CHAPTER II

MATERIALS AND METHODS

1. Materials

- DTZ HCl (Lot No. 0690798) distributed by Siam Chemical Product Co., Ltd., Thailand.
- ETHOCEL Standard 10 Premium and METHOCEL K4M Premium EP (Batch No. PB05012N01) distributed by Rama Production Co., Ltd., Bangkok, Thailand.
- Dibutyl phthalate (AR grade, Lot No. 61219343) obtained from MERCK-Schuchardt Limited, Hohenbrunn, Germany.
- Diethyl phthalate (AR grade, Analysis No. 325384/1 393) and Triethyl citrate (AR grade, Analysis No. 387266/1 34398) obtained from Fluka Chemie, Buchs, Switzerland.
- Isopropyl myristate (Commercial grade, Lot No. 453168) received from S. Tong Chemicals Co., Ltd., Bangkok, Thailand.
- Isopropyl palmitate (AR grade) supplied from Uniqema, Selango Darul Ehsan, Malaysia.
- N-methyl-2-pyrrolidone (AR grade) purchased from ISP Pharmaceutical, USA.
- Oleic acid (Commercial grade, Lot No. ACH01/89), Polyethylene glycol 400 (Commercial grade, Lot No. PH0901AAEC), and Propylene glycol (Commercial grade, Lot No. PL 45/363) distributed by Srichand United Dispensary Co., Ltd., Bangkok, Thailand.
- Tween 80 (Commercial grade, Lot No. 807870) distributed by B.L. Hua Co., Ltd., Bangkok, Thailand.
- Potassium dihydrogen orthophosphate (AR grade, Batch No. F2H145) supplied from Asia Pacific Specialty Chemicals Limited, NSW, Australia.
- Acetonitrile (AR grade, Batch No. 0252146) and Methanol (AR grade, Batch No. 0395698) supplied by Fisher Scientific UK Limited, UK.
- All other chemicals were analytical grade.

2. Methods

2.1 Preformulation of Free Film Formulations

2.1.1 Preparation of Free Film Formulations

Films composed of different ratios of EC, HPMC and various plasticizers were prepared by a plate casting method. The compositions of free film formulations that have been used for determining tensile testing are represented in Table 4. EC and HPMC were weighed and then dissolved in approximately 30 ml of solvent, respectively. An equal volume of methylene chloride and methanol was used as solvent for the formulations. The plasticizer concentration incorporated in each formulation was 30% of dry weight of polymers. After 30 min of plasticized, the solutions of formulations *F0-1*, *F2-4*, *F5-7*, *F8*, and *F9-10* were diluted to approximately 50, 45, 40, 35, and 30 ml, respectively, in order to reduce the viscosity. The resultant solutions were poured into a glass plate with 9-cm in diameter. They were set at an ambient temperature for 24 hr and were subsequently oven-dried at 45°C for 30 min to remove the residual organic solvents. The dried film was kept in desiccator until used.

Table 4 Compositions of free film formulations (weigh per plate).

Ingredients (g)	Formulations										
	F0	*F1*	*F2*	*F3*	*F4*	*F5*	*F6*	*F7*	*F8*	*F9*	*F10*
HPMC[1]	0.55	0.55	0.44	0.44	0.44	0.33	0.33	0.33	0.22	0.11	0
EC[1]	0	0	0.11	0.11	0.11	0.22	0.22	0.22	0.33	0.44	0.55
DBP[1]	-	0.165	0.165	-	-	0.165	-	-	0.165	0.165	0.165
DEP[1]	-	-	-	0.165	-	-	0.165	-	-	-	-
TEC[1]	-	-	-	-	0.165	-	-	0.165	-	-	-

[1] HPMC, EC, DBP, DEP and TEC are represented for hydroxypropyl methylcellulose, ethylcellulose, dibutyl phthalate, diethyl phthalate, and triethyl citrate, respectively.

2.1.2 Evaluation of Free Film Formulations

2.1.2.1 Thickness

A film specimen was cut into rectangular shape 0.5 cm in width and 4 cm in length. Six specimens were measured using a thickness tester and used in the following step. The thickness of each specimen was the average value of 5 separate measurements along the length of the specimen.

2.1.2.2 Tensile Testing

Tensile strength measurement was modified from the ASTM D-882 test (ASTM standards, D882; Okhamafe and York, 1987). The material testing machine (Instron 5500 Series, Instron Corporate Headquarters, MA) was used for measuring ultimate tensile strength, percent elongation at break and toughness of free film. The machine was equipped with a 1–kg-tension load cell. The cross-head speed was controlled at 10 mm/min. The cross-section area of the free film was calculated by multiplying the mean thickness with gauge width. The free film was clamped by an upper and lower grip then the machine was operated. Six determinations of each formulation (from **2.1.2.1**) were tested and calculated. The ultimate tensile strength and percent elongation at break were calculated from equations (9) and (10), respectively. Toughness or area under stress strain curve is a function of the work done (force × displacement) in breaking the film.

$$Ts = \frac{Bl}{Sc} \tag{9}$$

Where Ts is ultimate tensile strength, Bl is breaking load and Sc is cross-section area of the specimen.

$$\%E = \frac{Ls - Lo}{Lo} \tag{10}$$

Where E is elongation at break, Ls is length at breaking point and Lo is original length of the specimen.

2.1.3 Determination of DTZ HCl Solubility

Excess amount of DTZ HCl was added to various solvents such as phosphate buffer saline (pH 7.4), deionized water, ethyl alcohol, propylene glycol, isopropyl myristate, isopropyl palmitate, and polyethylene glycol 400, respectively, in air tight, light resistant container. The mixture was allowed to equilibrate in a shaking water bath at 32 ± 1°C for 24 hr. The supernatant was then filtered through 0.45 μm Millipore filter (Millipore, Bedford, MA). The concentration of DTZ HCl was measured by HPLC after appropriate dilution.

An HPLC system (CLASS-VP Software, Shimadzu, Japan) consisted of a UV detector (SPD-10A), a pump (LC-10AD), and an automatic injector (SIL-10AD). The wavelength of the UV detector was 240 nm. The flow rate was 1 ml/min. The retention volume of DTZ HCl was 8.6 ml using a reversed-phase column (Hypersil GOLD column, 5 μm, 150 x 4.6mm inner diameter, Thermo Electron Corporation, Bellefonte, PA). The mobile phase consisted of phosphate buffer, pH 3.0 / acetonitrile (50/50, v/v); the injected volume was 20 μl. Under these conditions, a good linearity and reproducibility were obtained between 0.2-100 μg/ml of DTZ HCl (modified from USP 25, Assay of diltiazem hydrochloride extended-release capsules).

2.2 Formulation Development of DTZ HCl TDDS

2.2.1 Preparation of Film Formulations Containing DTZ HCl

Film specimens composed of different ratios of HPMC and EC, DTZ HCl and plasticizer were prepared by a plate casting method (as described in **2.1.1**). Fixed amount of DTZ HCl was used as may be seen in Table 5. Hydroxypropyl methylcellulose (HPMC) and ethylcellulose (EC) were used as polymer matrix at various ratios of 10:0, 8:2, 6:4, 4:6, and 2:8, respectively. In the case of plasticizer, dibutyl phthalate (DBP), diethyl phthalate (DEP) and triethyl citrate (TEC) were used in various formulas as indicated in Table 5 at concentration of 30% of polymer matrix.

The dried film was kept in desiccator until used. The amounts of ingredients that have been used for preparation of film formulations as represented in Table 5.

2.2.2 Determination of DTZ HCl Content in Film Formulations

The drug content of the film formulations was evaluated by a HPLC method. A known weight of polymeric film as in Table 5 was dissolved and diluted subsequently with an equal volume of methylene chloride and methanol, and the content of DTZ HCl was determined.

Table 5 The amounts of ingredients used in film formulations containing diltiazem hydrochloride.

Ingredients	**Formulations**									
(ratio by weight)	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A9**	**A10**
DTZ HCl	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
HPMC	10	10	10	8	6	6	6	4	2	0
EC	0	0	0	2	4	4	4	6	8	10
DBP	3	-	-	3	3	-	-	3	3	3
DEP	-	3	-	-	-	3	-	-	-	-
TEC	-	-	3	-	-	-	3	-	-	-

2.2.3 Moisture Uptake Study of Film Formulations Containing DTZ HCl

Film specimens were cut into square shape (1 cm x 1 cm) and kept in a desiccator for 24 hr. Subsequently, the films were weighed (Ws) and transferred to another desiccator containing saturated sodium chloride solution (relative humidity 75%). After equilibrium was attained, the films were taken out and weighed (Wm). Moisture uptake capacity was calculated according to the following equation:

$$\text{Moisture uptake capacity (\%)} = \frac{(Wm - Ws)}{Ws} \times 100 \qquad (11)$$

2.2.4 Transparency of Film Formulations containing DTZ HCl

A strip of HPMC-EC film (12x30 mm) was mounted on the cell holder of the spectrophotometer (Model V-530, Jasco, Japan), and the film transmittance was measured at 600 nm against air as the blank. Three strips were determined to obtain the mean with standard deviation (Lin, et al., 1991).

2.2.5 Surface Topography

The surface topography of each HPMC-EC film prepared from each formulation was determined using a SEM (Jeol, JSM-5410LV Scanning Microscope).

2.3 An Evaluation of DTZ HCl TDDS

2.3.1 *In vitro* Drug Release Study

In vitro release of DTZ HCl from film formulations (in Table 5) were evaluated by means of dissolution tester (Erweka DT6 Dissolution tester, Erweka, Heusenstamm, Germany), using the paddle-over-disk method according to USP 25, <724> apparatus 5. Film specimens (12.57 cm^2) were fixed on the disk assemblies by means of pharmaceutical grade transfer adhesive (Cotran^{TM} PGTA, No.9871, 3M Pharmaceuticals, MN). The disk assemblies were put into vessels (500 ml water, 32°C) of dissolution tester (50 r.p.m.); a 5-ml sample was taken at 0.5, 1, 2, 3, 4, 6, 8, and 12 hr, respectively. An equal volume of water was immediately added after each sampling. The concentration of DTZ HCl was spectrophotometrically determined at 236 nm.

2.3.2 *In vitro* Skin Permeation Study

2.3.2.1 Skin Preparation

Porcine ears were obtained from a local slaughter house. The ears were cleaned with water to remove bloodstains. The epidermis was prepared by soaking the ear in water at 60°C for 45 seconds (Gao and Singh, 1998). The ear was removed from the water. The intact epidermis was teased off from dermis with forceps, washed with water and kept in the refrigerator at -40°C. The frozen skin was thawed at an ambient temperature before mounting on the diffusion cell.

2.3.2.2 Permeation System

The modified Franz diffusion cell with the diffusion area of 1.81 cm^2, was used. The receptor compartment (in contact with the dermis side of the skin) was filled with 14 ml of PBS, pH 7.4. The system was connected to a water bath to maintain the temperature at 37± 1°C.

2.3.2.3 Permeation Study

From section **2.2.2 - 2.2.5**, the suitable formulations were selected for further study. Various enhancers at a concentration of 10% (w/w) of dry weight of polymers were added to an appropriate formula. The amounts of ingredients used for preparation of film formulations in this section were represented in Table 6.

A thawed skin was mounted between the donor and the receptor compartments by means of a clamp and was hydrated in PBS for 1 hr. The receptor compartment was replaced with freshly prepared receptor medium, 37± 1°C. The film formulation was placed on the skin and then clamped between the donor and receptor compartments. At predetermined times, 1.0-mL sample was taken from the receptor compartment and equal volume of PBS was immediately added after each sampling. The concentration of DTZ HCl was analyzed by the HPLC method.

Table 6 The amounts of ingredients used in film formulations containing diltiazem hydrochloride for permeation study.

Ingredients	Formulations									
(ratio by weight)	A1	A4	A41	A42	A43	A44	A45	A46	A47	A5
DTZ HCl	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
HPMC	10	8	8	8	8	8	8	8	8	6
EC	0	2	2	2	2	2	2	2	2	4
DBP	3	3	3	3	3	3	3	3	3	3
IPM [1]	-	-	1	-	-	-	-	-	-	-
IPP [1]	-	-	-	1	-	-	-	-	-	-
NMP [1]	-	-	-	-	1	-	-	-	-	-
OA [1]	-	-	-	-	-	1	-	-	-	-
PEG [1]	-	-	-	-	-	-	1	-	-	-
PG [1]	-	-	-	-	-	-	-	1	-	-
Tw [1]	-	-	-	-	-	-	-	-	1	-

[1] IPM, IPP, NMP, OA, PEG, PG, and Tw are represented for isopropyl myristate, isopropyl palmitate, N-methyl-2-pyrrolidone, oleic acid, polyethylene glycol 400, propylene glycol, and Tween 80, respectively.

2.3.2.4 Determination of Permeation Parameters

As described by Barry (1983), the steady-state flux (J_{ss}), lag time (T_{lag}), diffusion coefficient (D), skin/vehicle partition coefficient (K), and apparent permeation coefficient (P_{app}) are defined by the following equations (12)-(14).

$$J_{ss} = \left(\frac{dQ}{dt}\right)_{ss} \bullet \frac{1}{A} = \frac{DKC}{h} \qquad (12)$$

$$D = \frac{h^2}{6T_{lag}} \qquad (13)$$

$$P_{app} = \left(\frac{dQ}{dt}\right) \bullet \frac{1}{A} \bullet \frac{1}{C_s} \qquad (14)$$

Where, A is the effective diffusion area;

h, the thickness of skin;

C, the constant concentration of the donor solution;

C_s, the drug concentration in the saturated solution and

$(dQ/dt)_{ss}$ is the steady-state slope.

2.3.2.5 Statistical Analysis

Results are expressed as the mean ± S.D. of at least four experiments. Kruskal-Wallis one way analysis of variance was used to test the statistical significance of differences among groups. Statistical significance in the differences of the means was determined by Mann-Whitney Rank Sum Test.