solid mixtures.

(IV) It acts as a polyelectrolyte that will flocculate suspended particles or unstable colloids.

(V) It acts as an efficient tableting aid and binder.

Pharmaceutical products Gelatin is used to manufacture capsules in the pharmaceutical industry. Two types of capsule are produced. The hard capsules or two-piece ensembles into which the pharmacist adds powers are made entirely of gelatin with no other additives except, in some cases, colouring. The soft or elastic capsules contain a plasticizer, usually glycerol or propylene glycol, and are often used to contain cod liver oil and other vitamin products. Photochemical deterioration of light sensitive substances may be prevented by incorporating suitable dyes in the gelatin composition (Wood, 1977). Gelatin is also used to coat pills to help eliminate crumbling, sticking, evaporation of moisture, and taste when swallowing. Gelatin is used in making tablets as a moisturing agent, a binder, and a disintegrator. Gelatin is also used as a carrier or binder for various drugs and is often protects the drug from atmospheric oxidation. Gelatin has also been employed in delaying the absorption of a number of drugs (e.g. heparin, adrenocorticotrophic hormone, and epinephrine) (Ockerman and Hansen, 1988).

Photographic products Gelatin is important in photography and is also used to make baryta-coated paper. Paper is first coated with a suspension of barium sulphate in gelatin which gives a smooth bright surface to the paper. The physical properties of gelatin such as its protective-colloidal, setting, swelling, and film-forming properties are used for the photographic process (Kragh, 1977). Films are coated with gelatin, which contains the light-sensitive silver reagent. The gelatin controls the size of the silver

halide grain and protects it from the reducing action of the developer so that the reduction is proportional to the exposure to light (Ockerman and Hansen, 1988).

Microbiological culture media Special grades of gelatin may be used as a bacterial culture media. Nutrient gelatin is useful for the differentiation of micro-organisms by their proteolytic effects. Gelatinase activity is determined by gelatin charcoal discs, which consists of finely powered charcoal and formalin denatured gelatin are completely sterile (Wood, 1977). Gelatin is also used to determine the strength of enzymes in culture media (Ockerman and Hansen, 1988).

Miscellaneous uses Gelatin is used as a binder in the engobe composition used to mask coat a pure colour over the fireclay ware before glazing for the sanitary fireclay industry (Wood, 1977). The sticking power of gelatin can be used in insecticide sprays. Gelatin can also be used in microencapsulation, as a foamer in ore floatation for separation of minerals, and as a foamer in fire extinguishers. Gelatin is used in cosmetics and in wave-set lotions. Some of its properties that are beneficial in cosmetics are its adhesive and emulsifying powers. Moreover, It can be used in making smokeless gunpowder (Ockerman and Hansen, 1988).

2.8 Electrospinning process

Electrospining technique was introduced in early 1930s. It is a novel method to produce fiber in a nano scale with simple apparatus (Ki *et al.*, 2005). This process is remarkably efficient, rapid, and inexpensive. The suitable polymer and solvent can produce nanofibers with their diameter in the 40-200 nm range (Grafe and Graham, 2003). Fibers production using electrospinning technique is shown in Figure 2.5.
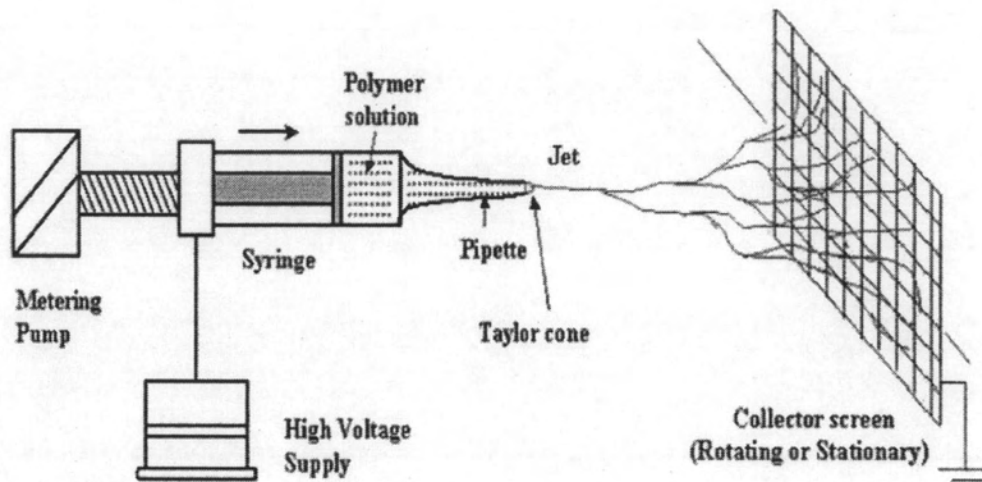
Figure 2.5 Schematic of the electrospinning set up.

Source: Grage and Graham (2002)

The mechanism of the fiber formation produced by electrospining technique can be summarized as follows: (Fong and Reneker, 2001)

(1) Initiation of the jet and the extension of the jet along a straight line.

(2) Continuous flow of the jet, which allows the jet to become very long and thin while it follows a looping and spiraling path.

(3) Solidification of the jet into fibers.

In this process, electrically charged polymer are created by applied voltage from a high voltage supplier. Electric field is using to control the formation and deposition of polymers (Matthew *et al.*, 2002). When applying high voltage to polymer solution or melted polymer, the electrical field is made between the tip of a pipette and the grounded collector. Upon increasing the electrostatic filed strength up to but not exceeding a critical value, surface charge is induced on a polymer fluid deforming a hemispherical pendant droplet into a conical shape which is named the Taylor's cone (Figure 2.6) (Neamnark, Rujiravanit and Supaphol, 2006).
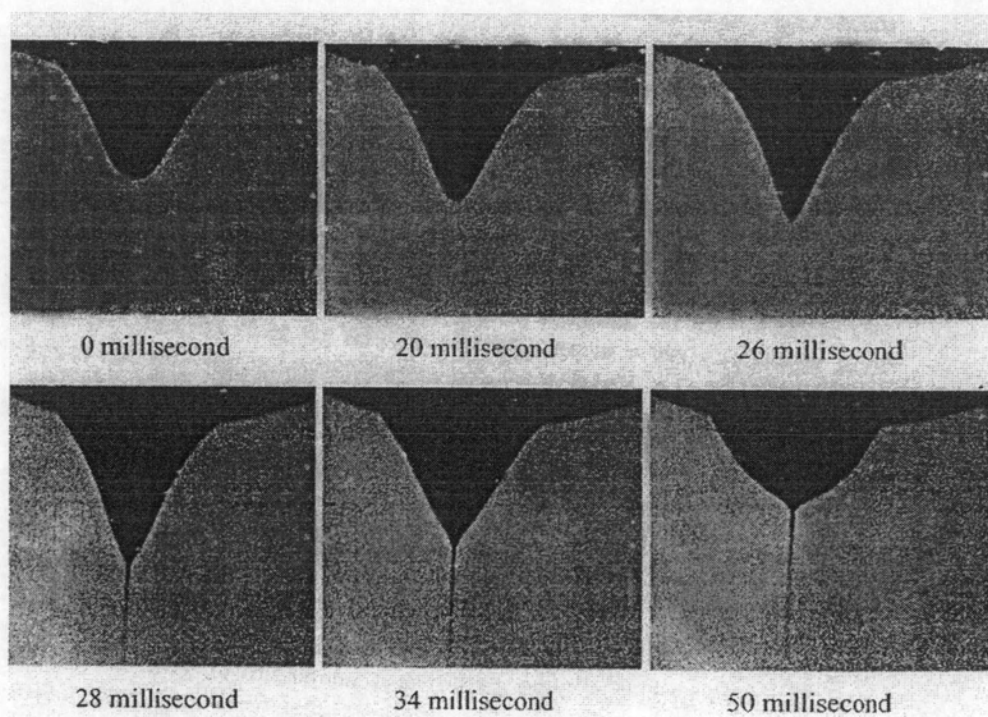
Figure 2.6 A series of droplet shapes takes at frame rate of 500 frames per second and
a shutter speed of 2 ms.

Source: Fong and Reneker (2001)

As a critical voltage where electrostatic repulsion force of surface charges overcome surface tension, the charged fluid jet is ejected from the tip of the Taylor's cone and the charge density on the jet interacts with the external field to produce an instability (Figure 2.7) (Geng, Kwon and Jang, 2005). After the charged jet is ejected, the conical protrusion becomes a rounded shape. At a lower potential, a jet can also be initiated by mechanically pulling a jet out of the pendent droplet since the voltage required for maintaining the jet flow was lower than that required for initiation (Fong and Reneker, 2001).
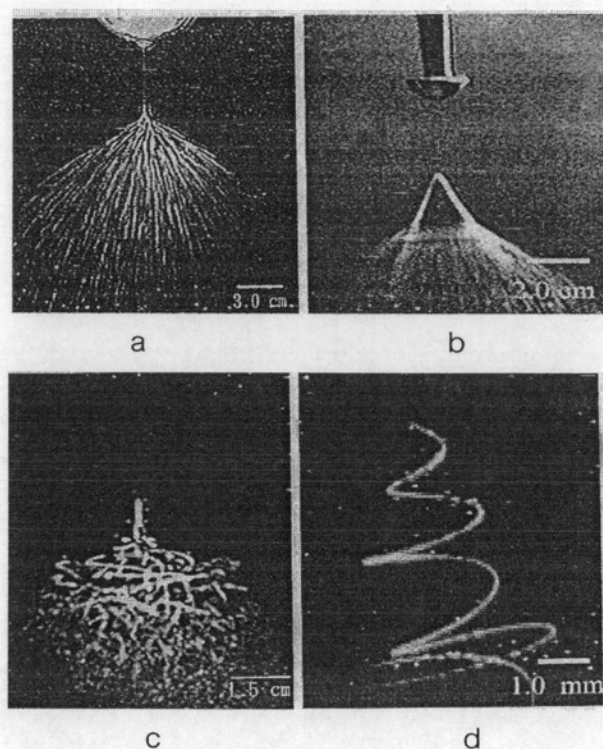
Figure 2.7 Images of electrospinning jet with different exposure times. (a), (b), and (c),
shutter speed was 16.7 ms (d) shutter speed was 1.0 ms.

Source: Fong and Reneker (2001)

After initiation, the route of the jet is straight for a particular distance. Then, an electrically driven bending instability grows at the bottom end of the straight segment. The bending allows a large elongation to occur in a small region of space. The electrically driven bending instability occurs in self-similar cycle. Each cycle has three steps and is smaller in scale than the pre-cycle. The three steps in each cycle are: (Fong and Reneker, 2001)

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

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

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

Two cycles of bending instability are shown in Figure 2.8. The first electrically driven bending instability that produces an array of helical bends is the jet near the end of the straight segment. While the jet runs continuously, it shifts through a series of similar but changing paths. Most loops move downward at a velocity of about 1 m/s, but some loops with larger diameters move slower. The slightly curved thin segment that runs horizontally across the left image in Figure 2.8 is part of such a loop that remain in view for over 15 ms. This segment is smooth until, in a time interval of only one ms, the bends and loops shown in the right image of Figure 2.8 develop. During this 15 ms period, many bends and loops of the first cycle of bending instability form and move downward through the field of view. The diameter of every segment of the jet becomes smaller, and the length of every segment increases. The loops grow larger. Bending instabilities develop and grow (Fong and Reneker, 2001).
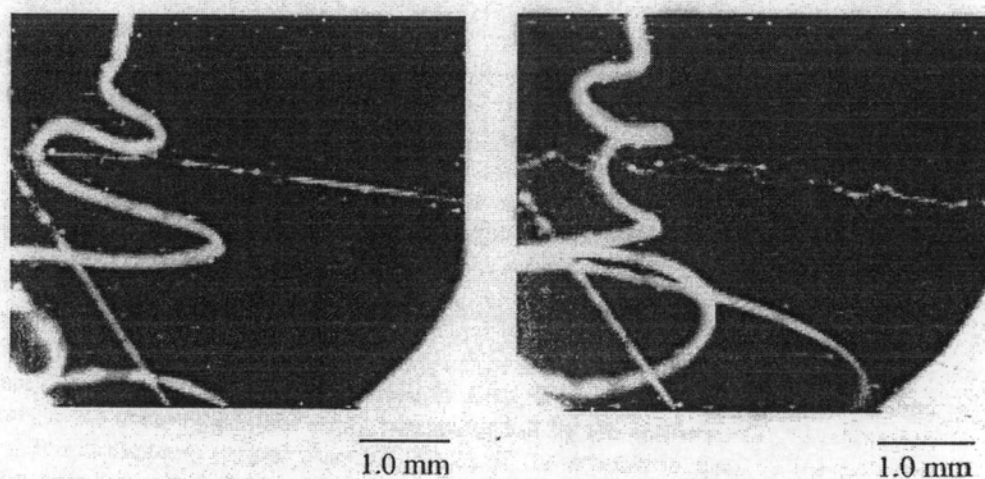
Figure 2.8 The development of the second cycle of bending instability. The time interval between these two images was 15 ms. The camera shutter speed was 0.25 ms.

Source: Fong and Reneker (2001)

The jet within the electric field is directed toward the grounded target, during which time the solvent evaporates and fibers are formed. It produces a randomly single continuous filament that collects on the grounded target as a nonwoven fabric (Matthew *et al.*, 2002).

It was found that morphology such as fiber diameter and the uniformity of the electrospun polymer fibers are dependent on many processing parameters. These parameters can be divided into three groups as shown in Table 2.3 (Tan *et al.*, 2005).

Table 2.3 Processing parameters in electrospinning.

Solution properties	Viscosity
	Polymer concentration
	Molecular weight of polymer
	Electrical conductivity
	Elasticity
	Surface tension
Processing conditions	Applied voltage
	Distance from needle to collector
	Volume feed rate
	Needle diameter
Ambient conditions	Temperature
	Humidity
	Atmospheric pressure

Source: Tan *et al.* (2005)

2.9 Literature Reviews

Due to their broad applications, nanofibers from various polymers have been remarkably increasingly produced. Electrospinning technique is chosen in widespread for the production of nanofibers because of its simplicity. Generally, synthetic polymers are used as the raw material for nanofibers production but they have limited applications in biomedical and some food applications. Natural polymers are more suitable as the raw material for nanofibers production for those fields. Many researchers have investigated electrospinning of nanofibers. Most researches aimed to study the effect of various parameters on morphology and diameter of the obtained nanofibers.

Matthews et al. (2002) described that electrospinning can be adapted to produce tissue-engineering scaffolds that are composed of collagen nanofibers. Type I collagen from calfskin and type I and type III collagens isolated from human placenta were used and dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol at various concentrations. The structural properties of electrospun collagen varied with the tissue of origin, the isotype, and the concentration of the collagen solution used to spin the fibers. Their experiments demonstrated that fiber orientation can be controlled by a rotating mandral. The inherent properties of the electrospinning process make it possible to fabricate complex, and seamless, three-dimensional shapes. The electrospun collagen was found to promote cell growth and the penetration of cells into the engineered matrix.

Huang et al. (2004) investigated electrospinning of porcine skin gelatin, and the mass concentration-mechanical property relationship of the resulting nanofiber membranes. 2,2,2-trifluoroethanol was used as the solvent. The resulting solution with a mass concentration between 5 and 12.5% was successfully electrospun into nanofibers having diameters in the 100 to 340 nm range. Lower or higher gelatin concentrations were not suitable in electrospinning at ambient conditions. The nanofiber mat that had the finest fiber structure and no beads on surface was obtained from 7.5% gelatin solution. The mat also had 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.

Ki et al. (2005) characterized gelatin nanofiber prepared from porcine skin gelatin in formic acid solution. Degradation of gelatin occurred in formic acid but this did not significantly affect the spinning ability and morphology of the electrospun gelatin nanofibers. At below 7 wt% gelatin concentration, the electrospun nanofibers could not be formed. It was found that 0.75 kV/cm was the critical electric field for stable electrospinning at 10 cm distance. The electrospun gelatin nanofiber exhibited a mixture of a-helical and random coil conformation, which was amorphous structure with very low crystallinity. The structural transformation, from a helical (a-helix and triple-helix) to random coil conformation, might occur when formic acid was used for the dissolution of gelatin for electrospinning process.

Zhang et al. (2006) made the as-electrospun porcine skin gelatin nanofibers water insoluble through a glutaraldehyde crosslinking treatment so as to preserve their fibrous morphology and enhance their thermal and mechanical performance. 2,2,2-trifluoroethanol was used as the solvent. The crosslinking was carried out in a saturated glutaraldehyde vapor. After crosslinking, both tensile strength and modulus of nanofiber mat were enhanced to nearly 10 times higher than those of the as-electrospun gelatin membrane.

Rho et al. (2006) produced electrospun fiber from type I of calfskin collagen in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) in order to fabricate a biomimetic nanofibrous extracellular matrix for tissue engineering. The average diameter of collagen nanofibers electrospun from 8% collagen solution in HFIP was 460 nm (range of 100–1200 nm). The as-spun collagen nanofibrous matrix was chemically cross-linked by glutaraldehyde vapor. As cross-linking time increased up to 9 h, the weight loss values of collagen matrices gradually decreased and they swelled less in water. The tensile strength of the collagen matrix was above 10 MPa. The tensile strength of the collagen nanofibrous matrix was comparable to that of two commercial tissue regenerative membranes and a

wound-dressing material. This indicated that the cross-linked collagen nanofibrous matrix provided a similar level of mechanical stability when applied in the wound-healing procedure.