CHAPTER III

EXPERIMENTAL

1. Materials

The following materials obtained from commercial sources were used.

1.1 Model drug

- Diclofenac sodium BP (bromine free) (Batch No. 20031109, Xinhui Medicine, China, purchased from Utopian Co.,Ltd.)
- Diclofenac sodium reference standard (Control No. 245004, Department of Medical Sciences, Ministry of Public Health)
- Diclofenac related compound A, USP reference standard (CAT No. 18881, Rockville)

1.2 Additives

- Lactose hydrous USP/NF/BP/EP/JP 200 mesh (Lot.No.00087527, Whydale, New Zealand)
- Dibasic calcium phosphate (Lot.No. 25489, Total chemical Co.,Ltd. Bangkok, Thailand)
- Glyceryl monostearate (flake) (Lot & Control No. 20609, Belgium, Srichand United dispensary Co.,Ltd., Bangkok, Thailand)
- Glyceryl behenate (Compritol 888 ATO®, Lot.No. 29482, supplied by Gattefosse, France)
- Glyceryl pamitostearate (Precirol® ATO5, Lot.No. 28994, supplied by Gattefosse, France)
- Glyceryl tristearate (Tristearin, Lot.No. 435283/1, Fluka, Germany)
- Saturated polyglycolized glycerides (Gelucire 50/02, Lot.No. 26422, supplied by Gattefosse, France)

1.3 Chemicals

- Methanol, HPLC grade (Batch No. 0441366, Fisher, UK)
- Hydrochloric, AR grade (Lot.No. K31937917 315, Merck KGaA,Darmstadt, Germany)
- Sodium hydroxide (Lot No.B247498 249, Merck KGaA, Germany)
- Tri-sodium orthophosphate (Batch No. AF 403101, Ajax Finechem, Australia)
- Sodium dihydrogen phosphate (Lot No. A 768946 407 , Merck, Germany)
- Orthophosphoric acid (Batch No. A1B021, Asia Pacific Specialty Chemicals Limited, Australia)

2. Equipment

- Planetary Mixer (Model A701A, Kenwood MFG.Ltd., Japan)
- Water bath (Model WB4P,Thermotek, USA)
- Analytical balance (Model A200s, Sartorius GmbH, Germany, Model UMT2, Mettler Toledo, Switzerland and Model PB3002, Mettler, Switzerland)
- Dissolution apparatus I (Model VK 7000, Vankel®, USA)
- High performance liquid chromatography (Model SCL-10AVP, Shimadzu, Japan)
- Ultraviolet/visible spectrophotrometer (Model V-530, Jasco, Japan)
- pH meter (Model 210 A+, Thermo orion, Germany)
- Friabilator (Erweka TAR 20, Germany)
- Sieve shaker (Filtra, Spain)
- Ultrasound transonic digital sonicator (Model T680/H, Elma, Germany)
- Differential scanning calorimeter (Model 822^e, Mettler, Switzerland)
- Scanning electron microscope (Model JSM 5410LV, Joel Ltd., Japan)
- X-ray diffractometer (Model JDX-3530, Jeol Ltd., Japan)
- Fourier transform infrared spectrometer (Model 1760X, Perkin Elmer, Germany)
- Ultrapycnometer 1000 (Quantachrome, USA)
- Viscometer DV II+ (Brookfield, USA)
- Laser diffraction particle sizer (Model 2601 Lc, Malvern Instrument, UK)

3. Methods

1. Material characterization

1.1 Scanning electron microscope (SEM)

The materials were examined under a scanning electron microscope (SEM) for morphological evaluation. The samples were prepared by gold sputtering technique before SEM examination. The shape and surface topography of materials were determined.

1.2 Particle size

The geometric mean particle size of the distribution by weight was determined by laser diffraction technique. The air pressure was 1.0 Bar. The feed rate was 25 g/sec. The geometric mean particle size of materials was averaged from three determinations.

1.3 Melting point

The melting point of materials was determined by a differential scanning calorimeter.

1.4 Viscosity

The viscosities of molten binders were determined at 5°C above the melting point of binders, except for GMS the viscosity of which was also measured at 15°C and 25°C above its melting point, by a viscometer with the spindle number 31. The viscosities of molten binders were averaged from three determinations.

1.5 True density

The true density of materials were determined by using helium gas displacement. The true density averaged from five determinations was reported in term of g/cm^3.

2. Pelletization

2.1 Preliminary study

Attempts were made to form blank pellets by two methods as following :

Method A : Binders were added as powdered form

Lactose and dibasic calcium phosphate were passed though a 30 mesh (595μm) sieve before use. The amounts of lactose or dibasic calcium phosphate and binder as presented in Table 1 were weighed and put into a jacketed planetary mixer. The temperature of circulating water was set at 10°C above the melting point of the studied binders. This allowed the temperature of mass within the bowl to be controllable around 5°C above the melting points for all the binders studied. The dry powders were mixed at 100 rpm for 10 minutes. When the temperature of the mixture reached the equilibrium, i.e. 5°C above the melting point of the binders studied, the mixture was then mixed at a paddle speed of 200 rpm. The formation of pellets was observed every 3-5 minutes. The process was stopped when the products were stable for 3 minutes and the processing time was recorded.

Method B : Binders were added as molten form

Lactose and dibasic calcium phosphate were passed though a 30 mesh (595μm) sieve before use. The 250 g of lactose or dibasic calcium phosphate were weighed and put into a jacketed planetary mixer. The 70 g of binder as presented in Table 1 was melted at the temperature of 5°C above its melting point. When the temperature of the powder reached the equilibrium, i.e. 5°C above the melting point of the binders studied, the binder was slowly added with constant mixing at a paddle speed 200 rpm. The formation of pellets was observed every 3-5 minutes. If the pellets had not occurred, the binder was slowly added more. The process was stopped when the pellets were formed and the products were stable for 3 minutes. The mixing time was the time taken

from beginning of binder addition until the products were stable for 3 minutes. The amounts of binder were calculated.

Table 1 Properties of binders that were used in the preliminary study

Binders	HLB	Melting point (°C)	Viscosity at MP + 5°C (mPa.s)
Glyceryl monostearate	3.8	53	60.8 ± 0.46
Precirol ® ATO5	2	57	26.7 ± 0.30
Compritol 888 ATO®	-	74	22.6 ± 0.46
Gelucire 50/02	2	53	21.3 ± 0.17
Tristearin®	-	56	25.3 ± 0.42

HLB = hydrophilic-lipophilic balance

MP = melting point

2.2 Preparation of diclofenac sodium pellets

In this study, the jacketed planetary mixer had limit of mixing speed in range of 100 – 200 rpm. The mixing temperature was limited by a water bath which in a range of 30°C - 92°C. As results of preliminary studies, the mixing time less than 10 min, gave high amount of fines. The mixing time more than 17 min, gave uncontrollable growth rate of agglomerate, generating large balls.

All materials in Table 2, except for GMS, were passed though a 30 mesh (595μm) sieve before use. Pellets containing 10% w/w of diclofenac sodium (DS) were prepared by addition of molten binders, as described in section 1.1 (method B). The amounts of DS and lactose or dibasic calcium phosphate, were mixed in a jacketed planetary mixer at a paddle speed of 100 rpm for 10 minutes. GMS was melted in a porcelain disk at the temperature of 5°C or 25°C above its melting point prior to added into the dry mixture. According to the formulation designated in Table 3, when the temperature of the powder reached the temperature of 5°C (T_1) or 25°C (T_3) above the melting point of GMS, a given amount of molten GMS was slowly added at once with constant mixing. The accurate weight of GMS added was obtained by weighing a slightly excess amount of GMS in a tared porcelain disk and substraction of the quantity after

addition. The speed was then kept at 100 or 200 rpm and the mixing time, the time beginning after binder addition was completed, lasted for 10 (S_1) or 15 (S_3) minutes. The total number of formulation studied were 16 (LA-1 – LA-8 and CA-1 – CA-8). In addition, formulation LA-9 and CA-9 presented in Table 4 was also processed at a speed of paddle 150 rpm, temperature of the powder 15°C (T_2) above the melting point of GMS and mixing time 12.5 min (S_2) for extra information. For other binders presented in Table 5, the pellets were formed with lactose at the mild conditions studied i.e. paddle speed of 100 rpm, mixing temperature of T_1 and mixing time of S_1 as presented in Table 6. The conditions used were, in fact, the same as for formulation LA-5 when using GMS as binder.

When the process completed, diclofenac sodium pellets were removed from the mixer to an aluminum tray. The pellets were allowed to cool at ambient temperature, weighed and kept in a desiccators containing silica gel at ambient temperature for further analysis.

Table 2 Compositions of diclofenac sodium pellets prepared with glyceryl monostearate

Formulation	**Compositions**		
LA-1 – LA-9	DS (10%)	GMS	Lactose
CA-1 – CA-9	DS (10%)	GMS	dbcp

LA = formulation with lactose

CA = formulation with dibasic calcium phosphate

DS = diclofenac sodium

GMS = glyceryl monostearate

dbcp = dibasic calcium phosphate

Table 3 Conditions for preparing of diclofenac sodium pellets using GMS as a binder by melt technique (LA-1 – LA-8 and CA-1 – CA-8)

Speed of paddle (rpm)	**Mixing temperature (°C)**			
	MP_{GMS} + 5°C (T_1)		**MP_{GMS} + 25°C (T_3)**	
	Mixing time		**Mixing time**	
	10 min (S_1)	**15 min (S_3)**	**10 min (S_1)**	**15 min (S_3)**
200	LA-1 CA-1	LA-2 CA-2	LA-3 CA-3	LA-4 CA-4
100	LA-5 CA-5	LA-6 CA-6	LA-7 CA-7	LA-8 CA-8

LA = formulation with lactose

CA = formulation with dibasic calcium phosphate

MP_{GMS} = melting point of glyceryl monostearate = 53°C

Table 4 Conditions for preparation of diclofenac sodium pellets using GMS as a binder by melt technique (LA-9 and CA-9)

Speed of paddle (rpm)	**Mixing temperature (°C)** **MP_{GMS} + 15°C (T_2)**
150 rpm	**Mixing time 12.5 min (S_2)**
	LA-9 CA-9

LA = formulation with lactose

CA = formulation with dibasic calcium phosphate

MP_{GMS} = melting point of glyceryl monostearate = 53°C

Table 5 Compositions of diclofenac sodium pellets prepared with other binders

Formulation	**Compositions**		
PR	DS (10%)	Precirol® ATO5	Lactose
CP	DS (10%)	Compritol 888 ATO®	Lactose
GL	DS (10%)	Gelucire 50/02	Lactose
TS	DS (10%)	Tristearin®	Lactose

PR = formulation with Precirol® ATO5

CP = formulation with Compritol 888 ATO®

GL = formulation with Gelucire 50/02

TS = formulation with Tristearin®

DS = diclofenac sodium

Table 6 Conditions for preparation of diclofenac sodium pellets using other binders by melt technique

Speed of paddle (rpm)	**Mixing temperature (°C)** MP_{binder} **+ 5°C (T_1)**
100 rpm	**Mixing time 10 min (S_1)**
	PR
	CP
	GL
	TS

PR = formulation with Precirol® ATO5

CP = formulation with Compritol 888 ATO®

GL = formulation with Gelucire 50/02

TS = formulation with Tristearin®

MP_{binder} = melting point of Precirol® ATO5 = 57°C

melting point of Compritol 888 ATO® = 74°C

melting point of Gelucire 50/02 = 53°C

melting point of Tristearin® = 56°C

3. Characterization of diclofenac sodium pellets

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

3.1 Morphology

The pellets were examined under a scanning electron microscope (SEM) for morphological evaluation. The samples were prepared by gold sputtering technique before SEM examination. The shape and surface topography of pellets were determined. The pellets were also cross-sectioned for internal texture.

3.2 Particle size distribution

Particle size distribution was determined by sieve analysis, consisted of set of US standard sieves, ranging from 7 mesh (2,800μm), 8 mesh (2,360μm), 10 mesh (2000μm), 12 mesh (1,700μm), 14 mesh (1,400μm), 16 mesh (1,180μm), 18 mesh (1,000μm), 20 mesh (850μm), 25 mesh (710μm), 35 mesh (500μm), 50 mesh (300μm), 80 mesh (180μm) sieves and collection pan. The total amount of pellets obtained were put on the top of the sieve series. The sieves were placed on the sieve shaker and shaked for 10 minutes. The pellets retained on each sieve size were weighed and calculated in percentage of total weight. The cumulative percentage weight undersize was plotted against the diameter on the log-probability paper, having a logarithmic scale for the diameter and a probability scale for the cumulative percentage. According to Edmundson (1967), from this graphical representation, the geometric mean diameter, d_g (i.e.median or 50% diameter) and the geometric standard deviation, s_g (i.e. the slope or the spread of the normal curve; being equal to the square root of quotient of diameter at 84.13% undersize divided by the diameter at 15.87% undersize) can be determined. The value of s_g of 1.0 signifies no variation in size or only one size of pellets produced.

In practical, the data can be computed with probit analysis (Statistical Package for Social Sciences, SPSS 11.5, SPSS UK Ltd.), from which the value of d_g is the diameter at the probit of 0.5 and the value of s_g can be derived from the square root of the quotient of the diameter at the probit of 0.6 divided by the diameter at the probit of 0.4.

3.3 Determination of the angle of repose

The angle of repose was measured from a heap carefully built up by dropping the pellets samples of 10 g through a glass funnel with stem diameter of 12 mm to the horizontal plate. The angle of repose was averaged from three determinations. Each angle of repose was calculated from the following equation:

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

Where α is the angle of repose, H is the height of heap, R is the radius of heap

Table 7 Relationship between the angle of repose and flowability (Nagel and Peck, 2003)

angel of repose (θ)	**flowability**
≤ 38 °	Good
38-42 °	Fair
≥ 42 °	Poor

3.4 Determination of flow rate

Accurately weighed amount of about 10 g of pellets were filled in a glass funnel with 1.2-mm internal stem diameter fixed on the clamp. The time was recorded when the pellets started to flow until finished. The flow rate averaged from three determinations was reported in term of g/sec.

3.5 Bulk density, tapped density and compressibility index

The bulk density (B) of the pellets was determined by pouring 10 g of the pellets into a 25 ml graduated cylinder and the volume of pellets was measured. The graduated cylinder was tapped on a tap density tester through distance of 1.3 cm until no further decrease in the pellets volume was seen (approximately 300 times). The tapped density (T) was then calculated. The bulk density and tapped density were averaged from three determinations.

$$\text{Bulk density(g/ml)} = \frac{\text{weight of pellets(g)}}{\text{bulk volume (ml)}}$$

$$\text{Tapped density(g/ml)} = \frac{\text{weight of pellets(g)}}{\text{tapped volume (ml)}}$$

Both densities were averaged from three determinations. The Carr's compressibility was calculated from the following equation.

$$\% \text{ Compressibility} = (T - B) \times \frac{100}{T}$$

Classification of flowability by Carr's Indices (Davies, 2001)

Carr's Index (%)	Flow
5-12	Free Flowing
12-16	Good
18-21	Fair
23-33	Poor
35-38	Very Poor
> 40	Extremely Poor

3.6 True density

The true density (T) of the pellet was determined by Ultrapycnometer 1000, Quantachrome by gas displacement (He gas). The true density averaged from five determinations was reported in term of g/cm^3

3.7 Percent Friability

Five grams of pellets passed through a 16 mesh (1,180 μm) sieve and retained on a 20 mesh (850μm) sieve were filled with five 5 mm diameter metal spheres into the PVC container. The container was firmly closed with the cap and rotated at 25 rpm for 4 minutes. After that, pellets finer than 30 mesh (595μm) was sieved off. The result averaged from three determinations, reported as percentage of weight loss.

3.8 Sphericity of pellets

The sphericity of prepared pellets was determined by using image analyzer. One hundred sample pellets of each formulation were analyzed by software program Image Pro Plus® of Image analyzer. Longest diameter or Feret maximum (R_1) and smallest diameter or Feret minimum (R_2) of pellets were divided to perform the aspect ratio, i.e. ratio between major axis and minor axis of ellipse equivalent to object that referred to the sphericity of pellets. Another parameter used to define the sphericity of pellets in this study was the value of roundness which was derived from perimeter^2 devided by 4 π area of the projected image.

3.9 The X-ray diffractometry

The X-ray diffractometer is used to determine the diffraction angle of the substance, from which the interaction between each component in mixing and melt pelletization process is determined.

The samples for X-ray diffraction studied were milled and were firmly packed into the cavity of thin rectangular glass plate. The sample was taken to expose to the X-ray diffraction chamber. The X-ray diffraction patterns were recorded from 5° - 30° terms of 2Ø angle.

3.10 The IR spectroscopy

Infrared spectroscopy is used to comfirm the functional groups of substances and products after production process by observing the intensities of IR peaks.

The IR spectra of pellets were examined using the potassium bromide disc (KBr) method with an infrared spectroscopy in the range of the wavelength 4000-400 cm^{-1}.

3.11 The differential scanning calorimetry

Themal analysis is the most common approach to study physicochemical interactions of two or more component system. The thermograms of pellets were recorded on a thermal analyzer. About 8-10 mg, corresponding to the amount in the formulation, of sample was put onto the aluminum pan. The sample was taken into the condition that had been purged with liquid nitrogen gas. The condition used the heating rate of 10°c/min and temperature between 25°C and 320°C.

3.12 Drug content and uniformity of drug content of DS in pellets.

Pellets retained on the sieves ranging from 16 mesh (1,180μm), 18 mesh (1,000μm) and 20 mesh (850μm) sieve fractions were mixed. The drug content and uniformity of drug content of DS in the pellets was quantitatively determined by mean of absorption peak area ratio from HPLC method.

HPLC Analysis

HPLC chromatographic conditions :

Column	:	Hypersil® C18 column (250 x 4.6 mm), 5 μm (UK)	
Detector	:	UV detector was set at 254 nm	
Flow rate	:	1.0 ml / min	
Injection volume	:	20 μl	
Mobile phase	:	A mixture of phosphate buffer pH 2.5 and methanol, 35:65 % v/v	
Retention times	:	diclofenac sodium	19 min
		diclofenac related compound A	9 min

The calibration curve of DS and diclofenac related compound A is shown in Figure 120 and Figure 121, respectively, in appendix A. Each concentration was determined in triplicate.

Preparation of DS standard solution

About 25 mg of DS was accurately weighed into a 25 ml of volumetric flask. Seventeen milliliter of methanol HPLC grade was added to dissolve the drug. After dissolution, the solution was adjusted to volume with the solvent. The final concentration of this standard stock solution was 1 mg/ml.

The 1, 2, 3, 4 and 5 ml of the standard stock were separately pipetted and transferred into five 10 ml of volumetric flasks. All flasks were subsequently diluted to volume with methanol HPLC grade so that the final concentrations were 100, 200, 300, 400 and 500 µg/ml, respectively.

Preparation of pH 2.5, phosphate buffer

Mixture of equal volumes of a 0.1% w/v solution of ortho-phosphroric acid and a 0.16% w/v solution of sodium dihydrogen orthophosphate were mixed and adjusted to pH of 2.5.

Assay of DS content in pellets

Five grams of each formulation were weighed and pulverized by mortar and pestle. Then, the powder was accurately weighed equivalent to 250 mg into a 25 ml of volumetric flask, which was then filled with 20 ml of methanol HPLC grade and sonicated for 30 minutes. Afterwards, the volumetric flask was adjusted to volume by methanol HPLC grade and mixed thoroughly. The solution was filtered through 0.45 µm membrane filter paper. The 3 ml of solution was pipetted and transferred into 10 ml of volumetric flask. The solution was adjusted to 10 ml of methanol HPLC grade and mixed thoroughly. Finally, the final concentration of sample solution was 300 µg/ml. Each sample determined in duplicate.

Assay for uniformity of drug content DS in pellets

Ten capsules were taken by random sampling. Each capsule was filled into a 25 ml of volumetric flask, then dissolved with methanol HPLC grade and sonicated for 30 minutes. Each solution was adjusted to 25 ml with methanol HPLC grade and mixed thoroughly. The solution was filtered through 0.45 µm membrane filter paper. Then, three ml of this solution was pipetted and transferred into a 10 ml of volumetric flask. Methanol

was added to volume and mixed. The final concentration of sample solution was being 300 µg/ml.

Validation of HPLC method

The analytical parameters used for the assay validation were specification, accuracy, precision and linearity. (USP 27/ NF 22, 2004)

Specificity

Under the chromatographic condition used, the peak of DS has to be completely separated from and not interfered by the peak of other components in the sample. Non-active ingradients, including lactose, dbcp, GMS, gelucire 50/02, precirol, compritol, tristearin. The chromatograms were evaluated by comparing with the standard solution of DS.

Accuracy

Placebo solution with diclofenac sodium reference standard having concentrations of 100, 200,300, 400 and 500 µm/ml were prepared and injected. The percentage of the analytical recovery of each standard solution was calculated. The mean percentage of analytical recovery was 95-105% with percentage of coefficient of variation (%RSD) <2.00% indicated the high accuracy of this method.

Precision

Within Run Precision, The within run precision was determined by analyzing five sets of five standard solution of DS in the same day. Peak area ratios of DS was compared and percentage coefficients of variation (%RSD) of each concentration were determined. The percentage of coefficient of variation (%RSD) values of peak area ratio both within run and between run were <2.00% which indicated that the HPLC methods could be used to determine the amount of DS over period of time studied.

Between Run Precision, The between run precision was determined by comparing each concentration of DS standard solution that were prepared and injected on different days. The percentage coefficient of variation (%RSD) of DS peak area from the three sets of standard solutions having the same concentration was determined. The percentage of coefficient of variation (%RSD) values of peak area both within run and

between run were <2.00% which indicated that the HPLC methods could be used to determine the amount of DS over period of time studied.

Linearity

DS standard solution ranging from 100 to 500 µm/ml were prepared and analyzed. Linear regression analysis of peak area ratio versus their concentrations was performed.

3.13 Dissolution study

Pellets retained on sieve fractions #16 (1,180µm), #18 (1,000µm), #20 (850µm) were mixed and were filled in capsules No.1 equivalent to 25 mg of DS. Sample of six capsules for each formulation were evaluated.

Nine hundred milliliters of 0.1 N HCl was placed in a glass vessel specified in USP dissolution test (apparatus I). The medium was equilibrated to 37± 0.5°c. The apparatus was operated at a rotating speed of basket of 100 rpm. Two dissolution media were used to mimic the pH conditions in human gastrointestinal tract. First nine hundred milliliters of the medium was 0.1 N HCl in which dissolution of pellets was tested for two hours. Then, it was changed to pH 6.8 phosphate buffer and the solution test was carried out for six hours.

Ten milliliters of the specimen were withdrawn at the time interval of 30 min, 1 hr, 2 hr, 2 hr 5 min, 2 hr 10 min, 2 hr 15 min, 2 hr 30 min, 2 hr 45 min, 3 hr, 3 hr 30 min, 4 hr, 4 hr 30 min, 5 hr, 6 hr, 7 hr and 8 hr and the fresh medium of 37 ± 0.5 °c was replaced immediately after sampling to keep the volume of the medium constant during the experiment.

Each sample was filtered through paper filter (Whatman® No.1). The first one milliliters of filtrate was discarded. The absorbance of each sample was spectrometrically assayed at 275 nm using dissolution media as blank.

The amount of DS release at anytime internal was calculated from calibration curve. A corrective amount was made for the previously removed sample to determine the total amount of drug release at each time interval.

Calibration curve of diclofenac sodium

In 0.1 N HCl solution

DS 5 mg was accurately weighed into 50 ml of volumetric flask and dissolved with 1 ml of methanol, then adjusted with water to volume. The solution was used as the stock solution.

The 4, 5, 7, 10 and 12 ml stock solution was individually pipetted onto 50 ml of volumetric flask and then diluted to volume with 0.1 N HCl. The final concentration of each solution was 0.008, 0.01, 0.014, 0.02 and 0.024 mg/ml, respectively.

The solution was assayed spectrophotometrically at 275 nm. The absorbance and calibration curve of DS in 0.1 N HCl are shown in Table 23 and Figure 118, respectively in appendix A. Each concentration was determined in triplicate.

In phosphate buffer pH6.8

Five milligrams of DS was accurately weighed into 50 ml volumetric flask and dissolved with phosphate buffer pH 6.8, then adjusted to volume. The solution was used as the stock solution.

The 1 and 2 ml stock solution was individually pipetted into 10 ml of volumetric flask and 4, 6, 8 and 12 ml stock solution was individually pipetted into 50 ml of volumetric flask and then diluted to volume with phosphate buffer pH 6.8. The final concentration of each solution was 0.01, 0.02, 0.008, 0.016 and 0.024 mg/ml, respectively.

The solution was assayed spectrophotometrically at 275 nm. The absorbance and calibration curve of DS in phosphate buffer pH 6.8 are shown in Table 24 and Figure 119, respectively in appendix A. Each concentration was determined in triplicate.

Data analysis of dissolution profile

The percentage amounts of DS released from pellets were plotted as a function of dissolution time and the area under the curve (AUC) was calculated as follows:

Total area = percent drug content x total dissolution time*

Area under the curve (AUC) at each time internal = 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

* total dissolution time is 8 h

3.14 Stability of pellets

The DS pellets (LA-9, CA-9, PR, CP, GL, TS) were stored in a glass container which was placed inside stability chamber and exposed to the temperature of 45±2°C and relative humidity of 75% for 4 months. At time interval of 1, 2, 3 and 4 months, 250 mg of pellets, equivalent to 25 mg DS were randomly sampled for analyzing the remained amount of DS.

3.15 Statistical analysis

The statistical analysis was carried out by analysis of variance using Statistical Package for Social Sciences, SPSS 11.5, SPSS UK Ltd.