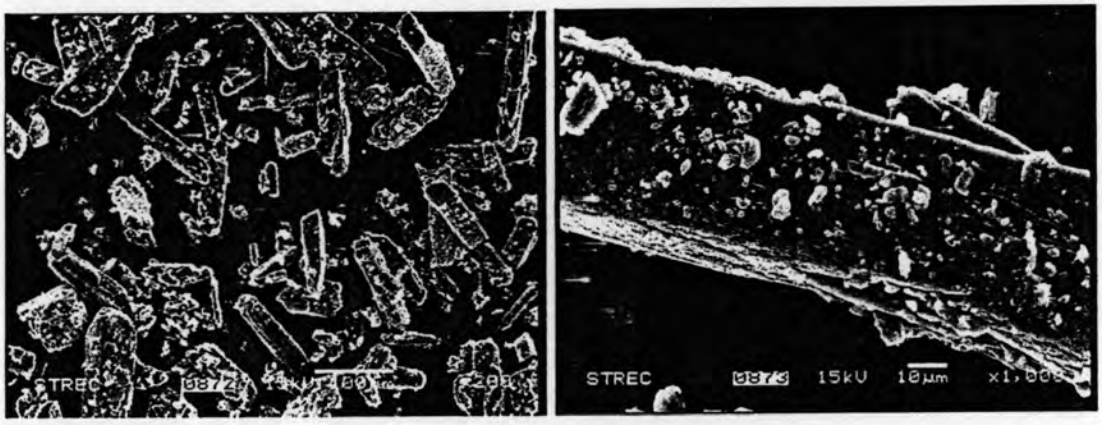
CHAPTER IV
RESULT AND DISCUSSION

1. Material characterization

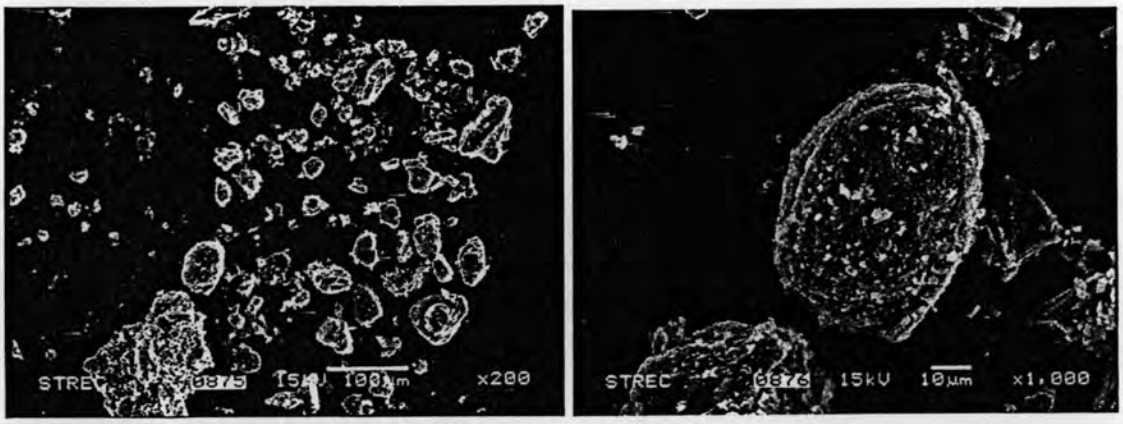
1.1 Morphology

The shape and surface topography of propranolol hydrochloride, diclofenac sodium and microcrystalline cellulose were examined by scanning electron microscope (SEM).The morphology of theirs were presented in Figures 28 to 30.



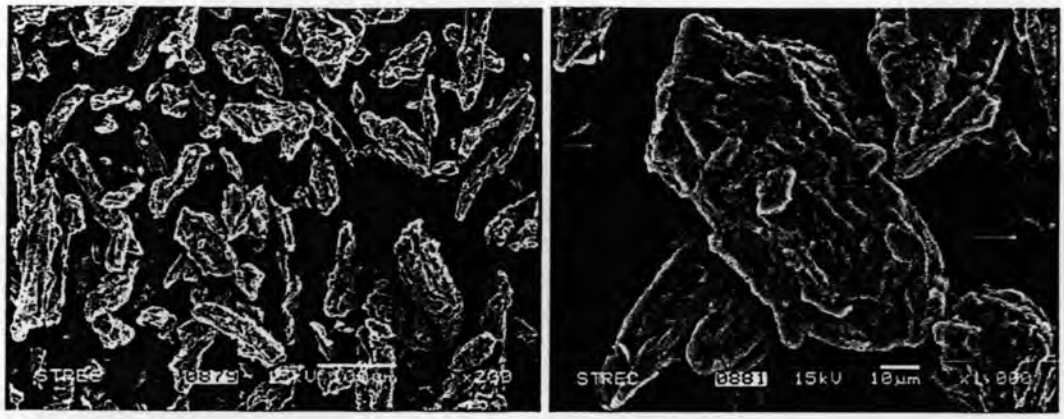
(a) (b)

Figure 28 Scanning electron photomicrograph of propranolol hydrochloride in magnification of (a) x200 and (b) x1,000.



(a) (b)

Figure 29 Scanning electron photomicrograph of diclofenac sodium in magnification of x200 and (b) x1,000.



(a) (b)

Figure 30 Scanning electron photomicrograph of microcrystalline cellulose in magnification of x200 and (b) x1,000.

Propranolol hydrochloride showed rod shape crystalline particles while diclofenac sodium and microcrystalline cellulose showed irregular shape. Diclofenac sodium was relative rounder shape than others. Moreover, particle size of diclofenac sodium compared with others was relatively small.

1.2 Particle size and size distribution

The particle size and size distribution of raw materials were determined by laser light diffraction technique and the results are shown in Table 12

Table 12 The particle size and size distribution of raw materials [mean (SD)].

Physical properties	Propranolol hydrochloride	Diclofenac sodium	Microcrystalline cellulose
d (v,0.1), μm	12.33 (0.35)	0.11 (0.00)	27.46 (0.39)
d (v,0.5), μm	67.54 (2.03)	22.51 (0.37)	91.43 (0.67)
d (v,0.9), μm	197.34 (7.42)	62.31 (0.74)	193.89 (1.41)
D[4,3], μm	88.71 (2.84)	26.43 (0.17)	102.26 (0.43)
Span	2.74 (0.03)	2.76 (0.07)	1.83 (0.00)
Uniformity	0.85 (0.01)	0.94 (0.03)	0.56 (0.01)

Note: - d (v,0.5) is the size at which 50% of the sample is smaller and 50% is larger (mass median diameter).

- d (v,0.1) and d (v,0.9) are the size of particle below which 10% and 90% of the sample lies respectively.
- D[4,3] is the volume mean diameter.
- The span is the measurement of the width of the distribution, defined as the differences between the diameter at the 90 and the 10 percentage points relative to the median diameter.
- The uniformity is a measure of the absolute deviation from the median.

From Table 11, it was found that microcrystalline cellulose was of narrower size distribution and more uniform than propranolol hydrochloride and diclofenac sodium. In addition, diclofenac sodium was smaller size than propranolol hydrochloride and microcrystalline cellulose. This result agreed with the results obtained from scanning electron microscope.

1.3 Angle of repose, bulk, tapped densities, percent compressibility and percent cohesiveness

Table 13 presents physical properties of raw materials measured by powder characteristic tester. Flowability of raw materials was poor and both drugs had high percent of cohesiveness than microcrystalline cellulose. Lower bulk and tapped densities of propranolol hydrochloride and microcrystalline cellulose were mainly due to larger size and irregular shaped particles (Sinko, 2006).

Table 13 Angle of repose, bulk, tapped densities, percent compressibility, percent cohesiveness and apparent density of raw materials [mean(SD)].

Physical properties	Propranolol hydrochloride	Diclofenac sodium	Microcrystalline cellulose
Angle of repose (deg)	60.23 (1.01)	54.70 (0.50)	42.37 (1.18)
Bulk density (g/cm^3)	0.38 (0.00)	0.45 (0.00)	0.30 (0.00)
Tapped density (g/cm^3)	0.61 (0.00)	0.80 (0.01)	0.43 (0.00)
% Compressibility	38.4 (0.85)	43.5 (0.40)	30.9 (0.57)
% Cohesiveness	53.13 (0.96)	38.40 (0.62)	2.53 (1.10)
Apparent density (g/cm^3)	1.20 (0.00)	1.48 (0.00)	1.47 (0.00)

1.4 Apparent density

The apparent density of raw materials was determined by using helium gas displacement. Because helium gas could penetrate into the smallest pores or crevices and was not adsorbed by the material, it was generally conceded that the helium method gave the closest approximation to true density. As shown in the Table 13, propranolol hydrochloride had lower apparent density than diclofenac sodium and microcrystalline cellulose.

2. Pelletization

2.1 Preparation of pellets

Core pellets were prepared by extrusion-spheronization process. Extrusion-spheronization was a multiple-step compaction process comprising dry mixing of the drug with microcrystalline cellulose, wet granulation of the mass using deionized water, which converted the powder into a plastic mass that could be easily extruded, extrusion of the wetted mass, charging the extrudates into the spheroniser to produce a spherical shape, drying the wet pellets in a dryer and finally, screening to achieve the required size distribution. (Erkoboni, 1997; Gazzaniga et al., 1998; Thoma and Ziegler, 1998; Schmidt and Kleinebudde, 1999).

The degree of liquid saturation of the granulation is one of the most critical factors in the formulation. Water was used as binding liquid in order to form a suitable dense and cohesive mass for extrusion. Moreover, the liquid content must be high enough to bring about the optimum surface plasticity required for spheronization (Swarbick and Boylan, 1998). If the moisture content is less than the lower limit, a lot of dust will be formed during spheronization resulting in a large yield of fines. Exceeding the range of the moisture content leads to an overwetted mass and agglomeration of the individual pellets during spheronization due to the excess of water at the surface of the pellets (Harrison et al., 1987).

In this study, extrudate was prepared by extruding wet mass containing various amounts of water. The amounts of water used for propranolol hydrochloride (PL) were 180 g and 195 g per 300 g of powder mixture (water:MCC ratio =1:1.2 and 1:1.3), while diclofenac sodium (DS) was 195 g, 210 g and 225 g per 300 g of powder

mixture (water:MCC ratio = 1:1.3, 1:1.4 and 1:1.5). The yield of pellets in the size range of 850 to 1180 μm obtained by adding water 180 g per 300 g (water:MCC ratio = 1:1.2) of PL mixture and 225 g per 300 g (water:MCC ratio = 1:1.5) of DS mixture was much higher than others as shown in Figure 31 and 32; and was used as core pellets in this study.

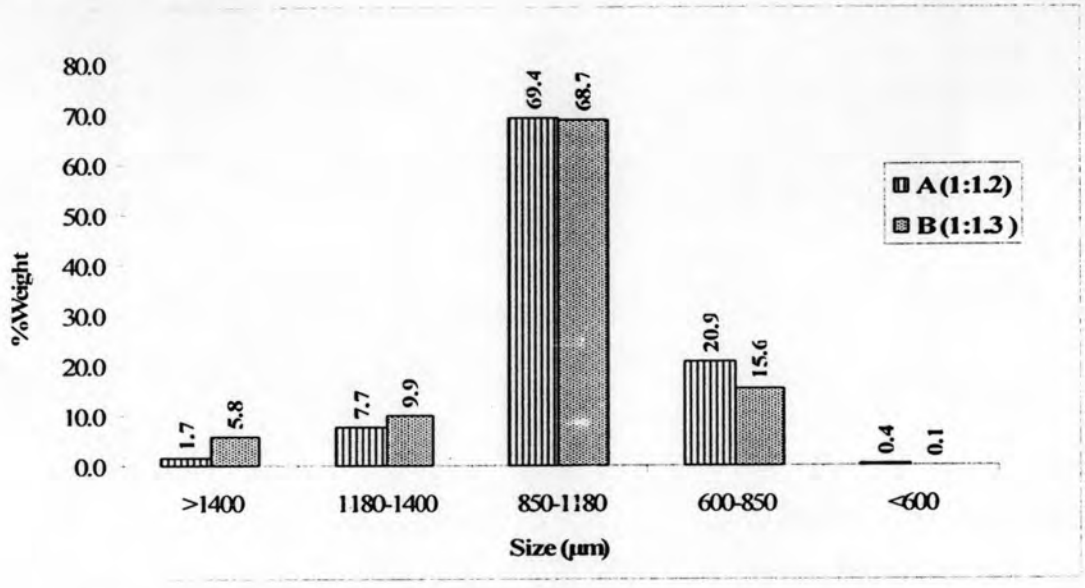


Figure 31 Percent yield of PL pellets obtained from various amount of water.

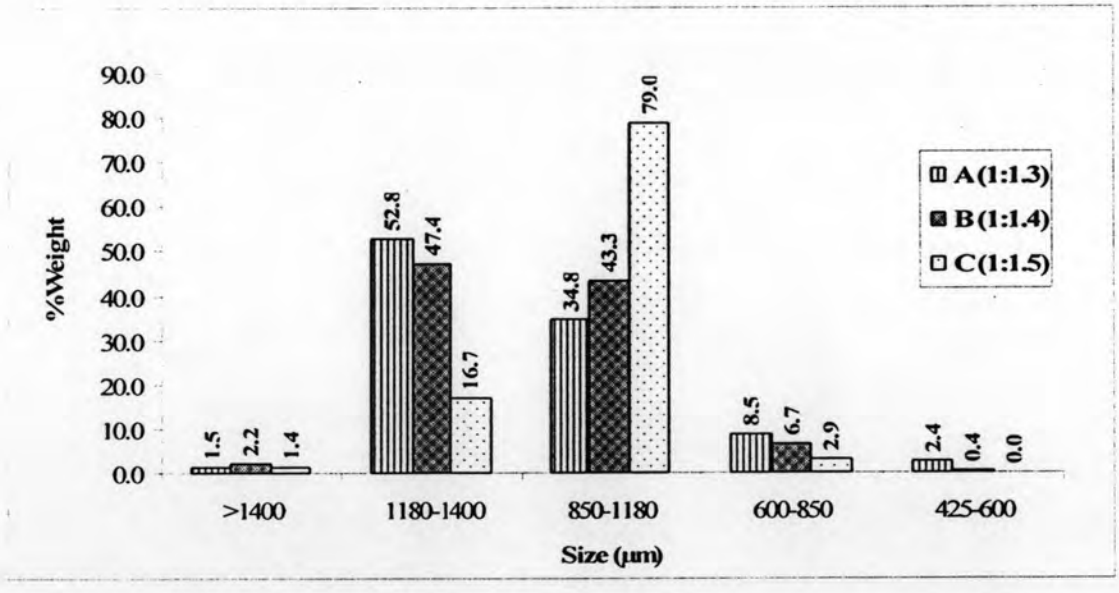


Figure 32 Percent yield of DS pellets obtained from various amount of water.

Water to microcrystalline cellulose ratio had influence on pellet size and friability (Vertommen and Kinget, 1997). Moreover, the solubility of the drug in the

granulation liquid has a dramatic influence on the amount of granulation liquid needed to obtain the proper plasticity. A soluble drug will dissolve in the granulation liquid increasing the volume of the liquid phase in contrast with a formulation containing a non-soluble drug (Baert et al., 1991). Due to higher solubility of propranolol HCl than diclofenac sodium in water, so it needed less amount of water to prepare suitable pellets.

2.2 Characterization of core pellets

The pellets which passed through a 16 mesh (1,180 μm) sieve and retained on a 20 mesh (850 μm) sieve were further characterized.

2.2.1 Morphology

The morphology, size, shape and surface topography of propranolol hydrochloride and diclofenac sodium pellets (PL and DS pellets) were observed using scanning electron microscopy at different magnifications (x30, x65, x100 and x1,000) as shown in Figures 33 and 34. For both drugs, the core pellets had almost spherical shape and rough surface filled with pores. The cross section of pellets illustrated that PL pellets were occupied with cavities and less dense than DS pellets.

The physical properties of starting material such as particle size, density and flowability have a profound influence on the extrusion characteristics of wet mass and resulting pellets (Fielden et al., 1989; O'Connor and Schwartz, 1985). In addition the different mechanism of spheronization process was affected on the morphology of the pellets; the mechanism proposed by Baert et al. (1993) hollowed pellets could be formed.

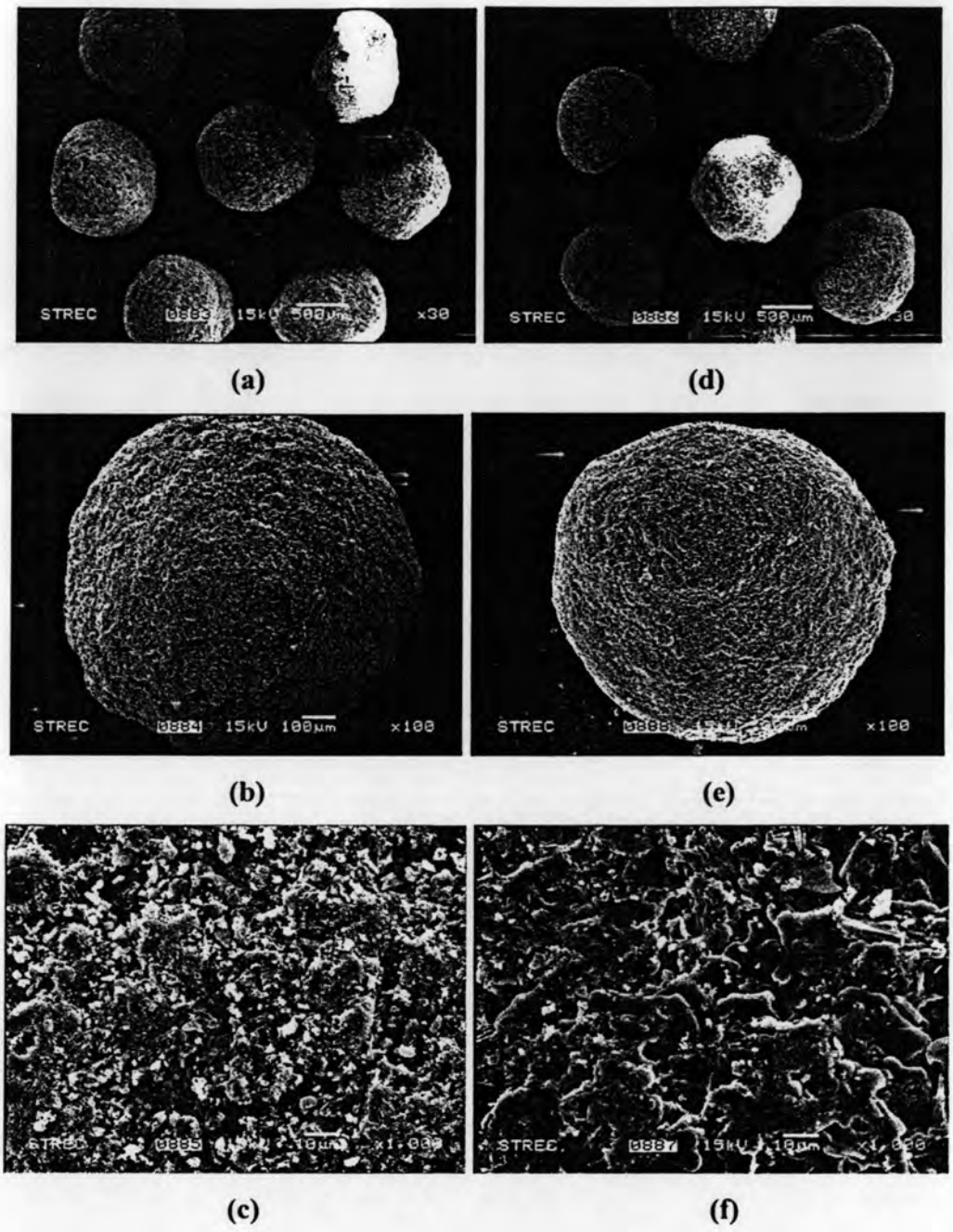
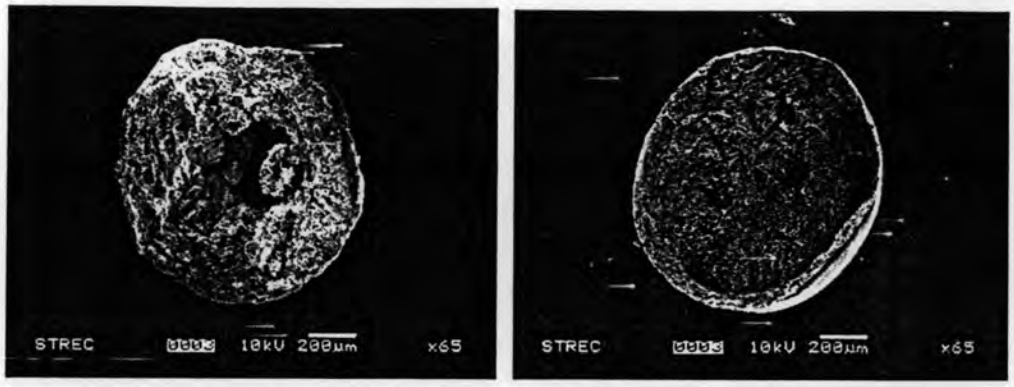


Figure 33 (a) (b) and (c) Scanning electron micrograph of propranolol hydrochloride pellets in magnification of x30, x100 and x1,000 respectively

(d) (e) and (f) Scanning electron micrograph of diclofenac sodium pellets in magnification of x30, x100 and x1,000 respectively



(a) (b)

Figure 34 (a) Scanning electron micrograph of propranolol hydrochloride pellets in magnification of x65, cross section

(b) Scanning electron micrograph of diclofenac sodium pellets in magnification of x65, cross section

2.2.2 Particle size and size distribution

Sieve analysis method was used to evaluate size distribution of the core pellets. The percent of weight retained on each sieve was calculated and plotted against sieve size as shown in Figure 35. The pellets produced were of narrow size distribution; about 70 to 80 % of pellets were within the range of 850-1180 µm. This highest fraction of pellets was characterized and used as core pellets in coating process.

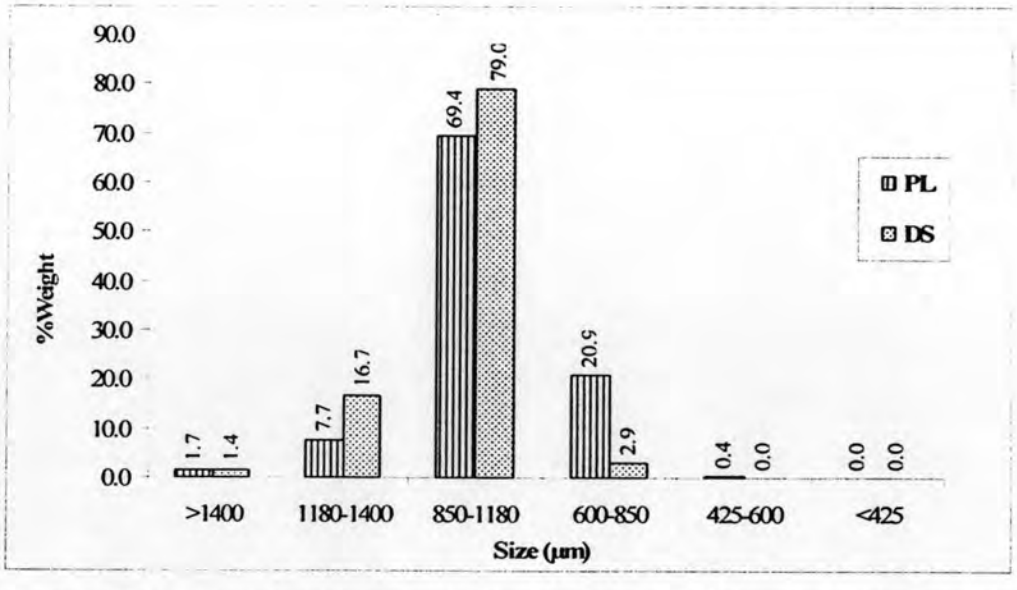


Figure 35 Size distribution of propranolol hydrochloride (PL) and diclofenac sodium (DS) pellets.

2.2.3 Moisture content

Moisture content of pellets was presented in terms of % loss on drying (%LOD) as shown in Table 14. DS pellets had higher % LOD than PL pellets, due to higher amount of water used in extrusion-spheronization process when drying time kept constant (Charoenkitpaiboon, 2003).

2.2.4 Bulk, tapped densities and compressibility index

Bulk and tapped densities were determined and evaluated for compressibility index. Bulk density is used to determine the space required for the storage bulk pellets, while, tapped density is used to investigate the packing property of the pellets. From the data shown in Table 14, it was found that the value of bulk density, tapped density and compressibility index of PL pellets were slightly lower than DS pellets. The small difference of bulk and tapped density, resulting in low compressibility index value (<10%), indicating free flowing behavior and good packing of pellets (Chopra et al., 2001). This result may be due to its spherical shape of the pellets.

Table 14 Physical properties of core pellets [mean (SD)]

Physical properties	**PL pellets**	**DS pellets**
Moisture content (%LOD)	0.43 (0.04)	0.76 (0.04)
Bulk density (g/cm^3)	0.67 (0.00)	0.74 (0.00)
Tapped density (g/cm^3)	0.69 (0.00)	0.77 (0.00)
%Compressibility	3.33 (0.00)	3.71(0.01)
Apparent density (g/cm^3)	1.33 (0.00)	1.51 (0.00)
Friability (%)	0.01 (0.01)	0.01 (0.00)
Crushing force (g)	889.67 (240.050)	948.93 (192.179)
Flow rate (g/s)	11.71 (0.08)	13.33 (0.16)
Angle of repose (deg)	17.81 (0.20)	16.92 (0.20)
Mean diameter (μm)	1057.97 (60.32)	1018.48 (65.45)
Aspect ratio	1.10 (0.05)	1.09(0.05)
Roundness	1.15 (0.05)	1.13 (0.04)

2.2.5 Apparent pellet density

From Table 14, apparent density of DS pellets was higher than PL pellets, corresponding to that of the drugs. It was possible that spheronization process densified DS pellets and PL pellets in different way (Rowe, 1985; Baert et al., 1993). The result agreed with the cross section photomicrographs of pellets in which PL pellets were hollowed and higher porous than DS pellets. Thus, it was possible that the properties of starting material and/or the pelletization process had influence on the density of final product.

2.2.6 Crushing force and Percent friability

Pellets strength or hardness can be correlated with the friability or can be measured directly by measuring the force required to break a pellet (Reynolds, 1970; Bataille et al., 1993). The crushing force needed to break the PL and DS pellets was 889.67 ± 240.05 g and 948.93 ± 192.18 g, respectively (Table 14). Higher crushing force of DS pellets solidly construct of the pellets.

The essential requirement of pellets is to have an acceptable friability to withstand further processing, especially the subsequent coating. A high amount of attrition during the coating procedure could modify the release behavior due to the incorporation of small particles in the film (Ghebre-Sellassie, 1989; Schultz and Kleinebudde, 1995). A friability of less than 0.8% is generally accepted for tablets, but for pellets this value could be higher due to the higher surface area/unit and subsequent involvement of frictional force (Ghebre-Sellassie 1985). As shown in Table14, the friability of both drug pellets measured by friabilator with metal spheres was negligible (0.01%) because the pellets formed by extrusion-spheronization processing, usually have very low friability (Lindner et al., 1994).

2.2.7 Flowability and angle of repose

Flow rate and angle of repose of core pellets were reported in Table 14. Quick flow rate and repose angle < 25 deg indicated that core pellets had excellent flowability, agreed with the result from % compressibility. This result might be due to rounded shape of the pellets.

2.2.8 Sphericity of pellets

Sphericity is an important particle characterization parameters due to shape of particles can affect other properties such as flowability and coating performance (Heng et al., 2002). The sphericity of the pellets was evaluated by measuring roundness and aspect ratio. For a perfectly round particle, the respective values are 1.0. Roundness and aspect ratio value (Table 14) of both core pellets shown that the pellets shape was similar to that of perfect sphere, this values being nearly 1.0.

2.2.9 Charge determination of fluidized core pellets

The occurrence of electrostatic charges is almost unavoidable in gas-solid fluidization. Positive and negative charges are generated inside a fluidized bed due to repeated particle contacts and separation, supplemented by the friction of particles rubbing against each other and the column wall.

Particle-particle collisions result in a separation of charges and the net charge over the two particles is conserved. Which particle gains or loses charge is difficult to predict, but size differences can play an important role, typically leading to identifiable classes of particles building up charge of one sign and other particles building up an opposite charge (Mehrani et al., 2005).

The second source of charge build-up results from particle-wall collisions. When this is the case, a strong preference for the direction of charge separation is usually displayed. That is, the particles will always charge selectively positive or negative. In a bed consisting of particles of very similar sizes, the particle-wall collisions will be the dominant mechanism for such charge build up. However, particles of a slightly different sizes or compositions may well show the opposite charge build up (Demirbas, 2006).

Propranolol hydrochloride (PL, cationic drug) and diclofenac sodium (DS, anionic drug) were selected to prepare core pellets and expected they should have different charges on the surface during fluidization process. Faraday cup method (Figure 25 in chapter III) was used to directly measure charges build up on fluidized

core pellets after 15 minutes of pre-heating time inside acrylic chamber. It was found that the total charge measured by electrometer was inconsistent. However, the most frequent charge obtained for PL pellets was positive, while that for DS pellets was negative. The charge coulomb was in a low range of pico-coulomb with high variation (data was not shown). This was possible that this method has some disadvantages, such as addition or loss of charge during the handling of pellets before entering the cup (Mehrani et al., 2005) and disturbance of outer chamber environment, especially relative humidity, as well as was unable to measure the charge build up inside the chamber during fluidization process.

2.2.10 Drugs content in core pellets

The drug content of propranolol hydrochloride and diclofenac sodium in the pellets was quantitatively determined by high performance liquid chromatography (HPLC) method. The HPLC method of both drugs were validated and the results as shown in Appendix B.

Drug content of propranolol hydrochloride (PL) and diclofenac sodium (DS) pellets were analyzed using validated HPLC method and the results were shown in Table 15. Drug content of PL and DS pellets was 100.01% and 97.26 % respectively. Drug content in both of the pellets complied with the standard specification of USP 28, which stipulates range of drug content in the formulation of both drugs was 90.0 – 110.0 % of the labelled amount.

Table 15 Drug content in core pellets (%).

Core pellets	Sample 1	Sample 2	Sample 3	Average (SD)
PL	101.99	98.16	99.89	100.01 (1.92)
DS	97.39	96.59	97.80	97.26 (0.61)

3. Preliminary study on the coating process

In preliminary study, atomizing air pressure and feed rate of coating agent were screened. The coating conditions and coating composition were previously described in section 3 of chapter III; sunset yellow lake was used as an indicator since visual observation was made easier on film with sunset yellow lake.

To select an appropriate coating condition for further studies, appearance, flowability in terms of compressibility and coating efficiency were evaluated.

First, PL pellets were used as the core pellets; and HPMC and EC were the coating agents. Atomizing air pressure and feed rate of coating agent were varied. All the coated pellets showed good flowability as value of the % compressibility < 10 (Table 16). For PL pellets, HPMC-A, HPMC-D, EC-B and EC-E conditions gave coated pellets with no or few agglomerations, less sticky to filter and relatively high percent coating efficiency were chosen to process the DS pellets (Table 17). The resulting pellets were evaluated and found that the appropriate coating conditions for the two coating agent were different. For HPMC polymer, HPMC-D: atomizing air pressure 2 kg/cm^2 and feed rate 5 ml/min, were used. For EC polymer, EC-B: atomizing air pressure 1 kg/cm^2 and feed rate 10 ml/min, were used.

Table 16 Evaluation of PL coated pellets in preliminary study

Condition	Color[a]	Agglome-ration[b]	Stick to filter[c]	Uncoated pellets[d]	Bulk density (g/ml)	Tapped Density (g/ml)	%Compressibility	% Coating efficiency
HPMC-A	++++	0	++	+	0.72	0.74	2.40	86.58
HPMC-B	+	0	+++	+	0.71	0.73	2.13	80.90
HPMC-C	+	++	++++	++	0.67	0.69	2.13	95.67
HPMC-D	+++++	0	+	+	0.72	0.74	2.67	94.98
HPMC-E	+++	0	++	+	0.72	0.74	2.53	82.05
HPMC-F	++	++	++	++	0.70	0.71	1.87	87.26
EC-A	+++	0	++	+	0.75	0.77	2.13	69.50
EC-B	+++++	0	+	+	0.74	0.75	1.20	82.55
EC-C	++++	+	+	++	0.73	0.74	1.47	91.22
EC-D	+++	0	+	+	0.74	0.75	0.80	66.02
EC-E	++++	+	++	++	0.76	0.77	0.80	68.60
EC-F	+	0	+++	+++	0.73	0.74	1.07	78.00

[a] Color (+ to +++++) : low to high intensity of dye on film.
[b] Agglomeration (0 to +++) : no to high agglomeratation.
[c] Stick to filter (0 to ++++) : no to high stickness.
[d] Uncoated pellets (0 to +++) : non to more pellets.

Table 17 Evaluation of DS coated pellets in preliminary study

Condition	Color[a]	Agglome -ration[b]	Stick to filter[c]	Uncoated pellets[d]	Bulk density (g/ml)	Tapped Density (g/ml)	%Compressibility	% Coating efficiency
HPMC-A	+++	++	+++	++	0.79	0.80	1.60	78.55
HPMC-D	+++++	0	++	+	0.80	0.80	0.67	78.51
EC-B	++	+	++	++	0.81	0.83	2.40	76.58
EC-E	++	0	++	++	0.80	0.82	2.53	74.91

[a] Color (+ to +++++) : low to high intensity of dye on film. [b] Agglomeration (0 to +++) : no to high agglomeratation.

[c] Stick to filter (0 to ++++) : no to high stickness. [d] Uncoated pellets (0 to +++) : non to more pellets.

4. Process development of coating technique using electrostatic enhanced fluidized bed

Film coating is a process in which the results obtained are attributable to the complex interaction of numerous factors. In this section, three factors: types of core pellets, film formers and applied electrical potential were studied. The selected coating conditions from the preliminary studies, as shown in Table 18, were used. Compositions of coating agent and experiment design were previously presented in Table 9 and 10 in chapter III.

Table 18 Coating condition for electrostatic coating

Parameter	Value
Pellets load (g)	100.00
Pre-heating time , temperature	15 min , 60°C
Inlet air temperature (°C)	60 ± 2
Fluidized air velocity (m/s)	5.5
Atomizing air pressure (kg/cm^2)	2 (HPMC) / 1 (EC)
Feed rate (ml/min)	5 (HPMC) / 10 (EC)
Post-heating time , temperature	30 min , 60°C
Curing time, temperature (Hot air oven)	12 h. , 60°C

Target weight gained = 20 %

The pellets were coated using bottom spray enhanced electrostatic fluidized bed. Post-heating and curing step subsequent to the coating process is often recommended to reduce stability problems due to enhanced film formation by further coalescence (Wesseling and Bodmeier, 2001).

5. Evaluation of coated drug pellets

The statistical analysis, general linear model ANOVA, was employed to discuss the importance of each factor.

5.1 Morphology

Electron photomicrograph of coated pellets and cross sectioned pellets from electrostatic coating technique are shown in Figures 36 to 39. In general, the size and shape of coated pellets were similar. The shapes of whole EC coated pellets were more irregular than that of HPMC coated pellets.

Photomicrographs of cross sectioned of pellets obtained from all coating conditions displayed the interface between the core pellets and coating film. The breach of the film and core pellets obtained from some coating conditions may be due to pressure during cross sectioning by a razor.

The cross sectioned pellets of both drugs showed variation in film thickness, although no difference between applied and non-applied electrical potential of coating process was observed. However, it was found that the film coating of DS pellets of each polymer was slightly thicker than PL pellets. This result was attributed to lighter density of PL pellets, resulting in different fluidization pattern and velocities within the coating process (Wesdyk et al., 1990). The heavier pellets were fluidized less and therefore circulated much more times through the coating zone when compared to the lighter beads as a result the thicker films observed.

With higher magnification (Figures 40 to 43), SEM photomicrographs show that film coated on the surface of pellets displayed rough surface. There was no difference in film surface of HPMC coated pellets for both drugs while the EC coated pellets showed some differences. PL pellets coated with EC were of smoother surface and likely to be homogenous or continuous film while the EC coated DS pellets were rough and scaled-like film on the surface.

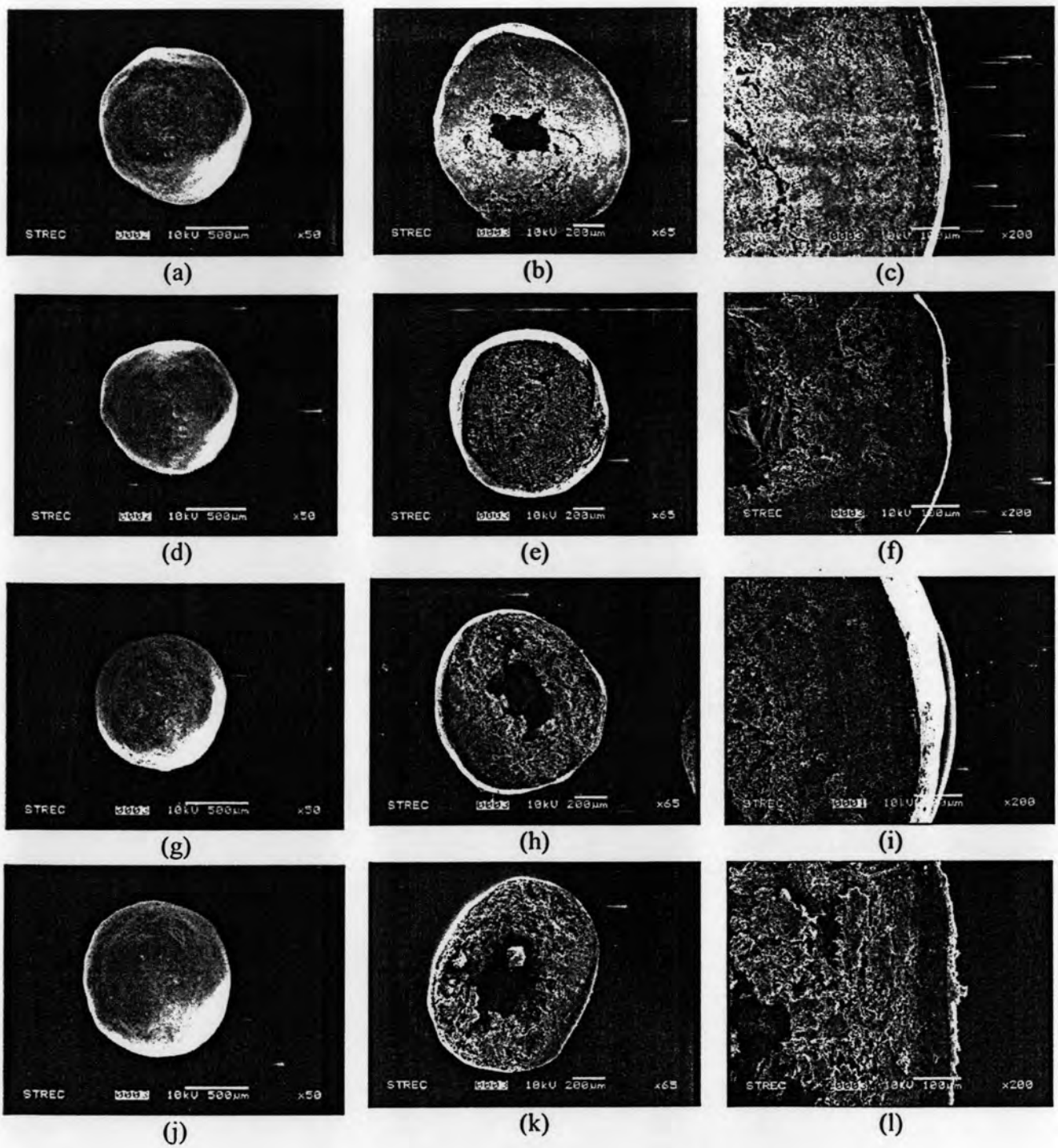


Figure 36 SEM photomicrographs of whole pellet x50, (a) (d) (g) (j); cross sectioned pellet x65; (b) (e) (h) (k); cross sectioned pellet x200; (c) (f) (i) (l) of HPMC coated PL pellets.

(a), (b) and (c) : applied positive potential (+)

(d), (e) and (f) : non applied potential (non)

(g), (h) and (i) : applied negative potential (-)

(j), (k) and (l) : switching applied potential (+/-)

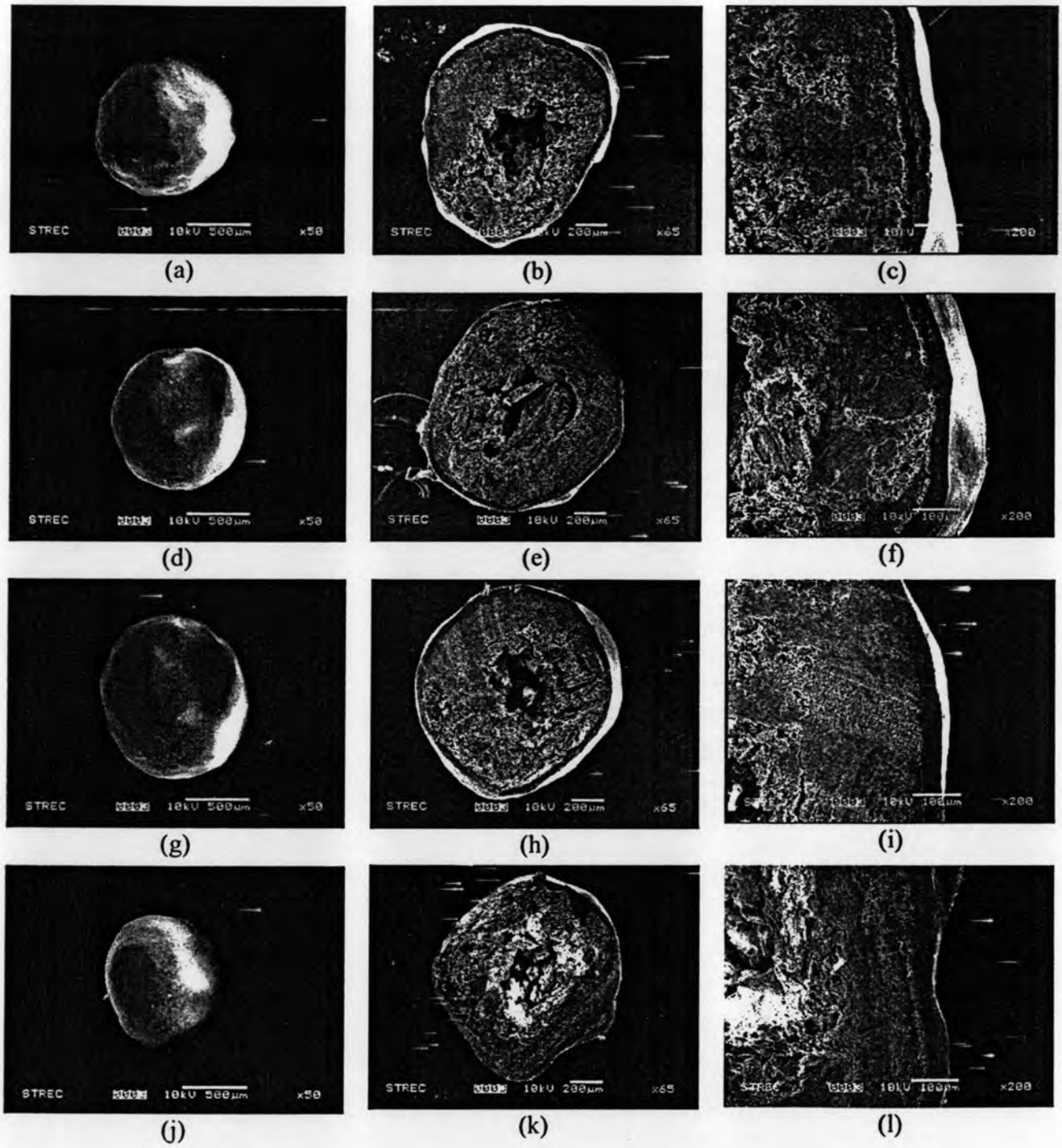


Figure 37 SEM photomicrographs of whole pellet x50, (a) (d) (g) (j); cross sectioned pellet x65; (b) (e) (h) (k); cross sectioned pellet x200; (c) (f) (i) (l) of EC coated PL pellets.

(a), (b) and (c) : applied positive potential (+)

(d), (e) and (f) : non applied potential (non)

(g), (h) and (i) : applied negative potential (-)

(j), (k) and (l) : switching applied potential (+/-)

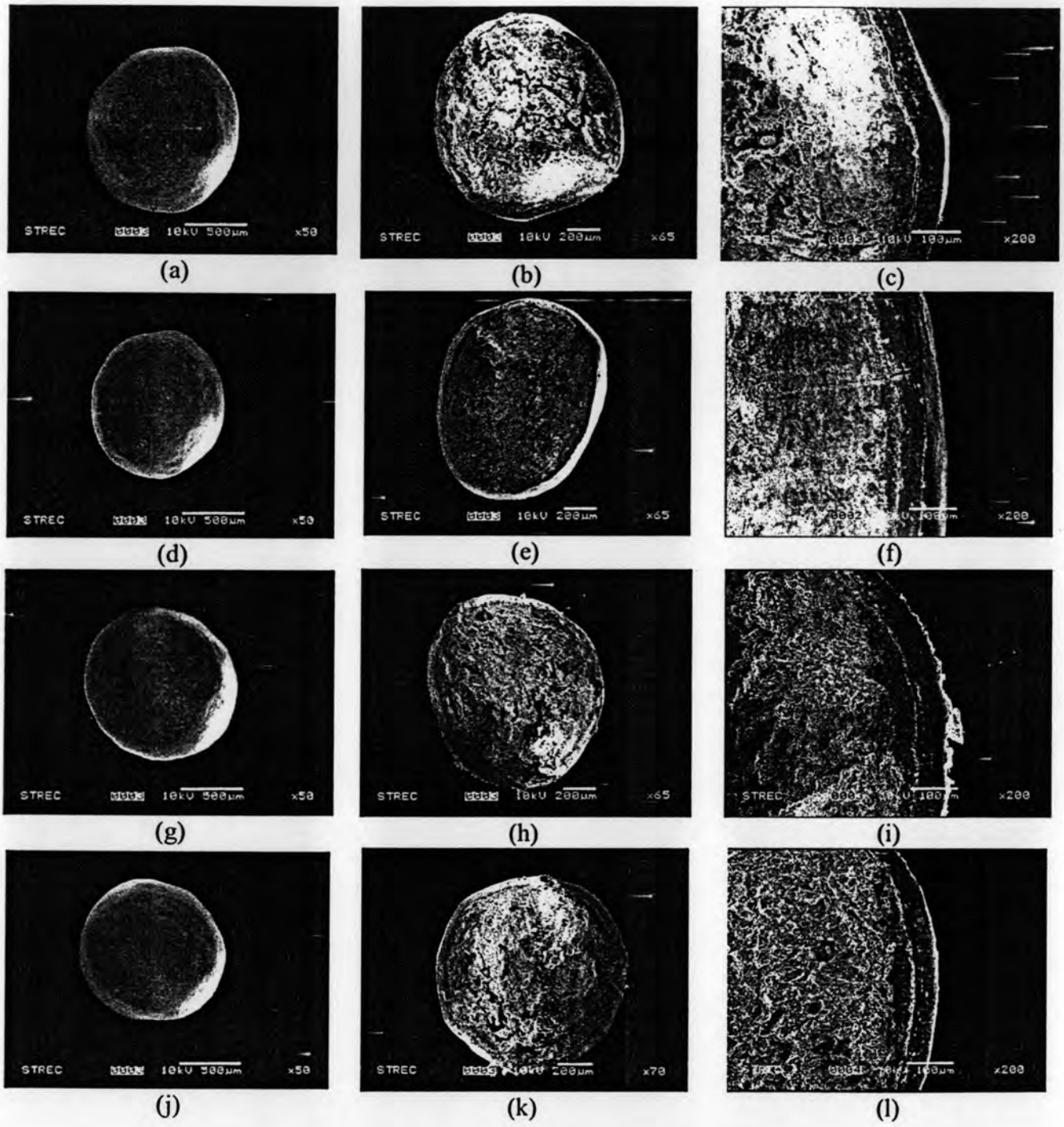


Figure 38 SEM photomicrographs of whole pellet x50, (a) (d) (g) (j); cross sectioned pellet x65; (b) (e) (h) (k); cross sectioned pellet x200; (c) (f) (i) (l) of HPMC coated DS pellets.

(a), (b) and (c) : applied positive potential (+)

(d), (e) and (f) : non applied potential (non)

(g), (h) and (i) : applied negative potential (-)

(j), (k) and (l) : switching applied potential (+/-)

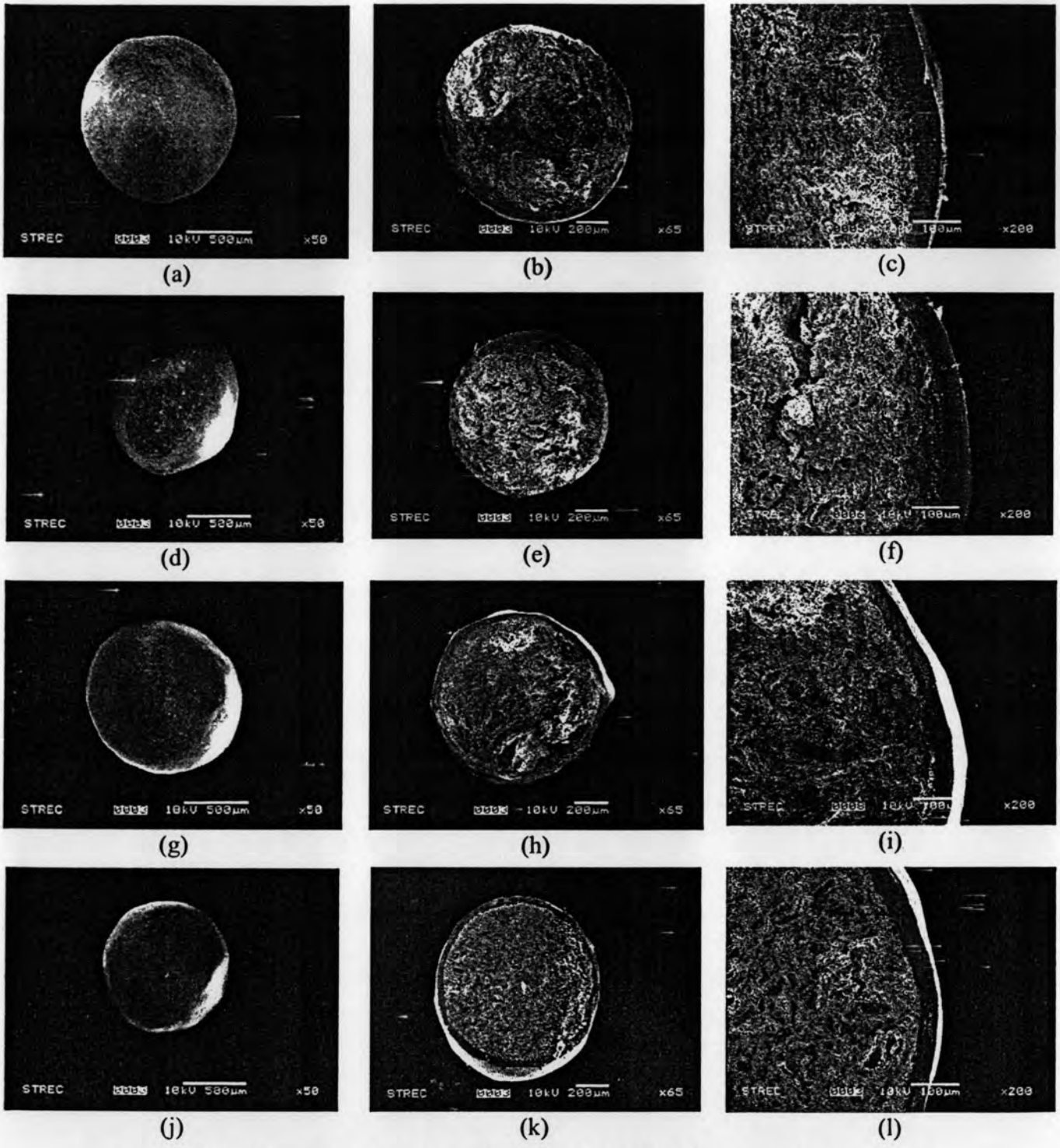


Figure 39 SEM photomicrographs of whole pellet x50, (a) (d) (g) (j); cross sectioned pellet x65; (b) (e) (h) (k); cross sectioned pellet x200; (c) (f) (i) (l) of EC coated DS pellets.

(a), (b) and (c) : applied positive potential (+)

(d), (e) and (f) : non applied potential (non)

(g), (h) and (i) : applied negative potential (-)

(j), (k) and (l) : switching applied potential (+/-)

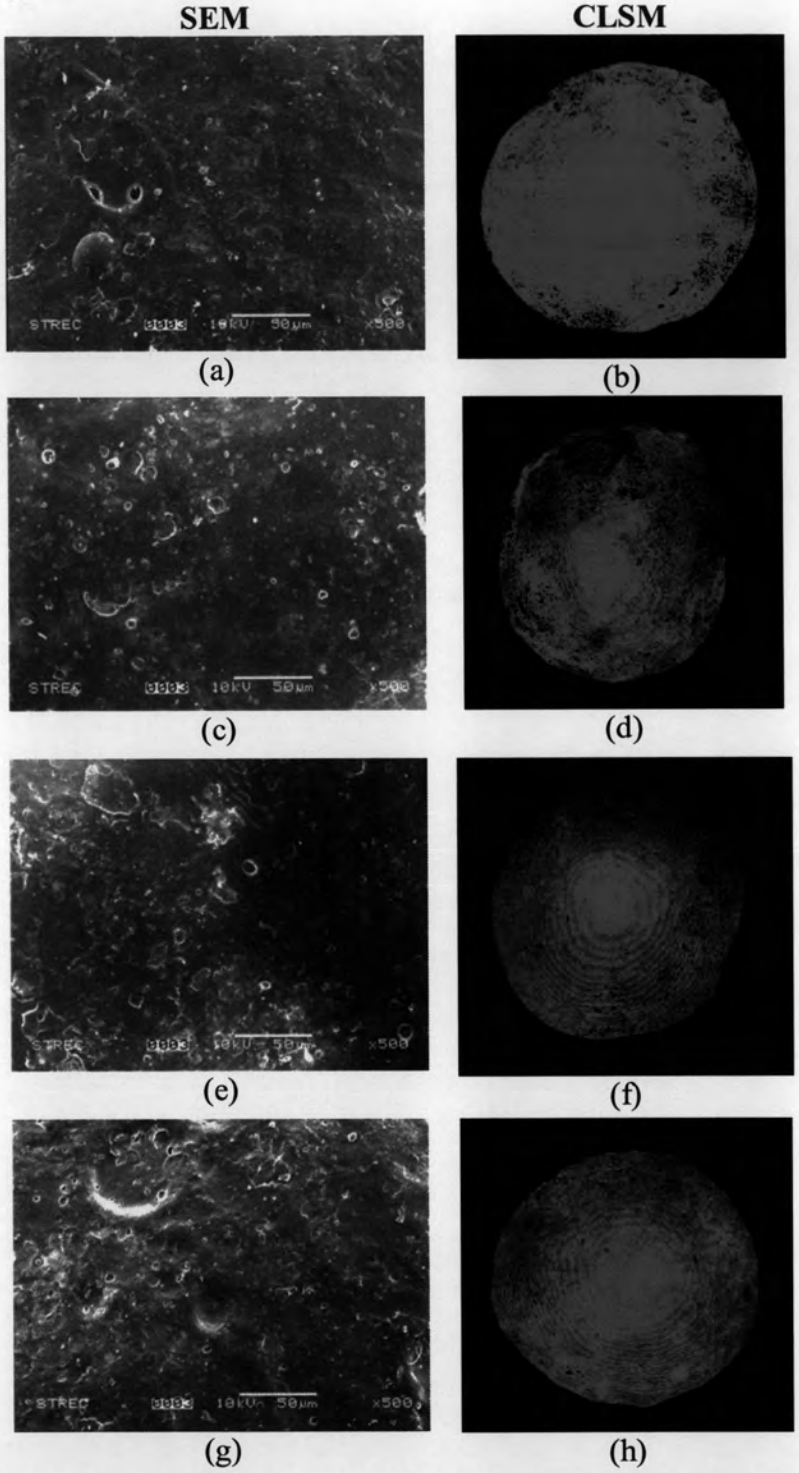


Figure 40 SEM photomicrographs at magnification of x500, (a) (c) (e) (g), and CLSM image at magnification of x10, (b) (d) (f) (h), of HPMC coated PL pellets.

(a) and (b) : applied positive potential (+)

(c) and (d) : non applied potential (non)

(e) and (f) : applied negative potential (-)

(g) and (h) : switching applied potential (+/-)

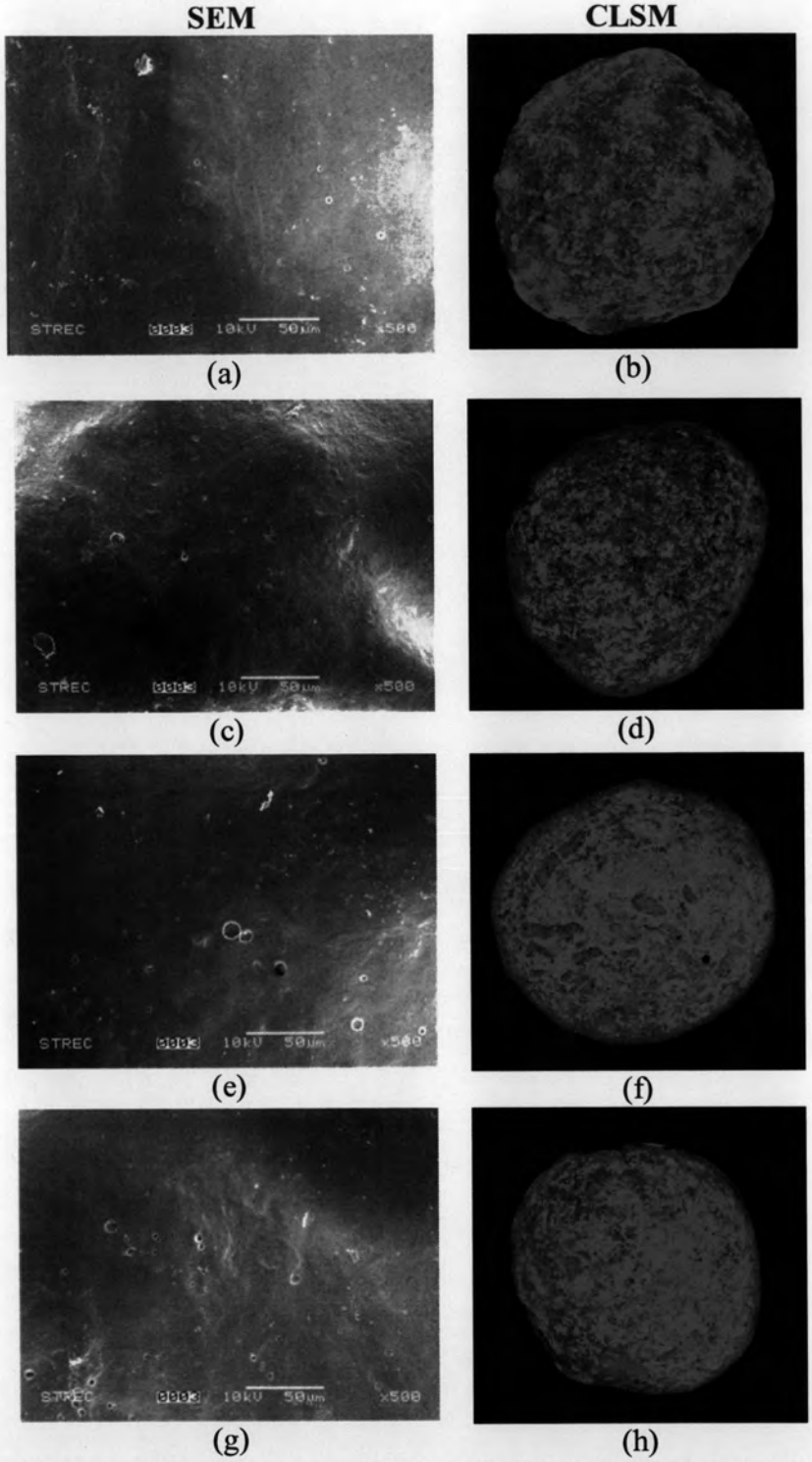


Figure 41 SEM photomicrographs at magnification of x500, (a) (c) (e) (g), and CLSM image at magnification of x10, (b) (d) (f) (h), of EC coated PL pellets.

(a) and (b) : applied positive potential (+)

(c) and (d) : non applied potential (non)

(e) and (f) : applied negative potential (-)

(g) and (h) : switching applied potential (+/-)

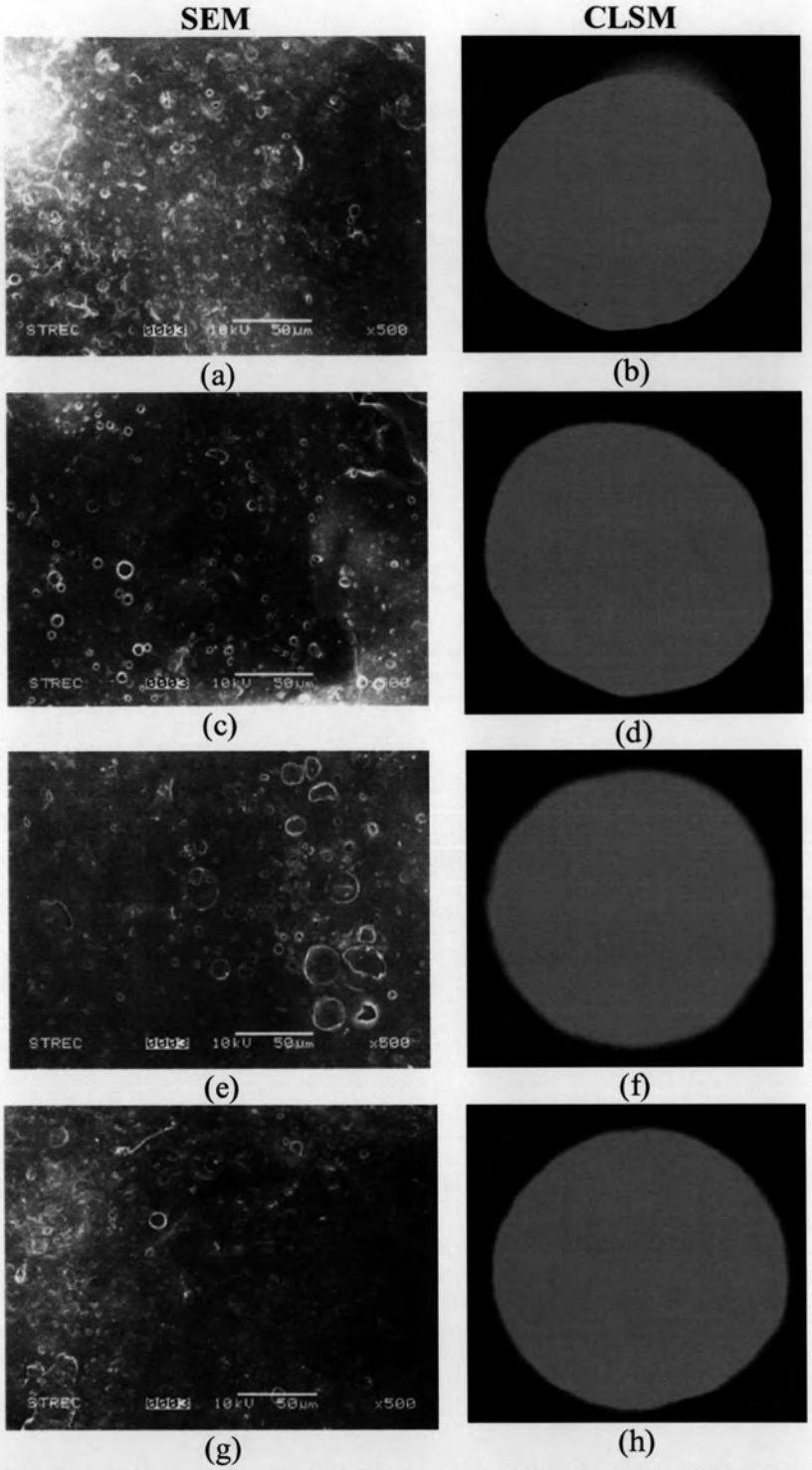


Figure 42 SEM photomicrographs at magnification of x500, (a) (c) (e) (g), and CLSM image at magnification of x10, (b) (d) (f) (h), of HPMC coated DS pellets.

(a) and (b) : applied positive potential (+)

(c) and (d) : non applied potential (non)

(e) and (f) : applied negative potential (-)

(g) and (h) : switching applied potential (+/-)

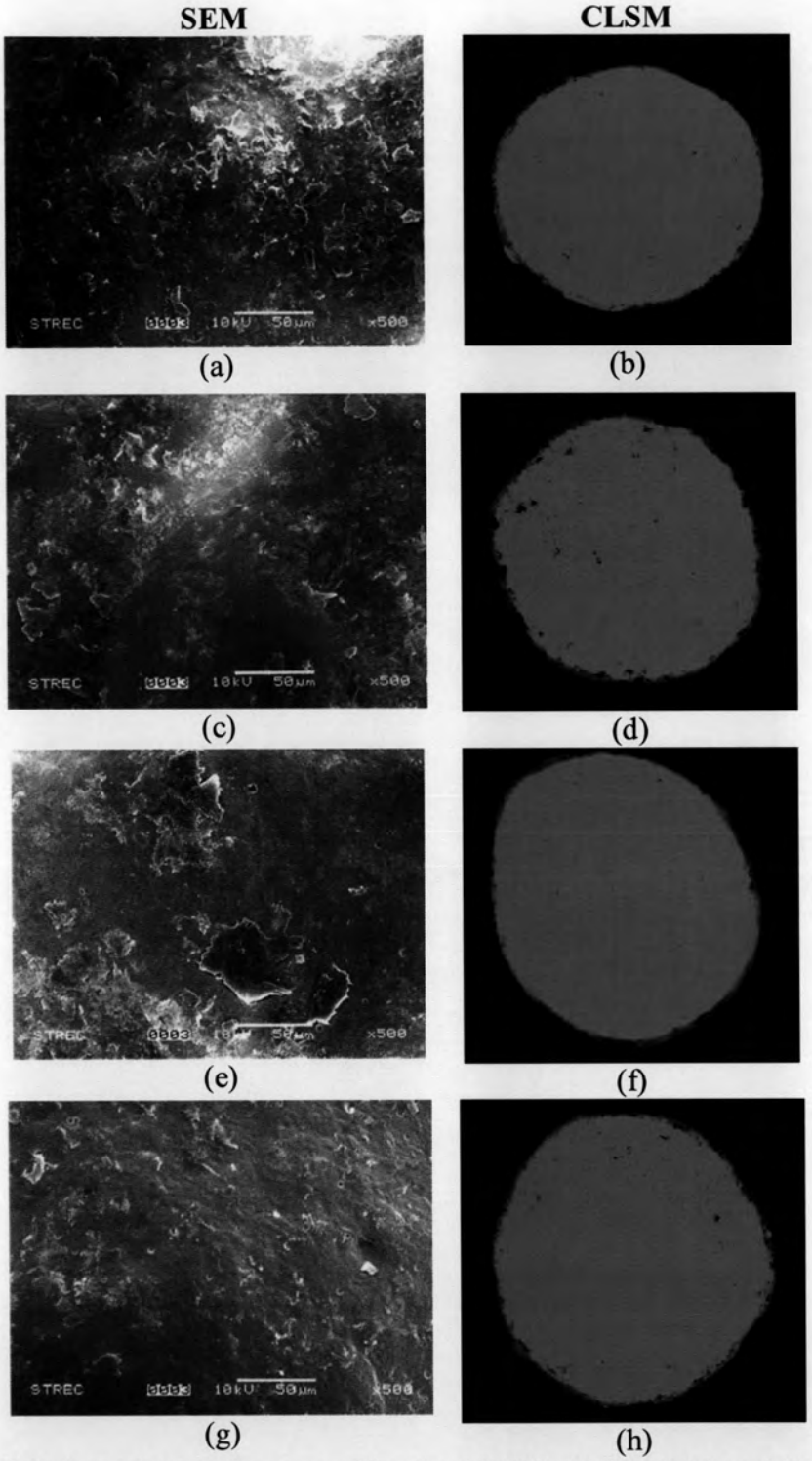


Figure 43 SEM photomicrographs at magnification of x500, (a) (c) (e) (g), and CLSM image at magnification of x10, (b) (d) (f) (h), of EC coated DS pellets.

(a) and (b) : applied positive potential (+)

(c) and (d) : non applied potential (non)

(e) and (f) : applied negative potential (-)

(g) and (h) : switching applied potential (+/-)

5.2 Moisture content

The moisture balance was used to measure the amount of residual moisture in the coated pellets after curing process. The moisture content in term of percent loss on drying (%LOD) of coated pellets in each condition was presented in Table 19. The values are in the range of 0.52 – 1.07 %.

In agreement with the core pellets, the higher moisture content was found in DS pellets. The maximum value was obtained from EC coated DS pellets which was applied with negative voltage. Beside to the moisture content in core pellets, the moisture content of coated pellets may be depend on the different capability of water evaporation in the drying and curing stage of coating process of each film former in the same temperature (Bodmeier, 1997).

5.3 Flow rate, bulk, tapped densities and percent compressibility

The flow rate was evaluated by monitoring the time taken for coated pellets to flow through an orifice of the funnel. Bulk and tapped densities were obtained by Jolting volumeter. The results were presented in Table 19.

Percent compressibility is an excellent indication of uniformity in size, shape and moisture content (Sanz, 2001). It was found that in term of flow rate, ranging in 10.96-14.74 g/s and percent compressibility less than < 10 %, the coated pellets obtained from all coating conditions indicated the excellent flowability. The coated pellets seemed to have better flowability as opposed to the core pellets.

For both film formers, the flow rate of DS coated pellets was likely to be higher than that of PL coated pellets. From the statistical analysis flow rate, bulk and tapped densities were mainly significant affected by types of drug core pellets in HPMC coating, while in EC coating, these properties of coated pellets were strongly affected by applied electrical potential However, types of drug core pellets was the major effect on percent compressibility of coated pellets for both film formers.

Table 19 Evaluation of coated pellets in electrostatic coating technique (I).

Variables			Mean (SD)						
Drug	Polymer	Applied potential**	Moisture (%LOD)	Flow rate (g/s)	Bulk density (g/cm^3)	Tapped density (g/cm^3)	%Compres-sibility	Aspect ratio	Round-ness
PL	HPMC	(+)	0.66 (0.03)	11.38 (1.03)	0.72 (0.01)	0.74 (0.01)	2.96 (0.64)	1.09 (0.05)	1.21 (0.06)
		non	0.81 (0.03)	12.18 (0.91)	0.72 (0.01)	0.73 (0.01)	1.85 (0.64)	1.08 (0.04)	1.19 (0.06)
		(-)	0.63 (0.03)	12.02 (0.65)	0.72 (0.00)	0.73 (0.00)	1.85 (0.64)	1.06 (0.04)	1.16 (0.04)
		(+/-)	0.70 (0.05)	10.96 (0.55)	0.72 (0.00)	0.72 (0.00)	0.37 (0.64)	1.08 (0.04)	1.19 (0.04)
	EC	(+)	0.53 (0.06)	12.55 (0.78)	0.75 (0.00)	0.76 (0.00)	1.48 (0.64)	1.07 (0.04)	1.16 (0.03)
		non	0.55 (0.07)	11.15 (0.60)	0.76 (0.00)	0.77 (0.00)	1.22 (0.00)	1.08 (0.04)	1.19 (0.06)
		(-)	0.53 (0.03)	12.01 (0.89)	0.75 (0.00)	0.76 (0.00)	0.37 (0.64)	1.08 (0.04)	1.17 (0.04)
		(+/-)	0.53 (0.07)	12.60 (0.38)	0.86 (0.01)	0.86 (0.00)	0.42 (0.72)	1.07 (0.04)	1.19 (0.04)
DS	HPMC	(+)	0.86 (0.06)	12.53 (0.45)	0.79 (0.01)	0.79 (0.01)	0.00 (0.00)	1.20 (1.07)	1.41 (2.10)
		non	0.63 (0.03)	13.97 (0.55)	0.78 (0.02)	0.79 (0.01)	1.48 (0.64)	1.08 (0.05)	1.20 (0.05)
		(-)	0.86 (0.06)	14.74 (0.52)	0.77 (0.00)	0.79 (0.00)	1.85 (0.64)	1.08 (0.04)	1.20 (0.03)
		(+/-)	0.98 (0.05)	14.25 (0.39)	0.80 (0.00)	0.80 (0.00)	0.37 (0.64)	1.07 (0.04)	1.17 (0.04)
	EC	(+)	1.07 (0.11)	12.31 (0.35)	0.77 (0.00)	0.78 (0.00)	1.11 (0.00)	1.10 (0.06)	1.18 (0.02)
		non	0.94 (0.10)	11.68 (0.00)	0.74 (0.01)	0.75 (0.00)	1.48 (0.64)	1.10 (0.06)	1.18 (0.03)
		(-)	1.53 (0.02)	12.55 (0.37)	0.78 (0.01)	0.79 (0.00)	1.48 (0.64)	1.09 (0.06)	1.18 (0.02)
		(+/-)	0.52 (0.10)	14.46 (0.50)	0.78 (0.01)	0.79 (0.00)	1.48 (0.64)	1.09 (0.05)	1.18 (0.08)

** (+) : applied positive potential
(-) : applied negative potential
non : non-applied electrical potential
(+/-) : switching applied potential

5.4 Sphericity

In this study, image analysis was used to evaluate two parameters, aspect ratio and roundness, indicating the degree of sphericity. The value of these parameters close to 1.0, pellets shape becomes more spherical. The mean value of the two parameters in various coating conditions were shown in Table 19.

There was no significant difference ($p>0.05$) on aspect ratio for all condition of HPMC coating, while there was a significant affect ($p<0.05$) of types of core pellets on aspect ratio when EC was the coating agent. The EC coated DS pellets had higher aspect ratio value than that of EC coated PL pellets.

In consistent with the result of aspect ratio, roundness of HPMC coated pellets obtained from all condition were no significant different ($p>0.05$). In contrast there was a significant effect of applied electrical potential on the roundness of EC coating ($p<0.05$).

5.5 Crushing force

The crushing force is an indicator of pellets strength or hardness, the higher value means the greater hardness. The crushing force value is given by the maximal force corresponding to the peak of the force-time plot. In the presence of plural peaks or notches, some authors adopted different approaches to assume the correspondence between a peak and the real crushing strength value. In some cases the last one was chosen (Horisawa et al., 2000), whereas in other cases the highest one (Wan and Lai., 1994) and yet in other works the first peak giving a preset drop of force was taken into account (Wikberg and Alderborn., 1992, Johansson et al., 1995). In this study the first drop of force was reported as crushing force of pellets.

Crushing force value of coated pellets was shown in the Figure 44 and Table 21. These results to be in line with crushing force of core pellets, coated DS pellets tend to have higher value. For both film formers, there was significant effects of the core pellets and applied electrical potential on the crushing force ($p<0.05$). From post hoc analysis (Table 26), crushing force showed significant difference between switching applied potential and others in HPMC coating, while in EC coating

there was significant difference between switching applied potential and positive or negative applied potential.

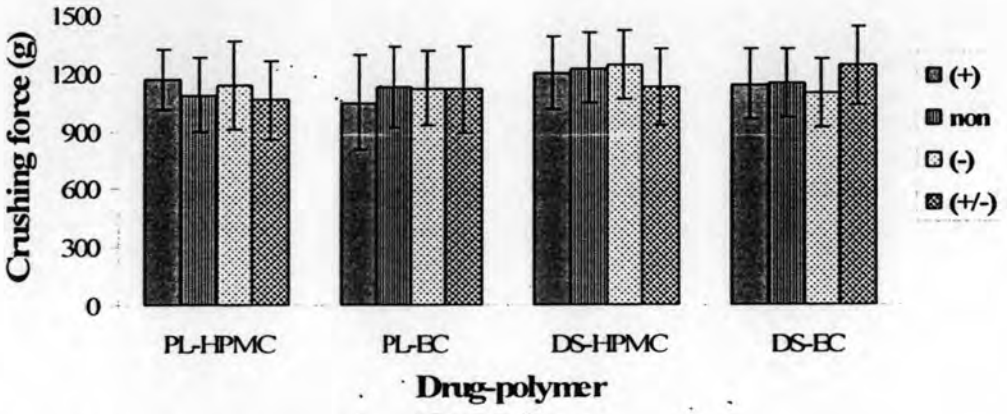


Figure 44 Crushing force of coated pellets.

PL-HPMC	: HPMC coated PL pellets	(+)	: applied positive potential
PL-EC	: EC coated PL pellets	non	: non-applied electrical potential
DS-HPMC	: HPMC coated DS pellets	(-)	: applied negative potential
DS-EC	: EC coated DS pellets	(+/-)	: switching applied potential

The highest crushing force value in HPMC coating was obtained when positive potential was applied to PL pellets and when negative potential was applied to DS pellets. In contrast results were obtained in EC coating; both conditions gave the lowest crushing value. This may be the difference in film forming in each type of film former.

5.6 Drug contents of coated pellets

Drug content of coated pellets was analyzed by high performance liquid chromatography and the data were shown in the Table 20. The average percentage of drug content of PL and DS coated pellets were in the range of 90.96-94.37 % and 91.50-99.85 % respectively. Percent drug content in both drugs pellets complied to the standard specification of USP 28, which stipulates the range of percent drug content in the formulation of both drugs is 90.0 – 110.0 %.

Table 20 Percentage of drug content in coated pellet

Condition		Sample 1	Sample 2	Sample 3	Mean	SD
PL-HPMC	(+)	91.90	94.30	93.09	93.10	1.20
	0	94.56	94.32	93.35	94.08	0.64
	(-)	91.63	93.56	91.27	92.16	1.23
	(+/-)	92.45	95.81	94.44	94.24	1.69
PL-EC	(+)	94.84	91.58	92.37	92.93	1.70
	0	90.43	89.84	92.60	90.96	1.46
	(-)	92.74	91.43	90.07	91.41	1.33
	(+/-)	94.88	96.21	92.02	94.37	2.27
DS-HPMC	(+)	95.75	96.13	95.80	95.89	0.21
	0	95.11	93.97	95.00	94.69	0.63
	(-)	94.75	95.66	97.12	95.84	1.20
	(+/-)	98.29	99.24	102.04	99.85	1.95
DS-EC	(+)	91.65	90.80	92.05	91.50	0.64
	0	93.42	92.43	92.68	92.84	0.51
	(-)	93.58	91.31	91.79	92.23	1.20
	(+/-)	91.69	92.06	92.41	92.05	0.36

PL-HPMC : HPMC coated PL pellets
PL-EC : EC coated PL pellets
DS-HPMC : HPMC coated DS pellets
DS-EC : EC coated DS pellets

(+) : applied positive potential
non : non-applied electrical potential
(-) : applied negative potential
(+/-) : switching applied potential

5.7 Coating efficiency.

Percent coating efficiency was calculated by comparing percent weight gained of coated pellets with the total amount of solid contents of coating agent used in the process. Higher percent coating efficiency indicated that higher weight gained and lower amount of coating agent loss. Percent coating efficiency is presented in Figure 45 and Table 21

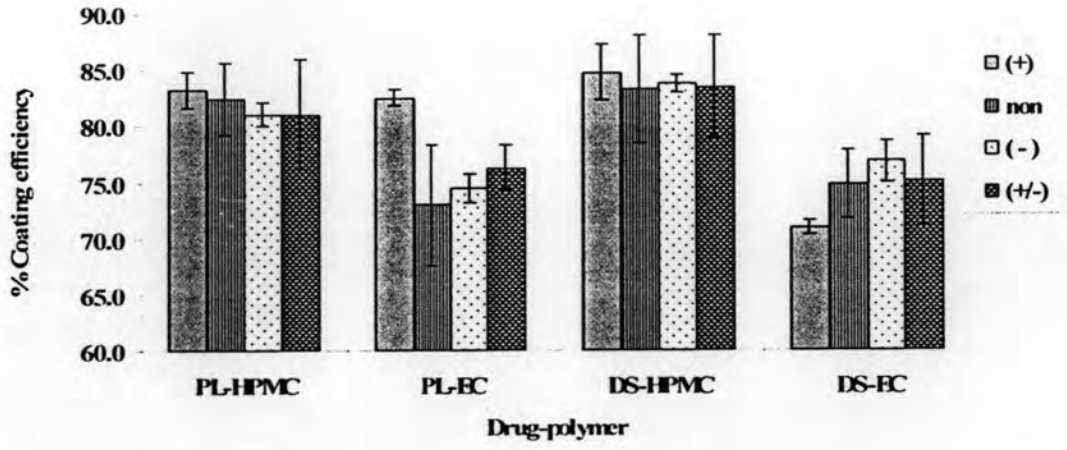


Figure 45 Percent coating efficiency of coated pellets

PL-HPMC	: HPMC coated PL pellets	(+)	: applied positive potential
PL-EC	: EC coated PL pellets	non	: non-applied electrical potential
DS-HPMC	: HPMC coated DS pellets	(-)	: applied negative potential
DS-EC	: EC coated DS pellets	(+/-)	: switching applied potential

From the data obtained, percent coating efficiency did not support the previous hypothesis about attractiveness between opposite charges. There was no significant difference (p>0.05) between the effect of applied and non-applied electrical potential on coating efficiency. However, it was found that non-applied potential yielded higher variation than applied potential to the nozzle. As shown in Figure 46, percent relative standard deviation of each set of drug-polymer pellets was lower for process of applied electrical potential except for switching electrical potential. It could be implied that applied electrical potential of any single type of charge to the nozzle, increased the consistency in the coating process.

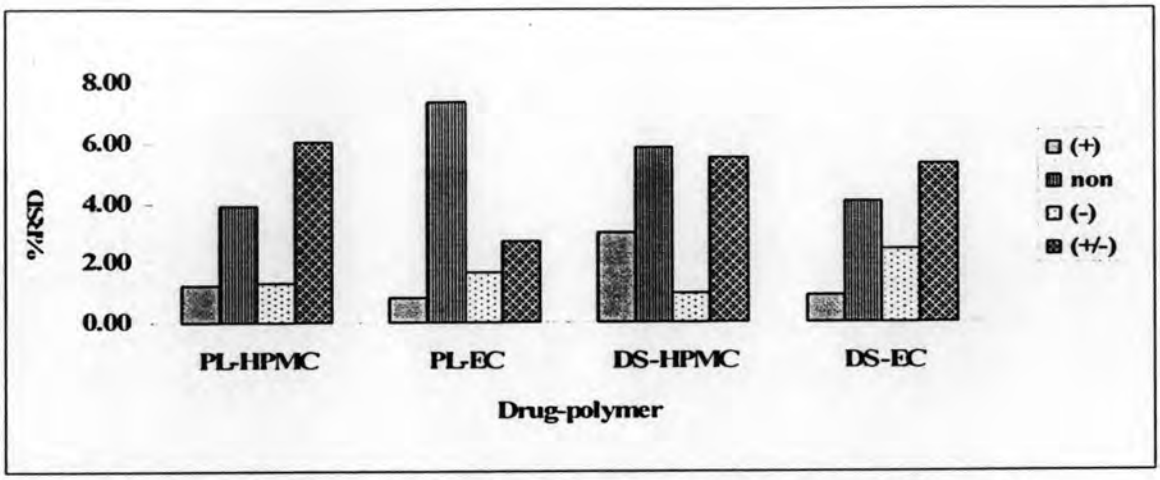


Figure 46 Percent relative standard deviation of percent coating efficiency of electrostatic coating technique

PL-HPMC : HPMC coated PL pellets
PL-EC : EC coated PL pellets
DS-HPMC: HPMC coated DS pellets
DS-EC : EC coated DS pellets

(+) : applied positive potential
non : non-applied electrical potential
(-) : applied negative potential
(+/-) : switching applied potential

5.8 Film thickness

The release rate of an active drug may be influenced by many factors. Important ones are the thickness of coating applied on the pellets. In this experiment, film thickness was determined by image analysis. Image analysis enables a direct measurement of thickness of the coating applied but has been concerned mostly with particle size and surface roughness parameter. However, it has been reported that image analysis can be used to determine film thickness for coated pellets of narrow size distribution (Andersson et al., 2000).

The film thickness of each coating condition was calculated by comparing their diameters to the mean diameter of the core pellets. Data of film thickness is shown in Table 21 and Figure 47.

From the statistical point of view, film thickness for both film formers were significantly affected by types of drug core pellets and applied electrical potential ($p<0.05$). Post hoc analysis (Table 26) showed the significant difference from each other for applied electrical, except positive potential applied and non-

applied potential in HPMC coating; and negative potential applied and non applied potential in EC coating.

Table 21 Evaluation of coated pellets in electrostatic coating technique (II).

Variables			Mean (SD)			
Drug	Polymer	Applied potential**	%Coating efficiency	Film Thickness (μm)	Crushing force (g)	AUC disso (%h)
PL	HPMC	(+)	83.3 (1.1)	86.86 (36.51)	1165.128 (159.666)	58.5 (0.6)
		non	82.4 (3.2)	90.61 (32.44)	1087.755 (195.143)	59.2 (1.0)
		(-)	81.1 (1.1)	70.87 (27.78)	1138.755 (229.894)	57.5 (0.7)
		(+/-)	81.1 (4.9)	77.23 (28.60)	1058.603 (203.858)	57.1 (0.4)
	EC	(+)	82.5 (0.7)	85.18 (31.82)	1046.533 (242.806)	156.4 (35.4)
		non	73.0 (5.4)	82.04 (32.35)	1126.449 (208.869)	352.9 (71.2)
		(-)	74.5 (1.2)	60.92 (29.42)	1118.633 (191.494)	295.8 (43.5)
		(+/-)	76.2 (2.0)	87.57 (35.08)	1110.186 (226.938)	157.3 (44.5)
DS	HPMC	(+)	84.8 (2.5)	108.55 (35.74)	1200.018 (188.188)	41.2 (0.7)
		non	83.3 (4.8)	112.94 (33.10)	1223.132 (182.850)	43.2 (1.1)
		(-)	83.8 (0.8)	63.71 (32.17)	1240.252 (175.480)	42.2 (0.5)
		(+/-)	83.5 (4.6)	104.91 (30.13)	1121.252 (198.202)	42.6 (0.4)
	EC	(+)	71.0 (0.6)	113.58 (31.87)	1139.750 (182.545)	222.9 (82.8)
		non	74.8 (3.0)	65.62 (28.79)	1145.942 (172.945)	716.1 (147.9)
		(-)	76.8 (1.9)	85.56 (35.98)	1097.683 (176.975)	718.4 (171.6)
		(+/-)	75.1 (4.0)	85.97 (27.77)	1235.566 (207.135)	305.2 (74.5)

** (+) : applied positive potential non : non-applied electrical potential

(-) : applied negative potential (+/-) : switching applied potential

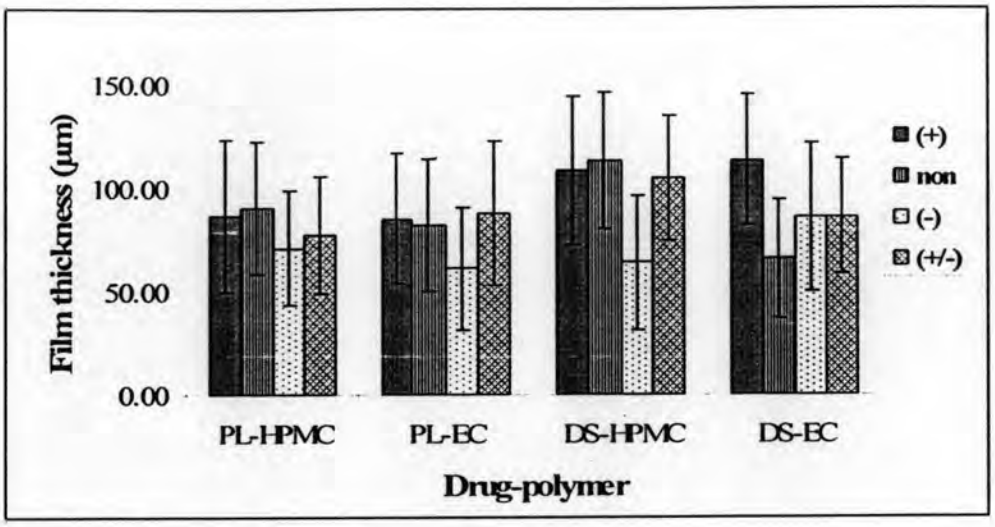


Figure 47 Film thickness of coated pellets.

PL-HPMC	: HPMC coated PL pellets	(+)	: applied positive potential
PL-EC	: EC coated PL pellets	non	: non-applied electrical potential
DS-HPMC	: HPMC coated DS pellets	(-)	: applied negative potential
DS-EC	: EC coated DS pellets	(+/-)	: switching applied potential

For HPMC coating, types of drug core pellets had greater effect on the film thickness than applied electrical potential as signify by the F value in Table 25. HPMC coated DS pellets possessed thicker film than that of PL pellets, except when the negative potential was applied. The maximum and minimum value of film thickness for both drugs of HPMC coated pellets were obtained from the process of non-applied electrical potential and applied negative potential respectively .

The thicker film coated for DS pellets may be resulting of higher density of DS pellets. As previous reported by Wesdyk et al.(1990), more dense or heavier beads received a thicker film due to their were fluidized pattern with slower velocities and lower height into the coating chamber, so that circulate much more times through the coating zone and received more coating agent.

On the contrary, for EC coating, the effect of applied electrical potentials seemed to be greater, as signify by the F value (Table 25). The maximum film thickness was obtained from switching applied potential for PL pellets and applied positive potential for DS pellets. While applied negative potential to PL pellets or

non-applied potential for DS pellets gave the minimum value of film thickness of EC coated pellets.

5.9 Fluorescence intensity

In this study, confocal laser scanning microscope (CLSM) was utilized as a tool to characterize homogeneity of film on the pellets surface because of its non-invasive nature and ability to visualize the internal structure of film coating. Fluorescence material (6-carboxyfluorescein, 6-FAM) was incorporated in the coating agent for investigation of film formation.

As shown in Figure 40 to 43, the superimpose images of fluorescence distribution on the surface of coated pellets. Samples were scanned in 190 μm section at the interval of 10 μm in the direction from the surface downwards to the core pellets. There was individual character in each set of drug-polymer pellets.

For both coating agents, the PL pellets were lower fluorescence intensity. It may imply that film layer of DS pellets was thicker than that of PL pellets. This agreed with SEM photomicrograph of cross sectioned pellets. Inhomogeneous spreading of fluorescence agent was also observed only on PL pellets coated with either film former.

As fluorescence intensity is directly related to the number of fluorophore molecules within observed data, fluorescence can provide quantitative measurements. Fluorescence intensity (I) in each layer of film coating was calculated by CLSM (Figure 48). Then, the relative intensity (I/I_0) of fluorescence probe was calculated and plotted as a function of distance of film layer from the initial surface (Figure 49), where I_0 was the fluorescent intensity of the initial layer of coated pellets.

The CLSM-thickness (Table 22) determined by the distance from the initial surface to the layer where it had the maximum value of I or I/I_0 showed significant difference ($p<0.05$) between the effect of types of drug core pellet and applied electrical potential for HPMC coating (Table 25). Meanwhile, only types of drug core pellets significantly affected on CLSM-thickness for EC coating ($p<0.05$). For both

film formers, mean value of the CLSM-thickness of DS pellets was deeper than that of PL pellets. These results were consistent with the result of film thickness obtained by image analysis and SEM photomicrograph.

In all cases, the CLSM-thickness values were lower than the film thickness values determined by image analysis. This may be attributed to how the initial surface in CLSM technique was defined. The identification of the initial surface in CLSM technique here was made in difficulty due to limitation of the machine which was not design for a curvature surface. Therefore, the layer which showed clear bordered image was considered as the initial layer for investigation. In this case, there might be some thickness that was ignored. The correlation between two thickness parameters is shown in Appendix E.

In addition, the results of analysis of variance for CLSM-thickness were different from those of the results of film thickness determined by the image analysis. This may be because of the number of sample pellets investigated and a more sensitivity of image analysis.

The slope of the linear portion of the curve between I_{max} and I_0, (I_{max}-I_0), and the distance up to I_{max} for both film formers (Table 22) were shown that types of drug core pellets significantly affected on the distribution of fluorescence molecule across the film coating ($p<0.05$). PL pellets had higher slope than that of DS pellets, agreed with the result observed from the CLSM image (Figure 40 to 43) which found more inhomogenous film on PL pellets for both film formers.

The area under the curve (AUC_{CLSM}) of between the average intensity of fluorescence and the distance of film layer calculated up to I_{max} was used as an insight measure of relative coating efficiency (CLSM-coating efficiency, Table 22). The result shown that types of drug core pellets was significantly affected ($p<0.05$) on the AUC for both film formers. The DS coated pellets had higher AUC_{CLSM} than that of PL coated pellets. In addition AUC_{CLSM} value in HPMC coating was affected by the applied electrical potential. The correlation of AUC_{CLSM} and %coating efficiency is depicted in Appendix E.

Table 22 Evaluation of coated pellets from confocal laser scanning microscopy (CLSM)

Variables			Mean (SD)					
Drug	Polymer	Applied potential**	I_0	I_{max}	Max I/I_0	CLSM-thickness (µm)	Slope (μm^{-1})	AUC_{CLSM} (µm)
PL	HPMC	(+)	368.505 (97.800)	469.421 (87.465)	1.295 (0.169)	10.0 (0.0)	10.09 (4.06)	4189.6 (905.3)
		non	210.221 (54.007)	207.475 (67.990)	0.977 (0.079)	6.7 (2.9)	1.27 (2.74)	1516.4 (1098.6)
		(-)	103.137 (30.607)	151.773 (57.001)	1.484 (0.328)	13.3 (5.8)	4.17 (4.32)	1585.9 (320.1)
		(+/-)	226.223 (59.169)	251.521 (60.411)	1.121 (0.112)	8.3 (2.9)	2.48 (2.75)	1939.2 (728.1)
	EC	(+)	114.410 (53.631)	262.219 (31.170)	2.663 (1.341)	26.7 (5.8)	5.46 (1.62)	5289.3 (456.3)
		non	148.204 (9.476)	265.096 (43.419)	1.781 (0.183)	30.0 (10.0)	4.08 (1.32)	7003.1 (3291.6)
		(-)	255.623 (121.009)	403.363 (121.986)	1.655 (0.244)	16.7 (5.8)	9.85 (4.28)	5234.0 (571.5)
		(+/-)	193.949 (14.760)	379.585 (175.095)	2.012 (1.095)	20.0 (10.0)	7.61 (5.13)	6702.1 (5186.1)
DS	HPMC	(+)	430.455 (132.666)	1343.230 (429.560)	3.331 (1.557)	46.7 (15.3)	20.45 (9.42)	50195.0 (19309.4)
		non	681.054 (109.247)	1492.198 (586.776)	2.177 (0.811)	40.0 (0.0)	20.28 (13.52)	51324.8 (18525.5)
		(-)	697.252 (160.983)	1637.377 (249.819)	2.401 (0.405)	63.3 (20.8)	17.00 (10.12)	87589.8 (19171.8)
		(+/-)	838.280 (80.781)	1412.319 (187.729)	1.700 (0.324)	33.3 (5.8)	17.45 (7.63)	42179.8 (11280.5)
	EC	(+)	317.755 (131.397)	659.306 (35.360)	2.407 (1.232)	36.7 (5.8)	9.30 (2.68)	18862.0 (2296.0)
		non	302.987 (91.638)	650.403 (42.568)	2.305 (0.830)	40.0 (10.0)	8.94 (2.94)	21063.9 (4566.2)
		(-)	462.905 (85.107)	772.350 (68.093)	1.691 (0.202)	36.7 (5.8)	8.71 (2.61)	24296.2 (6492.9)
		(+/-)	421.176 (37.984)	691.990 (80.894)	1.665 (0.359)	40.0 (17.3)	6.77 (0.61)	25164.5 (13382.0)

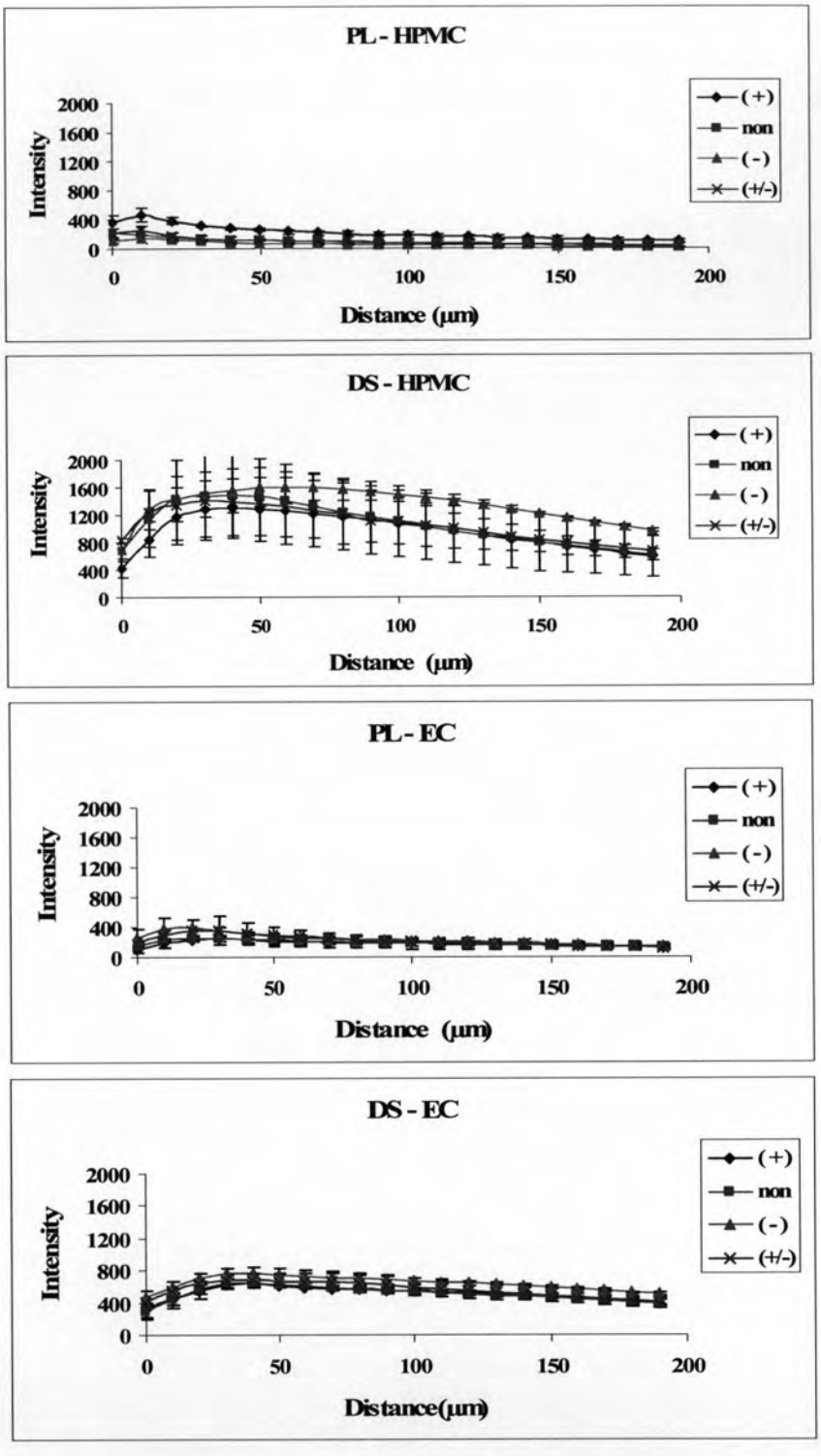


Figure 48 Fluorescence intensity (I) plotted against distance from initial surfaced layer (μm) of the coated pellets.

(+) : applied positive potential
non : non-applied electrical potential
(-) : applied negative potential
(+/-) : switching applied potential

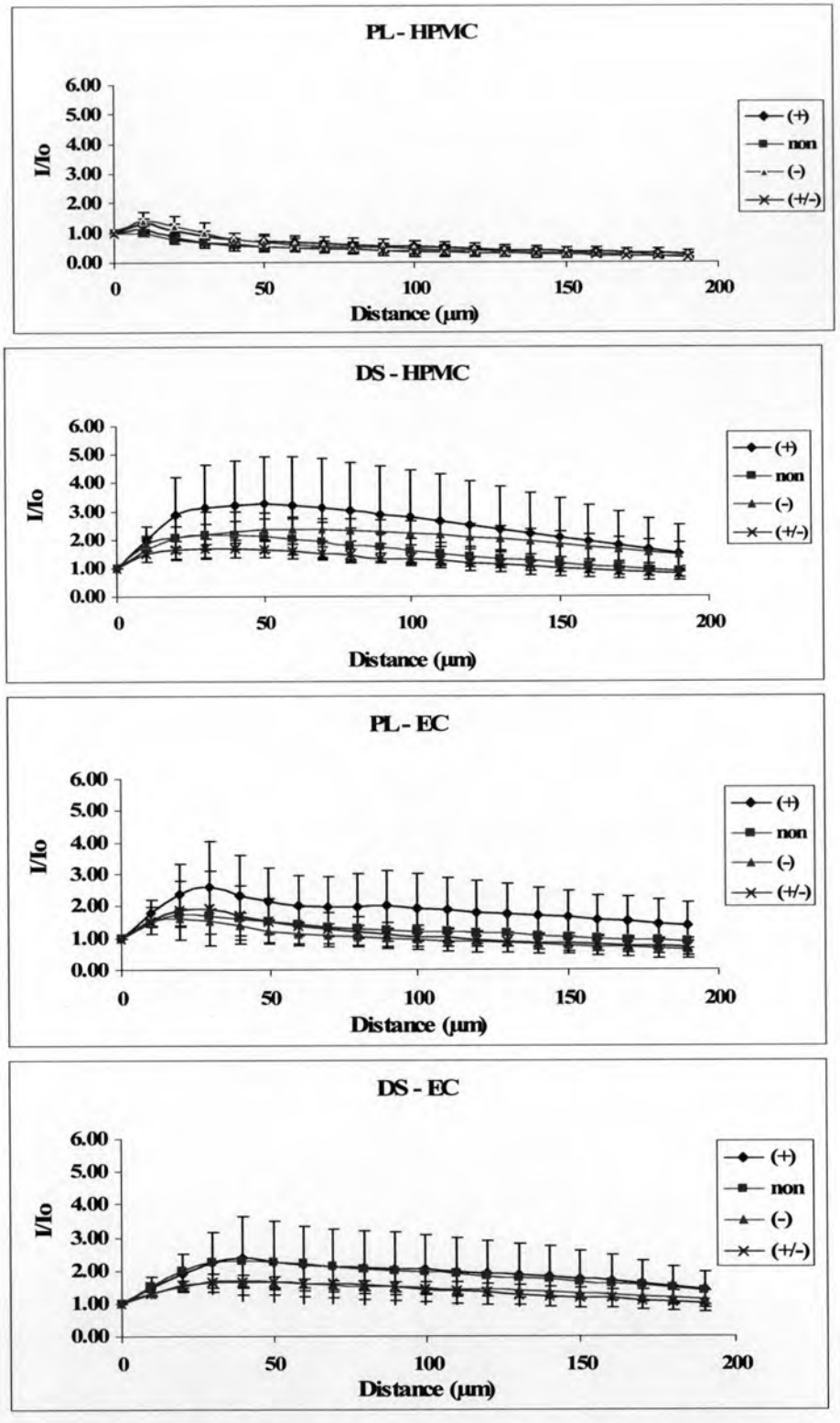


Figure 49 Relative fluorescence intensity (I/Io) plotted against distance from initial surfaced layer (μm) of the coated pellets.

(+) : applied positive potential non : non-applied electrical potential

(-) : applied negative potential (+/-) : switching applied potential

5.10 Dissolution studies

Dissolution profile of coated pellets was evaluated by USP/NF dissolution apparatus I (rotating basket method). Dissolution of PL coated pellets in diluted HCl (1:100) and DS coated pellets in phosphate buffer pH 6.8 are depicted in Figures 50 to 53.

EC is insoluble film former while HPMC is soluble film former. Therefore the drug release from EC coated pellets was sustained. The release of both drugs from HPMC coated pellets almost reached 100% in 40 minutes (Figures 50 and 51). On the other hand, less than 40 % of both drugs pellets were released from EC coated pellets in 24 hours (Figures 52 and 53).

Total area under the curve (AUC_{disso}) of percent of drug release was calculated based on trapezoidal rule up to 40 minutes for HPMC coating and 24 hours for EC coating. The average values from three replicate was presented in Table 23. From the obtained data, it was found that AUC_{disso} were significantly affected by types of drug core pellets and applied electrical potential ($p<0.05$).

In the case of HPMC coating, AUC_{disso} of PL coated pellets were higher than that of DS pellets in any case of applied electrical potential. This result may be due to lower film thickness and lower hardness of PL pellets. In contrast, AUC_{disso} of PL coated pellets were lower than that of DS pellets in any case of EC coating. However this result is inconsistent with film thickness and hardness studied of EC coated pellets.

Although the relationship between the properties of film obtained from varied coating conditions could not be established, it was observed that, among the results of the same core pellets and coating agent, the extent of drug release was likely higher when was not applied electrical potential to the coating process.

This could imply that applied electrical potential to the nozzle in coating process may be greater adhesion between core pellets and coating agent and the better coalescence of film were formed.

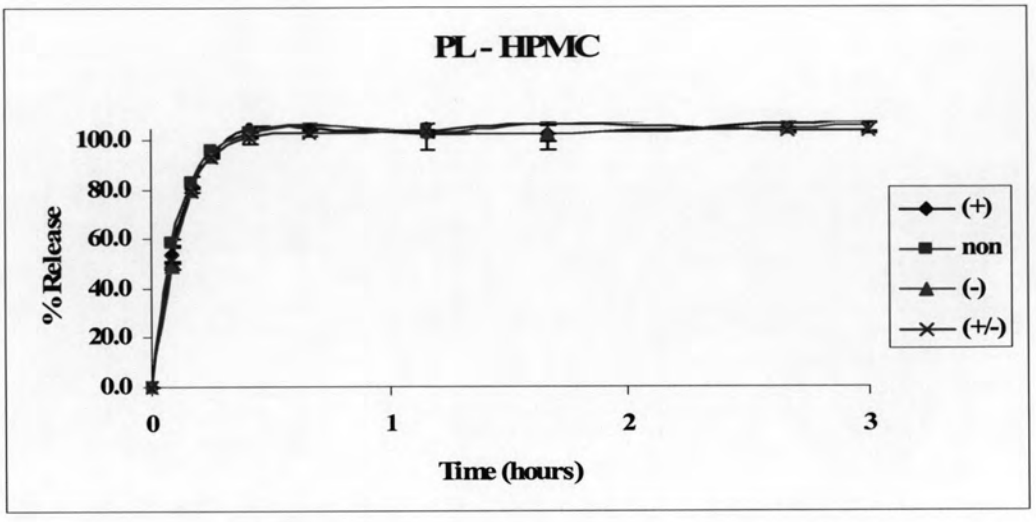


Figure 50 The dissolution profile of HPMC coated PL pellets (PL-HPMC) with various applied voltage.

(+) : applied positive potential
non : non-applied electrical potential
(-) : applied negative potential
(+/-) : switching applied potential

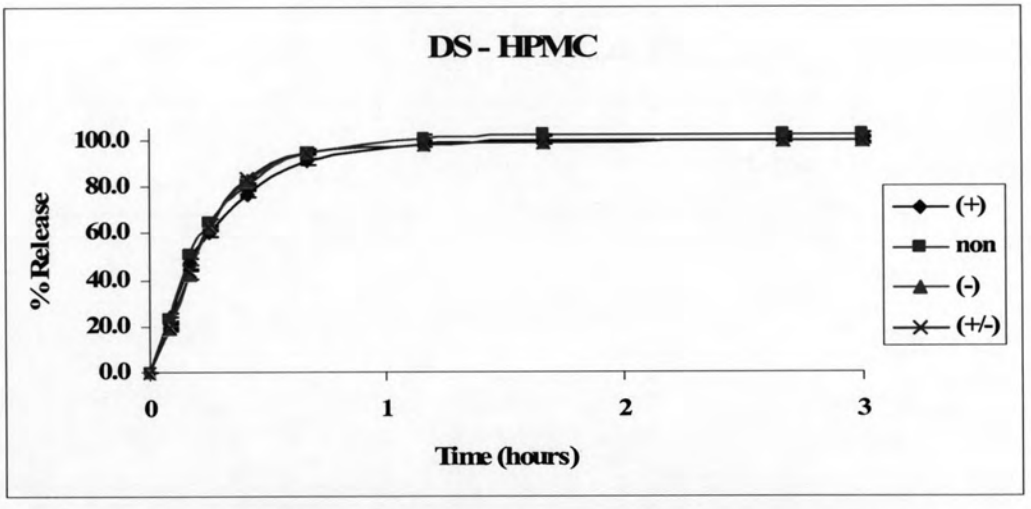


Figure 51 The dissolution profile of HPMC coated DS pellets (DS-HPMC) with various applied voltage.

(+) : applied positive potential
non : non-applied electrical potential
(-) : applied negative potential
(+/-) : switching applied potential

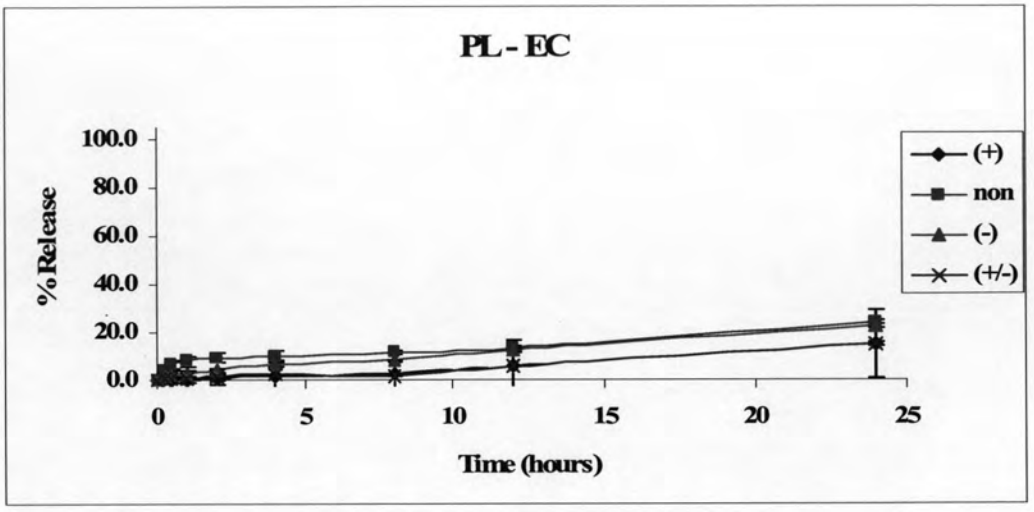


Figure 52 The dissolution profile of EC coated PL pellets (PL-EC) with various applied voltage.

(+) : applied positive potential non : non-applied electrical potential

(-) : applied negative potential (+/-) : switching applied potential

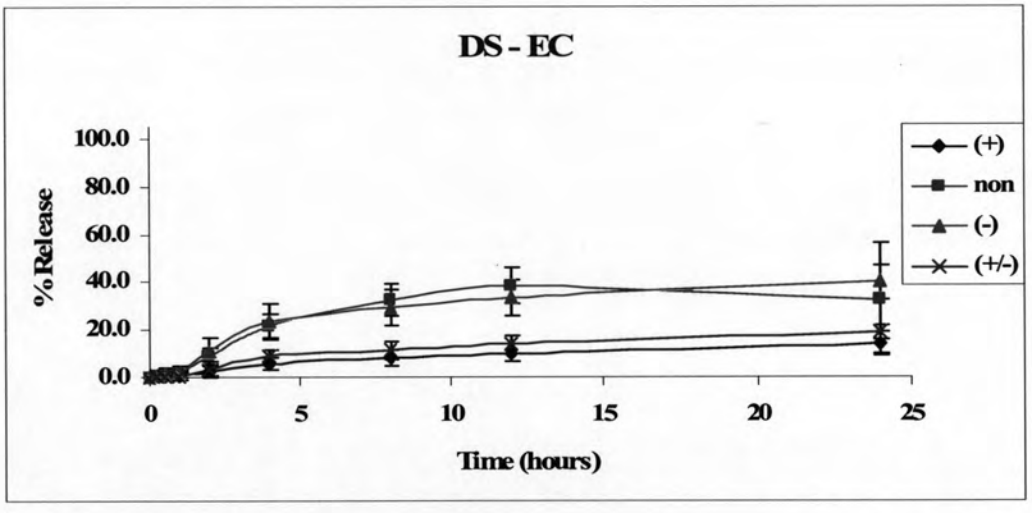


Figure 53 The dissolution profile of EC coated DS pellets (DS-EC) coated pellets with various applied voltage.

(+) : applied positive potential non : non-applied electrical potential

(-) : applied negative potential (+/-) : switching applied potential

Table 23 The area under the curve (AUC_{disso}) of propranolol hydrochloride pellets in diluted hydrochloric acid (1:100) and diclofenac sodium pellets in phosphate buffer pH 6.8, based on assayed content.

Formulation	Applied potential	Area under the curve (AUC) (%h)				
		1	2	3	Mean	SD
PL-HPMC	(+)	57.83	58.91	58.64	58.46	0.56
	non	59.37	60.09	58.18	59.21	0.97
	(-)	56.76	57.99	57.77	57.51	0.66
	(+/-)	57.34	57.31	56.68	57.11	0.37
PL-EC	(+)	191.18	157.66	120.32	156.39	35.45
	non	308.72	435.11	314.96	352.93	71.24
	(-)	250.10	300.58	336.64	295.78	43.47
	(+/-)	201.65	112.72	157.41	157.26	44.46
DS-HPMC	(+)	40.94	42.04	40.68	41.22	0.72
	non	44.29	43.17	42.11	43.19	1.09
	(-)	41.78	42.02	42.81	42.20	0.54
	(+/-)	42.17	42.88	42.87	42.64	0.40
DS-EC	(+)	313.71	203.21	151.66	222.86	82.79
	non	886.82	634.38	627.02	716.07	147.91
	(-)	910.72	663.65	580.97	718.45	171.57
	(+/-)	253.25	271.71	390.55	305.17	74.52

PL-HPMC : HPMC coated PL pellets
PL-EC : EC coated PL pellets
DS-HPMC : HPMC coated DS pellets
DS-EC : EC coated DS pellets

(+) : applied positive potential
non : non-applied electrical potential
(-) : applied negative potential
(+/-) : switching applied potential

5.11 Statistical analysis

From the data obtained, it was found that complex results were observed when types of film former was included in the analysis of variance. Due to the fact that changing the coating agent, the coating conditions were also changed. So, the statistic analysis were evaluated effect of types of drug and applied electrical voltage on the properties of coated pellets distinguished by types of film former as shown in Table 24 and 25.

Generally, types of drug core pellets and applied electrical potential in both types of film former significantly influenced on bulk density, flow rate, crushing force, film thickness and AUC_{disso} of coated pellets.

In addition, types of drug core pellets significantly influenced on tapped density, % compressibility, CLSM-thickness and AUC_{CLSM}, whereas applied electrical potential affected on %compressibility, CLSM-thickness and AUC_{CLSM} of HPMC coating; tapped density and roundness of EC coated pellets.

The significant difference between each of potential applied and non-applied electrical potential was analyzed by post hoc analysis and the result as shown in Table 26. In many cases, such as bulk density, flow rate and AUC_{disso}, the effect of applied electrical potential was significantly different from that of non-applied electrical potential.

According to the results of evaluation of coated pellets in present study, the result of applied opposite potential of core pellets to the nozzle in a bottom spray fluidized bed coating was not shown clearly difference from that of non-applied potential in any drugs core pellets or film formers. Although, the reproducibility of coating efficiency was improved by applied electrical potential, the effect was not dependent on the types of charge applied.

Many reasons could be raised to discuss. First, this may be according to small build-up charges on the drugs core surface. Also, unpredictable of charges on the surface of core pellets from tribocharging during fluidization has been observed by Mountain et al. (2001). In the present study, it was shown by Faraday method that positive charge and negative charge were not always detected on PL pellets and DS pellets, respectively. In addition, the amount of microcrystalline cellulose in the core pellets (50 %w/w) may affect on types of charges on the core surface.

Next, the charges of sprayed droplets could be possible opposite to the applied potential if they were charged by induction mechanism. For instance, by this mechanism applied positive voltage to the nozzle would induce negatively charged droplet, resulting in improved coating efficiency as shown in Table 21, for PL pellets whose charge was positive. Moreover, unpredictable charge or possibility of charge built-up or lost during spraying droplets of coating agent out of the nozzle with the

high pressure, as well as the distance of droplet travel before depositing on the surface of the pellets may be the other aspects.

The potential of random occurrence of charged type on core pellets and sprayed droplets may induce randomly deposit of sprayed droplets attracted to the surface of pellets. In addition, apart from that the charged droplets would prefer deposition on unlike charged to like charged materials. It has been reported that charged particles tended to be attracted to neutral surface (Barletta and Tagliaferri, 2006).

Moreover, some additives, such as sodium lauryl sulphate (an anionic surfactant) in the aqueous colloidal ethylcellulose dispersion (Aquacoat®) or 6-carboxyfluorescein (anionic fluorescence dye) which was used as an indicator in coating agent for CLSM evaluated should be considered. It might be possible that this excipients interfered the effect of applied electrical potential in electrostatic coating technique.

Besides the nature of polymer which is more favorable to film formation, high atomizing air and low feed rate primarily produce finer droplets and allow sufficient time for film forming before next droplets deposit on the surface of pellets. This resulted in better film formation in HPMC coatings.

Table 24 The results of analysis of variance with full model distinguished by types of film former (I).

Response / Factors		Bulk density		Tapped density		% Compressibility		Flow rate		Aspect ratio		Roundness		Crushing force	
		HPMC	EC	HPMC	EC	HPMC	EC	HPMC	EC	HPMC	EC	HPMC	EC	HPMC	EC
Drug	*F*	517.231	28.444	608.444	72.000	11.571	4.961	67.711	9.069	1.451	33.216	1.396	0.987	18.833	7.860
	p	**0.000**	**0.000**	**0.000**	**0.000**	**0.004**	**0.041**	**0.000**	**0.008**	0.229	**0.000**	0.238	0.321	**0.000**	**0.005**
Potential	*F*	3.385	144.741	0.889	630.667	7.381	0.936	5245	15.063	1.679	1.191	1.391	3.709	5.554	2.645
	p	**0.044**	**0.000**	0.468	**0.000**	**0.003**	0.446	**0.010**	**0.000**	0.170	0.312	0.244	**0.011**	**0.001**	**0.049**
Drug * Potential	*F*	3.385	101.630	10.815	409.333	8.524	2.335	3.073	3.743	1.080	0.754	0.807	6.673	1.305	3.034
	p	**0.044**	**0.000**	**0.000**	**0.000**	**0.001**	0.112	0.058	0.033	0.357	0.520	0.456	**0.000**	0.272	**0.029**
Levene's test		S	NS	NS	S	NS	S	NS	NS	S	S	S	S	NS	NS

HPMC : **Hydroxypropyl methylcellulose coating**

EC : **Ethylcellulose coating**

Drug : **Types of drug core pellets**

Potential : **Applied electrical potential**

F : **Mean square between group/ Mean square within group**

p : ***p*- value**

S : **Significant difference at a significant level ($p \leq 0.05$)**

NS : **No significant difference at a significant level ($p \leq 0.05$)**

Levene's test : **Levene's test of homogeneity of variance**

Table 25 The results of analysis of variance with full model distinguished by types of film former (II).

Response / Factors		% Coating efficiency		Film thickness		CLSM-thickness		Slope		AUC_{CLSM}		AUC_{disso}	
		HPMC	EC	HPMC	EC	HPMC	EC	HPMC	EC	HPMC	EC	HPMC	EC
Drug	*F*	1.861	3.438	50.258	16.807	84.100	14.727	19.763	1.882	121.901	44.593	2.994E3	40.600
	p	0.191	0.082	**0.000**	**0.000**	**0.000**	**0.001**	**0.000**	0.189	**0.000**	**0.000**	**0.000**	**0.000**
Potential	*F*	0.351	1.050	45.928	29.859	3.833	0.788	0.565	0.939	3.910	0.441	5.455	21.121
	p	0.789	0.398	**0.000**	**0.000**	**0.030**	0.518	0.646	0.445	**0.029**	0.727	**0.009**	**0.000**
Drug * Potential	*F*	0.093	7.794	11.983	23.345	1.730	0.545	0.264	1.615	4.239	0.347	4.152	4.693
	p	0.963	**0.002**	**0.000**	**0.000**	0.201	0.658	0.851	0.225	**0.022**	0.792	**0.024**	**0.016**
Levene's test		NS	S	NS	NS	S	NS	NS	NS	S	S	NS	S

HPMC : Hydroxypropyl methylcellulose coating

EC : Ethylcellulose coating

Drug : Types of drug core pellets

Potential : Applied electrical potential

F : Mean square between group/ Mean square within group

p : *p*- value

S : Significant difference at a significant level ($p \leq 0.05$)

NS : No significant difference at a significant level ($p \leq 0.05$)

Levene's test : Levene's test of homogeneity of variance

Table 26 The results of post hoc analysis for applied electrical potential

Response		Bulk density		Tapped density	% Compressibility	Flow rate		Round ness	Crushing force		Film thickness		CLSM Thickness	AUC_{CLSM}	AUC_{disso}	
Potential		HPMC	EC[b]	EC[a]	HPMC[b]	HPMC[b]	EC[b]	EC	HPMC[b]	EC[b]	HPMC[b]	EC[b]	HPMC[b]	HPMC[b]	HPMC[b]	EC[a]
(+)	non	-	**0.031**	0.393	0.600	**0.010**	**0.005**	-	0.320	0.134	0.207	**0.000**	0.384	0.915	**0.004**	**0.043**
	(-)	-	0.078	0.979	0.301	**0.002**	0.646	-	0.799	0.600	**0.000**	**0.000**	0.093	**0.026**	0.971	0.099
	swit	-	**0.000**	0.069	**0.005**	0.110	**0.003**	-	**0.001**	**0.009**	**0.040**	**0.000**	0.198	0.481	0.933	0.827
(-)	non	-	**0.001**	0.310	0.600	0.436	**0.014**	-	0.212	0.328	**0.000**	0.978	**0.016**	**0.021**	**0.004**	0.997
	swit	-	**0.000**	0.089	**0.001**	0.060	**0.001**	-	**0.000**	**0.038**	**0.000**	**0.000**	**0.006**	**0.006**	0.961	0.160
swit	non	-	**0.000**	**0.033**	**0.002**	0.237	**0.000**	-	**0.017**	0.269	**0.001**	**0.000**	0.661	0.548	**0.005**	0.073

(+) : applied positive potential

non : non-applied electrical potential

(-) : applied negative potential

swit : switching applied potential

\- : Tamhane, Dunnett T3 and Games-Howell can not detect difference

a : Games-Howell test (Equal variances not assumed)

b : LSD test (Equal variances assumed)