CHAPTER III

EXPERIMENTAL

Materials

The materials used and sources of supply are as follows.

1. Raw materials:

1.1 Diltiazem hydrochloride (Lot no. DIL/M-18104, Supplied by Siam Pharmaceutical Co., Ltd, Thailand)

1.2 DOWEX®88 Sulfonic acid cation exchange resin 4 % crosslinkage (Lot no. SL051815Q1, Dow Chemical Company, USA.)

1.3 DOWEX®HCR-S Sulfonic acid cation exchange resin 8 % crosslinkage (Lot no. TI231815Q3, Dow Chemical Company, USA.)

2. Additives:

2.1 Eudragit®RL PO (Lot no. G040636392, Rohm GmbH, Germany)

2.2 Eudragit®RS PO (Lot no. G031038154, Rohm GmbH, Germany)

2.3 Triethyl citrate (Lot no. 445571/1, Fluka, Swizerland)

2.4 Talcum (Wyndale, New Zealand)

2.5 Microcrystalline cellulose (Lot no. 2155, Asahi Chemical Industry, Japan)

2.6 Lactose monohydrate (Haichen, China)

2.7 Sodium starch glycolate (distributed by Rama Product)

2.8 Polyethylene glycol 6000 (Fluka, Swizerland)

2.9 Polyethylene glycol 4000 (Fluka, Swizerland)

2.10 Magnesium stearate (Asia Pacific PTE Ltd., Australia)

2.11 Hard gelatin capsule number 0 (distributed by Samchai Chemical, Thailand)

3. Reagents:

3.1 Potassium chloride (Lot No. AF501338, Ajax Finechem, Australia)

3.2 Potassium dihydrogen orthophosphate (Lot No. AF401428, Ajax Finechem, Australia)

3.3 Sodium chloride (Lot No. AF309070, Ajax Finechem, Australia)

3.4 Sodium hydroxide (Lot No. 247498249, Merck, Germany)

3.5 Calcium chloride (Lot No. AF602066, Ajax Finechem, Australia)

Methods

The experimental method was divided in 3 parts as follows.

Part I. Study of drug loading

1. Purification of ion exchange resins

Resins were classified to the size range of 25-30 mesh (US Standard sieves, Laboratory test sieve ASTM E11, Endecotts, Ltd., USA) and purified using the method described in previous report (Cuna et al., 2000). About 100 gm of resins were washed 3 times with 500 ml deionized water, 500 ml of 95 % ethanol, 500 ml of 50 % ethanol and 500 ml of deionized water to remove impurities. The resins were activated 2 times with 500 ml of 2 M NaOH and washed with deionized water until the elute was neutral. The resins in Na^+ form were dried overnight in a hot air oven at 50°C to constant weight and kept in a desiccator for further use.

2. Preparation of diltiazem resinates

The following factors affecting drug loading were investigated.

2.1 Effect of resin crosslinkage and quantity of resins

Cation exchange resins having different degree of crosslinkage, 4 % (DOWEX®88) and 8 % (DOWEX®HCR-S) were used. Drug loading onto resins was studied under varying quantity of resins 12.50, 5.00, 2.50, 1.25, 0.50, 0.25, 0.167 and 0.125 gm per 0.25 gm drug. Fifty millilitres of 0.5 % w/v drug solution was prepared in a temperature-controlled shaking bath at 30±1°C. Then, the accurate weight of dried resins (n=3) was added in drug solution and shaken for 24 hours. At equilibrium, the diltiazem resinates were separated from the filtrate by filtration, washed several times with deionized water until the filtrate showed no absorbance of diltiazem hydrochloride at 237 nm, dried overnight at 50°C and kept in a desiccator.

2.2 Effect of concentration of drug loading solution

From the result of the study in 2.1, the drug to resin ratio of 1:1 by weight was more suitable for preparing resinates. In order to study the concentration or solvent dilution of drug loading solution, diltiazem resinates were prepared by using different drug loading solution concentrations of 0.5 %, 1 %, 4 % and 8 % w/v. The 4 % and 8 % crosslinkage resins of 0.125, 0.25, 1.0 and 2.0 gm were mixed with 25 ml of 0.5 %, 1 %, 4 % and 8 % w/v drug loading solution, respectively, in a temperature-controlled shaking bath at 30±1°C for 24 hours. At equilibrium, the diltiazem resinates were separated, washed and dried as previously described in 2.1.

2.3 Effect of temperature during drug loading

2.3.1 The percentage of drug loading at equilibrium

The diltiazem resinates were prepared at various temperatures between 30 to 50±1 °C. The 4 % and 8 % crosslinkage resins were mixed with 25 ml of 1 %, 4 % and 8 % w/v drug loading solution (resin to drug ratio of 1:1) for 24 hours. At equilibrium, the diltiazem resinates were separated, washed and dried as previously described in 2.1.

2.3.2 The time required for system equilibrium

The diltiazem resinates were prepared at various temperatures between 30 to 50±1 °C. The 4 % crosslinkage resins were mixed with 50 ml of 8 % w/v drug loading solution (resin to drug ratio of 1:1) for 28 hours. The sample of 20 µl was withdrawn at the time intervals of 0.50, 1, 3, 6, 12, 24 and 28 hours. The remainder of drugs in the loading solution was assayed by spectrophotometer (ultraviolet/visible spectrophotometer, Model V-530, Jasco, Japan) at wavelength of 237 nm. The amount of drug loading onto the resinates was the difference of the initial and the remainder of drugs in the loading solution.

3. Characteristic of diltiazem resinates

3.1 Microscopic morphology of resinates

The resinates were examined under the stereomicroscope and Scanning Electron Microscope (Model JSM-5410LV, Joel Ltd., Japan) for morphological evaluation. The shape and surface topography of resinates were determined.

3.2 Moisture determination

The moisture content of resinates was determined by moisture analyzer (Model MA30, Sartorius, Germany). About 1 gm of sample was exposed to an IR lamp until constant weight was obtained. The moisture content was calculated automatically. The results were obtained from an average of three determinations.

3.3 Bulk density, tapped density and compressibility index

The bulk density (ρ_b) of the resinates was determined by pouring 15 gm of the resinates into a 50 ml graduate cylinder and measuring the volume of resinate. The graduate cylinder was tapped on a tap density tester until a constant volume was obtained. The tapped density (ρ_t) was then calculated. Both densities were averaged from three determinations. The Carr's compressibility, which expresses the flow property as presented in Table 5, was calculated from the following equation.

Table 5 Classification of flowability by Carr's index (Davies, 200)

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

$$\text{Carr's compressibility} = \frac{(\rho_t - \rho_b) \times 100}{\rho_t}$$

3.4 Determination of diltiazem hydrochloride content (% drug loading)

The amount of drug loading onto the resinates (% w/w of drugs in the resinates) was assayed by spectrophotometer. Diltiazem resinates were ground with mortar and pestle. The accurate weight of powder (30-50 mg) was placed in 250 ml volumetric flask to which 0.4 M potassium chloride solution about 240 ml was added for eluting diltiazem from resinate. The eluate was transferred by filter sampler and replaced with the same volume of fresh eluate. The total volume of eluate was collected and adjusted to 500 ml in volumetric flask. The eluate was diluted to a suitable concentration and assayed for the content of diltiazem hydrochloride by spectrophotometer (ultraviolet/visible spectrophotometer, Model V-530, Jasco, Japan) at wavelength of 237 nm.

$$\text{\% drug loading} = \frac{\text{drug weight}}{\text{resinate weight}} \times 100$$

3.5 Study of drug release

The diltiazem release studies of resinates were modified from the sustained release capsule monograph of USP27 (2000) and performed using USP dissolution test apparatus II (paddle method, Model DT-6R, Erweka, Germany). Nine hundred millilitres of dissolution medium was maintained at 37±1°C and the rotating speed of paddle was maintained at 50±1 rpm. The release study of each dissolution medium was done in triplicate.

The resinates equivalent to 90 mg of diltiazem hydrochloride were filled into a gelatin capsule number 0 and transferred into the vessel. The sample of 10 ml was withdrawn through a filter at the time intervals of 0.08, 0.25, 0.50, 1, 2, 4, 6, 8, 10 and 12 hours. The same volume of the fresh medium was replaced immediately after each

sampling to keep the constant volume of the medium in the vessel throughout the experiment.

The filtrate was diluted to a suitable concentration and assayed for the content of diltiazem hydrochloride using spectrophotometer at wavelength of 237 nm. The amounts of diltiazem hydrochloride release at any times were calculated from the calibration curve for each dissolution medium. A cumulative correction was achieved for the previously removed sample to determine the total amount of the drug release. Each of dissolution values reported was based on an average of three determinations of each dissolution medium.

This experiment was studied in different dissolution medium conditions as follows.

3.5.1 Effect of ionic strength and counter ions of dissolution medium

- 0.01, 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 M potassium chloride
- 0.01, 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 M sodium chloride
- 0.1 M hydrochloric acid
- 0.04 M calcium chloride (ionic strength= 0.1)

3.5.2 Effect of pH of dissolution medium

- 0.1 M hydrochloric acid
- phosphate buffer pH 6.8
- phosphate buffer pH 6.8 adjusted ionic strength to 0.1 with sodium chloride
- phosphate buffer pH 7.2
- phosphate buffer pH 7.2 adjusted ionic strength to 0.1 with sodium chloride

Part II. Study of resinate coating

The ion exchange resins had the advantage in controlled release properties. However, it could be coated with semipermeable membrane to modify drug release. The resins were in spherical shape with narrow particle size range, which were good properties for coating by fluidized bed technique (Ichikawa et al, 2001).

1. Formulation and preparation of coated resinates

In order to modify drug release, resinates were coated with semipermeable membrane. Bodmeier (1997) reported that acrylic polymer such as Eudragit® were more flexible and more suitable for coating of pellets or resins to be compressed into tablets. Plasticized Eudragit®RS and Eudragit®RL exhibited flexible films with elongation values in excess of 125 %. In this study, Eudragit®RLPO and Eudragit®RSPO were used to modify drug release and protect resinates from the compression pressure during tableting process.

1.1 Coating formulation

Table 6 shows the formulation for studying the effect of amount of polymer and polymer ratio between Eudragit®RLPO and Eudragit®RSPO. Coating solution was prepared by dissolving polymer in mixture of isopropyl alcohol and water. Triethyl citrate was added in this solution for plasticizing polymer. Then, talcum which was sieved through sieve size 40 mesh was dispersed in polymer solution as the antiadherent to prevented tackiness of the coated resinates.

Table 6 Composition of coating solution

Ingredients	Formulations per 100 gm resinates (gm)					
	C1	C2	C3	C4	C5	C6
Eudragit® RLPO	7.5	10	15	12	9	7.5
Eudragit® RSPO	-	-	-	3	6	7.5
Triethyl citrate	1.5	2	3	3	3	3
Talcum	7.5	10	15	15	15	15
Isopropyl alcohol	93.5	125	187	187	187	187
Deionied water	40	53	80	80	80	80

1.2 Fluidized bed coating apparatus and coating conditions

The resinates were coated using bottom spray fluidized bed coating apparatus (Model 95900450, Aeromatic fielder, Switzerland). The coating conditions were as follow.

Inlet air temperature	:	55°C
Outlet air temperature	:	52-55 °C
Atomizing air pressure	:	1.5 bar
Pump speed	:	5 rpm
Spray rate	:	2.4 ml/min
Air brower	:	level 7
Curing time	:	15 minutes

2. Characteristic of diltiazem resinates

2.1 Microscopic morphology of resinates

The resinates were examined using the method as previously described in Part I (3.1).

2.2 Moisture determination

The moisture content of resinates was determined by moisture analyzer according to the method as previously described in Part I (3.2).

2.3 Bulk density, tapped density and compressibility index

The bulk density (ρ_b) and tapped density (ρ_t) of the resinates was determined by the method as previously described in Part I (3.3).

2.4 Determination of diltiazem hydrochloride content

Determination of diltiazem hydrochloride content of the coated resinates was performed using the method as previously described in Part I (3.4).

2.5 Study of drug release

The coated resinates equivalent to 90 mg of diltiazem hydrochloride were filled into a hard gelatin capsule number 0. The diltiazem release study of coated resinates in 0.1 M potassium chloride solution was performed using USP dissolution test as previously described in Part I (3.5).

3. Study on the effect of compression pressure on uncoated and coated resinates

The uncoated resinates and coated resinate formulation C3 were compressed by hydraulic press tableting machine at compression pressure 3,000 psi. Then, the resinates after compression were characterized for microscopic morphology and drug release profile. The release profile was compared with the resinates before compression.

The mathematical method for the comparison of dissolution profiles are described in two equations, difference factor (f_1) and similarity factor (f_2) (Hara et al., 1998; Costa et al., 2003). The equations were as follow.

$$f_1 = \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum R_t} \times 100 \quad (19)$$

$$f_2 = 50 \log \{[1 + \frac{1}{n} \sum_{t=1}^{n} (R_t - T_t)^2]^{-0.5} \times 100\} \quad (20)$$

Where n is the number of dissolution time points, R_t and T_t are the dissolution values of two profiles in comparison at time t. For curves to be considered similar, f_1 values should be close to 0 and f_2 values should be close to 100. Generally, f_1 values up to 15 (0-15) and f_2 values greater than 50 (50-100), which means an average difference of no more than 10 % at the sample time points. Both equations were acceptable method for dissolution profile comparison. Generally, the f_2 equation was preferred. In this experiment, the similarity factor (f_2) was used.

Part III. Development of multiple-unit sustained release tablets

According to the study of resinate coating (Part II), the suitable coating formulation which presented diltiazem release more than 50 % in 12 hours (coating formulation C1-C3) were selected to formulate into disintegrating tablets.

1. Study on the effect of amount of resinates in formulation

In order to study the effect of amount of resinates in formulation of disintegrating tablets, microcrystalline cellulose (Avicel® PH101) and lactose were used in the formulation in ratio of 70:30. Three percent of sodium starch glycolate (Explotab®) was used as internal disintegrant. Two grades of polyvinyl pyrolidone (PVP) such as PVP K30 and PVP K90 were investigated for the optimum amount of binder to formulate tablets with 20-30 % resinates.

Microcrystalline cellulose and other excipients were produced in granular form before mixed with coated resinates to improve flow property and reduce segregation problem between resinates and MCC granules which might be caused by the different size between resinates and MCC particles.

1.1 Formulation and preparation of tablets

Microcrystalline cellulose granules were prepared by wet granulation method according to the composition presented in Table 7. Fraction of Avicel® PH101, lactose and Explotab® were mixed in a plastic box by geometric dilution method for 5 minutes and transferred in a mortar. The PVP solution was added and mixed until wet mass was obtained. The wet mass was screened though a sieve 16 mesh and dried at 60°C for 30 minutes. The dried granules were rescreened through sieve size 20 mesh. Then, the granules were characterized for their physical properties such as moisture content, bulk density, tapped density and particle size distribution.

To prepare the dried mixed granules, the fraction of coated resinate formulation C3 and MCC granules were mixed in plastic box by geometric dilution method for 10

minutes. Then, talcum and magnesium stearate were added and final mixed for 3 minutes. Hydraulic press tableting machine was used in this study. The granules of the formulation were compressed and maintained for 30 seconds and then the compression pressure was slowly released (Prapaitrakul and Whitworth, 1989). Ten millimetres in diameter round flat faced punch and die were used to compress the tablets. The granule weight 500 mg was compressed at compression pressure of 3000 psi.

Table 7 Composition of microcrystalline cellulose granules and tablets

Ingredients	Formulations (gm)								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Wet granulation of microcrystalline granules									
Avicel® PH101	64.4	64.4	64.4	60.9	60.9	60.9	64.4	64.4	64.4
Lactose	27.6	27.6	27.6	26.1	26.1	26.1	27.6	27.6	27.6
Explotab®	3	3	3	3	3	3	3	3	3
PVP K30	5	5	5	10	10	10	-	-	-
PVP K90	-	-	-	-	-	-	5	5	5
Deionized water	40	40	40	40	40	40	40	40	40
Dried mixed granulation									
Coated resinates C3	50	30	20	50	30	20	50	30	20
Microcrystalline granules	46	66	76	46	66	76	46	66	76
Talcum	3	3	3	3	3	3	3	3	3
Magnesium stearate	1	1	1	1	1	1	1	1	1

1.2 Evaluation of the granules

1.2.1 Moisture determination

The moisture content of granules was determined by moisture analyzer using the method as previously described in Part I (3.2).

1.2.2 Bulk density, tapped density and compressibility index

The bulk density (ρ_b) of the granules was determined by pouring 15 gm of the granules into a 50 ml graduated cylinder and measuring the volume of granule. The graduated cylinder was tapped on a tap density tester until a constant volume was obtained. The tapped density (ρ_t) was then calculated. Both densities were averaged from three determinations. The Carr's compressibility was calculated from the following equation.

$$\text{Carr's compressibility} = \frac{(\rho_t - \rho_b)}{\rho_t} \times 100$$

1.2.3 Particle size distribution

Particle size distribution was determined by sieve analysis, consisting of a set of US standard sieves, ranging from 75, 150, 180, 250, 425, 850 microns and a collection pan. Approximate 10 gm of granules were put on the top sieve. The sieves were placed on the sieve shaker and shake for 20 minutes. The granules retained on each sieve size were weighed and calculated in percent of total weight.

2. Evaluation of sustained release diltiazem tablets

2.1 Weight variation

The weight variation of tablets was determined by an analytical balance. Twenty tablets were individually weighed. The mean and standard deviation were averaged from twenty tablets determinations.

2.2 Thickness

The thickness of ten individual tablets was determined using a micrometer for each batch. The sample mean and standard deviation of each batch of tablets were calculated.

2.3 Hardness

The hardness of tablets was determined by hardness tester (Model 2E/205, Schleuniger, Switzerland). The mean and standard deviation were averaged from three tablets determinations.

2.4 Friability

The friability of tablets was determined by a friabilator (Model TA3, Erweka, Germany). Ten tablets were weighed by an analytical balance "w_0". Ten tablets were rotated at 25 rpm for 4 minutes. The tablets were reweighed again after the dust was eliminated, "w". The percent of friability was calculated based on the following equation.

$$\% \text{ Friability} = \{(w_0 - w) / w_0\} \times 100$$

2.5 Disintegration Time

Disintegration time was determined according to the USP XXV method (Disintegrating apparatus: Model ZT52, Erweka, Germany). The disintegration time of three tablets were evaluated in water at 37±1°C with disk.

2.6 Microscopic morphology of resinates after compression

The tablets were examined under the stereomicroscope and Scanning Electron Microscope (SEM) for morphological evaluation. The shape and surface of tablets and resinates were determined.

2.7 Determination of diltiazem hydrochloride content in tablets

Ten tablets were ground with mortar and pestle. The accurate weight of powder (100-150 mg) was prepared and determined by method previously described in Part I (3.4).

2.8 Study of drug release

The diltiazem release study of sustained release tablets in 0.1 M potassium chloride solution was performed using USP dissolution test previously described in Part I (3.5).

The placebo tablet was used in drug release study for correction the absorbance of the drug release at time evaluated. The procedure for preparation of placebo tablet was the same as the drug formulation. The concaved oblong 10.3x21.9 mm punch and die were used to compress the placebo tablet. The tablet weight was 1.5 gm and the compression pressure was the same as of the drug formulation. Dissolution test of placebo tablet was performed in the same dissolution medium as for drug formulation tablets. The sample was withdrawn at the same time intervals, volume of sampler and volume of replacement medium as drug formulation tablets. The concentrations of sample were determined with the same dilution as drug formulation tablets. The objective of the dissolution study of placebo tablet was to verify specificity of the method for quantitative analysis of drug release.

3. Study on the effect of soft material

In the previous study in Part III (1) the resinates compressed with microcrystalline cellulose granules exhibited poor physical property tablets, too soft to handle and capping. This might be caused by the size of resins (707-841 microns) which was used in this experiment. The binding property of granules was too low to hold the resinates. In this experiment, the plastic and soft material such as polyethylene glycol molecular weight 4000 and 6000 were used to improve cohesion within the tablets and to protect resinates from compression force. However, they had only limited binding action when used alone, and can prolong disintegration if present in concentrations greater than 5 % w/w (Rowe et al., 2003).

In practice, two or more compression aids were blended together because tablet appearance and their hardness are often better than the individual excipients. In this

experiment, microcrystalline cellulose and polyethylene glycol were used as the excipient of tablets. The MCC and PEG granules were prepared by wet granulation method. Fraction of Avicel® PH101, lactose and Explotab® from Table 8 were mixed in plastic box by geometric dilution method for 5 minutes and transferred in a mortar. The PVP solution and PEG solution was added and mixed until wet mass was obtained. The wet mass was screened though a sieve 16 mesh and dried at 60°c for 30 minutes. The dried granules were rescreened through sieve size 20 mesh.

From the study in Part III (1), the optimal quantity of resinates in tablets was about 20 %. Because addition of PEG caused prolonged disintegration, five percent of Explotab® was used as the external disintegrant. The dried mixed granules were prepared using the formulation in Table 8 by method as previously described in Part III (1). The total weight of tablets in this study was 1.5 gm which equivalent to 90 mg of diltiazem hydrochloride. The concaved oblong 10.3x21.9 mm punch and die were used to compress the tablets (Figure 9).

In order to study the effect of PEG in the tablet formulation, the tablet formulation F17 which had no PEG (control formulation) was produced to compare the dissolution profile with the tablets produced from PEG granules.

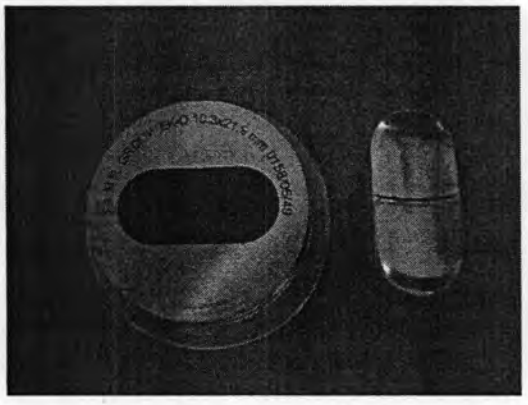

Figure 9 The concaved oblong 10.3x21.9 mm punch and die.

Table 8 Composition of granules and tablets

Ingredients	Formulations (gm)							
	F10	F11	F12	F13	F14	F15	F16	F17
Wet granulation of MCC and PEG granules								
Avicel® PH101	57.4	50.4	57.4	50.4	50.4	50.4	50.4	64.4
Lactose	24.6	21.6	24.6	21.6	21.6	21.6	21.6	27.6
Explotab®	3	3	3	3	3	3	3	3
PVP K30	5	5	5	5	5	5	5	5
PEG 4000	10	20	-	-	20	20	20	-
PEG 6000	-	-	10	20	-	-	-	-
Deionized water	40	40	40	40	40	40	40	40
Dried mixed granulation								
Uncoated resinates (Equivalent to 90 mg DTZ)	-	-	-	-	21.5	-	-	-
Coated resinates C1 (Equivalent to 90 mg DTZ)	-	-	-	-	-	24.5	-	-
Coated resinates C2 (Equivalent to 90 mg DTZ)	-	-	-	-	-	-	25.7	-
Coated resinates C3 (Equivalent to 90 mg DTZ)	26.8	26.8	26.8	26.8	-	-	-	26.8
Microcrystalline granules	64.2	64.2	64.2	64.2	69.5	66.5	65.3	64.2
Explotab®	5	5	5	5	5	5	5	5
Talcum	3	3	3	3	3	3	3	3
Magnesium stearate	1	1	1	1	1	1	1	1

4. Study on the effect of disintegrating time of tablets on drug release

In the previous study in Part III (3), using of polyethylene glycol in the tablet formulation might cause the prolonged disintegration time. In order to study this effect, the total coated resinates were manually separated from tablet formulation F11 and F14 from study Part III (3). The release profile of separated coated resinates was studied comparing with the tablets which had same formulation and compression pressure.

5. Study on the effect of compression force

To study the effect of compression force, the granules of the formulation F11 and F14 from study Part III (3) were compressed at three compression pressures of 1,500 psi (340 pound), 3,000 psi (681 pound) and 4,500 psi (1,022 pound) by the hydraulic press, and the relationships of compression force and release behaviors were evaluated.

6. Study on the effect of compression machine

Hydraulic press tableting machine and single punch tableting machine (EKO, Viuhang Engineering, Thailand) were used in this study with the same size of punch and die, concaved oblong 10.3x21.9 mm. The granules of the formulation F11 from study Part III (3) were employed in this study. The tablet weight and hardness were adjusted to 1.5 gm and 8-10 kp, respectively.

7. Calibration curve of diltiazem hydrochloride

Calibration curves for diltiazem hydrochloride assay were concluded in following mediums.

- in water
- in 0.1 M hydrochloric acid
- in phosphate buffer pH 6.8
- in phosphate buffer pH 6.8 adjust ionic strength to 0.1 with sodium chloride
- in phosphate buffer pH 7.2
- in phosphate buffer pH 7.2 adjust ionic strength to 0.1 with sodium chloride
- 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 M KCl
- 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 M NaCl
- 0.04 M $CaCl_2$

Forty milligram of diltiazem hydrochloride was accurately weighed into a 100 ml volumetric flask through the aid of a glass funnel. The powder was dissolved and adjusted to volume with each medium. This solution was used as the first stock solution. The 1, 3, 2, 5, 3 and 4 ml, of the first stock solution were individually pipetted into 100, 200, 100, 200, 100 and 100 ml volumetric flask, respectively. All solutions were adjusted to volume with each medium. The final concentrations of each solution were 4, 6, 8, 10, 12 and 16 μg/ml. The absorbance was determined by spectrophotometer at wavelength of 237 nm.