CHAPTER II
LITERATURE REVIEW

2.1 Electrospinning

Electrospinning is an interesting process for producing non-woven fibers with average diameters in the range of micrometers down to nanometers. In this process, a high voltage is used to create an electrically charged jet of polymer solution or melt, which dries or solidifies to leave a polymer fiber. One electrode is placed into the spinning solution/melt and the other attached to a collector. Electric field is subjected to the end of a capillary tube that contains the polymer fluid held by its surface tension. This induces a charge on the surface of the liquid. Mutual charge repulsion causes a force directly opposite to the surface tension. As the intensity of the electric field is increased, the hemispherical surface of the fluid at the tip of the capillary tube elongates to form a conical shape known as the Taylor cone. With increasing field, a critical value is attained when the repulsive electrostatic force overcomes the surface tension and a charged jet of fluid is ejected from the tip of the Taylor cone. The discharged polymer solution jet undergoes a whipping process wherein the solvent evaporates, leaving behind a charged polymer fiber, which lays itself randomly on a grounded collecting metal screen. (The experimental set up of electrospinning process was shown in Figure 2.1) The nanofibers produced by electrospinning method have showed amazing characteristics such as very large surface area-to-volume ratio and high porosity with very small pore size (Shin et al, 2001). Therefore, electrospun nanofibers have become promising materials for many biomedical applications such as wound dressing, drug delivery, and scaffold for tissue engineering (Li et al, 2002; Kenawy et al, 2003; Yoshimoto et al, 2003; Bhattarai et al, 2004; Zong et al, 2002).There have been efforts of fabricating continuous, single fiber strand using this technique. A simple technique suggested the use of a rotating device with angular velocity of up to thousands of round per minute (rpm). Researchers from Virginia Commonwealth University (Boland et al, 2001) used this technique to obtain aligned electrospun poly (glycolic acid) (PGA) (at 1000 rpm) and type I collagen fibers (at 4500 rpm rotating speed).

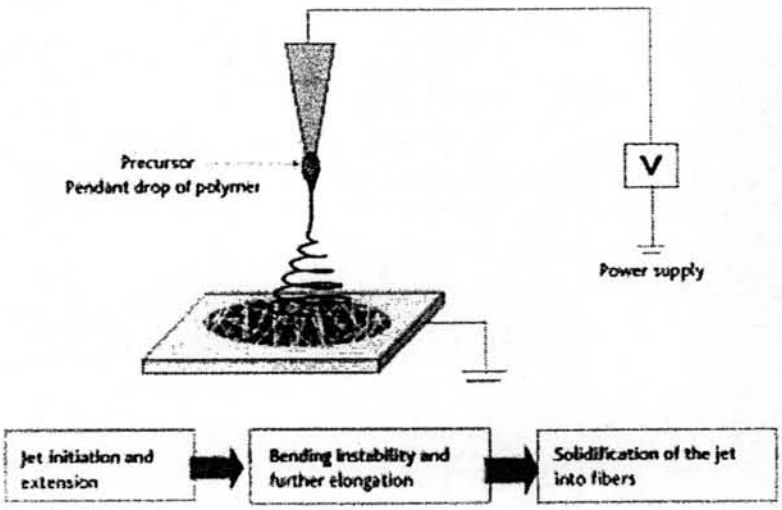

Figure 2.1 Schematic of the electrospinning setup.

There are several parameters that can influence the transformation of polymer solutions into nanofibers through electrospinning. These parameters include (1) the solution properties such as viscosity, elasticity, conductivity, and surface tension, (2) governing variables such as hydrostatic pressure in the capillary tube, electric potential at the capillary tip, and the gap (distance between the tip and the collecting screen), and (3) ambient parameters such as solution temperature, humidity, and air velocity in the electrospinning chamber (Huang et al, 2003) One of the most important quantities related with electrospinning is the fiber diameter. Since nanofibers are resulted from evaporation or solidification of polymer fluid jets, the fiber diameters will depend primarily on the jet sizes as well as on the polymer contents in the jets. It has been recognized that during the traveling of a solution jet from the pipette onto the metal collector, the primary jet may (Bergshoef et al, 1999) or may not be split into multiple jets(Reneker et al,2000), resulting in different fiber diameters. Another parameter influencing the fiber diameter is the solution viscosity. A higher viscosity results in a larger fiber diameter (Doshi et al, 1995). However, when a polymer is dissolved in a solvent, the solution viscosity is proportional to the polymer concentration. Thus, the higher polymer concentrations result in the larger

nanofiber diameters. Another parameter affecting the fiber diameters to a remarkable extent is the applied electrical voltage. Generally, a higher applied voltage ejects more fluid in a jet, resulting in a larger fiber diameter (Demir et al, 2002).

Geometric properties of nanofibers such as size and its distribution, orientation, and morphology (e.g. cross section shape and surface roughness) can be characterized using scanning electron microscopy (SEM), chemical integrity of nanofibers can be characterized by Fourier-transformed infrared spectroscopy (FTIR) (Grahlert *et al,* 1999) and nuclear magnetic resonance (NMR) techniques (Bourbigot *et al,* 2000). The configuration of the macromolecules in the fibers, which can be characterized by optical birefringence (Buchko *et al,* 1999), wide-angle X-ray diffraction (WAXD), small-angle X-ray scattering (SAXS) and differential scanning calorimetry (DSC) (Zussman *et al,* 2002).

With such versatility, electrospun fibers are being explored for use in many different applications like healthcare, biotechnology and environmental engineering, defense and security, and energy storage and generation (Figure 2.2).

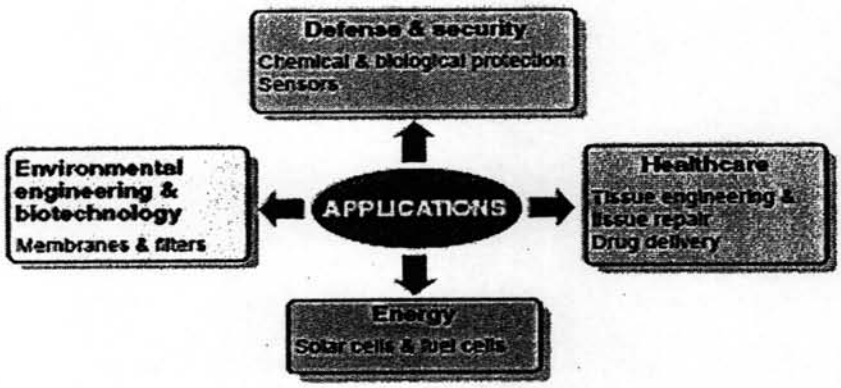

Figure 2.2 Schematic of potential applications of electrospun fibers.

The potential of these electrospun nanofibers in human healthcare applications is promising, for example in tissue/organ repair and regeneration, as vectors to deliver drugs and therapeutics, as biocompatible and biodegradable medical implant devices, in medical diagnostics and instrumentation, as protective

fabrics against environmental and infectious agents in hospitals and general surroundings, and in cosmetic and dental applications.

The nonwoven electrospun nanofiber meshes produce high porosity, interconnectivity, microscale interstitial space, and a large surface-to-volume ratio that are an excellent material for membrane preparation, especially in biotechnology and environmental engineering applications. Moreover, the electrospun nanofibers have also received great attention for sensor applications because of their unique high surface area that absorb more of a gas and change the sensor's conductivity more significantly.

Polymer nanofibers are considered as excellent membrane materials for detect chemical and biological warfare agents with sensitivity and selectivity, protect through filtration and destructive decomposition of harmful toxins and provide site-specific in vivo prophylaxis. Nanofiber membranes may be used to replace the activated charcoal in adsorbing toxins from the atmosphere. Active reagents can be embedded into the nanofiber membrane by chemical functionalization, post-spinning modification, or through using nanoparticle polymer composites (Figure2.3) as well as serving protection and decontamination functions. Nanofiber membranes will also have to provide the durability, washability, resistance to intrusion of all liquids, and tear strength required of battledress fabrics. Natural energy resources such as crude oil, coal, natural gas, and uranium are a necessity for everyday life. Some possible alternatives can replace current supplies: Polymer batteries, fuel cells, photovoltaic cells, wind power generators, and geothermal power generators. Given their high porosity and inherent large total surface area, electrospun nanofiber membranes are being considered for polymer batteries, photovoltaic cells, and polymer electrolyte membrane fuel cells.

2.2 Chitosan

Chitosan is a family of linear copolymers derived by *N*-deacetylation from nanofiber mesh. Chitin is a natural abundant polysaccharide and the supporting material of crustaceans, insect, etc., is well known to consist of 2-acetamido-2-deoxy-ß-D-glucose through a ß (1→4) linkage.

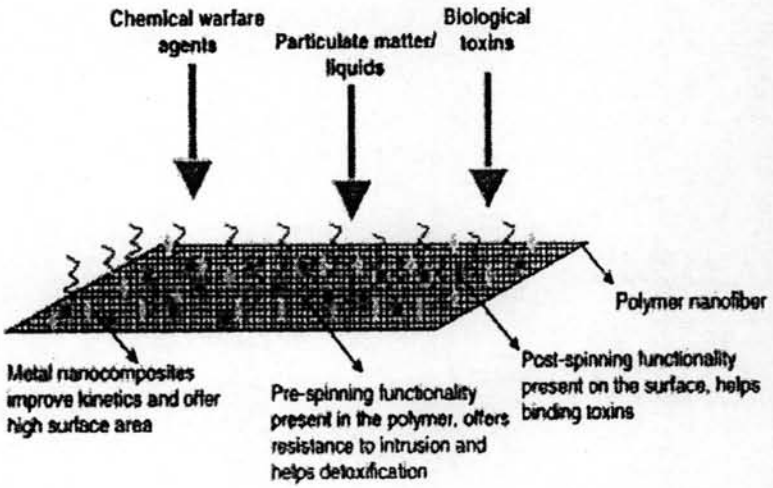

Figure 2.3 Schematic of the incorporation of functional groups into a polymer

Chitin can be degraded by chitinase. Its immunogenicity is exceptionally low, in spite of the presence of nitrogen. It is a highly insoluble material resembling cellulose in its solubility and low chemical reactivity. It may be regarded as cellulose with hydroxyl at position C-2 replaced by an acetamido group. Like cellulose, it functions naturally as a structural polysaccharide. Chitin is a white, hard, inelastic, nitrogeneous polysaccharide and the major source of surface pollution in coastal areas (The structures of cellulose, chitin and chitosan are shown in Figure 2.4). Chitin and chitosan are of commercial interest due to their high percentage of nitrogen (6.89%) compared to synthetically substituted cellulose (1.25%). This makes chitin a useful chelating agent. As most of the present-day polymers are synthetic materials, their biocompatibility and biodegradability are much more limited than those of natural polymers such as cellulose chitin, chitosan and their derivatives. However, these naturally abundant materials also exhibit a limitation in their reactivity and processability. In this respect, chitin and chitosan recommended as suitable functional materials because these natural polymers have excellent properties such as biocompatibility, biodegradability, non-toxicity, adsorption properties, etc. Recently, much attention has been paid to chitosan as a potential

polysaccharide resource. Although several efforts have been reported to prepare functional derivatives of chitosan by chemical modifications, very few attained solubility in general organic solvents and some binary solvent systems. Chemically modified chitin and chitosan structures resulted in improved solubility in general organic solvents.

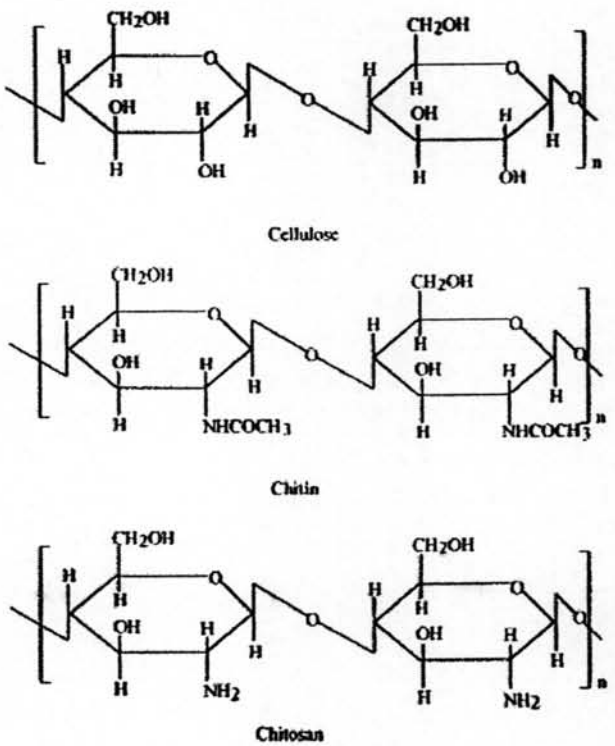

Figure 2.4 Structures of cellulose, chitin and chitosan.

Chitin is easily obtained from crab or shrimp shells and fungal *mycelia*. In the first case, chitin production is associated with food industries such as shrimp canning. In the second case, the production of chitosan–glucan complexes is associated with fermentation processes, similar to those for the production of citric acid from *Aspergillus niger*, *Mucor rouxii*, and *Streptomyces*, which involves alkali treatment yielding chitosan–glucan complexes. The alkali removes the protein and deacetylates chitin simultaneously. Depending on the alkali concentration, some soluble glyca ns are removed. The processing of crustacean shells mainly involves

the removal of proteins and the dissolution of calcium carbonate which is present in crab shells in high concentrations. The resulting chitin is deacetylated in 40% sodium hydroxide at 120°C for 1–3 h. This treatment produces 70% deacetylated chitosan as in Figure 2.5

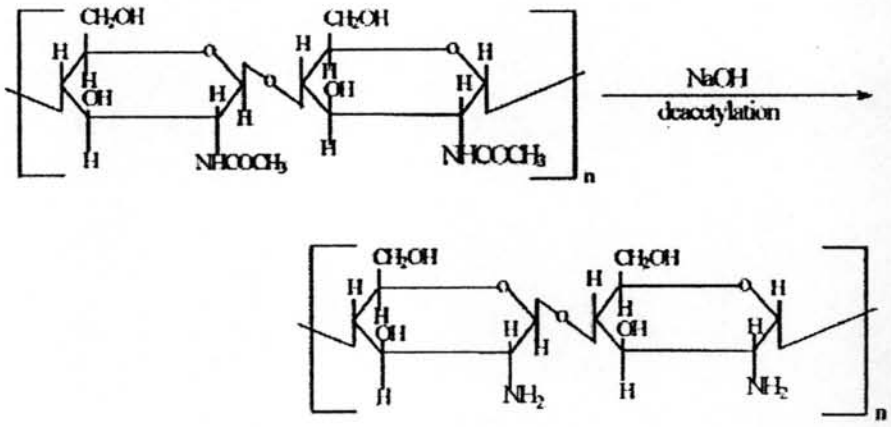

Figure 2.5 The structure of deacetylated chitosan.

Most of the naturally occurring polysaccharides, e.g. cellulose, dextran, pectin, alginic acid, agar, agarose and carragenans, are neutral or acidic in nature, whereas chitin and chitosan are examples of highly basic polysaccharides. Their unique properties include polyoxysalt formation, ability to form films, chelate metal ions and optical structural characteristics. Like cellulose, chitin functions naturally as a structural polysaccharide, but differs from cellulose in its properties. Chitin is highly hydrophobic and is insoluble in water and most organic solvents. It is soluble in hexafluoroisopropanol, hexafluoroacetone, chloroalcohols in conjugation with aqueous solutions of mineral acids and dimethylacetamide containing 5% lithium chloride. Chitosan, the deacetylated product of chitin, is soluble in dilute acids such as acetic acid, formic acid, etc.

Chitosan, which is polycationic in acidic environments, possesses an ability to form gels at acidic pH values because it is hydrophilic and can retain water in its structure. The acetylation of chitosan in hydroalcoholic media allows the selective modification of the free amino groups and is responsible for a process of gelation. It

has been shown that the charge density of the chain segments is an essential parameter for the formation of gels and all factors that lower this parameter favor deswelling and reversibility. The high hydration, the physicochemical and physical properties, as well as the polyelectrolyte behavior of this kind of gel allow applications such as bioactive dressing for wound healing. Gels can also be used as a slow-release drug-delivery system. The solubility of chitosan can be sharply reduced by cross-linking the macromolecules with covalent bonds using, for examples, glutaraldehyde. Swelling of the films decreases with an increase in the amount of cross-linking agent added. The swelling of enzyme-containing films decreases more significantly, probably because of formation of additional crosslinks due to the participation of the functional groups of the enzyme in the reaction.

Recently, the gel forming ability of chitosan in *N*-methylmorpholine *N*-oxide and its application in controlled drug release formulations has been reported (Dutta et al, 1997). The hydrolysis of chitin with concentrated acids under drastic conditions produces relatively pure D-glucosamine. The nitrogen content of chitin varies from 5 to 8% depending on the extent of deacetylation, whereas the nitrogen in chitosan is mostly in the form of primary aliphatic amino groups. Chitosan, therefore, undergoes reactions typical of amines, of which *N*-acylation and Schiff reaction are the most important. Chitosan derivatives are easily obtained under mild conditions and can be considered as substituted glucans. *N*-Acylation with acid anhydrides or acyl halides introduces amido groups at the chitosan nitrogen. Acetic anhydride affords fully acetylated chitins. Linear aliphatic *N*-acyl groups above propionyl permit rapid acetylation of hydroxyl groups. Higher benzoylated chitin is soluble in benzyl alcohol, dimethylsulfoxide, formic acid and dichloroacetic acid. The *N*-hexanoyl, *N*-decanoyl and *N*-dodecanoyl derivatives have been obtained in methanesulfonic acid (Nishi et. al, 1979).

At room temperature, chitosan forms aldimines and ketimines with aldehydes and ketones, respectively. Reaction with ketoacids followed by reaction with sodium borohydride produces glucans carrying proteic and non-proteic amino groups. *N*-Carboxymethyl chitosan is obtained from glyoxylic acid. Examples of non-proteic amine acid glucans derived from chitosan are the *N*-carboxybenzyl chitosans obtained from *o*- and *p*-phthalaldehydic acids. Chitosan and simple

aldehydes produce *N*-alkyl chitosan upon hydrogenation. The presence of the more or less bulky substituent weakens the hydrogen bonds of chitosan; therefore *N*-alkyl chitosans swell in water in spite of the hydrophobicity of the alkyl chains, but they retain the film forming property of chitosan.

An important parameter to examine closely is the degree of *N*-acetylation in chitin. This parameter has a striking effect on chitin solubility and solution properties. Chitosan is the universally accepted non-toxic *N*-deacetylated derivative of chitin, where chitin is *N*-deacetylated to such an extent that it becomes soluble in dilute aqueous acetic and formic acids. In chitin, the acetylated units prevail (degree of acetylation typically 0.90). Chitosan is the fully or partially *N*-deacetylated derivative of chitin with a typical degree of acetylation of less than 0.35. To define this ratio, attempts have been made with many analytical tools, which include IR spectroscopy, pyrolysis gas chromatography, gel permeation chromatography and UV spectrophotometer, first derivative of UV spectrophotometer, ^{1}H-NMR spectroscopy, ^{13}C solid state NMR, thermal analysis, various titration schemes, acid hydrolysis and HPLC, separation spectrometry methods and, more recently, near-infrared spectroscopy.

Chitosan molecular weight distributions have been obtained using HPLC. The weight-average molecular weight (M_w) of chitin and chitosan has been determined by light scattering. Viscometry is a simple and rapid method for the determination of molecular weight; the constants α and K in the Mark–Houwink equation have been determined in 0.1 M acetic acid and 0.2 M sodium chloride solution. The intrinsic viscosity is expressed as:

$$[\eta] = KM^{\alpha} = 1.81\times10^{-3}M^{0.93}$$

The charged nature of chitosan in acid solvents and chitosan's propensity to form aggregation complexes require care when applying these constants. Furthermore, converting chitin into chitosan lower the molecular weight, changes the degree of deacetylation, and thereby alters the charge distribution, which in turn influences the agglomeration. The weight-average molecular weight of chitin is 1.03×10^{6} to 2.5×10^{6}, but the *N*-deacetylation reaction reduces this to 1×10^{5} to 5×10^{5} (Lee V.F. et. al, 1974).

Both cellulose and chitin are highly crystalline, intractable materials and only a limited number of solvents are known which are applicable as reaction solvents. Chitin and chitosan degraded before melting, which is typical for polysaccharides with extensive hydrogen bonding. This makes it necessary to dissolve chitin and chitosan in an appropriate solvent system to impart functionality. For each solvent system, polymer concentration, pH, counterion concentration and temperature effects on the solution viscosity must be known. Comparative data from solvent to solvent are not available. As a general rule, the maximum amount of polymer is dissolved in a given solvent towards a homogeneous solution. Subsequently, the polymer is regenerated in the required form. A coagulant is required for polymer regeneration or solidification. The nature of the coagulant is used.

An electrospinning method was used to fabricate nanofibous matrix for wound dressing. Chitin was deplymerized by gamma irradiation to improve its slubility. The electrospinning of Chitin was performed with 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) as a spinning solvent. Morphplogy of Nanofibers was investigated by scanning electron microscopy. Although as-spun chitin nanofibers had the broad fiber diameter distribution. Most of the fiber diameters are less than 100 μm. For deacetylation, as-spun chitin nanofibous matrix was chemically treated with a 40% aqueous NaOH solution at 60 or 100 °C. With the deacetylation for 150 min at 100 °C or for 1day at 60 °C, chitin matrix was transformed into chitosan matrix with degree of deacetylation (DD) 85% without dimensional change (shrinkage). This structural transformation from chitin to chitosan was confirmed by FT-IR and WAXD (Min et al, 2004). Chitosan nanofibers were electrospun from aqueous chitosan solution using concentrated acetic acid solution as a solvent. A uniform nanofiber mat of average fiber diameter of 130 nm was obtained from the the optimum condition of 7% chitosan solution in aqueous 90% acetic acid solution was successfully electrospun in the electric field of 4 kV/cm. Average fiber diameters and size distribution decreased with increasing electric field and more bead defects appeared at 5 kV/cm or more (Geng et al, 2005).

2.3 Tetrahydrocurcumin

Tetrahydrocurcumin (THC) is an antioxidative substance, which is derived from curcumin, the component of turmeric (Leelavinothan Pari). THC has possessed strong antioxidant action among all the curcuminoids. Structurally, THC and curcumin (Figure 2.6) have identical β-diketone structures and phenolic groups, but differ in that THC lacks the double bonds (Sugiyama et al, 1996; Okada et al, 2001). In addition, THC exhibited similar physiological properties as the active form of curcumin in vivo. Furthermore, (Okada et al, 2001) THC has more potent antioxidant activity than curcumin.

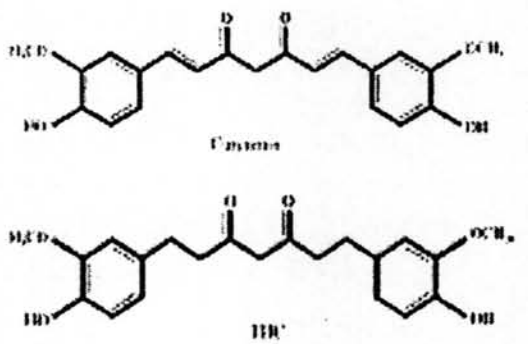

Figure 2.6 Chemical structures of curcumin and its major metabolite, tetrahydrocurcumin (THC). (Health. D. Dennish et al, 2005).

2.4 Drug Release Study

Controlled release of drugs is a very important process to achieve highest therapeutic efficiency. As a result, much research has been carried out in order to develop new controlled release systems that are more efficient and, most important, more cost-effective. Most conventional controlled systems provide a constant level of drugs in plasma. It, however, does not improve therapeutic efficiency of the drugs in some cases. In doing so, the suitable controlled release system should be able to administer drugs at a specified rate over a proper period of time during treatment. To achieve such characteristics, complete understanding over either the physical or

chemical properties is crucial. Controlled release of drugs from electrospun fibers has been of increasing interests in recent years and the release characteristics should depend on interactions between polymer and drug pair as much as on the sizes of the fibers. Kenawy et al. was the group to report release profiles of tetracycline hydrochloride (an antibiotic drug) from electrospun poly (ethylene-co-vinyl acetate) (PEVA), poly (lactic acid) (PLA), and PEVA/PLA blend fibers. (Kenawy et al, 2002). The solubility of drugs in the chosen polymer/solvent system was very important for successful incorporation of drugs within the as-spun fibers (Zeng et al, 2003).Electrospun methacrylate-based copolymers [i.e., poly(methacrylic acid-co-methyl methacrylate) (E-L100), poly(ethyl acrylate-co-methyl methacrylate chloride) (E-RLPO), and poly(butyl methacrylate-co-(2-dimethylaminoethyl) methacrylate-co-methyl methacrylate) (E-EPO)] loaded with indomethacin (10% by weight of the copolymer) were successfully prepared into fibers using an equivolume of EtOH and ethylacetate (EA) as the spinning co-solvent. The drug-loaded as-spun copolymer fibers appeared to be flat in their cross-sectional shape, with the size ranging between 1.2 and 2.5 μm. At 24 h, the amount of the drug released from these drug-loaded as-spun copolymer fibers was about 55%, 30%, and 18% for drug-loaded as-spun E-EPO, E-L100 and E-RLPO fibers, respectively (Pornsopone et al. 2006).