

CHAPTER II

LITERATURE REVIEW

2.1. Curcumin

Curcumin is a yellow pigment extracted from herbs called *Curcuma longa* or turmeric. Turmeric is commonly found and cultivated in India, South-East Asia, China and other tropical countries. Moreover, turmeric is also found in other parts of the world but is known by different names in different languages. (Goel, Kunnumakkara and Aggarwal, 2008)

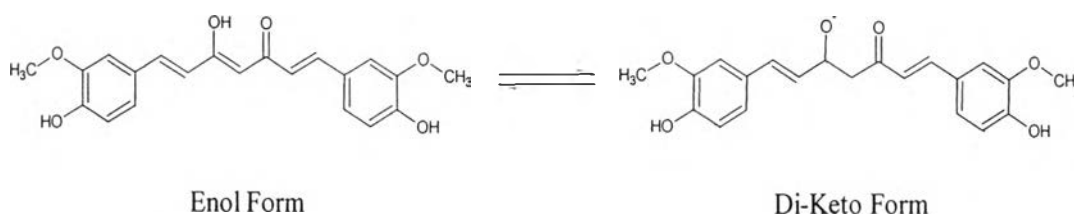


Figure 2.1 Chemical structures of curcumin in enol and di-keto form.

Curcumin is identified as 1,6-heptadiene-3,5-dione-1,7-bis(4-hydroxy-3-methoxyphenyl)-(1E,6E) or diferuloylmethane (Figure 2.1). It has melting point of 183°C. Furthermore, it has a molecular formula of $C_{12}H_{20}O_6$ and molecular weight of 368.38 g/mol. It is a polyphenol which is poorly soluble in water and ether; however, it is soluble in ethanol, dimethylsulfoxide and acetone. Also, it exists in two tautomeric conformations i.e. enol and di-keto forms (Figure 2.1). Curcumin appears bright yellow in acidic environments (pH 2.5-7) where most molecular constituents are in the di-keto form and acts as a proton donor. However, it changes to orange/red in basic conditions (pH >8) where the enol form dominates and curcumin acts as an electron donor (Lee W.-H. et. al, 2013). At high pH conditions, curcumin starts to degrade into vanillin, ferulic acid and feruloylmethane as shown in Figure 2.2. Also, curcumin has different degradation times depending on the solvent. For example, if

curcumin was placed in a phosphate buffer system at pH 7.2 or above, it degrades in 30 minutes. (Wang et. al., 1997)

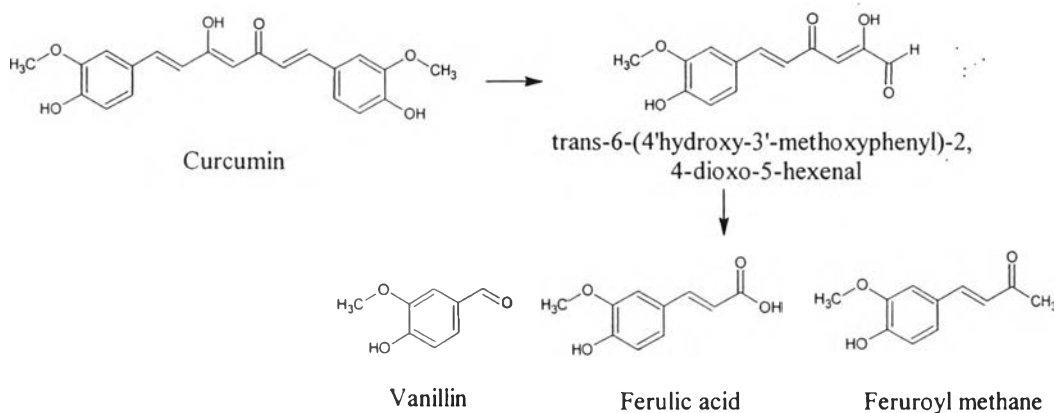


Figure 2.2 Chemical structures of products obtained from degradation of curcumin.

Currently, curcumin has been used in many applications ranging from food to medicine. In the kitchen, curcumin is used as a spice to add color and flavor in curries. Also, it is used as colorings in other foods. In medical industries, curcumin is used as a therapeutic ingredient all over the world due to its various properties i.e. anti-oxidant, anti-inflammatory, anti-carcinogenic, antimicrobials and others. It could be used as treatment for respiratory conditions, liver disorders, colds, diabetic wounds, sinusitis, sprains and swelling and even treating abdominal pain.

Curcumin is pH dependent where it has the ability to change color from yellow to orange and orange to red at neutral and basic environments, respectively. As a result, curcumin would be a good indicator for food spoilage by detecting the volatile amines produced by bacteria on the meat. Kuswandi et al. used this property to apply curcumin as food sensors by immobilizing curcumin onto bacterial cellulose membrane and testing the membrane on shrimp samples. The sensor was successfully used as a sticker for real-time monitoring of shrimp spoilage in ambient and cool conditions. In the future applications, this natural food sensor could be used in fish industries as a spoilage indicator due to it is low cost, safe, simple and biocompatible properties. (Kuswandi et al, 2012)

Pourezza and Golmohammadi studied the pH sensitivity of curcumin which could be applied as a lab on paper device for pH measurements. First, they observed the color change of curcumin solution. The graph is the UV spectra of curcumin at pH 7-13 (Figure 2.3).

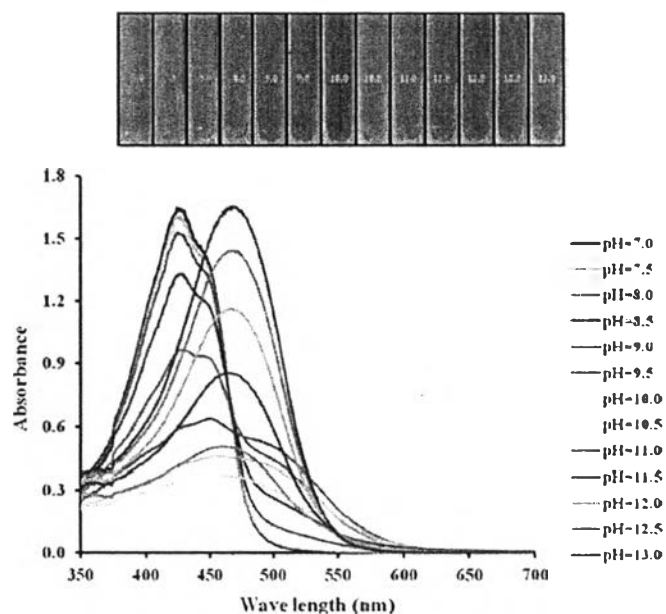


Figure 2.3 UV spectra of the curcumin solutions with various pH values in the range of 7-13 after 30 minutes (Pourezza, and Golmohammadi, 2015).

From pH 7-9 shows that the maximum absorbance is at 423 nm but after pH 9.5 the peak shift to 460nm which indicate change in color and the images show that it changes from yellow to orange (Pourezza, and Golmohammadi, 2015).

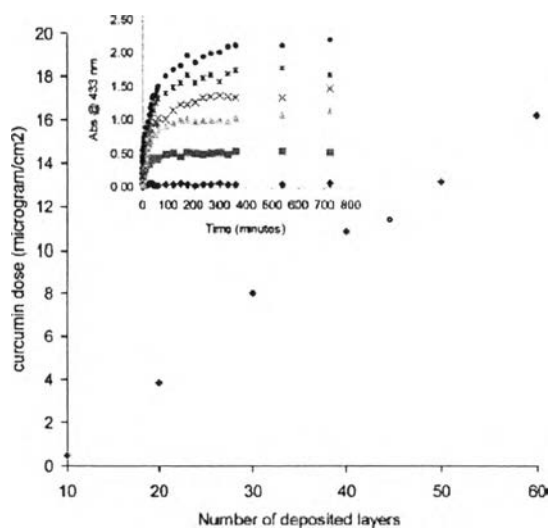


Figure 2.4 UV-vis absorbance of curcumin loaded into PEMU thin films with an increasing number of deposited layers as a function of time (Kittitheeranun, Sanchavanakit, Sajomsang and Dubas, 2010).

Kittitheeranun et al. studied the loading of curcumin on polyelectrolyte multilayer thin films for a potential use as an anticancer drug in a drug delivery patch. They studied the solvent composition, concentration of the loaded curcumin and film thickness using UV-Vis spectroscopy. Figure 2.4 shows that curcumin loading on to PEM was successful and the amount of curcumin loaded is controlled by the amount of film layers (Kittitheeranun, Sanchavanakit, Sajomsang and Dubas, 2010).

2.2. Layer-by-Layer Deposition

The layer-by-layer deposition (LbL) is a method used to build polyelectrolyte multilayer films which involves electrostatic interactions between oppositely charged molecules by adsorption onto a substrate. LbL has received a lot of attention due to its simplicity and versatile applications. This method was first discovered by Iler in the 1960s and was rediscovered by Decher and coworkers in the early 1990s (Iler, 1966). Presently, this technique has been the focus of many researchers because it

widens the fabrication pathway of polymer thin films with tunable properties and surface chemistry.

This method requires a total of four steps as shown in Figure 5. First, assuming that the substrate has a positively charged surface, the substrate is exposed to a polyanion solution. The polyanion interacts with the oppositely charged surface and forms a monolayer. Second, the monolayer is rinsed in deionized water to remove excess polyanion solutions and to avoid contamination of the next polyelectrolyte solution. Third, the monolayer is exposed to a polycation solution to form the second layer. Finally, the bilayer is rinsed to remove excess polymers. By following this procedure, multiple layers can be made by repeating the steps continuously until the desired number of layers is reached. The LbL can be performed by three methods: spinning, dipping and spraying (Decher, 1997).

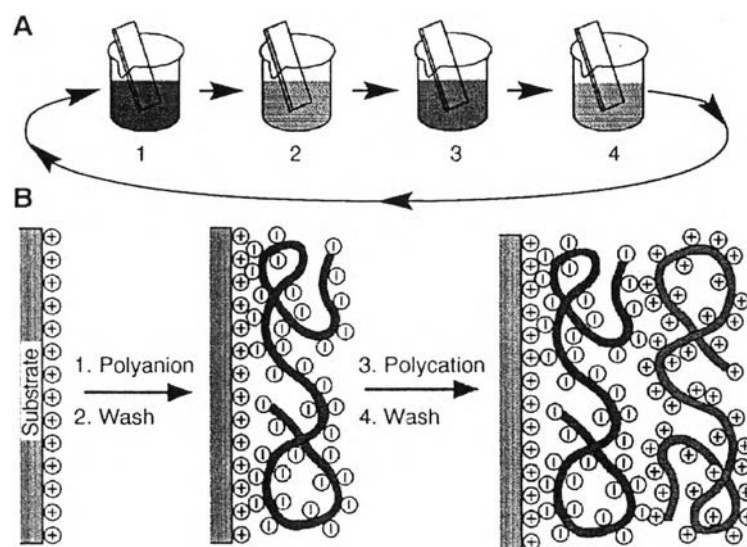


Figure 2.5 Schematic diagram of the Layer-by-Layer assembly (Decher, 1997).

The LbL deposition can be used in many systems including the incorporation of small organic colloids, clay particles, inorganic clusters and proteins. This gives a wide variety of applications ranging from solar cells to drug delivery and sensors.

Macdonald et al. studied protein drug delivery used as supplements for bone construction using LbL assemblies of Bone Morphogenetic Protein 2 (BMP-2). This study may provide new approaches to bone wound healing and multi-therapeutic release coatings (Macdonald et al, 2011).

Podiadlo, Shim and Kotov studied the incorporation of organic/inorganic films such as clay nano-sheets and carbon nanotubes in layer-by-layer assemblies. These two materials are commercially available and have structural and physico/chemical properties unique to each which enables the preparation of variety of functional composites. Carbon nanotubes can be used as electrical conductors and chemical sensors, where clay nanoparticles can be applied in bio-compatible materials for bio-electrocatalytic properties and catalysts (Podsiadlo, Shim, and Kotov, 2009).

The film fabrication method can be used in solar cells. Zhang et al. studied the use of poly (acrylate sodium) PAAS/Titanium (TiO_2) films that can be used in solar cells. This film showed promising results for dye-sensitized solar cells where the energy conversion of these cells increased by 1.29% (Zhang et al., 2010).

2.2.1 Polyelectrolytes

Polyelectrolytes are polymers with charged functional groups (Nagvekar, Tihminlioglu and Danner, 1998). In aqueous solutions, these polyelectrolytes dissociate into a charged polymer chain and its counter ion. Due to its ionic character, they are water soluble; unlike traditional polymers which are soluble in organic solvents (Dobrynin, Colby, and Rubinstein, 1995). Traditional polymers contain a relatively low concentration of charged functional groups while polyelectrolytes contain higher concentrations of charged groups. Polyelectrolytes can be polycations, polyanions or polyampholytes depending on the ionized groups present on the chain.

Polyelectrolytes can be found naturally, modified or produced synthetically (Nagvekar, Tihminlioglu and Danner, 1998). Natural polyelectrolytes are mostly biological polymers, such as nucleic acid, proteins and polysaccharides. These natural polymers can be further modified to form polyelectrolytes such as chitosan which was produced from the deacetylation of chitin, the exoskeleton of

crustaceans (Esquenet and Buhler, 2001). Moreover, due to the wide variety of functional groups, many combinations of synthetic polymers can be prepared. They can be either cationic or anionic depending on the charge of the functional groups. Figure 2.6 shows examples of polyelectrolytes.

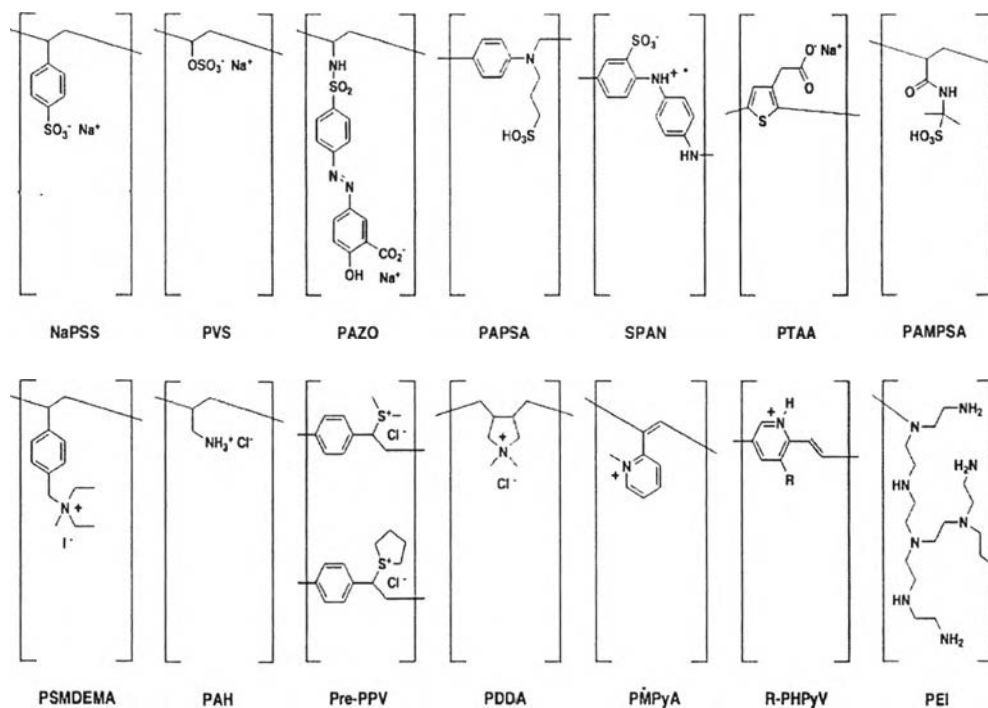


Figure 2.6 Example of polyelectrolytes.

Polyelectrolytes can be used to produce polyelectrolyte multilayers (PEMUs) and polyelectrolyte complexes (PECs) by combining two polyelectrolytes. PEMUs are nano scale thin films made of two oppositely charged polyelectrolytes deposited alternatively on a charged substrate. PECs are precipitates that form after mixing two oppositely charged polyelectrolytes. Both involve the ion exchange phenomenon where a charge segment replaces a salt ion compensating the surface charge as shown in equation 1:



Where Pol-Pol⁺ is an ion pair, M⁺, A⁻ are salt counter ions. The subscript “m” refers to the polymer surface (Dubas, and Schlenoff, 1999).

The most common synthetic polyelectrolyte pair is poly(diallyldimethyl ammonium chloride) (PDADMAC) and poly(sodium 4-styrenesulfonate) (PSS) which are classified as strong polyelectrolytes and is chemically stable. Another interesting polyelectrolyte pair is chitosan (CHI) and alginic acid (ALG) which are modified and natural polyelectrolytes that are commonly used in medical applications. This works will be focusing on PEMUs.

2.2.1.1 PDADMAC and PSS

The most commonly used in fabricating PEMUs are PDADMAC and PSS (Figure 2.7). Poly(diallyl dimethyl ammonium chloride) or PDADMAC and poly(sodium 4-styrenesulfonate) or PSS is a polycationic and a polyanionic polyelectrolyte, respectively. They are characterized as strong polyelectrolytes due to being fully ionized under a wide range of pH. Both polyelectrolytes are commonly used to produce polyelectrolyte multilayers or polyelectrolyte complexes for various applications such as cell culture, drug delivery and electrodes.

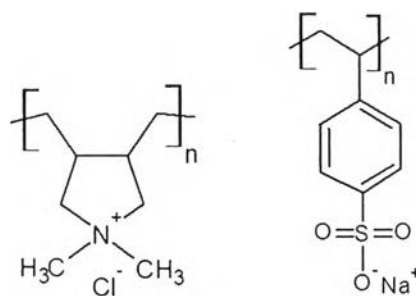


Figure 2.7 Chemical structure of (left) poly (diallyl dimethyl ammonium chloride) (PDADMAC) and (right) poly(sodium 4-styrenesulfonate) (PSS).

Dubas and Schlenoff studied the growth of PDADMAC/PSS thin films affected by parameters such as salt concentration, salt type, solvent quality, deposition time and polymer concentration. They found that salt concentration has the strongest influence in the PEMU buildup i.e. film thickness.

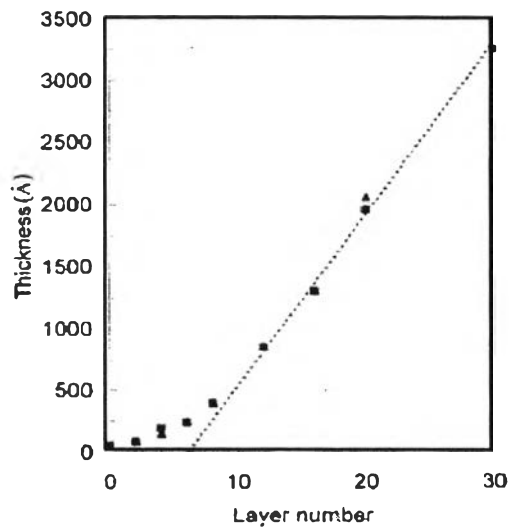


Figure 2.8 Thickness as a function of the number of layers for a PSS/PDADMAC multilayer (Dubas and Schlenoff, 1999).

They found that the thickness increases with the number of deposited layers (Figure 2.8). Additionally, the thickness is linearly dependent on the salt concentration from 0.02M to 2M. (Dubas, and Schlenoff, 1999)

2.2.1.2 Chitosan and Alginate Acid

CHI and ALG are modified and natural polyelectrolytes which are commonly used in biomedical applications due to its biocompatibility.

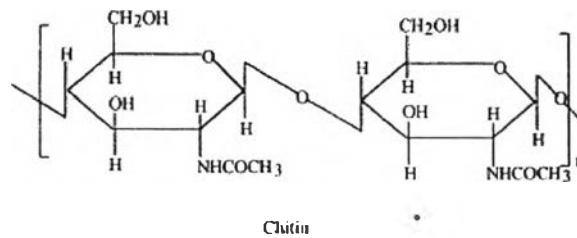


Figure 2.9 Chemical structure of chitin (Ravi Kumar M. N. V., 2000).

Chitin is an abundant polysaccharide which can be found in the exoskeletons of crustaceans (Figure 2.9). The processing of crustacean shells involves the removal of proteins and the dissolution of calcium carbonate, this produces chitin. Chitin can undergo deacetylation in 40% sodium hydroxide at 120 °C to produce 70% deacetylated chitosan, the modified product of chitin. (Ravi Kumar, 2000)

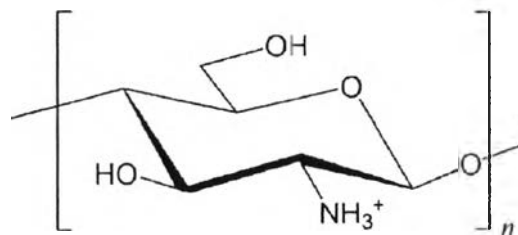


Figure 2.10 Chemical structure of chitosan (Fujie, and Takeoka).

Chitosan has antibacterial and antifungal properties (Figure 2.10). Furthermore, due to its biocompatibility it is commonly used in applications such wound dressing (Jayakumar et. al., 2011) and drug delivery (Tang C., Guan Y.-X., Yao S.-J., and Zhu Z.-Q., 2014). It can be used in industrial applications such as waste water purification (Reddy, and Lee, 2013).

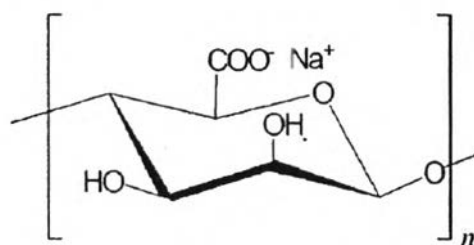


Figure 2.11 Chemical structure of sodium alginate (Fujie and Takeoka).

Alginic acid or sodium alginate is an edible polyelectrolyte extracted from brown algae and consists of the 1, 4- β -D-mannuronic acid and α -L-guluronic acid (Figure 2.11). It is water soluble but precipitates at acidic conditions. Mostly, alginic acid is crosslinked by calcium ions to form gels which are commonly used in drug delivery applications (Soares, Santos, Chierice, and Cavaleiro, 2004).

Caridade and coworkers studied the free-standing films made of the LbL assembly of CHI/ALG. These thick films are detached from the substrate and can be used in biomedical applications, specifically drug delivery. Since both CHI and ALG are weak polyelectrolytes, they are partially charged at moderate pH near their pKa. Thus, the film growth is dependent on the ionic strength and pH (Caridade et.al, 2013).

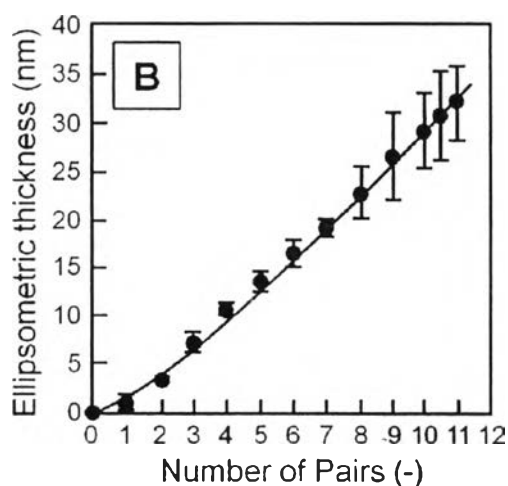


Figure 2.12 Thickness as a function of number of layers of CHI/ALG thin films (Fujie, and Takeoka).

Fujie and Takeoka also studied the free-standing films of CHI/ALG. As shown in figure 2.12 the thickness growth corresponds to the number of layers of the polyelectrolytes, similar to PDADMAC/PSS thin films. However, chitosan and alginate is pH-dependent and the suitable pH for PEMU build-up is pH 5 (Fujie and Takeoka).

2.2.2. Loading of Compounds onto PEMUs

The loading of compound onto PEMUs usually involves electrostatic interaction between the PEMU and the compound. For example, Sirorat and Dubas loaded silver nanoparticles capped with sodium alginate onto PEMU-coated surgical sutures to enhance its anti-microbial activity. The silver nanoparticles do not have a charge; as a result it was capped with sodium alginate in order to be adsorbed onto the suture surface (Dubas, Wacharanad, and Potiyaraj, 2011).

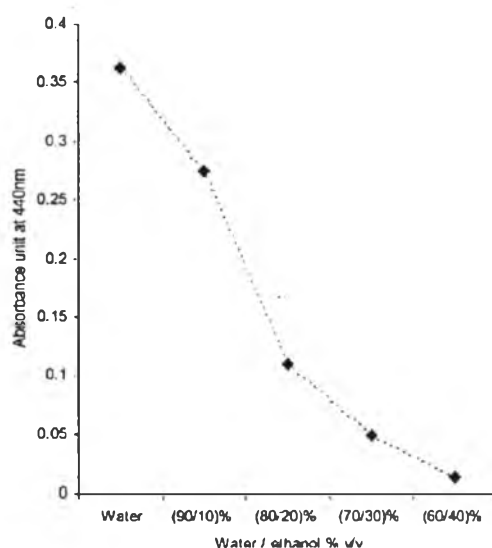


Figure 2.13 Absorption spectra of curcumin in PEMU thin films loaded from 0.00025% curcumin in solutions of various water/ethanol ratios (Kittitheeranun, Sanchavanakit, Sajomsang and Dubas, 2010).

Kittitheeranun and coworkers also studied the solvent effect towards loading curcumin onto PEMU. Curcumin is an organic compound that is not as soluble in water compared to solvents such as ethanol, methanol and acetone. In their work, they add curcumin into a mixed solvent composed of water and ethanol then immerse the PEMU-coated substrate. The curcumin particles will load onto the PEMU via solvent partitioning where curcumin behaves as the hydrophobic phase and the solvent as the hydrophilic phase. It is known that the PEMU is hydrophilic due to the charges of the polyelectrolytes and that the top layer is highly charged. However, the bulk of the PEMU is neutral; therefore, it is a favorable environment for the lipophilic molecules to diffuse into the PEMU. The results are shown in Figure 2.13 which indicates that the curcumin will load onto the PEMU at high water content. Additionally, since the loading of molecules were driven by partitioning mechanism where the PEMU is the non-polar phase and the mixed solvent was the polar phase. The curcumin will preferably diffuse into the PEMU because it is more hydrophobic (Kittitheeranun, Sanchavanakit, Sajomsang and Dubas, 2010).

2.3. Electrospinning

Electrospinning is a technique which produces fine fibers from viscous polymer solutions using electrostatic forces which produces fibers with thinner diameter (from microscale to nanoscale). As a result, the material cover higher surface area compared to conventional spinning processes. Electrospinning can be conducted at room temperature with atmosphere conditions. All three types of polymers can be fabricated into fibers using electrospinning i.e. natural polymers, synthetic polymers and modified polymers. There are many parameters in the electrospinning process which must be considered. A typical electrospinning setup consists of three major components: a high voltage power supply, a spinneret (needle) and a collector as shown in Figure 2.14. First the polymer solution is fed through a spinneret. The nozzle acts as an electrode where the high voltage more than 5 kV was applied. The repulsive force within the charged solution is larger than its surface tension and a jet would erupt from the tip of the spinneret. As the solvent

evaporates, the polymer solution repels itself due to electrostatic forces within the solution which leads to a bending instability stage with further stretching of the solution. The collector acts as a counter electrode which is used to collect the fibers which are deposited as a nonwoven mesh.

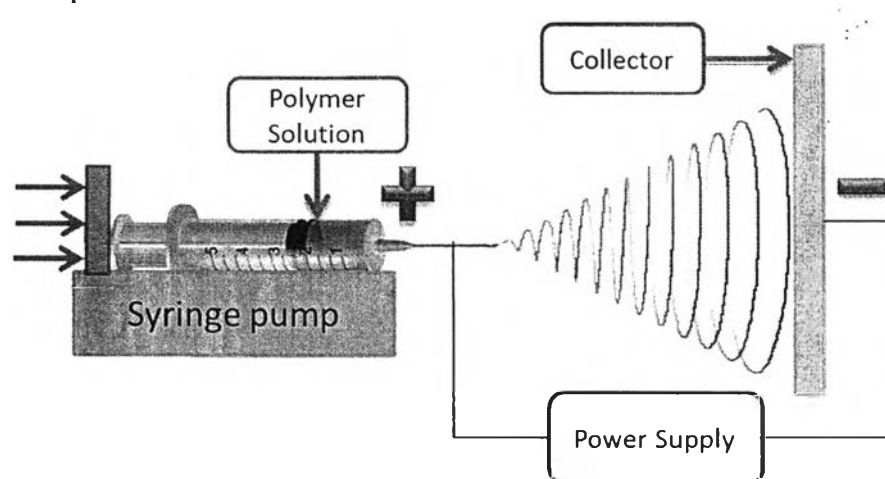


Figure 2.14 Diagram of the electrospinning process.

There are many parameters in the electrospinning process which must be considered. The parameters are characterized to three categories. First, solution parameters, including viscosity, conductivity, molecular weight, and surface tension, Second, process parameters i.e. electric field, gap distance and flow rate. Lastly, ambient parameters which are temperature and humidity. All of these parameters determine the fiber morphology and diameter of the electrospun fibers (Bhardwaj and Khundu, 2010).

2.3.1 Solution Parameters

First, solution viscosity or concentration plays a vital role in determining the fiber morphology and size during electrospinning. If low viscosity or concentration of polymer was used then electrospray would occur instead of electrospinning because very low polymer concentration results in the formation of polymeric nanoparticles. If the concentration is slightly higher, a mixture of beads and fibers will be obtained. Finally, if the concentration is suitable, smooth nanofibers can be obtained. The same effects also applied to molecular weight.

Second, surface tension is one of the main factors in electrospinning since it involves the solvent composition of the solution. By reducing the surface tension, the beaded fibers can be converted into smooth fibers. The surface tension can be adjusted by the ratio of the solvent mixture (solvent composition).

Third is the solution conductivity or the ionic strength of the solution. Ions on the polymer chain increase charge carrying ability of the polymer jet which may result in high surface tension; therefore, obtains poor fiber formation. The presence of ionic salts can produce nano-fibers with small diameters. Conclusively, an increase in solution conductivity results in thinner fibers.

2.3.2 Process Parameters

The applied voltage is the crucial component for electrospinning process. There must be sufficient amount of applied voltage to cause the polymer solution to be ejected from the spinneret. The fiber morphology is affected by the applied voltage; however, the morphology also depends on the polymer concentration and the gap distance between needle tip and collector.

The flow rate is another parameter that affects the fiber forming process. Low flow rate is commonly applied to achieve more time for polarization. If the flow rate is too high, bead fibers with thick diameter will be obtained due to the short drying time before the fibers reach the collector; therefore, has low stretching force. This relates to the gap distance between the needle and the collector. The further the gap distance, the more time the fibers were allowed to dry out the solvent and stretch (thinner diameters).

The collectors used in the electrospinning process are usually conductive substrates such as aluminum foil to collect the charged fibers. However, substrates such as aluminum foils are difficult to transfer for other uses. Therefore, other types of collectors can also be used such as, wire mesh, pin, grids, rotating rods or wheels, etc., depending on the type of application.

2.3.3 Ambient Parameters

Ambient parameters are such as humidity and temperature can also affect fiber morphology. Low humidity causes the solvent to dry quickly which leads to the formation of thin fibers and vice versa (Unnithan, Arathyram, and Kim, 2015).