CHAPTER III
EXPERIMENTAL

1. Materials

The following materials obtained from commercial sources were used.

1.1.Model drugs

- Diclofenac sodium (Batch No.051004-1, Henan, China, purchased from Utopian Co., Ltd.)
- Propranolol hydrochloride (Batch No.20050326, Jiangsu, China, purchased from Utopian Co., Ltd.)
- Diclofenac sodium DMSc Reference Standard (Control no.348004, Bureau of Drug and Narcotic, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand)

1.2. Excipients

- Ethylcellulose (Aquacoat® ECD Lot no. JN06816101, FMC BioPolymer, DE, USA)
- Hydroxypropyl methylcellulose (Methocel® E5-LV Lot no.TD23012406, Dow Chemical Company, LA, USA)
- Microcrystalline cellulose (Ceolus PH 101®, Lot no.1556, ASAHI KASEI Chemicals Corporation, Tokyo, Japan)
- Polyethylene glycol (PEG6000, Lot no. 427124/1 33901, Fluka, Buchs, Switzerland)
- Sunset yellow lake (Lot no. 6-601, Butterfield food ingredients Ltd., Norfolk, UK)
- 6-Carboxyfluorescein (Lot no.1284701, Fluka, Rehovot, Israel)

1.3 Chemicals

- Acetonitrile HPLC grade (Batch no.0565053, Fisher Scientific, Leicestershire, UK)
- Diethyl phthalate (Lot no. 325384/1 393, Fluka, Buchs, Switzerland)
- Hydrochloric acid (Lot no.B40076, J.T.baker, NJ, USA)

- Methanol HPLC grade (Batch no.0570489, Fisher Scientific, Leicestershire, UK)
- Methanol AR grade (Batch no. K31916809 314, Merck KGaA, Darmstadt, Germany)
- Ortho-phosphoric acid (Batch no. AA505024, Ajax Finechem, NSW, Australia)
- Procainamide hydrochloride (Lot no. 1310611, Fluka, Shanghai, China)
- Sodium dihydrogen phosphate (Batch no. AF502342, Ajax Finechem, NSW, Australia)
- Sodium hydroxide (Lot no. B464398 414, Merck KGaA, Damstadt, Germany)
- Sodium dodecyl sulphate (Lot no. Sep05, Srichand United Dispensary Co.,Ltd., Bangkok, Thailand)
- Tribasic sodium phosphate (Batch no. AF403101, Ajax Finechem, NSW, Australia)
- Triethyl citrate (Lot no. 1074419, Fluka, Steinheim, Germany)

2. Equipments

- Analytical Balance (Model PB3002, Mettler Toledo, Schwerzenbach, Switzerland and Model A200s, Sartorius Gbh, Goettingen, Germany)
- Confocal Laser Scanning Microscope (Model FV1000, Olympus, Tokyo, Japan)
- Differential Scanning Calorimeter (Model 822^e, Mettler Toledo, Schwerzenbach, Switzerland)
- Dissolution Apparatus (Model VK7000, Vankel, NJ, USA)
- Extruder (Model EXKS-1, Fuji Paudal Co.,Ltd., Osaka, Japan)
- Fluidized bed air suspension (Created by Department of Chemical Engineering, Faculty of Engineer, Chulalongkorn University, Bangkok, Thailand)
- Friabilator (Erweka TAR 20, Heusenstamm, Germany)
- High performance liquid chromatograph (Model SCL-10A VP, Shimadzu, Kyoto, Japan) assembled with
    - System controller (Model SCL-10A VP, Shimadzu, Japan)

  - Liquid chromatograph (Model LC-10AD VP, Shimadzu, Japan)
  - Degasser (Model DGU-14A, Shimadzu, Japan)
  - Auto injector (Model SIL-10AD VP, Shimadzu, Japan)
  - Column oven (Model CTO-10AS VP, Shimadzu, Japan)
  - UV-VIS detector (Model SPD-10A VP, Shimadzu, Japan)
- Hot air oven (Model UL80, Memmert, Munich, Germany)
- Image analysis software (Image-Pro® plus version 4.5 for Windows, Media Cybernetic, Inc.)
- Jolting volumeter (Modified by Department of Manufacturing Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand)
- Lazer diffraction particle sizer (Mastersizer 2000, Malvern instruments, Worcestershire, UK)
- Hotplate magnetic stirrer (Model M6, CAT, Germany)
- Moisture balance (Model HR83, Mettler Toledo, Schwerzenbach, Switzerland)
- pH meter (Model 210A+, Thermo orion, Germany)
- Planetary mixer (Model 5K5SS, Kitchen aid, Michigan, USA and Model EB20F, Crypto-peerless Ltd., London, UK)
- Powder tester (Model PT-N, Hosokawa Micron, Osaka, Japan)
- Scanning electron microscope (Model JSM-5800LV, Joel Ltd., Tokyo Japan)
- Sieve shaker (Filtra, Barcelona, Spain)
- Spheronizer (Model S320, Aeromatic-Field, Hamshire, England)
- Stereomicroscope (Model ML 9300, Meiji, Tokyo, Japan) linked with Digital camera (EOS100, Canon, Tokyo, Japan)
- Texture analyser (Model TA .XT Plus, Stable Micro Systems, Godalming, UK)
- Ultrasound transonic digital sonicator (Model T680/H, Elma, Singen, Germany)
- Ultrapycnometer 1000 (Quantachrome, NY, USA)
- Ultraviolet/visible spectrophotometer (Model V-530, Jasco, Tokyo, Japan)

3. Methods

1. Physical characterization of raw materials used in pelletization

The following physical properties of raw materials were evaluated.

1.1 Morphology

The shape and surface topography of raw materials were determined by scanning electron microscope (SEM). The samples were prepared by gold sputtering technique prior SEM examination. Photomicrographs of raw materials were taken in magnification of x200 and x1,000 at 15 kV.

1.2 Particle size and size distribution

The particle size and size distribution of raw materials were determined by laser light diffraction technique. The samples were dispersed in the medium (liquid paraffin), and measurement was made immediately to avoid agglomeration of the particles. The samples were determined in triplicate and the average of the mean diameters was calculated.

1.3 Angle of repose

Angle of repose was measured by using the Powder Tester. An excess quantity of raw material was passed through sieve 710 μm and filled in the glass funnel with 4 mm orifice positioned above a fixed diameter base. The angle of repose was determined by measuring the height of the cone of powder by laser light and calculating the angle of repose (α) from the following equation. Each sample was determined in triplicate and the results were averaged.

$$\alpha = \tan^{-1} \frac{H}{R}$$

1.4 Bulk, tapped densities, percent compressibility and percent cohesiveness

Bulk, tapped densities, percent compressibility and percent cohesiveness of raw materials were measured by using the Powder Tester. Each sample was determined in triplicate and the results were averaged.

Table 4 Flow properties and corresponding flowability parameters (USP28)

Flow character	Angle of Repose (degree)	Compressibility index (%)
Excellent	25-30	≤ 10
Good	31-35	11-15
Fair	36-40	16-20
Passable	41-45	21-25
Poor	46-55	26-31
Very poor	56-65	32-37
Very, very poor	> 66	> 38

1.5 Apparent density

Apparent densities of materials were determined by using helium gas displacement. The sample was dried at 45°C over night before analysis, then about 0.2-0.4 g of sample was weighed and filled in a micro cell. The apparent density averaged from five determinations was reported in term of g/cm^3.

2. Pelletization

2.1 Preparation of pellets

Pellets were prepared by extrusion-spheronization process using the formulation describes in Table 5.

Table 5 Formulation of propranolol hydrochloride and diclofenac sodium pellets

Ingredients	Amount of solids (%w/w)	Functions
Propranolol HCl or Diclofenac sodium	50	Active ingredient
Microcrystalline cellulose (Ceolus®101)	50	Extrusion aids
Deionized water	qs.	Binding liquid

Pelletization by extrusion/spheronization process consisted of five steps including dry mixing, granulation, extrusion, spheronization and drying. Active ingredient and microcrystalline cellulose were weighed and mixed for 15 minutes in a planetary mixer at the lowest speed. The necessary quantity of deionized water was gradually added and mixing continued for 20 minutes. Mixing was stopped to scrap the sides of the bowl at regular intervals. After that, the wet mass was extruded by a radial single screw extruder with a die of 1 mm diameter and 1 mm length. The extrudates obtained were transferred to a spheronizer. The extrudates were rolled into pellets on a spinning friction plate of the spheronizer at 800 rpm for 5 minutes. The product was then dried in a hot air oven overnight at 60°C.

2.2 Characterization of pellets

2.2.1 Morphology

Scanning electron microscope (SEM) was used to examine shape and surface topography of pellets. The samples were prepared by gold sputtering technique prior to microscopic examination. Photomicrographs of pellets were taken in magnification of x30, x100 and x1,000 at 15 kV.

2.2.2 Particle size and size distribution

Size distribution of pellets were classified by sieve analysis which consisted of a set of US standard sieves, ranging from sieve no. 14, 16, 20, 30, 40 mesh (passing apertures of 1,400, 1,180, 850, 600 and 425 μm respectively) and a collecting pan. One hundred grams of pellets were accurately weighed and placed on the top of the sieves. A set of sieves were placed on the sieve shaker and allowed to shake for 10 minutes. The retained pellets on each sieve size were weighed and calculated for the percentage of weight retained on sieve by following equation:

$$\% \text{ Retained} = \frac{\text{Retained weight (g)}}{\text{Total pellet weight (g)}} \times 100$$

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

2.2.3 Moisture content

The moisture content of pellets was determined by moisture balance. About 2 g of pellets were accurately weighed and uniformly spread as thin layer on an aluminium plate. Then, they were exposed to high temperature of approximately 105°C until constant weight was obtained. The moisture content in terms of loss on drying was calculated automatically. The results were obtained from an average of three determinations.

2.2.4 Bulk, tapped densities and compressibility index

The bulk density (ρ_b) of the pellets was determined by pouring 20 g of the pellets into a 50 ml graduate cylinder and measuring the volume of pellets. The graduate cylinder was tapped on a jolting volumeter until a constant volume was obtained. The tapped density (ρ_t) was then calculated. Both densities were average from three determinations. The Carr's compressibility, which expresses the flow property as presented in Table 4, was calculated from the following equation:

$$\% \text{ Compressibility} = \frac{(\rho_t - \rho_b)}{\rho_t} x 100$$

2.2.5 Apparent pellet density

The apparent density of pellet was determined by Ultrapycnometer 1000, Quantachrome by gas displacement (He gas). The sample was dried at 45°C over night before analysis, then taken in dessicator 15-20 minutes. About 0.7-0.8 g of sample was weighed and filled in a micro cell. The apparent density was average from five determinations and reported in term of g/cm^3.

2.2.6 Percent friability

Ten grams of pellets were filled into a polypropylene container. Five metal spheres of 5 mm diameter were added into the container and firmly closed with the cap. The container was rotated in the friabilator at 25 rpm for 4 minutes. After that, pellets finer than 40 mesh (425 μm) was sieved off. The average result from three determinations was reported as percentage of weight loss.

2.2.7 Flow rate

Accurately weighed amount of about 20 g of pellets were filled in a glass funnel with 1.2 cm internal stem diameter fixed on the clamp. When the pellets started to flow until finished, the time was recorded. Flow rate was average from three determinations and reported in term of g/s

2.2.8 Angle of repose

The angle of repose was measured from a heap built up by falling of 20 g of the pellets samples through a glass funnel with 1.2 cm internal stem diameter fixed on the clamp at 10 cm height from the smooth surface. Average result from five determinations was reported. The angle of repose was calculated from the following equation:

$$\alpha = \tan^{-1} \frac{H}{R}$$

2.2.9 Sphericity of pellets

Photomicrographs of one hundred sample pellets of each model drug were taken by stereomicroscope linked with digital camera in magnification of x10 before examinations. The software program Image Pro® plus of Image analyzer was used to evaluate the sphericity of pellets. The aspect ratio and roundness were determined to indicate the sphericity of pellets in this study. Both parameters were calculated based on the following equation:

$$\text{Aspect ratio} = \frac{\text{Longest diameter or Feret maximum } (R_1)}{\text{Smallest diameter or Feret minimum } (R_2)}$$

$$\text{Roundness} = \frac{\text{perimeter}^2}{4\pi \text{ area of projected image}}$$

2.2.10 Crushing force

The crushing force of 50 pellets was determined using Texture Analyser with a compression mode. The 4 mm diameter cylinder stainless probe was used and the parameters of the texture analyser for measuring crushing force were as follows: pretest speed 0.1 mm/s, test speed 0.1 mm/s, posttest speed 10 mm/s, and

distance 40% strain. The first maximum of the force-time curve was taken as the crushing force of the pellet.

2.2.11 Charge determination of fluidized core pellets

Faraday cup method, as presented in Figure 25, was used to directly measure charges built up on the core pellets. Faraday cup or faraday pail consists of two metal cups which are electrically insulated from each other. After 15 min of pre-heating time of core pellets fluidized inside acrylic chamber the pellets were immediately transferred to the inner cup of faraday pail. The charge inserted inside the inner cup generates a potential difference between the cups; and the potential was measured and transformed to coulomb unit as the degree of charges by electrometer (Model 617, Keithley®, USA). The measurement was repeated at least five times for core pellets of each drug.

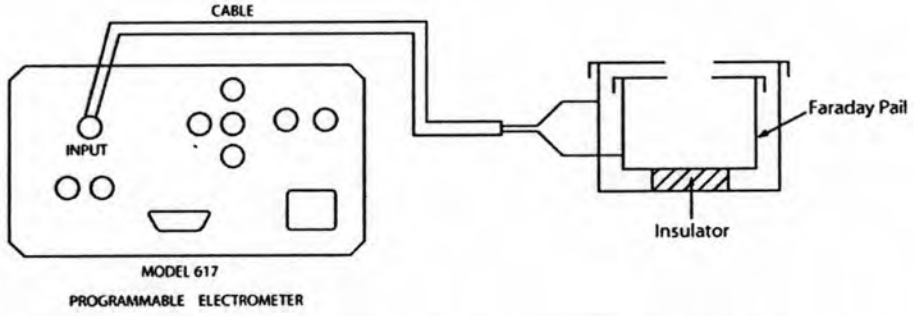

Figure 25 Schematic diagram of charge determination by Faraday cup method

2.2.12 Drugs content in pellets

The drug content of propranolol hydrochloride and diclofenac sodium in the pellets was quantitatively determined by mean of absorption peak area from high performance liquid chromatography (HPLC) method.

HPLC analysis

HPLC chromatographic conditions:

Propranolol hydrochloride pellets

Column	: Thermo® C8 column (4.6 x 250 mm) , 5 µm (USA)
Mobile phase	: SLS–0.15M phosphoric acid–Acetonitrile-Methanol
Flow rate	: 1.0 ml/min
Injection volume	: 20 µl
Detector	: UV 290 nm
Retention times	: Propranolol hydrochloride 9 min
	Procainamide hydrochloride 5 min

Diclofenac sodium pellets

Column	: Thermo® C8 column (4.6 x 250 mm) , 5 µm (USA)
Mobile phase	: Phosphate buffer pH 2.5 : Methanol (30:70)
Flow rate	: 1.0 ml/min
Injection volume	: 20 µl
Detector	: UV 254 nm
Retention times	: Diclofenac sodium 9 min
	Diethyl phthalate 5 min

Validation of HPLC method

The typical analytical characteristics used in method validation were specificity, accuracy, precision, linearity and range (USP28/NF23, 2006).

Specificity

The specificity of an analytical method is the ability to assess the peak of model drug from the sample without interfered by other components, presented in the sample. The excipients included microcrystalline cellulose, hydroxypropylmethyl--cellulose, Aquacoat®, PEG6000, triethyl citrate and 6-carboxyfluorescein. Their chromatograms were compared with the chromatogram for the standard solution of the model drug.

Accuracy

The accuracy of an analytical method is the closeness of the test results obtained by that method to the true value. The model drug standard concentrations of 100, 200, 300, 400 and 500 µg/ml were spiked in the placebo solution and injected. Accuracy was calculated as the percentage of recovery of each standard solution. The mean percentage of recovery of 95-105% with percent of coefficient of variation (%RSD) <2.00% indicates the high accuracy of the method.

Precision

The precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatly to multiple samplings of homogenous sample. The percentage of coefficient of variation (%CV) or relative standard deviation (%RSD) values of peak area of drugs both within run and between run of less than 2.00 % which indicates that HPLC methods can be used to determine the amount of model drugs over period of time studied.

Within run precision (Repeatability)

Five sets of five standard solution of model drug were analyzed on the same day to determine within run precision. The percentage of coefficient of variation (%CV) or relative standard deviation (%RSD) values of peak area of the drug from each concentration were determined.

Between run precision (Reproducibility)

The between run precision was determined by comparing each concentration of drug standard solution prepared and analyzed on different days. The percentage of relative standard deviation (%RSD) values of concentration of drugs from two sets of the calibration curves was determined.

Linearity

The linearity of an analytical method is the ability to elicit test results that are directly proportional to the concentration of drugs in samples within a given range. Five replicates of each concentration of standard solutions containing the model drugs in various concentrations ranging from 100 to 500 µg/ml were prepared

and analyzed. The linear regression analysis of the curve obtained by plotting the absorbance versus the concentrations was calculated.

Standard preparation

Propranolol hydrochloride (PL) and Diclofenac sodium (DS)

Working standard was accurately weighed about 125 mg to 50 ml volumetric flask, then diluted with methanol (for PL) or diluent (MeOH:H_2O =70:30, for DS) to volume. This solution was used as the standard stock solution. The 3.0 ml of stock solution were transfered to 25 ml volumetric flask, diluted with methanol or diluent to volume. The final concentration of standard solution was 300 µg/ml.

Resolution solution

Propranolol hydrochloride

Procainamide hydrochloride of was accurately weighed about 7 mg to 25 ml volumetric flask, then diluted with methanol (HPLC grade) to volume. Pipetted 5.0 ml of this solution and 5.0 ml of propranolol HCl standard stock solution to 25 ml volumetric flask, diluted with methanol to volume and mixed.

Diclofenac sodium

Ten microliters of diethyl phthalate was pipetted to 50 ml volumetric flask and diluted to volume with diluent (MeOH:H_2O =70:30). Transferred 1.0 ml of this solution and 3.0 ml of diclofenac sodium stock standard solution to 25 ml volumetric flask, diluted with diluent to volume and mixed.

Assay preparation

Propranolol hydrochloride (PL) and Diclofenac sodium (DS)

Two grams of pellets were weighed and pulverized by mortar and pestle. Then, accurately weighed powder equivalent to 50 mg of propranolol HCl or 75 mg of diclofenac sodium into 50 ml volumetric flask, diluted and adjusted to volume with methanol (HPLC grade). The 5 ml of solution was pipetted and transfered into 25 ml volumetric flask. The solution was adjusted to volume with methanol (for PL) or diluent (for DS) and mixed thoroughly. The final concentration of sample solution

was 200 µg/ml for PL and 300 µg/ml for DS. Each sample was determined in triplicate.

All solutions were filtered through 0.45 µm membrane filter before analysis.

3. Preliminary study on the coating process

In coating process, there may be many parameters involved and these process parameters may affect the character of final product. In preliminary, therefore, the effect of atomizing air pressure and feed rate of coating agent were screened. The coating parameters of coating condition as shown in Table 6.

Firstly, one hundred grams of the core pellets were pre-heated in the fluidized bed coater for 15 min at 60°C to obtain the appropriated core pellets temperature before coating. The inlet air temperature was 60 ± 2°C. Then, the coating agent was sprayed with the different atomizing air pressures and feed rates, as shown in Table 7. The expected weight gained of coated pellets was 10 % of initial pellets weight. The coated pellets were post-heated in fluidized bed coater for 30 min at 60°C and then the pellets were transferred to a tray and were cured in the hot air oven for 12 hours at 60°C to obtain the completed film formation. In this section, the electrical potential was not applied to the spray nozzle.

Table 6 Coating conditions for preliminary study.

Parameter	Value
Pellets load	150.00 g
Pre-heating time , temperature	15 min , 60°C
Inlet air temperature	60 ± 2°C
Fluidized air velocity	5.5 m/s
Post-heating time , temperature	30 min , 60°C
Curing time, temperature (Hot air oven)	12 h , 60°C

Target weight gained = 10 %

Table 7 Coating condition in the preliminary study (continue).

Coating condition	Atomizing air pressure (kg/cm^2)	Feed rate (ml/min)
HPMC-A	1	5
HPMC-B		10
HPMC-C		15
HPMC-D	2	5
HPMC-E		10
HPMC-F		15
EC-A	1	5
EC-B		10
EC-C		15
EC-D	2	5
EC-E		10
EC-F		15

Two coating agents with different type of film formers were prepared. Composition of coating agent is presented in Table 8. Coating solution (C1) was prepared by dispersing hydroxylpropylmethylcellulose (HPMC) in 70°C deionized water. Then, cold deionized water was added to obtain clear solution. After that, dissolved PEG6000 (32 % of solid weight of HPMC) and dispersed sunset yellow lake in deionized water were added into the solution.

The aqueous dispersion of ethylcellulose (Aquacoat® ECD, 30% solid content) was diluted 1:1 with water to prepare coating agent (C2). Triethyl citrate (24 % of solid weight of EC) and dispersed sunset yellow lake in deionized water were added into the diluted dispersion.

Both coating agents were stirred continuously for 18 hours at room temperature before use. Six coating conditions (A to F) according to those presented in Table 6 of each of the film formers were performed.

Table 8 Composition of coating agent in preliminary study.

Ingredients	%w/w	
	C1	C2
Hydroxypropyl methylcellulose (HPMC, E5)	5.00	-
Ethylcellulose (EC, Aquacoat® ECD)	-	47.44[a]
Polyethylene glycol 6000(PEG 6000)	1.60	-
Triethyl citrate (TEC)	-	3.41
Sunset yellow lake	0.60	1.71
Deionized water	92.80	47.44

[a] Weight of Aquacoat dispersion

In the first set of experiment, PL pellets were used as the core pellets. The coated PL pellets were evaluated in term of processability, appearance of film color, occurrence of agglomeration between pellets and % coating efficiency. The coating condition that yielded coated pellets with good appearance, no or few agglomeration, less sticky to filter and relative high percent coating efficiency were chosen to process the second set of experiment using DS pellets to be core pellets. The coating conditions selected for further study were the conditions optimized for coating both PL and DS pellets.

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

4.1 Composition of coating agent

In this section, the coating agents were prepared by the method previously described in the preliminary study, except for 6-carboxyfluorescein (6-FAM) was used instead of sunset yellow lake to provide the good contrast against the core material when film was characterized with confocal laser scanning microscopy. The compositions of two coating agents were presented in Table 9.

The 6-FAM was accurately weighed about 1.45 mg to 100 ml volumetric flask, then diluted with water to volume. This solution was used as 6-FAM stock solution (concentration 0.0145 mg/ml). The 7 ml of stock solution equivalent to

101.5 µg of 6-FAM was pipetted and added into the 400 g of coating agents C1 and 133.34 g of C2 respectively, which were used for each condition.

Table 9 Composition of coating agent in electrostatic coating

Ingredients	%w/w	
	C1	C2
Hydroxypropyl methylcellulose (HPMC, E5)	5.00	-
Ethylcellulose (EC, Aquacoat® ECD)	-	48.26 [a]
Polyethylene glycol 6000 (PEG 6000)	1.60	-
Triethyl citrate (TEC)	-	3.47
6- Carboxyfluorescein (6-FAM)	*	*
Deionized water	93.40	48.26

[a] Weight of Aquacoat dispersion

*cannot display percent value due to very low amount of 25.38 µg (C1) and 76.12 µg (C2) based on 100 g of coating agent.

4.2 Electrostatic coating process parameters

The experimental design used to study the effect of coating process variables: core pellets, film formers and applied electrical potential are shown in Table 10 and carried out in triplicate.

Table 10 Experimental design (n = 3)

Core pellets	Film formers	Applied electrical potential **
Propranolol hydrochloride (PL)	Hydroxypropylmethylcellulose (HPMC)	(+) 4 kV
		(-) 4 kV
		(0)
		Switching (+/-) 4 kV
	Ethylcellulose (EC)	(+) 4 kV
		(-) 4 kV
		(0)
		Switching (+/-) 4 kV
Diclofenac sodium (DS)	Hydroxypropylmethylcellulose (HPMC)	(+) 4 kV
		(-) 4 kV
		(0)
		Switching (+/-) 4 kV
	Ethylcellulose (EC)	(+) 4 kV
		(-) 4 kV
		(0)
		Switching (+/-) 4 kV

** (+) : positive charge (-) : negative charge (0) : non-applied electrical potential
switching : switch charge of applied electrical potential (+/-/+/-)

The pellets were coated using bottom spray enhanced electrostatic fluidized bed as depicted in Figure 26. The selected coating conditions from the preliminary study were used in this section. The expected weight gain of electrostatic coated pellets was 20 % of initial pellets weight. Total coating time validated was 80 min for HPMC and 16 min for EC; difference in coating time due to different concentration of film formers in coating agent. For switching to opposite electrical potential, coating time was divided into four constant intervals, always starting by applying positively charged potential.

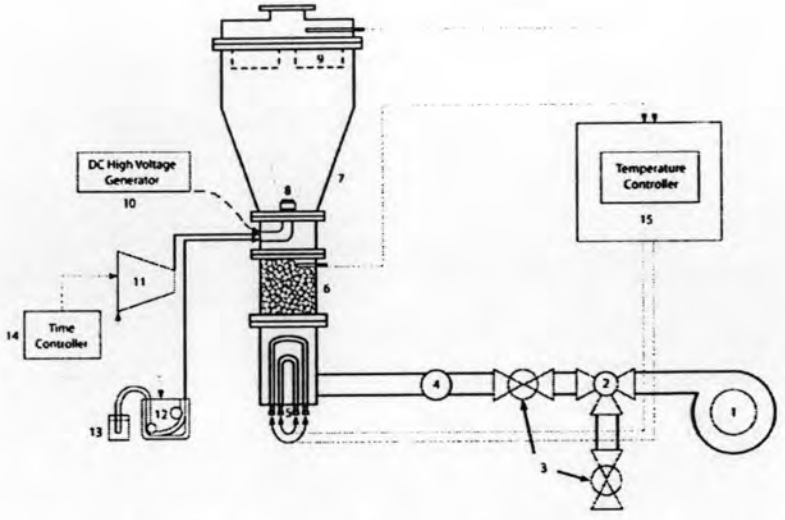

Figure 26 Schematic diagram of the bottom spray enhanced electrostatic fluidized bed 1) Blower 2) 3-ways Valve 3) Ball valve 4) Gauge 5) Heater 6) Air distributer 7) Fluidized bed chamber 8) Nozzle 9) Bag filter 10) High Voltage DC Generator 11) Air compressor 12) Peristaltic pump 13) Coating agent 14) Time controller 15) Temperature controller.

In general, the coating process was as described in section 3. After pre-heating pellets in fluidized bed, the electrical potential was applied to the nozzle simultaneously sprayed the droplets of coating agent until expected weight gain was obtained.

4.2.1 Types of drug core pellets.

Propranolol HCl (PL, cationic drug) and diclofenac sodium (DS, anionic drug) were selected to prepare core pellets and expected that they should have different charges on the surface during fluidization process.

4.2.2 Types of film formers.

Two non-ionic film formers commonly used in pharmaceutical industry, hydroxypropylmethylcellulose (HPMC) and ethylcellulose (EC), were selected to prepare coating agent in this study. However, they appeared in different forms. HPMC was available in powder form and prepared to be solution. EC is commercially available as an aqueous pseudolatex dispersion (Aquacoat®, 30% solid

content), containing various excipients for stabilization and improved film formation, which is ready to use by diluting with water.

4.2.3 Applied electrical potential.

Applied electrical potential was varied in three types: positive (+), negative (-) and switching between positive and negative (+/-) during coating process at the constant interval for each type of film formers. The magnitude of potential applied was 4 kV. For HPMC, switched applied electrical potential every 20 min while EC coating was switched every 4 min. Data obtained was compared to non-applied electrical potential (0).

It was hypothesized that the applied electrical potential onto the nozzle would induce charged droplets of coating agent. If it was opposite to the charge on fluidized pellets, the process gains improved coating efficiency by the attractive force between opposite charges as depicted in Figure 27

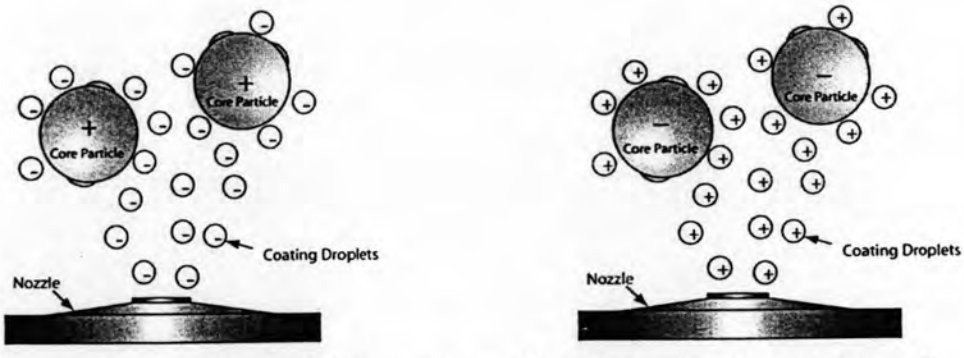

Figure 27 Attractive force between opposite charges in electrostatic coating

5. Evaluation of coated drug pellets

The physical properties of coated pellets such as morphology, moisture content, sphericity and crushing force of pellets compared to core pellets were evaluated. The methods used were the same as previously described in section 2.2. Other evaluations proceeded as the methods described below. Except for coating efficiency, sample of coated pellets for evaluation was taken from one experiment of each condition.

5.1 Percent coating efficiency

Core and coated pellets of each batch were accurately weighed before and after coating process. Percent coating efficiency was calculated from the following equation and average from triplicate.

$$\% \text{ Coating efficiency} = \frac{(\text{Weight of coated particles} - \text{Weight of uncoated particles}) \times 100}{\text{Total weight of coating material input (water-free)}}$$

5.2 Film thickness

To measure the film thickness of coated particles, the stereomicroscope linked with digital camera was used to capture images of one hundred sample pellets of each coating condition in magnification of x10. The mean diameter of each coated pellets was determined by image analyzing program (Image Pro® plus version 4.5). The averaged diameter of coated pellets was calculated. The film thickness of coated pellets was calculated by using the following equation:

$$\text{Film thickness} = \frac{\text{averaged diameter of coated pellets} - \text{averaged diameter of core pellets}}{2}$$

5.3 Film properties evaluated by Confocal Laser Scanning Microscopy (CLSM)

For CLSM analysis, three coated pellets from each coating condition were determined. The pellets were individually placed on a cover glass, then mounted with glycerol before evaluation. A CLSM (Olympus, Japan) equipped with a multi-line Ar laser (Olympus Fluoroview FV1000) and Olympus IX81 inverted microscope were used. Optical excitation was carried out using a wavelength of 488 nm and a laser power of 0.3 mW. The box size was 512 x 512 pixels. The pellets were scanned and recorded in 190 μm section from the surface of film coated pellet downwards to the core pellets at intervals of 10 μm in the Z direction. Kalman for N= 4 frames per Z level was set prior to initiation of the Z series. The relative intensity (I/I_0) and the average intensity (I) of fluorescence probe were plotted as a function of the distance

from the initial surfaced layer of pellet where I was the fluorescence intensity at any distance from the initial surfaced layer and I_0 was the fluorescent intensity of the "initial surfaced layer" of coated pellets.

The distance of which the maximum value of intensity (I_{max}) was obtained and considered to be the layer approaching to the core pellets. The distance from the initial surfaced layer to the layer of the maximum value of intensity may signify "CLSM- thickness" of the coating films.

The area under the curve (AUC_{CLSM}) of between the average intensity of fluorescence and the distance from initial surfaced layer of pellets calculated up to the peak value were compared and used as another measure of relative coating efficiency.

The slope of the linear portion of the curve between I_{max} and I_0, (I_{max}-I_0), and the distance up to the maximum peak was used to identify change in intensity and hence the distribution of fluorescence molecule across the film coating. The higher the slope, the more inhomogeneous film coating tended to be.

5.3 Drug contents of coated pellets

Determination of drug content of the coated pellets was performed using the method as previously described in 2.2.12.

5.4 Dissolution studies

The condition used for determination of drug release by using USP Dissolution Apparatus I (basket) as shown in Table 11.

Table 11 Dissolution conditions for coated pellets.

Condition	Propranolol HCl	Diclofenac sodium
Medium	diluted hydrochloric acid (1:100), 900 ml	phosphate buffer pH 6.8, 900 ml
Rotating speed (rpm)	100	100
Temperature (°C)	37 ± 0.5	37 ± 0.5
Total time (hours)	3 (HPMC), 24 (EC)	
UV wavelength (nm)	294	281

To obtain a sink condition, propranolol hydrochloride and diclofenac sodium coated pellets were accurately weighed equivalent to 24 mg and 48 mg of drug content respectively and then pellets were filled in the basket. Ten milliliters of specimens were withdrawn through filters at time intervals of 5, 10, 15, 25, 40, 70, 100, 160 and 180 minutes for HPMC coated pellets and at the time interval of 0.5, 1, 2, 4, 8, 12 and 24 hours for EC coated pellets. The same quantity of the fresh medium was replaced immediately after each sampling to keep the constant volume of the medium constant throughout the experiment.

The filtrate was diluted to a suitable concentration and assayed for the drug content by UV-VIS spectrophotometer using dissolution medium as blank. The amounts of drug release at any time were calculated from the calibration curve. A cumulative correction was achieved for the previously removed sample to determine the total amount of the drug release at each time interval.

The percent amounts of drugs released from pellets were plotted as a function of dissolution time and sum of area under the curve (AUC) was calculated based on trapezoidal rule as follow:

Area under the curve (AUC) at each time interval

= {½ x (%drug release at lower time interval + %drug release at higher time interval) x time interval}

Total area under the curve (total AUC)

= summation of AUC at each time interval

Each of drug release and AUC values reported was based on an average of three determinations of each formulation.

5.5 Statistical analysis

Data were analyzed using general linear model, ANOVA was performed.