CHAPTER III

EXPERIMENTAL

This work can be divided into two main parts. The first is to investigate the effects of polymer concentration and applied potential on morphological appearance of electrospun methacrylate-based copolymers. The optimal conditions for achieving fibers of uniformed shape and size, the conditions were chosen and used for further investigation whether these electrospun copolymers can be used as controlled release carriers.

3.1 Electrospinning of Methacrylate-Based Copolymers

3.1.1 Materials

Methacrylate-based copolymers used in this work were poly(methacrylic acid-*co*-methyl methacrylate) (Eudragit L100, Röhm GmbH, Germany), poly(ethyl acrylate-*co*-methyl methacrylate-*co*-trimethyl-ammonioethyl methacrylate chloride) (Eudragit RLPO, Röhm GmbH, Germany), and poly(butyl methacrylate–*co*-(2-dimethylaminoethyl) methacrylate-*co*-methyl methacrylate) (Eudragit EPO, Röhm GmbH, Germany), the weight-average molecular weight of which was 135,000 $g \cdot mol^{-1}$, 150,000 $g \cdot mol^{-1}$, and 150,000 $g \cdot mol^{-1}$, respectively. The chemical structures of these copolymers are shown in Figures 3 to 5, respectively. These copolymers were used to produce methacrylate-based electrospun fibers and methacrylate-based cast films and were further investigated to see whether the as-spun fibers could be used as drug delivery materials. Either ethanol (EtOH) or a mixture between EtOH and ethyl acetate (EA) were used as the solvent system. Indomethacin was used as the model drug and was loaded into as-spun fibers of selected conditions. With the same drug copolymer solutions, controlled release of the model drug from both as-spun fibers and cast films were compared.

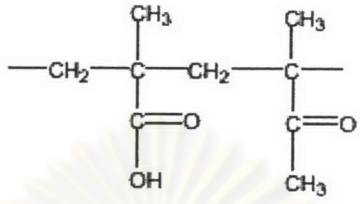

Figure 3 Chemical structure of Eudragit L100

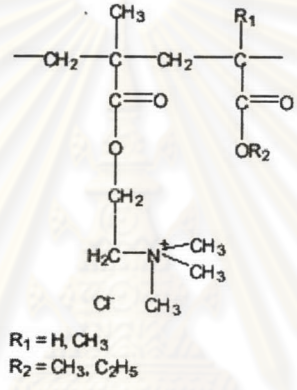

Figure 4 Chemical structure of Eudragit RLPO

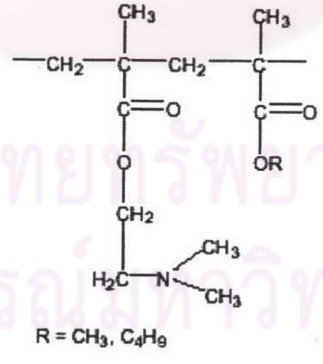

Figure 5 Chemical structure of Eudragit EPO

3.1.2 High Voltage Power Supply

A high voltage power supply (D-ES30PN/M692, Gamma High Voltage Research, Ormond Beach, Florida) was used to charge the as-prepared polymer solutions

between a needle tip and a grounded collector. The applied potentials used in this work ranged between 7.5 to 22.5 kV across a fixed collection distance of 15 cm.

3.1.3 Solution Preparation

In the first part, solutions of methacrylate-based copolymers were prepared in EtOH in the concentration range of 10 to 35 percentage of polymer weight by volume of the solution (i.e. % (w/v)). In the second part, solutions of selected concentrations and spinning conditions were prepared in a mixture of EtOH and ethyl acetate in a 1:1 volumetric ratio in order to decrease evaporation. Ten percentage by weight of indomethacin based on the weight of the copolymer was loaded in these as-prepared solutions.

3.1.4 Determination of Polymer Solution

As-prepared solutions of methacrylate-based copolymer in ethanol were determined for their viscosity using a Brookfileld DV-III programmable viscometer (see Figure 6). The temperature of the solutions was controlled at 30°C. The surface tension of the solutions were measured using a Krüss K10T tensiometer (see Figure 7). An average value for each solution was calculated from at least 3 readings. Again, the measurement was carried out at 30°C and the atmosphere in the measuring chamber was saturated with vapor of the solvent to limit evaporation of the solvent from the pendant drop samples. The conductivity of the solutions were also measured using a Orion 160 conductivity meter (see Figure 8) at 25°C.

3.1.5 Electrospinning Process

The experimental setup to study the effect of polymer concentration on morphological appearance of the as-spun fibers is as follows. A solution of methacrylate-based copolymers with the concentrations being between 10 and 35 % (w/v) was placed in a 50 mL syringe. The syringe was clamped vertically to a PVC stand. It was placed at 15 cm from the grounded collector which was an aluminum foil

sheet. The external electrical field was applied to the polymer solution by attaching a positive electrode to the needle tip. A constant pressure of nitrogen gas was fed into the syringe to control the flow rate of the polymer solution. In order to observe the effect of applied potential, the applied potentials of 7.5 to 22.5 kV were applied. The effect of copolymer type was also investigated by varying the type of the methacrylate-based copolymers used.

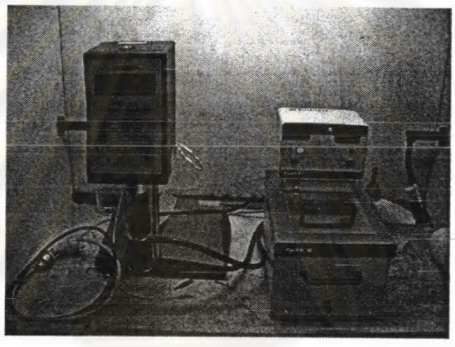

Figure 6 Brookfield DV-III programmable viscometer

Figure 7 Krüss K10T tensiometer

Figure 8 Orion 160 conductivity meter

3.1.6 Characterization of As-spun Fibers

To investigate morphology and size of the obtained as-spun fibers, a JOEL LS002 scanning electron microscope (SEM) was used. Three samples were obtained from each spinning condition and were coated with gold by an ion-sputtering device for 4 minutes prior to analysis. For each sample, about 20 SEM images were taken, just to obtained 8 best images for further analysis for the fiber shape and size as well as the size distribution. The size of the as-spun fibers was determined using a SemAfore program, while the size distribution was calculated from the obtained data using a SPSS Program.

3.2 Controlled Release Study of Acrylic Copolymer Electrospun Fibers

3.2.1 Preparation of phosphate buffer

Firstly, 0.2 M monobasic potassium phosphate solution was prepared by dissolving 27.22 g of monobasic potassium phosphate (KH_2PO_4) in water and later diluted with water to 1000 mL. Secondly, 0.2 M sodium hydroxide (NaOH) solution was prepared by dissolving 8.0 g of sodium hydroxide in water and later diluted with water to 1000 mL. Thirdly, the phosphate buffer solution was prepared by placing 50 ml of the as-prepared monobasic potassium phosphate solution in a 200-ml volumetric flask, adding 34.7 mL of sodium hydroxide solution, and then adding water to the

required volume. Lastly, the as-prepared phosphate buffer solution was tested by a pH-meter to attain a pH level of 7.2.

3.2.2 Drug-Loaded Samples

Drug-loaded electrospun methacrylate-based fibers were prepared by blending ten percentage weighed amount of drug in the spinning solution prior to electrospinning. For comparison, the drug-loaded spinning solution was also cast into films. These drug-loaded samples were left in room temperature for 24 hours to ensure that the samples were completely dry. The samples were then examined for the morphological appearance and size under SEM and later tested for their drug releasing profile.

3.2.3 Calibration curve

The standard drug solution was prepared by first putting 50 mg of indomethacin in a 100-mL flask, which was later filled up by methanol. Later, 12 ml of this solution was put in a 100-mL flask, which was later filled up by the as-prepared phosphate buffer solution (having a pH level of 7.2). The as-prepared standard drug solution was later diluted with the as-prepared phosphate buffer solution so that the amount of drug in subsequent solutions was varied in the range of 20 to 60 mg per mL. The UV absorbance of the drug at different concentrations was measured using ultraviolet-visible spectroscopy (UV-Vis) at the Petroleum and Petrochemical College, Chulalongkorn, to determine the drug at the wavelength of about 320 nm.

3.2.4 Drug Assay

Drug assay was carried to verify the existence of drug in either as-spun or as-cast samples. Transfer 25 mg of indomethacin, accurately weighed, to a 100-mL volumetric flask, add 10 ml of water, and allow to stand for 10 minutes, swirling occasionally. Add 60 mL of methanol, shake for 10 minutes, dilute with methanol to volume. Dilute a portion of the clear solution quantitatively and stepwise, if necessary,

with a mixture of equal volumes of methanol and pH 7.2 phosphate buffer to obtain a solution containing about 40 microgram of indomethacin per mL as a standard drug. To compare the sample with the standard drug, the sample is treated similarly to the procedure previously described. The amount of samples used equals that of the standard drug in a drug portion. A wavelength of maximum absorbance at about 320 nm was detected using an ultraviolet-visible spectroscope. A mixture of methanol and phosphate buffer at pH 7.2 was used as a blank. These procedures were used to assay the amount of drug within the samples.

3.2.5 Controlled Release of Drug

The samples with the same amount of drug were cut into three small pieces with the area of 1×1 cm^2 to study their controlled release character. Both the as-spun and as-cast samples were put into a bottle containing a known amount of phosphate buffer at pH 7.2. In this bottle, the buffer solution was mechanically stirred in order to achieve homogeneity. These buffer solutions were tested for the amount of soluble drug after a certain period time (i.e. 10 min, 20 min, 30 min, 1 hr, 3 hrs, 6 hrs, 12 hrs, and 24 hrs, respectively) was reached.

3.2.6 Determination of Drug Releasing

The drug release profiles of the copolymer samples and standard drug were characterized by using an ultraviolet visible spectroscopy. A wavelength of maximum absorbance at about 320 nm was detected. The maximum absorbance of the samples relates to the concentration of drug in the solutions. A relationship between the concentration of released drug and time was plotted to show the controlled release character of each sample.