

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Properties of Methacrylate-based Copolymer Solutions

4.1.1 Viscosity

The viscosity values of methacrylate-based copolymer solutions are measured and summarized in Table 1, while Figure 9 shows viscosity as a function of copolymer concentration for the three types of copolymer solutions.

Table 1 Viscosity of methacrylate-based copolymer solutions

Concentration (% (w/v))	Viscosity (cp.)	Shear Stress (D/cm ²)	Shear Rate (1/sec)	Torque (%)
Eudragit EPO				
10%	5.0	11.6	233	2.5
15%	14.2	32.6	233	7.1
20%	43.4	98.1	233	22
25%	117.0	273.0	233	58.7
30%	386.0	450.0	119	99.1
35%	1730.0	457.0	26	99.1
Eudragit L100				
10%	25.4	59.1	233	12.7
15%	162.0	377.0	233	81.4
20%	1055.0	460.0	44	99.0
25%	7294.0	464.0	6	99.4
30%*	n/a	n/a	n/a	n/a
35%*	n/a	n/a	n/a	n/a
Eudragit RLPO				
10%	6.8	17.2	233	2.7
15%	17.6	40.5	233	8.8
20%	47.2	110.0	233	23.7
25%	259.0	464.0	179	99.8
30%	520.0	464.0	89	99.9
35%*	n/a	n/a	n/a	n/a

*The value could not be measured since the viscosity is too high.

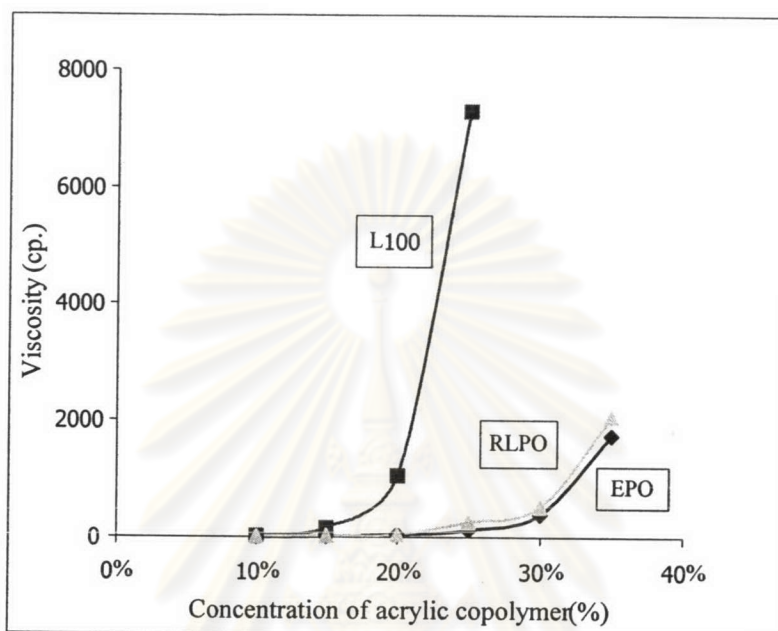


Figure 9 Viscosity as a function of copolymer concentration for the three types of copolymer solutions.

Obviously, for a given copolymer concentration, solution of Eudragit L100 exhibited much higher viscosity than those of Eudragit RLPO and Eudragit EPO, which, in turn, showed comparable values. Though the average molecular weight of Eudragit L100 was quite lower than Eudragit RLPO and Eudragit EPO, it showed a much higher viscosity than those of the other two powders. The presence of the carboxylic group causes Eudragit L100 molecules to form hydrogen bonding with an adjacent carboxylic group either intra- or intermolecularly. The hydrogen interaction causes mobility of Eudragit L100 molecules to be more limited than the other two powders. In addition, Eudragit RLPO showed a bit higher viscosity than Eudragit EPO, despite of having the same molecular weights. The present of the chloride counter-ion may act as a weak moiety linking different trimethylammonium groups between different Eudragit RLPO molecules, causing the viscosity to be greater than Eudragit EPO.

4.1.2 Surface Tension

The surface tension values of methacrylate-based copolymer solutions are determined and summarized in Table 2, while Figure 10 shows surface tension as a function of copolymer concentration for the three types of copolymer solutions.

Table 2 Surface tension of methacrylate-based copolymer solutions

Eudragit EPO						
Concentration (% (w/v))	10%	15%	20%	25%	30%	35%
1st	24.97	24.84	24.17	23.88	24.18	24.57
2nd	24.85	24.67	23.99	23.96	24.15	24.64
3rd	24.82	24.71	24.18	23.98	24.17	24.33
Avg.	24.88	24.74	24.11	23.94	24.17	24.51
Eudragit L100						
Concentration (% (w/v))	10%	15%	20%	25%	30%	35%
1st	25.21	26.33	26.37	26.05	24.48	24.12
2nd	25.24	26.32	26.39	25.88	24.51	24.38
3rd	25.41	26.30	26.38	25.99	24.53	24.55
Avg.	25.29	26.32	26.38	25.97	24.51	24.35
Eudragit RLPO						
Concentration (% (w/v))	10%	15%	20%	25%	30%	35%
1st	24.97	24.8	24.3	24.21	23.81	23.55
2nd	24.99	24.69	24.28	24.06	23.70	23.68
3rd	24.96	24.67	24.40	23.94	23.63	23.70
Avg.	24.97	24.72	24.33	24.07	23.71	23.64

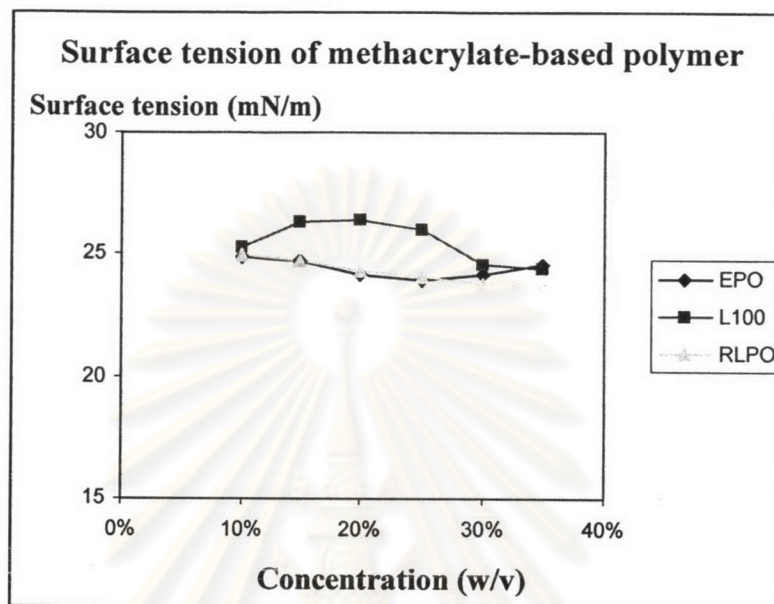


Figure 10 Surface tension as a function of acrylic copolymer concentration for the three types of copolymer solutions.

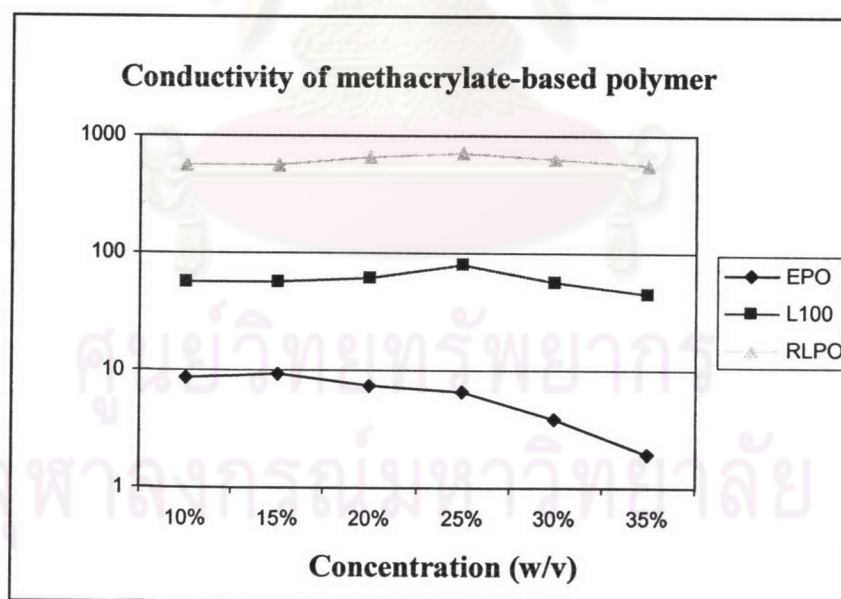
It was found that the surface tension was in the range of 23.55 to 26.39, which was quite identical. It may be concluded that the concentration did not affect appreciably the surface tension.

4.1.3 Conductivity

The conductivity values of methacrylate-based copolymer solutions are measured and summarized in Table 3.

Table 3 Conductivity of methacrylate-based copolymer solutions

Concentration (% (w/v))	Eudragit EPO ($\mu\text{s}/\text{cm}$)	Eudragit L100 ($\mu\text{s}/\text{cm}$)	Eudragit RLPO ($\mu\text{s}/\text{cm}$)
10%	8.54	55.3	557
15%	9.14	57.3	570
20%	7.44	61.4	652
25%	6.63	79.5	700
30%	3.81	56.8	620
35%	1.92	45.4	560

**Figure 11** Conductivity as a function of copolymer concentration for the three types of copolymer solutions.

Irrespective to the type of the methacrylate-based polymers used, the conductivity value increased initially, reached a maximum, and later decreased with increasing polymer concentration. Among the various polymers investigated, Eudragit RLPO showed the highest conductivity, while Eudragit EPO showed the lowest value. The possible reason for the observed highest conductivity values of Eudragit RLPO should be a result of the charges already present in its molecules. In case of Eudragit L100, the presence of ionizable $-\text{COOH}$ group under application may render the solutions of this polymer the second highest conductivity values. Logically, Eudragit EPO which does not have any charge nor ionizable moiety present on its molecules showed the lowest conductivity values.

4.2 Preparation and Characterization of As-spun Fibers

4.2.1 Preliminary Work

Before the experiment was setup, many polymers have been experimented in order to gain both skill and knowledge on the spinning technique. The systems investigated were, for examples, poly(ethylene phthalate) in *m*-cresol, poly(ethylene oxide) in distilled water, poly(caprolactone) in methylene chloride. From these preliminary studies, basic understanding towards the electrospinning process was gained, which ranged from the preparation of a spinning dope to some precautions that helped in getting good as-spun fibers. A number of parameters have been shown to affect the spinnability of a polymer solution: which are, for examples,

1. Despite the success in preparing a spinning solution, electrospinning of the solution may not produce fibers at all, since many parameters have been found to affect the spinnability of a solution. These are, for examples, some solution properties (e.g., viscosity, surface tension, conductivity, etc.), applied voltages, collection distance, solvent type, and needle size. If the viscosity of the solution is too high, the polymer solution could simply not flow. When the solution having right properties is prepared, a hemi-spherical drop is formed at a tip of a needle. Under an influence of a high enough electrostatic field, the Coulombic repulsion destabilizes the drop from the hemi-spherical

to a cone shape. A slightly higher electrostatic field causes a charged polymer jet to be ejected from the apex of the cone. If the condition maintains, continuous stream of charged jet thins down and simultaneously dries out to form electrospun fibrous webs on the collection screen. However, often times, during spinning, clogging of the spinning at the needle tip can occur if the solvent used is readily volatile. Such a problem can be easily solved by a reduction in the solution concentration or by mixing a harder-to-evaporate liquid to modify the volatility of the solvent.

2. In some cases, increasing applied voltage to a highly viscous solution can overcome the flowability properties of the solution, because increasing applied voltages leads to an increase in the electrostatic force on the hanging drop at the needle tip. Generally, applied voltages can be varied within the range of 5 to 30 kV. Beyond the applied voltages of 30 kV may cause undesirable ionization to the surrounding air. In addition, too high voltages may lead to irregularly-shaped as-spun fibers.

3. When the viscosity and the applied voltage were solved, the needles could be factor affecting the spinnability of the solution. It was found that smaller needles cause the solution to flow harder. If, however, bigger needles were used, undesirable drops may present on the collective screen. Depending on the polymer system investigated, a needle of appropriate size can be chosen, by trial-and-error basis.

4. When the charged drops were ejected, the droplets could result instead of the fibers. The reasons could be due to the low solution concentrations. Increased concentration of the spinning solution causes the molecules to be more entangled, which helps prevent the cohesive breakage of the ejected, charged jet. In addition, the solution should also exhibit reasonable conductivities. Too low or too high of the conductivity values lead to unsuccessful electrospinning.

4.2.2 Effects of Polymer Concentration on As-spun Fibers

The effects of polymer concentration and applied voltage on morphological appearance of the as-spun fibers were explained based on the results obtained for Eudragit EPO fibers. Figure 12 shows the effect of polymer concentration on

morphological appearance of the as-spun fibers. At 15% w/v, only discrete droplets with large size distribution were observed. Solutions with low concentrations did not have enough chain entanglement to withstand both of the electrostatic and Coulombic repulsion forces acting on an ejected, charged jet. Once the charged jet was broken up, surface tension resulted in the formation of the discrete droplets observed. It should be noted that the sickle-shaped droplets were formed as a result of the evaporation of the solvent within the droplets.

Increased the polymer concentration to 20% w/v resulted in the formation of beaded fibers. At 20% w/v, the chain entanglement was high enough to prevent the charged jet from breaking up into droplets. Further increase in the polymer concentration to 25 and 30% w/v resulted in smooth fibers, because of high enough chain entanglement to completely prevent the breaking up to the charged jet. Interestingly, increasing polymer concentration also resulted in larger as-fibers. The increase in the fiber diameters was a result of the increased viscoelastic force that counteracts the Coulombic repulsion force that tries to stretch the charged jet (which, in turn, causes the jet to thin down).

Instead of the round fibers that normally obtained in various other polymer systems, the as-spun Eudragit EPO fibers was flat. The formation of ribbon-like fibers could be a result of the rapid evaporation of the solvent (i.e. ethanol). Due to the small size of the ejected, charged jet, the outer surface of the jet could “dry” much faster than its inner core. Since the time during which the jet was on the flight to the target was very short, the inner core might not have enough time to “dry” completely. Once the jet deposited on the target, evaporation of the residual solvent continued. Since the outer surface of the depositing jets was already “dry,” the evaporation of the solvent from the inner core could result in the collapse of the jets, hence the flat fibers were formed.

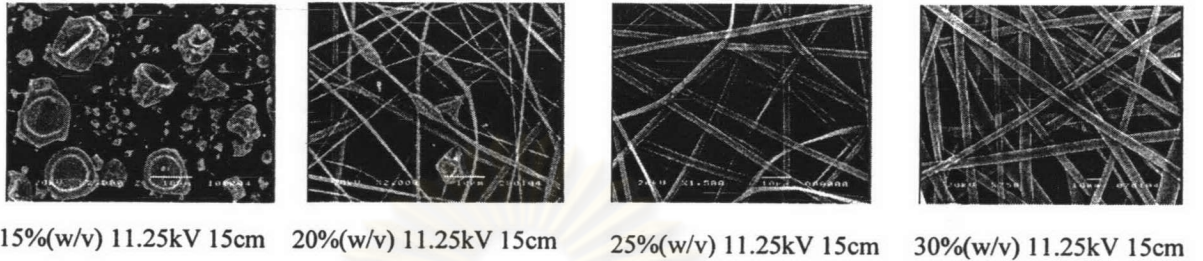


Figure 12 SEM images of Eudragit EPO at 11.25 kV 15 cm

4.2.3 Effect of Applied Voltage on As-spun Fibers

The effects of applied voltage on morphological appearance of the as-spun Eudragit EPO fibers are shown in Figures 13 and 14 for electrospun fibers from ethanol with concentrations of 25 and 30% w/v, respectively. The collection distance was fixed at 15 cm. Generally, the as-spun fibers were flat, irrespective to the applied voltage used. Obviously, the sizes of the as-spun fibers initially increased, reached a maximum values, and then decreased with increasing applied voltage. The initial increase in the fiber sizes could be a result of the increased mass throughput, while the observed decrease in the fibers sizes at high applied voltages might be a result of the increased surface charge density, causing the ejected, charged jet to split apart.

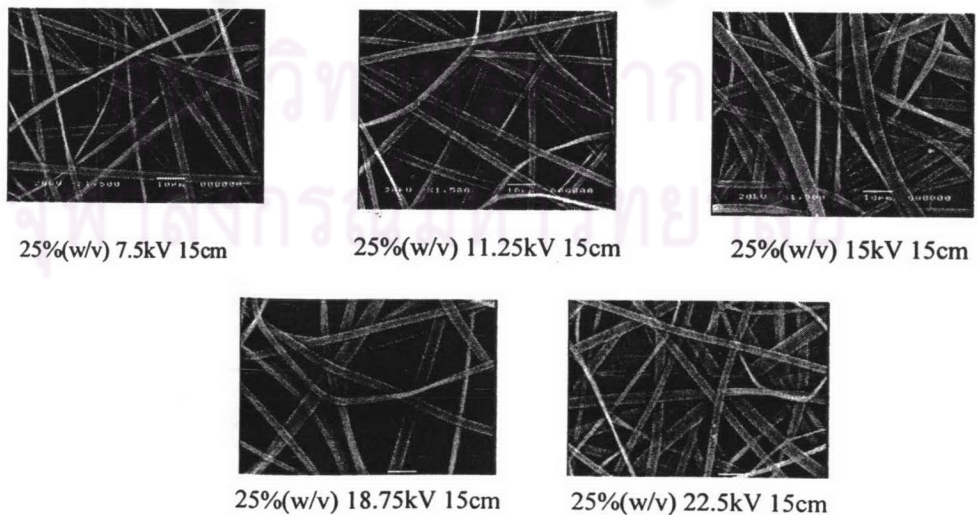


Figure 13 SEM images of Eudragit EPO at 25%w/v 15 cm

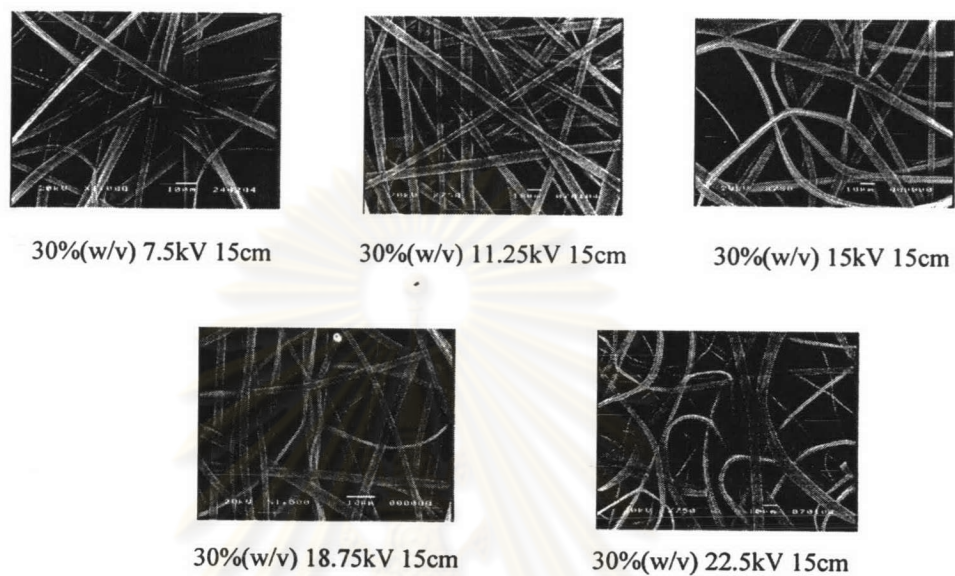


Figure 14 SEM images of Eudragit EPO at 30%w/v at 15 cm

Figures 15, 16, and 17 respectively, show SEM images of electrospun Eudragit EPO, Eudragit L100, and Eudragit RLPO fibers in ethanol while Figures 18, 19, and 20 show SEM images of Eudragit EPO/drug, Eudragit L100/drug, and Eudragit RLPO/drug in a mix solvent of ethanol and ethyl acetate (1:1) respectively.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

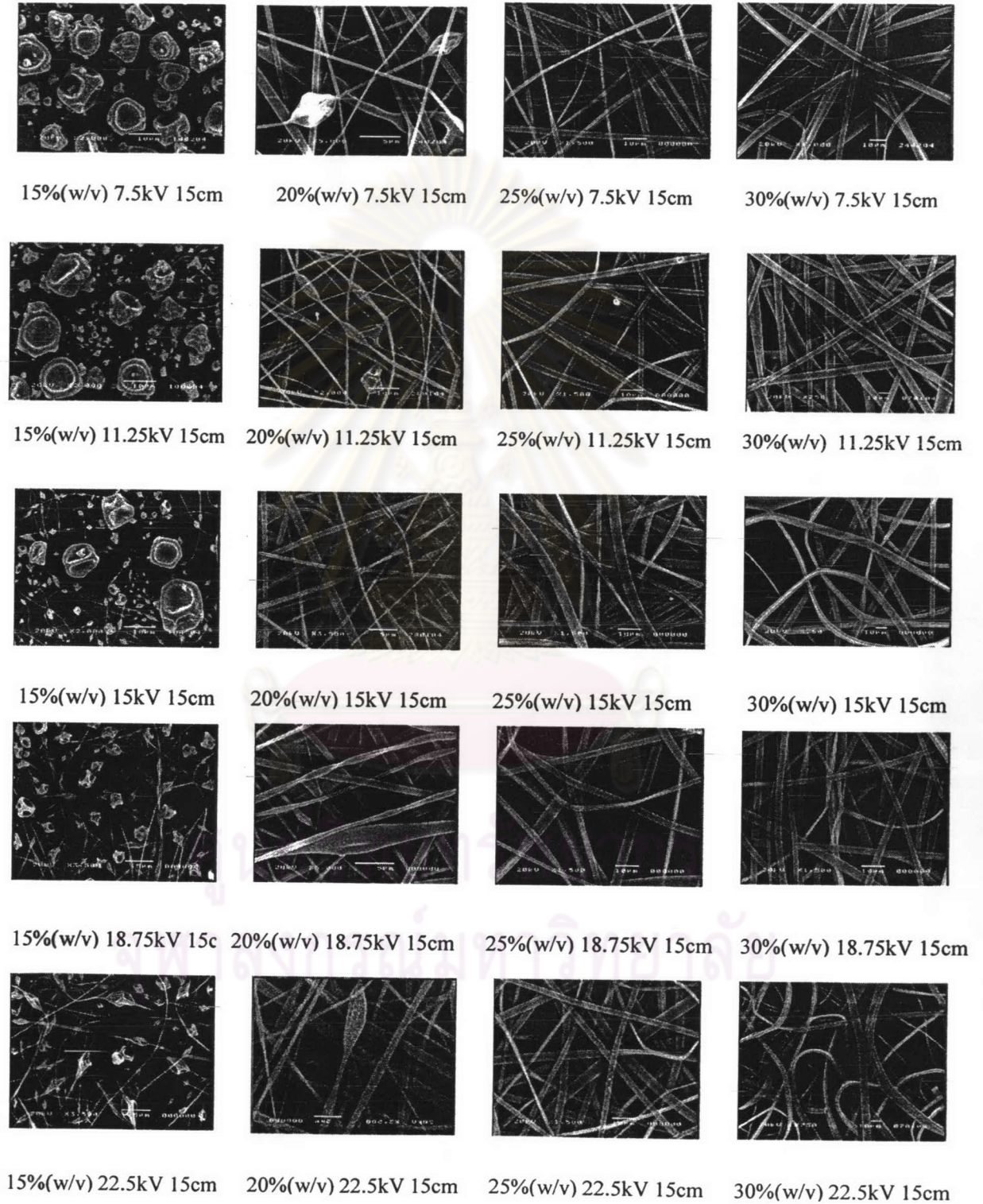


Figure 15 SEM images of electrospun Eudragit EPO fibers

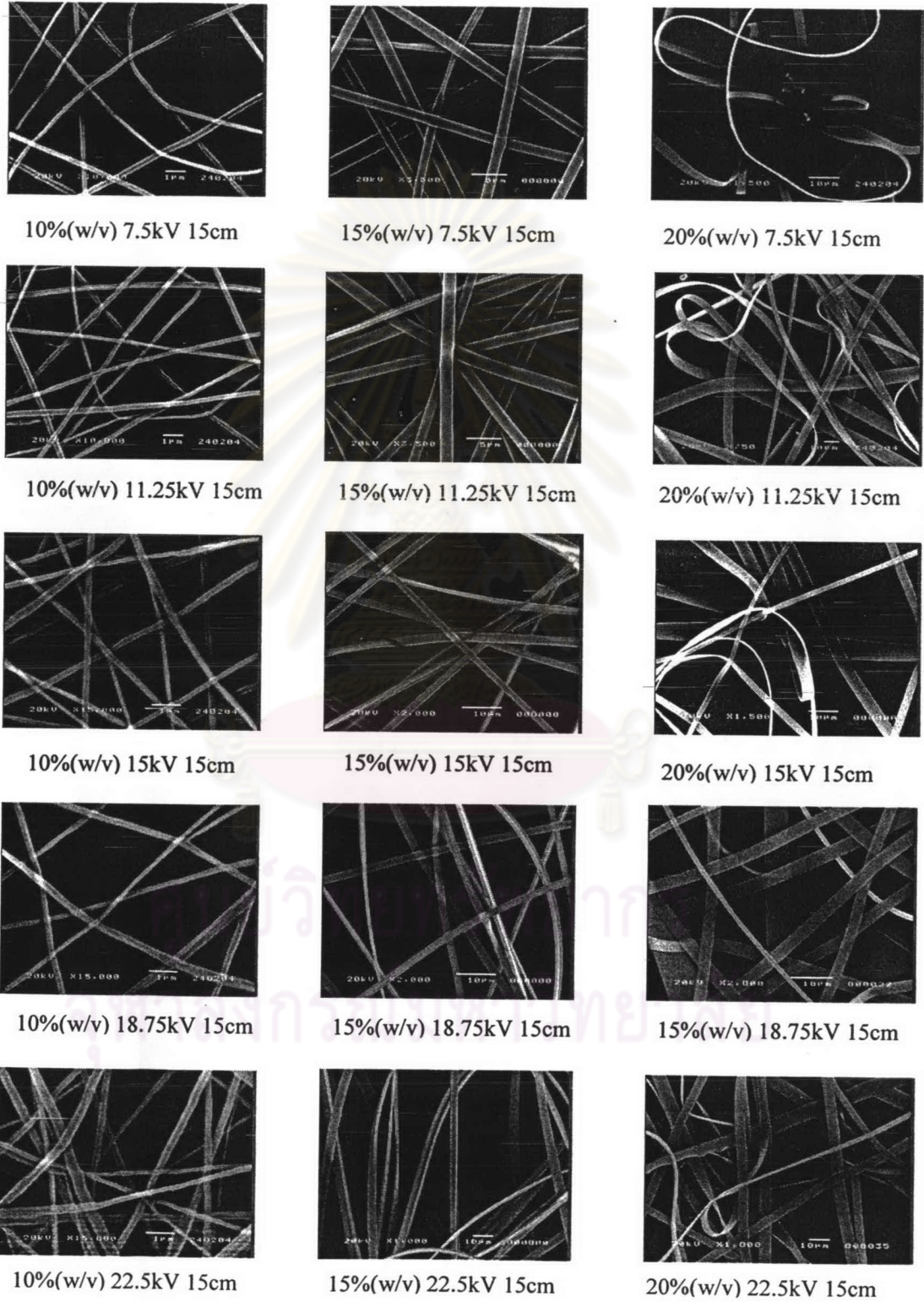


Figure 16 SEM images of electrospun Eudragit L100 fibers

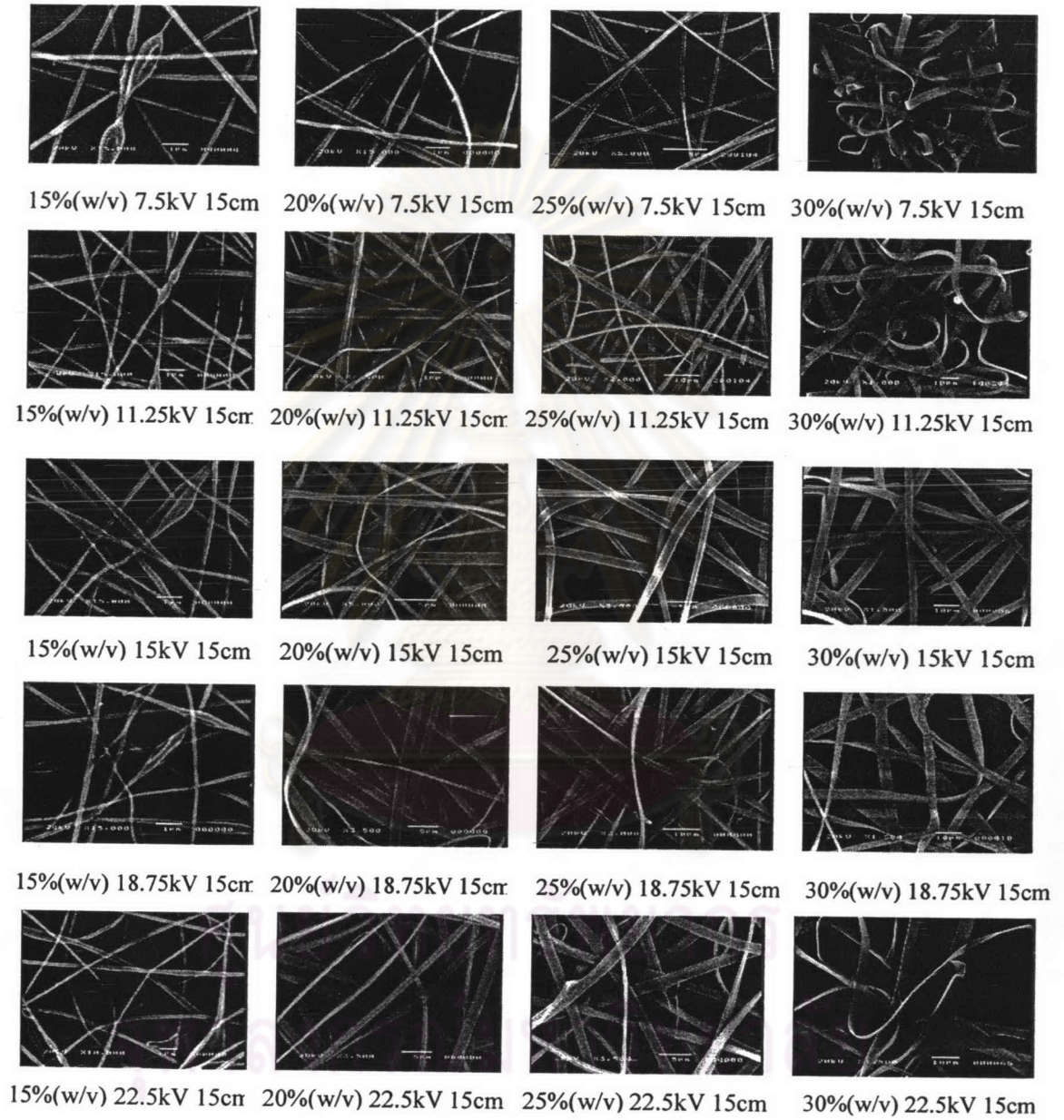


Figure 17 SEM images of electrospun Eudragit RLPO fibers

For Eudragit EPO, regardless of the applied potential used, only sickle beads and beaded fibers were observed when the polymer concentration was 15% (w/v). Obviously, the number of discrete sickle beads was found to decrease and beaded fibers with the beads being more elongated was more apparent with increasing applied potential used. Further increase in the polymer concentration to 10% (w/v), the number of beads was found to decrease and appeared more elongated. At the polymer concentrations of 25 and 30% (w/v), only smooth fibers were obtained. With regards to the sizes of the as-spun fibers, the fiber diameters were found to generally increase with increasing polymer concentration and applied potential. However, at 30% (w/v), it appears that fibers of bimodal distribution were obtained, which may be a result of the high viscosity of the solution at this concentration. Interestingly, the morphology of the obtained fibers appeared to be flat. At 35% (w/v), the viscosity is too high, then the polymer solution could not eject from the needle to form the fibers.

For Eudragit L100, only the solutions with the polymer concentration in the range of 10 to 20% (w/v) were spinnable. At higher concentrations, the solutions became too viscous to be spinnable. Obviously, only smooth fibers were observed. Generally, the fiber diameters were found to increase with increasing concentration of the solutions as well as with increasing applied potential. Similar to Eudragit EPO fibers, the as-spun Eudragit L100 fibers appeared to be flat.

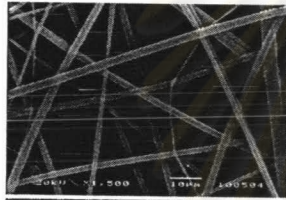
For Eudragit RLPO, though not shown, the solution containing 10% (w/v) of the polymer gave only discrete beads after electrospinning. At 15% (w/v), beaded fibers were formed with the beads appeared to be more elongated with increasing applied potential. With further increase in the polymer concentration, only smooth fibers were obtained. It should be noted that the 30% (w/v) Eudragit RLPO, though appeared to be spinnable, had some problems during spinning, due to the rapid evaporation of the solution at the tip of the needle. With regards to the fiber diameters, they were found to increase with increasing polymer concentration and applied potentials. Similarly, the fibers obtained appeared to be flat. At 35% (w/v), the polymer solution could not spin to form the fibers since this concentration has too high viscosity.

4.3 Drug-loaded As-spun Fibers

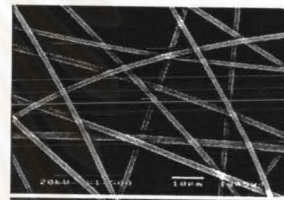
4.3.1 The Morphology of Drug-loaded As-spun Fibers



30%(w/v) EPO 7.5 kV 15 cm



25%(w/v) EPO 11.25 kV 15 cm



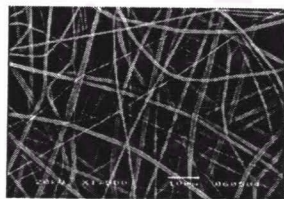
30%(w/v) EPO 11.25 kV 15 cm



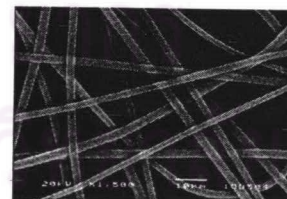
25%(w/v) EPO 15 kV 15 cm



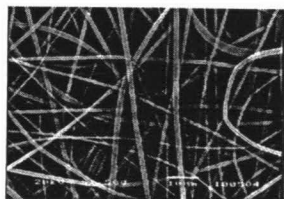
30%(w/v) EPO 15 kV 15 cm



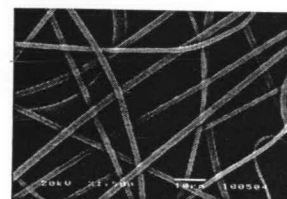
25%(w/v) EPO 18.75 kV 15 cm



30%(w/v) EPO 18.75 kV 15 cm



25%(w/v) EPO 22.5 kV 15 cm



30%(w/v) EPO 22.5 kV 15 cm

Figure 18 SEM images of drug-loaded electrospun Eudragit EPO fibers. The fibers were prepared from solutions of Eudragit EPO/drug in a mixed solvent system of EtOH and EA (1:1).

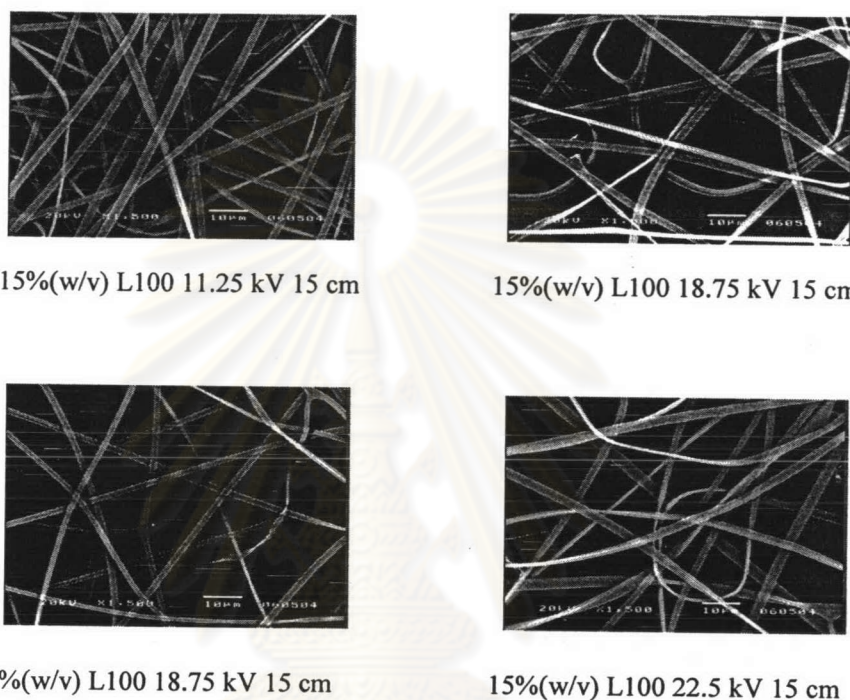


Figure 19 SEM images of drug-loaded electrospun Eudragit L100 fibers. The fibers were prepared from solutions of Eudragit L100/drug in a mixed solvent system of EtOH and EA (1:1).

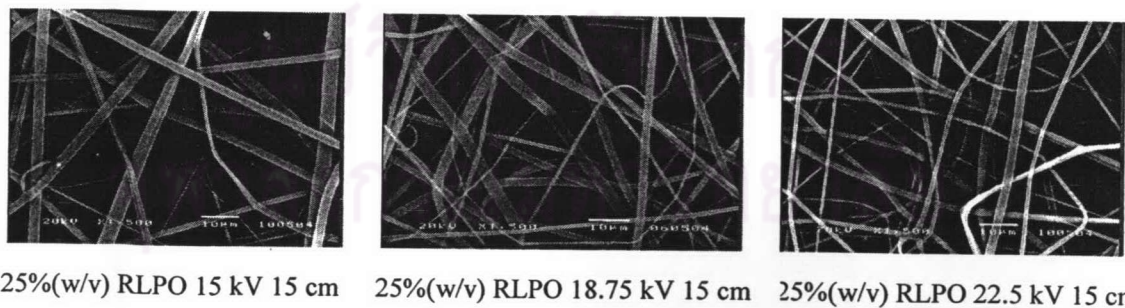


Figure 20 SEM images of drug-loaded electrospun Eudragit RLPO fibers. The fibers were prepared from solutions of Eudragit RLPO/drug in a mixed solvent system of EtOH and EA (1:1).

Figures 18, 19, and 20 show drug-loaded as-spun Eudragit EPO, Eudragit L100, and Eudragit RLPO fibers. It should be noted that the spinning of these drug-loaded fibers could only be achieved when EtOH and EA in the equivolumetric ratio were used as the solvent system. EA was added to the solution in order to deter the clogging at the tip of the nozzle through a reduction of the evaporation rate. Interestingly, only drug-loaded 25 and 30% (w/v) solutions of Eudragit EPO, 15% (w/v) solution of Eudragit L100, and 25% (w/v) solution of Eudragit RLPO were electrospinnable. The morphological appearance of the obtained fibers appeared to be rather flat.

To measure in quantitative of the fiber sizes, the fiber sizes are reported as the average values with the standard deviation in Figures 17, 18, and 19 for as-spun Eudragit EPO, Eudragit L100, and Eudragit RLPO fibers, respectively, while the raw data are summarized in Table B1. The effects of solution and spinning conditions on the obtained fiber sizes were investigated. Table 5 summarizes the average diameter of the selected as-spun fibers with drug. It should be noted, again, that EtOH and EA(1:1) were used as the mixed solvent for fibers with drug loaded.

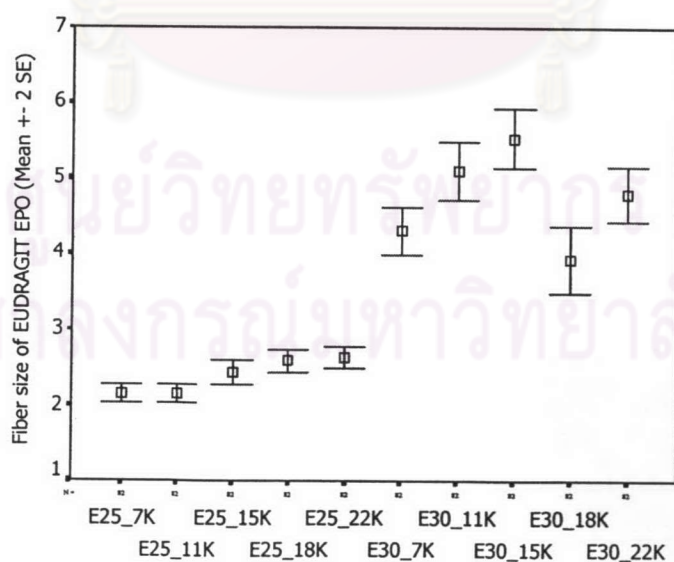


Figure 17 Average diameter of as-spun fibers from 25 and 30% (w/v) Eudragit EPO solutions in EtOH as a function of applied potential.

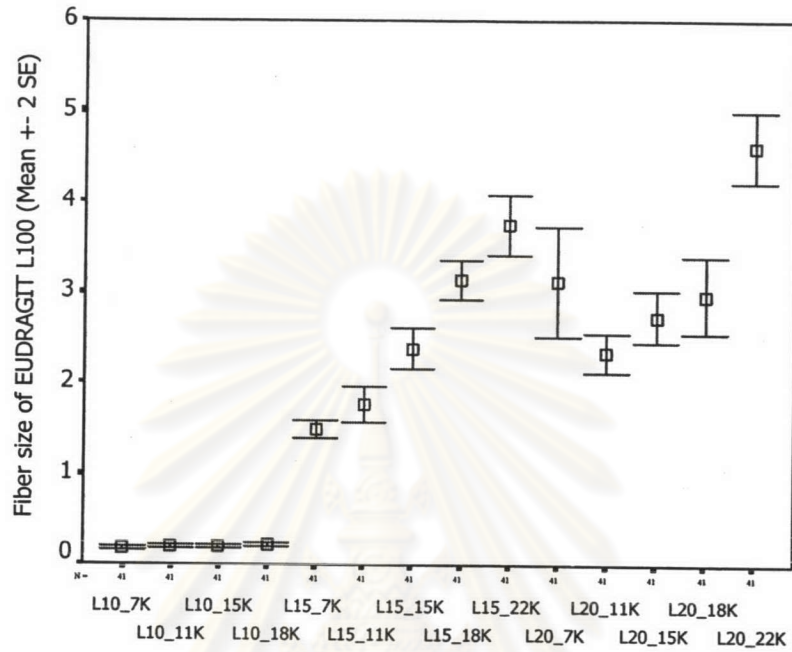


Figure 21 Average diameter of as-spun fibers from 10, 15, and 20% (w/v) Eudragit L100 solutions in EtOH as a function of applied potential.

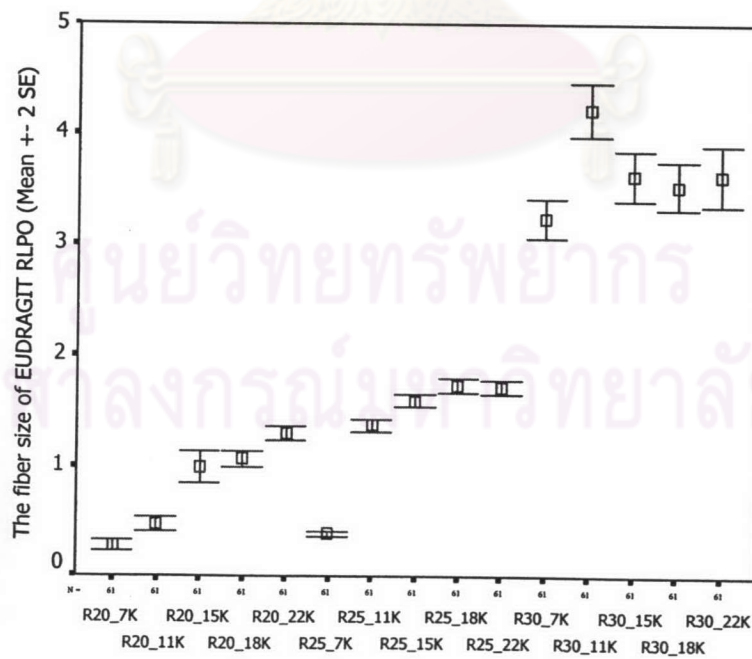


Figure 22 Average diameter of as-spun fibers from 20, 25, and 30% (w/v) Eudragit RLPO solutions in EtOH as a function of applied potential.

From Figures 17 to 19, the horizontal axis is the spinning conditions while the vertical axis is the average fiber sizes obtained in each condition. The abbreviations were used in the x-axis, the first letters are from the polymer type while the next digit are the concentrations of the polymer solution, and the last digits with K are the applied voltages used in each condition. According to these quantitative results shown and the data listed in Table B1, the average fiber diameters were, in general, found to increase with increasing applied potential and polymer concentration. The increase in the applied potential resulted in an increase in the electrostatic force acting on a jet segment. The increase in the electrostatic force could be responsible in an increase in the mass throughput (provided that a viscoelastic force was large enough to counter the electrostatic force), and the increase in the mass throughput was, in turn, responsible for the observed increase in the fiber diameters.

On another account, the increase in the polymer concentration resulted in an increase in the viscosity of the solution, which, in turn, was responsible for an increase in the viscoelastic force. The increase in the viscoelastic force deterred the deformation of a jet segment, which was caused by the Coulombic repulsion force acting in the jet segment.

In some occasion however, instead of observing an increase in the fiber diameter with increasing applied potential, an otherwise observation may be observed. An example is the as-spun fibers from 30% (w/v) Eudragit EPO solution in EtOH (see Figure 17). In the example shown, an abrupt decrease in the average fiber diameter was observed between the applied potential of 15 and 18 kV. The probable reason may be due to the multimodality of the data obtained at such high potential differences applied. At high applied potentials, residual charges within a thinning jet may be too large that the jet may split into smaller jets in order to reduce the surface charge density.

Table 4. Average fiber diameters of drug-loaded as-spun Eudragit EPO, Eudragit L100, and Eudragit RLPO fibers at selected conditions for comparing controlled-release of drug.

Conditions	Fiber size in μm (Mean)	Standard Deviation
EPO 30% at 11.25 kV (with drug)	1.700	0.443
L100 15% at 11.25 kV (with drug)	1.717	0.457
L100 15% at 22.5 kV (with drug)	2.208	0.361
RLPO 25% at 18.75 kV (with drug)	1.699	0.595

4.3.2 Comparison of As-spun Fiber Sizes

According to the results shown in Table 4, the drug-loaded as-spun fibers from 30% (w/v) Eudragit EPO in EtOH and EA at 11.25 kV, 15% (w/v) Eudragit L100 in EtOH and EA at 11.25 kV, and 25% (w/v) Eudragit RLPO in EtOH and EA at 18.75 solutions exhibited similar average fiber diameter values of about 1.7 μm . Drug release kinetics was then carried out on these fibers to compare the effect of copolymer type and size on drug releasing characteristic. Figure 20 shows drug release profiles from as-spun fibers from the aforementioned solutions and spinning conditions. Drug release profiles from films being cast from the same solutions were also included for comparison. By the way, the assay of drug is one of the important topic since the amount of drug loaded before processing (10%wt of copolymer) may be different from after processing. Then, the assay could show the amount of drug in the samples after processing.

Table 5. The assay of drug-loaded samples in as-spun fibers and cast films

As-spun Fibers			Cast Film		
EPO	RLPO	L100	EPO	RLPO	L100
54.5 %	105.0 %	21.3 % (small)	55.2 %	102.0 %	90.6 %
		22.4 % (big)			

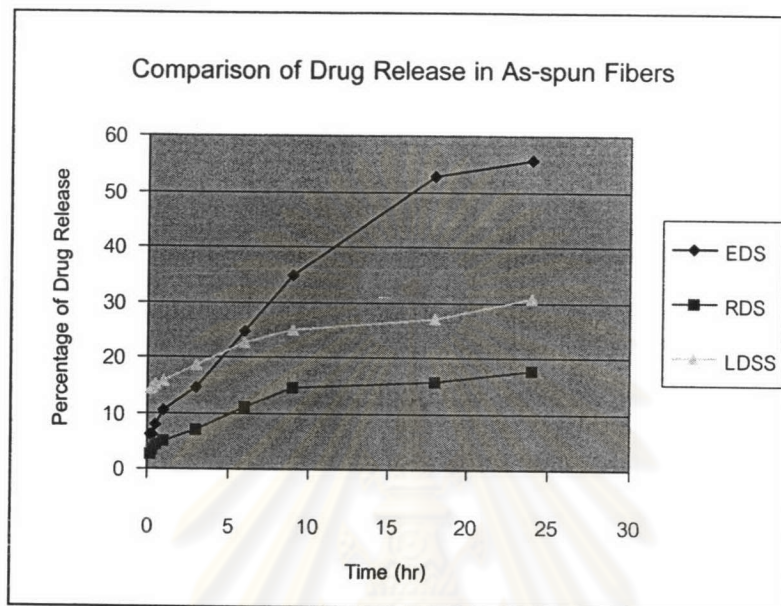


Figure 20 Drug release profiles of as-spun fibers of Eudragit EPO, Eudragit L100, and Eudragit RLPO.

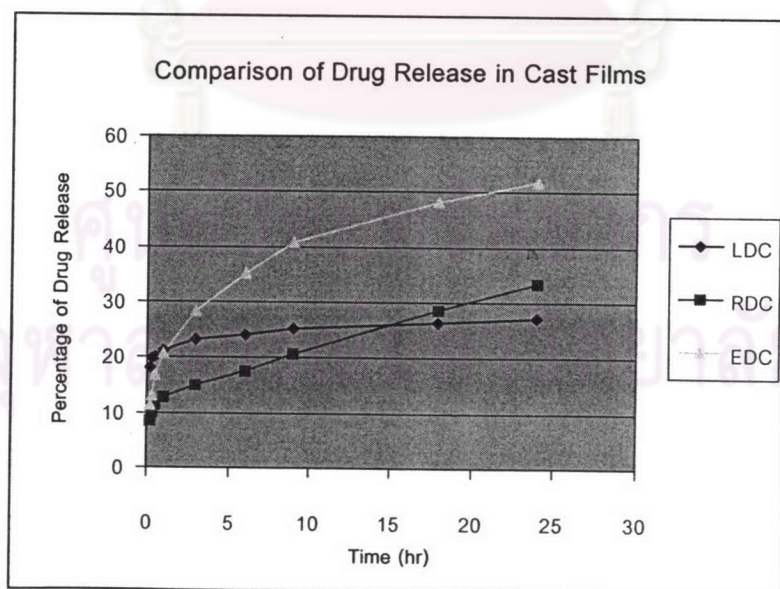


Figure 21 Drug release profiles of cast films of Eudragit EPO, Eudragit L100, and Eudragit RLPO.

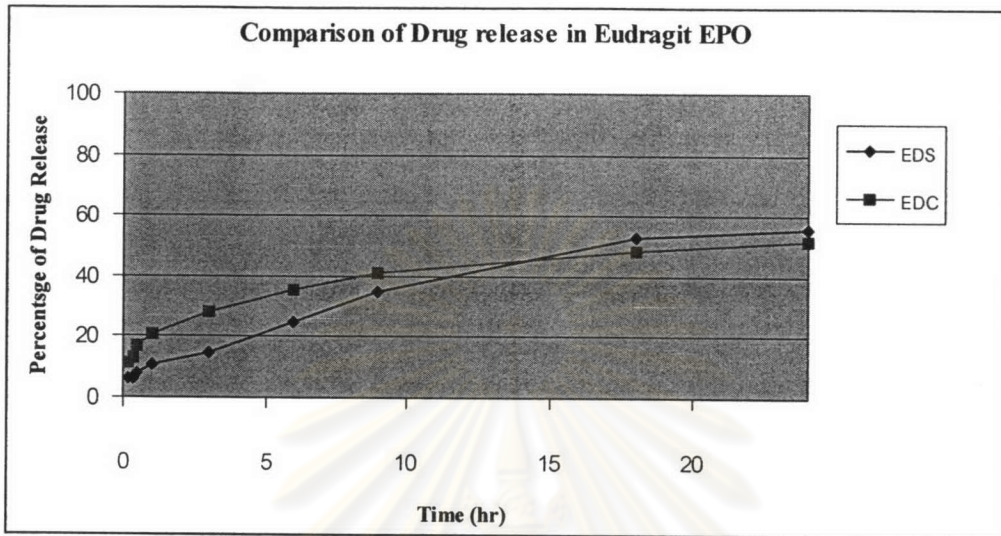


Figure 22 Drug release profile of as-spun fibers and cast films of Eudragit EPO

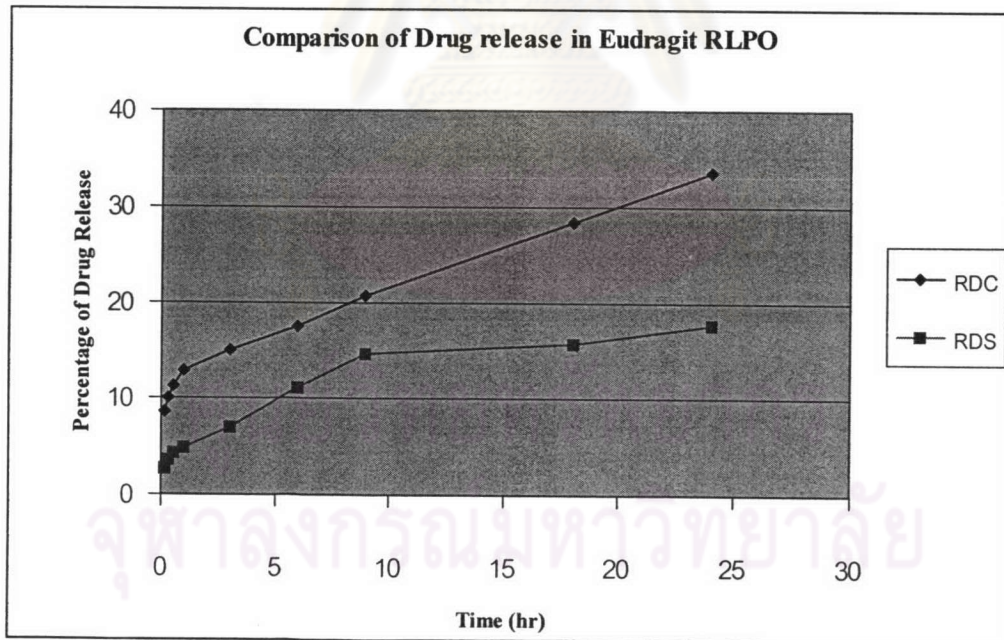


Figure 23 Drug release profile of as-spun fibers and cast films of Eudragit RLPO

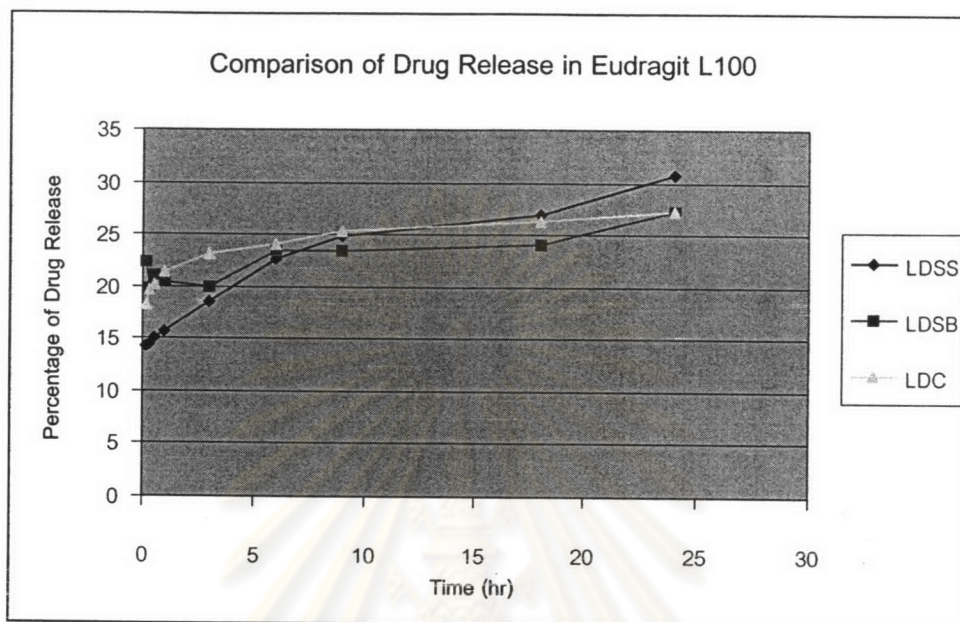


Figure 24 Drug Release profile of as-spun fibers and cast films in Eudragit L100

The drug releasing profiles were separated into two types of processing including electrospinning process to form electrospun fibers and cast process to form cast films. Three types of methacrylate-based copolymer were compared in drug releasing profile between the two processes by using UV-Vis spectroscopy. The concentration of the drug in buffer was detected at the different time. The same weights of drug and polymer were used in every sample to ensure the accuracy of comparison.

Firstly, it was seen that EPO electrospun released the drug in the first 10 hours less than in EPO cast film, but after that the electrospun released the drug more than in the cast film which of the same composition. The overall drug released in EPO electrospun samples are higher than in the EPO cast film.

Secondly, RLPO cast film started to release the drug at the higher level than RLPO electrospun and then released steadily with the time increase. On the other hand, RLPO electrospun steadily released in the first 9 hours and leveled off. It was seen that RLPO cast film released the drug in all over the time more than RLPO electrospun.

Lastly, L100 electrospun fibers released the drug at starting point less than in the cast films, but overall drug release in as-spun fibers were higher than in cast film

samples. Moreover, the profiles of drug releasing are shown that L100 can be released continuously until 24 hr while in L100 cast films samples tended to slow down in drug release characteristics.

However, from the result it can be concluded that the type of polymer affected on the drug released profiles. For example, from the profiles EPO can be used for drug controlled release, since it can be released the drug in long time and released the drug more than in the cast film which has all the same condition. L100 samples released the drug rapidly, then it can be used with the immediately acting. If these two types of polymer are incorporated use, it may give immediate and sustainability drug delivery material.



ศูนย์วิจัยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย